# **Chapter 6 Evaluation and Improvements of Egg and Larval Quality in Percid Fishes**

## **B. Schaerlinger and D.** Ż**arski**

 **Abstract** For a sustainable breeding process, the optimization of recirculating aquaculture system $(s)$  (RAS) fish rearing conditions and the control of out-of-season reproduction, it is important (i) to define intrinsic and extrinsic factors regulating the fish life cycle and (ii) to have a good knowledge of what makes a good ovum, embryo or larva. In the present chapter, we first describe the current knowledge on ova characteristics and the proper embryonic and larval development progress for several percid species. Indeed, it is important to well define the correct sequence of events in order to better characterize potential impairments. The characterization of ova defects or developmental failures (mortality or abnormalities occurrence) may allow the definition of different categories/levels of quality. This quality scale could help scientists and fish breeders to choose the most relevant quality indicators depending on their technical or scientific problem. Indeed, indicators could be either predictive, to assess ova quality, or studied after fertilization to determine embryonic and/or larval abilities to develop properly and reach key steps in addition to the ova quality. However, the possible indicators allowing precise determination of the egg and larvae quality are actually scarce. Some morphological parameters in most cases allow indicating, with high probability, high (e.g. intensiveness of cortical reaction in pikeperch) or low (e.g. oil droplets fragmentation in ovulated eggs of Eurasian perch and pikeperch) egg quality rather than quantifying the real quality. Moreover, there is still no clear molecular predictors of the egg and larvae quality due to the too few or ambiguous data obtained in the field. On the base of the most recent studies it seems that in many cases molecular analyses are one of the most promising methods possibly allowing an estimation of the ova, embryos and larvae quality. But the research activities in this field are still in progress.

 **Keywords** Percids • Egg • Embryo • Larva • Quality

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# <span id="page-1-0"></span>**6.1 Introduction**

As a whole, fish reproduction in captive conditions often leads to various levels of success. Indeed, the living conditions of breeders influencing gametogenesis on the one hand, and incubation and larval rearing conditions on the other hand can lead to reproduction failures. Assessing reproduction quality indicators represents an important field of research in aquaculture. Egg quality has recently been defined as its ability to be fertilized and develops into a healthy embryo and larva (Bobe and Labbe 2010; Migaud et al.  $2013$ ). One can add that the embryo and larva quality can be defined as their abilities to properly reach several key steps during the development. In order to evaluate properly ova, embryonic and larval quality, two questions need to be addressed. First, what is considered as good, medium or low quality? Second, what is the most relevant way to evaluate reproduction success, and if possible in a predictive manner? To answer the first question, it appears necessary to establish criteria including mortality stages, deformities occurrence or larval resistance. These criteria could help to define more properly some quality levels/categories. Indeed, in the literature, each study defined their own criteria and making a careful comparison become impossible.

 This chapter mainly aims to present methods currently available to predict, assess and improve reproductive performance success in percid species, but also briefly describes the developmental steps of the embryos and larvae allowing wider overview on the problem of the egg quality. The level of knowledge on this aspect is various depending on the studied species. However, in every case there are many variables needed to be improved in order to obtain good quality offspring. Here we first aimed at defining the morphological and biochemical composition of good quality ova and the proper sequence of the embryonic and larval development. This knowledge is necessary to properly define failures that can occur during oogenesis leading to poor quality ova and/or developmental process. Finally, the last part of the chapter presents several categories of factors that could affect reproductive success.

## **6.2 Ova Structure and Biochemical Composition**

# *6.2.1 Ova Structure*

The fish egg is the final product of the entire oogenesis process (Tyler and Sumpter 1996). In general, the ovulated egg consists of an external layer called the chorion (*zona radiata*) and an internal layer called the vitelline membrane directly sur-rounding the egg cell (see e.g., Cotelli et al. [1988](#page-26-0); Riehl and Patzner 1998; Quagio-Grassiotto and Guimaraes 2003; Mansour et al. [2009](#page-28-0)). Just beneath the plasma membrane, cortical alveoli (granules) are usually located, which play a very impor-tant role upon the activation (Hart and Yu 1980; Lee et al. [1999](#page-28-0)). In percids, the surface of the chorion is coated with structures forming a sticky (walleye and pikeperch) or thick jelly-like (Eurasian and yellow perch) layer (Riehl and Patzner 1998;



**Fig. 6.1** Structure of the Eurasian perch *zona radiata* (a) Embryo at that the blastula stage (b) higher magnification to show the structure of the *zona radiata externa* (Pictures of D. Zarski (a) and M. Alix ( **b** )). *Arrows* present the *zona radiata externa* and *arrowhead* the emplacement of the chorion. *Scale bars* correspond to 500  $\mu$ m in (**a**) and 200  $\mu$ m in (**b**)

Formicki et al. [2009 \)](#page-27-0) which is also called the *zona radiata externa* (ZRE, Fig. 6.1 ). Actually, only the ZRE creates the differences in the morphology of the eggs among the freshwater percids. Morphologically, egg cells of those species (without the ZRE) look very similar. The eggs are fully transparent with one, clearly-visible oil droplet (with a diameter of about 0.6 mm) within the egg cell and the main volume of the non-activated egg consists of yolk mass (Mansueti [1964](#page-28-0) ; Żarski et al. [2011a](#page-30-0) ,  $2012a$ , [b](#page-30-0)). The average diameter of such eggs ranged between 1.0 and 1.3 mm in pikeperch (Demska-Zakeś et al. 2005; Żarski et al. 2012b), between 0.86 and 0.91 mm (without ZRE) in Eurasian perch (Sulistyo et al. 1998; Żarski et al. [2012c](#page-30-0)) and approximately 1.2 mm in yellow perch (Mansueti 1964).

 Eggs of walleye and pikeperch are deposited (ovulated) as a batch of single eggs, similarly to other freshwater fish species, whereas eggs of Eurasian and yellow perch are situated within the cylindrical, gelatinous strand which was in the literature named as ribbon (e.g., Probst et al. [2009 ;](#page-29-0) Formicki et al. [2009 \)](#page-27-0). This structure is unique among the freshwater fish and is usually deposited in one piece with a length of up to 5.5 m (Korzelecka et al. 1998; Formicki et al. 2009). There is a relationship between the size of females and the one of egg ribbons that has been established within a size of breeders ranging from 150 to 350 mm (Dubois et al. 1996). Eggs within the ribbon are connected by a very thick ZRE, which may occupy from 25 % to 30 % of the entire egg diameter (Mansueti [1964 \)](#page-28-0).

 After activation, the eggs, due to the cortical reaction (where the content of cortical alveoli is released between the vitelline membrane and the chorion forming a perivitelline space), start to swell due to the infusion of water into the perivitelline space through the osmotic gradient, caused by content of cortical alveoli (for details see e.g., Coward et al. [2002](#page-26-0); Minin and Ozerova [2008](#page-29-0)). This process leads to form the final volume of the egg, and was also described as water hardening (e.g., Mansueti 1964; Czesny et al. [2005](#page-27-0)a; Demska-Zakęś et al. 2005). In the case of walleye and pikeperch, after the contact with water (Fig.  $6.2$ ), the eggs become

<span id="page-3-0"></span>

**Fig. 6.2** Eggs of pikeperch at the moment of contact with water (a) and after water hardening (60 min post activation) (**b**). The *scale bar* represents 1 mm (Pictures of D. Zarski)

sticky (e.g., Schlumpberger and Schmidt [1980](#page-29-0); Rinchard et al. 2005; Demska-Zakes et al. 2005; Kucharczyk et al. [2007](#page-28-0)), whereas in perch, large water acquisition by the gelatinous layer was observed (Formicki et al. [2009](#page-27-0) ). After the swelling process, percid eggs look the same, except the formed perivitelline space (and thus increased diameter). After water hardening, diameter of the walleye eggs ranges between 1.6 and 2.0 mm (Serns [1982](#page-29-0); Czesny et al. [2005a](#page-26-0)) and for pikeperch between 1.26 and 1.44 (Demska-Zakęś et al. [2005](#page-27-0); Żarski et al. 2012b). In the case of yellow perch, the internal (without ZRE) egg diameter ranges approximately between 1.6 and 1.8 mm (Mansueti [1964](#page-28-0)) and in Eurasian perch between 1.30 and 1.43 mm (Zarski et al.  $2011b$ ). The ZRE constitutes up to 50 % of the total egg volume (Mansueti 1964; Korzelecka et al. 1998).

## *6.2.2 Molecular Composition of the Ova*

Except for gas and few molecules, fish embryos in the egg don't have any exchange of material with the outside. Therefore, the process of incorporation of molecules in the oocyte during the oogenesis is extremely important since its correctness ensures the proper development and survival of the offspring. On the contrary, bad incorporation/expression of particular molecules can have a huge negative impact during the embryonic and larval development (mortality, abnormalities occurrence depending on the cellular function which is affected). These molecules are involved in three main cellular functions: (i) the energetic reserves for the embryo (glycogen, lipids, free

amino acids and Vitellogenins), (ii) the cellular process and the structure in the embryos (proteins, free amino acids, lipids, transcripts and several ions), and (iii) molecules necessary to keep the osmotic balance in the egg (water, free amino acids).

 In percids, the biochemical composition of ova has been studied in some targeted species. No large scale studies have been performed to investigate their transcriptome, proteome, glycogen or free amino acid composition. Most studies focused on lipids composition of ova. The most abundant lipids are neutral lipids (wax ester, triacylglycerols, cholesterol and free fatty acids (around 85 % of the total lipid content)) while the polar lipids (phosphoglycerlipids, sphingolipids) correspond to around 15  $\%$  of the total lipid content (Henrotte et al. 2010). Moreover the most abundant fatty acids are n-3 polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) while the saturated fatty acids (SFA) and the n-6 PUFA are less represented in *P. fluviatilis* (Blanchard et al. 2005; Żarski et al. 2012c; Henrotte et al. 2010). Moreover, it has been shown that the most represented fatty acids are not only docohexaenoic acid (DHA) but also 16:1 and 18:1 fatty acids. These last types of lipids have never been studied in ova so far. On the contrary, eicosapentaenoic acid (EPA) and arachidonic acid (AA) were poorly represented in eggs of wild Eurasian perch ( $\overline{Z}$ arski et al. 2012c). In the walleye n-3 PUFA is the most abundant fatty acid in ova followed by SFA and MUFA while n-6 PUFA are poorly represented (Moodie et al. [1989](#page-29-0) ). In the Eurasian perch, it has been shown that the diet of females can affect the fatty acid proportions in the ova and may be linked to the reproductive success (Henrotte et al. 2010). Percid ova are characterized by the presence of an oil globule in addition to the yolk. Moodie et al. [\( 1989](#page-29-0) ) have shown in the walleye that while the yolk is composed by lipoproteins and polar lipids (e.g. phospahtidylcholine and phosphatidylethanolamine), the oil globule are composed by neutral lipids, mainly triacylglycérols (TAG). In addition, the fatty acid composition of each compartment is different as the yolk is mainly composed by SFA and n-3 PUFA. The neutral lipids of the oil droplet are composed by n-3 PUFA and MUFA. It has been suggested that the polar lipids of the yolk are used before the neutral lipids of the oil globule during the embryonic development, mostly as energetic reserves (Wiegand 1996).

 Molecules necessary to keep the osmotic balance in the egg are usually important for marine species which eggs undergo a huge hydration just prior to ovulation. This mechanism has poorly been described in freshwater fish species such as percids. A recent study investigated the hydration level during *P. fluviatilis* oocyte maturation and showed an increase of around 4 % of water intake during this process. Up to now, this data corresponds to the fewer hydration process ever described  $(Zarski et al. 2012c)$  $(Zarski et al. 2012c)$  $(Zarski et al. 2012c)$ . Moreover, recent studies indicated that some populations of Eurasian perch *Perca fluviatilis* could spawn in brackish or even salty water (Tibblin et al. [2012 ;](#page-30-0) Skovring et al. [2013](#page-29-0) ), suggesting an adaptation of perch eggs to various salinity conditions. Same data have previously been observed in ruffe *Gymnocephalus cernua* (Vetemaa and Saat 1996). It could be interesting to investigate more carefully the potential hydration process in freshwater fish as percids.

# **6.3 Embryonic and Larval Development**

## *6.3.1 Embryonic Development*

 Fish embryonic development is exposed to several extrinsic and intrinsic factors that can affect the speed of the embryogenesis and consequently leads to asynchronous development within and between spawn. Among these factors the temperature of incubation is probably the most important and several studies take into account this parameter by using the degree day parameter (number of days elapsed since the fertilization at a given temperature ( $°C$ )). However, this classification has mainly two important drawbacks. First, it doesn't take into account other parameters that could modulate fish embryogenesis such as the water flow, oxygenation or the water composition, and second it doesn't allow any easy comparison of the embryonic development between fish species. In the current scientific context requiring more careful studies of embryonic stages (corresponding to the mortality or deformities apparition stages) or the comparison of the embryonic development of several species, it becomes thus more accurate to characterize fish embryogenesis through morphological criteria.

Fish embryogenesis elapses from the ova activation (described in the Sect. [6.2.1](#page-1-0)) to the first oral feeding which are easily observable and stable stages. The choice of the first oral feeding as the end of embryogenesis instead of the hatching period is done because hatching may occur at different embryonic stages depending on the fish species. Moreover, within a species, the total length of the embryos at hatching may be different depending on the environmental conditions and, to some extent, the size of the female being positively correlated with the size of the egg. It suggests that the hatching stage is not fixed even within a spawn. This has been shown in *Perca fluviatilis* for which embryos can hatch with size ranging from 4 to 9 mm (Konstatinov [1957 \)](#page-28-0) and with different eye pigmentations (M. Alix and B. Schaerlinger personal observations). Similar data have been observed in *Perca flavescens* (Mansueti  $1964$ ). On the other hand, the first oral feeding corresponds to the end of the period in the course of which the embryo depends only on its own reserves. Once the animals begin to eat, they enter into the larval stages (see Sect.  $6.3.2$ ) until they have the same morphology than the adults (juvenile stages). The embryonic development can be divided into five main steps (cell cleavage, gastrulation, organogenesis, hatching and the free embryo period). Even if several differences can be observed among fish species, these main periods of embryonic development chronologically occur in the same manner.

 Up to now, only few works carefully examined percids embryonic staging. As those studies were conducted with different aims, they pointed on diverse stages of the embryonic development. Most of them have been performed on North American species as the fantail (*Etheostoma flabellare*), rainbow (*Etheostoma caeruleum*) and banded darters (*Etheostoma zonale*) (Cooper [1979](#page-26-0); Paine 1984; Paine and Balon [1984a](#page-29-0); Mendelson et al. [2006](#page-28-0)), the American yellow perch (*Perca flavescens*) (Mansueti 1964), the logperch (*Percina caprodes*) (Paine and Balon 1984b; Cooper

[1978 \)](#page-26-0) and the walleye ( *Stizostedion vitreum* ) (Mc Elmann and Balon [1979](#page-28-0) ). These fish represent *Etheostoma*, *Perca*, *Percina* and *Sander* genders, respectively. Others have been done on European species as the ruffe ( *Gymnocephalus cernuus* ), Balon's ruffe (*Gymnocephalus baloni*) and the yellow pope (*Gymnocephalus schraester*) representing the *Gymnocephalus* gender (Kovac [1992](#page-28-0); Kovac 1993a, [b](#page-28-0), 1994) and the Danube streber (*Zingel streber*) for the *Zingel* gender (Kovac 2000). The reproduction traits of several of these species are given in the Table [6.1](#page-7-0) . As a whole, the total duration of the embryonic development of those percid species is highly variable from activation to the first feeding (Figs.  $6.3$  and  $6.4$ ). A compared analysis of each step allows the determination of common features and differences at each step.

#### **6.3.1.1 Cell Cleavage Stages**

 After the entry of the spermatozoa in the ova, the yolk, that was previously uniformly distributed, begins to segregate in the vegetative pole while the cytoplasm segregates in the animal pole  $(Fig. 6.3)$  $(Fig. 6.3)$  $(Fig. 6.3)$ . It corresponds to the first embryonic cell called the zygote period. Thereafter begins a series of cell cleavages at the animal pole to obtain a multicellular embryo called blastula. In percids, the duration of this period can last from 12 h (for the streber) to 30 h (for the yellow perch) (Fig. [6.4](#page-9-0) , Table 6.2) suggesting that the regulation of the speed of cell division is different between species. This phenomenon is interesting in regard to the fact that both species are those presenting the longest embryonic development among percids (Mansueti [1964](#page-28-0); Kovac 2000) (Table [6.2](#page-11-0)). The cell cleavage period can be subdivided into two phases: first cell divisions are synchronous while in the second phase, corresponding to the beginning of the blastula period, the cell divisions become asynchronous. Finally, the cell cleavage is characterized by the midblastula transition (MBT) that corresponds to a switching of genes expression from the maternal genes to the zygotic genes (expression of the embryonic genes). The timing of the MBT has not been determined in any studied percids species but it may correspond to the middle end of the cleavage period such as in *Brachydanio rerio* (Kimmel et al. 1994).

#### **6.3.1.2 Gastrulation Process**

 The gastrulation phase corresponds to a series of cell migrations called epiboly (Fig. [6.3 \)](#page-8-0), and allowing the cell distribution around the vitelline reserves. During that step, the cells migrate from the animal pole to the vegetative pole. The staging is indicated as a percentage of epiboly corresponding to the proportion of yolk surrounded. One key step corresponds to the germ ring step. During that step, the epiboly stops and an involution of the unique cell layer occurs to form two cell layers: (i) the epiblast (future ectoderm mainly precursor of the epiderm and the nervous system), (ii) the hypoblast (precursor of the mesoderm that will lead to the circulation system, muscles, bones and most of the internal organs and the endoderm, precursor

<span id="page-7-0"></span>

Table 6.1 Summary of the breeders and reproduction conditions of representative percid fish **Table 6.1** Summary of the breeders and reproduction conditions of representative percid fish

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**Fig. 6.3** Embryonic developmental table of the walleye (*S. vitreus*). (a) ova before activation; (b, **c** ) cell cleavage; ( **b** ) 2 cells step; ( **c** ) blastula step; ( **d** ) gastrulation steps, the orientation of the embryos varies while the cell movements process;  $(e-i)$  organogenesis;  $(e)$  beginning of the anteroposterior axis formation; (**f**) somites formation; (**g**) the tail is detached from the yolk, the somitogenesis proceeds to elongate the embryo, the eyes are developed and the otic vesicle is visible; ( **h** ) circulation system development ( **i** ) embryo at hatching. *AP* animal pole, *VP* Vegetative pole, *A* anterior, *D* dorsal, *P* posterior, *V* ventral (Drawings from Mc Elman and Balon [1979 \)](#page-28-0)

of the digestive system and its derivatives). Moreover, rapidly a cell accumulation appears on one side of the embryo called the embryonic shield. It marks the dorsal part of the embryos and is the first manifestation of the dorso-ventral axis.

 As for the cell cleavage, the gastrulation total duration is diverse among percids from 6 h for the rainbow darter to 23 h for the streber (Paine and Balon 1984a; Kovac [2000](#page-28-0)) (Fig. [6.4](#page-9-0), Table [6.2](#page-11-0)). The germ ring and embryonic shield formation occurs early at 25 % of epiboly in *Etheostoma* species, the timing seems to be important as premature or delayed involution may lead to lethality (Mendelson et al. 2006). This has not been described in any other percid, probably because the authors didn't pay attention on that particular point.

 In several percid species, due to the presence of the oil droplet and the cellular migration, the gastrulation may be accompanied by a rotation of the embryos  $(Fig. 6.3)$ . Indeed, while the first part of the epiboly occurs in the horizontal plane, from the stage corresponding to the 50  $%$  of epiboly, the embryo rotates by 90 $^{\circ}$  so the oil droplet protrudes and the embryonic shield takes a different position. This has been described in the walleye (Mc Elmann and Balon [1979](#page-28-0)), the northern log-perch (Paine and Balon [1984b](#page-29-0)) but not in darters (Paine and Balon 1984a; Mendelson et al. [2006](#page-28-0) ) or in the *Gymnocephalus* gender (Kovac [1994 \)](#page-28-0) (Fig. [6.4](#page-9-0) ). For other percids, the gastrulation observation was not described clearly enough to know whether this rotation occurs or not. It may be due to a difference of cell migration speed between the dorsal and ventral part of the embryos as proposed in other fish species embryos (Kimmel et al. [1994](#page-27-0)). As a consequence, the blastopore doesn't

<span id="page-9-0"></span>

 **Fig. 6.4** Comparison of the embryonic development of representative species of each percid gender *blue* E. *caerulum* (Paine 1984; Cooper 1979; Medelson et al. 2006; Paine and Balon 1984) (20 °C), *orange* G. *schraester* (Kovac [1992](#page-28-0) ) (16–20 °C), *black* P. *fl avescens* (Mansueti [1964 \)](#page-28-0) (6.7 °C, mean temperature at spawning), *green* P. *caprodes semifasciata* (Paine and Balon [1984](#page-29-0) ; Cooper [1978](#page-26-0) ) (20 °C), *red* S. *vitreus* (McElman and Balon [1979](#page-28-0) ) (9–14 °C) and *purple* Z. *streber* (Kovac ) (12–17 °C). The time scale of each step (cell cleavage, gastrulation, organogenesis, hatching, free embryo and larval) are represented in bold lines when the stage is properly documented in term of time or dotted lines when the stage is not well determined. The time scale <span id="page-10-0"></span>close at the vegetative pole but rather ventrally in these fish. Surprisingly, species undergoing early germ ring and embryonic shield apparition are not those presenting the early rotation while the two processes may probably be linked. One can thus conclude that data describing the gastrulation in percids are not precise enough because each author focused on diverse staging and didn't pay enough attention on others to properly understand these processes and thus make a general rule.

#### **6.3.1.3 Organogenesis**

This step can last from 5 days for *Percina caprodes* to 23 days for *Perca flavescens* (Mansueti 1964; Paine and Balon [1984b](#page-29-0)) (Fig.  $6.4$ , Table  $6.1$ ). During this step, tissues will begin to differentiate and most of organs will progressively appear. This process continues during the free embryos and larval stages until the animal become a juvenile.

 After the complete covering of the yolk at the end of the gastrulation, embryos begin to elongate and grow. Both antero-posterior and dorso-ventral axes become obvious. In addition, the Kupfer vesicles appears. It is a transient spherical ciliated organ specific to teleosts fish which seems to be involved in the left-right determination of the heart, brain and gut (Essner et al. 2005). The organogenesis subdivision can be staged thanks to the number of somites (repetitive units appearing sequentially from the anterior part of the trunk to the end of the tail). Moreover, the first cell differentiation occur leading to the organogenesis with the apparition of the neural plate giving rise to the future central nervous system. Interestingly, for the walleye and the rainbow darter the first step leading to the formation of the neural plate occurs before the blastopore closure thus showing that the first organogenesis manifestation is earlier in comparison to other fish species. As during the gastrulation, an embryonic rotation is observed in several species (walleye, logperch). This step is called translocation and is characterized by the decrease of the distance between the head of the embryo and the oil globule through embryonic shifts (Mc Elmann and Balon [1979](#page-28-0)). This phenomenon has not been observed in other percid species.

 As a whole, the chronology of the organ differentiation and development is the same in every species. However, some organs finish their development during the hatching period, free embryos and larval stages for some species while for others, their development is complete before hatching. One example is the gaping of the mouth that takes place early during organogenesis in *Perca flavescens* while it takes place later in other species (Mansueti 1964) (Fig. [6.4](#page-9-0)). Several organs easily observable with a binocular loupe begin to develop (e.g. the optic, otic and olfactory precursors, the heart beating, the melanophores apparition in the yolk and later on the

**Fig. 6.4** (continued) is presented as hours and days elapsed from the activation without taking into account the temperature. General features commonly studied in papers are presented below the time scale for each species as indicated in the legend. The larval stages are not complete due to the lack of information for some species

Scientific name	Common name	Embryonic incubation temperature $(^{\circ}C)$	Larval incubation temperature $(^{\circ}C)$	Egg size (mm)	Total embryonic duration
Etheostoma caeruleum	Rainbow darter	20	20	$1.9 - 2.2$	Around 12 days
Gymnocephalus schraetser	Yellow pope	$16 - 20$	$16 - 20$	1.22	Around 7.5 days
Perca flavescens	Yellow perch	$10 - 22$	$10 - 22$	1.6	29 days
Percina caprodes	Logperch	20	20	1.23	Around 8 days
Stizostedion vitreum	Walleye	15	15	1.85	$14.5$ days
Zingel streber	Danube streber	$12 - 17$	$15 - 17$	1.94	Around 24 days

<span id="page-11-0"></span>Table 6.2 Summary of the developmental characteristics of representative percid fish





*ND* non determined

embryonic body and the eyes pigmentation (eyed-stage)). The ability of embryo to reach these steps should be important to follow and thus predict the apparition of deformities or mortality occurrence. Indeed, up to now, the most studied developmental rates are checked at 72 h post-fertilization or eyed stages (with few other steps as shown in the Sect.  $(6.3.3.1.1)$  $(6.3.3.1.1)$  $(6.3.3.1.1)$  in works studying the reproductive performance quality. Investigating parameters cited above may allow the identification of developmental failures within this interval in order to be more precise.

#### **6.3.1.4 The Hatching Period**

 During the organogenesis, several granules called the hatching glands appear. They contain hatching enzymes that are released before hatching and weakens the chorion and/or the *zonula radiata externa* (Kimmel et al. [1994](#page-27-0) ). Firstly, the development duration within the envelope is highly variable among species. For example, the comparison between *Zingel streber* and *Perca flavescens* shows that while the total duration of the embryonic development (from activation to the first feeding) is quite the same (around  $23-29$  days), the yellow perch hatches after a long period in the envelope (around 23 days), while the streber development in the envelope is very short (around 12 days) (Mansueti [1964](#page-28-0) ; Kovac [2000](#page-28-0) ) (Fig. [6.4 ,](#page-9-0) Table [6.1](#page-7-0) ). By comparing the embryonic morphology of both species, there are mainly differences of the yolk sac utilization. Indeed, authors of both studies didn't pay attention to the same criteria to describe embryos. As proposed by Mansueti (1964), this difference can mainly be due to the spawning mode. The yellow perch eggs are surrounded by a thick jelly coat (as for the Eurasian perch *Perca fluviatilis*) protecting the embryos from the outside aggression and thus allowing a longer period of development in the envelope. In contrast, other percid species eggs are solely protected by a tough chorion and are either buried in the substrate or dermersal (Table [6.1 \)](#page-7-0). In those cases the developmental phase within the envelope is shorter, potentially avoiding predatory behaviors from other species. From a developmental point of view, the staging of embryos is diverse between species mostly thanks to the skeletal, branchial, intestinal and fins developments. For example, while some individuals hatch with a quite well developed mouth with teeth or fins allowing movements in every direction, others acquire these abilities during the free embryonic stages (Fig. [6.4 \)](#page-9-0).

 Interestingly, depending on the species, the hatching duration lasts from several hours to 5 days for the same spawn suggesting that even within a species the developmental stages can be variable. For most of percids, authors observed differences in the embryos total length at hatching and sometimes differences in the circulation or skeletal systems advancement. In *Perca fluviatilis*, Konstantinov characterized four developmental stages at hatching (Konstantinov [1957](#page-28-0) ) that differ in term of developmental advancement from those of *Perca flavescens* (Mansueti 1964). Criteria to distinguish each stage are mainly the yolk absorption level and the total length. However, other criteria as the eye development should be established in the future to better characterize these stages (M. Alix and B. Schaerlinger, personal observation). This difference of hatching development may confer an advantage for a given species because the dispersion of the size of free embryos allows a better

adaptation to the environment. Indeed, embryos of different sizes can, on the one hand, easily avoid predators and, on the other hand, take advantage of preys from diverse species and sizes. So, even if several embryos die during this phase, others may pass through this step, allowing thus the recruitment success of the species whatever the environmental conditions.

### **6.3.1.5 Free Embryos Stages**

Mainly this step allows the preparation of embryos to ensure an efficient transition to the larval stages (Fig.  $6.3$ ). Indeed, the free embryos phase corresponds to a period of final development of the intestinal, branchial, fins and circulatory systems which become progressively active (Fig.  $6.4$ ). The vitelline reserves have greatly reduced and the circulation system begins to be deviated from the yolk to the gut and branchial systems allowing a new way of oxygenation and alimentation of the body. Several studies shows in percid species a high rate of mortality at that stage that can mainly be due to an impairment of this circulation deviation. Moreover, the embryos continue to grow except for the logperch (Paine and Balon 1984b).

## *6.3.2 Larval Development*

 The onset of the larval development is controversial as some scientists rather use the hatching period, others the first oral feeding or the yolk resorption (Teletchea and Fontaine 2011). As explained above, we choose to determine the first oral feeding as the onset of the larval development. Indeed, hatching and yolk resorption can be dependent upon spawn characteristics or incubation conditions. They thus can't correspond to a developmental step. On the contrary, the first oral feeding elapses for a small period of the development and may probably correspond to a specific stage of maturation of the intestine.

This part of the chapter used mainly data from *Etheostoma*, *Percina*, *Gymnocephalus*, *Sander* and *Zingel* genders (Paine and Balon [1984a](#page-29-0), [b](#page-29-0); McElmann and Balon [1979](#page-28-0); Kovac [1993b](#page-28-0), [2000](#page-28-0)). However as for the embryonic stages, the larval development of each species has not been studied with the same purposes and don't focused on the same characteristics. The larval development can be divided into two large steps, the finfold phase and the finformed phases (Kovac [2000](#page-28-0)). Each of these stages could be further cut into several steps but we choose to present only the most important characteristics of each phase.

#### **6.3.2.1 The Finfold Phase**

This stage begins with the first feeding, but the larvae still need yolk supply, principally lipids from the oil globule. In the walleye, it has been shown that the oil globule fastly decreases during this period (McElman and Balon [1979](#page-28-0) ). In the meantime, the stomach and the intestine develop and replace the yolk in the ventral space.

Moreover, this phenomenon is accompanied by the final reduction of the vitelline circulation and a definitive switch toward the intestine circulation (Paine and Balon  $1984b$ ; Kovac  $2000$ ). Once the oil globule is entirely used, larvae depend exclusively upon exogenous food. As for the free embryonic stage, this period of mixed feeding may allow larvae to adapt their digestive physiology to the exogenous food. The time elapsing from the first oral feeding to the first defecation can be quite long, suggesting a progressive starter of the digestive function (e.g. in the walleye the first oral feeding takes place 15 days post fertilization in the walleye and the first defeca-tion was observed 2 days later which is quiet long (McElman and Balon [1979](#page-28-0))). Moreover, temperate fish larvae need to have a developmental synchronization with the preys availability in the nature. The transition period could be involved in this synchronization. Another important characteristic of this stage is the filling of the swim bladder allowing the control of buoyancy. This step is usually concomitant with the onset of oral feeding and it corresponds to an important key step during the larval development. Indeed, yellow perch larvae with uninflated swim bladders display lower survival rate than those with an inflated one due to a lack of preying efficiency (Czesny et al.  $2005<sub>b</sub>$ ). The respiration function is mediated by gills that are fully protected by the operculum and larvae display regular mouth movements demonstrating active breathing in every species. The finfold stage is also the time for the beginning of cartilage and bone formation. It begins with a progressive chondrification and ossification of the head and the body axis with the formation of vertebrae (Paine and Balon [1984b](#page-29-0); Kovac 2000; McElman and Balon 1979). Moreover first teeth begin to appear. Finally, the body pigmentation increases in the head and along the ventral side of the body axis.

#### **6.3.2.2 The Finformed Phase**

While during the first larval stage animals are mainly surrounded by the finfold, the second step of the development is characterized by the differentiation of fins. The finfold degenerates all around the larvae except in the part corresponding to the emplacement of fins in adults (anal, caudal, dorsal and pelvic). Moreover, rays begin to differentiate in every fins before undergoing an ossification (Paine and Balon [1984b](#page-29-0); Kovac 2000; McElman and Balon 1979). Finally, the body pigmentation is almost complete and larvae can present colour diversity. At the end of the step, larvae look like adults but sexually immature and thus become juveniles.

# **6.4 Evaluation of Egg and Larval Quality**

# *6.4.1 What Are the Egg Quality Indicators?*

 Prediction of the egg quality in aquaculture is one of the most important and challenging step of controlled reproduction. Objective indicators allow not only facilitating the commercial fish production (by choosing only the highest quality eggs for further steps of <span id="page-15-0"></span>reproduction) but also more efficient and faster development of the reproductive protocols through the research activity. Some indicators can be predictive (measured before fertilization) and characterize principally ova quality while other are evaluated after the fertilization (reproductive performance) and characterize ova quality in addition to embryonic and larval quality. Predictive indicators are often species specific and it can thus be difficult to find relevant ones for each species. However, assessing reproductive performance is very relevant but not predictive leading sometimes to long and expensive studies. On another level, indicators can be divided into morphological (Brooks et al. [1997](#page-26-0)) or biochemical indicators. Morphological are easy to evaluate but have to be determined for every species. Biochemical indicators can need access to specific equipments but have the advantage to lean on general molecular mechanisms the deregulation of which would affect the developmental success of every fish species. Moreover, even if the genome of a fish species is unknown (which is the case in most percid species), biochemical assessment of the quality could be performed with few technical adjustments. Finally, molecular indicators may mainly be investigated on ova and would thus be predictive. Performing the characterization of developmental failure could probably be important to define levels of quality which in turn would help to choose the most relevant quality indicator. This approach could thus help scientist and fish breeders to improve their assessment of reproductive success and finally their breeding practices.

# *6.4.2 Description of Developmental Defects*

## **6.4.2.1 Occurrence of Mortality During Embryonic and Larval Stages**

 Reproduction failures are often due to lethality occurrence among embryos and larvae. Sometimes, when rearing conditions are well established in captive conditions, the survival rate is higher than in the nature as shown for the walleye (Ivan et al. [2010 \)](#page-27-0). However, when the rearing conditions are not well controlled, mortality occurrence is very high in hatcheries. Lethality stages have poorly been studied in fish and almost no work has been performed on percids. However, this question needs to be addressed as the stage and the phenotype of mortality could reveal clues to understand rearing conditions problems. As a whole in the Eurasian perch several steps of mortality has been observed. The first one takes place during the first 24 h probably corresponding to cleavage defects and/or zygotic genome activation (MBT) impairment (M. Alix and B. Schaerlinger unpublished data). Moreover other lethality stages have been observed by the time of hatching or first feeding. It may be interesting to better characterize mortality occurrences in order to define general phenotypes and thus improving rearing conditions to avoid lethality. As proposed in the Sect. [6.3.1.3](#page-10-0) , assessing the proper developmental advancement by checking several specific stages within the interval between the fertilization and the eyed-stage should help to define other key steps of the embryonic susceptibility.

#### **6.4.2.2 Occurrence of Developmental Abnormalities**

 Developmental abnormalities are a very unwanted element of aquaculture production. The high rate of abnormal embryos and larvae leads to a reduced number of fry which could be intended for further culture purposes. Abnormal development in fish embryos and, consequently, freshly-hatched larvae, may be caused by several factors. Among the environmental factors, temperature and salinity have been observed to affect embryonic development negatively when they reach values exceeding the optimal range for a particular species (e.g., Bermudes and Ritar [1999](#page-26-0); Haddy and Pankhurst 2000; Kupren et al. 2011). In addition, it was proven that improper broodstock management (including diet composition) and/or reproductive procedure (including photothermal manipulations and hormonal treatment) were responsible for developmental abnormalities in embryos and freshly-hatched larvae (e.g., Kjørsvik et al. 1990; Aegerter and Jalabert [2004](#page-26-0); Bonnet et al. [2007](#page-26-0); Palińska et al. [2011](#page-29-0); Żarski et al. [2011a](#page-30-0)). Additionally, toxic substances, including heavy metals (e.g., Von Westernhagen et al. 1988; Jezierska et al. 2000; Ługowska [2007](#page-28-0); Ługowska and Kubik [2011](#page-28-0)), present in the water during incubation as well as in the natural environment of the spawners (Black et al. 1988; Cameron et al. [1992](#page-26-0); Von Westernhagen et al. 1988; Singh et al. 2008) were found to affect embryonic development and induce larval deformations.

 In the case of freshwater percids, data on deformations in newly-hatched free embryos are very limited. It has recently been reported that the procedure of artificial reproduction may affect deformations in freshly-hatched free embryos, where the most common scoliosis and lordosis of different parts of the spine, yolk sac malformation, yolk sac oedema, gape jaw and cardiac oedema were found  $(Zarski et al. 2011a)$  $(Zarski et al. 2011a)$  $(Zarski et al. 2011a)$ . However, in other fish species, many other deformations have been found, such as kyphosis, axial curvature in the abdominal and caudal region, severe spine curved-in axial and caudal region, C-shaped larva, pigmentdeficient eye, deformed skull, body shortened (for details see: Jezierska et al.  $2000$ ; Ługowska  $2007$ ; Palińska et al.  $2011$ ) (Figs. [6.5](#page-17-0) and 6.6). Each particular deformation may occur alone or together with other deformations (Jezierska et al. [2000](#page-27-0); Ługowska 2007; Palińska et al. [2011](#page-29-0); Żarski et al. [2012a](#page-30-0)). In Eurasian perch, deformation occurrence and frequency were found to be strictly related with the quality of eggs and ranged between 6.36  $%$  and 86.14  $%$  in the highest and lowest egg quality, respectively ( $\overline{Z}$ arski et al.  $2011a$ ). However, such a high deformation rate was probably a result of an artificial reproduction protocol, while deformation in perch spawned naturally in the natural environment did not exceed 1.78 % (Treasurer [1983 \)](#page-30-0).

 It has already been reported that larval deformations in pikeperch may be affected by the weaning protocol (from live to compound diet) and feeding regime. In such cases, the retraction of upper and lower jaws as well as scoliosis were described (Kestemont et al.  $2007$ ). Kowalska et al.  $(2006)$  reported mainly lordosis, whereas Hamza et al. (2008) recorded mainly kyphosis and lordosis. Authors very rarely

<span id="page-17-0"></span>

Fig. 6.5 Pikeperch, *Sander lucioperca*, larvae with normal shape (a) and with the most common morphological deformations ( **b-d)** . ( **b** ) spinal lordosis, ( **c** ) spinal lordosis and cardiac oedema, ( **d** ) cardiac oedema. *Arrows* indicate cardiac oedema. The *Scale bar* represents 500 μm (Pictures of D. Zarski)

<span id="page-18-0"></span>

**Fig. 6.6** Eurasian perch, *Perca fluviatilis*, with normal shape (a) and different type of deformations (**b–e**). (**b**) larva with several deformations (cardiac and yolk sac oedema, lordosis, jaw malformation), (c) larva with jaw malformation and tail deterioration, (d) larva with several oil droplets in the 'yolk-sac area', (e) 'c-shaped' larva. The *scale bar* represents 500 μm (Pictures of D. Zarski)

<span id="page-19-0"></span>noted scoliosis or incomplete jaw development, as compared to the data described by Kestemont et al. ( [2007 \)](#page-27-0). The frequency of total deformations reported by those authors ranged between 15.5 % and 35.5 % (Kestemont et al. 2007), 9.5–16.5 % (Kowalska et al. [2006 \)](#page-28-0) and 7.5–12.5 % (Hamza et al. [2008](#page-27-0) ).

## *6.4.3 Defi nition of Quality Indicators*

## **6.4.3.1 Egg Quality**

#### 6.4.3.1.1 Morphological Indicators

 In freshwater percids, the most common practice of egg quality evaluation was the determination of embryo survival rate shortly after fertilization (Migaud et al. [2004](#page-28-0) ,  $2006$ ;  $\overline{Z}$ arski et al.  $2011a$ , at the eyed-egg stage (Czesny and Dabrowski 1998; Kucharczyk et al. 1998; Czesny et al. [2005a](#page-26-0); Żarski et al. 2011b) or at hatching (Migaud et al.  $2006$ ). Egg diameter has also been reported to be correlated with embryo viability in the walleye (Malison et al. [1998](#page-28-0)). A simple general morphological examination was also very helpful in the evaluation of fish egg quality. Partially or totally opaque eggs or visible internal damage of the yolk structure were also usually reliable indicators of poor quality (e.g. Treasurer [1983](#page-30-0); Pavlov and Emel'yanova [2008](#page-29-0); Teletchea and Fontaine [2011](#page-30-0)).

 In the case of Eurasian and yellow perch, a very simple method of indication of low egg quality was egg-ribbon fragmentation (Dabrowski et al. [1994](#page-26-0); Overton et al. [2008](#page-29-0) ). Recently, it was reported that fragmentation of oil droplets in ovulated eggs of the Eurasian perch may be a useful tool for its quality evaluation, with more than one big oil droplet in an ovulated egg proven to be an indicator of decreased quality (Żarski et al. 2011a). However, this method may be used only when "dry" eggs (before contact with water) are evaluated, while many lipid droplets may coalesce into one large droplet after the contact of eggs with water. A similar morphological indicator of oil droplets in ovulated eggs was certified in pikeperch (Fig.  $6.7$ ). In the egg batches where oil droplet was found to be fragmented  $(n=7)$  the embryonic survival rate (at 72 h post fertilization) and hatching rate did not exceed 49 % and 20 %, respectively. Among the hatched larvae over 94 % of different types of deformities were recorded (D. Żarski unpublished data). However, this phenomenon has only been observed sporadically in this species. A much more reliable egg quality indicator of pikeperch eggs was the observation and determination of egg deformation (extreme chorion deformation) rate shortly (3–5 min) after egg activation in the water. This method is based on observations of the cortical reaction which, during the first few minutes, occurs very violently in eggs of this species and is an indicator of high egg quality. This phenomenon was clear and easily noticeable ( $\overline{\text{Zarski}}$  et al.  $2012b$ ). For the walleye, the turbidity of ovarian fluid (measured spectrophotometrically) caused by the increased amount of protein after short-term storage (8 h) of the eggs was negatively correlated with egg quality at the end of the spawning season (Dietrich et al. 2012). However, according

<span id="page-20-0"></span>

 **Fig. 6.7** Eggs of pikeperch, *Sander lucioperca* , with fragmented oil droplets. Eggs before activation ( **a** ) and during the early development ( **b** ). The *scale bar* represents 500 μm (Pictures of D. Zarski)

to the authors, this method seems to be an indicator of the changing proportion of over-ripened and/or broken eggs rather than egg quality itself. Schrader and Schrader  $(1922)$  reported that abnormal blastomere morphology (during the first cell divisions shortly after fertilization) were very good indicators of low egg quality in the walleye. Similar observations were made for other fish species, such as Atlantic cod, *Gadus morhua* L. (Hansen and Puvanendran [2010](#page-27-0)). The data on other non-biochemical egg quality indicators in freshwater percids is very scarce and more work is still needed in this field.

## 6.4.3.1.2 Biochemical Indicators

 Few works investigated potential biochemical indicators of egg quality that are mainly predictive. Some studies focused on the relationship between the fatty acid composition of the Eurasian perch eggs and their reproductive performance. However, the comparison of these data shows that conclusions cannot be generalized. Indeed, while Henrotte et al.  $(2008)$  show that the n-3/n-6 ratio above 2.5 in the egg could be an indicator of low quality gametes, data obtained by Blanchard et al.  $(2005)$  (ratio ranging from 2.9 to 3.5 in wild populations) contradict this hypothesis. In the same manner, the DHA/EPA ratio within the egg doesn't seem to be a reproducible egg quality indicator. Indeed, it can range from 3.5 (Abi-Ayab et al.  $2000$ ) to 8.4 (Henrotte et al.  $2010$ ) for good quality eggs. These data suggest that even if the yolk composition is crucial for the proper development of the embryo, other parameters should be studied in the meantime to predict ova quality. One of these parameters could be the yolk utilization during the embryogenesis. Another study performed on the same species showed that high activity of the Cathepsin L (enzyme involved in the Vitellogenin proteolysis during the embryonic and larval development) just before hatching is negatively linked to the hatching rate or the embryonic resistance to osmotic stress (Kestemont et al. [1999](#page-27-0) ).

The proteomic profile of *Perca fluviatilis* ova has been investigated on spawn of various qualities that have been estimated thanks to the study of reproductive performance (Castets et al.  $2012$ ). Data showed that proteins involved in cell protection against stress, protein degradation, Vitellogenins or cell metabolism display various expression level depending on the ova quality. They thus could be promising indicators of ova quality.

 Addressing the question of the molecular composition of the ova to predict its quality is important because it will give clues to find good predictive indicators that could be used for several species. Indeed, the affected cellular functions are, in general, conserved among fish species and data obtained in one percid species could be applied to others. This research should first be done at a large scale (e.g. transcriptomic, proteomic) but it is also important to confirm results with smaller scale techniques (e.g. western blotting, RT-PCR…) to choose the better indicators. Moreover, it appears that assessing gametes quality through molecular techniques would rather need to investigate the level/ratio of diverse molecules and the combination of all these data should give an indication of the quality rather than investigating only one molecule expression. This field of research deserves to be carefully studied.

## **6.4.3.2 Free Embryo and Larval Quality**

 Evaluation of egg quality on the basis of the survival rate of embryos (e.g. to the eyed-egg stage) was not always a reliable indicator of the effectiveness of reproductive procedures or induction of maturation processes, while the development of embryos in eggs of low quality could be observed even up to the hatching stage (e.g. Zarski et al.  $2011a$ , leading to the need for an evaluation of larval quality.

Evaluation of the larval quality in fish was mostly based on the determination of survival rate at particular life stages (Dąbrowski et al. [2000 \)](#page-26-0). Initially, the ability of free embryos to hatch was indicated as a reliable indicator of its quality (e.g., Bobe and Labbe [2010](#page-26-0) ) as it was suspected that mainly properly-developed larvae and/or larvae with appropriate energy content were able to get out of the egg shell. However, it was proven that even embryos with developmental malformations were able to hatch successfully (Ługowska and Sarnowski 2011; Zarski et al.  $2012a$ ). Next, the deformation rate of freshly-hatched embryos was used as an indicator of quality. But hatched embryos without evident deformities could still be of variable quality, which may be reflected in a failure of inflation of the swim bladder or the start of exogenous feeding (which were also considered to be quality indicators in percids) (Dąbrowski et al. [2000](#page-26-0); Żarski et al. 2011a). Recently, a test of salinity stress was developed and applied for evaluation of percid hatched embryos quality. This method indicated their ability to perform osmoregulation and energy mobilization to counteract stress (Kestemont et al. 2007). This method is very simple to use and seems to give a reliable indication of their quality (Kestemont et al. [1999](#page-27-0); Henrotte et al. [2010](#page-27-0)) for both research and aquacultural purposes.

# **6.5 Improvement of Egg and Larval Quality**

 Reproduction success depends upon breeders rearing conditions on the one hand, and egg and larval incubation environment on the other hand. The control of these conditions needs to be understood for every species in order to improve zootechnical practices and reproduction success in captive breeding. This part will mainly focus on data obtained for *Perca* and *Sander* genders.

# *6.5.1 Broodstock Management*

 Fish life cycle depends upon two main categories of factors: (i) determining fac-tors and (ii) modulating factors (Wang et al. [2010](#page-30-0)). Determining factors seem to be involved in triggering key steps of the gametogenesis (e.g. vitellogenesis, ovulation). Identifying and controlling determining factors will allow the establishment of environmental programs to perform out-of-season reproduction. However, even if determining factors are well controlled, the reproduction success remains unstable in recirculating aquaculture system(s) (RAS). Modulating factors are able to do fine-tuning of reproduction conditions. They correspond to parameters as the stress of manipulations, nutrition, intrinsic characteristics of the fish population or the individual breeders or hormonal injections. Thus, while determining factors could be seen as a switch for fish reproduction, modulating factors further act as dimmer switch to progressively improve or damage the quality of reproductive performance.

#### **6.5.1.1 Temperature and Photoperiod**

In the nature, temperate fish, among which most of percids belong, are submitted to important amplitudes of temperature and/or photoperiod variations (Taranger 2010; Wang et al. [2010](#page-30-0)). For Eurasian perch *Perca fluviatilis*, it has been shown that female's gametogenesis steps follow these variations suggesting that they could play the role of determining factors of egg quality (Sulistyo et al. [1998 \)](#page-29-0). Further works confirmed this hypothesis and allowed the establishment of a photo-thermal

program capable of controlling Eurasian perch reproduction cycle in RAS condi-tions although the reproductive performance remain various (Migaud et al. [2004](#page-28-0), [2006 ;](#page-28-0) Wang et al. [2006](#page-30-0) ; Fontaine et al. [2006 ;](#page-27-0) Abdlufatah et al. [2011 , 2013 \)](#page-25-0). However, the yellow perch (*Perca flavescens*) gametogenesis is principally controlled by the temperature (Dabrowski et al. [1996 \)](#page-26-0). For the pikeperch ( *Sander lucioperca* ) only few works investigated the effect of both parameters. Although, there is very limited data available on the effect of these variables on the spawning effectiveness in percids, it is already well established that photo-thermal manipulations are among the most important factors affecting gametes quality in domesticated broodstock of these species. This creates the necessity for more intensive studies in this specific field where more attention should be paid not only for the spawning performance in general, but more specifically to gametes quality.

## **6.5.1.2 Nutrition**

Breeders' nutrition influences greatly their fecundity and the gametes quality. Indeed, it has been shown in several species that the fecundity seem to be directly linked to the energetic value of their regime (e.g. forage fish vs. pellets) (Cerda et al. 1994; Bromage et al. 1992). Moreover, as previously explained, fish eggs contain large amount of nutrients among which lipids and amino acids. These are either taken from female's intrinsic reserves or directly from the food during the reproduction season. In percids, very few works focused on the improvement of the nutritional contribution for the reproduction success. A preliminary study performed on the pikeperch showed that commercial food choose in the study leads to fewer reproduction success in comparison to forage fish or mixed nutrition (Wang et al. [2009 \)](#page-30-0). This is mainly due to diverse lipid compositions between pellets and forage fish. Another study showed that a diet composed of  $3/2/2$  ratio of respectively DHA/ EPA/AA led to the Eurasian perch eggs and larvae of good quality (Henrotte et al. 2010).

## **6.5.1.3 Hormonal Treatments**

In order to synchronize fish spawning by triggering ovulation of females in the same time or provoke spawning earlier than the regular spawning season, hormonal injections are often performed. Those treatments include injections of pituitary extracts, human chorionic gonadotropin (hCG), luteinizing hormone-releasing hormone analog (LHRHa), pregnant mare serum gonadotropin (PMSG) or Gonadotropinreleasing hormone (GnRH). Several studies showed that pikeperch or Eurasian perch spawning can be induced by diverse hormonal stimulations (Żarski et al. [2011a](#page-30-0); Zakęś [2007](#page-30-0)). Depending on the nature and the doses of hormones, reproductive performances can sometimes be altered, although wild fish (which has completed the gametes maturation process in the wild or in the wild-like environment [i.e. earthen ponds]) were examined. For example, pond-reared pikeperch hatching rate was more negatively affected by the application GnRH analogous in compari-son to hCG injections (Krist'an et al. [2013](#page-28-0)). Moreover, those treatments could induce some stress in fish as it was reported in the pikeperch (Falahatkar and Poursaeid 2014). As considering the effect of hormonal treatment on the spawning effectiveness more details may be found in the Chap. [4](http://dx.doi.org/10.1007/978-94-017-7227-3_4).

## **6.5.1.4 Other Factors**

 Several other factors are able to modulate reproduction success. They can be divided into three main categories: (i) environmental (e.g. temperature, light intensity), (ii) populational (e.g. domestication level, females weight) and (iii) nutritional (e.g. feed composition, feeding rate). Up to now, almost no studies investigated the impact of these factors on percid reproduction success. Moreover, it is commonly known that fish are susceptible to stressors, such as inevitable manipulations, however, this aspect was hardly studied in the percids. And the recent study proved in pikeperch that handling affects the fertilization and hatching rates, or even may inhibit the spawning (Sarameh et al.  $2012$ ). Therefore, the stress should be also considered from the perspective of the modulating factors affecting gametes quality. This creates the need to consider the stress level as a factor affecting egg quality and thus as an important research priority in this field.

## *6.5.2 Egg and Larval Manipulation and Incubation*

As for breeders, egg and larval incubation conditions are of first importance for the proper control of fish life cycle. It includes physicochemical parameters of the water as the temperature, salinity, oxygen and pH, handling or incubation conditions (zoug bottles, tray-type incubators, etc.), the nature and the quality of substratum and the food supply for larvae. A detailed description of egg incubation optimal conditions is given in the Chap. [4](http://dx.doi.org/10.1007/978-94-017-7227-3_4). Some works investigated the effect of embryonic and larval rearing conditions on their quality. The optimal temperature of incubation has largely been documented in *Perca fluviatilis* and *Perca flavescens* with various data. Indeed, incubation temperature ranges can be 12–20 °C (Wang and Eckmann [1994 \)](#page-30-0), 8–16 °C (Swift [1965 \)](#page-30-0), 12–16 °C (Kokurewicz [1969](#page-28-0) ) or 10–16 °C (Guma'a 1978) for *P. fluviatilis* and 10–18 °C (Hokanson and Kleiner 1974) for *P. flavescens*. Moreover, for the Eurasian perch, the survival rate observed at 22  $\degree$ C was 75.7 %, 7.5 %, 2.3 % and 0 % respectively in Wang and Eckmann (1994), Hokanson and Kleiner (1974), Swift (1965) and Guma'a (1978) studies. This difference can be explained by fish population adaptation to various spawning temperatures depending to their natural living conditions (Wang and Eckmann 1994). For example, populations used by Swift and Guma'a studies had been taken in Windermere (NW of England) with an incubation temperature ranging from 9 to 18 °C. On the other hand, Wang fish population from the Lake Constance (at the border between <span id="page-25-0"></span>Germany, Austria and Switzerland, in the Alps) with temperature ranging from 12 to 18 °C. This hypothesis has been confirmed with northern European *P. fluviatilis* populations (Sandstrom et al. [1997 \)](#page-29-0). Moreover, there were some differences of susceptibility of developmental stages to the temperature (Wang and Eckmann 1994). As shown for the temperature, it is important to optimize egg and larval incubation conditions not only for every species but also depending on the fish populations used in the fisheries.

## **6.6 Conclusion and Prospects**

In conclusion, it appears that in most of percid fish species the reproduction practices still need to be more carefully studied. In some species as Eurasian perch, out-of-season reproduction of the RAS-reared broodstock is possible but the reproduction success still needs to be improved since the egg and larvae quality are still in many cases variable, although the same photo-thermal and feeding regimes are applied. In other species as the pikeperch, hatchery practices are not well established. It may be presumed that with clear egg and larvae quality indicators the development of aquaculture of this species will progress much faster. Especially, that in every case that it is important  $(i)$  to define determining and modulating factors regulating fish life cycle (ii) to properly describe the developmental process of each species to determine the proper timing to reach key steps (iii) to have a large overview of developmental failures (mortality, deformities) that can occur in the studied species (this knowledge could help to define some level of developmental impairment and thus categories of ova, embryos or larval qualities) and (iv) to choose relevant quality indicators specifi c to the studied species and depending upon the stage that need to be checked (ova, embryo, larva). Current methods to evaluate egg and larval quality are still infrequent. All of them are morphological and can either indicate high or low probability of the egg development but fail to predict the nature of the developmental impairment. Biochemical indicators seem promising but further work need to be done. Once these questions answered (either successively or in parallel), it could help to improve rearing practices of fish species and may help, in the future, to define a methodology to study new candidates for the diversification of aquaculture.

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