Patrick Williot · Guy Nonnotte
Mikhail Chebanov *Editors*

The Siberian Sturgeon (*Acipenser baerii*, Brandt, 1869) Volume 2 - Farming

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Foreword

This book on the Siberian sturgeon (*Acipenser baerii*) presented by my friend Patrick Williot is both a body of scientific data for almost 40 years on this species and its breeding and exemplary history of the development of a new species in aquaculture. But how has this "saga" started and how has Patrick Williot been able to devote his entire life of being a scientist to this species?

The story was born in the region that remained the last sanctuary of the European sturgeon (*Acipenser sturio*), Aquitaine, and its rivers and estuaries. In the 1970s, it seemed that this species was doomed to disappear if no protective measures were taken. This then led the CEMAGREF (old Irstea) team, of which at this time I had the responsibility, to launch with the support of national and regional authorities, as well as professionals, a research program on the biology of this species. This program would probably not have achieved its current development if a fortuitous event had occurred. In 1975, I was called by the deputy director of the Aquaculture Department of CNEXO (ancestor of IFREMER) Pierre Rouzaud, who asked me if we were interested in hosting 300 young Siberian sturgeon (300 g each) that CNEXO was to receive from its Soviet counterpart, VNIRO, in exchange for sea bass fry from the Palavas station. My response was immediate and positive, while we had no program for this operation!

We did have some advantages: an experimental breeding facility with warm waters from a thermal power station in Ambés, a young and motivated team in aquaculture, and an early knowledge of this family of fish through the work on local species. The reception of these early fingerlings was made at the Roissy Airport (where I rode in a van), with an immediate transfer with a transport container to our station. Then it was the start of a first work program on the diet of this species, for which we had no data. Collective work between CEMAGREF, INRA, and the subsidiary factory of Grands Moulins de Paris specializing in fish feed allowed to move quickly on this issue and even publish the first scientific paper on the subject.[1]

[1] Barrucand M., Ferlin P., Lamarque P., Sabaut J.J., 1979. Alimentation artificielle de l'esturgeon *Acipenser baeri*. Proc. World Symposium on Finfish Nutrition and Fish feed Technology, Hambourg, 20–23 juin 1978, Vol. 1, 411–421.

But everything remained to be known and learned to get the technical mastery of all the operations necessary to develop the breeding of this species. Indeed, until then, only the Soviet team had been working on it, but only on the reproduction and larval rearing, aiming on restocking of fresh and estuarine waters. Jointly conducted missions by CEMAGREF (P. Ferlin) and INRA (P. Lamarque) in the 1970s in the USSR and Iran showed that the larval rearing system (crucial phase for aquaculture) was absolutely not adapted to Western conditions. To feed the larvae and fingerlings of sturgeon, they first had to make a mashed cabbage (sort of "borsch") and then give it as food to small red worms (chironomids), which were recovered by hand in trays with light above (that they do not like!) and heating below (which attracts and concentrates them). On the other hand, in countries rich in native populations, obtaining eggs was done by sacrificing the females, while with our small population imported, we had to eventually reuse for other female pundits, developing a cesarean technique[2]. Many other and fundamental aspects for breeding also remained to be studied in terms of reproductive physiology, nutrition, diseases, etc.

This work started through an agreement between CEMAGREF and INRA, at the Donzacq station of INRA, but it was very soon necessary to create new facilities, such as the hatchery in Saint Seurin sur L'Isle, strengthen scientific laboratories, and train researchers and technicians, but above all find a leader to initiate and manage these actions. It is Patrick Williot who volunteered to take the lead in this ambitious research program that would be subject to his life of being a scientist.

His work had three consequences:

- An amount of scientific and technical knowledge on this species, subject of this book
- The development of a new aquaculture industry in France and abroad, creating jobs and producing high-quality products
- The ability to protect wild sturgeons, especially in Aquitaine, not only by controlling their capture but also by developing methods of breeding and larval rearing issued from the work of the team of Patrick

So I would like, as a prelude to this book, to pay tribute to the tenacious and efficient work of Patrick Williot and thank him for having contributed to the development of this sector and to the protection of these species that are so emblematic, the sturgeons. I also hope that this book will induce new vocations and develop new research in this area that has always fascinated me.

Boulogne-Billancourt, France

Philippe Ferlin
Former Chairman of OECD
Committee of Fisheries
General Inspector of Agriculture
and Fisheries (Hon.)

[2] The editor in chief of this two volumes (P. Williot) has been primarily trained in the former USSR in the 1970s. A publication (Charlon et Williot 1978) provided, among others, the different ways of rearing the live preys for sturgeon larvae (oligochete, dapnia, artemia) as well as basic surgery. In the early 1980s, ceasarian has been industrialized simultaneously in France and USA. Later on, obtaining of ovulated eggs without ceasarian has been developed in Russia (Podushka 1986, see also Chap. 27 in this issue).

Contents

Introduction to the Siberian Sturgeon Books with a Focus on Volume 2 Dedicated to the Farming of the Species

It has been recalled in the general introduction of the books (Williot et al. 2017) that the species first arrived in France in the mid-1970s as one of the preliminary examples of a French-Soviet cooperation network in the field of oceanology. Moreover, the very beginning of the story of this first arrival has been kindly described by one of the key persons (Ferlin 2017). Given that the species has became the support of sturgeon farming in France with the country as a pioneer, it seems useful to briefly present the main phases of the development of the farming of this non-native species in France.

Thanks to a quick survey on fisheries of migratory fishes in the Gironde-Garonne-Dordogne Basin (southwest of France) in the early 1970s (CTGREF 1973), some people were sensitized to sturgeon issues in France. In addition to previous alarms regarding the status of the European sturgeon (*Acipenser sturio*), the authors of the study assessed the loss of income for the professional fishermen and suggested some main lines to better manage future fisheries from an administrative point of view. Therefore, the eventuality of receiving some sturgeons in 1975 was immediately caught. Rapidly most of the juveniles of the Siberian sturgeon were held in a specific facility supplied by warm water coming from a power plant located along the Gironde Estuary some kilometers north of Bordeaux.

Despite the lack of experience and non-optimal rearing conditions, encouraging husbandry results were recorded regarding growth, feed acceptance, and food transformation by the fish. Additionally it has been shown that the species was able to tolerate unusual rearing conditions (Barrucand et al. 1978). In the meantime, two French scientists took the opportunity offered by the French-Soviet cooperation network to join a 3-month training course in the former Soviet Union in 1977 to get an experience of working with sturgeons. It is somewhat strange that this was achieved through the general policy of the Soviet Union considering sturgeon issues as extremely sensitive. The acquired experience has been published in detail (Charlon and Williot 1978). Thus, the accumulated knowledge on sturgeons allowed the setting up of a long-term conservation plan for the European sturgeon where the Siberian sturgeon has been used to set up methods and to acquire experience transferable to the European species (Williot et al. 1997, 2004, Williot and Castelnaud 2011). In other words, the Siberian sturgeon was thus considered as a biological model in the

late 1970s. But a key point remained; this was the mastering of the reproduction of the fish that arrived in 1975 in order to be completely independent from Soviet suppliers. During the same period, the French-Soviet network got in touch with P. Williot to request him to join the network as a French representative for aquaculture. The acceptance was a good opportunity to keep in touch with Soviet scientists and to improve our knowledge of the Soviet sturgeon plans that were synthesized later on (Williot 1984).

Regarding the French sturgeon research program, this decision proved to be effective soon after a time of new exchange of living material between both parties was on the table. The Soviet party proposed besters (*Huso huso* ♀ × *Acipenser ruthenus* ♂) and requested for a batch of sea bass, *Dicentrarchus labrax*. The French party could not envisage the bester for two reasons. The first reason is because of its hybrid status of which both parents, the beluga (*Huso huso*) and the sterlet (*Acipenser ruthenus*), were nonindigenous species in France even if the hybrid F1 was known to be fertile (Charlon and Williot 1978). The second reason is we already got some experience with the Siberian sturgeon, and we did not want to dilute our efforts with another sturgeon species. Indeed, a rapid agreement was obtained and a batch of fingerlings of Siberian sturgeon arrived in France in 1982. Despite several difficulties in different fields due to a lack of appropriate hatchery and of knowledge in the management of brood fish, successful reproductions and progenies were recorded in the first two consecutive years, 1981 and 1982 (Williot and Rouault 1982; Williot and Brun 1982). In the meantime, we learned from the Soviet counterparts that both batches of Siberian sturgeon originated from the Lena River population.

From this time, the French sturgeon research program consisted of two subprograms, one dealing with the restoration of the European species and the other one, the new one, focusing on the farming of the Siberian sturgeon. To develop the second subprogram, it was necessary to take precautions with the spreading of this nonnative species, particularly on the sanitary aspects. In addition to the strict control of sanitary status of specimens held in the research facility, it was decided that they should conduct preliminary tests for potential pathology in fish farms (with one exception, all were trout farms) supplied with different water temperatures, and these were carried out during the 1980s (Brun et al. 1991). As the results were considered acceptable (no species-specific pathology was recorded), some farmers decided to go into Siberian sturgeon production for the meat all the more so since a marketing study (1980s) concluded that there is a promising market regarding prices and volumes. And to supply the market, a large private hatchery was set up which benefited from the transfer of know-how with a well-identified brood stock from the research sector. The facility has operated since the very early 1990s, and the very beginning of farmed sturgeon occurred in Western Europe (Williot et al. 1993). Very rapidly, the fish market over Western Europe entered a crisis which made the previous basis of the economic functioning of the hatchery unrealistic due to lowest demand as previously expected. A solution for the managers has been to move the objective of production from meat to caviar with regard to the dramatic decline of wild Eurasian sturgeon populations which were the main suppliers for the market

(Williot et al. 2002). But this change requested a great amount of investment for stock constitution, and then most financial functioning of sturgeon farms changed as well. From this period, the production has slowly increased up to recently where the world caviar market entered a deep crisis with an important decrease of prices at farm gate due to an unexpected competition because the present assessment of the world market (the production) is still lower than the one assessed in the 1980s by Williot and Bourguignon (1991) of about 550 t.

In the former Soviet Union, the interest of Siberian sturgeon farming has been pinpointed in the late 1970s (Berdichevsky et al. 1979). In their review on sturgeon farming in the USSR, Barannikova (1987) did not mention the Siberian sturgeon as an object of production. As the species is reported by Chebanov and Billard (2000) as representing about one third of the Russian production, this strongly suggests that the development of this production started during the 1990s as for France (Williot et al. 1993). Due to (1) the easy availability (at the start thanks to the large French hatchery aforementioned) of its larvae, fingerlings, or fertilized eggs, (2) its freshwater status which made the species easily farmable in freshwater without any question as for a lot of other sturgeon species, and (3) its great plasticity already pointed out, the farming of the species has spread worldwide, e.g., from China to Uruguay (Wei et al. 2011; Bronzi et al. 2011).

The present volume is divided into six unequal parts, the last being devoted to some methods. The first deals with the reproduction and the early ontogenesis as illustrated by larval weaning. Reproductive cycles, reproduction sensu stricto, and sperm cryoconservation are the main issues. The second part is focused on growing with different approaches on food and feeding including some consequences of feeding such as excretion. In the field, a chapter deals with the effects of incorporation of soy in food. The third part aims at giving examples of production-quality-related issues by sensorial approach and off-flavor phenomena of caviar on one side. Quantity aspect is provided by the main figures of world production of sturgeon products (meat and caviar) on the one hand and by an example of fingerling production for restocking a large Siberian catchment on the other hand. Four chapters are gathered into a long-term management of brood stock because they deal with either genetic variability of brood stocks, the manipulation of the genome, or the interest of breeding hybrids with the Siberian sturgeon. Three chapters are under the health status umbrella as two are related to the welfare of the species, one being focused on plasmatic indicators and the other being a synthesis in the field, and the last chapter is an updated synthesis on the immunology of the species. The fifth part reports two documented analyses of the ecological risk of installation of the species elsewhere than its natural geographical extension. Finally, three methods are given: in vitro incubation of ovarian follicles, echography enriched with a lot of photos, and oxygen demand in farming.

Audenge, France — P. Williot
La Teste de Buch, France — G. Nonnotte
Krasnodar, Russia — M. Chebanov

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About the Editors

Mikhail Chebanov Mikhail Chebanov, Prof. Dr. sc., is professor of the Department of Aquatic Bioresources and Aquaculture of the Kuban State University and director of the State Regional Centre for Sturgeon Gene Pool Conservation "Kubanbioresursi" of the Ministry of Natural Resources (Krasnodar, Russia). He has developed the technology of all-year-round reproduction of different sturgeon species as well as the selection and breeding program and the formation of the largest in Russia sturgeon living gene bank. He has developed and widely implemented the method of ultrasonic diagnostics of sturgeon for optimization of brood stock management. In 2004 he was awarded the Russian Federation Government Prize in Science and Technology for the "development and implementation of technology of control reproduction and commercial rearing of sturgeon." For many years, Prof. Chebanov served as director of the South Branch Federal Center of Genetics and Selection for Aquaculture, consultant of FAO UN and convener of the ad hoc Working Party on Management of Sturgeon of the European Inland Fisheries Advisory Commission (EIFAC/FAO), and deputy chair and member of the Sturgeon Specialist Group of the World Conservation Union (IUCN). Prof. Chebanov is a cofounder and member of the board of directors of the World Sturgeon Conservation Society (www.wscs.info) and has chaired numerous workshops and conference sessions, including most International Symposia on Sturgeon (1993–2017). He is the author of more than 170 scientific papers, including 12 books.

Professor Guy Nonnotte Doctor of the University of Strasbourg, Guy Nonnotte was appointed research officer at the CNRS in the Laboratory of Comparative Physiology of the Regulations at F-Strasbourg in 1974. He conducted original research especially on cutaneous respiration and skin ionic exchanges on many models of freshwater and seawater fish. In 1986, he joined the Laboratory of Neurobiology and Comparative Physiology (CNRS and University of Bordeaux I, F-Arcachon) and pursued research on extracellular acid-base balance and cell volume regulation in fish exposed to environmental changes and pollutants with a specific interest for the model "Siberian sturgeon." He supervised a thesis on the toxicity of ammonia for the Siberian sturgeon in collaboration with Dr. Patrick

Williot. He took also the opportunity to initiate an important collaboration concerning the respiration, the acid-base balance, and the physiological effects of environmental stress for the Siberian sturgeon with the Laboratory of Animal Physiology at F-Brest. In 1994, he was appointed professor of animal physiology (fish physiology) at the University of Brest (France) and managed the Laboratory of Cellular Biology and Physiology. He wrote numerous publications in international reviews and managed several thesis on fish physiology. He was also appointed for numerous evaluations and as jury of thesis by INRA, IFREMER, INSERM, and CNRS. In 2002, he was designated as vice-president of the University of Brest, responsible for research, and in 2007, emeritus professor of the same university.

Patrick Williot Patrick spent the three quarters of his professional career primarily taking on the challenge of the disappearance of the European sturgeon, *Acipenser sturio*. To achieve this task, he mobilized all the means of research (both applied and fundamental lines, biological model, national and international cooperations) and management (private-public partnership, transfer of know-how, installation of a new experimental facility, search for regular financial support). Patrick has developed his activity around aquaculture-conservation biology. Patrick stimulated the development of sturgeon farming primarily in France based on the biological model, the Siberian sturgeon, *Acipenser baerii*. He has been the kingpin of the first International Symposium on Sturgeon held in Bordeaux in 1989 where he launched the concept of an international association for the conservation of sturgeons which was installed (the WSCS; wscs.info) some years later. Later on, he edited the peer-reviewed proceedings (*Acipenser*), served as scientific committee member of further International Symposia on Sturgeon as well as the sturgeon specialist group of the IUCN, and organized other symposia and workshops.He succeeded in obtaining for the first time the controlled reproduction of farmed European sturgeon in 2007 which saved the species from extinction and opened the door for restoration. Patrick initiated and carried out the edition of a book on Biology and Conservation of the European Sturgeon (2011) and on the biology and farming of the Siberian sturgeon which is in process. Patrick published about 90 papers in peer-reviewed journals or books.

Part I

Reproduction and Early Ontogenesis

Reproductive Cycles in Sturgeons with a Special Focus on the Farmed Siberian Sturgeon

26

Patrick Williot and Mikhail Chebanov

Abstract

The chapter reports the published data on biological cycles and/or puberty in sturgeons for both wild and farmed brood stocks. There are three main lines to document these data, the analysis of large-scale sampling or landings, the analysis of sampling with previously tagged fish and the echography which has a remarkable efficacy. Both biological characteristics are lower for farmed fish as compared with their wild counterparts. This is mainly due to water temperature and food availability which favour the growth in farmed conditions. There are observations which suggest that the more precocious and the more frequent the spawning, the smaller the fish. A yearly spermiation is the most common figure in farmed males, while 2-year period is the most frequent for the females. Thanks to a 5-year survey on a given cohort of farmed Siberian sturgeon, the figure of the biological cycles is as follows: there are two types of cycles, the single and the recurring. In the first batch, the 2-year cycle is the most frequent (32%), while the 3-year cycle accounts for nearly 11%. In the second batch, a 2-year cycle represents 20%, and the 1 + 2 or the 2 + 1 recurring cycles account for nearly 32%. Altogether the 2-year cycles may represent up to 84%. This explains that the relative number of yearly spawnable females exhibits a 2-year variation from a minimum of 35–45% up to a maximum of 59–63%. There are data showing that the yearly number of mature females depends on time lapse of domestication duration with variable percentages depending on generation and period.

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Keywords
Sturgeon • Siberian sturgeon • Biological cycles • Frequency • Puberty

Introduction

Sturgeons are long-lived fish with late puberty and non-yearly oogenesis in wild population (Williot et al. 2011a). This means they are able to spawn many times, and therefore the notion of biological cycles arrives naturally. This data on biological cycles are closely related to the puberty and therefore to environmental conditions, i.e. easy accessibility to spawning grounds, water temperature, food availability, and absence of pollution. In fish farming conditions, usually most of the aforementioned characteristics are, more or less, under control to allowing production planning. Despite an apparent simplicity, obtaining such population characteristics is not often an easy task.

The interest of documenting biological cycles was already underlined with regard to the European sturgeon (*Acipenser sturio* L.) by Williot et al. (2011b). Indeed, this is a key point with regard to population dynamic studies in fisheries to assess yearly reproductive potential in either sustainable management of population or in conservation biology (population viability analysis (PVA) and further minimum viable population (MVP)) as illustrated by Jaric et al. (2011) and in managing a farmed brood stock. With regard to the last objective, it is key point to perform an audit of the production of Siberian sturgeon fish farm (Williot unpublished) all the more so since a large part of sturgeon farming is devoted to caviar production. Due to the financial lure of this activity, some economical studies on the production as illustrated in the white sturgeon were carried out (*Acipenser transmontanus* R.) (Logan et al. 1995). And to do that, authors made the most probable hypothesis on biological cycles thanks to very preliminary results in the field.

The objective of the present short chapter is to provide a synthesis on the different aspects of the biological cycles of the Siberian sturgeon, i.e. how this can be determined? Which results are available on the species? And which is the sturgeon-related literature in the field? What differences are between wild and farmed populations?

26.1 The Means to Determining the Biological Cycles and Puberty

There are three main lines in achieving this task. The first one consists in analysing a large-scale sampling or landings further documented on size, sex and gonadal development as illustrated by Van Eenenaam et al. (1996) on a 2-year spawning migration period for the Atlantic sturgeon (*Acipenser oxyrinchus*) in the Hudson River where gonadal development was the focus issue thanks to a tissue gonad sampling protocol. The authors examined the iteroparity by the presence of atretic ovarian follicles in the samples was used as a tool to analyse the biological cycles. In the past, the studies were most likely restricted to the analysis of landings knowing

relationship between biometry and gonadal development. The second line deals with sampling fish previously tagged. Then, fish samples are checked for sex discrimination and staging gonads through hormonal and vitellogenin (Vtg) dosages. Among the hormones are steroid hormones which are linked to sex gender and to the development stage of the gonads of the fish, i.e. oestradiol (E2), testosterone (T) and 11-testosterone (11-KT) (see Williot 2011 for a review) or a combination of those aforementioned as in wild stellate sturgeon (*Acipenser stellatus*) with the use of ratios E2/11-KT or E2/T (Ceapa et al. 2002). The third line is based on echography of the fish. This method has two important advantages, there is no need to sample the fish, and for experienced people, the efficacy is remarkable. The method has been popularised by Chebanov and Galich (2009, Chap. 49 for an extensive presentation on the species). The two last lines rely on previous setup relationship between either hormone or Vtg content or echographic image and direct observation of gonad tissue sample as described in details for the species by Le Menn and Pelissero (1991, Chap. 14). This is achieved thanks to biopsies, and further tissue gonad observations and description are the standard reference for many studies including the new currently used methods such as echography. When individual time longitudinal is needed on a fish population (wild or farmed origin), tagging is a key tool, and for some year this has become easier with a PIT tag inserted carefully in a dorsal part of the fish under the skin and further read with the ad hoc device.

26.2 Biological Cycles and Puberty in Sturgeons

Overall published data are shown in Table 26.1. A general outlook has revealed an extreme diversity in the biological characteristics. However, a more in-depth analysis allows pointing out the following aspects. These characteristics are absent for a lot of sturgeon species, including those which were previously subject to an intense fishery and for the majority of wild populations of the species that are documented. This illustrates the difficulties to document some biological characteristics especially in a changing environment. Additionally, the impact of temperature is major as highest temperatures show a reduction in both the age at puberty and the length in the interval between two successive gametogenesis.

Both puberty and time lapse between two successive reproduction events show a great variation, i.e. the fish exhibit a large range of occurrence of this characteristics. Further, they decrease in farmed fish as compared with wild ones. In the field, sturgeons are no exception. This is a well-known observation which most likely results in better rearing conditions especially regarding water temperature and food availability. Males matured earlier than females. In farming conditions, the males exhibit mostly a yearly spermiation, while 2 years is the most frequent interval between two successive spawning. The only exception might be for the sterlet (*Acipenser ruthenus*) of which nearly half of females spawned each year. The species, being one of the smallest, is the most precocious among the sturgeon family and most likely its life span is shorter than most other sturgeon species. These observations suggest the more precocious and the more frequent the spawning, the smaller the fish.

Table 26.1 The biological cycles, puberty and duration of sexual activity in sturgeons

Species	Wild (catchment)/ farmed (origin)	Puberty (age in year) and frequency (%)	Biological cycles (years between two reproductions)	Length of sexual activity (years)	Observation	Source
Acipenser oxyrinchus	Wild (Hudson River)	12 (5%), 14 (33%) ♂ 14 (4%), 16 (18%) ♀	1 ♂ (suggested) >1 ♀ (suggested)	8 ♂ 22 ♀	Samples on two spring spawning migrations	Van Eenenaam et al. (1996)
Acipenser transmontanus	Wild	10–12 ♂ 15–32 ♀				PSMFC[a] in Doroshov et al. (1997)
	Farmed	3–4 (80–90%) ♂ 6–14 with 50% at 8 ♀	1 ♂ 2 (67%) ♀		20 °C and 10–14 °C for 6 months before spawning	Doroshov et al. (1997)
Acipenser ruthenus	Farmed (originated from Danube brood fish)	1.0 ♂ 2.2 ♀	1 (40–50%) ♀ 2 (27–34%) ♀ 0.6 ♂ 0.9 ♀	Declining trend in reproductive capacity for fish >14–16 years	5–27 °C Along a 5-year experimental period Warm water fish farm with annual sum of temperature >8600 degree days and artificial wintering	Williot et al. (2005) Chap. 49 Chebanov et al. (2008)
Acipenser gueldenstaedtii	Farmed	2.5 ♂ 5.2 ♀	1.2 ♂ 1.4 ♀		Warm water fish farm with annual sum of temperature >8600 degree days and artificial wintering	Chap. 49 Chebanov et al. (2008)

Acipenser stellatus	Farmed	2.5 ♂ 4.3 ♀	0.8 ♂ 1.4 ♀	Warm water fish farm with annual sum of temperature >8600 degree days and artificial wintering	Chap. 49 Chebanov et al. (2008)
Huso huso	Farmed	4.0 ♂ 7.2 ♀	1.5 ♂ 2.0 ♀	Warm water fish farm with annual sum of temperature > 8600 degree days and artificial wintering	Chap. 49 Chebanov et al. (2008)
Acipenser sturio	Wild	13–15 ♂ 19–22 ♀			Williot et al. (2011a, b)
	Farmed	13 ♀	2 (67%) with variable cycles ♂	10–12 to 23–25 °C First ovulating ♀	Williot and Rouault (2008) Williot et al. (2009)
Acipenser baerii	Wild (Ob River) (Lena River) (Lena River)	15–18 ♂ 16–20 ♀ 9–10 ♂ 11–12 ♀	1–2 ♂ 3–4 ♀ 2–3 ♂ 3–5 ♀		Kozhin (1964) Sokolv and Vasiliev (a) (1989)
	Wild (Siberian catchments)		3–5 ♂ And ♀		Ruban (2005) and Chap. 1 for more details
	Farmed (originated from Lena River brood fish)	4+ ♂ 7–9 ♀	1.5–2 ♂ And ♀	Konakovo warm water fish farm with annual sum of temperature > 5400 degree days	Akimova (1985) Petrova et al. (2008)

(continued)

Table 26.1 (continued)

Species	Wild (catchment)/ farmed (origin)	Puberty (age in year) and frequency (%)	Biological cycles (years between two reproductions)	Length of sexual activity (years)	Observation	Source
	Farmed (originated from Lena River brood fish)	6 ♂ and 7 ♀	1 (12%), 2 (47%), 4 (23%), 3 (17%) ♀ 1 (50%) ♂ 2 (64%) ♀		Two cohorts (1974 and 1981) 7 ± 2 – 16 ± 2 °C Given cohort with fish 8.5–12.5 year 5–8 to 25–28 °C	Williot et al. (1991) Williot and Brun (1998) See Table 27.2 for more details with ♀
	Farmed	2.5 ♂ 4.0 ♀	1.0 ♂ 1.3 ♀		Warm water fish farm with annual sum of temperature >8600 degree days and artificial wintering	Chap. 49 Chebanov et al. (2008)
Huso dauricus	Farmed	9 ♂ 10 ♀	1–2 ♂ 3 ♀(suggested)		Warm water fish farm with annual sum of temperature >4600 degree days	Rachek and Svirskiy (2008)
Acipenser schrenckii	Farmed	7 (43%)–8 (100%) ♂ 9 ♀	1 ♂ 2 ♀		Warm water fish farm with annual sum of temperature >4600 degree days	Rachek and Svirskiy (2008)
Acipenser nudiventris	Farmed	3.0 ♂ 5.0 (35–40%) ♀ 6.0 (75–80%) ♀	1–1.5 ♂ 1.5–2.0♀		Warm water fish farm with annual sum of temperature >8600 degree days and artificial wintering	Chebanov (unpubl.)

[a]Pacific States Marine Fisheries Commission, 1992

Thanks to a 5-year survey on the 1984 cohort of farmed Siberian sturgeon composed at the beginning of 575 tagged specimens (Williot and Brun 1998), it has been possible to describe all the combinations in the reproduction cycles which are reported in Table 26.2. Two types of oogenetic cycles were shown, single and recurring, the second being the most frequent with 56.6%. Within each of the aforementioned types, a 2-year period is far the most frequent interval between two spawning events with 32.2% and ~20% for simple and recurring biological cycles, respectively. When adding the recurring cycles 1 + 2 or 2 + 1, the maximum frequency of 2-year biological cycle may account for nearly 84%. This explains that drawing the percentage of spawnable females (females which can be successfully reproduced a few months later) of a given cohort along the 5-year experimental period, we got a 2-year cyclic figure of which the maximum is close to 59–63%, while the minimum fluctuates between 35 and 45% (Fig. 26.1). Half of the males matured each year.

Table 26.2 Frequency of different ovarian cycles post puberty observed on the 1984 cohort of the Siberian sturgeon along a 5-year period (Williot 1997; Williot and Brun 1998)

Ovarian cycle (years)	Frequency (%)
Single	
1	0.5
2	32.2
3	10.7
Recurring	
1	3.6
2	19.9
1 + 2 (or 2 + 1)	31.6
1 + 3 (or 3 + 1)	1.5
Total number of females = 196	Total 100%

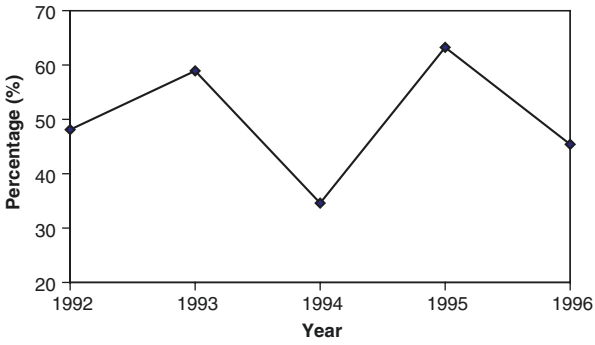

Fig. 26.1 Yearly changes in the relative number of spawnable female of Siberian sturgeon in the 1984 cohort along a 5-year experimental period (drawn from data in Williot and Brun 1998)

Table 26.3 Percentage of yearly maturing females of Siberian sturgeon of domesticated form and Lena-1 breed during different age periods (Petrova et al. 2008)

	Domesticated form (natural generations)		Lena-1 First generation			Lena-1 Second generation	
Age (years)	Average 1973–1976	1991	1981–1982	1984–1987	Average 1981–1987	Average 1988–1991	1991
7–10	45.0±7.8	57.5±12.3	75.5±8.2	51.9±7.9	59.8±5.8	53.2±6.2	71.7±7.1
11–15	72.7±8.5	56.2±13.0	67.9±7.8	56.9±4.0	60.6±4.0	63.8±4.8	80.2±4.6
Average long term[a]	60.2±5.8	56.8±12.0	65.6±5.3	55.3±4.0	58.6±3.0	58.8±5.3	76.4±4.3

[a]Average percentage of annual maturing females from the first spawning in 2006

A long-term study has been carried out by Petrova et al. (2008) to check the changes in yearly mature females of the species reared in warm water according to domestication time lapse (Table 26.3). Within the first generation, the percentage shows a decreasing trend from the first period (1981–1982) to the following one (1984–1987). In contrast, within the second generation, the percentage shows an increasing trend from 1988 to 1991. And these last results appear to be higher than the data collected from what the author call natural generations. Obviously, this trend should be confirmed to have a reference period of equal duration. Additionally, a standardisation of data collection should be set up in order to make possible comparison of these last findings with those aforementioned in the section.

26.3 Some Conclusions

Whatever the sturgeon species, reproduction or caviar production needs raising the fish as long as they reach their puberty (at the minimum). Due to the large range of time lapse to get this development stage as well as the heterogeneity even within a given cohort, several cohorts need to be raised simultaneously to allow a steady reproduction. Then the knowledge of biological cycles and related age at puberty for the most fish is a key point to plan and control the reproduction. And those characteristics depend on the rearing conditions, water temperature (being a crucial point) and on generation. Finally, our attention is paid to another factor which might be of importance; this is the potential changes in the yearly number of mature females that may depend on the duration of the domestication period.

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Controlled Reproduction of Farmed Siberian Sturgeon *Acipenser baerii* Brandt

27

Patrick Williot and Mikhail Chebanov

Abstract

The present chapter is a synthesis of the available data related to the management of the reproduction of the Siberian sturgeon under farmed conditions. Some reproduction-related biological characteristics are briefly quoted. Preselection of potential brood fish is related in detail as well as pre-spawning holding regime with a focus on water temperature. Definite selection of reputed ready-to-spawn brood fish is described. As for preselection, the usefulness for the definite selection of the rate of migration of the germinal vesicle towards the animal pole (the polarization index, PI) is presented. The two tools for males (echography and texture of testis) are evoked. With regard to females, plasmatic indicators and observations of ovarian follicles are described with a focus on the powerful of the in vitro maturation competence (IVMC). The different alternatives for hormonal stimulation, including some unusual practices when management somewhat failed, are described in conjunction with time scale working agenda. Collection, observations, stocking and management of gametes are successively presented. The many possibilities for the so-called de-adhesive treatment are described as post-fertilization treatment. Some data on yields and perspectives are discussed. In general, we provide practical comments and a lot of illustration both with photos and graphs.

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Keywords

Acipenser baerii • Controlled reproduction • Preselection criteria • Water temperature • Polarization index • Selection of ready-to-spawn brood fish • In vitro maturation competence • Hormonal stimulation • Collection of gametes • Management of gametes • De-adhesive treatment

Introduction

In captivity, usually sturgeons are not providing their gametes naturally; a hormonal stimulation is needed (Gerbilsky 1941; Ginzburg and Dettlaff 1969; Charlon and Williot 1978).

As a result, all sturgeon-farming purposes, whatever the progenies are destined for, restocking or long-term farming for human consumption, are in need of a set of methods allowing to reproduce the sturgeons with the best effectiveness as possible.

However, sturgeons are long-live fish with some different ecological traits depending on the species. They show a late puberty, mostly a non-yearly oogenesis, great heterogeneity in the gametogenesis within the specimen of a given cohort and an anatomical specificity which altogether complicate the setting up of the aforementioned set of methods. With an emphasis on the Siberian sturgeon, a synthesis was published in the field in the early 2000s (Williot 2002). More recently a very detailed set of methods dedicated to Ponto-Caspian sturgeon species was given (Chebanov and Galich 2011).

The aim of the present chapter is to update the data on the Siberian sturgeon with a chronological description of all the steps from the pre-adult until the fertilized eggs given that reproduction-related methods are similar whatever the age of the brood fish. Some perspectives are ending the chapter.

Finally, it is noteworthy that although the present chapter deals with the reproduction of the Siberian sturgeon, part of the present data might be also of use for the management of female brood fish destined to produce caviar especially in the countries where caviar is now produced with ovulated eggs (Chap. 38).

27.1 Some Reproduction-Related Sturgeon Biological Characteristics

Mostly, Siberian sturgeon stops feeding prior to spawning (Ruban 2005; Chap. 1). Given the phylogenetic position of sturgeons, one may wonder whether their endocrine functioning is similar to that of the teleost fish. Despite the far lower number of studies on sturgeon species, there are suggestions to show that sturgeon endocrinology is very similar to that of teleost fish (Webb and Doroshov 2011). Although many uncertainties are remaining as well as the role (if any) of some hormones (Chaps. 16 and 17), this strongly suggests that the two-cell layer (visualized together in Fig. 27.1) model, developed on the teleost fish to explain the endocrine pathways along the life span (Nagahama 1987), is likely to occur in sturgeon. The aforementioned statement is strongly supported by the fact that the GTH-1-FSH and GHT2-LH were clearly identified in the Siberian sturgeon (Quérat et al. 2000). At the end of the normal course of sexual cycle,

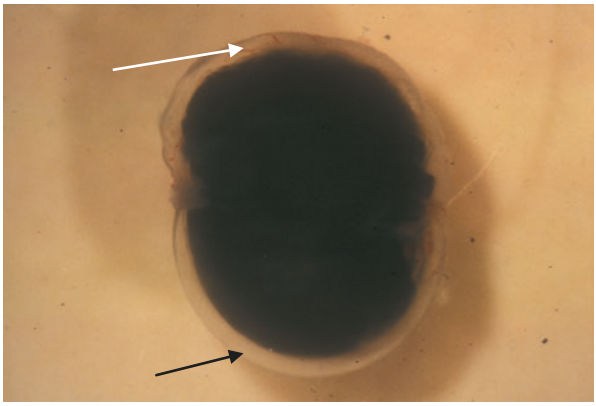

Fig. 27.1 Partially hand-denuded ovarian follicle. The two-cell layer (theca and granulosa external and internal layer, respectively) is visualized together at the upper part of the follicle (*white arrow*) which points the red blood vessels of the cells. The *dark arrow* shows the external layer of the Zona radiata that will be responsible for the adhesive property of post-fertilized eggs (credit Patrick Williot)

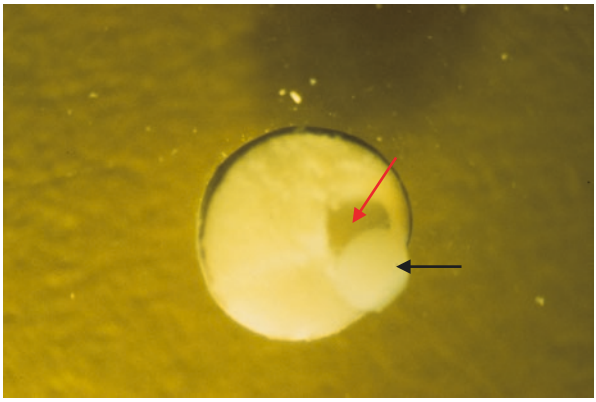

Fig. 27.2 Post boiling nonmature fully grown oocyte showing the nucleus (or germinal vesicle) (the *dark arrow* on the *right part* of the photo) out from its place materialized by a hole (*red arrow*) closed to the animal pole of the oocyte (credit Patrick Williot)

oocyte's maturation processes take place prior to ovulation. One of the major processes of the maturation is the breakdown of the envelopes of the nucleus shown in Fig. 27.2. This is needed to prepare the female gametes to be ready to be fertilized by spermatozoa. And this is the result of the indirect action of a progestagen-like hormone which is produced by the internal cell layer surrounding the oocyte, the follicular cells (Le Menn and Bennetau-Pelissero Chap 14). The action of this hormone, the so-called maturation-inducing steroid (MIS), will be mimicked in a biotest (IVMC)[1] to select the upcoming ready-to-spawn females (Sect. 27.5, Chap. 48). With regard to the Siberian sturgeon, the nature of the molecule is still debating (Chaps. 16 and 17).

The development of the gonads is asynchronous within a given fish (Williot and Brun 1998). This means that at a given time, one may observe different sized ovarian follicles during the most part of the oogenesis (Fig. 27.3) with the exception of the very end of oogenesis where only two very different sizes can be observed, the large one, i.e. those that will be ready to complete their maturation and further ovulate very soon, and the very small ones which represent the future generation (Fig. 27.4) (Williot and Brun 1998). And therefore the ovary should not present anymore greasy tissue as shown in Fig. 27.5.

[1] IVMC = in vitro maturation competence.

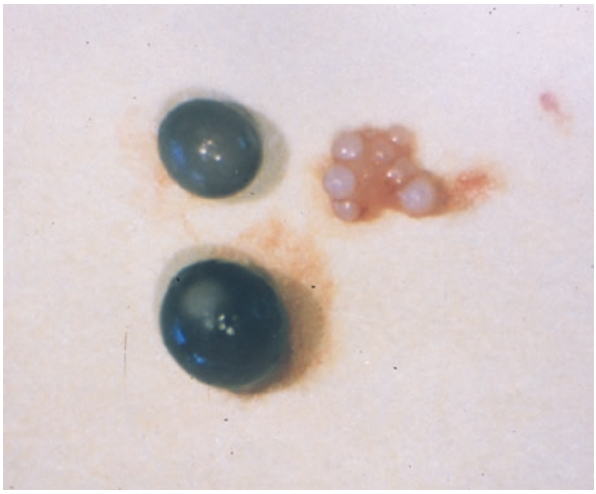

Fig. 27.3 Different sizes of ovarian follicles at a given time from an under-matured brood fish female (credit Patrick Williot)

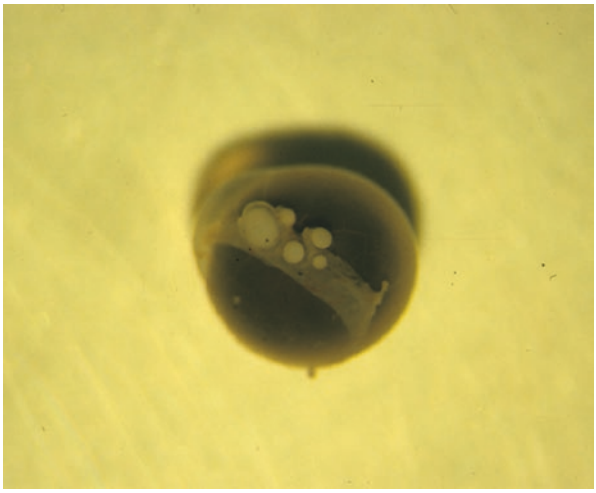

Fig. 27.4 Fully grown dark grey ovarian follicle after boiling with small white ones representing the next generation already exhibiting different sizes (credit Patrick Williot)

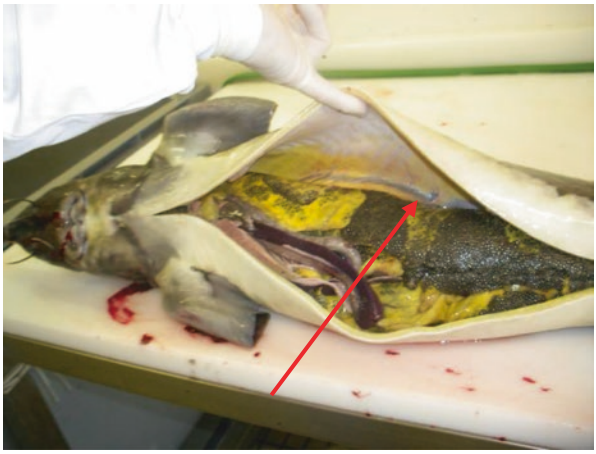

Fig. 27.5 Under-matured ovaries illustrated by some still greasy yellowish tissues. The *arrow* shows the left funnel, i.e. the entrance of the oviduct (credit Patrick Williot)

Most of the aforementioned processes are under the influence of water temperature. Naturally, sturgeon species inhabit northern hemisphere, and with the only exception of the Chinese sturgeon, *Acipenser sinensis* (Zhong-Ling and Zhao Yan 1991), they spawn from the end of winter (e.g. the beluga, *Huso huso*) up to the beginning of summer for the most northern species, namely, the Siberian sturgeon (Ruban 2005, Chap. 1). Due to the long duration of the oogenesis including the lasts steps, the time scale of the present chapter is successively months, weeks, days and hours.

The last point that has to be underlined is an anatomic characteristic. Sturgeon are equipped with rather long oviducts (Wolffian ducts) which represent about one third long of the body cavity (Fig. 27.5), the first part of which ends by a one-way valve, and then join together with the Müllerian ducts upstream the genital pore (Conte et al. 1988).

27.2 Preselection of Potential Brood Fish

Due to heterogeneity within a cohort, each specimen has to be checked individually. The objective is to discriminate the brood fish of which the gametogenesis is advanced enough to justify a peculiar management with the hope of further reproduction in the upcoming months (Akimova 1985).

There are two methods to observe the development of the gametogenesis (Table 27.1). The first one consists in carrying out sampling tissue gonads (ovarian follicles (Fig. 27.6) or testis sample) through a laparotomy (Williot and Brun 1998) which allows classifying the females depending on their mean diameter of the ovarian follicle (Fig. 27.7) and further depending on the advancement of migration of nucleus towards the animal pole as expressed by the polarization index (Fig. 27.8). This might be extremely useful in performing this screening a few months before the potential reproduction period as it allows keeping only the fish of which the ovarian follicle diameter is larger than the 2.9 mm (Williot and Brun 1998). The second is based on echography (Chebanov and Galich 2009; Chap. 49), as illustrated by a device in works at field (Fig. 27.9). The accuracy is far better for the second; however the primer remains the reference to standardize any other

Table 27.1 Comparison of the two methods (completed and updated after Williot and Sabeau (1999)) for sexually developed brood fish

Criterion	Sampling	Echography
Invasiveness	Yes	No
Yield (no of fish per day)	50–100	1300–1700
Effectiveness	~97%	~97%
Needed materials	Current surgical tools, stereomicroscope and microscope	Echograph
Financial aspect		Costly but redeemable
Accuracy of the observation	Very good	Very good

Fig. 27.6 Sampling ovarian follicles with a hollow probe (credit Patrick Williot)

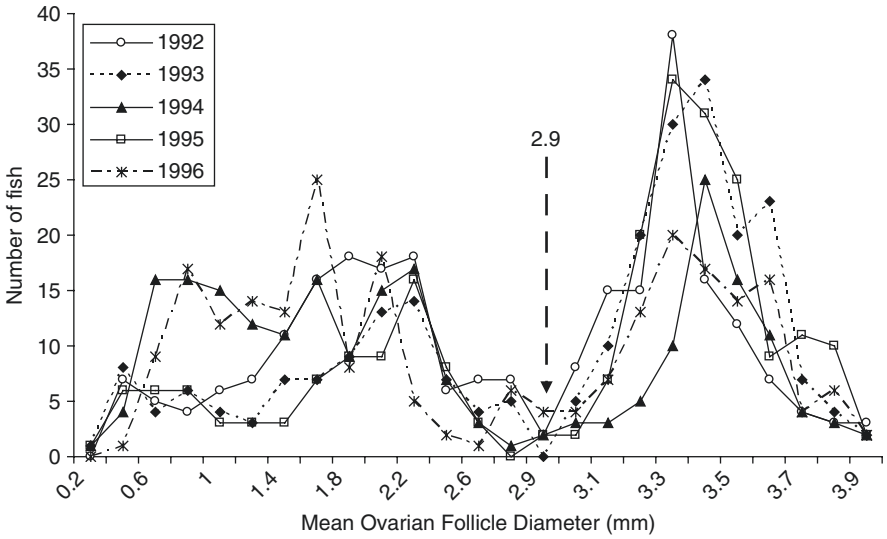

Fig. 27.7 Distribution of the mean ovarian follicle diameter from brood fish belonging to a given cohort at a time of preselection (October-November) along the 5-year experimental period (after Williot and Brun 1998). Diameter values of 2.9 mm were use as a minimum to preselect the potentially mature brood fish in the upcoming months

method. Moreover it provides some additional data such as the possibility to determine the degree of migration of the nucleus towards the animal pole (polarization index, PI) which partly explains that it is more time-consuming than the echography.

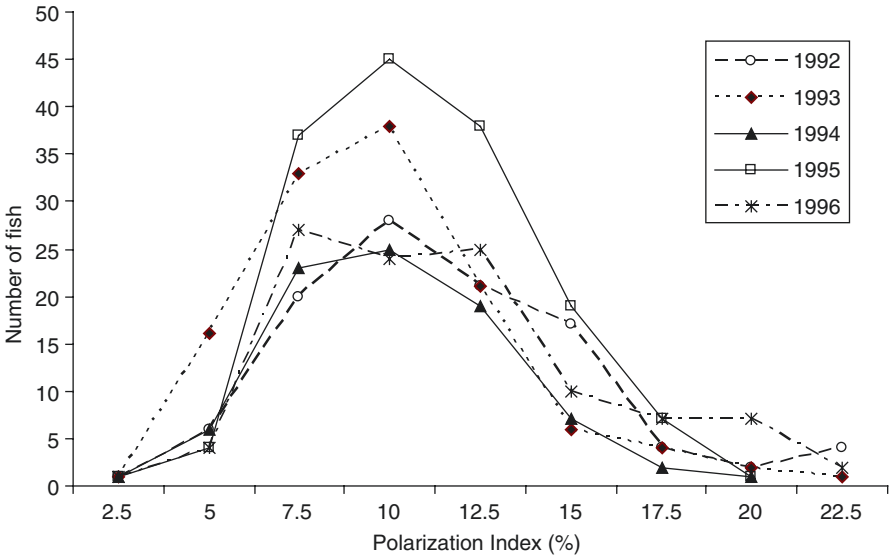

Fig. 27.8 Distribution of the polarization index (PI) from the ovarian follicles of preselected brood fish as mentioned in Fig. 27.5 (after Williot and Brun (1998)). The smaller the PI, the more advanced the migration of the germinal vesicle (GV)

Fig. 27.9 Operating echograph on site (credit Patrick Williot)

27.3 Management of Potential Future Brood Fish

Only the preselected females exhibiting a mean follicle diameter ≥2.9 mm are concerned in autumn as shown in Fig. 27.7, of which the distribution of the polarization index is shown in Fig. 27.8 with a modal value closed to PI ~10%. In fact, there are the female brood fish that will be potentially able to be spawnable in the upcoming months. Among those that have to be isolated are the females of which an ovarian follicle sample exhibits atretic figures (Fig. 27.10a, b). With regard to the retained females, their developmental stage corresponds to the end of stage 4 and the very beginning of stage 5 as defined by Le Menn and Pelissero (1991, Chap. 13). One of the key issues is the vernalization or the wintering period which is needed for the brood fish to achieve the last phases of their gametogenesis in the best conditions, i.e. to allow the production of good-quality gametes. That is why Kazanskiy (1981) proposed to hold the sturgeon brood fish (especially the Russian sturgeon, *Acipenser*

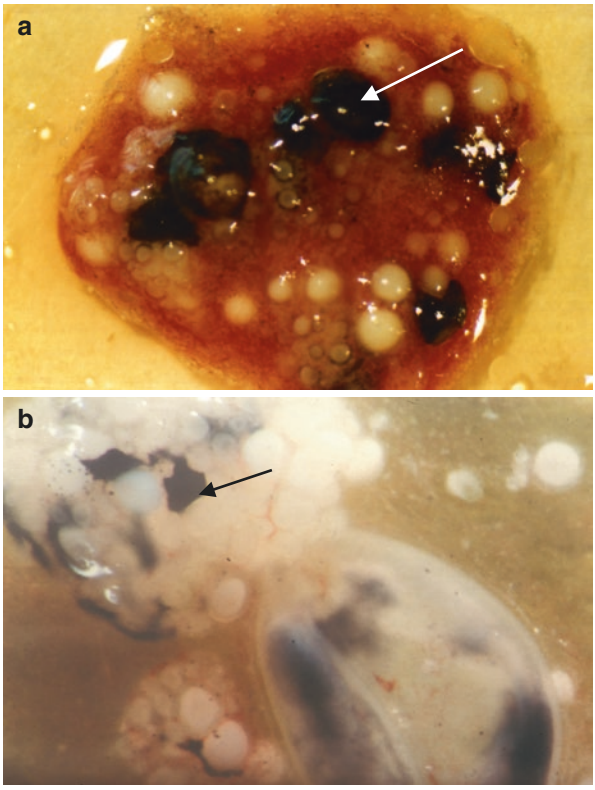

Fig. 27.10 (**a**) Advanced stage of atresia. Some ovarian follicles still exhibit a large size and a dark colour (*white arrow*) (credit Patrick Williot). (**b**) More advanced stage of atresia. The *white arrow* shows the remaining envelopes of a large atretic ovarian follicle. The *black arrow* shows a small black heap representing the last step of the atresia (credit Patrick Williot)

gueldenstaedtii) for about 3–4 months at a temperature 4–5 °C lower than the lowest spawning temperature for the species. The vernalization was successfully applied by Williot et al. (1991) on Siberian sturgeon in holding the brood fish in a water temperature of 7 ± 2 °C instead of the current yearly water temperature of 16 ± 2 °C. Later on, Williot and Brun (1998) reported a maximum range of 5–8 °C vs. 25–28 °C on the same species. Rather simultaneously, Chebanov and Savelyeva (1999) documented the wintering conditions for the Russian sturgeon, the stellate sturgeon, (*Acipenser stellatus*), the beluga (*Huso huso*) and the sterlet (*Acipenser ruthenus*). As sturgeon farming is developing worldwide partly by valorizing better rearing conditions, especially with regard to water temperature, the following information were reported by Chebanov and Galich (2011) in using warm water in raising the Siberian sturgeon. The duration of wintering can be reduced to 1–1.5 months. Along the course of overwintering, a short-term increase (up to 7 °C) or a decrease (up to 2 °C) is permissible.

Fish with skin damage should not be exposed to temperatures of lower range without prior holding at 8–10 °C for recovering. In addition, both decreasing and increasing temperature gradients (at the initiation and the end of the vernalization period) should not exceed 1–2 °C and 2–3 °C per day for females and males, respectively (Kazanskiy 1981).

During most of this wintering period, brood fish have to be food deprived to mimic the current ecological trait of the fish that stop feeding during their spring reproductive migration.

Fish holding facilities might be of varied types in shape sizes and material. Tanks, raceways and ponds might be used. They could be made of concrete, plastic film and earth. As an example, tanks of volume above 40 m^3 and depth above 1.5 m or "Kurinsky-type" running water concrete ponds of 105 m × 17 m size or ponds of 1000–4000 m^2, which can be divided into several sections by net walls, for holding of males/females of different stage of gonad development are used. The stocking density of brood fish in ponds during wintering period should be less than 25 kg/m^3. Whatever the holding structure, attention should be paid to the oxygen content that should be kept at a level consistent with the standard metabolism or over of the fish (Chap. 50).

27.4 Definite Selection

The time of the definite selection depends on production planning for progenies, and this depends on marketing demand. In fact, in temperate northern conditions, this might be organized from December to late April when water temperature control is available. As noted earlier, this is the time to choose a priori the best brood fish that will hormonally stimulated very soon. With regard to females, only preselected brood fish in autumn are concerned, i.e. those at stage 4 of the gonadal development. The different means are available to select males and females are going to be described. As much as possible, the accuracy of the methods will be provided.

Fig. 27.11 Upper-head spawning dress of a mature male (credit Mikhail Chebanov)

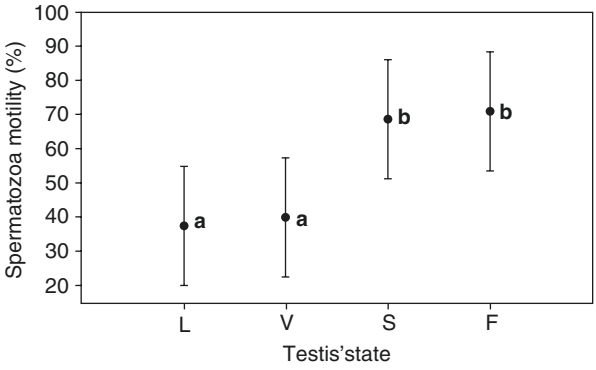

Fig. 27.12 Dependence of motility (mean) of fresh spermatozoa on the testis texture determined 1 month before the reproduction. Vertical bars are 95% confidence interval. *L* liquid, *V* viscous, *S* soft, *F* firm. Different letters correspond significant differences at $p < 0.05$ (after Williot et al. (2000))

27.4.1 Males

The definite selection of mature males can be performed, thanks to external appearance, echography and observation of a piece of tissue gonads. In some case, ripe males exhibit a whitish upper-head spawning dress (Fig. 27.11). For experienced people, echography proved to be an effective means. Finally, it has been shown that the texture of the tissue testis might be a very good indication as well in allowing to select the best males with regard to further motility of spermatozoa which is significantly better when the texture is either soft or firm as compared with liquid or viscous (Fig. 27.12). It is worth noting that this last finding can be in contrast with the common opinion.

27.4.2 Females

Many ways have been explored to select the best potentially spawnable sturgeon females, and Siberian species is one of the few sturgeon species on which the more extend studies were carried out. There are three means to achieve the objective, which are mostly complementary especially for the two last. The first group consists

in searching for plasmatic indicators of use in either assessing the potential readiness for spawning or in contrast to point out the brood fish which already entered in atresia. The second deals with the observation of ovarian follicles, and the third is focused on a biotest that mimics in vitro the oocyte's maturation, the in vitro maturation competence (IVMC).

27.4.2.1 Plasmatic Indicators

The first group of plasmatic indicators deals with the readiness of spawnable female. Indeed this supposes that the vitellogenic phase is achieved, i.e. the step of accumulation of reserves into the oocytes which is driven by estradiol (E_2) which leads to the production of vitellogenin (Vtg). These molecules should be at their lowest level as shown for E_2 in the Siberian sturgeon, where low level of the steroid corresponds to high level of embryogenesis. A similar outcome is shown for the Vtg by Fujii et al. (1991) in bester (*Huso huso* × *Acipenser ruthenus*) and later on in Siberian sturgeon where low level of Vtg is positively correlated with high level of embryogenesis (Fig. 27.13 and 27.14). An indirect dosage of Vtg could be assessed by Ca dosage, this ion being very closely linked to the Vtg as shown in the white sturgeon (*A. transmontanus*) by Linares-Cazenave et al. (2003). Even the aforementioned results might be used in the understanding of last phases of oogenesis; their usefulness in decision-making for female to be hormonally stimulated is not accurate enough because this might represent a rather long steady-state period for the females and does not really give information on the physiological status of the brood fish.

The second group of plasmatic indicators relies on the setting up of means able to detect early atresia, and they were developed on the white sturgeon (Lu et al. 2011; Webb and Doroshov 2011). This might be of use especially when brood fish are not regularly controlled for their advancement of oogenesis.

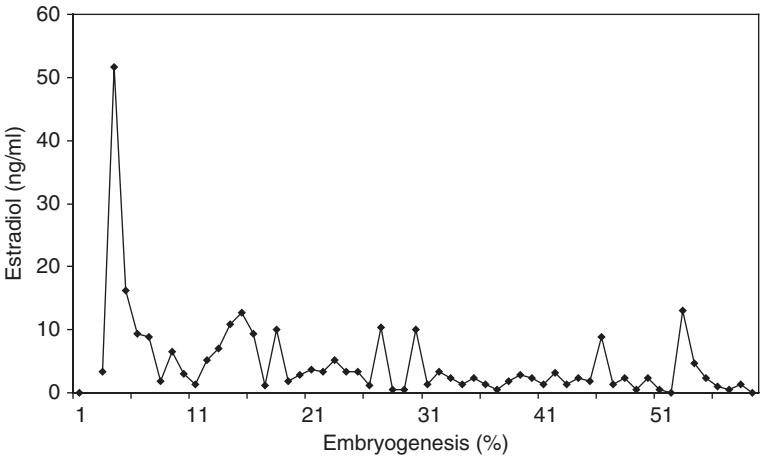

Fig. 27.13 Relationship between the plasmatic content of estradiol and embryogenesis success of the fertilized eggs of the corresponding females (after Williot (1997a))

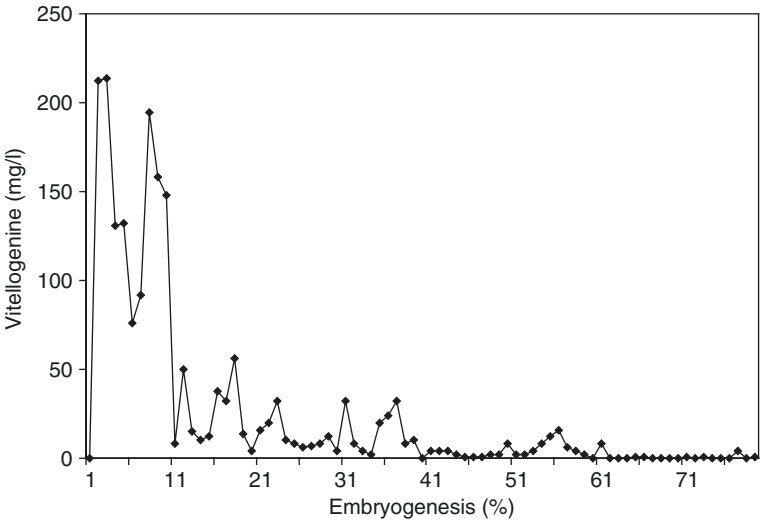

Fig. 27.14 Relationship between the plasmatic content of vitellogenin and the embryonic success of the fertilized eggs of the corresponding female (after Williot (1997a))

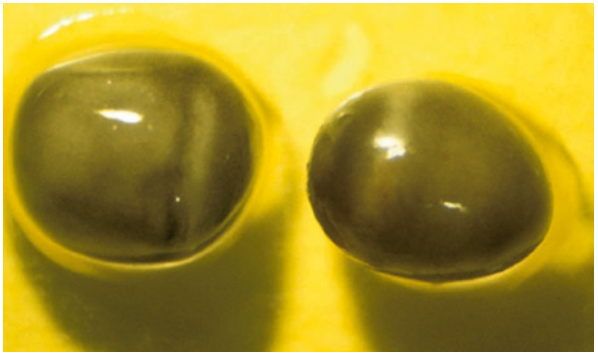

Fig. 27.15 Normal fully developed ovarian follicles. Note the rings surrounding the animal pole (credit Patrick Williot)

27.4.2.2 Observations of the Ovarian Follicles

The present group of means relies on the sample of ovarian follicles. This can be performed in a very secure way for the fish, and an appropriate realization does not require any post cross-stitching. To properly achieve this task, appropriate tools are very helpful as described by Kazanskiy et al. (1978).

The sample should not contain any greasy tissue as shown above (Fig. 27.5) for under mature ovaries, and it should exhibit large-sized homogenous ovarian follicles. Only the small whitish next generation should be present. The outlook of ovarian follicles should be "normal" as shown by example on Fig. 27.15. Indeed, different drawings can be encountered, from a rather clear greenish one to an almost dark colour except for the animal pole which mostly remains clearer and surrounded by coloured annulae. No abnormalities should be present such as a marble

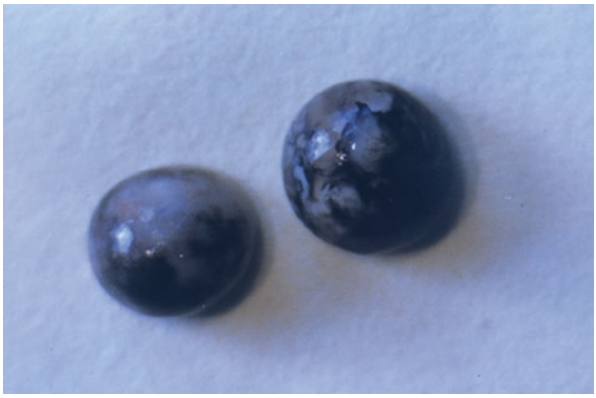

Fig. 27.16 Primary visible sign of atresia. Overmature fully grown ovarian follicles showing a marbled appearance (credit Patrick Williot)

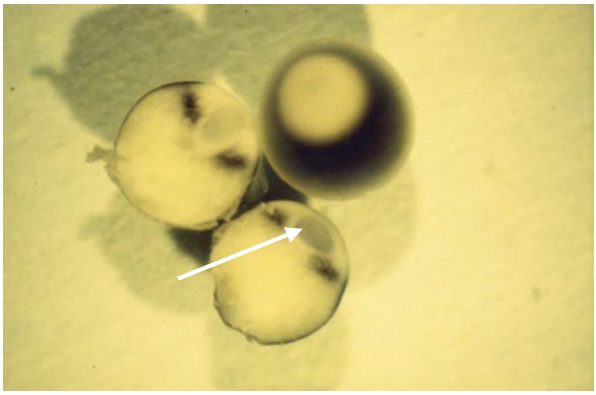

Fig. 27.17 Post boiling fully developed ovarian follicles exhibiting a depigmented animal pole together with dark spots in the yolk surrounding the germinal vesicle (*white arrow*) (credit Patrick Williot)

appearance which is a sign of over-maturation (first visible steps of atresia) (Fig. 27.16). Another somewhat unusual figure consists in a depigmented animal pole and incorporation of black pigments within the vitellus and surrounding the germinal vesicle (Fig. 27.17). The origin of the peculiar pigmentation outlook remains unknown even though signs of overmaturation have been suspected (Kornienko 1973). A comparative study of the influence of the blackish pigmentation of the vitellus on reproductive performances showed that pigmented eggs were more prone to mortality during the second part of the embryogenesis and no differences were recorded from hatching up to 14dph in terms of survival and growth (Williot 1998).

Once the external observations are realized, it is easy to prepare the ovarian follicles (boiling them for about 3 min and further rapidly chilling them to favour the coagulation of the vitellus) to measure the advancement of germinal vesicle towards the animal pole after cutting each piece by a median plan through the pole in order to reveal the nucleus (Figs. 27.18 and 27.19). Therefore it is possible to determine the polarization index (PI) according to Kazanskiy et al. (1978). And the last authors proposed to keep a threshold value PI ≤ 0.07 for further hormonal stimulation. Later

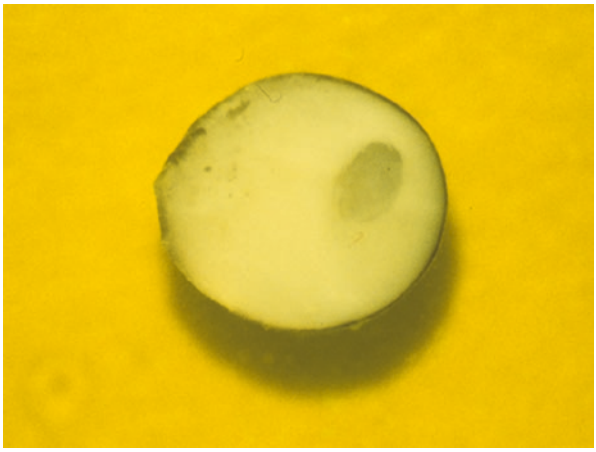

Fig. 27.18 Post boiling fully developed denudated ovarian follicle with migrated germinal vesicle. PI ~ 0.1 (credit Patrick Williot)

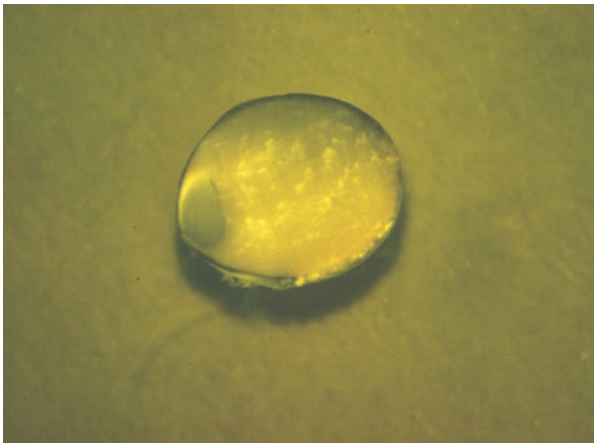

Fig. 27.19 Post boiling fully developed and denudated ovarian follicle with an almost completely migrated germinal vesicle. PI ~ 0.02 (credit Patrick Williot)

on, it has been shown that PI might be not always correlated with a more powerful way to examine the spawnability of Siberian sturgeon brood fish, a biotest based on the in vitro competence of ovarian follicles to mature (IVMC) (Fig. 27.20) (Williot et al. 1991). Do in practice, within a batch of ovarian follicles, those pieces that exhibited the incapacity to mature revealed by the presence of the hole of the germinal vesicle which is still visible (called GV[2]) and those that matured (called GVBD[3]) of which the hole is not anymore visible are counted separately. The more elevated the GVBD, the more mature the female of which the ovarian follicles were extracted from. PI and IVMC were shown to be the most potent predictors for ovulation in stimulated white sturgeon as well (Lutes et al. 1987). Indeed, PI values $\leq$0.08 may correspond to values of IVMC in a large range, from 42 to 100% (Fig. 27.20) which

[2] GV = germinal vesicle.

[3] GVBD = germinal vesicle breakdown.

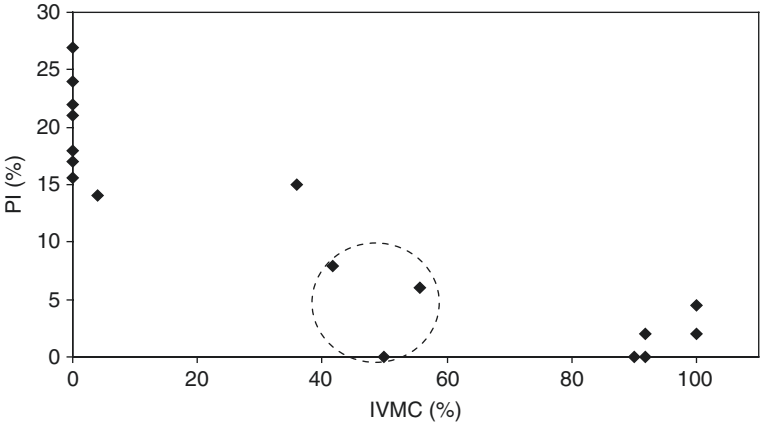

Fig. 27.20 Relationship between polarization index (PI) and in vitro maturation competence (IVMC) (modified after Williot et al. (1991)). The *three dots* within the *dotted circle* correspond to advanced migratory germinal vesicle and moderate IVMC

Table 27.2 Grouping of females on the basis of the oocyte polarization index (PI) and recommendations on programming of reproduction (modified from Chebanov and Galich (2011))

Group	PI range (%)	Group of females	Recommendations on programming of reproduction
I	PI < 0.04	Overripe	Subjected to fattening till next spawning season
II	0.04 ≤ PI < 0.10	Ripe 1 (hormonal response)	Instant gonadotropic stimulation while reaching low spawning temperature
III	0.10 ≤ PI < 0.12	Ripe 2	May be held for 2–3 days upon reaching the spawning temperatures
IV	0.12 ≤ PI < 0.15	Near to spawn	Hormonal injections administrated after 7–14 days holding at spawning temperatures
V	0.15 ≤ PI ≤ 0.18	Spawnable	20–40 days holding at spawning temperature prior to injection

means that females with rather low PI may exhibit very variable physiological status and then lead to a large range of yield post the hormonal injections. Indeed, three (i.e. 37%) out of the eight females, of which the PI is ≤0.08, showed a moderate value of IVMC 42–56% (dotted circle), too low to expect a good results after the hormonal stimulation. Other IVMC usefulness will be extensively developed later on in the sub-section.

Whatever, in case of fish reared in warm water with well-controlled artificial wintering, the availability of PI allows to classify (*allow + to + infinitive*) the females in different groups according to the values of PI and then to their degree of advancement of the oogenesis as already mentioned earlier (Fig. 27.7) or below with more accuracy (Table 27.2). Females from groups II and III might be used subsequently without recurrent biopsy. The PI in mature oocytes of females from groups IV and

Table 27.3 Duration of pre-spawning holding period of Siberian sturgeon broodstock based on the oocyte polarization index (PI) and temperature holding conditions (modified from Chebanov and Galich (2011))

PI (%)	Sum of effective temperatures, degree days	Length (days) of holding at different temperatures			
		9–10 °C	12–13 °C	14–16 °C	17–18 °C
0.10	30–50	5–8	3–6	2–5	1–3
0.11	60–70	7–10	4–7	3–6	2–4
0.12	90–100	9–12	5–9	4–7	3–5
0.13	120–150	10–14	9–12	7–8	5–7
0.14	170–200	12–15	10–14	9–12	7–10
0.15	210–250	15–18	12–17	10–14	9–12
0.16	260–300	18–22	15–20	12–16	–
0.17	350–400	21–25	17–22	14–21	–
0.18	410–500	30–40	25–30	20–25	–

V should be further re-examined, depending on timing of their readiness to spawn. The specimens from group V, which exhibit high values of PI which has not changed after holding at spawning temperatures for 20–40 days, should be considered as immature and destined for fattening. With regard to fish of which PI value is ≥0.12, i.e. groups IV and V in Table 27.2, an appropriate pre-spawning management is recommended. It is based on the effective water temperature sum (expressed in growing degree days) (Chebanov and Galich 2011). Holding should be conducted within the optimal range of spawning temperatures, excluding any short-term temperature elevations beyond this range. For this purpose the less mature specimens should be held at lower spawning temperatures, and the thermal gradient (as rate of temperature increase) should be lower before the hormonal stimulation. Failure to follow this requirement causes desynchronization in oocyte maturation that results in poor hatchery quality of eggs. Recommendations are given below on holding duration depending of PI and water temperature (Table 27.3).

As regards the males to males, they are capable for spawning even after short-time holding at spawning temperatures; therefore, the most effective way to keep their reproductive capacity is to hold them at low temperatures. In the case of extended postponed holding after males at spawning temperature, they can get overripe.

It has been mentioned above that in vitro maturation competence (IVMC) is more powerful than PI. Briefly the method consists in incubating ovarian follicles in a given media completed with a hormone in standardized conditions. When ovarian follicles are mature, the action of the hormone results in the breakdown of the envelopes of the germinal vesicle shown in Fig. 27.2, and then its trace is getting invisible and is therefore qualified of GVBD for germinal vesicle breakdown. The more elevated the GVBD rate, the more mature the ovarian follicles are and the more spawnable is the female. Details on the implementation of the method are given in Chap. 48. Not only might the powerful of the biotest be better than PI, but also it was shown that its value may change in a few days, then being representative of a changing physiological status of brood fish and therefore improving the decision-making time for hormonal stimulation (Williot et al. 1991). Later on, the efficacy of the method has been

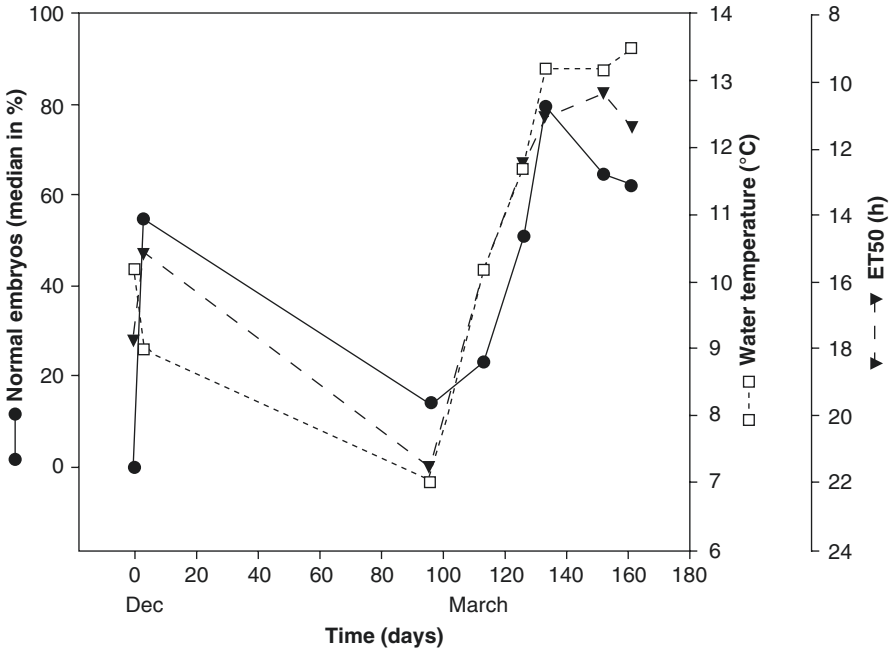

Fig. 27.21 Relationship between water temperature from which female brood fish are coming from, the in vitro maturation competence (IVMC) of the ovarian follicles expressed by the time (h) to get 50% (ET_{50}) of maturation represented by the germinal vesicle breakdown (GVBD) and the embryogenesis (after Williot (1997a, b), Goncharov et al. (1999))

increased in taking into account of the delay in observing the potential GVBD in vitro. From the response curves, it is possible either to interpolate or compute the effective time to get 50% of maturation (ET_{50}) allowing comparisons between females (Williot 1997a, b) and observing that the lower the ET_{50}, the better the results post hormonal injection in terms of the embryogenesis rate (Williot 1997a, b; Goncharov et al. 1999) as shown in Fig. 27.21. The figure also highlights that there is a correlation between ET_{50}, the embryogenesis rate and the difference between both rearing temperatures of brood fish females and that retained for spawning. Indeed, the worst results, the greater the difference between the two temperatures because fish were transferred rapidly from one to another, which points out again the management of water temperature as a key factor, as it has been aforementioned in the section.

27.5 Hormonal Stimulation

27.5.1 Possible Pre-spawning Corrections of Reproductive Status of Brood Fish

When holding duration at spawning temperatures slightly differs from those presented in Table 27.3 or when changes in water temperature has not been in the admissible range, it is expedient administrating triiodothyronine (T3) prior to

hormonal stimulation in order to enhance ovulatory success at a daily dosage of about 20 mg kg^{-1} BW over 2–4 days (Dettlaff and Davydova 1979; Dettlaff et al. 1993; Smol'anov 1979). The authors reported that there were no positive effects when atresia was already engaged.

Sorokina (2004) suggested the administration of ascorbic acid (vitamins C) and α-tocopherol (vitamin E) during the pre-spawn holding of brood fish to increase reproductive performance, enhance fecundity and accelerate the synchronization of oocyte maturation in wild female and hence attain higher egg fertility rates. This technique involves the application of above-mentioned pharmaceuticals: ascorbic acid (10% solution, 100 mg mL^{-1}) and α-tocopherol acetate (30% solution, 300 mg mL^{-1}). For 2-week course (four injections per day) of C and E vitamins, single administration (10 mg and 15 mg per kg of female weight, respectively) was recorded the best result.

Matishov et al. (2007) advise to perform cyanocobalamin (vitamin B12) 500 μg/mL injections (or 50 μg per kg of body weight) the day following E and C vitamins administration. The cyanocobalamin amplifies protective functions and enhances immune status and consequently responses to stressors in females, alongside with improvement of their hatchery characteristics (e.g. egg fertility and survival rates of progeny).

27.5.2 Hormonal Stimulation

There were two distinct sources of hormones that can be used to stimulate maturation-ovulation and spermiation for female and male sturgeon brood fish, respectively: sturgeon and carp pituitaries and analogous of luteinizing hormone-releasing hormones (LHRH). The use of sturgeon pituitary was primarily used in the former USSR (Gerbilsky 1941; Charlon and Williot 1978). Later on as the sturgeon pituitaries were difficult (if not unavailable) to obtain in Western countries, carp pituitaries were successfully applied on the Siberian sturgeon (Williot and Rouault 1982; Williot and Brun 1982; Williot et al. 1991; Goncharov et al. 2001) and on the white sturgeon (Doroshov and Lutes 1984). The currently applied dosages are 1–2 μg kg BW^{-1} and 4–6 μg kg BW^{-1} for males and females, respectively. Rapidly, several teams turned about new more securing and stable sources such as the analogous of luteinizing hormone-releasing hormone (LHRH) of which most recent generalized spelling is GnRH (gonadotropin-releasing hormone). This is a decapeptide produced by the hypothalamus which acts on the hypophysis, and the advantage of which is the possibility to be artificially produced. Depending on biological groups, the formula may change. It was demonstrated that one of the GnRH of the brain of the Siberian sturgeon was similar to that of the mammalian mGnRH (pGLU-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH2) (Leprêtre et al. 1993) and later on in the Russian sturgeon (Lescheid et al. 1995). In the field, more details are given by Kah and Adrio (Chap. 12). Several analogous of GNRH proved to be effective in farmed sturgeon, e.g. Doroshov and Lutes (1984) in the white sturgeon and Williot (1986, 2002) and Williot et al. (2002) in the Siberian sturgeon. However, regarding the above-mentioned results, it is recommended to use the mGnRH at the current dosage of 2–5 μg kg BW^{-1} and 5–10 μg kg BW^{-1} for males and females, respectively. Whatever the hormone, when (1) the migration of the germinal vesicle is not completely

achieved, (2) the water temperature management was inappropriate and (3) there are preliminary signs of deterioration of the ovarian follicles, a two-injection sequence is recommended with 12 h apart as shown in the European sturgeon (Williot et al. 2000a, 2009). The first injection corresponds usually to a tenth of the total dose.

Prior to injection, hormone should be carefully dissolved in physiological solution with characteristics similar or close to that of plasma to favour its diffusion. It is not necessary to handle the fish; this can be done directly on the fish being in the tank. The syringe should be then progressively emptied before being taken off.

Regarding the male brood fish, if necessary, they can be hormonally stimulated several times at a few weeks interval as reported for the *A. sturio* by Williot (1997). In order to optimize the success of such a practice, it is recommended to simulate brief vernalization in first reducing the water temperature and then increasing it over a 2–3 week's cycle.

Finally, when the hormonal injection should be performed? It is recommended to organize the working timetable as follows taking in mind that the water temperature is a key factor. The first decision-making factor depends on the water temperature that leads to an assessment of the latency in ovulation (see next section). Second, favour work at day time, and then given the shorter latency duration and the time you accept to start working with female give you by back calculation the time at which the females should be hormonally stimulated. With regard to the male, there are two useful indications: the first is that as much as possible, the availability of the best sperm batches should be determined before any work with the female, and the second is provided by the changes in spermatozoa motility which is at its highest level when collected 36 h post hormonal stimulation (Fig. 27.22). Therefore, knowing the

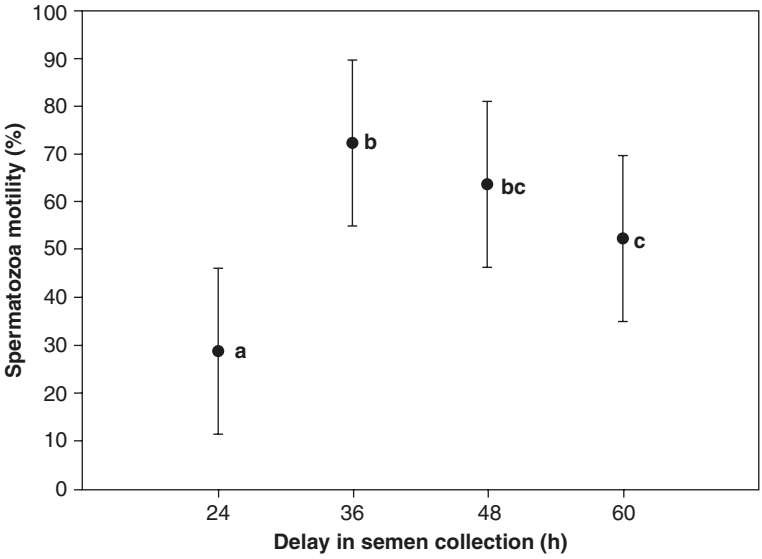

Fig. 27.22 Dependence of motility of fresh spermatozoa (mean) on the delay in semen collection post hormonal injection (after Williot et al. (2000b))

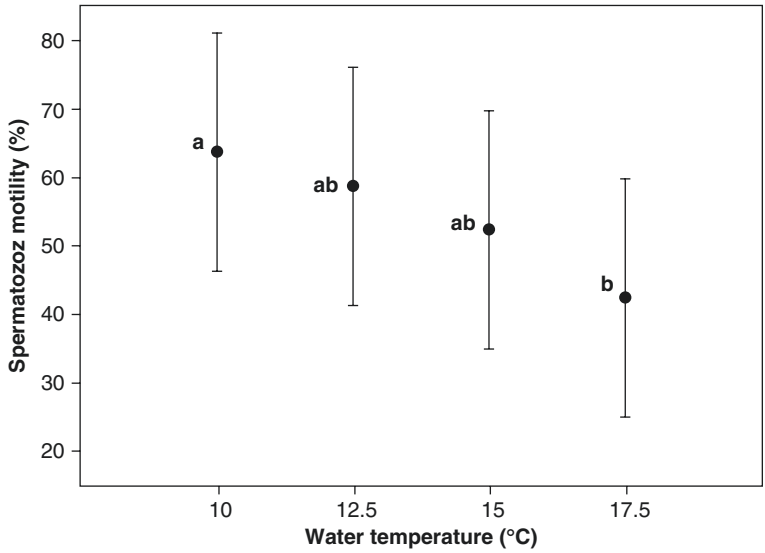

Fig. 27.23 Dependence of motility of fresh spermatozoa on water temperature during hormonal treatment for Siberian sturgeon reared at 10 °C (after Williot et al. (2000b))

needed time to collect and observe the sperms before potentially starting working with the females gives the proximate time to hormonally stimulate the male.

The last key factor at this stage of the controlled reproduction process is again the water temperature. The optimal range for the species, suggested by Sokolov and Malyutin (1977), is 11–16 °C. A study on the influence of the water temperature on sperm motility was performed by Williot et al. (2000a, b) who demonstrated that the motility declined from 10 °C up to 17.5 °C with no significative differences from 10 to 15 °C (Fig. 27.23). The last value was retained at the experimental level to standardize all the process for both sexes (Williot 2002). It is worthy to note that some moderate reproduction success might be carried out closed to the upper level admissible for the species (18–19 °C) as shown by Vizziano et al. (2006).

27.6 Collection of Gametes

There are different ways to observe the onset of ovulation. The first is the observation of ovulated eggs on the bottom of the tank. This is made easier when females are each in one tank. The second is to handle the fish (Fig. 27.24) by starting from about 2 h before the minimum expected time for ovulation. It happened that the ovulated eggs cannot be expelled because of a plug within the oviducts that should be taken off.

The available series of data of the latency in ovulation are plotted in Fig. 27.25. The three upper curves are closed to each other especially. However, it remains that the two curves (upper and lower limits) reported and modified by Chebanov and Galich (2011) delimit a lower range of values than the two others.

Fig. 27.24 Evaluation of the onset of ovulation in a mature Siberian sturgeon female (credit Mikhail Chebanov)

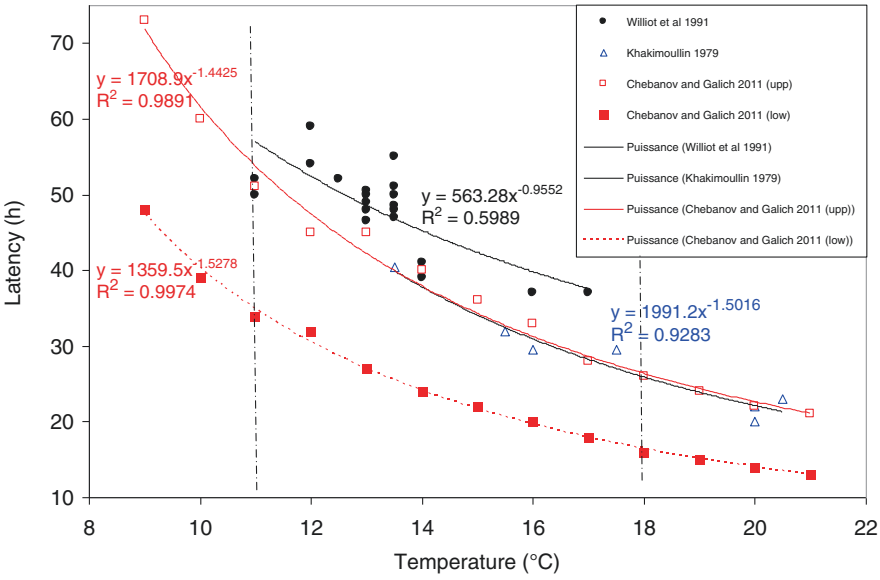

Fig. 27.25 Latency in ovulation depending on temperature. The two vertical axes (11 and 18 °C) delimit the optimum range of temperature

Ovulated eggs often cannot be expelled directly through a genital pore from the abdominal cavity to the outside because they have to go through the long oviducts equipped with a one-way valve as recalled in Sect. 27.2. The collection of gametes might be achieved by three different ways. One consists in stripping the females

Table 27.4 Comparisons of the disadvantages of the three procedures in collecting the ovulated eggs in the Siberian sturgeon

Procedure	Invasiveness	Handling	Risks of injury (potential)	Time-consuming
Stripping (2–7)	No	High rate	Protector effect of mucus removed Physical consequences of massages unknown	Very high
Laparotomy with further suture	High	Moderate	Healing Opening for stranger elements	Moderate
Incision of oviducts	Minor	Low	Low	Extremely low

Fig. 27.26 Collection of ovulated eggs by a laparotomy which consists in a 3–4 cm opening of the abdominal wall (*dark arrow*) about 5 cm in front of the urogenital opening (masked by the hand of the operator) and further closed by sutures (Patrick Williot)

many times at about 2-h intervals as long as needed, as illustrated by Williot et al. (1991). Two to seven collections were performed depending of females aged from 7 to 15 years in the Siberian sturgeon. The main advantage is that there is no invasive process but it is highly time-consuming (Table 27.4). The second relies on the "a shunt" of the natural pathways, the oviducts; by opening (3–5 cm long) the abdominal wall about 5 cm in front of the urogenital opening (Fig. 27.26), this is the laparotomy (Williot 2002). Apart from its high rate of invasiveness, the key point of this method is to optimize the time in practising the opening. Thanks to multiple stripping experiments, it has been possible to study the influence of time of collecting the ovulated eggs on the fertilization rate (Fig. 27.27). One of the outcomes is the higher fertilization rates are recorded when the ovulated eggs are collected between 4 and 14 h post observation of the onset of the ovulation. It is worth noting that the poor results obtained with the female 3 are due to the fact that the fish has not been vernalized. The third procedure is due to Podushka (1986, 1999) and consists in making an incision of about 2 cm long inside one of the oviducts (Fig. 27.28), thus allowing the ovulated eggs in going directly from the abdominal cavity straight upstream the genital opening; therefore the collection is being made through the

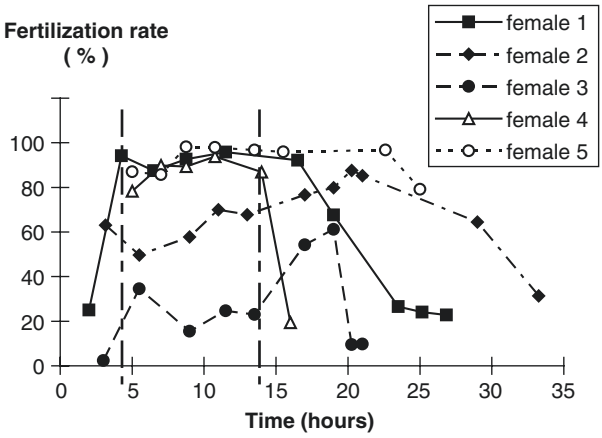

Fig. 27.27 Fertilization rate according to the delay between the beginning of the ovulation and the time at which ovulated eggs were collected. The poor results of female 3 are most likely due to the fact that the female was not vernalized (after Williot et al. (1991)). The range (4–14 h comprised between the two vertical axes) represents the optimum with regard to the best expected fertilization rate

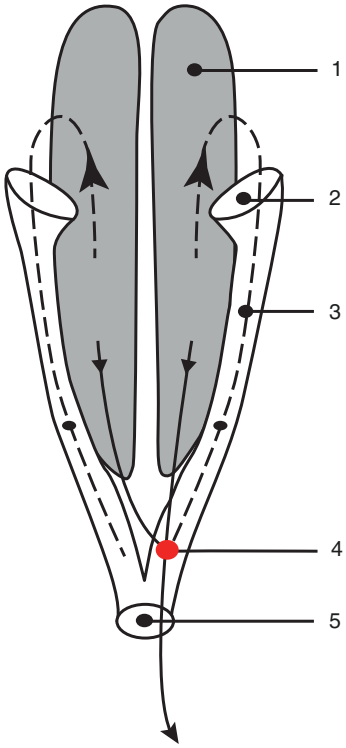

Fig. 27.28 Schematic drawing presenting the relative location of the ovaries and oviducts in the body of sturgeon (modified from Podushka (1999)): *1* ovary, *2* oviduct funnel, *3* oviduct, *4* incision location, *5* genital opening. The *dotted line* represents the pathway of ovulated eggs at natural spawning; the *solid line*, at stripping after oviduct incision

Fig. 27.29 Collection of ovulated eggs after incision of the oviduct according to Dr. Podushka's method. The scalpel is still in the right hand of the operator (credit Patrick Williot)

genital opening (Fig. 27.29). While applying this method, the female should be placed on a sloping table with the head upwards and the tail not hanging down. To open the abdominal cavity, the scalpel with its cutting edge upward (blade width should be less than the diameter of the genital opening) is inserted into the genital opening, and an incision (1–2 cm in length) is made at the caudal area of the oviduct wall in one or both oviducts, thus making a potential passage into the body cavity (Chebanov and Galich 2011). Stripping is conducted, while eggs are freely flowing from the body cavity (typically from 2 to 20 min depending on female size). One hour after the first stripping, during which 80–90% of the eggs are collected, the second stripping procedure, which does not involve an additional oviduct incision, is performed. In the case of larger and highly productive females, a third stripping sometimes may large that all ovulated eggs can be stripped in one or two portions without incision and additional efforts, in a way similar to applying Podushka's method. A danger to be avoided is the accidental incision of a kidney or the blood vessels of the rectum; however, this potential damage is not associated with an increased risk of spawners mortality. In addition, unskilled operators can injure the spawner's rectum with the scalpel. In this case, ovulated eggs will pass out through the anal vent. Usually the scalpel wound heals quickly, but very rarely an inflammation may take place. Usually this damage is not risky to the spawner's life. Due to its several advantages (Table 27.4), the method is now generalized out of Russia on different *Acipenseridae* (e.g. Stech et al. 1999; Kabir and Bani 2011).

However, despite disadvantages of other methods, it is worth noting that when carefully applied, no deleterious effects were recorded whatever the applied method.

Fig. 27.30 Collection of semen into a beaker (credit Patrick Williot)

The collection of semen may be performed thanks to a syringe equipped with a short polypropylene tube carefully inserted into the genital opening (Williot et al. 2000a, b) or directly into a beaker by stripping (Fig. 27.30). It has been mentioned above that the best time, with regard to motility, to collect the semen is 36 h post hormonal injection (Williot et al. 2000a) (Fig. 27.22). When needed, many collections may be performed along the time as shown by Kopeika et al. (1999) which is accompanied by a decrease in spermatozoa concentration (Williot et al. unpublished).

27.7 Management of Gametes and Mating

Semen samples are observed for volume, aspect, motility and density. At field, motility is assessed, thanks to a microscope with magnification of 400 for the intensity of movements together with the relative number of moving spermatozoa along three durations: 30 s, 1 min and 2 min (Williot 2002). This can be easily performed by a trained people and then provide repetitive results. With regard to density, a more precise measurement might be done thanks to a counter cell. The semen samples collected in beaker should be covered prior to being put in refrigerator (4–8 °C) to avoid any external contamination. The larger the beaker, the longer the conservation as this allows increasing the surface of exchange between sperm and surrounding air. A much longer conservation might be provided by storing the sperm in small plastic bags inflated with oxygen and then hermetically closed.

Ovulated eggs collected in *ad hoc* bowls are observed for their appearance (drawings), fluidity and the presence of clusters (should be taken off as much as possible), are sieved if there is an excess of coelomic fluid and then finally weighed. In case of doubtful quality, maturation can be controlled for GVBD test (see Sect. 27.27.5.2.2). Even rarer ovulation may occur without prior maturation, and then GV is observed instead of GVBD (Williot 2002). The bowls can be covered and keep at 15 °C for up to 4 h without any deleterious effects on further early ontogenesis (Gisbert and Williot 2002).

The fertilization is carried out according to prescription from Ginzburg and Dettlaff (1969) and further Dettlaff et al. (1993). Because of multiple micropyles in sturgeon eggs, and *A. baerii* is no exception (Debus et al. 2002), it is necessary to avoid polyspermy. The above-mentioned authors recommend the use of 10 mL of sperm for 1 kg ovulated eggs and to dilute the sperm in water (1/200) to optimize the fertilization rate together with a minimization of polyspermy. The same authors also showed that almost 90% of fertilization occurred in the first 20 s of mixing; therefore it is not necessary to maintain the mixing of sperm-watery suspension with ovulated eggs for more than 2 min maximum. Then, the fertilized eggs should be separated from the suspension by sieving and placed into the ad hoc container for the anti-adhesive treatment. Prior to that treatment, it is recommended to put a batch of fertilized eggs into a large petri dish placed in the same thermal conditions as for the eggs to allow assessing the effect of de-adhesive treatment and later of the incubation. If needed, the counting of alive/dead eggs might be delayed by stopping the embryogenesis with a 10% formaldehyde solution and then putting the petri dishes in the refrigerator.

The mating program depends on the objective; when the hatchery aims at producing fish for the human consumption market, any mating could be carried out. In contrast, when the hatchery also targets using some of the progenies for future broodstock, family or half family mating should be favoured. At present, in the absence of published data on heritability, fish with rare alleles should be privileged, and, in the absence of any background, random mating with well-known repaired brood fish has to be favoured with subsequent observation on progenies.

27.8 Treatment of Fertilized Eggs and Incubation

The external layer of fertilized sturgeon eggs rapidly becomes sticky in order to ease the eggs to fix on gravels in the river bed to allow the eggs to achieve their embryogenesis in well-oxygenated conditions. In farming conditions, this could be an obstacle except when one successfully arranges the eggs in monolayer on a tray to avoid the suffocation of the eggs which may very easily stick together. Therefore to overcome the disadvantage, i.e. to avoid the egg's adhesion, several means have been proposed (Table 27.5). The previous one (mineral silt) has been utilized for long by the former soviet scientists (Charlon and Williot 1978). Large specific jars was developed for this purpose (Fig. 27.31). A variant is the "blue clay". Later on, scientists and/or practitioners have looked for standardized products without the need of long preparation; there are industrial dry clay powder, talcum, fuller earth and tannin. The first has the advantage of being pathogen-free due to its manufacturing process. It has been used with simple conic jars (Fig. 27.32). The last quoted product, the tannin, is rather difficult to use because of its positive action and should be strictly limited in time to avoid deleterious effects on the eggs. Once the de-adhesive treatment is achieved, eggs should be rinsed many times and placed into incubators (Figs. 27.33 and 27.34), while the clay powder has proved to be more suitable for large quantities of eggs. The duration of the incubation, i.e. from the fertilization up to the beginning of the hatching, is under the dependence of water temperature as shown in Fig. 27.35. It seems that from 15 °C onwards, the duration

Table 27.5 Different techniques and substances for sturgeon egg de-adhesion

Substance	Preparation	Concentration of eggs per kilogramme or per litre	Duration of treatment	De-adhesion technique
Mineral silt	Prepared in autumn, purified, heat disinfected, stored as a creamy suspension	1 L of suspension per 5 L of water	35–45 min	In an anti-adhesive treatment unit or by hand in an enamel-coated, aluminium or plastic container
"Blue clay"1[a]	Stored in dry state, diluted with boiling water 1 day prior to application as a creamy liquid	300 g of dry clay per 5 L of water	35–45 min	–
Industrial fine clay[b]	Heated and stored in dry state Free of pathogens	280 g per L of water Verify the pH and buffered at ≥7 when needed	~1 h	Bubbling thanks to compressed air by underneath
Talcum	Diluted in water immediately prior to de-adhesion	100 g per 5 L of water	45–60 min	–
Milk[c]	Dilution rate in water 1/10 (v/v)		15–20 min	Cleaning, rinsing ~40 min
Tannin	Diluted in water immediately prior to application	2.5 g per 5 L of water	40 s	By hand (only)

[a]Podushka (1999)
[b]Williot (2002)
[c]Khakimoullin et al. (1980)

Fig. 27.31 Large jars currently used in Russia for the de-adhesive treatment based on silt suspension (or "blue-clay") (credit Patrick Williot)

Fig. 27.32 Conic jars used for the de-adhesive treatment based on clay suspension with compressed air underneath (credit Patrick Williot)

Fig. 27.33 Fedchenko incubators ("Osyotr") currently used in Russia (credit Patrick Williot)

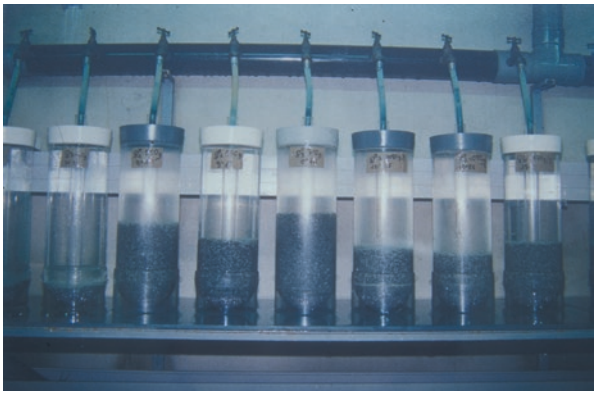

Fig. 27.34 MacDonald jars (7 L) currently used in Western countries for the incubation (credit Patrick Williot)

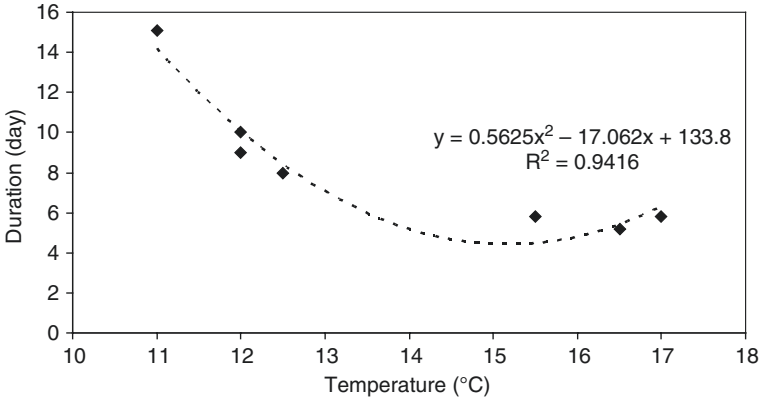

Fig. 27.35 Length of incubation from fertilization to beginning of hatching (after Williot et al. (1991))

of the embryogenesis does not continue to shorten. Within the most current thermal conditions, the length of the incubation lasts from about 9–10 days at 12 °C to about 5–6 days at 15 °C. An independent thermoregulated closed water system equipped with UV treatment and saturated oxygen level is of use. Along the incubation, control of success of fertilization rate (~4–8 cells, stages 4–5) and embryonic rate (small plug stage, stages 16–17) might be performed based on a careful sample of 200–300 pieces (Dettlaff et al. 1993).

27.9 Trends in the Yield of Ova

The plot and the weight of ova obtained with females of different age are shown in Fig. 27.36. The best fit is a two-order polynomial curve that shows a weak increasing trend ($R^2 = 0.358$) and might be observed of the weight of ova with the age of the females. This is due to two unusual experimental dots, one giving an oosomatic index over 20% and the other an oosomatic index closed to 3%. By taking off those two dots, we got Fig. 27.37 of which the best fit is three-order polynomial curve with a far better determination coefficient ($R^2 = 0.765$). Therefore the weight of ova shows an increasing trend of the female with a plateau from a female's age closed to 14 kg. Same data of weight of ova were plotted with the age of the females (Fig. 27.38). The best fit is one-degree curve order with a very poor determination coefficient ($R^2 = 0.117$) and a very low slope. The above observations confirmed those previously reported by Williot et al. (1991) who stated that the quantity of ova is more correlated with the age (r_s[4] = 0.68, $p < 0.001$) than with the weight of the female ($r_s = 0.43$, $p < 0.05$) computed with the whole set of data.

[4] r_s = rank correlation of Spearman.

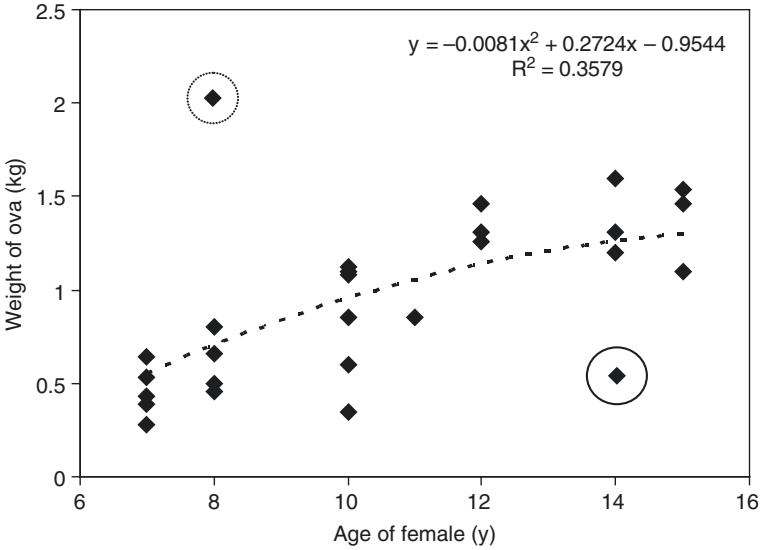

Fig. 27.36 Weight of ova depending on the age of the females. *Encircled dots* are unusual records (modified after Williot et al. (1991))

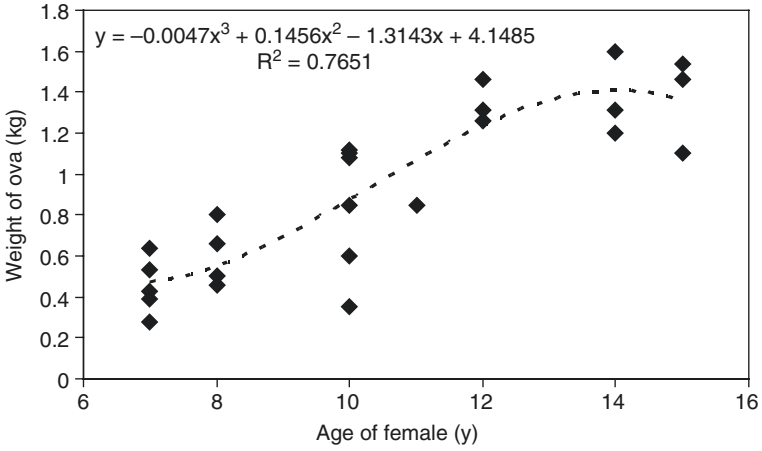

Fig. 27.37 Weight of ova depending on age of females with two extreme dots taken off as they are very seldom recorded

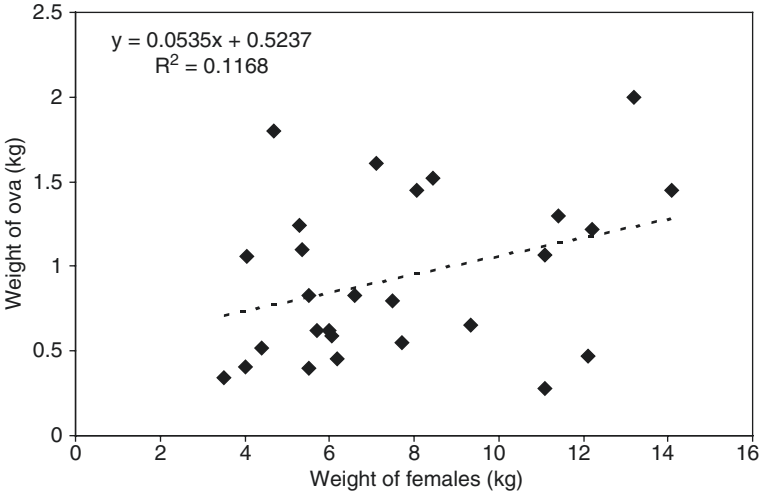

Fig. 27.38 Weight of ova depending on weight of females

27.10 Perspectives

There are three main domains that are in need of further investigations and/or setting up. The first deals with the building of a large data bank of available characterized genetic resources on the species. Sperm cryoconservation should be part of it. This would need the setting up of common methods to collect, identify and store the samples. The second one embraces the reproduction in a broad sense, i.e. how can we manage the brood fish to obtain progenies without hormonal stimulation? How can we get progenies by mimicking the wild? Preliminary approaches were carried out long time ago by building holding tanks with special water flow to reproduce the conditions in spawning grounds, the so-called Kazanskiy tanks (Charlon and Williot 1978). At that time results were not good enough to go further in that direction though the tanks are still in use. More recently, a more integrated approach in a larger scale was set up to improve the hydrological conditions with an artificial spawning channel focused on both Russian and stellate sturgeons (Chebanov 1998, Chebanov et al. 2002). Later, new tank's management was reported on green sturgeon (*Acipenser medirostris*) by Van Eenennaam et al. (2012). This would need a global approach of diverse disciplines dealing among others with biology, behaviour, hydrodynamics and techniques.

The third concerns the influence of food and feeding on final products quality, i.e. the eggs whatever their use, reproduction or caviar. The main obstacle to date is the great difficulty to find the means to maintain long-time experiment regarding the late puberty of the species. A partial alternative would be work with already mature fish within a two-spawning interval which is mostly a 2-year interval.

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Siberian Sturgeon Sperm Cryoconservation

28

Andrzej Ciereszko and Martin Pšenička

Abstract

Sperm cryopreservation can potentially be used as a management tool for better organisation of spawning and improvements of breeding programs, providing a security strategy against decline in natural populations and incorporating of genes from wild fish into hatchery populations. Two extenders are presently available to insure high-quality cryopreserved Siberian sturgeon semen: (1) an extender consisting of 30 mM Tris, 23.4 mM sucrose, 0.25 mM KCl, 10% methanol and pH 8.0 and (2) a simplified extender consisting of just 0.1 M glucose and 15% methanol. Cryopreservation protocols secure good level of post-thaw motility (20–60% vs. 40–80% for fresh semen). A high variability in usefulness of semen for cryopreservation is a main problem in successful freezing. As such, it is difficult to predict a priori which semen samples would be successfully cryopreserved. Sperm characteristics, such as motility, have only limited usefulness, and more research towards identification of reliable markers of cryopreservation is needed. At present, implementation of cryopreserved semen into hatchery practices and breeding programs is limited despite the fact that this challenging task can greatly improve breeding programs for cultured Siberian sturgeon and help to protect diversity of wild populations. So far, the main application for cryopreservation of sturgeon semen has been for hybridization.

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Spermatozoa • Sperm motility • Cryopreservation • Cryoinjuries

Introduction

Sperm cryopreservation is an efficient means of long-term ex situ conservation of germplasm so as to protect biodiversity of wildlife model species and to improve breeding of domestic animals, including fishes (Mazur et al. 2008; Martínez-Páramo et al. 2009; Robles et al. 2009). Sperm cryopreservation has been developed for more than 200 species of teleost fish (Tiersch 2011) and can potentially be used as a management tool in hatcheries, for example, to enable better organisation of spawning, improve breeding programs, provide security against disease and transfer genes from wild fish into hatchery populations (Cloud et al. 1990). Cryopreserved sperm has potentially high importance in the conservation of sturgeons, as illustrated by Willot et al. (2004) for the European sturgeon, *Acipenser sturio*, and initiated as soon as in the 1990s and further by Kopeika et al. (2000), including Siberian sturgeon (Billard and Lecointre 2001). This chapter describes main issues related to current knowledge concerning cryopreservation of Siberian sturgeon semen. Two effective protocols are described, and semen characteristics (both physiological and biochemical) of fresh and cryopreserved semen are provided. The problem related to high variability of semen quality is also addressed.

28.1 Cryopreservation and Cryodamage

Development of effective cryopreservation technology is a challenge because of the numerous factors that cause damage to spermatozoa during freezing-thawing; the most important include temperature effects on lipids, intracellular and extracellular formation of ice crystals, oxidative stress and osmotic stress (Leung 1991). Cryogenic damage results in a marked reduction in DNA integrity and membrane permeability which influence basic sperm functions such as motility, fertilizing ability and quality of progeny (Leung 1991; Suquet et al. 1998). Therefore, cryopreservation methodology has to be carefully optimized for each species.

28.2 Procedures Developed for Teleosts Are Not Efficient for Sturgeon

Sturgeon spermatozoa are morphologically, physiologically and biochemically distinct from teleostean sperm. These features include the presence of an acrosome and the acrosomal reaction, an elongated nucleus and midpiece with

several mitochondria, prolonged duration of motility and low osmolality of the seminal plasma. Presumably because of these characteristics, cryopreservation methods developed for teleost fishes are not efficient for sturgeon sperm. Earlier attempts focused on the development of cryopreservation methodology that used post-thaw sperm motility or early embryo development as endpoints (Tsvetkova et al. 1996; Billard et al. 2004; Alavi et al. 2012). For example, cryopreservation of sturgeon spermatozoa using DMSO[1]-sucrose extender resulted in the recovery of spermatozoa with basic motility characteristics similar to that in fresh semen (Ciereszko et al. 1996). Unfortunately, neither sperm motility nor early embryo development is a good predictor of cryopreservation success in sturgeons (Glogowski et al. 2002). These early studies also suggested that DMSO and/or ethylene glycol are not good cryoprotectants for semen of sturgeons (Brown and Mims 1999; Jähnichen et al. 1999; Linhart et al. 2006). However, the low usefulness of DMSO for cryopreservation of sturgeon spermatozoa is related to its harmful effect on the acrosome, probably by causing a precocious triggering of acrosome reaction, much before any egg contact (Pšenička et al. 2008).

28.3 High Quality of Semen Is Prerequisite for Successful Cryopreservation

Cryopreservation is very stressful technology which produces sperm-damaging effects (see above). Thus it is vital to secure the best quality of semen possible, thereby better withstanding the stresses of freezing and thawing. Semen from sturgeons is usually obtained after hormonal treatment; it has been demonstrated that sperm quality significantly varies in relation to maturity state of testis, water temperature during hormonal treatment and time from hormone injection to milt collection (Dzyuba et al. 2012; Shaliutina et al. 2012, 2013).

For Siberian sturgeon, 10 °C is the optimal temperature for hormonal treatment and that the best semen motility can be obtained 36 h after hormone injection (Williot et al. 2000). Morphologically, firm or soft testis (contrary to viscous or liquid) is best suited for hormonal stimulation (Williot et al. 2000). Billard et al. (2004) indicated that high quality of sperm left in genital tract 24 h after hormonal injection (in vivo storage) was better than sperm stripped from the same fish and stored in vitro. In vitro storage can be improved through oxygenation of semen in order to counteract anoxic conditions during storage. These recommendations are important prior to cryopreservation. Preliminary results also suggest that Siberian sturgeon sperm can be obtained out of season and cryopreserved (Judycka et al. 2015a).

[1] Dimethyl sulfoxide.

28.4 Methanol Is Well Suited as Cryoprotectant for Sturgeon Cryopreservation

28.4.1 Basic Protocol Developed by Horváth and Urbányi (2000)

The breakthrough in cryopreservation of sturgeon sperm was achieved by introducing the use of methanol as a cryoprotectant (Horváth and Urbányi 2000) and the use of modified Tsvetkova's extender (MTE). These authors presented preliminary results for sterlet which were later successfully applied to Siberian sturgeon by Glogowski et al. (2002).

Basic steps in cryopreservation methodology include (Horvath et al. 2009):

- Milt collection with elastic tube connected to a syringe
- Extension at 4 °C of milt at a ratio 1:1 with extender (MTE) consisting of 30 mM Tris, 23.4 mM sucrose, 0.25 mM KCl, 10% methanol and pH 8.0 (adjusted with HCl)
- Suction of extended milt to 0.25 or 0.5 mL plastic straws
- Freezing of straws 3–4 cm over surface of liquid nitrogen for 3 min
- Storage of straws in liquid nitrogen

Sperm is thawed by placing straws in water bath at 40 °C for 6 s for 0.25 mL straws or 13 s for 0.5 mL straws.

This basic protocol has been consistently reliable for sturgeon sperm (Urbanyi et al. 2004; Horváth et al. 2005, 2006, 2009, 2010).

28.4.2 Simplified Protocol Developed by Judycka et al. (2015b)

A simple extender consisting of 0.1 M glucose and 15% methanol (GM) has recently been developed and compared with MTE extender for cryopreservation of Siberian sturgeon semen (Judycka et al. 2015b). Efficacy of both extenders appears to be similar (with the exception of 20% higher post-thaw motility for GM extender) when used with the cryopreservation techniques described above. This simplified extender appears to be a practicable modification for cryopreservation of Siberian sturgeon semen. A 30-min equilibration period before freezing and a 30-min post-thaw storage were tested using the GM extender, and it was found that both procedures did not change semen quality. The use of prolonged equilibration period may allow filling a large number of straws, which would be beneficial due to high volume of Siberian sturgeon semen. Also, a long post-thaw storage time can greatly improve organisation of hatchery work because a high number of straws can be thawed and used for fertilization of a large volume of eggs.

28.5 Effects of Cryopreservation on Semen Characteristics of Siberian Sturgeon

Cryogenic damage caused by freezing-thawing leads to lower quality of Siberian sturgeon cryopreserved semen as compared to fresh semen; these qualitative effects are a reduction in sperm motility, fertilizing ability and biochemical changes (Table 28.1). A decrease in the number of motile sperm always occurs, similarly, it is clear that low motility before cryopreservation always results in low post-thaw motility. A decrease in the percentage of motile sperm is correlated with a decrease in other sperm qualitative characteristics as measured by CASA[2] such as VAP[3], VCL[4], VSL[5], LIN[6] and ALH[7] (Judycka et al. 2015b). However changes in these

Table 28.1 Semen characteristics (mean ± SD) of fresh and cryopreserved semen of Siberian sturgeon

Feature	Fresh semen	Cryopreserved semen	References
Sperm motility			
% Motility	88 ± 4.4	23 ± 8.8	Tsvetkova et al. (1996)
	80	58	Judycka et al. (2015b)
	61.7 ± 17.6	15.6 ± 2.9	Glogowski et al. (2002)
	41.3	25.3	Sieczyński et al. (2015)
VCL	121.9	112.2	Sieczyński et al. (2015)
ALH	10.4	5.4	Sieczyński et al. (2015)
Fertilization			
% Fertilization	89 ± 7.6	53 ± 8.3	Tsvetkova et al. (1996)
	21.9 ± 5.7	29.6	Glogowski et al. (2002)[a]
Seminal plasma			
Protein conc. (mg mL^{-1})	0.39 ± 0.19	0.80 ± 0.31	Sarosiek et al. (2004)
Arylsulfatase (U L^{-1})	319 ± 235	529 ± 311	Sarosiek et al. (2004)
Acid phosphatase (U L^{-1})	2.71 ± 1.97	8.26 ± 5.74	Sarosiek et al. (2004)
β-N-acetylglucosaminidase (U L^{-1})	24.7 ± 17.9	30.9 ± 20.1	Sarosiek et al. (2004)

[a]mean ± SEM

[2] Computer-assisted sperm analysis.

[3] Average path velocity.

[4] Curvilinear velocity.

[5] Straight-line velocity.

[6] Linearity.

[7] Amplitude of lateral head displacement.

parameters are not consistent, because Sieczynski et al. (2015) recorded significant changes only for percentage of motility and VCL. It is likely that these differences reflect high variability in the quality of fresh semen. A decrease in sperm motility can be caused by mechanical damage to sperm membranes and flagella (broken or bent) which produce changes in swimming effectiveness (Billard et al. 2004). Moreover, depletion of sperm ATP stores after cryopreservation will affect sperm energetics for movement. This decrease is highly variable regarding individual males (Billard et al. 2004). Further studies are necessary to evaluate a practical use of these parameters, for example, to assess a usefulness of fresh semen to cryopreservation (see below).

Fertilization rates of cryopreserved semen are usually lower than those for fresh semen, although values as high as 58% were reported (Judycka et al. 2015b). Similarly to sperm motility, fertilizing ability of spermatozoa is variable due to inconsistent usefulness of semen quality for cryopreservation. It has to be underlined that that high fertility rates observed during early stages of development may not always indicate high hatching rates (Glogowski et al. 2002).

Sperm enzymes that can be released from spermatozoa after freezing-thawing are likely to be found to be biochemical indicators of cryogenic damage to sperm cells (Table 28.1). Lactic dehydrogenase and acid phosphatase also can potentially be employed as markers of damage to plasmalemma and midpiece. It is assumed that the sperm acrosome is affected by cryopreservation. Billard et al. (2004) have reported that up to 10% of frozen-thawed spermatozoa of Siberian sturgeon exhibited acrosome reaction (projection of acrosomal filament). Injuries to sperm acrosome can be evaluated through monitoring of activities of acrosin, arylsulfatase and β-N-acetylglucosaminidase. Sarosiek et al. (2004) have tested usefulness of a few enzymes to evaluate cryodamage to Siberian sturgeon spermatozoa. Activity of arylsulfatase appeared to be especially useful for this purpose, followed by acid phosphatase activity. On the other hand, β-N-acetylglucosaminidase activity appeared not to be useful for monitoring cryodamage.

Fertilization ability is the most important feature of spermatozoa to be protected during cryopreservation. So far, results obtained for Siberian sturgeon studies suggest that when methanol is used as the cryoprotectant, fertilizing ability is well protected after freezing-thawing (Glogowski et al. 2002; Judycka et al. 2015b). However, data are still fragmentary due to the low number of fertilization trials, difficulties in securing appropriate controls and low absolute fertilizing rates. Therefore, more studies are necessary in order to confirm good fertilization rates for more males.

28.6 The Use of Cryopreserved Semen

Introduction of cryopreserved semen into hatchery practices and breeding programs of Siberian sturgeon can potentially improve breeding of this species. Unfortunately, at this time implementation of cryopreservation has not been achieved. Most likely, one of the main reasons for this problem is a high variability in usefulness of semen

for cryopreservation. As such, it is difficult to predict a priori which semen samples would be successfully cryopreserved. Sperm characteristics, such as motility, have only limited usefulness, and more research towards identification of reliable markers of cryopreservation is needed. This challenging task can provide a necessary breakthrough leading to control of variability of cryopreservation results. Implementation of cryopreservation can greatly improve breeding programs for cultured Siberian sturgeon and help to protect diversity of wild populations. Until now usefulness of cryopreserved semen has been demonstrated only in hybridization programs.

Acipenseriformes are characterized by a high capacity for hybridization (Rochard et al. 1991; Billard and Lecointre 2001). Hybridization is frequently used in sturgeon culture to combine complementary parental characteristics. Cryopreserved sperm greatly facilitates hybrid production, because sperm can be used out of usual reproductive periods, so there is less need to synchronize spawning time of parental species. Urbanyi et al. (2004) have demonstrated the usefulness of cryopreserved semen for successful production of sturgeon hybrids, including crossing Siberian sturgeon and sterlet. This opens an exciting opportunity for improvement of sturgeon breeding.

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Weaning in Siberian Sturgeon Larvae

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Abstract

In this chapter we have revised the available information regarding feeding practices with Siberian sturgeon larvae from the onset of exogenous feeding to the early juvenile stage, data gathered from published studies and compared and/or complemented with current practices from French hatcheries. Different feeding strategies based on feeding larvae with inert diets (crumble or micropellets) or combining them with a period of feeding based on live preys (*Artemia nauplii*) is also discussed, since the transition to one feed to another is still a very critical moment for the life of the animal, which requires gradual and specific dietary protocols for the success of the process in terms of growth performance, survival and fry quality. Although Siberian sturgeon larval rearing may be considered easier when compared to other sturgeon and freshwater species, special attention is needed for optimizing larval feeding in order to maximize larval performance, since this phase of the productive cycle has a considerable impact on the economic profitability of the activity.

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Introduction

Generally, weaning is commonly considered as the switch from one type of feed to another type. The transition from one feed to another is still a very critical moment for the life of the animal, which requires gradual and specific dietary protocols for the success of the process (Parma and Bonaldo 2013). In aquaculture practices, this term has often been used for different activities. For example, weaning wild specimens to establish a broodstock or on-growing them for productive purposes is generally considered as the change from live prey to a nonliving food item, whereas in fish larviculture, weaning consists of replacing live feeds with inert food early in life (see examples for different sturgeon species in Monaco et al. 1981; Bardi et al. 1998; Pourali-Fashtomi and Mohseni 2006; Williot et al. 2005; Chèvre et al. 2011 among others). As the above-mentioned authors reported, weaning is not simply a change from one food to another, but is a phase of adaptation where both types of food are provided for a certain period of time with a slow decrease in the administration of live feed, generally zooplanktonic preys, and a progressive increase of inert diet (Williot et al. 2005, 2011; Chèvre et al. 2011). This gradual transition from one type of live prey to the inert diet will allow the larva to progressively adapt to the new feed type.

To evaluate different weaning strategies and/or feeding protocols, larval mortality and quality (i.e. low incidence of skeletal deformities, tolerance to environmental stressors, good growth performance) are the most important variable for hatchery managers, even though this is not the only parameter generally considered for assessing the success of this process. In this context, the best weaning protocol is that based on a combination of several variables such as larval survival, growth, size dispersion and fingerling quality, variables that have a direct impact on fingerlings' performance during the on-growing period. In addition to the above-mentioned biological variables, productive variables such as labour, production costs and the use of facilities need also to be considered, and consequently, the final choice should be based on their correlation and economic yield. Although sturgeons do not generally require the extended use of live foods that many marine and freshwater larvae require (Hamlin and Kling 2001), the costs and labour associated with even short-term feeding can be considerable (Le Ruyet et al. 1993). Thus, eliminating the reliance on live foods will reduce material and labour costs, further increasing profitability margins. In this chapter we will present a synthesis of available information about the scientific knowledge and actual farming practices regarding the onset of exogenous feeding and early nutrition in Siberian sturgeon larvae.

29.1 The Onset of Exogenous Feeding

The initial time of first feeding for fish larvae is considered to be a critical period because it influences their subsequent survival and growth. Periods of food deprivation after the consumption of the endogenous reserves contained in the yolk sac during the prelarval phase can result in abnormal behavioural and morphological larval development such as the degeneration of the digestive system and trunk musculature, and a decrease in the efficiency with which food is utilized and feeding activity (Heming et al. 1982). In natural environments, a delay in the onset of exogenous feeding may increase larval vulnerability to predation and also result in reduced growth and stamina caused by starvation or inadequate nutrition, leading ultimately to increased mortality rates. Similar effects may be found under aquaculture practices if fasting may occur, even though their severity should be lower due to the control of food supply by hatchery managers. Consequently, knowing the age or size when first feeding normally occurs is very important, as is knowing how long larvae are able to withstand food deprivation before reaching the point of no return (PNR), when the cumulative effects of starvation become irreversible and 50% of starved larvae are still alive but are unable to feed even when food resources become available, and also evaluating the effects of starvation on fish condition and digestive system development (Blaxter and Hempel 1963). In culture conditions, the PNR is important as it allows the synchronization of the rearing process to the physiological state of the larva, thus maximizing larval survival and growth as well as minimizing size dispersion at further stages of the rearing process (Gisbert et al. 2004). In natural environments, the period of time from hatching to exogenous feeding for Siberian sturgeon takes 12–14 days at 14–15 °C (population from the Lena River) and 8–10 days at 18–19 °C (Kozlov and Abramovich 1986; Petrova et al. 1991), whereas first feeding occurs at 5–6 days at 16.4–17.5 °C in the Ob River population (Chepourkina and Golubkova 2006). Mortality due to starvation after the consumption of yolk sac in Siberian sturgeon larvae dramatically increases between 15 and 19 days post-hatch (dph) at 17.5–18.0 °C (Dabrowski et al. 1985; Gisbert and Williot 1997). However, larvae that were not fed until 17 days post-hatch (7–8 days after the onset of exogenous feeding) were able to start feeding and kept their ability to grow, but they grew at a slower rate than those fish first-fed between 9 and 11 dph. This resilience to prolonged starvation in sturgeon larvae is due to the accumulation of fat reserves in the liver and the anterior and intermediate regions of the intestine (Gisbert et al. 1998; Wegner et al. 2009; Chai et al. 2011; Asgari et al. 2014). Under experimental conditions, larval mortality from the onset of exogenous feeding to the end of the larval period (20–30 days after hatching at 18 °C) may vary between 2.1 and 23.5% of the total number of fish larvae (Gisbert et al. 2000), which is dependent on the quality of the batch of eggs and generally occurs mainly between 9 and 18 dph (Fig. 29.1). These mortality rates are similar to those reported earlier within different rearing

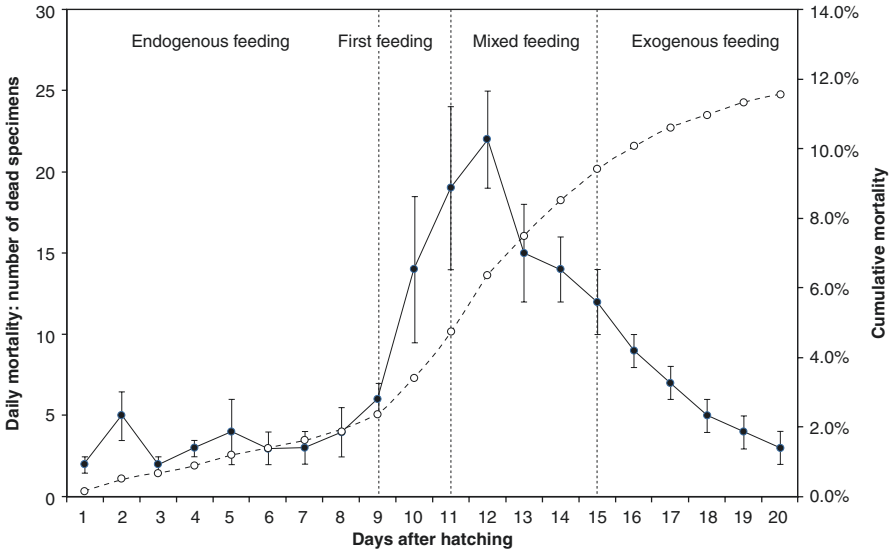

Fig. 29.1 The daily mortality pattern (*filled circle*) and cumulative mortality (*white circle*) of Siberian sturgeon from hatching to the early juvenile stage (20 days after hatching) obtained from 20 different females. Data regarding daily mortality are expressed as the number of dead specimens per day (1350 prelarvae per experimental tank at the beginning of the rearing period), whereas cumulative mortality values are expressed as a percentage. Data are reported as ± standard deviation calculated from 20 different mattings as reported in Gisbert et al. (2000). The *dotted line* represents the period of transition to exogenous feeding and the mixed nutrition stage

regimes (Gisbert and Williot 1997; Williot 1998) and comparable to those obtained by Dabrowski et al. (1985) who fed larvae with live food. According to the former authors, losses during this period are unrelated to egg size and are attributed to the transition from endogenous nutrition to artificial feeding. The reason behind such a wide range of larval mortalities observed among the progeny that were originated from 20 different females is unclear, but it seems to indicate that there are some batches of larvae, which more readily accept, digest and assimilate artificial diets (Gisbert et al. 2000).

Behavioural observations can serve as a useful visual guide to detect the onset of exogenous feeding age for Siberian sturgeon (Gisbert and Williot 1997; Gisbert et al. 1999). In this context, the beginning of external feeding corresponds with the ending of the schooling behaviour during daylight hours and the dispersal of larvae across the bottom of the tank and the apparent depletion of endogenous reserves contained in the large yolk sac typical of this species. Larvae show typical searching behaviours: they move the head to both sides from its main trajectory and increase their swimming activity when food is detected. Yolk absorption could be a useful visual guide to determine the best time to offer sturgeon larvae their first diets, and it is used commonly in other species such as salmonids (Piper et al. 1983). Furthermore, first feeding in Siberian sturgeon larvae is related to the

expulsion of the melanin plug, which is eliminated after first feeding with the first faeces (Gisbert and Williot 1997). The melanin plug is considered an accumulation of melanin granules in the spiral valve formed as a waste product originated by the consumption of the endogenous reserves contained in the yolk sac, as well as by the participation of these yolk reserves in the digestive system formation (Gisbert and Sarasquete 2000). In contrast, the end of the endogenous feeding phase is considered to occur in other sturgeon species like *A. gueldenstaedtii*, *A. transmontanus* and *A. oxyrinchus* larvae with the extrusion of the melanin plug prior to the onset of exogenous feeding (Dettlaff et al. 1993; Gawlicka et al. 1995; Ostaszewska et al. 2011). In this sense, Dettlaff et al. (1993) noted that pigment plug ejection could provide a practical guideline to determine the onset of exogenous feeding in *A. gueldenstaedtii*; however, in Siberian sturgeon larvae, the extrusion of melanin plug cannot readily be used as a criterion to determine the timing of the transition to active feeding. Thus in Siberian sturgeon, the end of the endogenous feeding stage occurs with the depletion of the yolk sac and the resorption of the yolk mass that separates the oesophagus and the cardiac stomach. This occurs at between 8 and 9 days post-hatching at a rearing temperature of 18 °C. Until this yolk mass is absorbed, the fish are unable to ingest food particles (Gisbert and Williot 1997). As in other sturgeon species, when larvae begin to feed, they possess an anatomically complete and functional digestive tract with a high degree of morphological organization (Gisbert et al. 1998) and a full repertoire of digestive enzymes (Zóltowska et al. 1999), which allows them to be feed directly on artificial diets (Dabrowski et al. 1985; Gisbert and Williot 1997; Gisbert et al. 2000 among others). Although precautionary first feeding of Siberian sturgeon larvae before 9–10 dph (17–18 °C) was not recommended by Gisbert and Williot (1997), in contrast to previous suggestions for other sturgeon species that recommended the earlier introduction of feed in the rearing tanks in order to allow first-feeding larvae to get used to the new diet (Conte 1988), at Nurseteich Sarl, a French hatchery specialized in the production of Siberian sturgeon fry for on-growing purposes, the administration of feed into larval rearing tanks generally starts at 5 days post-hatching at a rearing temperature of 16–17 °C based on the previous experience of hatchery managers rearing other freshwater species like carp and catfish, although the efficiency of this feeding strategy has never been evaluated by the company. In this sense, this feeding strategy is also supported by past studies that have shown that administration of feed in small doses before the completion of the yolk sac stimulates the transition to exogenous feeding and significantly increases both survival ability of larvae and growth rates (Mironov 1994). In any case, overfeeding before the onset of exogenous feeding may increase systemic bacterial loading, the potential for gill infections (MacPhee et al. 1995), and contributes to poor water quality. Siphoning in order to remove the excess of feed can be extremely labour intensive and introduces risks of accidental damage to the fish during the siphoning process.

The effect of egg diameter on the size of newly hatched prelarvae (0 dph) and the first-feeding age in Siberian sturgeon was assessed by Gisbert et al. (2000) by means of evaluating different egg batches obtained from 20 females aged 13–14 years old in a 2-year study. The former authors concluded that the age of first feeding in this

Table 29.1 Linear relationship ($Y = a + bX$) between first-feeding age in days post-hatching (dph) and egg diameter, size in standard length (SL) and wet body weight (BW) of newly hatched prelarvae in Siberian sturgeon (Gisbert and Williot 2002)

Y	*X*	*a*	*b*	*N**	*r2*	*P* value
First-feeding age (dph)	Egg diameter (mm)	4.08	1.68	20	0.39	0.003
First-feeding age (dph)	SL (mm)	1.34	0.75	20	0.52	<0.001
First-feeding age (dph)	BW (mg)	7.88	0.103	20	0.46	0.001

*N** corresponds to different progeny obtained from different mattings as reported in Gisbert et al. (2000)

sturgeon species takes place between 9 and 11 days post-hatching in larvae reared at 18 °C, and this age is correlated with egg diameter (mm), yolk sac volume (mm^3) and total length and wet body weight of newly hatched prelarvae (Table 29.1). Thus, the larger the egg diameter, the larger the size of prelarvae at hatching, thus the later the onset of exogenous feeding as a consequence of a larger amount in endogenous reserves contained in the yolk sac. The age at first feeding reported by the former authors is in agreement with the findings of other studies conducted at similar rearing temperatures (Dabrowski et al. 1985; Park et al. 2013), whereas exogenous feeding has been reported to occur at 14 dph at lower water rearing temperatures (15–16 °C) (Hamlin et al. 2006). According to Park et al. (2013), teeth that were seen first at 5 dph in both upper (maxillary) and lower (mandibular) layers were sharpest and longest coinciding with the onset of exogenous feeding, whereas they become shorter and blunted in shape some days later and completely disappeared at 21 dph. Thus, transient changes in teeth development may be used as a complementary visual guide for evaluating the optimal moment for first-feeding Siberian sturgeon larvae, as well as assessing the success of the complete transition to exogenous feeding.

29.2 Feeding Procedures and Larval Nutrition

The first large-scale attempts to culture sturgeon from the larval stage were carried out in the former USSR at the end of the nineteenth century using live food organisms, e.g. oligochaetes (*Enchytraeus* sp. and *Tubifex* sp.), and zooplanktonic organisms, such as cladocerans (*Daphnia* sp. and *Moina* sp.) (Charlon and Williot 1978; Dabrowski et al. 1985), which is in agreement with the feeding habits of this species at this developmental stage in the wild (Ruban 2006). However, this practice mainly relied on wild available zooplankton usually growing in ponds nearby indoor larval rearing facilities that were regularly harvested and the live preys transferred into the indoor larval rearing facilities. In addition to limitations in the regular supply of live prey produced in ponds, their biochemical composition may be quite variable, depending on pond's primary production and climate conditions. These limitations may be overcome with the use of *Artemia* sp. as a food source (Monaco et al. 1981; Buddington and Doroshov 1984), since the supply and regular production of this

type of live prey are assured year-round and its biochemical profile can be easily manipulated by means of the use of commercial or tailor-made emulsions (Øie et al. 2011). However, the procurement of live food is a labour-intensive process, and live prey is sometimes difficult to rear or harvest in commercial quantities, especially when it is naturally produced in ponds. In addition, the nutritional profile of live food may be inadequate to complete the growth-out phase of cultured sturgeons, since their nutritional requirements change along ontogeny. Further, the exclusive use of only one type of food organism (particularly worms of the genus *Enchytraeus*) can result in apparent metabolic disorders and poor subsequent health in juveniles (Buddington and Doroshov 1984; Hung 1991).

Although moist diets based on natural products (blood and bone meals, silkworms, mineral and vitamin supplemented) were formulated in the former USSR during early development of sturgeon hatcheries, their use had limited success and did not replace cultured live feeds. First attempts to raise Siberian sturgeon larvae with artificial dried diets were carried out by Semenkova (1983) and Dabrowski et al. (1985). A comparison of the growth performance and survival of larvae fed with live food (*Tubifex* sp.) with those fed with different artificial dry diets (e.g. a dry diet based on single-cell protein with freeze-dried liver, a commercial salmon starter and a casein-based diet) demonstrated that artificial larval diets can be used successfully for intensive commercial culture of several sturgeon species from the onset of exogenous feeding (Dabrowski et al. 1985). These results are similar to those reported for *A. transmontanus* (Monaco et al. 1981; Buddington and Doroshov 1984; Lutes et al. 1990; Hung 1991), *A. nacarii* (Giovannini et al. 1991), *A. ruthenus* (Napora-Rutkowski et al. 2009) and *A. persicus* (Pourali-Fashtomi and Mohseni 2006). Today, the practice of feeding only formulated feeds is becoming more common for many sturgeon species, despite some reports of decreased growth and survival (DiLauro et al. 1998; Mohler 2000; Ebrahimi 2006; Zhang et al. 2009). This shift in management strategy is fuelled by advances in artificial diet formulation (Hung 1991; Medale et al. 1991; Gawlicka et al. 1996) and identification of optimal feeding regimes (Cui et al. 1997; Köksal et al. 2000; Rad et al. 2003). In this sense, growth in total length (TL) and wet body weight (BW) in Siberian sturgeon larvae from hatching to 30 dph fed with inert feeds (50% protein, 15.5% fat; Lansy A2, W3, Artemia Systems™, Belgium) and reared under small-scale hatchery conditions (18–18.5 °C) may be described according to the following regression equations: $TL_{(mm)} = 0.125 \times Age_{(dph)}^{0.046}$ ($r^2 = 0.96$) and $BW_{(mg)} = 8.08 \times Age_{(dph)}^{0.15}$ ($r^2 = 0.91$) (data calculated from 21 different mattings as reported in Gisbert and Williot 2002).

Although Siberian sturgeon may be fed from the onset of exogenous feeding with inert diets, as the main three hatcheries in France are currently doing, until recently there were still some commercial hatcheries, especially in Russia, where live feed (*Artemia* sp.) was still commonly included as part of a first-feeding regime (Vedrasco et al. 2002). Thus, during the first days of transition to exogenous feeding, it is a good practice to lower the water level in the tank when administering live food, thus decreasing the energy expenditure of fry while seeking feeds and avoiding loss of live organisms with water draining from the tank. In other sturgeon

species, *Artemia* nauplii are complemented with minced oligochaetes and a small proportion of zooplankton on the basis of 3–5 g of feed per 1000 larvae. *Tubifex* sp. and other oligochaetes are fed in minced form (the amount depending on the fry weight) diluted with water and administered along the tank wall (perimeter) in two or three portions (Chebanov and Galich 2013). In this context, several authors have attempted to evaluate whether long-term growth and survival of Siberian sturgeon were influenced by the administration of live feeds at first feeding and determine if overfeeding is beneficial at early developmental stages. In particular, Hamlin et al. (2006) showed that feeding Siberian sturgeon with a mixture of newly hatched *Artemia* nauplii (1 nauplius/mL from first feeding to 35 dph and 0.5 nauplius/mL from 35 to 40 dph when larvae were weaned onto an inert diet) and a soft-moist starter diet (Silver Cup™, Nelson and Sons Inc., USA; 44–50% protein, 16–18% fat) did not reported any beneficial long-term effect on the growth performance and survival of fry at 125 dph in comparison to larvae fed exclusively on the above-mentioned starter diet. In contrast, the former authors found that larvae fed with a mixture of live prey and the inert diet showed slightly heavier body weights (1.2 times) at 35 and 75 dph in comparison to those solely fed with the inert diet, whereas this difference disappeared at older ages. However, no differences in survival were found at 35, 75 and 125 dph between both experimental groups. Thus, the former authors concluded that given the higher cost of live food production and consequent labour (3.4 times for feeding 10,000 larvae) in comparison to feeding larvae with just the inert diet, there did not appear to be an advantage to the administration of live foods for long-term growth or survival for this species.

There exist few formulated diets especially nutritionally designed for sturgeon larvae. In particular, there exists a formulated diet (crumble presentation form; ADVANCE®, Coppens, The Netherlands) for sturgeon larvae (<0.2 g in wet body weight) that is recommended to be administered 3 days after feeding larvae with *Artemia* and during a period of 10–11 days, when larvae are completely weaned onto the inert diet. This diet is recommended by the manufacturer from 0.2 to 6.5 g of wet body weight, contains 56% proteins and 15% fat (gross energy, 21.3 MJ/kg feed; digestible energy, 19.7 MJ/kg feed) and is supplemented with several hydrosoluble (C) and liposoluble (A, D, E) vitamins, as well as with β-glucans as an immune stimulant to support disease resistance in larvae and fry. The above-mentioned feed manufacturer has also another inert diet for young sturgeons (START PREMIUN®, Coppens) that is recommended to be administered when larvae are larger than 0.5 g of wet body weight until they reach 10 g. This diet has the same properties as the previous one (supplementation with vitamins and β-glucans), although its levels in proteins are lower (54%). Other inert diets especially formulated for first-feeding sturgeon larvae are manufactured by BioMar. In particular, LARVIVA ProStart 300 & 400® (67% proteins, 12% fat) is recommended as a diet for first-feeding sturgeon larvae, or it may be administered in a co-feeding regime. This diet is supplemented with vitamins C, D, E and A and with the probiotic Bactocell®, which has extensive documentation of improved survival and reduced occurrence of deformities in fish larvae from other species (Lamari et al. 2013). For

older ages and when larvae are well adapted to inert feed, LARVIVA ProWean 300 & 500® (58% proteins, 10% fat) is recommended. Similar to the former diet, this one is also supplemented with vitamins and the probiotic. In addition to the above-mentioned diets, other hatchery managers may use other feeds for sturgeon larvae, a decision that is generally made based on their own experience.

Laboratory experiments have demonstrated that food odour stimulates feeding responses in sturgeon larvae and juveniles (Kasumyan 1999). Thus, the organoleptic characteristics of the feed become an important factor in determining fish feeding. In addition, Kuzmin et al. (1999) found that the efficiency of diet consumption is increased by using chemical stimulants that improve the taste characteristics of the diet. Free amino acids have been tested as diet stimulants in the larvae of different sturgeon species (Kasumyan and Taufik 1994) who reported that out of the 20 amino acids tested, only two (glycine and L-alanine at a threshold of 1 μM) were able to induce food-searching behaviour. Kuzmin et al. (1999) found that lysine, methionine and alanine were the most important amino acid attractants, while the low molecular weight nitrogenous substances, sodium glutamate and fish protein concentrates also stimulated feeding behaviour in larvae. However, the effectiveness of these feed attractants in commercial larval diets is yet to be established (Hung and Deng 2001), since there are several raw materials (i.e. protein hydrolysates, krill meal) that may exert similar effects.

Feeding rate, water temperature and fish size are among the three most important factors affecting the growth of fish (Brett 1979), and thus determining the optimal feeding rate is important to the success of any aquaculture operation. This is particularly true for larval fish because they are very susceptible to over- and underfeeding, both resulting in increased incidences of disease and mortality due to improper larval nutrition or water quality (Deng et al. 2003; Hamlin et al. 2006; Zheng et al. 2015). In addition, as feed cost is one of the most important factors affecting the bottom line of an intensive aquaculture operation, including the larval period, because it constitutes at least 50% of the production cost (Rana et al. 2009), the knowledge of the optimum feeding rate is also important in terms of reducing the operational costs associated with fish rearing. In this sense, the quantities of feed offered to larval fish have considerable impact on growth and survival (Charlon and Bergot 1991; Gisbert and Williot 1997). At the risk of underfeeding or starving young fish, which are more vulnerable to short-term durations of malnutrition, it is a common practice to overfeed hatchery animals to ensure they are getting adequate amounts of feed, but this practice is not without consequence. As Hamlin et al. (2006) reviewed, excess feed can exacerbate larval mortality by encouraging protozoan growth (*Chilodonella* sp.) in sturgeon rearing tanks (Mohler et al. 2000) and bacterial contamination (*Myxobacter* and *Flexibacter* sp.) (Conte 1988; Brun et al. 1991), as well as increase the likelihood of gill infection (MacPhee et al. 1995). Conversely, food-limiting fish especially at these early developmental stages introduces risks of starvation and cannibalism. Under normal conditions, cannibalism has not been shown to be especially problematic for most species of sturgeon (Gisbert et al. 2000; Gisbert and Williot 2002; Hamlin et al. 2006), but inadequate amounts of feed have been shown to encourage this phenomenon

(Charlon and Bergot 1991). Thus, Charlon and Bergot (1991) found a significant and positive correlation between the quantity of distributed feed and larval survival and recommended that feeding rates for Siberian sturgeon larvae should be 20% of the stocked fish biomass per day. Similar results were reported by Deng et al. (2003). Feeding ratios change depending on the feed manufacturer's instructions and the experience of hatchery managers. For instance, in French hatcheries, Siberian sturgeon is fed at 5 days post-hatching at 6% of the stocked fish biomass per day (16–17 °C), whereas feed rate is progressively decreased and fish are fed at 2.3% at 30 days post-hatching. In the case of sturgeon larval feeds manufactured by Coppens, they are recommended to be distributed between 4 and 6% of the stocked biomass per day at the onset of exogenous feeding (16–18 °C), and it is a figure that is reduced to 2.5% when fish are 2.5 g. In *A. transmontanus* where growth performance and body composition of larvae were significantly affected by feeding rate, the optimum feeding rates based on specific growth rates as determined by the broken line analysis were 26%, 13%, 11% and 6% body weight per day, respectively, for each of the first 4 weeks after initiation of feeding (Deng et al. 2003). However, in a recent study, Hamlin et al. (2006) recommended reducing the above-mentioned feeding rates for Siberian sturgeon and suggested feeding larvae at low feeding rates (a slight or negligible amount of uneaten feed remaining in the bottom of the tanks in a 24-h period). The former authors showed that at lower feeding levels, sturgeon larvae were able to attain comparable weights and survival to higher-fed animals and concluded that these feeding practices would translate into reduced costs in terms of labour associated with tank maintenance. As Chebanov and Galich (2013) recommended, after each feeding delivery, checking of the feed consumption should be performed; if a large quantity of uneaten feed is recorded, the feeding schedule and the state of the fish should be checked. The daily rate should then be adjusted after determination of possible causes of the weak feeding activity. According to the former authors, feeding frequency for sturgeon larvae of 0.04–0.06 g should be 24 times per day, whereas feeding frequency is reduced to 12 times per day when fish are 0.07–0.5 g and up to 6 times per day between 0.5 and 2 g of wet body weight. Finally it is important to mention that although feeding larval fish on a percent body weight basis is a beneficial guideline, varying cohorts of fish may exhibit differential feeding behaviours, and this practice may be prejudicial to production efficiency if optimal feeding levels are above or below this recommendation; thus, hatchery managers are recommended to establish their own feeding practices based on their own know-how and experience.

Conclusions

Although Siberian sturgeon larval rearing may be considered easier when compared to other sturgeon and freshwater species, special attention is needed for optimizing larval feeding in order to maximize larval performance, survival and quality, since this phase of the productive cycle has a considerable impact on the economic profitability of the activity. Special attention may be taken for not overfeeding larvae, since an excess of feed may deteriorate water quality and exacerbate larval mortality by encouraging protozoan growth, bacterial contamination, as well as increase the likelihood of gill infection. However, undernourishing fish especially at these early

developmental stages introduces risks of starvation and cannibalism. Although there exist several diets especially manufactured for sturgeon larvae, further research is needed in order to provide insight into the potential link between early nutrition and the development of deformities in the axial skeleton as it was indicated by Leprévost and Sire (2014).

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Part II

Ongrowing

Food Characteristics and Feeding Management on Sturgeon with a Special Focus on the Siberian Sturgeon

30

Ryszard Kolman and Andrzej Kapusta

Abstract

Information on nutrition and feeding is scarce for most sturgeon species. In natural waters, the Siberian sturgeon, *Acipenser baerii* Brandt, feeds predominantly on benthic organisms. Information on Siberian sturgeon nutrition and feeding is needed because of increased interest from hatcheries and farms producing fish for release, meat, and caviar. Feeding of fish is one of the most important components in the process of sturgeon breeding. At early stages of fish life, the special sturgeon feed with raised content of protein is applied. In the initial period of larval Siberian sturgeon feeding and during the first 6 days of exogenous feeding, it is beneficial to use natural feed, for example, larval *Artemia* sp., followed by dry food. Juvenile Siberian sturgeon with a mean body weight of up to 10 g can be fed appropriately selected granulated trout feed or specialty sturgeon feed. This feed is highly digestible that promotes fast growth of juvenile sturgeon.

Keywords

Acipenser baerii • Diet • Feeding • Growth • Rearing • Sturgeon

Introduction

Siberian sturgeon, *Acipenser baerii* Brandt, like most fish of the genus *Acipenser*, is typically benthophageous. Within its natural range of occurrence, which is the great Siberian rivers, its food includes benthic organisms such as the crustaceans (Copepoda, Cladocera, *Pontoporeia* sp., *Mesidothea* sp.), insect larvae (Chironomidae, Plecoptera, Trichoptera, Ephemeroptera, Ceratopogonidae, Simuliidae, and others), mollusks

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(*Sphaerium* sp., *Pisidium* sp.), and small benthic fish and fish eggs (Egorov 1961; Karasev 1987; Ruban 1999). Of course, the composition of the natural sturgeon diet changes depending on its developmental stage, the season, and location of the foraging grounds. For example, in the lower reaches of the Ob' River during the summer, the Siberian sturgeon food is dominated by the freshwater clams *Sphaerium* sp., while in winter the diet comprises primarily larval Simuliidae (Ruban 1999). However, in the upper reaches of the Ob' and the Irtysh Rivers, the diet of sturgeon was dominated by larval Chironomidae and Oligochaeta. The size of the sturgeon, obviously, has a significant impact on its diet composition. The food consumed by juvenile (up to a body length of 50 cm) comprises mainly larval insects and small clams of the genus *Sphaerium*. Older individuals feed mainly on mollusks, including clams, and also sometimes on fish juvenile and eggs (Dryagin 1948). Similar differences are attributed to the occurrence of various food organisms in the Yenisei basin, and the gut contents of sturgeon examined from the delta region of this river were dominated by *Mesidothea* sp. In the upper reaches of the river, the basic food component of the Siberian sturgeon comprises larval Chironomidae with the addition of mollusks and small lamprey and fishes. Studies of the Siberian sturgeon feeding have identified a clear link between the composition of organisms found in the stomach and their occurrence in the river (Dryagin 1948; Egorov 1961). The diet of Siberian sturgeon under natural conditions is varied with a high protein content and a relatively low fat content. In addition to the content of these basic nutritional components, their source is very important as it determines the amino and fatty acid profiles of the lipids. Knowledge of the food and feeding of sturgeon is fundamental because of increased interest from hatcheries in producing juveniles for release and farms in producing meat and caviar. Within this issue, a detailed knowledge of sturgeon feeding could be relevant.

30.1 Sturgeon Feeding Under Aquaculture Conditions

One of the cornerstones of providing fish with the appropriate environment for growth while maintaining them in good condition with high survival rates is ensuring appropriate feed quality in terms of composition, aroma, and taste. Appropriate feed quality not only guarantees high growth rates but also higher fish survival rates, and following specific feeding treatments is required by juvenile sturgeon stages as well as selects and broodstock (Kolman 2010).

30.1.1 Feeding of Larvae

Immediately after hatching, larval sturgeon, including the Siberian species, feed on the contents of the yolk sac for a period of approximately 6–9 days (Detlaf et al. 1981). During this period, the posterior end of the alimentary tract fills with a melanin substance (Fig. 30.1). Immediately before the end of this period, the melanin plug is ejected, which is a signal that the sturgeon larvae are ready to be fed intensively. Gisbert and Williot (1997) found that exogenous feeding was positively correlated with the expulsion of the melanin plug.

Fig. 30.1 Larval Siberian sturgeon during endogenous feeding (credit M. Szczepkowski)

During the period when they are transitioning to exogenous feeding, sturgeon hatchlings have well-developed stomachs (Detlaf et al. 1981; Ostaszewska and Dabrowski 2009). The larvae of these fish adapt relatively easily to feeding on formulated feeds (Dabrowski 1984). The moment the sturgeon transitions to exogenous feeding, enzymes occur in the digestive tract that enables protein digestion in the stomach, while pancreatic enzymes (alkaline protease and α-amylase) are found in notable quantities directly after the initiation of feed intake (Gisbert et al. 1999). This is why the feed delivered during the transition to exogenous feeding should have an advantage of low molecular weight proteins (about a 25% share), while the share of the fraction of long-chain polypeptides should comprise from 4 to 7.5%. Feeds with such a protein structure ensure not only high survival rates during the critical phase of transitioning to exogenous feeding but also during the subsequent period of high hatchling growth rates (Dabrowski 1979; Gershanovich et al. 1991).

In order to determine precisely the proximate composition of feeds for larvae stages of Siberian sturgeon, experimental rearing was performed during which the occurrence of digestive enzymes in the larval digestive tract was identified and then in larvae aged from 9 to 44 days post-hatch (dph) (Żółtowska et al. 1999, 2002). Immediately following the initiation of exogenous feeding, i.e., from 9 dph, only alkaline protease was active in the larval digestive tracts. In the first week of exogenous feeding (16 dph), lipolytic enzymes and maltase were active as was low-level acidic protease activity, but without pepsin. The first pepsin activity was not confirmed in the digestive tracts of Siberian sturgeon larvae until 44 dph. Amylolytic activity gradually increased from 3 to 6 weeks post-hatch. Maltase and trehalase activity peaked at 30 dph. The results of this study confirm earlier observations by Gershanovich et al. (1991) regarding the composition of food for sturgeon hatchlings and early juvenile stages. The results of these indicate primarily that in the early stages of exogenous feeding, the larval sturgeon diet should contain short-chain protein, while in the later phases (after 44 dph), the feed must contain long-chain protein originating from fish meal.

In the initial period of larval Siberian sturgeon feeding and during the first 6 days of exogenous feeding, it is beneficial to use natural feed, for example,

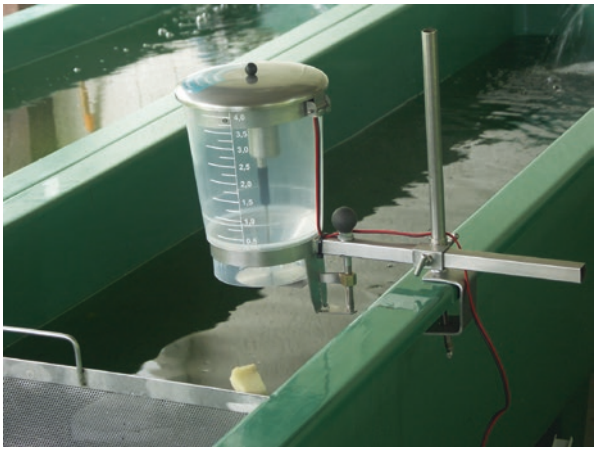

Fig. 30.2 Automatic feeder distributing *Artemia* nauplii or other planktonic feeds (credit G. Wiszniewski)

larval *Artemia* sp., followed by mixed feed, e.g., Perla Larva Proactive and Aller Futura EX (Kolman et al. 1999; Najdegerami et al. 2015). Each of the feeds was recommended as sufficient diets for fish larvae as a sole food due to its stability in water and long-term suspension in the water column. This results in considerably higher survival rates during this crucial period of life. To ensure the continual, high concentration of natural feed in rearing tanks, an automatic feeder construction was developed that contains a dosing mini-pump (Fig. 30.2). The feeders are controlled by a steering mechanism that permits changing the feed ration and the frequency of delivery. However, to ensure that feed is rationed precisely, the automatic feeders have to be equipped to deliver small granulated feeds precisely (Fig. 30.3).

The formula for feeds used during intense sturgeon culture, and especially starter feeds for the initial hatchling feeding period, must be nutritionally balanced, but attention must also be paid to the aroma and taste of the feed. This is important because sturgeon find food primarily using chemoreceptors (Kasumyan 1999). It has been demonstrated that the olfactory organ in sturgeon is developed in the embryonic stage; however, the ability to receive taste stimuli is not active until the initiation of exogenous feeding (Devitsina and Kazhlaev 1992). Thus, behaviors governing the search for and consumption of food begin to take shape in the larval stage, which is why using feed that is inappropriate in terms of odor and/or taste, especially in early rearing, can have a negative impact on hatchlings and lead to differentiated sizes or, in extreme cases, starvation and increased mortality (Kolman 2010). Not meeting these conditions leads to drastic differentiation in larval sizes. Special attention should be given to the transition to exogenous feeding, where cannibalism, difficulties in adaptation to a new diet, overfeeding, and resulting bacterial infections dramatically reduce survival to the fingerling stage (Gisbert and Williot 2002). This is especially dangerous because during the initial period, sturgeon willingly attack prey of larger sizes, which can be smaller starving individuals. In this instance, usually both fish die, since the attacking fish is

Fig. 30.3 Automatic feeder distributing small granulated feeds (credit L. Kopański)

unable to swallow its prey that it has taken from the tail end and ends up choking on the prey's head. To reduce losses of sturgeon linked with the transition to exogenous feed, small quantities of feed should be delivered even before exogenous feeding commences (initially from 4 to 5 dph). This is to permit the hatchlings to grow accustomed to the odor of the feed and to shorten the adaptation period to formulated feed. In the end, this has the effect of a smaller number of larvae starving and less size differentiation among individual fish. A small portion of larvae (fewer than 2–3%) do not take up feed from the tank bottom; rather, they swim belly up at the tank surface searching for food. These individuals are not appropriate for further rearing as they have lower growth rates than others that feed intensively on formulated diets. When either inappropriate feed or too little of it is supplied, larval pectoral fin anomalies are noted that appear to prompt the fish to stage mutual attacks (Chikhachov et al. 1981; Semenkova 1983; author's own observations). Selecting the appropriate feed in terms of odor and flavor can be determined easily with the following practical method: lightly moisten the feed and then flatten the granules slightly before tossing them into the tank with the sturgeon that are beginning to feed—if no fish gather around the feed, then it is inappropriate.

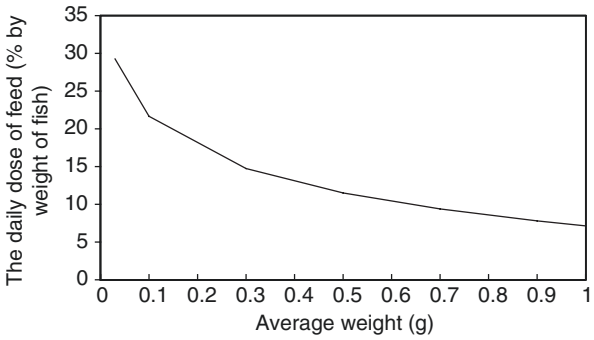

Fig. 30.4 Size of daily feed ration for larval and early juvenile of sturgeon depending on mean fish body weight (according to Kolman 2010)

During the transition period to exogenous feeding, feed should be supplied in excess, which means that the daily ration should exceed by 50% the biomass of the hatchlings. After active feeding begins, then the feed ration should be reduced to about 30%, and in further rearing, it should be further reduced in accordance with the feeding curve (Fig. 30.4) (Kolman et al. 1996; Kolman 2010). Feed should be delivered to the fish around the clock at frequent intervals which is facilitated by automatic and/or band feeders operating continually.

30.1.2 Feeding of Juveniles

An important aspect of the technology of feeding sturgeon hatchlings and juvenile is the application of the appropriate feed particle size and its gradual adjustment along with fish growth (Table 30.1). Changes in feed particle size should be done gradually over 2–3 days. Most sturgeon species hatchlings, including those of Siberian sturgeon, that are beginning to feed exogenously can take up and consume feed of a granulation size of a diameter of 0.2–0.4 mm. Hatchlings that have been reared to a weight of 0.2–0.5 g can be fed with a granulation size of 0.4–0.6 mm and that with a weight of 1.0 g can consume feed with a diameter of 0.6–0.8 mm. Juvenile with a mean weight of 2.0 g is capable of ingesting feed granules from 1.0 to 1.5 mm and larger from 1.5 to 2.0 mm (Shcherbina et al. 1985).

Juvenile Siberian sturgeon with a mean body weight of up to 10 g can be fed appropriately selected feed containing less than 11–15% lipid and 50–56% protein. During this period, the feed should be supplied at least ten times daily. The daily ration depends on current fish body weight (Fig. 30.5), and it should be updated at least once weekly through measurements of mean fish body weight. Nevertheless, the ration supplied should be gradually increased daily to the target quantity. Since Siberian sturgeon also feed intensively in the dark, they should also be supplied with feed at night. When delivering feed, it is good to bear in mind that sturgeon are mainly bottom feeders so the food should be distributed equally over the tank bottom as this helps counteract fish size differentiation.

One important factor contributing to the results of culture is frequent fish size sorting, especially in the juvenile stage up to a mean weight of approximately 50 g. Sturgeon is characterized during this period by very high growth rates and very

Table 30.1 Optimal feed particle size in relation to sturgeon body weight

Fish body weight (g)	Feed granulation (mm)
<0.2	<0.4
0.2–0.5	0.4–0.7
0.5–1.0	0.7–1.0
1–5	1–1.4
5–50	1.5–2
50–100	2–2.5
100–200	2–3
200–500	3–5
500–1500	5–6
>1500	>6

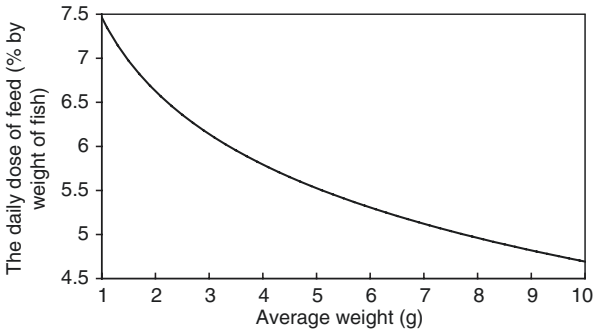

Fig. 30.5 Daily feed ration for juvenile sturgeon depending on mean body weight (according to Kolman 2010)

intense metabolism. Accordingly, the occurrence of size variation leads quickly to the creation of starving individuals and losses. Slowly growing individuals that have been sorted out and then fed accordingly can also be full-value cultured fish (Georgiadis et al. 2000a, b; Kolman 2010). The phenomenon of growth rate differentiation can be especially distinct among sturgeon hybrids: second-generation bester (*Huso huso* (L.) × *Acipenser ruthenus* L.), *H. huso* × bester hybrid (BBS), *A. ruthenus* × bester hybrid (SBS), and others. Among the population of this sturgeon line, slow-growing individuals can comprise as much as 20% of the total number of fish. However, with parental species, including Siberian sturgeon, differentiation is less frequent, and it occurs primarily during the early juvenile stage until a mean body weight of 10 g (Kolman et al. 1997).

30.1.3 Feeding Sturgeon for Meat and Caviar

Marketable sizes are usually 1.5–2.5 kg for Siberian sturgeon in Poland (Kolman et al. 2007). Feeding of that size sturgeon can be done with typical commercial trout granulated feed or with feeds specifically formulated for sturgeon that contain 40–52% protein and no more than 15% lipid. As the fish grow, the frequency of feeding can gradually be lowered to four times daily. Since sturgeon also feeds intensively when it is dark, the fish should also be fed during the night. The daily feed ration for juvenile sturgeon and commercial-sized fish, which, in Siberian sturgeon, is a body weight in excess of 2.5 kg, should be updated depending on mean

fish body weight at least twice weekly (Figs. 30.6 and 30.7). Kaushik et al. (1989) reported that 1.45% of body weight per day for Siberian sturgeon weighing between 90 and 400 g was optimal daily food delivery rate.

The delivery of the feed should be done bearing in mind that sturgeon mainly take up food from the bottom so attention should be focused on ensuring that the feed is distributed equally over the tank, or pond, bottom surface area as this helps

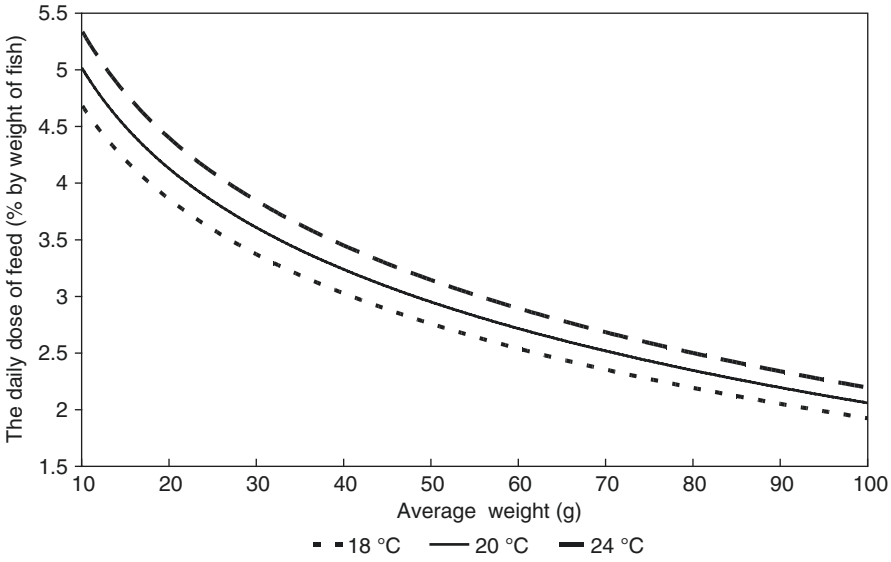

Fig. 30.6 Daily feed ration for older juvenile sturgeon depending on mean fish body weight (according to Kolman 2010)

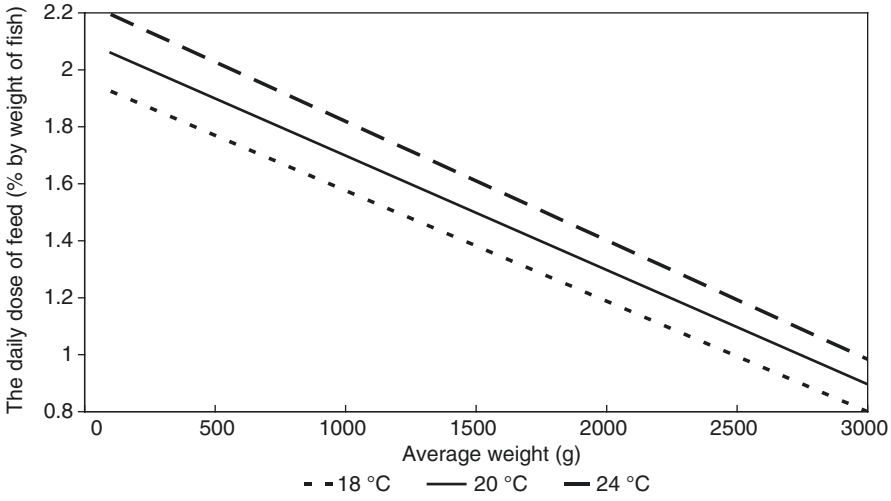

Fig. 30.7 Daily feed ration for older juvenile and commercial-sized sturgeon depending on mean fish body weight (according to Kolman 2010)

counteract the occurrence of fish size differentiation. That allows to decrease the potential completion between fish and therefore the potential domination of large ones. This is also why the stocking density is very important, and with fish with mean body weights ranging from 50 to 100 g, this should not exceed 10 kg/m^2, while at mean body weights of up to 0.5 kg, stocking density should not exceed 20 kg/m^2. Fish larger than 1 kg can be stocked to a density of 50 kg/m^2 (Kolman 2010).

Broodstock and female caviar stocks should all be fed special diets produced specifically for sturgeon broodstock. Siberian sturgeon broodstock feed should contain a large percentage of high-quality fish meal, which is especially important to facilitate qualitative and quantitative reproduction (Kolman 2010). Protein requirement for optimum growth of Siberian sturgeon was 40% (Médale et al. 1991; Hung and Deng 2002). Kaushik et al. (1994) stated that the growth performance and apparent digestibility coefficient (ADC) of protein were higher with casein or casein and soybean diets than with a fish-meal diet in Siberian sturgeon. Further requirements are a well-balanced amino acid profile, vitamin level, and the right fatty acids. The optimal dietary lipid level has not been determined in any species of sturgeon (Hung and Deng 2002); however, sturgeon fed diets with 12.5–14.0% lipid showed a rapid growth and high feed efficiency (Médale et al. 1991; Kolman 2010). The presence of phytoestrogens needs to be kept in mind when Siberian sturgeon diets are selected (Francis et al. 2001). Pelissero et al. (1991) emphasize that attention must be paid to potential long-term effects of phytoestrogens from soya bean extract. Therefore, the protein in feeds for sturgeon broodstock should not be plant based.

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Reasons and Possibilities of Fish Meal Replacement in the Siberian Sturgeon

31

Benedetto Sicuro

Abstract

Although several fish feed companies currently produce commercial sturgeon feeds, in Siberian sturgeon farming, most farmers usually use nonspecific fish diets, particularly high-energy salmonid diets. Considering the general situation and trend of finfish nutrition, it is clear that forage fish meal should be reduced at minimum quantity in the future of fish artificial nutrition. For this reason, new ingredients already tested with other species have been progressively introduced in Siberian sturgeon nutrition. Fish meal replacement has substantially followed the path of salmonid artificial feeding. Most commonly used vegetal protein alternative to fish meal has been initially soybean meal and successively soybean meal included in blends of vegetal proteins. Between animal protein sources, blends of rendered animal proteins have been recently used with success. The replacement of fish meal with rendered terrestrial animal products added with lysine and methionine appears the most interesting possibility in the future as no adverse effect of fish growth and substantial modifications on fish body composition have been observed, while replacement with vegetal protein appears less attractive solution as soybean meal can interfere in fish reproduction for the presence of phytoestrogens.

Keywords

Fish meal replacement • Soybean meal • Antinutritional factors • Rendered animal protein • Blend of vegetal proteins • Blend of rendered animal proteins

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An Introduction to Fish Meal Replacement

To sustain the rapid growth of aquaculture, it is necessary to use nutrient resources efficiently. The impact of nitrogen and phosphorus loading from aquaculture farms has been extensively investigated in the freshwater ecosystems, and several solutions have been proposed such as integrated aquaculture and promotion of environmentally compatible ingredient utilization (Ali et al. 2016; Edwards 2015; Ottinger et al. 2016; Zhang et al. 2015). In this context, fish nutrition plays a key role, and in these last years, several attempts to reduce the use of fish meal have been carried out, in all farmed species. It is very well known that the main ingredient in artificial feeds is fish meal, and it is clear that in the future it must be reduced at minimum possible level or possibly abandoned (Kaushik et al. 2004; Tacon and Metian 2008, 2015; Tacon et al. 2011).

Due to the limited diffusion of sturgeon farming with respect to other forms of fish farming, sturgeon artificial feeding until this moment has been essentially based on commercial salmonid feeds, even if it is clear that sturgeons have anatomical and physiological substantial differences from other fish (Fauconneau et al. 1986; Moreau et al. 1996; Daprà et al. 2009). Salmonid feeds have resulted in good productive results, and this fact has initially not encouraged specific research in sturgeon nutrition. However, as with other farmed fish species, the future of sturgeon farming must be based on sturgeon nutritional needs.

Main alternative feed ingredients to fish meal are commonly divided into two main categories: plant and animal proteins. The most common plant proteins used in aquafeed industry are soybean meals (both full-fat and solvent extract meal), gluten cereal meals, algal proteins, and pulses by product meal (Tacon et al. 2011; Bendiksen et al. 2011). Animal proteins used in fish meal are aquatic proteins as fish by-product meal (trimming fish meal), fish silage products and hydrolysates or terrestrial animal proteins as meat by-product meal, poultry by-product meal, and blood by-product meal (Tacon et al. 2011).

The use of alternative ingredients to fish meal is not only affected by fish nutritional needs but also by economic factors such as fish meal price increase (Zhu et al. 2011), market availability of feedstuffs, and market concerns about food safety issues (Tacon and Metian 2015). Protein is the most expensive component of fish feeds. For this reason, in the non-European countries, fish feed manufacturers largely use terrestrial animal meals, while in Europe consumers' safety issues limit the utilization of these ingredients (Tacon et al. 2011). Considering the use of fish meal in aquafeed industry, an important clarification should be made: there are different types of fish meal and not all fish meals have negative environmental impact. In fact, recent scientific literature suggests that in the future the forage fish meal use must be reduced at minimum possible level, while the use of trimming fish meal, obtained by fish discards and by-product processing, should be encouraged and promoted (Ytrestøyl et al. 2015). The case of the Atlantic salmon in Norway is enlightening on this matter. Due to the progressive inclusion of vegetal protein and trimming fish meal in the artificial nutrition of Atlantic salmon, the [fish in/fish out] ratio has been reduced from 4.4 in the 1990s to 0.7 in 2013 (Ytrestøyl et al. 2015).

Not only in Norway is this a relevant issue but also in the entire world; in fact in 2008 it was estimated that 33% of fish meal used in Europe and 25% at global level were obtained by trimming fish meal (Tacon et al. 2011). Given that assumptions, it is clear that this is the direction that must follow also sturgeon artificial feeding.

31.1 Alternative Proteins Currently Used in Siberian Sturgeon Nutrition

Siberian sturgeon is one of the most common species utilized in sturgeon aquaculture worldwide (Bronzi et al. 2011; Wei et al. 2011; Williot et al. 2001), and it is the dominant species of farmed sturgeon in China (Wei et al. 2011); therefore its artificial nutrition has been extensively investigated. Most sturgeon farmers usually use existing commercially available diets, particularly those of high-energy salmonid diets with 40–50% crude protein, 12–20% crude lipid content, and 18–22 MJ kg^{-1} gross energy for Siberian sturgeon (Mèdale and Kaushik 1991; Kaushik et al. 1994; Liu et al. 2009; Mazurkiewicz et al. 2009; Docan et al. 2012; Eslamloo et al. 2012; Williot 2009), beluga sturgeon (*Huso huso*) (Mohseni et al. 2006; Paschos et al. 2008; Vali Hosseini et al. 2010), green sturgeon (*Acipenser medirostris*) (Zheng et al. 2015), lake sturgeon (*Acipenser fulvescens*) (Moreau and Dabrowski 1996), white sturgeon (*Acipenser transmontanus*) (Ng and Hung 1995), and hybrid sturgeons (Guo et al. 2011; Qiyou et al. 2011). Commercial salmonid feeds and starter diets for marine fish have been also used during larval stages (Dabrowski et al. 1987; Fauconneau et al. 1986; Gisbert and Williot 2002; Deng et al. 2003; Zheng et al. 2015). Crude protein requirements for Siberian sturgeon have been recently updated to lower values; in fact Ronyai et al. (2002) found effective dietary protein level of 29–33%, and Xue et al. (2012) stated that Siberian sturgeon fed with massive replacement of fish meal can reach 36% of crude protein.

The relatively short history of sturgeon nutrition has followed the path of main piscivorous farmed species, and ingredients already tested with other species have been progressively introduced in sturgeon nutrition (Table 31.1). First researches expressly targeted on Siberian sturgeon artificial nutrition have been carried out in the late 1980s and the early 1990s in France (Dabrowski et al. 1985, 1987; Fauconneau et al. 1986; Kaushik et al. 1989, 1991; Mèdale and Kaushik 1991).

Fish meal replacement in fish feeds has been studied for more than 20 years in several piscivorous fish species (Lazzarotto et al. 2015), and the scientific literature often showed fish growth reduction in case of high levels of plant protein inclusion in some recent species for aquaculture such as cobia (Chou et al. 2004), sharpsnout sea bream (Hernandez et al. 2007), and turbot (Yun et al. 2014). Some of these detrimental effects are related to low digestibility of plant proteins, imbalanced amino acid composition, and the presence of antinutritional factors (Francis et al. 2001; Gatlin et al. 2007). In other fish species more commonly farmed such as rainbow trout or Atlantic salmon, the fish meal replacement has progressively increased in these last years, and there are several researches reporting 100% of fish meal

Table 31.1 Alternative ingredients used in Siberian sturgeon nutrition

Ingredient	Productive parameter[a]	Size (g)	Duration (days)	Source
Blend-rendered animal protein	FCR from 1 to 1.08	28.0 ± 0.2	56	Zhu et al. (2011)
Blend-rendered animal protein	FCR from 1 to 1.08	39.0 ± 0.2	56	Xue et al. (2012)
Cottonseed meal	Protein digestibility 87.6%	8.4 ± 0.2	42	Liu et al. (2009)
Fermented feather meal	Protein digestibility 87.7%	8.4 ± 0.2	42	Liu et al. (2009)
Hydrolyzed feather meal	Protein digestibility 90.9%	8.4 ± 0.2	42	Liu et al. (2009)
Meat and bone meal	Protein digestibility 84.5%	8.4 ± 0.2	42	Liu et al. (2009)
Poultry by-product meal	Protein digestibility 90.4%	8.4 ± 0.2	42	Liu et al. (2009)
Rice protein concentrate	Good fish growth	19.1 ± 6.7	113	Sicuro et al. (2015)
Soybean meal	FGR 1.06–1.32	160	90	Kaushik et al. (1994)
Soybean meal	Protein digestibility 87.7%	8.4 ± 0.2	42	Liu et al. (2009)
Soybean meal	n.r.	n.r.	241	Luo et al. (2015) (broodstock diets)
Soybean meal	PER 1.49–1.62	66.7 ± 0.1	56	Guo et al. (2011)
Soybean meal	FCR 1.87–2.27	32.8 ± 2.5	42	Ronyai et al. (2002)
Soy protein concentrate	FCR 0.6–1.82	140 ± 1.3	50	Mazurkiewicz et al. (2009)
Spirulina meal	FCR 1.0–1.39	92.1 ± 3.6	42	Palmegiano et al. (2005)
Vegetal protein blend: soybean meal and wheat meal	FCR 1.29–1.4	39.0 ± 0.2	56	Yun et al. (2014)
Wheat meal	FCR 0.6–1.82	140 ± 1.3	50	Mazurkiewicz et al. (2009)
Yeast (single-cell protein)	Larval growth	n.r.	30	Dabrowski et al. (1985); Fauconneau et al. (1986); Dabrowski et al. (1987) (larval diets)

[a]Available productive parameter in the cited article is reported
FCR food conversion rate, *FGR* feed growth rate, *PER* protein efficiency ratio, *n.r.* not reported

substitution (Sales 2009; Lazzarotto et al. 2015). Numerous studies, especially in rainbow trout and Atlantic salmon, have reported that quality of final product can be affected by fish meal replacement, in terms of quality of final product (Gatlin et al. 2007). Therefore, if the complete replacement of forage fish meal is an imperative necessity and a crucial target in the next years for Siberian sturgeon nutrition and in aquaculture feeds in general, it is also evident that quality for consumers must be

considered. Some results obtained in aquafeed research have been transferred to Siberian sturgeon, and the first ingredient used as potential alternative to fish meal has been soybean meal. An inclusion of 6–9.5% of soybean meal was already tested in experimental diets in the early 1990s (Kaushik et al. 1991). Similarly to salmonid artificial feeding (Badillo et al. 2014; Lazzarotto et al. 2015), research on fish meal replacement showed that soybean meal can be efficaciously used in Siberian sturgeon nutrition (Ronyai et al. 2002); in fact soybean meal showed high protein digestibility (91.8%) (Liu et al. 2009) that was similar to salmonids (Refstie et al. 2000). Soybean meal is currently tested also in the artificial feeding of other sturgeon species, such as beluga (Mohseni et al. 2006; Vali Hosseini et al. 2010; Ta'ati et al. 2011), sterlet (Ustaoglu and Rennert 2002), green sturgeon (Zheng et al. 2015), and hybrid sturgeons (Guo et al. 2011; Qiyou et al. 2011). The main negative aspect of dietary soybean inclusion in Siberian sturgeon nutrition is the presence of phytoestrogens (Latonnelle et al. 2002a, b; Pelissero et al. 1991a, b). Siberian sturgeon showed high sensitivity to phytoestrogens with respect to other fish species (Latonnelle et al. 2002b); in fact sturgeons synthesized vitellogenins irrespective of the season or their sexual status (Latonnelle et al. 2002a). Not only has soybean meal (and soybean products) been tested in Siberian sturgeon nutrition but also other vegetal and animal protein potential alternatives to fish meal (Table 31.1). High inclusion levels of vegetal ingredients without detrimental effects on fish growth have been obtained with spirulina meal (Palmegiano et al. 2005); however a total replacement of fish meal has been recently reached only using a blend of vegetal ingredients, such as soybean meal and wheat gluten meal, eventually added with amino acid supplementation (Yun et al. 2014).

Rendered animal proteins have been successfully proposed in piscivorous fish species both in freshwater such as sunshine bass (Muzinic et al. 2006), rainbow trout (Badillo et al. 2014), chinook salmon (Fowler 1991), and tilapia hybrid (Xue et al. 2003) and in saltwater such as Malabar grouper (Wang et al. 2008). In Siberian sturgeon feeds, meat and bone meal, poultry by-product meal, hydrolyzed feather meal, and fermented feather meal have been introduced (Table 31.1). Siberian sturgeon showed high digestibility of rendered animal protein (Liu et al. 2009; Zhu et al. 2011; Xue et al. 2012) that is deficient in some essential amino acids, especially lysine and methionine. Similarly to the case of vegetal ingredients, a practical method often used to compensate eventual deficiencies is to introduce a blend of ingredients supplemented with crystallized amino acids (Zhu et al. 2011; Xue et al. 2012). Siberian sturgeon showed similar characteristics to other piscivorous fish species, such as rainbow trout, turbot, cobia, and European sea bass, that can efficiently use crystallized amino acids in the artificial feeds (Fournier et al. 2004; Peres and Oliva-Teles 2006; Nang Thu et al. 2007; Salze et al. 2010). Blend of rendered animal proteins has been successfully tested at 75% level of fish meal substitution in Siberian sturgeon (Xue et al. 2012). Also heat treatment, such as Maillard reaction, during fish feed processing is known to lower utilization of dietary amino acids (Deng et al. 2005). As regards to the possible effects of fish meal replacement on flesh quality, there are few studies on fillet fatty acid composition of Siberian sturgeon, but it is clear that it mirrors its diet (Badiani et al. 1997;

Nieminen et al. 2014). Liu et al. (2014) in Chinese sturgeon (*Acipenser sinensis*) found that muscle lipid level affects meat quality in terms of nutritional value and sensory properties.

The replacement of fish meal with rendered terrestrial animal products did not substantially change the fish body composition (Zhu et al. 2011; Xue et al. 2012), and this fact confirms the effectiveness of this nutritional strategy. Luo et al. (2015) showed that DHA/EPA dietary ratio in broodstock nutrition has a direct effect on egg and larval quality of Siberian sturgeon; in particular a DHA/EPA ratio of 1.9/1 improved hatching rate, larval survival, and larval weight after 35 days post-hatching. Fish feed treatments, such as extrusion, improve starch utilization by fish that can utilize cooked starch better than raw starch (Gong et al. 2015). Extruded or pregelatinized starches can be readily used by Siberian sturgeon (Kaushik et al. 1989) and by white sturgeon (*Acipenser transmontanus*) (Lin et al. 1997). However Deng et al. (2005) showed that in white sturgeon, Maillard-type reactions can occur, thus reducing the feed nutritional quality caused by destruction in essential amino acids, decreased digestibility, and eventually production of antinutritional and toxic components.

Finally, fish meal reduction can be indirectly obtained not only by protein substitution but also by macronutrient substitution, thus improving the carbohydrates and lipid inclusion in the diet (Médale et al. 1991; Vali Hosseini et al. 2010). Carbohydrates provide an inexpensive source of energy in fish diets, and Siberian sturgeon and other sturgeon species (Liu et al. 2014; Deng et al. 2005) can readily use dietary carbohydrates, especially in the adult phase (Gong et al. 2015). This fact can be explained as adaptive response to environmental and nutritional possible modifications of these primitive species with respect to teleosteans.

31.2 Perspectives

Considering the general situation and trend of finfish nutrition, it is clear that forage fish meal should be reduced at minimum quantity in the future of Siberian sturgeon nutrition. Thus considering with attention lysine and methionine levels and dietary phytoestrogens, scientific literature indicates that almost complete substitution of fish meal is possible using a mixture of vegetal protein or rendered animal proteins. In order to make Siberian sturgeon farming environmental sustainable, it is clear that the future must be "forage fish meal-free." On the light of the extensive literature in farmed fish nutrition, it is possible to suggest some possible ingredients for the future of Siberian sturgeon feeding (Table 31.2), thus keeping in mind that the optimal solution for commercial diets will be a mixture of those feedstuffs.

Considering the great attention focused on caviar production, sturgeon broodstock nutrition is rather neglected. In this sector, the replacement of fish meal will be presumably limited considering that the ideal nutrients for broodstock, such as polyunsaturated fatty acids, are principally contained in fish meal. After the environmental impact and productive issues, which are extensively studied, the quality of the product should

Table 31.2 Potential alternative to fish meal for future Siberian sturgeon nutrition

Potential ingredient	Advantages	Disadvantages
Soybean meal; soybean derivatives meal	Large availability, medium/low price, environmental sustainability	Effects on reproduction, antinutritional effects
Trimming fish meal	Environmental sustainability, ethically acceptable respect forage fish meal, medium price	Minor nutritional value respect forage fish meal, possible presence of contaminant
Vegetal proteins blend	Medium availability, medium/low price, environmental sustainability	Antinutritional effects
Meat and bone meal; other animal by-products	Medium availability, medium/low price, environmental sustainability	Minor nutritional value respect fish meal

be considered with attention in order to promote Siberian sturgeon consumption. Replacement of fish meal protein affects the quality for consumers, and it is a crucial issue for the future of Siberian sturgeon farming as sturgeon flesh is commonly eaten in the Western countries where consumers are usually more exigent in terms of quality. Moreover sturgeon meat is largely consumed in China and Russia, and the future sturgeon farming should be targeted to a medium price product in this area, thus utilizing fish feeds with high replacement of fish meal. This different productive target will be presumably obtained putting more attention on final product price rather than organoleptic characteristics, in order to meet the needs of a larger sector of consumers.

In this moment, there is a need to transfer these results from academia to aquafeed industry and to start testing modern Siberian sturgeon feeds "in field." Paraphrasing a famous Steve Jobs quote, it can be stated that "Fish feed is not just what it looks like and feels like. Fish feed is how it works."

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Endocrine Disruption in the Siberian Sturgeon *Acipenser baerii* Fed with a Soy-Containing Diet

32

Catherine Bennetau-Pelissero and Françoise Le Menn

Abstract

Under the South-West climate conditions, the fish reproductive maturity was reached within 6–8 years allowing the development of a local caviar production. While studying the reproductive physiology of this fish, an estrogenic endocrine disruption was discovered. We managed showing that it was mainly due to soy-based diet containing estrogenic isoflavones. Because the rainbow trout reared in the same conditions did not exhibit such a disruption, we studied the effects of soy isoflavones at different steps of the estrogen endocrine pathways in both species. We managed to show that sturgeon was 50 times more sensitive to estrogenic soy isoflavones than trout. We also showed that this difference of sensitivity was not linked to differences at the estradiol synthesis step nor at the estradiol blood transport step. It was neither due to differences at the estradiol receptor levels nor at the liver cell level. The 50 times difference of sensitivity between the two species was linked to a difference in isoflavone bioavailability due to different xenobiotics detoxifying efficiency of the liver. Genistein, the main soy estrogenic compound, was shown to exhibit deleterious effect on trout reproduction. Until now the effect of this compound and of others from soy is still unknown on sturgeon reproduction due to the late reproductive maturity of this species. Studies are required to check for better reproductive efficiency of this species in the French fish-farm conditions.

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Siberian sturgeon • Vitellogenin • Estradiol • Phytoestrogens • Soy • Metabolism • Estradiol receptor • Aromatase • Hepatocyte • Steroid- binding protein Fish reproduction

Introduction

The first batch of Siberian sturgeon, *Acipenser baerii* Brandt, arrived in France in 1975 within a French-Soviet cooperation network as a biological model for the restoration of the threatened European sturgeon, *Acipenser sturio* (Williot and Rouault 1982; Williot et al. 1997). By side of setting up appropriate husbandry methods (including reproduction), some questions remained unanswered such as the ability to discriminate both genders especially for young specimens by absence of sexual dimorphism. Based on the literature on the rainbow trout (Akimova et al. 1979; Akimova et al. 1985; Le Bail and Breton 1981), the proposal was made to investigate the vitellogenin content as this molecule was supposed to be female sex-specific. Doing that, an endocrine-disrupting phenomenon was observed due to the fish-feeding practices. This chapter relates the story of this discovery in the Siberian sturgeon and its validation by comparison to another fish-farming species, the rainbow trout *Oncorhynchus mykiss.*

The text below will present the progressive exploration of the food-related endocrine disruption observed in the Siberian sturgeon in the French fish-farming conditions. The results were validated by a comparison to the situation observed in the rainbow trout following the same rearing practices. Namely, this chapter will go successively with the following subparts: (1) The blood biomarkers of fish reproductive endocrine physiology used to study the fish reproductive physiology will be presented. (2) The discovery of the phytoestrogen contamination of fish in the French fish-farm conditions will be presented. (3) The identification of the differences between sturgeon and trout toward their sensitivity to phytoestrogens will be assessed. (4) Then, the discovery that phytoestrogen bioavailability is the reason of the difference of sensitivity between the two species will be explained. (5) The evolution of sex steroid levels in the Siberian sturgeon reared under endocrine-disruptive conditions will be presented. (6) The effects of soy phytoestrogens on fish reproduction will be presented. (7) Finally, the recent data about the effect of isoflavones in reared fish will be presented. Some conclusions and perspectives will be drawn at the end of this chapter.

32.1 Blood Biomarkers of Fish Reproductive Endocrine Physiology

While the fish were reared for the first time in France, biomarkers helping following the reproductive physiology were crucially needed to validate morphological observations (Williot and Brun, 1998). Therefore, it was decided to follow the main steroids implicated in fish reproduction and to develop an assay to appreciate the blood

vitellogenin levels. This protein being an estrogen-dependent lipo-glyco-phosphoprotein produced by the liver of oviparous females could also help distinguishing between male and female fish (Pelissero 1988). Sex steroids including estradiol, estrone, and testosterone were assayed in male and female plasmas for more than 4 years with sampling orchestrated every 3 months (Pelissero et al. 1988). Steroid profiles correlated to ovarian development stages are presented in Fig. 32.1. This figure shows estradiol, estrone, and testosterone plasma levels measured by specific RIAs in the plasma of female Siberian sturgeon at different stages of ovarian development (Pelissero 1988). These stages were determined by histology of the ovary performed on biopsies according to the classification reported in the chapter by Le Menn and Bennetau-Pelissero in this book and in Le Menn et al. (2007). The fish were from the 1982 cohort of fish and the samplings were performed in summer 1987 while the fish were on a fish diet only based on fish meal. It can be observed that the estrogen levels were relatively low compared to that of testosterone. Estradiol's highest concentration was recorded in fish plasma while the ovary was in stage IV and therefore when yolk accumulation was at its highest efficiency. Testosterone plasma levels rise at stage IV and V. This is also observed in other fish species and is currently explained by a progressive reduction of the transformation efficiency of testosterone into estradiol by aromatase (Fostier et al. 1983). However, in teleost species, the magnitude of these concentrations is usually of the same order. It is not the case here since testosterone plasma levels (means ± SD) were found to be almost ten times higher than that of estrogens (22.03 ± 5.6 ng mL^{-1} for estradiol at stage IV and 142 ± 32.5 ng mL^{-1} for testosterone at stage V). Values are means ± standard deviation. Yolk protein was purified (Pelissero and Le Menn 1987). Immunological methods allowed identifying it in fish plasma (Fig. 32.2). Unexpectedly vitellogenin was found in all male and female plasmas although no estradiol was significantly detected in immature fish.

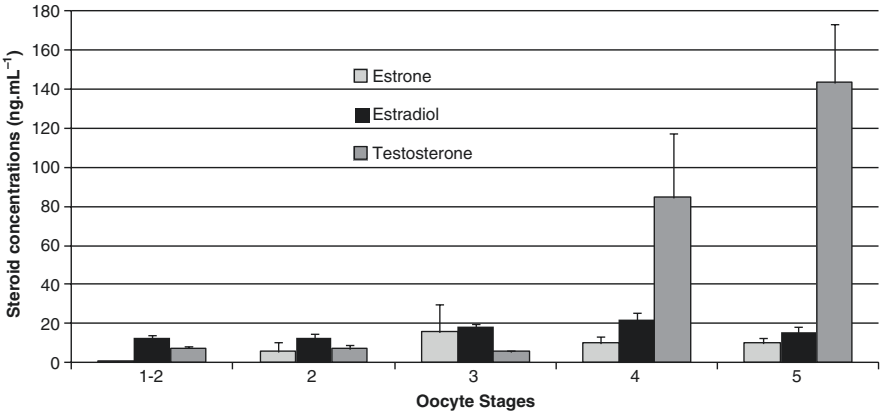

Fig. 32.1 Estradiol, estrone, and testosterone plasma levels in female Siberian sturgeon fed with a diet essentially containing protein from fish origin (From Pelissero et al. (1988) Annal Comp Rend Acad Sci 307(14): 749–754)

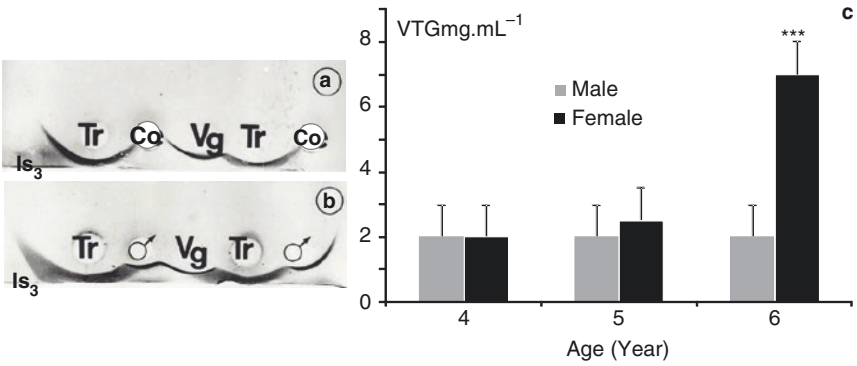

Fig. 32.2 Vitellogenin characterization and levels in the plasma of male and female Siberian sturgeon reared in French fish farm. (**a**, **b**) Ouchterlony test. *Tr* treated fish, *Co* control fish, *Vg* purified Siberian sturgeon vitellogenin, ♂ plasma from male Siberian sturgeon, Is_3 immune serum to Siberian sturgeon vitellogenin n°3. (**c**) Vitellogenin plasma levels measured using a specific ELISA for Siberian sturgeon vitellogenin (From Pelissero 1988, PhD Thesis)

32.2 Estrogen Contamination in the French Fish-Farm Conditions

An ELISA test was developed for vitellogenin measurements in fish plasma (Pelissero et al. 1989; Cuisset et al. 1991). Using this new tool, the presence of yolk protein in male and immature female plasmas was confirmed (Fig. 32.2). This phenomenon was noticed on all reared fish including fish bred in different locations of the southwest of France in the Aquitaine region.

Vitellogenin is known to be produced by the oviparous female liver under estrogen induction (Söffker and Tyler 2010). Vitellogenin can also be a marker of estrogenic endocrine disruption in oviparous species (Arukwe and Goksøyr 2003). Vitellogenin gene is a model of estrogen-dependent protein and was studied for many years in amphibians like *Xenopus* sp. (Mosconi et al. 2002), in fish (Navas and Segner 2006), and in chicken (Beato et al. 1989) models. The synthesis of this protein is possible in both sexes under estrogen stimulation although naturally males do not produce significant levels of estradiol. When our work was in progress in the late 1980s, nobody had studied yolk production in the Siberian sturgeon. This species is the result of several successive polyploidizations (Fontana et al. 2008). The number of chromosomes in the Siberian sturgeon is 249 ± 7 according to Vasil'ev et al. (1981) and Birstein et al. (1993), and no sex chromosome can be identified on metaphase plates (Vasil'ev et al. 1981); a doubt could then rise on the sex segregation of vitellogenin synthesis in the Siberian sturgeon since the presence of a vitellogenin inductor could not be excluded in the genome of all fish. Because of that the demonstration that vitellogenin was a sex-segregated protein had to be done accurately, in this species. Indeed, vitellogenin in this species is a 200 Kda

lipo-glyco-phospho-protein (Pelissero and Le Menn 1987) of which the synthesis is highly energy costing, as is its liver secretion and ovary sequestration. Siberia living conditions are very hard and the local sturgeon is able to live in frozen rivers for durations as long as 6 months saving its energy. In that specific context, it was not plausible that this species would produce a protein highly energy costing for no use. Therefore, the presence of vitellogenin in immature and male fish plasma could either indicate that the detection tool was not specific enough or that fish were contaminated by environmental estrogens (Hinfray et al. 2010). Analyzing male fish plasma, the protein detected by the ELISA tool was shown to exhibit the same molecular weight, the same electrostatic charge, and an equivalent lipid, protein, and carbohydrate composition (Pelissero and Le Menn 1987). Therefore, it was identified as vitellogenin. This detection could indicate that fish were submitted to an estrogenic contamination.

Because all reared fish, whatever their rearing location, exhibited this contamination, we thought that the endocrine-disruptive contamination could not come from the water. Indeed, the water was different in quality in all rearing sites. The contamination should then come from a source common to all fish in all locations. Because we found that yearling sturgeon kept unfed for several months did not exhibit vitellogenin in their plasma (data not shown), the source of contamination was thought to be the commercial fish diet used in fish farms. The latter was known to contain fish meal and soybean meal both known to potentially contain estrogenic compounds. In soybean meal, the estrogens are phytoestrogens, namely, essentially genistein and daidzein, while in fish meal, steroidal estrogens could be detected (Table 32.1).

To check for the estrogenic contamination of fish via the fish diet, an experiment was conducted comparing a soy-based diet, a commercial fish diet containing fish meal and soybean meal, and a control diet based on ultrapure casein meal free of all estrogenic compounds (Pelissero et al. 1991b). Two-year-old fish which were immature were fed during 15 weeks in controlled conditions, and blood samples were collected every 3 weeks for steroid and vitellogenin levels in plasma. Although the sex steroids were undetectable, vitellogenin plasma levels which were not nil at the beginning of the experiment due to the previous contamination in fish farm decreased in casein-based diet-fed fish. The vitellogenin plasma levels were undetectable by the end of the experiment (Fig. 32.3). Meanwhile, vitellogenin plasma levels went from 0.6 to 1.6 $mg.mL^{-1}$ in fish fed with the commercial diet for trout. Unexpectedly, in fish fed with the soy-based diet, vitellogenin plasma levels increased by a tenfold factor from 0.6 to 6 $mg.mL^{-1}$ within only 15 weeks (Fig. 32.3). This experiment clearly demonstrated that the diet was responsible for the estrogenic contamination in fish reared in French fish farms (Pelissero et al. 1991b). It clearly showed the tremendous estrogenic effect of soy phytoestrogens in the Siberian sturgeon. It showed that the commercial fish diet was estrogenic in the Siberian sturgeon without solving the question of the identity of the estrogen source in the commercial diet. Indeed, both fish meal containing steroidal estrogens and soybean meal containing phytoestrogens could be involved in this process (Pelissero and Sumpter 1992). However, considering the tremendous estrogenic effect of the

Table 32.1 Steroid and soy isoflavones in different fish diet brand available in the 1990s on the French market

Commercial fish diets of the French market	Testosterone (ng g^{-1})	Estrone (ng g^{-1})	Estradiol (ng.g^{-1})	Genistein (ng g^{-1})	Daidzein (ng g^{-1})	Total phytoestrogens (ng g^{-1})
Diet 1	Na	490 ± 95	40 ± 9.5	92,750 ± 6290	76,190 ± 1550	168,950 ± 7940
Diet 2	Na	910 ± 165	105 ± 25	98,340 ± 9060	73,740 ± 1540	172,070 ± 10,600
Diet 3	790 ± 220	350 ± 60	100 ± 20	1180 ± 120	1710 ± 280	2880 ± 400
Diet 4	1330 ± 415	520 ± 95	1490 ± 320	800 ± 30	500 ± 10	1302 ± 40
Diet 5	Na	560 ± 100	1510 ± 310	Na	Na	Na
Diet 6	875 ± 240	940 ± 180	1490 ± 330	Na	Na	Na
Diet 7	Na	430 ± 75	375 ± 80	Na	Na	Na

Na not analyzed

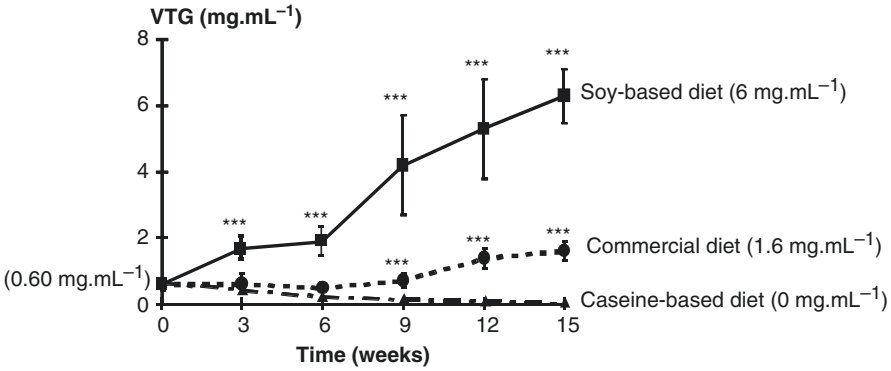

Fig. 32.3 Vitellogenin plasma levels in immature Siberian sturgeon (2-year-old fish) fed three diets. Between parentheses are the mean vitellogenin levels (From Pelissero et al. (1991b) Gen Comp Endocrinol 83(3):447–457)

Table 32.2 Vitellogenin synthesis induced by *i.p.* injections of phytoestrogens in yearling Siberian sturgeon (20 g mean weight)

Compound tested	Doses (mg mL^{-1})	Vitellogenin (µg/mL^{-1})	Number of fish
Control	–	<0.1	(n = 5)
Formononetin	0.5	<0.1	(n = 4)
Daidzein	0.2	2.00 ± 0.06	(n = 4)
Equol	0.05	8.8 ± 2.8	(n = 3)
Biochanin A	0.5	98 ± 14	(n = 4)
Genistein	0.2	213 ± 56	(n = 4)
Coumestrol	0.05	272 ± 98	(n = 4)
Estradiol	0.0001	246 ± 97	(n = 4)

Fish fed on maggots 2 weeks before and during the treatments
From Pelissero et al. (1991a)

soy-based diet on sturgeon vitellogenin synthesis, it was decided to study the effect of the legume phytoestrogens in *Acipenser baerii*.

The main compounds previously known to be present in legumes as well as in animal diet and exhibiting estrogenic properties were synthesized and tested *in vivo* in yearling sturgeon (20 g body weight) fed maggots free of estrogens (Pelissero et al. 1991a). Namely, these compounds were genistein, daidzein, biochanin A, formononetin, equol, and coumestrol. They were tested in parallel to estradiol. Each compound was tested at a unique dose defined on data obtained in sheep and published previously (Braden et al. 1967). The compounds were tested by intraperitoneal injections every 2 days for 10 days. The results obtained are mentioned in Table 32.2. They show that although the estrogenic efficiency described in mammals was not strictly followed in sturgeon, all compounds except formononetin were active by *i.p.* injection (Pelissero et al. 1991a). In all cases except for formononetin, the vitellogenin plasma levels were significantly increased above the levels of control fish.

32.3 The Differences Between Sturgeon and Trout on Their Sensitivity to Phytoestrogens

The demonstration of the estrogenic effect of a commercial fish diet based on fish meal and soybean meal was shown in the Siberian sturgeon. However this commercial diet was also fed to the rainbow trout, and this fish did not exhibit such an estrogenic reaction. Therefore, the question became "Why is there a difference between the two species toward the estrogenic effect of soy phytoestrogens?" The following paragraphs will expose the studies undertaken to understand and demonstrate this specific aspect of the story.

First, two experiments were undertaken. The first one showed that Siberian sturgeon were sensitive to genistein in their diet, exhibiting a significant increase of their vitellogenin levels when fed with a semisynthetic diet enriched with 20 ppm genistein for 8 weeks (Fig. 32.4) (Latonnelle et al. 2002b). Meanwhile, the rainbow trout was only sensitive to 1000 ppm genistein incorporated in a semisynthetic diet (Bennetau-Pelissero et al. 2001). Therefore, it appeared that in French fish-farm conditions, the Siberian sturgeon was 50 times more sensitive than the rainbow trout to the main soy phytoestrogen, namely, genistein.

To understand the origin of this difference, the effect of legume phytoestrogens was examined at different steps of the estrogen endocrine pathway (Fig. 32.5). This includes the estradiol synthesis, the estradiol blood transportation system, the hepatocytes, and the estradiol receptor affinity.

Estradiol is mainly synthesized in the ovary of female fish. Estradiol is the end product of a complex cascade of enzymatic steps, the latter being orchestrated by aromatase responsible for the conversion of testosterone into estradiol and of Δ4-androstenedione into estrone (Fevold 1983; Guiguen et al. 2010). The effect of the legume phytoestrogens was tested on the Siberian sturgeon (Rafini et al. 2002)

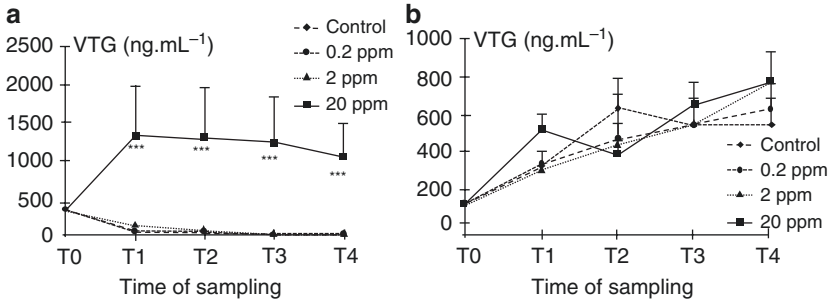

Fig. 32.4 Vitellogenin plasma levels measured in (**a**) immature 500 g Siberian sturgeon plasma and (**b**) female 500 g rainbow trout at the beginning of their first vitellogenesis. Samples are collected at 3-week intervals. In both cases, each treatment is applied to ten fish which are spread in different groups each fed with a semisynthesized diet based on casein supplemented with 0, 0.2, 2, or 20 ppm genistein. In sturgeon 20 ppm induced a significant increase of vitellogenin plasma levels. This was not seen in trout (From Bennetau-Pelissero et al. (2002) Rev Med Vet 153(7):513–516)

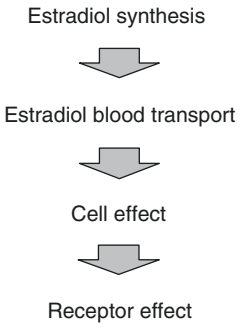

Fig. 32.5 Diagrammatic representation of the different steps of hormone information transfer at which the endocrine-disrupting effects of soy phytoestrogens were examined

and the rainbow trout ovarian aromatase *in vitro* (Pelissero et al. 1996). Briefly, tritiated [1β,2β-^{3}H] Δ4-androstenedione was incubated with a microsome fraction of the ovary obtained after crushing the tissue at 4 °C with antiprotease inhibitors and serial ultracentrifugations. This microsome fraction is known to contain aromatase. When acting, aromatase performs the aromatization of the estrone A-ring from Δ4-androstenedione liberating tritiated water. After precipitating the protein, remaining steroids were removed from the supernatant using activated charcoal, and the tritiated water was counted on a β-counter. Positive and negative mixtures were run in parallel with samples of tritiated Δ4-androstenedione incubated with microsomes and with different concentrations of flavonoids. If the aromatase is inhibited, the tritiated water production is prevented and decreased when the flavonoid concentrations rise. On the opposite if the compound has no effect on the aromatase activity, tritiated water is produced always at high levels. Inhibition curves were obtained which allowed determining inhibiting concentration of 50% (IC_{50}) values. These concentrations are those which provoke the inhibition of 50% of tritiated water production when compared to the production in control condition. The results obtained are presented in Table 32.3. The compounds were shown to slightly inhibit the enzyme prepared from ovaries. Such effects were more recently reported in fish by other authors (Cheshenko et al. 2008). The Scatchard analysis of the inhibiting curves allowed determining that the inhibition when occurring was nonspecific. In addition, although differences appeared in the sensitivity of the two species to various compounds, these differences could not explain the differences of sensitivity to phytoestrogens recorded for the trout and the sturgeon *in vivo*.

Then the sex hormone-binding protein (SBP), responsible for the transportation of testosterone and estradiol in the blood, was studied. The question was to determine if this plasma protein was able or not able to bind legume phytoestrogens (Bennetau-Pelissero et al. 1998, 2001). This time again, the work was performed on both species, and the results obtained are presented in Fig. 32.6. Briefly, SBP from plasma was incubated with, and adsorbed to, a DEAE Biogel. This Biogel was then incubated with known tritiated testosterone together with increasing doses of the compounds to be tested. If the compound interacts with SBP, it replaces the tritiated testosterone and the radioactivity linked to the DEAE-SBP complex is reduced. The complex was separated from the incubation medium by filtration under vacuum. All

Table 32.3 Comparison of the inhibiting activity and relative activity of several compounds on microsomal aromatase from the Siberian sturgeon and the rainbow trout ovary and from human placenta

Compounds tested	IC_{50} (μM) sturgeon[a]	Relative activity sturgeon	IC_{50} (μM) trout[b]	Relative activity trout	Relative activity human[b]
DL aminoglutethimide	0.375	146.7	39	19	4.5
Flavanone	7.5	6.9	>1000	<0.7	1
Flavone	55	1	731	1	1
Biochanin A	125	0.44	>1000	0.3	–
Equol	125	0.44	793	0.9	–
Daidzein	≫50	0	≫1000	≪0.7	–
Formononetin	≫50	0	≫1000	≪0.7	–
Genistein	≫50	0	3500	0.2	–
Apigenin	≫50	0	84	8.7	6.7
Quercetin	≫50	0	139	5.3	0.7
7-Hydroxyflavone	≫50	0	>1000	<0.7	20
7,4-Dihydroxyflavone	≫50	0	200	3.7	5
α-Naphtoflavone	≫50	0	227	3.2	114
Chrysin	Activator	–	≫1000	≪0.7	16
Estradiol	Activator	–	>1000	<0.7	–

Inhibiting activity is observed when the amount of tritiated water revealing the aromatase activity and normally produced in controlled condition is blocked by the addition of the tested compound. IC_{50} is the concentration of a tested compound responsible for 50% inhibition. Relative activity is expressed with respect to that of flavones taken as the reference
From [a]Rafini et al. 2002; [b]Pelissero et al. 1996

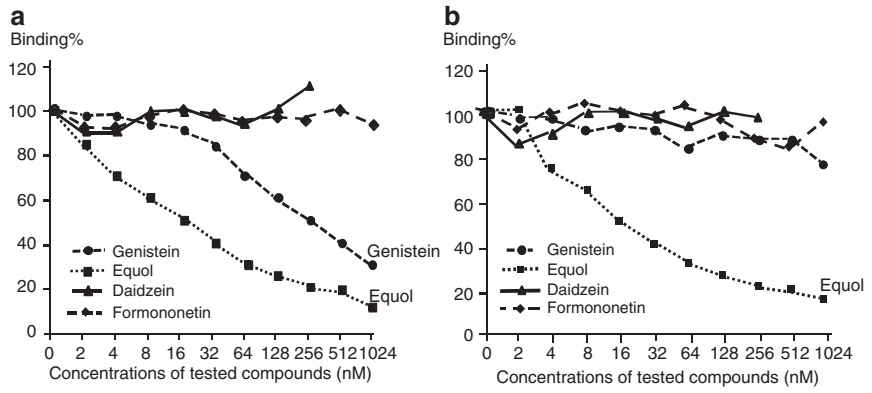

Fig. 32.6 Binding of soy phytoestrogens to (**a**). Siberian sturgeon steroid-binding protein (**b**) rainbow trout steroid-binding protein (From Bennetau-Pelissero et al. (1998) Bull Fra Pêche Pisc 350–351:571–583)

steps were performed at 4 °C using antiprotease inhibitors. From these experiments, an IC_{50} value could be assessed as well as Scatchard plot analyses. From these data, it was shown that the legume phytoestrogens were able to bind to SBP in a nonspecific way. This means that their presence induces a modification of the protein conformation which prevents the binding of steroid. Isoflavones are not competitive inhibitors because they do not strictly compete with the SBP-binding site for estradiol or testosterone (data not shown). These results are in accordance with those obtained by Martin et al. (1996) on human SBP and those obtained later in rat (Hillerns et al. 2005). In these conditions it was shown that the steroid-binding protein from the Siberian sturgeon was able to bind equol and genistein when, in the rainbow trout, the SBP was only able to bind equol (Bennetau-Pelissero et al. 1998). However, the binding capacity was noticed to be significant only for doses higher than the micromolar concentration. Therefore, this difference, although it may contribute to the difference of sensitivity observed *in vivo* between the two species, did not explain it totally.

The ability of the legume phytoestrogens was tested on yolk protein synthesis using hepatocyte primary cultures *in vitro*. Briefly, hepatocytes were collected from fish anesthetized and killed under the laminar flow hood. The liver was first perfused with a rinsing solution and then with a solution containing collagenase allowing the liver cells to separate one from each other. After perfusion, the liver was removed and gently shacked in a bath of culture medium in sterile conditions. Hepatocytes were filtered on gauze and rinsed with culture media three times by gentle subsequent centrifugations and resuspended in the culture medium. After counting for viable cells, a suspension of known cell concentration was prepared and distributed in cell culture plates. After 3 days of gentle shaking in rotation, liver cell aggregates formed in the middle of the cell culture wells and the compounds could be tested by addition to the culture medium every 2 days for duration going from 10 to 15 days. Positive and negative controls were run in parallel**.** The results obtained are shown in Fig. 32.7. This figure shows that in both species all phytoestrogens including formononetin were able to induce vitellogenin synthesis. There were no differences between trout and sturgeon in their ability to respond to phytoestrogens compared to estradiol. It should be noted that in the Siberian sturgeon, formononetin was found not to be a potent estrogen when administrated via *i.p.* administration (Pelissero et al. 1991a), while it was potent *in vitro* in rainbow trout hepatocytes (Pelissero et al. 1993) and in Siberian sturgeon hepatocytes (Latonnelle et al. 2002b). This can be explained because cultured hepatocytes conserve the majority of their enzymatic activities including the demethylation activity which can be responsible for the potentiation of formononetin estrogenic activity via its demethylation into daidzein.

To close this investigation, the affinity of estradiol receptors prepared from liver of rainbow trout or from that of Siberian sturgeon was investigated using competitive binding of legume phytoestrogens toward tritiated estradiol *in vitro* (Latonnelle et al. 2002a). Namely, if the compound competes with tritiated estradiol for the

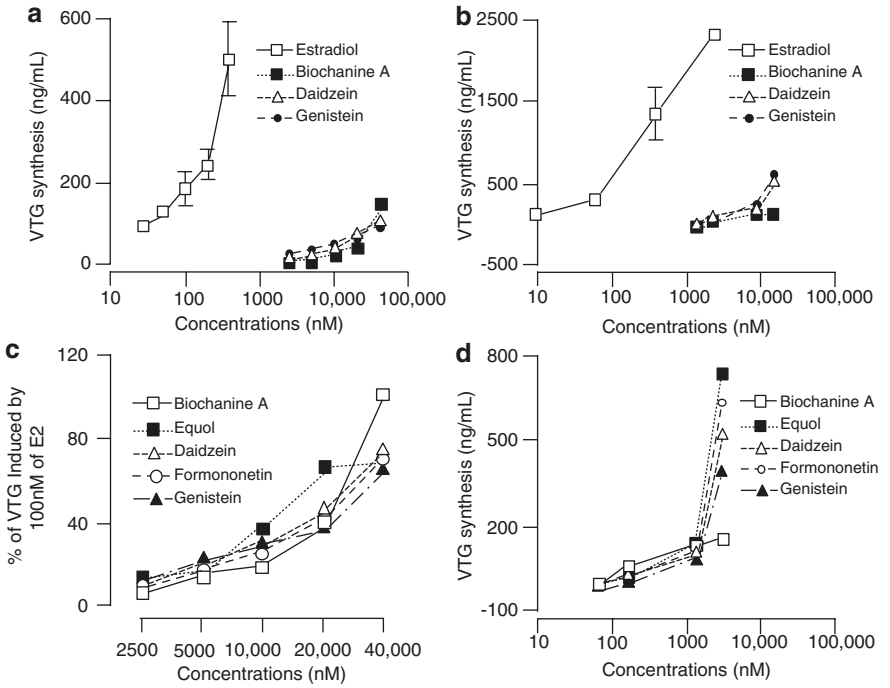

Fig. 32.7 Vitellogenin synthesis induction in (**a**, **c**) rainbow trout primary cultured hepatocytes and in (**b**, **d**) Siberian sturgeon primary cultured hepatocytes. Soy phytoestrogens are tested in comparison to estradiol as reference compound (From Pelissero et al. (1993) J Steroid Biochem Mol Biol 44(3):263–272 and from Latonnelle et al. (2002b) Gen Comp Endocrinol 126(1):39–51)

receptor, the amount of radioactivity bound to the receptor declines. This allows the calculation of IC_{50} binding curves. The results are shown in Fig. 32.8 and separately for trout and sturgeon according to the level of affinity of the compounds for the estradiol receptors. This time again the estradiol receptors of the two species exhibited rather similar affinities to phytoestrogens indicating (Table 32.4) that the differences of sensitivity observed *in vivo* for the two species toward genistein did not lay at the estradiol receptor level.

32.4 Bioavailability of Phytoestrogens Is the Clue

All these results demonstrated that the difference between the two species could not be shown using *in vitro* tests and demonstrated their limits (Latonnelle et al. 2002b). Then it became obvious that the difference could come from the metabolism of these compounds in the two species. At that time, it was impossible to assay the isoflavone plasma levels in fish. Therefore a complete comparative pharmacokinetic study was undertaken on trout and sturgeon of both sexes using tritiated genistein and genistein labeled with ^{14}C.

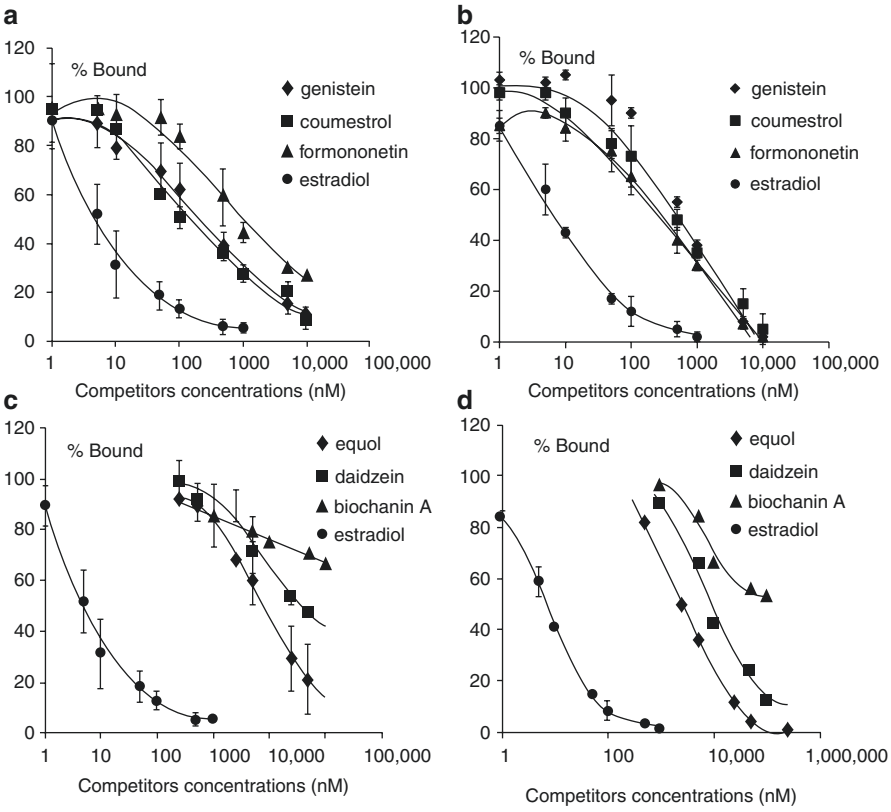

Fig. 32.8 Binding affinities of soy phytoestrogens for rainbow trout estradiol receptor (**a**) and (**c**) and for Siberian sturgeon estradiol receptor (**b**, **d**) (From Latonnelle et al. (2002a) Gen Comp Endocrinol. 129(2):69–79)

The same amount of tritiated genistein was injected in the dorsal aorta of rainbow trout and of Siberian sturgeon, and blood samples were withdrawn in standardized conditions at defined times postinjection and for 50 h. The results are plotted in Fig. 32.9. This figure shows that the Siberian sturgeon eliminates the tritiated genistein at a much lower rate than the rainbow trout does. Twenty hours postinjection, when there were only 8 dpm[1]/100 μL of tritiated genistein remaining in the trout plasma, there were still 500 dpm of tritiated genistein remaining in the sturgeon plasma. An analysis of the nature of genistein metabolites in both species was undertaken using ^{14}C-labeled isoflavones. It showed that in the rainbow trout, the main metabolites were glucuronides when they were sulfates and orobol in the Siberian sturgeon (Fig. 32.9). Finally when each species was fed with the appropriate feeding ratio containing the same amount of genistein, sturgeon was found to exhibit 50 times

[1] Dpm: disintegrations per minutes applied to the transformation of radioactive compounds.

Table 32.4 Concentrations displacing 50% of [^{3}H]estration (DC50) and relative affinity of phytoestrogens based on 100 (DC_{50} estradiol/DC_{50} competitor) for rtER and ssER from hepatic nuclear extract

	Trout		Sturgeon	
Compounds	**DC_{50}**	**Relative affinity**	**DC_{50}**	**Relative affinity**
Estradiol	7	100	5	100
Estradiol glucuronide	7000	0.1	7400	0.07
Estradiol sulfate	>10,000	<0.07	10,000	0.05
Biochanin A	>100,000	<0.007	>100,000	<0.005
Coumestrol	400	1.75	150	3.5
Daidzein	9050	0.077	83,000	0.006
Equol	5300	0.13	8300	0.06
Formononetin	260	2.69	1090	0.5
Genistein	570	1.22	220	2.3
Testosterone	>1000	<0.7	>1000	<0.5
Tamoxifen	1500	0.5	21,900	0.02
ICI 182,780	10	70	60	8.5

From Latonnelle et al. (2002a, b)

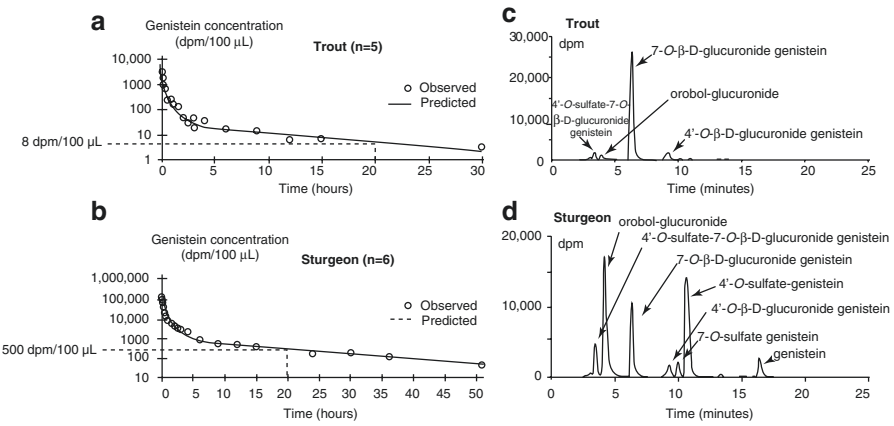

Fig. 32.9 Plasma kinetic of elimination of tritiated genistein from (**a**) rainbow trout plasma, (**b**) Siberian sturgeon plasma. (**c**) HPLC analysis of rainbow trout plasma after ingestion of tritiated genistein. (**d**) HPLC analysis of Siberian sturgeon plasma after ingestion of tritiated genistein (From Gontier-Latonnelle et al. (2007) Gen Comp Endocrinol 150(2):298–308)

higher plasma levels of genistein than did trout. Indeed, these data clearly showed that the difference of bioavailability of phytoestrogens was responsible for the difference of sensitivity of the two species to the estrogenic effect of soy-containing diet. As a matter of fact, the 50 times ratio between genistein plasma levels existing in sturgeon *vs.* trout fed with the same diet explained the 50 times ratio of sensitivity to soy phytoestrogens.

32.5 Sex Steroids in the Siberian Sturgeon

32.5.1 Estradiol

As mentioned previously, the commercial diet for trout fed to fish in fish farms contains both soybean meals and fish meals. Fish meals are prepared from fish caught in the wild and being at various stages of their reproductive cycle. All parts of the fish can be used to prepare fish meals, but if the fish is of certain commercial value (cod, tuna, salmon, hake, or sea bass), only their viscera including their gonads are used for fish meals. Gonads are the site of the main sex steroid production, and therefore, as mentioned in Table 32.1, sex steroids can be found in fish meal and fish diet containing fish meal. As a matter of fact, first looking at fish of the two cohorts we had in France at that time, we observed a progressive increase of plasma estradiol in male Siberian sturgeon when they get older (Table 32.5). This observation was made in 1987. In addition, estradiol, estrone, and testosterone were assayed using specific RIA in the blood of Siberian sturgeon reared in the experimental fish farm of Donzacq (southwest of France) for 5 years. The data obtained on the last 3 years were published in Pelissero and Le Menn (1989). These assays were performed as markers of the reproductive function of fish and correlated to the gonad development. One hundred and fifty fish were sampled every 3 months in the fish-farm conditions. Estrogen levels recorded on 15 randomly chosen females and 15 randomly chosen males were brutally seen to decrease after the first year of reported experiment (1988) with no correlation with gonad development (Fig. 32.10). It was discovered that the fish diet brand was changed at the time the plasma estradiol levels decline. In the new brand, it appeared that a significant part of protein from fish origin was substituted by protein of plant origin. The first diet contained 350 ± 26 ng of estradiol per 100 g of diet (measured on four different batches) was fed to Siberian sturgeon until December 1987. The second commercial fish diet was fed to the same fish until June 1989. It was shown to contain much less estradiol, *i.e.*, 50 ± 4.3 ng.100 g^{-1} measured on four different batches. As seen in Fig. 32.10, the steroid plasma levels recorded on these fish brutally decline in both males and females. On the first diet brand, the estradiol levels were not significantly different (30 ± 14.5 ng.mL^{-1} vs. 80 ± 20.8 ng.mL^{-1}, in males *vs*.

Table 32.5 Steroid levels in male and female Siberian sturgeon plasma at different gonadal stages

Age	Sex and stage	Testosterone (ng.mL^{-1})	Estrone (ng. mL^{-1})	Estradiol (ng.mL^{-1})
4-year-old fish	♀ Stage II	8.2 ± 0.29	5.1 ± 3.21	11.6 ± 1.2
	♂	119 ± 43.0	6.6 ± 1.89	12.8 ± 1.15
5-year-old fish	♀ Stage II–III	5.8 ± 0.54	10.8 ± 7.18	16.6 ± 1.34
	♂	104.4 ± 47	3.3 ± 1.52	14.4 ± 0.95
10-year-old fish	♀ Stage IV–V	109.7 ± 25.5	10.3 ± 1.53	19.3 ± 2.68
	♂	178.0 ± 6.43	4.1 ± 1.15	26.7 ± 4.7

From Pelissero (1988)

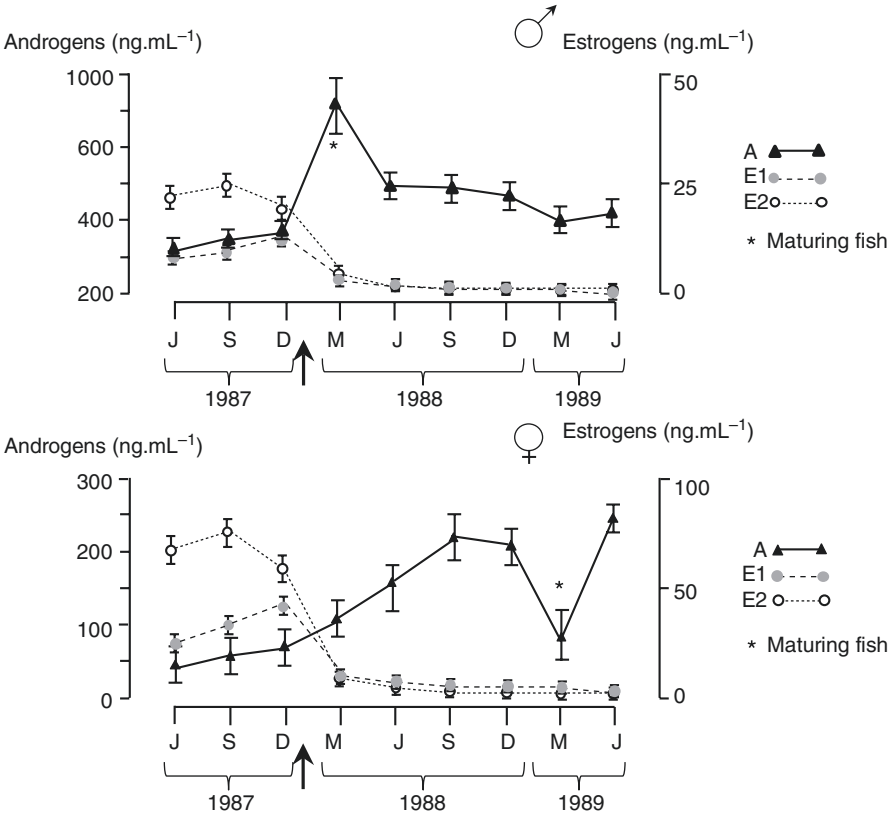

Fig. 32.10 Estradiol, estrone, and testosterone plasma levels in Siberian sturgeon reared in French fish farm between 1987 and 1989. The *upper* graph concerns males and the lower graph concerns females. The *black arrow* at the abscise axis between December 1987 and March 1988 indicates a change of the fish diet brand. The first one, essentially based on fish meal, contained 350 ng.g^{-1} estradiol. The second one contained 50 ng.g^{-1} estradiol and was partially based on legume meals (From Pelissero 1990 PhD Thesis n°427, Univ Bordeaux 1)

females, respectively). In females the estrone levels were significantly higher than that of males during this period (45 ± 10.2 ng.mL^{-1} vs. 6.2 ± 1.3 ng.mL^{-1} in females vs. males, respectively). After the change in the diet, the estrogen levels were decreased to undetectable values in both sexes, confirmed by gas chromatography mass spectrometry (GC-MS) analysis (data not shown). Finally, only testosterone plasma levels could be correlated to gonad development. In March 1988, they revealed the maturation of males and were correlated to fully mature testis. In March 1989, still in males, testosterone plasma levels were significantly lower than the year before because the gonad examination revealed that the sperm production period was already over. In females, the decrease of testosterone in March 1989 is due to a decrease of the

hormone to a value close to 1 $ng.mL^{-1}$ in females reaching the spawning stage. These results indicated that the estrogen levels were disturbed in the Siberian sturgeon of both sexes. They could not be used as markers of the reproductive activity. Estrogens were too high when fish were fed with a commercial fish diet based on fish meal and were too low when fish were fed with a commercial fish diet based on plant protein.

The first part of the profile tended to indicate that estradiol could accumulate progressively in Siberian sturgeon plasma. Indeed, it is considered by some authors that the Siberian sturgeon does not usually eat fish in its natural environment except when it is old (over 50 years old) (Sokolov et al. 1986). It was therefore interesting to investigate if the Siberian sturgeon was able to eliminate sex steroids from fish diet which normally, in other species, requires the activities of specific liver cytochrome P450 enzymes (Scornaienchi et al. 2010). Therefore, a bioavailability study was undertaken in the Siberian sturgeon using tritiated estradiol to study the time needed for estradiol elimination after a diet intake. The study was performed to know if the time needed for estradiol elimination after an oral intake was not or greater than 12 h. If it was, because fish were fed on a regular basis in fish farm (twice a day: once in the morning and once in the evening), estradiol from a meal would not be eliminated before the subsequent meal would be distributed. In this following meal bringing a new batch of estradiol, the hormone could accumulate progressively explaining why the estradiol plasma levels progressively raise in Siberian sturgeon plasma as they get older. The results of the study are presented in Fig. 32.11 and correspond to the study by Pelissero et al. (1991c). This study showed that estradiol could be maintained in fish plasma for durations over than 24 h which is much longer than that was observed by Baroiller et al. (1987) for the rainbow trout where the elimination was done within 5 h. Three doses were tested and they showed that the higher the dose the less efficient the elimination process. The hypothesis drawn out at the time of the study was the involvement of the steroid-binding protein levels increased under estradiol stimulation (Pelissero et al. 1991c) and participating to the increase of estradiol bioavailability in sturgeon plasma. Nowadays, the question which remains is to know whether this accumulation would occur in natural condition, *i.e.*, with a diet corresponding to that found by the Siberian sturgeon in its natural environment. In other species including humans, it was shown that the estrogenic isoflavones could be conjugated in the liver by the same enzymes as estradiol (Seppen 2012). In addition, genistein was shown in human to activate significantly the estradiol glucuronidation (Pfeiffer et al. 2005). These data indicate that isoflavones can act directly on their own elimination process as well as on that of estradiol. If this could be confirmed in sturgeon, it could explain the results obtained in the Siberian sturgeon fed with a soy protein-rich diet. Indeed, this diet bringing few steroids would not increase their plasma levels in fish, but at the same time, isoflavone if they increase the elimination of steroids would be responsible for the undetectable plasma levels recorded after 1987 (Pelissero and Le Menn 1989). However, our hypothesis still remains to be confirmed by an appropriate experiment.

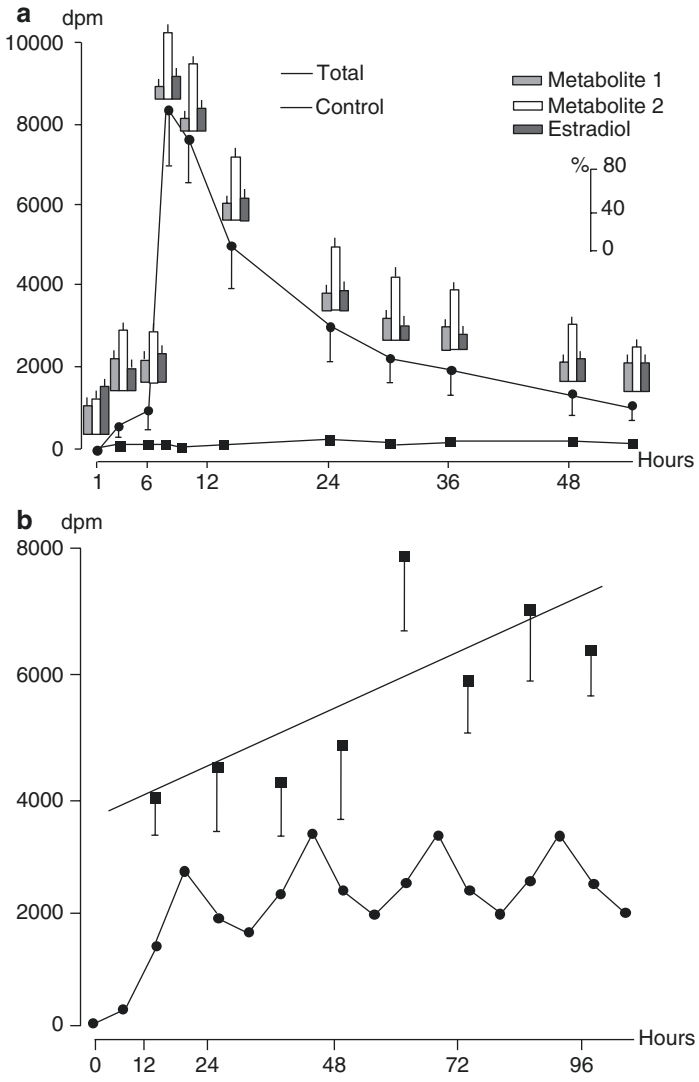

Fig. 32.11 (**a**) Time evolution of three tritiated fractions collected on LH20 chromatography from the plasma of Siberian sturgeon after the ingestion of 2 μCi of [(4, 6)-^{3}H]estradiol (15 ng estradiol). $N = 5$ in both treated and control fish. (**b**) Experimental accumulation curve (*plain line*) of the tritiated estradiol fraction during chronic administration twice a day for 5 days of 2 μCi of [(4, 6)-^{3}H] estradiol (15 ng estradiol). In comparison, the model curve is presented in dotted line (From Pelissero et al. (1991c) Fish Physiol Biochem 9:231–245)

32.5.2 11-Ketotestosterone

Because vitellogenin could obviously not be used for sturgeon sexing, another blood biomarker was investigated. Namely, 11-ketotestosterone was known to be synthesized in male to promote spermiogenesis, *i.e.*, the transformation of

Table 32.6 11-ketotestosterone plasma levels in 4-year-old male and female Siberian sturgeon

Age	Sex and stage	11-Ketotestosterone (ng.mL^{-1})	Mean (± SD)
4-year-old fish	♀ Stage II	0.64	0.92 ± 0.19
		0.89	
		0.99	
		0.86	
		0.67	
		1.07	
		1.19	
		1.06	
	♂ Spz d	43.38	60.27 ± 43.08
	♂ Spz e	115.24	
	♂ Spz d	59.19	
	♂ Spz c	28.72	
	♂ Spz e	107.35	
	♂ Spz a	7.74	

From Pelissero (1988)

spermatid into spermatozoa (Fostier et al. 1983). This androgen is not aromatizable into estrogens and therefore is not produced by the trout ovary. We thought that this androgen could be used to distinguish between male and female Siberian sturgeon. A first exploration was made on a small number of fish. The results are presented in Table 32.6 and in Pelissero (1988). They showed that the steroid was hardly detected by a radioimmunoassay in females, while it was present in all 4-year-old male fish tested. However, as expected, the 11-ketotestosterone levels are linked to the progression of spermatogenesis and increase in older fish. An ELISA was raised further by B. Cuisset and published in Cuisset et al. (1994). In her work, B. Cuisset also showed that contrarily to what is observed in the rainbow trout female Siberian sturgeon were able to produce 11-ketotestosterone at spawning. Nevertheless, the 11-ketotestosterone assay was used to distinguish between male and female Siberian sturgeon for nearly 10 years by the French sturgeon farmers (Cuisset et al. 1995).

32.6 Soy Phytoestrogens and Teleost Fish Reproduction

At the time of this work, looking at the effect of soy isoflavones on sturgeon reproduction appeared not feasible because fish reach their reproductive maturation when they are between 6 and 8 years old. Without any other clue, an experiment designed to check for an effect of soy isoflavones on this fish species would have needed to feed at least two groups of fish for 8 years. One could have received a commercial diet containing soy, and the other group could have received a semisynthetic diet with no soy and no other source of estrogens. This would have been very expensive and no diet supplier would have accepted to finance such a program. Therefore, it was decided to check for the effect of soy phytoestrogens on fish reproduction, using

the rainbow trout as a model. In this species, the reproduction physiology was better known, and we decided to check for the effects of genistein during the first vitellogenesis and first maturation and spawning. We decided to synthesize enough genistein to feed a reasonable number of male and female trout for 1 year. Two doses were compared to a control diet based on fish meal. One diet based on fish meal was enriched with 500 ppm genistein and the other with 1000 ppm genistein. The 500 ppm level can be achieved in commercial fish diet. These diets were compared to the same control diet with no genistein. Therefore, the group of experimentally fed fish will be further named G0, G500, and G1000 (Bennetau-Pelissero et al. 2001). Sampling was organized for the first time when the fish were 1-year-old in winter. The following were done at the previtellogenic stage at the end of the following February. The next sampling was organized at the end of May during the early vitellogenic stage. Then the fish were sampled at the end of July at the beginning of the yolk-efficient accumulation. The next sampling took place at the end of October at the end of the full vitellogenic stage. The last sampling occurred in December at spawning. See Bennetau-Pelissero et al. (2001) for a specific scheme of the experimentation. Many different incomes were analyzed including plasma yolk protein and sex steroids (Fig. 32.12) as well as the gonadotropin plasma levels together with the maturation-inducing steroid (Table 32.7). The reproductive efficiency was also examined looking at several criteria in both sexes as well as on fecundation success. The results are given in Table 32.8. Although the effects do not follow a classical dose-response curve, some coherence could be noted between the LH levels and the reproductive parameters examined. Indeed, it appeared that the 500 ppm dose impaired greater the spawning efficiency and the resulting fecundation efficiency and survival at embryo stage. Normally male gametogenesis is synchronized with that of female to insure the best success of reproduction. The main outcome of this experiment is that spawning and spermiation were phased shift. Under G500, males were ready for reproduction earlier than control and females were ready later than control. The effect on LH was expected based on the data obtained previously in mammals (Findlay et al. 1973) and confirmed later in human (Cassidy et al. 1995).

32.7 What Did We Learn Recently from Soy Isoflavone Effects in Fish?

Zhang et al. (2002) showed that, in medaka (*Oryzias latipes*), changes in vitellogenin, circulating steroids, and *ex vivo* steroidogenesis occur in response to genistein. These changes were similar to that induced by 17-β-estradiol. The doses tested were 0.32 $\mu g.g^{-1}$, 3.2 $\mu g.g^{-1}$, or 130 $\mu g.g^{-1}$. However, some endpoints were more sensitive to estradiol treatment (vitellogenin), while others are more sensitive to genistein (male testosterone and ovarian estrogenesis). Kiparissis et al. (2003) also reported in *Oryzias latipes* that exposure to 1000 $\mu g.L^{-1}$ genistein caused an increased incidence (12%) of gonadal intersex in male medaka. Equol and genistein treatments induced delayed oocyte maturation, atretic oocytes, an enlarged ovarian lumen, proliferation of somatic stromal tissue, and primordial

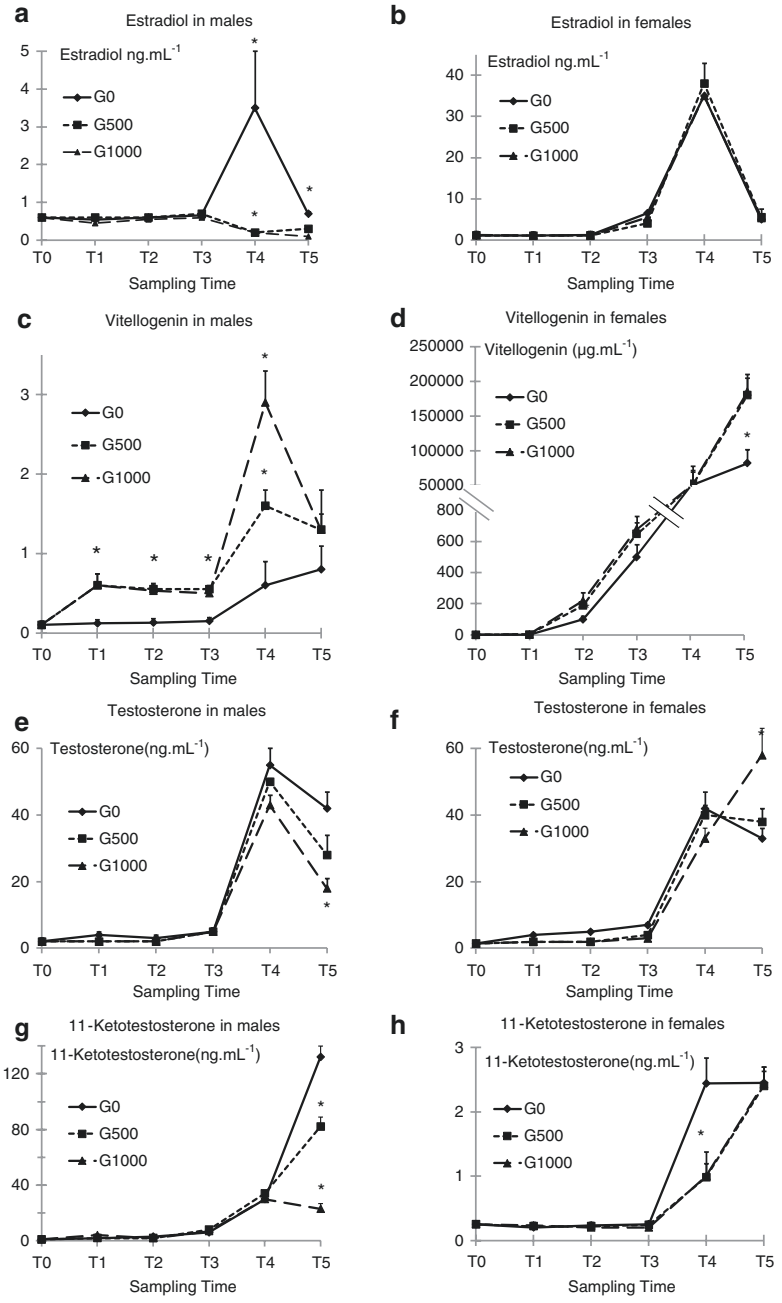

Fig. 32.12 Steroid and vitellogenin profiles in male and female rainbow trout submitted to G0, control fish meal-based diet; G500, diet with 500 ppm genistein; G1000, diet with 1000 ppm genistein. Values are means of 20 measurements; bars are SEM; * indicates a significant difference at a = 0.05. (From Bennetau-Pelissero et al., (2001) Gen Comp Endocrinol 121:173–187)

Table 32.7 FSH, LH, and 17α,20β(OH)2-progesterone plasma levels in males and females rainbow trout fed diet with or without genistein at the end of gametogenesis (T4) and at spawning (T5)

Sex Hormone	Sampling time	Diets G0	G500	G1000
Males				
FSH	T4	6.96 ± 1.59	5.76 ± 1.72	6.34 ± 0.52
LH		ND	ND	ND
FSH	T5	2.16 ± 0.39	1.47 ± 0.23	1.83 ± 0.29
LH		0.44 ± 0.09	0.31 ± 0.09	0.53 ± 0.09
MIS*		10.93 ± 0.88[a]	5.46 ± 0.92[b] (*)	7.62 ± 0.90[a,b]
Females				
FSH	T4	6.14 ± 0.86[a]	4.91 ± 0.68[a]	3.46 ± 0.50[b](*)
LH		0.33 ± 0.10	0.39 ± 0.10	0.35 ± 0.07
FSH	T5	6.38 ± 1.55[a]	3.44 ± 0.82[b](*)	3.37 ± 1.04[a,b]
LH		15.18 ± 3.00[a]	6.93 ± 0.99[b](*)	8.02 ± 0.97[b](*)
MIS		251.22 ± 21.40[a]	183.22 ± 13.48[b](*)	211.77 ± 18.58[a,b]

Figures are means ± SEM obtained on 20 fish per diet. Means within the same row not sharing a common superscript letter are significantly different.
[a,b]G0 contains no genistein; G500 contains 500 ppm genistein; and G1000 contains 1000 ppm genistein. *MIS* maturation-inducing steroid (17α,20β$(OH)_2$-progesterone), *T4* full vitellogenesis, *T5* spawning time. (*) means that α = 0.05
From Bennetau-Pelissero et al. (2001)

germ cells in female medaka. The responses were concentration-dependent. Both compounds induced alterations to externally visible secondary sex characteristics with feminized secondary sex characteristics in 72% of the male medaka treated with 1000 μg.L^{-1} genistein. Inudo et al. (2004) still in the medaka (*Oryzias latipes*), assessed the effect of a 28-day feeding period with diet enriched in soy phytoestrogens on reproduction. Fish were paired and the treatment did not induce significant differences in the number of eggs or fertility parameters. However, hepatic vitellogenin was significantly increased in males with the highest dose of phytoestrogens, (genistein, 58.5 ± 0.6 μg.g^{-1}; daidzein, 37.3 ± 0.2 μg.g^{-1}). The authors concluded that fish diets with high amounts of phytoestrogens had the potential to induce vitellogenin production in male medaka, even if reproductive parameters were unaffected.

Pollack et al. (2003) reported that genistein fed to fingerlings striped bass (*Morone saxatilis*) induced a significant vitellogenin response at 2 mg.g^{-1} and 8 mg.g^{-1} doses in diet. Genistein induced a U-shaped response curve characteristic of the low-dose effects of some endocrine-disrupting chemicals.

In Atlantic salmon parr, 6-month feeding diets containing genistein at four concentrations, 0 (control), 500, 1000, and 3000 ppm, had no effect on growth or feed conversion (Malison et al. 2005). However, the serum levels of vitellogenin were higher in the genistein-treated fish, and genistein at all doses inhibited the process

Table 32.8 Reproduction criteria in male and female rainbow trout fed diet with or without genistein

	Diet		
Criteria	**G0 (n = 10)**	**G500 (n = 12)**	**G1000 (n = 15)**
Percentage of males in spermiation à T4	71.48	76.92	80
GSI at spawning (%)	2.6 ± 0.21	2.5 ± 0.26	2.8 ± 0.36
Sperm volume collected at spawning (mL Kg^{-1})	5.84 ± 1.36[a]	13.8 ± 1.73[b](*)	11.2 ± 0.90[b](*)
Spermatocrit (%)	36.8 ± 2.49[a]	36.9 ± 3.46[a]	32.7 ± 1.98[b](*)
Motility (s)	42.2 ± 1.05[a]	38.3 ± 0.99[b](*)	37.2 ± 1.3[b](*)
	G0 (n = 19 males)	G500 (n = 19 males)	G1000 (n = 16 males)
Relative fecundity (n = 10 females)[1]	1240 ± 80	1448 ± 105	1491 ± 135
Absolute fecundity[2]	148.6 ± 5.27	166.5 ± 7.10	156.9 ± 5.73
Fresh weight of 100 eggs (g) (n = 10 females)	7.97 ± 0.98	9.07 ± 0.85	7.6 ± 1.06
Egg diameter (n = 10 females)	3.52 ± 0.047	3.53 ± 0.084	3.56 ± 0.068
% of residual gonad	1.83 ± 0.95[a]	3.85 ± 1.45[a]	0.80 ± 0.051[b](*)
% of spawning female at T4	89[a,b]	68[b](**)	100[a]
% of spawning female at T5	100[a]	79[b](**)	100[a]
Percentage of females with viable eggs (n = 10)	100	80	100
Percentage of survival at embryo stage[3]	77.8 ± 5.44	70.40 ± 11.42	88.8 ± 3.18

Figures are means ± SEM obtained on 100 fish per diet. Means within the same row not sharing a common superscript letter are significantly different.

[a,b]G0 contains no genistein; G500 contains 500 ppm genistein; and G1000 contains 1000 ppm genistein

[1]Number of eggs per female; [2]Egg mass per kg body weight; [3]% of embryo development >40%. (*) $\alpha = 0.05$; (**) $\alpha = 0.1$. From Bennetau-Pelissero et al. (2001)

of smoltification. This may be linked to an acceleration of reproductive maturation which is known to antagonize the smoltification process in *Salmo salar*.

Sassi-Messai et al. (2009) studied the effect of genistein in a zebra fish model. They showed that genistein acted through at least two different pathways in zebra fish embryos. It was able to induce apoptosis in an ER-independent manner and to regulate *aromatase-B* expression in the brain in an ER-dependent manner.

Mai et al. (2012) reported that high dietary soy isoflavones level (8 $g.Kg^{-1}$) significantly depressed weight gain, feed efficiency ratio, whole-body crude lipid content of fish, and apparent digestibility coefficients of nutrients ($P < 0.05$). However, the content of soy isoflavones in soybean meal is 2.5 times lower, and the authors consider that these adverse effects might be neglected.

Bagheri et al. (2013) reported that the result of a long-term feeding of soy-rich diet in the goldfish (*Carassius auratus*) was a decrease in plasma testosterone and an increase in 17β-estradiol. The average number of eggs spawned and sperm quality was reduced with impacts on both oocyte maturation and spermatogenesis. In addition, a reduction in fertilization and hatching rates was dose-dependently observed. The food contained 0, 35, 65, or 100% of soybean meal. Isoflavones were controlled and their levels were in the range of normal soy protein concentrations.

Stevenson et al. (2011) showed that exposure to genistein did not affect circulating levels of the 11-ketotestosterone or estradiol of sexually mature male of *Betta splendens*. The phytoestrogens tested did affect neither GSI nor sperm concentration or motility and fertilization success. However, fish exposed to phytoestrogens showed some modifications in sperm quality. The authors concluded that these males of *B. splendens* were not very sensitive to the estrogenic action of phytoestrogens. These results were then confirmed by Brown et al. (2014) who showed that genistein or β-sitosterol had no effect on the steroid levels even though a significant decrease of aggressive behavior was observed previously (Clotfelter and Rodriguez 2006).

Although these results may seem discrepant among fish species, they can be interpreted as the result of metabolic differences especially at the liver xenobiotics elimination level. They also underline the importance of *in vivo* investigations as long as endocrine-disrupting effects have to be studied on reproductive issues.

Conclusions and Perspectives

The effects of soy phytoestrogens in fish were first discovered and described in the Siberian sturgeon. Our work was innovative in different aspects. (1) It showed that fish diet could contain estrogens from animal or plant sources which could potentially affect fish reproductive endocrinology. (2) It showed that an impact on vitellogenin synthesis may not always impair completely fish reproduction. (3) It showed that *in vitro* tests developed on hepatocytes, aromatase, sex hormone-binding globulin, or estradiol receptors may not reflect the *in vivo* situation and should be considered with caution when species comparisons have to be addressed. (4) It showed that the metabolism of endocrine-disrupting compounds is one of the main issues to consider when studying their effects *in vivo*. (5) Our work showed for the first time that an endocrine disruption not only concerns a receptor interaction but can concern all the different steps of a hormone pathway from its synthesis to its elimination including its transportation in biological fluids. (6) Our work also allows comparison between models and not only among fish. Some of our results can be explained looking at highly conserved endocrine systems like the gonadotropin pathway which is known to be affected by phytoestrogens in fish as it is in mammals including humans.

The question of a physiological disruption of reproduction in the Siberian sturgeon by the dietary estrogens is still pending. Although fish can be reproduced in the French farms rearing conditions, it is still unknown if they would do better in the absence of these endocrine disruptors (steroids and isoflavones from diet) in their environment. Indeed, the trout example shows that the impairment can be partial still allowing a certain degree of fertility. The only way to answer this question

would be to compare the Siberian sturgeon in strictly equivalent thermal conditions either in fish farm or in the wild with no dietary estrogen contamination. Until now this approach has been considered as impossible due to the time needed to reach sexual maturity in the Siberian sturgeon. The data obtained on the rainbow trout would however suggest a reduction of fertility.

Few studies were undertaken so far on other fish species. The results *in vitro* are usually more consensual than *in vivo* reflecting the results observed in our studies comparing the rainbow trout and the Siberian sturgeon steroid metabolism. Nevertheless, soy isoflavones could well exert endocrine-disrupting effect in many reared animal species, and the reduction of isoflavones in animal and human diets could now be a crucial pending issue.

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Pre- and Probiotics and Immunostimulants in Siberian Sturgeon: Gut Microbiota and Immunomodulation

33

Zahra Geraylou

Abstract

The endogenous gut microbiota serves a variety of functions in the host. They protect the host against pathogen invasion to the gastrointestinal tract, in mediating the development, maintenance and effective functionality of the intestinal mucosa and gut-associated lymphoid tissue. Gut microbiota is capable and able to support host digestive function via the production of wide range of exogenous digestive enzymes and vitamins. Therefore, manipulation of the gut microbiota through dietary supplementation of beneficial microbe (probiotics), and non-digestible substances (prebiotics) that selectively stimulate the growth of one or limited health-promoting bacteria in the intestine of the host, not only provides benefit to the host from nutritional point of view but also as an alternate viable therapeutic modality to overcome the adverse effects of antibiotics and drugs.

The use of immunostimulants for the prevention of fish disease has become more promising over the last decades. Immunostimulants enhance the innate defence mechanisms and increase resistance to specific pathogens by conferring the signals to the animal's neuro-immune-endocrine system or various cell signalling pathways.

The effects of pre- and probiotics or their combination, synbiotic, and immunostimulants on Siberian sturgeon growth performance, intestinal microbiota modulation, innate immune response and health have been discussed in this chapter.

Keywords

Prebiotic • Probiotic • Symbiotic • Immunostimulants • Gut microbiota • Immune response • Siberian sturgeon

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Introduction

Siberian sturgeon is one the most important sturgeon species due to its rapid growth, reaching to the sexual maturation within a very short period and exhibition of wide range of feeding habits (Williot et al. 2001; Bronzi et al. 2011). Siberian sturgeon is cultivated for a large proportion of caviar and meat production. Although the rate of sturgeon rearing is steadily increasing (Bronzi et al. 2011; Wei et al. 2011), intensive sturgeon culture is usually faced with several acute and chronic stress agents such as poor water quality, high density and handling (Rafatnezhad and Falahatkar 2011) which resulted in disease outbreaks, including several viral, bacterial and fungal diseases, some of which were unknown prior to cultivation (Williot et al. 2001; Bauer et al. 2002). Sturgeon fish is sensitive to bacterial diseases such as *Yersinia ruckeri*, *Vibrio anguillarum*, *Flexibacter columnaris*, *Flavobacterium johnsoniae*, *Flavobacterium hydatis*, *Aeromonas hydrophila*, *Aeromonas sobria*, *Pseudomonas* spp. such as *Pseudomonas fluorescens*, *Edwardsiella tarda* and *Streptococcus* spp. such as *Streptococcus dysgalactiae* (Vuillaume et al. 1987; Brun et al. 1991; Francis-Floyd 2000; Soltani and Kalbassi 2001; Bauer et al. 2002; Brunetti et al. 2006; Ma et al. 2009; Cao et al. 2010; Karatas et al. 2010; Timur et al. 2010; Meng et al. 2011; Chen et al. 2012; Shaowua et al. 2013).

The problems of diseases, particularly bacterial infections of the cultured organisms due to the deterioration of water quality, have emerged as the major constraints in aquaculture industry (Bondad-Reantaso et al. 2005). In the last decades, antibiotics and chemotherapeutics are used for disease prevention and control, which are extensively criticized for potential development of antibiotic-resistant bacteria, destructive effect on both microflora of aquatic organisms and environmental microbial flora, the accumulation of antibiotic residues in aquatic organism tissue and human and animal health issues. As an alternative strategy, vaccination is an effective prophylactic treatment for infectious diseases which introduced for long-term specific immunity against a specific pathogen by applying a specific antigen (Ellis 1988); however, vaccines are not suited for use with larvae (Nikoskelainen et al. 2001) as the larvae are only dependent on the non-specific immune system. Further vaccines are only available against a limited range of pathogens (Ellis 1999).

Therefore, there is great interest in developing alternatives to traditional means of prevention and combating diseases. Formulated feeds can provide essential nutrients to support growth of the cultured organism and may be one of the most promising means to affect the health and disease resistance (Gatlin 2002). In recent years heightened research has been concentrated on food-based strategies to modulate the composition of the intestinal microbiota, boost the innate (non-specific) defence mechanisms and increase resistance of cultured organism to specific pathogens.

Microbiological modulators and immunostimulants are believed to be ideal and effective disease control strategies that foster sustainability in aquaculture.

Manipulation of microbial populations by microbiological modulators in fish host or the rearing environment has been used as a means of reducing the presence of

opportunistic pathogens and simultaneously stimulating the host immunological responses (Gatesoupe 1999; Verschuere et al. 2000; Gatlin 2002). The most well-known microbiological modulators to regulate and modulate the composition of the intestinal microbiota are the dietary use of prebiotics, probiotics and their combination, synbiotics.

Another alternative technique to prevent the diseases has been proposed that is the strengthening of fish immune systems through the application of immunostimulants (Sakai 1999). Immunostimulants are dietary additives that enhance the innate defence mechanisms and increase resistance to specific pathogens by conferring the signals to the animal's neuro-immune-endocrine system or various cell signalling pathways.

The present chapter summarizes and discusses on the role of intestinal microbiota and the potential application and challenges of probiotics, prebiotics and immunostimulants in Siberian sturgeon with focus on the interaction between sturgeon and those feed additives from an immunological viewpoint looking.

33.1 The Role of Gut Microbiota in Nutrition, Health and Immunity of Fish

The alimentary tract of fish is a very complex and dynamic microbial ecosystem, which interacts with the internal and external environment. The microbial colonization, establishment, composition and diversity in the gastrointestinal (GI) tract of fish is a complex process and starts immediately after hatching and is completed within a few hours (Ganguly and Prasad 2012). Bacteria (aerobic, facultative anaerobic and obligate anaerobic forms) are the main colonizers in the GI tract of fish (Spanggaard et al. 2000; Pond et al. 2006; Ward et al. 2009; Wong and Rawls 2012; Larsen et al. 2014), which are composed of two primary groups—those that are permanent colonizers (autochthonous or indigenous bacteria) and transients (allochthonous bacteria). The bacteria present in the aquatic environment influence the composition of the gut microbiota as the host and microorganisms share the ecosystem (Verschuere et al. 2000). However, gut microbiota composition of fishes is not a simple reflection of the microorganisms in their local habitat but may result from host-specific selective pressures (Sullam et al. 2012). One of the most important features of GI microbiota in fish is variability. Trophic level, habitat salinity, diet, age, geographical location and environmental factors affect strongly composition and activity of the fish GI microbiota (Ogbondeminu 1993; Verschuere et al. 2000; Refstie et al. 2006; Skrodenyte-Arbaciauskiene et al. 2008; Lozupone and Knight 2007; Kesarcodi-Watson et al. 2008; Sullam et al. 2012).

The GI microbiota serves a variety of functions in the host. Influences of microbiota on the fish host have been conventionally studied by comparisons of the physiological characteristics of germ-free and conventional fish. In recent years, the effect of colonization by components of the microbiota in zebra fish (*Danio rerio*) was evaluated at genomic level (Rawls et al. 2004). The authors reported that some

genes were always expressed, independent of the type of bacteria used, while the expression of other genes was bacteria-specific, suggesting that at least a subset of zebra fish genes is sensitive to unknown factors induced by specific bacteria present in the gut microbiota.

The autochthonous bacteria are resident populations that colonize the epithelial surface of the gastrointestinal tract (GIT), including the microvilli. They may protect the GIT against the attack of bacterial pathogens by taking up space and resources along the mucosal lining of the GIT, driving pathogenic bacteria in a transient state and declining the chance of harming the intestinal cells or causing infection (Gutowska et al. 2004; Saha et al. 2006; Skrodenyte-Arbaciauskiene et al. 2006; Sugita and Ito 2006). The bacterial flora of the GI tract of fishes in general may influence a wide variety of metabolic processes through the stimulating epithelial proliferation and expression of numerous genes. They are capable of producing the wide range of digestive enzymes such a proteolytic, amylolytic, cellulolytic, lipolytic and chitinolytic enzymes, which are important for digestion of proteins, carbohydrates, cellulose, lipids and chitin (Gutowska et al. 2004; Syvokiene 1989; Cahill 1990; Sugita et al. 1990, 1997; Mickeniene 1999; Skrodenyte-Arbaciauskiene et al. 2006; Burr et al. 2005). The anaerobic bacteria might play a role in the digestion and absorption of nutrients by supplying it with volatile fatty acids (Clements 1997; Ramirez and Dixon 2003).

33.2 Gut Microbial Composition in Sturgeon Fishes

Sturgeon is considered as a carnivorous fish, presenting a short digestive tract containing oesophagus, large stomach (cardiac, fundus and pyloric stomach), pyloric caeca and intestine (Fig. 33.1).

However, compared to the other carnivorous fish, sturgeons have a unique gastrointestinal tract. The pyloric stomach wall of sturgeons is hypertrophied to a gizzard-like organ (Buddington and Doroshov 1986), their intestine has a functional ciliated epithelium and the hindgut is modified into a spiral valve. This spiral

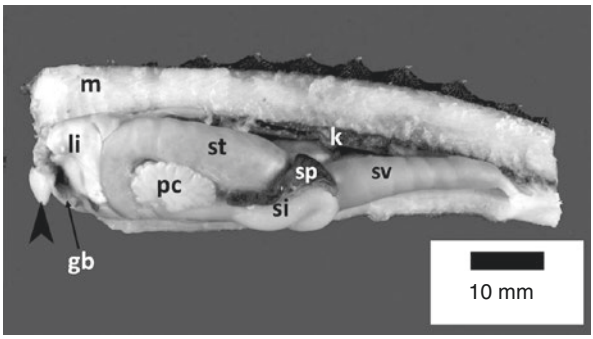

Fig. 33.1 Left oblique view (45° angle). The sketch shows the dorsal muscles (m), kidney (k), heart (*grey arrowhead*), liver (li), gall bladder (gb), stomach (st) and pyloric caecum (pc) followed by the small intestine (si) and spleen (sp) and the caudal dome of the swim bladder (*black Asterisk*) with the spiral valve (sv) on the *right*. Adapted from Daprà et al. (2009)

structure, which is typical for this group, increases the surface and facilitates digestion and nutrient absorption. Specific gut anatomy of sturgeon fishes may influence microbial composition within the gut.

Most of the existing knowledge on the intestinal microbiota of fishes has been mainly obtained from culture-based approaches, which often reveal only a limited range of microbial diversity, and identification of the fish microbiota has typically relied on phenotypic and biochemical key characteristics.

The relatively recent introduction of molecular techniques for the identification and quantification of microorganisms has led to a better understanding of microbial diversity and its function.

Despite the importance of gut microbiota in sturgeon culture and disease resistance, little information is available regarding gut microbiology. One of the primary researches on sturgeon gut microbiota was carried out on white sturgeon (*Acipenser transmontanus*) by Callman and Macy (1984). The authors studied anaerobic and aerobic bacterial flora from the spiral intestine of hatchery-raised sturgeon and reported a new *Bacteroides*-like organism as the predominant strict anaerobe. A diverse autochthonous microbiota was identified in the distal intestine (DI) of beluga using 16S rDNA PCR-denaturing gradient gel electrophoresis (PCR-DGGE) (Salma et al. 2011). *Aeromonas* spp. such as *Aeromonas allosaccharophila* and *Aeromonas media*, *Acinetobacter* sp., *Escherichia coli*, *Plesiomonas* spp. such as *Plesiomonas shigelloides* and *Shewanella* spp. such as *Shewanella putrefaciens* constituted the most microbial population in the DI of beluga. In another study, Bacanu and Oprea (2013) compared the allochthonous gut microbiota of wild and domestic *Acipenser ruthenus*. The results displayed different microbial profiles with higher diversity in domestic fish.

In recent years, some studies have been focused to isolate and characterize lactic acid bacteria (LAB) on sturgeon fishes. Lactic acid bacteria were isolated from different sections of sturgeon's gut and often found as subdominant microbial components in the GIT. Askarian et al. (2009) isolated and characterized indigenous LAB in different parts of the digestive tract of beluga (*Huso huso*) and Persian sturgeon (*Acipenser persicus)* [oesophagus, stomach, proximal intestine (PI) and distal intestine (DI)] in which LAB community differed between the species and the highest LAB levels were found in the DI of both sturgeon species. 16S rRNA gene sequence analysis from beluga and Persian sturgeon revealed *Lactobacillus curvatus*, *Lactococcus raffinolactis*, *L. lactis* subsp. *cremoris* and *Streptococcus* spp. *Leuconostoc mesenteroides* and *Enterococcus seriolicida* constitute LAB population.

Isolation and characterization of cultivable allochthonous lactobacilli were also carried out in beluga and Persian sturgeon by Ghanbari et al. (2009). Their results showed relatively high levels of LAB in the intestine of beluga (log 5.3 CFU[1]g^{-1}) and Persian sturgeon (log 6.4 CFU g^{-1}). Based on phenotypic and biochemical characteristics, *Lactobacillus sakei* and *Lactobacillus plantarum* were most common in both species. In addition, *Lactobacillus coryniformis*, *Lactobacillus alimentarius*, *Lactobacillus brevis, Lactobacillus casei* and *Lactobacillus oris* were also isolated as minor components of the LAB community. The presence of LAB in Persian sturgeon was confirmed by Soltani et al. (2013). The authors reported that two species

[1] Colony-forming unit.

of *Lactococcus garvieae* and *L. lactis* formed 42.5% and 36.1% of the LAB population, respectively, while *Pediococcus pentosaceus*, *Weissella cibaria* and *Enterococcus faecalis* were a minor part of the LAB community.

33.2.1 Microbial Composition in the GI Tract of Siberian Sturgeon (*Acipenser baerii*)

Investigation of cultivable bacteria in different gut sections, foregut, midgut and hindgut (HG), of Siberian sturgeon using culture-dependent approach revealed the higher bacterial density and diversity in HG in comparison with the other gut segments (Geraylou et al. 2014). Preliminary study on Siberian sturgeon's gut microbiota was performed by Mahious et al. (2006). The author isolated and identified the gut microbiota of fish using culture-based and molecular techniques. The majority of isolates belonged to the *Bacillus subtilis* group (19.8%) and are members of the *Enterobacteriaceae* family (29.1%); moreover the presence of *L. lactis* was also reported.

The comprehensive study on gut microbiota composition in Siberian sturgeon and their manipulation by pre- and probiotic diets was conducted by using high-throughput molecular screening of the 16S rRNA genes (Geraylou et al. 2012, 2013a, b).

Next-generation sequencing techniques, including amplicon and shot-gun approaches, and associated bioinformatic tools have developed our ability to quantify and classify commensal bacteria. According to the results achieved by 16s rRNA amplicon sequencing carried on gut microbiota of Siberia sturgeon, *Fusobacteria*, *Firmicutes* and *Proteobacteria* compose the main population of bacteria in the hindgut of Siberian sturgeon. *Fusobacteria* possessed the highest relative abundance among the phyla, almost exclusively represented by *Cetobacterium* at the genus level with a high similarity to *Cetobacterium somerae* at species level (Geraylou et al. 2013a). The *Firmicutes* phylum dominated by genera of *Eubacterium*, *Bacillus*, *Clostridium*, *Lactococcus*, and *Lactobacillus* was predominant. *Proteobacteria* was another predominant phylum in the hindgut of Siberian sturgeon, mostly represented by *Rhodobacteraceae*. The phyla *Chlamydiae*, *Actinobacteria* and *Bacteroidetes* were represented at low relative abundances in the hindgut of Siberian sturgeon. At the level of species, *Cetobacterium somerae* (56–76% of sequences) was the most dominant species found in the HG of Siberian sturgeon followed by *Plesiomonas* sp., *Eubacterium budayi*, *Clostridium* spp. and *Bacillus* spp. (Geraylou et al. 2013a).

33.3 Sturgeon Immune System

Sturgeons possess several primitive features and are supposed to be phylogenetically related to extinct ancestors of teleostean fishes. They have some unique characteristics of immune system (Fäuge 1986; Lundqvist et al. 1996, 1998), but it seems that they possess all types of defence responses like other vertebrates (Lukyanenko 1971, 1989; Kolman et al. 1999). Long-living fishes like sturgeons presumably have to survive many attacks of microbes, viruses and parasites and

ought to have developed efficient defence mechanisms to a large extent based upon leucocyte-producing tissues (Lukyanenko 1971, 1989; Kolman et al. 1999). The immune systems of fish have two integral components: (1) the innate, natural or non-specific and (2) the adaptive or specific immune system. The innate immune system is quite important for fish since the synthesis of antibodies in fish is relatively slow in comparison with antibody production in the higher vertebrates. It is formed by physical barriers, a series of cellular and humoral components. The barriers include the skin and the mucous membranes of the gills and the intestinal tract. If this barrier defence is compromised, then the pathogen is faced with a dam of underlying defences which include cellular and soluble components. The key cells of the innate immune system are the phagocytic cells (granulocytes (neutrophils) and monocytes/macrophages) and the non-specific cytotoxic cells' various lytic enzymes and components of the complement pathways, agglutinins and precipitins (opsonins, primarily lectins), natural antibodies, cytokines, chemokines and antibacterial peptides (Magnadóttir 2006).

Adaptive immunity is a more complex component of the immune system and activated by the innate immune system. The adaptive or specific immune system is characterized by the humoral immune response through the production of antibodies and by the cellular immune response, which is mediated by T-lymphocytes, capable of reacting specifically with antigens (Warr 1997).

These various responses are intended to kill a wide variety of foreign or invading microorganisms, and enhancing them may significantly reduce the mortality of the aquatic organism.

The main lymphomyeloid tissues in sturgeon fish are the thymus, the spleen, the anterior part of the kidney (head kidney), the meningeal myeloid tissue, the pericardial tissue and lymphoid masses of the intestine, especially in the spiral valve (Fäuge 1986).

The gut immune system, which is known as gut-associated lymphoid tissues (GALT), has developed mechanisms to distinguish between potentially pathogenic bacteria and the normal autochthonous bacteria. If potentially pathogenic bacteria are detected, the cellular and humoral mechanisms of the GALT activate the innate immune system and, subsequently, the adaptive immune system to prevent bacteria from causing infection (Gomez and Balcazar 2008). GALT not only provides defence against infectious agents but also regulates immunity in the alimentary tract. In teleost, the level of GALT organization in fish is lower than in mammals, and unlike mammals, fish lacks Peyer's patches, secretory IgA and antigen-transporting M cells in the gut (Buddington et al. 1997). However, it consists principally of lymphocytes, eosinophil granular cells, several types of granulocytes and mucus IgM (Zapata and Amemiya 2000; Zapata et al. 2006). The involvement of GI microbes in the epithelial proliferation, development and maturation of GALT by stimulating B cell proliferation in GALT through a classical antigen-specific immune response (Nilson et al. 1992; Silverman and Goodyear 2002) or by directly stimulating the innate immune system (Medzhitov and Janeway 1997; Leadbetter et al. 2002) and immunity of fish has already been discussed in the gnotobiotic studies (Rawls et al. 2004; Rekecki et al. 2009; Rhee et al. 2004).

The kidney of sturgeons consists of both excretory and lymphoid tissue (Gerogi and Beedle 1978). Sturgeons have the ability to live in both freshwater and marine environments, so their kidney has great ability for adaption process (Ojeda et al. 2003). The kidney of such fishes usually is a fused organ lying in a retroperitoneal location just ventral to the spinal column. Lymphoid, or rather haemopoietic, tissue is found mainly in the anterior part of the kidney (head kidney), where erythrocytes, granulocytes, lymphocytes and macrophages develop. The head kidney is a haematopoietic organ in several fish species, analogous to the bone marrow in mammals (Catton 1951; Zapata and Amemiya 2000), and therefore contains proliferative and plastic cells, i.e. cells that bear the capabilities to divide and finally differentiate. The meningeal tissue is bone marrow-like (myeloid), mainly granulopoietic, but it also contains lymphoid elements. The pericardial tissue is predominantly lymphoid. The pericardial tissue has a lymph node-like appearance. It seems to be the site of interaction between lymphocytes and vascular endothelium. The thymus contains cortex and medulla. The spleen, as in higher vertebrates, is differentiated into white and red pulp. The highly diversiform and well-developed lymphoid tissues of sturgeon may serve as basis of efficient immune mechanisms (Fäuge 1986). Chondrostean possess a major site for the production of granulocytes within a mass that is associated with the meninges (membranes surrounding the central nervous system) (Drzewina 1905; Scharrer 1944), and their heart is frequently covered with tissue that contains lymphocytes, reticular cells and a small number of macrophages (Drzewina 1905; Scatizzi 1933).

33.4 Application of Microbiological Modulator and Stimulants in Sturgeon Fishes and Their Relevance to Host Immunity

33.4.1 Probiotics

Probiotics are defined as live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance (Fuller 1989). This terrestrial-based definition of probiotics has been modified over the years particularly on its applicability in aquaculture based on the complex relationship of an aquatic organism with the external environment (Verschuere et al. 2000; Spanggaard et al. 2000; Irianto and Austin 2002; Wang et al. 2008; Kesarcodi-Watson et al. 2008). According to the authors, ‘probiotic for aquaculture is a live, dead or component of a microbial cell that, when administered via the feed or to the rearing water, benefits the host by improving either disease resistance, health status, growth performance, feed utilization, stress response or general vigor, which is achieved at least in part via improving the hosts or the environmental microbial balance’. They offer benefits to the host primarily via the direct or indirect modulation of the intestinal microbiota, which will in turn promote the host’s immune system and growth, stimulate its enzyme activity and improve its disease resistance (Verschuere et al. 2000; Brunt and Austin 2005; Kesarcodi-Watson et al. 2008; Merrifield et al. 2010).

Among the numerous beneficial effects of probiotics, modulation of the immune system is one of the most commonly purported benefits of the probiotics. Understanding the potential of probiotics in the modulation of fish immune system may be an important basis for pointing manipulation of the microbial composition. Various factors like source, type, dose and duration of supplementation of probiotics can significantly affect the immunomodulatory activity of probiotics.

Probiotics modulate various immunohaematological parameters in teleost. Probiotics interact with the immune cells such as mononuclear phagocytic cells (monocytes, macrophages), neutrophils and natural killer (NK) cells to enhance innate immune responses and stimulate the proliferation of B-lymphocytes in fish. The enhancement of phagocytic activity, respiratory burst activity, lysozyme, peroxidase and anti-protease activity, complement activity and cytokines by probiotics has been reported in many studies (Nayak 2010). Elevation of immunoglobulin level by probiotic supplementation is reported in many animals including fish (Al-Dohail et al. 2009; Nayak et al. 2007; Panigrahi et al. 2004).

Several probiotics and/or their components interact with the GALT to induce immune responses. The promotion of the immune response by probiotic bacteria may also occur in adherence with GALT and may therefore directly affect immune cells like leukocytes (De Simone et al. 1986).

The effect of probiotics in stimulating the sturgeon systemic immune responses is documented in a few studies, but that of local gut immunity is lacking. Limited attempts due to lack of suitable tools are made to access the gut immune response following probiotic treatment.

The use of probiotics in fish culture has recently appeared as a promising biological control strategy. Some microbes have been evaluated as probiotics for different Sturgeon species (Table 33.1). The involvement of probiotics in nutrition and digestive enzymes (Askarian et al. 2011; Hoseinifar et al. 2011a; Faramarzi et al. 2011), histological and bacteriological changes (Salma et al. 2011), haemato-immunological parameters (Hoseinifar et al. 2011a) and modulation of the intestinal microbiota (Hoseinifar et al. 2011b) in some Sturgeon species has been proven.

33.4.2 Prebiotics

The term ‘prebiotic’ was first defined in 1995 by Gibson and Roberfroid as ‘a non-digestible food ingredient that selectively stimulates growth and/or activity of one or a limited number of bacteria’ Gibson and Roberfroid (1995).

Among the established prebiotics such as fructooligosaccharide, transgalactooligosaccharide, inulin and mannan oligosaccharide, mannan is most commonly used as the dietary supplementation for fish and crustacean species (He et al. 2003; Staykov et al. 2007; Ringø et al. 2010; Ringø et al. 2014; Merrifield et al. 2010). Enhancement of growth, survival, number of beneficial gut bacteria, immune responses and reduction of susceptibility to pathogenic bacteria upon ingestion of prebiotics have been reported for several aquatic animals.

Table 33.1 Potential probiotic application for sturgeons

Identity of the probiotics	Fish species	Effects	References
Lactobacillus curvatus	*H. huso* *A. persicus*	Growth enhancement, higher survival Improving the intestinal enzyme activities of *H. huso* at a dose of $9*10^9$ *L. curvatus*/gram feed	Askarian et al. (2011)
Leuconostoc Mesenteroides	*Huso huso* *A. persicus*	Growth enhancement, higher survival and improved intestinal enzyme activities of *A. persicus* at a dose of $2*10^9$ *L. curvatus*/gram feed	Askarian et al. (2011)
Saccharomyces cerevisiae	*A. Persicus*	Improving the growth and feeding parameter	Iranshahi et al. (2011)
Staphylococcus aureus *Leuconostoc mesenteroides* *Lactobacillus plantarum*	*H. huso*	No signs of cellular damage for distal intestine exposed only to *L. mesenteroides* and a combination of *L. mesenteroides* and *S. aureus* Damaging the epithelial cells and disorganizing the microvilli for distal intestine composed of *S. aureus* and *L. plantarum*	Salma et al. (2011)
Saccharomyces cerevisiae var. *ellipsoideus*	*H. huso*	Improving the growth and feeding parameter at a dose of 2% Elevation of autochthonous LAB levels at a dose of 2% per kg of feed No effect on haematological parameters and serum biochemical parameters	Hoseinifar et al. (2011a)
Protexin (*B. licheniformis, B. subtilis* and *B. circulans*)	*A. persicus*	Enhancement of growth performance and feeding parameters for the fish fed with bioencapsulated *Bacillus* bacteria within *Daphnia magna* at different times and different concentrations	Faramarzi et al. (2011)
Protexin (*B. licheniformis, B. subtilis* and *B. circulans*)	*A. persicus*	Reduction in ammonia and urea excretion for the fish fed with bioencapsulated *Bacillus* bacteria within *Daphnia magna* at a dose of 2×10^7 bacteria per millilitre	Faramarzi et al. (2012a)
Protexin (*B. licheniformis, B. subtilis* and *B. circulans*)	*A. persicus*	Higher survival against salinity, temperature, ammonia and pH stresses	Faramarzi et al. (2012b)

H Huso, *A Acipenser*

Several studies in sturgeon fish revealed that prebiotics are promising and have beneficial effects on growth performance, gut microbiota, immunity and disease resistance (Delaedt et al. 2008; Rurangwa et al. 2008; Akrami et al. 2009; Hoseinifar et al. 2011b, c; Geraylou et al. 2012a; Akrami et al. 2013a; Geraylou et al. 2013a, b) (Table 33.2).

Table 33.2 Potential prebiotic application for sturgeons

Prebiotic	Dose and length of administration	Fish species	Results	Reference(s)
Mannan oligosaccharides (MOS)	0.3% 5 weeks	*A. oxyrinchus desotoi*	No effect on growth performance, feed conversion and gross gastrointestinal morphology	Pryor et al. (2003)
Inulin	1, 2 and 3%	Juvenile	Elevation number of intestinal LAB in fish fed with 2% and 1% inulin after 4 and 8 weeks	Akrami et al. (2009)
	4 and 8 weeks	*H. huso*	Reduction of the total number of bacteria Negative relationship between growth performance, feed utilization and inulin supplementation	
Oligofructose (OF)	1, 2 and 3% 7 weeks	Juvenile *H. huso*	Affecting the fish blood profile including haemoglobin concentration, leucocyte levels and the proportion of lymphocytes	Hoseinifar et al. (2011b)
Oligofructose (OF)	1, 2, 3% 7 weeks	*H. huso*	No effects on autochthonous gut bacteria level No effect on growth performance in fish fed with diets supplemented with oligofructose at 1 and 2% Increase of survival and number of lactic acid bacteria at 2% OF	Hoseinifar et al. (2011c)
Inulin	1.0, 2.0 and 3.0% 8 weeks	Juvenile *H. huso*	No effects on serum enzymes Decrease in mean values of alkaline phosphatase with the increase in supplementation level of inulin Increase of white blood cell count in group treated with 1% inulin No effects on red blood cell count, mean corpuscular haemoglobin and glucose	Ahmadifar et al. (2011)

(continued)

Table 33.2 (continued)

Prebiotic	Dose and length of administration	Fish species	Results	Reference(s)
Immunoster or Immunowall	1, 2% 8 weeks	*H. huso*	Enhancement of the final weight and length in both levels	Ta'ati et al. (2011)
Mannan oligosaccharide (MOS)	2, 4% 46 days	*Juvenile* *H. huso*	No effects on growth and survival No effects on LAB population	
Fructooligosaccharides (FOS)	1 and 2%	Juvenile	Increase of the total heterotrophic aerobic bacteria and presumptive LAB levels at a level of 1% FOS	Akrami et al. (2013a)
	11 weeks	*A. stellatus*	Reduction of total heterotrophic aerobic bacteria and presumptive LAB at a level of 2% FOS	
Mannan oligosaccharide (MOS)	0.2, 0.4% 46 days	Juvenile *H. huso*	No effects on serum enzyme activity Enhancement of lymphocytes and eosinophils at 0.2% MOS	Akrami et al. (2013b)
Mannan oligosaccharide (MOS)	0.2, 0.4%	*H. huso*	No differences in survival rate, intestinal lactic acid bacteria and in hematological parameters	Razeghi-Mansour et al. (2012)

33.4.3 Pre-, Pro- and Synbiotic Supplements in Siberian Sturgeon

A large number of studies have been performed on Siberian sturgeon concentrating on growth performance, toxicology, normal rearing conditions, reproduction, haematology, stress responses and immunostimulation (Hamlin et al. 2008; Kolman 2002; Ruchin 2007; Sadati et al. 2011); however, information on the effect of pre- and probiotic and synbiotics on Siberian sturgeon is limited.

33.4.3.1 Prebiotic Application in Siberian Sturgeon

Positive effects of some prebiotics have been presented on the growth performance, innate immunity, microbial fermentation and Siberian sturgeon's gut microbiota.

A study of the effects of inulin and oligofructose on performance of Siberian sturgeon by Mahious et al. (2006) indicated that feeding Siberian sturgeon with feed supplemented by 2% inulin or oligofructose resulted in significantly higher specific growth rate and a better feed conversion ratio. Inulin or oligofructose dietary did not affect total short-chain fatty acids (SCFAs) and lactate production in the hindgut of Siberian sturgeon juveniles (Mahious et al. 2006).

In recent years, comprehensive studies were performed to investigate the prebiotic potential of arabinoxylooligosaccharides (AXOS) under in vivo and in vitro condition in different organisms including juvenile Siberian sturgeon. AXOS are a newly discovered class of candidate prebiotic that exert different properties depending on their structure (Courtin and Delcour 2002).

AXOS are fragmentation products of arabinoxylans (AX), which occur in the cell wall of many cereal grains, consisting of a main chain of β-1,4-linked D-xylopyranosyl units to which O-2- and/or O-3-L-arabinofuranosyl units are linked (Swennen et al., 2005, Swennen et al., 2006). Based on the fragmentation processes, different preparations of AXOS with varying degree of polymerization (DP) and arabinose to xylose ratio (DS) are produced. AXOS compounds are characterized by both the average DP (x) and DS (y) and reflected in their denomination as AXOS-x-y.

Beneficial effects of AXOS on growth performance, gut microbiota composition and fermentation have already been reported for pigs (Niewold et al. 2012) and humans (Grootaert et al. 2009; Sanchez et al. 2009).

Preliminary investigation on prebiotic properties of AXOS on juvenile Siberian sturgeon indicated that administration of 1% AXOS-15-0.26 for 18 weeks affected the microbial hindgut community and acetate, propionate and total SCFA production (Delaedt et al. 2008; Rurangwa et al. 2008).

Prebiotic potential of different concentrations (2% or 4% AXOS-32-0.30) and structures (AXOS-32-0.30 and AXOS-3-0.25) of AXOS in juvenile Siberian sturgeon is evaluated by Geraylou et al. (2012, 2013a). The authors indicated that AXOS improves sturgeon's health through prebiotic action, but the induced effects depend on the specific structure of AXOS. The impact of AXOS on Siberian sturgeon is dose

dependent, as supplementation with 4% of AXOS-32-0.30 did not lead to the same responses. Authors indicated that prebiotic potential of AXOS in Siberian sturgeon also strongly depends on the average degree of polymerization. A higher degree of polymerization of AXOS had a stronger beneficial impact in this sturgeon species.

The growth performance of Siberian sturgeon improved by feeding 2% AXOS-32-0.30 diet. High-throughput sequencing-based analysis of the hindgut revealed hindgut community composition affected by AXOS. The sequences identified were mainly distributed between three phyla: *Fusobacteria*, *Firmicutes* and *Proteobacteria*. *Fusobacteria* showed the highest relative abundance among the phyla, most notably in the control treatment. This phylum was almost exclusively represented by *Cetobacterium* at the genus level with a high similarity to *Cetobacterium somerae* at species level. AXOS-32-0.30 (2%) diets significantly shifted the *Firmicutes-Fusobacteria* ratio in favour of *Firmicutes*. The relative abundance of *Eubacterium*, *Clostridium*, *Lactobacillus*, *Bacillus* and *Lactococcus* increased significantly in the hindgut of fish fed with different preparations of AXOS. Incorporation of AXOS with higher average DP in fish diet resulted in the highest increase in relative abundance of certain species of LAB, *Clostridium* and *Bacillus*. However, overall diversity of the hindgut microbiota was not affected by AXOS. This could be explained by the fact that higher relative abundances of beneficial bacteria including *Clostridium* and LAB, potentially leaving less niche space for other bacteria. These data support the idea that prebiotics can have a positive influence on growth or metabolism of bacteria without significant changes in bacterial diversity of the gut but simply by a shift in the community composition itself towards health-promoting bacteria.

Functional foods such as prebiotics that target the colon and affect the internal environment and bacterial community composition enhance the concentration of SCFAs such as acetate, propionate and butyrate. Immunomodulatory properties of short-chain fatty acids have been demonstrated in several studies (Pratt et al. 1996; Meijer et al. 2010). SCFAs, the main acidic products of bacterial fermentation, contribute towards a low colonic pH, have a direct inhibitory activity towards important gastrointestinal pathogens (Gibson 2004) and provide a major source of useful energy and nutrients for the host and energy for the colonocytes (McNeil 1984). Acetate, propionate and butyrate were the main fermentation products in the hindgut of Siberian sturgeon fed with different preparations of AXOS (Geraylou et al. 2012, 2013a). Production of acetate, butyrate and total short-chain fatty acids increased in fish fed with 2% AXOS-32-0.30, while acetate was the dominant SCFA. Authors suggested that this higher production of acetate in fish fed with AXOS-32-0.30 (2%) could be explained by the increased relative abundance of acetate producers such as *Clostridium* spp. and LAB in the hindgut of sturgeon species fed with this AXOS diet.

There is increasing evidence that fermentable dietary fibres and prebiotics can modulate various properties of the immune system (Schley and Field 2002). Geraylou et al. (2012) indicated the potential of AXOS to enhance the innate immune responses of Siberian sturgeon. Both AXOS-32-0.30 and AXOS-3-0.25 (2%) enhanced the immune responses in some content; however, the effects of AXOS on the innate immune response were more pronounced with the AXOS

compound having a longer avDP. Immunomodulatory effects of AXOS could be attributed to the stimulation of LAB growth and higher production of short-chain fatty acids in the hindgut, which could then be taken up by the fish.

33.4.3.2 Probiotic Application in Siberian Sturgeon

Probiotic studies focusing on Siberian sturgeon are less investigated. Although the ability of probiotics to modulate the intestinal microbiota and immune responses is of high importance to investigate, no information is available on these topics in Siberian sturgeons. In an 8-week study with Siberian sturgeon fed with six experimental diets formulated with different levels (1, 2, 4, 8 and 16 g kg^{-1}) of *Bacillus* sp., Gao et al. (2009) revealed that inclusion level of 2 g kg^{-1} significantly increased growth performance and digestibility.

33.4.3.3 Synbiotic Application in Siberian Sturgeon

The combined application of probiotics and prebiotics is known as synbiotics, which work by improving the survival and colonization of live microbial dietary supplements (probiotics) in the gastrointestinal tract and can promote health and thus improve the welfare of the host (Rodriguez-Estrada et al. 2009, Ai et al. 2011, Ye et al. 2011, Geng et al. 2011, Cerezuela et al. 2012 a).

The effects of administrating putative endogenous probiotics *Lactococcus lactis* spp. *lactis* and *Bacillus circulans*, alone and in combination with potential probiotics, arabinoxylan oligosaccharides (AXOS-32-0.30), are investigated in juvenile Siberian sturgeon (Geraylou et al. 2013b). Those candidate probiotics were selected from HG microbiota of Siberian sturgeon through screening the gut microbiota for antagonistic activity against five fish pathogens and subsequent characterization using different in vitro tests (Geraylou et al. 2014). Combined administration of 2% AXOS-32-0.30 + *L. lactis* spp. *lactis* ST G45 resulted in improvement of growth and immune responses of Siberian sturgeon and important alterations in the intestinal microbiota by a significant decrease in bacterial diversity and increase of the relative abundance of lactic acid bacteria. Strong synergism between *L. lactis* strains and AXOS was observed, since higher abundances of *L. lactis* were present in the hindgut when the symbiotic was administrated.

33.4.3.4 Polyhydroxyalkanoates as Microbial Polymers

Polyhydroxyalkanoates (PHAs) are polyesters of various hydroxyalkanoates that are synthesized by many Gram-positive and Gram-negative bacteria (Reddy et al. 2003). Because of inherent biodegradability, PHAs have attracted the worldwide attention of scientists, and applications of PHAs have been reported in packaging industry, agriculture, automotive sector, food industry and medical implantation devices (Kalia et al. 2011).

Different aerobic and anaerobic microorganisms isolated from a variety of ecosystems are able to degrade extracellular PHAs.

Polyhydroxybutyric acid is an important member of family of PHAs, and it can be degraded by bacteria (Kato et al. 1992; Patnaik 2005). It is a polymer belonging to the polyester class that is produced under conditions of physiological stress and excess of carbon as a form of energy storage by bacteria such as *Alcaligenes*

eutrophus and *Bacillus megaterium*. Poly-β-hydroxybutyrate (PHB) has been shown to be able to protect *Artemia* nauplii (*Artemia franciscana*) against vibriosis (Defoirdt et al. 2007a; Halet et al. 2007; Van Cam et al. 2009). In another study, Liu et al. (2010) isolated PHB-degrading bacteria from a gastrointestinal environment of Siberian sturgeon, European sea bass and giant river prawn. Two of the most promising PHB-degrading strains isolated from sturgeon belonged to genus *Acidovorax*. The results showed that PHB-degrading bacteria isolated from Siberian sturgeon are able to inactivate acyl homoserine lactones, a type of quorum-sensing molecule that regulates the virulence of different pathogenic bacteria. Improvement of weight gain, survival and SCFA concentration in the GI tract of European sea bass (*Dicentrarchus labrax*) juveniles and the larvae of the giant river prawn (*Macrobrachium rosenbergii*) (Defoirdt et al. 2007b; Nhan et al. 2010) has been reported.

Najdegerami et al. (2012) examined the effects of three commercial diets containing 2% and 5% PHB on growth performance and GI tract microbial community of Siberian sturgeon fingerling. Analysis revealed that PHB affected the bacterial community diversity and community organization so that higher species richness and diversity in terms of metabolic potential were noticed once the fishes were fed with 2% PHB, while the lowest species richness was revealed at 5% inclusion level. Some specific bands became apparent and were phylogenetically similar to *Bacillus* and *Ruminococcaceae*. Najdegerami et al. (2015) reported that enrichment of *Artemia* nauplii by PHB for 4 weeks resulted in modulation of total bacterial community, combining allochthonous and autochthonous bacteria in the DI of Siberian sturgeon. PCR-DGGE analysis revealed that the bands of two bacteria species were dominant, irrespective of the treatment; however, no further information on these two bacteria species were presented.

33.4.3.5 Immunostimulants

Immunostimulants can be grouped under chemical agents, bacterial preparations, polysaccharides, animal or plant extracts, nutritional factors and cytokines (Sakai 1999). Nutritional factors such as Vitamins B and C, growth hormone and prolactin have also been reported to be immunostimulators. These immunostimulants mainly facilitate the function of phagocytic cells and increase their bactericidal activities. Several immunostimulants also stimulate the natural killer cells, complement, lysozyme and antibody responses of fish (Sakai 1999).

A number of different biological and synthetic compounds such levamisole (Siwicki 1989) or glucans (Jeney and Anderson 1993; Jeney et al. 1997) or high levels of vitamin C (Hardie et al. 1991; Duncan and Lovell 1994) have been found to enhance the non-specific system in fish and simultaneously to increase barrier against a series of pathogens, both specific and opportunistic (Anderson 1992; Raa et al. 1992; Jeney and Anderson 1993). The potential of some immunostimulants has been examined in Siberian sturgeon. Jeney and Jeney (2002) investigated the potential of glucan, vitamin U and vitamin C on non-specific cellular activity in sturgeon hybrid *Acipenser ruthenus* × *Acipenser baerii*. Authors reported that the application of glucan and vitamin U in diets modulated the non-specific defence

mechanisms in sturgeon. Leukocyte activity enhanced within 2 weeks after feeding 0.5% glucan/food kg. Incorporation of vitamin U (100, 200 or 300 p.p.m.) to fish feed for 4 weeks resulted in higher phagocytosis and respiratory burst activity of circulating leukocytes, while fish fed with vitamin C had significantly lower erythrocyte and leukocyte counts compared with the control group.

Xie et al. (2006) studied the effects of dietary ascorbic acid (AA) in Siberian sturgeon immune responses. Authors reported that although *Acipenser baerii* is one of few fish species capable of synthesizing AA, however, dietary AA may be conditionally necessary for Siberian sturgeon to achieve optimal immune response, particularly in early developmental stages.

In another study Eslamlooa et al. (2012) investigated the effects of different levels of dietary lactoferrin (LF) on growth performance, physiological status, iron absorption and innate immune response of juvenile Siberian sturgeon. The different rations of LF were tested (100, 200, 400, 800 and 1600 mg LF kg^{-1} diet) for 8 weeks. This study revealed the ability of dietary LF to sequester iron, which is an essential nutrient required for the growth of bacteria. Plasma iron in LF treatments greatly declined, and the total iron-binding capacity increased in fish fed with 800 mg LF kg^{-1}. However dietary LF didn't affect fish growth performance, haematological parameters, serum proteins or hepatic enzyme and stress indicators (plasma cortisol, glucose and lactate). LF was also shown to improve some physiological and immunological parameters of Siberian sturgeon, to some extent. The amount of mucus secretion and serum bactericidal activity rose in fish fed on dietary LF, although other non-specific immune responses such as mucus bactericidal activity, serum and mucus lysozyme activity, serum peroxidase, serum natural haemolytic complement activity and serum IgM were not influenced by LF.

The effects of different levels of dietary choline on growth rate, body composition and total liver lipid of juvenile Siberian sturgeon were investigated by Yazdani Sadati et al. (2014). Fish were fed by different levels of choline chloride (2000, 4000 and 8000 mg kg^{-1} diet) for 12 weeks. The authors reported the optimum dietary choline chloride level for normal growth performance of juvenile *Acipenser baerii* appears to be 1500 mg kg^{-1}. Weight gain, feed conversion ratio, specific growth rate and protein efficiency ratio were affected by dietary choline concentrations. Total lipid of the liver and plasma, plasma cholesterol and triglyceride and phospholipid levels showed an increasing trend with increasing levels of dietary choline.

Conclusion

The beneficial effects of pre- and probiotic dietary supplements for better growth, digestion, immunity and disease resistance have been recorded in Sturgeon fishes. Considering the immunomodulatory properties of pre- and probiotics in sturgeon fish, expanding the knowledge of interactions between gut microbiota, the intestinal epithelium and the gut immune system is essential in advancing the appropriate strategy for stimulating the gut immune system as well as systemic immunity through manipulation of gut microbiota without altering the intestinal homeostasis.

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Nitrogen Excretion in Sturgeons with Special Emphasis on the Siberian Sturgeon: Methods and Effect of Food, Feeding and Size of Fish

34

Mirosław Szczepkowski

Abstract

Chapter 34 discusses the methods of excretion of nitrogen compounds from sturgeon and the most important factors influencing their size. In sturgeons, a majority of metabolic products of nitrogen transformations are eliminated in the form of ammonia. To a lower degree, nitrogen is excreted in the form of urea. The contribution of urea in total excretion of nitrogen can be however considerable, at a level from 17 up to 50%. Excretion of ammonia occurs particularly through the gills and transport through cell membranes of the gill epithelium and excretion of urea through kidneys or gills. Main factors which affect the excretion of nitrogen compounds are the size of fish, water temperature and food ration. The stabilisation of the amount of excretion (endogenous level of ammonia excretion) in the Siberian sturgeon occurs after approximately 1 week of starving.

Keywords

Siberian sturgeon • Ammonia • Excretion • Nitrogen • Urea

Introduction

Metabolic transformations of proteins and other molecules absorbed from food result in the development of nitrogenous compounds which have to be eliminated from the organism. The basic products of nitrogenous transformations in the fish organism are ammonia and urea (Wright 2007). The former is particularly highly toxic for fish (Ip et al. 2001). In sturgeons, excretion in small

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amounts can occur in the form of ketone bodies, typical of excretion in elasmobranches (Singer et al. 1990).

Ammonia in the organism of fish particularly develops through the transamination of various amino acids (Watts and Watts 1974) and deamination of adenylates in the muscles (Mommsen and Hochachka 1988). High content of nitrogenous compounds in water can be a factor restricting the growth of sturgeons (Falahatkar 2011). Therefore, knowledge of the processes of excretion is of high practical significance. This is particularly important in intensive rearing systems, where it is necessary for designing water treatment facilities, e.g., in recirculation systems. This is equally important during transport of fish, when constant water exchange is not possible.

Chapter 34 discusses the methods of excretion of nitrogen compounds from sturgeon and the most important factors influencing their size.

34.1 Different Forms of Nitrogen

The maximum concentration of non-ionised ammonia in water, tolerated by Siberian sturgeon, has not been investigated in detail. Sturgeons, however, and particularly their juvenile stages, are considered sensitive to high ammonia concentration, potentially leading to gill necrosis and mass losses (Chebanov and Galich 2013). The authors determine the threshold level of non-ionised ammonia for juvenile stages of various species of sturgeons as 0.05 mg L^{-1}. According to research on fry of shortnose sturgeon (*Acipenser brevirostrum*), the lethal concentration of non-ionised ammonia (96 h LC50) amounts to 0.58 mg L^{-1} (Fontenot et al. 1998).

34.2 Methods for Assessing the Excretion in Sturgeons

Sturgeons are included in the group of ammonotelic fish, similarly as teleost fish, in which the majority of metabolic products of nitrogen transformations is eliminated in the form of ammonia (Wood 2001). Its excretion occurs particularly through the gills (Singer and Ballantyne 2004) and transport through cell membranes of the gill epithelium. It occurs in the presence of rhesus glycoproteins (Wilkie 2002; Weihrauch et al. 2009). These mechanisms, however, have not been fully investigated so far. To a lower degree, nitrogen is excreted in the form of urea in fish through kidneys or gills (McDonald et al. 2006). In sturgeons, the contribution of urea in total excretion of nitrogen is considerable and can be at a level from 17 up to 50% (Medale et al. 1991; Gershanovich and Pototskij 1992, 1995; Altinoka and Grizzleb 2004). The ratio of the amount of nitrogen excreted in the form of ammonia and urea depends among others on feeding fish: in fed sterlets (*Acipenser ruthenus* L.), excretion in the form of ammonia was 1.4 times higher than urea, and in starved fish, it was even 2.6 times higher (Gershanovich and Pototskij 1992). Another factor determining the proportions of nitrogen excretion

in the form of these two compounds is water salinity: in hybrids of sturgeons at an increase in salinity from 0 to 10‰, excretion of ammonia decreased and that of urea increased (Gershanovich and Pototskij 1995). In gulf sturgeon (*Acipenser oxyrinchus desotoi*), at an increase in salinity from 0 to 9‰, excretion of ammonia also decreased, but excretion of nitrogen in the form of urea was constant (Altinoka and Grizzleb 2004).

34.3 Effect of Food

Excretion of ammonia in Siberian sturgeon is determined by the type and composition of its food. In larvae of Siberian sturgeon, varied absorbability of amino acids from natural food and fodder was determined (Dabrowski et al. 1987). This can affect the amount of excreted ammonia. The effect of food on excretion was also determined in larvae of the Persian sturgeon (*Acipenser persicus*). Larvae fed with zooplankton supplemented with probiotic excreted both less ammonia and urea in comparison to fish fed with unenriched zooplankton (Faramarzi et al. 2012).

In older sturgeons, a correlation was determined between nitrogen excretion and amount of protein in food. In the hybrid of Siberian sturgeon with Russian sturgeon, an increase in its content in food from 25 to 55% was determined to cause a considerable increase in the amount of non-faecal excretion of ammonia and to have no effect on faecal excretion (Guo et al. 2012). In Siberian sturgeon fed with isoprotein foods containing varied components (with fish meal and fodder with fish meal replaced with an addition of plant proteins), it was determined that the amount of faecal excretion depended not only on the amount of protein but also on the source of its origin (Yun et al. 2014).

34.4 Effect of Feeding

Feeding is one of the most important factors determining the amount of excretion. It influences both the amount of excreted ammonia and the fluctuations of its changes. In general, an increase of the amount of ingested food causes an increase in the rate of ammonia production. In the case of white sturgeon (*Acipenser transmontanus*) fed with food in commercial conditions, the correlation was of exponential character at an amount of protein of up to 1.5% g $fish^{-1}$ day^{-1} (Thomas and Piedrahita 1998). In sterlet fed with chironomid larvae (*Chironomus* sp.) in doses increasing up to 8.4% of body mass per day, an increase in the amount of excreted ammonia was linear. Further increase in the amount of food caused no adequate increase in ammonia excretion (Gershanovich and Pototskij 1992). This may result from its incomplete eating.

The course of daily profiles of ammonia excretion depends among others on the feeding schedule. In Siberian sturgeon fed for a period of 17 h during 1 day, an increase in the

excretion of ammonia nitrogen was observed after 3–5 h from the commencement of feeding. Its maximum values were recorded after the completion of feeding (Jatteau 1997). Very similar results were obtained in juvenile atlantic sturgeon *Acipenser oxyrinchus* fed for 18 h during 1 day (Szczepkowski et al. 2011). In various species of sturgeons (Siberian sturgeon, Amur sturgeon (*Acipenser schrenckii*) and white sturgeon) fed in a non-continuous manner, an increase in the amount of excreted ammonia was observed immediately after feeding and the maximum value after 2–6 h from the moment of feeding (Salin and Williot 1991; Thomas and Piedrahita 1998; Lin et al. 2004).

High fluctuations of the amount of excreted ammonia are unfavourable. They may lead to periodical exceeding of its value in water above levels tolerated by fish. This particularly concerns intensive rearing systems such as recirculation systems. The amplitude of changes in the excretion of ammonia is considerably higher than in the case of oxygen consumption (Zakęś 1999). In certain cases, the amount of excreted ammonia after the commencement of feeding increased even more than 11 times (Jobling 1981). In fry of Siberian sturgeon, in groups with body mass from 0.8 to 73 g, even during constant feeding for 24 h, considerable fluctuations occurred in the amount of excreted ammonia, with the maximum values exceeding the minimum of 1.6–5 times (Szczepkowski et al. 2000a).

Starving of fish has a strong effect on excretion. After 24 h of no feeding, the amount of excreted ammonia in fry of Siberian sturgeon with a body weight from 1 to 303 g varied from 26.4 to 0.7 mg kg^{-1} h^{-1} and was lower by 60–80% than in fish fed continuously (Szczepkowski et al. 2000a). It should be emphasised that in fish starved for 24 h, strong daily fluctuations were also recorded in changes of ammonia excretion. The stabilisation of the amount of excretion (endogenous level of ammonia excretion) in the species occurs after approximately 1 week of starving (Salin and Williot 1991).

34.5 Effect of Size of Fish

The unitary value of ammonia excretion decreases along with fish body weight. At a temperature of 20 °C, ammonia excretion in Siberian sturgeon fed with artificial food decreased from 1625 mg kg^{-1} day^{-1} in fry with an average body weight of 0.8 g to 40.8 mg kg^{-1} day^{-1} in fry with a body weight of 449 g (Szczepkowski et al. 2000a). At a temperature of 18 °C (fish body weight from 40 to 1700 g), ammonia excretion decreased from 530 to 239 mg kg^{-1} day^{-1} (Jatteau 1997). The dependency is equally strong in fish fed with natural food. In lake sturgeon (*Acipenser fulvescens*) with an average body weight from 10 to 120 g, fed with *Artemia nauplii*, ammonia excretion in the smallest fish (10 g) was four times higher than in the group of average-sized fish (50–65 g) and five times higher than in the largest fish (Bharadwaj et al. 2008). The level of endogenous ammonia excretion, not related to the ingested food, did not depend on the size of fish. According to Salin and Williot (1991), the endogenous level of ammonia excretion in Siberian sturgeon observed after 8 days of starving did not differ in various size groups from 60 to 2000 g and amounted to approximately 32 mg NH_4-N kg^{-1} day^{-1}.

34.6 Other Factors Determining Excretion

Other factors determining nitrogen excretion in sturgeons particularly include water temperature. In sterlet and sturgeon hybrids, increasing temperature from 12 to 28 °C increased both the excretion of ammonia and urea (2.7 and 2.9 times, respectively), but their proportion in the analysed range of temperatures remained stable (Gershanovich and Pototskij 1995). In Amur sturgeon, the amount of ammonia excretion was considerably higher at a temperature of 28 °C than at 23 °C and 18 °C (Lin et al. 2004). The authors also observed that at a lower temperature (18 °C), the maximum of ammonia excretion occurred later (after 8 h from feeding) than at higher temperatures at which it was observed already after 4 h.

In the case of water pH, in different species and hybrids of sturgeons, the dependence of ammonia excretion is of the opposite character than in the case of temperature. An increase in water pH from 4.0 to 9.6 is accompanied by a decrease in ammonia excretion (Gershanovich and Pototskij 1995). Under such conditions, the contribution of urea in excretion also increased.

Other factors with potential effect on the amount of excretion include high concentration of ammonia in the environment, but in the case of sturgeons, this has not been investigated in detail.

No effect was recorded of the stocking density on ammonia excretion. In the rearing of Atlantic sturgeon with an increase of stocking density, the mean value of ammonia excretion was at a similar level, despite the fact that the efficiency of feed utilisation expressed as the feed conversion ratio was considerably lower (Szczepkowski et al. 2011).

34.7 Summary

The knowledge of the process of nitrogen excretion in sturgeons, including Siberian sturgeon (Table 34.1), is still very incomplete. A lot of fragmentary data exist concerning different species and hybrids of sturgeons. It has still not been determined, however, to what degree they are specific to particular species and to what degree they are characteristic of the entire group of the fish. It has been evidenced that the values of metabolic indices, including ammonia excretion, and course of changes in their daily profiles, can differ considerably in different sturgeons. This may be related to, e.g., their different activities in the daily cycle. This was evidently observed in the comparative research of Siberian sturgeon and its hybrid with Sakhalin sturgeon, *Acipenser mikadoi*, showing high nocturnal activity (Szczepkowski et al. 2000b). It is also of high importance that sturgeons occupy an indirect place between primitive and teleost fish, with a considerable contribution of excretion in the form of urea which, as indicated above, depends on various physiological and environmental factors.

Table 34.1 Ammonia excretion in Siberian sturgeon in different conditions

Value of nitrogen excretion (mg kg^{-1} day^{-1})	Body weight (g)	Water temperature (°C)	Daily food ration (% BW per day)	Source
1625[a]	0.8	20	10.0	Szczepkowski et al. (2000a)
633.6[a]	1.0	20	0	Szczepkowski et al. (2000a)
530[b]	40	18	2.5	Jatteau (1997)
32[c]	60–2000	17.2	0	Salin and Williot (1991)
368[b]	160	18	1.5	Jatteau (1997)
16.8[a]	303	20	0	Szczepkowski et al. (2000a)
40.8[a]	449	20	1.9	Szczepkowski et al. (2000a)
368.2[a]	827	20	1.0	Szczepkowski et al. (2000b)
273.6[a]	1146	20	1.0	Szczepkowski et al. (2000b)
239[b]	1700	18	1.5	Jatteau (1997)

[a]NH_4 + NH^3
[b]NH_4–N + NH^3–N
[c]Endogenous level of excretion NH_4–N

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Part III

Production

Caviars: How to Describe and Compare Their Qualities? The Sensorial Approach

35

Mireille Cardinal

Abstract

How do we describe the qualities of caviars found on the market? How do we evaluate their sensory properties? Are there great differences between caviars? Which criteria can be used? To answer these questions, the methodology of quantitative and descriptive analysis used for a European project on "Production of caviar from roe and ovulated oocytes from farmed sturgeon species" is described. The sensory characteristics of caviar were evaluated in reared and wild sturgeon using a trained panel. The most relevant and discriminative sensory descriptors were chosen to describe appearance, odour, flavour and texture. This list of criteria is discussed in comparison with recent work. Overall, sharing a common vocabulary and evaluation procedure, the profiling method allows having a better evaluation of caviar characteristics and can be used as a tool to control a production.

Keywords

Caviar • Sturgeon • Sensory characteristics • Sensory profile • Descriptors

Introduction

As already mentioned by Williot et al. in 2001, the most significant changes in the sturgeon industry during the last years of the XX century have been in the emergence of the pond as a production system and in the increasing caviar production from farm sturgeons. The potential of caviar market and its likely change was identified with the question of the consequence on product quality. Nowadays, and since

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2011 with the prohibition import of wild sturgeons from the Caspian Sea and the real development of a sturgeon-farmed production (Bronzi and Rosenthal 2014), the need of tools to describe the caviar quality is an important topic for producers and consumers. Along the years, producers of sturgeons and stakeholders have developed their own vocabulary and references to describe the different qualities of caviar and facilitate trades for this product, but during a long time, there was no general and common method to describe and quantify the sensory criteria of caviar.

It is the reason why we propose here to present some works that intended to describe the caviar quality and especially the sensory characteristics. Indeed, food product quality goes over different meanings. Generally, the first quality required is the sanitary quality to prevent the consumer from any health trouble, but the term quality covers also a nutritional quality, a technological quality and of course a sensorial quality. In this latter case, two different approaches need to be under consideration: on one hand, the point of view of the consumer who can express some preferences for certain ranges or qualities of products and, on the other hand, the point of view of the producer who needs to qualify the sensory characteristics in order to have a better control of his production and also to be able to adapt his product to the consumer demand. The development of the sensory science and methodologies associated allowed to giving a better insight on what the consumer likes while trying to explain the reasons of observed preferences using sensory characteristics previously described.

The literature allows to illustrating these two approaches. In the case of consumer perception, Toussaint-Samat (1997) refers to a famous consumer, the French king, Louis XV (1715–1774), who knew an extreme reaction with caviar consumption. Even if the caviar has a status of being a luxurious product which develops expectations, a positive image and laudatory comments, most people rarely or have not ever tasted the product and did not have any reference in mind. It happens sometimes that the first sensory experience produces a violent reaction: for example, it was reported that Louis XV spat the precious caviar given as a present by the ambassador of Russia. If this history reveals that a training step is often necessary in order to accept new perceptions and open the range of liked products, it does not give any explanation on the reasons of dislike.

Two centuries later, attempts were found in literature, not to find explanation of the Louis XV dislike but to describe the main characteristics of caviar in order to have a vocabulary available to discriminate different qualities and to be more objective to qualify the product.

According to Gödecken (1986), for a same process and a same shelf life, the taste of caviar produced from four different species seemed very close: the beluga (*Huso Huso*), osetra (*Acipenser gueldenstaedtii*), sevruga (*Acipenser stellatus)* and ship (*Acipenser nudiventris*). However he noticed that the prices could be very different on the market due to the importance of appearance attached by consumers. A list of criteria (Table 35.1) was established by this author according to the species.

Sternin and Doré (1998) have also presented and used criteria of colour, appearance, egg size and odour to characterise the product: the colour was different according to the species, from grey to black, with sometimes golden yellow marl, and from brown to green. The egg form was a criterion which was taken into account (round to slightly

Table 35.1 Characteristics of caviar according to Gödecken (1986)

Caviar	Sensory characteristics
Beluga	The biggest eggs, colour from steel grey to clear grey
	Notice of author: *the biggest are the eggs, the clearest are the colour*
Osetra	Eggs smaller than beluga eggs but firmer
	Different kind of colours: black, grey, steel, dark grey, shade from brown to gold yellowish
	Dry appearance
	Particular taste close to hazelnut taste
Ship	Smaller eggs than osetra eggs
	Colour from grey to brown
Sevruga	Smaller eggs than osetra eggs
	Eggs surrounded with a soft membrane
	Extreme delicate taste

elongated) as well as their integrity. The facility to separate eggs or to have a sticky behaviour was also a quality criterion. Size and homogeneity of eggs were evaluated, and the odour could be qualified as typical of sturgeon, sweet or sour, not fresh or with a yeast odour. Regarding criteria of taste, the typical taste of sturgeon caviar was described as close to those of egg yolk, delicate, tasty and persistent. A taste of grass has been cited, sometimes muddy, acrid, sour and bitter. The texture was also considered as an important factor of quality as these authors noticed that "the egg membrane had a nice melting texture and let a sensation of creamy liquid" or also that the product was "dried with a loss of melting on the egg membrane".

At the same period, a European project, INCO-COPERNICUS (Contract IC 15CT 96-1005), gave the opportunity to apply a sensory methodology, well known in the food sector, in order to have a tool for the comparison of the caviar quality. The objective of this project entitled "Production of caviar from roe and ovulated oocytes from some farmed sturgeon species" was to develop research on alternative production of caviar and especially with farmed sturgeons. Various scientific institutes were involved in this research, including the Museum of Natural History, CEMAGREF (now IRSTEA), IFREMER from France, the Institute of Fresh Water Ecology and Inland Fisheries from Germany, a fish farming research station from Moldavia, a centre of research for fishing fish culture and fish processing and the University of Galati from Romania. The objective was not to define a level of quality for commercial products but to identify and quantify potential differences between products. In order to reach this aim, the study was conducted in accordance with sensory profile methodology (Stone et al. 1974; Stone and Sidel 1985) using a selection step for the most objective and relevant descriptors. This type of sensory technique has already proved efficient in different fields of fish farming (Ostrander and Martinsen 1976; Thomassen and Rosjo 1989; Johnsen and Kelly 1990; Skonberg et al. 1993).

In 1998, the overall objective of the project was to establish the technologies to make high-quality caviar from farmed sturgeons in order to reduce the high fishing pressure on wild endangered sturgeons. In this context, it was necessary to analyse the economic feasibility of this new production way but also to characterise the caviar quality produced in different conditions: a traditional caviar taken from an

ovarian follicle at the right stage of maturity and a "caviar" made out of ovulated eggs (fertilised or not with spermatozoa). The different steps followed during this project to elaborate a grid of sensory evaluation are presented.

35.1 Methodology of Sensory Profiling: The Setting Up of the Method for Caviars

To bring to a successful work, the methodology requires a trained panel with an experience on sensory description and takes into account different steps as product description, quantification and selection of descriptors (Billard, 2002). This work performed in the context of the European COPERNICUS project is presented here.

- Panel
  Sensory analysis was carried out by the internal panel of the French Research Institute for Exploitation of the Sea (IFREMER). Twenty volunteers, previously selected on their sensory capacities and trained on sensory methods applied to marine products, were invited to participate in caviar analysis. Once the product is known, it was relatively easy to convince people to integrate the pool of panellists! Interest and curiosity for the product allowed the panellists to be present for the sessions.
- Evaluation conditions
  Sensory tests were performed in a special room designed according to the recommendations of ISO (2007). Panellists are placed in individual partitioned booths with controlled temperature (20 °C) and light (day light, $T = 6500$ K). Data were collected with a computerised system (Fizz, Biosystèmes, Dijon, France).
- Selection of descriptors
  The first step of the work was to generate and to select sensory descriptors which would be used to compare different samples all along the study. These descriptors had to be relevant and discriminative.

  The methodology of selection was based on a search and a quantification of criteria used to characterise and discriminate caviar on aspect, odour, flavour and texture. The objective was to use these selected attributes to elaborate a sensory profile for a product.

  Two steps are generally required with this methodology (ISO 13299; ISO 2003):
  - A step of **description** which allows to screen all the qualitative characteristics of products.
  - During this first step, the objective was to present to the panel a large range of products in order to take into account all the sensory dimensions and avoid to miss some criteria specific to product or processing condition. Different caviars were prepared by project partners or bought from retailers (Table 35.2). The aim was to present products whose sensory characteristics were as contrasted as possible and representative of usual products. Different species were used: *Acipenser baerii* from CEMAGREF, *Acipenser stellatus* from Romania and retailers and *Acipenser gueldenstaedtii* from retailers. Caviar

Table 35.2 Caviars presented for description and quantification steps

Samples	Species	"Eggs" origin	Conditions of storage (°C)	Origin country
1	*Acipenser stellatus* (sevruga)	Ovarian follicles	+2	Iran (retailer)
2	*Acipenser baerii*	Ovarian follicles	+2	Aquitaine (Estudor company)
3		Fertilised ovulated eggs	+2	CEMAGREF
4	*Acipenser stellatus* (sevruga)	Ovarian follicles	+2	Russia (détaillant) (retailer)
5	*Acipenser gueldenstaedtii* (osetra)	Ovarian follicles	+2	Iran (retailer)
6	*Acipenser stellatus* (sevruga)	Ovarian follicles	Frozen at −20	Romania

from *A. baerii* was produced by two different methods, either directly from ovaries or from ovulated eggs.

– A **quantitative step** where each panellist scores the intensity of each descriptor generated during the first step. Three kinds of data treatment, presented further, gave information to choose the more relevant and discriminative attributes and allowed to remove descriptors with the same meaning:
– To reduce the descriptors number, the frequency of the score 0 was calculated. Indeed if this frequency was high, whatever the products may be, that means that the attribute was not a characteristic of the product. It was not **relevant** so it was rejected.
– When a descriptor presented a different intensity according to the products, then it was a **discriminative** criterion. So, to evaluate the discriminative power of each descriptor, it was necessary to compare the score distributions of each product. These distributions were all the more different that the descriptor was evaluated differently according to the product.

In order to answer to this question and to have a tool of discrimination, we tested the distances between the frequency distributions, using the CHI 2 distance defined as:

$$\text{CHI}\,2 = \sum_{pi}\sum \left(f_{ip} - e_i\right)^2 / e_i$$

where p represents a product, i represents a score, f_{ip} was the frequency of observed answers for the score i and the product p and e_i was the mean frequency for the score i, for all the products.

The CHI 2 distance gave us information on the discriminative power of this criterion. The higher the index was, the stronger the discriminative power.

To reduce this list generally too long for an easy use in profiling test, statistical tools were used as multivariate analysis which show the relative importance and contribution of attributes to discriminate products. The treatment generally used is a

Table 35.3 Sensory descriptors selected to describe caviar

Odour	Appearance	Flavour/taste	Texture in mouth
Global intensity	*Eggs colour*:	Global intensity	*Overall perception*:
	Darkness	Anchovy	Separated eggs
Anchovy	Homogénéity of colour	Salted	Melting texture (melting of eggs under the tongue)
Sour/vinegar	Bronze colour	Sour	Sticky texture
Yeast	White spots on eggs	Sweet	*First bite of eggs*:
Butter	Translucent aspect	Butter	Crunchy texture
Raw potato	*General appearance of eggs*:	Metallic	Eggs seem to slide under the teeth
	Egg size	Bitter	
	Filled eggs	Rancid	
	Evaluation of jelly:	Earthy taste	
	Quantity of jelly	Astringency	
		Persistence	

principal component analysis. The aim of this analysis was to point out the synonym or opposite attributes. The treatment was realised from the score mean for each criteria and each product.

A **final step of selection** with the panel. When various criteria appeared to be correlated, a discussion was organised with the panel in order to select those which seemed clearest and most relevant. Once they reached a consensus, each criterion was described by a definition. Finally, 6 descriptors for odour, 8 for appearance, 12 for flavour and 4 for texture in the mouth were considered relevant and discriminative to describe the caviar sensory properties. These descriptors are presented in Table 35.3. Even if the work intended to take into account a large range of products, it is possible that the list given is not exhaustive. However, these criteria allowed to make objective comparisons between the ranges of samples chosen.

- Profiling session
  Once the step of descriptor generation is performed, generally various sensory sessions are organised in the conditions already described, and panellists rate the sensory attributes on a continuous scale presented on a computer screen, from low intensity (0) to high intensity (10). Products are assigned three-digit numbers, randomised and served simultaneously for a comparative evaluation. The data are immediately transferred by the network to a central computer for statistical processing (Fizz, Biosystèmes, Statgraphics Plus for Windows V.1 and Uniwin Plus V.3).
- Data treatment on profiling data
  Data collected from the profiling test are generally submitted to a two-way analysis of variance (ANOVA) with panellists and products as independent factors. This treatment allows to identify for each descriptor if a significant difference exists between samples. A multivariate data processing as a principal component analysis (PCA) presented previously to reduce the numbers of criteria can be used to give a synthetic view of the main characteristics of the

evaluated products. This analysis is performed on scores averaged over assessors and is chosen to summarise in few dimensions the key information in sensory data while explaining the differences between samples. In the presented work, PCA is based on the correlation matrix, so all attributes were given the same importance.

35.2 Results: Sensory Profiles of Caviars

The panel was used to provide a first approach to characterising the caviar produced from farmed sturgeons (*A. baerii* and *A. stellatus)* in comparison with caviar from wild sturgeons (*A. gueldenstaedtii* and *A. stellatus*). At the same time, the quality of "caviar" obtained from ovulated and recently fertilised eggs was studied.

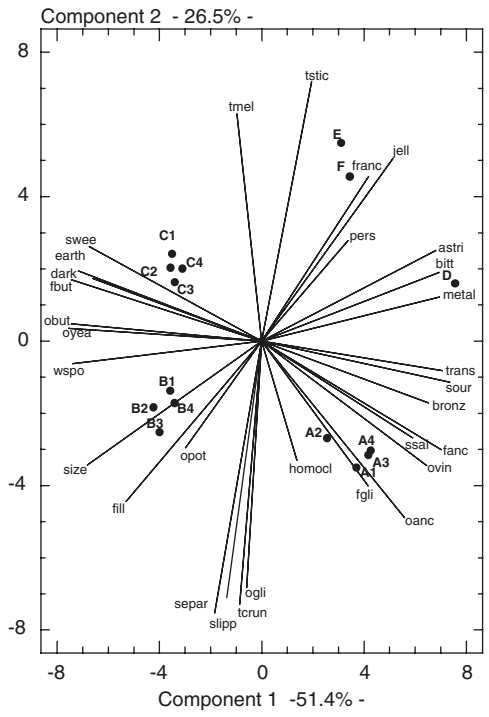
– Sensory characteristics of caviar produced from farmed sturgeon (*A. baerii*) compared to other caviar

Different qualities and origins of caviar were presented to the panellists (Table 35.4). Four sessions were proposed to characterise the different kinds of caviar. After a session in which products A, B and C were evaluated, these samples were presented with each of the other samples. The detail of the results and the main conclusions can be found in Cardinal et al. (2002). Figure 35.1 gives a synthetic view of the results through the simultaneous representation of products and descriptors on the two first dimensions of standardised principal component analysis (PCA). The first plane of PCA accounted for 80% of the total information and allows to show the main properties of the different samples. The first axis (51.4% of inertia) separates *A. baerii* caviar from *A. gueldenstaedtii* caviar. Caviar produced from farmed *A. baerii* (B et C) was mainly considered to be buttery and yeasty, with a sweet and earthy taste compared to the caviar of oscietre—*A. gueldenstaedtii*—(A) which had a bronze colour, a translucent appearance, an odour and flavour of anchovy and a salted and sour taste. Criteria of texture were mostly involved in the creation of the second component (26.5% of the inertia). Sample C, with a melting, sticky and low crunchy texture, was similar to sevruga—*A. stellatus*—(D, E, F) and rather unlike oscietre (A), whereas the caviar produced with fertilised ovulated eggs

Table 35.4 Characteristics of caviar samples used in sensory profiling

Product	Origin
Product A	Caviar of *A. gueldenstaedtii* of retail origin (Iran), quality no.1
Product B	"Caviar" of farmed *A. baerii* from the CEMAGREF research farm, produced with fertilised ovulated eggs and salted at 4%, 45 s after fertilisation (Aquitaine, France)
Product C	Caviar of farmed *A. baerii* from the Estudor Co. (Aquitaine, France)
Product D	Caviar of *A. stellatus* of retail origin (Russia), quality no. 1
Product E[a]	Caviar of farmed *A. stellatus* from the Brates polyculture pond (Romania)
Product F[a]	Caviar of wild *A. stellatus* from the Danube River (Romania)

[a]Preparation and packaging were performed at the Brates experimental farm in Galati, but not in very strict conditions, and transport conditions were not optimal (plastic bags on ice)



Identification of caviar
A: *A. gueldenstadtii* (Iran),
B: farmed *A. baeri*, fertilized ovulated eggs (Aquitaine),
C: farmed *A. baeri* (Aquitaine),
D: *A. stellatus* (Russia),
E: farmed *A. stellatus* (Romania),
F: wild *A. stellatus* (Romania)

abbreviation used

Odour: global intensity (ogli)
anchovy (oanc)
vinegar / acid (ovin)
yeast (oyea)
butter (obut)
raw potato (opot)

Appearance: light (dark)
Homogeneity of colour (homocl)
bronze colour (bronz)
white spot (wspot)
translucent aspect (trans)
egg size (size)
filled eggs (fill)
quantity of jelly (jell)

Flavour: global intensity (fglo)
anchovy (fanc)
salted (ssal)
sour (sour)
sweet (swee)
butter (fbut)
metallic (metal)
bitter (bitt)
rancid (franc)
earthy taste (earth)
astringency (astri)
persistence (pers)

Texture: separated eggs (separ)
melting texture (tmel)
sticky texture (tsti)
crunchy texture (tcrun)
eggs sliding under the teeth (slipp)

Fig. 35.1 Synthetic view of the results through the simultaneous representation of products and descriptors on the two first dimensions of standardised principal component analysis (PCA)

(B) had a very crunchy texture and seemed to slide under the teeth. Russian sevruga—*A. stellatus*—(D) was also unlike samples B and C, mainly because of its strong anchovy and vinegar odour. The difference between anchovy taste and salted, bitter, acid and metallic tastes also accounted for the opposite impressions given by these two samples. With regard to products E (farmed *A. stellatus*) and F (wild *A. stellatus*), both showed very similar sensorial properties, with a light overall odour and a sticky texture for crushed eggs. However, owing to production and transport problems, no clear conclusions could be made about the possible differences between these two products. "Caviar" processed with ovulated and recently fertilised eggs (B) showed characteristics slightly different than true caviar made with ovarian follicles with a very crunchy texture and a slipping movement under the teeth.

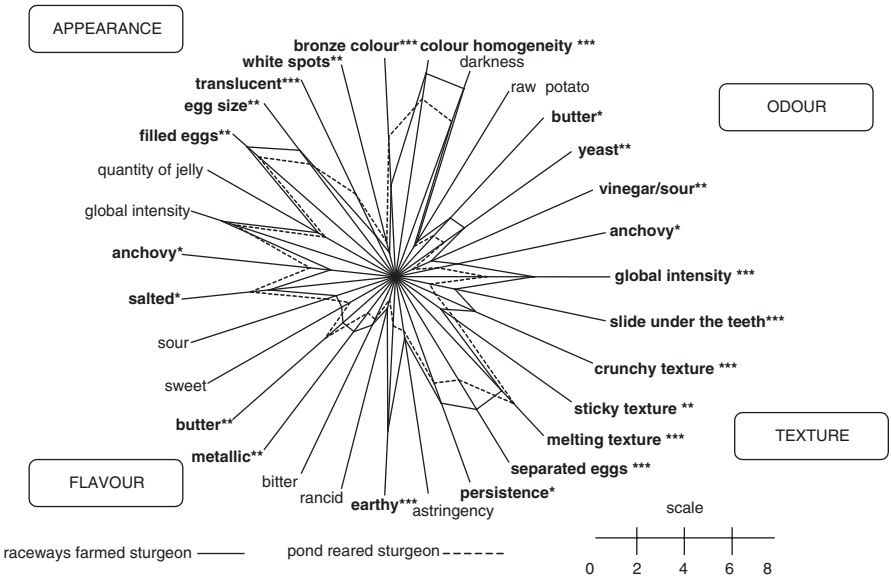


Fig. 35.2 Sensory profile of two types of caviar from *A. baerii* reared in a raceway or in a pond. Significant level: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Mean score of 18 panellists, 2 repetitions. Source: Cardinal et al. (2002)

The results obtained in this study were not conducive to making a general conclusion and explaining the sensory differences observed among the different types of caviar or distinguishing a species effect, rearing effect or processing conditions effect. A species influence was likely for the presence of some odours and flavours, and earthy values might be attributable to rearing conditions, and some specific characteristic of texture such as a high degree of crunchiness could be linked to the processing in the case of fertilised ovulated eggs.

– Sensory characteristics of caviar from *A. baerii* reared in two different conditions: in a pond and in intensive conditions in a raceway

To complete information on sensory characteristics of caviars from *A. baerii* and focus on earth taste identified previously, a comparison was undertaken on caviar from sturgeons cultured in raceways (Estudor Co.) and caviar produced from sturgeons reared in ponds (Sturgeon Scea Co.). It was shown (Fig. 35.2) that only caviar from females in raceways had a more prominent global odour and an earthy taste, suggesting that these characteristics were not related to the pond culture system itself. Since then, investigations on different species have allowed to identify the problem and to study some procedures to eliminate the earthy taste (Schrader et al. 2005; Robin et al. 2006; Percival et al. 2008), which is strongly rejected by the consumer. A special attention is given to the overall environmental conditions in the farm and the effect of feeding on sensory characteristics of caviar. The sensory methodology described and used in this work allowing an objective characterisation

of caviar may serve as a tool to identify the most determinant quality parameters in the farm and contribute to improve the quality of farmed caviar.

This preliminary study did not allow to give a hierarchy between species effect and process parameters on sensory characteristics of caviar; nevertheless, it seemed that the sensory properties of caviar are determined in a certain extent by species factor, and probably diet can be modified according to environmental conditions and especially when muddy taints occur in sturgeon farms.

35.3 Recent Developments in Sensory Evaluation of Caviar

Since this work is aimed on sensory characteristics of caviar where the quantitative and descriptive analysis was performed (Cardinal et al. 2002), very few works focused on this topic. But recently, Baker et al. (2014) published an article on sensory properties of caviar and reviewed the last studies found in literature such as the work of Jonsdottir et al. (2004), the work of Ueda et al. (2009) on cod roe, and the work of Wang et al. (2012) on the influence of flavonoids on sturgeon caviar. In their introduction, Baker et al. (2014) noticed that in spite of these works, no standard method was reported and no reference standards were described for the illustration of the different sensory attributes. Therefore they proposed to create a lexicon for sensory evaluation of caviar. The second objective of their study was to relate consumer acceptance of caviar to these attributes. This work has been carried out with caviar from farm-raised sturgeons fed with varying diets, and the description phase also included eggs from other species such as salmon, trout, capelin or herring. Even the sturgeons species used in this study were not detailed in the paper, this last work of 2014 gives the opportunity to compare their results with the attributes selected in our previous study. In these two examples, the same methodology of descriptive and quantitative analysis was used. Table 35.5 summarises descriptors listed in the two cases. The same general description is obtained, and most of the attributes are common, but a few differences can be observed. For example, "anchovy" and "sour/vinegar" are present in the work of Cardinal et al. (2002) to describe odour or flavour but are not identified in Baker et al. (2014); some specific attributes such as "white spot on eggs," "translucent aspect," "filled egg" or "quantity of jelly" for appearance or "eggs that slide under the teeth" for texture in the mouth are certainly related to the kind of products used during the project. This difference between the two lists is likely a consequence of the range of products presented to the panellists during the step of description of sensory characteristics. In 2002, the work of Cardinal et al. was carried out with only caviar from sturgeons (*A. stellatus*, *A. gueldenstaedtii*, *A. baerii*) and with specific conditions of processing for some samples such as fertilised ovulated eggs or storage which could explain some particular characteristics such as "anchovy note" or "eggs that slide under the teeth".

The lexicon developed by Baker et al. (2014) has a number of smaller sensory criteria, 16 instead of 31 in the paper of Cardinal et al. (2002), that could be a great advantage to reduce time of sensory sessions with trained panellists. Indeed, in a

Table 35.5 List of sensory attributes selected to describe caviar in two studies using profiling methodology

Sensory attributes from the study of Cardinal et al. (2002) (20 trained panellists)	**Characteristics and Evaluation order**	**Sensory attributes from the study of Baker et al. (2014) (6 trained panellists)**
Global intensity Anchovy Sour/vinegar Yeast Butter Raw potato	***Odour***	Seafood fresh Others attributes identified by Check all that apply technique (CATA): Butter, earthy, faecal, fishy, old linseed paint, oxidised, rubbery, sea fresh, yeast/fermented
	Tactile texture (spoon and fingers)	Firmness between fingers Separated eggs (using spoon)
Eggs colour: Darkness Homogeneity of colour Bronze colour White spots on eggs Translucent aspect *General appearance of eggs:* Egg size Filled eggs *Evaluation of jelly*: Quantity of jelly	***Appearance***	*Eggs colour*: Black Green Mustard Marbled Ringed *General appearance of eggs:* Egg size
Overall perception: Separated eggs Melting texture (melting of eggs under the tongue) Sticky texture *First bite of eggs*: Crunchy texture Eggs seem to slide under the teeth	***Texture in mouth***	Firmness Dissolvability (how quickly it dissolves once in the mouth)
Global intensity Anchovy Salty Sour Sweet Butter Metallic Bitter Rancid Earthy taste Astringency Persistence	***Flavour***	Salty Sea fresh Fresh butter Oxidised Earthy Yeasty Bitterness *Persistence and aftertaste (CATA)*: Butter, earthy, faecal, fishy, metallic, old linseed paint, oxidised, rubbery, salty, sea fresh, yeast/fermented
No reference standard		Reference standards presented

perspective of definition of quality classes, the possibility given to assessors to use the "check all that apply" method can be sufficient to detect off odours or off flavours in products instead of scoring all the characteristics. Moreover, the reference standards presented allow a more accurate scoring for the panellists. However, for some characteristics, it is often difficult to present the same reference standards from one laboratory to another, and some adaptations are often necessary. The panel training phase remains a key step in this kind of evaluation all the more that the number of panellists is reduced. A minimum number of ten panellists are generally recommended for this kind of evaluation. If the range of products chosen is large enough in the first steps of description, good results of discrimination should be obtained on samples collected from the market, for example.

These studies confirm the interest of such a grid to describe and discriminate different samples of caviar and could aid professionals of the caviar industry to conduct sensory evaluations. Moreover as noticed in introduction, the description of sensory characteristics of caviar using the profiling techniques is a useful tool to give a better insight on what the consumer likes. Indeed, the reasons of consumer preferences can be found through sensory characteristics previously described with a panel.

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The Off-Flavors Management in the Production of Farmed Sturgeon

36

Emmanuel Bonpunt

Abstract

The rearing of sturgeon from its beginnings faces the problem of earthy taste in caviar and flesh. After a short presentation of the origins of this problem, a certain number of solutions possible in farming are presented, like depuration stocking in pure water or in recirculated systems and caviar selection before sales. Nevertheless, a global thought against this problem is now indispensable for the survival of each sturgeon freshwater fish farm.

Keywords

Sturgeon fish farming • Off-flavors • Depuration • Sensorial asting • Caviar

Introduction

Off-flavor taste problems are numerous in aquaculture, mainly in freshwater.

For centuries, the practice of having earth pond fishes spend several days in clear water basin to decrease the mud taste before slaughter is known. In the north of the Caspian, in the 1980s, beluga (*Huso huso*) and ossetra (*Acipenser gueldenstaedtii*) fisheries in the fall were avoided because of the earthy taste associated with wastewater flowing into the Volga in this period (Gödecken 1986). Sturgeons caught in shallow and stagnant water, with a strong presence of algae, generally tended to give caviar with the same characteristics. Given that caviar from aquaculture production develops in a global current context of strong competition, most producers were forced to put in place arrangements to limit or eliminate these unpleasant tastes. The aims of the present chapter are to briefly update the knowledge on (1) the origin of

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the unpleasant tasting, (2) how this happens, and (3) how to eliminate this inconvenience with a special emphasis on recirculated aquaculture systems (RAS).

36.1 The Origin of the Off-Flavor

It is related to the presence of bacteria, *Actinobacteria* of the genus *Streptomyces* and cyanobacteria, and a wide variety of fungi and molds that produce molecules yielding an unpleasant taste or odor (Berni and Billard 2002) at very low concentrations, of the thousandth of a milligram per liter. The well-known responsible molecules in aquaculture are geosmin and methylisoborneol (MIB), also responsible for the earthy-musty taste in drinking water or wine. This is because soil leaching will allow compounds produced by bacteria and soil fungus to be in the waters of river; coloring of the waters can become an indicator. And this leaching may also have an impact on the most superficial aquifers, from which we can draw clear but contaminated water. Additionally, the heat allows the development of populations of cyanobacteria in the water, even in a very clear lake or river, which will produce these compounds. Finally the bottomof ponds or pools with low water renewal promoting settling, rich in mineral or organic solids, or bottom of pools with clear waters, but with irregularities where uneaten food or feces can be deposited, is also an area for production of these compounds. Indeed, Guttman and van Rijn (2008) showed that particulate organic matter in the presence of abundant oxygen is very favorable to this production environment. Closed recirculated systems in freshwater are prone to provide off-flavor fish flesh, whatever the species reared. Most likely, this is because it has been shown recently that a part of every bacteria population is able to produce the aforementioned molecules responsible for the earthy taste. The mechanical and biological filters are important production areas of these compounds. The quality of a food inducing more or less fragmented feces may have an impact on the amount of geosmin present (Comeau et al. 2013).

36.2 Effect of Off-Flavor

After some hours spent in water containing tens of nanograms per liter of these components, fish absorb through their gills, so their muscles and their eggs are in charge of several hundred ng kg^{-1}, the sensory detection threshold being 200 ng mL^{-1} for the flesh. These molecules are very lipophilic. Tastes obtained in caviar are very varied, (earth, mud, grass, earthy, musty, bitter (green walnuts), cellar, etc.). If within wines 20 of various origins and perfectly identified molecules are clearly responsible for varied tastes parasitic characteristics, such a study has been conducted in caviar or farmed trout flesh (Robin et al. 2006) and concluded that the two aforementioned components are responsible for the earthy-musty taste.

Attempts to explain the transfer of these molecules in the fish are as follows according to A. Lautraite (com pers): geosmin and methylisoborneol (MIB) are quite lipophilic (their index lipophilia defined as log (octanol/water) and

abbreviated as log K_{ow} is 3.70 and 3.1, respectively) according to Boardman and Flick (2013), and their solubility in water is thus low (150–194 mg mL^{-1}, respectively). So they easily pass through cell membranes; it is a passive transfer without enzymatic reaction. If the concentration in water is higher than in the blood of fish, the compounds will be absorbed through the gills or the digestive tract, travel in the blood, and set in the fat of myotomes or into the oocytes. Concentration in muscle and fat and in the oocytes will thus be much greater than in water or blood from the fish. This accumulation process will be fast (a few minutes or hours). If you put the fish in the water where the concentration of geosmin and MIB is very low or zero, these compounds will tend to passively diffuse, but very slowly (several days or weeks), from muscle lipids or oocytes to fish blood and then passively diffuse through the gills (or the digestive tract) into the water column.

Preliminary results from E. Schram suggest that Nile tilapia bio-concentrates more geosmin in its ovaries than in its fillets and that the elimination of geosmin from the ovaries is slower than from the fillets. Higher initial geosmin levels combined with a slower elimination increase the time required for geosmin elimination in the depuration phase. This finding is especially relevant for the practice of off-flavor depuration when not only the fillet but also the ovary is of commercial interest, e.g., aquaculture of sturgeons. Off-flavor depuration time should be based on the elimination of geosmin and probably 2-MIB from the ovary rather than the fillet. Using the sensory quality of fillets as indicator for the sensory quality of the ovary may result in the harvest of still off-flavored caviar.

So, the rinsing applied to fish flesh may not be sufficient for the production of caviar.

36.3 How to Get Rid of Off-Flavor?

There are varied ways to both eliminate the off-flavors and earthy tastes and assess the results prior to any marketing decision.

36.3.1 The Rinsing

36.3.1.1 Open Circuit

The best solution is stored in clear water in open circuit, if one has sufficient resource of drilling water (Fig. 36.1), river, or lake bottom devoid of any contamination. The waters of shallow groundwater, river, or closed circuit may be infected without warning, after years without problems. The duration of the depuration is a function of the load in the off-flavor of the fish, the concentration of off-flavor in the rinsing water to be reabsorbed then released by the fish and slow down the process, so the pure water renewal. Also the temperature intervenes directly in affecting metabolism under the rule of Q10. The general rule is at least 2 weeks for lightly loaded fish and up to 6 weeks for heavily loaded fish, at a temperature of 17 °C. There is a wide

Fig. 36.1 Depuration tank in sturgeon farm supplied with well water inside a concrete basin supplied with colored river water (Esturgeonnière Farm, France) (Photo: Bonpunt)

variability in sturgeon for the concentration of molecules in the oocytes and for the purification speed. Furthermore, caviar batches are often obtained from several females, with only one fish contaminating a batch or more without the dilution effect taking place. We assume that concentrations of geosmin in unpleasant caviar may not be detectable in the flesh of the same fish.

36.3.1.2 Recirculated Circuit

With the objective of eliminating the unpleasant earthy taste, two routes have been tested or used successfully. The first one is to avoid the presence of the producers of these molecules in circuit, avoiding the presence of rotating filters, biological filters, fluidized beds (Fig. 36.2), and areas rich in organic matter, so a peculiar circuit devoted to the depuration has to be installed.

Even if fish are not fed during this period, the off-flavor compounds released by the fish purifications will come and infect the others. The second route is to eliminate these molecules from the circuit regularly. Among the existing treatments, the treatment with peroxide could be effective enough to ensure sustainable levels in the fish (Davidson et al. 2014). However, a similar weekly treatment applied on sturgeon females has caused oogenesis disturbances or atresia which then has to be currently avoided. An alternative treatment is using the ozonation. The ozone

Fig. 36.2 Water treatment devices in sturgeon farm (Nurseteich Farm, France). *Red arrow* shows the rotative filter (mechanical filter) (Photo: Mauduit)

treatment, being effective only with lethal concentrations to the fish, may be done very carefully. The renewal of water purification basin must be totally devoid of off-flavor components. All the outflow of the basin must pass through an ozone generator and then directed to a very large buffer tank for a sufficient period in order to lower both the levels of residual ozone and oxidant molecules acceptable for fish. The required volume of this buffer basin will likely be very large, between two and four times that of the depuration basin. In order to avoid any risk of poisoning fish by these oxidant molecules of which the level will change with the time, it is needed to follow continuously the redox potential at the inflow of the rearing tanks in order to adapt the ozone generator production. Instead of using peroxide or ozone, activated carbon or zeolite could be used as trapping component in filters (Boardman and Flick 2013). Additionally, the search for a bacterial reactor able to consume these compounds might be envisaged as illustrated by Hebrew University of Jerusalem.

36.3.2 Sorting Before Slaughter

It is to taste the oocytes[1] obtained by biopsy at the time of the selection of females ready to produce caviar.

36.3.3 Selection After Salting

It should be noted that caviar production batches from the same batch of fish will have different off-flavor intensities. Some lots with low intensity can see disappear in three weeks the taste of earth and be sold after a tasting of verification. Others

[1] In this chapter, the expression of oocytes corresponds to the biological wording of ovarian follicles (editor's note).

will have to wait several months for storage and regular verification. Still others will have to be permanently decommissioned.

It is worthy to note that the reliable detection of off-flavor requires several people of the production laboratory are entrained for tasting the caviar, tasting facility with grids and blind tests confirming the choice of candidates. This can easily be obtained by taking advantage of the sensorial method described by Cardinal (Chap. 35). Given the availability of a more and more important number of actors on the caviar market with different origins (species, rearing conditions, etc.), the absence of interference taste becomes an essential condition of marketing.

Conclusion

Although the effect of the scarcity of luxurious caviar hinders the objectivity of neophyte tasters, professional buyers know how to detect caviar defects, which give essential control to breeders of this problem parasite taste. However, it does not seem desirable to achieve a uniform product that is bland and tasteless, and farmers may be able to 1 day promote their circuit, rinsing the presence of bacteria and producing a pleasant aroma.

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37

Artificial Production of Siberian Sturgeon Fingerlings for Restocking the Siberian Rivers of the Ob'-Irtysh Basin: A Synthesis

Marina Korentovich and Alexander Litvinenko

Abstract

Optimization of works on the artificial reproduction of Siberian sturgeon *(Acipenser baerii* Brandt) by improving technological and organizational methods will be able to increase significantly the efficiency of sturgeon farm reproduction, serve a prerequisite for preserving the genetic diversity, and restore its stocks in natural reservoirs of Western Siberia.

The results of researches on the rearing of Siberian sturgeon larvae and juveniles with use of live feeds (*Artemia* nauplii) enriched by highly unsaturated fatty acids (DHA, EPA, linen seed oil, sunflower seed oil, etc.), probiotics, and vitamins are discussed in the article. The data on the biochemical composition of highly essential fatty acids in enriched live feeds are given. A comparative analysis of the rate of sturgeon linear-weight growth and survival at different rations of feeding for larvae and juveniles is presented.

Due to rapid transferring of Siberian sturgeon fry with the help of nonself-propelled vessel from Abalak Sturgeon Hatchery Farm (ASHF) which is situated in the Irtysh River, 200,000 fingerlings with average weight 3.9 g in 2007 were released in nursery areas—the mouth and inlet of the Ob' River with rich natural food supply. In all period (24 days) of sturgeon fry transportation in distance 1700 km, juveniles were not fed. Starvation of fry in the period of transportation had a significant impact on the value of hematological parameters. The color index and viscosity of the blood cells decreased. The rate of erythrocyte sedimentation increased 2.3 times on average (from 4.3 ± 1.1 mm/HR to 8.4 ± 2.3). Protein content after 24 days of starvation decreased 1.24 times (from 10.58 to 8.51%), and fat content decreased from 0.31 to 0.25%. The moisture content of

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fingerlings increased from 87.56% on the first day of fasting to 88.4% on the 24th day. Ichthyopathological examination of transported sturgeons showed the absence of parasites: protozoa of genera *Trichodina* and *Diplostomum*.

Keywords

Sturgeon • Fingerlings • Artificial reproduction • *Artemia* • Enrichment • HUFA • Rearing • Starvation • Transportation • Hematological and biochemical indices

Introduction

Reservoirs in Western Siberia, mainly rivers of Ob'-Irtysh basin (Fig. 37.1), have been known with their fish resources for a long time. As for sturgeon fish, it is primarily related to the Ob' population of Siberian sturgeon (Fig. 37.2). The whole life cycle of the sturgeon takes place entirely in the territory of the Russian Federation. In the Ob'-Irtysh basin, conditions for fish feeding historically are the most favorable due to the presence of rich feed estuaries—the Ob' and Taz. Another hydrological feature of the basin is the lack of conditions for sturgeon breeding in downstream. So Ob' sturgeon population is characterized by high rate of linear-weight growth (in comparison with sturgeon population from the River Lena) and long-distance spawning migration—more than 2000 km.

The maximum catches of sturgeon last century reached 1,400 tons (1935) with the average value of the annual catch of 600–700 tons or 5% of the total catch in the country (Mamontov et al. 2000). The sharp decline in commercial stocks in the Ob'-Irtysh basin began in the 1980s of the twentieth century (Fig. 37.3). Primarily, it was

Fig. 37.1 Ob'-Irtysh Basin in the Map of Russia. The Federal State Unitary Enterprise "Gosrybcenter" (Tyumen) (*circle*); sturgeon experimental-Hatchery plot (*triangle*); Abalak Sturgeon Hatchery Farm (*star*)

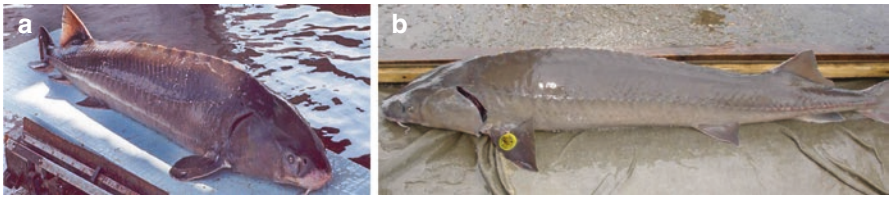

Fig. 37.2 Female (**a**) and male (**b**) of Siberian sturgeon from the Ob' river population; Abalak Sturgeon Hatchery Farm

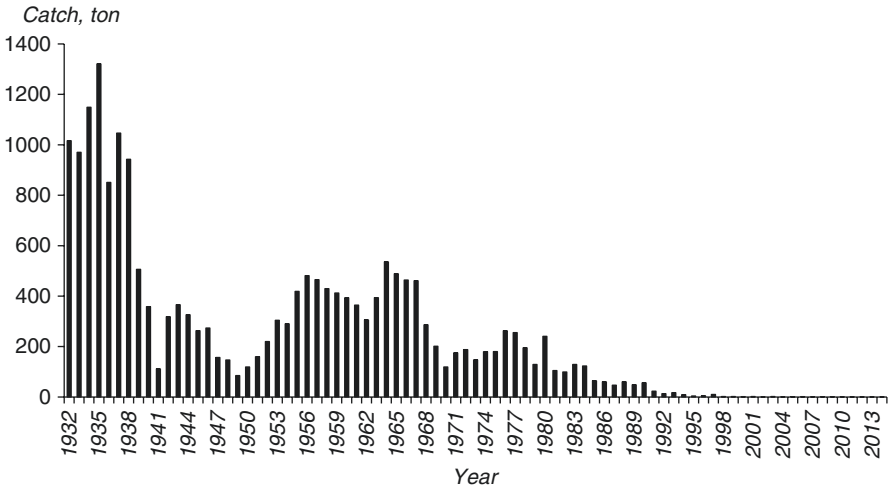

Fig. 37.3 Dynamics of sturgeon catching (ton) in the Ob'-Irtysh basin in the period from 1935 to 2013

due to the construction of Novosibirsk hydroelectric power station in the Ob' River in 1956 and construction of Shulbinskaya and Ust-Kamenogorskaya HES in the Irtysh River. After that Ob'-Irtysh sturgeon population lost about 40% of spawning area (Kasyanov 1975), and 20 years ago (since 1995) new sturgeon generations had only a few individuals. Construction and operation of West Siberian oil and gas industry especially in the Middle Ob' River basin and in some parts of the Gulf of Ob', where oil content is ten times higher than maximum allowable concentrations, also led to a reduction of sturgeon stocks and their food supply and worsen conditions of reproduction. By the mid-1990s, difficult socioeconomic situation in the country led to a sharp increase of illicit catch of spawners on migration directions as well as in the Gulf of Ob'. Threat of extinction of Ob' sturgeon population originated. In 1998 a decision to include this population in the Red Data Book of the Russian Federation was made.

If current trends are reducing the inventory in 10–15 years, the Ob' sturgeon population may disappear entirely from the commercial catches. The plight of the sturgeon in the Ob'-Irtysh basin will certainly require a significant increase in the efficiency of work on the artificial reproduction; rearing of fingerlings, especially during the early post-embryogenesis; transportation of fry; preparation for restocking; and so on.

37.1 Sturgeon Nutrition in Larval Period

It is known that for sturgeon fish during their larval and post-larval periods, there is an expressed staging of their development. Growth and formation of larvae are followed by development of gastrointestinal tract. Larval stages in the development of sturgeon are the most vulnerable in terms of adverse factors of water environment, because the formation of the digestive system of these fish has continued for 30–60 days (Shmalgauzen 1975). The low survival rate for some species and populations of *Acipenser* family (Siberian sterlet of the Irtysh River population, Siberian sturgeon of the Ob' River and Baikal Lake populations, Amur sturgeon *Acipenser schrenckii*) in this period is related primarily to the lack of specialized artificial feed. Especially low survival of larvae is noted for individuals received from spawners which are caught from natural reservoirs, and they aren't adapted to feeding by artificial food (Rachek and Skirin 2004; Afanasyev 2006; Chepurkina 2010; etc.). Often, oligochaetes (*Enchytraeus* sp. and *Tubifex* sp.) and some species of zooplankton (*Daphnia*, *Moina*, and others) are used as food for sturgeon larvae (Buddington and Doroshov 1984; Dabrowski et al. 1985; Gershanovich et al. 1987; Gisbert and Williot 2002). However, the study of larval physiological state after feeding with the help of these nutritional organisms shows the inadmissibility of their application in monoculture (Buddington and Doroshov 1984; Gisbert and Williot 2002; Ebrahimi 2006; Ebrahimi and Zare 2006). Therefore, the researches to replace these live feeds with other ones, high grade and physiologically appropriate to the sizes of the fish, are being conducted.

Feasibility of successful use of *Artemia* nauplii as a starter live food was proved with sturgeon larvae over 50 years ago (Pleskachevskaya 1963). It was shown that the addition of *Artemia* nauplii in tanks in the amount of 20% of the body weight of Siberian sturgeon larvae for 1–2 days before the beginning of active feeding has contributed to the acceleration of the mass emissions of melanin plugs and reduces the loss of fish to 3.7% instead of permissible 20–30%. In the absence of feed in a specific time release of melanin plugs lingered in the complete formation of digestive system. The almost complete lack of damage to the blades of pectoral fins and gills led to significant decreasing of mortality of Siberian sturgeon larvae. The proposed method of transfer larvae to the consumption of external food contributed to obtaining viable individuals with their high survival (Krasnodembskaya 1999).

Despite all *Artemia* advantages, it is not a kind of food which can give optimal amount of nutrients for excellent larval condition. Especially the content of highly unsaturated fatty acids (HUFA): eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are necessary in the process of larvae growth but at the same time they cannot be synthesized, is not enough in shrimps. Several techniques of increasing the nutritional value of *Artemia* metanauplius with the use of method of HUFA enrichment were developed (Sorgeloos and Leger 1986). Sea algae, microencapsulated and compound feed, ώ-acids, and emulsions are used to achieve this purpose. The nutrient mixtures for the enrichment of brine shrimp nauplii (*Artemia franciscana*)—Super Selco, Selco-DHA, Super Artemia, Super HUFA,

etc.—were developed by specialists of the company INVE (Belgium) and American scientists (Lavens and Sorgeloos 1996).

It should be noted that there are some works devoted to feeding larvae of different sturgeon species with live feeds enriched by emulsion Selco and other treatments (vitamin C, probiotics, etc.) (Hafezieh et al. 2010; Chebanov et al. 2011; etc.). Supervisions of the Iranian experts have shown a decline in mortality (5–12% less) for beluga (*Huso huso*), Persian (*Acipenser persicus*), and Siberian (*A. baerii*, the Lena River population) sturgeon larvae after feeding of *Artemia* nauplii (*Artemia urmiana*), enriched with treatment DHA, EPA, and vitamin C (20%) (Nouri et al. 2005; Fashtomi and Mohseni 2006) and also HUFA with poly-β-hydroxybutyrate (PHB) (Najdegerami et al. 2013; etc.) or suspensions of yeast (Jafaryan and Aghilinejad 2013), respectively, in comparison with feeding by a non-enriched *Artemia*. Twenty-day feeding of Persian sturgeon larvae with the help of shrimps *A. urmiana* encapsulated by linseed oil (DHA, 0.001 mg/g; EPA, 0.03 mg/g of dry weight) has revealed significant weight growth of fish in comparison with larvae after feeding with nauplii enriched by cod liver oil (DHA, 7.64 mg/g; EPA, 11.39 mg/g). Use of the enriched solution of ovarian liquid of sturgeon females (DHA, 3.13%; EPA, 12.10%) was characterized by higher survival of Persian sturgeon larvae (age—20 days) till 93.3 ± 1.6% in comparison with the individuals who are grown up with use of feed bioencapsulated with cod liver oil (91.7 ± 0.8%) and linseed oil (87.2 ± 0.2%) (Hafezieh et al. 2010).

In another hand, there are no enough researches which are devoted to use *Artemia* as a live food for Siberian sturgeon of the Ob'-Irtysh population enriched with different vegetable oils, probiotics ("NARINE-FORTE"), and complex of vitamins ("TRIOVIT"). Some refined vegetable oils, linen, sunflower, cedar, sesame, oil of grape seed, germs of wheat, thistle, and others, are considered as the best natural sources of HUFA (Ipatova et al. 2009). All of these oils are rich of the most important for sturgeon larvae HUFA—oleic (*n*9), linoleic (*n*3), and linolenic (*n*6) acids. Linoleic and linolenic types of acids are indispensable. They are not synthesized in the fish body, and they must be obtained together with food in accordance with the need of individuals. These fatty acids are converted to other highly unsaturated acids by elongation and denaturation. Analysis of the content of HUFA in dry *Artemia* nauplii from the lakes of the Altai region showed a fairly high amount of linolenic acid (31.37%) and relatively low (4.1%) concentration of eicosapentaenoic acid (Solovov and Studenikina 1990).

37.2 Transportation, Preparation, and Selection of Sites for Releasing the Fingerlings

Besides creating modern intensive technology for rearing of sturgeon larvae and fry based on the method of preparation of enriched live feeds, the increase in the number of juveniles which are released into natural water reservoirs is possible due to accelerated transportation of fry to habitats with more favorable conditions for fattening with the help of self-propelled vessels.

Studies conducted on the Caspian Sea in the late 1960s of the last century proved the efficiency of short-distance transportation of fingerlings from hatcheries to feeding areas (Mikhailova et al. 2004). At the same time, it was unknown how long multiday transportation would affect the physiological condition of juveniles.

The idea of rapid transferring of Siberian sturgeon fry in nursery areas with rich natural food supply (mouth and inlet of the Ob' River) appeared repeatedly. Based on many years of researches (Stepanova 2008), it was found that the region of delta and the southern part of inlet of the Ob River are the most rich in zoobenthos organisms (up to 74–200 g/m^2). Furthermore, the chemical composition of water in the inlet of the river remains invariable within the last decades. It belongs to low-mineralized water of hydrocarbonate class of calcium group in the salt composition. The water is very soft with neutral reaction of environment (Knyazeva 2008). Observations of Siberian scientists (Votinov and Kasyanov 1978) conducted in 1976 have shown that sturgeon juveniles migrated into the Gulf of Ob' River mainly during the first 2 years after their hatching; some of them stayed in the river for a longer time. Some individuals become mature without feeding migration to the inlet, and they are representatives of local resident fish stock. The main aged group of fish in downstream migration was fingerlings (85–90%); 2-year-old fish were 5–10%, and 3–5-year-old fish were less than 5%. The length of fish body (L) fluctuated from 5 to 46 cm and weight from 5 to 715 g (Votinov and Kasyanov 1978).

In 2005 the scientists of State Scientific-and-Production Centre for Fisheries ("Gosrybcenter") made a decision about the design of nonself-propelled experimental fish vessel (Fig. 37.4) for the purpose of transportation of Siberian sturgeon juveniles reared in tanks and ponds, to distance 1800 km from the hatchery sturgeon farm to the mouth of the Ob River.

Fig. 37.4 Nonself-propelled experimental fish vessel for fry transportation (created in Gosrybcenter 2007)

It is known that a detailed study of ecology and physiology of sturgeon juveniles showed high adaptive plasticity of individuals during prolonged fasting (Gershanovich et al. 1987). The maximum duration of starvation in terms of temperature fluctuations from 13 to 18 °C in experiments with Russian sturgeon larvae (at average weight 36 mg) from the river Volga accounted to 32 days after hatching; for sturgeon larvae from Baikal Lake (average weight 26 mg), it was 24 days; for sturgeon from the Lena River (average weight 24 mg), it was 17 days (Bogdanova 1969). Differences in the resistance to larval starvation of different *Acipenser* species Bogdanova L. are associated with different amounts of fat reserves. It was known that the maximum period of reversible hunger for pond Siberian sturgeon juveniles (the Ob' River population) with biomass 3 g at water temperature 20 °C consisted of 11 days (Khakimullin 1984).

Our previous researches conducted with sterlet fry in 2006 (Chepurkina, et al. 2009; Chepurkina 2010) expanded the idea of the perfection of sturgeon fish physiological adaptation in early stages of ontogenesis. Sterlet fingerlings reared in ponds of Abalak Sturgeon Hatchery Farm during 1.5 months with the help of natural food supply have been replaced in lab pools of Gosrybcenter in quantity 1000 individuals to check their ability to long starvation. It has been found out that pond sterlet fingerlings (aged 55 days, average weight 3.62 ± 0.49 g, total length 10.8 ± 1.44 cm) were able to endure saturation during 60 days at water temperature 14–15 °C with the minimum mortality (less 1.0%). The maximum period of reversible fasting for yearlings reached 103 days; the mortality reached 18.6%; the fingerlings lost on average 31.2% of their weight. The difference between reversible and irreversible starvation was 1–3 days. After fasting the fish for 3 months fingerlings began to eat live food (larvae of Chironomidae family) in 3–5 hours after the start of feeding. Restoration of body weight to initial values occurred within 30 days (*SGR* = 0.012) (Chepurkina 2010).

37.3 Biochemical Changes in Sterlet Fingering Body after Starvation

In the period of prolonged starvation (100 days) under laboratory conditions, moisture content in sterlet fingerling increased from 78.5% to 88.7% of body weight. The amount of fat decreased significantly—3.3 to 7.5 times (from 1.9% to 0.3–0.5%). The protein content was reduced 2.5 times—at the first day of experiment, it was 14.9%, and at the 100th day, no more than 6%. The caloric content of fingerlings decreased 2.1 times—from 892 Cal/g to 418 Cal/g (Chepurkina 2010).

So, during preliminary experimental works carried out in vitro with sterlet fingerling, it has been found out that feeding of pond sturgeon fry with artificial food during long-term transportation (till 60 days) with average weight of fry 3 g at average water temperature 15 °C is inexpedient.

37.4 Materials and Methods

Researches were conducted in 2002–2015 in the Laboratory and Sturgeon Experimental-Hatchery Plot of the Federal State Budgetary Scientific Institution "Gosrybcenter" and also in Abalak Sturgeon Hatchery Farm (ASEHF) (Fig. 37.5). The full chemical analysis of river and geothermal water from wells was carried out in the Hydrochemical Laboratory of "Gosrybcenter" according to standard techniques (Alekin et al. 1973).

37.4.1 Sturgeon Larvae Rearing with the Help of Enriched Artemia Nauplii

In ASHF, Siberian sturgeon larvae were reared in tanks (5.0 × 0.75 × 0.5 m) with running water (the Irtysh River) (Fig. 37.6). In Sturgeon Experimental-Hatchery Plot (with running water from the Balda River), the square tanks (2.1 × 2.1 × 0.6 m) were used for rearing fish (Fig. 37.7). In the period of researches, the quantity of larvae has made more than 20,000 pieces. The initial weight of larvae was 22.0 ± 0.1 mg (mean ± sd). The period of rearing with the help of live food was 20–25 days.

Water temperature during larvae rearing was in average 17.0 °C (fluctuations from 16.4 till 18.6 °C); content of oxygen dissolved in water didn't fall lower than 8 mg/L (8.1–9.8 mg/L). The efficiency of dissolved oxygen was 80–95%. If there was a need, running river water was passed previously through water-cooling installation "Angara" series EBSV (made in Russia). Control of water temperature,

Fig. 37.5 The view of the Irtysh River and artificial ponds for rearing of sturgeon fry in Abalak Sturgeon Hatchery Farm (ASHF)

Fig. 37.6 The tanks for sturgeon larvae rearing at Abalak Sturgeon Hatchery Farm

Fig. 37.7 The tanks for sturgeon larvae rearing at Sturgeon Experimental Plot of the Federal State Budgetary Scientific Institution "Gosrybcenter"

oxygen regime, and saturation (%) was carried out with use of handheld meter for the measurement HACH HQ40d field case (made in the USA).

Artemia nauplii of Siberian populations (*Artemia parthenogenetica*, Barigozzi 1974; Bowen and Sterling 1978) were used as live food for sturgeon larvae. The moisture content of dry cysts did not exceed 6–8%; mass fraction of shell was less than 2%; mass fraction of impurities was less than 0.01%. Daily product was 14.6 g/L; the ratio of dry weight of cysts to wet weight of crustaceans was 1:2.5.

Incubation of *Artemia* cysts was carried out during 24 h with constant light (2000 Lux) and aeration (in a bottom of apparatus) according to standard methods (Sorgeloos et al. 1986; Litvinenko et al. 2000). As incubation equipment, we used 150 liter apparatus made of plexiglass or stainless steel (Fig. 37.8) with working volume of the apparatus 100 L (all equipment was made in Tobolsk Experimental-Mechanical Plot (design of "Gosrybcenter"). Stainless steel apparatus for *Artemia* cysts incubation consisted of three conical flasks placed in one container. In the

Fig. 37.8 Department for incubation of *Artemia* cysts in Abalak Sturgeon Hatchery Farm (ASHF) (in the background of the picture there is *Artemia* incubation system consisting of three stainless steel flasks placed in one container)

container, there was heated water with stable temperature 29–30 °C. A solution for *Artemia* cysts incubation was placed in every flask. After incubation hatched nauplii were carried out through the rubber tubes located at the bottom of the flask. The temperature of cyst incubation was 26–28 °C; the content of non-iodized salt (NaCl) was 20 g/L. To increase the buffering capacity of the medium, baking soda ($NaHCO_3$) was used for the incubation of *Artemia* cysts in an amount of 2 g/L. The loading density of *Artemia* cysts was 10–12 g/L.

The salinity of the incubation solution was determined by refractometer Reef Octopus WY-100 (made in China). The measurements of acidity (pH) have been made with a handheld meter HQ40d18 (Hach Company).

Artemia enrichment has been executed in the following ways. The culture of crustaceans of the first metanauplial stage was added to saline solution (NaCl 20 g/L). Before that incubation was conducted at temperature 26–28 °C for 24 h; the density of nauplii in apparatus was 200–300 ind./mL. Then an emulsion of fatty acids—Selco (produced by the firm INVE) in two versions (Experiment 1, Selco-DHA (high content of docosahexaenoic acid) (Fig. 37.9a), and Experiment 2, Selco-Experimental (the ratio of DHA to eicosapentaenoic acid (EPA), 2:1))—was added to solution with 24-h nauplii (nauplii of the first larval stage). The concentration of emulsion was 0.3 g/L.

Besides some vegetable oils (sesame, cedar, grape seed, germs of wheat, thistle, sunflower, and linseed oil), probiotic "NARINE-FORTE" (made in Russia, Krasnogorsk, company "Ferment") (Fig. 37.9b) and (/or) a solution of vitamins (A, D_3, E) "TRIOVIT" (KRKA d.d., made in Slovenia) were used as enriched

Fig. 37.9 Treatment for *Artemia* nauplii enrichment: (**a**) Selco-DHA (INVE); (**b**) Probiotic "NARINE-FORTE" and Vegetable Linseed Oil (both of them are made in Russia)

treatment. The main signs of the selection of oils were high content of HUFA and survival of crustaceans in solution of fatty acids during enrichment and after that.

After 6 h, re-enrichment was spent. Through 6 h after the second treatment *Artemia* were wash out carefully in fresh water. Then enriched nauplii were stored in saline solution (5 g/L) at temperature 13–15 °C and constant aeration of water (the saving period was less 24 h). Living enriched shrimps were filtered through a sieve and made into tanks with fish larvae every hour for 19–25 days. The Control groups of sturgeon were fed with live non-enriched nauplii. Each experiment was carried out in four replications.

The biochemical analysis of HUFA content in *Artemia* cysts, non-enriched nauplii, and shrimps enriched by fatty acids was carried out on frozen material in the laboratory of biochemical researches of Artemia Reference Center (Ghent, Belgium). Weight of one sample was 50–100 mg (dry cysts) and 200–500 mg (crude weight of nauplii) (three frequencies). For determination of HUFA content, the gas chromatograph Chrompack CP 9001 was used (Coutteau and Sorgeloos 1995; Lepage and Roy 1986). Processing of statistical data was carried out by means of computer program ANOVA of the 16th version (SPSS Inc., IL. USA).

After *Artemia* enrichment by different vegetable oils feeding sturgeon larvae was conducted only with *Artemia* enriched by linseed oil and complex (linseed oil + "TRIOVIT" + "NARINE-FORTE"). For feeding sturgeon larvae in Control groups, artificial starter feed Aller Futura (made in Denmark) and $BM_S55/13$ (production of Germany) were used.

To estimate maximum theoretical growth of sturgeon larvae, the following equation was used (Gershanovich et al. 1987):

$$W_t = \left[N\left(1 - a/b\right)\left(t - t_0\right) + W_0^{1-a/b} \right]^{b/b-a}$$

where N is a constant.
a/b is the exponent of weight in the equation of dependence of energy metabolism from the body weight.
$t - t_o$ is the period of time, days.
W_o is the initial body weight (mg or g).

To estimate the rate of sturgeon larvae growth, daily rate of feeding, feeding coefficient, and specific growth rate (SGR) were used. Currently specific growth rate was used to compare growth: $SGR\ (\%day^{-1}) = 100\ (\ln Wt - \ln W_o)/t$,

where W_t—initial weight.
W_o—final weight.
t—period of fish growing, days.

To assess the chemical composition of larvae, caloric content of dry matter (dry weight – DW) by the method of bichromate oxidation and percentage content of DW in fish body was determined.

Statistical processing of the obtained data was carried out with use of methods of variation statistics and computer programs Microsoft Excel and Statistika (Lakin 1980).

37.4.2 Conditions for Sturgeon Fry during Long-Term Transportation

A long-term transportation of Siberian sturgeon fingerlings with the help of nonself-propelled vessel from Abalak Sturgeon Hatchery Farm (the Irtysh River) to the mouth of the river Ob' was in August–September 2007 to the distance of 1800 km (Fig. 37.10). Catching and loading of juveniles into stationary containers with a volume of 2.5 m^3 were carried out for 3 days. Before putting into the reservoirs fish were treated with a solution of potassium permanganate, using portable cargo containers (Fig. 37.11). After that fingerlings were kept in running water in tanks which were installed near by the vessel. The average weight of fish was 3.9 ± 0.8 g. The total amount of fish was 200 thousand of sturgeon fingerling (785 kg or 52% of standard loading). The loading in three reservoirs was 100% (30 kg per container).

At all time of being fish in the containers (24 days), sturgeon juveniles were not fed. Water temperature was maintained in the range of 15–16 °C. During loading and the first days of transporting, the water temperature in the tanks ranged from 17.6 to 20.0 °C. The nearer vessel moved to the mouth of the river, and the lower

Fig. 37.10 Schematic map of the Tyumen region: Abalak Sturgeon Hatchery Farm (*downstream closed to Tyumen*). The area of sturgeon fingerlings release (settlement Salemal) (*downstream Salekhard*)

water temperature was in the containers. It decreased gradually from 20.0 to 11.7 °C.

During the voyage, the content of dissolved oxygen averaged 6.40–6.68 mg/L with minimum value 3.49–4.61 mg/L and maximum 9.14 mg/L. In the period of fish transportation cooling and aeration of water in the compartment of water treatment, automatic control of dissolved oxygen and temperature and water circulation with the use of two schemes (direct flowed and recirculated system) were provided.

To determine the migration directions of released juveniles and estimate survival and amount of population in the commercial stock of the Ob'-Irtysh basin, the part of the largest individuals with weight more than 10 g and in an amount of 9.65

Fig. 37.11 The loading container for short-term transportation of sturgeon fry (V – 300 L)

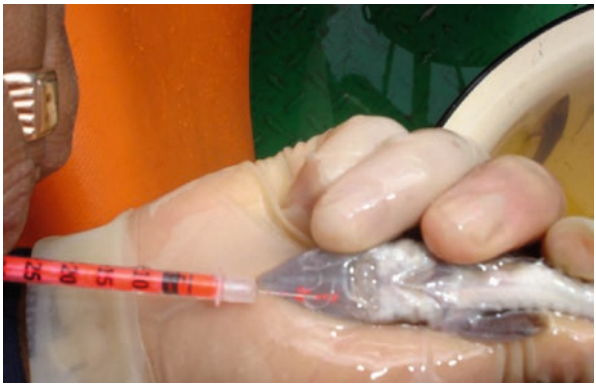

Fig. 37.12 Tagging of sturgeon fingerlings with visible implant elastomer tags (production of North West Technology, the USA)

thousand fingerlings during the voyage was marked by fluorescent tags (Fig. 37.12). Tagging was conducted in low temperature (14–16 °C) for fixing substance and removing the excess from the body. Release of sturgeon juveniles in inlet was performed for 2 days (Fig. 37.13) after draining water alternately from each container (Figs. 37.14 and 37.15).

To assess the physiological condition of Siberian sturgeon juveniles during the long transportation (24 days), blood samples were carried out on live fish (Fig. 37.16). Because it was almost impossible to make blood sampling for the hematologic analysis (in particular, on determination of speed of subsidence of erythrocytes) and to

Fig. 37.13 The region of loading sturgeon fingerlings; the mouth of the Ob' River

Fig. 37.14 The pouring out sturgeon fingerlings through the hatchway of container into the tray

estimate extent of pathological changes of erythrocytes from fish with weight 4 g or less, we used fingerlings with bigger sizes. Average weight of yearlings was 10.95 ± 1.26 g (mean ± sd) (fluctuations from 8.26 to 12.60 g); the average length was equal to 10.71 ± 1.35 cm; the amount of fish was 50 individuals.

The main diagnostic indicators of blood cells were amount of hemoglobin, erythrocytes, and leukocytes; speed of subsidence of erythrocytes (SSE); and leukocyte formula (Zhiteneva et al. 1981; Musselius 1983). Calculation of uniform elements of blood cells was carried out by the unified method (Ivanova 1983), and their detailed identification was carried out on the standard classification (Ivanova 1983; Zhiteneva et al. 1981).

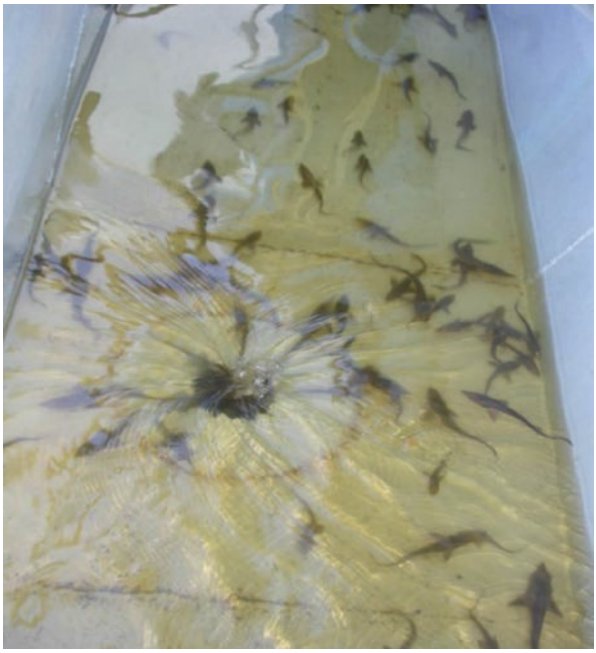

Fig. 37.15 The loading of sturgeon fingerlings through the hole of tray into the Ob' River

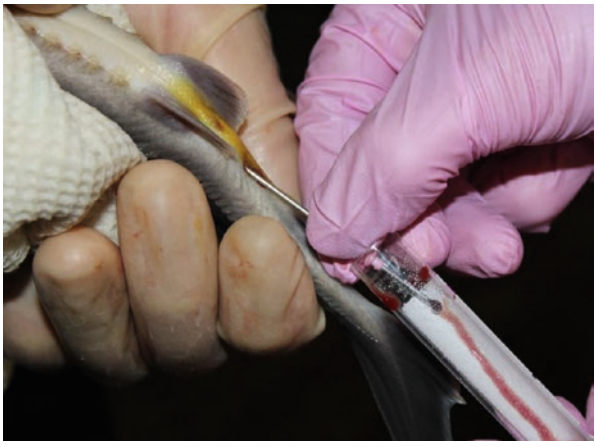

Fig. 37.16 Blood sampling at Siberian sturgeon fingerlings

Some manipulations have been done in a certain sequence:

Taking blood issue from fish caudal vein
Preparing blood dab
Loading mixing pipette for quantitative calculation of uniform elements of blood
Loading Sahli's hemoglobinometer for determination of content of hemoglobin in blood
Putting the reaction of subsidence of erythrocytes (RSE)
Stirring up a mixing pipette and using Goryaev's chamber

Determining the content of hemoglobin in blood by Sahli's method
Counting number of erythrocytes in Goryaev's chamber
Fixing and painting the taken blood dabs
Counting quantity of leukocytes and platelets by an indirect method
Defining a leukocyte's formula

The counting of blood cells was performed with the use of unified method (Ivanova 1983). The morphological characteristics of white blood cells and their detailed identification were carried out according to the conventional classification (Musselius 1983; Zhiteneva 1999).

37.5 Results

The emulsion Selco and analogs represent complexes of extracted fats of seafood and vitamins. After enrichment with the help of emulsion Selco, nauplii contain high level of essential highly unsaturated fatty acids—DHA (22:6ω3) and EPA (20:5ω3) (Sorgeloos et al. 1986). Use of the method of bioencapsulation of *Artemia* nauplii from the Great Salt Lake (*A. franciscana*) has led to an increase of the speed of weight growth of Russian sturgeon (*Acipenser gueldenstaedtii*) larvae 2.9 times in comparison with the previous method of feeding (within 3 days, *Artemia* nauplii (five times a day), then feeding with artificial starter feed Aller Aqua). Besides mortality of juvenile fish was reduced 1.3 times (the survival of 18-day-old larvae had reached 97.6%) (Chepurkina, et al. 2014). These researches were extremely important in the conditions of industrial rearing of sturgeons.

In the course of experimental works on the feeding of Siberian sturgeon larvae (embryos have been received from wild spawners of the Ob' population in conditions of ASHF) with live feeds, enriched by various preparations of Selco, it was revealed that the highest rate of the weight growth was observed when fish was fed by nauplii which had been enriched previously by Selco-DHA. During 25-daily feeding, larvae body weight reached 1312 ± 43 mg in Experiment 1, i.e., 1.62 times higher than in Control groups (810 ± 35 mg); in Experiment 2 it was 1.37 times higher in comparison with Control fish (1109 ± 46 mg) (data statistically significant at $p < 0.01$) (Table 37.1).

The average *SGR* during rearing was 0.12 in Control groups. After feeding fish nauplii enriched by Selco-Experimental the meaning of this index was 0.13, with use of emulsion Selco-DHA—0.15. Maximum *SGR* indices were registered on the 13th day of feeding for 17-day-old larvae as in Control and Experimental groups: in Control, 0.24; in Experiment 2, 0.30; and in Experiment 1, 0.33. The coefficient of variability of body weight *CV* during feeding was identical in Control and in Experiment 1, making 14.46 and 14.01%, respectively. A slight increase in the variability of this feature till 17.0% was observed in Experiment 2. The maximum range of fluctuations (28.97%) was observed on the 10th day of larvae feeding with nauplii enriched by Selco-Experimental. The survival of fry at the end of rearing regardless of the method of feed enrichment amounted to 52.0–52.5%. The highest

Table 37.1 Weight growth of sturgeon larvae after feeding with *Artemia* nauplii (*A. parthenogenetica*)

Age (days)	Sturgeon larvae feeding with the help of non-enriched Artemia nauplii (Control groups)											
	Weight of larvae (mg)				Daily gain of fish			Survival (%)	Quantity of dead fish (%/days)	Days of feeding	N fish in sample	Water T (°C)
	$X \pm m_x$	Min–max	CV (%)	σ	mg	% from fish weight	SGR					
3	22 ± 0.1	20–23	2.9	0.65	0.7	3.2	0.05	100.0	0	–	25	17.0
5	25 ± 0.2	23–27	3.8	0.96	1.5	6.0	0.06	87.6	6.2	1	25	18.2
8	31 ± 0.7	25–40	10.7	3.33	2.0	6.5	0.07	56.8	10.3	4	25	18.1
14	82 ± 3.3	45–112	20.3	16.63	8.5	10.4	0.16	55.0	0.3	10	25	18.6
17	169 ± 3.9	100–202	11.6	19.61	29.0	17.2	0.24	53.1	0.6	13	25	18.1
20	233 ± 6.9	174–321	14.9	34.69	21.3	9.1	0.11	52.4	0.2	16	25	18.0
22	296 ± 13.5	218–425	22.8	67.35	31.5	10.6	0.12	52.4	0	18	25	20.3
25	449 ± 13.9	314–610	15.5	69.39	72.0	16.0	0.14	52.0	0.2	21	25	22.0
28	763 ± 30.5	550–1150	20.0	152.40	104.7	13.7	0.18	52.0	0	24	25	22.5
29	810 ± 35.9	610–1300	22.1	179,34	47.0	5.8	0.06	52.0	0	25	25	22.4
	Sturgeon larvae feeding with the help of *Artemia* nauplii enriched by Selco-experimental (**experiment 1**)											
3	22 ± 0.1	21–23	2.3	0.5	0.7	3.2	0.05	100.0	0	–	25	17.0
5	26 ± 0.1	24–27	2.7	0.70	2.0	7.7	0.08	86.3	6.9	1	25	18.2
8	36 ± 1.4	27–60	20.1	7.22	3.3	9.2	0.11	56.9	9.8	4	25	18.1
14	84 ± 4.8	50–118	29.0	23.75	8.0	9.5	0.14	55.2	0.3	10	25	18.6
17	208 ± 5.6	163–280	13.5	28.07	41.3	19.9	0.30	53.2	0.7	13	25	18.1
20	305 ± 13.7	220–445	22.5	68.47	32,3	10.6	0.13	52.5	0.2	16	25	18.0

21	335 ± 12.4	222–450	18.6	62.15	30.0	9.0	0.09	52.5	0	17	25	20.0
25	641 ± 20.4	438–812	15.9	101.77	76.5	11.9	0.16	52.5	0	21	25	22.0
27	860 ± 42.5	619–1540	25.0	212.31	109.5	12.7	0.15	52.0	0.3	23	25	22.3
29	1109 ± 46	725–1640	20.9	232.17	124.5	11.2	0.13	52.0	0	25	25	22.4
	Sturgeon larvae feeding with the help of *Artemia* nauplii enriched by *Selco-DHA* (**experiment 2**)											
3	22 ± 0.1	21–23	1.85	0.41	0.7	3.18	0.05	100.0	0	–	25	17.0
5	27 ± 0.1	25–28	2.41	0.65	2.5	9.26	0.10	85.6	7.2	1	25	18.2
8	37 ± 1.0	29–50	13.43	4.97	3.3	8.92	0.11	57.0	9.5	4	25	18.1
14	90 ± 2.2	67–125	12.38	11.14	8.8	9.78	0.15	55.1	0.3	10	25	18.6
17	244 ± 9.2	174–410	18.75	45.74	51.3	21.03	0.33	53.6	0.5	13	25	18.1
20	371 ± 11.3	270–516	15.19	56.35	42.3	11.40	0.14	52.6	0.3	16	25	18.0
22	536 ± 19.0	410–762	17.75	95.16	55.0	10.26	0.18	52.6	0	18	25	20.3
25	757 ± 28.5	558–1070	18.80	142.29	73.7	9.74	0.12	52.6	0	21	25	22.0
27	923 ± 42.6	680–1615	23.10	213.22	83.0	8.99	0.10	52.5	0.1	23	25	22.3
29	1312 ± 43.0	1000–1745	16.4	215.15	194.5	14.83	0.18	52.5	0	25	25	22.4

intensity of deviation was observed from the first day till the eighth day of feeding (6.2–9.8%/day) with a top of intensity on the fourth day in all variants (Control, 10.3%; Experiment 1, 9.5%; Experiment 2, 9.8%). It should be noted that mortality of Siberian sturgeon larvae after feeding with live feeds is reduced dramatically to 0.2%/day when they reach the age of 14 days or more (if the temperature is 18.6 °C, it happens on the tenth day of feeding) (distinctions are significant at $p < 0.01$).

The next series of experiments were focused on the development of biotechnological processes for enrichment of *Artemia* nauplii Siberian populations with the help of emulsion Selco-DHA proven more effective to use for Siberian sturgeon larvae released from spawners of mature stock reared in aquaculture conditions (Sturgeon Experimental Plot of Gosrybcenter). In addition, during sturgeon feeding by enriched and non-enriched live feed, there was a task to undertake a comparative analysis of the following parameters: the rate of linear-weight growth of larvae, survival, energy value, and dry matter content of fish body.

During the experiments, it was established that in the period of endogenous feeding of sturgeon larvae, specific rate of weight growth is small and consists of 0.05–0.06 (Tables 37.2 and 37.3). On 5–7 days after transition to active feeding of live feeds, the speed of growth rate increased significantly till 0.24 in Control and 0.29–0.33 in Experiment groups (Table 37.3). The maximum values were observed for 16-day-old larvae – 0.38 after feeding with enriched *Artemia* nauplii and 0.34 after *Artemia* feeding. The average *SGR* ratio for the period of rearing (17 days) was 0.24 in Experiment groups and 0.22 in Control ones. Average daily gain of individual as specific growth rate of fish weight amounted to 18–18.4% of larvae body weight. Maximum values of this index were marked on the 16th day of rearing in Experiment (26.8%) and on the 17th day in Control (26.7%) (distinctions are significant at $p < 0.01$).

Variability of sturgeon larvae growth just after transition to exogenous feeding was insignificant: the coefficient of variation *CV* for the length was equal to 5.2–5.7% and for weight 13.9–14.3% (Table 37.2). Starting from the third day of rearing, the values of the coefficient of variation increased reaching a maximum for 12–14-day-old individuals. *CV* for weight in Control groups was 30.2% and in Experiment 27.1% and for length 13.1% and 9.6%, respectively. Throughout the period of larval rearing (17 days), the coefficient of variation for body length, on average, was 2.8 times less than the coefficient of variation for weight (correlations from 2.2 to 4.0). Variability of sign during rearing with the use of enriched live feed was 8.5% lower than for larvae the same generation fed by *Artemia* nauplii. The average values of *CV* in Experiment reached 8.0% for length and 21.7% for weight and in Control 9.3 and 23.5%, respectively.

Both in Control and in Experiment groups, it was indicated that the growth rate increased when there was a decline in the variability of the sign. The growth rate of sturgeon larvae increased with increasing daily rates of feeding and body weight: at the daily rate of 100% and weight more than 200 mg, the maximum average daily gains were observed (till 26.8%) (Fig. 37.17).

The dependence of body weight on the length of sturgeon larvae during feeding with non-enriched *Artemia* nauplii (Fig. 37.18a) and enriched *Artemia* (Fig. 37.18b) is extremely high in both cases: $R^2 = 0.9867$ and $R^2 = 0.9652$, respectively.

Table 37.2 Weight growth of Siberian sturgeon (mature stock was reared in aquaculture conditions) larvae after feeding with *Artemia* nauplii (*A. parthenogenetica*)

Age (days)	Sturgeon larvae feeding with the help of non-enriched Artemia nauplii (Control groups, N = 366 ind.)													
	Length of larvae (mm)				Weight of larvae (mg)				N fish in sample	Days of feeding	Survival (%)	Mortality (%/day)	N fish in tank (pieces)	Water T (°C)
	$X \pm m_x$	Min–max	CV (%)	σ	$X \pm m_x$	Min–max	CV, (%)	σ						
6	16.3 ± 0.2	14.2–18	5.66	0.81	24 ± 0.8	22–28	24.23	1.92	21	1	98.4	0.6	5311	21.3
8	17.1 ± 0.2	15–18.8	5,45	0.93	27 ± 1.1	19–43	21.26	5.74	30	3	95.4	1.5	5153	22.6
10	19.0 ± 0.3	13–23	10.47	1.99	44 ± 1.3	21–72	27.13	11.94	85	5	92.7	1.4	5006	23.3
12	23.0 ± 0.4	16–28	11.04	2.54	73 ± 2.7	28–115	26.31	19.21	50	7	91.7	0.5	4953	23.4
14	28.0 ± 0.5	19–33	12.98	3.63	128 ± 5.0	53–209	30.20	38.66	60	9	91.2	0.3	4924	24.7
16	36.0 ± 0.4	26–41	9.22	3.32	253 ± 6.9	136–390	20.98	53.07	60	11	90.6	0.3	4892	25.1
17	39.0 ± 0.5	29–45	10.57	4.12	345 ± 10.8	161–486	24.34	83.97	60	12	90.4	0.1	4881	23.6
6	16.6 ± 0.2	13–17.8	5,20	0.76	24 ± 0.4	21–28	13.57	2.04	22	1	98.7	0.5	5331	21.3
8	16.7 ± 0.3	13–19.5	8,83	1.48	26 ± 1.1	15–41	22.95	5.97	30	3	94.7	2.0	5114	22.6
10	20 ± 0.3	16–24	9.18	1.84	46 ± 1.7	22–71	26.56	12.22	50	5	88.7	3.0	4787	23.3
12	25.2 ± 0,3	20–29	9.59	2.42	86 ± 3.3	50–121	27.21	23.40	50	7	87.8	0.5	4741	23.4
14	32 ± 0.4	27–36	8.37	2.68	167 ± 5.0	99–252	23.02	38.45	60	9	87.4	0.2	4722	24.7
16	40 ± 0.4	34–45	7.57	3.03	358 ± 1.0	241–600	20.47	73.30	60	11	87.2	0.1	4706	25.1
17	45 ± 0.4	38–51	7.52	3.39	472 ± 10.9	292–700	17.95	84.72	60	12	87.1	0.05	4701	23.6

Table 37.3 Indices of speed of weight growth of Siberian sturgeon (Ob' population) larvae (embryos have been received from mature stock in aquaculture conditions) after feeding by *Artemia* nauplii (*Artemia parthenogenetica*); Sturgeon Experimental Plot of Gosrybcenter

Age (days)	Days of feeding	Weight growth indices of larvae after feeding with non-enriched Artemia nauplii (Control groups; N = 366 ind.)					Weight growth indices of larvae after feeding with Artemia nauplii enriched by Selco-DHA (Experiment groups; N = 332 ind.)				
		$W \pm m_w$ (mg)	SGR	Average daily gain of fish		N fish in sample (ind.)	$W \pm m_w$ (mg)	SGR	Average daily gain of fish		N fish in sample (ind.)
				(mg)	(%)				(mg)	(%)	
6	1	24 ± 1	0.05	1	4.2	21	24 ± 1	0.05	1	4.2	22
8	3	27 ± 1	0.06	2	7.4	30	26 ± 1	0.06	1	3.9	30
10	5	44 ± 2	0.24	9	20.5	85	46 ± 2	0.29	10	21.7	50
12	7	73 ± 3	0.23	15	20.5	50	86 ± 3	0.31	20	23.3	50
14	9	128 ± 5	0.28	28	21.9	60	167 ± 5	0.33	41	24.6	60
16	11	253 ± 7	0.34	63	24.9	60	358 ± 5	0.38	96	26.8	60
17	12	345 ± 11	0.31	92	26.7	60	472 ± 11	0.27	114	24.2	60
Mean			**0.22**		**18.0**			**0.24**		**18.4**	

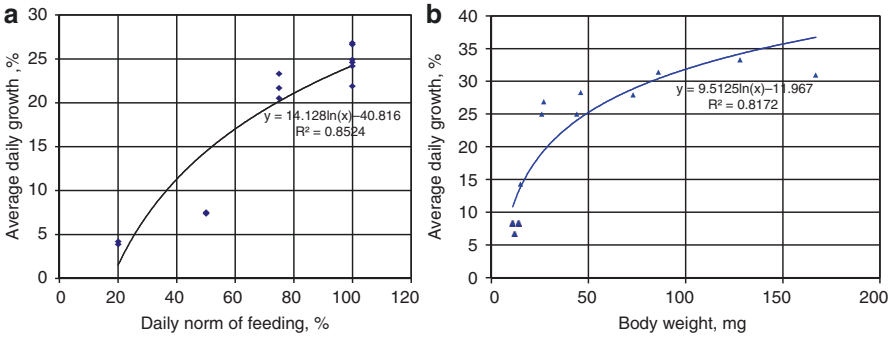

Fig. 37.17 Dependence of average daily growth of sturgeon larvae: (**a**) from Daily Norm of Feeding (%); (**b**) from body weight of sturgeon larvae (mg)

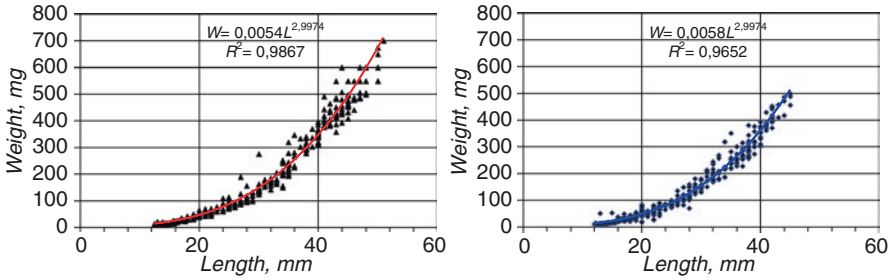

Fig. 37.18 Dependence of body weight on the length of sturgeon larvae during feeding with: (**a**) non-enriched *Artemia* nauplii ($n = 350$); (**b**) enriched *Artemia* nauplii ($n = 555$)

Studies have shown that the rate of larvae weight growth after 3 weeks of rearing with feed of *Artemia* nauplii enriched by Selco-DHA was 3.9 times higher than after using artificial starter feed Aller Futura and BM_S 55/13. So, on the 17th day of feeding with enriched nauplii, larvae reached length 45.0 ± 0.4 mm and weight 472.0 ± 10.9 mg and after using artificial feed, 21.8 mm and 120 mg, respectively (distinctions are significant at $p < 0.01$).

Assessing the percentage of fat and protein in sturgeon larvae body, it was observed that with increasing of sizes and fish weight, the content of dry substance in their body increased. The decrease of dry substance occurred only on the first 8–11 days after transition fish to active feeding (Table 37.4). On the seventh day of feeding, the content of indicator *DM* in Control was 10.8% and in Experiment 10.7%; on the 11th day, the amount of *DM* in Control decreased to 9.2% and in Experiment to 10.5%. After 17-daily feeding of sturgeon with brine shrimp nauplii enriched with Selco-DHA, caloric content of larvae was 1.2 times higher than caloric content of larvae fed with non-enriched crustaceans, making at the end of rearing 5.29 Cal/mg in Experiment and 4.60 Cal/mg in Control (Table 37.4).

So the comparative analysis of experimental works on feeding of Siberian sturgeon larvae with live *Artemia* nauplii enriched with *Selco* treatment has allowed to estimate survival, rates of linear-weight growth, and the caloric content of larvae. At

Table 37.4 Caloric content of dry matter in the body of Siberian sturgeon larvae (Ob' population) in Control and Experiment groups

Age (days)	Days of feeding	Variability of group	N fish in sample (ind.)	Wet weight of larvae (mg)		Dry weight of larvae (mg)		Content of dry matter (%)	Caloric content (Cal/mg)
				General	Average	General	Average		
12	7	Control	50	3628	72	389.9	7798	10.83	5.01
		Experiment	50	4319	86	449.7	9188	10.66	5.77
16	11	Control	50	14,101	282	1279.8	25,595	9.23	4.60
		Experiment	50	18,248	365	1907.4	38,148	10.49	5.29

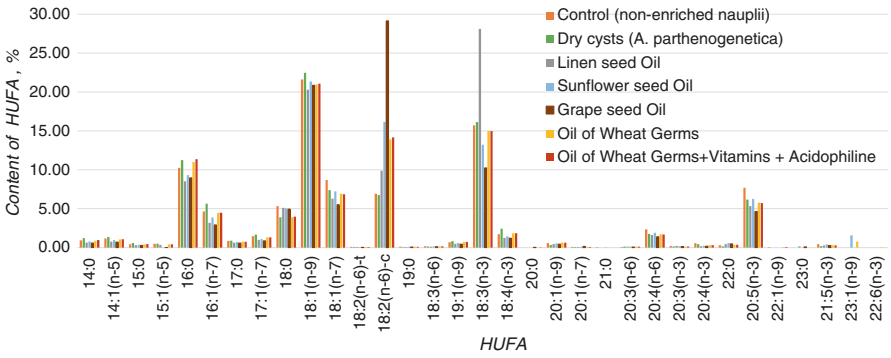

Fig. 37.19 Content of HUFA (%) in dry *Artemia* (*A. parthenogenetica*) cysts, nauplii and metanauplii enriched by different vegetable oils

the same time, the possibility of using for enrichment *Artemia* nauplii the other available preparations with high level of fatty acids has been investigated, in particular some vegetable oils (linseed and sunflower oil, oils of wheat germs and grape seed, sesame, and thistle oils). Using data of general biochemical analysis and the maintenance of HUFA in *Artemia* nauplii as sources of linoleic, linolenic, and olein acids which are irreplaceable for larvae of freshwater fish species, three vegetable oils can be used (linen, sunflower, and wheat germs) and also two complexes (oil of wheat germs or linen, vitamins "TRIOVIT", probiotic "NARINE-FORTE"). The main criteria of the choice of oils were the following indicators: high content $\omega 3$ and $\omega 6$ irreplaceable fatty acids in *Artemia* after enrichment; the caloric content; high content of proteins, fats, and mineral substances in solid body of nauplii after enrichment; linear growth of shrimps; and also survival of *Artemia* nauplii in the enriching solution and in freshwater after bioencapsulation.

Due to comparative biochemical analysis, 33 fatty acids were selected in enriched *Artemia* nauplii (Fig. 37.19). High content of irreplaceable highly unsaturated $\omega 3$ and $\omega 6$ fatty acids—eicosapentaenoic (20:5(n − 3)), 5.33% (9.48 mg/g dry weight); α-linolenic (18:3(n − 3)), 28.11% (50.03 mg/g dry weight); and arachidonic ($\omega 6$), 1.62% (2.88 mg/g)—was noted there (Fig. 37.20). The content of linoleic acid (18:2(n − 6)) in enriched feed was 1.4 times higher than in non-enriched nauplii—9.89% (17.59 mg/g dry weight) and 6.92% (9.34 mg/g dry weight), respectively.

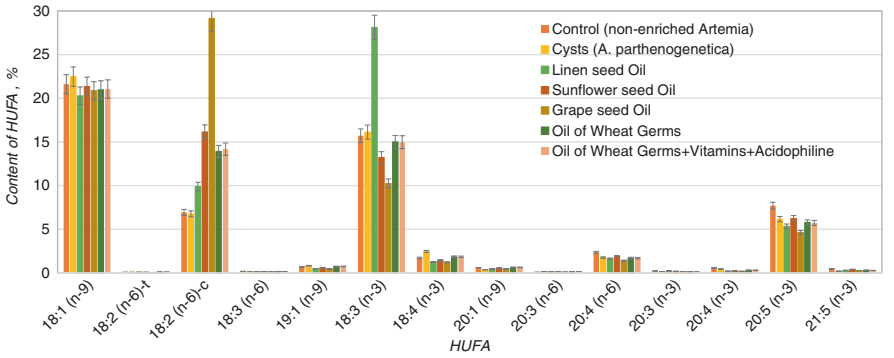

Fig. 37.20 Content of main ω^3, ω^6, and ω^9 fatty acids (mg/g dry weight) in dry *Artemia* (*A. parthenogenetica*) cysts, nauplii, and metanauplii enriched by different vegetable oils

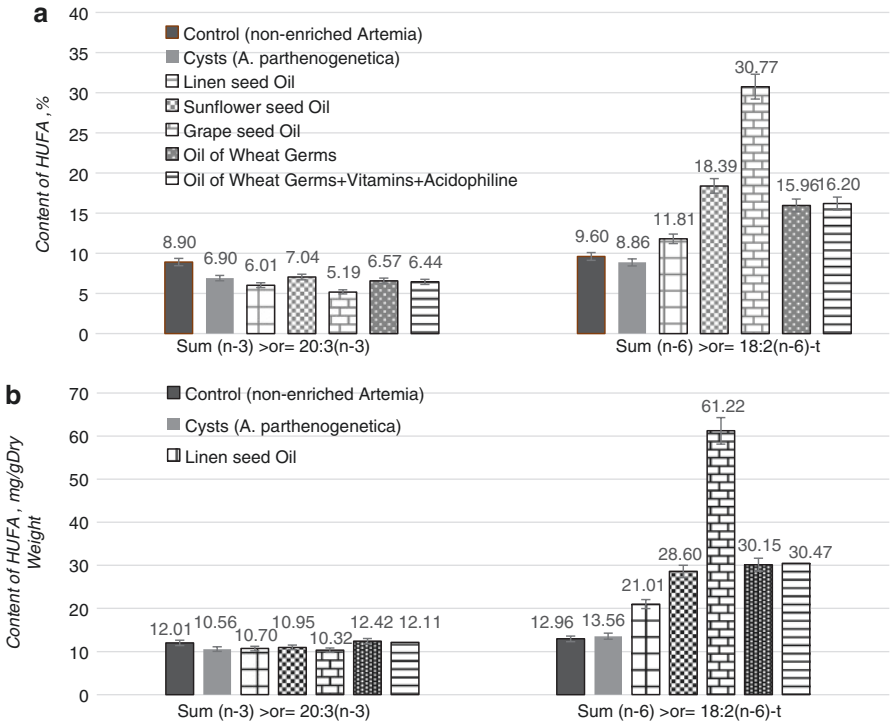

Fig. 37.21 Total content (*A*, %; *B*, *mg/g DW*) ω-3 and ω-6 HUFA in dry *Artemia* (*A. parthenogenetica*) cysts, nauplii, and metanauplii enriched by different vegetable oils

The amount of oleic acid (18:1(n − 9)) was 20.29% (36.10 mg/g dry weight) (Fig. 37.21). The sum of main fatty acids in *Artemia* emulsified with linseed oil was 1.9 times higher than in newly hatched nauplii—15.01/100 mg and 7.79/100 mg DW, respectively.

Table 37.5 The general chemical composition of *Artemia (A. parthenogenetica)* cysts, non-enriched nauplii, and metanauplii enriched by different vegetable oils, vitamins "TRIOVIT," and probiotic "NARINE-FORTE"

Treatment	Caloric content, kCal/100 g dry matter	Content (%)		Content, % from dry matter		
		Wet	Dry matter	Protein	Fat	Mineral substances
Cysts	378.9	10.87	89.3	62.7	0.34	5.7
Control (non-enriched nauplii)	470.0	85.7	14.3	71.3	19.6	7.0
Sunflower oil	475.5	90.8	9.2	63.0	23.9	12.0
Linseed oil	454.0	90.1	9.9	63.6	21.2	13.1
Oil of wheat germs	432.3	90.3	9.7	71.1	15.5	11.3
Linseed oil + "TRIOVIT" + "NARINE-FORTE"	450.0	90.0	10	70.0	18.0	10.0
Oil of wheat germs + "TRIOVIT" + "NARINE-FORTE"	449.2	90.6	9.4	66.0	19.2	11.7

During the biochemical analysis, it has been found out that in the period of bioencapsulation (12 h), there were changes in the content of the main organic substances of *Artemia* body. After enrichment of crustaceans with the help of sunflower, linen oils, and oil of wheat germs, the content of proteins and fat reached 70.0–71.1% and 21.2–23.9%, respectively (Table 37.5). Caloric content of metanauplii after the use of sunflower oil emulsion was higher than in nauplii (Instar I)—475.5 kCal/100 g and 470.0 kCal/100 g, respectively. It should be noted that the high content of mineral substances in *Artemia* enriched by different vegetable oils was 11.7–12.0% and after the influence of sesame oil till 13.3%.

The highest rates of linear-weight growth of sturgeon larvae were observed after feeding fry with *Artemia* nauplii (*A. parthenogenetica*) pre-enriched with linseed oil (*oleum lini*) in conjunction with probiotic "NARINE-FORTE" and vitamin complex with microelements "TRIOVIT" (Table 37.6).

Use of this enriched live food has contributed to statistically significant increasing of juveniles' weight 2.12 times in comparison with feeding by non-enriched *Artemia* and 1.51 times higher than after feeding with nauplii enriched by emulsion Selco-DHA. The use of preparations had no impact on improving the survival of reared juveniles. In Table 37.6 there are the results of sturgeon feeding within 20 days after using *Artemia* nauplii enriched by different biological preparations (initial age of larvae—20 days).

Table 37.6 The Effect of Siberian sturgeon (Ob' population) larvae feeding during 20 days with non-enriched and enriched *Artemia* nauplii (*A. parthenogenetica*) from Siberian lakes

Index		Control	Variants of emulsions for *Artemia* enrichment				
		Non-enriched *Artemia*	Selco-DHA	Selco-DHA + "TRIOVIT"	Selco-DHA + "NARINE-FORTE"	Oleum lini	Oleum lini + "NARINE-FORTE" + "TRIOVIT"
Initial sturgeon larval weight (g)		0.209 ± 0.025	0.205 ± 0.010	0.200 ± 0.024	0.202 ± 0.031	0.201 ± 0.035	0.204 ± 0.043
Final sturgeon larval weight (g)		0.954 ± 0.120	1.336 ± 0.090	1.972 ± 0.130	1.853 ± 0.240	1.557 ± 0.230	2.020 ± 0.180
Length of *Artemia* nauplii, mm	Average	0.58 ± 0.03	0.68 ± 0.04	0.69 ± 0.10	0.79 ± 0.17	0.68 ± 0.01	0.81 ± 0.24
	Min–max	0.50–0.70	0.63–0.70	0.63–0.73	0.64–0.83	0.64–0.72	0.65–1.34
Specific growth rate (%/day) of sturgeon larvae body weight		0.076	0.094	0.114	0.111	0.102	0.115
Sturgeon larvae survival, %		85.2	84.4	85.6	84.8	85.1	85.3

Note: The values of statistically significant differences are shown at $p < 0.01$

37.5.1 The Results of the First Experimental Voyage

The number of juvenile fish transported without feeding for a voyage (24 days) was supposed to be not less than 500 thousand fingerlings with an average weight of 2.5–3.0 g or 3–4000 ind./m^3. The total mortality during the loading and transportation was 2.3%, and from that quantity 2.0% died during the loading. In the period of transportation, few individuals (0.3%) with weight 2.13–5.86 g have died.

Overall, visually the physiological state of juvenile fish throughout transportation and during releasing was assessed as satisfactory. Data of biochemical researches showed that protein content after 24 days of starvation decreased 1.24 times (from 10.58 to 8.51%) and fat content decreased from 0.31 to 0.25%. The moisture content of fingerlings increased from 87.56% on the first day of fasting to 88.4% on the 24th day (Fig. 37.22). Ichthyopathological examination of transported fish showed the absence of contamination of their typical parasites: protozoa of genera *Trichodina* and *Diplostomum*.

Changes of Hematological Parameters During Transportation of Sturgeon Juveniles. Starvation of sturgeon juveniles in the period of transportation had a significant impact on the value of hematological parameters. For the observation period both qualitative and quantitative changes in the composition of the blood occurred. The color index and viscosity of the blood decreased. The rate of erythrocyte sedimentation increased 2.3 times on average (from 4.3 ± 1.1 mm/HR to 8.4 ± 2.3). At the beginning of the experiment, this index was characterized by higher variability of sign than at the end (CV_1 = 153.95%, CV_{24} = 86.19%). The number of red blood cells to 17 days of starvation grew slightly from 4.20 ± 0.92 mLn./mm^3 to 4.49 ± 1.07 mLn./mm^3; on the next 24 h, it was a sharp reduction of their number to 2.82 ± 1.11 million pieces/mm^3 (1.6 times). This indication ranged 1.8 times stronger on the 24th day of fasting than on the first day (CV_{24} = 124.82%, CV_1 = 69.05%, respectively) (Table 37.7).

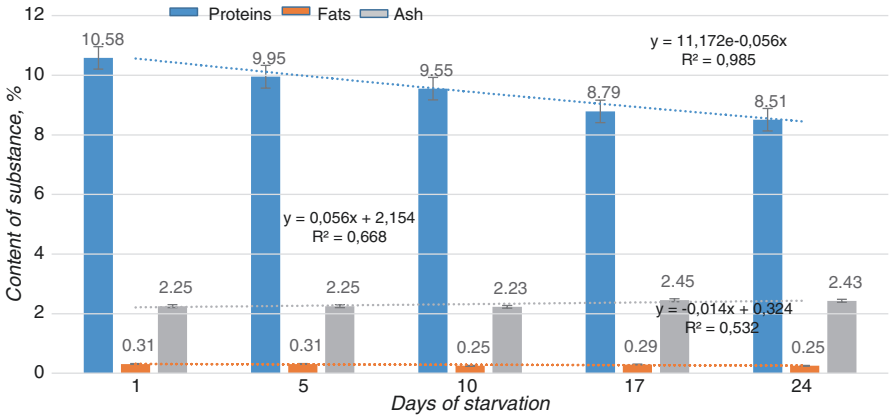

Fig. 37.22 Dynamic of content (%) of proteins, fats, and ash during the starvation of Siberian sturgeon fingerlings

Table 37.7 Red blood cell indices of Siberian sturgeon fingerlings during their long-term starvation; laboratory conditions

Indices	Days of starvation														
	1			5			10			17			24		
	M ± m	σ	C_V	M ± m	σ	C_V	M ± m	σ	C_V	M ± m	σ	C_V	M ± m	σ	C_V
Number of erythrocytes; mln. cells/mm^3	0.42 ± 0.09	2.9	1.45	0.17 ± 0.07	2.1	0.83	0.43 ± 0.1	3.2	1.35	0.46 ± 0.11	3.3	1.37	0.28 ± 0.12	3.7	0.76
RSE, mm/h	4.30 ± 1.14	3.6	1.18	4.60 ± 1.09	3.4	1.34	2.80 ± 0.59	2.8	1.50	4.11 ± 0.97	3.1	1.33	8.40 ± 2.41	7.6	1.20
Quantity of analyzed fish, ind.	10			10			10			10			10		

In the study of morphological characteristics of white blood cells, the following results were obtained. The number of leukocytes before starvation amounted to 186.05 ± 42.94 thousand/mm^3; on the 24th day of fasting, they decreased to 175.08 ± 70.57 thousand/mm^3 (Table 37.8). By the end of fasting, asymmetry of distribution of the character increased (from 72.99% to 127.46%).

In the composition of leukocytic formula, it was revealed that at the first day of fasting, the most numerous fractions were occupied by lymphocytes (41.93%). Lymphocytes have been presented by the following groups: big, small, middle, and holo-nuclear ones (Table 37.8). The amount (%) of big (6.22 ± 1.42), middle (13.5 ± 6.46), and small (18.07 ± 3.59) lymphocytes at the first day of starvation was less than on the 24th day of fasting: for big and small lymphocytes 1.5 times, 9.41 ± 1.47 and 27.14 ± 3.49, respectively, and for middle 1.2 times (15.48 ± 2.07). The quantity of holo-nuclear lymphocytes decreased 1.5 times (from 5.45 ± 1.59 to 4.14 ± 0.94).

The second large group was represented by neutrophils (34.44 ± 5.42%) and the third one by eosinophils (23.31 ± 5.43%). From agranulocytes, in addition to lymphocytes, there were a few monocytes; their percentage did not exceed 0.71%. By the end of fasting, the content of lymphocytes increased till 53.5% (1.3 times), and the number of eosinophils decreased to 19.16%. Among granulocytes too sharp decline occurred with neutrophils—main phagocyte cells of blood—their percentage has decreased 1.5 times (till 22.78%).

Before fasting a very strong positive correlation between fish body weight and number of erythrocytes was observed (r = 0.953). Also there was a strong correlation between weight and number of leukocytes (r = 0.837). On the 24th day of fasting, the coefficient of correlation between these varying signs was weak: in the first case, it amounted to 0.260 and in the second 0.186. As at the beginning of fry fasting as on the 24th day, a very strong positive correlation between the number of erythrocytes and leukocytes was revealed: at the beginning (the first 24 h), the ratio was equal to 0.919, and at the end of the experiment, this correlation was close to functional (r = 0.992).

37.6 Discussion

As a result of complex fish breeding and biological researches, some new biotechniques for larvae rearing and long-term transportation of Siberian sturgeon fry to the natural reservoirs of Western Siberia (inlet of the Ob' River) were developed. For the first time in Russia, some experimental works directed to optimization of ways of sturgeon larvae rearing by means of *Artemia* nauplii enriched with preparations with the high content of HUFA were carried out. This biotechnology allows to use rationally and effectively natural stocks of *Artemia* crustaceans whose potential in Western Siberia is rather essential (the latest researches of G. van Stappen (Van Stappen et al. 2002) are described in 18 *Artemia* habitats in Siberian lakes).

Table 37.8 White blood cell indices of Siberian sturgeon fingerlings at the first and the last (24th) days of starvation ($n = 20$); laboratory conditions

Formula of white blood cells (%)		1 day of starvation				24 days of starvation				Standard
		M ± m		σ	C_V	M ± m		σ	C_V	
Eosinophils		23.31 ± 5.43		17.15	73.57	19.16 ± 3.10		9.81	51.2	2–9
Neutrophils		34.44 ± 5.42		16.50	47.91	22.78 ± 5.61		17.73	77.83	14–25
Lymphocytes	Big	6.22 ± 1.42	43.24 ± 3.27	4.49	72.19	9.41 ± 1.47	56.17 ± 1.99	4.64	49.31	72–77
	Middle	13.5 ± 6.46		6.46	47.85	15.48 ± 2.07		6.55	42.31	
	Small	18.07 ± 3.59		11.36	62.87	27.14 ± 3.49		11.03	40.64	
	Holo-nuclear	5.45 ± 1.59		2.96	71.50	4.14 ± 0.94		5.02	92.11	
Monocytes		0.71 ± 0.22		0.68	95.77	0.58 ± 0.11		0.35	60.34	0.5–1.0
Leukocytes, thousand cells/mm^3		186.05 ± 42.94		135.79	72.99	175.08 ± 70.57		223.15	127.46	

The experiment works with the use of *Artemia* nauplii (*A. parthenogenetica*) from Siberian lakes enriched by highly unsaturated fatty acids for feeding Siberian sturgeon larvae showed statistically significant increase of juvenile weight 1.4 times after applying emulsion with high content of eicosapentaenoic acid (Selco-Experimental) compared with feeding by non-enriched live feed. Feeding of sturgeon larvae with brine shrimps enriched by emulsion with high content of docosahexaenoic acid (Selco-DHA) contributed to statistically significant weight gain of Siberian sturgeon juveniles 1.6 times. The ratio of larvae length and body weight at the initial stages of feeding shows that the values of exponent *b* and the parameter *a* in equation of the regression are closed after using both non-enriched and enriched live feeds.

Enrichment of *Artemia* nauplii by linseed oil with the addition of vitamin complex "TRIOVIT" and probiotic "NARINE-FORTE" allowed to obtain maximal daily increases of sturgeon larvae weight. Fry weight after 20 days of rearing reached 2.02 g, or it was 2.12 times higher in comparison with feeding with non-enriched brine shrimps. Specific rate of weight growth during rearing amounted to 0.115. Maximum daily increases (to 27%) were observed at the daily rate of feeding with bioencapsulated feed, equal to 100% of larvae body mass (over 200 mg). High daily growths in the experiments are understandable. During intensive breeding, when the fish are kept in a confined space in case of excessive feeding, the energy forces for obtaining food are minimized. The main task of cultivation is to ensure the maximum growth for cultivated object. The growth of individuals follows a certain trajectory, whose shape is determined genetically (Gershanovich et al. 1987). According to Gershanovich et al. (1987), the sizes of sturgeon at this age are 20–25% of maximum possible. The results of the experiments showed that the larvae of Siberian sturgeon after feeding *Artemia* nauplii realized their growth potency till 23.1%; when they used enriched nauplii in Selco diet, till 31.6%; and in complex with linseed oil, vitamins (TRIOVIT), and probiotics ("NARINE-FORTE"), 41.9%. The curves of larvae weight growth in last cases are closed to exponential: $W = 0.004e^{0.2671T}$, ($r = 0.99$).

The use of emulsions had no impact on improving juvenile survival in comparison with fish fed with non-enriched *Artemia.* After the development of this method in Gosrybcenter, the Patent for the Invention № 2,577,478 has been taken out (Chepurkina 2016).

It is known that linseed oil (*oleum lini*) is a liquid from golden-yellow to brown color. It is insoluble in water; in the presence of oxygen it is polymerized easily and forms a durable transparent pellicle (Ipatova et al. 2009). This ability is due to the high content of unsaturated fatty acids (triglycerides): linoleic (15–30%), linolenic (44–61%), and oleic (13–29%). The structure also consists of proteins (up to 24%), glycoside linamarin, carbohydrates, organic acids, enzymes, ascorbic acid, and carotene (Shikov et al. 2004). "NARINE-FORTE" (acidophilus milk) is a probiotic; it consists of concentrated milk, fermented by symbiotic cultures *Lactobacillus acidophilus* of strain "NARINE" and the active ferment of bifidobacteria containing strains *Bifidobacterium bifidum* and *B. longum*. *Lactobacillus acidophilus* helps to normalize the natural microflora in the gastrointestinal tract and enhance digestion

and absorption of food; they have a tonic and immunostimulating action. "TRIOVIT" contains antioxidant vitamins C, E, and β-carotene (provitamin A) and oligoelement selenium. The protective properties of antioxidant vitamins increase the body's resistance to adverse environmental factors; they increase protective properties of the organism during inflammatory processes (Nikolaev 1985).

Enrichment of *Artemia* nauplii with polyunsaturated fatty acids (DHA or linoleic acid) with the addition of vitamin complex "TRIOVIT" and probiotic "NARINE-FORTE" was carried out within 5 years to feed Siberian sturgeon larvae received from spawners as from natural populations as from aquaculture mature brood stocks. Over 20,000 sturgeon fingerlings were reared, most part of them was released in the Ob'-Irtysh basin.

Further, for sturgeon larvae feeding with enriched nauplii, the prospects of the use of sunflower oil and oil of wheat germs should be noted.

Thus, in the course of experimental works it was found that the realization of growth potentialities of Siberian sturgeon juveniles in industrial cultivation could be achieved by optimizing the conditions of feeding. The proposed method of preparing live food and biotechnical methods of larvae feeding promoted the success of the most important phase of larvae rearing, including their high survival rate. It was found that the feeding of sturgeon larvae with *Artemia* nauplii until the weight of the fish reaches 200–250 mg takes 7–8 days at the temperature 18–20 °C. Using the method of *Artemia* bioencapsulation, it was possible to increase the speed of larvae weight growth from 1.3 to 2.1 times and to reduce the feeding period with live feed in few days, which is extremely important in the conditions of juvenile industrial rearing. At that time, the intensity of the daily mortality was close to zero. Therefore, a gradual transition to artificial starter feed on the next 10–11 days most often occurs at high daily increases with preservation of low fish mortality.

The second important problem considered in this article concerned to increasing the efficiency of works on sturgeon artificial reproduction in Western Siberia with the help of fry transportation to natural habitats with optimum food supply.

According to the results of the first experimental voyage, it was found that at an average water temperature 15.8 °C and dissolved oxygen 7.1 mg/L, long-term transportation (within 24 days) of starving sturgeon fingerlings in quantity 500.000 individuals with average weight 4 g at stocking density 30 kg/L was possible. Visually prolonged fasting did not affect the physiological condition of transported fish. The behavior of starving juvenile fish during transport and release was quite active. Total fish mortality for transportation time did not exceed 0.3%.

The value of hematological parameters of sturgeon juveniles was determined largely by the period of transit. During the period of fasting, the number of erythrocytes decreased 1.5 times, and the rate of subsidence was reduced 2.3 times. Despite the fact that the number of leukocytes changed little during the period of starvation, there was a redistribution of formed elements of white blood in the direction of increasing the proportion of lymphocytes and significant reduction in the number of granulocytes (eosinophils and neutrophils). Since lymphocytes are the part of immune system which protects the body from outside influences and retains its genetic constancy (Ivanova 1983), the increase of their percentage content in the

blood of examined Siberian sturgeon juveniles indicates enhanced immunity to stress environmental factors (in this case, prolonged fasting).

The transportation of Siberian sturgeon yearlings was allowed to put them on the Delta of the Ob' River with rich food supply and optimal hydrochemical regime. In addition, through the transportation of juveniles, there was a possibility to avoid massive catch of fish by different fishing gears (set nets, wicks, etc.) in the channel of the Ob River.

It should be noted that despite successful tests of the first long-term sturgeon fry transportation in Western Siberia, the voyage was the only one. Nevertheless, it is necessary that these researches would be continued in the future for performance of several purposes: identification of sturgeon fry migratory ways and extent of adaptation to places of food supply, studying of fish food requirements, an assessment of survival and quantity of individuals in brood stocks in the Ob'-Irtysh basin, etc.

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An Assessment of the Characteristics of World Production of Siberian Sturgeon Destined to Human Consumption

38

Mikhail Chebanov and Patrick Williot

Abstract

This chapter provides an overview on the world gross production of Siberian sturgeon and its related farmed caviar. The different production structures (flow-through raceways, cages, and recirculated aquaculture systems (RAS)) used in the world for the cultivation of Siberian sturgeon are briefly given. Then, the history of farming the species in as much countries as possible is reported according to two periods, namely, the initiation (till 2000) and the developmental (after 2000). The production trends and price dynamics (for some occasional periods) in the various countries in the last 40 years are described. In 2014–2015, the yearly gross production was assessed to be closed to 27,000–28,000 tons and that of caviar to about 150 tons. The new methods of caviar production from ovulated eggs of the Siberian sturgeon and the trend of production are given. Undoubtedly, the geographical distribution of Siberian sturgeon farms has been much broader than that engaged in farming of other sturgeon species. While most of the enterprises that are producing the larger part of the overall capacity are located in China, Russia, and France, a lot of new countries (Armenia) worldwide are involved in this production. There is Uruguay at the south to some in the Polar Circle near Murmansk (Russia) and Norway and from the USA (Northern Carolina and Florida) at the west to South Korea at the east. Some economic characteristics of the Siberian sturgeon production are given (selling prices,

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production costs, etc.). Finally, the analysis of the context allows suggesting some perspectives for the farming of the species.

Keywords
Siberian sturgeon • Farming • Geographical distribution • Caviar • Gross production • Meat • Production structure • Market • Economics

Introduction

The first experiments on the commercial cultivation of various sturgeon species were carried out in Russia in the 1940s–1950s and performed in concrete basins and ponds (Stroganov 1951, 1957, 1968; Sukhoverkhov et al. 1952). Widespread commercial rearing of bester (beluga × sterlet) had been initiated in the late 1960s primarily in ponds and cages (Burtsev 1969). In 1969 the first batches of marketable bester in the amount of 3.4 tons were grown in Aksai fish farm and above 4 tons in the fish hatchery (both the Rostov region, the USSR) (Afanasiev 1971; Chernomashentsev and Chernomashentseva 1971; Milshtein, Slivka 1972; Burtsev 2015). The preliminary investigations on the possibilities of farming Siberian sturgeon were performed in Russia in the mid-1970s. These attempts were performed either in ponds up to weight of about 2.9 kg (in Rostov region) or in cages till a mean weight of 2 kg (Pyalovsky water reservoir near Moscow) and in tanks supplied by a warm water from a power station (Mikheyev 1974; Berdichevsky et al. 1979). The most impressive results were gained at Konakovo experimental hatchery (former Kalinin (now Tver') region) in sturgeon farm established in connection with a power station (Berdichevsky et al. 1979; Sokolov et al. 1976, Smoljanov 1979, 1981, 1987, 1989); Burtsev et al. 1984; Barannikova et al. 1972). Indeed, experimental rearing of Siberian sturgeon started in 1973 in tanks of Konakovo sturgeon hatchery (Russia) based on fingerlings of the same origin as those sent to France for the first time in 1975 (Melchenkov and Kanidieva 2015; Williot et al. 2017a, b). Therefore Russian and French brood stocks have a global common origin (Smoljanov 1979; Petrova et al. 1990, 2008; Melchenkov and Kanidieva 2015). The rearing in cages of the Siberian sturgeon was carried out both in warm-water farms at power stations (Elektrogorsk, Volgorechensk, Cherepovets, Petchora, Luchegorsk) including atomic plants (Leningrad, Kursk, Kolsky, etc.) and in many natural temperature regime freshwater bodies, primarily water reservoirs (Korneyev et al. 1969; Mikheyev 1974; Kharitonova and Lyuliev 1978; Popova 1979). Unfortunately, the results of these works were published only in Russian. Only Charlon and Williot (1978) reported an amount of yearly production of commercial sturgeon in Russia of a few tons of bester. During this initiation period of sturgeon farming in the former USSR, and Siberian sturgeon is no exception, fish were fed either trout food or on-site composed food mainly based on frozen marine fish complemented with starch, fish or soy meal, and yeast (Charlon and Williot 1978).

The very early 1980s has been a key date regarding the development of the rearing of the Siberian sturgeon. Indeed, independently from each other but simultaneously, P. Williot (Williot and Rouault 1982) and I. Smoljanov, in France and in former USSR, respectively, obtained in 1981 the first progenies from a farmed brood stock originated from the Lena population and thus opened the farming of the species. As a matter of fact, the Siberian sturgeon production has been starting in

France and later on in Western Europe (namely, Germany, Hungary, and Italy) in the late 1980s and early 1990s with the objective of meat production (Williot et al. 1993). The turn to caviar production occurred in Western Europe by the mid-1990s and started to be effective by the late 1990s (Williot et al. 2001).

There are reviews devoted to sturgeon farming, respectively, in Western (Williot et al. 1993, 2001; Williot 2000; Arlati and Bronzi 1995, Bronzi et al. 1999; Bronzi and Arlati 2002; Jones 2001; Arndt et al. 2002) and Central Europe (Steffens et al. 1990), in Russia (Barannikova 1987; Barannikova et al. 1995; Chebanov 2000, Chebanov and Billard 2001; Chebanov et al. 2009, 2011; Mamontov and Gepetskiy 2001), and in the USA (Beer 2001). Development of sturgeon activities was also produced in Japan (Gribanova 1970; Sajto 1970; Katsumi and Genjiroi 1977; Fujii and Maruyama 1997), Greece (Paschos et al. 2008), Turkey (Ercan 2011; Memiş 2014), and Iran (Pourkazemi et al. 2016). More detailed papers covering the huge development of sturgeon aquaculture in China (Li et al. 2009; Podushka and Chebanov 2007; Wei et al. 2011; Shen et al. 2014) were published. Status and prospects of sturgeon meat and caviar production on the world level were assessed (Williot and Bourguignon 1991; Bronzi et al. 2009, 2011; Bronzi and Rosenthal 2014, 2016).

Unfortunately in all mentioned papers, no one is focused on the Siberian sturgeon; more, there are very seldom species-specific data on the Siberian sturgeon except the only review devoted to the species presented by P. Williot in a special FAO survey (Williot et al. 2005). In addition, the development of the farming of the species worldwide encourages the attempt to carry out a synthesis on the farming of the species; this is the aim of the present chapter at assessing the world production of the species and its main characteristics. This is a challenge because there is no current way to achieve the task.

The content of the present chapter is organized into two main subchapters: the first deals with the assessment of the world production and the second reports on some characteristics of its development. The first subchapter contains three sections; one consists in describing the production structures used worldwide to hold the fish during their ongrowing phase. The other gives an assessment of the volume of production in distinguishing an initiative phase until the late 1990s and a developmental one from the early 2000s onward. This is because that corresponds to two different phases having a major difference in the objective of production (meat and further caviar) in the one hand with the major changes thus observed in the financial structure of most part of the previous companies (Williot et al. 2001). In both subsection, as much as possible, country-specific data are reported.

However, before presenting the results of our investigations, a short subsection below referred as method explains how it has been performed as well as the difficulties that were encountered.

38.1 Method

There are two main ways to get data on production; one supposes use already published data in the field, while the other consists in performing a survey given that the second is extremely difficult in practice with a worldwide distribution. However, both

ways were mobilized in the present study. Especially with regard to already published data, it is necessary to ask: Where the data are coming from? How were they collected? And thus: Which truthfulness for the data? Indeed, to our knowledge, there is no declarative constraint for the production (from both quantitative and qualitative aspects) in any country. Each country feeds and updates International DataBases (EU, FAO, CITES, etc.) according to its own organization system. In some countries (e.g., France), specific administrative services are aware of the production, e.g., the services that examine and further deliver the authorization to produce fish in respect of the law about the threshold limit of some components in the water outlet to constrain the pollutant effect of fish farms. But these services have no constraint to communicate. It is worthy to note that, though constraining, this regulation system is a long-term security with regard to the quality of the production as the rearing water is then not too enriched in rearing pollutants. There is another way to obtain data; these are specific surveys as illustrated successively by Wei et al. (2011) and Shen et al. (2014) in China. Depending on the country, another collection of production's data may be encountered; this is through professional organization like SFAM[1] in France. This source may be considered as the more robust. This may explain discrepancies between published data for a given country. Further, some data may present inconsistencies; they are pointed out. A complementary mean to get data apart from those published (if any) is to ask leading sturgeon culture experts from different countries. They are gratefully acknowledged for their contribution, and the list is given at the end of the manuscript.

A last source of uncertainties is related to the wording. In the present case, the two main products are sturgeon as a whole and caviar. With regard to the former, the word "meat" was (and still is) often used, e.g., Williot et al. (2001), instead of using "gross production" which includes by definition viscera, gonads (ovaries or testis), head, skin, etc. Indeed, the last wording represents the easiest representation of the volume of production as in any animal activity. As much as possible, the precision is given in the present chapter.

To enable comparisons of production costs and prices of products for every country, a currency converter to a past date using official rates since 1953 (multicurrency calculator euro, dollar, etc.) is used http://fxtop.com/fr/conversion-devises-date-passee.php?A=4.12&C1=EUR&C2=USD&DD=01&MM=01&YYYY=2016&B=1&P=&I=1&btnOK=Chercher. And the results correspond to the evaluation of the currency in 2016 otherwise mentioned.

38.2 World Production Assessment

The section is composed of four subsections: the first deals with the structures built for holding the sturgeon, and the second presents the initiation period, i.e., from the beginning until the late 1990s (<2000) with a presentation by country (as much as possible) and by alphabetical order except for Russia and France that were the pioneer countries for the farming of the species. The third subsection focuses on the

[1] SFAM = Syndicat Français d'Aquaculture Marine, French association of which sturgeon farmers are members.

developmental period, i.e., from 2000 onward. Again, as much as possible, country-specific data are given. The fourth subsection gives a synthetic view of the most probable figures of the world production of the species.

38.2.1 Production Structures

There were no structures especially designed for sturgeon culture (Bronzi et al. 1999). Siberian sturgeon has been rearing in three main structures that are ponds (Fig. 38.1), raceways (Fig. 38.2), and cages (Fig. 38.3). Apart from the structures,

Fig. 38.1 One of the largest sturgeon farms in Russia, the Kuban Sturgeon Farming Institute, Krasnodar region. On the left is a group of concrete pond. On the right side are two series of earth raceways (credit M Chebanov)

Fig. 38.2 Classical sized concrete raceways arranged in parallel. The water inlet canal is on the right down corner. Primary step of building of the sturgeon farm L' Esturgeonniére, South West of France (credit Esturgeonnière Co)

Fig. 38.3 Overall picture of a cage sturgeon farm in Bulgaria established in a large reservoir (credit E. Galich)

Fig. 38.4 Operating aerators in raceways. Esturgeonnière sturgeon farm, South West of France (credit P. Williot)

the water flow can be more or less important, i.e., all the needed oxygen for the fish to achieve their activity metabolism is brought by the water renewal, this is the so-called flow-through system, or at the opposite, the water renewal is quasi null, and then the oxygen is brought by aerators (Figs. 38.4 and 38.5). Obviously all intermediate situations might be encountered. It is worthy to note that there is another solution to bring oxygen together with depuration water system also called recirculated aquaculture system (RAS) (Fig. 38.6). In addition to the above two important

Fig. 38.5 Small twin raceways with a low-water depth. Note that some are covered by a net to avoid avian predation, and one is enriched with oxygen by an aerator. Prunier Manufacture sturgeon farm, South West of France (credit P. Williot)

Fig. 38.6 Example of a large RAS (Recirculated Aquaculture System). The structure in the center of the photo is settled in between two series of raceways. It is mainly composed of biological filters and a depuration system to take out the nitrogen (fluidized beds). L' Esturgeonnière sturgeon farm, South West of France (credit P. Williot)

factors, shape and water-oxygen supplying, a third one has to be taken into account; this is the material used for the bank and the bottom; this can be earth (Fig. 38.1), concrete (Fig. 38.1). A mix of those three techniques might also be encountered. The last alternative with geo-membrane cover of the race-ways seems attractive because of its low cost, though there is a lack of relevant evaluations in terms of their practical efficiency and long-standing resistance. Below is a brief description of examples of raceways, ponds, cages, and RAS given that there are no simple criteria to discriminate ponds and raceways.

38.2.1.1 Raceways and Tanks of Other Shape

Their area ranged from 100 to 2000 m^2 and depth from 1 to 2 m. Their walls are mainly vertical and made of concrete; they can be arranged in a series comprising from 10 to 100 units (Figs. 38.1, 38.2, and 38.4). Many different examples may be encountered in the choice of dimensions for the raceways, one of the key questions being the ratio between length and width (Figs. 38.5, 38.7, 38.8, and 38.9). The last quoted example represents the largest raceways ever seen by the authors. This sturgeon fish farm has the advantage of being supplied by an important inflow of river water that allowed distributing the inflow on the whole width of the each raceway. This last example points out that a raceway is by definition a structure where the current of water flow supplies the oxygen demand of fish and favored the elimination of wastes.

Fig. 38.7 Small raceway separated into three parts and equipped with an aerator (left side of the photo). Small shadowed circular tanks are for holding juvenile sturgeon. Prunier Manufacture sturgeon farm, South West of France (credit P. Williot)

Fig. 38.8 Small twin raceways with a low-water depth separated into two parts. Prunier Manufacture sturgeon farm, South West of France (credit P. Williot)

Fig. 38.9 Very large size raceways. Les Esturgeon de l'Adour fish farm, South West of France (credit P. Williot)

Fig. 38.10 Large concrete circular tanks for rearing Siberian sturgeon in China (credit M. Chebanov)

The possibility of separating holding tanks or ponds in diverse parts more adapted, for example, to the size of the fish, seems an advantage (Figs. 38.4, 38.7, and 38.8). This presupposes both general and individual (for each water body) hydro-chemical, breeding, and ichthyo-pathological control. In addition, if this deem necessary, it would be possible to create open RAS within such complexes, to insure effective operation under conditions of restricted water supply (for instance season dependent). The smallest size of raceways with a 0.5 m depth might be illustrated (Fig. 38.7) and might be also used to produce live food such as daphnia (Charlon et Williot 1978) or *Artemia salina* as live food supply for sturgeon larvae.

Though less widespread worldwide, concrete circular tanks used for Siberian sturgeon rearing have to be mentioned (Fig. 38.10). Also sub-square tanks are used (Fig. 38.11) as in Vietnam.

38.2.1.2 Ponds of Small Area

They represent small running water bodies of area from 0.01 to 0.5 ha (Fig. 38.1), the bottom and walls of which are made of concrete, or covered with special film (geo-membrane), or in earth. The water supply of water bodies is performed forcedly or self-flowing from natural water bodies (river, lake, water body). Hydro-chemical and thermal regime of water bodies is closed to natural. Such farms are distributed in regions with long-term vegetative season (number of days with average water temperature above 12 °C) and good water supply, ensuring continuous

Fig. 38.11 Sub-square concrete tanks in Vietnam. Each of the four 90° angles is changed for a 45° wall to limit the accumulation of wastes and then the deterioration of water quality. Note that tanks are partially shadowed (credit V. Chipinov)

water delivery and drainage. Frequency of water exchange in such ponds ranged from one to three times per day (depending on the season of the year). Combined system "tank in the pond" or "RAS in the pond" used in Hungary (Laszlo Varadi's pers. comm.) and Russia (Chebanov et al. 2006; Chebanov and Galich 2011a, b) is a variety of the pond rearing of Siberian sturgeon.

Earthen ponds may present a drawback with regard to feed Siberian sturgeon. Indeed, specimens of the species are benthic feeders; thus in absence of changing feeding area places, the behavior of the fish may lead to deep hole at the bottom with subsequent accumulation of wastes and difficulties for fishing (Williot et al. 1988). However, with an adapted management, some sturgeon farms have developed large ponds (~ 0.5 ha) to hold the female during their last phase of ongrowing, e.g. in France (not shown).

38.2.1.3 Cages

Siberian sturgeon farming in cages has been primarily using in the former states of USSR (Fig. 38.12). Later on, the cage rearing system has been developing in many countries, e.g., Bulgaria (Figs. 38.3 and 38.13), China (Fig. 38.14), and Uruguay. While the former cages were (and still are) of small size (Fig. 38.12) arranged one after the other in canals supplied by warm water and then valorize the calories from the industry (e.g., power plants), the second group is floating cages usually of large size (Fig. 38.14). Those larger floating cages are installed in reservoirs mostly human-created upstream dams. The primary advantages of such systems have been

Fig. 38.12 One of the largest sturgeon cage farms in Russia, the Kuban Sturgeon Farming Institute, the Krasnodar Region. Cages are arranged by the side of the other in a canal supplied by industrial warm water (right part of the photo) (credit M. Chebanov)

Fig. 38.13 Detailed view of the sturgeon cage farm Ecetra Commerce in Bulgaria (credit E. Galich)

Fig. 38.14 Detailed view of sturgeon cages in "Kaluga Queen" China (credit M. Chebanov)

the low specific consumption of production inputs and cheap costs of the necessary equipments. In other words, this can be a mean to take profit of winter warm-water. Sometimes warm-water cage systems are used in combination with ponds and tanks, supplied from natural surface (Fig. 38.12) or underground water sources (Chebanov et al. 2006).

Especially with regard to large floating cages, there are two unanswered question. The first deals with the feeding behavior of the species which is mostly benthic. Which is the feeding efficiency of the food? Which is the lost part of the food, the one that is not consumed by the fish and then drops at the bottom underneath the cages? The second questioning is related to the impact on the water rearing conditions due to the biological transformation of both un-eated food and feces on the fish. It should be noted that the size of large floating cages ranged from 30 to 40 m^2 (Fig. 38.12). The majority of the large cage farms were constructed in the 1970s–1980s and had a yearly production capacity ranging from 70 to 300 tons for every farm.

38.2.1.4 Recirculated Aquaculture System (RAS)

The important advantage of such systems is the possibility to arrange fully controlled temperature and hydro-chemical regime in tanks (Kiselyov et al. 1995). RAS are based on the principal of the maximally full mechanical and biological purification (Fig. 38.6), as well as sterilization and oxygenation of water passed through the tanks with fish and possibly manifold reuse of water with minimal

requirement of extra "freshwater." The high cost of the equipment typically restricts application of such systems for production of Siberian sturgeon in regions, where favorable natural temperature regime allows to use less expensive production systems. Under this conditions it will be expedient to use small RAS (for prior to spawn holding rearing of mature brood fish, obtaining and incubation eggs, rearing of larvae and fry, especially out of the spring season) combined with cages and ponds and tanks with water supply from natural sources for rearing of Siberian sturgeon from fry to mature state.

Not surprisingly, RAS have the widest geographical distribution. This system is attractive in countries with a strong environmental regulation either on outlet water quality or on farming of non-native species, as illustrated in EU by France. This is also a good alternative to high production costs due to water temperature control in either Northern countries such as Norway (Dalsgaard et al. 2013) and Russia or at the opposite in Southern countries (Saudi Arabia or Thailand).

38.2.2 Initiation Period of the Siberian Sturgeon Farming (Till 2000)

We have mentioned that the former USSR and France have pioneered the farming of the species; therefore, both states' status are going to be detailed at first. Further, available data related to other countries are presented according to their spelling alphabetical order.

38.2.2.1 Russia

In the former USSR and later on in Russia, the largest sturgeon fish farms involved in rearing of Siberian sturgeon in cages and tanks were fish collective enterprise (kolkhoz) at Pavlopolsk water reservoir near Mariupol (Donetsk region) (Timoshenko and Popova 1985), Volgorechensk fish hatchery (Kostroma region), and sturgeon fish farm "Diana" at Cherepovets power station (Burtsev 2015).

Along with that, despite the successful start of experimental works in the USSR and elaboration of basic elements and temporal norms of Siberian sturgeon rearing in concrete tanks (under the direction of I. I. Smoljanov) and floating cages (under the direction of V.P. Mikheyev), during that period, the commercial rearing had not attained the wide development, and related research activity was not supported to the extent required. Some reasons of that are presented below.

First, as it was widely known, the maximum catches of sturgeon fishes in natural water bodies, foremost in the Caspian basin, were reported in the 1970s of the past century (above 27 thousand tons of sturgeon and on average 1700 tons of caviar in this region, which increased to about 2700 tons in the 1970s and then declined since the early 1980s) (Bourguignon 1989; Williot and Bourguignon 1991; Williot et al. 2001). That lead to stable and low prices on sturgeon meat and caviar on the domestic market, making unfavorable economical context for more extended engagement of acting state and collective fish farms into commercial rearing of Siberian sturgeon under lack of private fish farms.

Second, rearing of bester in Russia on a larger scale than Siberian sturgeon culturing was stipulated only by the feasibility of this hybrid rearing in earthen ponds on natural food resources or alternatively in small concrete tanks or cages with the use of coarse fish or made of it paste-like feeds. The ponds used for bester had been earlier constructed for carp rearing. Owing to this the dominated number of bester was reared in the vicinity of natural water bodies. The total production of the bester reared in the USSR in 1972 and in 1983 were 16 and 200 tons, respectively (Steffens et al. 1990).

In the late 1980s and early 1990s after the collapse of the USSR, the commercial stocks as well as official catches of sturgeon species in the Caspian basins declined sharply (Barannikova et al. 1995; Williot et al. 2002). Along with that, incremental rate of unregulated and unreported fishing of sturgeon, that dozen times exceeded the officially reported yield (Vaisman and Raymakers 2001), also stipulated the filling of the illicit market. Thus, despite the conditions of market-driven economy in states, former Soviet republics, the new established private or privatized ex-state sturgeon farms reared Siberian sturgeon only on a small scale, due to 1.5–2 times higher production cost of its rearing in comparison with the market price on sturgeon.

In view of that, the major part (above 75%) of Siberian sturgeon as distinct from bester were reared in intensive fish farms located long distance from the Caspian Sea and the Sea of Azov—in central and northern parts of Russia and in Siberian regions. Initially rearing was conducted in cages and tanks in warm-water farms at power stations. The farms activities had been financially supported by power-producing companies that allowed to offer Siberian sturgeon production at lower cost retail price. Those farms were not integrated into the system of Ministry of Fisheries of the Soviet Union; after privatization they were affiliated to joint-stock company "Aquatron" (Usenko 1990; Mamontov and Gepetskiy 2001).

During the 5-year period (1994–1999), the number of sturgeon farms in Russia increased several fold (from 19 to 70), that enabled 6 times increase in production up to 1200 tons in 1999 (Chebanov 2000). That rise occurred against the three times decrease in production volume of other cultured freshwater species. The share of Siberian sturgeon production was about one third of the total sturgeon production volume (Chebanov and Billard 2001), and more than 75% were produced in cages, 15% in tanks (raceways) of warm-waters farms, and only less than 3% in ponds (Chebanov 2000; Mamontov and Gepetsliy 2001). Vdovchenko and Rozhdestvenskiy (1999) reported that *A. baerii* cultured in geothermal brackish water in Siberia reached up to 2 kg in the first year. Abramenko (1999) described an example of this production system with the rearing of sturgeons in the waste-heat effluent of a pulp and paper plant in Arkhangelsk. In the 1990s the enhancement of Siberian sturgeon rearing in the USSR (as the world over) in addition to the mentioned above economic factors was stipulated as well by awareness of very successful R&D works on rearing and raising of this species in France and Russia, respectively (Williot and Rouault 1982; Williot and Brun 1982; Williot et al. 1988; Kozlov and Abramovich 1986; Shevtsova 1991).

38.2.2.2 France and Its Relative Place in the European Community (EC) and Further European Union (EU)

The start of Siberian sturgeon farming in France was initiated in the 1970s with the ongrowing trials of the first batch of juveniles of the species that arrived in France in 1975 (Barrucand et al. 1978). Thanks to attractive preliminary results on growth, food efficiency, and adaptability to unusual rearing conditions, the species then became potentially interesting for farming. The window has been completely opened with the successive two controlled reproduction of the fish in 1981 and 1982 (Williot and Rouault 1982; Williot and Brun 1982). At a time of these preliminary investigations, most of the rearing trials have been performed in raceways and the sturgeon were fed trout food. Along the 1990s rearing trials by private fish farmers were based on fingerlings produced by the research sector, eventually with the use of tap water at the water source. As a by-product of the experience gained, a sturgeon culture activity in France was accelerated in the 1990s, with the construction of a sturgeon hatchery which operated from 1991 onward, soon followed by the establishment of several new ongrowing units or by the changes of already operated fish farms from the primary species (mostly trout) toward the Siberian sturgeon. Those operations required a substantial financial placements as well as human resources. It is worth noting that the research sector transferred its know-how in sturgeon reproduction, management of brood fish, rearing of fingerlings, transferring well-identified brood fish originated from the Lena River population, and assistance to a private new established sturgeon hatchery. Some financial assistance was received from the European Union, the French government, the region of Aquitaine, and the various local provinces (Sabeau 1998). According to assessment in the early 1990s (Williot et al. 1993), the volume of the total annual production of the Siberian sturgeon in aquaculture was approximately 20 tons in France and 10 tons in Italy and Germany (Table 38.1). The last country that was initiating a Siberian sturgeon farming activity was Spain. One of the prerequisites for the Siberian sturgeon farming development has been the mastering of controlled reproduction with hormonal stimulation in France since 1981 (Williot and Rouault 1982), in Italy since 1992 (Arlati et Bronzi 1995), and in Germany—since 1993.

Review of the status of Siberian sturgeon commercial farming in France (in Aquitaine, South West of France) was provided by Sabeau (1998). In 1997 three enterprises were involved in ongrowing of Siberian sturgeon, and ten other ones were producing some small volumes of sturgeon meat (and caviar) as a supplement

Table 38.1 Most probable Siberian sturgeon production in the European Community (EEC) (after Williot et al. 1993)

Country	Hatchery (nb)	Grow-out farms (nb)	Yearly production (tons)	Installed capacity of production (tons)
France	1	6	20	200
Italy	1	3	10	30
Germany	–	3	10	20
Total	2	12	40	250

Table 38.2 Overall estimated production and standing stock biomass of Siberian sturgeon in European farms in 1996

Country	Hatchery (nb)	Major fattening plans (nb)	Yearly gross production (tons)	Total stock (tons)
France	2	4	100	200
Italy[a]	1	3	120	60
Austria[b]	1		10	
Germany	0	1	10	20
Poland	0	1	10	0
Denmark		1		
Hungary	1			
Total	5	10	250	280

Data are based on consultation (extracted from Bronzi et al. 1999)
[a]*A. baerii* and hybrids
[b]*A. baerii* and bester

to their prime production engagement. In the late 1990s as well, few programs were offered those supposed new sturgeon farm creation, conversion of traditional farms, and expansion of existing sites (Sabeau 1998). The former driver for the creation of ongrowing sturgeon fish farms has been the production and the marketing of sturgeon meat. During that period the consumption of sturgeon meat was regarded as a spin-off of caviar. Therefore, the producers decided to establish a collective structure GIE[2] "GIE L'Esturgeon d'Aquitaine" that was aiming at the propagation of information related to sturgeon and its products. The market for sturgeon meat was a newly established one with steady annual elevation from 15 tons (in 1993) to 300 tons (in 1997) (Sabeau 1998). The volume of Siberian sturgeon production in Aquitaine during that period had increased from 80 tons in 1994 till 150 tons in 1995 and 200 tons in 1996 (Jones, pers. com.). The overall estimated gross production and standing stock biomass of Siberian sturgeon and quantity of European farms in 1996 are shown in Table 38.2 (Bronzi et al. 1999). As compared with the former assessment (Williot et al. 1993), the number of producing countries increased as well as the yearly gross production. With regard to France, Sabeau (1998) and Jones (pers. com.) reported 3 ongrowing sturgeon farms and 10 farms producing small quantities. The discrepancies between the two assessments are mostly related to these 10 sturgeon farms reputed to produce small quantities. Even the explanation remains unknown; the example pointed out the necessity to define as precisely as possible the meaning of the words, e.g., which is the minimum volume of production for a sturgeon farm to be qualified as a hatchery?

The most significant change in Siberian sturgeon culture in the second half of the 1990s was the turn of the production from meat toward caviar because the market of meat did not respond as previously expected (Williot et al. 2017a, b). As a result, sturgeon farmers have been in need of liquid assets to support the constitution of

[2] GIE = Groupement d'Intérêt Economique.

brood stocks necessary to produce caviar, and then many financial structures of sturgeon fish farms changed as promoters have been unable to face with this abrupt and important demand in finances (Williot et al. 2001). The program was targeted on considerable volumes of caviar production (Sabeau 1998). In 1999 the number of plants involved in this activity in France increased till two hatcheries and six ongrowing farms, and annual production of the Siberian sturgeon reached to 200 tons of meat and 4 tons of caviar too (Williot et al. 2001).

The forecast for France, made in the year 1997 (Sabeau 1998), supposed (1) continuation of fresh sturgeon market development owing to intensification of its activity in the course of relevant awareness raising and creation of value-added products adapted to end-consumer habits and (2) development of market for "Aquitaine"-produced caviar via expanding the available that time ongrowing units with following improvement of caviar production techniques and by development of a specific image of "Aquitaine" caviar in contrast to one of wild origin.

The target volumes of annual sturgeon production for 2007, forecasted in 1997 (in one decade), were 1000—2000 tons of fresh meat and 10—15 tons of caviar (Sabeau 1998). This prognosis was made far too optimistic in considering not operating but designed production facilities. Siberian sturgeon along with white sturgeon (*A. transmontanus*) had been one of the two most commonly sturgeon species bred in captivity for meat sturgeon species in Western Europe. Captive breeding of *A. baerii* for caviar has been limited to France, with the majority of caviar being consumed within the European Union (EU) market. In 1997 capacity of sturgeon meat production amounted in Europe to 900 tons (Bronzi et al. 1999), 35% of that was the share of Siberian sturgeon.

38.2.2.3 Armenia

The experimental works on farming of Siberian sturgeon in ponds and raceways have been carried out in Armenia Republic since 1981 (Mailyan and Akopyan 1984). Many trout farms initiated rearing of sturgeon fish with the use of artesian water (of 13–19 °C temperature) firstly in small amounts just for assortment and only after 2000—in some considerably larger amounts.

38.2.2.4 Belarus

In April 1998 in Belarus, the progeny from the Siberian sturgeon (at the fifth year of life) was obtained for the first time at a collective farm in Minsk region.

38.2.2.5 Estonia

In the late 1970s, Siberian sturgeon culture was initiated in Estonia (that time a republic of the USSR); sturgeon stocking material (eggs and fry) for Estonian farms was delivered from the sturgeon farm in Konakovo, Russian Federation. Siberian sturgeon in Estonia was reared primarily in cages and raceways (Bronzi et al. 1999). In the first large warm-water fish farm at Estonian power station (in Narva), the females of Siberian sturgeon (progeny of 1977) reached maturity in 1983 (S. Podushka pers. com.).

The annual production of one recirculating farm which had operated from 1990 to 1996 was 2–8 tons of 2–4 kg sturgeon specimens. The fish reached this size in 18–20 months. By 1997, only a few remaining specimens of Siberian sturgeon were kept in three fish farms, and commercial production had ceased (Paaver 1999).

38.2.2.6 Germany

The sturgeon culture initially included pilot programs in German Democratic Republic in the late 1960s (Steffens et al. 1990; Müler 1992), using eggs of bester hybrid imported from the Soviet Union. The first successful reproduction of bester (F2) hybrid was attained in 1981. In Germany a number of fish enterprises had developed culture systems for several sturgeon species. These were performed either on a trial basis or on a small commercial scale. Sturgeons had been reared in both flow-through and RAS, utilizing (when it was applicable) waste heated water from power stations (one unit were located in Saxony) or other sources to diminish the costs of production. One farm in Schleswig-Holstein, operational unit at the end of 1997, circular deep tanks (of 4 m diameter), and self-feeders were used, although the success had not been so promising. Experimental units exist with flow-through systems at the coast of Mecklenburg-Vorpommern, holding some Siberian sturgeon (Bronzi et al. 1999). According to estimate of Williot et al. (1993), the volume of the total annual farming production of the Siberian sturgeon in two farms of Germany was approximately 10 tons, with total stock of about 20 tons. In 1999 the number of plants in Germany involved in this activity increased to three hatcheries and four ongrowing farms, and annual production of the Siberian sturgeon reached to 30 tons (Williot et al. 2001).

38.2.2.7 Greece

Starting of sturgeon farming in Greece dated from 1996 with the importation of 20,000 fertilized eggs of *A. baerii* that had been imported from Russian Federation. *A. baerii* specimens reared for controlled reproduction reach first maturity after 6–7 years in controlled conditions. Ten ongrowing sturgeon farms in Greece (Paschos et al. 2008) have been established and comprise three types: flow-through system farms with earthen ponds, flow-through system farms with rectangular concrete tanks (raceways), and farms with closed recirculation systems (previously used for eel rearing. One farm was established in 1995, three in 1998, one in 1999, and five rainbow trout farms rear sturgeons intermittently, when market demand for sturgeon fingerlings is high. Currently, only one large-scale farm (established in 1998) is in continuous operation. The remaining nine farms operate on an occasional basis, depending on market demands, fry availability, and the economic situation (Paschos et al. 2008). According to available data, Siberian sturgeon reaches a minimum marketable size of 1.5–2 kg within less than 18 months. At this not less than 70% of Siberian sturgeon specimens after 18–20 months of holding reach 2.5 kg weight in earthen ponds, 3.1 kg in flow-through concrete raceways, and 3.4 kg in circular tanks of RAS—(Paschos et al. 2008).

38.2.2.8 Italy

In Italy, the first experimental rearing of sturgeon took place in the early 1980s in the Lombardy region, in 1977 with *A. naccarii* at the Orzinuovi fish farm and in 1981 with the *A. transmontanus* at warm-water (steel factory effluent) fish farm in Calvisano (Bronzi et al. 1999). According to Arlati et al. (1988), the Siberian sturgeon, *A. baerii*, is mentioned for the first time as the support of a "nonsurgical collection of gametes through a laparotomy" in 1992. It is worth noting that a laparotomy cannot be considered as a nonsurgical technique.

The Siberian sturgeon seems to have properly adapted to environmental conditions of Italian Peninsula, of which inland water bodies are relatively cold (temperature 14–18 °C). Each farm in Italy is typically engaged in the production of more than one sturgeon species. And the Siberian sturgeon (*A. baerii*) is one of the preferable products. This type of farming in Italy, initially originated in Lombardia region, has been successfully expanding to other Italian provinces. Sturgeons are typically reared in raceways or ponds, primarily within the monoculture. Further trials were conducted in mostly closed recirculation systems and in floating cages. Nowadays the large hatcheries and fish farms, engaged in sturgeon culture, are located in one region (Lombardy). Above nine tenth of the whole Italian sturgeon production were concentrated there (Arlati and Bronzi 1995). In 1995 the annual sturgeon production in Italy was about 380 tons, with 7.5% of Siberian sturgeon and its hybrid. The estimated total production was obtained from 15 enterprises, comprising 11 ones involved in production, including 3 sturgeon hatcheries and 4 experimental plants (Arlati and Bronzi 1995). In 1999 (Williot et al. 2001), the number of plants involved in rearing Siberian sturgeon in Italy increased till two hatcheries and five ongrowing farms, and annual production of this species and its hybrids reached to 140 tons. There is an evident inconsistency in Table 38.2 that is related to Italy. For a sturgeon meat production, the stock (60 t) cannot be far lower than the yearly gross production (120 tons) as mentioned for this country.

38.2.2.9 Hungary

As indicated by Pinter (1991), introduction of Siberian sturgeon in aquaculture was started in Hungary in 1982 by the Fish Culture Research Institute at Szarvas (now is HAKI). Domestic brood stock has been raised successfully under intensive conditions (in small ponds) till maturation of females in 1987 (Ronyai et al. 1991). In the following years, different trials and comparative analysis are being carried out in Hungary not only with Siberian sturgeon but with some of its hybrids (Ronyai et al. 1991). During our (MC) several technical visits to Hungary's Ministry of Agriculture in 1993–1996, not less than five farms including a hatchery, small private and state warm-water farms at power station, and new RAS (Hungary-Norwegian joint venture "Propagen International") were involved in Siberian sturgeon farming. Along with this the total volume of Siberian sturgeon rearing in Hungary during that period was not considerable and amounted to 20 tons or less.

38.2.2.10 Japan

In Japan as it has been mentioned above, 500 fingerlings of *A. baerii* were introduced into aquaculture from the Soviet Union in October 1964 (Sajto 1970; Gribanova 1970; Fujii and Maruyama 1997). During 5 years of rearing in the circular tanks, fish reached the weight of 7 kg. In 1969 eggs in vivo from several females after 8 (each 5 days) injections of synachorine at a dose of 50 rabbit units via laparotomy were obtained (with further suturing of the abdominal cavity) (Gribanova 1970; Sajto 1970; Katsumi and Genjiroi 1977; Shevtsova 1991). Along with that, till 2000 commercial Siberian sturgeon farming had not been developed to wide scale in Japan, only experimental character of rearing this species was observed.

38.2.2.11 Moldova

In Moldova (that time it was a republic of the USSR) the commercial cultivation of the Siberian sturgeon was started in the late 1970s (Lobchenko and Vedrashko 1978; Vedrashko 1981; Sokolov et al. 1982). The species seems to be no longer of interest in the country as one of the main sturgeon producers did not mention the species as one of the reared and marketed sturgeon species (Aquatir Ltd. 2011).

38.2.2.12 Poland

In Poland Siberian sturgeon was cultured on an experimental basis in floating cages; those were installed directly in the discharge canal of a thermal power station. In 1999 the number of plants in Poland involved in rearing of Siberian sturgeon increased to one hatchery and five ongrowing farms, and annual production of the Siberian sturgeon and its hybrids reached to 30 tons (Williot et al. 2001).

38.2.3 Developmental Period (From 2000 Onward)

From the turn of the millennium, very deep changes have been occurring in the sturgeon farming and its related products. This is especially spectacular in Russia, in China, and, to a lesser extent, in some other countries of which some are newcomers. Before providing a world assessment of the production at the end of the paragraph, country-specific characteristics of the sturgeon production are presented in the following order: Russia, China, Western and Central Europe, and other countries for which we have some detailed information.

38.2.3.1 Russia

Not only the sturgeon production in Russia has very rapidly increased but also a new caviar processing has been generalizing.

38.2.3.1.1 Drivers of the Changes

In the year 2000, commercial catch of sturgeon was prohibited in the Sea of Azov basin and considerably restricted in the Caspian basin, due to catastrophic depletion in sturgeon stocks in natural water bodies. The breeders still captured in the sea

were intended exclusively for controlled reproduction at state sturgeon hatcheries and scientific purposes. The market demand and relative stabilization of economy had stimulated further intensive development of sturgeon culture in Russia (Chebanov and Billard 2001; Chebanov et al. 2006).

The severe shortage of caviar at the market had created very favorable conditions for farmed caviar-oriented companies. Along with that, as it was mentioned earlier, during that period, primarily warm-water farms at power works had been developed, and production of Siberian sturgeon, the species with the best studied gametogenesis, had raised considerably. However, there was a methodical problem associated with necessity of speedy, reliable, and noninvasive sexing method of Siberian sturgeon at early age (1.5–1.8 years), when the weight of fish reached 1.7–2.0 kg (the minimal market requirement). The lack of such techniques did not allow to conduct early culling of males for meat and rearing of separated females for caviar production, with the optimal use of feeding and water temperature regimes to accelerate the gametogenesis. Moreover, as known (Doroshov et al. 1997; Chebanov et al. 2006) the heterochrony of female puberty is observed at sturgeon farms (always), even when the females are the progeny of one family. Range of female ages at the first maturation can reach up to 9 years, with optimum utilization of abovementioned factors, while 6–10 years is needed in current conditions (Williot and Sabeau 1999). That was especially urgent for countries of the ex-USSR and Asian states with restricted feasibilities to gain long-term loans. Elaboration and wide implementation of express techniques of early sex and maturity stage determination (better known as echography technique) for Siberian sturgeon in the late 1990s (Chebanov and Chmyr 2002, 2005; Bonpunt 2006) and selection of early maturing females at a weight of 2–2.5 kg and regular noninvasive monitoring of reproductive system in females (Chebanov and Galich 2009, 2010, 2011a, b; Chap. 49) allowed to optimize management of sturgeon brood stock and significantly stimulated development of caviar-oriented production of Siberian sturgeon in Russia, China, and many other countries around the globe (Bronzi et al. 2009; Bronzi and Rosenthal 2011).

Caviar Produced from Ovulated Eggs

In Russia and the CIS[3] countries, the production of caviar from Siberian sturgeon in aquaculture has been developed since the late 1990s; herein the most widespread has been a business model, based on repeated intravital extraction of eggs from females and caviar production from ovulated eggs, as the most economically appropriate for Russia and CIS countries (as well as Latvia and some other countries), that allows to produce caviar at least five times from the same female. This technology has been used by the most currently functioning in Russia sturgeon enterprises, despite its key disadvantages (in terms of food production) as follows: the need to use natural hormonal preparations or synthetic superactive analogues of gonaditropin-releasing hormones (GnRHa) to stimulate eggs ovulation (Chebanov and Galich 2011); and necessity to strengthen egg membranes through some thermal or chemical process.

[3] CIS = Community of Independent States.

According to Podushka (1999) at the end of the past century in Russia, about 20 sturgeon farms were engaged in the production of ovulated eggs. The first industrial batch of ovulated eggs amounted to 200 kg was produced in 2004 at specialized processing enterprise OAO NPP (OJSC SPE) "Kasprybtsentr" (Podushka et al. 2005).

At present (in 2016), in Russia there have been in operation six major (with capacity range from 1 to 10 tons) manufacturers of Siberian sturgeon caviar and more than ten medium-sized producers (their capacity range—0.3–0.9 tons); only one third of the caviar are produced with the use of slaughtering technique. In addition to major manufacturers in Russia, there have been more than 100 small farms that have no caviar production facilities of their own, but pass the obtained intravital ovulated eggs its processing (from few dozens to few hundreds of kilogram annually) to the caviar processing plants. As a complement to sturgeon farms, there have been several companies engaged in caviar production that do not have their own fish sturgeon units, but are specialized in the processing caviar from ovulated eggs.

The eggs after their storage in the frozen condition (ranged from 50 to 500 kg from each farm) were delivered to four major companies ("Aqualife," Taganrog; Kasprybtestcenter, Astrakhan; Gosrybcenter, Tyumen; "Varex" and VNIRO, Moscow). These specialized enterprises have been certified properly and possess "know-how" (or patents) and technologists, as well as facilities (units) for pasteurized caviar production with packaging in glass cans. The scheme described has proved its expedience and efficacy for small sturgeon farms. In the early 2000s, the overall capacity of produced pasteurized caviar of Siberian sturgeon (from ovulated eggs) in Russia was at 100 tons level (Chebanov and Billard 2001).

Fundamentals of food production from ovulated sturgeon eggs were elaborated by Russian experts in 1990s (Podushka 1986, 2016; Podushka et al. 1990, 2008). Currently, only in Russia, not less than five patents (Kalinichenko et al. 2006) and several authors' certificates (inventions) in the field of ovulated egg processing for human consumption purpose have been registered lately in Russia. The processes imply various ways and thermal regimes of caviar processing before the salting (Kopylenko 2006; Kopylenko and Koryazova 2003, 2004). The key problems, which have been addressed by such inventions and technological improvements, include exclusion of egg adhesiveness, prevention of eggs destruction after the salting (ovulated eggs have considerably weaker membrane), and increase of shelf life of end products. The former problem has been addressed with the use of different schemes of thermal action, which provides protein coagulation of egg membranes and as a result their compressing. There also exists a method of egg membranes strengthening through the application of tannins, but on the downside of this method is the change in taste of the caviar that can attain astringent flavor, which disappears in the course of storage (Astakhova 2012). The longer shelf life of caviar produced from ovulated eggs is reached either with the application of preserving agents or by caviar pasteurization. At early stages, sodium tetraborate (borax) has been most widely used as a preservative for the traditional (obtained from slaughtered females) eggs. Currently, this chemical has been included in the list of CRM (carcinogenic, mutagenic, and toxic for reproduction substances) in accordance with Appendix 1 to directives 67/548/EWG, TRGS 905, and TRGS 906 (Novikov 2016). In Russia,

borax has been excluded from the list of permitted food additives for the manufacture of food products (SanPiN 2.3.2.1293-03). At present, other safer preservatives have been used. In Russia their list includes complex food additives LIV-1 and LIV-2 and Varex, made on the basis of sorbic acid, which have been widely used around the globe and possess among other the preservative effect and ensure long-term storage of caviar at positive temperatures (Gromova 2004a, b, 2005, 2016). Another way to increase the shelf life of caviar has been pasteurization of caviar in the process of salting or after packaging. Currently, the predominant part of the food products, made from ovulated eggs, is produced using this method. The shelf life of pasteurized caviar usually amounted to 9 months, in particular cases—up to 24 months, the storage of pasteurized caviar is recommended at temperatures below freezing (from −2 to −4 °C). Along with this, it is considered that long-term heat treatment and prolonged contact with the liquid at stages of caviar processing and salting are resulting in the leaching of nutrients, which adversely affects the taste of the final product.

In recent years, improved techniques of caviar processing and salting have been developed. Some of them are aimed on the considerable reduction in processing time and low temperature regime (Koryazova and Kopylenko 2014), providing long-term (2.5 months) storage of unpasteurized eggs without application of preserving agents.

The alternative direction comprises methods, elaborated in Germany and patented in the EU countries (and many others). These methods are based on the application of biological properties of sturgeon ovulated eggs and imply exposure to signaling molecules (instead of heat treatment or chemical affecting) to fix the membranes (Köhler 2007; Köhler and Ziegler 2016). This approach allows extending the shelf life up to 9 months without the use of preservatives. For instance, the production of such caviar has been preplanned at the new sturgeon farm in Switzerland.

It should be noted that in June 2016 in the Draft Code of Practice for Processing of Sturgeon Caviar, elaborated in the frame of Joint Food Standards Programme *Codex Alimentarius*, presented in the Report of the Codex Committee on Fish and Fishery Products (Rome, Italy, 27 June—1 July 2016), it was allowed to subsume to category "Caviar" the production of caviar from ovulated eggs by induction of ovulation using natural means as well as by the use of authorized products (Report of the Codex Committee on Fish and Fishery Products 2015).

Assessment of Production in Russia

In 2000, farming production of sturgeon in Russia consisted roughly of *A. baerii*,—30%; *A. gueldenstaedtii*,—30%; *A. ruthenus*,—5%; bester,—10%; and hybrid *A. gueldenstaedtii* × *A. baerii*,—nearly 20% (Chebanov and Billard 2001). Then the annual production of Siberian sturgeon in Russia reached to 500 tons (Chebanov 2000), in 2003, till 750 tons (Williot et al. 2005), and more than 80% of this volume was produced in warm-water fish farm at power station.

Table 38.3 Eight-year changes in some data on the production of commercial farming of Siberian sturgeon in Russia (Chebanov et al. 2006, 2008, 2009; Chebanov 2016)

Year	2006	2009	2010	2011	2012	2013	2014	2015
Gross production (10^3 tons)	0.8	1.0	1.1	1.2	1.3	1.4	1.8	1.8
Caviar produced from ovulated eggs (tons)	3	4	6	8	9.6	8.7	12	19*
Number of sturgeon fattening plants/number of plants involved in caviar production	100/8	100/10	120/10	150/14	170/14	180/15	210/18	230/20

In 2015 the relative species gross production indicative (in %) was as follows: sterlet,—35; Siberian sturgeon,—30; hybrid of Russian and Siberian sturgeons,—18; bester,—3; Russian sturgeon,—8; and the balance—beluga, stellate sturgeon, kaluga, Amur sturgeons, and other hybrids—6. The corresponding caviar production was Siberian sturgeon, 46; sterlet,—30; hybrid of Russian and Siberian sturgeons,—15; bester,—2; Russian sturgeon,—5; and beluga, Amur sturgeon, and other hybrids,—residual 2.

Recent changes in production and economics of the farming of Siberian sturgeon in Russia for the period 2006–2015 (Chebanov et al. 2006, 2008, 2009; Chebanov 2016) are presented in Table 38.3. Over the considered 10-year period, the production of Siberian sturgeon in Russia had raised more than two times from 800 to 1800 tons. In the meantime, the production of caviar increased more than six times and in 2015 reached to19 t. At this caviar production (till 2014), practically all of them were obtained from ovulated eggs. The number of farms involved in rearing of Siberian sturgeon increased considerably from 100 (for meat) and 8 (for caviar) in 2006 to 230 (for meat) and 20 (for caviar) in 2015.

Most recently (in 2015), a subsidy mechanism was firstly applied in Russia for loans, intended, namely, for aquaculture enterprises (from large fish farms to small subsistence ponds) engaged in particular in sturgeon farming. This allowed initiating a federal program for sturgeon farming development.

38.2.3.2 China

The production of farmed sturgeon in China leapt from 100 tons in 1998 to 2000 tons in the year 2000. And the trend has been continuing as sturgeon culture and trade in China have proven to be a rapidly developing industry. According to one of the very last completed surveys (Wei et al. 2011), more than 13 sturgeon species or hybrids were farmed in China, including *A. baerii* as one of the most important cultured sturgeon species. China became the world's largest producer of sturgeon meat and caviar

since early 2000 (Wei et al. 2004). In China, the first successful reproduction from farmed Siberian sturgeon brood fish dates to 2000 (Wei et al. 2011). Siberian sturgeon has become the dominant cultured species since at least 2002; its share was had been raised from 42% in 2009 (Wei et al. 2011) to 34% in 2012 (Shen et al. 2014). Siberian sturgeon has acquired good reputation for its vigorous growth and endurance to stresses associated with transportation and relatively good resistance to diseases. In 2006 total production of cultured sturgeon in China amounted to 17,424 tons; during that period the overall world production (both of wild captured and produced in aquaculture fish) excluding China was estimated to be only 4835 tons (FAO Fishstat Database 2007).

China's sturgeon culture persistently proceeded to achieve new records, according to official data as follows: 10,871 tons in 2003, 11,269 tons in 2004, 15,407 tons in 2005, 17,424 tons in 2006 (80% of the global production), and 12,862 tons in 2007 (FAO Fishstat Database 2007). It is worth noting that the two last records for 2006 and 2007 showing a dramatic decrease of circa 26% in the production are questionable. Not only there is no explanation to support the abrupt decline, but also the figure is not in agreement with another source of official data reported by Shen et al. (2014) that show an increase between 2006 and 2007 (Fig. 38.3). Which could be the origin (s) of this discrepancy?

The mentioned above figures seem to be more optimistic, than the prior made forecast, supposed that the annual total production in China should be rather stable at around 12,000 tons in 2004 and 2006.

In 2009 a steady increase in the production of farmed sturgeon was reported—21,000 tons (Wei et al. 2011).

The aforesaid values should be treated as more promising, than the prior made forecasts, those supposed rather stable level (about 12,000 tons in 2004 and 2006) of the annual production in China. In the early 2000s, commercial fish farms were distributed along the central and southern regions of China, namely, in Shandong, Hubei, Sichuan (including Chongqing), Guangdong, Guangxi, and Fujian provinces as illustrated by the respective mean number of future brood fish per farm by province (Table 38.4). Besides, brood stock culture and controlled reproduction were centered primarily in northern part of China: in Beijing, Liaoning, and Heilongjiang provinces. The sturgeon farms engaged in caviar production were foremost located in the provinces of Hunan, Hubei, Yunnan, Zhejiang, and Sichuan (Li et al. 2009).

Subsequent years, sturgeon farms were present in almost every province (except Xizang) and are concentrated mainly in Hubei (20%), Shandong (17%), Sichuan and Chongqing (15%), and Beijing (15%), accounting for 86% of the national sturgeon capacity (Shen et al. 2014). In the late 2000s, the relative part of the gross production for the Siberian sturgeon was 42% while that of caviar amounted to 40% (Wei et al. 2011).

Fertilized eggs obtained from controlled reproduction, according to estimates, accounted for 42, 54, and 60 million pieces in 2007, 2008, and 2009, respectively, whereas the supply of seeds from wild-captured fish had declined considerably (estimated 5, 4, and 3 million pieces from 2007 to 2009, respectively).

Table 38.4 Quantity (number of captive unsexed specimens of brood fish) of Siberian sturgeon maintained in the surveyed 83 sturgeon farms according to provinces (Li et al. 2009)

Province of China	Number of farms surveyed	*A. baerii* (nb)
Beijing	10	5500 ± 80
Guangdong	10	500 ± 20
Guizhou	5	1000 ± 35
Hebei	3	1350 ± 32
Hubei	17	1000 ± 35
Jiangsu	4	–
Shandong	10	1300 ± 24
Sichuan	10	1000 ± 22
Zhejiang	7	3000 ± 38
Heilongjiang	2	–
Fujian	5	1000 ± 20
Total	83	15,650 ± 100

The import of seeds had also decreased, and only fertilized eggs of Siberian sturgeon were imported during out-of-season periods. During the period from 2007 to 2009 the quantity of imported fertilized eggs decreased from 20 to 17 million pieces, while the amount of imported larvae increased from 8 to 10 million ones (Wei et al. 2011).

In 2014–2015 the total volume of Siberian sturgeon rearing in China at 120 farms amounted to about 17,000 tons gross production–24 tons of caviar within mostly cages and cement ponds with running water (Shen et al. 2014).

38.2.3.3 Western and Central Europe

France Within Western Europe

In 1999, sturgeon gross production from aquaculture in Western Europe was approximately 1,300 tons with an increasing trend. The Siberian sturgeon is the second sturgeon species by the volume of production (34%); the first position with 43% of the whole is due to the white sturgeon mainly, thanks to a large farm in Italy. In the early 2000s, the number of fish enterprises within Western Europe engaged in Siberian sturgeon farming amounted to 25 (Table 38.5) including 12 hatcheries (Williot 2000; Williot et al. 2001).

The wide scope of meat production should be highlighted, from fresh whole fish, (more than 200 tons) and caviar production was at 5 tons level in 2000. France is then the principal producer of farmed caviar in Europe (Williot et al. 2001; Jones 2001).

The dynamics of Siberian sturgeon caviar production in France during the period 1997–2014 is shown on Fig. 38.15. The figure shows a general increasing trend with two intense periods of growth, 1997–2001 and 2003–2007 separated by a 2-year plateau. A third increasing period exhibits a more irregular feature from 2009 onward. The two increasing phases are the results of previous investments realized 7–8 years before to increase the production potential.

Table 38.5 Estimate for 1999 of Siberian sturgeon meat and caviar production, depending on countries in Western Europe (modified after Williot et al. 2001)

Country	Plants (no.) Hatchery/ ongrowing	Yearly gross production (t)	Yearly production of caviar (kg)
Austria	2/2	5	–
Belgium	1/1	15	–
France[a]	2/6	200	4000
Germany[b]	3/4	30	Forecast
Italy (A.b. & hyb.)	2/5	140	–
Poland (A.b. & hyb.)	1/5	50	–
Spain	1/1	10	–
The Netherlands[b]	0/1	–	–
Total	**12/25**	**450**	**4000**

[a]One farm supplying the pet fish market
[b]Potential production not included

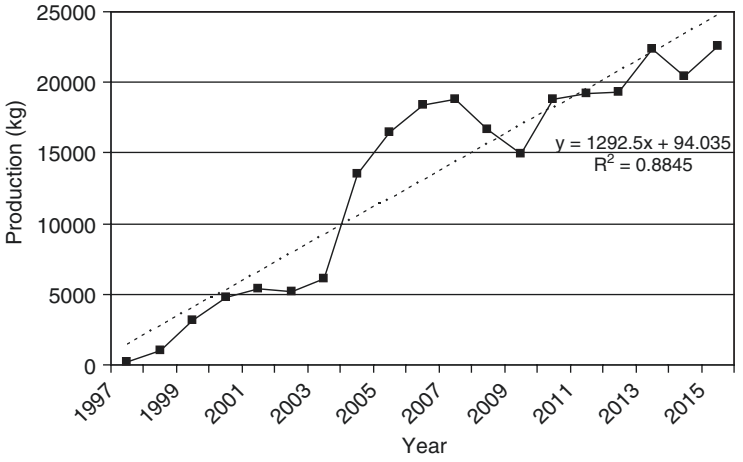

Fig. 38.15 Changes in the volume of production of caviar of Siberian sturgeon in France over the period 1997–2015 (sources: SFAM 2016)

This has corresponded to either the expansion of already existed farms or to the installation of new ongrowing sturgeon farms. It is worth noting the pronounced decreasing (2-year) period (2007–2009) that corresponds to the first notable crisis in world caviar market. In fact the fish farmers did not produce caviar as they were not stimulated by the demand of the market. This led to the following consequences. The volume of brood stock increased greatly of which the main part was not any more productive for at least the following 2 years due to the most probable recurring interval 2 years between two oogeneses (Chap. 26).

This would have generated crucial arbitrage within husbandry management as other younger cohorts were growing. Fortunately unsold females did not die even though they were not allowed to achieve their oogenesis. However, were there enough rearing structures to hold these unsold brood fish? Were there possibilities to build new structures? Were there enough financial resources to launch new investments in a recession period? The very last trend for the period is a new abrupt increase of the production. About 60% of the French caviar production is supplying the national market through wholesaler (SFAM), but this national market is extremely competitive. As the yearly data of meat production are available, both caviar and meat data were plotted on the same graph (Fig. 38.16). Data on meat represent the yearly production of meat from the caviar-producing females together with that of the 2–3 year old males, slaughtered at age, when sex identification became possible by using echography (Chap. 49). The 12-year (2004–2015) production of caviar exhibits an increasing trend with a slope two times less than in the previous similar graph with a high correlation coefficient even lower that of the one on the whole period (1997–2015) (Fig. 38.16). The regression line for the meat production shows a very low slope and a low correlation coefficient. Thus and surprisingly, this means that the two curves were diverging over the period of time. In other

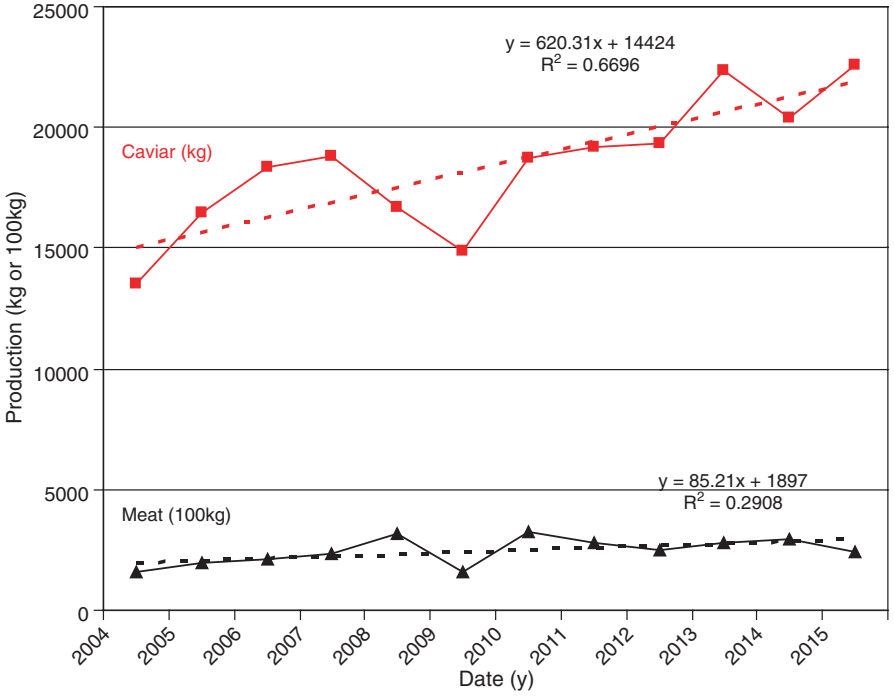

Fig. 38.16 Changes in the volume of production of both meat and caviar of Siberian sturgeon in France over the period 2004–2015 (sources: SFAM 2016)

words, the quantity of produced caviar has increased more rapidly than the produced meat. One of the reasons for that divergence could be a better constant effectiveness in managing the sturgeon stocks; females are exploited at a younger age as possible as well as for males. Whether the observation is stable over the time, further explanations must be searched for. More, not only both curves are diverging, but the ratio (caviar/meat) computed over the period 2004–2015 shows an apparent random variation from 0.05 to 0.095 (not shown). Again, a more in-depth analysis by the sturgeon farmers is needed.

Apart from the Siberian sturgeon which is the key sturgeon species in France, it is interesting to mention that new production is occurring, e.g., new species (*A. gueldenstaedtii*) in RAS. Similarly, there is a rearing trial with the beluga (*Huso huso*). This was made possible because the French legislation has become more flexible with the possibility of rearing non-native species in case they are reared in RAS to avoid any risks of escapement in the wild. So doing, this makes the competition between European countries more equilibrated for French sturgeon farmers.

Belarus

Before 2007 only private enterprises had been involved in rearing of commercial sturgeons. In 2007, the state fish enterprise "Selets" in Brest region started a commercial production of Siberian sturgeon. And the same year, for the first time, progenies of Siberian sturgeon were obtained in Belarus (Mamedov 2005; Mamedov and Lashkevich 2007). According to data of Konchits and Mamedov (2008), the overall capacity of commercial sturgeon production in Belarus amounted to 28 tons in 2007 and to 49.5 tons in 2008. The volume of sturgeons reared in 2007 by state enterprises amounted to 32.7 tons, 14.2 t (that is to say 43%) of which was due to the Siberian sturgeon. The total annual production of Siberian sturgeon during the period 2004–2007 was estimated as 20 tons (Mamedov 2005; Konchits 2008).

In 2008, eight enterprises had been involved in sturgeon farming, including five state ones (primarily pond) and three private ones. A few sturgeon species are cultivated in Belarus, the Siberian sturgeon (Lena population) and two hybrids, the GUBA (*A. gueldenstaedtii* × *A. baerii*) (Chap. 39) and the bester (*H. huso* × *A. ruthenus*). Three state farms have a brood stock (Konchits 2008). The private farms had been using the RAS intensive technology. According to data of FISHSTAT, the volume of Siberian sturgeon production in Belarus during the period 2011–2014 was rather stable and amounted to 74 tons (in 2011), 67 tons (in 2012), 43 tons (in 2013), and 83 tons (in 2014).

In 2016, seven farms were engaged in production of Siberian sturgeon: three RAS with an overall capacity of 30–50 tons, two cage farms supplied with warm water from power stations with a 40 tons production capacity, and two pond culture with a capacity of nearly 40 tons. Moreover, two RAS conducted extended holding of Siberian sturgeon females for further slaughtering, with total volume about 20 tons. Certified canned caviar of Siberian sturgeon has been produced by three farms (two RAS ones with production volume of 1 ton and one cage farm that produces 100-200

kg of caviar from ovulated eggs) (N. Barulin's pers. com). The limiting factor of sturgeon farming in Belarus Republic has been insufficient demand on this luxury product by the domestic market. In the mid-2010s, the annual sturgeon production, primarily of Siberian sturgeon and sterlet (*A. ruthenus*), amounted to over 300 tons, with some exports to Ukraine and Russia.

Belgium

In Belgium, rearing of Siberian sturgeon in RAS started in 1990, and in 1999 it already amounted to 15 tons (Williot et al. 2001) and in 2014 40 tons. In 2001 the Siberian sturgeon caviar was produced for the first time and in 2014 reached to about 1 tons.

Estonia

The private cage farm at warm channel at Estonian power station (Tartu) resumed its operation in 2004. In 2009, 120 tons of sturgeon was produced. In 2010 all the fish died during summer period. In 2012 a new project was initiated, with pre-planned capacity of 50–100 tons of Siberian sturgeon gross production and 10 tons of caviar. But due to very small volume of sturgeon market in Estonia, while the European market has been saturated, and due to economic sanctions of EU and contra-sanctions of Russia since 2015, export restrictions took place and similar projects were stopped in Estonia.

Finland

Currently in Finland only one fish farm (Carelian Caviar Co.) has been involved in Siberian sturgeon production since 2006. This RAS-type farm has primarily focused on caviar, but it also produces some amount of meat. The first caviar was obtained in 2010. The estimated capacity of production of Siberian sturgeon gross production for the year 2016 has been 45 tons and 3.8 tons of caviar. The expectations for 2017 amount to 50 t and 4.5 tons, respectively (pers. com. P. Hannelin).

Germany

In Germany, market demand for sturgeon products tended to be restricted in the context of prices varying over a greater range than that in Italy, depending on the type of product. Smoked sturgeon fillet was of high demand in Italy, but offer was inconsistent, that hampered the establishment of constant group of consumers for this product (Bronzi et al. 1999; Williot et al. 2005).

Firstly caviar in RAS (Produced by UFT, United Food Technology AG) in amount of 200 kg was produced in Germany in 2001. In 2003 the volume of production of Siberian sturgeon amounted to 20 tons, for a production of caviar about 1 ton, while in 2004,—volume of this species rearing was 30 tons. In 2007 Siberian sturgeon was reared at nine farms (five of them were RAS) with capacity about 100 tons, primarily for domestic market. In 2014–2015 the number of farms for rearing Siberian sturgeon ranged from 12 (Peter Steinbach pers. com.) to 17 (P. Gross' pers. com.) with overall production volume up to 300 tons, while 11 companies were

involved in caviar production with total volume of about 19 tons (P. Steinbach pers. com.). It is worth noting the somewhat large difference in the assessment of the number of farms (12 and 17) depending on the origin of the information. Obviously those two people were trustworthy. Is it the fact that the assessment relies on a 2-year period that could be responsible for such a gap? This is another illustration for the difficulties for achieving the aim of the present chapter. Some part of mature females was supplied from other fish farms from Germany and from other countries (France, Poland, and others) for processing and caviar production. According to some estimates (P. Steinbach pers. com.), these transfers accounted for a yearly amount up to 40–60 tons of meat and 3–5 tons of caviar to local German rearing of Siberian sturgeon and caviar production.

The main production systems have been ponds (not less than 15) of small area (P. Gross's pers. com.) and RAS (not less than 6 large ones) (J. Gessner's pers. com.). Main production system consists in combining RAS with small ponds or flow-through raceways. The fish are held in these systems up to 1.5–2 kg average weight, being safe from getting predated from birds after getting such weight. Quite a number of specimens are then transferred to ponds (mainly those deep for carps). When the fish get ready for harvest, in case of males at 3–6 kg and females—at 8–18 kg, they are taken out of the ponds. The males are destined for processing or angling. The females are brought into raceways (primarily) with clean water and kept there for at least 6 weeks. The half of the fish (50–60%) intended for caviar are grown using such technology. According to J. Gessner (pers. com.), at least 26 out of the 30 farms involved in production of sturgeon species also produce Siberian sturgeon. Actually, the caviar production has been more strongly concentrated (may be eight to ten producers), but officially there have been only five licensed producers/packers of caviar registered.

Overall production capacity of sturgeon farms was estimated at 500 tons (Peter Gross' pers. com.). At present it is difficult to provide accurate estimate of Siberian sturgeon production in Germany, due to the fact that large producers have been purchasing stocking material from small farms, including foreign ones (France, Hungary, Poland, etc.). In this regard, P. Steinbach (pers. comm.) estimated the total volume of Siberian sturgeon production in Germany as 140–180 tons (gross production) and 12–14 tons (caviar).

It is "Desietra" (with warm water) that has been the largest sturgeon RAS farm left, and there are some farms (three carp pond farms, one trout farm) located in Eastern Germany that are working together under the name of Saxenstör. In Southern Germany there are about five smaller farms (involved in carp and trout raising). In the north there are 2 farms: one of them keeps about 80 tons brood fish for own purpose and grow-out for "Desietra" and another one—about 40 tons of breeders for "Desietra" and "Diekmann and Hansen" company. Both harvest the males for need of these farms (P. Steinbach's pers. com.). A specialized farm is the one owned by Peter Gross (Frankfurt), that produces several million of fertilized eggs for export. According to Peter Gross (pers. com.), a price of 4 €/kg for sturgeon males possibly delivered from some French farms does not constitute an economical motivation for German farmers.

Table 38.6 Estimation of annual Siberian sturgeon gross production in Germany 2006–2015 given the whole sturgeon gross production and the relative part of the species (Joern Gessner's pers. com.)

Gross production	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Total sturgeon gross production (tons)	233	267	298	252	255	254	209	229	265	260
% A. baerii	65	65	60	60	50	50	45	40	40	40
Production *A. baerii* (tons)	151.4	173.5	178.8	151.2	127.5	127	94.05	91.6	106	104

Considering climate conditions in Germany, the period of the intensive growth is relatively short ~7 months throughout a year or less, and then the pond rearing of Siberian sturgeon for meat at natural temperatures proved to be practically not profitable. Along with that, RAS require more intensive power consumption, while the energy prices in Germany (0.22 €/kwh) is higher than in other countries. Evaluation of the German production is complicated since at least all six major producers rely on a network of farms producing for them and delivering females for caviar production (J. Gessner, pers. com.). More, a lot of the RAS fish are moved to ponds for ongrowing, and little production remains until the end in RAS. The annual Siberian sturgeon gross production is obtained given the whole sturgeon gross production and the relative part of the species (J. Gessner, pers. com.) (Table 38.6). The whole sturgeon gross production did not much change over the period as it varied from 233 to 260 tons by the end of the period. But the relative part of the species is constantly declining from 60% to 40%. As a result the production of Siberian sturgeon was declining from 151 tons in 2006 to 104 tons in 2015. It is worth noting that the total production per species remains somewhat obscure as most farms do sell ornamentals, pond fish, stocking material, and pre-grown fish. In contrast, the relative part of the Russian sturgeon (*A. gueldenstaedtii*) increased due to its better marketability of the caviar (J. Gessner's, pers.com.). It has to be noted that the present figure is most likely conservative. Whatever, the present assessment gives a yearly German gross production of the species closed to 100 tons.

At present, with the threat of the ESA (Endangered Species Act) promulgated in the USA and listing of *A. baerii*, the interest of rearing the pure species has been reduced due to the anxiety of producers to get future ban on exporting the pure species in the USA especially the Siberian sturgeon. It is more probable that more hybrids (Chap. 39) will be included in the sturgeon culture production systems in Germany again (as in the early 1990s).

Currently, one more typical German peculiarity has been the fact that sturgeon meat is marketed locally only. There are no large producers that supply supermarkets. There is little supply into supermarkets via large distribution chains. Up to the onset of the EU—Russia embargoes, massive amounts of the meat production have been sold to CIS countries deep frozen. This had been a reason for a decline in

production in Germany in the early 2010s when marketing of sturgeon meat has to search for new outlets (J. Gessner, pers. com.).

Technology of caviar processing from ovulated eggs of Siberian sturgeon was developed and patented by A. Köhler, and the relevant patents were sold out to different countries, including Germany and some Asian countries (Köhler and Ziegler 2016). The harvested ovulated eggs are exogenously treated in an aqueous solution by adding at least one signal transduction molecule. Accordingly the membrane of the egg is physiologically stabilized by ovoperoxidase activation. The signal transduction molecule is naturally occurring in the egg cell. The eggs are then preserved (Köhler-Gunther 2007).

Hungary

The volumes of Siberian sturgeon production in Hungary during years 2012–2014 were as follows: 28.1 tons (in 2012), 18.3 tons (in 2013), and 30.0 tons (in 2014). Not many farms in Hungary have been so far involved in Siberian sturgeon production; primarily it has been cultured as supplementary species or for sport fishing in ponds (P. Lengyel's pers. com.). As reported by Bronzi and Rosenthal (2014) in 2012, it produced 2.5 tons of sturgeon caviar with forecasted 15 tons in 2016. According to FAO (FISHSTAT 2015), the overall sturgeon production in Hungary had been stable (at a level of 21–24 tons) for the period 2005–2009, and then the maximum (81 tons) was reached in 2010, followed by decrease to 48 tons in 2014 (FISHSTAT, 2014). The total *A. baerii* caviar production is less than 1 tons/year (estimated).

In 2016 five farms have been engaged in Siberian sturgeon farming; all of them have been using warm water (from geothermal wells), including three farms using RAS and two ones using flow-through systems. Two farms from the list have been involved in the production of Siberian sturgeon meat and caviar for restaurants, hotels, and large retailers, while two other farms have been delivering mature females for other companies and some males for angling clubs, aquaria etc. The fifth enterprise deals with Siberian sturgeon meat (both fresh and smoked), purchasing it to restaurants and supermarkets.

At present in Hungary, there are two sturgeon hatcheries (of RAS type) with overall Siberian sturgeon fry annual production of about 100–120,000 specimens ranging in weight from 10 to 50 g.

The total Siberian sturgeon meat production is about 80–90 tons estimated in 2015. (For instance, in the year 2015, the total sturgeon production in Hungary was 142 tons.) (Á. Rideg's pers. com.)

The Hungarian Fish Culture Research Institute in Szarvas has been the owner of a sturgeon living gene bank, comprising Siberian sturgeon among other species. The gene bank at Szarvas has been so far involved only in reproduction of some sturgeon primarily for scientific purpose (Á. Rideg's pers. com.).

Italy

Currently, in Italy there are ten major sturgeon farms and about five to ten small farms. The types of farms are water flow-through systems. Many farms are ex trout farms converted into sturgeon culture, raceway systems with long and narrow

concrete tanks; others are earthen ponds of different surface and volume; other farms have earthen ponds covered with polyethylene or similar material. The water source is mainly from surface, small rivers and artificial channels; only a couple has been using discharge warm water coming from industry (electric or steel factories); many have also the support of well water (S. Marturano's pers. com.).

Roncarati and Melotti (2007) indicated, that from 1991 to 2006, the commercial production of all sturgeon species in Italy had been stable and amounted to 1000 tons with a major production of white sturgeon (Data elaborated from Associazione Piscicoltori Italiani (A.P.I.), Federation of European Aquaculture Producers (F.E.A.P.), and Istituto di Servizi per il Mercato Agricolo Alimentare (ISMEA). In our opinion, the absence of growth in the production of sturgeon meat along this period can be explained both by European market saturation (in particular, to white and Siberian sturgeon species) and by a higher demand for beluga and Russian and stellate sturgeon. In this context, development trends in the future are not encouraging due to the long-term maturation of sturgeon females; thus to attract investors was more difficult.

No differences exist between male and female meat; nevertheless, as the female size is larger, the meat may be processed in more convenient ways, offering sliced, smoked, or other preparations to the market.

Recently, the Italian production of sturgeon caviar (mainly from white sturgeon) in aquaculture had increased, almost twice, from 22 tons reported in 2008 by Bronzi et al. (2011) to over 40 tons in 2011 and 50 tons in 2016 (Bronzi and Rosenthal 2016). But volumes of Siberian sturgeon production had not raised considerably and till 2003 amounted to 100 tons (meat), while in 2012 it was about 170 tons (meat) and 3 tons (caviar); in 2014 it can be estimated at about 165 tons, that is to say about 20% from total sturgeon production in Italy – 825 tons (P. Bronzi's pers. com.)—and Siberian sturgeon caviar production reached to 4 tons. According to the same author, the difficulties in collection of data on production of Siberian sturgeon in Italy (and not only) are also associated with the differences between production of a given year (weight gain) and the quantity sold in that year.

Some producers give the annual production as increasing in biomass; some consider production correctly but also the fish bought in the previous year from another producer, and until last year the term "for human consumption" was not included in the definition; therefore also the fish sold to another farm were considered as production and again the year after when sold again (P. Bronzi's pers. com.).

Latvia

At present in Latvia, there are two companies that have been involved in sturgeon rearing. The annual capacity of these enterprises has been evaluated as 50 tons of meat and 2 tons caviar (D.Tracuks' pers. com.). There is also one state hatchery that deals with rearing of sturgeon. But in Latvian villages and small settlements (hamlets), there are many occasional producers (farmers) somehow engaged in small-scale sturgeon production. The past years dynamics of sturgeon production in Latvia have shown the descending trend. More and more producers switched over

purchasing (for instance from Lithuania) rather than rearing of sturgeon. This cause some additional problems associated with double counting of the same fish (D. Tracuks' pers. com.).

Netherlands

In the Netherlands, Siberian sturgeon was initially reared in small quantities in 1999 (Williot et al. 2001). In 2009 a new RAS was constructed. Its production capacity was 100 tons, while volumes of Siberian sturgeon meat and caviar reached approximately 50 tons and 1 tons, respectively (Bronzi and Rosenthal 2014).

Poland

In 2003, the Polish volume of Siberian sturgeon meat production in 2003 amounted to 180 tons (Williot et al. 2005). Later on in 2012, the annual caviar production from all sturgeons and their hybrids was estimated to be 0.4 tons (Bronzi and Rosenthal 2014). Anyway, pursuant to official Polish data and to R. Kolman (pers. com.), the production of Siberian sturgeon during the past few years tended to be about 50% of the whole sturgeon meat and caviar production. According to the mentioned author, the recent volumes of production have been 220 tons and 114 kg in 2013, 113.5 tons and 934.5 kg in 2014, and 199 tons and 5.7 tons in 2015 of meat and caviar, respectively. This short series shows an increase in the yield of caviar from 6.10^{-4} to $2.9.\ 10^{-2}$ that reveals a yearly increase in the number of mature females. It should be noted that in addition to the six specialized sturgeon hatcheries, about 15–18 enterprises (primarily trout farms with flow-through raceways) produced Siberian sturgeon as a supplementary species (R. Kolman's pers. com.). The clear increase in production capacity became available owing to establishment of two up-to-date sturgeon farms. It should be noted that mentioned above figures (related to years 2014–2015) were substantially higher than those predicted (Bronzi and Rosenthal 2014).

Romania

Despite the fact that experimental rearing of Siberian sturgeon has been performed since the early 2000s, the apparent increase in volume of commercial rearing of this species in Romania, as well as the formal registration, was initiated in 2013.

One of the main reasons of the raising interest to commercial rearing of Siberian sturgeon during the past few years in Romania, where (as it is known) for a long period commercial fishery and rearing of Danube sturgeons had been conducted (*A. stellatus*, *A. gueldenstaedtii*, *A. ruthenus*, *H. huso)* according to opinion of D. Tabacaru, was strict measures against black market (and sturgeon mafia), derived from dramatic reduction of sturgeon stocks in natural water bodies.

Hence under these conditions, when the responsible authorities stop to issue permits for capture of wild sturgeon breeders in Danube for control reproduction, rearing of Siberian sturgeon seems the one and only opportunity to develop sturgeon culture in Romania, especially in the Danube Delta. In 2016 the total stock of Siberian sturgeon in 8 Romanian farms was 300 tons (considering specimens aged up to 5 years). According to official statistics, from 2013 to 2016, totally about

700,000 live specimens of Siberian sturgeon were delivered to Romania primarily from Hungary, Italy, and Poland (D. Tabacaru's pers. com.). Along with this in 2016, only one company was engaged in Siberian sturgeon sales in small amount. There has been a lack of reliable data on Siberian sturgeon caviar production in Romania before 2016.

38.2.3.4 Some Other Countries

The countries ranged among the present subsection belong to either Eastern Europe[4] (Turkey) or South Caucasus (Armenia and Georgia) or Central Asia (Kazakhstan, Kyrgyzstan, and Uzbekistan) or Far East Asia (Japan and Vietnam). They are evoked by alphabetical order. These are countries for which there are some data concerning the production of Siberian sturgeon. There might be other countries for which no data has been available to our knowledge.

Armenia

Armenia has been engaged in large-scale production of Siberian sturgeon, as the main sturgeon species for about 30 past years. At present circa 20 sturgeon farms are producing meat and caviar.

Commercial production of Siberian sturgeon in Armenia in 2013 amounted to 2,000 tons, with more than 10 farms involved. In 2015 volume of Siberian sturgeon gross production amounted to 3,000 tons at 20 farms, involved in rearing of sturgeon, while caviar production—to 4 tons. The exports to Russia, Ukraine, and Georgia, which seemed negligible in 2011, had doubled again from 500 tons in 2013 up to 1,000 tons in 2015.

Georgia

In Georgia there have been two large sturgeon farms (the total capacity of the largest one—up to 300 tons) and few (less than 10) small farms involved primarily in holding and growing up of Siberian sturgeon specimens (2 kg) delivered from Armenia. The overall production capacity of all sturgeon farms is about 500 tons. The farms of the pond type typically use flow-through water supply from natural sources.

The construction of RAS of 100 tons capacity should be completed in 2017. The main object of sturgeon farming is Siberian sturgeon (90% of total sturgeon production). The stocking material has been delivered primarily from Armenia.

Marketing of commercial sturgeon (grown up to 4–6 kg) amounted to about 30 tons per year in 2014 (FISHSTAT 2015). The commercial production of caviar has not been conducted so far.

Japan

Japan's Miyazaki Fisheries Research Institute (on the island of Kyushu) began Siberian sturgeon farming trials in 1983, using stocking material delivered from

[4] The present geographical classification of countries does not represent any political engagement from the authors.

Soviet Union. According to some reliable forecast, production of Siberian sturgeon caviar in the prefecture should rise five times – from 60 kg in 2014 to 0.3 tons in 2016 (Sakamoto Motoo's pers. com.).

Kazakhstan

In the early 2010s, commercial sturgeon farming in Kazakhstan gained more substantial government assistance. In 2011 production of Siberian sturgeon in Kazakhstan was at rather low level of 50 tons. The target production in 2016 was estimated of 300 tons. This has been preplanned on the bases of low loan rate (up to 1.5%); the residual 7% should be covered in the frame of national subsidy program. In 2015, the number of farms in Kazakhstan involved in this activity increased to five ongrowing farms, and annual production of the Siberian sturgeon reached to 80 tons and 1 tons caviar.

Kyrgyzstan

In Kyrgyzstan production of Siberian sturgeon on an industrial scale has been conducted since 2011. In 2016 three caviar-oriented companies reared 90 tons of Siberian sturgeon (Dosayev's pers. com.) Two of these three companies were supplied with warm water from thermal plant, while the third one uses RAS with underground water supply. The prime aim of the rearing in Kyrgyzstan has been to obtain caviar from ovulated eggs; at this the first females reached their maturity in late 2016. The projected volume of caviar (from ovulated eggs) production in 2017–2018 is 2 tons.

Turkey

The first experimental production of Siberian sturgeon in Turkey was performed in 1996 in flow-through concrete raceways at Ankara University. The juveniles (1,300 ind. 75 days old) imported from France as a source of potential diversification in Turkish aquaculture (Köksal et al. 2000; Memiş 2007). Currently, the sturgeon culture in Turkey has been developed at ten farms (except institutes and universities). Two farm investments have been engaged in caviar-oriented culture of Siberian sturgeon using production systems combining recirculating and flow-through systems (Ercan 2011). The FIRST one was established in 2008 in Adana (southeastern Mediterranean province), while the second one - in 2013 in Antalya (same province).

Their target annual production capacity was initially about 29 tons of meat and 3 tons of caviar. The enterprise in Antalya produced 500 kg and 1 tons of caviar in 2013 and in 2015, respectively (Memiş 2014). Moreover, according to FAO data in 2014, total production of Siberian sturgeon at eight sturgeon farms amounted to 17 tons (FISHSTAT 2015).

The forecast for 2020 presupposes production of 6–8 tons of caviar of Siberian sturgeon. Data that confirm the large potential of sturgeon aquaculture development in Turkey was presented as well by Ercan (2011).

Uzbekistan

Rearing of Siberian sturgeon in Uzbekistan was initiated in 1990 when it was a part (as a Republic) of the Soviet Union. Culturing of sturgeon has been conducted at two farms, one of which was state, with total capacity of about 200 tons. In

2010–2014 the annual volume of Siberian sturgeon meat production amounted to 25 tons. The operation of the large RAS with a capacity of 100 tons of sturgeon stopped in 2015.

The first small batch of eggs from farmed females was obtained in 2015 and used further for control reproduction purpose. Quantity of captive brood fish of Siberian sturgeon in two fish farms accounted for about 500 specimens (D. Abdunazarov's pers. com.).

Vietnam

In Vietnam, commercial sturgeon farming has been an intensively progressing sector of aquaculture. The first sturgeon farms were established in the beginning of 2000s with support from Russian sturgeon experts. At present, few dozen farms, located all across the country, have been engaged in rearing of sturgeon. The concrete raceways and earthen ponds (with the bottom covered with geomembrane) have been the mostly widespread rearing structure. The cages for rearing of Siberian sturgeon (as distinct from beluga) have been used less frequently. Vietnam possesses good prospects for sturgeon culture development owing to high temperatures and long-term vegetative season that provide rapid growth of fish. An important factor of successful sturgeon farming development has proven to be a local manufacturing of production feeds, arranged by one of the leading feed producers "Skretting."

In 2015 the volume of sturgeon meat production amounted to 1,500 tons, 30% of which was the share of Siberian sturgeon (Viktor Chipinov and Nguyen Viet Thuy' pers. com.).

Only one company (Ca Tam Vietnam) has been involved in sturgeon caviar production. This enterprise has realized its caviar, produced from eggs of Russian sturgeon through slaughtering, using Caviar De Duc brand. The data on caviar production from eggs of Siberian sturgeon have not been reported. It should be noted that two main regions of Siberian sturgeon rearing are located in the north of the country near the Chinese border, nearby province Lao Cai, and in the south,—province Lam Dong. We have been told that the farming of the Siberian sturgeon is declining in the regions where the temperature might be too high for the species.

38.2.3.5 Assessment of World Production of the Siberian Sturgeon

Data related to sturgeon gross productions together with some information on the system of production are reported at first and later on those dealing with caviar.

Gross Production and Systems of Production

Global sturgeon production, i.e., nonspecific data, either at the world scale or at the European scale, is given in order to make the visibility and thus the interpretation of Siberian sturgeon-specific data easier.

As aforementioned, in the early 2000s, only one publication was devoted to the production of the Siberian sturgeon (Table 38.7). This table illustrates how much surveys might be incomplete. However, for the first time China and Russia are recognized to have become major producers especially concerning the gross production as caviar production is mostly not documented. Additionally and for the first

Table 38.7 Estimated gross production of Siberian sturgeon in 2003 (after Williot et al. 2005)

Country	Production of meat (tons)	Production of caviar (tons)	Production of eggs (a) and alevins (b)
Russia	750[1] and 500[3]	–	$20 \cdot 10^6$ (a) and $5 \cdot 10^6$ (b)
China	350[1]–2.200[2]	–	–
France	350	7	Large potential
Poland	180	–	Large potential
Germany	120	2	$4 \cdot 10^6$
Italy	100	–	$3.5 \cdot 10^5$ (a)
Belgium and Netherlands	20	2	–
Spain	6	0.4	$3 \cdot 10^6$ (b)
Ukraine	5	–	–
Uruguay	1	–	0
Hungary	0	–	$5 \cdot 10^6$ (a)
USA	0.5	–	–
Total	**1.700[1]–4.400[2]**	**12**	–

[1]Pure species; [2]pure species and hybrids; [3]hybrids

time as well, the importance of hybrids is clearly highlighted; this is for Russia and China. In 2008, the Siberian sturgeon was the most commonly used sturgeon species in 22 countries, reaching a total world production of about 8,800 tons of gross production per year (Bronzi et al. 2011).

Thanks to all collected data to build the present chapter, the elaboration of Table 38.8 was made possible. The number of sturgeon farms involved in Siberian sturgeon production (the criterion is used to rank the countries in the decreasing order), the gross production of the species, and the main types of production systems are reported by country for the year 2014. The first and most evident outcome from the table is the huge number of countries and farms involved worldwide in the production of this sturgeon species; there are 50 countries represented by about 530–550 farms. Note the rapid increase of Siberian sturgeon producing farms from 22 to 50 in the 6-year period, from 2008 to 2014. More and undoubtedly, the geographical distribution of farms involved in rearing of Siberian sturgeon and production of caviar has been much broader than that engaged in farming of other sturgeons. While most of enterprises dealing with Siberian sturgeon and providing the larger part of the overall capacity has been allocated in China, Russia, and France, Siberian sturgeon farms can be found from Argentina, Chile, and Uruguay at the south to some in the Polar Circle near Murmansk (at Kolsk Nuclear Power Plant), Russia, and Norway and from the USA states (Northern Carolina and Florida) at the west to South Korea at the east. There are new comers such as Armenia, Vietnam, and a group of Central-Eastern European countries involved in that production; there are Turkey, Ukraine, Romania, Belarus, and Hungary. In the same way, it is worth noting that two countries from Central Asia, Kazakhstan and Kyrgyzstan, are now in the top 20 of the world producers for the species. The two first producers, Russia and

Table 38.8 Number of farms, gross production, and production systems used worldwide for the commercial rearing of Siberian sturgeon in the year 2014

Country		Number of sturgeon farms	Quantity of commercial Siberian sturgeon (t)	Types of production systems			
				RAS	Cages	Flow-through raceways	Small ponds
1	Russian Federation	210	1800	+	+	+	+
2	China	120	17,000	+	+	+	+
3	Poland	21–24	210[a]	+	+	+	+
4	Armenia	20	3000	+		+	+
5	Italy	15	200	+		+	+
6	Vietnam	15	500	+	+	+	+
7	Germany	12[b]–17[c]–26[d]	106[d]–200[b,c]	+		+	+
8	France	10	315	+		+	+
9	Turkey	8	29	+		+	+
10	Ukraine	8	300	+	+	+	+
11	Romania	8	15	+		+	+
12	Belarus	7	83[e]–120[f]	+	+	+	+
13	Hungary	6	90	+	+	+	
14	Greece	5	30	+		+	
15	Kazakhstan	5	80	+	+	+	+
16	USA	4	50	+			
17	Kyrgyzstan	3	90[g]	+	+	+	+
18	Latvia	3	50	+		+	
19	Georgia	3	30			+	+
20	Korea	3	50	+		+	
21	Uzbekistan	3	25	+	+		
22	Bulgaria	2	20	+	+	+	+
23	Czech Republic	2	40–50[h]	+		+	+
24	Denmark	2	2	+			
25	Finland	2	45	+			
26	Japan	2	10	+		+	
27	Lithuania	2	20	+		+	+
28	Moldova	2	5	+			+
29	Spain	2	12	+		+	
30	Estonia	2	20	+	+		
31	Cyprus	2	1	+		+	
32	Slovenia	1	5	+			
34	Switzerland	2	70	+		+	
35	Argentina	1	20	+		+	
36	Austria	1	5	+			
37	Belgium	1	40	+			
38	Chile	1	5			+	
39	Laos	1	5	+			
40	Malaysia	1	5	+			

Table 38.8 (continued)

Country	Number of sturgeon farms	Quantity of commercial Siberian sturgeon (t)	Types of production systems			
			RAS	Cages	Flow-through raceways	Small ponds
41 Myanmar	1	5	+			
42 Netherlands	1	50	+			
43 Norway	1	1	+			
44 Portugal	1	10	+			
45 Saudi Arabia	1	50	+		+	
46 Serbia	1	5			+	
47 Thailand	1	20	+			
48 UK	1	5	+		+	
49 UAE	1	100	+			
50 Uruguay	1	180		+	+	
Total	**528–545**	**27,517–27,658**				

[a]R. Kolman's pers. com (2016)
[b]P. Steinbach's pers. com. (2016)
[c]P. Gross's pers. com. (2016)
[d]J. Gessner pers. com. (2016)
[e]FISHSTAT (2015)
[f]N. Barulin's pers. com. (2016)
[g]R. Dosaev's pers. com. (2016)
[h]O. Linhart's pers. com. (2016)

China, represent together 61% of all sturgeon farms. Finally, four European countries, Poland, Italy, Germany, and France, are in the top 10. Logically, the position of the gross production by country is not very much different than that abovementioned for the number of farms with two noticeable exceptions in the four first producers with China being the first, with 17,000 tons, i.e., 62%, and Armenia the second with 3,000 tons, i.e., 11%, which shows that the largest farms are in those two countries. Altogether, the world production of the Siberian sturgeon was assessed to be closed to 27,500 tons in 2014. It is noticeable that both assessments of the number of farms (from 12 to 26) and of the gross production (from 106 to 200 tons) show a large uncertainty is for Germany. Some reasons were already exposed earlier in the chapter (Sect. 38.3.3.3.6); they may, at least partially, explain these important discrepancies. To a lesser extent, the aforementioned comment is also valid for Belarus of which the yearly gross production is assessed to be in the range 83–120 tons.

The most recent assessment for nonspecific sturgeon production similar data at the world level is an inference of Bronzi and Rosenthal (2014) who prognosed 51,500 tons for 2011 based on the fact that Chinese production of 44,200 tons accounted for 86% in the world production. At the European level, there are the data from the FEAP (2016) which gather the main producers according to the

Table 38.9 Sturgeon gross production (tons) in main European country producers (FEAP Production Report 2016) (data collected by Dr. Alan Jones, FEAP (Federation of European Aquaculture Producers) (FEAP 2016)

	Year								
Country	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**
France	250	250	250	380	280	250	280	298	315
Germany	228	214	106	120	120	240	150	300	350
Hungary	21	24	34	14	14	51	56	56	120
Italy	1200	1350	1350	1900	1900	1700	1900	2000	1480
Poland	250	270	148	200	240	241	95	140	170
Spain	183	370	166	35	40	66	66	100	120[a]
Total	**2132**	**2478**	**2054**	**2649**	**2594**	**2548**	**2547**	**2894**	**2555**

[a]Uncertain assessment

association (Table 38.9). The evaluation of the gross production in 2014 is then 2,894 tons given that the evaluation minimizes the reality for EU as all the countries are not taken into consideration. From Table 38.8, the total gross production of the Siberian sturgeon for EU countries is then 1,185–1,232 tons which gives a ratio of production of the Siberian sturgeon at the EU level in the range of 41–42.5% in 2014 keeping in mind that this evaluation is then maximized given the aforementioned observation. A few additional comments can be pointed out from Table 38.8. The general trend over the 9-year period (2007–2015) is country dependent. The present authors discussed earlier in the chapter the changes for two countries, France (Sect. 38.3.3.3.1 and Figs. 38.15 and 38.16) and Germany (Sect. 38.3.3.3.6). It comes that, up to now, there is no dominant supranational driver as each country show a different trend in reaction to its own context. It remains that even the international part of the market represents a minority; it may play a major role in impacting the prices. A last remark is worth noting with regard to Table 38.8. The data for Germany do not agree with those already reported in Table 38.6, and the differences are noticeable. There is a great deal of clarification and standardization in the presentation of primary data, their collection and their aggregation.

Thanks to the data from Table 38.8, it has been possible to draw the distribution of the use of the different production systems around the world (Fig. 38.17). This is a qualitative indication, and the results are given by three large geographical areas, CIS, Asia, and Europe. RAS has been the prime systems applied for rearing of Siberian sturgeon. They have been distributed practically in all CIS[5] countries and in the main part (above 70%) of European and Asian states. The farms, used small ponds, and cages also have the largest distribution in CIS countries, while in other regions the share of these systems is rather small (18–31%). The share of flow-through raceways in different regions ranged from 62 to 83%.

[5] CIS = Community of Independent States.

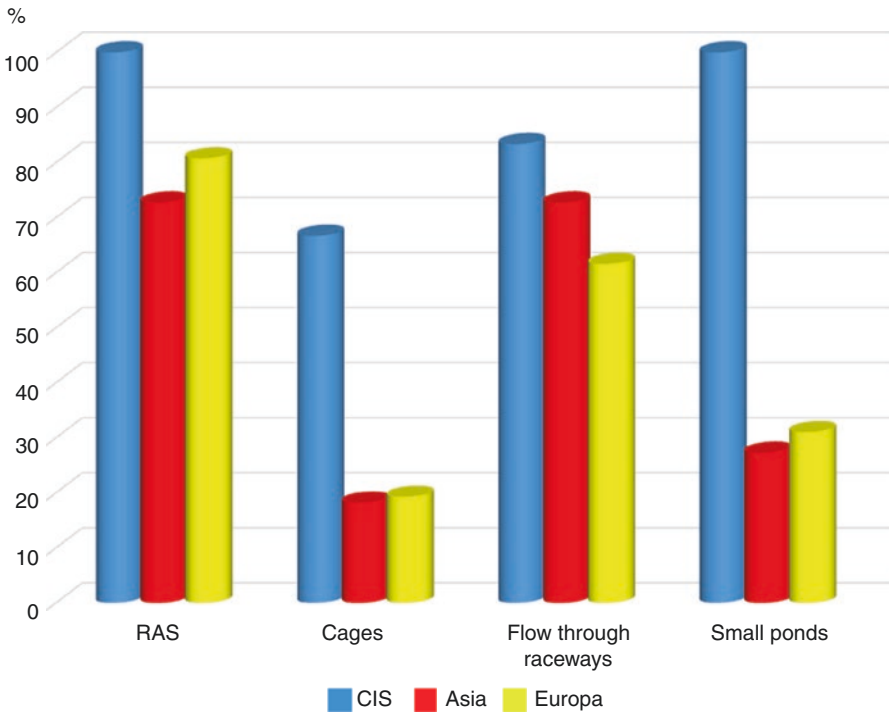

Fig. 38.17 Geographical distribution (large areas) of production systems used for farming the Siberian sturgeon (Table 38.8). Areas are CIS (Community of Independent States composed of Russia and some geographically and politically closed states), Asia, and Europe

Along with that, in Russia 80% of Siberian sturgeon has been reared in cages in warm-water farms, while in China—in cages – installed into natural water bodies. In Western Europe and the USA, rearing of Siberian sturgeon as exotic species in cages is prohibited.

Volume of Production of Caviar from the Siberian Sturgeon

In 2008, the estimated world production of farmed caviar for all sturgeon species (jointly produced in 80 farms in 16 countries) was within the range of 110–120 tons (Bronzi et al. 2011). There was an estimation of the overall production capacity of farmed caviar of about 260 tons in 2012 according to Bronzi and Rosenthal (2014). But there is no key to properly compare those two assessments except this illustrates an increasing trend in the world production.

Thanks to the present survey, it is possible to give an estimation of the yearly production of caviar from farmed Siberian sturgeon (2014–2016) (Table 38.10) where the countries are ranked according their respective decreasing order of production. Hence, the total volume of Siberian sturgeon caviar production in 32

Table 38.10 Estimated global annual production (tons) of caviar from farmed Siberian sturgeon depending on the country (2014–2016)

Country	Production (tons)
China	24
France	22.6[a]
Russian Federation	21
Germany	12–14
Vietnam	2
Uruguay	7
Poland	5.7[b]
Armenia	5
Italy	4
Ukraine	4
Korea	4
Finland	3.5
Saudi Arabia	3
Turkey	3
Greece	2
Latvia	2
Belarus	1.2[c]
Belgium	3
Kazakhstan	1
USA	1
Hungary	1[d]
Spain	1
Netherlands	1
Thailand	0.5
Lithuania	0.4
Estonia	0.4
Austria	0.4
Moldova	0.3
Japan	0.2
UK	0.2
Cyprus	0.05
Czech Republic	0.02[e]
Total	**149.5–152.5**

[a]SFAM (French Association of Marine Aquaculture) (2016). Effective production for 2015
[b]N. Barulin's pers. comm. (2016)
[c]R. Kolman's pers. comm. (2016)
[d]A. Rideg's pers. comm. (2016)
[e]O. Linhart's pers. comm. (2016)

countries was ranged from 147 to 150 tons. The first four countries, China, France, Russia, and Germany, represented 80.6 tons, i.e., about 54% of the total. Keeping in mind that the assessment for Germany is questionable, those aforementioned evaluations should be carefully used. However, this would not dramatically change the main conclusions that are that the four first countries totalize about half of the world production. This would change in the near future with the entrance in production of some newcomers as pointed out earlier in the chapter with regard to the gross production. This is most likely one of the reasons which supports the huge increasing forecast to 500–750 tons for the world production of caviar by the year 2020s by Bronzi and Rosenthal (2014).

38.3 Economic-Related Data

There exists some available economic-related data from sturgeon farming in three different fields, the products, the production costs, and the selling prices, which are going to be, respectively, detailed below. Different types of products might be based on sturgeon; besides the most two renowned are the caviar and the meat, the first one being by far the most valuable among all the eatable products. However, other products have to be mentioned such as the fingerlings for restocking as developed in Chap. 37 or as pet fish (Williot et al. 2001), the last one being marginal. There are also other end products, the use of which was well developed in the past such as the swim bladder used to prepare the isinglass (fish glue) and the skin for the leather (Williot et al. 2011). There has been undertaken a few attempts worldwide to value again the skin of sturgeon as leather, e.g., from the Siberian sturgeon in France (Fig. 38.18). The present chapter is focused on the caviar and the meat.

Fig. 38.18 Sample of Siberian sturgeon leather (Source: Femer, Marielle Philip)

38.3.1 Sturgeon Products with a Focus on Caviar and Meat

It has been mentioned earlier (Sect. 38.2) how ambiguous might be the use of the term meat, and some clarifications were thus given. A description of the main steps of production has been given (Williot and Sabeau 1999; Williot et al. 2001). The fish ready to be processed, i.e., after being usually starved before their capture from the tanks, correspond to the gross production. This is the first most significant and current step that allows comparing the volumes of production. After being stunned and then bled, mature females are hung for a while to be drained (Fig. 38.19). Later on, the abdomen of the females is opened (Fig. 38.20a, b) to check the quality of the eggs, color, size, and crisp texture. This is also the step that initiates the traceability by recording the number of the pit tag, identifying each animal. The main chronological phases for caviar processing to get malossol-type caviar have been described (Williot and Sabeau 1999). The last one consists in canning in large can of about 1.5–2 kg each (Fig. 38.21) allowing to hold them for up to some months in a thermoregulated room (Fig. 38.22) at a low positive temperature. Once the ovaries are taken out from the abdominal cavity, last phase of carcass preparation is engaged and may result in obtaining of peeled fillets (Fig. 38.23). They represent the final net weight of the sturgeon meat. In order to get the yearly whole volume

Fig. 38.19 Mature females hung post euthanasia and bleeding before opening (credit: P Williot)

Fig. 38.20 (**a**) Mature females with an opened abdomen prepared for a comparison of the apparent quality of their eggs (credit P. Williot). (**b**) Mature females prepared for a comparison of the apparent quality of their eggs and showing the fullness of the ovaries (credit P. Williot)

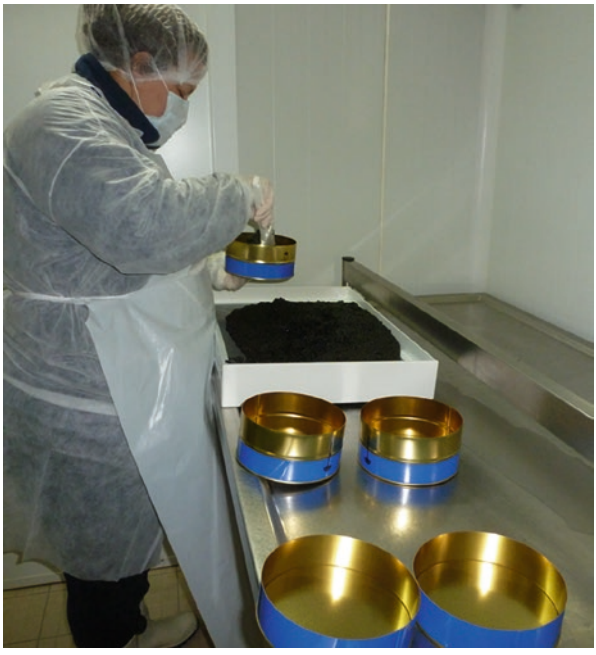

Fig. 38.21 Filling in the large metallic can with processed caviar of malossol type (credit P. Williot)

Fig. 38.22 Thermoregulated room for holding malossol-type caviar in Bulgaria. The two air conditioners in the top middle of the picture control the temperature (credit E. Galich)

Fig. 38.23 Peeled fillets of mature females of Siberian sturgeon (credit: P Williot)

of sturgeon production at a farm level, the quantity of young males is eliminated from the farm after sexing along the same period. Those male specimens can be sold alive or processed in the same ways of that of the females. They also can be sold eviscerated and headed (Fig. 38.24). Usually male specimens should be sold earlier than females, just when the sex has been identified. Along with the aquarium market, the sale of this male product yields an early income (Williot et al. 2001). The term "meat" supposes all sorts of marketable products, including fresh or processed ones (e.g., smocked), packaged or not.

Fig. 38.24 Young eviscerated and headed males of Siberian sturgeon post sex determination (credit: P Williot)

Marketable sizes are maximum 1–4 kg per fish for young Siberian sturgeon. The standing crop for 7-year-old females destined for caviar production is three times that for a production of fish with 4 kg mean weight (4-year-old fish) or older in Siberian sturgeon. At present the smallest commercial size is for fish weighing from 2 to 3 kg for fresh market and preferably bigger, up to 8 to 10 kg for smoking purposes (Arlati and Bronzi 1993). The largest size quoted above might be taken with care as a reference for the Siberian sturgeon as the Italian market is dominated by the white sturgeon (*A. transmontanus*) of which the fish are currently larger.

As far as sturgeon meat is concerned, various sizes may be offered, whole, steak, or fillet along with different preparation procedures, implying fresh, processed, and other meat as the end product. Even not strictly included in the present section, other products based on Siberian sturgeon are marketed; there are pate, rillettes, soup, etc. which allowed to valorize nondirectly marketable parts of the fish.

As to caviar, in order to extend the duration of its conservation, the traditionally processed caviar, malossol, could compete with some variants of processing (flash pasteurized, pasteurization). As in the course of caviar processing, sturgeon female can be used few times in a row, and some attempts to elaborate "caviar-like" products from ovulated eggs could be undertaken. Some indications on the present processes adapted to ovulated eggs are given below in Sect. 38.3.3.1.2.

38.3.2 Production Costs

There exist a lack of available relevant data. The first batch is an economic study carried out in Latvia on the financial efficacy of aquaculture-farming species (not shown), one of them being the Siberian sturgeon (Veveris et al. 2016). The authors provide a detailed table on the relative importance of the main items with the exception of investments of the production costs in the Latvian context (Table 38.11). Three levels of production of meat are considered, 5, 10, and 45 t, which lead to a total production costs that decrease from 4.27 to 4.2 to 3.73 €/kg, respectively, without taking into account the investments that are assessed to be about 142, 250, and 700.10^3 € for the three levels of production of meat considered and 1060.10^3 € for the yearly production of 2 tons of caviar. Apart from the investments, the items that are taken into consideration are: feed, energy, labor, fingerlings, and maintenance. When the production targets 45 tons, the most important items are feed (43%) and energy (34%), all others being lower than 10%. With regard to caviar production objective of 2 tons, the total is about 149 € with a somewhat different repartition, i.e., 1.1%, 16,7%, 30,2%, 28,5%, and 23,5%, respectively. The total production costs for caviar is very low. Some items are astonishing; i.e., the relative part of the energy is high and that of labor for the meat is low. Similarly, the dramatic increase of purchase price of fingerlings that jumps from 8–9% for meat to 28% for caviar is not understandable. In fact, the authors neither provide the reader with data nor with methods used for computations in both husbandry and accountancy fields. More, the authors quoted that "for producing caviar, in the Table 38.11 are presented only the time after when the first production starts appearing but prior to that at least 5 extra years are required to rear and grow the females." The evaluation of production cost is then under-evaluated.

Table 38.11 Approximate estimate of the main items of production costs for the live Siberian sturgeon (€/kg) reared in Latvia (extracted and completed from Veveris et al. 2016)

Products/ item	Volume of production (t/year)	Food	Energy	Labor	Fingerlings or larvae	Maintenance	Total cost without investment (€/kg)
Sturgeon meat	5	1.61 (38%)	1.64 (45%)	0.38 (9%)	0.39 (9.1%)	0.25 (5.8%)	4.27
	10	1.61 (39%)	1.54 (37.4%)	0.33 (8%)	0.39 (9.5%)	0.25 (6.1%)	4.12
	45	1.61 (43.2%)	1.27 (34%)	0.29 (7.8%)	0.31 (8.3%)	0.25 (6.7%)	3.73
Sturgeon roe	2	1.61 (1.1%)	24.83 (16.7%)	45.00 (30.2%)	42.50 (28.5%)	35.00 (23.5%)	148.94

Percentages of respective total costs are indicated between round brackets

Table 38.12 Eight-year changes in some data on economics (wholesale price and costs of production) of commercial farming of Siberian sturgeon in Russia (Chebanov et al. 2006, 2008, 2009; Chebanov 2016)

Criterion/year	2006	2009	2010	2011	2012	2013	2014	2015
Production costs for meat (€/kg)	5.5	5.5	4.5	5.6	5.8	5.0	5.2	6.5
Production costs for caviar from ovulated eggs (€/kg)	300	350	380	400	430	430	330	300

The second batch of data on the production costs comes from Russia (Table 38.12) and is a complementary set of data from Table 38.3. The production costs for meat increased slowly (5.5–6.5 €/kg), and that of caviar processed from ovulated eggs is remarkably stable with 300 €/kg with the exception of the last recorded year 2015 that shows a raised due to price elevation (in €) on import foods primarily used at sturgeon farms in Russia. It is worth noting that these data are much higher than those reported above for Latvia.

It was mentioned above (Sect. 38.3.3.5.1) that RAS are the most equally distributed production systems worldwide. This results in an increasing in the costs of production.

Detailed analysis of production costs and economic efficiency of Siberian sturgeon rearing and caviar production in RAS with capacity 50–100 tons, on the examples of three different types of such RAS production systems in Hungary, Romania, and Turkey with consideration of separate items of cost production, was presented by Schneider et al. (2009). Three different collaborators, namely, HAKI, AQUA, and CULTI (two last names codified for confidentiality compliance as Schneider et al. (2009) reported), presented three independent RAS projects of sturgeon farms. The mentioned RAS designs implied the complete cycle of production. The estimated gross investment for each of the three projects differed owing to different requirements related to applied materials and based units (pond, concrete, or plastic tank). Therewith, the estimated investment ranged from 1,652,000 tons to 3,862,000 € (Schneider et al. 2009). For example, according to calculations for three mentioned above RAS, the relative consumption of energy in RAS ranged from 36 to 61 kwh/kg meat of sturgeon and from 418 to 873 kwh/kg caviar. As well in that work, it was established that at price for caviar above 417 €/kg (580.34 $US/kg in 2009 or 453.39 $US/kg in 2016), production would be profitable (supposing 10% discount rate), and payback period from 7 to 10 years could be decreased to 6–7 years at price 600 €/kg (835.02 $US/kg in 2009 or 653.2 $US/kg in 2016). The potential advantages of RAS sturgeon farm against FT farm or pond system comprise (a) up to 1000 times reduction of water requirements, (b) better FCR, and (c) 2–3 times higher rate of return.

The key constraints affecting the sturgeon farms in Romania and Hungary were of economic nature. Due to the number of problems (energy and feed costs, financial inputs, license costs, taxes, market demand), RAS could hardly provide a direct solution, due to their higher dependence on national/international policies and administrative regulations. Alternatively, RAS could offer indirect solutions,

supposing positive economic consequences in the course of used resources reduction (Schneider et al. 2009).

Analysis of Siberian sturgeon rearing in RAS was also presented in Kitashin et al. (2016) and Tabacaru (2015). As it was shown in Kitashin et al. (2015), production of sturgeon caviar at target monthly volumes during a full-year cycle can be profitable owing to feasibility of Siberian sturgeon rearing and breeding in RAS under conditions of warm-water aquaculture (in Saudi Arabia) with exclusion of wintering periods.

According to Tabacaru (2015), a well-designed RAS can be profitable only if initial capital costs would be reimbursed by a consistently achieved high level of performance in terms of fish growth, stocking density, feed efficiency, conversion rate and especially survival rate. Actually, the profitability of the system does not depend on the market price, although in the case of caviar, this price is in full growth trend over the next 15 years, but rather on the margin between the production costs and the market price, which determines the profitability and therefore the commercial viability. Obviously, a product with market price of 600 Euros/kg and close production cost of 570 Euro could hardly compete with a product, the selling price of which (25 Euro/kg) is dozen time less than its production cost (2 Euro/kg) (Tabacaru 2015). For small RAS (up to 5 tons of fish mass), two to three workers seem sufficient, while for large systems (100–300 tons), the need for personnel does not increase linearly. Hence a large system is easier to complete than a small one and more profitable. Along with positive aspects, as the caviar possesses the status of unique delicacy, Geosmin (a major strong scent contributor) become a challenging issue in caviar production, especially when RAS is used. Due to this, it is urgent to consider and eliminate this problem properly in the course of RAS sturgeon farm operation. Chapter 36 is dedicated to the issue. To our opinion, as was mentioned above, under conditions of southern and temperate regions, the most economically feasible sturgeon caviar and meat system can be a combination of RAS (for reproduction and rearing of larvae and juveniles) with small ponds or flow-through raceways or concrete tanks.

38.3.3 Selling Prices

There are few data on prices, and then very few constitute a series allowing comparing with the volume of production, or volume of consumption (taking into consideration the potential importation), or any other potential influencing factor. More, sometimes, selling prices might be difficult to interpret by lack of precision on the level they are related in the marketing chain: farm gate (FG), wholesale (WS), or retail (RET).

There are two series of wholesale prices over the time. The primary available data are related to Italy at the very beginning of the development of the sturgeon production (Table 38.13). Though the comparison is somewhat biased due to the fact that the size of pieces have changed over the period from 1985 to 1997, the wholesale price exhibited a slow decreasing trend over the year when the production

Table 38.13 Production and wholesale prices of meat (*A. baerii*) from 1985 to 1997 in Italy (drawn after Bronzi et al. 1999)

	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997
Price ($)/(€)	17.5 (25.4)	17 (19.6)	15.5 (14.5)	15 (11.6)	14 (12.8)	13 (10.9)	14[a] (10.3)	13[a] (9.7)	13[a] (10.6)	13[a] (11.7)	12[a] (9.6)	12[a] (8.9)	11[a] (8.7)
Production (tons)	–	–	–	–	–	5	5	25	25	50	90	120	100

[a]Big size

Table 38.14 Eight-year changes in some data on economics (wholesale price and costs of production) of commercial farming of Siberian sturgeon in Russia (Chebanov et al. 2006, 2008, 2009; Chebanov 2016)

Criterion/year	2006	2009	2010	2011	2012	2013	2014	2015
Wholesale price of meat (average) (€/kg)	11.5	13	11	10.5	10	9.5	8.5	10
Wholesale price of caviar (average) (€/kg)	800	900	720	700	660	600	560	400

increases. Secondly and over a more recent period (2006–2015), there are data on wholesale prices for meat and caviar in Russia (Table 38.14). The average wholesale (WS) price for meat was at a high level (10–13 €/kg or 11.8–15.34 $US in 2006 to 12.14–15.78 $US in 2015) with some fluctuations with changing in currency rates and showed a slow decreasing trend.

Only in 2016, some decrease in price was recorded till 8 €/kg; at this the retail prices exceeded the wholesale 1.8 times. That of caviar at farm gate (FG) decreased by two times (800 €/kg or 943.76.48 $US in 2006 to 400 €/kg or 485.64 $US in 2015) along the period. This was associated mainly with the ruble rate.

Other scattered data are gathered below; they are organized by couple date-country for meat and caviar, respectively. It is worth noting that prices have been mostly not species specific with the exception of France and Latvia:

Caviar			
<1989	France	200–500 FF/kg (33.5 €/kg–33.2 $US/kg; 33.2 €/kg–83 $US/kg) RET smocked sturgeon	Bourguignon (1989)
<1992	Germany	>20 ECU/kg (26.82 $US) FG	Müller (1992)
<1993	France	10–13 ECU/kg (12.27–15.96 $US/kg) FG	Williot et al. (1993)
		1 ECU/piece of 2–5 g fry	Williot et al. (1993)
1992	Italy	11–12 $/kg (8.2–8.95 €/kg) whole fresh small-sized FG	Arlati and Bronzi (1995)
		13 ECU/kg (9.69 €/kg) whole fresh medium-sized FG	Arlati and Bronzi (1995)
		98% was sold fresh, 2% smocked fillets at 50 $/kg	Arlati and Bronzi (1995)
		A precooked slice at about 33 $/kg	
		A lot of sturgeon were (are) sold alive for restocking and then to support sport fishery	Arlati and Bronzi (1995)
		(Editor note: this is mainly true for *A. naccarii*)	
<1993	Italy	10–11 ECU/kg (12.27–13.5$US) FG	Williot et al. (1993)
1996	Italy	WS price dropped below half of that in 1985	Bronzi et al. (1999)
1997	Estonia	8.5 $/kg (6.75 €/kg) WS	

(continued)

(continued)

Caviar			
1998	China	1400 Chinese Yuan (CNY)/kg (165 €/kg–150.500A0$US/kg) RET	Shen et al. (2014)
>2001	China	50–60 CNY/kg (6.7 €/kg–7.2 $US/kg) and then remained unchanged for many years	Shen et al. (2014) Shen et al. (2014)
1998–2002	China	Price of meat remained stable for the next 5–6 years	Li et al. (2009)
2010s	Italy	2.5–3.2 €/kg (3.6$US–5.19$US) FG	Parisi et al. (2014)
2010–2012	China	45–25 CNY/kg (5–3 €/kg; 7.3–4 €/kg)	Shen et al. (2014)
		4.5 to 3.0 CNY/piece of fry (0.54€ to 0.36€; 0.7$ to 0.5$) Price in Western provinces was generally higher than that in Southern China	Shen et al. (2014) Shen et al. (2014) Shen et al. (2011)
2015	Poland	5€ (5.44 $US) WS; 5€ (5.44 $US) RET	(R Kolman's pers. com.)
2015	Japan	500 yen (4.14 $/3.41 €) per a 5-month-old fish	Takada (2016)
2016	France	5–11 €/kg WS depending on quality (Highest price corresponds to the appearance showed Fig. 38.23)	
<2016	Kyrgyzstan	500 rub/kg (6.2 €/kg–6.75 $US/kg) WS and RET 1100 rub/kg (13.63 $US/kg–14.84 €/kg) smocked meat.	(R. Dosayev's (Renad Dosayev's) pers. com.)
<2016	Vietnam	High demand partly satisfied by imports from China 7 $US/kg (6.43€/kg)	(Nguyen Viet Thuy's pers. com.)
Caviar			
<1989	France	700–1190 FF/kg (106.7 €/kg–116.2 $US/kg; (181.4 €/kg–197.6 $US/kg) Importation price in UE depending on species and country	Bourguignon (1989)
2013–2015	Italy	350–550 €/kg (381.05–598.79 $US/kg), FG 3200 €/kg (4410 $US/kg) RET depending on species and quality	Parisi et al. (2014)
2015	Poland	300–400 €/kg (326.61 $US/kg–435.48 $US/kg) depending on quality	(R Kolman's pers. com.)
2015	Japan	1000 yen/g (8.28 $US/g-6.82 €/g) RET	Takada (2016)
2016	Europe	600 €/kg (653.22 $US/kg)	Veveris et al. (2016)
2016	Latvia	700–1500 €/kg (762.09–1633.05 $US/kg) RET	Veveris et al. (2016)
2016	France	600 €/kg (653.22 $US/kg) WS or higher	

(continued)

Caviar			
		depending on quality	
2016	Kyrgyzstan	37,000 rub/kg (499.32 $US/kg; 458.64 €/kg) WS 60,000 rub/kg (809.712 $US/kg; 743.74 €/kg) RET	(R Dosayev's pers. com)

It has to be reminded that in case of delivery of alive fish to be sold at the retail (RET) level (especially for China, but not only), some of the quoted prices have to include the additional cost of transportation for alive fish. In 2006 the transportation costs (by water tank truck) were about 6.0 CNY/kg (0.63 €/kg; 0.74 $US/kg). And after 2006, prices elevated to about 8.0 CNY/kg (0.84 €/kg; 0.99 $US/kg) (Li et al. 2009).

Several times, selling prices (WS and RET) decreased abruptly in some countries (e.g., China, European countries). This instability of markets for commercial products had been associated with the subcritical size of the industry that failed to attract larger distribution systems and due to the fact that image of sturgeon as a luxury food item curbs the target consumer group to very specialized restaurant chains or customs (such as airlines), requiring an expensive infrastructure and distribution system for small quantities only (Bronzi et al. 1999). Also invoked has been the customer's unfamiliarity with this product.

38.4 Perspectives for Siberian Sturgeon Farming

It is possible to draw two main lines with regard to the most probable evolution in the Siberian Sturgeon aquaculture industry: (1) the market and (2) the assets of the species. The situation of the species cannot be evaluated independently from that of the global sturgeon farming world characteristics.

38.4.1 The Market

With regard to the market, three aspects are going to be pointed out, the volume, the diversification of the products, and the conditions of the exchanges.

The world sturgeon market is expanding, and the Siberian sturgeon being one of the major farmed sturgeon species participates to this increase. This comes after the dramatic decrease of the offer of the two major exporting countries Iran and Russia became quasi null in the 1990s, but the demand has not changed significantly, that stipulated changes in sturgeon caviar markets (Adeli and Namdar 2015; Engler and Knapp 2008). This evolution boosted the sturgeon farming worldwide. For example, in 2000 the overall sturgeon meat market capacity in Russia amounted to 10,000 tons (Chebanov and Billard 2001). In 2015 the overall market of sturgeon meat in Russia was estimated in 14,000–15,000 tons. And this is true for most of the producing countries, especially China. According to reliable forecasts (considering existing installations involved in sturgeon culture), the overall production of Siberian

sturgeon caviar should tend to be advancing and reach 200 tons toward the 2020s. More, this increase in the global production is not only due to the increasing capacity of some already involved countries but also to the increasing number of producing countries (e.g., Vietnam, Laos, Malaysia, Thailand, Myanmar, Portugal, Chile, Madagascar) as shown in Table 38.8. Accounting tendency of speedy market globalization, the current status quo (meaning all new EU countries, China, South America, etc.) is to be changed, supposing strong diversification of potential novel markets. The estimates of future production volumes has been based on:

- conservative data for traditional producers, who had been already consolidated;
- declared planned productions, provided by few countries and some companies;
- extrapolations for some fast-growing producers like China. It should be noted, that China has shown a linear growth over the last 4 years, while the market of traditional luxury and expensive products would persist at a fairly decent level.

Reflecting on the past (from 1970s to the 1990s), the traditional market would be able to absorb a yearly caviar production of about 3,000 tons (Bronzi and Rosenthal 2014). It is worth noting that, in the same paper, the authors suggested that a projection of 500–750 tons of yearly production of caviar for the next 10 years would be a reasonable forecast as compared with the present 260 tons before 2014. In absence of sound analysis, such statements are not only highly questionable but also bring confusion.

As far as the diversification of sturgeon products is concerned, the present synthesis highlights perfectly this trend which has been anticipated for long (Williot et al. 1993). As compared with the former times (from the sturgeon market point of view) where the three Ponto-Caspian sturgeon species dominated the market, these species practically disappeared from the statistics of production, and new ones took their place. Obviously, the former species, at least some of them, will reappear in the near future, but nobody is able to predict their future relative importance mainly because husbandry limits are not well known. A second road for diversification concerns the presentation. The best example comes from China where it exists a strong tradition for the consumption of live fish (with preference for juveniles of 1–1.5 kg weight, which) have restricted sturgeon market and lead to waste of related resources as judged by the authors of the survey (Shen et al. 2014). The authors explained that this is the main driver for a limitation to the expansion of the domestic consumption. By reading carefully the paper, it is possible to suggest another hypothesis. Indeed the authors pointed out that the knowledge in sturgeon husbandry is weak to such an extent so that it is a limiting factor to the expansion of the farming of the species. More evidently, the distribution of alive young sturgeons requires a specific logistics which might be not fundamentally different from that set up for the other fish species.

Therefore, the aforementioned remarks allow to suggest that this market might be considered as a chance instead when regarded from sturgeon farming activity. And the authors strongly suggest that many efforts have to be done to improve the efficacy of all this production including the marketing aspect. The third diversification road that has to be highlighted is the noticeable appearance of a “new caviar” product with the processing of ovulated eggs which does not require the

slaughtering of the females. Though essentially limited to Russia and a very few other country, this new processing way to produce caviar should be regarded with great attention. The new process includes together advantages and drawbacks. An advantage is the fact that the females may be used several times in their life with the subsequent positive impact in the production cost (Sect. 38.4.2). In the opposite there are two main drawbacks; the biological cells are different depending on the method, ovulated eggs in the new process, and ovarian follicles in the traditional process which is known as malossol-like caviar. This means that the treatment of the ovulated eggs is different as well as the end product. The second drawbacks might be the fact that ovulated eggs are obtained after a hormonal stimulation to mimic the natural phenomena. This may fear some people sensible to campaign aiming at discrediting the process in omitting to remind the nature.

The third aspect of the market deals with some of the conditions that regulate the exchanges. Caviar market has been for long globalized because some of the main consumers can not be considered the main producers. This is well illustrated by the recent example of China that was faced with export. In 2006, Hangzhou Qiandaohu Xunlong Sci-tech Development Co., Ltd. was the first company exporting cultured caviar. From that time, China has started to export farmed caviar with a steady growth (about 1, 2, 5, and 8 tons for years from 2006 to 2009, respectively). Actually, the number of caviar processing plants in China has tripled from one (in 2006) to three (in 2009) (Wei et al. 2011). At present, nine enterprises have been entitled to process caviar or to attempt caviar processing in China. In 2012, these enterprises produced 56.6 tons caviar, 90% of which was intended to be exported. This is the result of the regulations decreed by some national or international organizations, EU being one of them, the basic reason being that EU represents the main consumer in the world. In 1999–2007, imports of *A. baerii* caviar within EU were slightly above 1 tons (1004 kg) (Engler and Knapp 2008). Cases of exceeding the quantities in the EU import permits were not reported for Siberian sturgeon caviar. Permit tracking for *A. baerii* did not reveal any instances of reexporters exporting greater quantities of caviar than were reported on the relevant import permits. Additionally, in the great majority where the species is grown, it is a non-native species which is mostly classified as endangered in its natural geographical area; thus its trade is strictly controlled by an international commission, the CITES.[6] It is worth noting that this is a quasi-general situation with regard to sturgeon production at present. The objective of the CITES is to ensure that wild-originated specimens are not sold through black market. The global pathways of caviar trade frequently seem entangled, implying several agent countries within the EU in the course of goods delivery. This is associated with the necessity to harmonize the quantity of reexported caviar with that of imported caviar. Domestic EU trade does not supposed CITES permits and has not therefore been fixed in CITES Annual Reports. One shipment of *A. baerii*, for example, was produced in aquaculture in France, reexported by another EC Member State (Germany) to the UAE (United Arab Emirates), reimported via Luxembourg to the EU before

[6] CITES = Commission for the International Trade of Endangered Species.

being reexported from the EU for the second time to Iceland (Engler and Knapp 2008). The mechanism of relevance to and fro quantity checking has been elaborated by UNEP-WCMC (The United Nations Environment Programme's World Conservation Monitoring Centre), but this tool so far can be applied only at the initial stage of reexporting. So, not that reliable "mere eye" technique has been used alternatively to perform the checking. This means that improvements in the regulations as well as in their enforcement should be brought.

38.4.2 The Assets of the Species

Which are the assets of the species? Indeed, this is the key question given that several sturgeon species are already farmed, but their relative importance is extremely variable. More, it has been reminded that the situation may change along the time with a consequence extremely delayed as compared to the switch of species due to the long biological cycle. In this last subsection, the ambition is to highlight some of the main points which may incline toward the Siberian sturgeon as compared with the other sturgeon species.

Even though it is not species specific, it has to be reminded that sturgeon has no bones which is a considerable marketing advantage as compared with other fish species toward the consumers. The species support well the transportation due to its respiratory capacity and its resistance to low oxygen content (Chaps. 18 and 50).

Several approaches related to the quality of the Siberian sturgeon products have been developed. For long now, professionals recorded that size, color, and taste of Siberian sturgeon caviar might be similar to those of the Russian sturgeon, i.e., one of the very reference in the field (Sabeau 1998). With regard to taste and flavor, based on the Siberian sturgeon, peculiar approaches have been developed to either characterize the product, thanks to the sensorial method currently applied in food products (Chap. 35). The method is also of use to check for the crunchiness of the caviar which might be a concern as it have been recently recalled by Parisi et al. (2014). More, in case of unsatisfied taste with regard to the demand, origin and treatment of the couple Fish-Water has been developed to get rid of this inappropriate taste which might be especially of concern in RAS (Chap. 36). And it was stated earlier in the chapter (Sect. 38.3.1.4) how these production systems have become popular worldwide.

For some years now, fish welfare has become a concern with two classical types of arguments. One deals with the wish to abolish pain and fear of the fish, and the other points out that the quality of the fish product is better whether welfare is taken into account along the production cycle. Two chapters are focused on the subject in the farming-related volume: Chap. 45 reports updated results on the usefulness of a few blood parameters, and Chap. 44 constitutes, to our knowledge, the foremost attempt to build a broad sturgeon welfare synthesis document, with a focus on the Siberian sturgeon. The fact that it has been possible to document all the

welfare-related aspects means that there are a lot of available specific results that even some would gain in new in-depth experiments. To achieve this task, it has been necessary to mobilize not only the classical literature but also the gray one as well as the experience of the authors in the field.

Aiming at sturgeon farming, especially the Siberian sturgeon, the populations of which are under a protective status all over its natural geographical extension, supposes that great care should be paid to genetic characteristics of the fish that are going to be bred. More, the origin of the specimen should be known as there are different Siberian sturgeon populations depending on the catchment the fish are coming from, e.g., from the Yenisey River in the Western part of Siberia up to the Kolyma River basin in the far East of Siberia with Lake Baikal basin in between (Chap. 1). As an example to this above comment, Shen et al. (2014) underlined this mismanagement as one of the explanations to some poor results in breeding the species in China. Data (methods and results) are available with regard to the genetic characterization of brood stocks of the species in Poland (Chap. 41) and in Russia (Chap. 42). More, regarding the last a large development is provided in connections between the farming stocks and the wild ones. Regarding France, it has been stated many times that the former initiation of the farming was founded at the beginning on specimen originated from the Lena River population. Altogether, traceability and methods for genetic variability control of the Siberian sturgeon are available. Again this may be regarded as an advantage as compared with other sturgeon species.

It has been mentioned several times that the Siuberian sturgeon is a non-native species in most of countries where it is farmed. Therefore, it is founded to ask for ecological risks in case of escapements of individuals from the farms. Among the risks is that of installation, i.e., a long-term adaptation of the species to a new environment with potential impacts on both native species and biotopes. Thanks to two documented examples, one in Russia (Chap. 46) and another one in France (Chap. 47), no installation has been recorded. This means that the risk of installation of the species is quasi null, and then the species cannot be considered as a potential invasive species. There are so few documented results in the field to not highlighting those two.

Finally, in a survey of the publications on sturgeon research conducted during the period 1996–2010, the Siberian sturgeon arrives in the second position with the white sturgeon as first (Jarić and Gessner 2012). More generally speaking, the study is mainly oriented on ecology and conservation that favored the White sturgeon. And the species closed to the Siberian sturgeon are the lake sturgeon (*A. fulvescens*) and the paddlefish (*Polyodon spathula*) that are not reputed to be subject to great farming extension. This means that Siberian sturgeon is one of the very few sturgeon species which has been the support for many diversified researches. Alike for the genetic variability evoked above, there is more chance to find directly the needed information when faced a question, as compared with other sturgeon species. This rich network of information is a considerable advantage in favor of the species.

In sum, the species is at present one of the major actors of world sturgeon market and has some potential advantages in the course of the competition between the sturgeon species. This prognosis (if any) will be only verified on a long-term basis because other trials will be tested in the meantime.

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Part IV

Long-Term Management of Brood Stock

Hybrids of the Siberian Sturgeon

39

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Abstract

It is known that all sturgeon species when crossed (both interspecific and intergeneric crossings) allow to produce viable offspring, whereupon breeding of various hybrids of Siberian sturgeon is of practical importance for commercial or caviar sturgeon farming in various climatic and technological conditions.

The current chapter provides a brief overview of the experimental and commercial works on the hybridization of sturgeon with Siberian sturgeon used as one of the parental species. The history and methods of breeding and rearing of the various interspecific and intergeneric hybrids of Siberian sturgeon (with Russian and Amur sturgeon, with Sakhalin and the Adriatic ones, with Beluga, Kaluga, and sterlet) are described. The main morphological, breeding and reproductive characteristics are also presented, and options for economic use of hybrids are considered.

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Generalization of data from different studies allows to conclude that the Siberian sturgeon in terms of commercial features and morphometric characteristics has a "dominant phenotype," while the hybrids of Siberian sturgeon occupy diverse intermediate positions relatively to the parental species or show similarities to the Siberian sturgeon.

Keywords
Siberian sturgeon • Intergeneric hybrid • Interspecific hybrid • Reciprocal hybrid • Morphology • Reproductive characteristics

Introduction

The use of interspecific sturgeon hybrids in commercial sturgeon breeding proved to be, in many cases, more economically effective than the rearing of pure species (Burtsev 1983) due to reasons as follows:

- Possibility of combining the desirable traits of two or more species in a hybrid, for instance, a large growth potential for anadromous species with freshwaterness, fast ripening, and flavor qualities of sterlet with adaptive capacities of Siberian sturgeon
- Despecialization—failure of conservative species adaptations and, as a consequence, increasing plasticity of the hybrids and their adaptability to unusual environmental conditions
- Use of formative processes, i.e., increase in genotypic variability to improve the efficiency of selection
- Use of the effect of heterosis, i.e., increase in the viability, productivity, and the rate of growth and maturation of the first generation hybrids and complex hybrids

The choice of Siberian sturgeon as one of the parental species to cross hybrids derives from a number of biological as well as technological features of this species:

- As indicated above, the Siberian sturgeon is highly adaptable to different conditions of holding and handling.
- "Freshwaterness" increases the survival rate of hybrids with anadromous species hybrids when grown under conditions of freshwater aquaculture.
- The existence of several intraspecific groups (populations) (Chap. 1), that have a wide variability of characteristics and properties, allows to use various breeding techniques (including remote crossing, etc.).
- It is a popular aquaculture species worldwide—high numbers of brood stock (breeders) and a well-designed biotechnology of breeding and rearing.

Breeding works on the use of Siberian sturgeon as the initial form at hybridization were launched in 1979 (Burtsev et al. 1985; Filippova 1985).

Works on the selection of different interspecific hybrids with Siberian sturgeon have been conducted in Russia (Chebanov 2000; Chebanov and Billard 2001;

Podushka 2011; Podushka and Armyaninov 2006; Rachek et al. 2010). Research has been carried out practically on all possible variants of interspecific crosses. However, the following hybrids have been distinguished as the most promising: a hybrid of Russian sturgeon with Siberian sturgeon of Lena population (GUBA), a hybrid of the Siberian sturgeon of Lena population with beluga (BAH) and Kaluga (BAD), and reciprocal ones between Siberian sturgeon and sterlet (BAR), Adriatic sturgeon and Siberian sturgeon (Lena population) (AL), ship sturgeon (Saber et al. 2015), and even Sakhalin sturgeon (BAM). It should be noted that when the names of a hybrid are mentioned in this chapter, the first species will always be females.

The most promising hybrids for commercial sturgeon and caviar appear to be ones between genetically closer species (e.g., between multi-chromosome Russian and Siberian sturgeon), rather than hybrids between low and multi-chromosome species. For the latter ones, the decreases of viability for the hybrids of the second generation and the emergence of disturbances in development and of generative system anomalies are typical (Burtsev et al. 1978).

The aim of the chapter is to present an overview of the papers devoted to hybridization of sturgeon with Siberian sturgeon as one of the paternal species. The description of hybrids in the present works is presented with different degree of detail, depending on the level of study and the extent of use of each in sturgeon culture.

39.1 Interspecific Hybrid of Russian Sturgeon (*A. gueldenstaedtii*) with Siberian Sturgeon (*A. baerii*)—"GUBA"

39.1.1 History and Methods of Breeding

For the first time, a hybrid between Russian sturgeon (of Azov population) and Siberian sturgeon (of Lena population)—"GUBA" was obtained in 1979. Experimental crossings were carried out between 1979 and 1983 in the "Aksay" fish farm and "Vzmorie" sturgeon hatchery in Rostov region (Burtsev et al. 1985a, b; Filippova 1985). Later on, works with this hybrid were conducted in Astrakhan region (Shevchenko 1989; Shevchenko et al. 1989; Shevchenko 1991), as well as in Vologda region—at a warmwater fish farm (Filippova 1988; Aref'yev and Filippova 1993; Safronov and Filippova 2002) and the South Branch of Federal Centre of Selection and Genetics for Aquaculture in Krasnodar region (Chebanov and Billard 2001; Chebanov et al. 2008). The experimental works on growing of reciprocal hybrids between Siberian and Russian sturgeon in ponds have been carried out in Armenia since 1981 (Mailyan and Akopian 1984).

Rearing of reciprocal hybrids between Siberian sturgeon of Lena population and Russian sturgeon in RAS has been conducted in Poland (Kolman and Szczepkowski 2001; Szczepkowska and Kolman 2002), while that of the hybrid between Russian sturgeon and Siberian sturgeon was carried out in Belorussia (Barulin et al. 2008).

Experimental works on the rearing of GUBA hybrids were performed under different conditions: in ponds (Burtsev et al. 1985a, b), cages (Shevchenko 1991; Chebanov and Billard 2001; Safronov and Filippova 2002), and cages of pond type (Chebanov and Billard 2001; Chebanov et al. 2008).

Large multi-aged brood stocks of GUBA hybrids were established at several fish farms in Russia: in the Krasnodar, Vologda, Ryazan, Kostroma, and Moscow regions (Chebanov and Billard 2001; Safronov 2003; Mailkova and Novosadov 2005; Krivoshein 2007).

The aim of brood stock creation was to obtain fertile hybrids with commercial features similar to Russian sturgeon (including "caviar"), combined with higher rates of growth, maturation, and survival.

39.1.2 Morphological and Biological Characteristics

In its appearance, the hybrid is more similar to the Siberian sturgeon (Figs. 39.1a, b and 39.2a, b). It has an elongated body, a wider head, and prolonged rostrum. The body color varies from gray brown to dark brown with a purple tinge; the belly has a bright or yellowish coloration.

Due to most plastic and meristic characters, the hybrid is intermediate with a bias toward the paternal species (Table 39.1).

Of the 38 features and body indices, studied in hybrid juveniles and original parental species, 17 are inherited matrilineally, while 21-patrilineally (Efimov 2004).

The second (1999), third (2006), and fourth (2013) generations of the GUBA hybrids were obtained in the South Branch of the Federal Centre of Selection and Genetics for Aquaculture (Krasnodar, Russia) (Chebanov unpublished). Evaluation of the breeding quality of the hybrid specimens of first and second generations was also

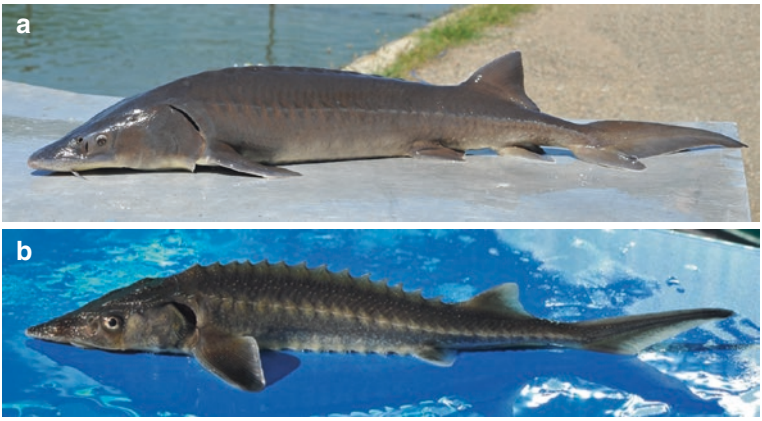

Fig. 39.1 (**a**) Hybrid between Russian sturgeon and Siberian sturgeon of Lena's population, side view (4 years, age; 4.2 kg, weight). (**b**) Juveniles of hybrid between Russian sturgeon and Siberian sturgeon of Lena's population, side view (120 days, age; 30 g, weight)

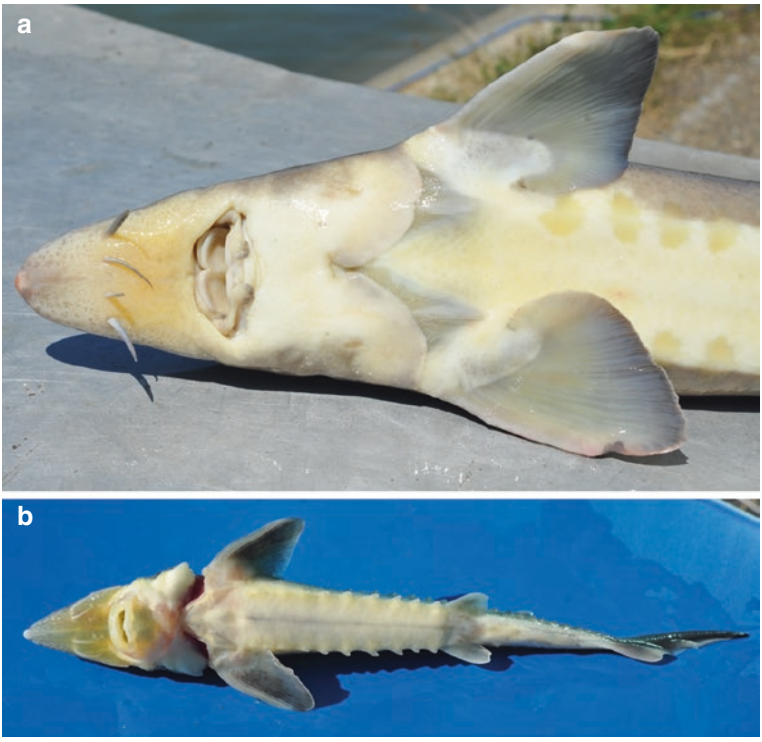

Fig. 39.2 (**a**) Hybrid between Russian sturgeon and Siberian sturgeon of Lena's population, a ventral view (4 years, age; 4.2 kg, weight). (**b**) Juveniles of hybrid between Russian sturgeon and Siberian sturgeon of Lena's population, a ventral view (120 days, age; 30 g, weight)

undertaken in Kadui fish farm. It was revealed that the viability of F1 was slightly higher than that of F2 and was close to the viability of Siberian sturgeon. The mean weight of F1 and F2 fingerlings was not significantly different, and under conditions of kadui fish farm amounted to about 180 g, while the mean weight of Siberian sturgeon fingerlings under the same conditions was 150 g (Safronov and Filippova 2004).

Thus, both the first (F1) and successive generations of the hybrids can be used with equal efficiency for the purposes of commercial sturgeon culture and caviar production.

39.1.3 Basic Breeding and Biological Parameters

The hybrid is characterized by a high growth rate that significantly outpaces that of Russian sturgeon at all stages of development (Efimov 2004). The best growth potential is manifested in the hybrid being grown at high temperatures and sufficient oxygen supply. With low content of dissolved oxygen in water, the daily gain, for example, for 300–800 g specimen, could decrease from 1.8% to zero per day (Gorsky and Yarzhombek 2003).

Table 39.1 Comparative morphometric characteristics of the GUBA hybrid and its parental species (according to Efimov 2004)

Character	Species, hybrids		
	Russian sturgeon	**Hybrid GUBA**	**Siberian sturgeon**
	Percentage of total body length		
Antedorsal distance	62.79	64.06	62.10
Anteventral distance	54.35	56.69	54.89
Antenatal distance	64.83	68.11	66.44
	% length head basis		
Snout length	44.32	51.23	53.45
Maximal head height	34.21	32.61	31.59
Minimal head height	25.45	22.41	20.13
Maximal head width	31.08	26.51	24.79
Distance from end of snout till midline of the middle barbels	21.69	28.84	32.99
Distance from end of snout till cartilaginous mouth roof	48.65	53.96	56.98
Length of the largest barbel	18.18	19.91	21.88
Meristic characters			
Number of dorsal scutes	10–13 (11.6)*	9–14 (12.4)	12–18 (15)
Number of lateral scutes	25–34 (29.6)	32–42 (36.7)	34–43 (39.6)
Number of ventral scutes	9–12 (10.5)	9–11 (10.2)	9–10 (9.2)

*the mean value (in the brackets).

In industrial conditions of hybrid rearing under an optimal temperature regime, the survival rate of 5–10 g juveniles, from the 1-day larvae, amounts to about 50%. Elevated temperatures allow a significant (twofold and above) increase in the growth rate of juveniles, but in this case the survival rate decreases down to 30–40% (Table 39.2).

If the first generation hybrids and the parent species (Russian sturgeon) have been reared in the same conditions throughout all the period of growth till maturity, the index of weight gain of the hybrids will be higher than that of Russian sturgeons of Azov population by an average of 20% (Filippova 1985). It can even reach over 40% in the Caspian population (Table 39.3).

At a mean temperature of about 20° C, under the age of 1 year, the growth rate of the hybrid is less than that of the reciprocal hybrid GUBA (*A.b.* × *A.g*), but with an increase in temperature to 24–25° C, the weight gain of the hybrid between Russian and Siberian sturgeon of Lena population is higher than that of the reciprocal hybrid (Szczepkowska and Kolman 2002; Filippova 2009).

As shown by Chebanov and Billard (2001), the hybrid GUBA, under optimal thermal regime, under the conditions of a warmwater fish farm with RAS, exhibited a considerably higher growth rate than Siberian sturgeon. The hybrid specimens reached 4.1–8.9 kg by the age of 3 years and 11–17 kg by the age of 5 years.

Table 39.2 Influence of the rearing temperature on the growth and survival rate of the hybrid (according to Safronov 2003)

Character	Temperature (°C)		
	18–22	25–26	26–28
Mean weight at the end of rearing, g	5–6	12	26
Survival rate (from 1-day prelarvae)	47–49	30–40	30

Table 39.3 Comparative characteristics of growth rate of hybrids and their parental species (according to Filippova 1985)

Age	Growth indices						
	Siberian sturgeon		GUBA hybrid		Population of Russian sturgeon		
					Caspian	Azov	
	Mean weight (g)	Weight gain (%)	Mean weight (g)	Weight gain (%)	Mean weight (g)	Mean weight (g)	Weight gain (%)
0+	220.0	–	300.0	–	160.0	240.0	–
1+	900.0	309.1	1270.0	323.3	400.0	1060.0	341.7
2+	1740.0	93.3	2600.0	104.7	900.0	2200.0	107.5

39.1.4 Reproductive Peculiarities

Under conditions of Kadui rybkhoz warmwater fish farm cages at the Cherepovetskaya power station male hybrid specimens reach maturation at the age of 7 years, while female matured at the age of 10 years (mid-annual temperature of the water at the farm is 14.5 °C).

The higher rate of maturation is typical for the hybrid under the conditions of Southern Russia. Thus, even at the natural temperature regime, males get mature at 3–4 years of age and females at 5–6 years. When using a RAS water system or under conditions of warmwater aquaculture, maturation of some individual hybrid females can be reached by the end of 4 years and almost all females reach maturity at 5 years old (Chebanov and Billard 2001; Chebanov et al. 2008).

The interspawning interval in females depends on the temperature of the region of holding and amounted about 9000 degree days (Filippova 1985). Males of the hybrid get mature annually.

Observations at the experimental RAS module in the Moscow region revealed that the smaller even aged fish reached their maturation earlier and spawn on average more often than large ones (Filippova 1985).

Long-term observations of the gonad development and monitoring of the reproductive system with application of an express technique of ultrasound diagnostics (Chebanov and Galich 2011) showed that the abnormal development of the gonads, including obesity, which is typical for Siberian sturgeon under industrial rearing conditions, is quite rarely (two to three cases per 1000 females) observed in the case of hybrids.

Hybrid brood fish (both females and males) adequately respond to hormonal stimulation of spawning through injections of a synthetic super analogue of gonadotropin-releasing

Table 39.4 Comparative characteristics of generative performance hybrids and parental species in the optimum conditions

Parameter	Species, hybrids		
	Russian sturgeon	GUBA hybrid	Siberian sturgeon
Age of puberty	5–7	4–6	4–6
Interspawning interval, year	2	1 (40%)–2(60%)	1–2
Relative fecundity, %	8–11	15–18	12–15
Number of ovulated (infertile) eggs in 1 g	45–47	42–46	50–54

hormone—GnRHa. The number of females reacting positively to the injection amounts to, on average, 88–95% (Chebanov et al. 2004, 2008).

The hybrid has a high relative fecundity. Egg yield, when using the in vivo stripping method of Podushka (1999), amounts to12–15% of body weight for the first-time spawning females and up to 18% for females for a second time (Table 39.4).

Eggs of the hybrids appear similar to those of Russian sturgeon: they have a dark brown coloration; the animal pole is more intensely pigmented than the vegetative one. High palatability of processed caviar is also consistent with that of Russian sturgeon caviar.

39.1.5 Commercial Features

The combination of the high rates of growth and maturation of females and the short interspawning interval when reared under industrial aquaculture conditions make this hybrid a promising hybrid for rearing in sturgeon farms focused primarily on the caviar production.

At present, the practical economic use of the hybrid has been performed in two directions: the annual obtaining of first-generation hybrids and the reproduction of hybrids “in itself.” The hybrid has a clear heterosis with respect to both the parental species. The effect of heterosis by weight in relation to the parental species amounts to 15–20% at 2 years of age. The heterosis effect on fertility amounts to about 3–5% (Safronov 2003).

The hybrid when compared with the Russian sturgeon exhibits a higher resistance to adverse effects, including bacterial diseases (Efimov 2004). An important factor that contributes to the efficiency and attractiveness of using hybrids in commercial rearing is its fertility and the homogeneity of the reproduction “in itself” in the second and third generations. The volume of this hybrid production in Russia amounted to about 1400 mt in 2015.

39.1.6 Reciprocal Hybrid of Siberian and Russian Sturgeon (A.b. × A.g.)

Works on the intensive rearing of reciprocal hybrids (*A.b.* × *A.g.*) were carried out in Poland (Szczepkowski et al. 2002). In its appearance, the hybrid is similar to the Siberian sturgeon, but on the majority of plastic and meristic characters, it demonstrates patroclinal inheritance and proximity to the Russian sturgeon (Table 39.5).

The comparison of growth rates of Siberian sturgeons and hybrid specimens, held under similar conditions, was conducted for the evaluation of the breeding qualities of the hybrid. During the first 60 days of observation, Siberian sturgeons and hybrids showed similar daily growth rates: 1.61% and 1.63%, respectively. Then, the growth rate of the Siberian sturgeons slowed, and by the age of 9 months, the difference in weight between the hybrid and Siberian sturgeon specimens amounted to above 23% (Table 39.6).

The hybridization between anadromous and potamodromous species allows to suggest a considerable heterosis effect in the first generation, that demonstrates higher growth rate than that of the hybrid. Hence, the hybrid between Siberian and Russian sturgeon can be evaluated as a promising hybrid for commercial sturgeon culture. But it should be noted that, on the whole, this hybrid has been less popular in sturgeon farming than GUBA.

Table 39.5 Values of meristic features of Siberian sturgeon (*A.baerii* Brandt) and its hybrid with Russian sturgeon (*A. gueldenstaedtii* Brandt) (mean values and standard deviations) (according to Szczepkowski et al. 2002)

Meristic feature	*Acipenser baerii*	Hybrid of *A. baerii* with *A. gueldenstaedtii*
Number of dorsal bony plates—Sd	14.4 ± 1.2	11.9 ± 1.6
Number of lateral bony plates—Sl	47.5 ± 2.1	37.9 ± 2.8
Number of ventral bony plates—Sv	9.0 ± 0.5	8.3 ± 0.9
Number of rays in the dorsal fin—D	46.5 ± 2.4	41.6 ± 2.1
Number of rays in the anal fin—A	33.4 ± 2.6	26.4 ± 1.8

Table 39.6 Dynamics of growth rates of Siberian sturgeon and the hybrid Siberian sturgeonRussian sturgeon

	Initial weight (g)	Weight in 60 days (g)	Daily weight gain (%)	Weight at age 9 months (g)	Weight gain (%)
A. b.	206.9 ± 29.8	410 ± 62	1.61	872 ± 145	323.3
A. b. × *A.g.*	216.6 ± 36.2	428 ± 76	1.63	1.078 ± 157	399.1

39.2 Interspecific Hybrid of Siberian Sturgeon (*A. baerii*) and Sterlet (*A. ruthenus*)

39.2.1 History and Method of Rearing

The wild specimen of this hybrid was morphologically described for the first time by Berg in 1911 (Berg 1948). The first comparison of growth and survival rates of this so-called "baeru" hybrid *(A.b.* × *A.r.)* and reciprocal one (*A.r.* × *A.b.*) called "rubaeus" (Sect. 39.4.1) in aquaculture conditions was conducted by Ronyai et al. (1991a, b). It was revealed that the hybrid, obtained with the use of Hungary reared females of Siberian sturgeon (progeny from specimens of Lena population) and males of sterlet (progeny from Danube River specimens) under conditions of intensive rearing at high stocking density, showed a growth rate similar to the one of the Siberian sturgeon (Table 39.6). A high diversity of sizes of individual fishes was also recorded. The coefficient of variation (Cv) of individual weight of "baeru" was 0.21, while for "rubaenus" and for Siberian sturgeon, it was 0.19 and 0.16, respectively. More detailed results of comparative analysis were presented in Ronyai et al. (1991b).

System works on the rearing of an interspecific hybrid of the Siberian sturgeon with sterlet were launched in the Konakovo branch of the commercial sturgeon VNIIPRKh in 1990. The purpose of this creation was to get a hybrid that combines a high adaptive capacity and the enhanced rate of growth of the Siberian sturgeon (under warmwater aquaculture conditions) with the resistance to technological effects and diseases, as well as the early maturation of the sterlet. To obtain that, the hybrid interbreeding of females of domesticated Siberian sturgeon with males of domesticated sterlet was performed.

39.2.2 Morphological and Biological Characteristics

The hybrid appears differently from the parental species on several features as follows: the body coloration, size of the body head and rostrum, the number of scutes, etc. By its morphometric characters, the hybrid takes an intermediate position when compared with the parental species both at the first and the second year of life, with a bias toward the maternal species, that justifies the matroclinal type of inheritance (Table 39.7) (Petrova et al. 2008).

The comparison between the hybrid and its parental species in terms of counting also confirms its matroclinal type of inheritance (Table 39.8) (Petrova et al. 2008).

In order to establish more accurately the similarity of the hybrid with the parental species, values of the Hubbs hybrid index (modified by Verigin and Makeyeva 1972) were calculated, and a diagram of their distribution was compiled (Fig. 39.3).

The analysis of this diagram has shown that the vast majority of characters are inherited matroclinally, i.e., with a bias toward the Siberian sturgeon, except for the following indices: the number of dorsal and ventral scutes (number 13 and 15 on the diagram, respectively).

Table 39.7 Comparative morphometric characteristics of hybrids between Siberian sturgeon and sterlet and its parental species (according to Petrova et al. 2008)

Indices	Species, hybrids					
	Yearlings (0+)			One-year-old fish (1+)		
	Siberian sturgeon	Hybrid	Sterlet	Siberian sturgeon	Hybrid	Sterlet
1. Weight of fish, g	260.3 ± 6.5	230.4 ± 5.8	190.2 ± 4.8	1250.1 ± 32	920.0 ± 23.4	380.2 ± 9.8
2. Length of fish, cm	43.5 ± 1.1	41.4 ± 1.03	35.2 ± 0.88	63.4 ± 1.5	58.6 ± 1.5	40.5 ± 1.1
3. Length of fish till the end of mid-rays, cm	37.1 ± 0.9	34.0 ± 0.9	30.2 ± 0.76	55.7 ± 1.4	52.1 ± 1.3	36.1 ± 0.9
4. Fish length till bases of mid rays, cm	34.1 ± 0.8	31.7 ± 0.8	28.2 ± 0.7	51.5 ± 1.3	48.6 ± 1.2	33.7 ± 0.8
5. Trunk length, cm	23.7 ± 0.6	24.5 ± 0.6	22.5 ± 0.57	38.6 ± 0.9	36.5 ± 0.9	26.9 ± 0.7
6. Snout length, cm	5.4 ± 0.1	5.2 ± 0.1	3.1 ± 0.07	6.8 ± 0.2	5.8 ± 0.1	3,9 ± 0,1
7. Mouth width, cm	2.4 ± 0.1	2.3 ± 0.1	1.46 ± 0.03	2.6 ± 0.1	2.4 ± 0.1	1.5 ± 0.1
8. Head length, cm	9.3 ± 0.2	7.3 ± 0.2	5.69 ± 0.14	12.8 ± 0.3	11.8 ± 0.3	6.8 ± 0.2
9. Maximal body height, cm	4.5 ± 0.1	4.4 ± 0.1	3.75 ± 0.09	7.1 ± 0.2	5.6 ± 0.1	4.2 ± 0.1
10. Maximal body width, cm	3.4 ± 0.1	3.5 ± 0.1	2.96 ± 0.07	5.9 ± 0.1	6.4 ± 0.2	3.4 ± 0.1
11. Maximal girth, cm	11.9 ± 0.2	11.6 ± 0.3	10.9 ± 0.27	19.8 ± 0.5	18.7 ± 0.5	13.3 ± 0.3
12. Minimal body height, cm	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.8 ± 0.1	1.6 ± 0.1	1.1 ± 0,1

Table 39.8 Comparative characteristics of meristic characters of hybrid and parental species (according to Petrova et al. 2008)

Character	Species, hybrids		
	Siberian sturgeon	Hybrid	Sterlet
13. Dorsal scute count	16.0 ± 0.29	14.8 ± 0.3	13.6 ± 0.24
14. Lateral scute count	44.9 ± 0.69	49.3 ± 0.81	61.8 ± 0.7
15. Ventral scute count	11.0 ± 0.21	12.8 ± 0.33	12.5 ± 0.26
16. Count of rays in dorsal fin	44.9 ± 0.47	42.6 ± 0.46	39.6 ± 0.52
17. Count of rays in anal fin	25.9 ± 0.52	24.2 ± 0.35	21.8 ± 0.45
18. Gill raker count	31.2 ± 0.29	28.2 ± 0.41	21.0 ± 0.52

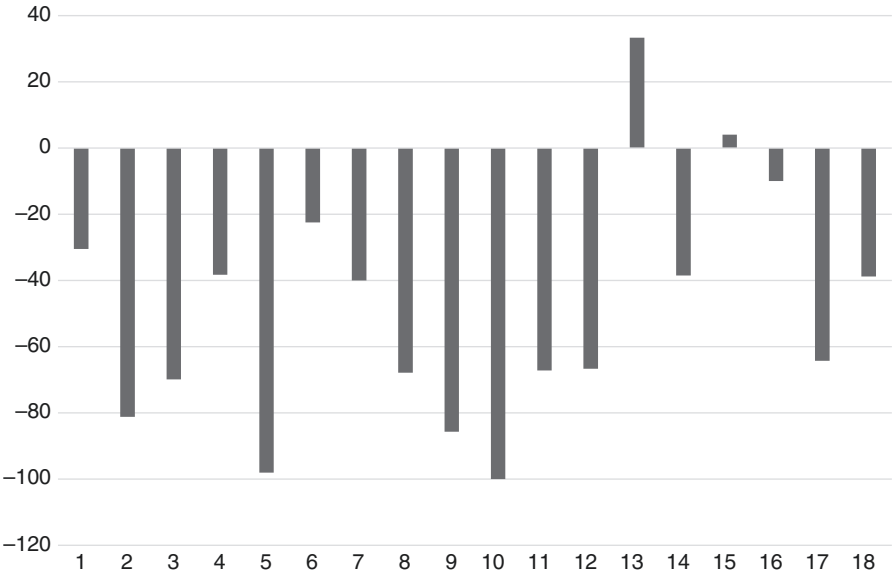

Fig. 39.3 Diagram of the hybrid index distribution (compiled after data of Petrova et al. 2008) (on the abscissa (*X*), characters; on the ordinate (*Y*), corresponding values of the hybrid index)

39.2.3 Main Hatchery and Biological Indices

The results of the comparison of fish breeding and biological characteristics also confirm an intermediate position of the hybrid with respect to its parental species. The highest survival rate, including high resistance to bacterial diseases, is typical of sterlet, followed by the hybrid and Siberian sturgeon (Petrova et al. 2008). The growth of hybrid juveniles was mostly higher than that of both the original species. With age, the growth rate of the hybrid approaches that of the Siberian sturgeon, exceeding the one for sterlet (Table 39.9).

Table 39.9 Comparative hatchery and biological characteristics of the hybrid and its parental species (Petrova et al. 2008)

Index	Value at different age					
	0+			1+		
	Siberian sturgeon	Hybrid	Sterlet	Siberian sturgeon	Hybrid	Sterlet
Stocking density, ind/m^2	300	300	300	75	75	75
Mean weight at start of rearing, g	19	23	16	338	303	242
Survival rate during rearing period, %	72	76	81	92	97	98
Mean weight at the end of rearing, g	245	230	190	1213	908	376
Weight gain, r	226	207	174	875	605	134
Growth rate, %	1189	900	1087.5	259	200	55
Index of weight gain	0.056	0.054	0.051	0.049	0.042	0.016
Feed conversion rate	1.6	1.3	1.3	2.8	2.5	3.0
Fish production, kg/m^2	53	51	47	84	66	28

39.2.4 Reproductive Peculiarities

The parental species of the hybrid have different ploidy (Vasil'ev 1985): Siberian sturgeon refers to multi-chromosome species ($2n = 238 \pm 7$), while sterlet to low-chromosome species ($2n = 118 \pm 2$). A hybrid between Siberian sturgeon and sterlet is characterized by an intermediate number of chromosomes: 180, that corresponds to the triploid state, which is often accompanied by sterility. Detailed studies of the reproductive system of hybrids has not, to the best of our knowledge, been conducted, thus the question of sterility of hybrids remains open. In this context, information about possible hybridization of native sterlet and Siberian sturgeon ("rubaenus") observed in the Upper Danube (Ludvig et al. 2009) has caused anxiety.

39.2.5 Commercial Features

The hybrid has a clear heterosis for viability, expressed in increased resistance to myxobacteriosis during the first year of life. The manifestation of the effect is amplified under conditions of high stress and higher stocking density. The increase in stocking density of hybrids by 25% as compared with the Siberian sturgeon allows getting higher fish productivity while maintaining a high quantitative yield. Fish productivity of commercial 2-year-old hybrids amounts to 65–70 kg/m^2 (Petrova et al. 2008).

The effect of heterosis for weight in the hybrid as regards the Siberian sturgeon is low and is ranged between 2 and 9% for specimens from the age of 1 month till 2 years. Regarding sterlet on under-yearlings, it rises by up to 165% and for 2-year-old fish—by up to 105%. The commercial weight of 2-year hybrids is 1.8–2.0 kg. It

is favorably different from hybrid parental species in terms of feed conversion ratio. The feed-related expenses in the course of rearing are decreased by 20–25% (Baranov 2000).

When considering the ratio of the edible and non-edible parts of the body, the hybrid also occupies an intermediate position with a bias to the maternal side. The total amount of the edible parts of the body amounts to 58.2% for 2-year-old hybrids.

39.3 Interspecific Hybrid of Sterlet (*A. ruthenus*) and Siberian Sturgeon (*A. baerii*)

Experimental works on hybrids were conducted at Volgorechensk fish farm (Kostroma city) under conditions of warmwater aquaculture. Sterlet and Siberian sturgeon of Lena population were used as the maternal and paternal species, respectively (Krivoshein 2006).

39.3.1 History and Method of Rearing

As mentioned in Sect. 39.2.1, the hybrid of sterlet and Siberian sturgeon rubaenus was obtained in May 1987 in Hungary. A comparative analysis of growth rate between this hybrid and the reciprocal hybrid baeru as well as the Siberian sturgeon, all reared under equal industrial conditions, showed (Table 39.10) that despite the fact that the mean weight of rubaenus was 13–14% less than that of other hybrids and of the Siberian sturgeon, the difference ($p = 0.05$) was not significant (Ronyai et al. 1991).

The aim of crossing is to get a hybrid that can withstand a wide range of water temperature during rearing with a predominance of high temperatures during the whole period of holding.

Table 39.10 Comparative characteristics of the growth rate of the hybrid and its parental species (Krivoshein 2006)

Index	Siberian sturgeon	Hybrid *A.r.* × *A.b*	Sterlet
Body weight at age 1+, g	55.0	50.0	36.0
Index of weight gain at 1 year of age	0.112	0.097	0.090
Body weight at age 1+, g	454 ± 35	436 ± 41	287 ± 27
Body weight at age 2+, g	1740 ± 117	1523 ± 97	452 ± 46
Weight gain, g	1286	1087	165
Growth rate of 3-year-aged fish, %	283	249	42,6

39.3.2 Morphological and Biological Characteristics

According to its morphological and biological characteristics, the hybrid occupies an intermediate position as regards the parental species and demonstrates a patroclinal type of inheritance (Table 39.10).

Determining of the nature of inheritance and similarities between the hybrid and the parental species was carried out by calculating the values of the hybrid index of Hubbs modified by Verigin and Makeyeva (1972) with successive plotting of their distribution chart (Rechinsky 2003).

The hybrid index distribution diagram (Fig. 39.4) shows that the vast majority of characters except the index "count of dorsal and ventral scutes" (number 13 in the chart) are inherited patroclinously, i.e., with a bias toward the Siberian sturgeon.

Thus, multi-chromosome Siberian sturgeon, when hybridized with sterlet, exhibits a dominant influence on the phenotype of offspring, regardless of its use as maternal or paternal species.

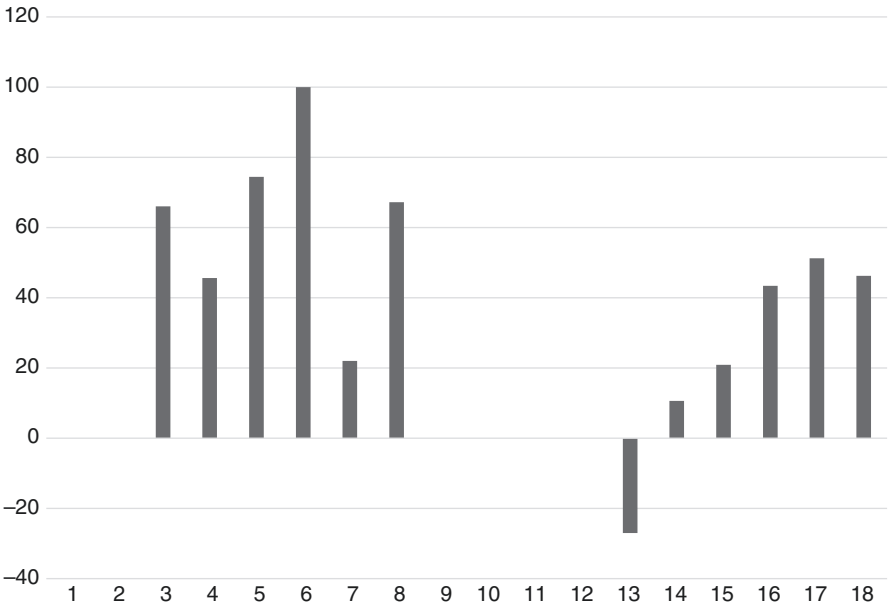

Fig. 39.4 Diagram of hybrid index distribution (list of indices is presented above, in the section "hybrid between Siberian sturgeon and sterlet" in (on the abscissa (X), characters; on the ordinate (Y), corresponding values of the hybrid index) (Complied after data of Rechnisky 2003)

39.3.3 Reproductive Peculiarities

The hybrid has a triploid chromosome set, which is typically accompanied by sterility. Along with this, trials on rearing of this hybrid males till their maturation were conducted at Karmanovsky fish farm in February 1998.

In order to test the ability of hybrids to produce mature gametes, four specimens were selected with mostly evident spawning dress, and hormonal stimulation of their maturation was conducted. The seminal fluid that was obtained from the three stimulated males demonstrated, on microscopic examination, a high mobility of the sperm after its activation with water (Podushka 2004). Spermium heads of the hybrid specimens had an elongated shape, while no anomaly in their structure was recorded. Further studies on genetic performance of hybrid spermium and their ability to fertilize the eggs have not, to the best of our knowledge, been conducted.

39.3.4 Commercial Features

The apparent effect of heterosis in relation to the sterlet and the patrocline nature of inheritance make this hybrid promising for rearing as a commercial object at sturgeon farms. The hybrid specimens reach their commercial weight of 1500 g at 2 years old. The ratio of edible and non-edible parts in this hybrid is also close to that of Siberian sturgeon, surpassing those characters of sterlet.

39.4 Interspecific Hybrid of Siberian Sturgeon (*A. baerii*) with Beluga (*Huso huso*)

39.4.1 History and the Method of Rearing

The work on the breeding of interspecific hybrid between Siberian sturgeon and beluga were carried out in 2004–2008, at the Elektrogorsk sturgeon fish farm, near power plants (the Moscow region) (Novosadov 2011).

The purpose of this creation was to obtain a highly productive hybrid, which would allow a significantly reduced duration of the production cycle of commercial sturgeon rearing.

In order to get the hybrid, an intergeneric mating of Siberian sturgeon females with beluga males (both from hatchery-reared brood fish) was performed.

39.4.2 Morphological and Biological Characteristics

A typical hybrid body coloration of the hybrid is completely obvious at the age of 2 months and is similar both to Siberian sturgeon (from light to dark gray) and beluga (bronze “beluga” hue).

The hybrid inherits primarily maternal exterior characters. Its main differences from the Siberian sturgeon occur (Novosadov 2011), firstly, in the features of the head: the barbels look like those of the Siberian sturgeon, without any fringe, and are oval in their cross section. They are all of the same length and do not reach the edge of the upper lip. The shape of the mouth of the hybrid is slightly semilunar, similar to that of beluga, as the mouth of the Siberian sturgeon takes an intermediate position between the one typical for parental and maternal species (*A. baerii* and *H. huso* respectively), with some bias toward the beluga. The lower lip is interrupted and the break of the lower lip is 23%. That occupies an intermediate position between the Siberian sturgeon and the beluga, this value being, respectively, 11% and 39% for this latter species.

On the majority of the 39 studied characters, the hybrid has a bias in the direction of the parental species (Siberian sturgeon). In this, the difference with the parental species is statistically significant by 33 characters. The hybrid significantly differs from beluga by 23 plastic and 4 meristic characters and from the Siberian sturgeon by 18 and 5 characters, respectively. At the same time, the hybrid differs from both parents by 14 plastic and 4 meristic characters (Table 39.11).

Table 39.11 Morphometric characteristics of the hybrid *A.b.* × *H.h.* (Novosadov 2011)

Index	Value	Cv	Significance of differences		
			With beluga	With Siberian sturgeon	With band Siberian sturgeon
Weight, g	185.7 ± 9.33	35.5	9.94	6.04	−5.53
Zoological length, cm	36.8 ± 0.58	11.2	9.82	7.47	−4.2
% Body length basis					
Head length, cm	8.8 ± 0.12	9.97	9.92	8.98	−2.39
% head length basis					
Rostrum length, cm	4.5 ± 0,07	11.5	12.14	6.31	−6.68
Maximal head width, cm	3.4 ± 0.07	13.8	7.49	8.2	0.55
Mouth width, cm	2.2 ± 0.03	9.11	−4.03	11.13	14.78
Width of lower lip break, cm	0.5 ± 0.01	15.5	−19.95	23.8	38.11
Maximal body height, cm	4.0 ± 0.08	15	7.68	5.13	−3.4
Minimal body height, cm	1.1 ± 0.03	19.9	7.51	8.13	0.2
Maximal body coverage, cm	12.7 ± 0.26	14.5	−42.94	4.49	−47.43
Meristic characters					
Dorsal scute count	12.9 ± 0.13	7.05	0.09	−3.14	−2.69
Lateral scute count	42.6 ± 0.37	6.21	0.49	1.91	1.46
Ventral scute count	9.0 ± 0.13	9.89	−6.24	−8.21	−0.84
Count of rays in dorsal fin	45.0 ± 0.42	6.67	−28.51	4.57	45.42
Count of rays in anal fin	24.2 ± 0.32	9.33	−17.43	3.99	21.55
Count of rakers at the first gill arch	27.4 ± 0.35	8.9	8.82	−2.97	−10.5

Analysis of the data presented in Table 39.11 confirms the higher level of similarity between the hybrid and the Siberian sturgeon and, consequently, the matroclinal way of inheritance.

39.4.3 Main Production and Biological Indices

The duration of embryonic development of the hybrid and of the maternal species (Siberian sturgeon) was similar and, at a water temperature of 15 °C, amounted to 6 days. Beluga, at this temperature, tended to develop more slowly. On this index the hybrid exhibited matrocliny.

The survival rate of Siberian sturgeon and hybrid "*A.b.* × *H.h.*" embryos prior to hatching did not differ significantly and amounted to an average of 77% and 50%, respectively.

By the end of the rearing period, fingerlings of the maternal species (Siberian sturgeon) reached a mean weight of 220 g, while the hybrid specimens reached 300 g (Table 39.12). The index of weight gain was also higher in the hybrid. The heterosis effect was manifested in somatic growth, i.e., the hybrid weight was 1.4 times higher than that of the Siberian sturgeon (Novosadov 2011).

By the end of the rearing period, the 2-year specimens of the hybrid "*A.b.* × *H.h.*" reliably exceeded, by that index (at 118 g or 12%), the 2-year ones of Siberian sturgeon. By the end of the second year of rearing, the fish productivity amounted to 148 kg/m^3 for the hybrid "*A.b.* × *H.h.*" and 121 kg/m^3 for Siberian sturgeon.

39.4.4 Reproductive Peculiarities

The parental species of the hybrid have a different number of chromosomes: the Siberian sturgeon has more chromosomes (2n = 238 ± 7) than the beluga (2n = 118 ± 2). A hybrid between these species should have an intermediate number of chromosomes: approximately 180 (the triploid state).

Table 39.12 Key rearing indices of the hybrid and its parental species (Novosadov 2011)

	0+			1+		
Index	**Siberian sturgeon**	**Hybrid**	**Beluga**	**Siberian sturgeon**	**Hybrid**	**Beluga**
Mean weight at the start of rearing, g	3.5	4,6	6,7	200,0	200,0	400,0
Mean weight at the end of rearing, g	220.0	300.7	400.0	898.0	1016.0	2600.0
Weight gain, g	216.5	296.1	393.			
Weight gain, %	6185.7	6436.9	5870.2			

A histological study of the gonads of the hybrid between Siberian sturgeon and beluga (Novosadov 2011) was conducted. It showed that this hybrid had a typical reproductive system. Anomalies at the second stage of development were not revealed. Thus, the theoretical sterility of the hybrid (as a consequence of triploidy) has not been confirmed yet.

39.4.5 Commercial Features

Intergeneric hybrids between Siberian sturgeon and beluga are characterized by a high weight gain, this feature inherited from beluga, and a good adaptability to the conditions of industrial fish rearing and disease resistance.

The hybrid is well adapted to the different methods of rearing: good results were obtained both when rearing was done in cages and in ponds. The hybrid reaches the commercial weight of 1800–2400 g at the end of the second year of rearing. Fish productivity of hybrids amounted to 90–100 kg/m^3. By the fifth year, the hybrid reaches an average weight of 6.7 kg (maximum value 10.5 kg) (Shishanova and Lippo 2008).

39.5 Interspecific Hybrid of Siberian Sturgeon (*A. baerii*) with Amur Sturgeon (*A. schrenckii*)

39.5.1 History and Method of Breeding

After the building of the brood stocks of Siberian sturgeon (*A. baerii*) of Lena and Baikal populations (*A.b.*L and *A.b.*B), those were delivered from the Kostroma and Trans-Baikal regions as larvae, experimental mating of native Amur sturgeon and introduced species were conducted at a warmwater farm in Primorsky region (Far East of Russia).

Works on experimental rearing of hybrids of Siberian sturgeon of Lena and Baikal population were carried out from 2000 to 2015 in cages of the research station of "TINRO Center," located close to the warm waters of the Primorskaya regional power plant in the Luchegorsk settlement, Primorsky Region (Rachek et al. 2010). For the last 15 years, the annual amount of heat in the cages of the station ranged from 4210 to 4720 heating degree days (HDD). Temperature of water varied from 1–3 °C in winter to 27–33 °C in summer. The materials for the research were reciprocal hybrids between Siberian sturgeons of Lena and Amur sturgeons (*A.b.*L × *A.sch.* And *A.sch.* × *A.b.*L), and Siberian sturgeons of Baikal population and Amur sturgeon (*A.b.*B × *A.sch.* And *A.sch.* × *A.b.*B) at ages varying from the yearling to 4 years old. Production parameters were studied in mature females of hybrid forms *A.b.*L × *A.sch.* And *A.sch.* × *A.b.*L during the process of long-term exploitation. Initial brood stocks intended for hybridization were provided by 1992 generation *A.b.*L breeders, 2001 generation breeders of *A.b.*, and 1993 generation *A.sch.* breeders.

Every spring, the fish were sorted and stocked at a lower density. The survival rate of yearlings was calculated from the larvae that went to active feeding until 2-, 3-, and 4-year-old specimens, for each year of the experiment. During autumn assessment, weighing and measuring of live hybrid specimens aged 0+, 1+, 2+, and 3+ from each experimental cage were performed with an accuracy of 5 g and 2 mm. The coverage indices (O/l_1,%), condition factors (K_y) in length l_1 and coefficient of weight accumulation ($K_м$), feed expenditures per year (kg per kg of weight gain), and fish capacity of cages (kg/m^2) were reported. General meristic and plastic characters were determined for 30–50 2-year-old individuals. To provide characteristics of females, weight of obtained eggs, and weight of one egg, "working" fecundity and gonadosomatic index (GSI) were assessed. The terms of female maturity and their interspawning intervals were also studied.

Hybrid juveniles of more than 20 g were transferred to 10 m^2 net cages of nylon mesh, where they were fed with pellets produced by TINRO Center and grown both to mature specimens or market size. The protein content in the feeds ranged from 38 to 42%.

39.5.2 Morphological and Biological Characteristics

During autumn assessment, weighing and measuring of live hybrid specimens aged 0+, 1+, 2+, and 3+ from each experimental cage were performed with an accuracy of 5 g and 2 mm. The coverage indices (O/l_1,%), condition factors (K_y) in length l_1 and coefficient of weight accumulation ($K_м$), feed expenditures per year (kg per kg of weight gain), and fish capacity of cages (kg/m^2) were reported. General meristic and plastic characters were determined for 30–50 2-year-old individuals. To provide characteristics of females, weight of obtained eggs, and weight of one egg, "working" fecundity and gonadosomatic index (GSI) were assessed. The terms of female maturation and their interspawning intervals were also studied.

The results of experiments are presented below (Table 39.13).

By the end of the experiment, the highest weight was recorded for the 4-year specimens of reciprocal hybrids of Siberian sturgeon of Baikal population with one of Amur population. On the whole, this was associated with initial advantage in weight and age of fingerlings and lower fish stocking density in cages throughout the experiment due to extremely high elimination of fry of both hybrid forms during rearing in tanks. The highest weight was typical for hybrid *A.sch.* × *A.b.*B. The condition factor and relative girth of both hybrids were lower than that of *A.sch.* × *A.b.*A and *A.sch.* × *A.b.*L.

The weight of 4-year-old hybrids *A.b.*L × *A.sch.* And *A.sch.* × *A.b.*L specimens was lower than that of Siberian sturgeon hybrids on the basis of Baikal population. However, they differed significantly by a higher survival rate. Fish capacity of cages with these hybrids exceeded 85–91 kg per m^2 of cage.

For the control, the pure line of generation 2000 and 2004 *A.b.*L was grown in cages. The average weight of 4-year-old specimens of both generations was 2.31 kg.

Table 39.13 Results of experimental (years 2000–2012) rearing of hybrid forms of Siberian sturgeon of Lena and Amur populations up to the age of 4 years

	Characteristics						
Hybrid form	Length l_1 (cm)	Weight (kg)	Girth index (%)	K_y	$K_м$	Feed expenditures per weight gain (kg/kg)	Survival rate (%)
0+							
*A.b.*L × *A.sch.*	27.0	0.113	40.3	0.59	0.080	3.2	59.2
A.sch. × *A.b.*L	26.7	0.112	40.4	0.64	0.079	3.1	69.7
*A.b.*B × *A.sch.*	31.2	0.155	37.1	0.63	0.107	2.7	5.6
A.sch. × *A.b.*B	31.4	0.167	39.1	0.64	0.108	6.5	9.0
1+							
*A.b.*L × *A.sch.*	48.2	0.720	40.0	0.74	0.034	3.6	84.2
A.sch. × *A.b.*L	45.7	0.602	40.9	0.71	0.030	5.3	52.4
*A.b.*B × *A.sch.*	54.4	0.980	39.4	0.73	0.037	3.2	86.1
A.sch. × *A.b.*B	48.5	0.797	41.1	0.76	0.032	2.4	82.2
2+							
*A.b.*L × *A.sch.*	61.1	1.583	42.4	0.80	0.022	3.0	95.7
A.sch. × *A.b.*L	58.2	1.396	43.8	0.82	0.022	3.6	87.7
*A.b.*B × *A.sch.*	69.7	2.116	39.6	0.76	0.023	2.9	91.6
A.sch. × *A.b.*B	63.6	1.892	44.6	0.81	0.025	2.6	92.0
3+							
*A.b.*L × *A.sch.*	70.5	2.472	42.3	0.81	0.015	3.4	95.0 (45.3)
A.sch. × *A.b.*L	69.7	2.528	43.7	0.88	0.020	3.4	98.0 (31.4)
*A.b.*B × *A.sch.*	76.7	2.746	39.9	0.69	0.010	4.8	98.6 (4.4)
A.sch. × *A.b.*B	75.3	3.167	41.8	0.80	0.019	2.1	100.0 (6.8)

Notes: *A.b.*L, Siberian sturgeon of Lena population; *A.b.*B, Siberian sturgeon of Baikal population; *A.sch.*, Amur sturgeon; the survival rate of 4-year-aged fish from transition to active feeding are in parentheses

The survival rate from larvae amounted to 39.3%, while fish capacity of the cages was 64.2 kg per m^2. During rearing in the control of pure line of generation 2007 *A.b.*B, 4-year-old fish rose to 2.78 kg with a survival rate from larvae amounting to 11.7% and a fish capacity of 35 kg per m^2 of cage.

Thus, the reciprocal hybrids *A.b.*L × *A.sch.* and *A.sch.* × *A.b.*L exceeded the specimens of pure line *A.b.*L in weight by 7% and in fish capacity by 34–42%. The survival rate of the hybrid *A.b.*L × *A.sch.* is 6% higher than that of specimens from maternal line. The survival rate of the hybrid form *A.sch.* × *A.b.*L, on the contrary, was 8% lower than that of pure line *A.b.*L.

The weight of 4-year-old specimens of pure line *A.b.*B was close to that of hybrid form *A.b.*B × *A.sch.* And 14% lower than that of hybrid *A.sch.* × *A.b.*B. The pure line *A.b.*B had a higher survival rate during rearing in cages than that of two hybrid forms with *A.sch.*

The main meristic and plastic characteristics of four reciprocal hybrid forms were shown in Table 39.14.

Table 39.14 Main morphometric characteristic of 2-year-aged specimens of Siberian sturgeon hybrid of two populations (Lena and Baikal) with Siberian sturgeon of Amur population

Character	Hybrid forms			
	A.b.*L × *A.sch.	***A.b.*L × *A.sch.***	***A.b.*B × *A.sch.***	***A.sch.* × *A.b.*B**
	Meristic characteristics			
Number of dorsal scutes, *Sd*	13.6 ± 0.1	13.7 ± 0.2	13.2 ± 0.2	13.3 ± 0.2
Number of lateral scutes, *Sl*	40.3 ± 0.4	41.4 ± 0.6	45.2 ± 0.6	45.0 ± 0.8
Number of abdominal scutes, *Sv*	9.4 ± 0.1	10.1 ± 0.2	10.8 ± 0.2	10.7 ± 0.2
Number of rays in dorsal fin, *D*	39.8 ± 0.4	43.8 ± 0.5	40.0 ± 0.6	42.9 ± 0.5
Number of rays in anal fin, *A*	22.5 ± 0.2	23.3 ± 0.3	19.2 ± 0.2	23.5 ± 0.3
	Plastic characteristics, % of total body length			
Head length, cm *C*	21.5 ± 0.1	21.3 ± 0.2	17.9 ± 0.1	20.2 ± 0.2
Length of caudal peduncle, cm *pl*	14.8 ± 0.1	9.1 ± 0.1	10.6 ± 0.1	10.1 ± 0.1
Coverage of body, cm *CC*	31.4 ± 0.2	33.1 ± 0.3	32.4 ± 0.3	34.8 ± 0.4
	Plastic characteristics, % of head length			
Length of snout to front edge of eye, cm *R*	51.5 ± 0.3	52.4 ± 0.3	48.2 ± 0.3	48.0 ± 0.5
Distance from tip of snout to middle barbels, cm ***rc***	32.3 ± 0.3	33.7 ± 0.4	25.3 ± 0.3	28.3 ± 0.6
Distance from tip of snout to cartilaginous mouth arch, cm *rr*	52.4 ± 0.3	53.8 ± 0.3	49.7 ± 0.4	52.0 ± 0.6
Width of snout at cartilaginous mouth arch, cm *SRr*	34.5 ± 0.2	34.3 ± 0.7	42.3 ± 0.4	42.5 ± 0.4
Width of mouth, cm *SO*	22.7 ± 0.2	19.3 ± 0.2	27.5 ± 0.3	26.6 ± 0.2
	Plastic characteristics, % of mouth width			
Length of lower lip break, cm *il*	22.5 ± 0.5	25.2 ± 0.7	31.1 ± 0.7	25.6 ± 0.8

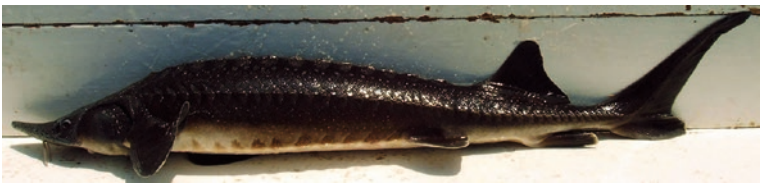

Fig. 39.5 Hybrid between Siberian sturgeon of Lena population and Amur sturgeon, side view

During the analysis of morphometric characteristics of hybrids, it appeared that the greatest differences with other hybrid forms were observed in the hybrid *A.b.*B × *A.sch.* This hybrid had a maximal number of lateral and dorsal scutes, a minimal number of rays in the anal fin, a minimal head size, a minimal distance from the end of snout to middle barbels and mouth, and the largest width of cartilaginous mouth arch and break in the lower lip.

Side images of hybrids and their underside views of their heads are presented on Figs. 39.5, 39.6, 39.7, 39.8, 39.9, 39.10, 39.11 and 39.12.

Fig. 39.6 Hybrid between Siberian sturgeon of Lena population and Amur sturgeon, underside view of the head

Fig. 39.7 Hybrid between Amur sturgeon and Siberian sturgeon of Lena population, side view

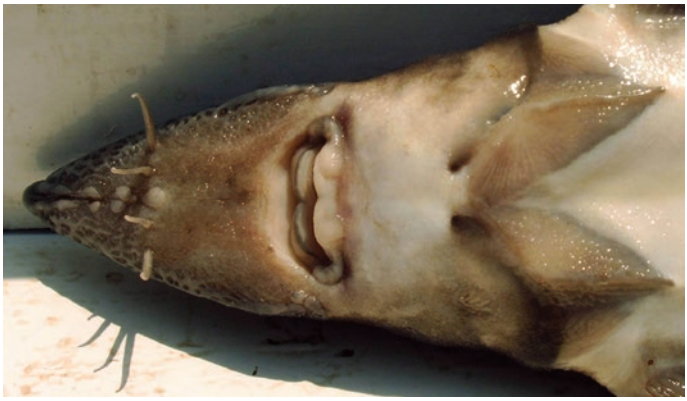

Fig. 39.8 Hybrid between Amur sturgeon and Siberian sturgeon of Lena population, underside view of the head

Fig. 39.9 Hybrid between Siberian sturgeon of Baikal population and Amur sturgeon, side view

Fig. 39.10 Hybrid between Siberian sturgeon of Baikal population and Amur sturgeon, underside view of the head

Fig. 39.11 Hybrid between Amur sturgeon and Siberian sturgeon of Baikal population, side view

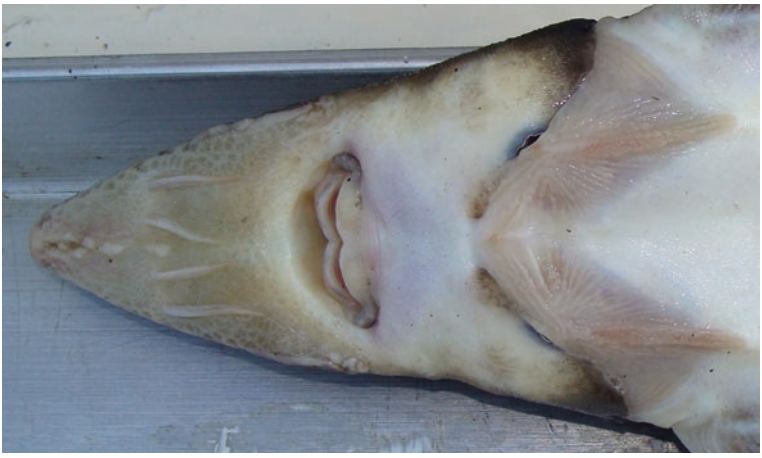

Fig. 39.12 Hybrid between Amur sturgeon and Siberian sturgeon of Baikal population, underside view of the head

39.5.3 Reproductive Peculiarities

Some specimens of *A.b.*L × *A.sch.* (generation 2000) and *A.sch.* × *A.b.*L (generation 2004) commercial hybrids were used to build of brood stock. Afterward, female of both hybrid forms were used for sturgeon caviar production.

The breeding, biological and production characteristics of hybrid females are shown in Table 39.15.

The first females of the hybrid *A.b.*L × *A.sch.* Reached their maturity at the age of 7 years old, with a higher body weight than specimens of hybrid *A.sch.* × *A.b.*L. Those reached maturity 1 year later. The maturation of females representing the entire generation in both hybrids occurred within 4 years. The percentage of mature females in each age category was similar. The average quantity of eggs, obtained from females of hybrid *A.b.*L × *A.sch.* For 9 years of exploitation, exceeded 3.2 kg, while for the reciprocal hybrid *A.sch.* × *A.b.*L, this value was less than 2.0 or 1.6 times lower. For the similar period and for the first use of 4-year-old *A.b.*L × *A.sch.* Hybrid females, the average quantity of eggs obtained amounted to 2.45 kg, that was 0.5 kg or 25% higher than that of *A.sch.* × *A.b.*L females. While the quantity of eggs was similar, the gonadosomatic index of the hybrid form *A.b.*L × *A.sch.* Was 20% higher than the same characteristic of the hybrid form *A.sch.* × *A.b.*L, with 56% higher "working" fecundity.

39.5.4 Commercial Features

The conducted studies showed the advantage of the hybrid form *A.b.*L × *A.sch.* as compared with the pure line of *A.b.*L in terms of weight, survival rate, and fish capacity. This hybrid form can be successfully applied for production of sturgeon caviar. The hybrid form *A.sch.* × *A.b.*L exceeded *A.b.*L in terms of weight and fish capacity but has a lower survival rate than the pure line *A.b.*L. Females of hybrid *A.sch.* × *A.b.*L are fertile and can be used for caviar production. Both hybrid forms can be recommended for rearing under conditions of warmwater farms.

Cultivating reciprocal hybrids *A.b.*B × *A.sch.* And *A.sch.* × *A.b.*B in warmwater farm has proven to be of low efficiency due to the low survival rate of fry at the stage of rearing in tanks.

39.6 Intergeneric Hybrid of Siberian Sturgeon *(Acipenser baerii)* with Kaluga (*Huso dauricus*)

39.6.1 History and Method of Rearing

The commercial rearing of Amur sturgeons began a quarter of a century later than that of the Siberian sturgeon (Sokolov et al. 1976; Rachek and Svirsky 2001). In 2005, at the Karmanovsky fish farm (Bashkiria) males of Kaluga reached sexual maturity for the first time, and there was a possibility of obtaining interspecific hybrids of Kaluga with other sturgeons. From several variants of crossing, a hybrid

Table 39.15 Breeding and biological and production characteristics of females of the hybrid between Siberian sturgeon of Lena population and Amur sturgeon

Hybrid	Mean weight of female at first maturation	Distribution of female on age of maturity		Mean value and range of weight of eggs, obtained from females (kg)	Interspawning intervals	Duration of female use (years)	Mean value and range of GSI (%)	Mean value and range of working fecundity (thous. eggs)	Mean value and range of an egg weight (mg)
		Age (years)	%						
Amur sturgeon × Sibetian sturgeon (Lena population) (2000)	16.2	7 8 9 10	17 33 33 17	3.21 ± 0.57 (0.6–5.2)	Mature annually or missing 1 year—50%; Missing 1–2 years—50%	9	14.7 ± 2.2 (4.5–21.6)	195 ± 32 (39–314)	16.5 ± 0.6 (14.6–19.6)
Amur sturgeon × Sibetian sturgeon (Lena population) (2004)	14.4	8 9 10 11	15 40 30 15	1.95 ± 0.21 (1.8-2.6)	Mature annually—29%; Mature annually or missing 1 year—7%; Missing 1 year—64%	4	12.2 ± 0.49 (11.0–13.4)	125 ± 13 (88–144)	16.4 ± 2.4 (12.4–22.2)

produced by insemination of Siberian sturgeon eggs by Kaluga sperm appeared to be the most promising commercially hybrid (Podushka and Armyaninov 2006). This hybrid was named "LenKa," with the use of the first syllables in the names of the parental species (Lena sturgeon and Kaluga sturgeon).

39.6.2 Morphological and Biological Characteristics

The "LenKa" hybrid has a dark back and sides and a white belly (Fig. 39.13). It differs from the Lena sturgeon by its big mouth (Fig. 39.14), the width of which is on average 29% of the length of the head.

At the time of crossing, new data on the karyotype of Kaluga showing that this species belongs to the sturgeon group with a multi chromosome set had not been published yet (Vasil'ev et al. 2009). So we relied on earlier data from the report of Vasil'ev (1985), in which Kaluga was considered a species with a low chromosome set and the nearest relative of the beluga sturgeon. Therefore, we assumed that the obtained hybrids, being hybrids between species of sturgeon with different numbers of chromosomes, could

Fig. 39.13 Hybrid between Siberian sturgeon of Lena population and Kaluga sturgeon ("LenKa"), ventral side view

Fig. 39.14 Hybrid between Siberian sturgeon and kaluga, underside view of the head

have abnormalities in the reproductive system development, up to full sterility described for such variants of crosses (Nikolyukin 1972; Serebryakova 1979).

Although it is known from Chinese sturgeon culture that hybrids of Kaluga and the Amur sturgeon are fertile (Podushka and Chebanov 2007), we were not sure that a hybrid of Kaluga with the Lena sturgeon would also reach sexual maturity without violations of gametogenesis. Therefore, initially we did not consider this hybrid as an egg producer and assumed that it would be used exclusively for the production of sturgeon meat (flesh). As expected, LenKa hybrids grew significantly faster than the Lena sturgeon under identical growing conditions, overtaking twofold in terms of mass (Podushka and Armyaninov 2008). It allowed to reduce the period of rearing of fish by 1 year.

39.6.3 Reproductive Peculiarities

When several LenKa males had reached their puberty at age of 3 years, mature sexual products were obtained from them via pituitary injections. Sperm was well activated when water was added to the seminal fluid. No noticeable morphological anomalies were observed at the light microscope level. Data on the fertility of LenKa males were consistent with the revised data on the ploidy of Kaluga (Vasil'ev et al. 2009). It was found that both parental species used in the crossing are multi-chromosomal, and the hybrid has no genetic preconditions for sterility.

LenKa males responded well to injections of superactive synthetic GnRHa analogue, producing a large amount of milky white semen. After puberty, the maturation of males occurs annually.

In 2013, the first females of LenKa hybrids reached maturity at the age of 8 with an average weight of 18 kg. Ten selected females were injected twice with GnRHa (dose: 15 + 15 mg per fish with an interval of 12 h between the first and the second injection). Obtained from an alive fish were 18.8 kg of ovulated eggs, which is about 10% of the body mass of females. The hybrid egg coloration appears similar to that of the eggs of Kaluga and Amur sturgeon. The eggs have a yellowish brown tint. The animal pole is pigmented more intensely than the vegetative pole. Some variation in the intensity of pigmentation was observed in different females (Fig. 39.15). There were 53–66 eggs (60.1 on average) in 1 g. The abdominal fluid is colorless and quite viscous. After puberty, the ovarian cycle of this hybrid typically takes 2 years.

Attempts to reproduce LenKa hybrids "in itself" have failed. Although eggs had a high rate of fertilization, very few larvae hatched, and all of them died later.

39.6.4 Commercial Features

In China, hybrids between the Amur sturgeon (*A. schrenckii*) and Kaluga (*H. dauricus*) are very popular in commercial sturgeon rearing. These hybrids are also fertile and yield about 30% of sturgeon caviar in aquaculture of China (Wei et al. 2011).

Fig. 39.15 Samples of eggs obtained from different female hybrids

Therefore, the LenKa hybrid is no less technological than the dominant hybrids in China. It has a high growth rate, reaches sexual maturity at a relatively early age, and is characterized by a synchronous maturation (Podushka et al. 2014). Hybrid breeders respond to GnRHa well and, unlike the Siberian (Lena population) sturgeon, are not prone to obesity. Ovulated eggs can be easily extracted from live mature LenKa females. After processing, the caviar has a high consumer quality. The spawners of LenKa have the same average size as Siberian sturgeon. Therefore, in due course, this hybrid will take its rightful place in the caviar-oriented sturgeon culture.

39.7 Hybrids of Siberian Sturgeon with Other Sturgeon Species

39.7.1 The Hybrids of Adriatic (*A. naccarii*) and Siberian (A. baerii) Sturgeons

The hybrid between *A. naccarii* and *A.baerii* was obtained for the first time in 1993 in Italy (Arlati and Bronzi 1995; Arlati et al. 1999) and was named as "AL," using the first letters in the names of the parental species origin regions (Adriatic Sea and Lena River).

In subsequent years, the hybrid of AL has been widely used in commercial sturgeon farms in Italy and to some extent in Bulgaria (Fig. 39.16) (Chebanov and Galich 2008), as well as for caviar production during the last decade also (Vaccaro et al. 2005; Bronzi and Arlati 2009; Pazzaglia and Giovannini 2009).

Fig. 39.16 Hybrid between Adriatic sturgeon (*A. naccarii*) and Siberian sturgeon

AL was recognized as a fertile commercial "species" of Acipenseriforms in Italy (GU 2008; Petochi et al. 2011). Arlati et al. (1999) analyzed eight meristic characteristics of 30 AL individuals (2+ and 3 + −year classes). Generally speaking, this hybrid shows the most typical morphological characteristics of Siberian sturgeons. Petrochi et al. (2011) applied different methods for sexing and reproductive staging: echography, histology, and sex steroids. The sex (male/female) ratio was found to be 1/1.5. On the basis of the results obtained, these authors indicate echography is an express and noninvasive tool to sex and indicate AL at its maturity stage. It is therefore very useful for farmers for sexing and breeding aims.

Vaccaro et al. (2005) revealed a high growth rate and flesh quality (fatty acid composition study) of the AL hybrid. In the year 2000, in the "VIP" fish farm in Orzinuovi (Nord of Italy), 130 tons of juveniles, previously obtained from domestic brood stock, were reared. Despite the lower price of caviar obtained from hybrids as compared with the price of caviar from pure sturgeon species, during 2007–2008, the sales of hybrid AL caviar amounted to 4.5 tons, whereas less than 400 kg caviar from GUBA were sold (Pazzaglia and Giovannini 2009). A reciprocal hybrid (*A.b* × *A.n*), also known as BACARII, has proven to be less popular in sturgeon culture. The same information relates to the GUNA hybrid, obtained by crossing Russian sturgeon (*A. gueldenstaedtii*) females with Adriatic sturgeon (*A. naccarii*) males. This also applies to the ADAM hybrid, obtained in Italy, by crossing Adriatic sturgeon (*A. naccarii*) females with aquaculture-produced white sturgeon (*A. transmontanus*).

It is essential to note that caviar, produced from all these hybrids, can now be identified by new molecular genetic methods based on nuclear markers (Boscari et al. 2014; Boscari and Congue 2016). This seems very urgent for gene pool conservation, as in the last several decades, the occurrence of non-native species and hybrids of sturgeons was observed in the different rivers (Ludvig et al. 2009; Friedrich 2013). For instance, in July 2013, after a record size flood on the Danube River, three individuals of (16.8–19.2 cm) fertile hybrid AL were caught (Weperth et al. 2014) between the mouth of River Ipoly and the Danube at Szub (1708–1707 km).

39.7.2 Hybrid of Siberian Sturgeon (*A. baerii*) with Sakhalin/Green Sturgeon (*A. mikadoi*)/*A. medirostris**

The hybrid between Siberian sturgeon of Lena population and Sakhalin sturgeon (*A. micadoi*) was obtained for the first time in spring 1995 in the Konakovo branch of VNIIPRKh by fertilization of eggs of the Siberian sturgeon with sperm of hatchery-bred male of Sakhalin sturgeon. The biological characteristics of the hybrid should comprise the inheritance of behavior and lifestyle of its parental species—Sakhalin sturgeon (Krylova et al. 1997).

Some of these hybrid eggs and eggs of Siberian sturgeons fertilized at the same time were transported to the Dgal experimental hatchery (Poland), where they were incubated. Comparative morphometric studies (Jankowska et al. 2002, 2006) were performed during fry rearing to define the characters that reliably distinguish pure-bred and hybrid fish (Table 39.16).

The results of rearing of an experimental batch showed a higher growth rate than that of Siberian sturgeons (at 2 years of age, the weight of the hybrid reached 2.4 kg) (Kolman et al. 1997) and a high nutritional value of meat (Jankowska et al. 2005).

Comparative morphometric studies were performed during fry rearing to define the characters that reliably distinguish purebred and hybrid fish (Table 39.16). The changes of morphometric features of the hybrid Siberian sturgeon and green sturgeon that occur during the period prior to achieving sexual maturity were investigated by Szczepkowska et al. (2011). Twenty-nine measurable body characters and six meristic characters (Krylova and Sokolov 1981) of 186 fish were assessed. The main morphometric characters of the hybrid are presented in Table 39.17.

Table 39.16 Values of meristic characters of Siberian sturgeon (*Acipenser baerii* Brandt) and its hybrid with green sturgeon (According to Jankowska et al. 2002, 2006)

Meristic character	*Acipenser baerii*	Hybrids of Siberian sturgeon and green sturgeon
number of dorsal bony plates—Sd	14.4 ± 1.2	9.16 ± 0.82
number of lateral bony plates—Sl	47.5 ± 2.1	33.05 ± 2.09
number of ventral bony plates—Sv	9.0 ± 0.5	8.69 ± 0.86
number of rays in the dorsal fin—D	46.5 ± 2.4	35.67 ± 2.59
number of rays in the anal fin—A	33.4 ± 2.6	22.92 ± 1.91

*In the analyzed below (39.8.2) papers (Kolman al. 1997; Kolman , Szepkowski 2001; Krilova et al 1997; Jankowska et al. 2002, 2005, 2006), by separate species Green sturgeon A.medirostris. The taxonomic status of these species has only recently been clarified. Sakhalin sturgeon A. mikadoi was considered conspecific with North American Green Sturgeon (A. medirostris) (Shmigirilov et al. 2007).

Table 39.17 The measurable characters in hybrids of Siberian sturgeon and green sturgeon at age 1+ from pond culture (according to Szczepkowska et al. 2011)

	Hybrids of Siberian sturgeon and green sturgeon
In % of L (Total length)	
l1 (Fork length)/L	89.76 ± 1.41
l2 (Body length)/L	83.30 ± 1.13
aD (Predorsal distance)/L	63.65 ± 1.17
aV (Preventral distance)/L	53.59 ± 1.07
aA (Preanal distance)/L	67.93 ± 1.07
C (Head length)/L	27.91 ± 1.01
H (Maximum body depth)/L	9.10 ± 0.58
h (Minimum body depth)/L	2.60 ± 0.18
pl (Length of caudal peduncle)/L	11.09 ± 0.91
lD (Dorsal fin length)/L	7.49 ± 0.55
hD (Height of dorsal fin)/L	7.33 ± 0.42
lA (Anal fin length)/L	3.70 ± 0.30
hA (Anal fin height)/L	7.32 ± 0.46
In % of C (Head length) or SO (Width of snout)	
R (Rostrum length)/C	57.75 ± 1.62
op (Postorbital distance)/C	36.92 ± 1.54
o (Diameter of the eye)/C	5.69 ± 0.54
HC (Head depth)/C	30.09 ± 1.22
hCo (Head depth at the center of the eye)/C	18.72 ± 1.01
iO (Interorbital distance)/C	23.76 ± 1.54
BC (Maximum head width)/C	37.57 ± 1.98
bC (Head width at the upper edge of the operculum)/C	25.58 ± 1.18
rc (Distance from the rostrum end to cartilaginous snout edge)/C	40.51 ± 1.88
rr (Distance from the rostrum end to base of the middle barbs)/C	60.05 ± 1.47
lc (External barb length)/C	18.46 ± 1.48
SRc (Mouth width at barb base)/C	19.44 ± 0.94
SRr (Mouth width at cartilaginous vault)/C	28.96 ± 1.11
SO (Width of snout)/C	22.15 ± 1.02
il (Width of the lower lip gap)/SO	22.70 ± 3.25

The most commercially advantageous body proportions of this hybrid fish were achieved at the age 4+, when head length (C) stabilized at 24% L, and the maximum body depth (H) was 11.82 ± 0.72% L (Szczepkowska et al. 2011). For this reason, authors conclude that the commercial value of the hybrids (*A.b.* × *A.m.*) increased during fish growth until age 4 +.

Hence, the selective breeding of Siberian and green sturgeons has a positive impact on the hybrid tissue quality with regard to level of *n*-3 acid, which is obvious owing to its elevation as compared with the maternal species, as well as due to

increase in total fat content being compared with the paternal species. It was concluded that the selective breeding of the abovementioned sturgeon species allows to get a hybrid with as high fillet gain similar to that of the parental species (Jankowska et al. 2005). Despite the lost (not inherited) pleasant coloration of tissue, the hybrid has acquired polyunsaturated fatty acids (*n*-3, LC *n*-3 PUFA) with prolonged chain (especially in contrast to its maternal species).

Conclusions

It is known that all sturgeon species, when crossed (both interspecific and intergeneric crossings), allow to produce viable offspring (Nikolyukin 1972), whereupon breeding of various hybrids of Siberian sturgeon is of practical importance for commercial or caviar sturgeon farming in various climatic and technological conditions.

Generalization of data from different studies allows to conclude that the Siberian sturgeon in terms of better quality and morphometric characteristics has a "dominant phenotype," while the hybrids of Siberian sturgeon occupy diverse intermediate positions relatively to the parental species, or show similarities to the Siberian sturgeon (Vasil'eva and Grunina 2011) The inheritance of some quantitative morphological characters in reciprocal hybrids of sturgeon were analyzed in detail by Vasil'eva et al. (2010).

For commercial sturgeon rearing, it is rational to use the first-generation hybrid that shows significant yield heterosis (up to 165%). In this case, it is expedient to take into account the cytogenetic characteristics of the parental species, as not only more viable but also fertile hybrids can be obtained by crossing within a genetically close group (Nikolyukin 1972, Burtsev et al. 1978).

The results of analysis showed that, as a rule, the most effective in intensive rearing conditions were reciprocal hybrids of potamodromous (freshwater) Siberian sturgeon with anadromous sturgeon species. Such hybrids show higher growth and survival rates, tolerance toward diseases and early puberty, as well as high relative fecundity.

As practical experience has shown, the most popular and abundant in sturgeon farms are hybrids of Siberian sturgeon with Russian sturgeon (GUBA) and Adriatic sturgeon (AL), as well as with Amur River sturgeons.

Along with this, considering the fertility of most hybrids, their extensive use in aquaculture requires great care and necessity to avoid invasion of hybrids into natural water bodies. Moreover, the aspect of hybrid production identification acquires special relevancy. As experience shows, in many cases, less worthy hybrid caviar is mispresented as more valuable caviar of pure sturgeon species. It is essential to note that caviar, produced from all these hybrids, can be identified at present with the use of new molecular genetics methods based on nuclear markers (Boscari et al. 2014; Boscari and Congiu 2016).

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Genome Manipulation and Sex Control in the Siberian Sturgeon: An Updated Synthesis with Regard to Objectives, Constraints and Findings

40

Dorota Fopp-Bayat

Abstract

The genome manipulations, such as gynogenesis or androgenesis, are widely applied in fish for artificial modification of chromosome set and allow the production of monosex stocks. Moreover, such manipulations enable rapid production of inbred populations that can be applied in crossbreeding programs. The application of the gynogenesis in sturgeons seems to be very important in creation of all-female stocks for caviar production. The Siberian sturgeon *Acipenser baerii* is the species most frequently cultured in European fish farms for black caviar. The production and all-female stocks of this species are highly desirable and commercially reasonable. Unfortunately, in Siberian sturgeon, the available sex identification methods are not effective for fish younger than age 3 years because these fish have no morphological sex specific features and no sex chromosomes were identified in this species. Therefore the direct production of all-female stock is very important. Although genome manipulations were successfully applied in the production of monosex populations of some fish species, especially with the XY sex-determination system, in Siberian sturgeon, such manipulations were characterized by low efficiency due to the low hatching rate. The present review provides a summary of genome manipulations in the Siberian sturgeon.

Keywords

Acipenseridae • Genome manipulations • Gynogenesis • Siberian sturgeon

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Introduction

Sturgeons (Acipenseriformes) are one of the oldest groups of fish, existing since the Late Cretaceous (Grande and Bemis 1991) and believed to have diverged from an ancient, pre-Jurassic ancestor approximately 200 Mya (Patterson 1982). The populations of sturgeons are extremely endangered in a natural environment, and 85% of them are at risk of extinction, making them the most threatened group of animals on the IUCN Red List of Threatened Species. Because the sturgeon populations are decreased, there is a need for production of these fish in aquaculture. The aquaculture production of sturgeon, in controlled condition, has increased over the last few decades, and it is conducted for commercial (Williot et al. 2001; Wei et al. 2011; Bronzi et al. 2011) as well as conservation purposes (Williot et al. 2002; Takahashi and Officer 2010). The sturgeon farm profile may include the production of juvenile or adult fish and requires appropriate breeding and farming technology that can be focused on meat or caviar production (or both).

The biology of sturgeons is very interesting because of valuable data on mechanisms underlying the evolution of vertebrates. Studies focused on the biology, physiology, genetics, and sex control to produce all-female populations are urgently needed not only for the understanding of the biology of reproduction of sturgeons but also to use this knowledge in aquaculture for caviar production. All Acipenseriformes are long-lived vertebrates and typically do not reach sexual maturation until they are at least 8–10 years of age, excluding the sterlet (Rochard et al. 1991; Mims et al. 2002; Hurvitz et al. 2007; Stahl et al. 2009). In aquaculture, sturgeons reach sexual maturation earlier due to optimal water condition and balanced feeding together with veterinary support.

Siberian sturgeon *Acipenser baerii* (and its interspecies hybrids) is the main sturgeon species cultured in Eurasia (Williot et al. 2001). In aquaculture conditions, this species is bred for meat and caviar, based on spawner brood stock. The domestic brood stock of Siberian sturgeon appear to produce offspring with gonochoristic sex ratio 1:1 (females/males). The similar sex ratio was observed in white sturgeon from San Francisco Bay (Chapman et al. 1996) and from domestic brood stock maintained in the University of California, Davis (Van Eenennaam et al. 1996 after S.I. Doroshov, pers. comm.). The selection of females, during Siberian sturgeon farming, is the most important stage and the "key point" in caviar production. Because sturgeons are sexually monomorphic, the application of the most accurate method to sex determination (through examination of the gonads) is essential in aquaculture (Hurvitz et al. 2007). Another way, leading to a formation of the monosex sturgeon stocks, is the application of the specific genome manipulation. Genome manipulation, especially gynogenesis (meiotic or mitotic), is attractive in sturgeons and for production of all-female progeny and for study of the sex-determination mechanism (Mims et al. 1997; Devlin and Nagahama 2002). In sturgeons, where females are heterogametic, the gynogenesis is the first step to the formation of the superfemale stock focused on the production of all-female offspring (Omoto et al. 2005; Flynn et al. 2007; Fopp-Bayat 2010).

The purpose of the present review was the description of the applied techniques of the genome manipulations in the Siberian sturgeon *Acipenser baerii*.

40.1 Sex Control in Aquaculture of Siberian Sturgeon

The application of the reliable methods of unisexual brood stock production and early separating fish by sex are very important in Siberian sturgeon aquaculture (Williot and Brun 1998; Chebanov and Galich 2009). In most commercial sturgeon farms, sturgeon males are harvested before maturity (until 3 years), for meat, and females are bred for about the next 5 years until maturity. Therefore, there is a need to use the noninvasive methods for sex identification in Siberian sturgeons. In aquaculture, the sex of Siberian sturgeon is usually identified in about 3–5 years of age with the application of biopsy, ultrasonography, endoscopy, or biopsy with microscopic observations (Chebanov and Galich 2009). The gonad biopsy technique is the most popular method for sex and gonad stage identification in sturgeon aquaculture. This technique, based on sampling a small piece of gonadal tissue (using trocar) with anesthesia, is time consuming and stressful for examined fish and human operators (Vecsei et al. 2003). Additionally, the biopsy is usually applied as the verification method of reference to "standardize and scale" the other methods (Williot 2011).

Endoscopy as minimally invasive technique was applied in sturgeon aquaculture, for sex identification, and enables direct observation with low incidents of morbidity and mortality (Camus 2009; Divers et al. 2009; Trested et al. 2010). In sturgeon endoscopic techniques demonstrated high accuracy rates for sex identification and determination of the stage of maturation (Wildhaber et al. 2005; Divers et al. 2009). The examination of one specimen using this technique takes about 5–10 min in anesthesia (Chebanov and Galich 2009). In Siberian sturgeon, the sex identification by ultrasound is reliable in fish of age 2–2+ years and 2.0–2.5 kg of weight (Chebanov and Galich 2009). Endoscopy and ultrasonography require having proper equipment, knowledge, and experience of the operator. The direct comparison of accuracy between ultrasonography and endoscopy in juvenile Siberian sturgeon has demonstrated both the reliable methods for successful sex identification (Jiménez et al. 2014). Ultrasonography (as the noninvasive method) is the faster method and less accurate than endoscopy, while endoscopy proved to be more invasive in comparison to the USG during gender identification (Jiménez et al. 2014). The sex identification of sturgeons was also conducted based on the plasma sex steroid analyses, but this technique requires specialized laboratory and is relatively expensive and time consuming (Webb et al. 2002; Hurvitz et al. 2005). This technique requires a 2 mL blood sample for analysis and allows further analysis on plasma components such as steroid hormones, VTG, proteins, calcium, etc. (Williot 2011).

40.2 Genome Manipulations in Sturgeons

The genome manipulation in sturgeon fish has been described by many authors (Grunina et al. 1995; Van Eenennaam et al. 1996; Mims and Shelton 1998; Recoubratsky et al. 2003; Omoto et al. 2005; Flynn et al. 2006; Fopp-Bayat et al. 2007; Fopp-Bayat 2010; Saber et al. 2014). Such techniques are powerful tools in

creation of a new genotype and phenotype of a progeny and achieve some important traits, e.g., sterility and paternal inheritance (Chourrout and Quillet 1982; Pandian and Koteeswaran 1998; Arai 2001).

40.2.1 Sex-Determination System in Sturgeons

The sex-determination system of the Acipenseriformes has received little study. Moreover, none of the sturgeon species so far investigated exhibit external sexual dimorphism, and no morphological difference has ever been found between any chromosome pair to indicate the presence of heteromorphic sex chromosomes.

The gynogenesis experiments were applied in studies of the genetic basis of the sex determination in white sturgeon, shortnose sturgeons, Siberian sturgeon, and ship sturgeon as a critical step in developing sex control strategies to maximize caviar production in aquaculture (Van Eenennaam et al. 1996; Flynn et al. 2006; Fopp-Bayat 2010; Saber et al. 2014). The results of applied meiotic gynogenesis in the four sturgeon species (white sturgeon, shortnose sturgeon, Siberian sturgeon, ship surgeon) indicated the heterogametic sex in female (Van Eenennaam et al. 1996; Flynn et al. 2006; Fopp-Bayat 2010; Saber et al. 2014). Flynn et al. (2006) observed the sex ratio of 35% male to 65% female in gynogenetic diploids of shortnose sturgeon, while Fopp-Bayat (2010) noticed the ratio of 20% male to 80% female in offspring of Siberian sturgeon gynogenetic. The identified percentage of female and male of ship sturgeon were 73% and 27%, respectively (Saber et al. 2014).

In species where female is characterized by homogametic sex (XX genotype), gynogens are always female. Alternatively, in species having the female as the heterogametic sex (WZ genotype), gynogenesis can be used to create "superfemales" (WW genotype). If the WW superfemale is viable and fertile, then this female will produce all-female offspring when crossed with a normal ZZ male. Siberian sturgeon is the species that the female is heterogametic, and gynogenesis can be applied for production of "superfemales" (Fopp-Bayat 2010).

40.2.2 Gynogenesis in Sturgeons with Particular Emphasis on Siberian Sturgeon

Gynogenesis (meiotic or mitotic) is a chromosomal manipulation whereby offspring is formed exclusively from maternal genetic information (Thorgaard et al. 1985; Mair 1993; Arai 2001; Devlin and Nagahama 2002). The embryo is developed by activating oocytes with genetically inactivated sperm cells (e.g., UV irradiation). The production of gynogenetic diploids involves two steps: (1) inactivation of paternal genome and activation of the eggs with inactivated homologous (or heterologous) sperm and (2) restoration of diploidy by a shock (physical or chemical), to retain the second polar body or suppress the first mitotic cleavage (Table 40.1) (Arai 2001; Devlin and Nagahama 2002).

Table 40.1 The basic treatment in gynogenesis and androgenesis manipulations

Treatment	Genome manipulations		
	Gynogenesis		
	Meiotic	Mitotic	Androgenesis
Sperm inactivation	UV irradiation	UV irradiation	–
Eggs inactivation	–	–	(X-, gamma, UV irradiation)
Genome duplication	Physical or chemical shock – Retain the second polar body	Physical or chemical shock – Suppress the I mitotic cleavage	Physical or chemical shock – Suppress the I mitotic cleavage – Application the diploid sperm from tetraploid fish donor

Table 40.2 The parameters of meiotic gynogenesis in sturgeons

Lp	Female species	Male species	UV dose J/m^{-2}	Diploidy restoration	References
1.	*Acipenser transmontanus*	*Acipenser transmontanus*	2160	12–15 min p.a. 32–34 °C for 1–5 min	Van Eenennaam et al. (1996)
2.	*Acipenser ruthenus*	*Acipenser ruthenus*	135	18 min p.a. 34 °C for 2 min	Recoubratsky et al. (2003)
		Bester hybrid	135	18 min p.a. 34 °C for 2 min	Fopp-Bayat et al. (2007)
3.	*Acipenser gueldenstaedtii*	*Acipenser gueldenstaedtii*	297	19–22 min p.a. 37 °C for 2.5 min	Recoubratsky et al. (2003)
4.	*Acipenser stellatus*	*Acipenser stellatus*	243	16.5 min p.a. 37 °C for 2.5 min	Recoubratsky et al. (2003)
5.	Bester hybrid	Bester hybrid	2100	15 min p.a. 34 °C for 3–6 min	Omoto et al. (2005)
6.	*Acipenser brevirostrum*	*Acipenser brevirostrum*	1200	20 min p.a. pressure shock of 8500 psi for 5 min	Flynn et al. (2007)
7.	*Acipenser baerii*	*Acipenser baerii* × *Acipenser gueldenstaedtii*	288.75	18 min p.a. 37 °C for 2.5 min	Fopp-Bayat (2007)
8.	*Acipenser nudiventris*	*Acipenser baerii*	473	10 min p.a. 2.5 °C for 2.5 min	Saber et al. (2014)

Min p.a. minutes post-activation

The induction of meiotic gynogenesis has been applied in aquaculture of some sturgeon species (Table 40.2) (Van Eenennaam et al. 1996; Recoubratsky et al. 2003; Omoto et al. 2005; Flynn et al. 2006; Fopp-Bayat et al. 2007; Fopp-Bayat 2010; Saber et al. 2014). The earliest work using this technique for the production

of meiotic gynogenetic diploids of sturgeon was reported by Romashov et al. (1963). In their study, none of the gynogenetic larvae survived beyond 192days after hatching. Techniques of induction of the meiotic gynogenesis was described in white sturgeon, *Acipenser transmontanus*; shovelnose sturgeon, *Scaphirhynchus platorynchus*; stellate sturgeon, *Acipenser stellatus*; Russian sturgeon, *Acipenser gueldenstaedtii*; sterlet, *Acipenser ruthenus*; bester (beluga, *Huso huso* x sterlet *Acipenser ruthenus*); shortnose sturgeon, *Acipenser brevirostrum*; Siberian sturgeon, *Acipenser baerii*; and ship sturgeon, *Acipenser nudiventris* (Table 40.2) (Van Eenennaam et al. 1996; Mims and Shelton 1998; Recoubratsky et al. 2003; Omoto et al. 2005; Flynn et al. 2006; Fopp-Bayat et al. 2007; Saber et al. 2014).

The sex ratio 1:1 (females:males) is uneconomical during the rearing of Siberian sturgeon stock for caviar production. Therefore the alternative method of production of all-female stock, based on genome manipulation, is required. Currently, the genomic manipulation on the Siberian sturgeon is still at an early stage, and researchers together with sturgeons breeders are attempting to produce superfemales for formation of caviar stocks.

The first successful induction of meiotic gynogenesis in Siberian sturgeon, verified by molecular markers, was described by Fopp-Bayat (2007), and in this research, the viable gynogenetic diploids were produced. In this experiment, UV-irradiated sperm from hybrid of Siberian sturgeon and Russian sturgeon was used for Siberian sturgeon eggs activation (Table 40.2). A total of 80 meiotic diploids of known parentage from two different experimental treatments were screened using microsatellite DNA, and uniparental transmission in meiotic diploids was confirmed. The experiment was characterized by low offspring survival (Fopp-Bayat 2007). Researchers involved in genome manipulation in fish emphasized that the low rate of obtained gynogenetic diploids is a consequence of the critical conditions of the heat-shock treatment as well as possible inbreeding (Ihssen et al. 1990).

40.2.3 Androgenesis

Androgenesis is the genome manipulation method resulting in development of progeny with exclusively paternal inheritance of nuclear genome information. This manipulation involves inactivation of the maternal nuclear genome in oocytes using irradiation (gamma, X, or ionizing UV) followed by fertilization with normal sperm (Table 40.1). To obtain viable diploid androgenetic individuals, either diploid sperm from tetraploid donors is used or physical shock (thermal or hydrostatic pressure) is applied to duplicate a single chromosome set by blocking the first mitotic division of the zygote (Thorgaard et al. 1990; Arai et al. 1995; Pandian and Koteeswaran 1998). Androgenesis involves inactivation of the egg's genome, by dispermic (Grunina et al. 1995) or monospermic activation by haploid (Scheerer et al. 1986) or diploid (Thorgaard et al. 1990) gamete. In dispermic androgenesis, the diploid status of androgenetic individuals is achieved by the fusion of the chromosome sets of two spermatozoa. In the spermatozoa that originated from different males, the level of heterozygosity in a given androgenetic individual will be similar to the

usual hybridization. For diploid androgenesis, it is necessary to inactivate the egg nucleus and duplicate the male chromosome complex. The resulting androgenetic individuals are homozygous and homogametic (Babiak et al. 2002). Androgenetic "supermales" YY can be viable in fish, providing the opportunity to investigate the sex-determination mechanisms and production of monosex stocks (Pandian and Koteeswaran 1998; Scheerer et al. 1991). Androgenesis is also a convenient manipulation for creation of inbred isogenic and clonal lines (Scheerer et al. 1991).

In sturgeons the androgenesis was induced only in scientific experiments (Grunina et al. 1995; Recoubratsky et al. 1996; Grunina and Recoubratsky 2005), and there is no information about application of such manipulation in aquaculture. Grunina and Neyfakh (1991) induced diploid androgenesis in the Siberian sturgeon by X-irradiation of the egg nuclei and diploidization of the male chromosome complex by late 37 °C heat shock. No genetic or biochemical proof was offered to support their inheritance that larvae produced were fully derived from the paternal genome. These putative androgenetic larvae died before the transition to active feeding (Grunina and Neyfakh 1991). The intraspecific dispermic androgenesis was induced in the Siberian sturgeon, Russian sturgeon, Persian sturgeon, stellate sturgeon, and beluga, and obtained androgenetic progeny were fully viable (Grunina et al. 1995; Recoubratsky et al. 1996; Grunina and Recoubratsky 2005).

40.2.4 Confirmation of Ploidy and Uniparental Inheritance after Genome Manipulation

Determination of the ploidy level is very important approach for assessing genome-manipulated progeny (e.g., gynogenetic or androgenetic diploids). Various methods have been applied to confirm ploidy level in genome-manipulated fish. In sturgeons, confirmation of ploidy level was conducted by using cytogenetical method, flow cytometry, coulter counting, or measurement of nucleus in erythrocytes. Flow cytometry and measurement of cell size and nucleus in erythrocytes are the most widely used methods to confirm triploidy (Varadaraj and Pandian 1988; Woznicki and Kuzminski 2002; Fopp-Bayat et al. 2006) and tetraploidy (Arai et al. 1991), but they are not useful to identify the gynogenetic or androgenetic diploids. In recent years, a precise and sensitive method based on microsatellite DNA analysis has been successfully applied in verification of uniparental inheritance after meiotic gynogenesis in sturgeons (Flynn et al. 2006; Fopp-Bayat and Woznicki 2006; Fopp-Bayat et al. 2007; Fopp-Bayat 2007). In practice, application of two carefully selected diagnostic loci is sufficient to verification of gynogenetic offspring with only maternal inheritance. In addition, selection of two loci is recommended, taking into consideration the mutation rate at these loci and the possible existence of paternal residual transmission in some offspring analyzed (Thorgaard et al. 1985).

In Siberian sturgeon, the genome manipulations (gynogenesis and androgenesis) were induced only in scientific experiments, and there is no information about the application of such manipulation in aquaculture. However the success of genome manipulation is very low in Siberian sturgeon; further studies are needed in order to

obtain experimental gynogenetic superfemale which would provide stocks of female sturgeon for caviar production.

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Genetic Variability in Farmed Brood Stocks of the Siberian Sturgeon in Poland

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Abstract

The Siberian sturgeon *Acipenser baerii* is one of the most common and most important sturgeon species cultured in Europe, being the main source of sturgeon meat and black caviar produced by large fish farms and international aquaculture companies. The present paper describes the genetic characteristics of Siberian sturgeon *Acipenser baerii* brood stock from the Polish fish farm. The genetic analysis, based on six polymorphic microsatellite DNA analysis, revealed a high level of genetic diversity in studied broodstock of Siberian sturgeon (PIC = 0.504–0.837 and I = 1.036–2.150). The observed values of allelic richness (A_r) varied from 6.000 to 13.500 in examined fish group. Observed (*Ho*) and expected (*He*) heterozygosity across the studied loci showed values from 0.723 to 1.000 and from 0.586 to 0.857, respectively. Overall, the examined broodstock were not in Hardy-Weinberg equilibrium (H-WE), where five of the six microsatellite loci deviated from H-WE. The estimated effective population size (*Ne*) values by the linkage disequilibrium and the molecular coancestry methods were at the level of 47.3 (95% CI = 39.6–57.2) and 41.3 (95% CI = 3.0–128.7), respectively. A total number of 38 rare alleles within investigated microsatellite loci were found, which consisted 51% of qualitative composition of all detected alleles. All the analyzed genetic indicators suggested the good genetic condition and high genetic value of studied Siberian sturgeon farmed broodstock.

Keywords

Acipenser baerii • Aquaculture • Broodstock • Genetic variability • Microsatellite DNA

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Introduction

Currently, most of the Siberian sturgeon (*Acipenser baerii*) populations in Eurasia are endangered because of water pollution, overfishing, damming, poaching, and the commercial fisheries, which contributed to the complete extirpation of the species in the basins of the Ob and Yenisei Rivers as well as in the Lake Baikal (Ruban and Akimova 2001; IUCN 2015). Due to the economic importance together with a reduction of the natural populations of Siberian sturgeon, fishermen began to produce this species in aquaculture. In Europe, this species is the main source of sturgeon meat and black caviar produced by large fish farms and international aquaculture companies (FAO 2015).

The Siberian sturgeon is one of the most common and most important sturgeon species cultured in Poland. The production of this sturgeon species (under controlled conditions) was started in the early 1990s on the basis of stocking material, imported from three sturgeon farms from Russia: Konakovo near Moscow, Krasnodarskij Yar near Krasnodar, and Gorachij Kluch near Krasnodar (Kolman personal communication). Then, Siberian sturgeon breeding has been started in two aquaculture centers: the Experimental Fisheries Department "Dgał" in Pieczarki (Inland Fisheries Institute in Olsztyn, Poland) and the Wasosze Fish Farm near Konin, Poland. Since that time, the two main broodstocks of Siberian sturgeon were formed on the basis of fish material originated from the same source, which are constantly maintained in those two sturgeon centers. Actually, both of these broodstocks are the main source of Siberian sturgeon material (eggs, hatch, and fry) in Poland.

The breeding programs, based on maintaining fish broodstock and focusing on morphological fish identification, are insufficient for selection and management of valuable fish species. During selection programs, without genetic monitoring, the original (initial) genetic diversity of broodstock may be reduced, reaching a critical value. Therefore, in such breeding programs, the genetic investigations of broodstock should be conducted. The genetic protocol for monitoring the genetic diversity in broodstock should be easy to implement, reliable, and feasible and result in obtaining important genetic indicators. The application of 5–6 highly polymorphic microsatellite loci could be sufficient if the fish material is genetically variable. If the genetic variability in the studied broodstock is low, the additional analysis should be carried out, based on a larger number of markers. Moreover, the genetic monitoring in fish broodstock should include several important indicators that help specify the genetic variation of the studied group of fish. Such study should consider the following indicators: number of alleles; allele frequencies; exact test for Hardy-Weinberg equilibrium (H-WE) and linkage disequilibrium (LD) for all loci within each broodstock examined; estimation of the inbreeding coefficient of an individual relative to its subpopulation (*Fis*), observed (*Ho*), and expected (*He*) heterozygosities; effective population sizes (*Ne*); Polymorphism Information Content (*PIC*) and Shannon's-Weiner's index (*I*); indicators of the bottleneck events (*IAM*, *SMM*, *TPM*); as well as the Garza-Williamson index (*M*).

Permanent genetic monitoring of spawners during the maintenance of the spawning broodstock for production of stocking material is a very important tool in modern aquaculture focused on biodiversity. Such approach of genetic-based management enables to avoid numerous adverse changes in a gene pool of fish stocks reared under captivity conditions, which contribute to loss of genetic diversity, decrease of heterozygosity, inbreeding, or outbreeding depression. Genetic analysis of broodfish should be focused on the molecular identification of the spawner species and genetic variation of the broodstock. Many examples of studies proved that indices of genetic polymorphism are lower for the hatchery broodstocks than for wild populations (Hansen 2002; Bernas et al. 2014; Panagiotopoulou et al. 2014). Therefore, determination of optimal selection breeding protocol under hatchery conditions is essential in sustainable aquaculture.

For these reasons, the main objective of the present study is to assess the genetic variability of Siberian sturgeon broodstock maintained in one of the major sturgeon aquaculture centers in Poland.

41.1 Materials and Methods

The fin clips were sampled from 94 adult individuals of Siberian sturgeon, reared in the Wasosze Fish Farm near Konin, Poland. The 54 studied specimens were directly imported from Russia, while the 40 specimens were randomly chosen from the selects of the F1 progeny, aged from 5+ to 7+, obtained during artificial breeding. The genomic DNA for amplification of six microsatellite loci *Afu-19*, *Afu-39*, *Afu-68*, *AfuB-68* (May et al. 1997), *Spl-163*, and *Spl-168* (McQuown et al. 2000) was extracted using Chelex 100 (Walsh et al. 1991). Reaction mixes (for amplification of the selected loci) were prepared in a total volume of 25 μL with 40 ng DNA template, 1× PCR reaction buffer (50 mM KCl, pH 8.5; Triton X-100), 0.4 mM of each primer, 0.25 mM of each deoxynucleotide triphosphate (dNTP), 3.3 mM $MgCl_2$, and 0.6 unit Go *Taq* DNA polymerase (Promega, Madison, WI, USA). Redistilled water was used to bring the reaction mixture to the desired final volume. Amplification was conducted with a Mastercycler gradient thermocycler (Eppendorf, Germany), with an initial denaturation at 94 °C for 5 min, followed by 35 amplification cycles (94 °C, 1 min; 52–57 °C, 30 s; 72 °C, 30 s), and a final elongation at 72 °C for 5 min.

In order to enable genotyping of PCR products with an Applied Biosystems 3130 Genetic Analyzer, forward primers were 5′-labeled with the different fluorescent reporter dyes (*Afu-19*-PET, *Afu-39*-FAM, *Afu-68*-FAM, *AfuB-68*-VIC, *Spl-163*-NED, *Spl-168*-PET). Fluorescent-labeled primers as well as PCR products were stored in the black boxes to be protected against sunlight. The lengths of the amplified DNA fragments were determined using the Applied Biosystems 3130 Genetic Analyzer sequencer against GeneScan 500 [LIZ] size standard (Applied Biosystems, California, USA). Fragment size and alleles were determined using the GeneMapper and the Genetic Analyzer software (Applied Biosystems, California, USA) according to the manufacturer's recommendations.

The matrix of the microsatellite raw data was established and filed in Microsoft Excel as well as subsequently checked for microsatellite null alleles, inconsistent values, scoring errors due to stuttering, and large allele dropout in samples using the Micro-Checker software (version 2.2.3) (Van Oosterhout et al. 2004). After that, the computer software Convert (version 1.31) was used for transformation of established microsatellite allele data into the input files for applied genetic analysis programs (Glaubitz 2004). Microsatellite allele frequencies, the observed number of alleles per locus (A_o), the number and percentage qualitative composition of rare alleles (A_{rare}), the number of effective alleles (A_e), allelic range, and Shannon index (*I*) for each loci within investigated specimens of Siberian sturgeon were calculated by GenAlEx computer software (version 6.5) (Peakall and Smouse 2012). Every detected allele which appeared at frequencies lower than 0.05 is considered as rare. The number of effective alleles (A_e), being defined as the number of equally frequent alleles which would take to achieve a given level of heterozygosity, was applied for basic characteristics of allele frequency distribution within the examined microsatellite DNA loci. Application of this measure in genetic studies allows obtaining the simple characteristics and comparison of numerous fish populations or broodstocks based on the number of detected alleles and its distribution. In a practical sense, lower values of this indicator suggest that a set of determined alleles within particular microsatellite locus are distributed with very different frequencies (Frankham et al. 2010). The observed (*Ho*) and expected heterozygosity (*He*) and the exact Hardy-Weinberg equilibrium (H-WE) test for each locus were calculated using Arlequin software (version 3.5) (Excoffier and Lischer 2010). The number of steps in the Markov chain was 1,000,000 and the number of dememorization steps equaled to 100,000. In order to assess a global H-WE, probability tests were carried out by Fisher's method and Smouse's multilocus analysis implemented in Genepop (version 2.9.3.2) and Popgene (version 1.3.2) (Yeh and Boylet 1997; Rousset 2008) software, respectively. The Arlequin software was also used to calculate the Garza-Williamson index (*M*), which is considered as the ratio of the number of identified alleles to its allelic size range within each microsatellite locus. Additionally, the test for bottleneck assessment was conducted using the Bottleneck software (version 1.9), which tests for departure from mutation-drift equilibrium based on heterozygosity excess or deficiency (Piry et al. 1999). This method is based on the assumption that a recently bottlenecked population should exhibit higher gene diversity than expected from the number of alleles at mutation-drift equilibrium because the allele numbers decrease faster than gene diversity in populations recently experiencing a bottleneck event (Cournet and Luikart 1996). Therefore, in non-bottlenecked populations, the value of expected heterozygosity (*He*) is equal to *Heq* (heterozygosity expected in a mutation-drift equilibrium). The excess of *He* over *Heq* is the evidence of severe reduction in population effective size that may occur because of a bottleneck event. For this purpose, the infinite allele model (*IAM*), stepwise mutation model (*SMM*), and two-phase model of mutation (*TPM*) were tested for examined specimens of Siberian sturgeon. *TPM* model was used with the default settings of 30% variation from IAM model and 70% from SMM model. Statistical

tests were performed using the one-tailed Wilcoxon signed-rank test. Additionally, the allele frequency distribution analysis was performed. The population that does not suffer any bottleneck is expected to show a normal L-shaped distribution. In contrast, a bottlenecked population exhibits mode shifts (Luikart and Cournet 1998). Moreover, allelic richness (A_r), the polymorphic information content (*PIC* value), and fixation index (*Fis*) were calculated using PowerMarker (version 3.25) and Fstat (version 2.9.3) computer software (Goudet 2002; Liu and Muse 2005). The fixation index (*Fis*) is known as the inbreeding coefficient (*f*), which is commonly defined as the probability that two alleles in a specimen are identical by descent. In other words, it is a measure of the deviation of genotypic frequencies from panmictic frequencies in reference to heterozygous deficiency and excess. The observed negative values of *Fis* indicate heterozygote excess (which may be related with outbreeding). Contrastingly, positive values of this indicator evidence heterozygote deficiency (which may be caused by influence of inbreeding events) compared with H-WE expectations (Wright 1965). The effective population size (*Ne*) was estimated for examined broodstock of Siberian sturgeon by NeEstimator computer program (version 2.01) (Do et al. 2013). The linkage disequilibrium and the molecular coancestry methods were used for computing *Ne*, where the lowest allele frequency used was 0.05 and 95% parametric confidence intervals (95% CI) were calculated. To accommodate the obtained genotypic data to the requirements of used computer software, every tetrasomic locus was analyzed as two disomic loci, and as a result, the mean values were considered for estimation of genetic parameters.

41.2 Results

In the present study, the six microsatellite DNA fragments were applied to assess the level of genetic variability of Siberian sturgeon broodstock maintained under controlled conditions at the Wasosze Fish Farm in Poland. All of examined microsatellite loci were considered as tetrasomic and were highly polymorphic. Overall, 74 different alleles were found which proved to be variably distributed in each microsatellite loci: from 8 in locus *Afu-39* to 18 alleles in locus *AfuB-68* with an average value of 12.3 alleles. A total length of identified alleles in the studied loci varied between 100 and 252 base pairs (bp) (Table 41.1). Additionally, a total number of 38 rare alleles within investigated microsatellite loci were found, which consisted 51% of qualitative composition of all detected alleles. The number of rare alleles per locus ranged from 5 (*Afu-39*, *Spl-163*, *Spl-168*) to 11 (*AfuB-68*), at an average of 6.3 alleles per locus (Table 41.1).

All of assessed genetic diversity parameters (*Ho*, *He*, A_r, A_o, A_e, *I*, and *PIC*) of the studied Siberian sturgeon individuals are presented in Tables 41.1 and 41.2. The number of effective alleles (A_e) ranged between 2.425 and 6.758. The polymorphic information content (*PIC*) and the rate of Shannon's index (*I*) indicated the value ranges 0.504–0.837 and 1.036–2.150, respectively. The observed values of allelic richness (A_r) varied from 6.000 to 13.500 in studied fish (Table 41.1). The values of

Table 41.1 Genetic diversity parameters of Siberian sturgeon: A_r, allelic richness; A_o, observed number of alleles; A_e, number of effective alleles; A_{rare}, number of rare alleles (in parentheses percentage qualitative composition of rare alleles); *I*, Shannon's index; *PIC*, polymorphism information content; *Fis*, fixation index

Locus	Allele size range (bp)	A_r	A_o	A_e	A_{rare}	*I*	*PIC*	*Fis*
Afu-19	123–156	6.000	9	2.425	*6 (67%)*	1.036	0.504	*−0.297**
Afu-39	124–148	6.500	8	2.601	*5 (63%)*	1.207	0.556	*−0.185**
AfuB-68	108–228	13.500	18	5.780	*11 (61%)*	1.973	0.800	*−0.200**
Afu-68	140–252	11.500	14	6.635	*6 (43%)*	2.016	0.829	*−0.175**
Spl-163	100–248	12.000	13	6.758	*5 (38%)*	2.150	0.837	*−0.131**
Spl-168	100–248	11.500	12	6.616	*5 (42%)*	2.037	0.829	*−0.031*
Mean	**–**	**10.166**	**12.3**	**5.136**	***6.3 (51%)***	**1.737**	**0.726**	***−0.170****

**Fis* values statistically significant at $P \leq 0.01$

Table 41.2 Comparison of observed (*Ho*) and expected (*He*) heterozygosity, expected heterozygosity (*Heq*) in an infinite allele model (*IAM*), stepwise mutation model (*SMM*), and two-phase model of mutation (*TPM*) as well as Garza-Williamson index (*M*) in examined specimens of Siberian sturgeon. *P*, level of significance

				IAM		*SMM*		*TPM*		
Locus	***Ho***	***He***	***P***	***Heq***	***P***	***Heq***	***P***	***Heq***	***P***	***M***
Afu-19	0.755	0.586	0.000	0.503	0.318	0.680	0.173	0.593	0.276	0.219
Afu-39	0.723	0.614	0.004	0.555	0.439	0.737	0.050	0.653	0.300	0.298
AfuB-68	0.989	0.826	0.003	0.768	0.287	0.880	0.050	0.836	0.329	0.115
Afu-68	1.000	0.852	0.000	0.723	0.089	0.855	0.111	0.799	0.245	0.111

Deviations statistically significant at $P < 0.05$

fixation index (*Fis*) within each loci were between −0.031 and −0.297. The average value of *Fis* was stated at −0.170, evidencing considerable excess of heterozygotes in the studied group of fish. Almost all of obtained *Fis* values were statistically significant with the exception of *Spl-168*. Furthermore, a mean value of this indicator was also highly significant (Table 41.1).

Observed heterozygosity (*Ho*) in the studied loci showed values between 0.723 and 1.000, while the expected heterozygosity (*He*) ranged between 0.586 and 0.857. All of the examined microsatellite loci deviated from Hardy-Weinberg equilibrium (H-WE), with the exception of *Spl-168* microsatellite locus. Both of the applied H-WE probability global tests detected highly significant ($P < 0.001$) global deviation of examined loci from H-WE expectations (Table 41.2). The estimated effective population size (*Ne*) values by the linkage disequilibrium and the molecular coancestry methods were at the level 47.3 (95% CI = 39.6–57.2) and 41.3 (95% CI = 3.0–128.7), respectively. Moreover, Table 41.2 shows the expected heterozygosities under the applied models of a mutation-drift equilibrium (*Heq*) for each of examined microsatellite loci. Within the studied fish group, tendencies toward

heterozygosity excess were detected under an *IAM*, *SMM*, and *TPM* for all of tested microsatellite loci. However, statistically significant *He* > *Heq* differences were observed under an infinite allele model (*IAM*) and stepwise mutation model (*SMM*), where two loci, *Spl-163* and *Spl-168* and *Afu-39* and *AfuB-68*, exhibited significant heterozygosity excess, respectively. Similarly, applying the Wilcoxon sign test showed significant ($P < 0.05$) overall heterozygosity excess exclusively under the *IAM*. On the other hand, the analysis of allele frequency distribution revealed an L-shaped distribution. All investigated loci differed in terms of the Garza-Williamson index (*M*), ranging from 0.077 to 0.298. The mean value of *M* in the investigated group of Siberian sturgeon equaled to 0.150 (Table 41.2).

41.3 Discussion

The genetic markers applied in fishery research and aquaculture are very important in the study of genetic variability of broodstock, parentage relationships, and the performances of lines during breeding programs (Fopp-Bayat 2010; Kucinski et al. 2015).

Numerous genetic studies evidenced that the fish stocks identified by genetic diversity parameters *PIC* and *I* close to 0.5 and 1.0, respectively, are considered as genetically moderate differentiated (You-Yi et al. 2009; Weiss et al. 2011). Lower values of such indicators (*PIC* and *I* lower than 0.4 and 0.9, respectively) are specific for broodstock with low genetic diversity (Ayllon et al. 2004; Liang et al. 2004). Contrastingly, higher values of these parameters (*PIC* and *I* higher than 0.6 and 1.3, respectively) indicate the high level of genetic diversity (Froufe et al. 2004; Fopp-Bayat 2010). The investigated broodstock of Siberian sturgeon was characterized by a high level of genetic diversity with the number of effective alleles (A_e) ranging between 2.425 and 6.758. The polymorphic information content (*PIC*) and the rate of Shannon's index (*I*) indicated that the value ranges from 0.504 to 0.837 and 1.036 to 2.150, respectively. Similar high levels of genetic variability were described for the Russian sturgeon (*Acipenser gueldenstaedtii*) and sterlet (*Acipenser ruthenus*) specimens reared at the Wasosze Fish Farm near Konin, Poland (Fopp-Bayat and Furgala-Slezniow 2010).

It is considered that small and isolated fish stocks kept under captivity conditions are much greater threatened by the negative influence of genetic drift than inbreeding effect (Frankham et al. 2010). Analysis of the effective population size (*Ne*) parameters of farmed fish stocks is a powerful tool for assessment of their susceptibility to genetic drift effect, enabling for monitoring of held breeding procedure effectiveness (Tringali and Bert 1998; Hoarou et al. 2005). Assessed values of effective population size (*Ne*) for studied group of fish are close to those reported for natural populations of lake sturgeon (*Acipenser fulvescens*) from the Kaministiquia River in Canada (Welsh et al. 2014). Such results evidence both the good genetic condition of studied Siberian sturgeon broodstock and high effectiveness of currently held breeding procedures for preservation of broodstocks genetic diversity at sturgeon's farm in Wasosze. Additionally, observed high qualitative proportion of rare alleles, at the level of 51%, implies that the examined broodstock is genetically

a very valuable and can be used as source material for establishment of new or supplementation of existing broodstocks of Siberian sturgeon in Poland.

According to the current results, the studied group of Siberian sturgeon was not in H-W equilibrium. The average *Fis* value was negative in the examined specimens, displaying significant excess heterozygotes against Hardy-Weinberg expectations. Most probably, observed significant excess may be a consequence of the use of a nonrandom subset of breeders for artificial reproduction in the hatchery conditions as was hypothesized by Luikart and Cournet (1999). Moreover, general deviation of the studied broodstock from Hardy-Weinberg equilibrium seems to support this hypothesis. Similar negative values of *Fis* indicator have been frequently reported for another fish stocks kept under controlled conditions (Kim et al. 2004; Ditlecadet et al. 2006; Fopp-Bayat et al. 2010; Kaczmarczyk et al. 2012; Kucinski et al. 2015).

The present paper has distinctly illustrated the importance of genetic analysis of the Siberian sturgeon broodstock in aquaculture, providing a reliable tool for monitoring of various indicators significant in production and management of sturgeon broodstocks. The proposed genetic study is characterized by some advantages; for example, proposed analysis can be performed noninvasively on the basis of a fin clip or a small amount of sperm (Fopp-Bayat and Ciereszko 2012). Additionally, the genetic analysis enables the genetic separation of individuals (if they are tagged) or spawning pairings based on the genetic characteristics (Kaczmarczyk and Fopp-Bayat 2013).

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Genetic Variability in Wild Populations and Farmed Broodstocks of the Siberian Sturgeon in Russia

42

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Abstract

In this chapter, we cover different genetic aspects of *A. baerii*. Phylogenetic position of Siberian sturgeon among other Acipenseriformes, genomic organization, and events of polyploidization are discussed. Genetic variation in four natural populations of the Siberian sturgeon (rivers Ob, Yenisei and Lena and Lake Baikal) is assessed for mitochondrial (control region or D-loop) and nuclear (microsatellite loci) markers. Most of *A. baerii* stocks reared at sturgeon farms in Russia have decreased genetic variation compared with wild populations. Two genetically distinct groups of stocks, both originated at Konakovo hatchery, are now widely distributed across sturgeon farms in Russia. Origin of "*baerii*-like" haplotype in the Caspian population of the Russian sturgeon is discussed in context of *A. baerii* paleogeography as well method for identification of each species by mtDNA analysis.

Keywords

A. baerii • Mitochondrial DNA • Microsatellite analysis • Genetic management • DNA-barcode

Introduction

Siberian sturgeon is a potamodromous species, inhabiting all major Siberian rivers—from rivers Ob and Irtysh to Kolyma and Indigirka—as well as the Lake Baikal (Ruban 2005). In 1950 numerous attempts were taken to introduce this species in

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lakes and rivers on the European part of USSR, which were mostly unsuccessful (Malyutin and Ruban 2009). However, recent findings of two adults specimens in River Pechora (Zakharov et al. 2007), could indicate small sustaining population as a result of previous introduction, conducted in 1956. There are also reports on this species or its hybrids in Danube River (Ludwig et al. 2009; Weiperth et al. 2014).

The Siberian sturgeon was described by Johann Friedrich Brandt from the rivers Ob and Lena (Brandt 1869). Later Nikolsky described fish from the Yenisei River as a distinct species—*A. stenorrhynchus*—and sturgeons of Lake Baikal were described as a form of this species (*A. stenorrhynchus var. baikalensis* (Nikolsky 1896)). In the following years, several revisions were published, and three subspecies were accepted: *A. baerii baerii* (the Ob-Irtysh basin), *A. b. stenorrhynchus* (Yenisei, Lena, and smaller rivers East from Lena), and *A. b. baikalensis* (Lake Baikal and rivers flowing to the lake—Selenga, Barguzin, and Upper Angara) (Sokolov and Vasil'ev 1989). However, recent study of morphology variation, conducted by Georgy Ruban, revealed extensive variation among populations from different parts of the same river, which was found to exceed variation among designated subspecies (Ruban 2005). It was later supported by lack of clear subspecies differentiation based on analysis of DNA variation of cytochrome oxidase I (COI) gene region (Birstein et al. 2009).

Siberian sturgeon is the first sturgeon species, introduced in the industrial scale aquaculture, and remains one of the leading species in sturgeon aquaculture in Russia and in the world (Malyutin and Ruban 2009; Bronzi et al. 2011). Introduction to aquaculture of this species began in 1973, when the first 61,000 fertilized eggs were obtained from wild sturgeons from Lena River and were brought to Konakovsky experimental warm-water fish farm near Moscow (Konakovo farm), and more embryos were transferred there from Lena in the following years (Malyutin and Ruban 2009). Progeny of these fish grown at Konakovo farm later seeded as numerous aquacultured broodstock of commercial farms. In 1975 the first Siberian sturgeon fingerlings were transferred from Konakovo to France (Williot and Rouault 1982) which spin off aquaculture of this species in the Western Europe. The vast majority of aquacultured broodstocks of the Siberian sturgeon, the currently existing worldwide (Williot et al. 2001; Wei et al. 2011), originated from the descendants of a limited number of specimens of sturgeon from Lena bred at Konakovo sturgeon farm. In 1995 100 thousand eyed embryos were imported from Russia to Uruguay, where *A. baerii* remains the major (together with bullfrog) commercialized species (Clavelli 2005). Because of its origin, aquacultured Siberian sturgeon is often called the Lena sturgeon. A limited number of state-running restocking farms and private aquaculture farms (growing fish for meat and caviar) in the Asian part of Russia have sturgeon stocks originated from local populations—Ob, Yenisei, and Baikal.

42.1 Sturgeon Mitochondrial DNA Phylogeny

Acipenseriformes are different from other vertebrates by very slow evolution of its nuclear genome (Herran et al. 2001; Krieger and Fuerst 2002), high and variable nuclear ploidy (Birstein et al. 1997; Ludwig et al. 2001; Fontana et al. 2007; Vasil'ev 2009), amazing ability for interspecies hybridization, as well as high morphometric plasticity (Nikolyukin 1972).

Molecular genetic data changed views on the taxonomy of this family. Previously the family Acipenseridae Bonaparte, 1831 was formed of two subfamilies, Acipenserinae (with two genera—*Acipenser* Linnaeus, 1758 and *Huso* J. F. Brandt and Ratzeburg, 1833) and Scaphirhynchinae, also contained two genera—*Scaphirhynchus* Heckel, 1835 and *Pseudoscaphirhynchus* Nikolskii, 1900. According to phylogeny reconstruction based on mtDNA, three species of genus *Pseudoscaphirhynchus* (flattened fishes from two murky rivers of Central Asia (Amu Darya and Syr Darya)) are not related to North American species of the genus *Scaphirhynchus* (Mississippi, Missouri, and Alabama Rivers) but instead are close to stellatus sturgeon (*Acipenser stellatus*). Striking morphological similarity between *Scaphirhynchus* and *Pseudoscaphirhynchus*, which was the reason for placing it in the same subfamily, is thought to be due to convergent evolution caused by adaptation to similar environment, particularly to the murky waters of the major rivers of central Asia and North America (Birstein et al. 2002; Hilton 2005). Also it was shown that two *Huso* species (*H. huso* and *H. dauricus*) positioned separately among the Acipenser species tree (Birstein and DeSalle 1998; Krieger et al. 2008).

Phylogenetic tree of the order Acipenseriformes based on complete mitochondrion genome sequences (without hypervariable control region, total alignment length 15,762 bp) is presented in Fig. 42.1. European (*A. sturio*) and Atlantic (*A. oxyrinchus*) sturgeons form the basal clade within the family Acipenseridae, and the next branch of the tree is pallid sturgeon, *Scaphirhynchus albus*, representing the subfamily Scaphirhynchinae. The rest of the tree split into two major branches—Pacific clade and Atlantic (sometimes called Ponto-Caspian) clade; Atlantic clade also includes genus *Pseudoscaphirhynchus.* In both clades, there are anadromous and potamodromous species, inhabited rivers or lakes, drained into the Pacific or

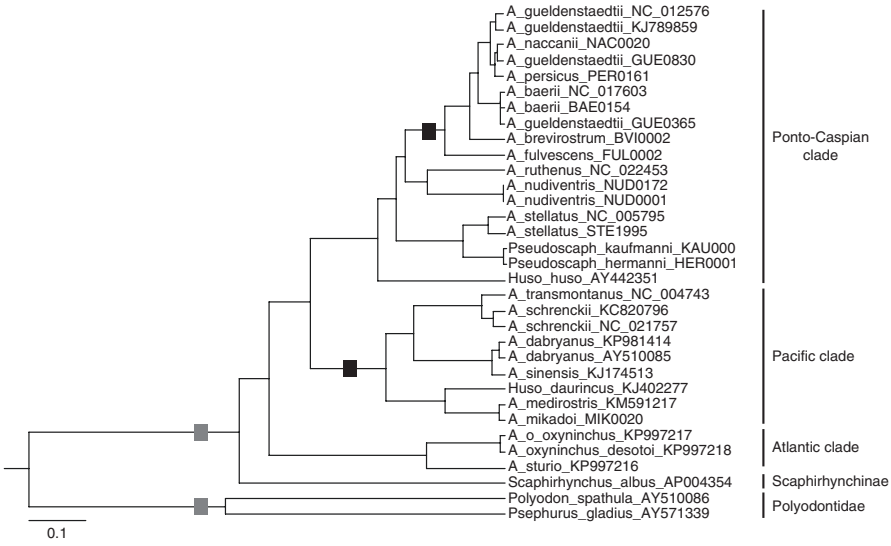

Fig. 42.1 An Acipenseriformes phylogenetic tree based on complete mitochondrial DNA sequences. *Black* and *gray boxes* indicate proposed recent and ancient whole genome duplication events

Atlantic Oceans. Indeed, the Caspian Sea was previously the part of ancient Thetis Sea, which also included Black and part of Mediterranean Seas, and sturgeons from that basin belong to the Atlantic clade (Fig. 42.1). Siberian sturgeon has a peculiar position on this tree. Being distributed further to the East, then some Pacific clade species, such as Amur sturgeon (*A. schrenkii*) and Kaluga (*Huso dauricus*), Siberian sturgeon form phylogenetic cluster with Russian (*A. gueldenstaedtii*), Persian (*A. persicus*), and Adriatic (*A. naccarii*) sturgeons. Once migration from the Caspian into the Arctic basin has led to formation of separate species from the Caspian Sea ancestors of Siberian sturgeon in the rivers of the Arctic basin. It happened in the distant past, and geographically isolated population beyond the Ural Mountains has since emerged as the Siberian sturgeon which is clearly different from the Russian sturgeon both morphologically and by mitochondrial DNA. According to molecular estimates (*cytochrome b* molecular clock), split between Russian and Siberian sturgeons was approximately 24 Mya (Peng et al. 2007).

However, despite the substantial divergence in DNA sequence between Russian and Siberian sturgeons, it was found that high proportion of Russian sturgeons in the Caspian Sea basin possess mitochondrial DNA sequence very similar to sequence found in *A. baerii*. It was proposed to call "*baerii*-like" haplotype of *A. gueldenstaedtii* (Birstein et al. 2000; Jenneckens et al. 2000). Origin of these haplotypes in the Caspian population of Russian sturgeon will be discussed later.

42.2 Different Ploidy Levels in Acipenseriformes

Ploidy level is an important factor in understanding sturgeon genetics. Several rounds of whole genome duplication took place during formation of modern species. All sturgeons are divided into "low-chromosome number" and "high-chromosome number" species (approximately 120 and 250 chromosomes in each cell nucleus (Ludwig et al. 2001)). Majority of microsatellite loci have up to two alleles in the "low chromosome," but the same loci have up to four alleles per individual in the "high-chromosome" group of species. The fact that these loci were duplicated in "high-chromosome" species indicates polyploidization event somewhere during evolutionary history. It proposed one WGD (whole genome duplication) event in Ponto-Caspian group of species (ancestor of Lake, Shortnose, Russian, Persian, Adriatic, and Siberian sturgeons) and up to three independent WDG events in the Pacific group (Peng et al. 2007; Vasil'ev 2009). Recent studies changed view on ploidy level and number of polyploidization events in the Pacific group. Kaluga was previously reported as a "low-chromosome" species, and for the Sakhalin sturgeon *A. mikadoi*, the number of chromosomes was estimated as ~500 (Birstein et al. 1993). Recently, it was shown that all sturgeons from the Pacific clade, including Kaluga and Sakhalin sturgeon, are "high-chromosome" species and possess about 250 chromosomes (Vishnyakova et al. 2008; Vasil'ev et al. 2009), supporting the hypothesis of single polyploidization event in the ancestral lineage leading to Pacific clade (black boxes in Fig. 42.1).

But there is also an evidence that species with ~120 chromosomes are themselves of tetraploid-origin and "high-chromosome number" species, including *A. baerii*, and others are, in fact, octoploids, while true diploid ancestor of Acipenseriformes,

possessed ~60 chromosomes, is now extinct. This suggestion is supported by the number of loci, which are tetraploid (up to four loci in individual fish) in 120 chromosome species and up to eight in "high-chromosome" sturgeons. According to genomic data, WGD events took place independently in ancestral lineages of both families—sturgeons Acipenseridae and paddlefish (gray boxes in Fig. 42.1). Yet another round of genome duplication was proposed for shortnose sturgeon (estimated number of chromosomes is about 380 (Kim et al. 2005; Peng et al. 2007; Havelka et al. 2013)), but ploidy level for this species needs further confirmation by different techniques.

Although *A. baerii* is a tetraploid (and ancient octoploid) species, spontaneous shift in ploidy level is frequently observed in aquacultured stocks (Havelka et al. 2014; Havelka et al. 2016). This shift is likely due to incorrect chromosomal segregation during gametogenesis, which could be a result of accelerated gametogenesis possibly caused by inadequate water temperature or other conditions during gonad maturation.

42.3 Genetic Variability of Mitochondrial DNA in Wild *A. baerii* Populations

Mitochondrial DNA variation is often used for study of phylogeny, population structure, and identification of species origin of caviar. Several regions of mtDNA, including genes coding cytochrome b (*CytB* gene), cytochrome oxidase subunit I (*COI*), and few other genes (Birstein et al. 1998; Zhang et al. 2013), were studied, and the control region of mitochondrial DNA, also called D-loop, was found as the most polymorphic part of mitochondrial DNA. Control region contains the only noncoding sequences of mitochondrial genome in vertebrates and is composed of central conserved section that is flanked by two hypervariable (HVRI and HVRII) regions (Brown et al. 1986). Two types of sequence variability are most commonly found in the control region of teleost fishes: single nucleotide polymorphisms and variable numbers of copies of tandem repeated sequences (Lee et al. 1995). Tandem repeated sequences are found in HVRI of many vertebrates, but Acipenseriformes are different from most other vertebrates by having long (80–82 bp) repeats and by number of repeats which vary from two to six not only among different specimens but also within single organism (state, called "heteroplasmy") (Buroker et al. 1990; Miracle and Campton 1995; Brown et al. 1996; Ludwig et al. 2000). Duplicated stretches of DNA sequences are usually all identical, and therefore this region is not informative for identification and population genetics analysis. On the other hand, HVRII, located between the central conserved section of the control region and phenylalanine tRNA gene, is the most polymorphic region of sturgeon mtDNA and is valuable to study of genetic variation.

To assess genetic variation in major *A. baerii* populations (Ob-Irtysh, Yenisei, and Lena river basins and Lake Baikal), we have sequenced HVRII of the mtDNA control region (680 bp) in fish of wild origin from the sturgeon reference genetic sample depository, maintained by Russian CITES Scientific Authority for Acipenseriformes at VNIRO Institute, Moscow.

Fifty different DNA sequences (haplotypes) (Tables 42.1 and 42.2) have been found among 357 Siberian sturgeons studied (sequences are deposited in NCBI GenBank under accession ## KU375049-KU375098). Seven haplotypes are shared among different

Table 42.1 Distribution of mitochondrial haplotypes in four wild populations of Siberian sturgeon

N	Haplotype	Population (origin)	Number	N	Haplotype	Population (origin)	Number
1	Hap1	Ob-Irtysh	1	24	Hap25	Lena River	1
2	Hap2	Yenisei River	1	25	Hap26	Lena River	1
3	Hap3	Lena River	9	26	Hap29	L. Baikal	1
		L. Baikal	3	27	Hap30	Ob-Irtysh	1
4	Hap4	Ob-Irtysh	3	28	Hap31	L. Baikal	1
		L. Baikal	4	29	Hap32	L. Baikal	4
5	Hap5	Ob-Irtysh	4			Yenisei River	2
		Yenisei River	3	30	Hap33	L. Baikal	1
		L. Baikal	1	31	Hap34	L. Baikal	1
6	Hap6	Lena River	1	32	Hap35	L. Baikal	1
7	Hap7	L. Baikal	3			Ob-Irtysh	1
		Lena River	12	33	Hap36	L. Baikal	3
		Ob-Irtysh	2			Ob-Irtysh	2
		Yenisei River	4	34	Hap37	Yenisei River	1
8	Hap8	L. Baikal	3	35	Hap38	L. Baikal	1
9	Hap9	Ob-Irtysh	1	36	Hap39	L. Baikal	1
10	Hap10	Ob-Irtysh	1	37	Hap40	Ob-Irtysh	1
11	Hap11	Lena River	1	38	Hap42	Yenisei River	1
12	Hap12	Yenisei River	1	39	Hap43	Lena River	1
13	Hap13	Ob-Irtysh	1	40	Hap44	Ob-Irtysh	1
14	Hap14	Lena River	1	41	Hap45	Ob-Irtysh	1
15	Hap15	Lena River	1	42	Hap47	Ob-Irtysh	2
16	Hap16	Ob-Irtysh	1	43	Hap48	Ob-Irtysh	1
17	Hap17	Lena River	2	44	Hap49	Lena River	5
18	Hap18	Ob-Irtysh	1	45	Hap50	Lena River	1
19	Hap19	Lena River	1	46	Hap51	Ob-Irtysh	1
20	Hap20	Lena River	1	47	Hap52	Yenisei River	1
21	Hap21	Lena River	1	48	Hap53	Ob-Irtysh	1
22	Hap22	Ob-Irtysh	1	49	Hap54	Lena River	1
23	Hap24	Yenisei River	1	50	Hap55	Ob-Irtysh	1

Table 42.2 Nucleotide and haplotype diversity in four natural populations of *A. baerii*

	Baikal	Yenisei	Lena	Ob-Irtysh
Number of specimens studied	94	62	60	48
Number of mtDNA haplotypes	14	9	16	21
Number of polymorphic sites	18	15	32	36
Haplotype diversity (H) (%)	81.7 ± 2.07	65.26 ± 3.76	77.85 ± 3.74	89.01 ± 2.98
Nucleotide diversity (π)	0.41 ± 0.24	0.13 ± 0.1	0.26 ± 0.17	0.49 ± 0.29

(2–4) populations, and other haplotypes are limited to only one population. Every population possesses few abundant haplotypes and large number of rare haplotypes, found in one or two specimens. Major haplotypes are not unique for particular river basin but shared among several populations. This distribution supports the severe bottleneck in population size in the past glaciation and may indicate a single common refugium for this species in the past. Most parsimonious network of haplotypes is presented in Fig. 42.2.

In Ob-Irtysh population, we found 18 unique haplotypes and 4 haplotypes shared among other populations, Baikal, 7 (and 6 shared), and Yenisei, 5 unique and 3 shared, and Lena River population has 13 unique and 2 shared haplotypes (Table 42.1, Fig. 42.2). Analysis of variances (AMOVA) indicates that all four populations are significantly different by F_{ST} values (Table 42.3).

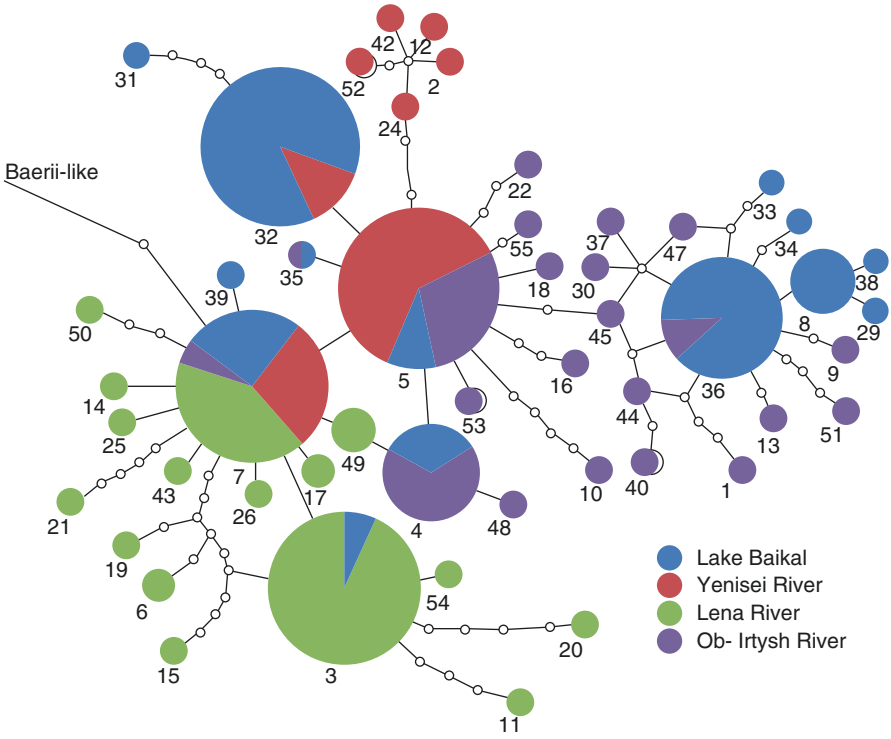

Fig. 42.2 Haplotype network of four natural populations of *A. baerii*. *Color filling* indicates proportion of specimens from each population. *Small white circles* indicate predicted haplotypes not detected in the dataset. Distance between any two neighbor circles equals one mutation (nucleotide substitution, insertion or deletion)

Table 42.3 Genetic distance (F_{ST}) among populations studied

	Baikal	Yenisei	Lena	Ob-Irtysh
Baikal	0			
Yenisei	0,13,246	0		
Lena	0,22,072	0,24,977	0	
Ob-Irtysh	0,07158	0,10,802	0,26,139	0

42.4 Decrease in mtDNA Variation in *A. baerii* Aquacultured Broodstock

Because one female gives thousands of eggs and fingerlings, all bearing the same maternal mitochondrial DNA, the most severe effect on genetic variability in aquaculture is seen in drastic decrease in mtDNA haplotype variation. Majority of Siberian sturgeons, reared now in commercial aquaculture in Russia, have only two mitochondrial haplotypes—BaeHap3 and BaeHap7 (Table 42.4). In the Asian part of Russia, two other haplotypes, BaeHap 4 and BaeHap5, are prevailing in some aquacultured stocks. These haplotypes are common in the Ob-Irtysh population but absent in Lena River. This distribution of haplotypes is a consequence of the origin and distribution of aquacultured stocks—if in the European part of Russia all stocks were bought from Konakovo

Table 42.4 mtDNA haplotypes in *A. baerii* aquaculture of different origin

N	HAP	Known origin (population)	N of specimens
1	Hap1	Ob-Irtysh	1
2	Hap3	Lena	69
		Baikal	3
3	Hap4	Ob-Irtysh	10
		Baikal	7
4	Hap5	Ob-Irtysh	12
		Yenisei	38
		Baikal	1
5	Hap7	Baikal	18
		Lena	73
		Ob-Irtysh	5
		Yenisei	70
6	Hap8	Baikal	9
7	Hap11	Lena	1
8	Hap16	Ob-Irtysh	1
9	Hap17	Lena	2
11	Hap19	Lena	1
12	Hap21	Lena	1
13	Hap29	Baikal	1
14	Hap30	Ob-Irtysh	1
15	Hap31	Baikal	1
16	Hap32	Baikal	6
17	Hap33	Baikal	1
18	Hap34	Baikal	1
19	Hap35	Baikal	1
20	Hap36	Baikal	16
21	Hap38	Baikal	1
22	Hap39	Baikal	1
23	Hap49	Lena	5
		Total	367

hatchery (originated mainly from Lena population), beyond the Ural Mountains, some aquacultured sturgeons originated (mostly, for restocking purposes) from domesticated local populations: from Ob-Irtysh and Yenisei rivers and from Lake Baikal. Mitochondrial haplotype diversity in wild Siberian sturgeon populations and in aquacultured stocks (both commercial fish and caviar producing farms and restocking farms) is shown in Fig. 42.3. While two stocks originated from Baikal population (Gusinoozersk and

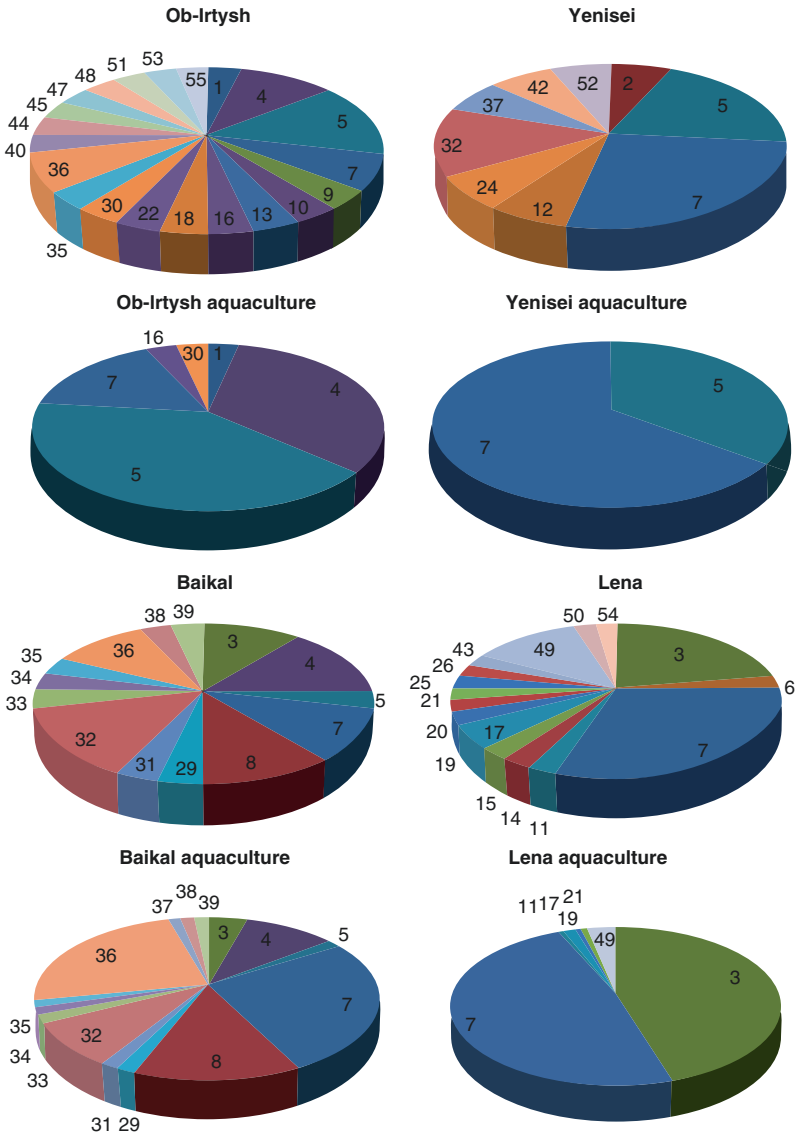

Fig. 42.3 Mitochondrial haplotypes diversity in four wild populations of *A. baerii* and in aquacultured stocks derived from these populations. Numbers at pie diagram sectors show mtDNA haplotypes, described in Table 42.1

Selenga sturgeon restocking farms) have haplotype diversity similar to wild population, sturgeon farms growing Lena, Yenisei, and, at lesser degree, Ob-Irtysh sturgeons demonstrate drastic loss of genetic diversity. There is a high probability that all aquacultured sturgeons from Yenisei area are descendants of just two females.

42.5 Use of Microsatellite Loci in Assessment of Genetic Variation of *A. baerii* and Other Sturgeon Species

Microsatellite loci are short (2–6 nucleotides long) repeats in DNA located in the cell nucleus. Because of high evolution rate, these loci are polymorphic and serve as genetic markers for many purposes—from identification of individuals and pedigree analysis to estimation of genetic differentiation among existing populations and even species. Microsatellite loci are described and extensively used in sturgeon genetic studies. At Russian CITES Scientific Authority for Acipenseriformes (VNIRO Institute), a set of five microsatellite loci are used for all species (Afug41, Afug51, An20, AoxD161, AoxD165) (Zane et al. 2002; Welsh et al. 2003; Henderson-Arzapalo and King 2002). Microsatellite loci analysis is used to verify origin of sturgeon samples to particular population. Also allelic content of unfertilized eggs, such as processed caviar, is identical to profile of maternal female specimen. This is because of large amount of somatic nuclear content in every egg cell including ovulated eggs for the reason not exactly evident. Caviar samples declared for CITES export permit are routinely tested by Russian CITES laboratory with set of microsatellite loci for concordance with previously genotyped producers.

Genetic variation in ten species of sturgeon inhabiting the Russian Federation (Russian, Siberian, Amur, Sakhalin, Persian, ship, and stellate sturgeons, Sterlet, Beluga and Kaluga, a total of 3591 individuals) by five microsatellite loci (Afug41, Afug51, An20, AoxD161, AoxD165) is summarized by Barmintseva and Mugue (2013). These loci were amplified successfully with the same set of primers in all species (except locus Afug41in Kaluga) and demonstrated high degree of allelic diversity.

Microsatellite loci analysis does not reveal any diagnostic alleles, specific for particular *A. baerii* population. All natural populations possess about the same set of alleles; however, allelic frequency does vary in different populations, allowing identifying origin of an aquacultured stock. Assignment test, performed with STRUCTURE software (Pritchard et al. 2000) for all samples, revealed that the dataset is most likely represented by four genetically distinct clusters. However, the distribution of samples in groups does not meet the accepted division of the Siberian sturgeon into subspecies—Baikal and the basins of three major Siberian rivers (Ob-Irtysh basin, Yenisei, and Lena). Samples from Lake Baikal and Yenissei River (and aquacultured stock of corresponding origin) are not different and belong to one cluster (shown in green in Fig. 42.4). Samples of the Ob River and aquacultured stocks consisting of domesticated sturgeon from this population also form a single group (red filling). Surprisingly, the samples of aquacultured stocks originated from the Lena sturgeon populations (all—of Konakovo origin) split into two distinct clusters. One cluster consists of old domesticated sturgeons of wild origin,

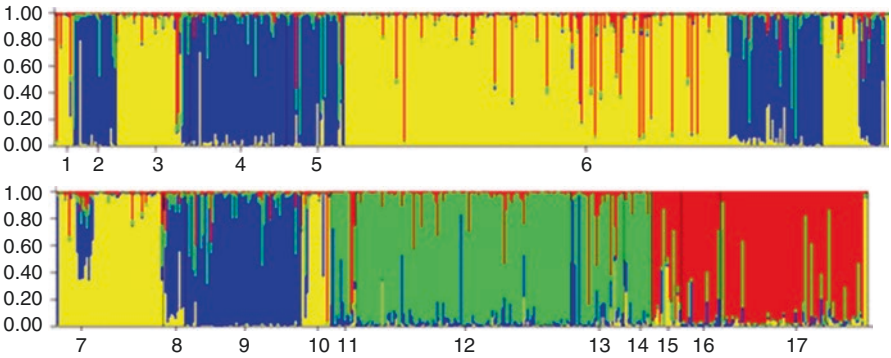

Fig. 42.4 Population assignment of wild populations and aquacultured stocks of Siberian sturgeon with five microsatellite loci (structure, $k = 4$). *Every bar* represents one individual, probability of assignment to cluster shown by proportion of each color. Aquacultured stock samples are *1–9*, of Lena (Konakovo) origin; *11*, Republic of Buryatia (Baikal origin); *12*, Krasnoyarsk District (Yenisei origin); and *16*, *17*, Tyumen district (Ob-Irtysh origin). Natural populations samples: *10*, Lena River; *13*, Yenisei R.; *14*, L. Baikal; and *15*, Ob-Irtysh

founders of Konakovo experimental sturgeon plant broodstock, as well as stocks from a number of aquaculture farms (yellow bars). At the same time, there is a second cluster of stocks, also from several commercial sturgeon farms (marked in blue, Fig. 42.4), which is genetically distinct from the first group of stocks. These stocks are apparently descended from a small number of individuals from the Konakovo farm and spread to a number of commercial sturgeon aquaculture. There is a registered breed named "Lena-1," the first sturgeon breed officially registered in Russia by Konakovo farm (other registered aquacultured sturgeon breeds in Russia are in fact different varieties of bester (*H. huso* × *A. ruthenus*) and respective backcrosses, registered under names "Aksai," "Burtzev," and "VNIRO" breeds). Genetically distinct group of *A. baerii* stocks could be representatives of "Lena-1" breed.

42.6 Phylogeography of *A. baerii* and "*baerii*-like" Mitochondrial Haplotype in *A. gueldenstaedtii*

At the end of the last century, all sturgeon species became listed in the Appendices of the CITES, and molecular genetics became widely used in control of international caviar trade. First molecular test was developed in 1996 (DeSalle and Birstein 1996; Birstein et al. 1998; Birstein et al. 1999) and was relied on species-specific primers targeting unique sequences in mitochondrial gene *cytochrome b*. However, toward the end of the last century, evidence for presence of mitochondrial DNA variant of Siberian sturgeon among fish was accumulated, identified by morphology as a Russian sturgeon and caught from the Caspian Sea (Birstein et al. 2000; Jenneckens et al. 2000). Before this discovery, a number of caviar shipments entered the United States and labeled as osetra (*A. gueldenstaedtii*) were considered as

mislabeled, because molecular test indicated its similarity with *A. baerii* DNA. Later, extensive study revealed that "baerii-like" haplotype is at high abundance among *A. gueldenstaedtii* from the Caspian Sea basin and about every third Russian sturgeon there has "*baerii*-like" haplotype (Birstein et al. 2005; Mugue et al. 2008).

Several hypotheses were proposed to explain occurrence of two distinct mitochondrial DNA lineages in the Caspian *A. gueldenstaedtii* population (Birstein et al. 2005). One proposed possibility was that "baerii-like" mitotype was introduced into the Caspian Sea population in the Soviet era by human activities. Indeed, number of *A. baerii* fingerlings were released into Oka River (Volga basin) and different water reservoirs built for hydroelectric purposes on Volga River and its tributaries (Ruban and Khodorevskaya 2011), but number of fish released were insignificant and there is no record that released fish reached maturity there.

The second scenario is that an ancestral to both *A. baerii* and *A. gueldenstaedtii* species, inhabited the Ponto-Caspian basin and harbored all types mtDNA variation, including "*baerii*-like" haplotype. When ancestral form invaded Siberian plains and evolved into *A. baerii*, it accidentally brought only "*baerii*-like" type of mitochondrial DNA (Birstein, et al. 2005). In this case "*baerii*-like" haplotype should be ancestral to all other mtDNA found in *A. baerii*, however, it is not supported by DNA study.

Based on mitochondrial gene *cytochrome b* sequence, *A. baerii* split from *A. gueldenstaedtii-A. persicus-A. naccarii* clade at 24 Mya (95% credibility intervals 7.5–51.5 Mya) (Peng et al. 2007) by spread into Siberian rivers from the Ponto-Caspian basin. Analysis of control region mtDNA haplotype data indicates that during the last glacial period populations of *A. baerii* went through bottlenecks, a sharp decrease in population size. Extant populations of the great Siberian rivers (Lena, Yenisei, Ob) do not show complete segregation and, having numerous population-specific haplotypes, share common haplotypes among populations. This distribution could be explained by periodical admixture among populations followed by period of geographic isolation. Absence of strong genetic segregation indicates that Siberian sturgeon had possibly single refugium during the first phases of the glacial period. Lake Baikal population possesses the most diverged haplotypes shared with all river populations, which makes plausible a hypothesis that this lake served as a refugium for *A. baerii* during glaciation maximum. A distribution of nucleotide site differences between pairs of individuals (mismatch distribution; (Rogers and Harpending 1992)) was calculated for all *A. baerii* populations and for "*baerii*-like" cluster in the Caspian Sea using Arlequin version 3.1. These mismatch distributions were compared with that expected under a model of sudden population expansion. Pairwise mismatch distribution shows that smallest mean number of pairwise differences (K2P) is in the Yenisei population. Coalescence analysis for "demographic expansion" model indicates that Baikal population is substantially older than all others, including set of "*baerii*-like" haplotypes found in *A. gueldenstaedtii* from the Caspian Sea. According to phylogeographic reconstruction, approximately 90 kya (thousands years ago), during local interglacial warm period (Henriksen et al. 2008), Siberian sturgeon left Baikal by Yenisei River and populated Ob-Irtysh and Lena River basins through the periglacial Great West Siberia

Lake. This huge lake was developed by blocking the river flow by glacier (Fig. 42.5), and at some point, it had water discharge into the Aral Sea via Turgay Valley and further toward the Caspian Sea by Uzboy pass (Mangerud et al. 2001; Mangerud et al. 2004). It is likely that Siberian sturgeon could migrate through Turgay Valley and Uzboy pass into the Caspian basin.

This phylogeographic scenario best explains presence of "*baerii*-like" haplotype in Caspian population of the Russian sturgeon. Hybridization *A. baerii* immigrants with *A. gueldenstaedtii* took place approximately 90 kya. This migration of the Siberian sturgeon into the Caspian Sea followed by hybridization with *A. gueldenstaedtii*, left "*baerii*-like" haplotype in the Caspian sea population as a footprint of the past phylogeographic event.

Relationship among "*baerii*-like" haplotypes is shown on Fig. 42.6. Haplotype network has star-like shape where numerous rare haplotypes are different from central common one by one or few substitution. This star-shape network is typical for young and growing population founded by very few individuals and descendant from only one ancestral haplotype—the central circle on graph (Fig. 42.6) and the

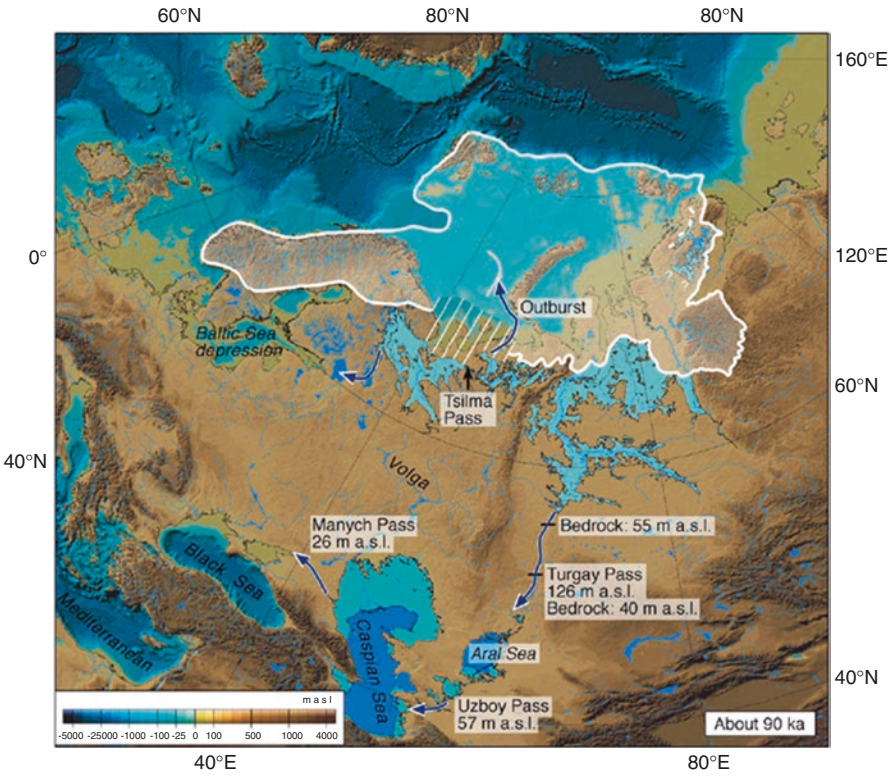

Fig. 42.5 Great West Siberian Lake connected Ob, Irtysh, and Yenisei river basins approximately 90,000 years ago (90 kya) and waterway toward the Caspian Sea. This waterway could be a route for *A. baerii* dispersion from Siberia to the Caspian (as discussed in the text) (after Mangerud et al. 2004)

most abundant in population. New mtDNA variants originated with time from the single ancestral haplotype by accumulation of spontaneous mutations. All "*baerii*-like" haplotypes in the Caspian Sea appears to be descendants of the single maternal line (haplotype G26, Fig. 42.6).

Not only mitochondrial "*baerii*-like" haplotypes are frequent in the Caspian population of *A. gueldenstaedtii*, but nuclear loci, common in the *A. baerii*, are also present at approximately the same proportion (30%) in the Caspian population of Russian sturgeon (Boscari et al. 2014). Such a high incidence of both mitochondrial and nuclear markers indicate that Siberian sturgeon, when penetrated into the Caspian basin, gained a substantial population size before it hybridized and genetically swamped in Russian sturgeon. Cold rivers flowing from the glacial lake toward the Aral and Caspian seas was likely an optimal environment for Siberian

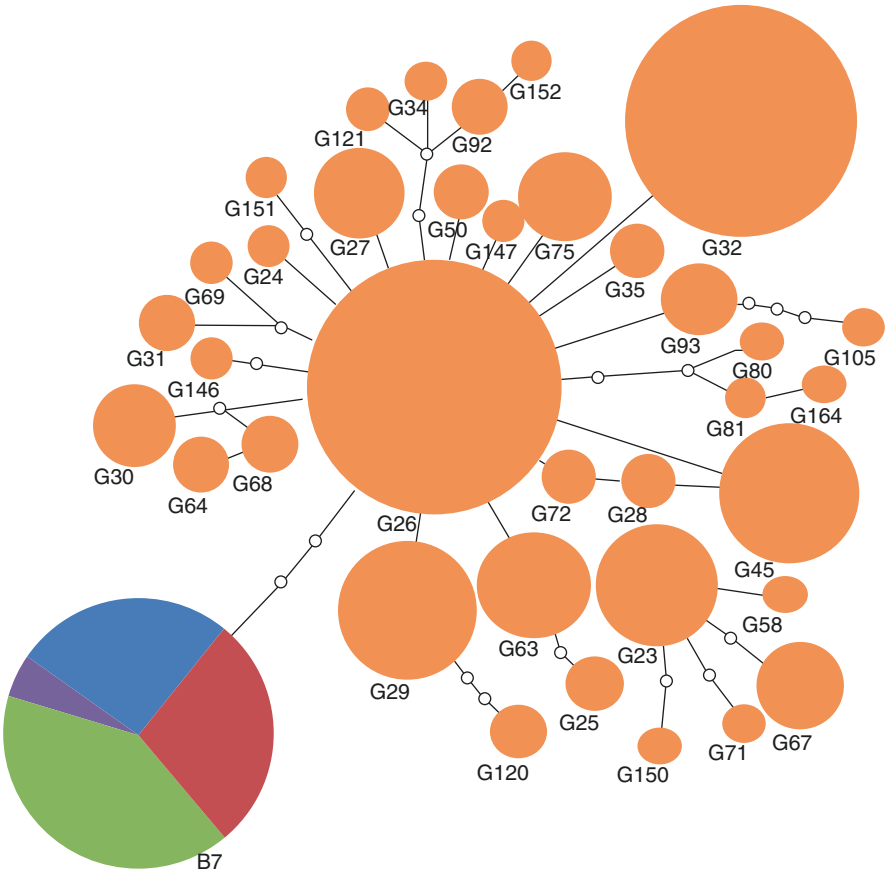

Fig. 42.6 Minimum spawning tree (haplotype network) of "baerii-like" haplotypes of *A. gueldenstaedtii* (*orange*). Haplotype B7 of *A. baerii* is shown to indicate connection and relationship to haplotype network of the Siberian sturgeon (Fig. 42.2)

sturgeon, while Russian sturgeon experienced hostile and far from its optimal climatic regime at that time. This would explain an abundance of *A. baerii* genetic markers in the *A. gueldenstaedtii* population. The absence of baerii-like haplotypes in *A. persicus* (Mugue et al. 2008) indicates that this species was already reproductively isolated from *A. gueldenstaedtii* and did not hybridize with *A. baerii*. Because baerii-like haplotypes were not present in the Azov Sea *A. gueldenstaedtii* population before mass releases of Caspian fingerlings started at the Soviet times (Timoshkina et al. 2009), it is likely that *baerii* × *gueldenstaedtii* hybridization took place after the Manych straight, connected periodically Azov and Caspian sturgeon populations, was finally closed.

42.7 Molecular Genetic Identification of *A. baerii* and Other Sturgeon Species

In spite of the great ecological plasticity and morphological variation, species identification of adult sturgeons is usually simple (with the exception of green (*A. medirostris*) and Sakhalin (*A. mikadoi*) sturgeons) by external characters (Vecsei et al. 2001). In addition, differentiation of Russian, Adriatic, and Persian sturgeons is also considered to be problematic. Species identification of juvenile sturgeons and fingerlings is not straightforward and requires special knowledge. Traditionally, species identification of black caviar is organoleptic and rather subjective. The caviar taste, color, and roe grain size substantially vary; the grain size ranges of different species overlap and are thus unreliable for species identification. Structural analysis of the egg envelope has shown that roe grains of different species differ in the shape and structure of micropyles fields, but these traits vary and are hardly distinguishable after roe is treated to produce caviar (Debus et al. 2002).

Mitochondrial DNA variation is the most widely used genetic marker for molecular identification of animal species, including sturgeons (Ludwig 2008). DNA barcoding, based on sequence variation of the 3'region of cytochrome oxidase subunit I, became very popular in scientific and forensic community (Hebert et al. 2003), but when it is applied for sturgeon identification, it faces several limitations (Birstein et al. 2009). Birstein and colleagues were the first to develop a DNA test for species identification of sturgeons (DeSalle and Birstein 1996; Birstein et al. 1998; Birstein et al. 2000). The method took advantage of species-specific substitutions in a region of the *CytB* gene and was patented in the United States and Europe as a set of primers allowing species-specific amplification of the *CytB* gene fragment. Ludwig and coauthors (2002) further developed the method based on the *CytB* polymorphism. Another method was developed for species identification involved consecutive restriction enzyme analyses of a PCR-amplified fragment of the *CytB* gene with seven enzymes and visualization of the restriction fragments in agarose or polyacrylamide gel. Direct sequencing of 270 bp CytB fragment was also proposed and used by the US Fish and Wildlife Service forensic laboratories (Fain et al. 2000). However, neither of these methods allows to distinguish Siberian sturgeon from the Russian sturgeon with "*baerii*-like" haplotype.

We developed fast and inexpensive method for ten sturgeon species identification, which is based on control region (D-loop) variation (Mugue et al. 2008). Multiple sequence alignment of the consensus sequences was used to determine the species-specific regions of the mtDNA D-loop and to design a set of species-specific primers. The primers were chosen to harbor a region that was characteristic of all individuals of the given species. For the convenience of genotyping, the species-specific primers were at different distances from anchor (common) primer so that the products amplified for different species would differ in size and would be easily distinguishable in 2% agarose gel (Fig. 42.7). The set of diagnostic primers is listed in Table 42.5.

Haplotype G26, ancestral to all "*baerii*-like" haplotypes, is different from all Siberian sturgeon haplotypes by substitution G-A in position 16,178 of *A. baerii*

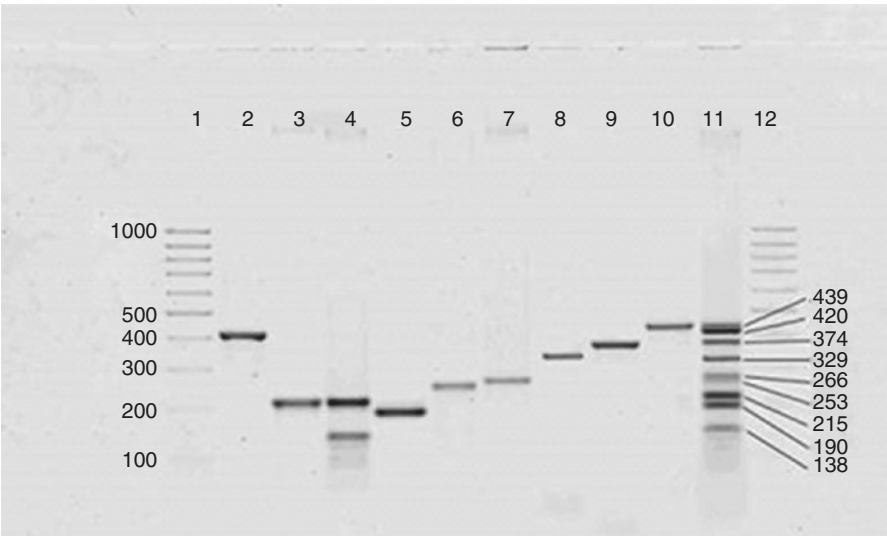

Fig. 42.7 Agarose gel electrophoresis of the PCR fragments obtained for various sturgeon species. Lanes: *1*, marker (100–1000 bp); *2*, *A. gueldenstaedtii* (AGF–AHR, 420 bp); *3*, *A. gueldenstaedtii* with the "*baerii*-like" mitotype (ABF–AHR, 215 bp); *4, A. baerii* (ABF–AHR, 215 bp and ABF–ABRM, 138 bp); *5*, *A. ruthenus* (RutF–AHR, 190 bp); *6*, *A. schrenkii* (SchF–AHR, 253 bp); *7*, *A. stellatus* (SteF–AHR, 266 bp); *8*, *A. nudiventris* (NudF–AHR, 329 bp); *9*, *H. huso* (HusF–AHR, 374 bp); *10*, *H. dauricus* (DauF–AHR, 439 bp); *11*, molecular weight marker of the sturgeon PCR products; *12*, marker (100–1000 bp) (from Mugue et al. (2008))

Table 42.5 Primers for species identification of sturgeons (Mugue et al. 2008)

Primer	Sequence	Used with	Product size (bp)	Species
AHR	TATACACCATTATCTCTATGT			All species
AGF	GCACAGACTATGTGGTATCCAGAA	AHR	420	*A. gueldenstaedtii*
ABF	CAGATGCCAGTAACAGGCTGA	AHR	215	*A. gueldenstaedtii* ("baerii"-like) and *A. baerii*
ABRM	TGTCTGTCTAGAACATAtG	ABF	182	*A. baerii*
HusF	TATCTATTACCTGCGAGCAGGCTG	AHR	374	*H. huso*
DauF	CCTCTTATGTACGCGGTGT	AHR	439	*H. dauricus*
NudF	TGTCTTTTCTGAAGGAGCTTTGC	AHR	329	*A. nudiventris*
RutF	GGGAATAACCGTTAATTTGG	AHR	190	*A. ruthenus*
SteF	GGGGTTCTTGGCATGTTGTGAGCG	AHR	266	*A. stellatus*
SchF	TGTGGGGTCACGGAcTTTACAG	AHR	254	*A. schrenkii*

reference genome NC_017603 (Chen et al. 2012, Fig. 42.8). Taking an advantage of this substitution, we constructed primer ABRM, which annealed exclusively to *A. baerii* mtDNA and, in pair with primer ABF, allowed amplification of a diagnostic 182-bp fragment.

Direct sequencing of D-loop is more expensive and time-consuming but also is widely used to distinguish Russian ("baerii-like") and Siberian sturgeons. Because sequencing technology is straightforward and does not require adjustment of PCR conditions to particular PCR machine, as it is needed for species-specific primers, sequencing is becoming the method of choice when caviar tests are running occasionally. We use routinely a primer pair AHR3M13R (TCACACAGGAAACAGCTATGACATACCATAATGTTTCATCTACC) and DL651 (ATCTTAACATCTTCAGTG) for PCR reaction (2.5 mM Mg^{2+}, annealing temperature set to 52 °C) and M13R (TCACACAGGAAACAGCTATGAC) primer is used for sequence of cleaned PCR product in one direction. Alignment with reference haplotype set allows assigning correct haplotype number for each sequence. Alternatively, sequences could be blasted against NCBI sequence database, and if BLAST result indicates relation to baerii-baerii-like cluster, then examination of A-16178-G substitution gives reliable identification of either one species. Sequences of 50 haplotypes, found so far among wild and aquacultured *A. baerii* in Russia, are deposited in GenBank (http://www.ncbi.nlm.nih.gov/nuccore) under KU375049-KU375098 accession numbers.

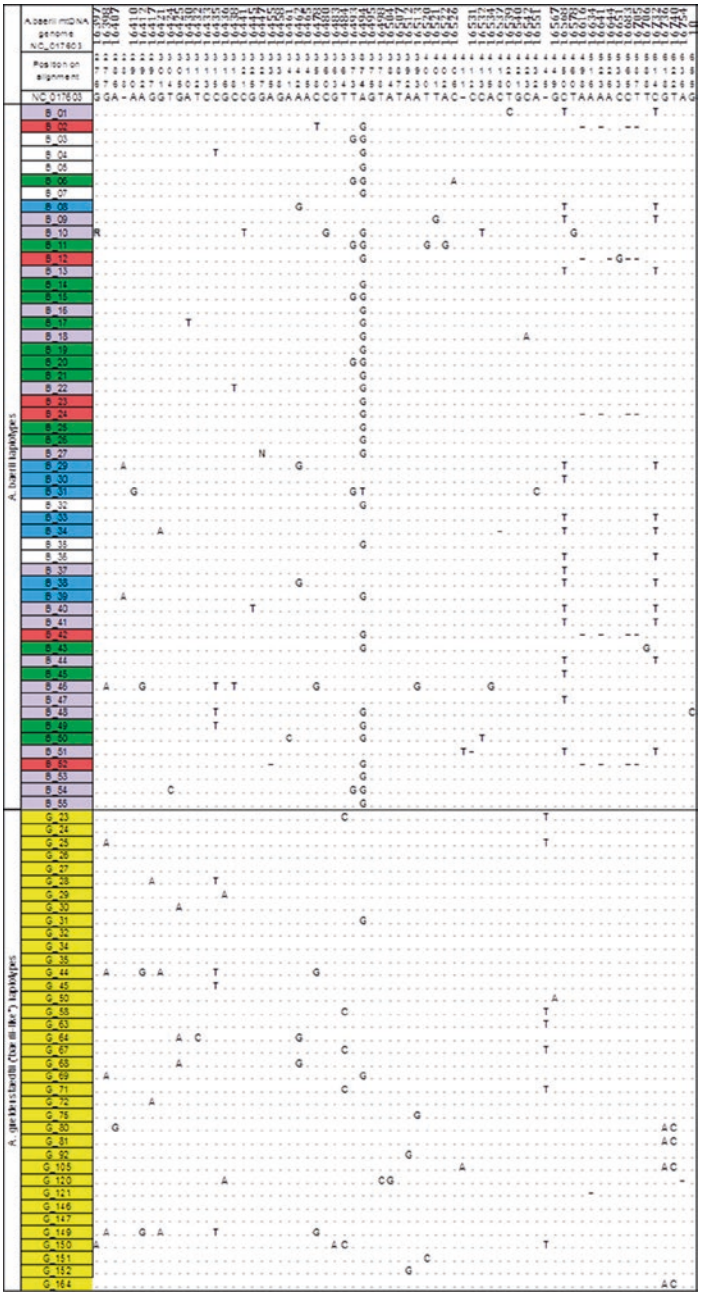

Fig. 42.8 Variable sites in 54 mtDNA haplotypes of Siberian sturgeon (*A. baerii*) and 37 haplotypes of Russian sturgeon (*A. gueldenstaedtii*) with "*baerii*-like" mtDNA. Haplotype IDs observed in only one population marked by color: Ob-Irtysh, *violet*; Yenisei, *red*; Lake Baikal, *blue*; Lena R., *green*; and Caspian Sea, *yellow*. Seven shared haplotypes (observed in more than one population) left uncolored. Sequences are aligned against RefSeq complete *A. baerii* mitochondrial genome (GB# NC_017603, (Chen et al. 2012)) and only variable sites are shown (identical with reference bases are shown as *dots*, deletions are marked as -)

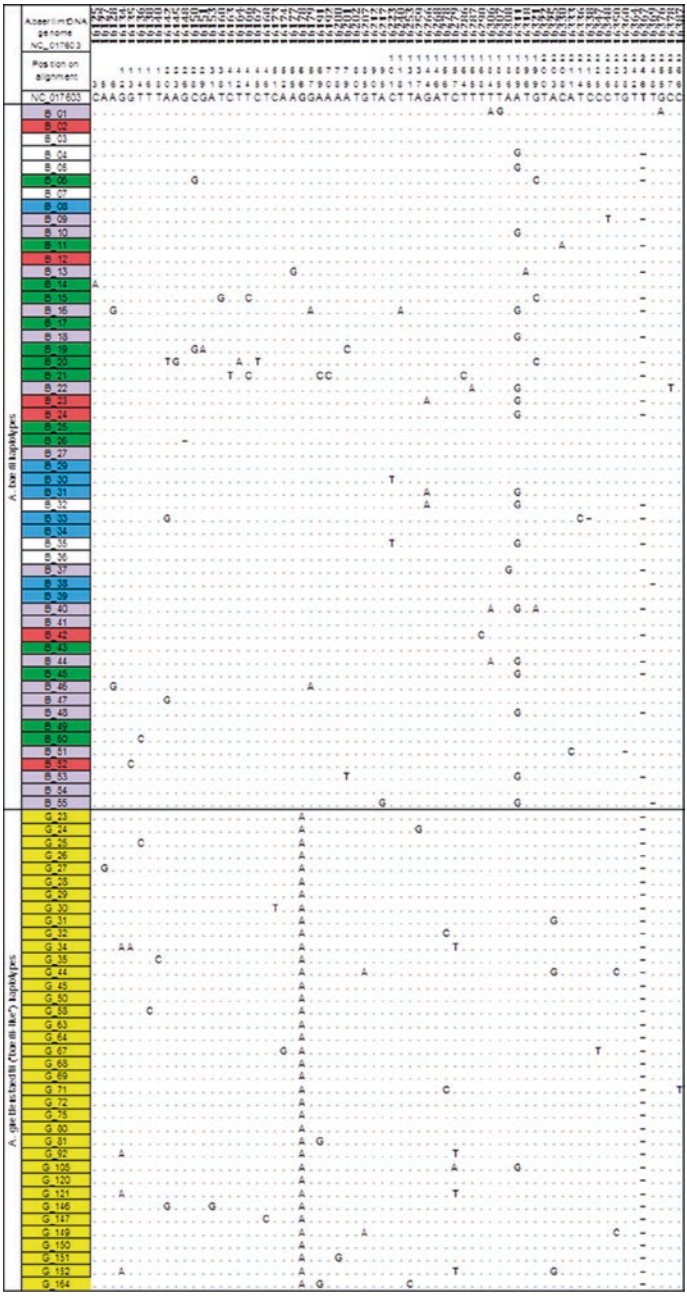

Fig. 42.8 (continued)

Conclusions

Siberian sturgeon is an important and valuable species in the freshwater aquaculture. Because of limited number of founder of aquacultured stocks, loss of genetic variation is already observed and at some extremes could be manifested by decrease of productivity and by abnormalities revealed during embryo development. Genetic management of existing aquacultured stocks should include steps to minimize inbreeding, admixture with unrelated stocks, use of large number of producers in propagation, and other measures.

Longevity of sturgeons also helps to maintain genetic diversity. Konakovo farm still keep domesticated Siberian sturgeons, brought over 40 years ago from Lena River, and these fish carry genetic variation from original wild population and could improve genetic quality of progeny if they are used wisely in breeding program. However, by analysis of genetic diversity of existing stocks in commercial sturgeon farms, we can see that most fish stocks are depleted by rare microsatellite alleles and uniformed by mtDNA. Implementation of proper genetic management, establishment of devoted breeding centers to supply farms with high quality and genetically healthy fingerlings, and development of genomic selection program is advised for prosperous future of Siberian sturgeon aquaculture.

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Part V

State of Health

Immunology in Sturgeons with a Focus on the Siberian Sturgeon Mechanisms, Responses to Stress and Stimulation

43

Valérie Chesneau

Abstract

This chapter aims to explore the state of knowledge about the immune mechanisms in sturgeon with a focus on the Siberian sturgeon (*Acipenser baerii*), stress factors that can disrupt the immune system and the sources of stimulation. Studies conducted carried out for several decades on sturgeon suggest specificities of their immune system compared to other fish species: special organs (meningeal myeloid tissue, tissues surrounding the heart), particularity of cells and components of immunity (larger white blood cells, lack of myeloperoxidase in neutrophils that are classified as heterophils). Other features have also been shown, i.e. the slow development of organs of immunity, the rapid response to acute stress, but also the great capacity for recovery from stress, all of which give a particular character to the sturgeon in the family of farmed fishes. Stress factors that can influence the immune system of sturgeons have also been researched in the last decade, with strong certainties about the influence of temperature, oxygen levels, pathogens and the presence in water of chemical substances. More and more programs on the research of solutions to boost the immune system have been implemented in recent years, with proven stimulatory actions on immunity factors (vaccines, probiotics, prebiotics, symbiotics, certain vitamins polysaccharides, plants and their components) and more mixed results (proteins, amino acids and certain vitamins). However, it seems that one domain is much less explored: the correlation between the pathogen, the host immunity and its environment. Nevertheless, this correlation is essential in the choice of solutions, which can be proposed, in particular in the field of immunostimulation.

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Acipenser baerii • Innate immunity • Acquired immunity • Immunity boosters • Stress factors

Introduction

Naturally, living beings carry a flora composed of multiple microorganisms (bacteria, viruses, parasites and fungi), which change according to different environmental or genetic factors, which are closely related to each lifestyle. There are also a lot of pathogens in the environment, namely, aquatic environments. These microorganisms do not necessarily induce a pathological state. However, when a disruption of the balance between host, environment and pathogen, induced by variations on one or more of these elements, occurs, it may lead to a pathological state (De Kinkelin et al. 1985). If the agent takes advantage of the host or if the host immune system is compromised, it may increase its susceptibility to the pathogen.

According to various studies, stress in fish is known to cause immunosuppression and result in a rising susceptibility to disease. Stress is a set of behavioural and physiological reactions in response to a threat of external origin. The perception of the situation by the individual plays an essential role in the stress response. Stress appears only if the animal perceives danger or discomfort. Stress can have multiple origins: (1) husbandry practices (transportation, sorting, food, etc.), (2) environmental factors (water quantity and quality, temperature, brightness, oxygenation, etc.) and (3) pathogens. It is common knowledge that fish stressed by one of these factors are more susceptible to infection (Kum and Sekkin 2011). The effects of stress on the immune response vary depending on the type of stress factors, fish species and physiological status, i.e. physiological well-being, rate of growth, ability to maintain natural and acquired resistance and immunity (Eslamloo and Falahatkar 2014). Thus, the health of the fish depends on the interrelationship of some major components of the fish, i.e. the environment in which they live and the pathogenicity and virulence of pathogens.

The immune system functions protect the body from damage caused by the invasion of microorganisms (bacteria, viruses, fungi and parasites). Leukocytes and a number of accessory cells perform this defensive function. These cells circulate throughout the body by using the blood and lymphatic circulation, but are preferably grouped in the lymphoid organs.

According to some studies, however, the lymphatic system is either absent or rudimentary in fish. Nonetheless, for some authors, lymphatic vessels make their way through many organs and could be differentiated (under the skin near the spine, the head and tail, the gill organs, the oral cavity, the intestine, etc.). These vessels have a structure similar to veins of the same calibre, but thinner wall that can be limited to a simple endothelium. There are valves which allow to reinject the lymph into the venous system. It is possible that the transporting vessels of lymph were confused with those of the secondary circulation: on this topic, uncertainty remains (Genten et al. 2010).

This chapter aims to briefly present the state of knowledge on the organs, cells and mechanisms of immunity in the sturgeon. It also aims to show the results of some work on the stress factors that affect the immune system and the means to boost the latter. This is not a comprehensive collection of bibliography but a summary opening many avenues of thought on the matter.

43.1 Immunity Organs

There are several types of immune organs which vary between different types of fish. Lymphoid (lymphomyeloid) tissues in sturgeons (hybrid sturgeon: *Huso huso* x *Acipenser ruthenus*, *Acipenser transmontanus* (Fänge 1986) and *Acipenser oxyrinchus oxyrinchus* (Gradil et al. 2014a, b) and *Acipenser naccarii* (Icardo et al. 2002)) were investigated by dissection, histology and transmission electron microscopy. The main lymphomyeloid tissues are the thymus, the spleen, the anterior part of the kidney, the meningeal myeloid tissue, the pericardial tissue and lymphoid masses of the intestine, especially in the spiral valve (Fänge 1986; Lange et al. 2000). Thus, *Chondrosteans* (*Acipenseridae* and *Polyodontidae*) and *Polypterus* are the only ones that possess a main site of mass production of granulocyte (for the most part), erythrocyte and lymphocyte cells that can be compared to the meninges in humans, called meningeal myeloid tissue. Their heart is often covered with a tissue, which contains lymphocytes, fibroblasts and macrophages.

The highly diversified and well-developed lymphoid tissues of sturgeons may serve as a basis for efficient immune mechanisms (Fänge 1986). However, the slow development of these immune organs may render sturgeon more vulnerable to viruses or other waterborne pathogens and may contribute to high mortalities seen in early life stages (Gradil et al. 2014a, b).

Indeed this study has demonstrated that these immune organs are first visible with an optic microscope between 541 degrees day (dd) (spleen) and 768 dd (meningeal myeloid tissue and thymus), approximately 400 dd after the onset of feeding. With the exception of an uncategorized granulocyte found in low numbers, all observed cell types are similar to those of other fish species. Thus, no lymphocytes have appeared in any of the analysed immune organs up to 768 dd. Other factors such as maternal protective immunity, the content of the yolk sac as well as the role of other relevant organs, particularly the gut, should also be considered in future developmental studies when assessing the overall immunity in sturgeon early life stages. Most of the sturgeon species concerned may certainly be considered as having a fully functional immune system within 3 months (1.5 months in the best cases). This may, however, vary as a function of the temperature.

43.1.1 Skin

The skin mucus of fish acts as the first line of self-protection against pathogens in the aquatic environment with a bacteriostatic activity. The skin mucus has a number of innate immune components such as complement molecules, lectins, proteolytic

enzymes and antimicrobial peptides (AMPs), which have been well documented for several fish species. Lectins are proteins that bind specifically and reversibly to certain carbohydrates. They are involved in various biological processes at the level of recognition between cells as in immune mechanisms. Antimicrobial peptides are proteins which are naturally synthesized to act as defences against bacteria, both gram-positive and gram-negative, fungi and viruses.

Firstly mucus prevents bacteria from adhering to epithelial cells. Then, due to the presence of the complement system, mucus has a dose-dependent bacteriostatic action, as demonstrated in the Siberian sturgeon (*Acipenser baerii*). In a study, it was shown that the skin mucus heating, treatment with EDTA (ethylenediaminetetraacetic acid is used as an inhibitor of Ca^{2+}/Mg^{2+}) and anti-C1q could significantly reduce its bacteriostatic activity (Fan et al. 2015). The complement protein, C1q, initiates the classical complement pathway by binding to the antibody-antigen complex and activating other complement factors leading to cell lyses.

43.1.2 Thymus

The thymus is next to the gill openings. The pharyngeal epithelium covering the thymus constitutes an effective barrier against the entry of both antigenic and non-antigenic materials from the pharyngeal cavity into the thymic parenchyma. A continuous layer of epithelial cells accomplishes this with lateral intercellular spaces tightly sealed by intercellular junctions such as tight junctions (Castillo et al. 1998).

The thymus is lobulated, and it has an outer cortex and an inner densely packed medulla with round, basophilic cells identified as T-lymphocytes (T-cells)—"T" for thymus—and reticular cells. Thus, the thymus is more specialized in lymphopoiesis that is lymphocyte haematopoiesis.

The similarity between the morphological structure of the sturgeon thymus and that of higher vertebrates suggests that they are functionally similar (Gradil et al. 2014a, b).

It has been observed that in the thymus, the percentages of heterophils, eosinophils, lymphocytes, epithelial reticular cells, undifferentiated cells, mitotic cells and necrotic cells do not vary with the age of the sturgeon. In the study, the only significant difference found was in undifferentiated cell percentages (highest in the oldest group), which might reflect a more active and proliferative thymus in the older animals.

43.1.3 Meningeal Myeloid Tissue

As for bone marrow in mammals, the meningeal myeloid tissue is responsible for haematopoiesis, i.e. the production of all types of blood cells including formation, development and differentiation of blood cells. All cellular blood components are derived from haematopoietic system cells. Nevertheless, most of the cells that are produced are granulocytes: neutrophils, eosinophils, basophils and heterophils (i.e. neutrophils that lack myeloperoxidase and have a reduced ability for oxidative bursts) (Dove et al. 2010; Palić et al. 2011). The meningeal myeloid tissue is located within the cranial cavity and surrounded dorsolaterally by the cartilaginous skull

and medio-ventrally by the brain or spinal cord, in a saddle-like manner (Gradil et al. 2014a, b; Scharrer 1944).

An ontogeny study demonstrates that this immune organ is first visible with an optic microscopic in 768 dd fish, approximately 400 dd after the onset of feeding. In 768 dd fish, the meningeal myeloid tissue consists mainly in reticular cells and undifferentiated cells. Most cell categories are present in 950 dd and older fishes.

In this tissue, erythrocyte, thrombocyte and mesenchymal reticular cell (i.e. multipotent stromal cells that can differentiate into a variety of cell types) percentages seem to decrease over time. In addition, erythrocytes and thrombocytes are absent in the oldest fishes. Even if there is no heterophil present in 768 dd fishes, they are the predominant cell type in the meningeal myeloid tissue. Large fishes have greater heterophil and eosinophil percentage when compared to smaller fishes. Lymphocytes are the least dominant immune cell type observed. They are absent in the 768 dd fish. There is no significant difference in lymphocyte percentages between ages.

Immune-like cells in the brain ventricles of larval lake sturgeon (*Acipenser fulvescens*) have also been described (Evans and Li 2014). Morphologically, these cells appear to be dendritic and pigmented macrophage cell clusters, moving freely within the cerebrospinal fluid (CSF). The melanomacrophage (MMCs) are also found in hatching eggs. In older specimens, an association with the hypothalamus, a region that integrates endocrine and nervous systems, has been suggested. This region could possibly be "educating" the acquired immune system.

43.1.4 Pericardial Tissue

The subepicardium is characterized by the presence of nodular structures separated from one another by connective tissue. Each smaller compartment is filled with lymphocytes, reticular cells, granulocytes and scattered macrophages. The pericardial tissue contains lympho-haemopoietic (thymus-like) tissue in the young sturgeons and a large number of lymphocytes after the sturgeons reach sexual maturity. It seems to be the site of interaction between lymphocytes and the vascular endothelium. This tissue is likely implicated in the establishment and maintenance of the immune responses (Icardo et al. 2002). The subepicardial tissue has two separate components, thymic and haemopoietic, or is a modified thymus with medullary haemopoietic capabilities; this point is not clear. Therefore, sturgeons present a cervical thymus, and the presence of subepicardial thymic tissue is not a singular case. However, the atrophy of the subepicardial tissue by the time the sturgeons reach sexual maturation has been observed.

43.1.5 Kidney

The fish kidney, unlike that in mammals, is divided into two sections. The posterior kidney is an excretory organ with the same function as the mammalian kidney. The head kidney assumes haemopoietic functions and, unlike higher vertebrates, is the main immune organ responsible for phagocytosis, antigen processing and formation of IgM as well as immune memory through melanomacrophage centres. The kidney

in fish is a disperse organ with a Y shape that is placed along the body axis. The lower part, situated parallel to the vertebral column, is a long structure most of which works as a renal system. The active immune part, the head kidney or pronephros, is formed by the two Y arms, which penetrate underneath the gills. In fish, this structure has a unique feature: the head kidney is also an important endocrine organ, homologous to mammalian adrenal glands, releasing corticosteroids and other hormones. In addition, it is a well-innervated organ. Thus, the head kidney is an important organ with key regulatory functions and the central organ for immune-endocrine interactions and even neuro-immuno-endocrine connections (Tort et al. 2003).

43.1.6 Spleen

In sturgeon, the spleen serves as a blood reservoir, and it has immune functions, trapping circulating antigens and being involved in lymphopoiesis. It is, therefore, a major secondary lymphoid organ.

The spleen has an elongated triangular shape, and it is located on the ventrolateral abdominal wall behind the pancreas and both adjacent and lateral to the intestinal wall and caudal portion of the stomach and pancreas. The spleen is characterized by the presence of myeloid follicles with mainly basophilic cells (white pulp) surrounding large arteries. The erythrocytes forming the red pulp are found within ellipsoidal blood vessels and scattered between follicles. No melanomacrophage centres are visible in any of the spleen samples studied (Gradil et al. 2014a, b). The most relevant changes during splenic development in sturgeon are in the heterophil percentages, which are significantly higher in the oldest fish when compared with the younger fish (2895 and 768 dd, respectively). This reflected a less developed spleen in the younger animals and progressive cell differentiation and maturation over time. Despite their size, the relatively high percentage of splenic undifferentiated cells in the 950 dd fish might indicate that they are less immunologically developed than the equivalently sized 2895 dd fish. This suggests that age might also play an important role in immunity at such early life stages.

43.1.7 Intestine

In studies (Korneva and Bednyakov 2011), cells with large bounded secretion, which are found in the intestinal epithelial layer, belong to immunocompetent blood cells, able to move freely from the bloodstream and to penetrate into various organs and tissues. Ultrastructurally, they belong to granulocytes, which are responsible for the non-specific reactions of the organism. The size and morphology of the cells observed are similar to those of eosinophils and neutrophils described for the haemopoietic tissue of sturgeons. While neutrophils occurred quite seldom, eosinophils have been found both in the epithelial layer and below the basal matrix of the intestinal epithelium. Acipenseridae have a distinctive feature: their spiral valve in the intestine is internally twisted or coiled to increase the surface area of the intestine,

thereby increasing nutrient absorption. The spiral valve has a villous aspect of the mucosa, and it is possible to distinguish nodular structures inside its axis that correspond to lymphoid organs.

43.2 Immune Cells and Mechanisms of Immunity

As in mammals, the immune system of fish is composed of a non-specific system (innate immunity) and a specific system (adaptive or acquired immunity). When an infectious agent penetrates the body, non-specific (innate) defence mechanisms are stimulated first. This activation alone may be sufficient to halt the infection. Otherwise, the disease develops and induces specific mechanisms, in particular the production of immunoglobulins. In vertebrates and most fishes, the resident immune cells in tissues are mastocytes, macrophages and dendritic cells. The immune cells, which patrol the body via the blood, are neutrophils, heterophils, eosinophils, basophils, monocytes, natural killer (NK) cells and T- and B-lymphocytes (T-cells and B-cells).

43.2.1 Non-specific System (Innate Immunity)

Innate immunity refers to a set of non-specific and identical responses whether the aggression be physical or microbial. Thus, the non-specific system is the support of natural immunity. This is the first line of defence against foreign substances entering the body. Mechanisms of innate immunity provide protection by blocking the binding, invasion or proliferation of microorganisms on or in a target tissue. The production or expression of both humoral and cellular innate parameters is commonly amplified or upregulated during immune response, but there is no memory. This means that a second encounter with the same pathogen will not result in enhanced responses as is seen in acquired immune responses. However, these responses are faster because they do not require learning or clonal expansion. Unlike mammals, the non-specific system of the fish represents the majority of the immune response.

43.2.1.1 Components of Innate Immunity

The innate immune system is generally divided into three elements: (1) the physical and chemical barriers, i.e. epithelial and mucosal (gills, skin, intestine) barriers with mucus secretion, (2) the cell components, and (3) the humoral parameters such as cytokines (i.e. chemokines, interleukins, interferons), lysozymes, complement proteins, lectins and antimicrobial peptides.

The main cells of innate immunity are:

- Phagocytes, which have the phagocytic ability to absorb (endocytosis) and to "digest" pathogens by different mechanism: enzymatic (superoxide dismutase, acid phosphatase, alkaline phosphatase), acidic and oxidative free radical pro-

duction (called "respiratory outbreak" because it requires increased O_2 consumption by the cell). This family consists in macrophages, dendritic cells and granulocytes (neutrophils so-called heterophils in sturgeon, eosinophils and basophils).

- The family of white blood cells (leukocytes), which includes monocytes, granulocytes, lymphocytes and mastocytes. The monocyte is immature blood cells from which macrophages and dendritic cells stem. In Siberian sturgeon (*Acipenser baerii*) (Docan et al. 2012) and Chinese sturgeon (*Acipenser sinensis*) (Zexia et al. 2007), it has been observed that the white blood cells, despite their morphology being similar to that of other fish species, are fewer in number but larger in volume. Furthermore, neutrophils are classified as heterophils, partly due to the lack of peroxidases present normally in these cells. Myeloperoxidase (MPO) is used as an indication of phagocytic, chemotactic and bactericidal functions of fish neutrophils in the degranulation process. The lack of MPO to form complexes—in order to reduce damage caused by free oxygen radicals—and the inability to produce an oxidative burst may limit their ability to kill a wide variety of bacterial organisms (Palić et al. 2011). Mastocytes (or mast cell) are a type of white blood cell. The fish mast cell, also known as eosinophilic granular cell, originates from haematopoietic organs, migrates to sites of maturation and increases in injured tissues. Although there is confusion arising from the different distribution of these cells in fish species and from discrepancies in their morphological and staining properties, there is a general agreement that their main functional role in immunity is quite similar (Sfacteriaa et al. 2015). Mast cells express piscidins, i.e. antimicrobial peptides (AMPs), and the abundance of these cells in various tissues at high risk of infection suggests that piscidins play a significant role in the non-specific immune defence of many teleosts (Silphaduang et al. 2006). No studies were found on sturgeon.
- Natural killer cells (NK), which are part of the family of lymphocyte as fellow lymphoid progenitor. They are, however, different from other T- and B-lymphocytes involved in acquired immunity. NK cells have the ability to induce apoptosis, i.e. programmed cell death, in cells infected with intracellular pathogens such as intracellular bacteria and viruses.
- Thrombocytes, which are generally recognized as the main cellular mediators of haemostasis in addition to being observed in various inflammatory reactions. That said, consistent data accumulated over the years indicate that platelets would not only be present in the process of inflammation but also contribute directly thereto. For example, thrombocytes contain and synthesize many biological mediators that play no obvious role in haemostasis. However, they significantly alter the local innate immune response by attracting neutrophils to sites of inflammation. In addition, they bind avidly to microorganisms expressing a variety of Toll-like receptors. It is now believed that thrombocytes could act as circulating sentinel cells that come into contact with infectious agents carried by the blood to prime antigen presentation and activate the innate immune response (Berthet 2011). Thrombocytes would also directly regulate the humoral

adaptive immune responses by the expression and secretion of inflammatory molecules. Although recently found in humans, this has never been explored in sturgeons.

The main humoral components are:

- Cytokine, which is a very large family of chemical molecules synthesized by cells to communicate with each other and influence each other. Many cytokines are involved in immune mechanisms and play very different roles such as the recruitment and attraction of other cells to the site of infection, cell differentiation, inflammation, etc. In addition, they can have an activating or an inhibitory action which makes it particularly complex to understanding. Depending on their action, they have different names.
  - Chemokines are a family of small cytokines, or signalling proteins, secreted by cells. Their name is derived from their ability to induce directed chemotaxis in nearby responsive cells: they are chemotactic cytokines.
  - Interleukins (IL) are a group of cytokines which are primarily expressed by white blood cells (leukocytes). Innate and acquired immune systems depend in a large part on interleukins.
  - Interferons (IFNs) belong to the large class of cytokines. They are released by host cells in response to the presence of several pathogens, such as viruses, bacteria, parasites and also tumour cells.
  - Tumour necrosis factor-α (TNF-α) is a cytokine involved in systemic inflammation and is one of the cytokines that make up the acute-phase reaction. It is produced chiefly by activated macrophages, although many other cell types such as CD4 T-lymphocytes, NK cells, neutrophils, mast cells and eosinophils can produce it.
- Lysozyme which is a bactericidal peptide enzyme that is release by various types of leukocytes. Lysozyme separates peptidoglycan layers in the cell walls of gram-positive bacteria, preventing them from invading the host cells. Lysozyme has been reported in sturgeon species. There are species differences, but in most cases, serum has the lowest level of lysozyme, followed by the liver, spleen and kidney, which have the highest levels in all sturgeon species studied (Subbotkina and Subbotkin 2003).
- Complement is a set of plasma proteins capable of self-activating chains, especially after contact with certain components (polysaccharides) of the membrane of microbes. Its activation gives rise to complementary pathways, facilitating the opsonisation, the phagocytosis and inflammatory responses that result in the pathogen's killing which is known as the "alternate pathway". However, its main action is in the context of the specific immune defence along "classical pathway" and lectin "pathway" (see below). The complement activity in sturgeon is much higher than in teleost species (Docan et al. 2012).
- Antimicrobial peptides (AMPs), naturally synthesized proteins, which act as defences against bacteria, both gram-positive and gram-negative, fungi and viruses. These molecular factors of innate immunity are of particular importance

in providing protective functions to lower vertebrates like fish, because the system of adaptive immunity in poikilothermic animals cannot ensure a sufficiently rapid and effective response (antibody formation) to infection at a low temperature in the environment. Sets of antimicrobial peptides called acipensins that are histone H2A fragments were for the first time isolated from leukocytes of the Russian sturgeon (*Acipenser gueldenstadtii*). These peptides have a broad spectrum of antibacterial activity and do not exhibit toxic properties towards host cells (Shamova et al. 2014).

43.2.1.2 Mechanisms of Innate Immunity

The innate immune response involves five main steps (1) the physical barrier, (2) the detection, (3) the chemical mediation, (4) the recruitment and the inflammation and (5) the phagocytosis.

Macrophages, dendritic cells and mastocytes have receptors called pattern recognition receptors (PRRs), which recognize polysaccharides, lipopolysaccharides (LPS), peptidoglycans, bacterial DNA, double-stranded viral RNA and other molecules not normally found on the surface of multicellular organism. These molecular patterns characteristic of microbial pathogens are called pathogen-associated molecular patterns (PAMPs). PRRs can also be soluble components like complement protein C3, lectins and various other humoral innate components. Thus, the innate immune system uses only a limited number of receptors that are active against as wide a variety of pathogens as possible. This strategy is in stark contrast to the approach used by the adaptive immune system, which uses large numbers of different receptors, each highly specific to a particular pathogen.

Following recognition of PAMPs, receptors initiate an intracellular signal transduction that results in the expression of genes involved in immune responses, such as antimicrobial peptides (AMPs), antiviral interferons (IFNs) and transcription factors that result in the activation of interferon regulatory factors (IRFs). In a typical scenario, an infected cell releases interferons that activate an increase in antimicrobial defences in neighbouring cells.

The activation of receptors expressed by macrophages, dendritic cells and mastocytes, present near the pathogen entry site, induces the production of chemical mediators called cytokines. Cytokines, such as tumour necrosis factor-α (TNF-α), interleukins (IL) and chemokines, play a crucial role not only in the innate immunity but also in the acquired immunity. In innate immunity, they specifically allow the recruitment and the attraction to the site of the original stimulus of other blood circulating immune cells (monocyte, macrophage, granulocyte), the activation of differentiation cells (monocytes in macrophages) and the activation of the acute-phase response. At the same time, the pathogen by binding to PRRs directly activates NK cells and the complement system. Complement is a system of plasma proteins that activates a cascade of proteolytic reactions on microbial surfaces but not on host cells, coating these surfaces with fragments that are recognized and bound by phagocytic receptors on macrophages. The cascade of reactions also releases small peptides that contribute to inflammation.

The last step is the destruction and apoptosis of pathogens by various mechanisms associated with involved cells. Macrophages, dendritic cells and heterophil destroy the foreign body by phagocytosis, NK cells lyse infected cells with catalytic enzymes that perforate the membrane and digest the protein, and the complement punctures the membrane.

Recent studies show a strong presence of chondroitin sulphate, a glycosaminoglycan in the bones of sturgeon well beyond that which can be found in other fish species. In mammals, the chondroitin sulphate increases the production of IFN, cell differentiation in CD4 T-lymphocyte (acquired immunity) and the expression of certain IL genes (Zhou et al. 2010). In mice, it increases the weight of the thymus and the mast cell number (Huang et al. 2014). Chondroitin sulphate in sturgeon may play an important role in immunity, as can be the case in mammals.

43.2.2 Specific System (Acquired Immunity)

The innate immune response can be ineffective in completely controlling pathogen growth. However, they slow pathogen growth and allow time for the adaptive immune response to strengthen and either control or eliminate the pathogen. The innate immune system also sends signals to the cells of the adaptive immune system, guiding them in how to attack the pathogen (Biowiki). The adaptive immune system can amplify the immune response and confers both, a specific response to antigen, which is therefore particularly suitable for the infectious agent, and a memory response for a more efficient removal of the same infectious agent.

So, acquired immunity is a second body defence line. This response is specific to the pathogen. It requires collaboration between antigen-presenting cells (phagocytes), T-lymphocytes (T-cells) and B-lymphocytes (B-cells). This collaboration allows clonal expansion of specific T-cells and B-cells of the pathogen as well as their differentiation into effector cells. The acquired immune response is delayed in time. It takes several days to develop in the case of a first exposure to an antigen, but only few hours if the infectious agent has already been met and if the body has a memory of the lymphocytes.

The immune system's first exposure to a pathogen is called a primary adaptive response. Symptoms of a first infection, called primary disease, are always severe because it takes time for an initial adaptive immune response to a pathogen to become effective.

Upon re-exposure to the same pathogen, a secondary adaptive immune response is generated, which is stronger and faster than the primary response. The secondary adaptive response often eliminates a pathogen before it can cause significant tissue damage or any symptoms. Without symptom, there is no disease, and it is impossible to know that the fish is infected. This secondary response is the basis of immunological memory, which protects fishes from getting diseases repeatedly from the same pathogen. By this mechanism, an individual's exposure to pathogens early in life spares the fish from these diseases later (Biowiki).

A third important feature of the adaptive immune response is its ability to distinguish between self-antigens, i.e. those that are normally present in the body, and foreign antigens, i.e. those that might be on a potential pathogen. As T-cells and B-cells mature, there are mechanisms in place that prevent them from recognizing self-antigens, preventing a damaging immune response against the body. This will not be detailed in this chapter.

43.2.2.1 Components of Acquired Immunity

The primary cells that control the adaptive immune response are the lymphocytes, the T-cells and the B-cells. B-cells are involved in the humoral response, while T-cells are responsible for the cell-mediated response. B-cells and T-cells recognize different substances such as antigens, which may occur in different forms.

- B-lymphocytes (B-cells) use surface immunoglobulins as receivers. Their specificity is identical to that of the immunoglobulin secreted from the B-cell after activation. B-cells recognize many soluble compounds as antigens: proteins, nucleic acids, polysaccharides, some lipids and small chemical molecules. There are different kinds of B-cells:
  - Mature B-cells and/or naive B-cells. Once exposed to an antigen, the naive B-cell either becomes a memory cell or a plasma cell (plasmocyte). These B-cells are characterized by the dual expression of IgM and IgD on their membrane. They are derived from immature B-cells that only have the IgM on their surfaces.
  - Plasmocytes which are antibody-producing cells and the final stage of differentiation of B-cells. Unlike other B-cells that have their antibodies on their membrane surface, plasmocytes are capable of producing soluble antibody.
  - Memory B-cells derived from B-cells after antigen recognition (during the primary immune response). Some of the B-cells—prior to antigen recognition—differentiate into memory B-cells, and while others differentiate into plasmocytes. Memory B-cells have the function of storing the antigen properties, to create a faster, longer and more intense immune response and more specifically in the case of a second infection with the same antigen (secondary immune response). In addition, the memory B-cells have a much greater lifespan than plasmocytes.
- T-lymphocytes (T-cells) are particularly important: they directly control a multitude of immune responses, as well as, in many cases, B-cell immune responses. Many of the decisions about how to attack a pathogen are therefore made at the T-cell level: knowledge of their functional types is crucial to understanding the function and regulation of adaptive immune responses. Each T-cell produces only one type of receptor specific to a single particular antigen. In contrast with B-cells, the vast majority of antigens recognized by T-cells are proteins that need to be fragmented before being recognized in association with MHC molecules on the surface of nucleated cells: these antigens are presented in a non-soluble form. T-cells are functionally grouped according to the class of MHC molecule that associates the peptide fragments:

  - Helper T-cells express a unique antigen on their surface called CD4. They recognize peptides associated with class II molecules of the MHC. They play a role in B-cell and cytotoxic T-cell activation.
  - Cytotoxic T-cells express a unique antigen on their surface called CD8. They recognize peptides associated with MHC molecules class I. They are able to recognize the specific antigen of the target cell and destroy its membrane.
  - Memory T-cells have a role in secondary immune response against an antigen previously encountered before. This response is characterized by its speed and efficiency.
- Antigen-presenting cell (APC) or accessory cells are cells that display antigens complexed with major histocompatibility complexes (MHCs) on their surfaces. Almost all cell types can serve as APCs. They are found in a variety of tissue types. Professional antigen-presenting cells include macrophages, B-cells and dendritic cells. They present antigens foreign to helper T-cells.

The main humoral parameters are:

- γ-Globulins (gamma globulins) which is a class of globulins. The most significant γ-globulins are immunoglobulins (antibodies), although some immunoglobulins are not γ-globulins and some γ-globulins are not immunoglobulins. Immunoglobulins (Ig) are glycoproteins endowed with antibody function. They are present in soluble form in plasma, in many secretions and in membrane form as part of the antigen receptor on the surface of B-cells. Antibodies are generally composed of four polypeptide chains: two identical heavy (H) chains and two identical light (L) chains. Both the heavy and light chains are composed of a variable (V) and a constant (C) domain. The V domain is responsible for antigen binding, whereas the C domain is responsible for binding to effectors molecules, which by triggering complex signalling pathways eliminate the antibody-coated foreign material (Das et al. 2012). Each type of antibody is specific to an antigen, such as a lock is to a single key. On the one hand, the antibodies not only contribute to the destruction of the infectious agent, and, on the other hand, they remain "in memory" to block any new infection caused by the same agent. The antibodies persist several months into the bloodstream and thus provide specific protection; this is the effect sought by vaccination after introduction of an attenuated or killed pathogen in the fish body.

In sturgeon, IgM-like antigens (Lundqvist et al. 1998) and IgDs (Zhu et al. 2014) have been described (Drennan et al. 2007). Of the five antibody classes described in mammals, it is noteworthy that only these two Igs can function as the antigen receptor for naive B-cells. Indeed, in addition to IgDs, three IgM variants have also been identified in sturgeons, whereas no IgT/Z-encoding genes were observed.

- Antigens on pathogens which are usually large and complex and consist of many antigenic determinants. An antigenic determinant (epitope) is one of the small regions within an antigen to which a receptor can bind. Antigenic determinants are limited by the size of the receptor itself. A typical protein antigen has multiple antigenic determinants, corresponding to the ability of T-cells to bind to different parts of the same antigen in three different ways (Biowiki).

- Interleukins (IL) which is a group of cytokines. The function of the immune system depends in a large part on interleukins. The majority of interleukins are synthesized by helper CD4 T-cells, as well as through monocytes, macrophages and endothelial cells. They promote the development and differentiation of T-cells, B-cells and haematopoietic cells.
- Major histocompatibility complex (MHC). With the exception of nonnucleated cells (including erythrocytes), all cells are capable of presenting intracellular (endogenous) antigens through the function of MHC molecules of class I. Some cells are specially equipped to present extracellular (exogenous) antigens through the function of MHC molecules of class II.

43.2.2.2 Mechanisms of Acquired Immunity

The trigger for the activation of the acquired immune system as well as for the activation and proliferation of lymphocytes is present in organized lymphoid tissues. The acquired immune response involves four main steps: (1) the antigen presentation, (2) the lymphocyte selection and proliferation, (3) the differentiation and (4) the specific response that results in the neutralization and phagocytosis of the infective agent or destruction of the infected cell.

The antigen processing and the presentation are processes that occur within a cell and which result in the fragmentation (proteolysis) of proteins, the association of the fragments with MHC molecules and the expression of the peptide-MHC molecules at the cell surface where they can be recognized by the T-cell receptor. However, the path leading to the association of protein fragments with MHC molecules differs for MHC class I and class II. MHC class I molecules present degradation products derived from intracellular (endogenous) proteins in the cytosol. MHC class II molecules present fragments derived from extracellular (exogenous) proteins that are located in an intracellular compartment (Mayer and Hudrisier 2016).

All nucleated cells express class I MHC. Proteins are fragmented by proteasomes (a complex of proteins having proteolytic activity) or by other proteases. The fragments are then transported by transporter proteins, assembled from a class I heavy chain and a beta-2 microglobulin (β2m) to the cell surface. Viruses replicate within nucleated cells in the cytosol and produce endogenous antigens that can associate with class I MHC. By killing these infected cells, cytotoxic T-cells (CD8) help to control the spread of the virus.

In a study (Stet et al. 1999), four β2m genes, which were characterized in the Siberian sturgeon (*Acipenser baerii*), can be divided into two groups, each encoding a slightly different β2m molecule. The cDNA sequence obtained encodes a protein of similar length compared with those of mammals. In contrast, all teleostean fish β2m sequences studied were different.

Only a limited group of cells, which includes antigen-presenting cells (APC), express class II MHC. Exogenous proteins taken in by endocytosis are fragmented by proteases in an endosome. The alpha and beta chains of MHC class II, along with an invariant chain, are synthesized, assembled and transported. Then the invariant chain is digested, and the peptide fragments from the exogenous protein are able to associate with the class II MHC molecules, which are finally transported to the cell

surface. Bacteria mainly reside and are replicated extracellularly. By being taken up and fragmented inside cells as exogenous antigens that can associate with class II MHC molecules, helper T-cells can be activated to assist B-cells to generate antibody against bacteria, thereby limiting the growth of these organisms. Some bacteria grow intracellularly inside the vesicles of cells like macrophages. T-cells help to activate macrophages to kill the intracellular bacteria.

During an infection, professional APCs detect signals characteristic of the infectious agent. Based on these signals, APCs can produce various cytokines and express different proteins on their surface. When these cells interact with naive T-cells, differentiation programs are induced depending on the cytokines produced. T-cells with a given specificity can therefore have different effects depending on the signals received by antigen-presenting cells. Very schematically, the antigen/MHC complex accompanied by specific signals will be recognized by T-cells with the CD4 marker or by T-cells with the CD8 marker. This will induce their proliferation (clonal expansions) and their differentiation into effector cells: helper T-cells or cytotoxic T-cells.

Unlike CD4 T-cells, where clonal expansion is activated immediately after recognition of the antigen/MHC complex, CD8 T-cells need the helper T-cells to proliferate. This activation will be done by the release of an interleukin (cytokine). Once the proliferation and differentiation of CD8 T-cells into cytotoxic T-cells have occurred, they will bind to the infected cells, release toxic molecules and induce cell death.

B-cells directly capture the infective agent using membrane antibodies. The B-cell proliferation requires the intervention of helper T-cells, which bind to membrane receptors, and the release of interleukins. B-cells then differentiate into plasma cells secreting antibodies. These antibodies released into the plasma bind specifically to their antigen to form immune complexes (key/lock). These complexes activate macrophages and complement, causing the destruction of the antigen-carrying microbe covered with their specific antibodies (as the microbe is "bristling" with immune complexes).

In this paragraph, the mechanisms have been very simply presented. Living organisms have very complex systems of regulation (activation and inhibition) of this immunity either through cellular or humoral mechanisms: the latter can't be described briefly. In addition, although extensively studied in mammals and to a lesser extent in teleost fish, much research remains to be conducted on sturgeons.

43.3 Stress Factors and Their Influence on Immunity

The pathogen can both be the cause of stress, thereby causing immunosuppression of the individual, and also take advantage of the weakening of the fish due to stress of various origins. In fish farming, we are often faced with the situation where different pathogens are found, and in this case, it is not always possible to determine who is actually causing the disease. Effects of stress on the immune response depend on the type of stress factor, fish species and physiological status (physiological

well-being, rate of growth, ability to maintain natural and acquired resistance and immunity, etc.) (Eslamloo and Falahatkar 2014). Stress responses are divided into three categories called primary, secondary and tertiary stress responses.

When a fish is exposed to stressful conditions, there is activation of the primary response, which is twofold: the brief response and extended or prolonged response. (1) The brief response (nerve response) is the development by the chromaffin cells of catecholamines (adrenaline = epinephrine and noradrenaline = norepinephrine). (2) The prolonged response (humoral response) is the activation of the hypothalamic-pituitary-interrenal (HPI) axis. The hypothalamus through the release of corticotropin-releasing hormone (CRH) activates the production of adrenocorticotropic hormone (ACTH) by the pituitary gland which itself activates cortisol production by the interrenal gland. This results in an elevation in plasma cortisol. Cortisol acts by negative feedback on the secretion of hormones of the pituitary and thyroid glands.

The combined action of catecholamines and cortisol on different tissues increases the breathing capacity (heart rate and volume), the gill blood perfusion but also the mobilization of available energy by using glucid and lipid reserves: it is the secondary response. Two mechanisms are involved: (1) neogluconeogenesis which is a metabolic pathway that results in the generation of glucose from certain non-carbohydrate carbon substrates (proteins, amino acids, lipids, etc.) and (2) glycogenolysis which is the breakdown of glycogen (n) to glucose 6-phosphate and glycogen (n-1).

Levels of cortisol and catecholamine concentrations can remain elevated for several days after the fish has been stressed, inducing tertiary stress response. They bind not only to receptors of mucus epithelial cells and to non-specific (innate) immunity cells but also to the Na^+/K^+ pumps of the branchial membrane. This results in increased mucus production, inhibition and/or activation mechanisms of the immunity (it depends) and the modification of the extracellular osmotic balance of fish. It causes effects on swimming, general behaviour (colour, folded fins, prostration, anorexia, etc.), the overall metabolism, immunity, growth and reproduction. This is the most easily identifiable form of stress. The intensity of the stress response is different in acute and chronic patterns. In these two patterns, cortisol and glucose are determining factors used for studies as indicators of the stress level (Balm et al. 1989).

Studies conducted in recent years have shown that sturgeon not only has a rapid response to acute stress but also a great capacity for recovery from stress, as shown in returning physiological parameters (cortisol, glucose and lactate) and lysozyme activity to prestress level (Eslamloo and Falahatkar 2014; Hoseini et al. 2016a, b; Falahatkar and Poursaeid 2013). However, the return to an initial rate varies as a function of the species, the type of stress (acute or chronic) and other conditions (Eslamloo and Falahatkar 2014). The above summarized mechanisms are those described in the case of acute stress. Chronic stress can have more serious consequences by altering the nervous system irreversibly.

The effect of stress on immunity does not obey to simple rules. It calls for mechanisms that sometimes have an inhibitory action on immune cells and sometimes activate a cascade of events. However, studies have shown that, most of the time, the influence on immunity is transient. On land animals, the stress can, in some cases,

have an immunosuppressive action that prevents the animal from fighting against a pathogen and, in other cases, limit the uncontrolled acceleration of the inflammatory response. More complex pro- or anti-inflammatory action may differ depending on the source of stress but also on the tissue involved (Merlot 2004). The adaptive immune response works in the same way wherein stress inhibits cell response and activates the humoral response, particularly the antibody production. However, current knowledge indicates that if the organism is not faced with any pathogen in a stressful situation, immunological alterations induced by stress can have no effect on its health. Conversely, if the organism must simultaneously face a viral or bacterial infection and stress, the latter can harm the implementation of an adequate immune response.

However, responses to different stress factors are specific to species. Thus, it is particularly difficult to make assumptions about the reaction of sturgeon when it is faced with stressful situations that were investigated for other species. In this section, some results of studies conducted on various factors related to environment, husbandry practices or pathogens are listed. The idea here is not to make an exhaustive inventory but to apprehend the consequences of stress factors on the sturgeon, knowing that many paths still remain to be explored.

43.3.1 Temperature

Water temperature is one of the main environmental factors that influence behaviour, activity and physiological functions of the sturgeon, such as feeding, reproduction, egg development (Kieffer and Kynard 1993; Piper et al. 1982), etc. It appears that the immune response of many fish species is less effective at low temperatures.

Data suggests that sturgeon species used on farms have a thermal preferendum around 20–25 °C. Studies on shortnose sturgeon (*Acipenser brevirostrum*) (Gradil et al. 2014a, b), Russian sturgeon (*Acipenser gueldenstaedtii*) (Kolman et al. 2000) and Chinese sturgeon (*Acipenser sinensis*) (Feng et al. 2012) have shown that temperature can influence the immune system. Some results indicate an increase of different markers, molecules or mechanism of immunity, at higher temperature: percentage of thrombocytes, lymphocytes and eosinophils, lysozyme activity, expression of IRF-1 and IRF-2 genes, potential killing activity and Na^+/K^+ ATPase activity (Krayushkina et al. 2006). But another study on great sturgeon (*Huso huso*) juveniles has shown that the white blood cell count, lymphocytes, the cortisol and the glucose concentrations were decreased slightly with increasing temperature (Zarejabad et al. 2010). In some studies, cumulative influences on growth of sturgeons have also been described. Temperature is an important element of the results obtained in many studies. It is especially vital during vaccination because it facilitates the humoral immune response (Kalbassi et al. 2000). However, some humoral factors like γ-globulins, ceruloplasmin (Kolman et al. 2000) or complement are elevated in sturgeons in response to seasonal decreasing temperatures.

It is clear that the temperature has a positive influence on the immune system. Nevertheless, some contradictory results could cast doubt on that finding. This is certainly due to the complexity of immune mechanisms, with sometimes given the same molecule, inhibiting and activating effects.

43.3.2 Stocking Density and Hypoxia

In a study, three immune indicators are assessed: serum total immunoglobulin level and lysozyme and complement activities. The results of these three parameters show that transient and moderate confinement stress (low water level) can induce alterations in the innate immune response of Persian sturgeon (*Acipenser persicus*) (Hoseini et al. 2016a, b).

It has been shown on Siberian sturgeons (*Acipenser baerii*) that were exposed to an air stress (Eslamloo and Falahatkar 2014) that sturgeon not only has a rapid response to acute stress but also a great capacity for recovery from stress, thus returning physiological cortisol to prestress levels after only 6 h. Moreover, the increase in lysozyme activity after stress demonstrates that acute stress can elicit positive reactions on the immune parameters.

On the Amur sturgeon (*Acipenser schrenckii*) (Meng et al. 2014), a significant increase of serum cortisol and glucose levels during a period of hypoxia stress is noticed; similar to that observed on Siberian sturgeon (*Acipenser baerii*) (Maxime et al. 1995) has been noticed. However, there is no significant difference with stocking density stress. An increase trend of white and red blood cells has been observed after a hypoxia stress, showing an activation of the immune response and the mobilization of the oxygen resource to supply tissues. Finally, an upregulation of CYP1A gene in some immune organs both in high stocking density and hypoxia stress has been observed. CYP1A gene products are used as biomarker when assessing exposure to contaminants in environmental system.

43.3.3 Natural or Synthesise Chemical Substances

Whether regarding human or animal health, the issue of chemicals in the environment is essential. In fish, this topic has been the subject of few studies that have shown effects of organophosphates on immunity. In sturgeon, a decrease in white blood cells (WBCs) and lymphocyte has been observed in individuals exposed to diazinon (a nonsystemic organophosphate insecticide formerly used to control cockroaches, silverfish, ants and fleas in residential, non-food storage buildings). However, the mechanisms are still largely unknown and worth exploring (Khoshbavar-Rostami et al. 2006).

A study investigated the effects of an anaesthetic (tricaine methanesulfonate) on haematological parameters including haemoglobin (Hb), erythrocyte number (RBCs), haematocrit (Hct), and white blood cells (WBCs) on sterlet (*Acipenser ruthenus*). The haematological parameters were assessed immediately and 24 h after anaesthesia. The results have shown that, in spite of the absence of mortality after anaesthesia, this chemical substance influences some haematological parameters. Immediately after anaesthesia, the values of Hb, Hct and RBCs increase, while these values decrease significantly 24 h after anaesthesia. The WBC concentrations did not show significant changes immediately after anaesthesia, while its values decrease significantly 24 h after exposure (Bishkoul et al. 2015). The 10-min exposure of juvenile of great sturgeon (*Huso huso*) to clove powder caused a significant increase in the haematocrit, in

the haemoglobin and in the total erythrocyte count after anaesthesia. These values returned back to normal within 24 h. Results of the examinations suggest that the use of clove powder does not cause irreversible damage to the blood parameters in juvenile great sturgeon (*Huso huso*) (Mohammadizarejabad et al. 2010). One can, thus, imagine quite easily that chemicals, be they natural or synthetic in origin, may have some influences on immunity through action on white blood cells.

43.3.4 Pathogens

This topic is certainly the one that has been the least investigated so far. Indeed, in the basic concept being laid as the presence of pathogenic agents which induces a cascade of events for activating the immune system, it does not seem obvious to look in detail at what happens when the animal is faced with a particular pathogen. Yet it would certainly be interesting and necessary to know for each pathogen encountered in farms and causing high mortality what its effect on the immune system of the fish is. Is the reaction and intensity the same for all pathogens? Are there any immunosuppressive phenomena and joint situations with immunostimulation and immunosuppression? This would have some interest, the least not being able to tailor treatment knowing that the immunostimulants all act at different levels. To date, few studies exploring the correlation between changes in components of immunity and pathogens have been conducted on sturgeon. Furthermore, not only are the effects certainly different from one pathogen (procedure, pathogenicity, etc.) to another but also from one species to another. Moreover, as seen later, many studies have shown the efficiency of some components as immunity boosters, but few researchers have pursued their investigation to prove their effectiveness in the presence of pathogens and particularly in situ. Can there be some inhibition of immune boosters in the presence of pathogens?

Observations on recent cases may nevertheless lead to think that mechanisms are very complex and more detailed studies should be conducted. These observations have not yet been objectified. The presence of a virus recently discovered on European farms, *Acipenser iridovirus-European* (AcIV-E) (Bigarré et al. 2016), rarely results in clinical signs on Siberian sturgeon (*Acipenser baerii*) under certain conditions but may have more serious consequences on Russian sturgeon (*Acipenser gueldenstaedtii*) on the same farm. However, in other farms with other conditions, the observations are different. Thus, everything suggests that there is an effect related to the species and its farming conditions. The young stages are the most affected, and the results seem to show a gradual increase in viral load as well as a gradual decrease correlated with the growth of the fish. However, all this remains to be explored. Other observations were made regarding the origin of bacterial pathology. On a same farm, an increase in mortality was observed on Siberian sturgeons (*Acipenser baerii*) between 4 and 8 years old, which was not the case on younger stages and on Russian sturgeons (*Acipenser gueldenstaedtii*). At this stage, the immune system is in place and normally effective, and one wonders why young stages are not affected. Again, it seems to be species-related. Yet, is this observation rather not due to farming conditions and the layout of the site? All this remains to be objectified by analysis.

Following infection with the parasitic copepod *Dichelesthium oblongum* of the Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*), gill Na^+/K^+ ATPase and alkaline phosphatase activity increase with the number of pathogen (Sokolowski et al. 2012). This has already been demonstrated on shortnose sturgeon (*Acipenser brevirostrum*) and green sturgeon (*Acipenser medirostris*) but to a lesser extent. The percentage of eosinophilic and neutrophilic granulocytes increases, while lymphocyte percentages decrease with the increasing of the parasite.

43.4 Leads to Boost the Immune System

Traditional disease control strategies include chemical disinfectants, vaccination and as a last resort antibiotic treatments. However, the emergence of bacterial resistance, environmental concerns and the lack of interest in developing vaccines for fish farming by industrial pharmaceutical companies to develop vaccines are barriers to the use of these methods. To enable the development of fish farms in the best conditions, the search for alternative effective ways to stimulate the immune system and prevent infectious diseases is essential. As seen above, it is not simple, and the same factor can have a positive or negative effect on immunity. This is the case for stress. In the same way, the action of many molecules on the immune system is dependent on many factors: species, farming conditions and environmental requirements.

Dietary supplements such as probiotics, prebiotics, symbiotics, vitamins and other immunostimulants should act by improving the innate immune response and sometimes acquired immunity.

43.4.1 Vaccine

The most obvious method appears to be vaccination: administration of an antigenic substance prepared from the causative agent of a disease or a synthetic substitute and used to provide immunity against one or several diseases. Although its effectiveness on certain species and for some pathogens is well proven, this method has a major drawback: the identification and the obtaining of an isolate of the enemy. The vaccine must be developed specifically for the pathogen in question. In addition, a small sector like fish farming, and this is particularly true in France, does not always attract the interest of laboratories for which vaccine development does not appear profitable. In this case, only the development of autogenous vaccines, which are much more expensive than vaccines with authorization, is possible. The regulation on autogenous vaccines is very restrictive because it does not take into account the fact that fish may be destined for other sites and therefore face other strains. Moreover, the search for an efficient vaccine against some pathogens, like *Streptococcus dysgalactiae*, is hindered by the lack of knowledge about their pathogenesis and virulence determinants (Abdelsalam et al. 2013).

Some studies have shown that, whatever the mode of vaccination, be it intraperitoneal or by bathing, it results in the activation of the mechanisms of non-specific immunity (Docan et al. 2012; Kolman et al. 1999a, b). It has also been demonstrated that white sturgeon (*Acipenser transmontanus*) can generate a specific antibody response

following immunization with *white sturgeon iridovirus* (WSIV) or white sturgeon gonad (WSGO) tissue culture cells (Drennan et al. 2007). A patent on a method for evaluating the immunization effect of a sturgeon bacterial disease (*Aeromonas hydrophila*) whole-cell inactivated vaccine was proposed (technology-x.net).

43.4.2 Dietary Supplements

43.4.2.1 Probiotics, Prebiotics, Symbiotics and Equivalents

Gut microbiota play an important role in fish health by protecting them against pathogens. This flora depends on the environment in which the fish live but also on its feed. The search for substances to change the intestinal bacterial flora with the development of favourable bacteria for fish health is therefore essential for the future of fish farming.

Probiotics are live microbial additives that are known for their beneficial effects by modifying the microbial composition of the host gut. They improve the digestibility and the absorption of nutrients, the growth performance and the resistance against pathogens. Although increasingly used in human health and in terrestrial animals, their use in fish is more recent (Gatesoupe 2008; Kesarcodi-Watson et al. 2008; Merrifield et al. 2010; Nayak 2010). On sturgeon, few studies have been conducted on probiotics (Geraylou et al. 2013a, b; Jafarzadeh et al. 2015).

Prebiotics are not digested by enzyme of the upper gastrointestinal tract, but selectively fermented by some types of intestinal bacteria in the large intestine, and they alter the gut bacterial composition. So, prebiotics are substrates that selectively promote the growth of one or more bacteria in the host intestine.

Their effects are associated with the production of short-chain fatty acids (SCFAs), which provide a beneficial environment for beneficial bacteria (Kolida and Gibson 2011; De Schryver et al. 2010). SCFAs, acetate (C2), propionate (C3) and butyrate (C4) are the main metabolic products of anaerobic bacteria fermentation in the intestine. In addition to their important role as fuel for intestinal epithelial cells, SCFAs modulate different processes in the gastrointestinal (GI) tract such as electrolyte and water absorption. SCFAs regulate several leukocyte functions including production of cytokines (TNF-α, interleukins), eicosanoids and chemokines. The ability of leukocytes to migrate to the foci of inflammation and to destroy microbial pathogens also seems to be affected by the SCFAs (Vinolo et al. 2011).

Prebiotics participate in the improvement of the microflora of the gut, improving the well-being of the host (Yousefan and Amiris 2009; Ringø et al. 2010). Again few studies have been conducted on sturgeon. Nevertheless, those that were conducted with inulin, oligofructose (OF), Immunoster, Immunowall and arabinoxylan-oligosaccharides (AXOS) show encouraging results (Geraylou et al. 2013a, b, 2014; Akrami and Hajimoradloo 2009; Hoseinifar et al. 2011; Mahious et al. 2006; Mohajer Esterabadi et al. 2010; Ta'ati et al. 2011). For example, results of a study (in vitro) suggest that AXOS and OF change microbial fermentation activity of hindgut microbiota of Siberian sturgeon (*Acipenser baerii*). The hindgut microbiota of Siberian sturgeon (*Acipenser baerii*) has a good fermentation capacity. Increased levels of fermentation of AXOS and OF might therefore influence gut health characteristics in Siberian sturgeon (*Acipenser baerii*) through the local supply of energy

by produced SCFA and by affecting microbial composition through altered pH as well as SCFA levels and patterns (Geraylou et al. 2014). Another study (in vitro) shows that Inulin and oligofructose are used as substrates by microbiota for the production of organic acids (Mahious et al. 2006).

Poly-b-hydroxybutyrate (PHB) is a natural polymer that can be depolymerized into water-soluble short-chain fatty acid monomers. These monomers can act as microbial control agents. Although PHB should not necessarily be considered as prebiotic (De Schryver et al. 2010), a study (Najdegerami et al. 2012) has shown that PHB could generate similar effects. Results of this study indicate that a well-balanced diet with PHBs increases bacterial species richness in the fish GI tract. PHB degradation in the sturgeon gut alters the GI tract pH, most probably because of b-hydroxybutyrate (SCFA) production, inducing changes in the composition of the bacterial community. So, the inclusion of PHB in the fish diet could be used as a microbial control agent.

A recent study has highlighted some positive effects (growth performance and immune indices) of dietary administration of *Lactobacillus plantarum* in the Siberian sturgeon (*Acipenser baerii*). The highest levels of lysozyme activity, total immunoglobulin (IgM) and complement component 3 (C3) have been observed in fish fed the diet containing *Lactobacillus plantarum*, but there was no significant difference in the level of complement component 4 (C4) in fish fed the experimental diets or the control diet (Pourgholam et al. 2016). In another study, this same team has shown that Siberian sturgeon (*Acipenser baerii*), the diet of which was supplemented with *Lactobacillus plantarum*, have more white blood cells and monocytes (Pourgholam et al. 2015).

A study that aimed to evaluate the effects of Grobiotic®-A, a commercial prebiotics, on great sturgeon (*Huso Huso*) (Adel et al. 2016) has demonstrated that the activity of lysozyme and alkaline phosphatase in skin mucus is significantly enhanced in prebiotic-fed groups. Furthermore, inhibitory activity of the skin mucus against pathogens, particularly *Streptococcus iniae* and *Yersinia ruckeri*, was significantly improved following prebiotic feeding.

More recently, it has been demonstrated that the simultaneous use of probiotics and prebiotics, generally called symbiotic supplement, may be more effective than if each was applied. However, there is only rare information about the effects of symbiotic supplements on fish especially in sturgeon. A study (Jafarzadeh et al. 2015) has shown that using a symbiotic (Biomin IMBO) has no effect on the indices of red blood cells. However, except lymphocytes, the values of white blood cells (WBC) count, neutrophils, monocytes and eosinophils are higher than in the control group. The symbiotic supplement stimulates more innate immunity of Russian sturgeon (*Acipenser gueldenstaedtii*) than adaptive immunity. This study has also shown increases of IgM concentration and lysozyme activity in symbiotic treatments compared to the control group. In another study (Geraylou et al. 2013a, b), the ability of *Lactococcus lactis* spp. *lactis ST G45* to colonize and modify the intestinal microbiota as a potential probiotic strain has been confirmed. These candidates are isolated from the hindgut of Siberian sturgeon (*Acipenser baerii*) (Geraylou et al. 2012). The selected probiotic strains isolated from Siberian

sturgeon (*Acipenser baerii*) are safe and capable of surviving and colonizing the fish intestinal mucus, as well as antagonizing the resident microbiota. The results of the same study strongly suggest that the dietary combination of *L. lactis* ssp. *lactis ST G45* and arabinoxylan-oligosaccharides (AXOS) is effective to promote growth performance and to boost some immune responses of the Siberian sturgeon (*Acipenser baerii*), compared to their separate supplementation.

Thus, probiotics, prebiotics, symbiotic supplements and equivalents seem to be good candidates to improve the well-being and the health of fish, particularly sturgeon. However, it is still necessary to conduct field studies to confirm the findings of the studies conducted so far.

43.4.2.2 Vitamins

Some animals can synthesize vitamin C, which is the case for sturgeon. That differentiates it from the teleosts that lack an essential enzyme (L-gulonolactone oxidase) involved in the synthesis of vitamin C. A study has shown that in a 6-month-old white sturgeon (*Acipenser transmontanus*) fed a diet devoid of vitamin C, the total tissue vitamin C concentrations were not decreased, thereby suggesting that white sturgeon (*Acipenser transmontanus*) GLO present in the kidney produced adequate amounts of vitamin C (Dabrowski 1994). However, endogenous vitamin C is not sufficient to satisfy the subsequent anti-oxidative immune requirements because the inflammatory cascade induced by infection inhibits GLO activity. Thus, under stress, it may be necessary to add exogenous vitamin C (Xie et al. 2006).

A higher number of white and red blood cells is observed in sterlet (*Acipenser ruthenus*) fed with diet containing vitamin E and vitamin C (Tatina et al. 2010). These findings about vitamin C have been confirmed in other studies (Falahatkar 2005). Supplemental ascorbic acid in Siberian sturgeon (*Acipenser baerii*) food increased serum lysozyme concentrations. However, it had no significant impact on circulating leukocytes and phagocytic activity (Xie et al. 2006). All these studies have also shown that vitamin C does not influence the growth performance, feed efficiency and survival of sturgeon. Regarding vitamin E results are much more mixed. In fact, one study (Safarpour Amlashi et al. 2011) indicated that different dietary vitamin E levels have no effect on haematological parameters, erythrocytes, cortisol and glucose concentration, lysozyme and complement activities of subyearling beluga sturgeon (*Huso huso*). But this vitamin has a direct effect on growth performance, and a deficiency of this vitamin in the diet resulted in the deterioration of growth.

43.4.2.3 Proteins

The amount of protein in the diet could have positive or negative impacts on the number of white cells in the blood, particularly lymphocytes, leukocytes and monocytes. A diet which would not contain enough protein induces a decrease of these cells, which results in a first weakening of the immune response. Lactoferrin is a non-heme iron-binding glycoprotein that is a part of the transferrin protein family and is found in high concentration in mammal milk and other body secretions. Based on results obtained on mammals or other fish, a study on Siberian

sturgeon *Acipenser baerii* (Eslamloo et al. 2012) tried to demonstrate the influence of lactoferrin on non-specific immune response. The studies that led to this reflection have shown that lactoferrin has the ability to increase the generation of granulocytes and neutrophils, phagocytic and lysozyme activities and the respiratory burst activity. Other biological functions of lactoferrin include bactericidal, antifungal, antiviral, antiparasitic, enzymatic, anti-inflammatory and anticarcinogenic activities. However, this study showed no effect on haematological factors (white and red blood cells), stress indicators (cortisol, glucose and lactate), serum and mucus lysozyme activities and the serum IgM. Only two effects have been observed: the enhancement of mucus secretion and the inhibition of alternative complement pathway activity (ACH50). It should be noted that in this study, the most beneficial effects were observed for an intermediate amount of lactoferrin, thereby motivating the author to conclude that excessive and long-term administration of some immunostimulants might lead to a decline in defence responses or reverting the immune parameters to a previous status by negative feedback systems.

43.4.2.4 Amino Acids

Knowing that tryptophan, an amino acid, helps to raise the rate of serotonin, which is involved in immune mechanisms, a study (Hoseini et al. 2016a, b) was conducted to see if this essential amino acid could have a positive effect on the immunity of the Persian sturgeon (*Acipenser persicus*). This study was also based on the fact that it had been shown that stress suppresses the thyroid hormones level in Persian sturgeon (*Acipenser persicus*) (Hoseini et al. 2016a, b) and that sturgeons have more widely distributed serotonin-reactive elements than other fish in their nervous system. This study has shown that tryptophan-supplemented diet extends the duration of cortisol response to stress in Persian sturgeon (*Acipenser persicus*) and consequently inhibits immunosuppression following a confinement stress. But it cannot be used as a preventative treatment because it has no significant effects on serum innate immune response in unstressed sturgeons.

43.4.2.5 Polysaccharides

Based on numerous studies that have demonstrated an immunostimulatory effect of orally administered β-glucan (polysaccharide), the author of a recent study (Aramli et al. 2015) has shown that this polysaccharide is a beneficial dietary supplement for improving the innate immune response and growth performance of Persian sturgeon (*Acipenser persicus*). White blood cells and lymphocytes count, lysozyme activity and alternative complement pathway activity (ACH50) of sturgeon fed with β-glucan diet are significantly higher than the control.

This was confirmed by a study (Song et al. 2009) that investigated the effect on non-specific immune responses of four polysaccharides (chitosan, stachyose, yeast polysaccharide and lipopolysaccharide), which were injected intraperitoneally into Amur sturgeons (*Acipenser schrenckii*). Among the four polysaccharides, chitosan was the most effective immunostimulator, with significant increase in α-naphthyl acetate esterase (ANAE) positivity in peripheral blood and bacteriolytic and

haemolytic activities compared to the control. Stachyose, yeast polysaccharide and LPS showed no significant improvement in ANAE activities and bacteriolytic activities, but all displays higher haemolytic activity than the control.

43.4.2.6 Plants and their Components

Steroidal plant hormone 24-epibrassinolide, EPIN, has been shown to produce an immunomodulating effect in 3-month-old Siberian sturgeon (*Acipenser persicus*) treated with its solution (Kolman 2001). Thus, the γ-globulin level in the experimental group was from 10 to 65% higher than in the control group. Furthermore, this difference was statistically significant during certain periods. Simultaneously, the bacteriolytic activity of lysozymes increased up to 50% in treated group compared with the control fish. Results of this study indicated that EPIN has an immunostimulatory effect and it influences immunoglobulin and/or immunological complex levels in the blood serum as well as non-immunoglobulin humoral immune factors. EPIN is certainly a promising compound for the fish farming industry because it has been shown to have positive effects on cholesterol control in humans, but above all to fight viruses.

For great sturgeons (*Huso huso*), the diet of which was supplemented by *Spirulina platensis* (mainly at 10% level); the respiratory burst activity of leukocytes, the serum IgM and the lysozyme activity are higher. On the contrary, the blood lymphocyte count was lower in experimental groups than in the control group. This same study (Adela et al. 2016) has shown, in vitro, a significantly high bactericidal activity in skin mucus (against *Streptococcus iniae*, *Yersinia ruckeri*, *Aeromonas hydrophila* and *Lactococcus garviea*). Furthermore, fish infected with *Streptococcus iniae* had an increased mortality, but it was alleviated by a diet supplemented with *Spirulina platensis.*

In 2012, a patent (CN 102552411A) was filed proposing an herbal preparation specifically for sturgeon fingerlings. This decoction consisted of plants having multiple properties: enhancement of immunity, hepato-protection and improvement of the liver function and regulation of the circulatory, antiviral, antibacterial, antiulcer, anti-inflammatory and detoxifying effects. Tests in laboratory and in units have shown efficiency on immune system activation.

Conclusions

Studies conducted on sturgeon suggest specificities of their immune system—special organs, cells and components of immunity—compared with other fish species, certainly because the sturgeon comes from a more ancient species group. In bibliographic studies, the difficulty lies in the incomplete knowledge and the risk of extrapolation from knowledge about other species. These characteristics explain why knowledge about the reaction of the fish confronted with diseases and associated clinical protocols cannot always be applied to the sturgeon. This non-exhaustive, exploratory work allows for a level of knowledge, but there still remain certain areas to explore. The immunity mechanisms were already laid several decades ago. Stress factors (temperature, stocking density and hypoxia, natural or synthesise chemical substances) that can influence the immune system of sturgeon were also the subject

of researches in the last decade. There have been an increasing number of programs on the research of solutions to boost the immune system (vaccines and dietary supplements) in recent years. The most numerous publications focus on dietary supplements such as probiotics, prebiotics, symbiotics and equivalents, vitamins, proteins, amino acids, polysaccharides, plants and their components. However, although some studies on sturgeon provide interesting evidence on the potential for immunostimulation of these compounds, results are sometimes mixed. This is certainly due, in large part, to the small number of studies conducted on this species. It seems that a domain that has been much less explored is the correlation between the pathogen, the host immunity and its environment. Farms are regularly confronted with pathologies, and the farmer often uses the experience and common sense to try solutions to face the crisis (changing farming conditions, sanitary prophylaxis, preventive treatments and possibly curative treatments). To determine among these solutions those that could be most effective, especially in immunostimulation, it would be necessary to objectify observations through in situ tests. This opens up great prospects for research in this area.

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Welfare in the Cultured Siberian Sturgeon, *Acipenser baerii* Brandt: State of the Art

44

Patrick Williot, Mikhail Chebanov, and Guy Nonnotte

Abstract

The chapter aims at synthesising both the knowledge and the practices that are sturgeon welfare related. This allowed the authors to outline some main lines that should be investigated to improve the welfare of farmed sturgeon with a focus on the Siberian sturgeon. Thus, in the first part, the general principles of welfare are recalled (the so-called Five Freedoms) illustrated by a few sturgeon-related examples. As welfare might be wrongly restricted to pain perception of animals (sturgeon at present), a very brief synthetic knowledge of neuroanatomy with available data in sturgeon is given. The chapter is then focused on the anaesthetics on fish with a peculiar extension on sturgeon. The chapter also devoted to the external signs that can be used to detect painful situation. Available non-invasive methods as well as the characteristics of the environment that may impact the fish (including their physiology) are presented. A brief overview of stunning-slaughtering methods is listed. Finally, a synthetic overview of the available welfare-related tools for sturgeons is provided. As an end, some future directions to be investigated are given.

Keywords

Siberian sturgeon • Welfare • Anaesthetics • Non-invasive methods • Behaviour • Environmental factors • Physiology • Management • Stunning • Slaughtering

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Introduction

Sturgeon farming is a rather recent aquaculture activity that has increased steadily over the past 25 years (Williot and Bourguignon 1991; Williot et al. 1993, 2001) with now the main producer being China (Bronzi et al. 1999, Wei et al. 2011; Bronzi and Rosenthal 2014; Chap. 38). The main objective of this farming activity is to produce caviar even in some countries that have a long tradition either of sturgeon meat consumption (Russia) or of consuming fresh aquatic animals including sturgeon recently (China). This development might be explained by two simultaneous factors, the decreasing landings of worldwide sturgeon fisheries (Williot et al. 2002) and with the attractiveness of high prices for caviar. Because of the late puberty of sturgeons, farmers have to keep them healthy (the females at the least) for many years. Therefore, they have to take care of the valuable fish for long, and then, so doing they have been applying, at least partly, the concept of "animal welfare".

Animal welfare was recently defined by OIE[1] (2014) as "animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear, and distress". And the organisation adds "good animal welfare requires disease prevention and appropriate veterinary treatment, shelter, management and nutrition, humane handling and humane slaughter or killing. Animal welfare refers to the state of the animal; the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment". Beyond the ethics dimension of animal welfare, the two-part definition of the OIE recalled above strongly suggests the potential advantages of such practises in farming (Huntingford et al. 2006). This led Sneddon (2006) to assume that "it is of course in the interests of industry to maintain good welfare since animals in 'optimum' condition grow better and give a better economic return".

So writing, OIE clearly evokes other dimensions of the animal welfare which are related to human sciences (ethics, politics, economics). Thus, the animal welfare is not restricted to scientific dimensions as already were pointed out by Le Neindre et al. (2009) and Servière (2014).

With regard to aquatic animals, the situation is so different depending on species (many fish species, molluscs, crustaceans, etc.) that no general rules can be promulgated. OIE prefers to regularly update some recommendations, e.g. welfare, transport, stunning and killing that can be found on the site of the OIE (www.oie.int).

Despite the international status of the OIE, which is basically sustained by scientific expertise, it remains that some of the aforementioned ideas (pain, fear and distress) are still in debate for fish. Indeed there are two central questions: Do the fish experiencing the pain? Are they conscious of the pain? More generally speaking, should fish welfare be restricted to those questions or are there other incentives in taking into account the fish welfare? In the last 10–15 years, a lot of papers were focused in fish welfare. This is not the objective of the chapter to give a synthesis but to recall some general principles

[1] OIE = Office International des Epizooties (French original acronym) known as the World Organisation for Animal Health.

as a guide to list the Siberian sturgeon welfare-related issues, given that some of them have been taken into account for long by either scientists or farmers. Altogether, welfare corresponds to the less stressful conditions in sturgeon farming that should allow the fish to keep their homeostasis status and develop their species-specific behaviour. Extensive studies focused on the impact of stress on welfare were already published, e.g. FSBI (2002), Conte (2004) and Ashley (2007). But none was focused on sturgeon, especially on the Siberian sturgeon. And more surprisingly, no peculiar welfare-related study targeted sturgeon with the exception of a very recent work investigating mainly the consequences on heat shock protein and oxidative stress of heat stress and dietary supplementation (Simide et al. 2016). Additionally, classical stress indicators in the blood (catecholamines, cortisol, lactate, glucose) are nonspecific and then may not allow pointing out the origin of the stress. As a result, the stress is not directly an apparent part of the chapter, but the concept is constantly underlying as a main line along the chapter.

Therefore, the aims of the present chapter are:

1. Giving the general principles of animal welfare with some corresponding sturgeon-related issues,
2. Comparing the neuroanatomy of the involved nervous system from primates to fish and sturgeon as well as reporting the last results on fish pain,
3. Giving an overview of the anaesthetics used in fish and in sturgeon,
4. Describing the available external criteria helpful in detecting potentially painful situations in sturgeon and their remediation,
5. Listing the non-invasive methods,
6. Listing the characteristics of the watering environment which can impact the sturgeon, including their physiology,
7. Giving the main methods for stunning-slaughtering,
8. Synthesising the means to avoid deleterious consequences of the noxious stimuli in the sturgeons, i.e. how to ensure the Siberian sturgeon with welfare,
9. Ending by proposing some lines to be investigated in the field.

44.1 General Principles of Welfare in Animals with Some Corresponding Sturgeon Fish Issues

The central question is how to define the welfare of animals. As there is no consensus in the field, the solution has been in defining what it should be, and this is the so-called five domains of freedom which now constitute the official framework (FAWC 1996; EC-DGF 2004) (Table 44.1). Obviously this was set up with reference mainly to mammals, so that some adaptation to fish especially to sturgeon might be done. Along the subsection, some sturgeon-related examples will be provided and even more detailed points will be given further in the chapter. The ready access to clean water quoted in domain 1 is referring to drinking water for mammals or birds and not to the environmental usual medium as for fish. Taking into account that most sturgeon species, and Siberian sturgeon is no exception, are benthic feeder, the bottom of rearing structure should not be abrasive. Further, resting areas evoked in domain 2 might be astonished for sturgeon except thinking on new naturally oriented means for reproduction as already investigated by Chebanov et al. (2002). Domain 3

Table 44.1 Five domines of freedom, characterising the animal welfare of freedoms for farmed animals (FAWC 1996; EC-DGF 2004)

Domain	Characteristics
Domain 1	Water and food (deprivation and malnutrition)
	Animal should have ready access to clean water and an appropriate diet in sufficient quantities and with a composition that contains full health and vigour
Domain 2	Environmental challenge
	Animal should have a suitable environment including shelter and resting area, whether outdoors or indoors
Domain 3	Disease, injury and functional impairment
	Disease should be prevented or rapidly diagnosed and treated
Domain 4	Behavioural/interactive restriction
	Animal should have sufficient space, proper facilities and, where appropriate, the company of the animal's own kind
Domain 5	Mental and physical suffering
	Conditions that produce unacceptable levels of anxiety, fear, distress, boredom, sickness, pain, thirst, hunger and so on should be minimised

referring to health status applied to fish appears to be much more complex than for aerial animals because fish environment, the water, is part of the problem. Density is the main question involved in domain 4. Finally, domain 5 is still on the table as some scientist contest the acceptability of such a domain in fish (Rose 2007).

Recently, the World Organisation for Animal Health (OIE) gave detailed contents on the above five domains as reported by Fraser et al. (2013). Again, those general principles were established for aerial animals. They are listed below with an adaptation to fish, especially for sturgeon (Table 44.2). There are only few published data on genetic selection in Siberian sturgeon (Petrova et al. 2008); however, shadow selection might happen when selecting brood fish, building on growing batches and rearing conditions are not carefully controlled and/or performed. This might result in an involuntary domestication (Boguerouk 2005; Bilio 2007, 2008). We should be attentive as any selective genetic program may produce unexpected consequences on progenies. With regard to the influence of the environment, this is one of the key points which will be developed later on especially with water parameters. As far as light is concerned, Siberian sturgeon prefers shadow atmosphere as compared with direct lighting (Williot et al. 1988). The sizes of the rearing structures should be adapted to these long fish in allowing them to freely move (Chap. 12). To our knowledge, there is no study focused on social relationship in sturgeon. Most likely stocking density might be a key factor in the field. Cannibalism was noted in larvae when feeding delivery was inappropriate (see review in Gisbert and Williot 2002). Further dominance by larger specimen is likely to occur and should be taken into account in feeding delivery and/or grading. As aforementioned, water quality is a key factor that will be developed later on in the chapter (Sects. 44.6 and 44.7). Food and feeding constitute an important issue (Chaps. 30, 31 and 33). Disease-related issues have been receiving much attention from the farmers in the last 10–15 years with the occurrence of some bacterial and viral pathogens (Chap. 43). A daily supervision may allow to triggering off a quick intervention. Preventive

Table 44.2 General principles of welfare in animals (adopted by OIE in 2012) with corresponding sturgeon issue (completed and/or adapted (italics) after Fraser et al. 2013)

General principles		Corresponding sturgeon issue
1	How genetic selection affects animal health, behaviour and temperament	Domestication Genomics
2	How environment influences injuries and the transmission of diseases and parasites	Nonabrasive building materials
3	How environment affects resting, movement and the performance of natural behaviour	Environmental characteristics (light, water, bottom, shape of rearing structures, etc.)
4	The management of groups to minimise conflict and allow positive social contact	Behaviour (social relationships) Stocking density Homogeneity in size to lower cannibalism Feeding management
5	Air quality, temperature and humidity in confined spaces should support good animal health and not be aversive to animals	
	Water quality (oxygen, temperature, pH, ammoniac, nitrate, nitrite, CO_2 and so on) on animal health and comfort	Physicochemical characteristics of water, water renewal, gradient of variations (Sects. 44.6 and 44.7)
6	Ensuring access to feed and water suited to the animal's needs and adaptations	Fish feeding (quality, quantity, delivery, presentation)
7	Prevention and control of diseases and parasites, with human euthanasia if treatment is not feasible or recovery unlikely	Routine observation Vaccination Preventive feeding Non-invasive methods (Chap. 49, Chebanov and Galich 2009; Fig. 44.1)
8	Prevention and management of pain	Anaesthesia Handling Human slaughtering
9	Creation of positive human-animal relationships	Ethics, empathy
10	Ensuring adequate skill and knowledge amongst animal handlers	Training of practitioners
11	*Fish management along the biological cycle*	Stressless methods, anaesthesia, non-invasive methods, grading, sex determination, control of oogenesis, tagging for females for management and traceability

actions limit the development of pathogens such as using vaccination and probiotics for stimulating the immunology (Chaps. 33 and 43), generalising non-invasive methods (Fig. 47.1; Chap. 49) and keeping much precaution to avoid dispersion of potential pathogens at the entrance of the farms and within (from tank to tank). Whether sturgeon feel pain or not, it is recommended to use anaesthesia (Sect. 44.3) each time fish should be handled for long and to set up specific management to limit aerial transportation (Figs. 44.2 and 44.3). Finally, human slaughtering should be used. Human-animal relationships and skill in animal welfare should be promoted

Fig. 44.1 A typical non-invasive echography on site presently used for sex determination. Please note the floating baskets in which fish are shortly stocked in water (credit Williot P)

Fig. 44.2 General view of large pipes to ease transferring Siberian sturgeon from one raceway to another post-sex determination on site (credit Williot P)

Fig. 44.3 Detailed view of a transferring pipe of Siberian sturgeon post-sex determination on site. Please note that the pipe is supplied by water, thanks to the pump in order to make the transfer automatic and stressless for the fish (credit Williot P)

within practitioners. Indeed, animal welfare, sturgeon fish in the present case, is a global attitude towards fish which embraces the whole biological cycle and then involves all the questions which arrive along the biological cycle, some of them being related with stressless methods, anaesthesia, non-invasive methods, grading, sex determination, control of oogenesis and tagging for females to ease for management and traceability.

Before developing more specific sturgeon welfare-related issues, a very brief synthesis on the available knowledge on the neuroanatomy is needed as one could argue that fish suffer and perceive pain or not.

44.2 Some Characteristics and Evolution of the Central Nervous System Involved in the Perception of Pain with a Focus on Fish and Sturgeon

It has been long for humans to admit that animals may not be so different from them and they started to change in enlarging their scope to the primates which is not restricted to human. A second changing scale was to consider the mammals, and more recently the changing level is to address the fish.

In the primates, a very condensed description of the knowledge of the route for the final perception of pain is as follows. There are specific receptors, called nociceptors, distributed both outside (skin) and inside the body (muscle, articulation, viscera) constituted of nervous fibre without any surrounding myelin gain. There are different types of nociceptors (end of sensorial neurones), the polymodal, the mechanothermal and the chemical receptors. The signal detected by the nociceptors is then transferred by two types of fibres, the Aδ and C, to the spinal cord (lateral route for the sensation and median route for the emotion) regarding stimuli from the body (Le Neindre et al. 2009). Signals from the face are transferred via the cranial nerves (trigeminal nerves). Aδ fibres (mechanothermal stimuli such as high pressures, punctures, pinching and high temperatures) are myelinised, and C fibres (polymodal stimuli, i.e. those described previously, plus chemical stimuli of algogen type) have a diameter of 1–5 μm and 0.5–1.5 μm and a speed transfer of 4–30 $m.s^{-1}$ and 0.2–1 $m.s^{-1}$, respectively (Becamel, com pers).

All signals finally reach the cerebral cortex where sensation (somesthesic cortex) and emotion (limbic cortex in the telencephalon) take place. The genesis of emotion implies complex processes allowing the assessment of situations, and these processes are correlated to the activation of structures of the central nervous system which are the most recent, phylogenetically speaking, i.e. those of the telencephalic cortex (Le Neindre et al. 2009). The same authors stated that a comparative analysis of the structures of the central nervous system, as well as the behavioural capacities, leads to the conclusion that other mammals, like primates, experience the pain as well.

What about the fish? Are they equipped with neural apparatus to sense, process and respond to potentially painfully stimuli? Most of the responses are recent and are related to teleosts fish.

Nociceptors were identified on the head of the rainbow trout (Sneddon 2002) as well as Aδ and C fibres at the level of the trigeminal nerve (Sneddon et al. 2003). Then fish possess all the areas of the brain involved in the processing of pain in mammals. Additionally, trout submitted to noxious stimulation did not show the appropriate fear response, suggesting that the pain they experienced masked their normal behaviour. Also, administration of a pain attenuation factor, morphine, to noxiously stimulated trout, carp and zebrafish ameliorated the adverse behavioural and physiological responses, thus demonstrating that these were pain-related responses (Sneddon 2006). This was reinforced by a study that showed that nociception stimulated both trout and carp and candidate genes were identified alike in mammals (Reilly et al. 2008). In a recent synthesis in the field, Sneddon (2011) extended and confirmed previous results.

What about sturgeon? To our knowledge, very few data are available in the field. However, trigeminal nerve projections were described for long in two sturgeon species, the Mexico Gulf Atlantic sturgeon (*Acipenser oxyrinchus desotoi*) and the shovelnose sturgeon (*Scaphirhynchus platorynchus*) (New and Northcutt 1984). Afterwards, medial trigeminal nucleus was mentioned in the brain of the Siberian sturgeon (Leprêtre et al. 1993), and more recently, trigeminal characteristics (motor nucleus and motor root) were also shown in the Siberian sturgeon (Graña et al. 2012). These findings might be interpreted as the potential existence of nociceptors in the head of the Siberian sturgeon. Does that mean that fish are sentient? Are they able to perceive pain,

i.e. being conscious? Many scientists in the field are inclined to think so especially thanks to behavioural experiences aforementioned (Chandroo et al. 2004), while some others refute and claim it is a posture or anthropomorphism as fish have a primitive neocortex (forebrain) (Rose 2007). More recently the controversy extended with Key (2016) who claimed that fish cannot feel pain as they "lack of necessary neurocytoarchitecture, microcircuitry, and structural connectivity for the neural processing required for feeling pain". Opposite this position is that of Seth (2016) who reports that non-mammalian consciousness—if it exists—may depend on different mechanisms. Alike Seth, Wadiwel (2016) points out that "there is a great deal of doubt about Key's thesis that fish do not feel pain" and confirms previous review (Huntingford et al. 2006). These positions are those of recent synthesis in the field by Sneddon (2011) and Brown (2015). Though important, the debate on consciousness of fish is only a part of the welfare as recalled in Table 44.1 where pain issues are classified in domain 5.

44.3 The Anaesthetics

44.3.1 Very Brief Synthesis of Anaesthetics Used in Fish

The main means to suppress the nociceptive feelings and/or pain and to minimise stress in fish is to use the anaesthesia, particularly at a time where handling is needed. Indeed, neurotropic drugs (either for sedation or for immobilisation) have been widely used in fish farming (Durve 1975; Klimonov et al. 1995; Kolman et al. 1997; Trzebiatowski 1970). And despite the considerable differences in the mechanisms of drugs effects on fish, such substances, not always accurately, are called anaesthetics. Classically, anaesthesia is divided into five steps from step 1 (no anaesthesia) until step 5 (irreversible anaesthesia or death). Step 2 corresponds to sedation, step 3 to tranquilisation and step 4 to anaesthesia appropriate to surgery (Feng et al. 2011a).

There are two categories of anaesthesia, one using a molecule (water soluble or gas) and one utilising a physical process such as electronarcosis. Molecules can be administered to fish in three ways, (a) bathing, (b) spraying the gills, and (c) injection, the previous one being the most commonly used because it allows a precise dosing and mass treatment. Spraying, mainly used on large fish, requires that a concentrated drug has no local irritant effect. The mechanisms of various drugs effecting on the central nervous system can be quite different. There exists an ichthyological classification of neurotrophic substances in accordance with their chemical structure and physiological effects on fish (Klimonov et al. 1995).

Two main types of products are available in obtaining anaesthesia, chemical and non-chemical. Amongst chemical anaesthetics are the aminobenzoates (MS-222, benzocaine, lidocaine, etc.) and some others molecules (metomidate, quinaldine, 2-phenoxyethanol, eugenol, propofol, ketamine and carbon dioxide) (Table 44.3) (completed from Feng et al. 2011a, b). Name, gross formula, presentation, effects and observations are provided. A variety of other molecules is given according to their usage: in bathing solution (metomidate, quinaldine, phenoxy-2-ethanol,

Table 44.3 Name and action of main chemical anaesthetics used in fish.

Name	Names and formula (presentation)	Actions—observations	Sources
Aminobenzoates			
Mesilate de tricaine	Tricaine methanesulfonate Ethyl 3-aminobenzoate methanesulfonate (white crystalline powder) (MS-222™) $C_9H_{11}O_2N + CH_3SO_3H$	Lowers the pH ($NaHCO_3$ used as buffer) Jam on axonal conduction	Feng et al. (2011b)
Benzocaine	Ethyl-4-aminobenzoate $C_9H_{11}NO_2$ (Benzoak®) 200 $g.L^{-1}$	Bradycardia Rapid elimination Low solubility in water Keep	Iversen et al. (2003)
Lidocaine	2-(Diethylamino)-*N*-(2,6-dimethylphenyl)acetamide $C_{14}H_{22}N_2O$ Often marketed as xylocaine	Inhibition of nervous influx by fixation activated molecule on specific sodium canal surrounding the nervous fibre	
Novocaine	Procaine chlorhydrate 2-(Diethylamino)ethyl 4-aminobenzoate $C_{13}H_{20}N_2O_2$		
Others			
Metomidate (etomidate)	Ethyl 3-[(1*R*)-1-phenylethyl] imidazole-5-carboxylate (Marinil™), Propiscin Powder $C_{14}H_{16}N_2O_2$	Bradycardia Respiratory depression Reduce heart rate For ornamental fish only	Iversen et al. (2003)
Quinaldine	2-Methylquinoline Colourless or slightly yellow oily liquid $C_{10}H_9N$ Quinaldine sulphate is the form used as an anaesthetic	Low toxicity in sturgeons	
Phenoxy-2-ethanol	$C_8H_{10}O_2$ Aqueous solution	Inhibitor biosynthesis of DNA and RNA Rapid elimination Hypercapnia Low toxicity in fish Not recommended for marketing products Allergenic Neurotoxic Bad smell	Blanc (2003)

Table 44.3 (continued)

Name	Names and formula (presentation)	Actions—observations	Sources
Eugenol	Clove oil (85–95% eugenol) $C_{10}H_{12}O_2$ (4-allyl-2-methoxyphenol)	Neurotoxic and hepatotoxic properties Rapidly absorbed and metabolised Inhibition of respiratory centre in the medulla oblongata Does not require any withdrawal period Antiseptic action Very high lethal concentration Safe for practitioners	Javahery et al. (2012) Iversen et al. (2003) Hamáčkova et al. (2006)
	Aqui-S™ (50% (540 g.L^{-1}) isoeugenol (4-propenyl-2-methoxyphenol))		Javahery et al. (2012) Iversen et al. (2003)
Propofol	Disopropylphenol $C_{12}H_{18}O$	Intravenous injection (i.v.) Has to be associated with an analgesic	Fleming et al. (2003)
Ketamine	$C_{13}H_{16}ClNO$ (ketamine chlorhydrate) Crystalline powder, water and ethanol soluble	Injection (i.v.) Glutamate inhibitor Low permeability through gills and skin	
Carbon dioxide (CO_2)		Lowers the pH ($NaHCO_3$ used as buffer)	
Electronarcosis	Chemical-like anaesthesia		

eugenol and carbon dioxide) and in injection alone (propofol and ketamine) or in association with another molecule. Eugenol, a non-chemical product, is mainly the essential oil from clove (*Eugenia caryophyllus*) obtained by distillation and can be found within two commercial forms: clove oil (solution) and Aqui-S™ (powder) that may lead to using two different concentrations expressed either by volume or by weight, respectively (Iversen et al. 2003). The last molecule sometimes used is the carbon dioxide (CO_2), the interest of which is that no xenobiotic molecule is introduced in the medium. So doing, there is no restriction on the delay for marketing the fish. Finally, there are very few physically based ways to anaesthetising the fish: (a) the electronarcosis and (b) the hypothermia. Whether both methods could

be used and/or recommended in fish farming as a mean to allow further human slaughtering, the first one (electronarcosis) is currently applied in nondestructive surveys of wild populations in relatively small water systems. Further, and independently from the actual impact of the method, lowering the temperature for large quantities of fish at fish farm level might be difficult to apply.

44.3.2 Anaesthetics Used in Sturgeon

Two series of data are available, those resulting from studies on the consequences of anaesthesia on some blood parameters and/or other biological characteristics and those mentioned in material and methods section of other studies on sturgeon (Table 44.4). Species, biometry, product, concentration and observations (temperature, anaesthesia condition and consequences) are given. There are a few main comments. Not surprisingly, the great majority of the main chemicals mentioned in Table 44.3 are documented with the clove oil the most currently studied (Fig. 44.4). However, it has to be kept in mind that the figure of the anaesthetics used in sturgeon through this table might be biased by legal aspect; some products and methods developed are under the dependence of the law which allows or not the use of the different means to anaesthetise the fish. The effective concentration is inversely proportional to the size of the fish. With regard to clove oil, except for fingerlings, the optimal range is 25–120 $mg.L^{-1}$, i.e. somewhat larger than the one previously reported by Javahery et al. (2012) with 50–100 $mg.L^{-1}$ and perfectly surround the previous most effective dosage of 0.70 $mL.L^{-1}$ (i.e. ~74 $mg.L^{-1}$) reported by Hamáčkova et al. (2006). For fingerlings, clove oil and aminobenzoates are documented with very high dosage for the primer. Regarding the large fish, namely brood fish, a large spectrum of possibilities is offered from chemicals including carbon dioxide to spray or intramuscular injection of ketamine, hypothermia (Fig. 44.5) and electronarcosis (Fig. 44.6). The last seems to be favoured in the recent year. One of the difficulties in the field is that side effects of the methods are often under documented if any as illustrated by carbon dioxide. Carbon dioxide is a colourless, odourless gas with a water solubility of 1.7 L/L water at 0 °C and 760 mmHg. The hydration of CO_2 will acidify water which should be buffered to reduce this potential stress to the fish. The recent interest of fisheries and aquaculture professionals in the use of CO_2 anaesthesia is based on its gaseous nature and the fact that it leaves no residues in the tissues. Exposure of fish to hypercapnia (1 to 5% CO_2 in air) induces respiratory acidosis in fish, as it produces a consistent decrease in blood pH upon exposure of the fish as demonstrated by Shaughnessy et al. (2015) in *Acipenser transmontanus*. CO_2 anaesthesia is often associated with the hypothermia. Therefore, this method needs more research into the best method of administration since it is a method that can significantly disturb the acid-base and ionic balances of all fishes (Iwanna and Ackerman 1994).

Indeed, we have to wonder whether anaesthetics are non-aversive to fish. More, which are their impacts on physiology? A recent study tested the most commonly used molecules on the zebrafish, thanks to a video-tracking software quantifying the

Table 44.4 Synthesis of anaesthetics on sturgeon otherwise mentioned

Species—size		Anaesthetic			
Species	**Biometry (mean)**	**Product**	**Concentration**	**Observations**	**Sources**
A. baerii	7–12 cm 2.1–6.4 g	Clove oil Benzocaine	EC[a]50 330–380 $mg.L^{-1}$ EC50 33–40 $mg.L^{-1}$	Anaesthesia in 3 min Feed intake within 10 min	Akbulut et al. (2012)
A. baerii	235 ± 59 g	Clove oil	120 $mg.L^{-1}$	16.5 °C Anaesthetised in 3–4 min Recovering in 6–7 min	Feng et al. (2011a)
A. baerii	19.9 cm 48 g	Clove oil MS-222	70–90 $mg.L^{-1}$ 80–120 $mg.L^{-1}$ [b]	~16 °C Concentration leading to minimum effects on blood parameters	Feng et al. (2011b, c)
A. baerii	25.3 cm 94.9 g	Clove oil MS-222	0.075 $mL.L^{-1}$ (79.5 $mg.L^{-1}$)[c] 125 $mg.L^{-1}$	17 °C Recovery time ~13 min Swelling and haemolysis of erythrocytes Severe depletion in leucocytes 24 h post-anaesthesia	Gomulka et al. (2008)
A. naccarii x A. baerii	12.4 ± 3.4 kg 120 ± 8.3 cm	Clove oil (85% eugenol) MS-222 [Medetomidine + ketamine hydrochloride (MK)] i.v.	100 $mg.L^{-1}$ 150 $mg.L^{-1}$ 0.04 $mg.kg^{-1}$ + 4 $mg.kg^{-1}$	Stress and blood physiology MS-222 induced the greater impact, attenuated with clove oil MK proved to be a safe and effective anaesthetic protocol	Di Marco et al. (2011)

(continued)

Table 44.4 (continued)

Species—size		Anaesthetic			
Species	**Biometry (mean)**	**Product**	**Concentration**	**Observations**	**Sources**
A. baerii *A. ruthenus*	Brood fish (0.7–8 kg)	Clove oil (90% eugenol)	40 ppm = 40 mL.L^{-1} (~42 mg.L^{-1}) (Fig. 44.4)	A preliminary dilution (1/10; v/v) of clove oil in ethanol is recommended especially at low temperature Anaesthesia in ~5 min	Williot (2002) Williot et al. (2011a, b)
A. baerii	100–180 g	Clove oil	0.070 mL.L^{-1} (~74 mg.L^{-1})	4–20 °C Anaesthesia in 10 min	Hamáčkova et al. (2006)
A. fulvescens		Clove oil	60 mg.L^{-1}		Peake (1998) in Javahery et al. (2012)
A. transmontanus	206–363 g	Clove oil	25 mg.L^{-1} (10 min) Median lethal concentration 526 mg.L^{-1}	Effective for 120 min	Taylor and Roberts (1999)
A. baerii	Juvenile 100–180 g	Clove oil	0.07 mL.L^{-1}	4–20 °C 10 min exposure	Kouřil et al. (2003)
A. baerii	10–15 kg	Clove oil	0.07 mL.L^{-1}	14–16 °C	Podushka and Chebanov (2007)
Acipenser sp. *A. schrenckii, hybrid* *A. schrenckii* x *Huso dauricus*	10–25 kg	Clove oil (5% eugenol solution (95% ethyl alcohol))	200 mg.L^{-1} 100 mg.L^{-1}	15 °C >20 °C	Podushka and Chebanov (2007) Mohler (2003)
A. gueldenstaedtii, *A. persicus*, *A. ruthenus*, *A. stellatus*	18–32 g 15–40 g 17–28 g 7–18 g	MS-222	50 mg.L^{-1} 75 mg.L^{-1}	24 °C 25–30 min Fitness indices estimation	Galich (2000), Chebanov et al. (2004), Chebanov and Galich (2013)

A. transmontanus	Sperm	Clove oil MS-222	150 mg.L^{-1} 225 mg.L^{-1}	Modest decline in motility No effects	Holcomb et al. (2004)
A. oxyrinchus oxyrinchus	3.1 kg (88 cm) 4.2 kg (95 cm)	MS-222	250 mg.L^{-1} + 500 mg.L^{-1} $NaHCO_3$	10 °C 22 °C Anaesthesia has greater effect than surgery	Matsche (2013)
A. oxyrinchus	4-yr.-old fish	Propofol (i.v.)[d] [Medetomidine + ketamine] (i.m.)[e]	3.5–7.5 mg.kg BW^{-1} 0.03–0.07 mg.kg BW^{-1} 3–7 mg.kg BW^{-1}	Adequate short-term immobilisation (shorter with propofol) Mild bradycardia Apparent respiratory depression	Fleming et al. (2003)
A. baerii (Lena)	5.8–9 kg 20–50 g	Etomidate	2 mL.L^{-1} 6–8 mL.L^{-1}	14 °C Narcotisation within 2 min 7 °C	Kolman et al. (1997), Kolman (2006)
A. baerii (Lena and Ob) *A. gueldenstaedtii* *A. stellatus,* *H. huso* x *A. ruthenus*	5.8–40.7 kg 20–50 g	Etomidate (Propiscin)	3–4 mL.L^{-1} 6–8 mL.L^{-1}	12–24 °C Narcotisation within 3–5 min 7 °C	Nikonorov et al. (2005)
A. baerii (Lena), *A. gueldenstaedtii,* *A. stellatus,* *H. huso*	8–60 kg	Etomidate (Propiscin)	10–14 mL.L^{-1}	11–19 °C 5–20 min Broodstock assessment and collection of ovulated eggs	Chebanov and Galich (2009), Chebanov and Galich (2013), Kolman (2006)

(continued)

Table 44.4 (continued)

Species—size		Anaesthetic			
Species	**Biometry (mean)**	**Product**	**Concentration**	**Observations**	**Sources**
H. huso	41.2 cm 198.3 g	2-Phenoxyethanol	0.7–0.9 mL.L^{-1}	19 °C Anaesthesia within 3 min Low effects on haematological and biochemical serum properties	Shaluei et al. (2012)
A. baerii	1.8 kg	2-Phenoxyethanol	1/200 (10 min)	15 °C	Nonnotte et al. (1993)
A. gueldenstaedtii	0.1–3.0 g	Benzocaine Lidocaine Novocaine	55–90 mg.L^{-1} 50–65 mg.L^{-1} 100–200 mg.L^{-1}	20–25 °C	Nikonorov et al. (2005)
A. stellatus	1.3–2.6 g	Quinaldine	35–50 µL L^{-1}	20–27 °C	
A. gueldenstaedtii *A. stellatus* *A. nudiventris* *H. huso*	1.0–3.0 g	Quinaldine hydrochloride	40–80 mg.L^{-1} 30–40 mg.L^{-1} 45–60 mg.L^{-1} 50–80 mg.L^{-1}		
H. huso	800–1200 g	Ketamine	6–10 mg.kg BW^{-1} 12–14 mg.kg BW^{-1}	Intramuscular injection 6–8 min delay Active during 10–12 min 1–3 min delay Active 15–20 min	Vasilieva et al. (1999)
Sturgeon		Ketamine	5% ketamine solution (physiological solution 1:3) 5% hydrochloride solution	Spray on gills 4–10 mg.kg BW^{-1} (i.m.)	Chebanov and Galich (2013)
A .baerii	4.1–4.5 kg 89–92 cm (♀)	Carbon dioxide (CO_2)	Bath with CO_2	5 °C Bath buffered with $NaHCO_3$ ($6.8 < pH < 7.5$) $8.0 < [O_2] < 12$ mg.L^{-1}	Hamlin et al. (2007)

Marine fish species		Carbon dioxide (CO_2)	0.33 $g.L^{-1}$ $NaHCO_3$ + 0.75 $mL.L^{-1}$ glacial acetic acid	Within 10 min	Oberg et al. (2015)
Oncorhynchus mykiss		Carbon dioxide (CO_2)	230–280 $mg.L^{-1}$		Blanc (2003)
A. fulvescens *A. brevirostrum*	31–39 cm	Electronarcosis	Low voltage direct current 20–30 V	Immobilised in 5 s Anaesthesia-like for 5 min	Henyey et al. (2002)
A. baerii	Juvenile	Electronarcosis	20–30 V (Fig. 44.6)	Lower impact in haematological biochemistry	Feng et al. (2011a, b, c, d)
A. oxyrinchus	29–47 cm (85–500 g)	Electronarcosis	0.54 V cm^{-1}	16–18 °C Lower cortisol content than anaesthetised fish (MS-222)	Balazik et al. (2013)
A. baerii	Brood fish	Hypothermia	Ice (Fig. 44.5)	Extraction of eggs (caviar production)	Podushka and Chebanov (2007)

[a]*EC* effective concentration
[b]Best concentrations to minimize impact on blood parameters
[c]Density of the product = 1.06 g cm^{-3} (Gomulka com. Pers.)
[d]*i.v.* intravenous injection
[e]*i.m.* intramuscular injection.

Fig. 44.4 Clove oil anaesthesia of Siberian sturgeon breeders in China before microsurgery extraction of ovulated eggs (courtesy by Podushka S)

Fig. 44.5 Siberian sturgeon specimen in a container with ice–water mixture slaughtered for subsequent extraction of eggs for caviar production (credit Podushka S)

swimming behaviour (Readman et al. 2013). The results suggest that MS-222 and benzocaine are aversive. In contrast, etomidate and 2,2,2-tribromoethanol did not induce aversive behavioural response. According to the same authors, the relative costs are increasing from isoeugenol, rated as 1, phenoxyethanol, etomidate and quinaldine sulfonate together as 2.5, benzocaine as 20 to MS-222 as 35. In the same subject as the one aforementioned (Readman et al. 2013), a recent work strongly suggests that the consequences of anaesthesia might be more effective than the only surgery (Matsche 2013). This means that side effects of anaesthetics have to be

Fig. 44.6 Electronarcosis applied for stunning young males of Siberian sturgeon post-sex discrimination (credit Williot P)

taken into account. In an attempt of using an anaesthetic for the first time, it is highly recommended to carry out a preliminary test on a small number of individuals.

44.4 Available External Criteria Aiming at Detecting Painful Situation with Potential Remediation

The most common way to detect painful situation is to observe changes in behaviour and/or external evidence for a painful physiological status or abnormalities in shape or injuries. This hypothesises that normal patterns of behaviour are quite well known and described with the threshold limits. Given the great variability within teleost fish species, behavioural patterns are different (Sneddon 2011) so that direct extrapolation might be unrealistic. Even within the same fish family, e.g. the sturgeon family, which is represented by a rather limited number of species (~25), there exist some very pronounced differences such as feeding behaviour, e.g. *Huso* sp. vs. *Acipenser* sp., the previous being very rapidly piscivorous in contrast with the second that are mostly benthic feeders (crustaceans, molluscs, worms, etc.). For a given species and a given criterion/parameter (feeding, temperature tolerance, etc.), different responses might be observed depending on the ontogenetic development of the species as already pointed out for sturgeons (Buddington 1991). A similar attempt to synthesise behavioural changes and abnormalities induced by stressors was carried out essentially for teleost fish (Martins et al. 2012). A sturgeon-related synthesis is proposed below with a focus on the Siberian sturgeon accompanied by the remedies (Table 44.5).

Most sturgeon species are benthic and prefer deep and turbid environments, and the Siberian sturgeon is no exception. This is why a shadowed environment has their preference. At a juvenile stage, in case of rearing in outside shallow waters, herons and rats may cause dorsal injuries. Abrupt swimming might be observed with sometimes subsequent shocks on vertical wall of raceways. Acoustic causes are mostly invoked and then circulation around rearing structure should be carefully organised. Ventral injuries especially visible on the two lines of scutes are the consequences of a rough bottom. Though the present chapter deals with juvenile-adult phase, it is worthy to note that at the beginning of the rearing of young

Table 44.5 Behavioural changes and abnormalities as poor welfare indicators and remedy in the juvenile and adult Siberian sturgeon

Behaviour and/or external abnormality	Cause	Sources	Remedy
Regrouping at the outlet end of the raceways	Deeper part are preferred	Williot et al. (1988)	Design of the rearing structures
Regrouping under shadowing part	Direct lighting is avoided by fish		Shadowing the rearing tanks
Narrow holes usually on the top head or closed to it	Bites by rats or herons		Careful covering of rearing tanks
Abrupt and quick swimming	Abrupt disturbance (acoustic,?…)		Avoiding any direct disturbance
Ventral injuries on scutes	Rough bottom		Adopt smooth bottom
Other injuries			
Inflated fingerling (Fig. 44.7)	Inappropriate food delivery	Williot unpublished	Increase the delivery frequency
Bend juvenile (Fig. 44.8)	Most likely ill-adapted food formula	Brun et al. 1991	
Ulcers (Fig. 44.9)	Pathogens (*Streptococcus dysgalactiae*)	Williot unpublished	Vaccination
Increasing of ventilatory patterns (frequency and amplitude)	Hypoxia	Nonnotte et al. (1993)	Injection of oxygen or operate aerators Take off the fish Check the water monitoring process
Successively Calm and increasing ventilation Jumps, tetany and anarchic movements Loss of equilibrium Oedemas and dilatation of blood space of gill epithelium	Acute ammonia toxicity	Salin and Williot (1991)	Take off the fish Check the water monitoring process Reinvestigate the water circulation system and water treatment in case of RAS[a]

[a]*RAS* recirculated aquaculture system

Fig. 44.7 Inflated fingerling of Siberian sturgeon (credit Williot P)

Fig. 44.8 Bend specimen of Siberian sturgeon (credit Williot P)

of the species (in the 1980s), we have been in trouble with fingerlings which exhibited a loss of equilibrium as a result of the inflation of swim bladder (Fig. 44.7). The reason is that the fish came at the surface to search for food and then swallowed air because of a connection between stomach and swim bladder in sturgeon which are thus called physostoma (Williot et al. 2011a, Chap. 3). Large bend juveniles and/or adults might be observed (Brun et al. 1991, Fig. 44.8, Chap. 4). Although there is no proof, a nutrition reason is suspected. Large ulcers (Fig. 44.9) are due to a bacteria (*S. dysgalactiae*), of which the treatment needs a previous vaccination. Most likely, other bacteria might be responsible for a similar injury. Increasing ventilatory patterns primarily reveals a deficit in oxygen which leads to hypoxia. Anarchic movements and subsequent loss in equilibrium may be due to ammonia toxicity (Salin and Williot 1991).

Fig. 44.9 A brood fish of Siberian sturgeon exhibiting an important ulcer caused by a bacteria (*Streptococcus dysgalactiae*) (credit Williot P)

44.5 Non-invasive Methods

Accurate management of farmed sturgeon stocks or wild-originated individuals of endangered species needs that one is able to document the health status of the fish with a minimum stressful consequences. To achieve this task, non-invasive methods have to be favoured. It is possible to distinguish two types of non-invasive methods depending on preliminary handling (Table 44.6). The first type might be divided into two groups, the first being a truly non-invasive one, i.e. it does not need any fish capture. It corresponds to either a direct or indirect (via a video-tracking procedure) observation of the behaviour of the fish. The second group needs a preliminary capture either to conduct an internal observation via echography (Chap. 49) or to put fish in a special chamber in order to carry out further observations. Temperature preference (Staaks et al. 1999), challenge test (Carrera-Garcia et al. 2016) and cardiorespiratory motion (Hafner and Lubecke 2012) can then be studied. The second type includes all the methods that need to install a sensor/captor/transmitter inside the fish to further record data without handling fish. Therefore, the methods classified in the second type might be called quasi non-invasive method as they first need the fish to be equipped. This can be a cannula to allow studying blood parameters (pH, gases, hormones, ions, blood cells, flow rate, etc.) or a specific sensor of which a preliminary list was already given by Cooke et al. (2004). Thanks to cannula, consequences on blood parameters of (a) hypoxia (Nonnotte et al. 1993; Maxime et al. 1995) and (b) handling and stress (Williot 1997; Williot et al. 2011b) were studied. Electromyogram allowed assessing muscle and then swimming activity (McFarlane et al. 2004; Cooke et al. 2004; Martins et al. 2012).

44.6 Environmental Factors that May Impact the Sturgeon

Environmental factors, especially those related to water characteristics, are key factors. However, other factors such as light and noise, even if rarely documented—especially the noisy atmosphere—should be taken into consideration. The available data is gathered in Table 44.7.

Table 44.6 Synthesis on non-invasive methods used and/or tested in sturgeon species otherwise mentioned

Methods	Species	Objectives	Level of investigation	Sources
I. Non-invasive methods				
Behaviour	All species	Comparing behaviour to stereotypes (video-tracking camera and software)	Field	Martins et al. (2012)
External observations	Sturgeon species	Abnormalities and/or injuries	Field	Table 44.5
Echography	All sturgeon species	Sex determination Anatomy Abnormalities Staging of gonads Selection for caviar production	Field	Chebanov and Galich (2009, 2013); Chap. 49
Experimental shuttle box	*A. sturio*	Temperature preference	Laboratory	Staaks et al. (1999)
Experimental chamber	*A. baerii*	Cardiorespiratory motion (non-contact Doppler radar)	Laboratory	Hafner and Lubecke (2012)
	A. sturio	Behaviour, growth, survival, crossing, rearing environment	Laboratory	Carrera-Garcia et al. (2016)
II. Quasi non-invasive methods				
Thermostated respirometer and cannulation	*A. baerii*	Consequences of hypoxia on oxygen-related blood parameters	Laboratory	Nonnotte et al. (1993)
Thermostated respirometer and cannulation	*A. baerii*	Consequences of hypoxia on ventilatory activity and heart rate and blood stress parameters	Laboratory	Maxime et al. (1995)
Two-metre diameter tank and cannulation (catheterisation)	*A. baerii*	Profiles of hormones and stress indicators	Laboratory	Williot (1997, Williot et al. (2011a, b), Chaps. 17 and 25
Electromyogram (EMG)	Fish	Muscle activity Swimming activity	Field	McFarlane et al. (2004); Cooke et al. (2004) Martins et al. (2012)

Table 44.7 Environmental factors with potential impact on *A. baerii* otherwise mentioned

Factor	Consequences—optimum range and/or threshold	Sources
External factors		
Light	Sturgeon are afraid of direct radiation Under 100 lux of illumination, better growth of 1.5–15 g fish for longer photoperiod (12 L/D–24 L/D)	Williot et al. (1988) Ruchin (2007)
Aquatic plants	They can be a trap for sturgeon	Williot et al. (1988)
Water characteristics		
Oxygen (activity metabolism, Standard metabolism)	Minimum values (percentage of saturation) 10 °C (47%; 30%); 15 °C (59%; 38%); 20 °C (70%; 45%); 25 °C (82%; 53%)	Klyashtorin (1976) Williot et al. (1988) Chap. 50
Carbon dioxide	0.3–0.4 kPa (normal range) 0.10 kPa–1.5 kPa acid-base regulation ≥6 kPa hypercapnia and lethal respiratory acidosis 1 kPa < in water <6 kPa in using CO_2 anaesthesia	In *Acipenser transmontanus* Shaughnessy et al. (2015) Cech and Doroshov (2005)
pH	Optimum values 6.5–8.1	Williot et al. (1988)
Salinity	350–850 g Tolerance values, 5–10‰ Short period, 12.5–17‰ In summer	Ait-Fdil (1986) Bennouna (1986) Krayushkina and Moiseenko (1977) Krayushkina (2006)
Temperature (°C)	Optimum range (18–20) threshold (~1; ~32)	Williot et al. (1988) Amerio et al. (1999)
Water flow (speed)	*U* crit[5] = 1.5–1.9 $BL^6.s^{-1}$ BL = 62 ± 3 cm *U* crit[5] = 2.3±0.1 $BL^6.s^{-1}$ BL = 16–21 cm	Chap. 12 Yuan et al. (2016)
Nitrogen components		
Ammonia (pH ≈ 8.1)	24 h LC50[a] Larvae 60–260 mg: NH_3–N, 0.82 $mg.L^{-1}$ NH_4^+–N, 36.5 $mg.L^{-1}$ 450 g NH_3–N, 1.82 $mg.L^{-1}$ NH_4^+–N, 136.2 $mg.L^{-1}$	Salin and Williot (1991) Salin (1992)
Nitrate-N	96 h LC50 7 g, 1028 $mg.L^{-1}$ 67 g, 601 $mg.L^{-1}$ 675 g, 397 $mg.L^{-1}$	Hamlin (2006)
Nitrite-N	72 h LC50 175 g, 130 $mg.L^{-1}$ Chloride content In FW, 130.5 $mg.L^{-1}$ (3.7 mEq L^{-1})	Huertas et al. (2002) Chap. 23

[a]*24 h LC50* lethal concentration for 50% of the fish at the end of 24 h
[b]Critical swimming speed
[c]*BL* body length in cm

Regarding light, it has already been mentioned that Siberian sturgeon prefers a shadowed atmosphere and turbid waters (Sect. 44.4). Indeed, their outlook depends on the environment, nontransparent waters lead to normal colouration (Fig. 44.10), while a dark colouration reveals rearing in transparent waters (Fig. 44.11). In a search for the effects of photoperiod on growth of small juveniles of the species (1.5–19.5 g), Ruchin (2007) showed that maximum growth was recorded for 12, 16 and 24 h light as compared with darkness condition with a difference in the range of 8–18%. Furthermore, the author reported that the experiment was performed with

Fig. 44.10 Normal colouration of Siberian sturgeon specimen held in nontransparent water without cover in Russia (credit Galich K)

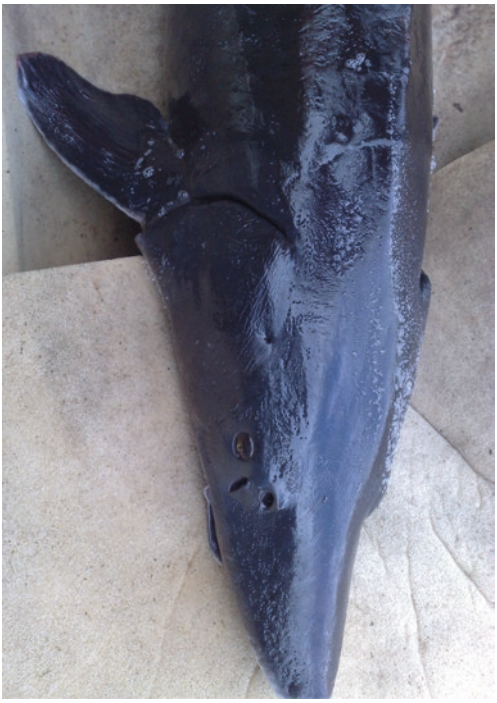

Fig. 44.11 Dark colouration of Siberian sturgeon specimen held in transparent water without cover in Armenia (credit Chebanov M)

100 lux illumination based on a previous experiment that demonstrated that maximum growth for those small fish was obtained with an illumination range of 30–800 lux.

Due to the anatomy of the species, the large quasi horizontal pectoral fins make the fish unable to keep out of vertical plants which act as a trap for the fish. This means that low slope of earthen pond banks have to be avoided.

The species is essentially a freshwater species which achieves long migrations from spawning grounds to deltas or upper estuaries of Siberian Rivers (Ruban 2005) where the salinity of the waters is low. Experimental studies have confirmed that the species is able to support water salinity in the range 5–10‰ (Ait-Fdil 1986) and possibly higher values (12–17‰) but only for a short period in summer (Bennouna 1986; Krayushkina and Moiseenko 1977; Krayushkina 2006).

Sturgeons are considered as oxygen resistant, i.e. they are able to survive low water oxygen concentration as expressed by the standard metabolism. However, it is the activity metabolism that should be taken into account to allow the fish to express all its potential especially growth. Minimum threshold values of these two metabolisms are given in percentage of saturation in oxygen depending on temperature. In practice this means that oxygen content is a key indicator for the fish welfare.

Fish produce a carbon dioxide quantity equivalent to the oxygen quantity consumed ($MO_2/MCO_2 \approx 1$). However, if the fish produce an oxygen uptake in water and need to use 5 to 30% of the total energy for muscle breathing to keep water passing over the gills, the CO_2 excretion is made easier by (1) the diffusibility of CO_2 through the gills and in water which is better than that of O_2, (2) the solubility coefficient of CO_2 in water which is higher than that of O_2, (3) one part of CO_2 being excreted as bicarbonate and (4) the CO_2 conversion in bicarbonate and carbonate.

In fish farming, the production of CO_2 by the fish is not a major problem except in acid water and in water recirculation circuits where pH can be low, PCO_2 increased and the bicarbonate cannot play the role of CO_2 absorber. The usual maximum threshold is 10 $mg.L^{-1}$ usually or 0.4–0.5 kPa (in air, the partial pressure of CO_2 is 0.03 kPa). High CO_2 partial pressures in water provoke excitation of the nervous system of the fish, hyperventilation, CO_2 loading in the blood and acidification of the blood which can be lethal if the CO_2 partial pressure increase is extended and higher than 5–6 kPa (Table 44.7).

Many fish species exhibit varying degrees of euryhalinity, being able to cope with short-term fluctuations of salinity. But very few migrate between the extremes of seawater and freshwater. Amongst the fishes which do, there are fishes (e.g. European sturgeon, *Acipenser sturio*, salmon, lampreys) which undergo anadromous (sea to freshwater) spawning migrations and some (e.g. eels) which undergo catadromous (fresh to seawater) spawning migrations.

In every case, the fishes use major osmoregulatory organs (gills, gut, kidney, salt glands in a few species) and several processes including metabolic processes to adapt to such aquatic environments and to keep the level of salts and other substances that dissolve in the blood solution of the body at a constant level.

Several species of sturgeon group such as the European sturgeon have moved to high seas as a part of their life story. As for the Siberian sturgeon, a species originating from Siberian Rivers, its euryhalinity capacity and its specificity have been more specifically examined by Krayushkina (2006). Although Siberian sturgeons from Lena River have short feeding migrations to estuaries, their capacities in osmotic and ionic regulation are limited and vary as a function of the season (Bennouna 1986; Ait-Fdil 1986). The maximum salinity seems to be near 10‰ (Table 44.7).

Water temperature is the primary factor when searching for an adequate site for Siberian sturgeon farming. And this supposes that ecological temperatures are known (Chap. 1) as well as the optimum range and threshold limits for the species. The optimum for growth seems to be close to 18–20 °C and the threshold values from ~1 °C to ~32 °C (Williot et al. 1988; Amerio et al. 1999) given that as soon as water temperature is ≥26–28 °C, care with feeding (low delivery of easily digestible food) is applied (Williot unpublished). Interestingly, neither difference was shown in growth or in food performance for 1.3–1.6 kg BW between 18 °C and 24 °C (Amerio et al. 1999). It is worth noting that these values do not take into account the specific needs for reproduction which are extensively developed in Chap. 27.

In aquatic environments, nitrogen enters in numerous forms, including inorganic and organic forms. Inorganic N is predominantly the sum of the nitrite, nitrate, ammonia and ammonium-N. Most inorganic N is typically in dissolved form in water. Organic N includes all substances in which N is bound to carbon. It occurs in soluble forms and is found in proteins, amino acids and urea. In nature, organic N can be biologically transformed into the ammonium form and then into the nitrite and nitrate forms.

Ammonotelic fish excrete ammonia NH_3 as the main product of the protein metabolism.

Amongst the different inorganic nitrogenous compounds (NH_4^+, NH_3, NO_2^-, NO_3^-) that fish may be exposed to in ambient waters, unionised ammonia (NH_3) is the most toxic, while in comparison, ammonium (NH_4^+) and nitrate ions are less toxic. Ammonium (NH_4^+) is the predominant form in the pH range of most natural water and is less toxic to fish as compared to NH_3. As the pH increases above 8, the ammonia (NH_3) fraction begins to increase rapidly. At pH 9, both forms would be nearly equal. Ammonium tends to be oxidised to nitrate in a two-step process ($NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$) by aerobic chemoautotrophic bacteria (*Nitrosomonas* and *Nitrobacter*, primarily), even if the level of oxygen is low. The toxicity of NH_3 increases with high temperatures and low levels of oxygen at low pH in water.

The toxicity of ammonia has been extensively studied in sturgeon (*Acipenser baerii*) by Salin and Williot (1991) and Salin (1992) (Chaps. 19–22). 24 h LC50 (lethal concentration for 50% of the fish at the end of 24 h) is also reported in Table 44.7. It therefore appears that the sensitivity to ammonia of Siberian sturgeon is age dependent. The youngest fish are more sensitive with a 24 h LC50 (larvae 60–260 mg (NH_3–N, 0.82 mg.L^{-1} and NH_4^+–N, 36.5 mg.L^{-1}) and fish 450 g (NH_3–N, 1.82 mg.L^{-1} and NH_4^+–N, 136.2 mg.L^{-1}), respectively).

The results of tests indicate that the 24 h LC50 is situated in the range of values found in other species. Siberian sturgeon is less sensitive than trout (*Oncorhynchus mykiss*) but more sensitive than catfish (*Ictalurus punctatus*). The tolerance level of sturgeon to ammonia is about the same as that of carp (*Cyprinus carpio*). However, it is sometimes difficult to establish direct comparisons, because the methods of calculating the LC50 are not the same and can, in some cases, lead to uncertain results.

Nitrite (NO_2) is typically an intermediate product when ammonium is transformed into nitrate by microscopic organisms and is therefore seldom elevated in unpolluted waters (Jensen 2003; Hamlin 2006). However, it is also a matter of great concern for intensive aquaculture. The high density of fish is associated with a large production of waste products, including ammonia excreted by the fish, with the potential accumulation of ammonia and nitrite to toxic levels (Hargeaves 1998). The tolerance limits of nitrites for the Siberian sturgeon fingerlings have been extensively studied by Huertas et al. (2002) and in Chap. 23 by Gisbert (Table 44.7). The 72 h LC50 of nitrite-N was 130 $mg.L^{-1}$ in freshwater with high chloride content (130.5 $mg.L^{-1}$), an effective protector against nitrite toxicity (William and Eddy 1986).

Nitrate (NO_3) does not normally reach toxic concentrations in natural environments or in recirculating systems with high water exchange and has therefore received less attention than ammonia and nitrite toxicity. Hamlin (2006) has determined the tolerance limits for Siberian sturgeon (Table 44.7). Three 96 h LC50 tests were conducted by this author, using 7, 67 and 675 g Siberian sturgeons. The 96 h LC50 results for nitrate-N were 1028 $mg.L^{-1}$, 601 $mg.L^{-1}$ and 397 $mg.L^{-1}$, respectively, indicating an increased receptiveness to nitrate with increasing weight. These findings demonstrate that nitrate may be an important pollutant for Siberian sturgeon reared in recirculating circuits with limited water exchange.

The last factor linked to water that might impact the welfare of the fish is the flow rate or more precisely the speed distribution of the water flow. Indeed it is related to the design of the rearing structures. The critical speed (*U*crit) is comprised between 1.5 and 1.9 BL s^{-1} with body length in cm (Chap. 12). This means that the speed of the current should be lower to allow the fish to avoid spending extra energy.

44.7 Effects of Characteristics of the Water on Physiological Functions

Table 44.8 presents a summary of the physiological disturbances known to be induced in Siberian sturgeon by changes of various ambient factors. Mechanisms accounting for these disturbances and the nature of compensatory responses, when present, have been indicated.

For instance, oxygen availability is essential for the fish livestock in fish farming. Hypoxia and hyperoxia, which are often carried out in ponds, both give rise to dramatic stress for the fish and have numerous physiological consequences. Our results (Chaps. 18 and 50; Williot et al. 1988) clearly demonstrate that the sturgeon is able to maintain a standard O_2 uptake during progressive hypoxia down to a critical

Table 44.8 Potential impacts of water characteristics on multiple physiological functions and mechanisms in *Acipenser baerii*

Water characteristics	Disturbances	Mechanisms accounting for the disturbance	Responses of the organism	References
Respiratory gases	Respiratory alkalosis and metabolic acidosis, Respiratory acidosis, Gill epithelium hyperplasia, Hypercapnic acidosis	Hyperventilation, ↓PCO_2, anaerobic metabolism (lactate)	Oxygen debt, anaerobic metabolism	Chap. 18, (1) (2) (3)
Oxygen				
Decrease		↓Ventilation and CO_2 retention	pH restoration, ↑[HCO_3^-]	(4)[a]
Increase		CO_2 loading	pH restoration, ↑[HCO_3^-]	(5)[b]
Carbon dioxide				
Increase				
Salinity	Metabolic acidosis, ↑[cl^-], ↑ [Na^+], ↑ [Posm][c]	Ionic and acid-base disturbances ↓pH, PCO_2 and [HCO_3^-]	pH restoration, extracellular anisosmotic regulation Cellular metabolic adjustments	(6) (7) (8)
Increase				
Freshwater pH	Ionic disturbances, Metabolic acidosis, Hypoxia	↓pH and [HCO_3^-] ↓[Cl^-], ↓[Na^+] Gill mucus hypersecretion	Ionic and acid-base compensation	(1)
Decrease (pH ≈ 4)				
Ammonia (Sublethal doses)	NH_3 influx, gill epithelium hypertrophy and necrosis, Metabolic and respiratory alkalosis	↓PO_2, ↑PNH_3 ↑pH, ↓PCO_2, ↑[K^+]	Hyperventilation Ionic regulation	(9) (10) Chap. 19
			Detoxification ↑ [glutamine] ↑ [glutamate]	Chap. 20 Chap. 21 Chap. 22
Nitrite	Ion regulatory, Respiratory processes	↓ blood oxygen transport ↑ [MetHb][d] Inhibition of Cl^- transport ↑ [lactate], ↑ [K^+] in plasma	Detoxification by oxidising nitrite to low-toxic nitrate	(11) Chap. 23
Nitrate	Ion regulatory, Respiratory processes	Inhibition of gill Na^+ and Cl^- transport, abnormal swimming ↓ Blood oxygen transport	Chronic health Welfare impacts	(11) (12) Chap. 23

[a]Great sturgeon *Huso huso*
[b]White sturgeon *Acipenser transmontanus*
[c]Osmotic pressure
[d]Methaemoglobin
PO_2 oxygen partial pressure, PCO_2 carbon dioxide partial pressure, PNH_3 ammonia partial pressure, HCO_3^- bicarbonate ion, Cl^- chloride ion, Na^+ sodium ion, K^+ kalium ion
(*1*): Williot et al. (1988); (*2*) Nonnotte et al. (1993); (*3*) Maxime et al. (1995); (*4*) Bagherzadeh Lakani et al. (2013); (*5*) Shaughnessy et al. (2015); (*6*) Ait-Fdil (1986); (*7*) Bennouna (1986); (*8*) Krayushkina (2006); (*9*) Salin (1992); (*10*) Salin and Williot (1991); (*11*) Huertas et al. (2002); (*12*) Hamlin (2006)

ambient PO_2 of 20–40 mmHg or 2.5–5 kPa at 15 °C. This is made possible thanks to a marked hyperventilation, as shown by increases in both frequency and amplitude of gill respiratory movements, which lead to alkalosis and hypocapnia.

Hyperoxia effects (short or long periods) have been studied by numerous authors (Dejours 1973; Truchot et al. 1980; Höbe et al. 1984) and more recently in *Huso huso* by Bagherzadeh Lakani et al. (2013). Facing these constraints, the fish make use of numerous adaptive mechanisms (acid-base regulation and ionoregulation) that allow maintaining the physiological functions in the best possible equilibrium.

Carbon dioxide increases provoke several ionic and acid-base disturbances; the most marked are metabolic hypercapnic acidosis, blood pH decrease and CO_2 loading. Baker et al. (2009) showed that the white sturgeon, *Acipenser transmontanus*, is very tolerant with severe aquatic hypercapnia (up at to 6 kPa PCO_2 in water) and extracellular acidosis, although it has an impressive ability to regulate intracellular pH. Conversely, at lower PCO_2 (1.5 kPa), white sturgeon can regulate blood pH. In fact, Shaughnessy et al. (2015) demonstrated, for the first time, the interactions of osmoregulatory and acid-base compensations in this sturgeon species when exposed to high PCO_2 and various salinities. Therefore, there is a need for such research into *Acipenser baerii* especially with the recent interest of fisheries and aquaculture professionals in the use of CO_2 anaesthesia.

The comparative studies of osmotic and ionic regulations in different *Acipenseridae* species have been extensively studied (Krayushkina and Moiseenko 1977; Ait-Fdil 1986; Krayushkina 2006). In *Acipenser baerii*, it appears clearly that the changes in plasma cortisol levels and Na^+/K^+-ATPase activities in the gills and kidneys after transfer into brackish water explain the osmotic and ionic regulation capacities and limits. The interactions between the adaptive capacities of this species to salinity and temperature have also been demonstrated since 1986 by Bennouna. In numerous fish species, data is also available in terms of food intake, stimulation of food conversion and fish growth which are dependent on the environmental salinity and temperature. The energetic cost generated by osmoregulation mechanisms has also been discussed (Kirschner 1995; Morgan and Iwama 1999; Boeuf and Payan 2001). However, to the best of our knowledge, a lot of information on these aspects has yet to be uncovered for Siberian sturgeon.

Some aquatic habitats are naturally acid, but since the beginning of the twentieth century, many regions have been chronically polluted by acid rain and snowfall, resulting from industrial emissions of sulphur and nitrogen oxides into the atmosphere (see Truchot 1987; Haines 1981). Acidification of water may also be a problem in closed circuit fish farming if PCO_2 increases and $[HCO_3^- + CO_3^{--}]$ cannot play their buffer role (Chap. 25).

Because of this serious environmental problem, numerous recent studies have dealt with the physiological effects of acid exposure in fish especially on the rainbow trout (Leivestad 1982; Wood and Mc Donald 1982). Typically, exposure to water with a pH of about 4.0 causes ionic disturbances and metabolic acidosis which must be compensated by osmoregulatory and acid-base mechanisms. Unfortunately,

the carbonate alkalinity of the water is never given, and the complete acid-base state of the ambient water (pH, $[HCO_3^{-}]$, $[CO_3^{--}]$, PCO_2) is never known.

For *Acipenser baerii*, Williot et al. (1988) reported optimum values of pH from 6.5 to 8.1 for a better growth.

Numerous pollutants such as ammonia (Salin and Williot 1991; Chaps. 19–22), nitrites (Huertas et al. 2002; Jensen 2003, Kroupova et al. 2005; Chap. 23) and nitrates (Hamlin 2006) pose serious risks to fish especially either in natural environment or in intensive fish farming conditions. Accordingly, a great deal of previous research has characterised toxicity effects on physiological mechanisms, gill structural changes and behaviour in fish exposed to contaminants. Nitrate and nitrite can reduce the oxygen-carrying ability in fish, i.e. *Acipenser baerii*. Haemoglobin, in fish exposed to nitrite, is converted into methaemoglobin that is unable to release oxygen to tissues, thereby causing hypoxia. Nitrate provokes an increase in the number of immature red blood cells and a lower level of mature blood cells, thereby causing anaemia. Significant damage, affecting osmoregulatory processes, at the gill and kidney levels, has been observed. Other toxic effects have been described including electrolyte imbalance, heart functioning problem, formation of compounds which can be mutagenic and carcinogenic, damage to liver cells and tissue oxygen shortage and increased vulnerability to bacterial and parasitic diseases (Camargo and Alonso 2006). Physiological responses of fish to pollutants are dependent on the bioavailability, uptake, accumulation and disposal of contaminants within the organism and on the interactive effects of multiple contaminants. In this regard, physiological responses are integrators of cellular and subcellular processes and may be indicative of the overall fitness of the individual organism and of the welfare of the fish.

As a conclusion, during their rearing stay in a fish farm or in natural ecosystems, the fish are confronted with various constraints: nutritional availability, health conditions and environmental specificities. Facing these constraints, the fish make use of numerous adaptive mechanisms that allow maintaining the physiological functions in the best possible equilibrium. The adaptive capacities of the animals to a given environment define their metabolic potential and the distribution of the metabolic possibility between the priority processes (survival) and the discretionary processes (growth and reproduction), finally their welfare all the more so.

The survival capacity and moreover the welfare of a living organism, i.e. the fish, depend on its aptitude to fulfill both conditions: (1) increase its metabolic resources as much as possible to face up to natural environmental constraints and (2) minimise its routine energetic needs and those linked to the adaptation to the environment.

In these conditions, if the difference between the energetic available resources and the real needs increases, all the physiological functions are optimised. The growth and welfare of the fish are ensured. On the contrary, any exposure to various stresses decreases the metabolic capacity of the animal which can reach its physiological limits and die.

44.8 Stunning and Slaughtering

Prior to transformation and further consumption, the final step of farming is the slaughtering of animals. It is preceded by stunning, and the whole process has to be humanely performed because stress and painful situation may deteriorate the quality of the carcass. Fish and especially sturgeons are no exception. To our knowledge, there is no published study focusing on slaughtering of sturgeon. Most of published studies are related to teleost fish with a special mention on the salmon *Salmo salar* (Table 44.9).

Additionally, even mentioned, the primary and very final steps before and after any stunning and slaughtering procedure are seldom taken into consideration. These primary and further post-slaughtering actions are fasting (or starvation) to empty the gut, capturing, crowding, moving, stunning and killing and further bleeding, gutting, washing and filleting (Lines and Spence 2011; Borderías and Sánchez-Alonso 2011, respectively) of the fish. Though seldom studied, the aforementioned primary steps are stressful and then should be taken into consideration to assess the welfare status of the fish. Essentially two complementary criteria are currently used to document the consequences stunning methods (and/or anaesthesia). There are EEG (electroencephalogram) and VERs (visual evoked responses). The former "determines loss and return of consciousness following stunning" (Robb and Roth 2003), and the second "is the response in the brain to flashes of light directed towards the eyes" (Van de Vis et al. 2003). The last authors conducted such an investigation on three species, the salmon (*S. salar*), the gilt-head sea bream and the eel in testing the consequences of different stunning methods, some of them being species specific together with their influence on quality of flesh. Flavour, hardness and some other quality assessment criteria (including visual appearance) were used to discriminate the investigated stunning processes. Altogether, the main synthetic conclusion is that electrical stunning is (or might be) a humane method provided that correct conditions are applied though bloodspot might be observed. Further works are required to prevent this defect. In another study with *S. salar*, Robb et al. (2000) showed that regardless of the accuracy of shooting, VERs were still lost more quickly than after exsanguination or carbon dioxide narcosis. Further it is concluded that carbon dioxide anaesthesia and gill cut without prior stunning may cause the Atlantic salmon to suffer pain. Finally, percussive stunning or spiking of the brain provided they are carried out accurately may be humane methods to kill the salmon. Live chilling followed by exsanguination appears to be highly stressful in *S. salar*, and muscle properties are deeply modified (Roth et al. 2007). A study on *Solea senegalensis* (Ribas et al. 2007) investigated the effects of three methods of anaesthetisation (clove oil, hypothermia and asphyxia) before slaughter on stress responses and final product quality assessed by rigor mortis, muscle pH, ATP/IMP ratios, eye refraction index and sensorial attributes. For this highly resistant species to hypoxic conditions, 1 $mL.L^{-1}$ clove oil was the best method for stunning sole because it not only ensures a good final quality product but also is acceptable for a direct human consumption.

Table 44.9 Some examples of stunning and slaughtering methods documented in fish with their subsequent effects on quality

Method	Species	Observations	Comments	Sources
Gill cut Or exsanguination	*Salmo salar* (2–3 kg)	VERs not immediately lost Vigorous movements occurred Fish were not rendered unconscious	Non-humane stunning	Van de Vis et al. (2003)
Carbon dioxide (CO_2)		Fish were not rendered unconscious Fish move vigorously during application	Non-humane stunning	
Instrumental percussion or percussive stunning Or blow		Concussion might irrevocable; however, often fish are hit more than once Pneumatic gun	Non-humane stunning Humane stunning	
Hollow punch or spike		Pneumatic gun adapted with hollow bolt that penetrate 29 mm into the head show that brain function was not lost immediately A high level of precision is needed of which application is difficult	Non-humane stunning Might be humane	
Electrical stunning (50 nHz a.c., 4.6 A for 3 s in seawater)		VERs and self-initiated behaviour lost immediately	Can be humane	
Immersion in ice slurry (~1 °C)	Gilt-head sea bream (*Sparus aurata*) 262 g (2 days of fasting)	Ability to perform self-initiated behaviour and VERs were lost after 5 min Vigorous movements which may indicate stress were observed	Non-humane stunning	
Asphyxia in air and possible transfer in ice slurry		No immediate brain dysfunction	Non-humane stunning	
Percussive stunning		Pneumatic gun above-mentioned applied either laterally or to the top of the head Self-initiated behaviour lost immediately	Can be humane	
Electrical (head only) (50 Hz, 80 V)		Fish require more than 200 mA across the head Exact minimum current to achieve stunning remains to be determined	Further studies are needed	
Salt bath (for desliming) or with Na_2CO_3)	Eel (*Anguilla anguilla*)	VERs not lost immediately Vigorous attempts to escape occurred	Non-humane stunning	
Live chilling and further freezing in cold brine (−20 to −16 °C)		Vigorous attempts to escape occurred EEG revealed irregular heart activity which may indicate stress	Non-humane stunning	
Electrical stunning [17 A, (200 V, 50 Hz a.c.) for 1 s]		Immediate unconsciousness Self-initiated behaviour immediately lost	Humane stunning	

(continued)

Table 44.9 (continued)

Method	Species	Observations	Comments	Sources
Gill cut	Salmon *Salmo Salar* (3–7 kg)	Vigorous movements in 30 s and ceased after 7 min	Non-humane stunning	Robb et al. (2000)
Carbon dioxide (CO_2)		Fish shook vigorously their heads and tails for about 2 min After removal from the water, and further cutting gill arches, two fish out of ten showed some movements in response to the cut. They made weak head shakes and tail flaps for up to 4 min	Non-humane stunning	
Percussive blow		Some tail flaps may be observed depending on the shot	Depends on the shot	
Spiking		On removal of the water, the fish showed the same response as in gill cut and percussive blow group. After a good shot, fish instantly became still. In one fish the brain was missed		
Percussive stunning	*S. salar*	The proportion of fish stunned was significantly dependent on the applied force used in the system. Eye injuries as haemorrhaging and eye bursts increased with the effective force. A hammer-shaped cylinder proved to be the most suitable	May be humane method	Roth et al. (2007)
Electric stunning	*Oncorhynchus mykiss* (~200 g)	Sinusoidal current 1000 Hz 250 V/m r.m.s. during 60 s Absence of simultaneous measurements of VERs	Preferred than asphyxiation in ice slurry	Lines et al. (2003)

Percussive stun after crowding	*S. Salar*			Roth et al. (2007)
Percussive stun after crowding, pumping and live chilling		[live-chilled fish were exposed to seawater (2 °C) saturated with CO_2 (pH 5.5–5.7) for 40 min] After chilling, fish were calm but not unconscious, as eye rolling was observed in all individuals pH ↓ and earlier rigor mortis showed that high muscle activity occurred during the process	Live chilling either in combination with CO_2 or with subsequent exsanguination of the unstunned fish appears to be highly stressful	
Exsanguination after crowding pumping and live chilling				
Clove oil (1 mL.L^{-1})	Sole (*Solea senegalensis*) BW = 155 g; TL = ~23 cm	Several pre- and post-mortem indicators were considered: Rigor mortis, muscle pH, ATP/IMP ratios, eye refraction index, sensorial attributes tested the quality Cortisol, glucose and osmolality tested the stress	Good quality final product which may be directly consumable	Ribas et al. (2007)
Hypothermia (16 °C to 2–4 °C)				
Asphyxia				

VERs visual evoked responses. The method has been developed long time ago to analyse the reaction of mammals unable to speak to some stressful situation

Some outcomes might be suggested from this data set that (a) hypothermia (ice slurry or live chilling) and asphyxia do not make the fish unconscious and then cannot be considered as humane killing, (b) percussive stunning might be humane depending on the shot and (c) electrical stunning might be humane as well whether applied current is accurately determined. An illustration of the last comment is given by Robb and Roth (2003) who achieved this task, thanks to a specific investigation destined to define the best field strengths and pulsations by using EEG and VERs. However, it is worth noting that other authors (Poli et al. 2005) concluded that electrical stunning was more stressful than spiked, knocked and live-chilled fish. This outlines on the one hand that comparisons (and the extrapolations) should be very carefully carried out and on the other hand that first processing steps of fish are species dependent (Borderías and Sánchez-Alonso 2011).

In a previous review, Robb and Kestin (2002) concluded that despite killing methods are diverse, they can be classified into two categories, those that induce a loss of sensibility slowly and those that achieve that rapidly. Further, those that induce a loss of sensibility over a long period tend to impinge more on the welfare and are detrimental to overall quality of the carcass.

In the absence of any documented investigations in this field on sturgeon, the aforementioned brief synthesis may provide documented examples on stunning-slaughtering methods that do not impair the welfare of Siberian sturgeon. At present, at least three stunning methods are used by sturgeon farmers: (a) percussive stunning, (b) ice slurry (Fig. 44.5) and (c) electronarcosis (Fig. 44.6). Exsanguination by cutting the gill arches allows further fish processing, i.e. gutting, extraction of ovaries for further malossol-type caviar processing and filleting. It is worth noting that whether there are no anymore doubts on the impact of stunning practices on the quality of the flesh, there is no attempt to document the potential effects of all the final process around the stunning on the subsequent malossol-like caviar.

44.9 Synthesis on Siberian Sturgeon Welfare

Welfare is a complex task that needs to embrace all the rearing aspects and management actions along the production cycle. And this should be doable at each step of the production process. This is an integrating process that needs a good knowledge of the breeding on the whole to allowing detecting and/or solving as rapidly as possible symptoms of impaired welfare. In addition, especially in case of sturgeon devoted to reproduction or caviar production that needs numerous years, one should be able to also explain a posteriori abnormal situation which supposes that criteria are recorded on the one hand together with a good knowledge of involved physiological mechanisms (Table 44.8) and those involved in the immunology (Chap. 43) on the other hand. And the best way to do that is to anticipate the impaired welfare in order to limit their impact when they are lately highlighted. Two approaches were proposed recently. One is to set up Fish Welfare Assurance System (FWAS) at a farm level (Van de Vis et al. 2012). Quality Assurance (QA) provides a system focused on control of processes. And according to the authors, QA is focused on

identification of hazards and establishment of critical steps that need to be controlled in a given process to prevent, eliminate or minimise a hazard to an acceptable level. And the Quality Assurance system is known as Hazard Analysis and Critical Control Points (HACCP) that can be used as a framework for fish welfare assurance at farms that is encouraged at the European level to be incorporated by all food manufacturing (Van de Vis et al. 2012). The second approach, due to Müller-Graf et al. (2012), consists in determining the likelihood and consequences of an adverse event which is referred to as a hazard. This is a risk assessment methodology to fish welfare developed in the context of the European Food Safety Authority (EFSA). It is remarkable that both approaches aiming at improving the welfare status of fish are occurring in a European context, i.e. under European incentives.

Along the present chapter, most of the useful basic available data are provided to allow building such a welfare analysis on the Siberian sturgeon; they are synthesised in Table 44.10**,** with some complements. At a time of site determination, great care to water supply should be paid. With regard to the objective of production, stocks are then determined and further the demand in oxygen with data of Chap. 50. Whatever the production system, running water or RAS,[2] water quality is the primary key factor that should be controlled on a regular basis. Carbon dioxide level, water pH and buffering capacity must also retain a particular attention in RAS. It is worth recalling that peculiar requirements for brood fish and early rearing (fertilised eggs, larvae and young fingerlings) are given in their specific chapters (Chaps. 27 and 29). External characteristics that should take into consideration are given in Table 44.7 with a main focus on water characteristics. As far as possible, the range and/or threshold values are provided. The conception of rearing structures has to respect as much as possible the species requirements (shadowed atmosphere) and to make easier the handling of the fish and the task of workers. It has to remind that the size of the females by the end of the biological cycle might be in the range of 5–12 kg each which makes their care a concern.

The primary production factor in fish farming, and sturgeon is no exception, is the food and feeding management. The acceptability, i.e. the palatability, of compound diet by the Siberian sturgeon is considerably improved with extruded pellets. Gross composition in terms of proteins and lipids has been determined (Chap. 30) as well as both interest and care for some additive or replacement of ingredients (Chaps. 31–33). However, appearance of the liver is often abnormal, grey-brown instead of red-brown, therefore suggesting a suffering physiological status of hepatocytes due to an inappropriate food composition. Many managing tasks may be achieved in respect to fish welfare, thanks to echography; there are sex determination, control of gametogenesis, oogenesis in particular and internal abnormalities (Chebanov and Galich 2009; Chap. 49). Fish can thus be redistributed over the raceways by pipes supplied with water to avoid any risk of impairing the welfare of the fish as well as that of workers (Figs. 44.2 and 44.3). The aforementioned welfare redistributing fish within the farm may also be of use after weighing.

[2] RAS = Recirculated Aquaculture System.

Table 44.10 Synthesis of suggestions for farming welfare of Siberian sturgeon

Sturgeon farming issues	Suggested improvement	Sources
Water supply		
– Quantity and quality	Oxygen demand Regular survey of main parameters with current control of record devices	Williot et al. (1988), Chap. 50 Table 44.7
Rearing structures and conception		
– General conception – Shape, materials, hydraulics – Environment	Ease handling of the fish and work of farmers Tank's cover	Table 44.7
Fish management (husbandry)		
– Feeding process	Composition Presentation Delivering process	Chaps. 30–33
– Sex determination – Control of gametogenesis – Detection of internal abnormalities	Echography	Chap. 49, Chebanov and Galich (2009),
– Weighing, grading, sorting	Transferring of fish from tank to tank, thanks to pipes and water to avoid air transportation and limit risks of fall for fish and farmers Stress-free sorting device inside rearing tank	Figs. 44.2 and 44.3 Hufschmied et al. (2011)
– Handling	Anaesthesia in case of long operation (tagging, vaccination, surgery, cannulation, etc.) with continuous water supply in the month to allow them breathing normally	Tables 44.3 and 44.4 Doroshov et al. (1983)
– Density	No effect of density (~7 to 22 kg m^{-2}) in 608 g BW & 68 cm TL fish on stress indicators	Hasanalipour et al. (2013)
– Behaviour	Abnormal vs. normal Unusual	Table 44.5
– Abnormalities and injuries	Daily survey Monitoring and recording data systems	Figs. 44.7, 44.8 and 44.9 Table 44.5 Noble et al. (2012)
– Mortality	Daily survey Monitoring and recording data systems	Ellis et al. (2012)
– Stunning and slaughtering	Some methods to be improved	Table 44.9

Table 44.10 (continued)

Sturgeon farming issues	Suggested improvement	Sources
– Practitioners	Training courses	Table 44.2
Comprehensive knowledge of the breeding facility		
– Anatomy and anatomopathology	Non-invasive methods	Table 47.6
	Anaesthesia	Chap. 49 Tables 44.3 and 44.4
– Physiology	Physiological functions and mechanisms	Table 44.8
	Non-invasive methods	Table 44.6
	Anaesthesia	Tables 44.3 and 44.4
– Monitoring and recording systems	Water characteristics	
	Fish related data	This chapter
	Data bank system, software, data collection system	Williot, unpublished
	Fish tagging system	

Hufschmied et al. (2011) proposed to set up a sorting system on rearing raceway, thus to considerably improve the welfare of the fish by limiting handlings. Any handling should be preceded by anaesthesia (Tables 44.3 and 44.4) and safe organised working place. An important husbandry-related factor is quasi absent from any investigations; this is the density with the exception of the work from Hasanalipour et al. (2013) that showed that there were no differences on cortisol, glucose and cholesterol levels for densities in the range of 12–36 individuals m^{-2} (608 g BW) or about 7–22 $kg.m^{-2}$. Further, the cortisol level remained at a low level (7.8–13.1 $ng.mL^{-1}$) very close to the basic level (7 $ng.mL^{-1}$) reported by Williot et al. (2011b). Therefore none of the tested stocking density showed deleterious effects on some primary stress indicators in the experimental conditions reported by the authors. Behaviour is one of the very few factors to be favoured with regard to welfare assessment. A preliminary synthesis of observed changes in behaviour is provided by Table 44.5. It is worth noting that the field is poorly documented. There are some evident observations regarding abnormalities and injuries (Figs. 44.7, 44.8 and 44.9; Table 44.5). The reported examples are mostly sturgeon specific as compared to an already published similar synthesis (Noble et al. 2012). To our knowledge, the question of abnormalities in shape remains an unsolved issue. The authors highlighted that these observations allow potential short- and long-term farming practices and mitigation strategies to reduce the evidence and prevalence of these injuries. In a similar way, long-term mortality rates can be used as a retrospective welfare performance and short-term mortality rates as an operational welfare indicator (Ellis et al. 2012). According to the authors, "scrutiny of mortality records band determining causes of death will enable action to be taken to avoid further preventable mortality". With a focus on the last two preceding issues, a strong suggestion is to perform a steady survey of all the rearing structures with an attentive look at all the tanks in order to detect as soon as possible abnormal status of fish and/or of critical rearing points. By the end of the production cycle, the last steps of the production process are stunning and slaughtering of the fish.

This is true also for ovulated eggs-based caviar at a time when females are reformed. Significant examples are given in Table 44.9. The main outcomes from works on teleost fish are as follows. Gill cut, carbon dioxide and hypothermia do not render the fish unconscious and then are considered as non-humane stunning methods. Percussive stunning and electronarcosis can be considered as humane method when they are well defined and adapted. An important welfare-related task is the training courses for the workers. Indeed welfare, like quality issues, has to be considered as a general concern for all the people working on a given farm. The last group of welfare-related issues is a transversal one, with the aim of which is to get a comprehensive knowledge of the functioning of the breeding facility. To do that, one has to be able to collect data on fish, anatomy and morphology in the one hand and physiology in the other hand depending on precise objective. Anaesthesia of fish might be necessary as well as non-invasive methods. Collection of physiological parameters might be mandatory when searching for consequences of rearing factor (water characteristics, food, etc.) suspected to impacting the fish. Female sturgeons are often aged; thus, history of tagged specimen provides the possibility to build a several-year image enabling to make a diagnosis over time. Appropriate software and data system are then key points that are also used for traceability of ended products.

It has to be recalled that some selection of methods, e.g. anaesthetic, stunning and slaughtering process, is not only performed on a scientific basis but has to be in agreement with local regulation.

44.10 Some Conclusions

To our knowledge, the present chapter is the primary attempt to both synthesise and analyse the welfare-related data on sturgeon with a peculiar focus on the Siberian sturgeon. As recalled in the introduction, due to the long production cycle, this is the interest of the farmers to take care of the fish, mainly of the females destined to the production of caviar. Thus, some appropriate solutions that do not impair the welfare of the fish are already at works. However, with the development of the chapter, it became evident that there are some under-documented fields.

Addressing to welfare, one of the very first approaches that come in mind is the behaviour and its related issues. And this field is poorly documented on the ongrowing phase. As pointed out by Martins et al. (2012), studies on establishing a library of stereotypes (or patterns) that can be used as references to highlight deviations are needed. This has to be achieved with specific devices such as video camera and data treatment software. In addition, to what extent the behaviour is impacted by the stocking density? Which are the relationships between the density and the growth, between density and welfare? The maximum density (about 22 $kg.m^{-2}$) of the only published study on the couple density-blood characteristics (Hasanalipour et al. 2013), though interesting, is far lower than most of recorded stocking densities in ongrowing sturgeon farms with larger fish. In case density assessment is a concern,

it might be interesting to explore a solution that does not need to handle the fish and could be able to document the behaviour and growth as well (Conti et al. 2006). The process is based on the use of acoustical telemetry and was primarily tested on some marine fish. Noisy atmosphere is suspected to be potentially disturbing for the sturgeon. Very few are known in the field except that it has been suggested a relationship between production of acoustic signals during the reproduction period by sturgeon brood fish (*Acipenser nudiventris* ♂) according to Tolstoganova (1999).

It was recalled that sturgeon, namely, the Siberian sturgeon, was afraid from direct light (Sect. 44.1). This would mean that sturgeon is sensible at least to some wavelength (Ruchin 2007) or to an amount of light. It has been demonstrated that "the retina of the Siberian sturgeon has the colour-processing circuitry common for all ray-finned fishes" (Govardovskii et al. 1991) and thus suggesting they are anatomically equipped to detect colour light. This opens an additional potentially welfare indicator through eye darkening which is an easy and inexpensive indicator of stress (Freitas et al. 2014). This eventuality should be investigated in relation with lighting. Recently it has been designed a system based on the vision to manage the feeding of fish (Jer-Vui Lee et al. 2013). The authors declared that the system can automatically feed the fishes by estimating fishes' appetite through machine vision. "With the developed algorithm, the system is able to detect the presence of fishes and count the number of fishes, to estimate and infer the fish appetite and further planning the feeding time". Feeding time may affect fish welfare (López-Olmeda et al. 2012) and nothing is known in the field with regard to the Siberian sturgeon. A close-related issue deals with the feeding system itself, and some authors based on a review are inclined to suggest "that demand feeders could be used to improve welfare by allowing fish to meet their nutritional needs" (Attia et al. 2012). Similarly, it was reported that rainbow trout "may be able to control feed availability using their behaviour patterns, with feed being presented upon demand" (McFarlane et al. 2004). We highlighted that humane stunning-slaughtering processes might be achieved in sturgeon, thanks to some investigations and improvements on a few methods. Preliminary studies explore the usefulness of blood indicators as potential tools for assessment of welfare of the species (Simide et al. 2016; Chap. 45). The first study reported on heat shock protein but not only and the second gives an updated synthesis on blood indicators that might be of use in the field. More, in both studies, authors proposed the use of data analysis, namely PCA[3] as an appropriate method to treat together the collected data. All these approaches regarding indicators and analysis should be promoted, developed and thus encouraged.

A last subject has to be mentioned even at present no one knows how to easily take into consideration the long-term effect of low concentrations of pollutants such as the nitrogen components.

[3] PCA = Principal Component Analysis.

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The Blood Indicators of Siberian Sturgeon Welfare

45

Rémy Simide, Sandrine Gaillard, and Simone Richard

Abstract

Animal welfare science is expanding rapidly. The use of physiological indicators is one of the most investigated ways in which to evaluate welfare. Blood samples are minimally invasive; they enable successive sampling of the same individuals and provide access to a large range of indicators from numerous physiological functions. Thus, they provide all the useful features for welfare research. The welfare of Siberian sturgeon is a very recent consideration. This chapter gathers together indicators from bibliography and our analysis which have been or could be used to assess this. The current research on stress responses and health status in Siberian sturgeon aids in the collation of this extensive information. Blood samples enable monitoring of hormonal response, biochemical and hydromineral indicators, oxidative stress parameters, immune status, hematological markers, and molecular indicators. The integration of these numerous physiological parameters in the context of welfare assessment is discussed throughout this chapter. Measuring physiological indicators from blood samples analyzed by multivariate analysis could be one of the future standards in the monitoring of fish welfare.

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Welfare • *Acipenser baerii* • Blood sampling • Physiological functions • Multivariate analysis

Introduction

Animal welfare science can be ordered into three large concepts based on different facets of welfare (Huntingford and Kadri 2009). There is (1) the feeling-based definition which focuses on the mental and emotional state of the animal. Briefly, it is the current emotional state of the fish which has to be understood as a continuum between bad and good feelings (Ellis et al. 2012; Martins et al. 2012). (2) The function-based definition spotlights biological functioning at the physiological level. The simplest definition of welfare from the functional point of view is that an animal has to be able to cope with its environment (Ellis et al. 2012). The least explored definition is (3) the nature-based definition, which implies that an animal can lead a natural life and express its natural behavior (Huntingford et al. 2006).

The scientific debate on whether fish have feelings is not yet over (Brown 2015; Rose et al. 2014). Moreover, tools to assess feeling-based welfare of wild and farmed fish are still lacking, even if this field of application is moving toward development (Duncan 2005; Brown 2015). In this context, the function-based definition is currently the most investigated and documented scientific methodology for the assessment of fish welfare. Thus, this chapter will only focus on welfare from a physiological point of view. Studies conducted on biological functions monitor the physiological stress response and the health status of fish. Indeed, under good welfare conditions, any characteristic physiological response is expected because life is not threatened or difficult (Volpato et al. 2007). In such cases, monitoring of the stress response is the only identifiable means to assess welfare (Duncan 2005; Veissier and Boissy 2007). Recently, health status monitoring for the assessment of welfare has arisen as an essential axis that satisfies the very definition of functional-based welfare (Ashley 2007; Segner et al. 2012). This notion is not only restricted to monitoring and control of pathologies (Nicks and Vandenheede 2014; OIE 2015). Good welfare requires a comprehensive ability to supply all the energetic demands of the organism (Duncan 2005; Segner et al. 2012). Poor health leads to a decrease in welfare, and poor welfare could induce a decrease in health (Ellis et al. 2012). Furthermore, the allostasis concept (McEwen and Wingfield 2003), which includes the notion of energetic equilibrium between the different biological functions of the animal, is increasingly taken into account in assessment of welfare (Korte et al. 2007; Prunet et al. 2012). Monitoring of welfare should be carried out following an appraisal of the energetic balance between different functions of the organism in order to evaluate the presence or absence of physiological trade-offs which risk impairing its integrity. Without current capabilities to truly measure the energetic consumption of each function, the monitoring of indicators of several key physiological functions could be an adequate substitute. Analysis of these data through multivariate tools allows to efficiently represent

the variations among these indicators. To learn more about fish and sturgeon welfare, the previous Chap. 44 of this book by Williot et al. should be consulted.

In sturgeon, welfare has only recently been considered (Eslamloo and Falahatkar 2014; Simide et al. 2016). Thanks to data from published studies on stress response and health status, a great deal of informations is available about potential indicators in Siberian sturgeon (*Acipenser baerii*) welfare. Each biomarker is supposed to represent one or few physiological functions. In example, the lysozyme is generally used as an indicator of the immune system, but its activity also depends on numerous stressing situations. The cortisol is a hormonal stress indicator (mainly acute stress), while the heat shock proteins (HSP) are generally measured to assess the cellular stress response (mainly acclimation attempt), but they are also chaperone proteins involved in metabolism. Firstly, we will discuss the benefits of blood samples and make some recommendations about them. Then, we will make a bibliographic synthesis of the biomarkers measured in Siberian sturgeon that are suitable in a welfare context, and we will discuss their use with our own data.

45.1 Material and Methods

Three batches of fishes were used.

In the first batch, the Siberian sturgeon were raised in the recirculating aquaculture system (RAS) of French producers, Picton and Sturgeon SCEA Company. Blood sampling was performed every 3 months in 20 fishes selected randomly from a pond. Fishes had 2.5 years old and weight 2.5 kg at the beginning of the experiment. Any modification of the mortality rate or in appearance of pathology emerges in the period of the experiment like any zootechnical issues. *Hsp70* and *tgf-β* expressions were analyzed in erythrocytes. RNA was extracted with a column extraction kit (NucleoSpin 8 RNA, Macherey-Nagel, Germany) with modifications. An initial purification step that involved mixing Extract-All (Eurobio, France) and chloroform was done, and a supplementary rDNase treatment was added. Double-strand cDNA was synthesized from total RNA according to the manufacturer's manual (Omniscript, Qiagen, Germany). Real-time PCR quantifications were performed on a LightCycler 480 (Roche, Germany). The reaction mix consisted of 5 µL of master mix (Roche, Germany), 1 µL of each primer (1 µM final), 1 µL of molecular grade water, and 2 µL of cDNA. All assays were carried out in duplicate, and a calibrator was added in order to standardize runs with each other. The following primers were hsp70-forward (5′-CATCCTGAACGTTTCTGCA-3′), hsp70-reverse (5′-TTCTCACGCTGCA-CATCATC-3′), tgfb-forward (5′-CGAACCCAAAGGCTACTACG-3′), and tgfb-reverse (5′-CGATCATGTTGGAGAGTTGC-3′). Target gene expressions were normalized to two reference genes, *β-actin* and *B2m*, with the primers actin-forward (5′-TATCCTGACCCTGAAGTACCCAATC-3′), actin-reverse (5′-CACGCAGCT-CATTGTAGAAGGTGTG-3′), B2m-forward (5′-GCTTCCACCCTCCCAA-CATC-3′), and B2m-reverse (5′-GAGTAGTGCTCTCCCTCCTTGG-3′). Specificity of the quantification assays was confirmed by migration on agarose gel of 15 amplification products. The PCR conditions were an initial denaturation at 95 °C for 10 min

followed by 40 amplification cycles at 95 °C for 10 s, 58 °C for 20 s, and 72 °C for 35 s. After each experiment, melting curves for every sample were analyzed to verify whether there was any by-product amplification.

In the second batch, the Siberian sturgeon were raised in the experimental facilities of the Institut Océanographique Paul Ricard (IOPR). The rearing system was composed of twenty 100 L (60 × 50 × 34 cm) fiberglass tanks with continuous filtration and a water renewal of 50 L per tank each day. Water quality parameters were measured daily: dissolved oxygen ($O_2 \geq 90\%$), ammonium (NH_4^+ <0.25 mg L^{-1}), nitrate (NO_3^- <12.5 mg mL^{-1}), and nitrite (NO_2^- <0.3 mg mL^{-1}). Temperature was automatically adjusted at 20 °C. The 4-month-old fishes 52.2 ± 12.3 g (mean ± SD) were divided in three groups of ten replicates. One group was fed with the control diet (EFICO Sigma 841, BioMar, France), another was fed with the diet containing a β-glucan-type prebiotic (0.1%, MacroGard, Orffa, Netherlands), and the last was fed with the diet containing vitamins (0.5%, Alpifish, Alpifeed, France). This period of complementation lasted 1 month. Then, half of the fishes from all groups were stressed by an increased temperature of 30 °C during 1 month. This extreme temperature was chosen to push the animal to express an intense stress response. At the end of the experimentation, blood samples were collected from caudal puncture in anesthetized fish by 90 mg L^{-1} of clove oil (following Feng et al. recommendation in 2011). After centrifugation, plasma was conserved at −80 °C. Lysozyme activity was measured from the protocol described by Simide et al. 2016. Briefly, 100 µL of a suspension of *M. lysodeikticus* in PBS was mixed with 15 µL of plasma. Reduction in absorbance at 450 nm depends on the lysis of the cells by the lysozyme. Hematocrit (Hct; %) was measured by centrifugation of total blood freshly collected in standard heparinized microhematocrit capillary tubes. Twenty-four stressed fish were then sacrificed, and samples of gill, liver, and intestine were conserved in RNA later at −80 °C until further analysis. RNA extraction and qPCR were processed as detailed above. The primers for the *ubiquitin*, the *caspase 3,* and the *insulin-like growth factor 1* were ubq-forward (5′-ACTCCTTCTGGATGTTGTAGTCGG-3′), ubq-reverse (5′-TTGAGCCCAGTGACACCATTGAGAAC-3′), casp3-forward (5′-AATCTGCTTGCAGGTCTGGTC-3′), casp3-reverse (5′-GATGCGGGAAATCTCTTGAA-3′), igf1-forward (5′-TTGTAGTTCTGGGATCCATGGG-3′), and igf1-reverse (5′-ACATCACACAAGTGCCACTG-3′).

In the third batch, the sturgeon came from the experimental facilities of the IOPR and rose like the control fishes of the previous batch. 1 mL of blood was sampled from ten fishes of 5-month-old with weight 111.5 ± 28.3 g (mean ± SD) without anesthesia. These fishes were then marked with T-tags (Floy Tag & Manufacturing, Inc., USA). No mortality was observed. One month later, these tagged fish (resampled) and ten others (control) raised in the same tanks were sampled following the same procedure. Lysozyme activity, hematocrit, and *hsp* expressions were measured as above. The other parameters were measured following the protocols described in Simide et al. 2016. Briefly, the complement activity was associated to the capacity of plasma to lysis a solution of 2% rabbit red blood cells (in EGTA-Mg-GVB buffer) measured by absorbance at 450 nm. The dilution of plasma that would produce 50% lysis was determined by probit analysis, and results were expressed as ACH50 (units per mL). The number of leukocytes was determined following

the Natt-Herricks-TIC (Bioanalytic) protocol. The plasma concentration of oxidative metabolites (mostly hydroperoxides) was measured using the d-ROMs test (Diacron International) following the manufacturer's protocol with an adaptation to micromethod. Antioxidant defense was evaluated using the commercial OxiSelect Total Antioxidant Capacity (TAC) assay kit (Cell Biolabs, Inc.), according to the manufacturer's instructions.

For each measured parameter, normality was tested. Used statistical analysis is specified in the text. If necessary, post hoc comparison was Student-Newman-Keuls (SNK). Statistical analysis was carried out using the program R version 3.1.2 (R Core Team, 2014). Graphics were performed with GraphPad Prism 6 (GraphPad Software, Inc.). All tests were two-tailed, and P values ≤ 0.05 were considered significant. Results are given as mean ± SD.

45.2 Results

45.2.1 Blood Sampling

Different types of sampling can be performed to monitor physiological functions linked to sturgeon welfare. (1) Lethal sampling is achieved on organs or whole individuals (for larvae). Nonlethal sampling can be invasive (necessarily stressful) or noninvasive. (2) Noninvasive sampling can be conducted on feces (Ellis et al. 2012), on the mucus for immunitary monitoring (Alexander and Ingram 1992; Shephard 1994; Segner et al. 2012), or directly from water, which is a promising thematic for the monitoring of hormonal reactions in fish (Scott and Ellis 2007). (3) Blood sampling is invasive but nonlethal. Other invasive and nonlethal samples could be achieved in the future from the larval yolk sac or peritoneal fluid in adults, but further research needs to be conducted in sturgeon (Zuccarelli et al. 2008; Linares-Casenave et al. 2013).

Blood sampling has many advantages. The most obvious is that the fish do not need to be killed. This allows longitudinal sampling (i.e., repeated sampling of the same individuals) to be carried out. Nonlethal sampling is also advantageous when working with economically valuable female sturgeon in aquaculture, but also with highly ecologically valuable wild populations. Thus, it is certainly better from a philosophical and ethical point of view not to kill fish in order to monitor their welfare. Physiological parameters are conveyed by the blood in the body. Blood consists of two different parts separated by a centrifugation (e.g. 10 min, 2500 g), the formed elements with the cells and the plasma with the soluble elements (or serum if the sampling is achieved without anticoagulant). Blood samples can be conveniently conserved, and each sampling (in hundreds of microliters) is sufficient to follow various physiological compartments at the same time, which is a prerequisite for welfare assessment. Thus, blood samples provide all of the useful features for welfare research. Blood sampling is easily accomplished in sturgeon, generally from a caudal puncture. In yearling sturgeon, where a small needle is required (e.g. gauge 25), the sampling can be made easier by prior flushing of the needle and syringe with an anticoagulant. All these advantages make blood samples an ideal candidate for a sampling campaign (wild or aquaculture).

Handling, air exposure, and blood sampling induce stress in fish. In welfare research this is problematic, particularly for longitudinal studies. In order to limit unwanted stress, facilitate sampling, and increase the repeatability of the procedure, it is possible to anesthetize the fish. However, caution must be shown when using chemical anesthetic products for research purposes. They can induce bias in several physiological parameters such as the cortisol rate or osmolarity in fish (Zahl et al. 2012), including sturgeon (Cataldi et al. 1998; Matsche 2011). In Siberian sturgeon, anesthesia with clove oil (eugenol) or tricaine methanesulfonate (MS-222) induces rapid swelling and destruction of erythrocytes together with variations in numerous biochemical indicators (Gomulka et al. 2008). After 24 h, a strong immunosuppressive effect is observed through a diminution of leukocyte numbers (Gomulka et al. 2008). However, appropriate dosage of these two anesthetic products helps to limit their physiological impact in Siberian sturgeon (Feng et al. 2011). Thus, it is advisable to test the chosen anesthetic protocol in several individuals before beginning experimentation. Limiting induced stress in sturgeon could also be achieved through the use of a fixed cannula. The installation protocol in sturgeon has been described in detail (Williot 1997; Williot et al. 2011). A brief describing chapter can also be found in this book (Chap. 24). The cannula procedure is preferable when measuring highly reactive parameters like cortisol, in successive sampling or during establishment of biochemical reference parameters. Finally, it should be noted that sturgeon are highly tolerant to blood sampling allowing longitudinal studies. Weekly repeated blood sampling of 500 µL of blood under anesthesia, for a period of 11 weeks, was performed without apparent problems on 40-gram Siberian sturgeon (Kolman 2002). In order to assess the physiological impact of repeated blood sampling, we performed two caudal punctures of 1 mL of blood (≈1% of the body weight) at 1 month interval, in ten tagged 5-month-old Siberian sturgeon. The levels of eight physiological parameters from the second sampling were compared to the levels of ten other fish only sampling the second time (Fig. 45.1). Only two parameters increase in resampling fish, the complement activity (student test, $p = 0.004$) and the expression of *hsp70* (student test, $p = 0.0004$). Thus, longitudinal studies can be easily achieved in sturgeon, but a validation that the followed parameters are unaffected by the sampling procedure should be done first.

In the next section, we will collate and discuss the available data on physiological indicators useful for welfare assessment in Siberian sturgeon.

45.2.2 Plasmatic Indicators

45.2.2.1 Hormonal Response

Stress response monitoring is one of the ways of estimating the level of welfare. This response is divided into three stages. The primary stress response is the perception of stress, and it is described as the alert phase, characterized by a hormonal reaction. The secondary stress response corresponds to mechanisms of physiological acclimation to stress. If the organism is not able to cope with an intense and/or long stress

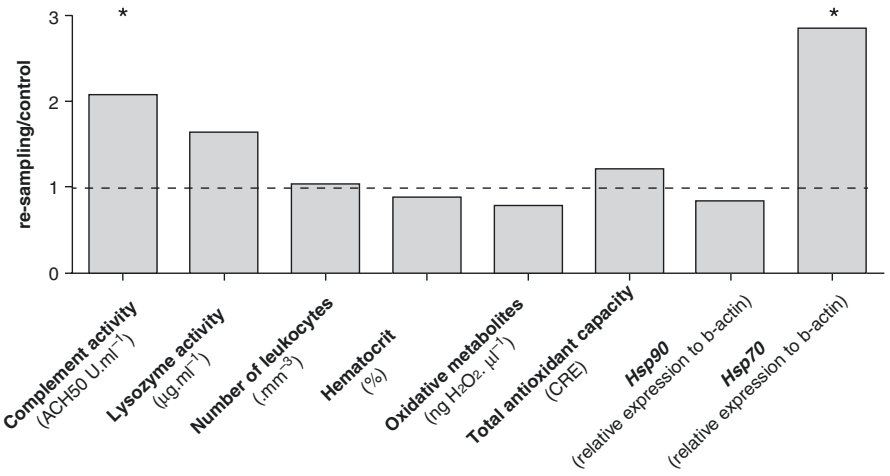

Fig. 45.1 Evaluation of the impact of a longitudinal study based on blood sampling from tagged fish (batch 3). The mean levels of the measured physiological parameters were compared to the physiological mean levels from fish only sampling once. Superscript indicates a significant difference between resampling and control fish (student *t*-test, $p \leq 0.05$)

event, it enters into a physiological exhaustion stage. This stage is the tertiary stress response (Barton 2002; Martínez-Álvarez et al. 2002; Schreck 2010).

The main hormones implicated in the stress response are catecholamines (adrenaline and noradrenaline) and corticosteroids. In sturgeon this is cortisol (Webb et al. 2007). Catecholamines are rapidly released in blood through stimulation of the central nervous system (Gallo et al. 2004). The release of cortisol is stimulated by adrenocorticotropin (ACTH). A cortisol peak occurs rapidly following stress induction in Siberian sturgeon (Maxime et al. 1995) before returning to basal levels within hours (Belanger et al. 2001). The brevity of this hormonal response does not make it a good functional welfare indicator, except in a context of experimental short-term challenging situation. In fact, a response to short-term stress should not be seen as a decline in welfare but as a potentially beneficial natural reaction that attempts to cope with changing conditions (McEwen and Wingfield 2003; Huntingford and Kadri 2009; Prunet et al. 2012). However, the cortisol rate does not necessarily return to its initial basal level during long-term stress in sturgeon (Lankford et al. 2003). In such cases, the monitoring of plasmatic cortisol rates could be considered as an indicator of chronic stress response and welfare.

Cortisol levels are low in sturgeon compared to other fishes. Unstressed sturgeon have a cortisol rate between 0.4 and 33.5 ng.mL^{-1} (Bayunova et al. 2002; Lankford et al. 2003). This wide range is partly due to daily and seasonal cycles of cortisol (Ellis et al. 2012) but also to the environmental context (Kynard and Horgan 2002; Lankford et al. 2003; Zubair et al. 2012), interspecies variations, the developmental stage of the fish but also in the quality of the sampling procedure. In Siberian sturgeon, the plasmatic cortisol rate is around 4–6 ng.mL^{-1} (Maxime et al. 1995; Hamlin

et al. 2008; Eslamloo et al. 2012; Eslamloo and Falahatkar 2014; Falahatkar et al. 2014). Moreover, various acute stress events induce an increase in the rate of cortisol between 11.8 and 50 $ng.mL^{-1}$ in juvenile or mature fish (Maxime et al. 1995; Williot et al. 2011; Falahatkar et al. 2014). This is a slight increase compared to the cortisol reaction following stress (variable in nature, intensity, and duration) for all other sturgeon species with a range between 4.9 and 466.6 $ng.mL^{-1}$ (Bayunova et al. 2002; Lankford et al. 2003). Chronic stress caused by nitrate intoxication (30 days) did not induce an increase in cortisol in Siberian sturgeon (Hamlin et al. 2008). Without the availability of new data on cortisol rates during chronic stress in Siberian sturgeon, it is difficult to conclude whether this parameter is of any relevance as a welfare indicator.

45.2.2.2 Biochemical and Hydromineral Indicators

The secondary stress response is partly induced by catecholamines and corticosteroids. The most commonly measured parameter of this response is certainly the plasmatic glucose concentration. It helps meet an increased energy demand in order to flee a potential threat, followed by active physiological acclimation mechanisms against the stress (Barton et al. 2002; Martínez-Porchas et al. 2009).

Glucose concentration in unstressed Siberian sturgeon rises with age. It is slightly less than 0.6 $g.L^{-1}$ in 7-month-old juveniles (Falahatkar et al. 2014); 0.8 $g.L^{-1}$ and 0.95 $g.L^{-1}$ in 3- and 4-year-old individuals, respectively (Hamlin et al. 2008); and 1.3 $g.L^{-1}$ in 11-year-old females (Williot et al. 2011). Caution must be taken when interpreting measured glucose concentration because it is dependent upon diet and the fasting protocol (Martínez-Porchas et al. 2009; Shi et al. 2010). This increases twofold after 1 h of nitrate intoxication (Hamlin et al. 2008) and more than 50% after acute hypoxia (Maxime et al. 1995). In a similar way to cortisol, measurements taken during chronic stress are lacking in the literature, and so the relevance of glucose concentration as a welfare indicator is uncertain.

Lactate concentration is also a common stress indicator. A combination of cortisol, glucose, and lactate are observed as indicators of stress in Siberian sturgeon (Maxime et al. 1995; Williot et al. 2011; Eslamloo et al. 2012; Eslamloo and Falahatkar 2014; Falahatkar et al. 2014). Plasmatic lactate concentration increases in cases of anaerobic metabolism in muscles during exercise (forced swimming or struggle) or following hypoxia (Warren et al. 2004; Allen and Cech 2007). Therefore, lactate is an acute stress indicator (Barton et al. 2000) that should not be used to assess functional-based welfare except in acute experimental challenge context.

Plasmatic lipid and protein concentrations including enzyme activities are parameters that are traditionally measured in human and veterinary medicines. Measured value(s) outside of its/their classical range(s) allows suspecting physiological disorders and pathologies. Unfortunately, little data is available in Siberian sturgeon (Gomulka et al. 2008; Feng et al. 2011). The most extensive database has been compiled by Di Marco et al. (2011) on sturgeon hybrids of *A. naccarii* × *A. baerii*. Similar collections of reference values exist for at least nine other sturgeon species (Di Marco et al. 1999; Asadi et al. 2006a, b; Shi et al. 2006; Shahsavani et al. 2010; Sepúlveda et al. 2012; DiVincenti et al. 2012; Matsche et al. 2014). All of this data confirms that

blood indicators are variable according to sex, maturity, age, and seasons (Matsche et al. 2013). Therefore, caution should be taken when comparing values in different groups of fish. Nevertheless, these blood parameters are useful for future studies on the evaluation of health and welfare in sturgeon (Di Marco et al. 2011).

More than half of the sturgeon species migrate in salted water, and most of the scientific studies dealing with hydromineral equilibrium are related to acclimation to salinity stress (Allen and Cech 2007; He et al. 2009). This issue is not often considered in Siberian sturgeon (Rodríguez et al. 2002) since most wild populations are restricted to freshwater (Rochard et al. 1990). Hydromineral balance is also linked to stress (Ellis et al. 2012). In fact, cortisol rate is the main regulatory hormone of ion uptake (McCormick 2001), and catecholamines increase gill permeability and branchial circulation, causing modifications in ionic concentration and osmolarity (Wendelaar Bonga 1997). Modulation of osmoregulation is one of the secondary stress response indicators (Segner et al. 2012). In lake sturgeon and Adriatic sturgeon, manipulations induce an increase in osmolarity (Cataldi et al. 1998; Baker et al. 2008; Allen et al. 2009). It is also a physiological process that is certainly highly energy demanding in fish (Bœuf and Payan 2001). Its implication in energy allocation between physiological functions is significant. If health status and welfare decrease, osmolarity could be disrupted.

45.2.2.3 Oxidative Stress Parameters

Free radicals or reactive oxygen species (ROS) mostly come from metabolism but also from other sources such as infections, pollutants, and stress in general. Through chain reactions, they have an impact on proteins, lipids, and nucleic acids (Monaghan et al. 2009). Defense mechanisms against ROS are called antioxidants. An imbalance between ROS and antioxidants in favor of the oxidants is called oxidative stress (Costantini and Verhulst 2009). Evaluation of oxidative stress enables us to simultaneously obtain information about the stress levels of an organism and its physiological capacity to cope with this stress. Oxidative stress is largely related to a decrease in long-term health (e.g. cellular senescence, pathology, or decrease in fecundity) (Martin and Grotewiel 2006; Costantini et al. 2010; Metcalfe and Alonso-Alvarez 2010). Thus, assessment of oxidative stress is a method by which welfare can be estimated.

In Adriatic sturgeon (*A. naccarii*), antioxidant enzymes appear in the early stages of life (Díaz et al. 2010), and a diet with oxidized lipids induces deformations and mortalities in Siberian sturgeon larvae (Fontagné et al. 2006). Thus, in sturgeon, the oxidative stress operates as in other organisms. In Siberian sturgeon, intoxication by selenium induces an increase in antioxidant enzymes in the liver and kidneys, which prevents lipid peroxidation (Pacini et al. 2013; Elia et al. 2014). Whatever the studies, oxidative stress is tissue dependent (Monaghan et al. 2009). Oxidative stress of only one tissue (blood) is maybe not representative of the whole body oxidative status. Yet, it was used with caution as an indicator of oxidative status. To our knowledge, only one study evaluates oxidative stress in Siberian sturgeon plasma (Simide et al. 2016). In this study we found that the total antioxidant capacity of plasma increases after a chronic heat stress challenge, which limits the appearance of oxidant

metabolites. Moreover, a dietary supplementation with a prebiotic, assumed to increase health status, leads to a reduction in oxidative stress under stress conditions.

45.2.2.4 Immune Status

As good health is necessary for good welfare, assessment of the immune system is also of interest (Segner et al. 2012; Huntingford and Kadri 2014). In fish, the evaluation of the immune system is essentially focused on the highly developed innate immunity (or natural or nonspecific), which does not require initial exposure to a pathogen (Magnadóttir 2006; Zhu et al. 2013). It is divided between humoral factors, inflammatory response, and cellular defenses. The humoral factors are noncellular defenses that specifically inhibit or annihilate the pathogens (Bols et al. 2001).

Lysozyme is certainly the most widely used indicator of the immune system in sturgeon. It can lyse bacteria and activate complement and phagocytes (Jollès and Jollès 1984). It is also identified as a stress marker. Indeed, the fish immune system, including lysozyme, is influenced (activated or repressed) by stress depending on its nature, intensity, and duration (Saurabh and Sahoo 2008; Tort 2011). Lysozyme activity increases in Siberian sturgeon fed with putative immunostimulant or ascorbic acid (Kolman 2001; Xie et al. 2006) but not fed with lactoferrin, β-glucan, or a candidate prebiotic (Eslamloo et al. 2012; Geraylou et al. 2012; Simide et al. 2016). Lysozyme activity is increased by direct stimulation of the immune system after intraperitoneal injection of lipopolysaccharides (LPS) or *Aeromonas salmonicida* (Kolman et al. 1999b; Xie et al. 2006). Under acute stress, lysozyme activity increases (Eslamloo and Falahatkar 2014), while it decreases after chronic stress exposure (Simide et al. 2016). Other than the developmental stage, the variation of lysozyme activity depends on many factors, such as nutritional status, seasonal variation, sex, salinity, pH, temperature, and infection (Saurabh and Sahoo 2008). Table 45.1 shows the high variability of the lysozyme activity in control groups (i.e., assumed good welfare) of Siberian sturgeon. Interestingly, lysozyme activities were very similar between two studies conducted in the same raising complex. It is

Table 45.1 Comparison of lysozyme activities in control groups of Siberian sturgeon of similar developmental stage

Weight (g) of Siberian sturgeon (mean ± SE)	Lysozyme activity in control group ($\mu g.mL^{-1}$)	References
76.0 ± 4.3	Around 20	Xie et al. (2006)
25.9 ± 0.9	8.62 ± 3.05	Geraylou et al. (2012)
26.3 ± 0.2	Slight under 8[a,b]	Eslamloo et al. (2012)
127.7 ± 3.4	Close to 7[a,b]	Eslamloo and Falahatkar (2014)
52.2 ± 1.3	4.61 ± 0.32	Simide et al. (2016)
40 ± 4[c]	Slight over 1	Kolman (2001)

[a]Fish raised in the same complex
[b]Equivalent unity in $U.mL^{-1}$
[c]Mean ± SD

clear that the developmental stage is not the only factor that can explain variations of the assumed basal range of values. The environmental and zootechnical conditions largely affect this value. Indeed, fish are poikilothermic organisms with a high phenotypic plasticity. This example with lysozyme indicates how reference values/ranges are a difficult notion to manage in fish. Evaluation such as this should be conducted in each different structure before commencing the monitoring of fish welfare in order to adjust basal values. Longitudinal studies with comparison through time of groups of fish are also a good way to get around the difficulty of lacking reference values.

The complement system helps in the phagocytosis or lysis of a pathogen. Fish have full complement activation pathways that include the classical, the alternative, and the lectin pathways (Whyte 2007; Zhu et al. 2013). In Siberian sturgeon it is the alternative hemolytic complement activity which is measured (activated independently of antibody by antigen). No increase of complement activity was observed with Siberian sturgeon fed with lactoferrin (Eslamloo et al. 2012) nor with β-glucan supplementation (Simide et al. 2016). Nevertheless, an increase was noted with pre- and probiotic complementation (Geraylou et al. 2012, 2013). Chronic stress also induces an increase in complement activity (Simide et al. 2016).

The immunoglobulins (Ig) are produced by B lymphocytes. In fish, IgM, which is characterized by low specificity, is the most represented (Zhu et al. 2013). The secretion of specific antibodies and natural antibodies did not differ in vaccination studies, suggesting that specific antibody and natural antibody responses are not clearly segregated in Siberian sturgeon (Kolman et al. 1999a, b; Kolman 2002). IgM or total Ig production was not improved by lactoferrin nor by pre- and/or probiotics (Eslamloo et al. 2012; Geraylou et al. 2013). While the cortisol induces a decrease in the number of lymphocytes, a stressing condition could affect the Ig production (Harris and Bird 2000; Davis et al. 2008).

45.2.3 Cellular Indicators

45.2.3.1 Immune Status

The other indicators described in this chapter are devoted to formed elements, the blood cells.

Cellular defenses characterized by leukocytes are generally classified as hematological in nature. Lymphocytes are involved in Ig production and modulation of the immune system. Eosinophils and basophils are linked with the inflammatory response. Monocytes and neutrophils play an important role in phagocytosis (Davis et al. 2008). The morphological features of the different leukocytes are well illustrated in lake sturgeon (*A. fulvescens*) (DiVincenti et al. 2012) and shortnose sturgeon (*A. brevirostrum*) (Knowles et al. 2006). These authors did not detect the presence of basophils in these species, whereas they have been found in other sturgeon species including Siberian sturgeon (Palikova et al. 1999; Kolman et al. 2000; Bahmani et al. 2001; Khoshbavar-Rostami et al. 2007). Figure 45.2 shows the different blood cells in Siberian sturgeon. The number of leukocytes in juvenile

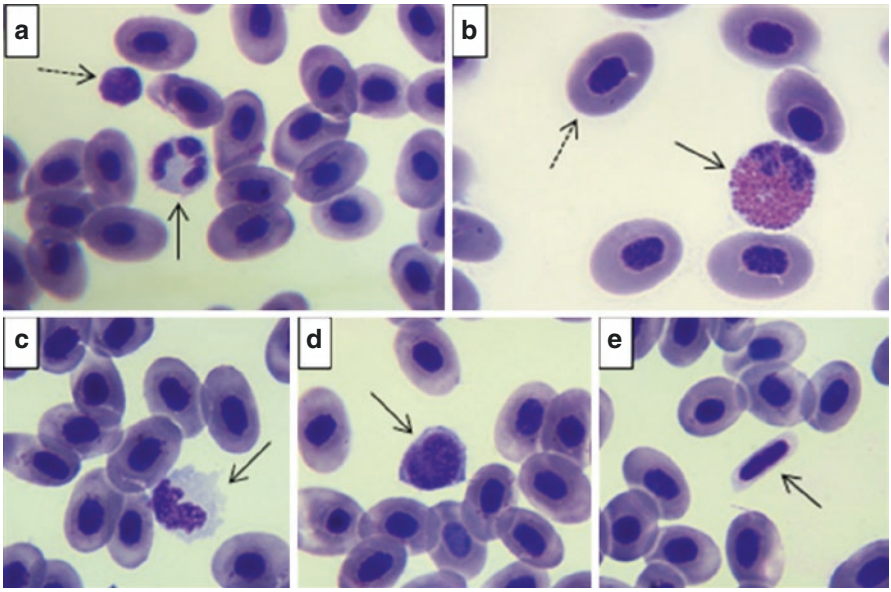

Fig. 45.2 Characteristic blood cells of Siberian sturgeon from smear stained by May-Grûnwald Giemsa (X 1000). (**a**) A neutrophil (*solid arrow*) and a small lymphocyte (*dotted arrow*), (**b**) an eosinophil (*solid arrow*) and an erythrocyte (*dotted arrow*), (**c**) a monocyte, (**d**) a large lymphocyte, (**e**) a thrombocyte

Siberian sturgeon is between 6.08 and 10.93 × 10^3 mm^{-3} (Gomulka et al. 2008; Eslamloo et al. 2012). We found a higher rate of 27.4 ± 9.9 × 10^3 mm^{-3} following a chronic heat stress. The differential leukocyte count for Siberian sturgeon is around two-thirds lymphocytes, a quarter of neutrophils, variable amounts of eosinophils, and close to zero monocytes (Palikova et al. 1999; Gomulka et al. 2008). Different proportions were found by Eslamloo et al. (2012) with a higher percentage of lymphocytes, a lower percentage of neutrophils, and more monocytes than eosinophils. The leukocyte profile is not only important as an immune indicator but also as a stress indicator. High cortisol levels induce a decrease in lymphocytes and an increase in neutrophil numbers (Davis et al. 2008). Variations observed between authors may be due to different initial stress levels. The activity of certain leukocytes can be evaluated in vitro. Phagocytic activity and respiratory burst activity of macrophages were generally assessed from the sampling of organs. The peroxidase activity, which measures neutrophil degranulation, can be achieved from serum (Quade and Roth 1997). A prebiotic and/or a probiotic enhances serum peroxidase activity in Siberian sturgeon (Geraylou et al. 2012, 2013).

45.2.3.2 Hematological Markers

The hematological parameters that are classically measured are erythrocyte count (cell per mm^3), hematocrit (%), hemoglobin (g/dL), mean corpuscular volume or hematologic indices (fL or μm^3), mean corpuscular hemoglobin (MCH, pg), and

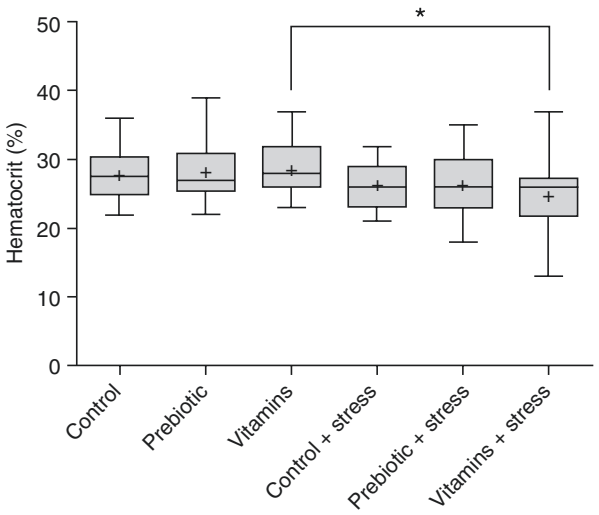

Fig. 45.3 Hematocrit of Siberian sturgeon (batch 2) after a dietary supplementation and a chronic heat stress (n = 20–30 per group). Crosses indicate means, while boxes indicate 75th percentile (*top line*), median (*middle line*), and 25th percentile (*bottom line*), and error bars indicate extreme values of data range. Superscript indicates a significant difference (SNK multiple comparison test, $p \leq 0.05$)

MCH concentration ($g.L^{-1}$). These markers have the advantage of being easily tested and inexpensive. They can indicate anemia or hemolysis (Jeney and Jeney 2002; Gomulka et al. 2008), thus a decrease in health. They may also fluctuate depending on stress (Kita and Itazawa 1990; Pierson et al. 2004) via the release of erythrocytes from the spleen, an increase in cell volume, and an increased cellular division rate (LeBlanc et al. 2012). They are therefore used on sturgeon as indicators of the overall physiological status or as stress markers (Jeney and Jeney 2002; Ahmdifar et al. 2011; Johnson et al. 2014). An overview of these different hematological parameters in juvenile Siberian sturgeon was carried out by Gomulka et al. (2008). In Siberian sturgeon, any modification of hematocrit was observed after acute stress (Gisbert et al. 2004), chronic stress (Rodríguez et al. 2002; Simide et al. 2016), or dietary supplementation (Eslamloo et al. 2012; Simide et al. 2016). We found only a small decrease (SNK multiple comparison test, $p = 0.05$) of hematocrit in fish supplemented by vitamins after a chronic heat stress exposure but no modification in other complemented and/or stressed groups (Fig. 45.3). Without additional available data in Siberian sturgeon, it would seem that these markers are not pertinent enough for welfare monitoring.

45.2.3.3 Molecular Indicators

Unlike in mammals, the erythrocytes of fish are nucleated. They not only have a role in carrying oxygen but are also important in the regulation of the immune system and the stress response (Lewis et al. 2010; Morera and MacKenzie 2011). The blood cell compartment offers many advantages for research in fish. Genomic studies provide many opportunities to study fish welfare (Prunet et al. 2012). From a single extraction, many physiological functions can be monitored. This is a promising topic to exponentially increase welfare indicators in a near future through transcriptomic and proteomic studies.

The heat shock proteins (HSP) are the most followed target genes to assess stressing conditions in sturgeon species. These genes encode chaperone proteins involved in cell metabolism and stress response (Roberts et al. 2010; Deane and Woo 2011). The HSP60 to HSP90 were monitored in whole larvae and various tissues under heat stress in sturgeon (e.g. Han et al. 2012; Linares-Casenave et al. 2013; Zheng et al. 2015). The levels of HSP60; HSP70, and HSP90 were measured in green sturgeon gills during salinity acclimation (Sardella and Kültz 2009) and after intoxication by selenium for HSP90 in green and white sturgeon larvae (Silvestre et al. 2010). The levels of HSP70 were also followed in these two sturgeon species in various tissues under cold shock stress and air exposure (Wang et al. 2013). The expressions of *hsp70* and *hsp90* were measured in the liver, spleen, and kidney of Amur sturgeon after crowding and hypoxia (Ni et al. 2014). Generally these stressing conditions induced an increase of HSP. However, the understanding of the physiological mechanisms which influence the modulation in the expression of these genes is still debated. We showed that an increase of *hsp90* and *hsp70* could be associated to an increase of other candidate target genes of the stress response (Table 45.2). Independently of their chaperoning activity, HSP plays several roles in the inhibition of apoptosis (Kalmar and Greensmith 2009). We showed a positive correlation between an apoptosis inducing protein, the caspase 3 (*casp3*), and *hsp70 and hsp90* expressions. The insulin-like growth factor 1 expression (*igf1*) was induced in the liver by the growth hormone in fish (Wood et al. 2005) and so influenced by the

Table 45.2 Spearman correlation matrix between gene expressions in the liver, gills, and intestine of juvenile Siberian sturgeon after a chronic heat stress

Correlation matrix (*r*; *p*-value)	***casp3***	***igf1***	***ubq***
Liver ($n = 21$)			
hsp90	**0.79**	−0.36	0.47
	<0.0001	0.11	0.04
hsp70	0.47	−0.45	0.22
	0.03	0.04	0.35
Gill ($n = 24$)			
hsp90	**0.80**	**−0.74**	**0.66**
	<0.0001	**<0.0001**	**0.0001**
hsp70	**0.73**	**−0.70**	**0.53**
	<0.0001	**0.0002**	**0.009**
Intestine ($n = 23$)			
hsp90	**0.80**	−0.40	0.48
	<0.0001	0.06	0.02
hsp70	**0.84**	−0.34	0.34
	<0.0001	0.12	0.12

In bold significant correlation with $r \geq 0.5$ and $p \leq 0.01$
Genes: *casp3* (caspase 3), *igf1* (insulin-like growth factor 1), *ubq* (ubiquitin), *hsp70* and *hsp90* (heat shock proteins 70 and 90)

metabolism rate. IGF1 was also already been used as stress indicator in fish (Deane and Woo 2009), and it was already known to induce a decrease of HSP70 or to not influence it (Deane and Woo 2011). We found a negative correlation between *hsp70*, *hsp90*, and *igf1* expressions in gills. *Hsp* expressions were also positively correlated in gills with the expression of the ubiquitin (*ubq*). While UBQ is dedicated to the degradation of damaged proteins, HSP are involved in both repair and degradation of the damaged proteins. Thus the modulation of the expression of HSP under stress can be linked to several physiological functions in Siberian sturgeon. Beyond being indicators of the cellular stress response, *hsp* expressions were also related to welfare.

Genome expression can also be evaluated in erythrocytes. To our knowledge, the use of erythrocytes in this way has been carried out only once in sturgeon (Simide et al. 2016). We followed the expression of *hsp70* and *hsp90* along a welfare gradient in Siberian sturgeon. We showed that the expression of *hsp70* and *hsp90* had complex variations and should not be interpreted only as a cellular stress response indicator but maybe as a putative indicator of health status.

Even if few information are currently available on Siberian sturgeon, these help to make some choices in future studies dedicated to the monitoring of Siberian sturgeon welfare (Table 45.3).

Table 45.3 Available physiological information on Siberian sturgeon which could be used in welfare assessment

Physiological indicators measured on Siberian sturgeon[a]				
Parameter	**Could be used to assess**	**Current observations**	**Main drawback**	**Main advantage**
Catecholamines	Acute stress response	↗	Not truly useful in welfare context	Precise stress response
Cortisol	Mainly acute stress response	↗	An high repeatability is needed in handling and sampling procedures	Lot of available data in fish
Lactate	Acute stress response	↗	Useful only for exercise, handling or hypoxia challenges	A way to estimate energy demand
Glucose	Mainly acute stress response	↗	Fasting procedure prior to sampling is easily achieved only in experimental condition	A way to estimate energy mobilization
Osmolarity	Salinity acclimation	↗	No study in stressed condition	A non specific indicator of physiological equilibrium
	Putative indicator of stress response	?		

(continued)

Table 45.3 (continued)

Physiological indicators measured on Siberian sturgeon[a]				
Parameter	**Could be used to assess**	**Current observations**	**Main drawback**	**Main advantage**
Reactive oxygen species	Health status Stress response	Depend to antioxidants response	No consensual indicators	Allows to estimate the oxidative balance, meaning the physiological potential to avoid oxidative damages.
Antioxidants	Health status Stress response	Depend to ROS response		
Lysozyme	Health status Stress response	↔ or ↗ ↘ or ↗ depend to stress	Modulations under stressing condition are largely unknown	Classical indicators of non specific immunity in fish
Complement	Health status Stress response	↔ or ↗ ↗ under chronic heat stress		
Immunoglobulin	Health status Putative indicator of stress response	↔ ?	No variation was observed yet	Promising indicator
Leukogram	Health status Stress response	↔ in quantity and proportion Maybe ↗	Unexpected variations in the different leukocyte types between studies	Inexpensive indicators
Hematocrit	Health status Stress response	↔ Mainly ↔ or small ↘	Generally no variation was observed	
Heat-shock protein	Putative indicator of health Stress response	↘ ↗	Variations should be deeper investigated	Promising indicator of global health status

Arrows describe the evolution of an indicator in Siberian sturgeon raised in experimental condition after a stress (stress response) or a dietary supplementation (health status) with increase (↗), decrease (↘), maintenance (↔), or lacking data (?)
[a]See the article for details

45.2.4 Highlighted Indicators for Welfare Assessment

Using the most suitable experimental protocol is important. It is more difficult to evaluate good welfare than it is to evaluate bad welfare (Volpato et al. 2007). In such cases, challenging fish through the induction of stress enables an estimation of the physiologic ability of an organism to cope with stress and to deduce the welfare level before that challenge. Figure 45.4 demonstrates the importance of a challenge

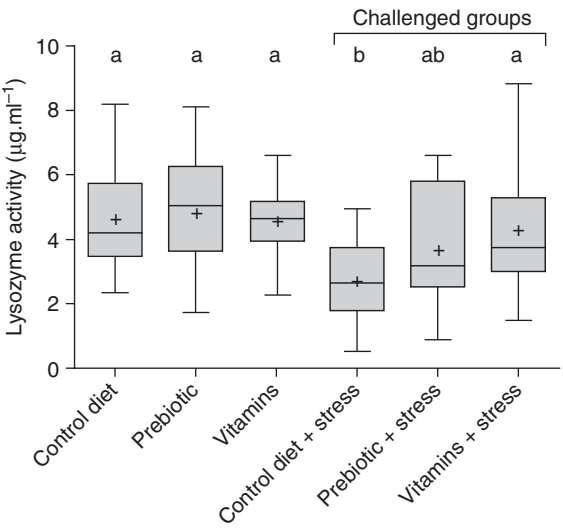

Fig. 45.4 Lysozyme activity in supplemented and/or challenged groups of Siberian sturgeon (batch 2, adapted from Simide et al. 2016). Crosses indicate means, while boxes indicate 75th percentile (*top line*), median (*middle line*), and 25th percentile (*bottom line*), and error bars indicate extreme values of data range. Different superscripts indicate a significant difference (SNK multiple comparison test, $p \leq 0.05$)

in health and welfare assessment. The positive effect of a 1-month complementation protocol by prebiotic or vitamins was only observed in challenged groups of fish by a chronic heat stress. The lysozyme activity only decreases in the challenged group fed with control diet (SNK multiple comparison test, $p = 0.0004$), while complemented fish resist to the same stress. Without challenge, this improved health status of complemented fish was not observed (Fig. 45.4; Simide et al. 2016).

Numerous indicators can be taken into account for welfare assessment. Selecting the most relevant indicators for a study is not easy. We should emphasize the fact that welfare assessment should only be considered when several physiological compartments are monitored together. It is the only indirect current way to validate the absence of physiological trade-offs. Thus, the level of health, a notion inseparable from welfare, should be discussed.

In this chapter we have presented indicators separately; however, it is important to understand the interactions between all of these measured parameters. First, their balance is partly determined by the distribution of energy (which may lead to trade-offs). Moreover, each parameter is involved in complex biochemical and physiological pathways or has roles in different physiological functions. Thus, one or few parameters can't be easily used like indicator of one physiological response (e.g. stress response). For a fuller understanding, we summarize the interactions between HSP, leukocytes, lysozyme, and oxidative stress. After a stress challenge, HSP are overexpressed in order to help in the establishment of a cellular stress response (Basu et al. 2002; Roberts et al. 2010). HSP also directly impact the immune system by the induction of leukocyte proliferation, for example (Srivastava 2002; Roberts et al. 2010; Deane and Woo 2011). In parallel, lysozyme belongs to the other parameters of innate immunity and induces an overexpression of HSP (Basu et al. 2002; Kalmar and Greensmith 2009). On the other hand, the accumulation of protein

damaged by oxidative stress also induces an overexpression of HSP. As HSP restore these damaged proteins, they can be directly associated with the antioxidant system (Finkel and Holbrook 2000). Immune system activation induces an increase in ROS via the respiratory burst activity of macrophages. These ROS are involved in the immune system by acting as antimicrobial agents, facilitating adhesion of phagocytes to the endothelium, and by stimulating the synthesis of cytokines (Bols et al. 2001; Sorci and Faivre 2009). In response to this increase in ROS, an adequate antioxidant response is required in order to limit damage in the organism (Costantini et al. 2010). Thus, the monitoring of numerous parameters enables a fuller understanding of the interactions between each parameter. We also showed that indicators of the same physiological compartment could be modulated in opposite manner by a stressing condition (e.g. increase in lysozyme activity and decrease in complement activity after a chronic heat stress). Moreover, many parameters are seasonal. Control groups have to be chosen carefully, and comparison between different datasets have to be made with caution. For example, in Siberian sturgeon raised in recirculating aquaculture system with few seasonality in environmental conditions, the *hsp70* expression has a coefficient of variation 3.3 times higher in April than in October (Fig. 45.5a), and the expression of *tgf-β* (involved in the regulation of the immune system) is 47 times higher in July than in October (Fig. 45.5b). Because of the relationship between the tested indicators, the interpretation of the results could be more relevant using multivariate analysis (Turnbull et al. 2005; North et al. 2006; Adams et al. 2007; Di Marco et al. 2008; Simide et al. 2016). Moreover, welfare is a continuum and a no fixed status (Ellis et al. 2012). Multivariate analyses allow gradients or distinct groups of fish to be discriminated according to the level of welfare. The amount of data provided to these analyses, like a principal component analysis (PCA), allows to select the most relevant variables and to better discriminate groups of fish. PCA is useful to visualize n dimensions (n = the number of indicators to analyze) in only two or three dimensions (principal components, PCs). Thus, it allows to represent the whole dataset in one single analysis. However, PCA should be interpreted with caution because it is only a representation of data. To take just a few examples, only a part of the entire dataset variation is taken into account, or some variables can have a high contribution compared to the others. Multivariate analysis has to be done after a good comprehension of the calculation is achieved by the analysis (e.g. Jolliffe 2014) to avoid risks of misinterpretation of the result. Two types of interpretation can be done thanks to a PCA: (1) a graphical observation and (2) the acquisition of a score. In Simide et al. (2016), the graphic of the variables (Fig. 45.6a) allows to see that the oxidants (ROMs) and the antioxidants (TAC) in plasma are inversely correlated as well as the activities of lysozyme and complement. The first PC which represents 43.85% of the total variation is largely explained by the oxidative stress, and the second PC which counts for 18.02% of the total variation is mainly explained by the expression of *hsp70* in the erythrocytes. The table of the contributions (Table 45.4) shows more precisely that the first PC is

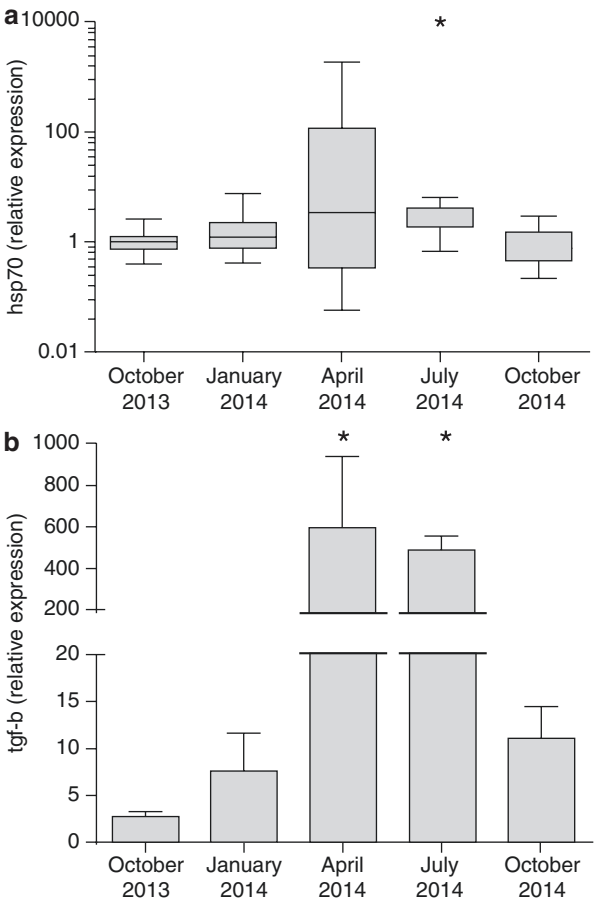

Fig. 45.5 Seasonality of physiological indicators of stress response (**a**) and immune system (**b**) in Siberian sturgeon (batch 1). (**a**) Boxes indicate 75th percentile (*top line*), median (*middle line*), and 25th percentile (*bottom line*), and error bars indicate extreme values of data range. (**b**) Error bars indicate SE. Superscripts indicate a significant difference with October 2013 (SNK multiple comparison test, $p \leq 0.05$)

explained by TAC, *hsp90*, the complement, ROMs, and then the lysozyme, while the second PC is largely explained by *hsp70*. The graphic of the individuals (Fig. 45.6b) allows to see that the four experimental groups (raised in different welfare conditions) are highly distinct. Indeed, each center of gravity and their corresponding confidence ellipse (which shows the possible area of the center of gravity) are in a different quarter of the PCA. Thus, adding supplementary fish in this analysis allows to place it in a welfare gradient particularly along the first PC with unstressed fish in the negative values (left) and positive values for the stressed fish. Turnbull et al. (2005) use these projection values in the first PC to determine a welfare score. Each Atlantic salmon (*Salmo salar*) had a welfare score that comes from a PCA built on four indicators. This method had the drawback to not account for a

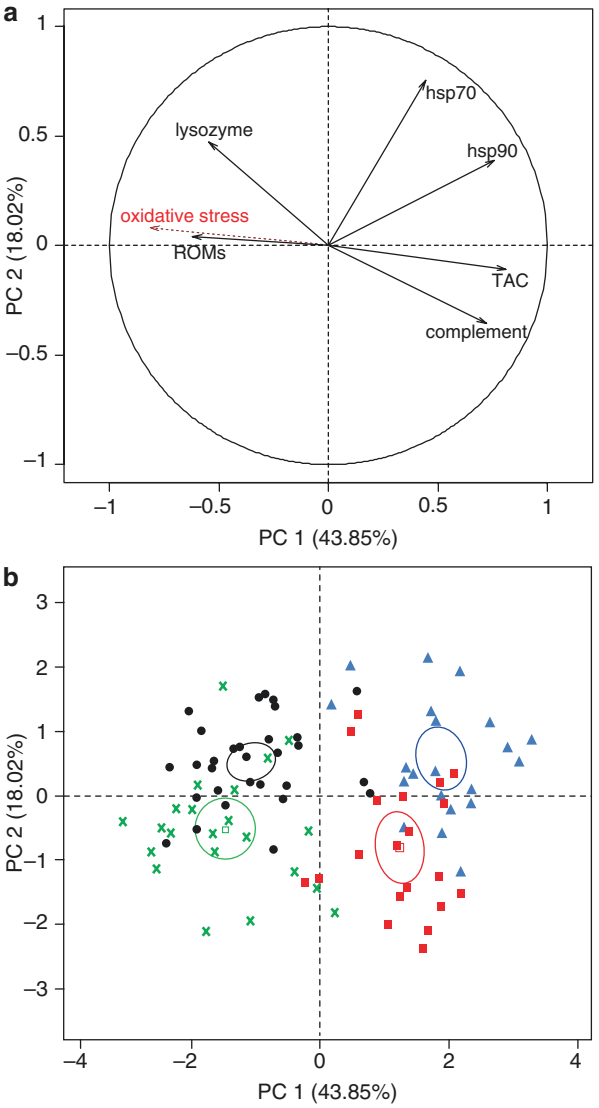

Fig. 45.6 Principal component analysis performed on six physiological indicators for four groups of Siberian sturgeon raised in different welfare conditions. Plotted according to the first two principal components (PCs). (**a**) Each active variable is represented by a *black arrow* and illustrative variables (not involved in the construction of PCs) by a *red dotted arrow*. (**b**) Each fish is represented by a *dot*. *Black circles* and *green crosses* are for fish fed with a control diet and a supplemented diet, respectively, in the absence of stress. *Red squares* and *blue triangles* are for fish fed with a control diet and a supplemented diet, respectively, in the presence of stress. The barycenter of each group of fish is represented by a square surrounded by a confidence ellipse (reproduced from Simide et al. 2016)

large part of the total variation of the dataset (29.9%) but had the interest to obtain a value supposed to represent the welfare of each fish. The highest score corresponds to the best welfare. The use of multivariate analysis is a promising technique to estimate a global physiological status linked to functional fish welfare. This approach is particularly interesting to create welfare reference dataset.

Table 45.4 Loadings and contributions of the indicators on the first and the second principal components (respectively, PC1 and PC2)

Indicator	Loadings on PC1	Contributions to PC1	Loadings on PC2	Contributions to PC2
Hsp70 (relative expression)	0.354	4.837	0.781	0.611
Hsp90 (relative expression)	0.743	21.298	0.401	0.161
Lysozyme ($\mu g.mL^{-1}$)	−0.554	11.816	0.477	0.228
Complement (ACH50 $U.mL^{-1}$)	0.714	19.629	−0.376	0.142
TAC (CRE)	0.821	25.993	−0.053	0.003
ROMs ($ng.H_2O_2.\mu L^{-1}$)	−0.653	16.427	−0.001	0.000

Conclusion

Blood sampling allows access to an important diversity of stress indicators, health status, and welfare. In Siberian sturgeon, available data is sparse. However, there are enough information to enable the observation of high variability within some parameters, according to environmental and zootechnical conditions, even if good welfare is assumed (control groups of fish). In fish, unlike in mammals, it is highly difficult to determine a universal reference range for a single parameter. In such cases, welfare assessment in a new raising complex should be carried out with appropriate caution. Because it's difficult to obtain reference values and to know the interactions between parameters and functions and because functional welfare is an integrated picture of physiological functions, welfare assessment should be achieved through the evolution between all the chosen indicators, meaning using multivariate analysis like PCA. The current available data demonstrates that evaluation of stress levels and health status is possible in Siberian sturgeon. An adequate selection of the indicators detailed in this chapter enables an estimation of welfare. For the first time, Eslamloo and Falahatkar (2014) began discussing the welfare of farmed Siberian sturgeon. They had monitored an indicator of the immune system and the primary and secondary stress responses from blood samples but only during acute stress. The impact of chronic stress in Siberian sturgeon welfare has been recently evaluated (Simide et al. 2016). We showed that using only physiological indicators from blood, analyzed by PCA, could be enough to estimate the level of welfare in Siberian sturgeon. Functional welfare should be only considered by monitoring several physiological functions together in order to estimate acclimation capacities of animals. Blood sample has an ethical dimension (by nonlethal sampling), allows to monitor fish (by longitudinal sampling), and provides access of numerous physiological indicators (from plasma and formed elements including nucleated erythrocytes) which makes it a promising procedure for further development in welfare research.

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Part VI

Ecological Risks

Synthesis of Introduction Trials of Siberian Sturgeon in North European Part of Russia

46

Mikhail Chebanov and Patrick Williot

Abstract

The aim of this chapter is to synthesize the documented facts of Siberian sturgeon introductions in the natural water bodies of the North European part of the USSR, carried out in 1964–1983. This chapter summarises the results of Siberian sturgeon juveniles of different origin (the Lena River, Lake Baikal) introduction and data related to its distribution and the growth and survival rates in the Gulfs of Finland and Riga of the Baltic Sea and Lake Ladoga. The carried-out introduction works "to expand the range of Siberian sturgeon" revealed the great potential of its growth under more favorable conditions and for the first time concluded about high level of its adaptive plasticity, which was the basis for this species as a promising object for wide commercial farming. The most important prerequisite for the introduction of Siberian sturgeon juveniles was a high food capacity of water bodies and a sufficiently large area, allowing fish to make food migration in the case of reduced quantity of food organisms in different areas. A mass bycatch of juveniles at the commercial fishing of other fish species and illegal poaching of adult sturgeon was also considered as a common cause of low efficiency of the introduction of both the Siberian sturgeon and other sturgeon species. To establish a self-reproducing sturgeon population, it was necessary first of all to create the conditions for natural propagation formulate- and formulate special regulations of commercial fishing in the water body—recipient. For a long time, numerous attempts of Siberian sturgeon introduction into various water bodies of the former USSR failed.

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Siberian sturgeons • Introduction • Baltic Sea • Ladoga Lake • Migration • Habitat • Monitoring, Russia

Introduction

The proposal related to feasibility of introduction of Siberian sturgeon of Baikal population (*A. baerii natio baikalensis* N.) in lakes Ladoga and Onega (Pushkarev 1927; Pravdin 1948; Dryagin 1954) as well as in other water bodies of northwest USSR regions was repeatedly expounded by prominent Russian ichthyologists (Berg 1949; Pravdin 1948, 1956, Gerbilsky 1954, etc.).

In 1956, 18 different age-graded Siberian sturgeon (of Ob population) (*A. baerii* Brandt) juveniles (average weight 0.4 kg) and 155 brood fish (average weight 13.4 kg) were released into the Pechora River. In 1956 and 1957, cases of Siberian sturgeon specimens capture were recorded in the middle and low reaches of the Pechora River, including the river mouth and in its tributaries Usa and Kolva. But self-reproducing population had not been established in the Pechora River basin (Rass 1971). But, later Kudersky (2005) expressed and substantiated an opinion regarding occurrence of Atlantic sturgeon (*A. sturio* or *A. oxyrinchus*) rather than Siberian one in Pechora River. Then Zakharov et al. (2007) reported about the first reliable capture since the last 150 years of two sturgeons in the basin of the Pechora River and suggested that Pechora River Basin should be included into the range of the Siberian sturgeon.

Biological justification of measures on expanding the geographical distribution of sturgeon fisheries of the Soviet Union was the so-called theory of biological progress of sturgeons and results of experimental analysis of adaptive plasticity and inherent system of phylogenetic adaptations (Gerbilsky 1962, 1967, 1970).

The results of a comparative study of the eurythermy, euryhalinity degree (Krayushkina 1967), growth rate, feeding range, and development of eurybiontic degree at the early stages of ontogeny (Sytina 1971; Zubova 1971; Bogdanova 1972) and data on adaptive plasticity of sturgeon populations at violation of the conditions of migration and reproduction (Barannikova 1968) served as the basis for the program on expanding the geography of sturgeon fisheries in the Soviet Union and, in particular, in the water bodies of the northwest of the European part of Russia (Gerbilsky 1970).

It was observed that in laboratory conditions, Siberian sturgeon of Lena population (*A. baerii* Brandt, "khatys") known in fishery and ichthyology literature as an example of extreme slow growing had completely the same growth rate as Russian sturgeon (Egelsky 1966).

In addition, according to data of an employee of Enoshima Aquarium (Japan) Dr. I Khirosaki, fingerlings of Siberian sturgeon (Lena population), transferred from Russia to Yokohama in October 1964 (of average weight 9 g and average length 12 cm), reached maximum weight and length (2095 g and 73.5 cm, respectively) in 1 year that corresponded to 11 years age of Siberian sturgeon of Lena population in Kolyma River and exceeded the average weight of sturgeon of commercial size in Lena River (cited by Gerbilsky 1970).

Table 46.1 The release of Siberian sturgeon juveniles into Lake Ladoga and the Gulf of Finland in the years 1964–1967 (Modified after Egelsky and Stepanova 1968)

Year	Water body	Age	Number of specimens
1964	The Gulf of Finland	Fingerlings, 0+	5000
	Ladoga Lake	Fingerlings, 0+	7540
1965	The Gulf of Finland	1 year	81
	Ladoga Lake	Fingerlings, 0+	462
		2 years	209
1966	The Gulf of Finland	Fingerlings, 0+	1247
	Ladoga Lake	1+[a]	140
		2+ years[a]	67
1967	The Gulf of Finland	1 year[b]	173
		2 years[b]	30
	Ladoga Lake	1 year[b]	240

[a]Tagged (vinyl chloride capsule with the label)
[b]Tagged (subcutaneous injected dyes under the left or right fins)

46.1 Release of Siberian Sturgeon in the Water Bodies of the Northwest of the European Part of Russia

General data related to production of different age-graded Siberian sturgeon juveniles in the years 1964–1967 by Egelsky and Stepanova (1968) are presented in Table 46.1.

In 1965–1967, also 8,000 and 32,000 juveniles (40,000 in total) were released, respectively, into the Gulf of Finland and into Lake Ladoga by Narva hatchery and Central water biological resources acclimatization service (TsPAU).

Results of juvenile introduction and data related to its distribution, growth and survival rates were presented by Gerbilsky (1962, 1967, 1970), Egelsky (1966, 1967, 1970), and Egelsky and Stepanova (1968, 1972). Short synthesis of these papers and results are presented below.

46.2 Data on the Distribution and Growth Rate of Sturgeon Juveniles in the Gulf of Finland

In 1964 to the Gulf of Finland, in total 5,000 Siberian (of Baikal population) sturgeon fingerlings of average weight 7.17 g were released, reared in ponds of Narva hatchery (Egelsky 1966). The release was conducted in the mouths of Narva and Luga Rivers at sections with sandy bed at depth of 0.5 m.

From Fig. 46.1, it is evident that by the end of 1964 after the release, sturgeon juveniles had been spread over long distances along the coast of the Gulf of Finland from Narva Bay to the coast of Finland (Gerbilsky 1967; Koli 1966; Egelsky 1970;

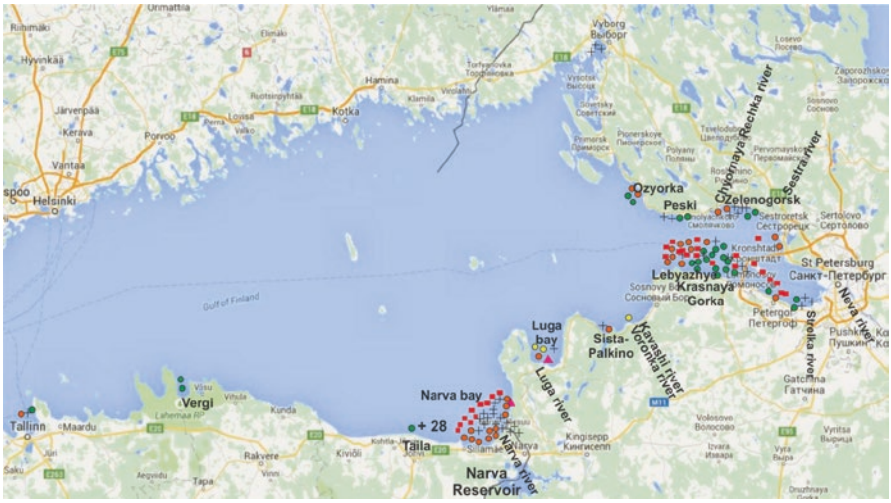

Fig. 46.1 The distribution of juvenile sturgeon in the Gulf of Finland: —sites of release (I) and distribution (2) of Siberian sturgeon (according to Gerbilsky 1967; Egelsky 1970; Egelsky and Stepanova 1972); sites of capture: —in 1964; +—in 1965; —in 1966 (+28 ind. in 31.07.1966); —in 1967; —in 1968

Egelsky and Stepanova 1972). Along with this, it can be noted that a significant portion of juveniles was caught by fishing gears near the places of its release. For instance, 16 different size-graded juveniles were captured in the Narva River and Narva Bay (Fig. 46.2).

According to the data of Egelsky and Stepanova (1968, 1972), in 1964, 3 individuals were caught along the coast of the Gulf of Finland, while in 1965, 24 individuals; in 1966, 20 individuals; and in 1967, 21 individuals. Table 46.2 showed that in September–October 1965, sturgeon juveniles (of age 1+) reached weight of 380–400 g at length of 46–47 cm.

In late July 1966 at the eastern part of the Narva Bay, 28 sturgeon juveniles (average length—50 cm) were caught by one setting of shore seine (Fig. 46.1 and Table 46.3).

According to Koli (1966) similar related to size values (250–650 g) were as well in juveniles caught in same period at the coast of Finland (Table 46.4).

Koli (1966) presented more detailed data on the growth of the Siberian sturgeon (Baikal population) in the Gulf of Finland—the 2-year-old specimens reached 46–50 cm till the end of 1965, while by October 1966, the 3-year-old individuals and 415–650 g with weight of 1200 g and length to 63 cm were occurred. One specimen weighing 1200 g was caught on August 15 at Söderskär, 25 km eastwards of Helsinki (Fig. 46.4).

Fig. 46.2 Distribution of sturgeon juveniles in the Baltic Sea (according to Koli 1966). Sites of capture: ■—in June to July 1965; ●—in August to October 1965; +—from March to October 1966; ●—in 1967. ▲—Sites of release

Table 46.2 Linear and weight growth and distribution of Siberian sturgeon juveniles (Baikal population) in Gulf of Finland (after Egelsky 1970)

Date of capture	Site of capture	Number of ind.	Weight (g)	Length (cm)
10–11.1964	Narva and Luga Rivers	2	–	–
14.06.1965	Narva Bay	1	50	–
14.06.1965		1	60	–
05.05.1965	Region of Strelna	1	43.8	25.2
05.1965		1	60	26
05.1965		1	75	29
06.1965	Luga River	1	–	–
10.06.1965	Narva Bay	1	93.2	–
10.06.1965		1	60	–
06.1965		4	–	–
06.1965	Region of Lomonosov	2	–	–
24.06.1965	Narva River	1	94	–
25.06.1965		1	90	
25.06.1965		1	73	–
06.1965	Region of Zelenogorsk	1	–	–
01.07.1965	Narva River	1	95	–
14.09.1965	Region of Zelenogorsk	1	380	46

Table 46.2 (continued)

Date of capture	Site of capture	Number of ind.	Weight (g)	Length (cm)
20.09.1965	Region of Vyborg	3	400	–
16.10.1965	Region of Lomonosov	1	380	47
04.06.1966	Region of Peski	1	650	55
23/VIII 1966	Narva Bay	1	1035	61
23.08.1966		1	735	52.5
23.08.1966		1	590	53

Table 46.3 Data on the growth rate of Siberian sturgeon juveniles, released as 0+ into the Gulf Finland in 1964 (after Egelsky and Stepanova 1972)

Year	Month	Captured (ind.)	Weighed and measured (ind.)	Weight (g)	Length (cm)
1965	May	6	5	43.8–75	25.2—29
	June	13	5	60–93.5	–
	August	1	1	95	–
	September	4	4	380–400	47
1966	June	2	2	450; 600	48.4; 55
	July	28	28	about 600	50 (on average)
	August	6	6	360–760	45.2–54
		1	1	1035	61
1966	September	2	2	415; 487	49; 52.5
	October	4	4	575–800	49.5–56.5
	November	3	3	520–930	51.2–59
1967	April	1	1	1690	69
	September	1	1	2650	78

Table 46.4 The growth of Siberian sturgeon juveniles in the Gulf of Finland (after Koli 1966)

Data	Number (ind.)	Length		Weight	
		n	cm	*n*	g
1965					
June	4	3	20	1	200
July	4	3	27–32	–	–
August	1	1	35	–	–
September	11	9	35–50	3	250–650
October	6	4	46–50	3	415–500
1966					
June	6	5	40–60	3	450–1050
July	6	4	45–60	2	500–1000
August	9	6	42–54	5	350–1200
September	2	2	50–58	1	850
October	2	2	47–63	1	1200

The length and weight of four Siberian sturgeon specimens, caught off the coast of Sweden (Hudiksvall and Söderhamn, cities Öland island) in 1967, amounted to 62 – 42.1 cm and 214 – 205 g, respectively; by 1969, a total of 19 sturgeons with the length of 35–70 cm was caught (Shristiernsson and Otterlind 1967).

Evidently sturgeons released in the region of Narva-Luga inhabited the southern coast of the Gulf of Finland, in its middle part. L. Koli (1966) reckoned that at the coast of Finland, sturgeons of the same releases were found. According to Koli's opinion, sturgeons entered the northern coast of the Gulf of Finland, migrating from the release cites along the southern and eastern coasts of the Gulf. Gradually moving to the west in 2 years post the release, they could be recorded at the Aland Islands and the southern part of the Gulf of Bothnia.

In April 1967, a tagged (Table 46.1) Siberian sturgeon (Baikal population) specimen of less than 3 years of age (of 1690 g weight and 69 cm length) was caught in Narva Bay. Similar sizes were observed in the Baikal Lake only for 9-year-old sturgeons (Egorov 1961; Chap. 1).

In late September 1967, a sturgeon juvenile (of 3+ years age) of 2650 g weight and 78 cm size (Table 46.3) was caught in the mouth of the River Kovashi (Egelsky and Stepanova 1968, 1972), while in June 1, 1967, a Siberian sturgeon of 2 kg weight and 70 cm length was captured off the coast of Finland (personal message of L. Koli).

At the same time in 1968 in the Gulf of Finland (mainly in the Neva Bay), a much less number of sturgeon juveniles were caught than before, due to significantly lower number of juveniles released in 1966–1967 (see Table 46.1).

46.3 The Capture of Tagged Siberian Sturgeon Juveniles in the Gulf of Finland

In the late 1967, 173 yearlings and 30 2-year Siberian sturgeon specimens (Table 46.1), tagged by means of injection of dyes and formed subcutaneous colored spots under the right and left pectoral fins following (Melnikova and Savostyanova 1968; Melnikova 1971, 1982), were released into the Gulf of Finland (Egelsky and Stepanova 1968).

On June 10, 1968, a Siberian sturgeon (of Lena population) specimen (of 220 g weight and 38 cm length), marked by dyes under its fin (see above), was caught in the area of Sestroretsk. Similar weight and length were reported in Lena River for 6- or 7-year-old fish (see Chap. 1). It should be noted that at its release in May 15, 1967, this marked juvenile had a weight of 85 g and length of 31 cm at 1 year age (Egelsky and Stepanova 1972).

In 1 year after the release, the marks were well of good quality also in four sturgeon juveniles caught in September and October at the southern part of the Neva Bay (settl. Lebyazhye) that allowed in due time Egelsky and Stepanova (1972) to recommend this marking technique for sturgeon juvenile. But unfortunately, this method has been used to a limited extent and only for sturgeon broodstock at fish farms.

46.4 Distribution and Growth Rate of Sturgeon Juveniles in Ladoga Lake

The relatively high growth rate was observed also for the 7,540 yearlings of Siberian sturgeon (5,000 psc., Lena, and 2,240 psc., Baikal populations), released in 1964 (Table 46.1) into the Ladoga Lake near cape Oryol (Egelsky 1966, 1970).

In 1965, along the eastern and southeastern shore, 83 Siberian sturgeon juveniles were captured by commercial fishing gear at depths ranging from 7 to 22 m, while from July 30 to October 3, 28 juveniles were caught along the coast from the settlement Lednevo to settlement Storozhno (Fig. 46.3) with weight from 125 (in July) to 300 g (in September and October).

In September and October 1965, 19 sturgeon juveniles of 200–350 g weight were caught at a depth of 30–35 m near the mouth of the River Olonka (east coast). According to Egelsky (1970), a tagged (Table 46.1) Siberian (Lena population) sturgeon individual was caught in the estuary of River Syas of 1+ year age, 170 g weight and 37 cm length. It should be noted that in wild conditions (natural habitat of Lena River), such weight and length are recorded only for 5-year-old specimens of Siberian sturgeon (Sokolov 1965 and Chap. 1).

In subsequent years (till 1970), along the southeastern and eastern coasts of the lake, about 301 captured sturgeon juveniles (78 in 1966, 12 in 1967, 9 in 1968, 202 in 1969) were recorded; of this more than 70% occurred in Volkhov Bay (Egelsky and Stepanova 1972), while seven Siberian sturgeon were caught in the river—mouth of the Volkhov, Svir, and Olonka rivers (Fig. 46.3).

In the northeastern part of the lake, sturgeons reached Mantsinsaari Strait. Captures were also reported in the southwestern part of the lake (from the Dalyokiy Point (cape) to the region of Osinovets), as well as to the north, along the western shore of the lake, near Priozersk.

46.5 The Capture of Tagged Siberian Sturgeon Juveniles in Lake Ladoga

In 1966 (September 15), for the study of the spatial distribution and growth rate of juveniles in Ladoga Lake, 140 age 1+ and 67 age 2+, Siberian (Baikal population) sturgeon specimens (Table 46.1) with the long-term individual tags (vinyl chloride capsule with a label attached to the dorsal scutes) were released in the area of Cape Oryol.

In addition at the end of 1967, 240 yearlings of Siberian sturgeon, marked by colored spots under the right and left pectoral fin following the technique of Melnikova and Savostyanova (1968) and Melnikova (1971, 1982), were released into the lake (Egelsky and Stepanova 1968).

By the end of 1967, 60 from 207 tagged Siberian sturgeon juveniles were caught mostly in the areas adjacent to the site of release (Volkhov and Svir Bays). Six

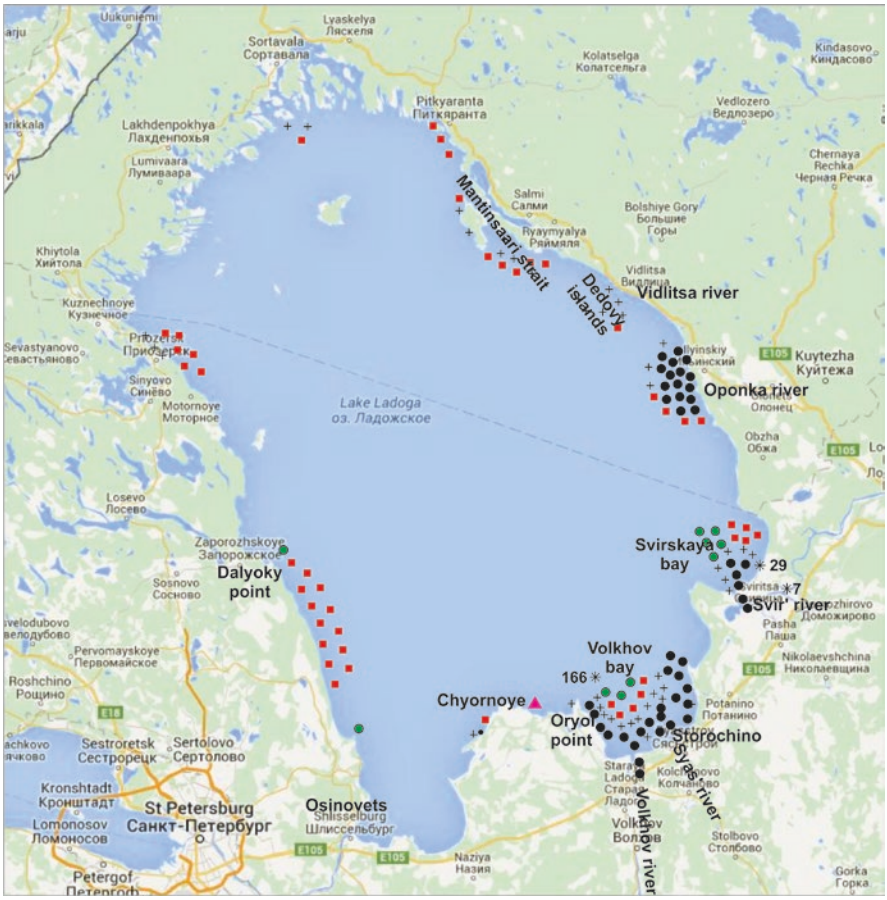

Fig. 46.3 Distribution of sturgeon juvenile in Lake Ladoga: ▲—sites of release; ●—in 1965; +—in 1966; ■—in 1967; ●—in 1968; ✳—in 1969 (the figure in front of the icon indicates the number of juveniles)

specimens were caught near Priozersk town, 2 in the area of Pitkyaranta, 5 in Mantsinsaari Strait, and 11 along the coast from the Dalyokiy Point to Osinovets (Fig. 46.3). It is important (Egelsky and Stepanova 1972) that one individual (whose weight increased from 300 to 344 g) was caught less than a month after the release at approximately 130 km from the release site (along the coast—about 200 km) at the northern part of Ladoga Lake (in the Mantsinsaari Strait).

In Table 46.5, data on the rate of growth and distribution of marked sturgeons (Egelsky and Stepanova 1972), caught in 1967, are present. This data allowed to assess the growth rate of the juveniles in the water body—recipient during the long period.

Table 46.5 Data on the distribution and growth of tagged sturgeon juveniles caught in 1967 in Lake Ladoga (after Egelsky and Stepanova 1972)

No	No of tag	Population	Date of hatch	Weight at release (g)	Length at release (cm)	Site of capture	Date of capture	Depth (m)	Weight at capture (g)	Length at capture (cm)	Average month gain	
											Weight (g)	Length (cm)
1	50	Lake Baikal	06.1965	85	33	Area of Osinovets	02.1967	–	700	48	123	3
2	51			130	38	Dalyokiy Bay		–	700	48	112	2
3	29			230	43	Svir Bay	03.1967	–	700	48	78	0.8
4	70			170	40.8	Svir Bay		–	700	48	88	1.2
5	22			90	36.7	Volkhov Bay	04.1967	–	650	46	80	1.3
6	118			180	39	Volkhov Bay	04.1967	–	700	48	74	1.3
7	5		06.1964	130	36	Svir Bay	05.1967	–	700	48	70	1.5
8	216		06.1964	420	52	Volkhov Bay	05.1967	–	700	48	35	0.5
9	208			400	49	Pitkaranta Island	18.05.1967	–	550	57	18	1
10	156			500	52	Mouth of Olonka River	15.05.1967	3	950	60	56	1
11	8		06.1965	120	39	Mouth of Svir River	01.06.1967	5–7	365	46	63	0.8
12	74	Lake Baikal	06.1965	150	39	Volkhov Bay	06.1967	–	700	48	61	1
13	38	Lena River	17.07.1965	130	36.5	Volkhov Bay	02.07.1967	3	350	42	22	0.6
14	173	Lake Baikal	15.06.1964	700	57.4	Pitkaranta island	30.07.1967	2.5	1021	68	32	1.1

As it can be seen from Table 46.5, monthly weight and linear growth increases for juveniles, released in Ladoga Lake at age 1+, were higher than that for juveniles released at the age of 2+ years. It should be noted that younger juveniles were caught primarily at earlier period (Egelsky and Stepanova 1972).

A 2-year-old Siberian sturgeon (Lena population) specimen (No. 13 in Table 46.5) had a weight of 130 g and a length of 36.5 cm at its release, while in 10 months after release, it reached the weight of 350 g and length of 42 cm. Weight of Siberian sturgeon (Baikal population) specimen (at 2+ age) released in 1966 amounted to 700 g and the length to 57.4 cm; by the time of the catch (June 30, 1967) its weight and length reached 1021 g and 68 cm, respectively (Egelsky and Stepanova 1968). Hence, the increase in length for this individual during 10 months of its life in a new water body was equal to 10.6 cm. Weight and length of other tagged specimen of Baikal sturgeon reached during 8 months to 950 g and 60 cm, respectively, from initially 500 g and 52.5 cm.

It was found that if 2-year-old fish had significant fluctuations in weight and length at its release, these differences had been leveled after several months of living in the lake. At the same time, Egelsky and Stepanova (1972) reported the high growth rate of sturgeon during winter period.

In the subsequent years, no cases of Siberian sturgeon capture in the Ladoga Lake were being reported (Kuderskii 1983).

46.6 Introduction of Siberian Sturgeon into the Gulf of Riga of the Baltic Sea

During the years 1962–1966, 8,200 Siberian sturgeon individuals representing the Baikal population and 900 ones representing Ob population were released into the Gulf of Riga. The average length and weight of juveniles were, respectively, 7.4–11.8 cm and 2.0–8.3 g. Data related to release of sturgeon juveniles in the Gulf of Riga are presented in Table 46.6.

Table 46.6 Data on release of Siberian sturgeon into the Gulf of Riga basin (after Kairov and Kostrichkina 1970)

Data (month and year)	Site of release	Siberian sturgeon population	Stocking material			
			Age, days	Average length (cm)	Average weight (g)	Number (thous. ind. ~)
08.1962	Daugava River, below Kegumsa	Ob River	63	7.4	2.0	0.87
08.1963	Mouth of Daugava River	Lake Baikal	77	8.7	3.2	1.55
			56	7.8	2.2	0.60
09.1965	Mouth of Lielupe River	Lake Baikal	93	11.8	8.3	0.55
08.1966	Mouth of Gauja River	Lake Baikal	63	9.3	4.0	7.05

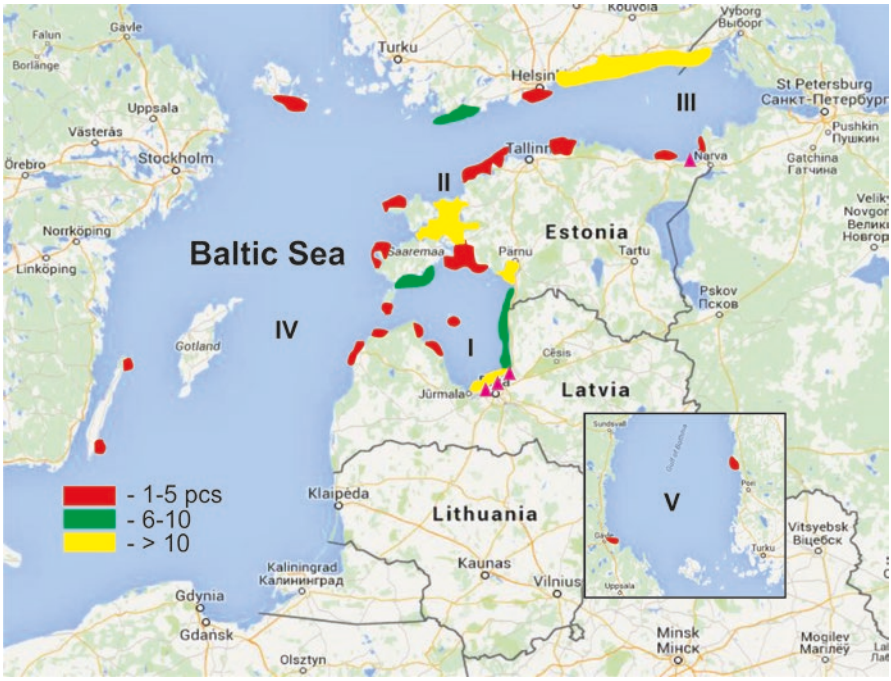

Fig. 46.4 Distribution of sturgeon in the Baltic Sea basin: I—the Gulf of Riga; II—Väina-Meri; III—Gulf of Finland; IV—the Baltic Sea; V—Gulf of Bothnia (after Kairov and Kostrichkina 1970)

Kairov and Kostrichkina (1970), on the basis of previously published data, compiled a sketch map of sturgeon distribution in the basin of the Baltic Sea (Fig. 46.4). According to their opinion, juveniles migrated from sites of their release (southern part of Gulf of Riga) along the eastern and western coasts to the North. A small part of the fish entered the Baltic Sea through straits. Catches of sturgeons (in the region of Ventspils, at the northern cost of Saaremaa and Hiiumaa islands, as well as at the southern part of the entrance to the Gulf of Finland) testified this.

By the end of 1969, more than 100 cases of catching of sturgeons in various parts of the Gulf of Riga, Väina-Meri (near the islands of Saaremaa, Hiiumaa, Muhu), and in the coastal area of the northeastern part of the Baltic Sea were observed. Siberian sturgeons of Baikal population were caught near the mouth of the Gauja River, at Ruhnu Island and in the area of Roya. Kairov and Kostrichkina (1970) noted that since not all cases of catch had been reported, so it can be assumed that actually sturgeons had been more widely distributed.

As it is clear from Fig. 46.4, most of the fish were caught in the Gulf of Riga and Väina-Meri. Kairov and Kostrichkina (1970) reported that the Siberian sturgeons were more frequently occurred along the east coast than along the west one. That was probably associated with the direction of main flow (counterclockwise) and with higher food productivity of the eastern coast. According to Kairov (1975),

sturgeons reached the Swedish shore through shallow waters, passing the Aland Islands. Direct crossing from the east to the west of the Baltic Sea, these authors found less likely, owing to the fact there were not any cases of their catches registered to the south of Ventspils.

Only fragmentary data related to the growth of Siberian sturgeon in the Gulf of Riga are available. In 2 weeks after the release to the lower reaches of Gauja River, yearlings of Siberian sturgeon (Baikal population) exhibited considerable growth. Their size increased from 9.3 cm to 14.1 cm, while their weight increased from 8 to 12 g. During this period, the juveniles consumed larvae of chironomids (*Tendipes plumosus*). The intensity of feeding is high; the relatedness (stomach fullness) reached 212 ‰ (Kairov and Kostrichkina 1970).

Long-term experiments on introduction of Siberian sturgeons into the Gulf of Riga basin proved the feasibility of further more extended activity in this course. As incidental bycatch, they were found in commercial catches. Part of the fish has been moving out of the bay to feeding grounds in Väina-Meri, while a small portion entered the northeastern part of the Baltic Sea.

In the following years, not any definite information related to recapture of Siberian sturgeon specimens in Riga Bay of the Baltic Sea had been reported.

Review and Conclusion

According to Gerbilsky (1970), Egelsky (1970), Kairov and Kostrichkina (1970), and Egelsky and Stepanova (1972), the data, obtained in the course of the acclimatization program, showed high potential for weight and linear growth of Siberian sturgeon in new, more favorable environmental conditions of the recipient water bodies the conclusion on the high level of adaptive plasticity of Siberian sturgeon was made for the first time.

In total, from 1956 to 1983 (Berdichevsky et al. 1983), the following number of different age-graded fry and juveniles of Siberian sturgeon of Baikal, Ob, and Lena populations had been released to the water bodies of the European part of USSR (Table 46.7).

In water bodies where the release of Siberian sturgeon had been performed regularly for several years, numerous cases of different age-graded individual capture were recorded (Ladoga and Peipsi Lakes, the Baltic Sea, the Oka River).

Till 1970, about 200 cases of Siberian sturgeon capture in Lake Ladoga and 380 cases of those in the Baltic Sea (Berdichevsky et al. 1983) were recorded, including 73 cases of this species capture alon the coasts of Finland, Sweden, and Estonia (Koli 1966; Christiernsson and Otterlind 1967, 1970; Paaver 1999). In Ladoga Lake, sturgeon juvenile had been widely spread throughout the whole water body, preferring however, primarily the western part of the lake. In Peipsi Lake, where exclusively Siberian sturgeon of Lena population was released, (in 1981), while 33 cases of its captures were registered (Paaver 1999) in the Oka River, 16 cases (Berdichevsky et al. 1983). In some water bodies, the results of the introduction were treated as negative because the release activity was not sufficiently biologically based (reservoir near Moscow, lakes of the Baltics).

Table 46.7 Introduction of Siberian sturgeon in water body of USSR (after Berdichevsky et al. 1983)

Water body	Age stage	Number, ind.	Years	Local population origin
Baltic Sea (the Gulfs of Finland and Riga)	Fry[a]	17,200	1962–1966	Baikal and Ob
	Fingerlings	1250	1966	Lena
	Yearlings and 0+	280	1967	Baikal
Ladoga Lake	Fry	8500	1964–1967	Lena
	Fingerlings	29,300	1964–1967	Baikal
	Yearlings and 0+	590	1965–1967	Baikal and Ob
	2+	64,000	1966–1967	Lena
Peipsi Lake	Fry	3200	1964	Lena
	Fingerlings	57,200	1975–1981	Lena
Seliger Lake	Fingerlings	72,400	1965–1966	Lena
Lakes of the Baltics	Fingerlings	105,000	1966–1974	Lena and Baikal
	0+	500	1967–1963	Lena and Baikal
	1+	37	1975	Lena
Pashozero (River Svir, basin of Ladoga Lake)	Fingerlings	8000	1977	Lena
Pashozero (River Svir, basin of Ladoga Lake)	0+	4200	1978	Lena
Zaozerie	Fingerlings	10,000	1977	Lena
Zaozerie	0+	300	1978	Lena
Pechora River	Different age graded	173	1956–1957	Ob
Oka River	Fry	92,000	1977	Lena
Oka River	Fingerlings	9800	1979–1981	Lena
Oka River	0+	2900	1977–1978	Lena
Oka River	1+	2000	1978	Lena
Water bodies in vicinities of Moscow (Pestovskoe, Istrinskoe, Ozyorninskoe)	Fry	24,000	1963	Lena
	Fingerlings	32,000	19641968–1970	Lena
Gorky Reservoir (Volga)	Fry	648,600	1961–1975	Baikal
Volgograd Reservoir (Volga)	Fingerlings	13,000	1960	Baikal
Sioni Reservoir (Georgia)	Fry	17,300	1981	Lena

[a]"Fry"—larvae, switched to an active feeding but did not reach the standard weight, i.e., less than 3 g

Obviously, in most water bodies of introduction, it was difficult to rely on the natural spawning of Siberian sturgeon; hence, its full naturalization had not been expected by the initiators of the acclimation program (Berdichevsky et al. 1983). The main purpose of the Siberian sturgeon release was the utilization of feeding base (benthos) that was frequently not that efficiently used or consumed primarily

by low-value fish and as well produced high-quality fish products based on natural food base (i.e., in modern terminology—pasture-based fish farming/ranching).

The most important prerequisite for the introduction of Siberian sturgeon juveniles was a high food capacity of water bodies and a sufficiently large area, allowing fish to make food migration in the case of reduced quantity of food organisms in different areas (Karpevitch 1975).

The introduction activities, carrried-out "to expand the range of Siberian sturgeon" have revealed the great potential of its growth under more favorable conditions, which was the basis for this species as a promising object of commercial sturgeon farming (Gerbilsky 1970; Sokolov et al. 1976; Berdichevsky et al. 1979; Charlon and Williot 1978; Williot 1984).

In subsequent years, the intensive development of commercial rearing and breeding of Siberian sturgeon in different aquaculture production systems, especially in warm water culture, was marked. After the first successful experience of progeny obtaining from the farmed breeders in Russian Federation (Smolyanov 1979) and in France (Williot et Rouault 1982), for about 20 years Siberian sturgeon being the most popular species of world sturgeon culture in a number of various countries from Uruguay to China (Williot et al. 1993, Bronzi et al. 1999, Chap 39).

A reported very frequent cases of this species specimens capture became a consequence of geographical expansion of Siberian sturgeon commercial rearing due to unintentional escape (especially during floods) in many European rivers and coastal marine water: from Szczecin Lagoon to the Pomeranian Bay, Odra River, and Regalica River (Gessner et al. 1999; Arndt et al. 2000, 2002; Kolman 2000), Slovak-Hungarian part of the Danube River (Holčik et al. 2006; Masar et al. 2006), Polish Reda River (Skóra 2012), and Austrian part of Danube River (Friedrich 2013). During December 1999 hurricane, more than 5000 Siberian sturgeons juveniles were accidentally introduced in the Gironde River estuary, after the submersion of a fish farm in France (Rochard et al. 2002; Maury-Brachet et al. 2008). In recent years, there were undertaken attempts to justify the continuation of the practice of acclimation of Siberian sturgeon in the waters of the North European part of Russia. So, the possibility of Baikal sturgeon introduction into inland water bodies of Kola Peninsula is considered by Muraveiko (2014). On the basis of the analysis of scientific papers and materials, author showed a great similarity of the abiotic factors in Lake Baikal and the inland water bodies of Kola Peninsula. The data on sturgeon introduction practice, water chemical composition in the compared water bodies, and adaptive capability of the introduced species and the possibility of interspecific food competition between indigenous species and an invading species is considered. According to Muraveiko (2014), the resumption of works to acclimatize marine organisms is necessary without any doubts and called-for at present and naturalization of Siberian sturgeon (Baikal population) in water bodies outside the impediment borders of the fish area will help to cope with a threat of disappearance of this rare Siberian sturgeon subspecies.

An analysis of the reviewed literature on attempts of introduction of the Siberian sturgeon into the water bodies of northwestern part of the Soviet Union, except for the above mentioned, allows making the conclusions as follows:

1. Unacceptability and illegal character in terms of modern concepts (Vinogradov 2005) and norms (EIFAC, FAO, IUCN) concerning the validity of alien species introduction, especially into the waters of international importance.
2. Opinion of Gerbilsky (1970), Egelsky and Stepanova (1972), Kairov and Kostrichkina (1970), and Berdichevsky et al. (1983) related to the great potential of its growth under more favorable conditions can be accepted.
3. The survival rate of different age-graded Siberian sturgeon juveniles would have been much higher if they were released in the northern water bodies in spring before the period of intensive development of natural food base, thus allowing to increase the length of feeding period being re-dispersed with simultaneous implementation of fisheries regulation measures.
4. A mass bycatch of juveniles at the commercial fishing of other fish species and illegal poaching of adult sturgeon should also be considered as a common cause of low efficiency of the introduction of both the Siberian sturgeon and other sturgeon species (Gessner and Arndt 2006; Podushka 2008; Williot et al. 2009) into freshwater bodies in the lack of natural propagation.

Thus, conducted analysis confirmed the conclusion of Kuderskii (1983, 2001) and Podushka (2008) that despite the huge efforts of the USSR, there had been lack of evidence for the installation of the Siberian sturgeon in the numerous recipient water bodies. To establish a self-reproducing sturgeon population, it is necessary (along with creation of conditions for natural propagation) to ensure homing of hatchery produced juveniles, after they reached sexual maturation. For this purpose it is urgent to arrange so-called imprinting of the progeny to water from the native river. This requires rearing of released juveniles (starting from transition to external feeding of larvae) in the water of the river destined for juvenile release (Boiko et al. 1993; Chebanov and Savelyeva 1999; Williot et al. 2002, 2009, 2011; Chebanov et al. 2002, 2011; Chebanov and Galich 2010, Boiko and Rudnitskaya, 2014).

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Synthesis of Escapements of Farmed Siberian Sturgeon in French Catchments: Some Extreme Events and a Lot Punctual Incidents

47

Marie-Laure Acolas, Chantal Gardes, Gilles Adam, and Eric Rochard

Abstract

To assess the escapement of *A. baerii* in the French catchments, we propose in this chapter to gather different sources of data related to (1) the escapes related in the French national press between 1990 and 2015, (2) the gray literature and scientific articles that were produced following the largely mediatized escape of thousands of *A. baerii* in the Gironde estuary after the 1999 hurricane, and (3) the bycatch declaration of exotic sturgeon within the frame of the bycatch declaration procedure set for the indigenous species *A. sturio* (2007–September 2015). We highlighted in this chapter that the level of escape at the national scale is poorly known and that no official synthesis exists. But thanks to press article and bycatch declaration, we also highlighted that *A. baerii* escapes occurred in all the main coastal rivers of the Atlantic French coast. A quantitative synthesis based on escape declaration synthesis by *A. baerii* owners would greatly help to measure the threat of such escape for the native species. The main risk highlighted in the Gironde for the native species would be trophic competition, species confusion risk, and diseases that could be spread. Fortunately since the large escape of 1999, it seems that *A. baerii* did not reproduce in the Gironde watershed. But

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escapes from farm or pond are still regularly occurring on the French Atlantic catchment, quantitative data being scarce.

Keywords
Press articles • Bycatch • Species introduction risk • Escape

Introduction

The beginning of the production in private fish farms of *Acipenser baerii* in France started in 1990, thanks to an official decree adopted in July 1990 which allowed the introduction of this exotic species in closed waters. Since then the number of farms has increased in France, the first ones being located in the South West of France (Williot et al. 2001; Chebanov et al. Chaps. 38 and 39). The initial objective was the production of flesh and it has extended to caviar production from 1995. Since the 1990s, small specimens of this species have been sold also in pet shops and for private ponds.

Concerning the escapement risk, a decree in February 2007 enforces the production farms to set a dual system of grids or other devices to prevent the escape and requires a written declaration of any escape of the owner of the fishes. In March 2013, another decree specifies the introduction procedure of exotic species in general for ponds with less restrictive measures to prevent escape; the ponds has to be equipped to avoid the escape but no double safety is required. Regulations do not enforce to individually tag the captive fish but the national action plan in favor of *Acipenser sturio* restoration advices to equip each captive exotic sturgeon with external tags to distinguish them from the indigenous species in case of escape (MEDDTL 2011).

In case of escape, the declaration has to be made to the local representative of the state, and there is currently no official national synthesis available because the state did not request it, the responsibility for any farming problem being at the local level.

In 1999, an escape of *A. baerii* occurred in the Gironde estuary as a consequence of a hurricane and got a large coverage in media. During this event called "the storm of the century," the winds exceeded 200 km/h and dykes breached which caused flooding of large amount of lands (Salomon 2002). A Siberian sturgeon farm situated in a marsh at the estuary border was submerged, and almost half of the production was missing and scattered in the marshes and in the open waters of the estuary. In this estuary, a recovery program of the indigenous species of West Europe *A. sturio* has begun (Williot et al. 1997), and a large amount of communication (press, associations, scientists, governmental representatives) dealing with the risk that such an escape presents for the indigenous species was carried out.

To assess the escapement of *A. baerii* in the French catchments, we propose in this chapter to gather different sources of data related to (1) the escapes related in the French national press between 1990 and 2015, (2) the gray literature and scientific articles that were produced following the largely mediatized escape of

thousands of *A. baerii* in the Gironde estuary after the 1999 hurricane, and (3) the bycatch declaration of exotic sturgeon within the frame of the bycatch declaration procedure set for the indigenous species *A. sturio* (2007–September 2015). This method is qualitative and not exhaustive but can be considered as a first step to arouse an official national synthesis in the coming years.

The presentation of this first synthesis is followed by a risk analysis about the introduction of *A. baerii* in French catchment.

47.1 Methods

The analysis of the press articles was carried out between 1990 and September 2015. A request was made on the following keywords in French in the database ©Pressedd (Press European Database: it is a press review database, access is restricted to a membership, here from Irstea Antony, France): *Siberian sturgeon* or *Acipenser baerii* and "escape" or "evasion." A press book was also consulted; it was constructed since 1990 by gathering all the press clippings where the words "sturgeon" and "Cemagref" (i.e., the former acronym of Irstea) or "Irstea" were mentioned.

For each relevant article, when mentioned, we have reported the year, the species of sturgeon, the number of fish escaped, the site or the watershed, the causes of the escape, the consequences highlighted by the article author, and the origin of the escape (hatchery, particular pond).

As a study case, the recapture rates and associated information of escaped *A. baerii* in the Gironde watershed following the 1999 tempest are presented. Local stakeholders and associations play an important role to sensitize the local people, and they gathered the data available (Mayer 2000). Through the Defense Association for Sturgeon (ADES), the fishermen were called up and largely involved in the recapture effort of the escaped sturgeons. The recaptures were either made by (a) the fish farm owners, (b) fishermen (professional and nonprofessional), or (c) scientific trawling campaigns in the estuary that were focusing on the indigenous population of *A. sturio* (Rochard et al. 2001). When a fish was captured, it was asked to measure its total length, to check if it was an immature or an adult and to locate its position in the watershed. Fish size was compared according to their localization (i.e., estuary or river) using nonparametric Kruskal-Wallis test followed by a post hoc test. Among the fish caught during the scientific sampling, some individuals were analyzed for metallic contamination (Maury-Brachet et al. 2008) and for stomach content (Brosse 2003) to evaluate either the potential contamination for the sturgeon species in the estuary or the trophic regime which could compete with the indigenous species. Thirteen individuals caught in the upper estuary between March and June 2003 were analyzed for metallic contamination (Maury-Brachet et al. 2008). In spring 2000 and 2001, 14 escaped *A. baerii* were analyzed for stomach contents (60–83 cm in total length) and compared thanks to gastric lavage method (Brosse et al. 2002) to 17 *A. sturio* sampled in Spring 1998 in the same sector (upper and median estuary) (Brosse 2003).

Since 2007 an increasing awareness campaign about the protected indigenous species *A. sturio* targeting mainly professional fishermen has allowed to gather bycatch data about sturgeon captures (www.sturio.eu). The main captures concern *A. sturio*, but sometimes exotic sturgeon species are declared with details of their localization and size. When a picture is sent, the species can be determined by the concerned biologists.

47.2 Results

47.2.1 Press Review Between 1990 and 2015

In total 236 articles were selected through the search criteria and among them 17 articles were relevant for this study.

Between 1990 and 2015, nine escaped events of Siberian sturgeon were relayed by the press (Figs. 47.1 and 47.2). The "tempest of the century" concerning the escape after the 1999 hurricane was the one with the highest number of mentions (nine articles) and concerns the larger number of fish, between 6000 and 8000 individuals depending on the articles.

Before 1999 the escape of this species was mentioned in different places in 1993, 1994, 1996, and 1997 in the Loire River, the Gironde, and in the mountains in East of France (Fig. 47.2). The reasons evocated for the origin of the fishes were escapes from farms without proper grids to avoid escape or pond cleaning without taking


Les "bæri" COURENT TOUJOURS

Deux ans déjà ! L'escapade des esturgeons sibériens

ACTUALITÉ • FRANCE

SAINT-FORT-SUR-GIRONDE (17) > Des milliers d'esturgeons d'élevage se sont échappés des bassins d'une pisciculture submergés par la crue du 27 décembre. Risque de pollution génétique

L'esturgeon en cavale

Avis d'évasion

Que sont devenus les esturgeons sibériens échappés lors de la tempête des 27 et 28 décembre dernier ?

Régions en bref

L'esturgeon pêché en Loire n'annonçait pas le retour d'une espèce disparue

On avait cru au retour en Loire de l'esturgeon européen disparu

Ce n'était qu'un esturgeon sibérien

Un poisson migrateur

Fig. 47.1 Press title extract concerning Siberian sturgeon escapes in France

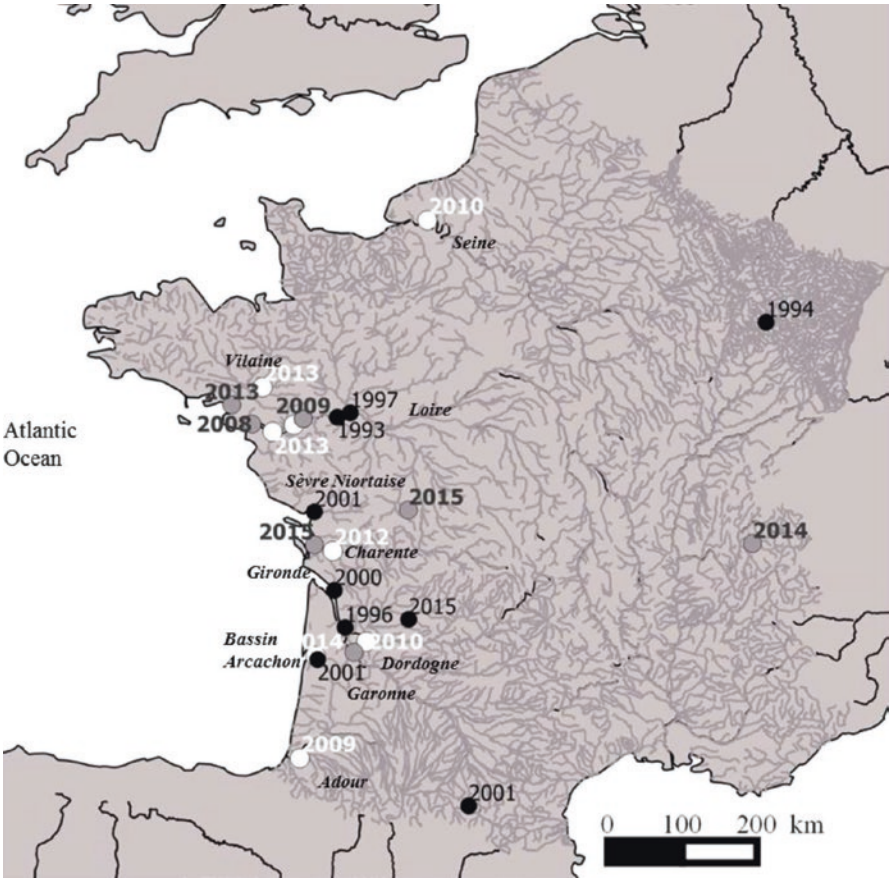

Fig. 47.2 Localization and year of declaration of exotic sturgeon species in the French catchments between 1990 and 2015

precaution. No fish numbers were indicated. In 2001, after the large escape of 1999, an article mentioned several escapes in different rivers of the southwest of France without precision about the numbers. Very recently in 2015, an article where a farm manager was interviewed mentioned a large escape (hundreds of individuals) in the Isle River which is a tributary of the Dordogne River where the indigenous species *A. sturio* is stocked regularly since 2007.

The consequences evocated by the press were mainly focusing about potential risks for the indigenous species (hybridization with *A. sturio* and risk for the recovery program, competition for food and space with other fish species) particularly following the 1999 large escape. But in 2015 the topic of the article was about farm production, and the consequences analysis of the escape mentioned a risk for the indigenous species (hybridization) and gain loss for the production. No other articles related this escape that was not mediatized despite the risk of threats for the indigenous population.

47.2.2 Recaptures Following the Escape in the Gironde Estuary

More than 8000 individuals, mainly juveniles but still 2400 potential spawners (85% females), were reported by the fish farm as escaped following the 1999 hurricane. The fish farm owners were able to catch 1045 individuals (11%) in marshes and ditches before they reached the Gironde estuary thanks to nets placed at strategic points until the end of April 2000 (4 months after the escape) (Mayer 2000). In total 319 other individuals (i.e., only 3.5%) were recaptured until more than 4 years after the escape (Fig. 47.3), and 84% of these recaptures occurred within the first 5 months after the escape. Fish were mainly captured in the estuary (80%) with two fishes that attended the wetlands in the estuary borders (Fig. 47.4a). Within the estuary, 76% of the captures occurred in the mesohaline section (middle part), 23% in the oligohaline section (upper part), and 1% in the polyhaline area. However the biggest fishes reach the two main rivers (Dordogne and Garonne), one fish swam even upstream a tributary of the Dordogne River (Figs. 47.4a and 47.5). Among the recaptured fish, 142 individuals were measured and checked for stage identification; the total length varied between 0.35 m and 1.30 m with significant differences between the fish caught in the estuary and those caught in rivers (Dordogne

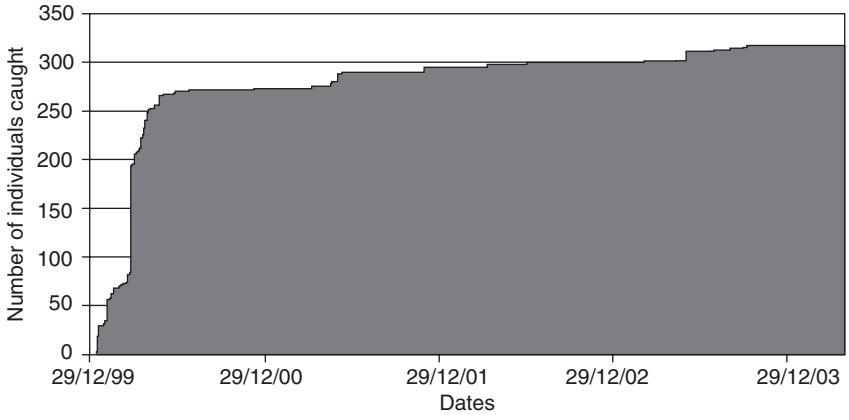

Fig. 47.3 Cumulative number of Siberian sturgeon caught after the escape of winter 1999 in the Gironde estuary

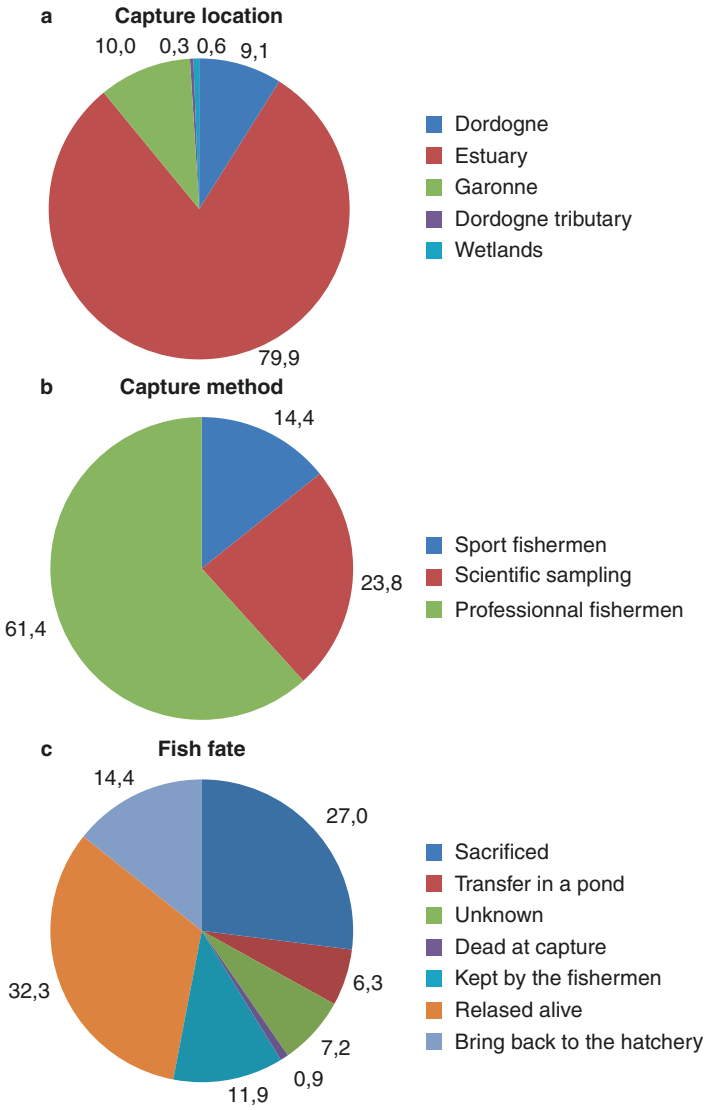

Fig. 47.4 Details about the capture of the escaped Siberian sturgeons from 1999: (**a**) capture location, (**b**) capture method, and (**c**) fish fate after the capture

and Garonne) ($P_{KW} = 0.03$, steel Dwass post hoc test Estuary-Dordogne $p < 0.01$; Estuary-Garonne $p < 0.01$; Dordogne-Garonne $p = 0.08$) (Fig. 47.5). Those individuals were mostly juvenile (110) but with some adults (24) and unidentified stages (8).

Most of the fish were captured by professional fishermen, then thanks to the scientific sampling in the estuary and finally by sport fishermen (Fig. 47.4b). Lots

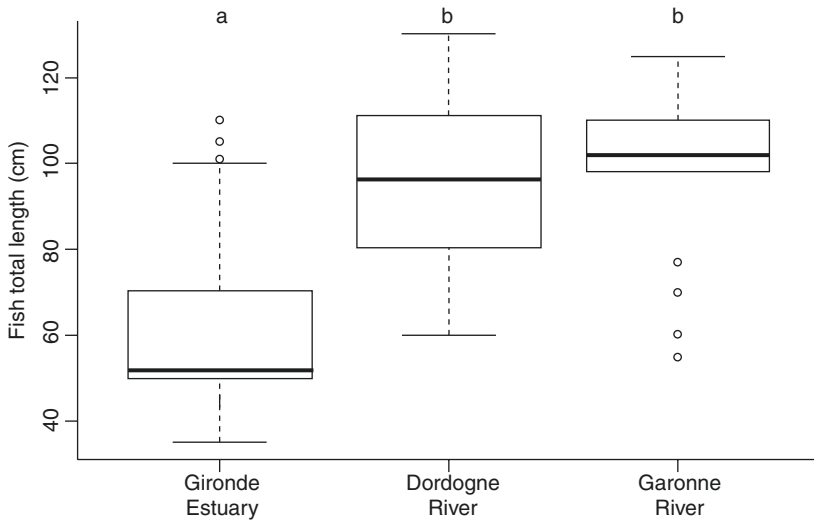

Fig. 47.5 Recaptured Siberian sturgeon total length boxplot per location (estuary vs. river) between 2000 and 2004 after the 1999 tempest. *NB: Different letter indicates a statistical significant difference. Median length, upper and lower quartiles and ranges are indicated by dark line, box and error bars respectively. Dots indicate outliers*

of fish (32%) were released alive in the river by fishermen, and the fate of 7% of the fish recaptured remained unknown. The other fish were sacrificed or kept for flesh by the fishermen or dead at capture (40%), and 20.7% were returned to the hatchery where they were coming from originally or transferred in a pond (Fig. 47.4c).

The comparison of stomach contents between Siberian sturgeon and the local species highlighted that during the first spring (2000), the Siberian sturgeon eat mainly Crustacean and some Polychaeta, but during the second spring in the wild (2001), they eat mainly Polychaeta like *A. sturio* (Brosse 2003). This finding highlights that *A. baerii* had the faculty to switch diet and that the sturgeons' diets overlapped which could lead to a trophic competition between benthic feeders.

The Siberian sturgeon caught in the upper part of the estuary was sacrificed and part of them was used as "living probe" to be able to assess their contamination level that could be representative of the contamination of the protected indigenous sturgeon species because they are benthic feeders and share a large part of their diet. After 3 years spent in the estuary, Maury-Brachet et al. (2008) observed a higher concentration level of Cadmium, Mercury, and Lead, but no significant differences for zinc and copper in the Siberian sturgeon compared to the corresponding fish still in the farm and fed with pellets. Those values were not considered to represent a physiological risk for those juvenile (mean standard length 70.8 ± 2.2 cm), but the study highlighted the road of trophic bioaccumulation of inorganic pollutants for *Acipenser* gender. Unfortunately the gonads were not sampled on older individuals, and this study cannot exclude any effect on reproductive physiology.

The trawling campaigns for *A. sturio* survey stopped in 2002, but they started again in 2009 and are still ongoing, and during these recent campaigns, no Siberian sturgeons were caught in the estuary (Acolas et al. 2011).

47.2.3 Recaptures in France Thanks to Sturgeons Bycatch Declarations Since 2007

By the mean of the declaration procedure of sturgeon bycatch mainly dedicated for European sturgeon, 18 individuals of exotic sturgeon species were reported since 2007 in France. Ten Siberian sturgeons were identified, thanks to pictures, four were suspected to be Siberian sturgeon but with no confirmation of the species (one in the Garonne, two in the Loire, and one in the Vilaine), three were unidentified sturgeon species, and one was *A. gueldenstaedtii* identified thanks to a picture. The origin of those individuals was unknown; they can either come from fish farm or pond or deliberate release from people. The bycatch declarations occur in low number but in all main rivers of the French Atlantic coast, i.e., from North to South: Seine River, La Vilaine, La Loire, La Sèvre Niortaise, La Charente, La Dordogne, La Garonne, Le bassin d'Arcachon, and L'Adour (Fig. 47.2).

The Siberian sturgeons caught were between 0.50 m and more than 1.2 m in the Dordogne River and in the Seine River (Fig. 47.6). Considering the size of the one caught in the Dordogne River, it could originate from the 1999 escape. We can notice that no juvenile *A. baerii* were declared in this catchment which may preclude that there was no reproduction success from the fish that escapes in 1999. However, there is no specific awareness campaign toward the fishermen concerning the exotic species, and those data can only be considered as occasional qualitative observations and not reliable survey to detect exotic species.

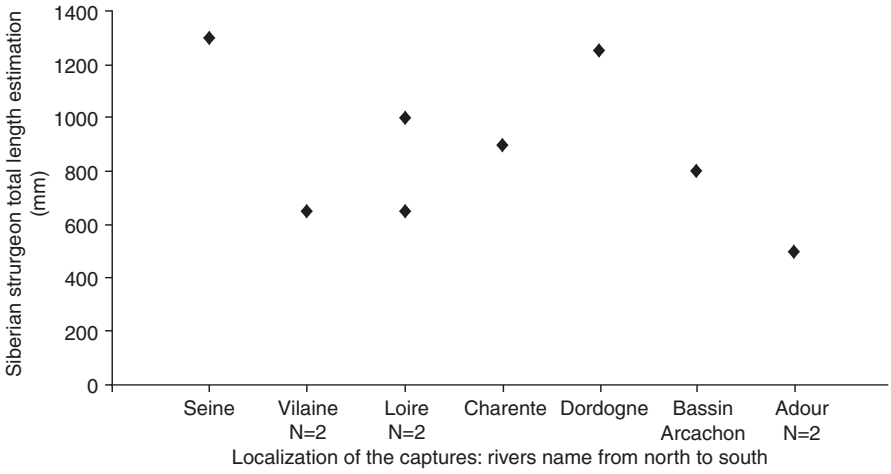

Fig. 47.6 Total length of Siberian sturgeons reported as bycatch declaration between 2007 and 2015

47.3 Discussion

47.3.1 Siberian Sturgeon Escapement Preliminary Synthesis

The press analysis shows that event with the highest coverage in media was the "tempest of the century" with the escape of thousands of Siberian sturgeons in 1999. The combination of events (1) a hurricane and (2) thousands of Siberian sturgeons escaped in (3) an area where the indigenous species was under a recovery program explains the number of articles and the mobilization of local stakeholders to communicate. Before and after that event, some articles mentioned the escape of this species (from 1993 to 2015) highlighting either the risk for the indigenous species (1996) or the loss of money for the fish farms (2015). Those data are sparse, but they concern all the main rivers of the Atlantic coast which could be worrying for the future in case of an exotic species settlement. Moreover, there are few probabilities that all the escapes would be relayed by the press, and we have clues that important escapes not mentioned by the press occurred: in 2001 an escape of 400 Siberian sturgeons of about 2 kg in an upstream tributary of the Garonne River was reported by the Agedra association, and in the same year, an escape of 1.2 tons happened near the Arcachon basin just below a fish farm and was reported by the fishery guards.

Few numbers of *A. baerii* were declared as bycatch since 2007, but the awareness campaign is mainly dedicated to *A. sturio*, not to exotic species, and oriented to fishermen at sea and in the Gironde basin. Few awareness campaigns are made for the fishermen in the other river catchments because the probability that one *A. sturio* enters the other river at the beginning of the restoration program was low. But now the occurrence of *A. sturio* in non-native watersheds could increase because we postulate that the homing rate of the stocked individuals may be weaker than those of the wild-born individuals. However the bycatch details corroborate the data from the press, escapes of *A. baerii* occur in all main rivers of the Atlantic coast, and new exotic species (*A. gueldenstaedtii*) are also encountered which claim for a national survey of the escapement of sturgeon species along the main coastal rivers.

The consequences of the escape in 1999 do not seem to have led to a detrimental impact on the native species or more particularly on the *A. sturio*. However at that time, the effects on the native species were not specifically addressed and *A. sturio* was at a very low level; no natural reproduction events seemed to have happen since then. Currently the stocking practices have allowed settling a juvenile population fraction in the estuary, the spawners being expected for the coming years. Since 2009 no Siberian sturgeons have been captured in the estuary despite that some individuals are still present in freshwaters (coming from the escape of 1999 or another). The success rate of the introduction of a new species (willingly or not) can be highly variable and difficult to predict (Williot et al. 2009). The questions of the potential risk of an exotic sturgeon species introduced in this watershed are still topical and important regarding the carrying capacity of the system, the species confusion risk, diseases that could be spread, and the genetic introgression risk that is more sensitive for small-size endangered population.

47.3.2 Risks Linked to Escapement of Siberian Sturgeon in France

Currently, in France, the main risks concern the Gironde-Garonne-Dordogne system, the only basin where the indigenous species is present and is supported by stocking (MEDDTL 2011). In the near future, if the stocking program succeeds, it is expected that the West European indigenous species spread in other watersheds (naturally or through stocking actions) and those risks can then be extending to other watersheds.

47.3.2.1 Trophic and Spatial Competition

The diet analysis in the Gironde highlighted that after 1 year in the estuary, the remaining Siberian sturgeon switches their diet to the available preys which are also used by other benthic feeders of the estuary (Brosse 2003). This illustrates that, if a population of Siberian sturgeon settled in the wild, a trophic competition could take place.

Concerning the spatial distribution, the life cycle being mainly in freshwaters for *A. baerii*, with a tolerance to salinity up to 9‰ (Rodriguez et al. 2002), it would have prevented this competition in the estuary. If an anadromous sturgeon species had escaped, the risk would probably have been higher. The spatial competition between *A. baerii* and *A. sturio* would be restrained to freshwater involving a potential competition for spawning ground and for food in the river at early stages. In fact, dams limiting the access to spawning grounds that could be more suitable for *A. baerii* upstream they would have used forced spawning ground as observed in other rivers (Ludwig et al. 2009), and the ones available downstream concern *A. sturio*. *A. sturio* juveniles are suspected to use the river habitats for less than 1 year (Acolas et al. 2012), and then the competition would occur mainly between young stages (from larvae to fish of about 35 cm).

47.3.2.2 Species Confusion and Sociological Difficulties

A sturgeon looks like another sturgeon for people that are not used to see these strange fish with no scales but bony scutes, long nose, and a protractile mouth. Siberian sturgeon is a freshwater species, so at sea there is no risk of confusion between the protected *A. sturio* and *A. baerii*. But as Siberian sturgeons however tolerate low levels of salinity (Rodriguez et al. 2002), highlighting a tolerance up to nine parts per thousand which were caught in the mesohaline part (5 to 18 parts per thousand) of the Gironde estuary after the escape, the confusion risks occur in estuaries and rivers. It is particularly true in the Gironde estuary where the native species is present and stocked, but it can also become a problem in other watersheds along the Atlantic French coast because the native species spends a large part of its life cycle at sea and can enter the mouth of other estuaries to feed (recent observation in the Loire mouth, Acolas et al., unpublished data).

It should be noted here that statutorily, a fisherman accidentally capturing a European sturgeon *A. sturio* must return it to the water (Ministerial order 20 December 2004 (légifrance.gouv.fr 2004)). Conversely, a sturgeon that is not native of French waters (Ministerial order 17 December 1985 (légifrance.gouv.fr 1985))

should not be returned to the water. In fact, according to the legal article L. 432–10 from the French environment code, it is forbidden to introduce nonindigenous species in rivers, channels, and lakes or ponds except lakes or ponds defined in articles L. 431.6 and L.431.7 (authorized fish farm and lakes or ponds resulted from damming with devices installed to avoid fish migration with the connected rivers). For pet fish, the ownership of exotic species is allowed but only in aquariums, not in the water types cited previously.

To prevent any risk of mortality for the protected species, the instruction given in the awareness campaign is to release the fish caught even if it gives more chances to the exotic species to live and settle in the novel area. In fact, the underlying assumption is that less risk is taken in respect of *A. sturio*, if an exotic sturgeon is released in the wild that if a European sturgeon specimen is extracted from its natural habitat by confusion with an exotic sturgeon. The risk of confusion being high, unintentional mortality of the protected species is a nonnegligible threat if the number of exotic sturgeon species increases.

When an exotic species arrives in a new environment, people react differently: it can be considered as an intrusion or an opportunity. Some people want to keep the indigenous species and in the case of *A. sturio* in West Europe to restore it to allow a functional population in the future (biodiversity preservation) which ensure at the same time a high quality habitat of the aquatic environment for the activities that depend of it (fishing, recreation, drinking water). Some other people could benefit from the installation of an exotic sturgeon which is not protected because it can be exploited rapidly (for trade or sport fishing) and its habitat is not protected and then the aquatic environment is less controlled (gravel extraction, pollution).

47.3.2.3 Disease Transmission

The disease transmission between exotic and native species is a high risk when species are introduced in the wild (Telfer and Bown 2012). In the case of sturgeon species, in addition to current pathology found in fish-rearing systems (Brun et al. 1991), the virus transmission such as herpesvirus would be critical such as described for *A. baerii* and bester (hybrid *Huso huso* × *A. ruthenus*) by Shchelkunov et al. (2009) which lead to significant mortalities in *A. baerii* farm (Doszpoly and Shchelkunov 2010). Sturgeon viruses such as nodavirus infection observed in *A. gueldenstaedtii* could also be transmitted to other fish species such as sea bass or mullets (Athanassopoulou et al. 2004).

47.3.2.4 Introgression Risks with the Native Species

When exotic species are released in the wild, the native species genome, particularly for endangered population of small size, is exposed to introgression which has been reported in some sturgeon species with fecund hybrids (Rochard et al. 1991; Jenneckens et al. 2000; Zhang et al. 2013). The different levels of ploidy between Siberian sturgeon (4*n*) and European sturgeon (2*n*) may prevent the hybrids to become fecund (Rajkov et al. 2014). Moreover the temperature known to be favorable for *A. baerii* is reported to be between 8 and 20 °C with an optimum between 11 and 15 °C (Ruban 2005), the optimum for *A. sturio* being supposed to be higher,

around 20 °C (Delage et al. 2014). In the Gironde basin after the escapement of 1999, no indication of reproduction (i.e., young of year caught in the river) of Siberian sturgeon was mentioned that may be explained by higher temperature than in their historical distribution range, and recent catches probably come from escapement of recent fish farms which have grown in number in the watershed.

Natural reproduction of Siberian sturgeon outside of its distribution range has been mentioned only once thanks to a genetic and meristic study (Ludwig et al. 2009). It was in the Danube River and hybridization occurred with the native population of Sterlet *A. ruthenus*. The ecology of these two freshwater species differed, and the authors explained the crossing by the larger thermal tolerance of Siberian sturgeon for their reproduction and the presence of a forced spawning ground below a dam which concentrates the Sterlet and the other fishes.

Natural hybrids of sturgeons are often rare (Ludwig et al. 2002); however, in hatcheries, the manipulation through hormonal injection and the control of environmental parameters could easily lead to the production of hybrids which are selected for particular performance (high growth rate, early age at reproduction) appreciated in aquaculture and trade. In the wild, only behavioral or physical barrier between populations could prevent from interbreeding. As many species can hybridize with Siberian sturgeon and give fecund individuals (Zhang et al. 2013), then it is the hybrid of Siberian sturgeon that could be released in the future and represent a threat for the native French species because hybridization may modify the behavior and the timing of the reproduction.

Conclusion

We highlighted in this chapter that the level of escape at the national scale is poorly known and that no official synthesis exists, the only data available being found in press article and in bycatch declaration made for the native species. But we also highlighted that *A. baerii* escapes occurred in all the main coastal rivers of the Atlantic French coast. A quantitative synthesis based on escape declaration synthesis by *A. baerii* owners would greatly help to measure the threat of such escape for the native species. Indeed, considering that 88% of the sturgeon species are under threatened status in their native area (IUCN criteria), the risk of escapement of exotic species in a particular area should be prevented at the European level (Arndt et al. 2002). In France, the last population of European sturgeon *A. sturio* count very few numbers of wild-born animals in the wild despite the species benefits from a stocking program. The presence of incidental escape of exotic sturgeon species can be considered as a serious threat for the recovery of the indigenous species and should be highly controlled.

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Part VII

Specific Methods

48

In Vitro Incubation of Ovarian Follicles of Cultured Siberian Sturgeon, *Acipenser baerii* Brandt: A Short Practical Implementation and Its Fundamentals

Patrick Williot

Abstract

This chapter is a practical description of the implementation of the in vitro maturation competence (IVMC) bioassay which is the decision-maker tool to choose the appropriate time (and then the suitable physiological status of the female) to hormonally inject the female with the best probability to get good-quality ovulated eggs. Brief recalls of involved organs, tissues, endocrine pathways. A detailed description of the procedure with the support of illustrations of the main tools is provided. A specific section is devoted to the incubation medium. The chronology of the diverse steps of the procedure is provided as well as the needed tools. Some comments on applications are given.

Keywords

Siberian sturgeon • Ovarian follicles • Oocyte • In vitro maturation competence (IVMC) • Endocrine pathways • Incubation medium

Introduction

As farmed sturgeons do not produce their gametes naturally, a hormonal injection is needed. With regard to the females, there is an aid in testing a priori the suitable physiological status of the fish, the result of which allows planning the hormonal stimulation. This aid consists in a bioassay to challenge the in vitro maturation competence (IVMC) of the oocyte by checking for the absence of envelopes of the nucleus which have been broken under the indirect action of a steroid. This is called the germinal vesicle breakdown (GVBD). When the oocyte is not ready for maturation, the envelopes of the nucleus are still present, and the trace of the nucleus is

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well visible after appropriate treatment; the status of the cell is then called germinal vesicle (GV) (Chap. 27). Then the female of which the ovarian follicles are collected from is considered as nonmature, and the eventual hormonal injection would be ineffective. This breakdown of the envelopes of the nucleus is one of the main mechanisms at a time when the ovaries trigger their development for the very last steps of the biological cycle. This is a way to prepare the female's genetic material to be fused with the male's genetic material once the spermatozoon has entered the oocyte through the micropyle. Indeed, the present objective through the IVMC is to set up a decision-maker tool for hormonal injection (Lutes et al. 1987; Williot et al. 1991; Williot 1997a). It is worth noting that IVMC might be used for two other purposes in addition to the present one; one deals with a search on the mechanisms involved in the last phases of development of the ovarian follicles, i.e. from the end of the growing period to ovulation. The other considers ovarian follicle maturation as a bioassay to test the potency of different hormonal preparation. One of the key issues in developing the IVMC deals with the choice of the incubation medium. This is the aim of the chapter to provide all details for the setting up of the whole procedure, including in reminding with some biological backgrounds.

48.1 Brief Biological Recalls

It is worth reminding that a sturgeon does not spawn naturally in captivity; a hormonal stimulation is needed. Therefore, the main question is how to select the best females to be injected, i.e. those for which we may expect the highest probabilities to ovulate and to get good-quality eggs.

At a time when the growth of the ovarian follicle is achieved, i.e. after the vitellogenesis is completed, the development of the ovaries is arrested. This status may last several months in sturgeon depending on environmental conditions, essentially temperature. The present chapter deals with the period that starts at a time the ovaries trigger their last phase of development of which the most visible mechanism is the breakdown of the envelopes of the nucleus, i.e. the oocyte's maturation. The endocrine pathways can be summarised as follows (Fig. 48.1). The hypothalamus is then producing gonadotropin-releasing hormone (GnRH) of which the target is the pituitary. In reaction, the gland produces gonadotropin hormones (GTHs) which will reach the ovaries by the blood system. The two cells surrounding the envelopes (thecal and granulosa cells from the outside to inside, respectively) of the oocyte transform the GTHs' signal in producing the maturation-inducing steroid (MIS) at the internal side of the granulosa cells of which the target is the oolemma layer (i.e. the outside of the oocyte). Further, the cytoplasm of the nucleus produces the maturation promoting factor (MPF) (Fig. 48.1) which is finally responsible for the breakdown of the envelopes of the nucleus. Indeed, this endocrine schema, known as the so-called two-cell model, mainly due to Nagahama (1987) and Nagahama et al. (1995) has been established based on studies performed on

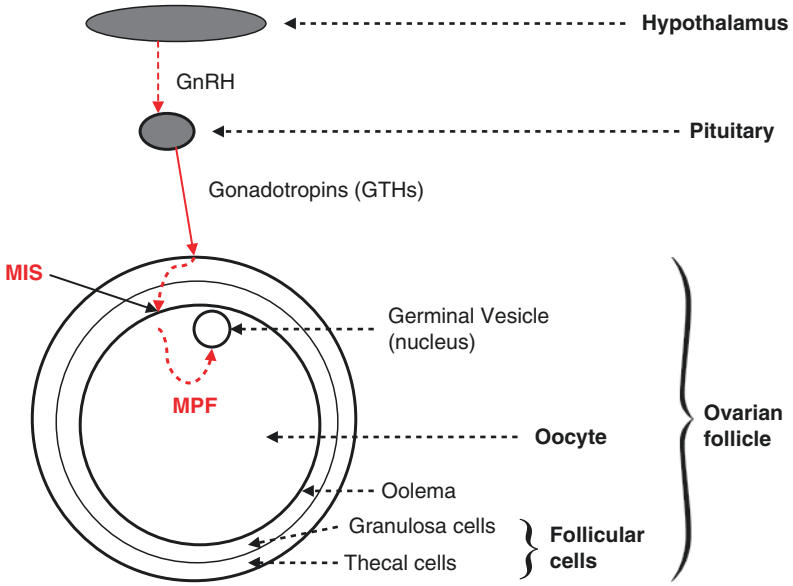

Fig. 48.1 Endocrine pathways by the final steps of development of the ovaries. Note that the position of the germinal vesicle is in the periphery which means that the growth period of the ovarian follicles is completed (credit: Williot P)

teleost fish. However, even in the absence of complete validity in sturgeons, many results and signs strongly suggest it can be applied in sturgeon. As an example, there is an MIS in the endocrine pathways of sturgeon, but the nature of the molecule is still debating (Chaps. 16 and 17). With regard to one of the two most current molecules identified as the MIS which is the 17α, 20β-dihydroxy-progesterone, a question of nomenclature and spelling due to Fostier (pers. com.) is worth recalling. In fact, this molecule does not exist because the progesterone has a cetone function in carbon 20, then a hydroxyl cannot exist at the same place. More, due to Kime, there is no choice for α or β in the plan of the molecule. As a result, the correct spelling is 17-hydroxy-20β-dihydro-progesterone which is a correct description of the molecule though not corresponding exactly to normalised spelling which is 17, 20β-dihydroxy-4-pregnen-3-one (Fostier com. pers.). The molecule is abbreviated as DOHP. It can be purchased from Sigma Co.

We would like to point out a last aspect that might be confusing. Speaking on the maturation of ovarian follicles is inaccurate from the strict biological point of view, because this is the oocyte that matures. It represents the central part of the ovarian follicle and contains the genetic material of the female. However, we cannot sample the oocyte alone but the ovarian follicles (Fig. 48.2), and then this is the reason why the wording ‘maturation of ovarian follicles’ may be used despite being not biologically founded.

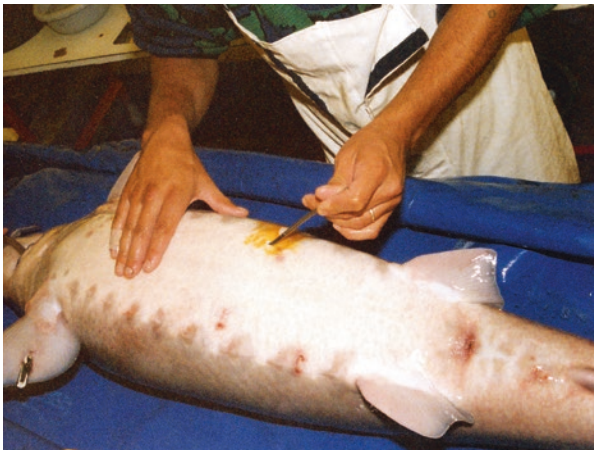

Fig. 48.2 Sampling a female for ovarian follicles. The fish is continuously supplied with water via a polypropylene tube in a month. Note that the angle of the probe is about 45°. The probe is similar to the small one shown in the next Figs. 48.4, 48.5 and 48.7 (credit: Williot P)

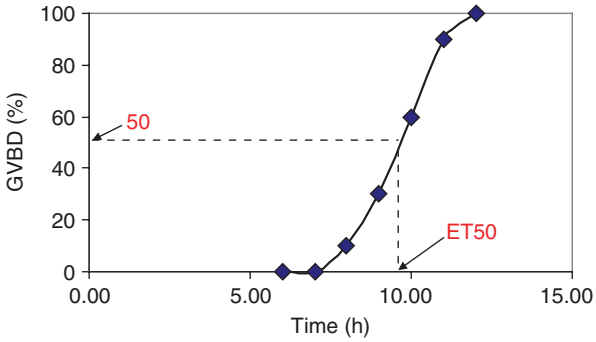

Fig. 48.3 An example of curve of time response is shown. The curve is a classic sigmoid-like shape which can then be used to determine ET50 (~09.30 h) as shown on the graph (credit: Williot P)

48.2 Decision-Maker Criteria

The objective of the chapter is to present why and how the bioassay known as IVMC is conducted. The aim of the bioassay is to mimic in vitro what happened in vivo after the fish are being hormonally stimulated (Sect. 48.1). To do that, ovarian follicles are placed in an incubation medium completed with a hormone that simulates the MIS. After a given delay (see Sect. 48.5), ovarian follicles of which GVBD (mature) or GV (nonmature) are counted.

The delay between the putting into incubation and counting may be approached in two ways, one static while the other may be dynamic. The primer consists in counting all the ovarian follicles of which GVBD or GV is achieved after a given delay previously tested and known to be over the possible expression of the phenomena. The second is a dynamic approach of the GVBD occurrence. Indeed, within a batch of ovarian follicles, all do not react exactly with the same rapidity; therefore, it is possible to determine the needed time to get 50% of GVBD called effective time to obtain 50% of maturing events (ET50) (Fig. 48.3). This is because the criterion proved its powerful prediction of embryogenesis rate, i.e. the shortest ET50, the highest embryogenesis rate (Williot 1997a; Goncharov et al. 1999).

It has been shown that a rate of GVBD ≥ 80% was necessary to expect ovulation and further good-quality products (Williot et al. 1991). Later on, an analysis of segmentation conducted within a group of 76 spawners, ET50 < 20.3 h allowed to select 69 females with a mean embryonic rate of 62%, while an ET50 < 13.7 h selected 38 females with a mean embryonic rate of 69.5%.

48.3 Incubation Media

With the objective being to allow the potential occurrence of in vitro oocyte's maturation, there are three different routes for the choice of an incubation medium: (a) one consists in adopting the simplest medium as illustrated by Ringer solution completed with sodium bicarbonate, (b) the second utilises a commercial product and (c) the third aims at elaborating a specific medium (Jalabert 1976; Williot 1997b). The last author investigated the three aforementioned routes. The tested specific incubation medium has been established based upon blood characteristics of the Siberian sturgeon and consequently has been called SIS medium. The main commercial medium was the Leibovitz (L-15) medium purchased from Sigma Chemical Co. (Lutes et al. 1987) and Ringer Solution (RS) according to Goncharov et al. (1999) and complemented with 1–2 $g.L^{-1}$ $NaHCO_3$ (Table 48.1). The main outcomes are as follows.

With regard to the three following criteria which are supposed to (a) discriminate females, (b) point out the lowest values, i.e. reveal the better sensibility, and (c) avoid 'abnormal' response (spontaneous maturation or false-negative response), the best medium is the SIS. L-15 is sometimes unable to point out the lowest sensibility. The worse medium is the Ringer family as they do not give secure results. The best

Table 48.1 Characteristics of the incubation media tested for Siberian sturgeon's ovarian follicle incubation (Modified after Williot 1997b)

Component or characteristic	RS1	RS1.5	RS2	SIS	L-15[a]
$NaHCO_3$ ($g.L^{-1}$)	1	1.5	2	0	0
NaCl ($g.L^{-1}$)	6.5	6.5	6.5	7.5	8
KCl ($mg.L^{-1}$)	250	250	250	200	400
$CaCl_2(2H_2O)$ ($mg.L^{-1}$)	400	400	400	285	185
$MgCl_2(6H_2O)$ ($mg.L^{-1}$)				170	200
$Na_2HPO_4(7H_2O)$ ($mg.L^{-1}$)					190
KH_2PO_4 ($mg.L^{-1}$)					60
Na_2SO_4 ($mg.L^{-1}$)				100	
Pyruvate sodium ($mg.L^{-1}$)					550
Hepes ($g.L^{-1}$)				4.76	
Others components	No	No	No	No	Yes
pH	8.02	8	8.03	7.55	8.14
NaOH(N) ($mL.L^{-1}$)				16	
pH				8.07	
Osmotic pressure ($mosmol.L^{-1}$)	235	245	255	275	320

[a]The complete formula of Leibovitz medium can be seen on Sigma book for example

pH values are 8 or over and preferably lower than 9. Associated with these pH values, the best osmotic pressure range is between 260–300 mosmol.L^{-1}. Indeed, it is suggested that the maximum rate of GVBD induced by a progestogen is dependent on a synergistic effect of pH and osmotic pressure of the incubation medium. Thanks to the modelling approach of the response that took into account both female and medium as independent variable as well as their interaction, it has been showed that the maximum of the explained variance depends on the simple interaction {medium x female} associated with the variable female (Williot 1997b). All these comments give sense to the approach in elaborating a specific medium.

48.4 Chronology of Operations

1. Preparation of:
   (a) Incubation medium. It is convenient to use deionised water in order to normalise the preparation. It has to be buffered with a biological buffer (Hépès[1]) adapted to the pH range. It is often necessary to add a small quantity of NaOH in order to obtain a pH $\geq$ 8 (Williot 2002). It can be stored in the refrigerator for a while.
   (b) Preparation of the hormonal solution. A preliminary solution in alcohol is needed.
   (c) Fish: starvation, capture and isolation if needed in a tank where fish became easy to handle.
   (d) Material and devices.
2. Anaesthetise the fish and place the fish in an adapted table with a continuous water renewal in the mouth.
3. Carry out of a pre-drilled hole with the pointed punch (lowest tool in Fig. 48.4).
4. Sample of ovarian follicles (Figs. 48.2, 48.5, 48.6, and 48.7).
5. Put the ovarian follicles in a beaker previously filled in with the incubation medium.
6. Wash the ovarian follicles and separate them from each other.
7. Fill in small Petri dishes (Φ = 55 mm) with 7.5 mL of incubation medium completed with 1 µg.mL^{-1} of progesterone except for control batch.
   One petri dish in case of static test, six to seven Petri dishes in case of dynamic test (ET50).
   Put 33 pieces (ovarian follicles) per Petri dish. This is the minimum number to apply parametric statistics.
   Petri dishes are then capped.
8. Place the Petri dishes in an incubator. This can be performed in rearing troughs supplied with larval rearing water exhibiting a constant temperature.

[1] With pK_a = 7.55, usable in the range of pH 6.8–8.2, far more stable than sodium bicarbonate under physiological pH especially in absence of control of PCO_2.

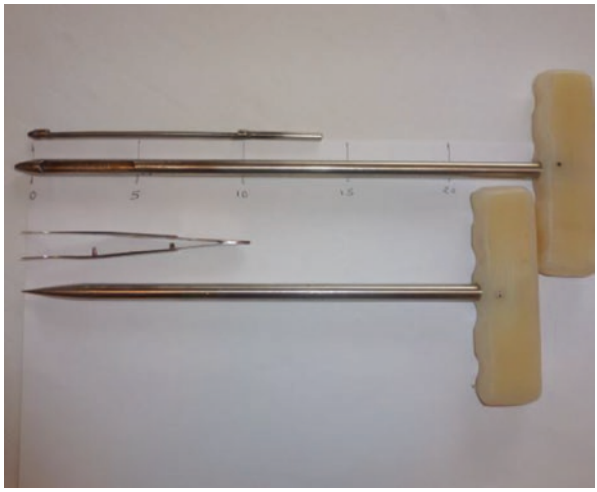

Fig. 48.4 The main needed tools to sample and further observe the ovarian follicles. *From top to the bottom*: A small sampling probe, a large sampling probe, pliers well adapted for ovarian follicles and a pointed punch to prepare the abdominal wall to be crossed by a sampling probe. The use of the punch has to be with a 45° angle to allow natural closing by internal pressure of ovaries on the internal side of the abdominal wall (credit: Williot P)

Fig. 48.5 An isolated photo of two probes showing the size of the probes with a cm scale. Note the rounded ends of both probes to avoid any injury to the tissues (credit: Williot P)

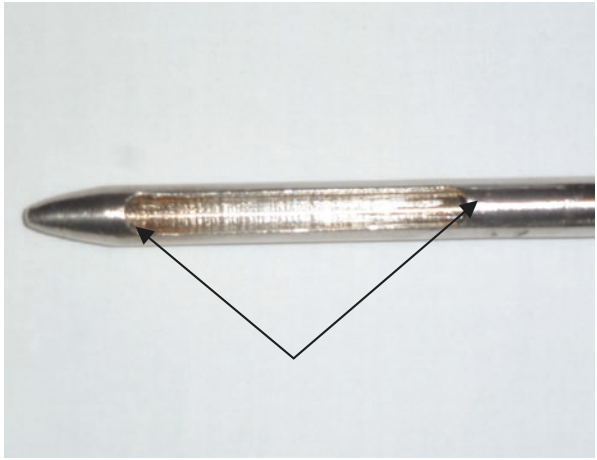

Fig. 48.6 Detail of the hollowed part of the largest probe delimited by the *two black arrows*. The diameter of the probe is ~8 mm, the width of the hollow is ~5 mm and the length is ~45 mm (credit: Williot P)

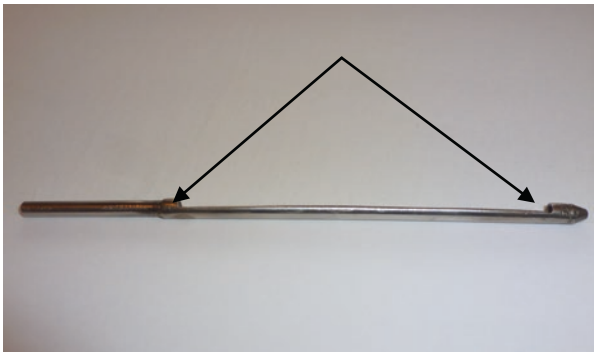

Fig. 48.7 Detailed view of the hollowed part of the smallest probe delimited by the *two black arrows*. The length is ~10 cm (credit: Williot P)

The standardisation was done at 18 °C which is in the upper range for incubation of eggs of the species and allows speeding up the involved biological processes.

In case of static test, the duration of incubation is 24 h. This time lapse allows the phenomena to be completely achieved.

In case of dynamic test, it is recommended to start observations about 6 h after the initiation of the incubation and with only 10–20 ovarian follicles. Depending on the results, this can be renewed each hour, eventually each half an hour.

9. Observations

   Observation and counting of GVBD/GV are completely similar to the procedure used for the determination of the polarisation index (PI) (Chap. 27). Ovarian follicles are boiled for about 3 min, rapidly chilled and then cut under stereomicroscope.

10. Computation of results according to methods mentioned above in Sect. 48.3.

48.5 Needed Materials

Stereomicroscope
Thermoregulated incubator
pH meter
Osmometer
Micropipette (addition of hormone into incubation medium)
Pipette (distribution of incubation medium into Petri dishes)
Petri dishes with their caps
Sampling probes for ovarian follicles
Pliers (Figs. 48.4 and 48.8), scalpel,…
Incubation medium (salts, water, hormone)
Beakers

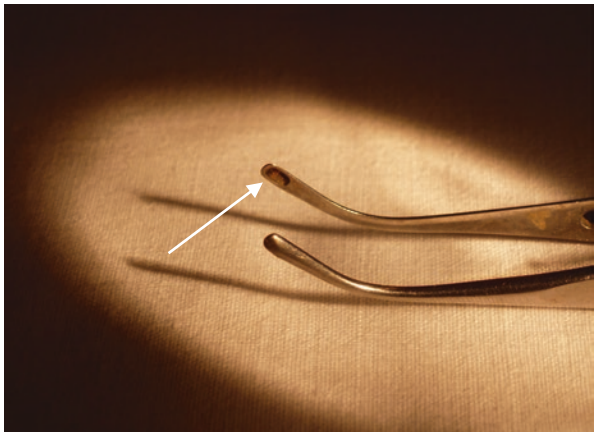

Fig. 48.8 Detailed extremity of both branches of the pliers well adapted to handle the ovarian follicles. The internal side of the branches is slightly hollowed (*white arrow* showing the *dark-grey spot*) to make easier the manipulations of the follicles (credit: Williot P)

48.6 Applications

The above description of the procedure might be considered as strictly limited to research due to the relative complexity of setting up the incubation medium and further computation. It has been developed in a context of extreme scarcity of brood fish, a highly endangered species, the Atlantic sturgeon, and a new nonindigenous species, the management of which had to be very careful. And the further results have justified the approach.

Indeed, the use of commercial media has been mentioned with a relatively high level of success with the Leibovitz medium (L15). Therefore, with some care L-15 may be used without the need of all the aforementioned controls such as pH and osmotic pressure as the recorded (Table 48.1) data are in the optimum range with ~8.1 and 320 mosmol.L^{-1}, respectively. Moreover, the graphic computation made easier the use of the procedure and needs neither software nor skill to get the result.

In the absence of thermoregulated incubator, this can be performed in placing the Petri dishes at the surface of rearing troughs supplied with larval rearing water exhibiting a constant temperature.

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Echography for Siberian Sturgeon (*Acipenser baerii*) Brood Stock Management

49

Mikhail Chebanov and Elena Galich

Abstract

The lack of external sexual dimorphism in sturgeons, including Siberian sturgeon, had been hampering the brood stock establishment and caviar production for a long time. The development of noninvasive express techniques of echography diagnostics of Siberian sturgeon has allowed to optimize sex structure of the sturgeon brood stocks, and to increase considerably the efficiency of caviar production and exclusive rearing of females.

In this chapter the procedure of express noninvasive echography scanning of Siberian sturgeon specimens for early sexing and maturity staging has been considered. This has enabled the completion of full echography study of gametogenesis and preparation of detailed atlas that presents echograms of different stages of gonad maturity in Siberian sturgeon males and females.

The new trends in effective application of the echography technique in sturgeon diagnostics, being developed during past few years, as follows, are described in this chapter:

- Identification of anomalies in the reproductive system (cysts, decreased fecundity of females, high testis lobularity, hermaphroditism) for culling of specimens with abnormalities and underdeveloped reproductive system

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- Conducting of different express measurements and calculation of gonadosomatic index, female fecundity forecast, and assessment of egg ovulation rate, aiming at identification of optimal time of egg maturity
- Assessment of different maturity stage duration to forecast final maturation of brood fish exposed to different rearing conditions (temperature, feeding), thus enabling the development of optimal holding regimes and food diets for different age groups
- It has been revealed that systematic functional echography diagnostics can considerably enhance efficiency of brood stock management and modern sturgeon culture technology in the course of sustainable development of caviar production from farmed breeders

Keywords
Acipenser baerii • Sexing • Echography • Maturity staging • Abnormalities

Introduction

For a long time, the lack of external sexual dimorphism in sturgeons (refer to Chaps. 32 and 33) had hampered the caviar production of Siberian sturgeon and establishment of their brood stock. An extensive noninvasive express ultrasound diagnostic technique has been properly developed and extensively applied in sturgeon culture practice. An application of this technique has subsequently allowed to diminish stresses and negative consequences of the domestication at high stocking densities and as well as optimize sex structure of sturgeon brood stock, including rare and endangered/threatened species (Williot 2011). These approaches also have enabled considerable increase in the caviar production efficiency owing to exclusive rearing of females (Chebanov et al. 2002, 2004).

An overview of different types of cheap and effective ultrasound scanning systems is provided. Echograms of more than one million sturgeons have been analyzed and processed (Chebanov and Galich 2009, 2010). This has made possible to conduct a full echography study of gametogenesis and prepare a detailed atlas with presented echograms of male and female Siberian sturgeon at different stages of gonad maturity. The aim of this chapter is to specify the protocol of ultrasound diagnostics, to assist in enhancement of the management efficacy of domestic brood stock of Siberian sturgeon. A survey of current advances in the application of the naming method in sturgeon culture is also presented.

49.1 Equipment for Sexing in Sturgeons Using Ultrasound

49.1.1 Biological Safety and Express Efficiency of Ultrasound Diagnostics

The diagnostic ultrasound has been established as biologically safe and has not been associated with harmful clinical effects.

A properly arranged sturgeon ultrasound diagnostic procedure typically is short time (up to 10 s), to say nothing of some rare or complicated cases.

In the course of the fish proper examining, their organs and tissues should not be destroyed, the fish should be exposed to open air for a short duration. This allows to reduce the stressors' influence to a minimum.

49.1.2 Transducers

Various types of transducers have been used in ultrasound diagnostics.

49.1.2.1 Linear Transducer

Ultrasound sections (echograms) upon the application of these transducers have a rectangular shape (Fig. 49.1). The scanning plane of the transducer is flat and tightly bound to the body of the fish; the size of the working surface ranges to 40–60 mm and the operating frequency is 5–10 MHz.

Such characteristics enable high-resolution ultrasound images to be obtained from fish weighing 2–20 kg, whatever the species. For larger specimens (above 50 kg), use of a convex transducer is recommended.

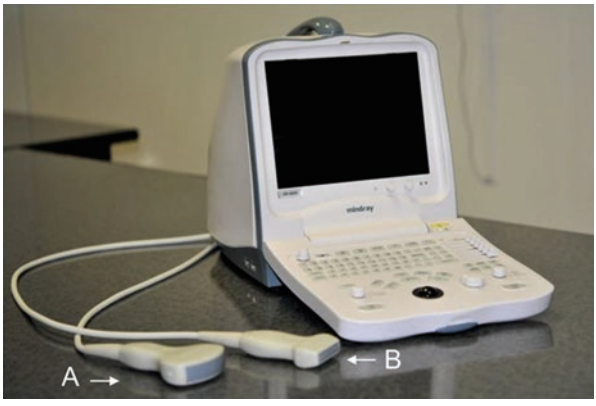

Fig. 49.1 Ultrasound portable scanning system Mindray DP-6600: *A* convex transducer and *B* linear transducer

49.1.2.2 Convex Transducer

The resulting section (echogram) has the shape of a trancated cone. Convex transducers usually have a low frequency (1.5–3.5 MHz)—and therefore low resolution, though still higher than that of linear transducers—and as well depth of scan above 20 cm (Chebanov and Galich 2008). Therefore, it is expedient to use them only for very large fish (above 50 kg).

49.2 Workplace Organization

To conduct speedy and accurate ultrasound sexing and staging of sturgeon specimens, it is essential to properly arrange a special workplace. In Fig. 49.2, an example of a mobile system for ultrasound diagnostics is presented.

The fish is to be placed on a specially designed table, holding the specimen at a relatively standstill position (Fig. 49.3). One person can hold a small fish, while two assistants are needed in case of a large fish examined (circa 3–4 kg).

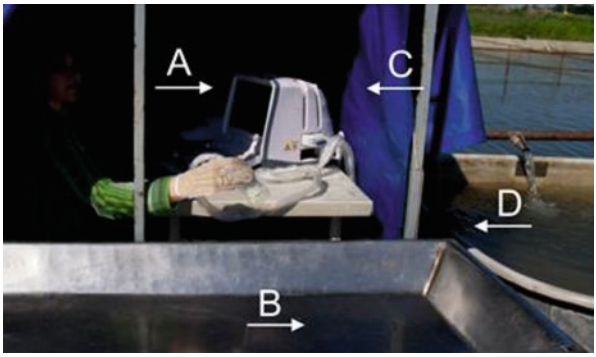

Fig. 49.2 Equipment of mobile data and analytical system on the basis of the ultrasound scanner Mindray DP-6600: *A* ultrasound scanner, *B* special stainless table for fish, *C* tent to protect the scanner monitor from direct sun beams, and *D* fish-collecting tanks

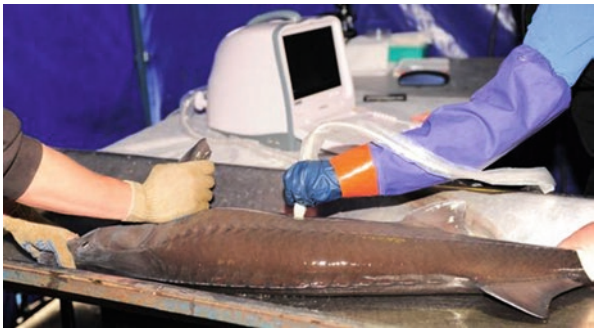

Fig. 49.3 Example of Siberian sturgeon specimen positioning and its holding during the ultrasound examination

49.3 The Resolving Capacity of the Echography Method for the Early Sex Diagnostics and Gonad Maturity Stage Determination of Sturgeons

The ability to identify an image and to diagnose the sex and maturity stage of Siberian sturgeon depends on the following factors: species membership, age, size, conditions, and rearing mode.

The diagnostic markers for sex determination are as follows:

- Localization of germinal tissue in the gonad (medial, lateral, etc.).
- Absence or presence of the gonadal tunic.
- Character of the surface and margins of the gonad; unbroken or broken margin, straight or curved margin.
- Echogenicity of generative tissue, which is revealed by different brightness on the screen image.
- Homogeneity or heterogeneity of gonad tissue structure.
- Relative distance from the genital opening and structure of the gonad caudal margin.

The best time to conduct the early noninvasive sex determination at sturgeon hatcheries with natural conditions is after over-wintering (temperature range from 8 to 12°C), while for warm water hatcheries it is after 2 months' holding at minimum water temperatures. Before sampling, the fish should be deprived of feed for 12 days (minimum).

Regardless of the higher growth rates, sex detection in fish reared at industrial sturgeon farms is difficult due to substantial fat accumulation and somatic growth prevalence over germinal tissue development. Thus, it is important to conduct ultrasound diagnostics to control optimal feeding and define the food deprivation period and timing of fish transfer to wintering.

Timely diagnostics allows to prevent obesity in Siberian sturgeon gonads, substitution of generative tissue (fatty and connective ones), and asynchronous development of the gonad.

During ultrasound control of gonadogenesis and fat accumulation, it is very important to consider ecological and biological characteristics of the Siberian sturgeon, especially its ability to accumulate fat intensively (Akimova 1985).

49.4 Noninvasive Detection of Organs and Tissues by Ultrasound Technique

49.4.1 Scanning Procedure Schedule

Noninvasive ultrasound express examination of sturgeon is conducted in the frontal or transverse planes. The transducer is pressed toward the body in the third–fourth

Fig. 49.4 Correct positioning of the transducer in the process of frontal scanning of a Siberian sturgeon specimen. The transducer is moving from the tail toward the head and backward

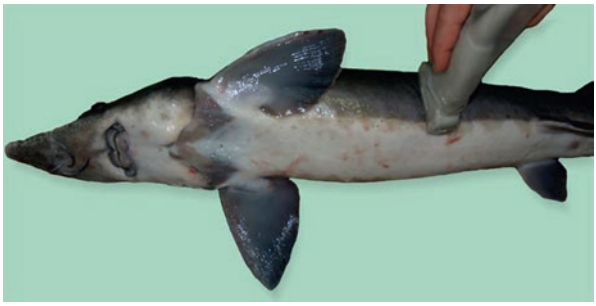

Fig. 49.5 Correct positioning of the transducer at scanning of Siberian sturgeon specimen in transversal plane. The transducer moves in direction from the tail to the head and backward

region of the ventral scutes (counting from the pelvic fins), so that one edge of the transducer is located above the scutes (Figs. 49.4 and 49.5).

The optimal frontal acoustic section angle is found by bending the transducer.

Then, if deemed necessary, the transducer is slowly moved in the proper plane toward the head till the mid-body axis (Fig. 49.5). The examination is conducted along the entire gonad length in this manner.

49.4.1.1 Peculiarities of Organ Visualization in Frontal Ultrasound Scanning

Starting from the age of 2 years, initial ultrasound diagnostics is conducted to preliminarily separated individuals with excessive weight.

The following tissues and organs are visible on the frontal scanning plane (from the transducer scanning plane). The anatomical structures located closer to the transducer are evident in the top part of the monitor, while more distant ones are evident in the lower part (Figs. 49.6 and 49.7):

1. Skin is a thin hyperechoic region, subcutaneous fat tissue, shown as a narrow (2–3 mm) stripe with moderate echogenicity.
2. Muscle tissue is the broad region of mixed echogenicity. The muscle fiber itself (a region of average brightness) alternates with the myotonic walls of connective tissue (on the screen appears as narrow inclined, almost vertical stripes, brighter than muscles).

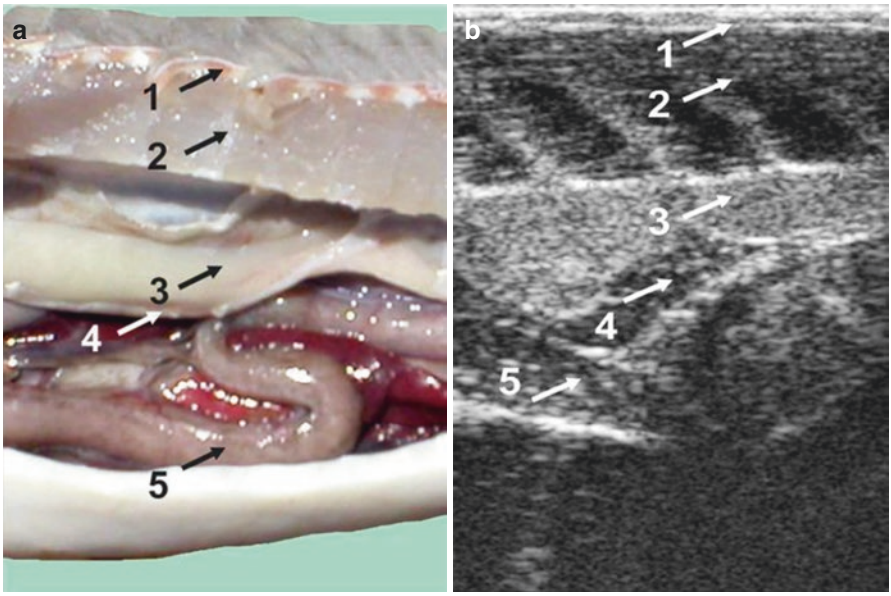

Fig. 49.6 Views of organ and tissue localization in the body cavity of Russian sturgeon male: (**a**) dissected view and (**b**) frontal ultrasound image (*1* skin and subcutaneous tissue, *2* muscle fiber, *3* gonad, *4* fat, *5* colon)

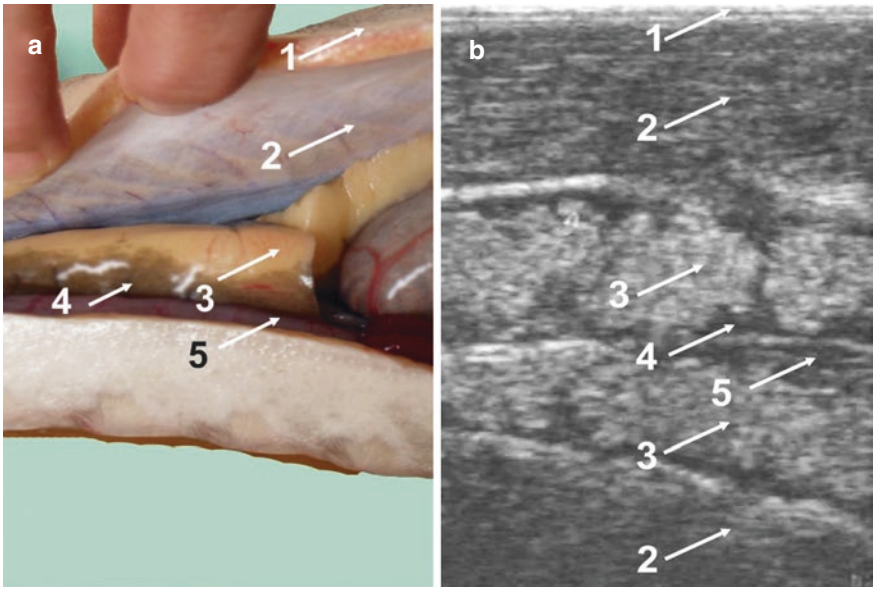

Fig. 49.7 Views of organ and tissue localization in the body cavity of Russian sturgeon female: (**a**) dissected view and (**b**) frontal ultrasound image (*1* skin and subcutaneous tissue, *2* muscle fiber, *3* gonad, *4* fat, *5* colon)

3. The serous membrane of the abdominal cavity appears as a bright, smooth, and distinct boundary line.
4. Gonads, that is the female and male reproductive glands, are visualized differently on the ultrasound scanner screen:
   – The gonad in males is an echo homogeneous structure, surrounded by a bright hyperechoic membrane, that visualized along the entire gonad length.
   – The gonad in females is a structure of heterogeneous echogenicity without clear boundaries; at dynamic examination it appears as an overlapping "cloudy" structure.
5. Intestine, represented as a longitudinal pipelike structure with clear enclosures, consists of two layers: external (hypoechoic) and internal (hyperechoic). In small fish (<4 kg), a second gonad and even muscles and skin (from the other side) are visible under the gonad in reverse order.

49.4.2 Peculiarities of Organ Visualization in Transverse Ultrasound Scanning

The organs localization on the monitor screen is different in transverse scanning compared to frontal scanning (Fig. 49.8).

Example of echograms with Siberian and Russian sturgeon gonad transverse scanning are presented below (Fig. 49.9).

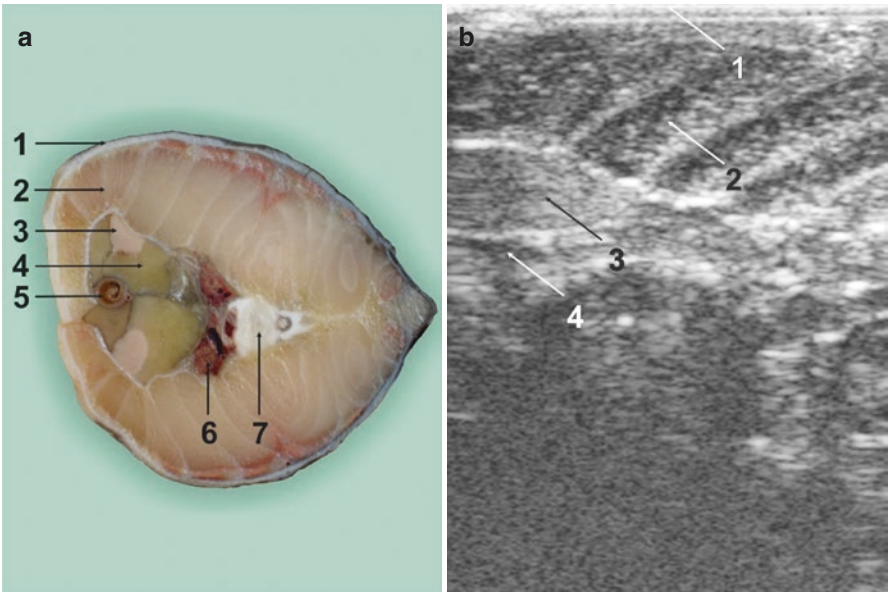

Fig. 49.8 View of organ localization in the body cavity of a male Siberian sturgeon: (**a**) dissected transverse view and (**b**) transverse ultrasound image (*1* skin and subcutaneous tissue, *2* muscle fiber, *3* gonad, *4* gonadal fat, *5* spiral intestine, *6* kidneys, *7* chord)

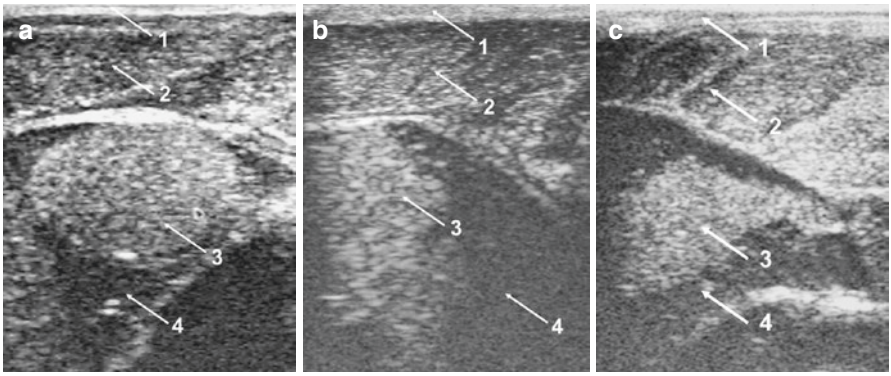

Fig. 49.9 Transverse ultrasound images: (**a**) male Siberian sturgeon, (**b**) female Siberian sturgeon, and (**c**) female Russian sturgeon (*1* skin and subcutaneous tissue, *2* muscle fiber, *3* gonad, *4* fat)

49.4.2.1 Skin

The skin, adipose layer, muscular tissue, and serous tunic of abdominal cavity are visualized in the same way as the lateral frontal scanning.

49.4.2.2 Gonads

The testicular tissue has an oval and almond-like shape enclosed within a clear hyperechoic borderline with uniform internal echo structure. At gonad maturity stage II, the generative part is surrounded partially (II, II semi-fatty, II–III) or completely (II fatty) by fat tissue, which looks like a hypoechoic (dark, almost black) zone on the monitor screen.

Ovarian tissue (region of mixed echogenicity) apparently grows into adipose, with no distinct margins, while at some stages, the gonad hyperechoic fat separates this part from the muscular tissue (Fig. 49.9).

The ultrasound images of male and female Siberian sturgeon with gonads at various maturity stages, used to assess fish reproductive status during season brood stock assessment, have been analyzed.

49.5 Analysis of Ultrasound Images of Different Stages of Testis Development

49.5.1 Male Maturity Stage I (M1)

The species-specific peculiarity of Siberian sturgeon is fat accumulation that have started from the earliest stages of gonadogenesis. Typically, at rearing under conditions of warm-water farms, the germinal and adipose tissue ratio ranges from 1:10 to 1:18 in females and males aged 2+ years, while in males at stage II fatty it amounts to 1:20 and above. The generative and adipose tissue ratio in

Siberian sturgeon varies depending on age, and till maturity stage III amounts to 1:6–1:5. For males at maturity stage IV, this ratio ranges from 2:1 to 10:1 (Akimova 1985).

The testis is evident as a thin taenia (of whitish gray or light pink color) enclosed in adipose tissue (Fig. 49.10).

The testicular tissue is poorly evident due to its small size in ultrasound scans of males at maturity stage I (Fig. 49.11).

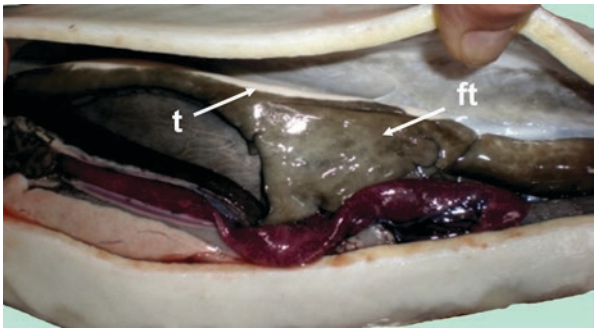

Fig. 49.10 Testes in Siberian sturgeon specimen at maturity stage I (M1): *t* testis, *ft* fat tissue

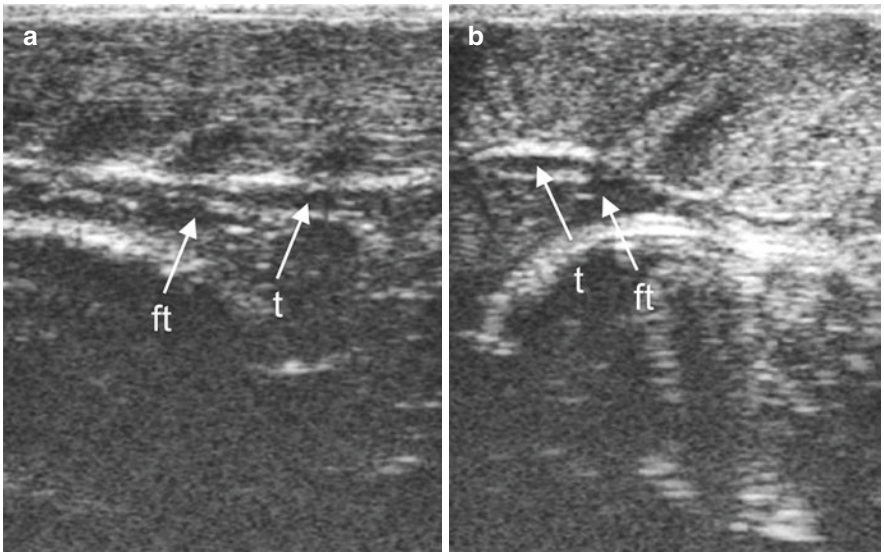

Fig. 49.11 Ultrasound images of testes in Siberian sturgeon specimen at maturity stage I (M 1): (**a**) frontal section and (**b**) transverse section (*t* testis, *ft* fat tissue)

49.5.2 Male Maturity Stage II (M2)

The testes at stage II (M 2) are well discernible, typically white or pink-white in color, being sunk into the adipose tissue. Maturity stage II in males differs from their stage I due to larger testicular tissue growth (Fig. 49.12). Starting from maturity stage II, the germinal tissue can be easily identified in frontal and transverse sections (Fig. 49.13). The testicular part is hyperechoic and has distinct margins.

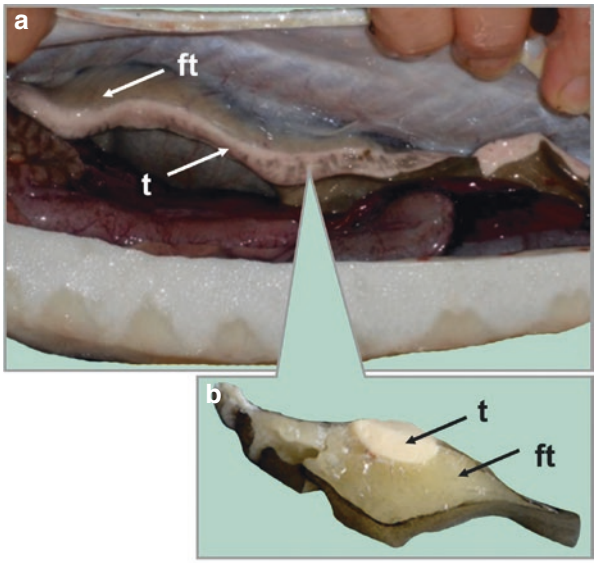

Fig. 49.12 Testes in Siberian sturgeon specimen at maturity stage II (M2): (**a**) localization of testes in the body cavity and (**b**) transverse section of the testis (*t* testis, *ft* fat tissue)

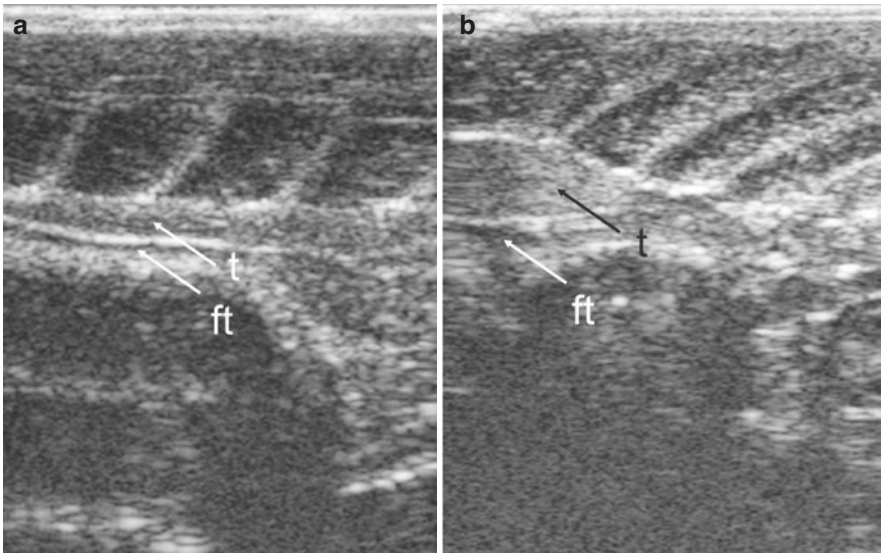

Fig. 49.13 Ultrasound image of frontal and transverse sections of male Siberian sturgeon at maturity stage II (M 2): (**a**) frontal section and (**b**) transverse section (*t* testis, *ft* fat tissue)

In terms of the echogenicity the fat part is practically not distinct from the testicular one. The gonad margins are smoothly curved, while the bright hyperechoic tunic of the testis is well evident.

49.5.3 Male Maturity Stage II Semi-fatty (M2sf)

Stage II (M2sf) semi-fatty occurs when fat covers testicular tissue up to one-half of its width (and is visible only from the lateral side).

Fat accumulation starts from the medial side and gradually spreads to the lateral side (Fig. 49.14).

During developmental stages II semi-fatty and fat, the testicular tissue exhibits a slight increase. The overall gonad volume expands to account for fat accumulation. Therefore, the color of the testicular and fat tissue on the echogram is practically identical. The testicular tissue at stage M2sf (Fig. 49.15) is hypoechoic and appears on the screen as dark regions divided by a light stripe. This line is the boundary

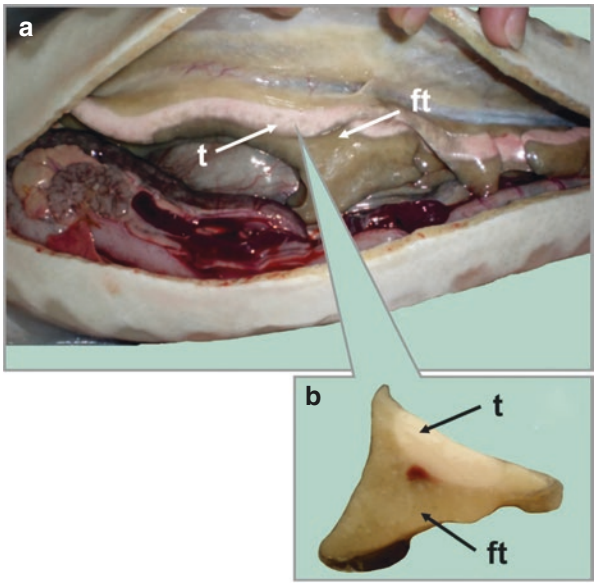

Fig. 49.14 Testes in Siberian sturgeon specimen at maturity stage II semi-fatty (M2sf): (**a**) localization of testes in the body cavity and (**b**) transverse section of the testis (*t* testis, *ft* testicular fat)

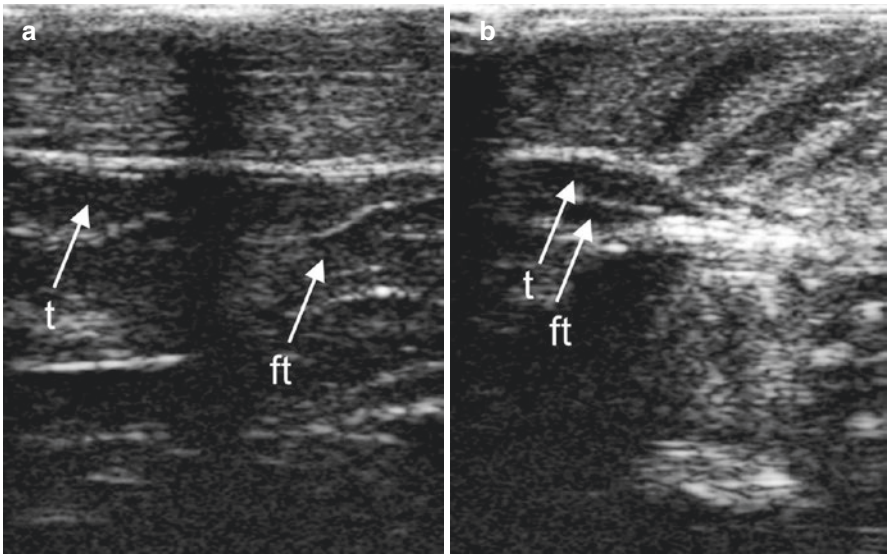

Fig. 49.15 Ultrasound image of frontal (**a**) and transverse (**b**) sections of Siberian sturgeon male at maturity stage II semi-fatty (M2sf) (*t* testis, *ft* testicular fat)

between the adipose and germinal tissues—conventional border between tissues of different density.

49.5.4 Male Maturity Stage II Fatty (M2f)

In the development process from stage II (M2) to stage II fatty (M2f), the weight of the testes exhibits a five- to tenfold increase (owing to fat). Fat amounted to 85–95% of the total gonad weight. Visually, fat completely covers the testicular tissue (Fig. 49.16).

The testicular tissue appears as a homogeneous fine-grained structure (of gray color on echograms), separated from the adipose (hypoechoic, dark) by the hyperechoic boundary, evident as a bright white line (conventional tissue border for different density and ultrasound signal velocity) (Fig. 49.17). The testis on the transverse section is tightly bound to the lateral muscles.

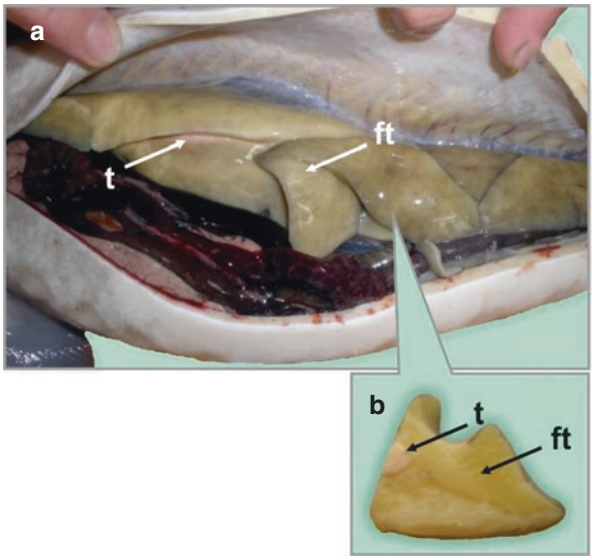

Fig. 49.16 Testes in Siberian sturgeon specimen at maturity stage II fatty (M2f): (**a**) localization of testes in the body cavity and (**b**) transverse section of the testis (*t* testis, *ft* testicular fat)

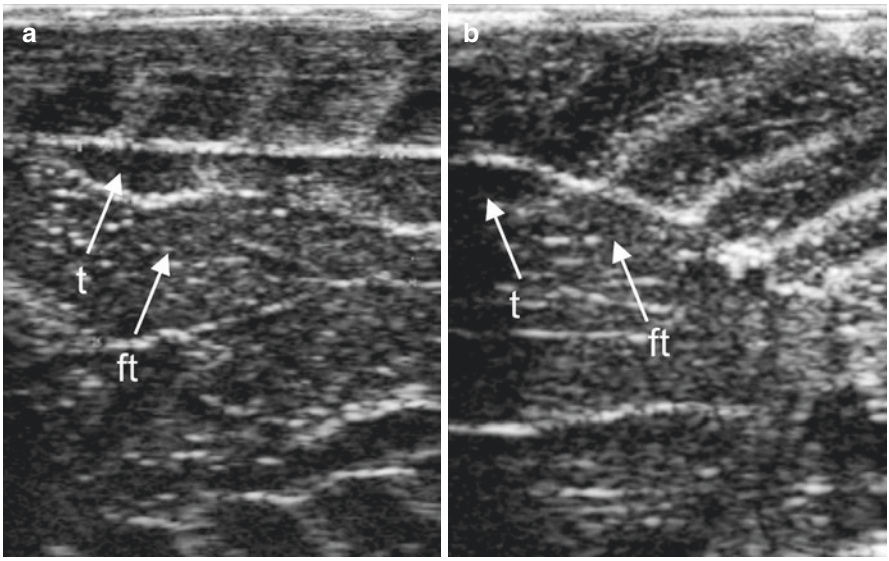

Fig. 49.17 Ultrasound images of frontal (**a**) and transverse (**b**) sections of Siberian sturgeon male at maturity stage II fatty (M2f): (*t* testis, *ft* testicular fat)

49.5.5 Male Maturity Stage III (M3)

Duration of this stage is very short. By the end of the stage, the fat is almost completely consumed for sexual cell (spermatogonia and spermatocytes) formation. The testicular part of the gonad exhibits a considerable increase (Fig. 49.18).

At maturity stage III, echogenicity of the testicular tissue shows a considerable increase. The testes appear on echograms as light gray homogeneous structures (in some cases almost white) with distinct hyperechoic margins (Fig. 49.19).

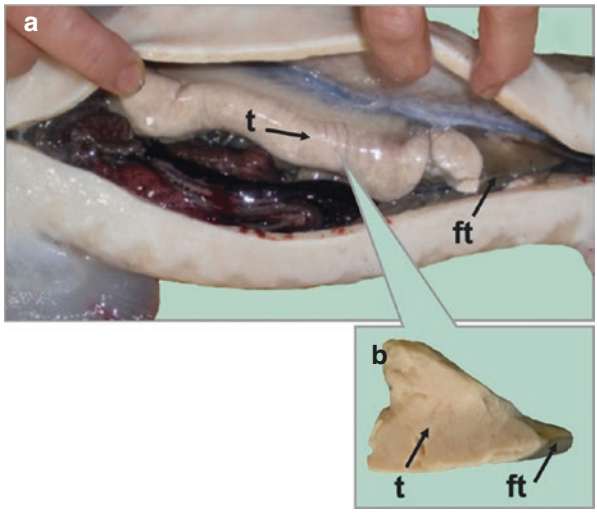

Fig. 49.18 Testes in Siberian sturgeon specimen at maturity stage III (M3): (**a**) localization of testes in the body cavity and (**b**) transverse section of the testis (*t*), testicular fat (*ft*)

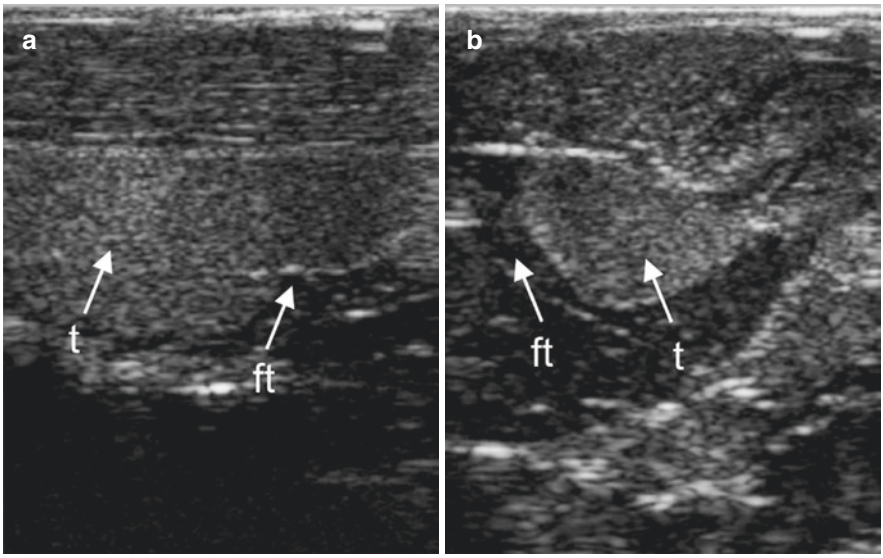

Fig. 49.19 Ultrasound images of frontal (**a**) and transverse (**b**) sections of Siberian sturgeon male at maturity stage III (M3): (*t* testis, *ft* testicular fat)

49.5.6 Male Maturity Stage IV (M4)

Spermatogenesis process completion characterizes this stage.

The testes become large in size and attain light, almost milky coloration when almost completely fat deprived (Fig. 49.20).

On echogram (Fig. 49.21), the testes at stage IV (M4) appear as bright hyperechoic fine-grained homogeneous structures with clear margins and well-defined tunics that are well discernible in the frontal and transverse sections.

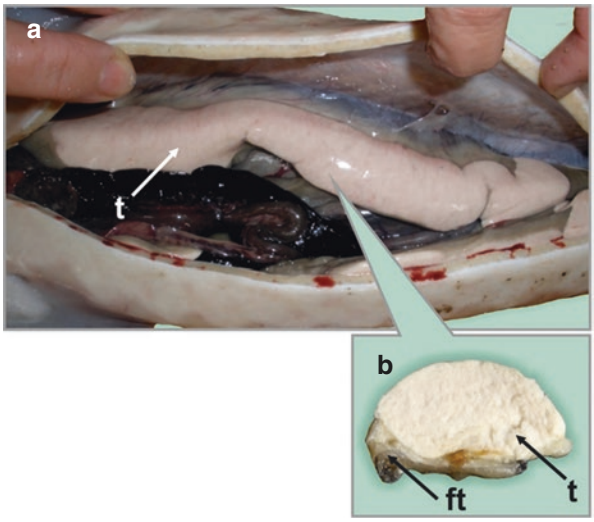

Fig. 49.20 Testes in Siberian sturgeon at maturity stage IV (M4): (**a**) view of the testis in the body cavity and (**b**) transverse section of the testis: (*t* testis, *ft* testicular fat)

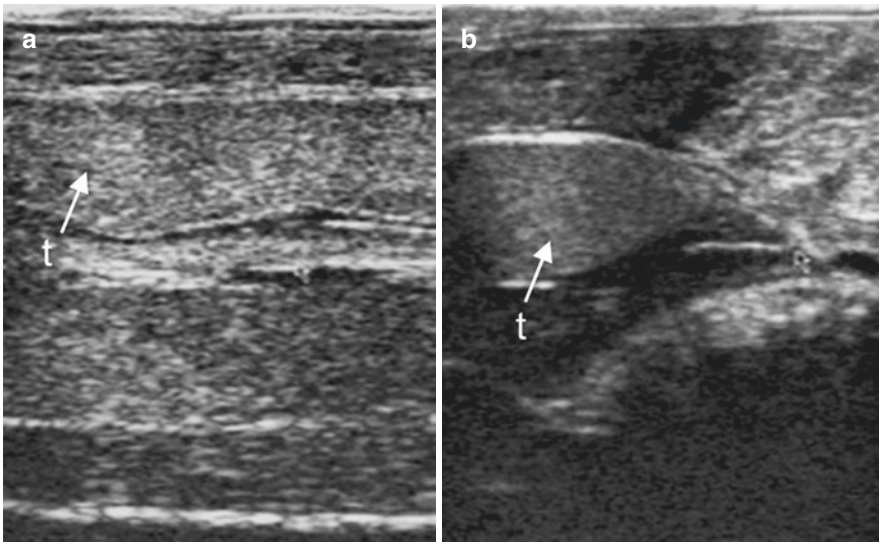

Fig. 49.21 Ultrasound images of frontal (**a**) and transverse (**b**) sections of Siberian sturgeon male at maturity stage IV (M4): (*t* testis)

Hyperechogenicity of the testis reaches its maximum at stage IV (image color is close to white). Ripe male maturity status and readiness to spawn can be assessed by the brightness of the testis image.

49.6 Analysis of Ultrasound Images of Ovaries at Different Maturity Stages

49.6.1 Female Maturity Stage I (F1)

Ovarian development at stage I in sturgeon females is characterized by the appearance of a longitudinal fissure on the gonad lateral side that is clearer on the caudal part. Individual oocytes at initial stages of protoplasmatic growth (previtellogenesis) are found in histological samples of female maturity stage I (Trusov 1972; Akimova 1985).

Siberian sturgeon sex and maturity stages can be determined through efficient ultrasound diagnostics application starting from maturity stage II.

49.6.2 Female Maturity Stage II (F2)

"Brain-like" folds (ovigerous lamellae) are notable in the lateral part of the ovary (Bruch et al. 2001). The ovary color varies from pinkish white to yellowish pink (Bahmani et al. 2005). The gonad fat amount is not that considerable (Fig. 49.22).

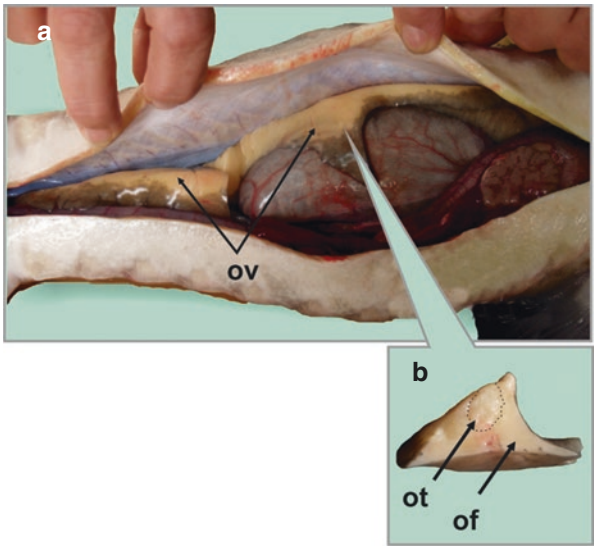

Fig. 49.22 Ovaries at maturity stage II (F2) in Siberian sturgeon specimen: (**a**) localization of the gonad in the body cavity (*ov* ovary) and (**b**) transverse section of ovaries (*ot* ovarian tissue, *of* ovarian fat)

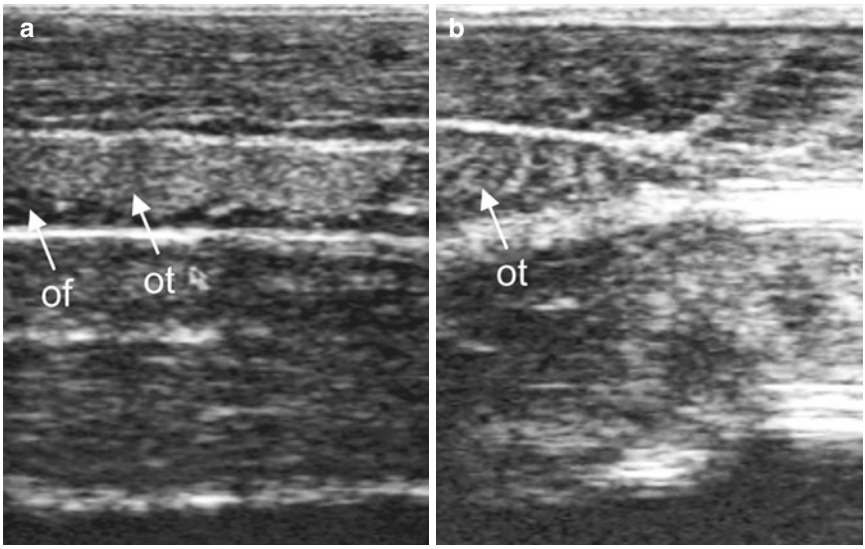

Fig. 49.23 Frontal (**a**) and transverse (**b**) ultrasound images of ovaries at maturity stage II (F2) in Siberian sturgeon specimen (*ot* ovarian tissue, *of* ovarian fat)

On ultrasound images, the ovarian tissue (Fig. 49.23) appears as a mixed echogenicity, grained "cloud-like" structure with uneven boundaries without a tunic.

At dynamic examination (cine-mode), the inner gonad structure changes with "cloud-like" lapping. The ovary fatty portion, on frontal and transverse images, is small and visualized as the darker areas are distinct from the lighter ovarian tissue.

49.6.3 Female Maturity Stage II Semi-fatty (F2sf)

Further ovary development at maturity stage II semi-fatty (F2sf) is associated with fat deposition that starts from the ovigerous lamellae (visually, the ovaries embedded in fat) and then continues on the medial and lateral sides (Fig. 49.24).

On the ultrasound image (Fig. 49.25), single ovigerous lamellae appear as higher echogenicity areas (gray or light gray color), alternating with hypoechoic (dark) fatty regions.

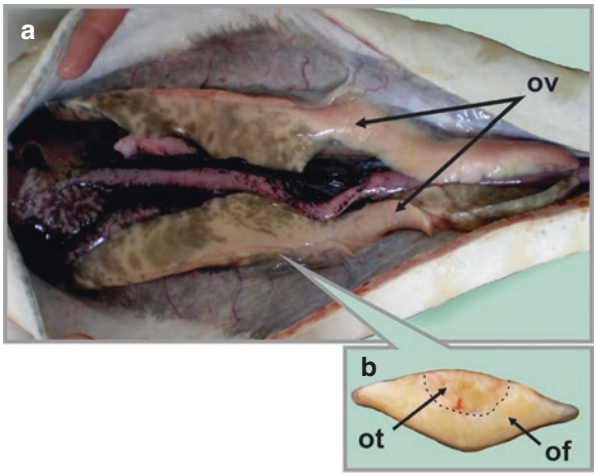

Fig. 49.24 Ovaries at maturity stage II semi-fatty (Fsf2) in Siberian sturgeon specimen: (**a**) localization of the gonad in the body cavity (*ov* ovary) and (**b**) transverse section of ovaries (*ot* ovarian tissue, *of* ovarian fat)

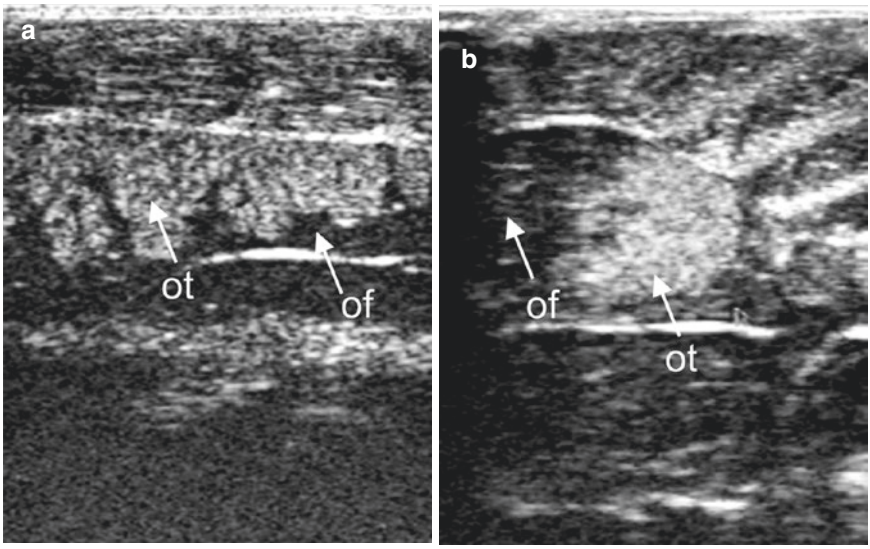

Fig. 49.25 Frontal (**a**) and transverse (**b**) ultrasound images of an ovary at stage II semi-fatty (F2sf) in Siberian sturgeon specimen: (*ot* ovarian tissue, *of* ovarian fat)

49.6.4 Female Maturity Stage II Fatty (F2f)

At female maturity stage II fatty (F2f), the ovaries accumulate fat on the lateral and medial sides to form a fat cover (Fig. 49.26).

Fat surrounds the ovarian tissue (light, of moderate echogenicity) from the medial and lateral sides (dark anechoic regions).

At female maturity stage II fatty (F2f) scanning (Fig. 49.27), the ovarian tissue (lighter) is encompassed by the fat (darker) tissue.

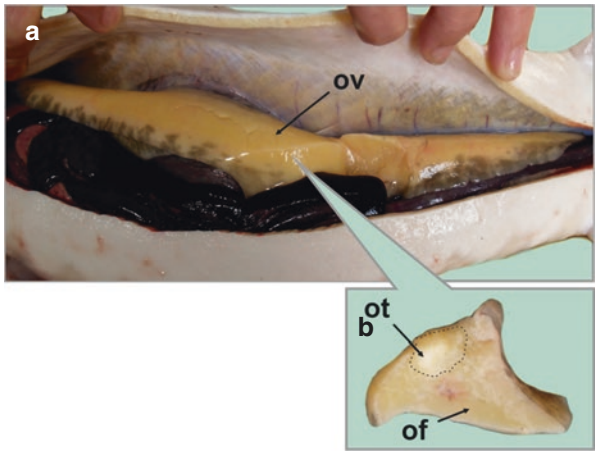

Fig. 49.26 Localization of ovaries at maturity stage II fatty (F2f) in the body cavity of a Siberian sturgeon female (**a**) localization of the gonad in the body cavity (*ov* ovary) and (**b**) transverse section of ovaries (*ot* ovarian tissue, *of* ovarian fat)

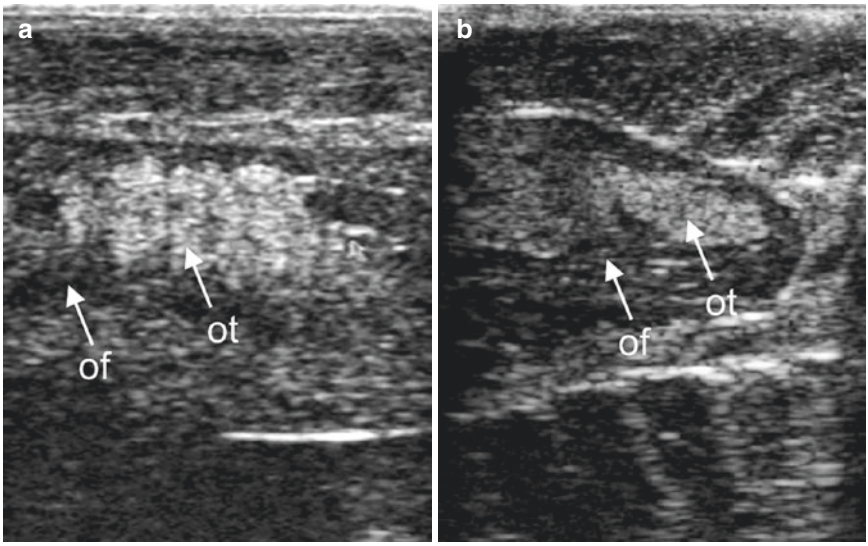

Fig. 49.27 Ultrasound images of frontal (**a**) and transverse (**b**) section of ovaries at maturity stage II fatty (F2f) in Siberian sturgeon specimen (*ot* ovarian tissue, *of* ovarian fat)

Under intensive Siberian sturgeon-rearing conditions, females that reach maturity stage II fatty (and decreased level of fat exchange) should be separated and transferred to an adequate temperature and feeding regime (Chebanov and Galich 2010, 2017). The optimal temperature range to hold selected females at maturity stage II fatty is 19–22 °C. The feeding rate for these females should be decreased (by 30%), and diets with fat content <12% preferably used. This hampers further fat accumulation and speeds up the transition to vitellogenesis (gonad maturity stage III).

Under natural conditions, the oocytes' protoplasmic growth period (maturity stages II and II semi-fatty) in Siberian sturgeon (Lena population) takes 8–10 years (Akimova 1985). In warm water farms the duration decreases and amounts to 3–5 years.

49.6.5 Female Maturity Stages II–III (F2–3)

At the onset of vitellogenesis occured at age 4–5 years and earlier (3 years) at optimum temperature and feeding regime, the further maturation of the ovaries is associated with the trophoplasmatic growth of oocytes due ongoing yolk synthesis.

Since gonads tend to possess less fat, the ovarian tissue becomes more evident (Fig. 49.28). The senior generation oocytes (of diameter about 500–600 μm) that protrude above the lateral part of the testis stages II–III (F2–3) are characterized by a nonpigmented elder generation oocyte with white eggs apparent on the yellowish white background. Lipoid residuals can be evident in some ovary sections.

An ovary on the ultrasound image shows (Fig. 49.29) moderate echogenicity (gray or light gray coloration). Ovigerous lamellae "penetrate" the gonad body and appear as a brachiate vertical structure ("coral-like" or "fringed" in shape), of higher echogenicity, which spreads to the dark hypoechoic region (the fat tissue).

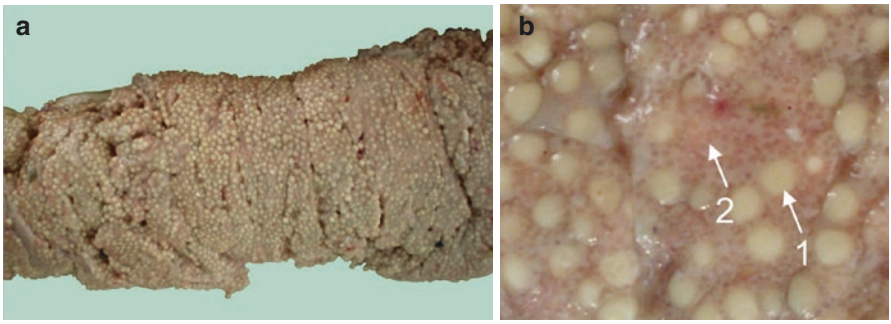

Fig. 49.28 View of an ovary (**a**) at maturity stages II–III (F2–F3), (**b**) zoomed view of (*1*) oocytes and (*2*) oocyte of senior generation

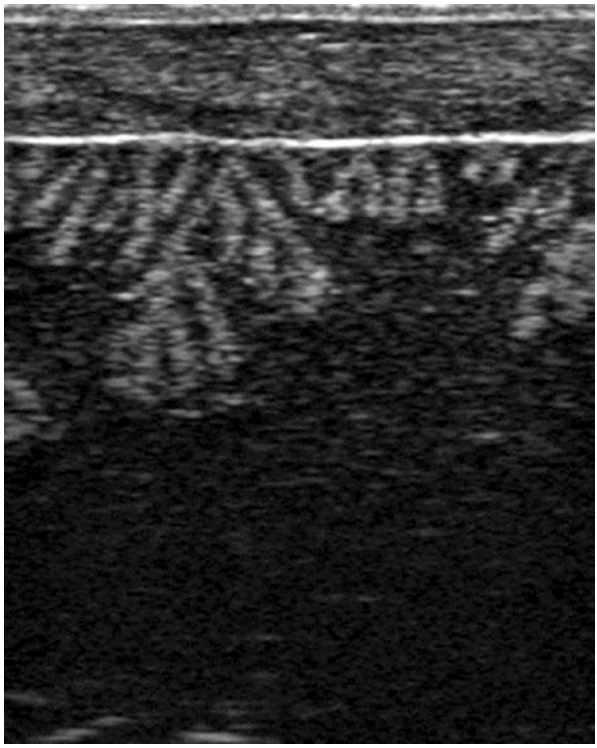

Fig. 49.29 Ultrasound images of frontal section of ovaries at maturity stage II–III (F2–F3) in Siberian sturgeon specimen

49.6.6 Female Maturity Stage III (F3)

White oocytes exhibit a size increase and grayish oocytes can be encountered. The nuclear polarization of pigmented oocytes is not well evinced. At the end of the stage, completely pigmented (gray) oocytes prevail among senior generation oocytes. This is an important visual sign of maturity stage III (F 3).

On the ultrasound image, ovaries at stage III appear with a clear granular texture (Fig. 49.30). Ovigerous lamellae are evident on the ultrasound image as light diffuse regions, embedded into the hypoechoic ovarian tissue, where small oocytes are visible.

On the frontal ultrasound image of a Siberian sturgeon ovary at late maturity stage III–stage IV onset, various sizes of small individual oocytes are discernible immediately below the muscle fiber (Fig. 49.31). The gonad can be visualized almost completely in small fish.

Fig. 49.30 View of an ovary (**a**) at maturity stages III (F3), (**b**) zoomed view of oocytes

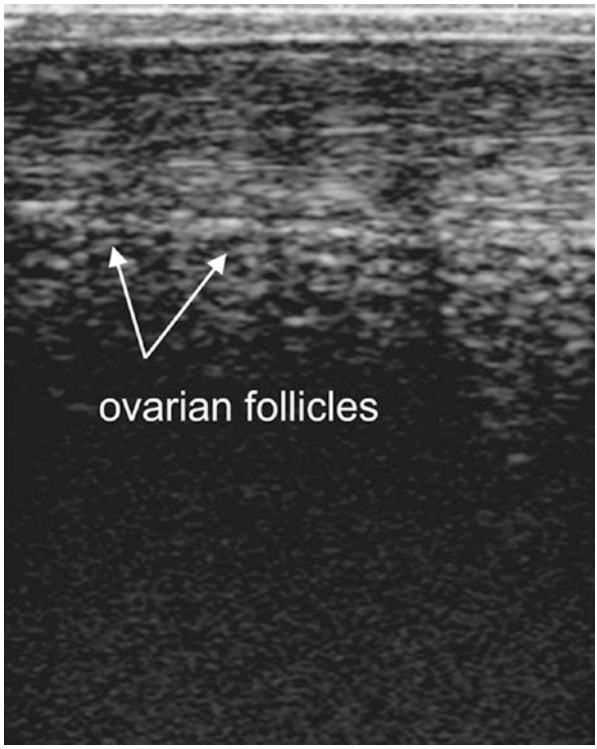

Fig. 49.31 Frontal ultrasound images of an ovary in Siberian sturgeon specimen at the end of maturity stage III and onset of stage IV (F3–F4)

49.6.7 Female Maturity Stage IV Incomplete (F4i)

At female maturity stage IV incomplete (F4i), all the dark gray oocytes are close to definitive sizes. The polarization of nucleus oocytes on the histological sections is clearly manifested. The small fat cover remnants surrounding the ovarian tissue are retained (primarily on the medial side).

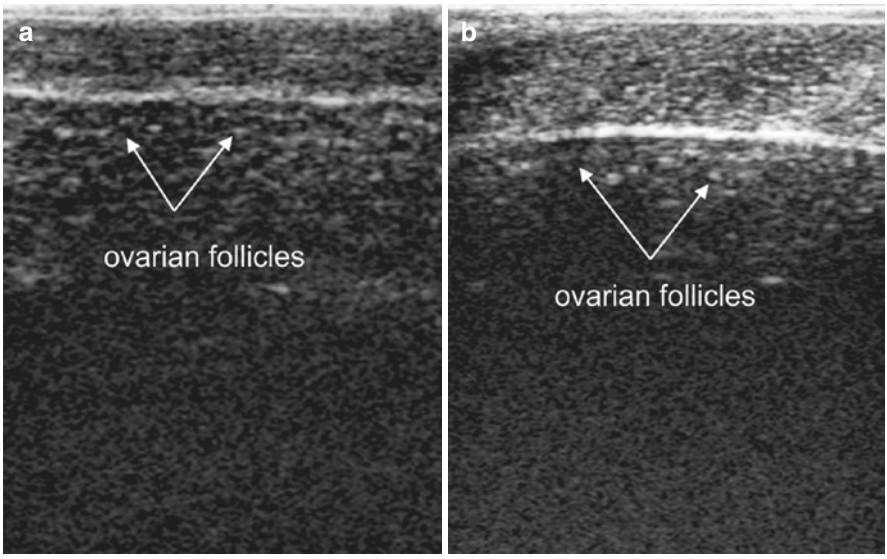

Fig. 49.32 Frontal (**a**) and transverse (**b**) ultrasound images of an ovary at the maturity stage IV incomplete (F4i) in Siberian sturgeon specimen

Single large oocytes of equal size are well discernible on the female Siberian sturgeon ultrasound image at this maturity stage (Fig. 49.32). The ability of the ovaries to let through ultrasound waves is considerably reduced, and thus the medial side of the gonad and organs located below could not be visualized.

49.6.8 Female Maturity Stage IV Complete (F4c)

Maturity stage IV complete (F4c) is a responsive (on hormonal injection) gonadal development state.

There is an evident lack of fat (Fig. 49.33), while the ovaries fill almost the entire body cavity, and completely pigmented oocytes (black in color) reach definitive size.

In the image (Fig. 49.34), single oocytes are evident as granular inclusions of almost equal size; the ovary shows granular heterogeneous appearance (Chebanov et al. 2004; Chebanov and Galich 2009). The oocyte lines become more apparent.

At this maturity stage, ultrasound is almost completely absorbed in the ovary upper (1 cm) layer; hence, the medial part of the gonad and organs below are completely not discernible.

Fig. 49.33 Localization of the gonad at maturity stage IV complete (F4c) in the body cavity of Siberian sturgeon specimen

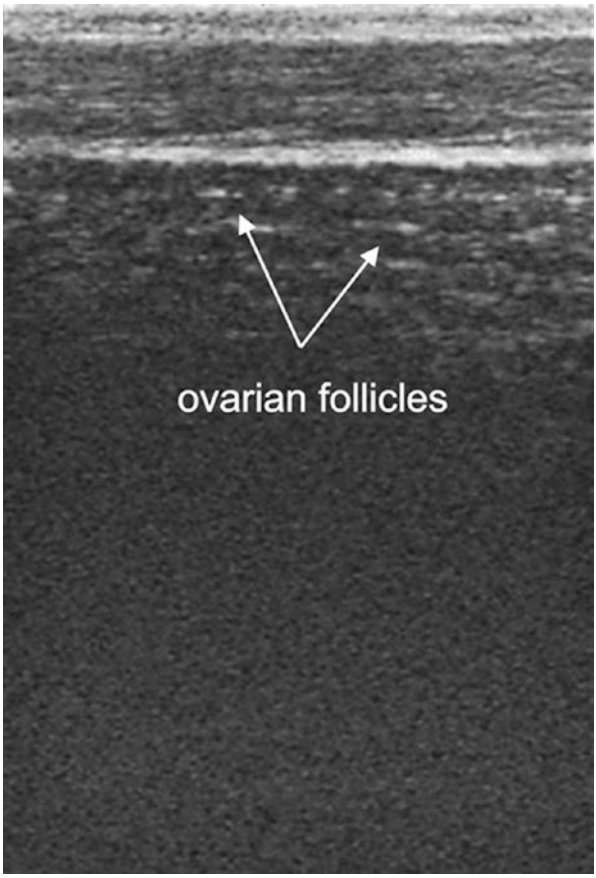

Fig. 49.34 Frontal ultrasound images of an ovary at the maturity stage IV (F4c) complete in Siberian sturgeon specimen

49.6.9 Female Maturity Stage V (F5): Spawn

On the stage V (F5) ultrasound images, the ovulated eggs of equal size arranged in rows are better defined than the ones at complete stage IV. This is a primary visual distinction for the ovary frontal scanning at maturity stage V from stage IV complete.

The effect of distal acoustic enhancement is registered due to considerable ovarian fluid accumulation under the oocytes rows (Fig. 49.35) that manifests as white (hyperechoic) dashes on the screen, providing more contrast than seen in stage IV images (Chebanov and Galich 2013).

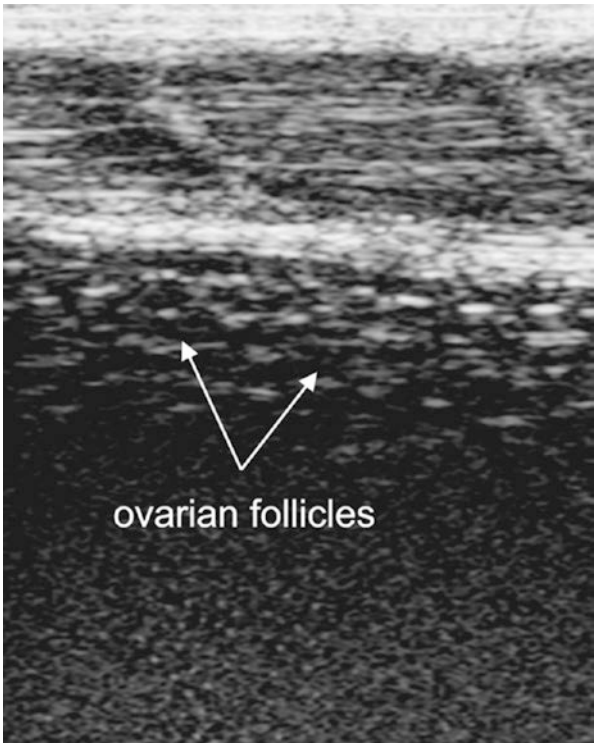

Fig. 49.35 Frontal ultrasound images of an ovary at the maturity stage V (F5) in Siberian sturgeon specimen

49.6.10 Female Maturity Stage VI (F6)

After the natural spawning or hatchery control non-lethal egg extraction from ripe females, resorbing mature oocytes and junior generation oocytes remain in the ovary. Post-spawn fish ovaries transit to maturity stage II.

The ultrasound images of the ovaries at maturity stage VI (F6) appear similar to stage II, while the residual resorbing mature oocytes are the prime difference between these echograms (Fig. 49.36).

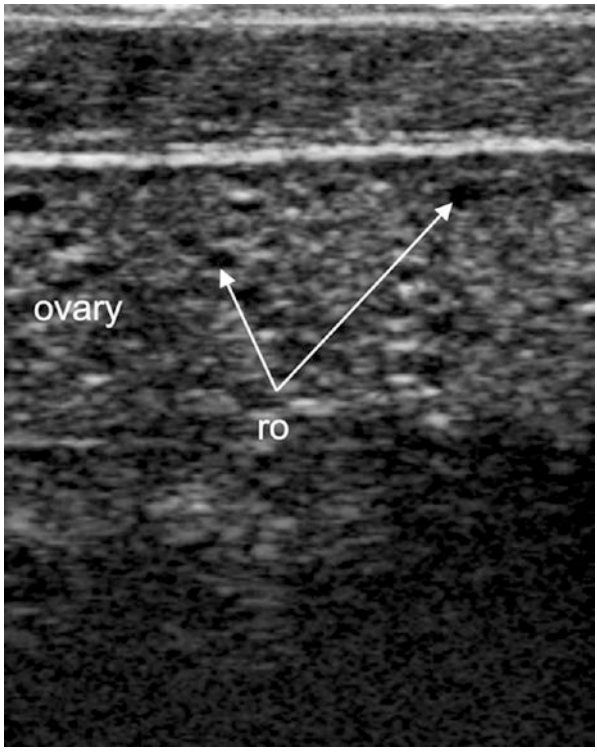

Fig. 49.36 Ultrasound image of frontal section of ovaries at maturity stage VI (F6) in Siberian sturgeon specimen (atresia): (*ro* resorbing mature oocytes)

49.7 Ultrasound Noninvasive Diagnostics of Developmental Anomalies in the Reproductive System of Sturgeon (Pathologic Echoanatomy)

Application of the noninvasive express method allows culling sturgeon individuals with reproductive system abnormalities, which are not promising for hatchery control reproduction.

Moreover, various malformation identifications on the ultrasound images enable monitoring of sturgeon reproductive system development. This is similar to that conducted in natural water bodies at bioecological indication of habitat degradation, as well as an assessment of breeder holding conditions (thermal regime, water quality, diet formulation, etc.) in aquaculture or at selection effect evaluation in sturgeon and domesticated pedigree breeding programs (Chebanov and Galich 2008).

Hence, the development of functional diagnostic methods allows efficiency enhancement of sturgeon health evaluation, especially in the domestic brood stocks of rare and endangered sturgeon species, when prolonged pellet feeding (e.g., with a high level of fat) can lead to serious liver and other organ damage (degeneration).

The express technique application will be especially useful for wild breeders and immature sturgeon preselection intended for pre-domestication (adaptation to artificial holding conditions) (Bilio 2007). In fact, pre-culling efficiency to detect individuals with inner organ "hidden" pathology using ultrasound diagnostics can hardly be overestimated due to the labor intensiveness and high cost of long-term "wild" fish domestication.

Asynchrony in ovarian tissue development in mature Siberian sturgeon females at long-term holding under high-temperature conditions is shown in Fig. 49.37a, b.

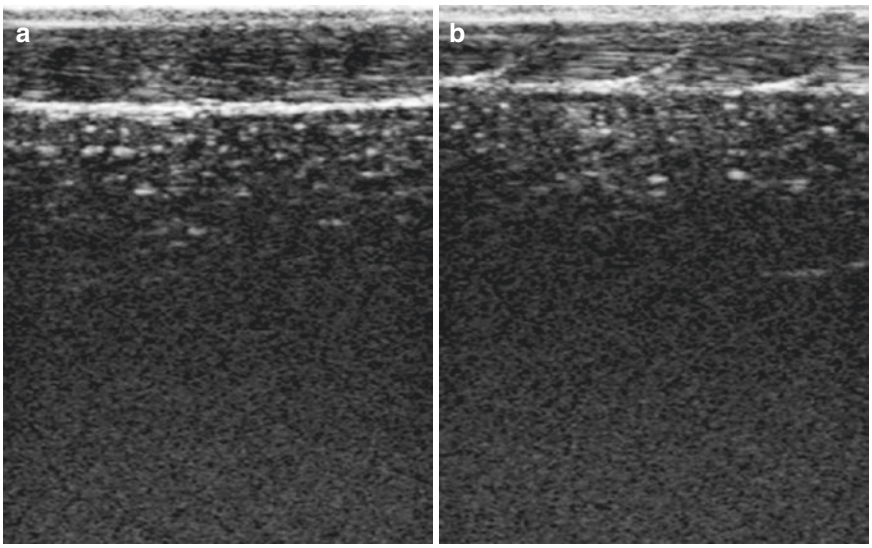

Fig. 49.37 Ultrasound images of ovaries in Siberian sturgeon females, held at constant high temperature (14–20 °C)

Inappropriate thermal regime during fish holding at late stages of gametogenesis leads to asynchrony in gonad development. The ovarian tissue is evident as irregular structures (oocytes of different generations).

49.7.1 Fatty Degeneration of Ovaries

Ovary fatty degeneration occurs due to the obesity of fish held in warm-water farms with year-round intensive feeding. Thus, single islets of germinal (testicular) tissue with a small quantity of mature oocytes are visible (Fig. 49.38).

Obligatory temporal (1–2 months) holding of females at low water temperatures (4–6 °C), associated with feed deprivation (starting from maturity stage II semi-fatty and II fatty), is recommended to avoid obesity, especially aiming at gametogenesis synchronization in the majority of females (Chebanov and Billard 2001; Chebanov and Savelyeva 1999; Chebanov et al. 2004; Chebanov and Galich 2008, 2009).

This is of great importance for brood stock management optimization and reproduction control in large sturgeon fish farms and hatcheries.

Unlike females of other sturgeon species (Russian sturgeon, stellate sturgeon (*A. stellatus*), sterlet (*A. ruthenus*)), females of Siberian sturgeon, that grown in farms with constantly high temperatures (from 10 °C and above) and a lack of wintering, accumulate a significant amount of fat. This subsequently hampers in vivo extraction of ovulated eggs (Podushka 1986), and considerably reduces the overall fecundity of the females (up to 1%), as compared with the expected one (8–15%).

On the ultrasound image, representing stage F4i, the border between ovarian tissue with oocytes and adipose tissue can be visualized by frontal scanning (Fig. 49.39).

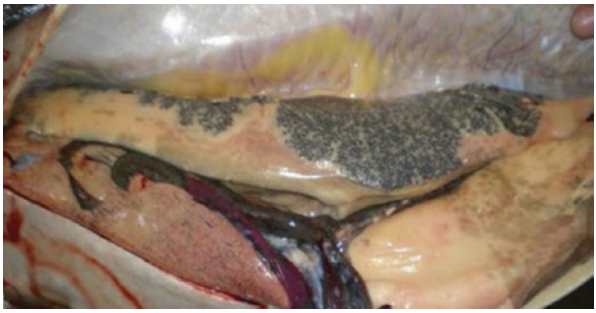

Fig. 49.38 View of fatty gonads in mature Siberian sturgeon female reared under conditions of constant holding at high temperature (15–23 °C) and feeding dissected view of a fatty gonad

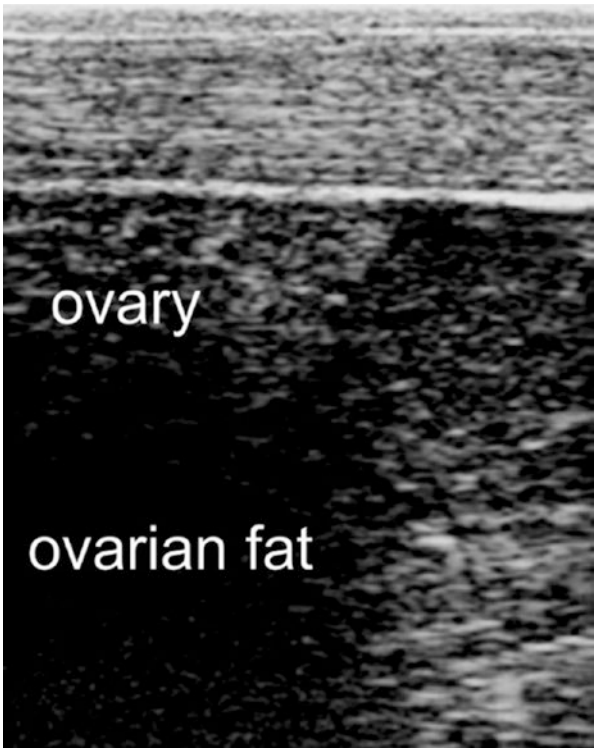

Fig. 49.39 Frontal ultrasound images of Siberian sturgeon specimen: an ovary at the incomplete maturity stage IV (F4i)

49.7.2 Cyst

A cyst (from Greek *kystis* bladder) is a pathological structure with tight walls, entirely filled with liquid. Cysts (2–4 mm in diameter) occurred in the germinal tissue of sturgeons at different maturity stages (Fig. 49.40a, b). On the echogram they appear as thick (wall)-rounded anechoic structures with acoustic enhancement below.

The cystic formations can be identified as organotypic tumors of human lipoma and fibroma type, which developed due to ovigerous lamellae degeneration (Moiseeva et al. 1997). Such malformations in gonad development affect the reproductive performance of fish, but do not hamper the normal development of gametes in the other gonad portion.

Similar neoplasms can be formed as well, at development of tumors, granuloma, and parasitic invasion.

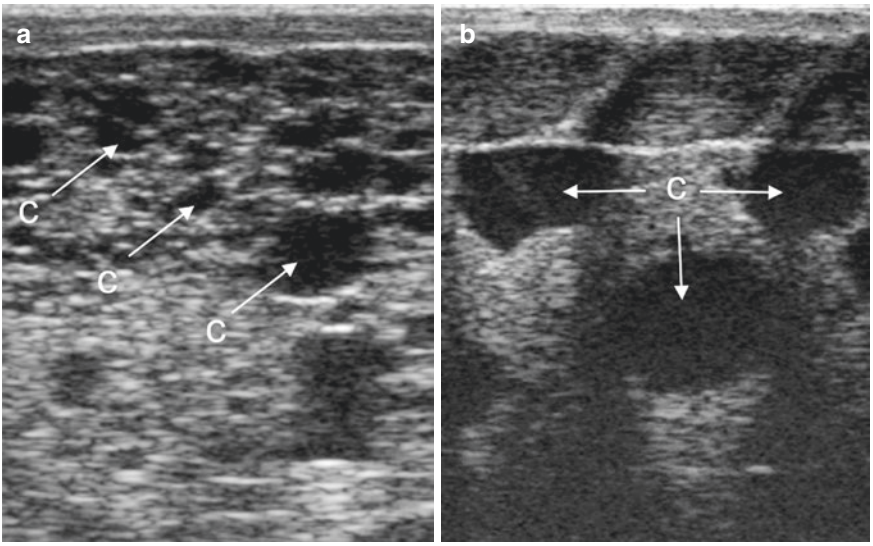

Fig. 49.40 Ultrasound images of cysts (*c*) with distal acoustic enhancement in the ovary of sturgeon

Polycystic gonads are diagnosed by the presence of multiple cysts of varying size, associated with increased gonad volume and architectonic violation. It is a consequence of the inflammatory process and improper brood fish handling during the spawning migration, usually on warm water farms.

49.7.3 Sclerotization

Sclerotization (from Greek *sklerosis*) is the hardening of the germinal tissue regions caused by a violation in blood circulation due to age changes, after obtaining gametes many times, or inflammation. It is expressed by alteration of gonad tissue with connective tissue and cartilaginous neoplasm formation.

49.7.3.1 Ascite (Dropsy)

Fluids in the body cavity of the fish may visually result in maturity misdiagnosis in females. In ultrasound diagnostics the ascites fluid is visualized with a minimum echo signal (echo-negative ascite), and inner organs (ovaries, testes) are diagnosed as areas of increased echogenicity (Fig. 49.41).

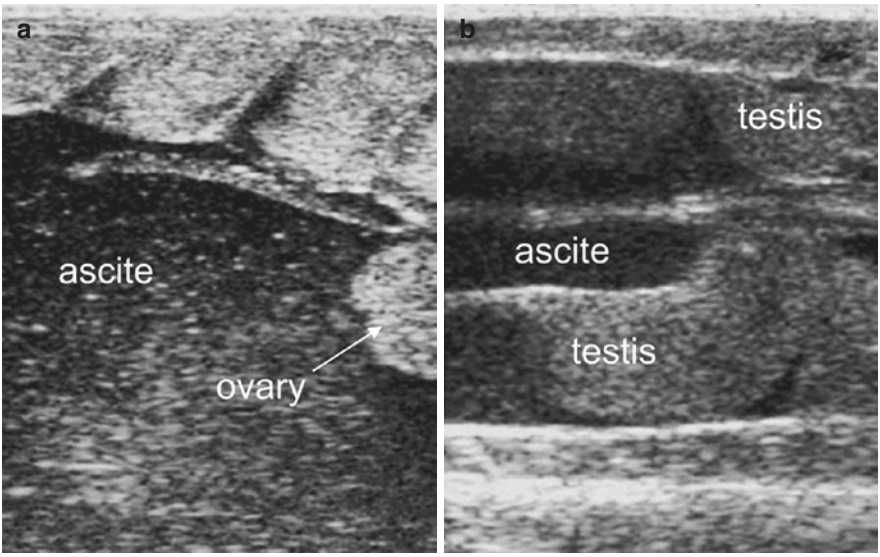

Fig. 49.41 Ultrasound images of frontal and transverse sections of ascite in the body cavity: (**a**) female and (**b**) male

Female and male gonads are well evident on the ultrasound images as bright hyperechoic structures, encircled with echo-negative ascite.

49.7.4 Hermaphroditism

Intersexuality is typically manifested by the presence of ovotestis. Specimens with one gonad represented by a testis and the second one by an ovary are rarely encountered.

Hermaphroditism is not a normal situation for sturgeon. Chirkina (1957) described ovotestis in hybrids between *Huso huso* and *A. ruthenus* (bester).

A few cases have been reported on wild sturgeons from polluted habitats (Chapman et al. 1996; Harshbarger et al. 2000; Van Eenennaam and Doroshov 1998).

According to Williot (2002), Williot et al. (2005), hermaphroditism can reach 5% for one generation of sterlet (older than 14 years). Cases of hermaphroditism with similar frequency have also been described for Russian sturgeon, both in aquaculture (Jackson et al. 2006) and for mature Russian sturgeon hatchery produced in natural water bodies.

According to different authors (Chapman et al. 1996; Van Eenennaam and Doroshov 1998; Harshbarger et al. 2000; Matshe and Rosemary 2012), the

occurrence of intersexuality in various sturgeon species in the wild has commonly been as low as 3% of the total population. At the same time, a more sound frequency of intersexes has been mentioned for some North American populations, 11.6% for the shortnose sturgeon from the Delaware River and Cooper River population (Matshe and Rosemary 2012) and 29% for pallid sturgeon males (*Scaphirhynchus albus*) from the Mississippi River (Harshbarger et al. 2000). The occurrence of intersexes in wild environment is often due to water contamination with endocrine-disrupting chemicals (EDCs) (Harshbarger et al. 2000; Mills and Chichester 2005; Matshe and Rosemary 2012). These chemicals interfere with the signaling pathways of endogenous hormones, which serve as the relevant mode of action (Swedenborg et al. 2009).

EDCs comprise, for instance, DDT, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers, and phthalates (Centers for Disease Control and Prevention 2009). The high content of PCB and DDT degradation products in the pallid sturgeon flesh and roe has been associated with the high-frequency ratio of intersexes among wild males from the Mississippi River population (Harshbarger et al. 2000).

Three cases of intersexes were reported for the white sturgeon (*A. transmontanus*) population from the Columbia River with DDT and its metabolites, as well as PCB and other pesticides, detected in the gonads and livers of these specimens.

An inverse correlation between the sturgeon androgens and the EDC plasma levels has been revealed (Feist et al. 2005). Instances of intersex have also been recorded in sturgeon aquaculture (Rzepkowska et al. 2014).

The occurrence of intersexes in controlled conditions is typically 5% of cultured fish, similar to that in natural populations (Flynn and Benfey 2007; Williot et al. 2005; Henne et al. 2006). So far, a higher frequency (14%) of intersexes has only been reported for Russian sturgeon (Jackson et al. 2006).

It should be noted that in relation to the presence of intersexual specimens, in some cases sexing using ultrasound, conducted in one part of the gonads, can lead to erroneous sex determination and consequent improper separation of males from females at sturgeon farms.

49.8 Express Diagnostics of Diseases and Traumas of Sturgeon's Internal Organs

Besides the above-described reproductive system anomalies, the noninvasive ultrasound technique may be effectively used for diagnostics of some other anomalies, frequently reported in the sturgeon hatchery and farming practice, e.g., cysts, tumors of different organs, notochord deformation, swimming bladder inflammation, liver and heart obesity, abdominal cavity dropsy, and foreign bodies (as well as stones) in the digestive system (Chebanov and Galich 2010, 2013, 2017).

49.9 Ultrasound Diagnostic for Optimization Brood Stock Management

Analysis of more than one million echograms enables us:

– To determine the sum of effective temperatures (degree-days) and optimal seasonal temperature changes necessary to reach early puberty for Siberian sturgeon and different species.
– To assess the duration of separate maturity stages depending on fish-holding conditions (temperature regime).
– To develop control feeding regimes to avoid gonad obesity and gonad lagging at maturity stage II fatty, ensuring timely onset of vitellogenesis.

The actual onset of vitellogenesis is determined by the well-balanced size of primary sexual cells and the ovary fat content that constitutes the energy basis for further oocyte development (Chebanov and Galich 2010).

Owing to this, in the course of ultrasound monitoring (in farms with natural temperature regime, only late autumn and spring, while in recirculation systems and warm-water farms, all year round), echograms serve as indicators for alteration in temperature and feeding regimes corresponding to different maturity stages.

It can be seen from Fig. 49.42 that under optimal conditions, Siberian sturgeon females begin to mature at ~4 years. Cases of subsequent maturation (at implementation of ultrasound monitoring feeding) have been reported in almost 90% of the females annually (Fig. 49.43).

From this, the range of effective temperatures (Chebanov and Galich 2013) for Siberian sturgeon is 9–25 °C.

Considerable differences in the age of puberty at different farms are caused by:

- Variations in weight of even-aged females determined by individual characteristics of metabolism.
- Stocking densities and lack of female size variations at holding.

Typically, lower weight females reach repeated maturation earlier, especially if at maturity stage IV (before spawn) they contain more fat in the gonad and a higher

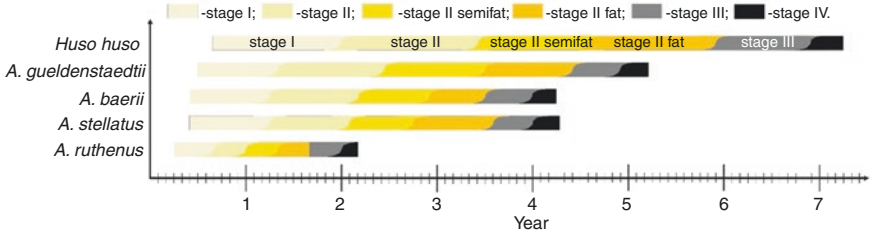

Fig. 49.42 Approximate onset and duration of different maturity stages prior to the first maturation of females of different sturgeon species at optimal temperature (total >8500 degree-days), controlled feeding, and "artificial wintering" of 1.5–2 months' length conducted annually during the last 2 years before foreseen maturation

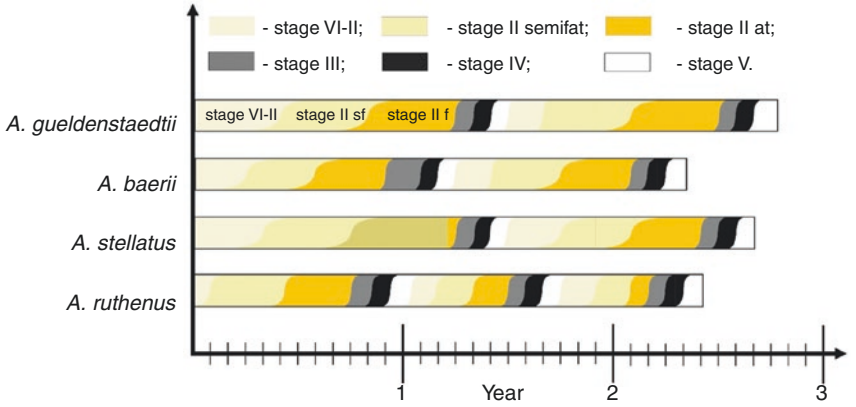

Fig. 49.43 Approximate onset and duration of different stages of gonadal development at second and successive maturations of females of different sturgeon species at optimal temperature (total >8500 degree-days), control feeding, and annual "artificial" wintering (1–2 months), conducted for 2 years prior to the foreseen maturation (Chebanov and Galich 2013)

amount (above 85% of overall oocyte quantity) of Siberian sturgeon pre-vitellogenic oocytes (reserve fund) with diameter not <0.6 mm (0.5 mm for stellate sturgeon, ship sturgeon, and sterlet).

Typically, at sturgeon hatcheries and farms with a technologically correct brood stock holding control regime (temperature and feeding regime) and proper management in compliance with the particular maturity stage, no problems with ultrasound diagnostics will arise.

Conclusion

Longstanding studies have acknowledged that the ultrasound diagnostics (echography technique) can be treated as a highly informative noninvasive express method to determine the sex and maturity stages of gonads, which allows to optimize sturgeon brood stock management on the basis of targeted early building for sexual structures.

It is important to note that nowadays this method allows not only to reject the "odd" males for early sale long before puberty (3–6 years, depending on the sturgeon species) but also selection of the early maturing females from all remaining specimens (Table 49.1). This allows acceleration of brood stock establishment as well as selection activity on creation of new breeds and domesticated forms. Moreover, as shown above, the ultrasound diagnostics being applied in sturgeon farming is not limited to the problem of sexing and staging. It can be successfully used to evaluate the health status of internal organs, including the identification of reproductive system developmental malformations, early diagnostics of diseases, calculation of gonadosomatic indices, fecundity of females, forecast maturation of breeders, determination of mature oocyte size, timing of full ovulation, onset, and completion of oocyte resorption (Chebanov and Galich 2009).

Table 49.1 Minimum weight and age requirements for Siberian sturgeon and when their sex can be non-invasively identified by ultrasonography

Species or hybrid	Industrial (warm water) fish farms		Hatcheries with natural thermal regime	
	Weight, kg	Age, years	Weight, kg	Age, years
Siberian sturgeon	2.0–2.5	2–2+	2.0–2.5	2+ −3
Russian sturgeon × Siberian sturgeon	2.0–2.5	1+ − 2	2.0–2.5	2–2+

This allows, in the continuous noninvasive monitoring modes, adjustment of the brood stock content (feeding, temperature, stocking density), to achieve the earliest ripening breeders.

It should be noted that in many respects owing to the introduction of the described echography techniques, one of the largest heterogeneous farmed brood stocks, comprising eight sturgeon species, has been established (Chebanov and Billard 2001).

Designed and implemented in the late 1990s, the express method of early ultrasound determination of gonad sex and maturity stages in sturgeon allowed this problem to be solved (Chebanov et al. 2002, 2004). Indeed, an experienced operator conducting ultrasonic diagnostics can determine the sex and maturity stage of more than 2000 Siberian sturgeon specimens in 1 day.

Over the past 15 years, the authors have regularly conducted training on ultrasound diagnostics for sturgeon culture specialists from virtually all fishery research institutes and the largest sturgeon farms in Russia and many other countries. In addition, as part of all recent International Symposia on Sturgeon and conferences on sturgeon fishes (the USA, Canada, France, Italy, Iran, Spain, China, Poland, Turkey, and others), the authors organized and conducted special workshops on training in modern methods of sturgeon sexing and maturity staging.

Application of the ultrasound diagnostics in practice allows to reveal new possibilities as follows:

- To determine the optimal temperature regime and feeding norms for holding of brood fish (and specimens saved for brodstock repair);
- To select early maturing individuals;
- To improve the efficiency of breeding and selection activities (under controlled conditions) with Siberian sturgeon, its breeds, and hybrids.

It can also evaluate the quality of population structure and revealing of violations in the process of sturgeon gamete maturation in natural water bodies (Chebanov and Galich 2009).

In recent years, the advanced modifications of ultrasound scanners, equipped with a computer program for image enhancement and scanning modes, have been used in medicine and veterinary. Modern ultrasound technology allows to examine the tissue structure (tissue harmonic modes, 3D and 4D echography,

matrix, and panorama scanning), which can significantly improve the quality of visualization and help carry out accurate and quick noninvasive disease diagnostics (Chebanov and Galich 2010). However, it should be noted that the purpose of this chapter was to show the possibility of widespread application of relatively inexpensive ultrasound scanners (≤5000 USD) to study and breed sturgeon.

It should be noted that the elaborated method of sturgeon ultrasound diagnostics has been included in two FAO publications, "Sturgeon Hatchery Practices and Hatchery Management for Release Guidelines" and "Sturgeon Hatchery Manual" in a series of Fisheries and Aquaculture Technical Papers (Chebanov et al. 2011; Chebanov and Galich 2013). This has raised a growing interest in this method and allowed the acceleration of its widespread implementation in the practice of sturgeon brood stock establishment and management.

Conducted studies have established that it is possible to effectively apply ultrasound diagnostics implementation to in vivo monitoring of sturgeon gonadal and gametogenesis morphological and functional abnormalities in natural water bodies.

Estimates of the occurrence frequency and manifestation degree of tumors, cysts, hermaphroditism, fatty degeneration of the gonads, presence of connective tissue growths, and partial or total atresia of gametes can all be obtained.

The frequency of such malformations due to negative changes in habitat conditions reached 7–8% for the Siberian sturgeon (Akimova et al. 1995; Ruban 2005).

In recent years, "grayscale" 3D image processing computer programs have been used in medicine (Nasnikova and Markina 2008). Some uses include multiplanar reconstruction, stratified use of longitudinal and transverse sections, and archiving of volumetric information on the internal organs. Compared with the methods of 2D ultrasonography mentioned by the authors, 3D ultrasonography in the future will enable a more accurate determination of the volume of various examined organs.

In connection with the above presentation, the early implementation of modern ultrasound diagnostics methods to study and breed Siberian sturgeon will not only improve the brood stock management efficiency for hatchery stock enhancement of rare and endangered species, but also accelerate the innovative development of commercial sturgeon caviar production.

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Oxygen Demand in Sturgeon Farming

50

Guy Nonnotte, Patrick Williot, Karine Pichavant-Rafini, Michel Rafini, Valerie Maxime, and Liliane Nonnotte

Abstract

O_2 is paramount in aquaculture to ensure growth and welfare in fish. But the diffusion of O_2 in water layers is very difficult, and suffocation may threaten the fishes continuously in intensive farming.

This study presents essential basic knowledge to appreciate the oxygen availability in water and oxygen demand in farmed sturgeons. In fish, because of the low O_2 solubility of the water, a large volume of water must come in contact with the gas-exchanging surface at the gill level. Moreover, water is also over 800 times denser than air and 50 times more viscous, so fish must use more energy (5–30% of total energy) than terrestrial animals to simply move water across their

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respiratory surfaces. To maximize the diffusion of oxygen, fish use a process known as countercurrent flow, in which water and blood flow in opposite directions across the gills. Activity metabolism in Siberian sturgeon and critical oxygen concentration in water were also assessed as functions of temperature and body mass. Finally, to prevent a decrease of the oxygen availability in water and hypoxic stress consequences on fish growth, it is important to record the oxygen concentration in outflowing water and to observe the activity level of the fish.

Keywords

Siberian sturgeon • *Acipenser baerii* • Respiration • Gill morphology • Activity metabolism • Critical oxygen level • Oxygen demand

Abbreviations

α_{CO_2}	Carbon dioxide solubility
α_{O_2}	Oxygen solubility
C_{sat}	C_{wO_2} at air saturation
C_{wO_2}	Concentration of oxygen in water
FW	Fresh water
HR	Heart rate
[Lact$^-$]	Lactate concentration
M	Body mass in g, kg called
MO_2	Oxygen consumption
Pa_{CO_2}	Arterial carbon dioxide partial pressure
Pa_{O_2}	Arterial oxygen partial pressure
P_B	Barometric pressure in kPa, mmHg, Torr
P_{CO_2}	Carbon dioxide partial pressure
P_{DA}	Blood pressure in dorsal artery
P_{diff}	Difference between systolic and diastolic pressure
pHa	Arterial blood pH
P_{O_2}	Oxygen partial pressure
P_{wCO_2}	Carbon dioxide partial pressure in water
P_{wO_2}	Oxygen partial pressure in water
Q_{10}	The ratio of MO_2 at temperature (t + 10) °C over MO_2 at temperature t °C
SW	Sea water
t	Temperature in °C

Introduction

Respiration is the process by which animals take in oxygen necessary for cellular metabolism and release carbon dioxide that accumulates in their body as a result of the expenditure of energy. When a fish breathes, water is moved on respiratory surfaces at the gill level in order to help with the process of respiration. Oxygen must

be continuously supplied to the animal, and carbon dioxide, the waste product, must be continuously removed for cellular mechanisms to function properly. To complete the oxygen demand at the gill level, cutaneous respiration must be taken into account and may represent an important part of the oxygen uptake for the eel, the flounder and the blenny, for example (Kirsch and Nonnotte 1977; Nonnotte and Kirsch 1978).

Oxygen is valuable because it is important in many ATP-producing cycles occurring throughout the body such as the Krebs cycle and the electron transport chain. Glycolysis breaks down glucose, a six-carbon sugar, into the three-carbon molecule of pyruvic acid. The series of reactions associated with glycolysis is necessary for aerobic and anaerobic pathways to work and is fundamental for cellular metabolism. In the presence of oxygen, the pyruvic acid is further oxidized. However, under anaerobic conditions the pyruvic acid is reduced to lactic acid.

In some animals, such as *Acipenser baerii*, the only source of additional ATP will be glycolysis if the supply of oxygen to active muscle cells is not sufficient to produce enough ATP to maintain intense activity (e.g. environmental hypoxia). Without sufficient oxygen, some of the pyruvic acid produced is reduced to lactic acid, which accumulates in the tissues. Excess lactic acid may also decrease blood pH and affect other tissues in the animal. When muscle activity decreases, extra oxygen is needed to convert the lactic acid back to pyruvic acid, which is then used by the Krebs cycle. This extra oxygen represents the animal's oxygen debt generated by the use of biochemical pathways to convert lactic acid to alcohol, which can then be excreted (Nonnotte et al. 1993; Maxime et al. 1995).

With respect to the relationship between oxygen consumption and ambient oxygen tension, aerobic organisms have classically been described as oxyconformers when their O_2 consumption varies directly with ambient P_{O_2} or oxyregulators if O_2 consumption is independent of this factor, at least within a certain range above a critical O_2 pressure P_c. Oxyregulation is made possible by a number of compensatory responses increasing the various O_2 conductances in the gas exchange system and allowing maintenance of an unchanged tissue O_2 supply, in spite of reduced ambient O_2 availability (see Dejours 1981). Oxyconformity throughout the whole P_{O_2} range, or failure of oxyregulation below the P_c, results from either the absence or the limitation of such mechanisms, so that oxygen supply is unable to match oxygen demand anymore. In these cases, tissue energy expenditure may either be depressed or more or less maintained by shifting to anaerobic metabolism.

Oxyconformity has rarely been described unambiguously in vertebrates (Prosser 1973; Ultsch et al. 1981). One of the best documented examples is the sturgeon *Acipenser transmontanus*, in which both O_2 consumption and gill water flow rate were reported to be reduced steadily with declining ambient oxygen tension (Burggren and Randall 1978). Furthermore, the absence of any O_2 debt repayment and of any decrease of blood pH indicates that a reduced total energy expenditure took place during hypoxic exposure, rather than a shift to anaerobic metabolism maintaining energy production (Dejours 1981).

Although based on clear and apparently consistent findings and fitting well the ancientness of the *Chondrostei* among vertebrate groups, this notion of oxyconformity being a characteristic of sturgeons was not supported by a large amount of previous data from Russian workers (see Vinberg 1956; Klyashtorin 1981) and has

also even been challenged by a more recent investigation on the same species (Ruer et al. 1987). However, these criticisms were only based on measurements of O_2 consumption as a function of O_2 tension. Therefore, in order to settle this problem, we have investigated (Nonnotte et al. 1993; Maxime et al. 1995) as completely as possible the respiratory and acid-base responses to declining oxygen tension in the Siberian sturgeon *Acipenser baerii* (see Chap. 18).

50.1 Oxygen Availability in Water

Water and air, as respiratory environments, are radically different in a number of ways. The most significant difference is that water contains only 1% O_2, whereas air contains 21% O_2 (by volume). Table 50.1 presents the solubility coefficient of O_2 and CO_2 in freshwater (FW), sea water (SW) and air (three different respiratory environments) as a function of temperature. In air this coefficient is the same for O_2 and CO_2 and is relatively constant at these temperatures.

This table clearly demonstrates that gas diffusion rates are lower in water than in air and the mean water/air rate is roughly 1/29. Salt water contains less oxygen than freshwater because the higher salt concentration decreases gas solubility. Finally, as water or air temperature increases, the amount of dissolved oxygen decreases. All of this provokes a great difference between aquatic and terrestrial animals in terms of energy expended to obtain oxygen. For example, the specific ventilation of a terrestrial animal is 0.6 L/mmol O_2, whereas it is 10 L/mmol O_2 for a fish (Dejours 1981; Belaud 1996).

Fish use a process known as countercurrent flow, in which water and blood flow in opposite directions across the gills, thereby maximizing the diffusion of oxygen. Moreover, water is also over 800 times denser than air and 50 times more viscous, so fish must use more energy to simply move water across their respiratory surfaces.

Fish use as much as 5–30% of the total energy for muscle breathing to keep water passing over the gills. Energy expenditure is also extremely variable and depends on different environmental factors, i.e. oxygen, temperature and salinity.

Table 50.1 The solubility coefficient α of O_2 and CO_2 in freshwater (FW), sea water (SW) and air as a function of temperature (Maxime and Nonnotte 1997)

	FW		SW 35 g/L		Air
Temp.	α_{O_2}	α_{CO_2}	α_{O_2}	α_{CO_2}	α_{gaz}
°C	µmol L^{-1} mm Hg^{-1}				
5	2.52	84.17	2.03	70.79	57.68
10	2.23	70.57	1.83	60.00	56.66
15	2.01	60.23	1.67	51.58	55.68
20	1.82	51.89	1.54	44.74	54.73

50.2 Oxygen Measurements in Water

Oxygen dissolved in water is usually measured with a Clark-type oxygen electrode largely described in the literature. The electrode is made up of a silver-silver chloride anode and a platinum cathode which are bathed in an electrolytic solution. An oxygen-permeable membrane is set at the electrode tip. When a polarizing voltage is applied through the anode and cathode, oxygen diffusing across the membrane is reduced at the cathode level, producing an electrical current through the electrode. This current is proportional to the partial pressure of oxygen in the water (P_{wO_2}). It can be amplified, recorded and analysed with computer interfaces. This electrode type does not consume oxygen but must be frequently calibrated to set up zero O_2 tension and/or air-saturated water O_2 tension.

If temperature and salinity of water are known, the dissolved oxygen quantity may be automatically given in $mgO_2\ L^{-1}$, and P_{wO_2} may be displayed in millimetres of mercury (mm Hg or Torr), in Pascal (Pa) or in bars. It is reminded that 1 kPa = 7.50 Torr = 7.50 mm Hg or 1 Torr = 133.32 Pa. These can be converted to O_2 concentration with the aid of a nomogram and oxygen solubility tables (see for details the review wrote by Belaud 1996).

For example, at 10 °C and at sea level, freshwater, well equilibrated with air, contains 7.896 mL L^{-1} O_2, that is, 7.896 × 32/22.4 = 11.28 mg L^{-1} or ppm. In aerated sea water at 25 °C and 35‰ salinity, there is only 4.725 mL L^{-1} O_2, that is, say 6.75 mg L^{-1} or ppm. These values correspond to saturation state and are in accordance with the physics law about gases' dissolution (Henry's law).

The concentration of oxygen in water (C_{wO_2}) is given by the following formulae:

$$C_{wO_2} = \alpha_{wO_2} \cdot P_{wO_2}.$$

with α_{wO_2} oxygen solubility coefficient in water and P_{wO_2} oxygen partial pressure in water.

α_{wO_2} value decreases with the increases of temperature and salinity (see Table 50.1).

P_{wO_2} at air saturation is the same in water and in air and can be calculated by:

Barometric pressure (P_B) − Vapour pressure of saturated water (P_{H_2O}) × 0.20946;

0.20946 is the fraction of oxygen in dry air. The vapour pressure of saturated water depends on the temperature. Its value is given by specific tables (Dejours 1981).

For example, in freshwater, at 13 °C and P_B = 756 mm Hg, P_{H_2O} = 11 mm Hg, α_{wO_2} = 0.06713 mg L^{-1} mm Hg:

P_{wO_2} is equal to (756 − 11.2) × 0.20946 = 156 mm Hg,

C_{wO_2} at air saturation (C_{sat}) is equal to 0.06713 × 156 = 10.47 mg L^{-1}.

C_{sat} is obtained after a long period of air bubbling in water and does not exist in fish farm structure with animals. However, it is the reference value to analyse the measures of oxygen concentration in water and to determine the best strategy of oxygenation.

The percentage of saturation is directly given by specific oximeters and is equal to (C_{wO_2}/C_{sat}) × 100. This is useful to know the absolute gap to saturation defined as $C_{sat} - C_{wO_2}$ (in mg L^{-1} or ppm) to manage the oxygenation devices. If positive, there is a deficit, and if negative, it's an oversaturation.

50.3 The Branchial Respiration

In fish, a large volume of water must come in contact with the gas exchanging surface because of the low O_2 solubility of water (see above). The two main respiratory surfaces in fish are the gills and the skin (Kirsch and Nonnotte 1977; Nonnotte and Kirsch 1978).

In a small volume, the gills considerably increase the gas exchange surface area (Figs. 50.1 and 50.2), and the blood-water barrier is very thin.

The gills of Siberian sturgeon (Salin 1992) are situated on either side of the pharynx and possess a series of arch-like structures that provide the physical support for gill filaments or primary lamellae. As in all teleosts, the gills of *Acipenser baerii* are

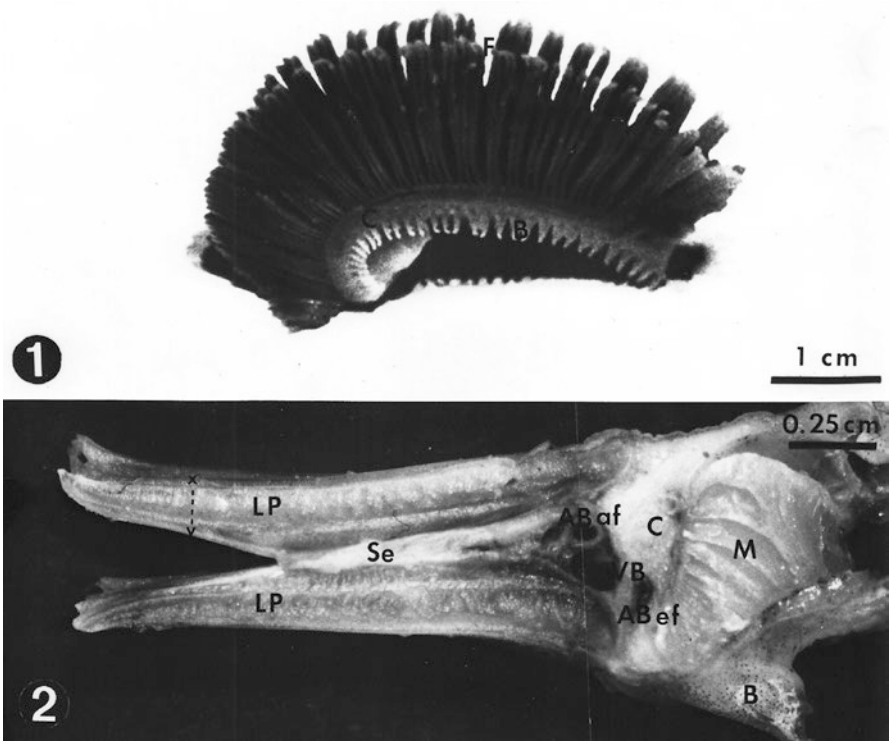

Fig. 50.1 Morphological studies of the gills of the Siberian sturgeon, *Acipenser baerii* (Salin 1992). (*1*) Morphology of the third holobranch. Each holobranch is supported by a cartilaginous axis (*C*). Note the gill rackers or branchiospins (*B*) and filaments (*F*) (×2.5). (*2*) Cross-section through a holobranch. The interbranchial septum (*Se*) runs on two-thirds of the length of the filament which is constituted by both primary lamellae (*LP*). We can also see the arterio-arterial system and the veino-lymphatic system. (*C*) cartilaginous ray; (*AB af*) afferent branchial artery; (*AB ef*) efferent branchial artery; (*B*) branchiospins; (*M*) muscle; (*VB*) branchial venous, venous-lymphatic system (×8)

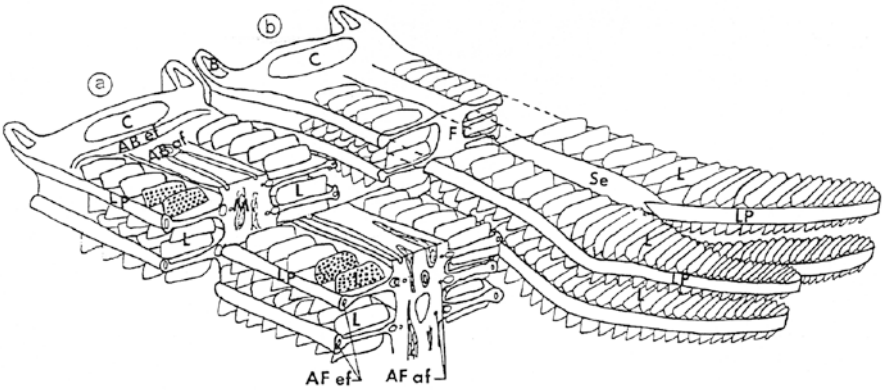

Fig. 50.2 Morphological studies of the gills of the Siberian sturgeon, *Acipenser baerii* (Salin 1992). Spatial representation of two branchial arches (*a* and *b*). Note the gill filaments (*F*), the interbranchial septum (*Se*) on two-thirds of their length, the primary lamellae (*LP*), the secondary lamellae (*L*), the branchiospins (*B*) and the cartilaginous rays (*C*). (*M*) muscle, (*AB af*) afferent branchial artery, (*AB ef*) efferent branchial artery (Salin 1992)

protected by an operculum which is a hard structure with a caudal opening (Figs. 50.1 and 50.2).

The gills of the Siberian sturgeon are composed of six paired arch-like structures which developed specifically. On each side, the first embryonic gill arch remains as a pseudobranch at the level of the spiracle which remains operational. The second embryonic gill arch gives rise to a hemibranch situated on the internal side of the operculum. The other gill arch-like structures constitute four holobranches spaced between five branchial chambers. The last chamber does not have a hemibranch on the caudal side. The holobranches represent 98% of the respiratory surfaces (Burggren and Randall 1978).

Each individual gill arch counts 200 filaments. Each filament is made up of two primary lamellae joined by an interbranchial septum on two-thirds of their length from the basis. Each filament is supported by a cartilaginous axis which partially constitutes the gill skeleton. These rays are associated with muscles which are important for the orientation of the filament in the water flow (Hughes 1984). Numerous gill rackers or branchiospins (around 50) are set on the inside of the branchial arches. They help to prevent solid substances from being carried outward through the gills. Each filament supports the secondary lamellae which are the basic respiratory unit.

As in all teleosts, two vascular systems are located at the basis of the filaments. The first is the arterio-arterial system which ensures the oxygenation of the blood flowing in the gills against the water flow through a countercurrent system. The second is the veino-lymphatic system, which is a nutritional system for the gills (Laurent 1984). Both systems are connected by anastomosis situated on each side at the basis of the secondary lamellae (Laurent and Dunel 1980).

50.4 Estimation of Respiratory Metabolism Levels

Usually, three levels of aerobic metabolism in fish can be defined: the standard metabolism, the routine metabolism and the active metabolism.

The standard metabolism corresponds to the minimum metabolic rate required to sustain life. It should be determined when fish do not expend energy for activity, food digestion, reproductive development and growth and are in a no stress situation. It is generally measured in the laboratory.

The routine metabolism is the fraction of energy used by unfed fish with movement of spontaneous swimming or routine activity. Routine metabolism is the mean oxygen consumption rate measured when precautions are taken against the fish being influenced by outside stimuli (Fry 1971).

The active metabolism already represents the metabolic rate at the maximum level of activity, and it is also the maximum aerobic rate associated with swimming at the greatest sustainable velocity. For example, Bret (1964) demonstrated that fast-swimming fish can increase their metabolism up to ten times the standard metabolism.

50.5 Measurement and Calculation of the Respiratory Metabolism

Respiratory metabolism is measured with a respirometer. There are essentially two types of respirometers: closed respirometers, in which the same volume of water is continuously used (Cech 1990), and open respirometers in which water is continuously replaced (Nonnotte et al. 1993). Both devices are used to determine the standard metabolism, but open respirometers allow maintaining the quality of water and preventing the accumulation of carbon dioxide and ammonia.

Closed respirometers can be adapted to evaluate the active metabolism or swimming metabolism. The most well-known swimming respirometer is the Brett-type respirometer. In this closed device, water is circulated past the fish, inducing it to swim (Bret 1964).

Figure 50.3 presents the open respirometer used by Nonnotte et al. (1993). Standard oxygen consumption (MO_2) in Siberian sturgeon was determined at steady state by measuring the oxygen partial pressure difference between water flowing into (P_{wO_2} inlet) and out of (P_{wO_2} outlet) the respirometer. A peristaltic pump allowed alternate sampling of the inlet and outlet water which passed at a constant flow rate (5 mL min^{-1}) through a thermostated P_{O_2}-measuring cell (Radiometer E 5046). Oxygen consumption was calculated according to the relation:

$$MO_2 = \left(P_{O_2}\ \text{inlet} - P_{O_2}\ \text{outlet}\right) \cdot Q_w \cdot \alpha_{wO_2} / \text{Body weight}$$

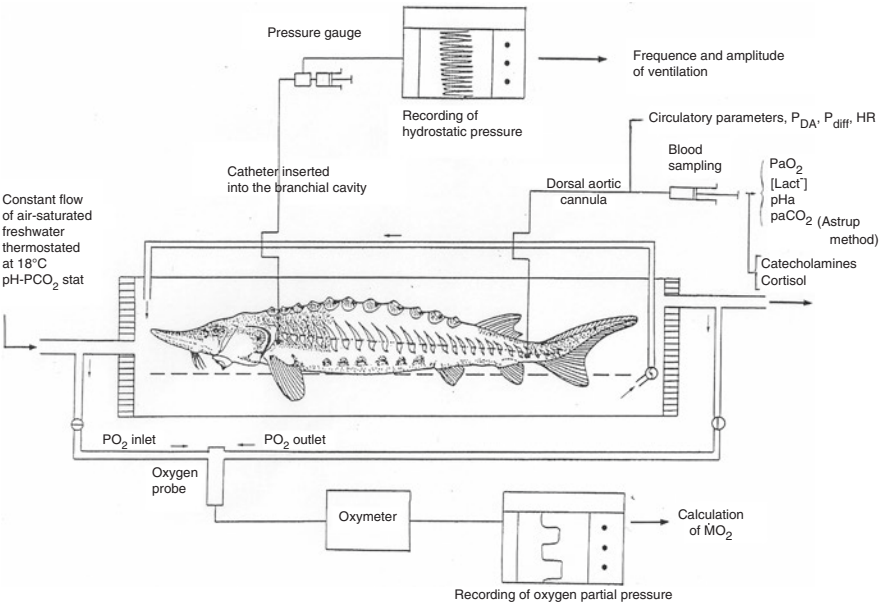

Fig. 50.3 Device for the measurements of the respiratory and circulatory variables in the Siberian sturgeon, *Acipenser baerii*. The respirometer was a plexiglass open-type respirometer. A continuous flow of water circulates over the fish to prevent hypoxia and accumulation of ammonia, CO_2 and other metabolic wastes. Automated designs incorporate computers with electrical valves to divert inflowing and outflowing water past a calibrated O_2 electrode. The water flow rate through the respirometer is constant. The difference in oxygen concentration between inflowing and outflowing water is about 1 mg L^{-1} except for the determination of oxygen critical partial pressure (see Chap. 18). Multiple physiological parameters may be determined simultaneously on blood samplings. In dorsal arterial blood, pHa; Pa_{O_2}, oxygen partial pressure; Pa_{CO_2}, carbon dioxide partial pressure (Astrup method); [Lact⁻], lactate concentration; P_{DA}, blood pressure; P_{diff}, difference between systolic and diastolic pressure; HR, heart rate, hormonal concentration

where Q_w is the constant water flow through the respirometer and α_{wO_2} is the O_2 solubility in freshwater (2.01 μmol L^{-1} mm Hg^{-1} or 0.0644 mg L^{-1} mm Hg^{-1} at 15 °C).

50.6 Variations of the Energetic Metabolism in Sturgeons

The energy metabolism of the fish depends on extrinsic and intrinsic factors which must be closely monitored in aquaculture.

Table 50.2 Comparisons of the standard metabolism MO_2 of the Siberian sturgeon with MO_2 of active and/or sluggish fish (12 °C ≤ temp. ≤ 20 °C)

	Standard metabolism		
Species	**µmol min^{-1} kg^{-1}**	**mg O_2 h^{-1} g^{-1}**	**References**
Siberian sturgeon	29.9	0.057	Nonnotte et al. (1993)
White sturgeon	42.1	0.079	Burggren and Randall (1978)
Atlantic salmon	44.1	0.084	Maxime et al. (1990)
Rainbow trout	32.5	0.062	Maxime et al. (1991)
Eel	15.7	0.037	Le Moigne et al. (1986)
Plaice	13.8	0.026	Steffensen et al. (1982)
Flounder	15.6	0.030	Duthie (1982)

Table 50.3 Standard metabolism in sturgeons

	Weight	Temperature	Standard metabolism		
Species	**g**	**°C**	**µmol min^{-1} kg^{-1}**	**mg O_2 h^{-1} g^{-1}**	**References**
Siberian sturgeon	1800	15	29.9	0.057	(1)
White sturgeon	63	16	105.3	0.200	(2)
White sturgeon	950	15	42.1	0.079	(3)
White sturgeon	2000	18	52.7	0.100	(4)
Atlantic sturgeon	69	19	105.3	0.200	(5)
Adriatic sturgeon	198	23	57.9	0.110	(6)
Green sturgeon	23	19	68.5	0.130	(7)
Green sturgeon	850	19	105.3	0.200	(7)

(*1*) Nonnotte et al. (1993); (*2*) Crocker and Cech (1997); (*3*) Burggren and Randall (1978); (*4*) Ruer et al. (1987); (*5*) Secor and Gunderson (1998); (*6*) McKenzie et al. (1997); (*7*) Mayfield and Cech (2004)

The intrinsic factors are essentially species, body weight and hormonal factors (physiological factors). The extrinsic factors correspond to the physicochemical characteristics of the aquatic environment. Those are mainly the oxygen concentration in water, the temperature and the salinity which interact with each other (see above). Pollutants (ammonia concentration, see Chaps. 19–22; nitrite, see Chap. 23), acid-base status and light must also be taken into account.

Various respiratory characteristics of the Siberian sturgeon fall into a range corresponding to that reported for poorly active, bottom-dwelling teleosts (Table 50.2). When appropriately corrected for body size (0.67 for the mass exponent) and temperature (Q_{10} = 2) differences, standard normoxic MO_2 at 15 °C of *Acipenser baerii* (Nonnotte et al. 1993) was lower than that of *A. transmontanus* (Burggren and Randall 1978) by about 25% but ranged between values reported for active fish, for example, salmon (Maxime et al. 1990), trout (Maxime et al. 1991) and sluggish fish, eel (Le Moigne et al. 1986), plaice (Duthie 1982) and flounder (Nonnotte and Kirsch 1978; Steffensen et al. 1982). This could

Table 50.4 Oxygen critical % saturation in water for Siberian sturgeon in two conditions, metabolism standard and metabolism activity, at different temperatures (Williot et al. 1988)

Conditions	Standard metabolism		Activity metabolism	
Temp (°C)	% Saturation	mg L^{-1}	% Saturation	mg L^{-1}
10	30	3.4	47	5.3
15	38	3.8	59	5.9
20	45	4.1	70	6.2
25	53	4.4	82	6.7

probably be related to the active growth of young Siberian sturgeon in spite of their relatively sluggish behaviour.

Additional information is given by Table 50.3 which presents the standard metabolism in different sturgeon species at various temperatures and with a large weight range of the individuals. It is remarkable that *Acipenser baerii* exhibits the lowest standard metabolism whatever the species.

50.7 Oxygen Critical Partial Pressure or Oxygen % Saturation in Water

Klyashtorin (1976) determined, in Siberian sturgeon, lethal levels of oxygen partial pressure in water (46 mm Hg at 10 °C). Nonnotte et al. (1993) showed that Siberian sturgeon was able to maintain standard oxygen consumption down to a critical level of ambient P_{wO_2} ($P_{wO_2} \leq 40$ mm Hg at 15 °C) and confirmed these results. But these studies were performed in standard metabolism conditions which are quite different from farming conditions.

Olifan in 1940 showed that the growth rate of young sturgeons at 21 °C was reduced by 20% if the water oxygen saturation level was 60% of the well-aerated water and 43% if it was only 48%.

Cech et al. (1984) demonstrated the influences of temperature and hypoxia on the growth rate of the white sturgeon, *Acipenser transmontanus*. Environmental hypoxia (P_{wO_2} = 90 mm Hg or 57% saturation) reduced the growth of the young sturgeons within each temperature level (15, 20 and 25 °C). Moreover sturgeon activity increased with each 5 °C temperature increase under normoxia and hypoxia, except in hypoxia at 25 °C where activity was not significantly different from that at 20 °C.

In 1996, Belaud demonstrated without any ambiguity that one needs to multiply MO_2 standard by 1.5 to approach the activity MO_2 of a fish fed ad libitum in fish farming.

It seems adequate, though, (Table 50.4) to propose for Siberian sturgeon, a mean critical oxygen saturation of water near 60–80% which corresponds to an activity

metabolism equivalent at 85 mg O_2 h^{-1} kg^{-1} at 15 °C and 100 mg O_2 h^{-1} kg^{-1} at 20 °C, respectively. But the evaluation of the activity oxygen consumption is approximate.

50.8 Effects of the Temperature

Fish, except for big pelagic species such as tuna, have a body temperature equal to that of the environmental water. They are poikilotherms.

In Siberian sturgeon, body temperature may vary over a range of 5–25 °C or more. This change in temperature is accompanied by a considerable change of the energy production.

To express the action of temperature on O_2 consumption (MO_2), it's usual to calculate what is called Q_{10}, which is the ratio of MO_2 at temperature (t + 10) °C over MO_2 at temperature t °C:

$$Q_{10} = \mathrm{MO}_2^{t+10} / \mathrm{MO}_2^{t} \left(\text{Arrhenius} - \text{Van't Hoff law}\right)$$

Generally, to determine the value of Q_{10}, it is not necessary to observe MO_2 at two temperatures that are actually different by 10 °C. The general formula for Q_{10} when the O_2 consumptions are measured at temperatures t_1 and t_2 is:

$$Q_{10} = \left(\mathrm{MO}_2^{t2} / \mathrm{MO}_2^{t1}\right)^{10/t2-t1}$$

The value of oxygen consumption Q_{10} is generally between 2 and 3. For a Q_{10} of 2.5, the oxygen consumption increases by 9.5% per °C.

Klyashtorin (1976) determined, in sturgeons, a mean value of oxygen consumption Q_{10} of 2.25 which corresponds to an increase of roughly 10–12% per °C.

This is corroborated by the results obtained by Ruer et al. (1987) in *Acipenser transmontanus* which determined an activity metabolism equal to 106 mg O_2 h^{-1} kg^{-1} at 18 °C and Williot et al. (1988) in Siberian sturgeon which measured 100 mg O_2 h^{-1} kg^{-1} at 17 °C and Nonnotte et al. (1993).

50.9 Effects of the Body Size

The energy expenditure of a fish increases with its size but not in direct proportion. However, any comparison of energy expenditures between organisms requires that the conditions of measurements be alike. Comparison must be made at the same temperature (see above), with similar activity and nutritional state. Moreover the stage of development and growth of the animals must be comparable.

The plot of the log of MO_2 vs. the log of body mass M gives a linear relationship of slope a.

We obtain the equation:

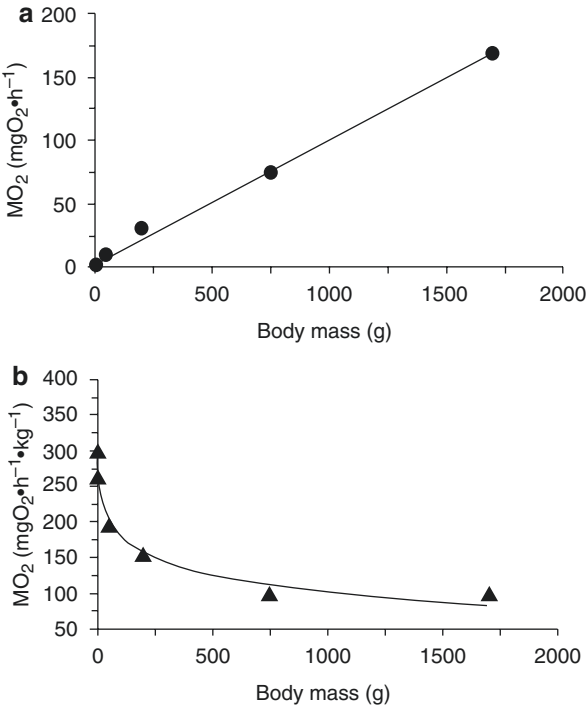

Fig. 50.4 (**a**) Activity oxygen uptake rate, MO_2 as a function of body mass in Siberian sturgeon (*Acipenser baerii*). A positive correlation exists between the body mass of the fish and their oxygen consumption: MO_2 (μmol h^{-1}) = 4.276*M* + 0.097 (*N* = 6, *R* = 0.99). *M*: body mass in g. (**b**) Activity O_2 consumption per unit body mass as a function of body mass. MO_2 is given in μmol h^{-1} g^{-1}. The oxygen demand is bigger for young fish than for adults. Log MO_2 (μmol h^{-1} g^{-1}) = 6.078–0.212 Log *M* (*N* = 6, *R* = 0.99). *M*: body mass in g. (from Williot et al. (1988)

$$\log \mathrm{MO}_2 = A + b \cdot \log M \quad \text{or} \quad \mathrm{MO}_2 = a \cdot M^b$$

a is the antilog of *A*. It represents the metabolic intensity coefficient and is characteristic of the species and the growth state. In fish, it depends also on the water temperature.

b is the weight exponent.

Interspecific allometric relations $MO_2 = f(\text{Mass})$ are the general rule in the animal kingdom. This allometric relation between O_2 consumption and body mass has been developed for fishes by Ege and Krogh (1915).

The allometric equation $MO_2 = f(M)$ cannot be calculated for a small range of body mass but only for a wide range. Consequently, for intraspecific comparison, it is usual to express the oxygen consumption per kg of body mass.

Many works refer to the effects of the fish body weight on the oxygen consumption.

In 1988, Williot et al. measured the activity metabolism of Siberian sturgeon in a wide range of body weight. Figure 50.4 shows two graphs built from the individual values of the oxygen consumption vs. the body mass of sturgeons in activity. There is a positive correlation (Fig. 50.4a) between MO_2 and their activity metabolism (mg h^{-1}). But when the activity metabolism of the Siberian sturgeon was given per unit of body mass (mg h^{-1} kg^{-1}), the oxygen demand of the small fish was higher than that of the big one.

The transformation of the preceding relation by dividing each member by the body weight leads to an exponential function (Fig. 50.4b). It's easy to understand

that the energy expenditure (expressed per unit of body weight) is bigger for the young fish than for the big one and decreases with the size of the sturgeons.

50.10 The Additional Oxygen Consumption

At first, if the fish oxygen consumption may be evaluated easily, the additional consumption is more difficult to quantify. It is linked to the microorganisms present in the rearing structures.

When the flow of water is important, it's negligible. But in closed recirculated circuit, all the activity of the microorganisms must be taken into account and must be fully added.

Secondly, fish excrete carbon dioxide and ammonia in water.

In aquaculture, one hardly encounters problems with CO_2. The excretion of carbon dioxide in water is made easier by a better diffusivity than that of oxygen through the gill epithelium and in water. The solubility coefficient for CO_2 in water is higher (25 times) than for O_2. Moreover, a part of the excreted CO_2 is converted in bicarbonate and carbonate.

In contrast, the ammonia excretion has two disadvantages: the toxicity of NH_3 (see Chaps. 19–22) and the oxidation of ammonia in nitrites and nitrates by the nitrification bacteria.

$$NH_3 + O_2 \rightarrow NO_2^- \text{ and } NO_2^- + \tfrac{1}{2}O_2 \rightarrow NO_3^-$$

The conversion of 1 g from ammoniacal nitrogen to NO_3^- costs about 5 g O_2 or 3.5 L O_2.

In case of low water flow or water recycling (such as fish farms), this oxidation takes place and must be taken in account to adjust the oxygenation devices to the total oxygen demand.

Conclusions

O_2 is paramount in aquaculture. But the diffusion of O_2 in water layers is very difficult, and suffocation threatens the fishes continuously in intensive aquaculture in raceways.

In *Acipenser baerii*, if supply of oxygen to active muscle cells is not sufficient (environmental hypoxia for example) to produce enough ATP to maintain intense activity (about 100 mg O_2 h^{-1} kg^{-1} at 20 °C), the only source of additional ATP will be glycolysis. Without sufficient oxygen, some of the pyruvic acid produced is reduced to lactic acid, which accumulates in the tissues. Excess lactic acid may also decrease blood pH and affect other tissues in the animal. When muscle activity decreases, extra oxygen is needed to convert the lactic acid back to pyruvic acid, which is then used by Krebs cycle. This extra oxygen represents the animal's oxygen debt generated by the use of biochemical pathways to convert lactic acid to alcohol, which can then be excreted (Nonnotte et al. 1993; Maxime et al. 1995). In this case, the fish growth can be strongly altered, and the welfare of the animals is not ensured.

For Siberian sturgeon, the oxygen demand seems to be less important than for salmonids.

Belaud (1996) reported, for rainbow trout aquaculture, that for 1 kg food, 200–220 g O_2 are directly consumed by the fish, 28 g $N_{ammonia}$ are excreted which costs 140 g O_2, 14 g phosphorus are rejected, 340 g suspended matter are added and 150 g O_2 are taken in the water for the biodegradation of the organic matter by microorganisms.

The total O_2 needs represent about 409–510 g O_2 per kg of food supply.

In water recycling, the total oxygen demand may be equal to twice the activity oxygen consumption of the fishes.

To prevent a decrease of the oxygen availability, it is important to record the oxygen concentration in outflowing water and to observe the activity level of the fish, two parameters which should be used to adjust automatically the oxygen supply.

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What Is the Future of Siberian Sturgeon Farming?

Asking the entitled question means that some doubts might be expressed on the future of the farming of the species as compared with other sturgeon species. The deep crisis in the world caviar market in the introduction of the present volume has been mentioned (Williot et al. 2017). These recent changes made precarious the functioning of most sturgeon farms worldwide especially those of which investments are still not redeemed and/or those of which production costs are incompressible. Among the diverse alternatives to go through is to look for a more in-depth choice for the species notwithstanding the legal aspect of rearing a non-native species which can be restrictive depending on the country. A very recent example unfortunately illustrates this comment with the status of the species in Florida (USA) in potentially changing the legislation.

Therefore, reasons to support the choice of a sturgeon species have to be rigorously conducted in comparing (1) biological and breeding characteristics of the species and (2) marketing aspect depending on species. More, there might be present evidence arguing to favour the choice of supporting a species that may not take into account in long-term perspective. For example, whatever their ecology, all sturgeon species are farmed in freshwater. But what could be the long-term impact of such farming conditions regarding species of which the ecology exhibits long spending period into high water salinity during ongrowing phase? No one knows. In contrast, there is no risk for those species which exhibit a quasi-exclusive freshwater status alike the Siberian sturgeon.

Regarding the biological characteristics, we have gathered in volume 1 the main documented issues; even some of them remained unsolved, e.g. the sex determination. As far as breeding is concerned, i.e. the present volume 2, the first two parts deal with two key issues for the farming of any species, i.e. reproduction and food-feeding-related issues. Once puberty is achieved, the main interval between two spawning events is 2 years, but the different recorded complicated occurrences are reported that allow building a long-term reproductive planning if needed (Chap. 26). This might be interpreted as an illustration of the plasticity of the species which is

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able to either reduce or increase this interval and then to increase or not its spawning frequency. Similarly, this allows planning caviar production within a given cohort. The management of brood fish for the reproduction is then extensively given in detail in order to provide the best quality products (eggs and further larvae) (Chap. 27). This includes the few months before the spawning sensu stricto up to the collection and management of gametes to further obtain fertilized eggs, viable embryos and good-quality larvae. In case farmers would like to separate the management of both sexes, effective cryopreservation of sperm is given (Chap. 28). The process might be of use when aiming at increasing the genetic variability of brood stock by acquiring other sperms. Due to lack of global traceability and references, great care should be brought about the origin and characteristics of sperm. Besides reproduction, food and the related feeding issues constitute a challenge from a long-term view. Despite the pertinence of each of the different issues of this group, there is an overlapping of the different chapters of this group (Chaps. 30–33) that reveals a high level of intricacy of these questions all the more so since that immunology (Chap. 43); a very new and updated chapter is also concerned by this remark. A brief overview of current practices on food and feeding is given (Chap. 30). A synthesis of available literature in the field of nutritional requirements is provided (Chap. 11) as has been given in volume 1. The optimum level for the main ingredients is provided. As the optimum level for protein is relatively high (~40%) of fish-originated ingredients, there were attempts to substitute plant protein for animal protein to lower the cost (Chaps. 31 and 32). There are risks in using some plant protein, e.g. soy which may be considered as an endocrine disruptor (Chap. 32). It is interesting from the historical point of view and for those interested in research planning to briefly recall the origin of these findings. In the early 1980s, a key question was to discriminate the gender as early as possible within the whole French research program. At that time, the search for plasmatic vitellogenin was considered to be the appropriate tool as this complex molecule produced in the liver under the stimulation of estradiol accumulates in the eggs and then was supposed to be female specific. Thanks to male control, it was shown that males also produced vitellogenin, and the reason was the presence of phytoestrogen components in the compound food (Chap. 32). Indeed, instead of using plant protein, there are suggestions to work preferably with terrestrial rendered animal protein (Chap. 31). This may suppose a change in legislation in some countries. There was another reason for such a search in substituting animal protein for plant protein that was related to the wastes. The wastes from fish and sturgeon are no exception, fed animal protein is more pollutant than plant protein, and some Western countries have obliged fish farmers to respect low threshold levels of nitrogen in the outlets (Chap. 34).

The third part of the volume deals with production. A broad overview of the world production of both meat and caviar for human consumption is given as well as an example fingerling production destined to be restocked with the hope of species preservation. With regard to the first type of production, it is worthy to note that obtaining species-specific data is complicated because (1) the species is not individualized in the statistics (if any), (2) sturgeon farms may produce several sturgeon species and (3) the main stakeholders are often reluctant to provide data and/or

information related to production and its related financial and marketing issues. Whatever, a tentative assessment of the world production for meat and caviar and its main characteristics is proposed. Among the characteristics are the recent trends in caviar production based on ovulated eggs instead of the traditional ovarian follicles and on the geographical extension of producing countries. Currently, Siberian sturgeon is the object of aquaculture in more than 50 countries; the whole volume of yearly world production is assessed about 22,000–23,000 thousand tons of gross production and 150 tons of caviar (Chap. 38).

Regarding the fingerling production, an example of restocking is given (Chap. 37) with the Ob-Irtysh catchment which is being impacted by oil and gas exploitation (Zaytsev 2012) so that the species is totally protected. The extremely long transportation and the related management of young fish from the hatchery up to the released areas downstream the river needed the help of an adapted boat that appears to be species specific. Out of the Siberian river catchments, that of the Lena River is the only one where the Siberian sturgeon population is not listed in the Russian Red Book, and this catchment, the Lena River, is where the two batches of Siberian sturgeon juveniles which arrived in France in 1975 and 1982 came from (Williot et al. 2017; Malyutin and Ruban 2009[1]).

In relation with the most attractive sturgeon product, the caviar, two chapters deal with quality aspect of the product. Biochemical origin and treatment of the potential unpleasant taste are provided (Chap. 36). Besides, a methodology to analyse the taste by the sensorial approach (Chap. 35) of the caviar is also described. There might be other quality aspects of the caviar production, one of them being the crushing character of caviar upon degustation. The main outcomes of a recent study on the matter (Augustin 2007) pointed out that washing and further salting are the main critical phases. The "ionic charge in the water results in tensions within external envelopes," and salting tends to break down the structure of the membranes. Additionally, the author gives a description of a transportable device able to measure the resistance to the breakdown of grain of caviar.

Four chapters constitute the fourth part of the volume 2; they deal with long-term management of brood stock. Two of them present how the genetic variability of brood stocks can be characterized, one in Poland (Chap. 41) and the other in Russia (Chap. 42). The former describes the genetic variability of polish brood stock thanks to microsatellites and the second study both mitochondrial and nuclear (microsatellites) markers for both farmed and wild populations of the species which constitute a great added value to this chapter. A chapter updates the studies aiming at controlling the sex by genome manipulation (Chap. 40) in describing in details the state of the art in the field. Up to now, we have been focusing our efforts on the Siberian sturgeon as a pure species, but given the very extended interspecies crossing in sturgeon, we may wonder which could be the advantages of farming hybrids with the Siberian sturgeon; this is the object of Chap. 39. The fifth part of the volume on

[1] The first batch of Siberian sturgeon arrived in France in 1975 and not in 1980 as mentioned by the authors. If necessary, a strong support to that is the fact that the first controlled reproduction of these fish has been obtained in France in 1981 (Williot and Rouault 1982).

farming includes three chapters which globally deal with the health status of the fish. All three are completely new approaches in sturgeon. The first out of the three consists in a synthesis on the immunology in the sturgeon with a focus on the Siberian sturgeon (Chap. 43). Mechanisms, responses to stress and stimulation are described. The two others tackle the welfare approach, one through plasmatic indicators (Chap. 44) and the second is a global approach of the matter for the species (Chap. 45). The authors of the last one (the three coeditors) tentatively gathered all the available data and methods (with their limits) at field; they show it is a complex approach that needs an extended knowledge of the fish, the environment and the husbandry altogether. A good illustration for that is the great number of references including other chapters of both volumes.

With the development of the farming of the species worldwide, one might be anxious with potential negative impact in case of escapements of specimen far away from their natural geographical distribution. From two examples of introduction of individuals of the species in non-native waters, no installation was recorded. Introduction was voluntary and repeated by numbers (Northwestern Russia, Chap. 46) and escapement from either fish farms or from the angling activities in close water ponds sometimes by number (France, Chap. 47). Thus, this means that the ecological risks of installation post-introduction of the Siberian species proved to be quasi-null. This is one of the very few documented examples in the field, and then it should be interesting to carefully analyse the reasons of this non-invasiveness in both Russia and France and to go further in depth of previous analysis in the field (Williot et al. 2009).

The last part aims at giving methods which focused on in vitro incubation of ovarian follicles (Chap. 48), on echography (Chap. 49) and on oxygen demand (Chap. 50). The first is used to test the in vitro maturation competence (IVMC) of the ovarian follicles as a decision maker bio-test for the selection of the best female to respond to the hormonal injection (Chap. 27). The second is an extremely well-illustrated chapter presenting a library of images (echographies) of the internal parts of the abdomen of fish to support the morphological description. The non-invasive method has been a considerable breakthrough in the early 2000s for early sex discrimination, i.e. for animals as young as 1.5–2.0 years weighing $\leq$2.0 kg (which corresponds to the minimum market requirement in Russia), and thus eases and speeds up the development of caviar-oriented farm (Chebanov and Chmyr 2002; Chebanov et al. 2004; Bonpunt 2006; Chebanov et al. 2006). The third method (Chap. 50), focused on oxygen demand, gives the detailed basements around the oxygen demand which is of the utmost importance to understand the whole physiological regulations involved in controlling the respiration. This allows computing the needed flow rates depending on the activity of fish and on environmental factors.

Altogether, the aforementioned compendium covers all the key items that are needed to carefully manage Siberian sturgeon farming. This means that even though there is still a great need of long-term research (e.g. long-term impact of food) which is not Siberian sturgeon specific, the species has supported a lot of studies worldwide, illustrating the interest focused on it. This is confirmed by a recent analysis on the publications focused on sturgeon during a 15-year period (1996–2010)

(Jarić and Gessner 2011). The species is second behind the white sturgeon (*Acipenser transmontanus*) of which a great part deals with environmental issues. This means that out of all sturgeon species, the Siberian sturgeon has been the most investigated. In other words, this is the species of which the library is the largest which is an advantage at a time when unexpected questions arrived. It has been observed by sturgeon farmers (Sabeau 1998) that the caviar from the species looks like that of the Russian sturgeon (*Acipenser gueldenstaedtii*) commonly known as osetra the species being one of the three of which the image is known worldwide. Finally, the most critical argument in favour of the species most likely relies on its remarkable adaptive capacity that makes the species very much securing from a long-term perspective once environmental conditions remain in the homeostasis range. Indeed, the species, which was not mentioned in the past in the declared landings for sturgeon by the former USSR the quantities of which were completely dominated by the three well-known wild exploited species in the Ponto-Caspian basin (*Huso huso*, *Acipenser gueldenstaedtii*, *Acipenser stellatus*), accounted for about 30% in the late 1990s (Chebanov and Billard 2001). Further, this relative part is similar to date (Chap. 38), which means that the species is very attractive in the sturgeon mother country, Russia.

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