Incubation and Hatching

10

Theoretical Background

Incubation of the developing embryos and control over the hatching process is the final step of controlled reproduction and the first step towards the control of fish life cycle (Schaerlinger and Żarski 2015). Incubation involves all methods that provide optimal conditions for the embryos to develop until they reach the hatching stage. In other freshwater species, such as for example pikeperch, the incubation process must be preceded by the specific procedures of removal of eggs adhesiveness, which is usually crucial for successful incubation (Zakęś and Demska-Zakęś 2009; Żarski et al. 2015b). However, in the case of Eurasian perch, due to specific characteristics of its eggs (spawned in the form of a ribbon), there is no need for any treatment following fertilization. Generally, the main concern during the incubation of Eurasian perch egg-ribbons is to provide suitable circulation of well oxygenated water around all the eggs. For that purpose many kinds of different incubators may be used (Kucharczyk et al. 1996; Żarski et al. 2011).

Hatching, is the set of procedures leading to acquiring hatched larvae without any egg debris and ready to transfer to the rearing unit. During hatching, special attention should be paid to removing the structure of the egg ribbon (jelly-like structures) in the most effective way. This is actually a crucial aspect since the ribbon structure loosens just before the larvae are ready to hatch (Formicki et al. 2009) and becomes a 'jelly-like' shapeless mass that is difficult to remove. Therefore, very careful supervision over the hatching process is highly advised. Especially, that hatching in percids is usually an asynchronous event lasting between 3 days at 19 °C and 5 days at 15 °C even if the same egg batch is incubated (Żarski et al. 2011, 2015a).

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Embryonic Development

At 12–13 °C, the first cell division can be observed within 3 h after fertilization. At 24 h following fertilization, the embryos should reach the blastula stage which is followed by the onset of the epiboly process heralding the beginning of gastrulation. This should be completed after the first 2 days of incubation, when the blastoderm completely covers the yolk (100 % epiboly). The subsequent steps are the organogenesis and tail segmentation that last until pigmentation of the eyes. The specific moment when pigmentation is clearly visible in the eyes of embryos takes place just before hatching in the Eurasian perch. At this moment, morphogenesis of many organ rudiments is almost complete and slows down considerably (Fig. 10.1).

Verification of the Effectiveness of Fertilization

Within the first 3 days of incubation, a significant embryonic mortality may be observed (Alix et al. 2013). Therefore, the success of fertilization should not be determined earlier than 72 h following fertilization, when the embryos reach the late neurula stage (when the body of the embryo can already be observed on the animal pole of the embryo – as described by Iwamatsu 2004). However, it should be highlighted, that at this stage the most common developmental abnormalities of embryo cannot be recognized, yet. Considering the fact that the hatching rate is usually much lower than the fertilization rate determined at earlier stages (Żarski et al. 2011; Alix et al. 2013; Schaerlinger and Żarski 2015), fertilization success (being

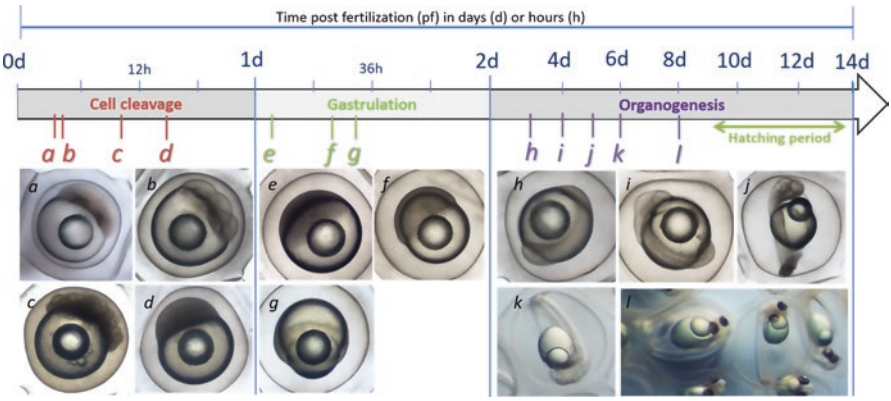

Fig. 10.1 Development of the Eurasian Perch (P. fluviatilis). The general features characterized by observation performed in living embryos during incubation at 13 °C (For more details see Alix et al. 2015). Description of the developmental steps: *a – 2 cells (3.5 hpf); b – 4 cells (4 hpf); c – 128 cells (10 hpf); d – High blastula (15 hpf); e – 30 % epiboly (26 hpf); f – 50 % epiboly (31 hpf); g – 90 % epiboly (41 hpf); h – Optic capsule (66 hpf); i – Otic vesicle (96 hpf); j – Tail elongation (5 dpf); k – Eye pigmentation onset (6 dpf); l – Eyed-egg stage (8 dpf)* (Photo: *a*, *k*, *l* – Palińska-Żarska K.; *b–j* – by courtesy of Alix M)

the overall result of gamete quality stemming from controlled reproduction procedures as well as fertilization effectiveness) should be verified at hatching. However, it should be emphasized that even those larvae that are able to hatch can be characterized by varying quality, as highly deformed individuals were also reported to hatch spontaneously (Żarski et al. 2011; Alix et al. 2013; Schaerlinger and Żarski 2015). Castets et al. (2012) indicated that the resistance of the larvae to starvation as well as their survival at day 7 post hatching were the indicators characterized highest egg quality. This additionally confirms that the larval performance can be more reliable egg quality indicator than fertilization and/or hatching rate. Therefore, a recently reported study suggests that the most reliable method for the verification of reproductive success in Eurasian perch is the determination of number of larvae with inflated swim bladder (Żarski et al. 2015c). This method of verification of the spawning outcome excludes from the overall mass of the larvae produced those with low biological quality whith the highest accuracy. This stems from the fact that the larvae characterized by lower quality are in most cases unable to inflate their swim bladders, which is a crucial event in the fish lifecycle (Woolley and Qin 2010; Palińska-Żarska et al. 2014). Therefore, to verify the productivity of spawning (in both scientific research and commercial production) the use of spawning efficiency index (SEI) is recommended to be used. This index is calculated by the number of larvae with inflated swim bladder in relation to body weight of the females spawned and returns the global overview on the final quality of the larvae stemming from the effectiveness of the entire controlled reproduction operation.

Spawning Efficiency Index (SEI)
The Spawning Efficiency Index developed for Eurasian perch (Żarski et al. 2015c) involves the determination of the number of larvae with inflated swim bladder in relation to the 1 kg of body weight of the females spawned.

$$SEI = \mathrm{N} / BWF$$

where:

N – the number of larvae with inflated swim bladder
BWF – the body weight of the spawned females (in kg).

Practical Advice
Non-developing eggs should be removed as soon as low egg quality is recognized. If it is impossible to recognize earlier, dead ribbons (or dead parts of the ribbons) should be removed if only white (non-transparent) eggs are present upon macroscopic observation. This will allow to keep the rearing unit in good sanitary conditions.

Incubation Devices

In fact, there are no standardized incubators for Eurasian perch eggs. For the purpose of incubation many different devices were constructed and tested and most of them were found to be effective. From the commercial point of view, however, the most suitable are those incubators that allow to 'suspend' the egg ribbons in the water column, which is very often the rearing unit intended to be used for initial rearing of larvae (Fig. 10.2). For this purpose, different floating cages or specific trays (floating or fixed just above the water surface) with the bottom replaced with a net (or any other riddled bottom) can be recommended (Fig. 10.3). In these incubators eggs can be washed from all sides allowing proper water exchange around all the eggs in the ribbon. However, it is important to provide the mesh size (or size of the holes in the bottom) that is large enough (about 3.0 mm) to allow freshly hatched

Fig. 10.2 Egg ribbons obtained from different females incubated in a separate floating 'cages' in a small scale experimental RAS, in which larvae were reared after hatching (Photo: D. Żarski)

Fig. 10.3 An example of an incubator for Eurasian perch egg ribbons with bottom, and part of the one wall replaced with a net (Photo: D. Żarski)

larvae to fall to the bottom of the tank, when transferred from the incubation to the rearing unit. However, the mesh should not be too large either, as ribbon debris can also fall through. The mesh size should allow to separate the larvae easily from the ribbon leftovers just by removing the cages (trays) from the tank (incubation unit).

Practical Advice
In order to facilitate the work with eggs it is recommended to use one hatchery unit for the incubation of all eggs, and transfer the eggs to the rearing system just prior to hatching. In this case, the temperature can be about 2–3 °C higher in the rearing unit. This will allow to synchronize the hatching process of the batches designated to a particular rearing unit and to minimize the amount of possible debris and/or pathogens originating from the low quality eggs in the rearing units.

Incubation Conditions

During the incubation process, apart from the oxygen dissolved in the water, another very important parameter to provide is adequate temperature and pH. In addition to the fact that temperature directly influences the developmental rate of the embryos, too low or too high temperature may also induce improper embryonic development leading to developmental abnormalities and/or embryo mortality (Kamler 2002). It was found that too low pH (below 5) can also be the cause for a prolonged

Table 10.1 Water quality requirements to be used in the hatchery unit

Dissolved oxygen (DO)	>3.5 ppm
CO_2	<5.0 ppm
pH	6.5–9.0
Calcium	10–160 ppm
Phosphorus	0.01–3.00 mg L^{-1}
Total hardness	50–400 ppm
Hydrogen sulfide	0
Nitrite (NO_2)	<0.1 mg L^{-1}
Unionized ammonia (NH_3)	< 0.0125 mg L^{-1}

According to Hart et al. (2006)

incubation period and embryo mortality (Rask 1983). Generally in case of the Eurasian perch, eggs can successfully be incubated at a thermal range between 8 and 18 °C, with 12.5 °C suggested to be the optimal one (Teletchea et al. 2009; Żarski et al. 2015a) at which the incubation lasts approx. 165 degree-days (about 13 days). However, the most commonly applied temperature for egg incubation of Eurasian perch ranges between 12 and 16 °C (e.g. Żarski et al. 2015a), which can be recommended to apply in the hatchery practice.

Generally, apart from the parameters such as temperature, pH and dissolved oxygen in the water there are no specific requirements for the incubation of Eurasian perch eggs. Therefore, water parameters in the incubation unit should meet the typical criteria for freshwater fishes. In the most ideal situation, the water should meet the general criteria for 'drinking water' authorized to be used for human consumption. However, if it is not possible to provide such a high-quality water, a special attention should be paid to using water that meets the typical criteria for fish hatcheries. In this regards the requirement given by Hart et al. (2006) for yellow perch, Perca flavescens¸ a closely related fish species to Eurasian perch, can be followed (Table 10.1). Overall, the water source should be free of any suspensions (transparent), odors as well as any dissolved toxic substances (including nitrogen and phosphorus compounds very often accumulating in RAS systems). In case of using open waters for supplying the hatchery unit it is important to clarify the water (to remove all the suspended particles such as mud, organic matter etc.) by mechanical filtration (e.g. with the use of drum filters) and effectively sterilize it prior to using for supplying the system. Special attention should also be paid to the direct surroundings of the water source (lake or river) to see whether intensive agricultural, industrial or even domestic pollutants will not penetrate the water with harmful substances. In case of using well water, special attention should be paid to purification of the water. Depending on the quality, it may be necessary to remove the iron and/or manganese compounds, decalcify and/or remove other excessively contained compounds prior to use. If tap water has to be used, it is important to make sure that it was properly dechlorinated before any use in the entire hatchery unit.

Important
Eggs should not lay on the bottom of the tank during incubation and should be maximally extended. This is due to the fact that from the eyed-egg stage the embryos may start to die due to their increased oxygen demand before hatching and looseness of the jelly-like structure making oxygen exchange more difficult.

Hatching

Prior to hatching, larvae develop hatching gland cells which are responsible for the production of a hatching enzyme called 'chorionase' (Luczynski et al. 1987; Rechulicz 2001). This enzyme allows to gently digest the internal structure of the chorion surrounding the developing embryo and as a results, the corion loses its rigidity. This, together with the loosening phenomenon of the 'jelly-like' structure of the external layer of the embryo (*zona radiata externa*) just prior to hatching (Formicki et al. 2009) allows the larvae to exit the eggs easily. This corresponds with a very active movement of the larvae inside the egg which provides additional help for the larvae to leave the egg envelope. It is very important to note, that the number and size of the hatching gland cells is also dependent on the water temperature where the highest chorionase production capacity is speculated to occur usually at optimal incubation temperatures (Rechulicz 2001). Although this requires further investigations in the Eurasian perch, it additionally justifies that the 'optimal thermal range' should be maintained during the incubation period.

Considering the fact, that the hatching process is dependent on the enzymatic activity, it is therefore recommended to assist hatching by increasing the water temperature which, in turn, will increase the enzymatic activity of chorionase. In the hatchery practice this involves an increase of water temperature by a few degrees (maximum up to 5 °C) when the first spontaneously hatched larvae are observed. This can shorten the hatching process and allow most of the larvae to hatch at the time of the mouth opening or shortly before (Alix et al. 2015) (Fig. 10.4).

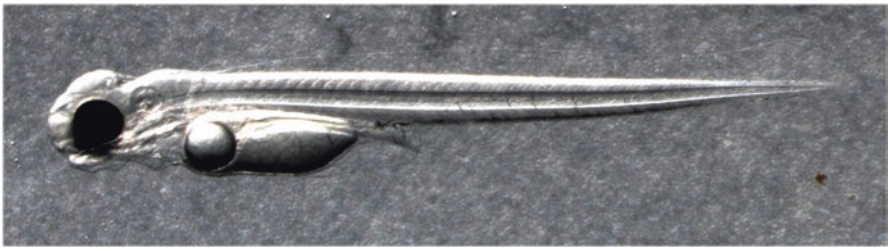

Fig. 10.4 Eurasian perch just after hatching (5.71 mm total length, incubated at 14 °C) (Photo: K. Palińska-Żarska)

Practical Advice

1. It is recommended to incubate the eggs at 12–13 °C and to support the hatching process with an increase of the temperature to maximum 15 °C.
2. Do not apply thermal treatment to the eggs when parallel incubation of the eggs obtained at different dates is conducted in the same hatchery unit.

Initial Larvae Rearing (General Advices)

After hatching larvae are recommended to be kept at 15 °C for first 14 days (Palińska-Żarska et al. unpublished). During that time the light conditions should be adjusted in such a way, that larvae are scattered all over the tank or they are gathering in the middle of the tank just under the water surface. It should be avoided to provide light conditions inducing gathering the larvae close to the tank walls and/or in the corners of the tank, what can alter the swim bladder inflation process (Palińska-Żarska et al. 2013). At such a temperature the first food should be offered between 5 and 7 days following hatching. As the first source of food larvae should have offered freshly hatched *Artemia* sp. nauplii.

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