



Sex Determination and Differentiation of the Siberian Sturgeon

6

Denise Vizziano-Cantonnet, Santiago Di Landro,
and André Lasalle

Abstract

This is a synthesis of the published information on sex determination and differentiation in sturgeons with special emphasis in Siberian sturgeon *Acipenser baerii*. The sex-determination system has been poorly studied in sturgeons and paddlefishes. Among 27 species of Acipenseriformes, the WZ-female model has been proposed in five species and one hybrid based on gynogenetic studies. The sex-determining gene remains unknown, and there are no studies on the influence from autosomal genetic factors or environmental cues. The lack of genetic sex marker is limiting the studies on sex determination and differentiation, as well as the application in the early sexing for culture purposes.

The gonad sex differentiation show similar histological features in different sturgeons studied and occur at juvenile stage (4–9 months of age). Siberian sturgeon reach the sex differentiation around the 8 months of age when reared in a range of temperature of 14–19 °C, coincident with the upper range of spawning temperature of species at wild. However, the potential effect of temperature on sex determination has not been studied in sturgeons. The molecular sex differentiation period precedes the sex differentiation and occurs between 3 and 6 months old in Siberian sturgeons. The factors involved in male and female pathways are currently studied at molecular level. Gonad transcriptomes are emerging in sturgeons stimulating the knowledge of factors that must direct a cascade of gene regulatory controls that provide sex-specific phenotypes. There is a lack of functional studies in sturgeons.

Keywords

Sturgeons • Sex determination • Sex differentiation • Gonad histology • Gene expression • *Acipenser baerii*

D. Vizziano-Cantonnet (✉) • S. Di Landro • A. Lasalle
Facultad de Ciencias, Laboratorio de Fisiología de la Reproducción y Ecología de Peces,
Iguá 4225, Montevideo 11400, Uruguay
e-mail: vizziano@gmail.com

Introduction

Acipenseriformes is an order of fishes in which are included sturgeons and paddlefishes. Some of their morphological characteristics as cartilaginous skeletons, heterocercal tail, scaleless skin, and the presence of the notochord in adults are unique of this order and make them different from all other bony fish (Birstein 1993; LeBreton et al. 2005). They are found only in the Northern Hemisphere more specifically in Asia, Europe, and North America (Magnin 1959; Bemis and Kynard 1997; Sokolov and Vasili'ev 1989) but were introduced in the Southern Hemisphere, particularly in Uruguay for their culture.

Sturgeons and paddlefishes are represented by two families, Acipenseridae and Polyodontidae, respectively. Twenty-five species of living Acipenseriformes are grouped in four genera (*Acipenser*, *Huso*, *Scaphirhynchus*, and *Pseudoscaphirhynchus*) included in the Acipenseridae family, while the Polyodontidae family is represented only by two extant species (Bemis et al. 1997). All those species have critical problems of conservation (Sokolov and Vasili'ev 1989; Birstein 1993) because of overfishing, incidental fishing, river pollution, dam construction, environmental disruptions, and the poor organization system of fisheries management, among others (Bacalbaşa-Dobrovici 1997; Bemis et al. 1997; Sokolov and Vasili'ev 1989; Wei et al. 1997).

The sturgeons and paddlefish are target species for caviar production and are considered one of the most valuable commercial fishes. The studies of sex determination and differentiation are key points for the understanding of their biology and have an application both in the biology of conservation and in fish production. Nevertheless, there are very scarce data on these topics as it was summarized in Table 6.1.

6.1 Sex Determination Mechanisms

The mechanism of sex determination has been extensively reviewed in fish (Penman and Piferrer 2008) and shows that gonochoric species has two main types of sex determination: a genetic sex determination (GSD) that occurs during conception and an environmental sex determination (ESD) determined after fertilization. Penman and Piferrer (2008) also summarized the types of sex-determining mechanisms in fish that are very variable. In fact the inheritance of sex is based on three main factors: (a) the presence of major genetic sex factors that determines a monofactorial system, (b) the presence of minor factors determining a polyfactorial system, and (c) the environmental differences that determine an environmental sex-determination system. A combination of these different factors can be observed in fish showing their variable systems of sex determination.

The mechanism of sex determination in basal and gonochoric species as sturgeons is not completely elucidated (Flynn et al. 2006). The presence of sexual chromosomes has not been demonstrated by cytological studies in sturgeons (Fontana and Colombo 1974; Van Eenennaam 1997) probably because these basal fish exhibit a relative primitive sex chromosome evolution (Volf 2005). In the absence of cytological demonstrable chromosomes, genetic approaches have been used to show whether the

Table 6.1 List of extant Acipenseriformes, their distribution area, status of conservation, sex-determination system, and sex differentiation time

Species name	Distribution area ^a	IUCN	Sex-		References	References
			determination system	differentiation time		
Asian species						
<i>Acipenser baeri</i>	Rivers of north coast of Russia (Ob, Aldan, and Lena rivers)	VU	ZZ-ZW ^b	Fopp-Bayat (2010)	Vizziano-Cantonnnet et al. (2016)	8 months 4 months
<i>Acipenser dabryanus</i>	Yangtze River and Tungting Lake	CR	-	-	-	-
<i>Acipenser mikadoi</i>	Siberia Pacific coast	CR	-	-	-	-
<i>Acipenser schrenckii</i>	Amur River (Russia, China)	CR	-	-	-	-
<i>Acipenser sinensis</i>	Yangtze and Pearl rivers	CR	-	-	Chen et al. (2006)	9 months
<i>Pseudoscaphirhynchus fedtschenkoi</i>	Syr-Darya basin	CR	-	-	-	-
<i>Pseudoscaphirhynchus hermanni</i>	Amu-Darya basin	CR	-	-	-	-
<i>Pseudoscaphirhynchus kaufmanni</i>	Amu-Darya basin	CR	-	-	-	-
<i>Huso dauricus</i>	Amur River drainage	CR	-	-	-	-
<i>Psephurus gladius</i>	Yangtze River	CR	-	-	-	-

(continued)

Table 6.1 (continued)

Species name	Distribution area ^a	IUCN	Sex-		References
			determination system	differentiation time	
European species					
<i>Acipenser gaidedenstaedtii</i>	Ponto-Caspian distribution	EN	-	-	-
<i>Acipenser stellatus</i>	Black, Caspian, and Azov seas	CR	-	-	-
<i>Acipenser ruthenus</i>	Rivers emptying into the Azov, Black, Caspian, Baltic, and Arctic seas and in the Amur River	VU	-	8 months	Wrobel et al. (2002)
<i>Acipenser nudiventris</i>	Danube River, Aral and Caspian seas	CR	ZZ-ZW ^b	-	Saber and Hallajian (2014)
<i>Acipenser persicus</i>	Caspian Sea and the Volga, Kura, and Ural rivers	CR	-	-	-
<i>Acipenser sturio</i>	Gironde the Atlantic and the North Sea	CR	-	-	-
<i>Acipenser naccarii</i>	Adriatic Sea	CR	-	6 months	Grandi et al. (2007)
<i>Huso huso</i>	Black, Caspian, Azov, and Mediterranean seas and Po and Danube rivers	CR	-	-	-

North American species									
<i>Acipenser medirostris</i>	North Pacific, North American rivers, from Southern California to Alaska	NT	-	-	-	-	-	-	-
<i>Acipenser transmontanus</i>	California, Oregon, Washington, and the province of British Columbia	LC	ZZ-ZW ^b	Van Eenennaam et al. (1999)	Before 18 months	Doroshov et al. (1997)			
<i>Acipenser fulvescens</i>	Mississippi River, the Great Lakes, and the Hudson Bay	LC	-	-	-	-	-	-	-
<i>Acipenser brevirostrum</i>	Rivers by the East Atlantic coast	VU	ZZ-ZW ^b	Flynn et al. (2006)	6 months	Flynn and Benfey (2007)			
<i>Acipenser oxyrinchus</i>	Gulf of Mexico to Labrador	NT	-	-	-	-	-	-	-
<i>Scaphirhynchus platyrhynchus</i>	Mississippi River	VU	-	-	-	-	-	-	-
<i>Scaphirhynchus albus</i>	Mississippi, Missouri, and Yellowstone rivers	EN	-	-	-	-	-	-	-
<i>Scaphirhynchus suttkusi</i>	"Mobile Basin" of the Mississippi and Alabama rivers	CR	-	-	-	-	-	-	-
<i>Psephurus spathula</i>	Mississippian Basin and contiguous rivers	VU	ZZ-ZW ^b	Shelton & Mims (2012)	-	-	-	-	-
<i>Huso huso female x Acipenser ruthenus male</i>	Sturgeon farming in Russia, Germany, Hungary, and Japan	/	ZZ-ZW ^b	Omoto et al. (2005)	6 months	Omoto et al. (2001)			

^aData mostly taken from Billard and Lecointre (2000), Bemis et al. (1997), and IUCN (2015)

^bSex-determination system suggested by sex ratios of meiotic gynogenetic progeny; "-", "no information found"; "r/" does not correspond VU vulnerable, CR Critically endangered, EN endangered, NT near threatened, LC least concerned

sex determination can be explained by sexual chromosomes. The gynogenetic studies support the contention of a female heterogametic genetic sex-determining system (ZZ/ZW) in many sturgeons (*Acipenser transmontanus*, Van Eenennaam et al. 1999; bester, *Huso huso* x *Acipenser ruthenus*, Omoto et al. 2005; *Acipenser brevirostrum*, Flynn et al. 2006; *Acipenser baerii*, Fopp-Bayat 2010; *Acipenser nudiventris*, Saber and Hallajian 2014) (Table 6.1). Recently it has been shown that *Polyodon spathula*, another fish of the order of Acipenseriformes, can have a ZZ/ZW sex-determination system (Shelton and Mims 2012). Collectively, these data suggest that sex determination among Acipenseriformes conforms to the WZ-female model (Shelton and Mims 2012). The emerging pattern of sex determination in Acipenseriformes is important for the long-term genetic management of cultured species of sturgeons and paddlefish. However, the number of species studied is scarce to consider that this is the common system of sex determination in this fish order.

For culture purposes the obtention of monosex populations is of great interest in particular a cohort of monosex genetic females producing caviar. In species with female heterogamety (ZZ male; ZW female) as proposed for Siberian sturgeon (Fopp-Bayat 2010), gynogenesis may produce ZZ males, WW “super” females, and/or ZW females (Van Eenennaam et al. 1999). These authors mentioned that the viability of “super females” WW after gynogenic experiments could not be identified in their experimental conditions (Van Eenennaam et al. 1999).

Another way for the production of super females (WW) is to obtain the sex inversion using hormones. More precisely the masculinization of females (ZW) is needed in order to obtain phenotypic and functional ZW neo-males to cross with normal females (ZW) to obtain super females (WW). However, very few efforts have been made to induce sex inversion by hormonal treatments in sturgeons, and most of them have been focused on feminization (Falahatkar et al. 2014; Flynn and Benfey 2007; Omoto et al. 2002). Only one report has been found in the literature on the masculinization of the hybrid bester (*Huso huso* female x *Acipenser ruthenus* male) using 17 α -methyltestosterone (Omoto et al. 2002), but this protocol has not been used in females of non-hybrid sturgeons, reared until adult and crossed to normal females to confirm the ZZ/ZW genetic system and to produce monosex genetic females.

The lack of sex chromosome-specific markers—searched for many years in sturgeons (Keyvanshokoo and Gharaei 2010; Khodaparast et al. 2014; Wuertz et al. 2006)—hinders the identification of the presence of super females (WW). Since the survival of WW super females has been observed in at least one species with ZZ/ZW genetic system (tilapia *Oreochromis aureus*) (Guerrero III and Guerrero 1975; Mair et al. 1991), there are no reasons to discard the possibility of survival of WW in sturgeons.

Finally, whether sturgeons use a monofactorial ZZ/ZW genetic system or has an influence from autosomal genetic factors or environmental influences remains to be elucidated. The sex ratio in wild (*Acipenser transmontanus*, Chapman et al. 1996) and in captivity (*A. baerii*, Williot and Brun 1998; *Acipenser ruthenus*, Williot et al. 2005) remains stable at 50:50 sustaining the idea of a monofactorial sex-determination system. However, studies on temperature effects on sex ratio or the sex ratio of progeny from masculinized females crossed to regular females have not been made in order to better understand the sex determination mechanism present in sturgeons.

6.2 Master Gene Driving Sex Determination

In fish the master gene driving sex determination (SDG) is not well conserved and has been discovered in species with male heterogamety (XX/XY genetic system) in which the SDG is linked to the Y chromosome and acts as male-dominant gene. In teleost fish SDG are transcription factors as *dmY* (Matsuda et al. 2002) and *sox3* (Takehana et al. 2014), members of the transforming growth factors-beta (TGF- β) family as *gsdf-Y* (Myosho et al. 2012) and *amh-Y* (Hattori et al. 2012; Yamamoto et al. 2014), or factors related to immune system as the *sdY* (Yano et al. 2012). There is no data on the master gene that control the sex in fish with ZZ/ZW sex-determination system, as it is the case of sturgeons. In other vertebrates with ZZ/ZW, two different mechanisms of sex determination have been described. The first one is a double dosage of the transcription factor *dmrt1* in Z that masculinizes some birds (Smith et al. 2009); and the second is the presence of a female-dominant gene associated with W chromosome in some frogs (*dm-W* a paralog coming from a duplication of the gene *dmrt1*) (Yoshimoto et al. 2008, 2010). This opens the question about the presence of a female-dominant master gene linked to the W chromosome or the presence of a double dosage of a gene in males (ZZ). In the Siberian sturgeons the autosomal *dmrt1* is expressed in gonads (Berbejillo et al. 2012, 2013), but it is not the factor that triggers gonad differentiation, since it is overexpressed 1 month after genes involved in male (*sox9*, *amh*) and female (*foxl2*, *cyp19a1a*) gonad differentiation (Vizziano-Cantonnet et al. 2016). However whether a similar W-linked *dmrt1* paralog described in Amphibia is active in sturgeons remains to be elucidated.

The sex-determining gene and sex genetic markers have been searched for many years in sturgeons at genetic level without success (recent review in Keyvanshokoh and Gharaei 2010; Yarmohammadi et al. 2011). The lack of genotypic markers linked to the sex prevented a genetic mapping or a sex genetic identification helping to discover the SDG as in other fishes. An alternative to genetic methodologies for SDG identification is the analysis of gonad transcriptome at undifferentiated stages in which the SDG is potentially upregulated. In teleost fish in which SDG was discovered, this gene has been found to be upregulated during the molecular sex differentiation period (Hattori et al. 2012; Matsuda et al. 2002; Myosho et al. 2012; Vizziano et al. 2007; Yamamoto et al. 2014; Yano et al. 2012). In the Siberian sturgeon the molecular sex differentiation period has been identified (Vizziano-Cantonnet et al. 2016), and the analysis of gonad transcriptome at this stage is under current investigation. Another recent methodology that could help to discover a genetic sex marker or the SDG is the RAD-seq that compares the genomic ADN of male and females to discover differences between sexes as it was done in different animals from invertebrates (copepod, Carmichael et al. 2013) to vertebrates (lizard, Gamble and Zarkower 2014).

The period of molecular sex differentiation goes before the time of sex differentiation recognized at histological level; thus the gonad morphology at early stages of development and the first signs of gonad sex differentiation are key points for the Siberian sturgeon biology. These topics are developed in Sects. 6.4 and 6.5.

6.3 Morphology of Gonads During Sex Differentiation and Early Gametogenesis

The sex differentiation time can be considered as the moment in which the first morphological signs can be recognized at histological level between male and female gonads. The morphological criteria used to distinguish the sex of the gonads have been reported and reviewed in many species of teleost fish (see reviews of Bruslé and Bruslé (1983); Devlin and Nagahama (2002); Nakamura et al. (1998)), and the first signs of gonad sex differentiation vary among species. In some species the first signs are observed in somatic tissues having special features or arrangements, and in other cases there is a clear and significant increase in germ cell numbers.

In contrast to the amount of information available regarding teleost fish, scarce investigation has been dedicated to sex differentiation in basal fish such as sturgeons. Early gonad morphology has been described for the Adriatic sturgeon (*Acipenser naccarii*) (Grandi and Chicca 2008; Grandi et al. 2007), the Chinese sturgeon (*Acipenser sinensis*) (Chen et al. 2006), the shortnose sturgeon (*Acipenser brevirostrum*) (Flynn and Benfey 2007), and more recently, the Siberian sturgeon (*Acipenser baerii*) (Rzepkowska and Ostaszewska 2014). The criteria used to recognize a presumptive female Adriatic sturgeon or Siberian sturgeon are the presence of invaginations at the gonadal surface (Grandi and Chicca 2008; Grandi et al. 2007; Rzepkowska and Ostaszewska 2014); however, the presence of mitotic or meiotic germ cell clusters is the unequivocal sign of ovarian development in the Siberian sturgeon (Vizziano-Cantonnet et al. 2016). On the other hand, fish that have gonads with a homogeneous distribution of non-meiotic germ cells are considered presumptive males of Siberian sturgeons (Vizziano-Cantonnet et al. 2016).

At histological level the undifferentiated gonad of *Acipenser baerii* shows the primordial germ cells restricted to the gonadal periphery among the epithelial cells or located inside the gonad, surrounded by somatic cells (Rzepkowska and Ostaszewska 2014; Vizziano-Cantonnet et al. 2016). As differentiation progresses, two distinct morphological patterns of primordial germ cell distribution were established in Siberian sturgeons: (a) the germ cells organized in clusters that indicates the presence of a female (Fig. 6.1a, b) and (b) the germ cells homogeneously distributed that indicates the presence of a male (Fig. 6.2a) (Vizziano-Cantonnet et al. 2016). The immunohistology using anti-cyp17 elevated against zebra fish protein showed a positive reaction in the cytoplasm of meiotic cells in clusters of the recently differentiated ovaries (Fig. 6.1b, d), while non-meiotic cells present in putative males have no reaction for this antiserum (Fig. 6.2b).

In late pachytene oocytes, the cysts are individualized by follicle cells, forming the ovarian follicles. In the ovarian follicles within the gonadal tissue, the now diplotene oocytes enter into the primary growth period. The ovary develops, and the ovigerous lamellae become prominent and develop on the border an active germinal epithelium, made up of somatic and proliferating germ cells. Some primary growth oocytes emerge among the less-developed germ cells (Fig. 6.3a).

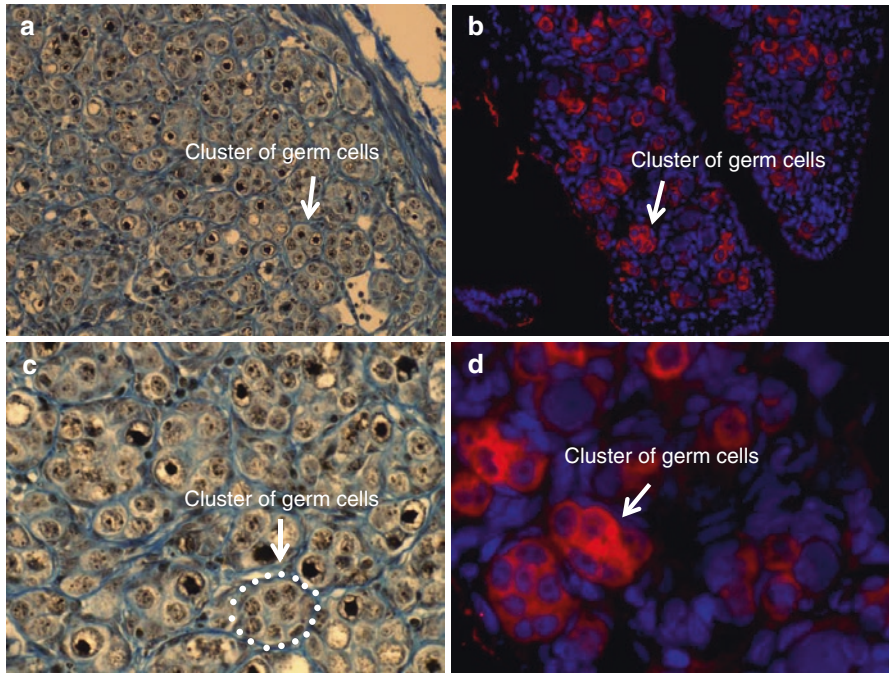


Fig. 6.1 Early differentiated ovary of *A. baerii*. The germ cells at different stages of meiotic prophase are surrounded by somatic cells and form cell nests or “clusters” (a, c). The cytoplasm of these germ cells in clusters is positive for the enzyme *cyp17a* as it is shown in orange in (b, d). The nucleus is colored in blue

In the putative male, the primordial germ cells differentiate into spermatogonia, proliferate, and increase in number, forming sinuous and cord-like structures that occupy the entire gonadal tissue. At this time, each spermatogonium is individualized by somatic cells, now differentiated into pre-Sertoli cells and form cysts. The interstitial tissue is organized among the cysts, consisting of fibroblasts, blood vessels, and amorphous mesenchymal cells. The testicular duct is formed in the dorsal gonad region (Fig. 6.3b).

The meiotic germ cells express the enzyme 17-hydroxylase (17OH or *cyp17*) that mediates two main steps of steroid synthesis: the conversion of progesterone into the precursor 17-hydroxy progesterone and the formation of androgens from progestogens. In teleost fish it has been proposed that meiosis is triggered by progestins as 17,20P (Miura et al. 2007), and the 17,20P is produced by spermatogonias that converts 17P into 17,20P by an activated 20 β HSD (Vizziano et al. 1996). The present data obtained in sturgeons comfort the idea of an autocrine regulation of germ cells by self-produced steroid produced. The absence of reactivity in male germ cells that remains at spermatogonial stage supports this idea.

The correct identification of the sex of gonads is essential to understand the process of sex determination and differentiation of sturgeons. The period of sex differentiation in the Siberian sturgeon reared in Uruguay has been identified based in

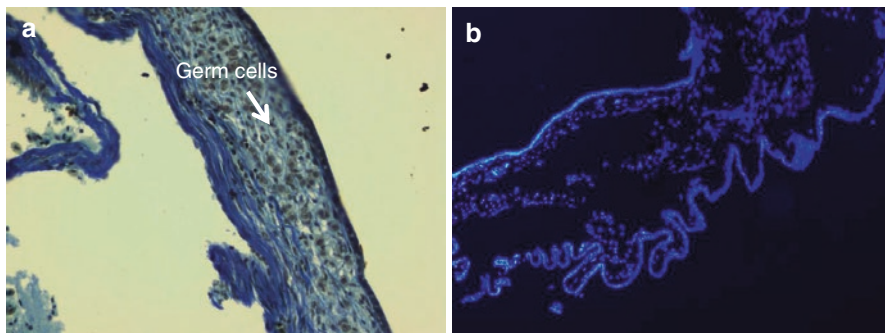


Fig. 6.2 Early differentiated testis of *A. baerii*. (a) Germ cells are located in the central region of the gonads and form cord-like structures delimited by somatic cells. (b) The cytoplasm of germ cell is not reactive for the *cyp17a* enzyme as it can be observed by the absence of *orange* color. The nucleus is colored in *blue*

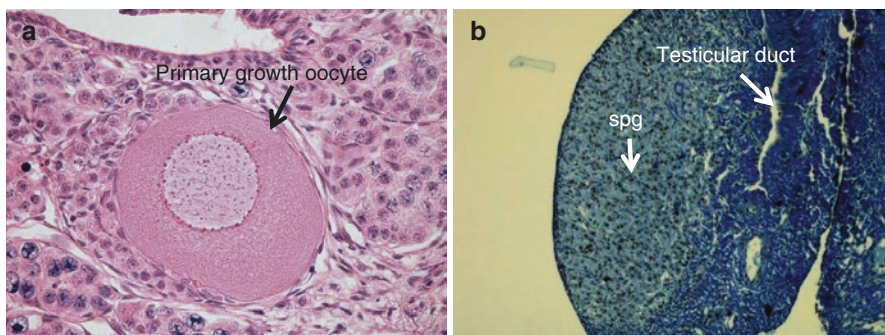


Fig. 6.3 Ovary (a) and testis (b) of *A. baerii*. A primary growth oocyte can be observed among less-differentiated germ cells in early developing ovaries (a). In testis a testicular duct is formed and the spermatogonia proliferate (b)

regular samplings of gonads during 3–27 months of age, and results were reported in the next section.

Gonad morphology is also a key point to consider when sex determination model needs to be discovered. In the Acipenseriformes as *Polyodon spathula*, it was concluded that the sex determination genetic model was XX/XY because of a wrong classification of the gonads of a cohort of gynogenote progeny (coming from gynogenesis) (Mims et al. 1997; Shelton and Mims 2012). The authors examined at 70 weeks-of-age fish (around 17 months old of age) and identified them as females, largely based on the developing lamellae in the anatomically differentiating gonads. Ten years later, the authors reinvestigate these fish and observed the presence of a significant number of males. Therefore, they revised their original hypothesis of female homogamety and corrected their earlier erroneous report (Shelton and Mims 2012). They conclude that *Polyodon* as sturgeons has a ZZ/ZW sex genetic-determining system.

6.4 Sex Differentiation Period

The sex differentiation is considered here as the time in which the fish has a gonad that can be recognized as a future testis or a future ovary by histological features.

The fish exhibit a great variability in the period of sex differentiation that can occur: (1) very early previous or around the hatching time (i.e., in the guppy *Poecilia reticulata*, the medaka *Oryzias latipes*), (2) during larval development (i.e., in the trout *Oncorhynchus mykiss* and tilapia, *Tilapia zillii*), or (3) very late during juvenile period between 1 and 2 years in the sea bass (*Dicentrarchus labrax*) or between 1.5 and 6 years in the eel (*Anguilla anguilla*) (Bruslé and Bruslé 1983). The sturgeons have a juvenile sex differentiation, and the age at sex differentiation varied from 4 to 9 months of age for the various sturgeons species analyzed (Chen et al. 2006; Grandi et al. 2007; Flynn and Benfey 2007; Grandi and Chicca 2008; Rzepkowska and Ostaszewska 2014; Vizziano-Cantonnet et al. 2016). It is not clear if the age at sex differentiation is species specific or depends on rearing conditions. For the Siberian sturgeon, when the presence of invaginations was the criterion used to define sex differentiation, age at sex differentiation was 4 months (Rzepkowska and Ostaszewska 2014). However, this distinction does not provide conclusive evidence of morphological sex differentiation in sturgeons (Hagihara et al. 2014; Vizziano-Cantonnet et al. 2016). The presence of mitotic or meiotic germ cell clusters was considered as a clear sign of ovarian development and the homogeneous distribution of non-meiotic germ cells as a sign of presumptive males. Using this criteria Siberian sturgeons are differentiated at 8 months of age in the Uruguayan rearing conditions (Figs. 6.1 and 6.2). It is interesting to note that the temperatures in Uruguay were warmer (from 12.5 °C in June to 26.5 °C in January, Table 6.2) than those of the original rivers in which the Siberian sturgeon lives as native species in the Northern Hemisphere (1–19 °C, Ruban, Chap. 1, this book). The lack of information on the time of sex differentiation at wild prevents a comparison between the wild and culture conditions. However the huge plasticity in the age of first maturity and in growth showed by Siberian sturgeons in the different rivers and lakes of Siberia (Ruban, Chap. 1, this book) suggests that the kinetics of sex differentiation can also be regulated by environmental factors.

At Uruguayan rearing conditions, the gonads remain undifferentiated until 6 months of age. We have no data on the 7 months of age, but we observed signs of sex differentiation at 8 months of age (Vizziano-Cantonnet et al. 2016). The kinetics of gonad development of Siberian sturgeons in Uruguay is shown in the Fig. 6.4a, and the temperature changes during the period of gonad development are shown in Fig. 6.4b. In the case of this cohort, the fecundation has been made in April in the Northern Hemisphere and transported and reared in Uruguay (Southern Hemisphere). In light gray, we are showing the period for which fish remained sexually undifferentiated and in dark gray the period of early gametogenesis (Vizziano-Cantonnet et al. 2016). The first 6 months of development occurred when the temperature ranged from 14 °C to 19 °C for this cohort (Fig. 6.4, Table 6.2), corresponding to the upper natural range of temperature at the spawning sites of the species (9 to 18 °C, Sokolov and Vasili'ev 1989). However, other batches of fertilized eggs can be received in February and March, when the temperatures are higher in Uruguay

Table 6.2 Annual average of temperature at the cages of sturgeons at the Rio Negro River

	2013												2014											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul					
<i>T</i> (°C)	26,48	24,91	23,37	23,37	17,57	15,57	14,45	14,02	15,24	19,03	22,65	25,74	26,20	25,03	23,3	20,05	16,14	12,5	13,18					
s.d. (±)	0,73	3,00	0,81	0,81	1,14	0,80	1,72	0,68	1,99	2,10	1,00	0,94	0,65	0,99	1,04	1,95	1,88	0,5	0,95					
<i>n</i>	25	19	31	31	31	30	31	31	30	31	30	31	31	28	31	30	31	30	31					

T (°C) = temperature; *n* = number of water temperature measures taken each month, s.d. (±) = standard deviation

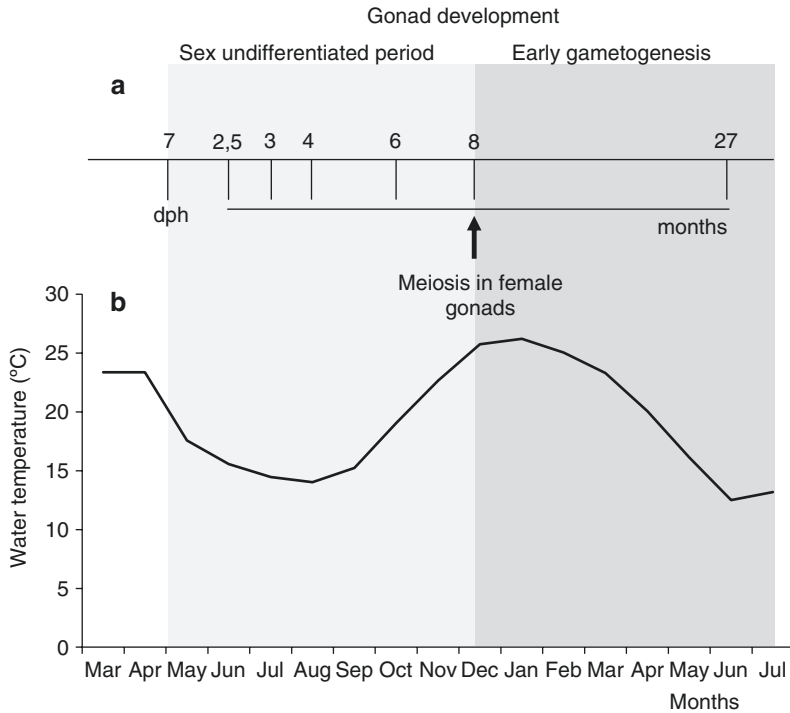


Fig. 6.4 Sex differentiation period and early gametogenesis in the Siberian sturgeon (a). dph = days post-hatching. The early meiosis in gonads with germ cells in clusters is one of the criteria to recognize the presence of a female gonad. Changes in temperature during the rearing conditions of embryos imported from the Northern Hemisphere are shown in (b)

(from 24 °C in February to 23 °C in April, Table 6.2). The sex ratio of Siberian sturgeons cultured in Uruguay has not been studied until now to understand whether high temperatures can affect the sexual development and sex ratio of the species. There are no comprehensive published data on the effect of temperature on sex differentiation of the Siberian sturgeon.

6.5 Period of Molecular Sex Differentiation

In fish, the period preceding gonad sex differentiation is characterized by early expression of sexually dimorphic genes involved in male or female pathways, controlled partly via molecular mechanisms; this stage is referred to as the “molecular sex differentiation period” (Vizziano et al. 2007). During the undifferentiated period, gonads show few histological changes and contain basically germ cells and somatic cells undergoing mitosis. Contrasting with that, they are very active at molecular level, and the pattern of male and female development can be recognized in particular when large-scale gene expression methodologies are applied to gonads of

monosex genetic populations (Baron et al. 2005; Ijiri et al. 2008; Vizziano et al. 2007). These works combined with the disruption of gonads to change the fish sex, provided an idea of the group of genes repressed or activated during the process of molecular sex differentiation that precede the gonad sex differentiation in trout (Raghuvver et al. 2005; Vizziano-Cantonnet et al. 2008; Vizziano et al. 2008). Several candidate genes were selected from large-scale gene expression studies, and their sex dimorphic expression was validated using monosex genetic populations (Baron et al. 2005; Cavileer et al. 2009; Guiguen et al. 1999; Lareyre et al. 2008; Vizziano et al. 2007; Yano et al. 2011b) or sex markers (Ijiri et al. 2008; Piferrer and Guiguen 2008). Recently, the period of molecular sex differentiation around 3–4 months for the Siberian sturgeon has been described (Vizziano-Cantonnet et al. 2016).

In teleost fish with an XX/XY sex-determining system, which is the type of fish in which the master sex-determining gene was discovered (*O. latipes*, Matsuda et al. 2002; *O. hatcheri*, Hattori et al. 2012; *O. luzonensis*, Myosho et al. 2012; *O. mykiss*, Yano et al. 2012; *O. dancena*, Takehana et al. 2014; *O. bonariensis*, Yamamoto et al. 2014), the male pathway is characterized by early dimorphic expression of transcription factors such as *dmrt1*, *sox9*, *nr0b1*, *dax1*, and *tbx1* (Baron et al. 2005; Marchand et al. 2000; Vizziano et al. 2007; Yano et al. 2011a) and type beta-transforming growth factors such as *amh* and *gsdf2* (Baron et al. 2005; Hattori et al. 2012; Lareyre et al. 2008; Myosho et al. 2012; Shibata et al. 2010; Vizziano et al. 2007; Yamamoto et al. 2014). Androgens appear to be involved in molecular mechanisms of sex differentiation in some teleosts (trout, Vizziano et al. 2007, 2008; pejerrey, Hattori et al. 2009, Blasco et al. 2013) but not in other fish (tilapia, Nakamura et al. 1998, Ijiri et al. 2008). In females, estrogens (mediated by *cyp19a1a*), and other factors as *foxl2* and *follistatin* (*fst*), are proposed as essential for ovarian differentiation (Guiguen et al. 2010; Piferrer and Guiguen 2008). Disruption of aromatase using anti-aromatase substances inhibits not only aromatase expression in all-female populations but also inhibits *foxl2a* expression during the ovary-to-testis transdifferentiation process (Vizziano et al. 2008), supporting the idea that *foxl2a* plays a key role in the early stages of ovarian development.

The gene candidates for regulation of sex differentiation in the Siberian sturgeon were *amh* and *sox9* for the masculine pathway and *cyp19a1* and *foxl2a* for the feminine pathway (Vizziano-Cantonnet et al. 2016). The expressions of *cyp19a1* and *foxl2a* were reinvestigated in the Siberian sturgeon in a larger period of development from 3 to 6 months of age following the methodology described previously (Vizziano-Cantonnet et al. 2016). We confirmed (a) the period of molecular sex differentiation is extended at least into 6 months of age (Fig. 6.5); (b) the presence of a group of fish with elevated *cyp19a1a* and *foxl2a* levels, presumably future females; and a second group with low *cyp19a1a* and *foxl2a* levels presumably indicating a male development (Fig. 6.5).

It is interesting to note that the percentage of fish with higher levels of *cyp19a1* and *foxl2* increases with the age of fish, being 31% at 3 months and 44–60% for 5–6 months (Table 6.3, Fig. 6.5) indicating that possibly the molecular sex differentiation occurs progressively during the period preceding the sex differentiation. This supports the idea of coexistence of fish engaged in the molecular sex differentiation

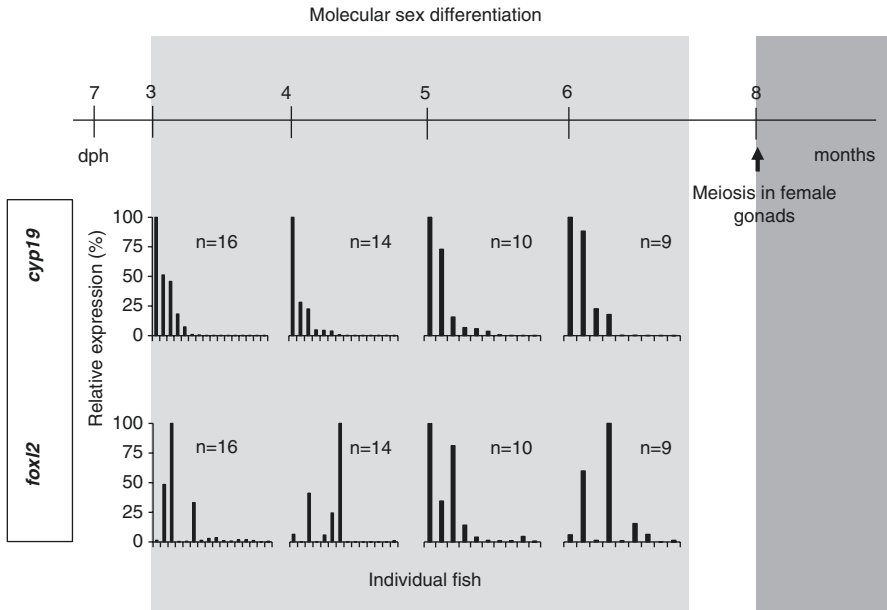


Fig. 6.5 Gonadal relative expression of *cyp19* and *foxl2* studied during the molecular sex differentiation period of Siberian sturgeon from 3 to 6 months of age. Relative quantification was carried out by normalizing the values to 18S ribosomal RNA gene abundance. Relative expression was calculated as a percentage of the highest expression level recorded for each gene tested. Dph = days post-hatching. The number of fish studied for each month is signaled as *n*

Table 6.3 Percentage of fish with higher levels of aromatase (*cyp19a1*) and *foxl2* during the molecular sex differentiation period (3–6 months old fish)

	3 M	4 M	5 M	6 M
<i>n</i>	16	14	10	9
<i>cyp19a1</i>	31%	43%	60%	44%
<i>foxl2</i>	31%	36%	60%	55%

M = months, *n* = number of fish studied

with fish undifferentiated at molecular level with bipotential gonads. The bipotential stage is the period in which the gonad is not sex differentiated at molecular level (mammals, Brennan and Capel 2004). This stage has not been studied in fish and sturgeons seem to be a good model for study this period.

Databases of gonadal transcriptome of sturgeon were published (*Acipenser fulvescens*, Hale et al. 2010; *Acipenser naccarii*, Vidotto et al. 2013; *Acipenser sinensis*, Yue et al. 2015), but these studies were unable in identifying the sex-determining gene or the pattern of sex differentiation at molecular level because they were based in one ovary compared to one testis. In order to better understand the process of molecular sex differentiation, fish at undifferentiated and differentiated stages are needed.

The knowledge on sex differentiation opens the possibility to study this period using multigenic approaches (i.e., using transcriptomics) in a key period in which the sex-determinant gene is known to be overexpressed in vertebrates to repress or activate the male or female pathways.

Conclusions

Sturgeons are basal and menaced fish very valuable for caviar production for which there is scarce information on sex determination and differentiation. The emerging pattern of sex determination is the WZ-female model (including Siberian sturgeon), but the sex-determining gene remains to be elucidated. The sex differentiation occurs at juvenile stage (4–9 months old) for different sturgeons studied. The Siberian sturgeon is sex differentiated at 8 months of age in Uruguayan rearing conditions (12–26 °C) and considering the clusters of meiotic germ cells in females as reference. The molecular sex differentiation occurs between 3 and 6 months of age, when *foxl2* and *aromatase* are taken into account. But the molecular pathway preceding the building of a testis or an ovary is largely unknown for sturgeons. The basic data to advance in this topic are emerging in the literature. The knowledge of sex determination and differentiation is less developed in sturgeons when compared to teleost fish, and an effort needs to be made to characterize at molecular level the changes that conduce to a male or a female gonad. There are no functional studies in sturgeons using the gene knock-out by mutagenesis or the gain of function by transgenesis. These methodologies need to be developed for these basal non-teleost and non-model fish of commercial interest.

Acknowledgments Many thanks are due to the enterprise Estuario del Plata (Uruguay) for their cooperation with Universidad de la República (UdelaR). Dr. Vet. Andrés Ryncowski gave us the temperature records. We are grateful with Dr. François Brion (INERIS, Paris, France) for the donation of the anti-cyp17 elevated against zebra fish protein.

Glossary

<i>amh</i>	Anti-Müllerian hormone
<i>amh-Y</i>	Y-linked amh
<i>ar</i>	Androgen receptor
<i>cyp17a1</i>	Cytochrome P450, family 17, subfamily a, polypeptide 1
<i>cyp19a1</i>	Cytochrome P450, family 19, subfamily a, polypeptide 1a
<i>dmrt1</i>	Doublesex and Mab-3-related transcription factor 1
<i>dm-W</i>	W-linked dmrt1 paralog
<i>dmY</i>	Y-specific DM-domain gene required for male development
ESD	Environmental sex determination
<i>figα</i>	Factor in germ line alpha
<i>foxl2a</i>	Forkhead box L2 a

<i>fst</i>	Follistatin
GSD	Genetic sex determination
<i>gsdf-Y</i>	Gonadal soma-derived growth factor on the Y
<i>gsdf2</i>	Gonadal soma-derived growth factor
GSI	Gonadosomatic index
<i>hsd17b1</i>	Hydroxysteroid (17-beta) dehydrogenase 1
<i>nrOb1</i>	Nuclear receptor subfamily 0, group b, member 1
SDG	Sex-determining gene
<i>sdY</i>	Sexually dimorphic on the chromosome Y
<i>sox9</i>	Sex-determining region Y-box containing gene 9
<i>sox9a1</i>	Sex-determining region Y-box containing gene 9
<i>sox9a2</i>	Sex-determining region Y-box containing gene 9
<i>star</i>	Steroidogenic acute regulatory protein
<i>tbx1</i>	T-box transcription factor gene family
17,20P	17,20 β -dihydroxyprogesterone
17P	17-hydroxyprogesterone
20 β HSD	20-hydroxysteroid dehydrogenase

References

- Bacalbaşa-Dobrovici N (1997) Endangered migratory sturgeons of the lower Danube River and its delta. *Env Biol Fish* 48(1–4):201–207
- Baron D, Houlgatte R, Fostier A, Guiguen Y (2005) Large-scale temporal gene expression profiling during gonadal differentiation and early gametogenesis in rainbow trout. *Biol Reprod* 73(5):959–966
- Bemis WE, Kynard B (1997) Sturgeon rivers: an introduction to acipenseriform biogeography and life history. *Env Biol Fish* 48(1–4):167–183
- Bemis WE, Findeis EK, Grande L (1997) An overview of Acipenseriformes. In: Birstein VJ, Waldman JR, Bemis WE (eds) *Sturgeon biodiversity and conservation*, 1st edn. Kluwer Academic/Plenum Publishers, New York, pp 25–71
- Berbejillo J, Martinez-Bengochea A, Bedo G, Brunet F, Volf J, Vizziano-Cantonnet D (2012) Expression and phylogeny of candidate genes for sex differentiation in a primitive fish species, the Siberian sturgeon, *Acipenser baerii*. *Mol Reprod Dev* 79(8):504–516
- Berbejillo J, Martinez-Bengochea A, Bedo G, Vizziano-Cantonnet D (2013) Expression of *dmrt1* and *sox9* during gonadal development in the Siberian sturgeon (*Acipenser baerii*). *Fish Physiol Biochem* 39(1):91–94
- Billard R, Lecointre G (2000) Biology and conservation of sturgeon and paddlefish. *Rev Fish Biol Fisher* 10(4):355–392
- Birstein VJ (1993) Sturgeons and paddlefishes: threatened fishes in need of conservation. *Conserv Biol* 7(4):773–787
- Blasco M, Somoza GM, Vizziano-Cantonnet D (2013) Presence of 11-ketotestosterone in pre-differentiated male gonads of *Odontesthes bonariensis*. *Fish Physiol Biochem* 39(1):71–74
- Brennan J, Capel B (2004) One tissue, two fates: molecular genetic events that underlie testis versus ovary development. *Nat Rev Genet* 5(7):509–521
- Bruslé J, Bruslé S (1983) La gonadogenèse des Poissons. *Reprod Nutr Dévelop* 23(3):453–449
- Carmichael SN, Bekaert M, Taggart JB, Christie HRL, Bassett DI, Bron JE, Skuce PJ, Gharbi K, Skern-Mauritzen R, Sturm A (2013) Identification of a sex-linked SNP marker in the Salmon louse (*Lepeophtheirus salmonis*) using RAD sequencing. *PLoS One* 8(10):e77832

- Cavileer T, Hunter S, Okutsu T, Yoshizaki G, Nagler JJ (2009) Identification of novel genes associated with molecular sex differentiation in the embryonic gonads of rainbow trout (*Oncorhynchus mykiss*). *Sex Dev* 3(4):214–224
- Chapman F, Van Eenennaam J, Doroshov S (1996) The reproductive condition of white sturgeon, *Acipenser transmontanus*, in San Francisco Bay, California. *FB* 94(4):628–634
- Chen X, Wei Q, Yang D, Zhu Y (2006) Observations on the formation and development of the primary germinal tissue of cultured Chinese sturgeon, *Acipenser sinensis*. *J Appl Ichthyol* 22:358–360
- Devlin RH, Nagahama Y (2002) Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture* 208(3):191–364
- Doroshov SI, Moberg GP, Van Eenennaam JP (1997) Observations on the reproductive cycle of cultures white sturgeon, *Acipenser transmontanus*. *Environ Biol Fish* 48(1–4):265–278
- Falahatkar B, Poursaeid S, Meknatkhah B, Khara H, Efatpanah I (2014) Long-term effects of intraperitoneal injection of estradiol-17beta on the growth and physiology of juvenile stellate sturgeon *Acipenser stellatus*. *Fish Physiol Biochem* 40(2):365–373
- Flynn S, Benfey T (2007) Sex differentiation and aspects of gametogenesis in shortnose sturgeon *Acipenser brevirostrum* Lesueur. *J Fish Biol* 70(4):1027–1044
- Flynn S, Matsuoka M, Reith M, Martin-Robichaud D, Benfey T (2006) Gynogenesis and sex determination in shortnose sturgeon, *Acipenser brevirostrum* Lesueur. *Aquaculture* 253(1):721–727
- Fontana F, Colombo G (1974) The chromosomes of Italian sturgeons. *Experientia* 30(7):739–742
- Fopp-Bayat D (2010) Meiotic gynogenesis revealed not homogametic female sex determination system in Siberian sturgeon (*Acipenser baeri* Brandt). *Aquaculture* 305(1–4):174–177
- Gamble T, Zarkower D (2014) Identification of sex-specific molecular markers using restriction site-associated DNA sequencing. *Mol Ecol Resour* 14(5):902–913
- Grandi G, Chicca M (2008) Histological and ultrastructural investigation of early gonad development and sex differentiation in Adriatic sturgeon (*Acipenser naccarii*, Acipenseriformes, Chondrostei). *J Morphol* 269(10):1238–1262
- Grandi G, Giovannini S, Chicca M (2007) Gonadogenesis in early developmental stages of *Acipenser naccarii* and influence of estrogen immersion on feminization. *J Appl Ichthyol* 23(1):3–8
- Guerrero RD III (1975) Use of androgens for the production of all-male *Tilapia aurea* (Steindachner). *Trans Am Fish Soc* 104(2):342–348
- Guiguen Y, Baroiller JF, Ricordel MJ, Iseki K, McMeel O, Martin S, Fostier A (1999) Involvement of estrogens in the process of sex differentiation in two fish species: the rainbow trout (*Oncorhynchus mykiss*) and a tilapia (*Oreochromis niloticus*). *Mol Reprod Dev* 54(2):154–162
- Guiguen Y, Fostier A, Piferrer F, Chang C-F (2010) Ovarian aromatase and estrogens: a pivotal role for gonadal sex differentiation and sex change in fish. *Gen Comp Endocrinol* 165(3):352–366
- Hagihara S, Yamashita R, Yamamoto S, Ishihara M, Abe T, Ijiri S, Adachi S (2014) Identification of genes involved in gonadal sex differentiation and the dimorphic expression pattern in undifferentiated gonads of Russian sturgeon *Acipenser gueldenstaedtii* Brandt & Ratzeburg, 1833. *J Appl Ichthyol* 30(6):1557–1564
- Hale MC, Jackson JR, Dewoody JA (2010) Discovery and evaluation of candidate sex-determining genes and xenobiotics in the gonads of lake sturgeon (*Acipenser fulvescens*). *Genetica* 138(7):745–756
- Hattori RS, Fernandino JI, Kishii A, Kimura H, Kinno T, Oura M, Somoza GM, Yokota M, Strüssmann CA, Watanabe S (2009) Cortisol-induced masculinization: does thermal stress affect gonadal fate in pejerrey, a teleost fish with temperature-dependent sex determination? *PLoS One* 4(8):e6548
- Hattori RS, Murai Y, Oura M, Masuda S, Majhi SK, Sakamoto T, Fernandino JI, Somoza GM, Yokota M, Strussmann CA (2012) A Y-linked anti-Müllerian hormone duplication takes over a critical role in sex determination. *Proc Natl Acad Sci U S A* 109(8):2955–2959
- Ijiri S, Kaneko H, Kobayashi T, Wang D-S, Sakai F, Paul-Prasanth B, Nakamura M, Nagahama Y (2008) Sexual dimorphic expression of genes in gonads during early differentiation of a teleost fish, the Nile tilapia *Oreochromis niloticus*. *Biol Reprod* 78(2):333–341
- IUCN (2015) The IUCN Red List of Threatened Species. Version 2015-4. www.iucnredlist.org

- Keyvanshokoh S, Gharaei A (2010) A review of sex determination and searches for sex-specific markers in sturgeon. *Aquac Res* 41(9):e1–e7
- Khodaparast M, Keyvanshokoh S, Pourkazemi M, Hosseini S, Zolgharnein H (2014) Searching the genome of beluga (*Huso huso*) for sex markers based on targeted bulked Segregant analysis (BSA). *Caspian J Env Sci* 12(2):185–195
- Lareyre J-J, Ricordel M-J, Mahé S, Goupil A-S, Vizziano D, Bobe J, Guiguen Y, Le Gac F (2008) Two new TGF beta members are restricted to the gonad and differentially expressed during sex differentiation and gametogenesis in trout. *Cybiu* 32(suppl):202
- LeBreton GT, Beamish FWH, McKinley SR (eds) (2005) Sturgeons and paddlefish of North America. Springer Science & Business Media, INC, New York
- Magnin E (1959) Répartition actuelle des acipenseridés. *Revue des Travaux de l'Institut Des Pêches Maritimes* 23(3):277–285
- Mair G, Scott A, Penman D, Beardmore J, Skibinski D (1991) Sex determination in the genus *Oreochromis*: 1. Sex reversal, gynogenesis and triploidy in *O. niloticus* (L.) *Theor Appl Genet* 82(2):144–152
- Marchand O, Govoroun M, D'Cotta H, McMeel O, Lareyre JJ, Bernot A, Laudet V, Guiguen Y (2000) DMRT1 expression during gonadal differentiation and spermatogenesis in the rainbow trout, *Oncorhynchus mykiss*. *Biochim Biophys Acta* 1493(1–2):180–187
- Matsuda M, Nagahama Y, Shinomiya A, Sato T, Matsuda C, Kobayashi T, Morrey CE, Shibata N, Asakawa S, Shimizu N (2002) DMY is a Y-specific DM-domain gene required for male development in the medaka fish. *Nature* 417(6888):559–563
- Mims SD, Shelton WL, Linhart O, Wang C (1997) Induced meiotic gynogenesis of paddlefish *Polyodon spathula*. *JWAS* 28(4):334–343
- Miura C, Higashino T, Miura T (2007) A progesterin and an estrogen regulate early stages of oogenesis in fish. *Biol Reprod* 77(5):822–828
- Myosho T, Otake H, Masuyama H, Matsuda M, Kuroki Y, Fujiyama A, Naruse K, Hamaguchi S, Sakaizumi M (2012) Tracing the emergence of a novel sex-determining gene in medaka, *Oryzias luzonensis*. *Genetics* 191(1):163–170
- Nakamura M, Kobayashi T, Chang XT, Nagahama Y (1998) Gonadal sex differentiation in teleost fish. *J Exp Zool* 281(5):362–372
- Omoto N, Maebayashi M, Mitsuhashi E, Yoshitomi K, Adachi S, Yamauchi K (2001) Histological observations of gonadal sex differentiation in the F2 hybrid sturgeon, the bester. *Fisheries Sci* 67(6):1104–1110
- Omoto N, Maebayashi M, Mitsuhashi E, Yoshitomi K, Adachi S, Yamauchi K (2002) Effects of estradiol-17 β and 17 α -methyltestosterone on gonadal sex differentiation in the F2 hybrid sturgeon, the bester. *Fish Sci* 68(5):1047–1054
- Omoto N, Maebayashi M, Adachi S, Arai K, Yamauchi K (2005) Sex ratios of triploids and gynogenetic diploids induced in the hybrid sturgeon, the bester (*Huso huso* female \times *Acipenser ruthenus* male). *Aquaculture* 245(1):39–47
- Penman DJ, Piferrer F (2008) Fish gonadogenesis. Part I: genetic and environmental mechanisms of sex determination. *Rev Fish Sci* 16(S1):16–34
- Piferrer F, Guiguen Y (2008) Fish gonadogenesis. Part II: molecular biology and genomics of sex differentiation. *Rev Fish Sci* 16(S1):35–55
- Raghuveer K, Garhwal R, Wang DS, Bogerd J, Kirubakaran R, Rasheeda MK, Sreenivasulu G, Bhattacharya N, Tarangini S, Nagahama Y, Senthilkumaran B (2005) Effect of methyl testosterone- and ethinyl estradiol-induced sex differentiation on catfish, *Clarias gariepinus*: expression profiles of DMRT1, cytochrome P450aromatases and 3 beta-hydroxysteroid dehydrogenase. *Fish Physiol Biochem* 31(2–3):143–147
- Rzepakowska M, Ostaszewska T (2014) Proliferating cell nuclear antigen and vasa protein expression during gonadal development and sexual differentiation in cultured Siberian (*Acipenser baerii* Brandt, 1869) and Russian (*Acipenser gueldenstaedtii* Brandt & Ratzeburg, 1833) sturgeon. *Rev Aquaculture* 6(2):75–88
- Saber MH, Hallajian A (2014) Study of sex determination system in ship sturgeon, *Acipenser nudiiventris* using meiotic gynogenesis. *Aquacult Int* 22(1):273–279

- Shelton WL, Mims SD (2012) Evidence for female heterogametic sex determination in paddlefish *Polyodon spathula* based on gynogenesis. *Aquaculture* 356:116–118
- Shibata Y, Paul-Prasanth B, Suzuki A, Usami T, Nakamoto M, Matsuda M, Nagahama Y (2010) Expression of gonadal soma derived factor (GSDF) is spatially and temporally correlated with early testicular differentiation in medaka. *Gene Expr Patterns* 10(6):283–289
- Smith CA, Roeszler KN, Ohnesorg T, Cummins DM, Farlie PG, Doran TJ, Sinclair AH (2009) The avian Z-linked gene DMRT1 is required for male sex determination in the chicken. *Nature* 461(7261):267–271
- Sokolov L, Vasili'ev V (1989) *Acipenser baerii* Brandt, 1869. In: Holcik J (ed) The freshwater fish of Europe, Vol I, Part II, general introduction to fishes-Acipenseriformes. AULA-Verlag, Wiesbaden, pp 263–284
- Takehana Y, Matsuda M, Myosho T, Suster ML, Kawakami K, Shin IT, Kohara Y, Kuroki Y, Toyoda A, Fujiyama A, Hamaguchi S, Sakaizumi M, Naruse K (2014) Co-option of Sox3 as the male-determining factor on the Y chromosome in the fish *Oryzias dancena*. *Nat Commun* 5:4157
- Van Eenennaam AL (1997) Genetic Analysis of the Sex Determination Mechanism of White Sturgeon (*Acipenser transmontanus* Richardson). Research Theses and Dissertations, California Sea Grant College Program UC San Diego. Available via <https://escholarship.org/uc/item/9x3272ww>. Accessed 1 Jan 1997
- Van Eenennaam A, Van Eenennaam J, Medrano J, Doroshov S (1999) Brief communication. Evidence of female heterogametic genetic sex determination in white sturgeon. *J Hered* 90(1):231–233
- Vidotto M, Grapputo A, Boscarì E, Barbisan F, Coppe A, Grandi G, Kumar A, Congiu L (2013) Transcriptome sequencing and de novo annotation of the critically endangered Adriatic sturgeon. *BMC Genomics* 14:407
- Vizziano D, Fostier A, Le Gac F, Loir M (1996) 20 beta-hydroxysteroid dehydrogenase activity in nonflagellated germ cells of rainbow trout testis. *Biol Reprod* 54(1):1–7
- Vizziano D, Randuineau G, Baron D, Cauty C, Guiguen Y (2007) Characterization of early molecular sex differentiation in rainbow trout, *Oncorhynchus mykiss*. *Dev Dyn* 236(8):2198–2206
- Vizziano D, Baron D, Randuineau G, Mahe S, Cauty C, Guiguen Y (2008) Rainbow trout gonadal masculinization induced by inhibition of estrogen synthesis is more physiological than masculinization induced by androgen supplementation. *Biol Reprod* 78(5):939–946
- Vizziano-Cantonnet D, Baron D, Mahe S, Cauty C, Fostier A, Guiguen Y (2008) Estrogen treatment up-regulates female genes but does not suppress all early testicular markers during rainbow trout male-to-female gonadal transdifferentiation. *J Mol Endocrinol* 41(5):277–288
- Vizziano-Cantonnet D, Di Landro S, Lasalle A, Martínez A, Mazzoni TS, Quagio-Grassiotto I (2016) Identification of the molecular sex-differentiation period in the Siberian sturgeon. *Mol Reprod Dev* 83(1):19–36
- Volff J (2005) Genome evolution and biodiversity in teleost fish. *Heredity* 94(3):280–294
- Wei Q, Fe K, Zhang J, Zhuang P, Luo J, Zhou R, Yang W (1997) Biology, fisheries, and conservation of sturgeons and paddlefish in China. In: sturgeon biodiversity and conservation. *Environ Biol Fish* 17:241–255
- Williot P, Brun R (1998) Ovarian development and cycles in cultured Siberian sturgeon, *Acipenser baeri*. *Aquat Living Resour* 11(02):111–118
- Williot P, Brun R, Rouault T, Pelard M, Mercier D, Ludwig A (2005) Artificial spawning in cultured sterlet sturgeon, *Acipenser ruthenus* L., with special emphasis on hermaphrodites. *Aquaculture* 246(1):263–273
- Wrobel K-H, Hees I, Schimmel M, Stauber E (2002) The genus *Acipenser* as a model system for vertebrate urogenital development: nephrostomial tubules and their significance for the origin of the gonad. *Anat Embryol* 205(1):67–80
- Wuertz S, Gaillard S, Barbisan F, Carle S, Congiu L, Forlani A, Aubert J, Kirschbaum F, Tosi E, Zane L, Grillasca J-P (2006) Extensive screening of sturgeon genomes by random screening techniques revealed no sex-specific marker. *Aquaculture* 258(1):685–688

- Yamamoto Y, Zhang Y, Sarida M, Hattori RS, Strussmann CA (2014) Coexistence of genotypic and temperature-dependent sex determination in pejerrey *Odontesthes bonariensis*. PLoS One 9(7):e102574
- Yano A, Nicol B, Guerin A, Guiguen Y (2011a) The duplicated rainbow trout (*Oncorhynchus mykiss*) T-box transcription factors 1, *tbx1a* and *tbx1b*, are up-regulated during testicular development. Mol Reprod Dev 78(3):172–180
- Yano A, Nicol B, Valdivia K, Juanchich A, Desvignes T, Caulier M, Zadeh AV, Guerin A, Jouanno E, Nguyen T (2011b) Sex in salmonids: from gonadal differentiation to genetic sex determination. Indian J Sci Technol 4(S8):60–61
- Yano A, Guyomard R, Nicol B, Jouanno E, Quillet E, Klopp C, Cabau C, Bouchez O, Fostier A, Guiguen Y (2012) An immune-related gene evolved into the master sex-determining gene in rainbow trout, *Oncorhynchus mykiss*. Curr Biol 22(15):1423–1428
- Yarmohammadi M, Pourkazemi M, Chakmehdouz F, Kazemi R (2011) Comparative study of male and female gonads in Persian sturgeon (*Acipenser persicus*) employing DNA-AFLP and CDNA-AFLP analysis. J Appl Ichthyol 27(2):510–513
- Yoshimoto S, Okada E, Umemoto H, Tamura K, Uno Y, Nishida-Umehara C, Matsuda Y, Takamatsu N, Shiba T, Ito M (2008) A W-linked DM-domain gene, DM-W, participates in primary ovary development in *Xenopus laevis*. Proc Natl Acad Sci U S A 105(7):2469–2474
- Yoshimoto S, Ikeda N, Izutsu Y, Shiba T, Takamatsu N, Ito M (2010) Opposite roles of DMRT1 and its W-linked paralogue, DM-W, in sexual dimorphism of *Xenopus laevis*: implications of a ZZ/ZW-type sex-determining system. Development 137(15):2519–2526
- Yue H, Li C, Du H, Zhang S, Wei Q (2015) Sequencing and de novo assembly of the gonadal transcriptome of the endangered Chinese sturgeon (*Acipenser sinensis*). PLoS One 10(6):e0127332