Chapter 4
Artificial Reproduction of Percid Fishes

D. Żarski, A. Horváth, J.A. Held, and D. Kucharczyk

Abstract Artificial reproduction, being a specific human intervention in the process of reproduction, is a key step in aquaculture of percid fishes. This group of fish, exhibits specific traits, considered amenable to artificial reproductive protocols. For example, this is the only extensively studied group of freshwater teleosts where application of human chorionic gonadotropin (hCG) and gonadoliberine analogues (GnRHa) alone, promotes final oocyte maturation (FOM) and spawning without any other hormonal therapy, whereas in other species (cyprinids, catfishes or salmonids) anti-dopaminergic treatment is also needed. Another characteristic trait is that percid females can release their eggs spontaneously in the tank, regardless of the presence of males. This makes artificial spawning of these fish relatively difficult. In the present chapter endocrine regulation as well as reproductive protocols applied to this group of fish are reviewed extensively, however, the focus of this review is on the final gamete maturation, spermiation and ovulation processes are the steps considered from artificial reproduction perspectives. The published data revealed that scientific activity was focused mainly on the problem of synchronization of ovulation and the effectiveness of different hormonal therapies. This evolved into the development of several specific protocols and methods (e.g. percid-specific preovulatory maturational stages of oocytes), which allowed improvement of that in these species. It was also established, that hCG or GnRHa applied alone are the most effective spawning agents in wild or pond-reared percids. However, there is still a considerable lack of data considering the effectiveness of these protocols in controlled reproduction of domesticated broodstocks. Apart from that, there are many other aspects to be investigated. Such as hormonal regulation of final gamete maturation and spawning, verification of some reproductive protocols as possible

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gamete quality determinants and gamete management protocols (prior to and following fertilization), which were relatively scarcely studied.

Keywords Hormonal regulation • Fertilization • Spermiation • Ovulation • Percids

4.1 Introduction

Spawning, either natural or artificial, is a key step in fish lifecycle. In the wild the act of spawning closes the development of the organism into adult specimen and in this way ensures the survival of the species. In captivity spawning is a necessary element securing constant production. All the commercially important percid fishes exhibit some differences considering the reproductive features, where the structure of the eggs laid (in the form of 'ribbon' or as a batch of single eggs) is the most distinctive one (for details see Chap. 2). However, there are also many similarities allowing consideration of these species as a one specific group representing comparable pattern of gonadal development as well as gametes maturation and spawning (see also Chaps. 2 and 3).

Artificial reproduction may be defined as a human intervention in the process of reproduction (Woynarovich and Horváth 1980). It takes into account a number of techniques aimed at producing high quality offspring suitable for further culture process or restocking. Generally, as considering artificial reproduction human intervention was concentrated on the final phases of the maturation of spawners (final oocyte maturation, ovulation and spermiation) and the methods of control over these processes as well as gametes management and other procedures (in vitro fertilization, egg treatment, incubation) up to hatching. This concerns, among others, manipulation of environmental conditions, hormonal therapies, gametes handling and other protocols which were proved to be crucial for successful reproduction.

From an aquaculture perspective, successful reproduction is one of the crucial steps in the culture process. Its effectiveness directly affects the production capacity and in this way may influence the production profitability. In all cultivated percids the reproduction of captive broodstock is feasible. Nevertheless, the artificial reproduction of wild or pond reared fish (which are exposed to natural food source and natural photo-thermal conditions) is still a major concern. Therefore, this chapter extensively described the procedures of artificial reproduction of wild (or pond reared) fish as well the one of breeders held in intensive conditions.

4.2 Endocrine Regulation of Spermiation

Endocrine regulation of sexual maturation and spermatogenesis in male percids is generally similar to that of other teleosts. As in most animal species spermatogenesis of fish is also controlled by the hypothalamic-pituitary-gonad axis. The

gonadotropin releasing hormone (GnRH) produced in the hypothalamus stimulates the release of gonadotropins (GtH) of the pituitary which in turn stimulate the steroid (androgen) production in the testis (Vizziano et al. 2008). While the function of androgens has been studied to some extent in percids, very little information is available on the male-specific actions of either GnRH or GtH-s in these species. GnRH is a decapeptide, a neurohormone that is mainly localized in the anteroventral preoptic region of the telencephalon (Peter 1983) which does not seem to exert male- or female-specific action. Its production is controlled by an antagonist, the dopamine, which is a unique feature in teleosts. Inhibition of GnRH action by dopamine is not observed in all teleosts, and particularly little information is available regarding this process in males. Fish gonadotropins are present in two forms characterized as either GtH I and GtH II being homologous with mammalian FSH and LH (Vizziano et al. 2008) or as FSH and LH proper (Schulz et al. 2010). Unlike in mammals where LH regulates sex steroid release by Leydig cells and FSH is responsible for the regulation of Sertoli cell activities in males, in fish the roles of the two forms are not clearly separated. Receptors of FSH display a preference for FSH, however, they can be activated by LH, too, although at higher concentrations. LH receptors seem to be more LH-specific in fish where cross-activation by FSH is possible only in non-physiologically high concentrations (Bogerd et al. 2005). Nevertheless, FSH is known to stimulate steroidogenesis in fish, suggesting that FSH receptors are present in the Leydig cells. The expression of FSH and LH receptor mRNA in Leydig cells was demonstrated for the first time in fish (and vertebrates) in the African catfish (*Clarias gariepinus*) by García-López et al. (2009). Generally, FSH concentrations are high during the onset of spermatogenesis (proliferation of spermatogonia, meiosis and early spermiogenesis) while LH levels start to increase close to spawning (spermiogenesis and spermiation) what was described extensively in Chap. 3.

The unique study on the gonadotropins of percid males has shown an increased expression of FSHβ and LHβ (the hormone specific β-subunit of gonadotropins) mRNA in pikeperch (*Sander lucioperca*) during early and mid-spermatogenesis compared to pre-spermatogenesis (Hermelink et al. 2011). The increase of LHβ mRNA expression was more pronounced than that of the FSHβ mRNA which corresponds with the findings in other teleost species. The main steroid hormones found in the testis include estrogens such as 17β-estradiol (E2), androgens such as testosterone (T) and 11-ketotestosterone (11-KT) and progestins such as 17α,20β-dihydroxy-4-pregnen-3-one (abbreviated as DHP or 17,20β-P) and 17α,20β,21-trihydroxy-4-pregnen-3-one (20β-S) (Schulz et al. 2010). The major sites of sex steroid synthesis in the testis are the Leydig cells that can be found in the interstitium between individual tubules or lobules. These are large polygonal cells characterized by extensive agranular endoplasmatic reticulum and mitochondria with tubular christae. These cells appear before or during sex differentiation depending on the species. These cells are believed to be homologous with the steroid-producing Leydig cells of higher vertebrates (Billard et al. 1982).

The function of estrogens in the testis is still largely unclear but it is suggested that it plays a role in spermatogonial renewal and multiplication (Vizziano et al. 2008). They

have also been reported to play a major role in gene expression in the testis (Schulz et al. 2010). In percid males E2 levels were rarely studied. As a notable exception, E2 concentration in males of yellow perch (*Perca flavescens*) was found to be low throughout the year with a five to tenfold increase in November–December to 50–100 pg/ml while decreasing just before the spawning season (Ciereszko et al. 1998). In contrast, in Eurasian perch (*Perca fluviatilis*) males E2 concentrations were found to be high during the spawning season (probably sustaining spermiation) and decreased significantly after the end of spawning (Rougeot et al. 2004). This corresponds with the findings of Hermelink et al. (2011) who reported that E2 levels remained at high and stable levels during pre-, early and mid-spermatogenesis in pikeperch.

Androgen receptors are expressed in the testicular somatic cells (primarily Sertoli and interstitial cells) but not in the germ cells (Schulz et al. 2010). They effectively support the entire process of spermatogenesis from spermatogonial multiplication to spermiation. Although both can be found in females, too, levels of 11-KT were found to be ten times higher in pikeperch males than in females (Teletchea et al. 2009a). Seasonal changes in plasma T and 11-KT levels have been relatively well described in percids and follow the same general pattern of decreasing abruptly following spawning and beginning to increase roughly 4–5 months before the subsequent spawning (described in greater details in Chap. 3).

Progestins have been shown to be present in elevated concentrations throughout the spermiation process in many fish species (Schulz et al. 2010). They play a particularly important role in sperm maturation and release, increasing pH of sperm ducts and probably exert their action through a membrane receptor of spermatozoa (Vizziano et al. 2008). In percid males the presence and concentrations of progestings are rarely measured. Interestingly, in the ruffe (*Gymnocephalus cernuus*) metabolites of 20β-S were found to act as pheromones that stimulated swimming activity and social interactions in conspecific males. When males were exposed to the urine of females injected with 20β-S they started swimming more actively and inspecting each other. In contrast, direct exposure to either progestins or to the urine of females injected with DHP caused no noticeable change in the behavior of males (Sorensen et al. 2004). In the pikeperch, concentrations of DHP stayed stable and low (below 100 pg/ml) during pre-, early and mid-spermatogenesis, suggesting that they only increase during active spermiation (as they do in other teleosts) which was not studied, yet (Hermelink et al. 2011).

4.3 Endocrine Regulation of Final Oocyte Maturation (FOM) and Ovulation

In teleosts process of oocyte maturation and ovulation is preceded by the oocyte growth (vitellogenesis) which involves incorporation of yolk proteins and lipids into oocytes (Tyler and Sumpter 1996; Devlin and Nagahama 2002; Patino and Sullivan 2002). During the vitellogenesis process oocytes are arrested in the first meiotic prophase (Nagahama 1994). The process is up-regulated by

hypothalamic-pituitary-gonadal axis, where the major role plays follicle-stimulating hormone (FSH). These processes are extensively described in Chap. 3.

After the complete vitellogenesis and just before ovulation the final oocyte maturation (FOM) process begins. During the FOM, the first meiotic division is completed and the second is started, which proceeds until the metaphase II stage (Nagahama and Yagashita 2008). The process is accompanied by a number of morphological changes involving, for example, migration and decomposition of the germinal vesicle, formation of yolk and coalescence of oil droplets into one large droplet (Migaud et al. 2003; Żarski et al. 2011a, 2012a, c). In percids the most characteristic feature during FOM was the oil droplet coalescence process. It was found that the small oil droplets coalesce into one big oil droplet, what was accompanied together with the GV migration (Żarski et al. 2011a, 2012a, c). During this phase the most important role plays second gonadotropic hormone which is luteinising hormone (LH) secreted by the pituitary gland during FOM (e.g., Kagawa et al. 1998; Patino et al. 2001; Patino and Sulivan 2002). However, Fontaine et al. (2003) suggest also that in the whole process E_2 and T may have been involved. Basically, FOM is triggered by the LH which stimulates theca cell layers of the ovarian follicle for production of 17α-hydroxyprogesterone which traverses the basal lamina and is then converted to the maturation inducing steroid (MIS) by the granulosa cells of the ovarian follicle (e.g., Nagahama 1994; Nagahama and Yamashita 2008). The MIS was proven to bind with the specific receptors of oocyte plasma membrane in postvitellogenic oocytes of teleosts (Nagahama and Yamashita 2008). In the case of freshwater percids, like in most fish species, the role of MIS the most probably plays 17α,20β-dihydroxy-4-pregnen-3-one (DHP) (Goetz and Theofan 1979; Goetz et al. 1989; Barry et al. 1995; Fontaine et al. 2003; Nagahama and Yamashita 2008; see also Chap. 3). The MIS promote the formation and activation of the maturation promoting factor (MPF) which is the complex of cdc2 kinase and cyclin-B (for details see: Yamashita et al. 1992; Nagahama and Yamashita 2008). The MPF is the final inducer of the oocyte maturation process (Nagahama and Yamashita 2008). The data on the MPF in freshwater percids are still missing, however already published data suggest that the pathways and mechanisms of this process is very similar to those described for other teleosts or even amphibians (extensively revised by Nagahama and Yamashita 2008).

In some of the fish species, such as cyprinids, maturation and ovulation are also controlled by the dopamine (DA), which acts as a LH inhibitory factor (Peter and Yu 1997; Mylonas et al. 2010). It is well reflected in the cases when GnRH analogues are administered for induction of maturation and ovulation *in vivo*, since elevated level of gonadotropins (as a result of GnRH administration) in the blood stream affect production of DA and in this way decreases effectiveness of such hormonal therapy (e.g., Peter and Yu 1997; Mylonas et al. 2010). In consequence, administration of GnRH alone does not affect maturation and ovulation. In such cases dopamine antagonist (e.g., metoclopramide, pimozide) administration is needed to affect ovulation (Krejszeff et al. 2009, 2010; Targońska et al. 2010; Kujawa et al. 2011). However, in the case of percids such additional treatment is not needed and administration of GnRH alone affects ovulation successfully (e.g.,

Kouril et al. 1997; Kucharczyk et al. 1998; Ronyai and Lengyel 2010; Żarski et al. 2013a). It may be then concluded that inhibitory effect of DA does not occur in the freshwater percids or it is very weak. Nevertheless, as considering endocrine regulation of FOM and ovulation of those species it seems that this aspect may be omitted.

Ovulation process includes expulsion of the matured oocyte from the ovarian follicles (Goetz 1983; Goetz and Garczynski 1997). Generally, this process is up-regulated by the LH secretion what affects consequently production of MIS (as described above and in Chap. 3). However, it is still not clear which factor directly affect process of ovulation. It was found that DHP plays an essential role in this process in yellow perch (Goetz et al. 1989; Goetz and Garczynski 1997). For the act of follicular rupture during ovulation the most probably proteinases are responsible (Nagahama and Yamashita 2008). However, the biochemical pathways between DHP (17,20β-P) and activation of proteinases remains unknown, although the arachidonic acid and prostaglandins were reported to be involved in this process (Bradley and Goetz 1994; Goetz and Garczynski 1997; Patino et al. 2003). It was reported, that indomethacin, a prostaglandin endoperoxide synthase inhibitor, has been responsible for inhibition of ovulation in yellow perch. It may be then suggested that cyclooxygenase metabolites are responsible in some way in regulation of ovulation (Goetz and Theofan 1979; Goetz and Garczynski 1997).

4.4 Artificial Spawning

4.4.1 Spontaneous Captive Spawning

One of the easiest methods of fish artificial spawning is so called ‘semi controlled’ (‘semi artificial’) spawning in tanks, small ponds or cages placed in the pond or lake (Steffens et al. 1996; Kucharczyk et al. 2007; Zakęś and Demska-Zakęś 2009). This method was widely used by inexperienced farmers, who wanted to produce larvae of those species. In general, the spontaneous spawning, regardless if performed in ponds, cages or tanks, is a simple method where spawners of both sexes are kept in a closed environment allowing them spawning without strict human control and stimulated only by the environmental conditions (e.g. Kucharczyk et al. 2007; Policar et al. 2008). The big advantage of this method is that fish are not disturbed by human, what limits the stress and they spawn whenever they are really ready to reproduce. The big disadvantages of such reproduction method are, very often, low fertilization rate, no possibility of controlled fertilization (for example specific crossbreeding, genome manipulation etc.) and limited possibilities to predict the moment of spawning. It may be improved by the application of hormonal treatment (e.g. Kucharczyk et al. 2000, 2007), but it still remains rather difficult to control the reproduction, although fish will spawn more synchronously.

In the case of yellow and Eurasian perch the spontaneous spawning was reported to be used many times (Hinshaw 2006; Ronyai and Lengyel 2010; Żarski et al.

2011a). This is caused by the fact that these species lay eggs in the form of ribbon (described in details in Chap. 2) which may be then easily removed and incubated in a regular hatchery devices. In this case, the fertilization rate should not be a problem if fish are kept in a small tank where spermiating male is able to produce a 'sperm cloud' which allows the spermatozoa surround and fertilize the ribbon effectively (Kucharczyk et al. 2000). However, there are no possibilities to perform selective breeding and/or other specific techniques such as fertilization of eggs with cryopreserved sperm or genome manipulation.

Application of this method of reproduction in the case of walleye and pikeperch is more complicated due to the fact that these species lay eggs as a batch of hundred thousands of sticky eggs strongly attached to any kind of substrate. Therefore, it is necessary to provide the nests in the spawning area. The nests are usually made of different natural (e.g. juniper branches, conifer branches, roots, sedges, alder, rice grass, wood wool, Fig. 4.1) or synthetic materials (Wojda et al. 1994; Skrzypczak et al. 1998; Kucharczyk et al. 2007). After the spawning the eggs together with the substrate were moved to the hatchery and incubated (Fig. 4.2). The incubation of the eggs attached to the substrate is quite problematic. The unfertilized or not developing eggs cannot be removed what usually leading to the fungal infection significantly reducing the incubation effectiveness. The substrate itself, when natural materials are used, is also a very good medium for fungal infection. Therefore, some improvement may be achieved by using the artificial substrate, although the problem of providing good water exchange around all the eggs still exists. Of course, this method of propagation may also be applied with or without hormonal treatment of spawners. However, this method is still much less effective than in the case of

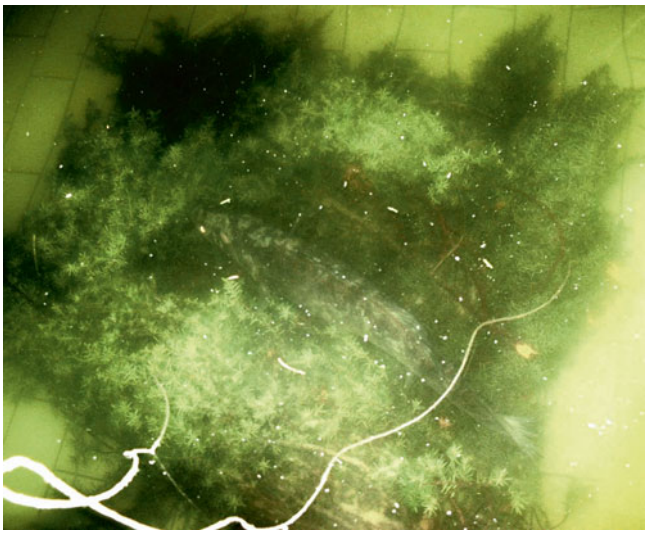

Fig. 4.1 A male pikeperch above the nest made of juniper branches with the eggs laid on it (Photo: D. Żarski)

Fig. 4.2 A juniper branch with the eggs of pikeperch laid on it. The abundance of white eggs indicates a low fertility and the whitish cloudy areas the beginning of fungal infection (Photo: D. Żarski)

yellow and Eurasian perch, since fertilization rate, and consequently survival rate, are usually much lower than the methods involving controlled fertilization.

Actually, the captive spontaneous spawning is the method which may be recommended to inexperienced farmers who has limited knowledge and skills in controlled reproduction. However, it has to be stressed that this method may generate low and unpredictable spawning effectiveness. While this method may be successfully applied in commercial farming of yellow and Eurasian perch, commercial aquaculture of pikeperch and walleye should not rely solely on this kind of propagation method.

4.4.2 Controlled Reproduction

Controlled reproduction involves the procedures of stimulation of spermiation and ovulation in already matured fish, gametes collection and in vitro fertilization. For culture purposes it is becoming a more and more required way for obtaining eggs and larvae. The biggest advantage of this method of propagation is the possibility of selective breeding of specimens with required pheno- and genotypes and to having control over the entire procedure of reproduction (Thorgaard 1995; Zohar and Mylonas 2001; Mylonas et al. 2010). It includes, among others, timing of fertilization, duration of incubation and timing of hatching. The latter, in the case of percids, may be crucial for larviculture effectiveness where size heterogeneity of larvae, caused by the different hatching times, may increase intensity of cannibalism and,

in consequence, production outcome (e.g. Baras et al. 2003; Kestemont et al. 2003; Kooten et al. 2010), as extensively described in Chaps. 9, 10 and 11

4.4.2.1 Sex Recognition

In all percids there is no clear sexual dimorphism. It was already reported that during the culture process some sex-dependent growth heterogeneity may be observed (for more details see Chap. 2). However, fish at the same size beyond the spawning season usually looks very similar and it is very hard to distinguish males from females. Only just before or during the spawning season the fish gender may be usually recognized as the males are spermiating after gentle pressure of their abdomen and females display distended abdominal part of body. However, it may still happen (especially prior to spawning season) that the sex be indistinguishable. In that case catheterization (Fig. 4.3) may be a useful method for gender verification (Ross 1984).

4.4.2.2 Determination of Maturity Stage of Females

The effectiveness of hormonal stimulation as well as latency time following hormonal treatment in females are strictly dependent on the maturity stage (Brzuska 1979, 1988). Until recently, in freshwater aquaculture, the germinal

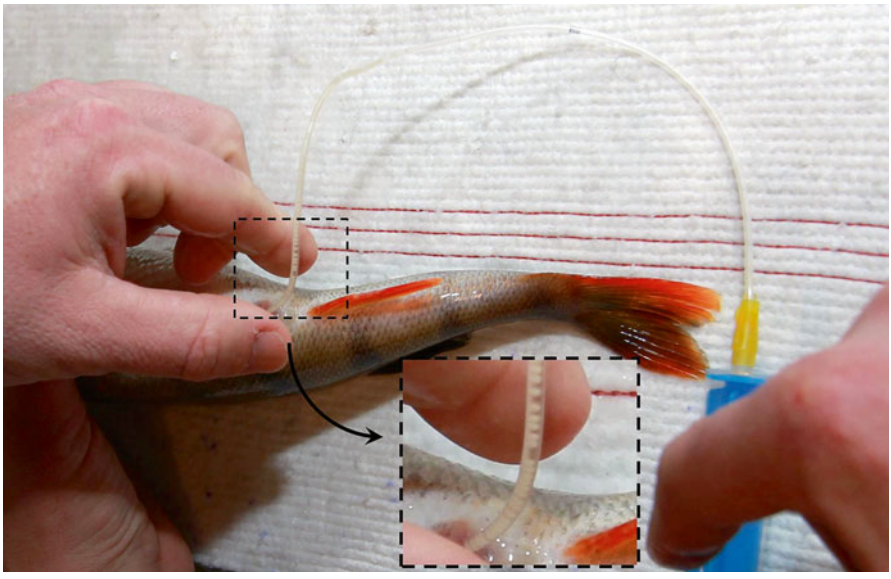

Fig. 4.3 Catheterization of the Eurasian perch female. Close to the genital pore oocytes may be seen in the lumen of the catheter (Photo: S. Krejszeff)

vesicle (GV) position in preovulatory oocytes was taken into consideration for determining the maturation stage of females. In effect four stages were distinguished: central position of GV (stage 1), early migration of GV (stage 2), late migration of GV (stage 3), peripheral position of GV or oocytes after the GV breakdown, i.e. GVBD (stage 4). However, the application of this method in freshwater percids in many cases created many ambiguities and consequently revealed weakness of this classification in this group of fish (Żarski et al. 2011a, 2012a). The largest ambiguity concerned the last stage (stage 4), where some authors to this stage qualified oocytes with the GV at a peripheral position (Kucharczyk et al. 1996, 1998, 2001; Targońska et al. 2014; Żarski et al. 2011b) and others oocytes which already undergone GVBD (Barry et al. 1995; Malison et al. 1998; Ronyai 2007; Zakęś and Demska-Zakęś 2009). Therefore, a verification of the final oocyte maturation (FOM, as described earlier in Sect. 4.3) process was made in order to distinguish a percid-specific features of preovulatory oocytes. Actually, six preovulatory oocyte maturation stages were distinguished, first in Eurasian perch (Żarski et al. 2011a):

- Stage I: the GV was situated in the oocyte centre, oil droplets were poorly visible (Figs. 4.4a and 4.5a);
- Stage II: beginning of GV migration, beginning of coagulation of clearly visible oil droplets (Figs. 4.4b and 4.5b);
- Stage III: migrating of GV (above half of the oocyte diameter), oil droplets were clearly visible (Figs. 4.4c and 4.5c);
- Stage IV: the GV is at the oocyte periphery, a large forming oil droplet was clearly visible; the droplet diameter was greater than the GV diameter and it reached the size of about one third of the oocyte diameter; also smaller droplets were visible (Figs. 4.4d and 4.5d);
- Stage V: the GV is situated at the oocyte edge, clearly visible one large (size of about half the oocyte diameter) oil droplet (Figs. 4.4e and 4.5e);
- Stage VI: oocyte samples taken for analysis were macroscopically transparent; no visible GV after they were placed in Serra's solution (following GVBD), oocytes at the pre-ovulation stage (Figs. 4.4f and 4.5f, g);

It may be found, that for the classification, not only the position of germinal vesicle but also the oil droplets coalescence rate were considered. This phenomenon, next investigated histologically (Żarski et al. 2012c, Fig. 4.5), seems to be very specific to percid fishes as this classification was later successfully transferred also to pikeperch (Żarski et al. 2012a). Therefore, it may be suggested that this classification may be used (with some minor modifications) in all percids allowing higher synchronization and more accurate prediction of ovulation.

4.4.2.3 Stimulation of Spermiation

Regarding hormonal induction of spermiation in percids, little information is available that is specific to this taxon. Percids are known to release an ample amount of sperm during the reproductive season. For example, non-treated Eurasian perch was

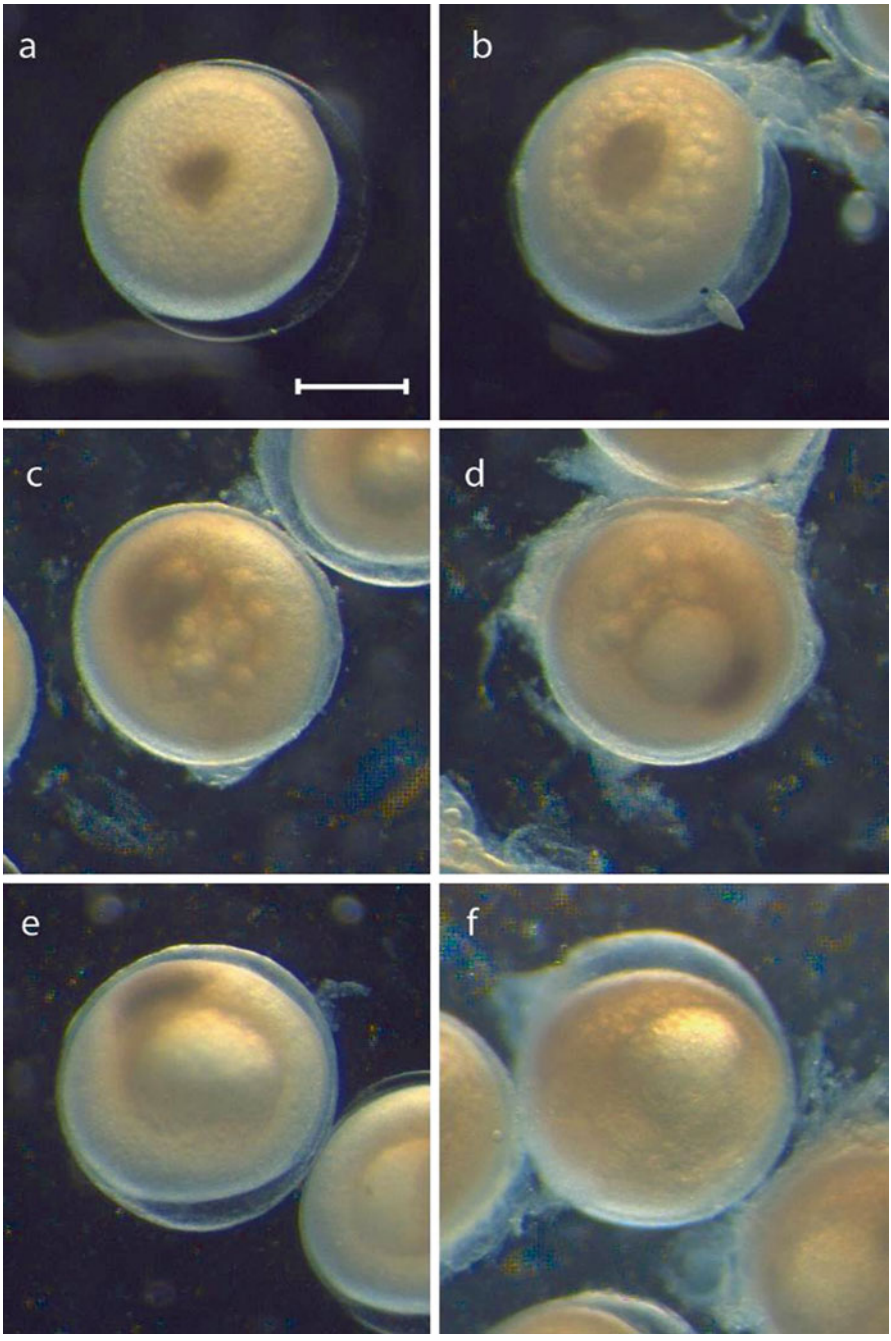

Fig. 4.4 Oocyte maturation stages for percids on the example of Eurasian perch (according to Żarski et al. 2011a); (**a**) stage I, (**b**) stage II, (**c**) stage III, (**d**) stage IV, (**e**) stage V, (**f**) stage VI; for details see the text; bar: 0.5 mm

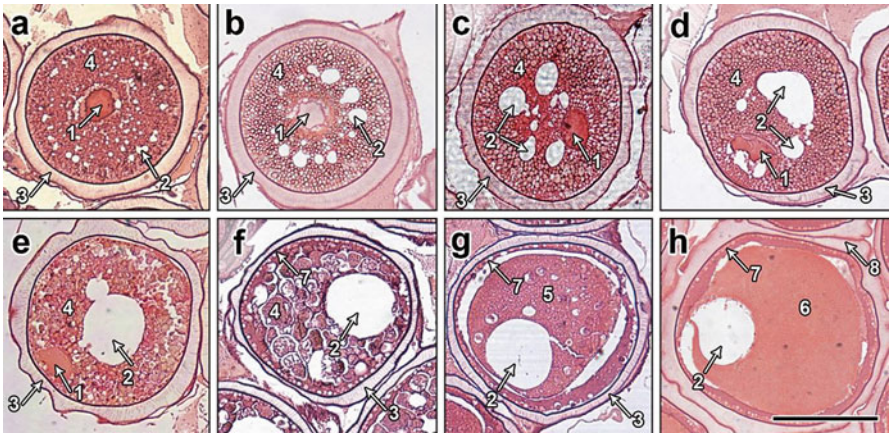

Fig. 4.5 Histological cross-sections of oocytes and ovulated eggs of Eurasian perch at different maturational stages (according to Żarski et al. 2012c): (**a**) stage I, (**b**) stage II, (**c**) stage III, (**d**) stage IV, (**e**) stage V, (**f**) early phase of stage VI, (**g**) late phase (after proteolysis) of stage VI, (**h**) stage VII (ovulated eggs). *1* germinal vesicle, *2* oil droplets, *3 zona radiata externa*, *4* yolk vesicles and globules, *5* homogenous (after proteolysis) yolk before hydration, *6* yolk after hydration, *7* cortical alveoli, *8 zona radiata externa* without visible microvillosities. *Bars* represent 0.5 mm

found to release approximately 13–20 mL of sperm per kg of body weight during the spawning season (Kucharczyk et al. 1996, 1998; Alavi et al. 2010). Therefore hormonal induction of males is only a supplementary measure to enhance milt production. Spermiation can be induced by a change in environmental conditions, such as temperature increase during transfer from one habitat to another as it was described for the walleye (Dąbrowski et al. 2000). Human chorionic gonadotropin (hCG), carp pituitary extract (CPE) or a combination of the two were used to stimulate spermiation in the pikeperch and Volga pikeperch (*Sander volgensis*) but none of them were applied male-specifically (Bokor et al. 2007, 2008). Żarski et al. (2013a) reported, that neither gonadotropins (i.e. hCG, CPE or combination of hCG and pregnant mare serum gonadotropin) nor neurohormone (gonadoliberine analog) affected spermiation rate and spermatozoa motility in pikeperch. In the yellow perch monthly injections of luteinizing hormone releasing hormone analog (LHRHa) starting in January increased production of milt in February and March (Dabrowski et al. 1994). Males of the Eurasian perch produced significantly higher volumes of semen following injection with either 2 mg per kg of body weight of carp pituitary extract or 0.5 pellet of the synthetic GnRH analog Ovopel as compared to the untreated control (Kucharczyk et al. 2001). Also combined injection of FSH (25 μg kg^{-1}) and LH (25 μg kg^{-1}) administered together with a dopamine antagonist (metoclopramide at a dose of 2.5 mg kg^{-1}) significantly increased sperm production in this species (Kucharczyk et al. 1996). During out-of-season spawning trials of Eurasian perch, smaller fish (individual weight: 30–70 g) displayed a higher percentage of spermiating individuals than did larger fish (200–400 g) following injection with various doses of Ovopel, however, it was unclear whether this was an

effect of hormonal treatment or that of the pre-spawning chilling period as these conditions were not investigated independently (Szczerbowski et al. 2009). Thus, administration of hormonal products to percid males can enhance spermiation parameters but it is not an inevitable procedure. However, it is very important to note that the sperm volume and quality may be dependent on the timing of its collection, with the highest parameters being recorded during the spawning season (Alavi et al. 2010). However, there is still missing data about the effectiveness of different hormonal treatment protocols and the latency time following hormonal treatment on the quantity and quality of sperm in percids, what has already been proven to have huge impact on the effectiveness of stimulation of spermiation in other fish species (Król et al. 2009; Cejko et al. 2012, 2013).

4.4.2.4 Stimulation of Ovulation

There are numerous differences in the methods and effectiveness of artificial reproduction of cultured and wild females of percid fishes. In the cultured stocks the most important aspect is to provide proper feeding regime and promote vitellogenesis with the use of proper photothermal manipulations prior to spawning (see Chaps. 3, 20 and 21 for details). These factors directly affect gamete maturation process and consequently eggs quality (Ciereszko et al. 1997, 1998; Sulistyo et al. 1998; Henrotte et al. 2008, 2010; Abdulfatah et al. 2011). Induction of FOM and ovulation in cultured females is possible with the application of photothermal manipulations only, even out-of the spawning season (Müller-Bellecke and Zienert 2008). So, there is no need for any hormonal therapy to obtain the eggs in contrast to wild fish, where hormonal stimulation is usually required. In the case of cultured stocks reproductive procedures are adjusted to the local conditions (within particular fish farm) and in accordance with specific production cycle.

Stimulation of FOM and ovulation under the controlled conditions is conducted mainly by the photo-thermal manipulations (Kolkovski and Dąbrowski 1998; Zohar and Mylonas 2001; Müller-Bellecke and Zienert 2008) and hormonal treatment (Brzuska 2005; Krejszeff et al. 2009, 2010; Mylonas et al. 2010). Additionally, some authors suggest that pheromonal stimulation can play a significant role in these processes (Stacey 2003; Żarski 2012). In the case of percids, among the environmental cues, induction of FOM (which includes morphological changes in oocytes after the completed vitellogenesis as well as germinal vesicle [GV] migration and its breakdown [GVBD]; for more details see Sects. 4.3 and 4.4.2.2) as well as ovulation are mainly controlled by the temperature (Dąbrowski et al. 1996). Photoperiod in those processes plays a minor role (Kayes and Calbert 1979; Dąbrowski et al. 1996; Ciereszko et al. 1997). Thus, in the practice of artificially induced reproduction more attention is paid to the thermal manipulations (Dąbrowski et al. 1994; Kucharczyk et al. 2007; Żarski et al. 2013a). Basically, in all percids similar thermal conditions are applied (Tables 4.1, 4.2, 4.3 and 4.4). The optimal temperature for artificial reproduction ranges between 10 and 15 °C (Kayes and Calbert 1979; Dąbrowski et al. 1994;

Table 4.1 Hormonal preparations, general reproductive methods applied and results of artificial reproduction of Eurasian perch *Perca fluviatilis*

Spawning agent	The dose (per kg of body weight)			Dopamine antagonist		Interval between injections (h)	Method of injection	Temperature (°C)[f]	Photoperiod	Maturity stage prior to hormonal stimulation	Spawning method	Ovulation rate (%)	Latency time (h)[g]	Embryonic survival (%)[h]	Reference
	I injection	II injection	III injection	Preparation	Dose (per kg of body weight)[h]										
hCG	200 IU	500 IU	5000 IU	–	–	24	IM	10–11	nd	2–3 [S4]	CS	50	96–128	84.1	1
	500 IU	–	–	–	–	–	IP	14	14L:10D	1 [S6]	TS	75	120–140	63	2
	500 IU	–	–	–	–	–	IP	12	14L:10D	1 [S6]	CS	67	110	53	2
	500 IU	–	–	–	–	–	IP	12	14L:10D	2 [S6]	CS	63	92	61.7	2
	500 IU	–	–	–	–	–	IP	12	14L:10D	3 [S6]	CS	78	68	76.2	2
	500 IU	–	–	–	–	–	IP	12	14L:10D	4 [S6]	CS	100	49	77.2	2
	500 IU	–	–	–	–	–	IP	12	14L:10D	5 [S6]	CS	100	29	81.2	2
	500 IU	–	–	–	–	–	IP	12	14L:10D	6 [S6]	CS	100	18	86.5	2
	500 IU[d]	–	–	–	–	–	IP	14	12L:12D	1 [S4]	CS	100	108	87.9	3
hCG + CPE	500 IU (hCG) + 0.4 mg (CPE)	3.6 mg CPE	–	–	–	24	IM/IP	10–11	nd	2–3 [S4]	CS	100	38–40	60.3	1
	200 IU hCG	500 IU (hCG) + 0.4 mg (CPE)	3.6 mg CPE	–	–	24	IM/IP	10–11	nd	2–3 [S4]	CS	100	62–64	72.1	1
	1000 IU (hCG) + 0.4 mg (CPE)	3.6 mg CPE	–	–	–	24	IM	10–11	14L:10D	2–3 [S4]	CS	92	38–64	53.3	4

CPE	0.4 mg	3.6 mg	–	–	–	24	IP	10–11	nd	2–3 [S4]	CS	100	38–40	61.9	1
	5 mg	–	–	–	–	–	IM	17–19	12L:12D	nd	TS	80	93	26	5
	4 mg[d]	–	–	–	–	–	IP	14	12L:12D	1 [S4]	CS	18	139	33.9	3
FSH + LH	75 IU (FSH) + 75 IU (LH)	–	–	PIM	5 mg	–	IM	14	14L:10D	2–3 [S4]	CS	50	16–22	55.5	6
	75 IU (FSH) + 75 IU (LH)	–	–	PIM	10 mg	–	IM	14	14L:10D	2–3 [S4]	CS	75	21.5–22	61.0	6
	75 IU (FSH) + 75 IU (LH)	–	–	MTC	5 mg	–	IM	14	14L:10D	2–3 [S4]	CS	100	20–21.5	66.8	6
	75 IU (FSH) + 75 IU (LH)	–	–	MTC	10 mg	–	IM	14	14L:10D	2–3 [S4]	CS	100	16–18	75.4	6
	75 IU (FSH) + 75 IU (LH)	–	–	–	–	–	IM	14	14L:10D	2–3 [S4]	CS	50	22–24	52.1	6

(continued)

Table 4.1 (continued)

Spawning agent	The dose (per kg of body weight)			Dopamine antagonist		Interval between injections (h)	Method of injection	Temperature (°C)[f]	Photoperiod	Maturity stage prior to hormonal stimulation	Spawning method	Ovulation rate (%)	Latency time (h)[g]	Embryonic survival (%)[h]	Reference
	I injection	II injection	III injection	Preparation	Dose (per kg of body weight)[h]										
mGnRHa	1 µg	–	–	–	–	–	IM	13–15	12L:12D	nd	TS	–	–	–	7
	5 µg	–	–	–	–	–	IM	13–15	12L:12D	nd	TS	11	94	98.5 (9)	7
	25 µg	–	–	–	–	–	IM	13–15	12L:12D	nd	TS	28	103	98.6 (9)	7
	125 µg	–	–	–	–	–	IM	13–15	12L:12D	nd	TS	78	115	94.1 (9)	7
	40 µg[d]	–	–	MTC	20 mg	–	IP	14	12L:12D	1 [S4]	CS	55	139	35.8	3
	0.2 µg[a]	20 µg	–	MTC	0.1 mg + 10 mg	24	IM	10–11	14L:10D	2–3 [S4]	CS	92	42–68	53.4	4
	0.2 µg[a, b]	20 µg	–	MTC	0.1 mg + 10 mg	24	IP	14	14L:10D	nd	CS	0	–	–	8
	40 µg[a, b]	–	–	MTC	20 mg	–	IP	14	14L:10D	nd	CS	0	–	–	8
	0.2 µg[a, c]	20 µg	–	MTC	0.1 mg + 10 mg	24	IP	14	14L:10D	nd	CS	95	144–168	36	8
	40 µg[a, c]	–	–	MTC	20 mg	–	IP	14	14L:10D	nd	CS	100	96–120	62	8
AN–GnRHa	40 µg	–	–			–	IM	17–19	12L:12D	nd	TS	100	79	29	5
	40 µg	–	–	MTC	10 mg	–	IM	17–19	12L:12D	nd	TS	60	83	25	5
sGnRHa	10 µg[d, e]	–	–	DOM	5 mg	–	IP	14	12L:12D	1 [S4]	CS	100	129	74.6	3
MTC	–	–	–	MTC	11 mg	–	IM	17–19	12L:12D	nd	TS	0	–	–	5

NaCl	+	+	–	–	–	24	nd	10–12	nd	2–3 [S4]	CS	25	72–120	34.1	1
	+	–	–	–	–	–	IM	14	14L:10D	2–3 [S4]	CS	25	22	44.1	6
	+	+	–	–	–	24	IM	10–11	14L:10D	2–3 [S4]	CS	17	64–68	7.5	4
	+	–	–	–	–	–	IM	13–15	12L:12D	nd	TS	–	–	–	7
	+	–	–	–	–	–	IP	14	14L:10D	1 [S6]	TS	–	–	–	2
	+	–	–	–	–	–	IM	17–19	12L:12D	nd	TS	100	108	43	5
	+[d]	–	–	–	–	–	IP	14	12L:12D	1 [S4]	CS	0	–	–	3
	+[b, c]	–	–	–	–	–	IP	14	14L:10D	nd	CS	0	–	–	8

References: 1. Kucharczyk et al. 1996, 2. Żarski et al. 2011a, 3. Targońska et al. 2014, 4. Kucharczyk et al. 2001, 5. Ronyai and Lengyel 2010, 6. Kucharczyk et al. 1998, 7. Kouril et al. 1997, 8. Szczerbowski et al. 2009

nd no data, *mGnRHa* mamalian analogue of gonadoliberine, *sGnRHa* salmon gonadoliberine analogue, *AN-GnRH* azagly-nafaraelin, *CPE* carp pituitary extract, *hCG* human chorionic gonadotropin, *MTC* metoclopramide, *PIM* pimozide, *DOM* domperidone, *S4* according to 4-stage classification of oocyte maturation stages, *S6* according to 6-stage classification of oocyte maturation stages (according to Żarski et al. 2011a), *TS* tank spawning, *CS* controlled spawning (eggs stripped manually, artificial fertilization etc.), *IP* injection applied intraperitoneally, *IM* injection applied intramuscularly (at the dorsal part)

[a]Analogue contained in the commercial preparation Ovopel (complex with metoclopramide)

[b]Spawning performed out-of season with 60 day-long period of temperature below 10 °C

[c]Spawning performed out-of season with 90 day-long period of temperature below 10 °C

[d]Out-of season

[e]Analogue contained in the commercial preparation Ovaprim (complex with domperidone)

[f]Temperature regime following initial injection

[g]Latency time between first injection and ovulation

[h]Embryonic survival determined at the eyed-egg stage

[i]Unknown moment of embryonic survival determination,

(+) fish were treated with saline (placebo group)

Table 4.2 Hormonal preparations, general reproductive methods applied and results of artificial reproduction of yellow perch, *Perca flavescens*

Spawning agent	The dose (per kg of body weight)		Dopamine antagonist dose (per kg of body weight)[h]	Interval between injections (h)	Method of injection	Temperature (°C)[j]	Photoperiod	Spawning method	Ovulation rate (%)	Latency time (h)[k]	Embryonic survival (%)	Reference
	I injection	II injection										
CPE	1 mg	–	–	–	nd	14	nd	TS	100	96–120	58.6–85	1[f, g]
hCG	230 IU	–	–	–	nd	14	nd	nd	100	96–120	nd	1[f, g]
mGnRHa	10 µg[a]	100 µg	10 mg[i]	48	IP	13	12L:12D	CS	50	nd[n]	nd	2
	10 µg[b]	100 µg	10 mg[i]	48	IP	13	12L:12D	CS	50	96–144	nd	2
	10 µg[c]	100 µg	10 mg[i]	48	IP	13	12L:12D	CS	100	96–144	82.7[m]	2
	100 µg[c]	–	10 mg	–	IP	13	12L:12D	CS	100	96–145	84.4[m]	2
	300 µg[c]	–	10 mg	–	IP	13	12L:12D	CS	100	96–146	78.3[m]	2
	10 µg[d]	100 µg	10 mg[i]	48	IP	12–13	15L:9D	CS	100	0–72[l]	48.8[m]	3
	10 µg[e]	100 µg	10 mg[i]	48	IP	12–13	15L:9D	CS	100	0–120[l]	25.3[m]	3
	10 µg[d]	100 µg	10 mg[i]	48	IP	12–13	10L:14D	CS	70	192–288	76.2[m]	3
	10 µg[e]	100 µg	10 mg[i]	48	IP	12–13	10L:14D	CS	80	144–240	87.1[m]	3

NaCl	+[a, b]	+	–	48	IP	13	12L:12D	CS	0	–	–	2
	+[c]	+	–	48	IP	13	12L:12D	CS	20	–	96.4[m]	2

References: 1. Kayes 1977 (following Craig 2000; Dąbrowski et al. 1996), 2. Dąbrowski et al. 1994, 3. Ciereszko et al. 1997

nd no data, '+' fish were treated with saline (placebo group) at a volume of 0.05 mL per fish, *mGnRHa* mamalian analogue of gonadoliberine, *hCG* human chorionic gonadotropin, *CPE* carp pituitary extract, *TS* tank spawning, *CS* controlled spawning (eggs stripped manually, artificial fertilization etc.), *IP* injection applied intraperitoneally

[a]Spawning performed in February

[b]Spawning performed in April

[c]Spawning performed in May

[d]For spawning 2-year old pond reared fish were used

[e]For spawning 3-year old pond reared fish were used

[f]Data taken from Craig 2000

[g]Data taken from Dąbrowski 1996

[h]As a dopamine antagonist pimozide was used

[i]Applied only with the first injection

[j]Temperature regime following initial injection

[k]Latency time between first injection and ovulation

[l]Spontaneous ovulation was recorded just before and just after first injection

[m]Embryonic survival determined at the eyed-egg stage

[n]Ovulation occurred within 3 weeks following injection

Table 4.3 Hormonal preparations, general reproductive methods applied and results of artificial reproduction of pikeperch, *Sander lucioperca*

Spawning agent	The dose (per kg of body weight)		Dopamine antagonist dose (per kg of body weight) [d]	Interval between injections (h)	Method of injection	Temperature (°C)[f]	Photoperiod	Maturity stage prior to hormonal stimulation	Spawning method	Ovulation rate (%)	Latency time (h)[g]	Embryonic survival (%)	Reference
	I injection	II injection											
hCG	500 IU	–	–	–	IP	13	12L:12D	3–4 [S6]	CS	100	96–120	88.3[h]	1
	500 IU	–	–	–	IP	15	12L:12D	3–4 [S6]	CS	100	72–96	84.4[h]	1
	500 IU	–	–	–	IP	12	nd	2 [S6]	CS	75	78–98	71.3[h]	2
	500 IU	–	–	–	IP	12	nd	3 [S6]	CS	100	57–78	73.3[h]	2
	500 IU	–	–	–	IP	12	nd	4 [S6]	CS	100	48–58	77[h]	2
	500 IU	–	–	–	IP	12	nd	5 [S6]	CS	83	32–49	76.5[h]	2
	500 IU	–	–	–	IP	12	nd	6 [S6]	CS	80	5–30	79.5[h]	2
	200 IU	200 IU	–	24	IP	14.5	nd	1–2 [S4]	CS	83.3	75	72.4[i]	3
	200 IU	500 IU	–	24	IP	14.5	nd	1–2 [S4]	CS	100	77	68[i]	3
	600 IU	–	–	–	IP	nd	nd	2 [S4]	CS/TS	100	60	nd	4
	150 IU	500 IU	–	48	IP	nd	nd	2 [S4]	CS/TS	100	133	nd	4
	250 IU	–	–	–	IM	15	14L:10D	2–4 [S4]	CS	71	85	70.9[k]	5
	500 IU	–	–	–	IM	16	14L:10D	2–4 [S4]	CS	100	78.1	84.2[k]	5
	750 IU	–	–	–	IM	17	14L:10D	2–4 [S4]	CS	100	78.6	86.8[k]	5
	1000 IU	–	–	–	IM	18	14L:10D	2–4 [S4]	CS	83	88	52.5[k]	5
PG–600	500 IU	–	–	–	IP	13	12L:12D	3–4 [S6]	CS	83	96–120	80.3[h]	1
	500 IU	–	–	–	IP	15	12L:12D	3–4 [S6]	CS	83	72–96	78.3[h]	1
hCG + CPE	200 IU[b]	3 mg	–	24	IM	15.5–16.7	12L:12D	nd	CS/TS	100	94	91[j]	6
CPE	4 mg	–	–	–	IP	13	12L:12D	3–4 [S6]	CS	67	72–96	78.2[h]	1
	4 mg	–	–	–	IP	15	12L:12D	3–4 [S6]	CS	67	48–72	74.9[h]	1
	3 mg[b]	3 mg	–	24	IM	15.5–16.6	12L:12D	nd	CS/TS	100	96	93[j]	6
	3 mg[b, c]	3 mg	–	24	IM	15.5–16.6	12L:12D	nd	CS/TS	94	81.5	86.5[j]	6

mGnRHa	5 µg	10 µg	2.5 + 5 mg	24	IP	14.5	nd	1–2 [S4]	CS	0	–	–	3
	5 µg	20 µg	2.5 mg + 10 mg	24	IP	14.5	nd	1–2 [S4]	CS	50	94	3.2[i]	3
	1 µg	–	–	–	IM	19	14L:10D	2–4 [S4]	CS	86	89.3	52.3[k]	5
	2.5 µg	–	–	–	IM	20	14L:10D	2–4 [S4]	CS	71	84.42	65.5[k]	5
	5 µg	–	–	–	IM	21	14L:10D	2–4 [S4]	CS	71	83.9	51.1[k]	5
	10 µg	–	–	–	IM	22	14L:10D	2–4 [S4]	CS	71	79.4	52.2[k]	5
	25 µg	–	–	–	IM	23	14L:10D	2–4 [S4]	CS	100	93	60.5[k]	5
	50 µg	–	–	–	IM	24	14L:10D	2–4 [S4]	CS	86	86.5	22.1[k]	5
	40 µg[a]	–	20 mg	–	IP	13	12L:12D	3–4 [S6]	CS	67	96–120	80.3[h]	1
	40 µg[a]	–	20 mg	–	IP	15	12L:12D	3–4 [S6]	CS	67	72–96	72.3[h]	1
	2 µg[a, b, c]	2 µg	1 + 1 mg	24	IM	15.5–16.7	12L:12D	nd	CS/TS	85	102	72[j]	6
O–GnRHa	5 µg[b]	15 µg	–	24	IM	15.5–16.5	12L:12D	nd	CS/TS	100	110	43[j]	6
	5 µg[b]	15 µg	10 mg[e]	24	IM	15.5–16.5	12L:12D	nd	CS/TS	50	117	56[j]	6
NaCl	+	–	–	–	IP	13	12L:12D	3–4 [S6]	CS	17	144	–	1
	+	–	–	–	IP	15	12L:12D	3–4 [S6]	CS	–	–	–	1
	+	+	–	24	IP	14.5	nd	1–2 [S4]	CS	50	100	70.5[i]	3
	+	–	–	–	IM	25	14L:10D	2–4 [S4]	CS	0	–	–	5

References: 1. Żarski et al. 2013a, 2. Żarski et al. 2012a, 3. Zakęś and Demska-Zakęś 2005, 4. Korbuly et al. 2010, 5. Kristan et al. 2013, 6. Ronyai 2007

nd no data, '+' fish were treated with saline (placebo group), *mGnRHa* mamalian analogue of gonadoliberine, *hCG* human chorionic gonadotropin, *PG-600* mixture of hCG and PMSG (pregnant mare serum gonadotropin) in the proportion of 1:2, *O-GnRH* ovurelin, *CPE* carp pituitary extract, *S4* according to 4-stage classification of oocyte maturation stages, *S6* according to 6-stage classification of oocyte maturation stages (according to Żarski et al. 2012a), *TS* tank spawning, *CS* controlled spawning (eggs stripped manually, artificial fertilization etc.), *IP* injection applied intraperitoneally, *IM* injection applied intramuscularly (at the dorsal part)

[a]Analogue contained in the commercial preparation Ovopel (complex with metoclopramide)

[b]Spawning performed out-of season

[c]Average of the two treatments

[d]Metoclopramide was used as a dopamine antagonist

[e]Applied with first injection only

[f]Temperature regime following initial injection

[g]Latency time between first injection and ovulation

[h]Embryonic survival determined 72 h following fertilization

[i]Embryonic survival determined at the eyed-egg stage

[j]Embryonic survival determined 16–18 h following fertilization

[k]Embryonic survival determined at hatching

Table 4.4 Hormonal preparations, general reproductive methods applied and results of artificial reproduction of walleye, *Sander vitreum*

Spawning agent	The dose (per kg of body weight)		Interval between injections (h)	Method of injection	Temperature (°C)[b]	Photo-period	Maturity stage prior to hormonal stimulation[g]	Spawning method	Ovulation rate (%)	Latency time (h)[c]	Embryonic survival (%)	Reference
	I. Injection	II. Injection										
CPE	13,4 mg	13,4 mg	72	IP	10	N	nd	ST	100	nd	nd	1
	3,1 mg	2 mg	72	IP	10	N	nd	ST	67	nd	nd	1
	13,1 mg	–	–	IP	10	N	nd	ST	50	nd	nd	1
hCG	500 IU	–	–	IM	9–10	nd	3	CS	40	96–120	92[e]	2
	500 IU	–	–	IM	9–10	nd	2	CS	25	96	nd	2
	150 IU[a]	500 IU	35	nd	nd	nd	nd	CS	50	155–179	nd	3
	150 IU	500 IU	48	IM	10	12L:12D	1	CS	60	120	41,4[f]	4
	150 IU	500 IU	48	IM	10	12L:12D	2	CS	100	120	71,7[f]	4
	220 IU	–	–	IP	10–14	N	nd	ST	69	24–120+	nd	5
	440 IU	–	–	IP	10–15	N	nd	ST	57	0–95[d]	nd	5
	660 IU	–	–	IP	10–16	N	nd	ST	74	0–120+[d]	nd	5
	880 IU	–	–	IP	10–17	N	nd	ST	68	0–120+[d]	nd	5
	1100 IU	–	–	IP	10–18	N	nd	ST	78	0–120+[d]	nd	5
	1320 IU	–	–	IP	10–19	N	nd	ST	75	24–119	nd	5
	1540 IU	–	–	IP	10–20	N	nd	ST	70	0–120+[d]	nd	5
hCG + DHP	150 IU	2 mg	48	IM	10	12L:12D	1	CS	40	48–120	1,4[f]	4
	150 IU	2 mg	48	IM	10	12L:12D	2	CS	100	120–144	0[f]	4
DHP	100 µg	–	–	IM	9–10	nd	2	CS	0	–	–	2
mGnRHa	100 µg	–	–	IM	9–10	nd	3	CS	60	72–120	90[e]	2
	100 µg	–	–	IM	9–10	nd	2	CS	0	–	–	2
	35 µg	100 µg	48	IM	10	12L:12D	1	CS	20	120	62,8[f]	4
	35 µg	100 µg	48	IM	10	12L:12D	2	CS	100	120	30,6[f]	4

NaCl	+	–	–	IM	9–10	nd	2	CS	0	–	–	2
	+	–	–	IM	9–10	nd	3	CS	0	–	–	2
	+	+	48	IM	10	12L:12D	1	CS	0	–	–	4
	+	+	48	IM	10	12L:12D	2	CS	0	–	–	4
	+	–	–	IP	10–13	N	nd	ST	40	24–119	nd	5
RS	2 mL	–	–	IP	11	N	nd	ST	0	nd	nd	1

References: 1. Lessman 1978, 2. Barry et al. 1995, 3. Dąbrowski et al. 2000, 4. Malison et al. 1998, 5. Hearn 1980

nd no data, '+' fish were treated with saline (placebo group), *mGnRHa* mamalian analogue of gonadoliberine, *hCG* human chorionic gonadotropin, *CPE* carp pituitary extract, *DHP* 17a,20b-di-hydroxy-4-pregnen-3-one, *RS* control group received 'Ringer's' solution (for details see Lessman 1978), *CS* controlled spawning (eggs stripped manually, artificial fertilization etc.), *ST* eggs were stripped manually and no fertilization was made, *IP* injection applied intraperitoneally, *IM* injection applied intramuscularly (at the dorsal part), *N* photoperiod was natural (coincident with the daily cycle)

[a]Spawning performed out-of season

[b]Temperature regime following initial injection

[c]Latency time between first injection and ovulation

[d]Some females ovulated at the day of the injection, i.e. up to 23 h post injection

[e]Embryonic survival determined 2 days following fertilization

[f]Embryonic survival determined at the eyed-egg stage

[g]Maturation stage was determined on the basis of four-stage classification

Kucharczyk et al. 1996, 1998; Kouril et al. 1997; Malison et al. 1998; Zakęś and Demska-Zakęś 2009; Żarski et al. 2011b, 2013a; Křišťan et al. 2012a). More recently it was shown that thermal manipulations could be a very useful tool in synchronization of ovulation in pikeperch (Żarski et al. 2013a). However, in order to evaluate possible reaction to such manipulation, and consequently apply it successfully in artificial reproduction, more studies are required to include species- and population-specific differences.

In the practice of hormonal stimulation two hormonal therapies are generally used. First, is to stimulate releasing of endogenous gonadotropins (GtH) from pituitary gland with the application of preparations containing gonadoliberine (GnRH, for more details see Sects. 4.2 and 4.3). The second one is to inject fish with GtH containing preparations (e.g. carp pituitary extract [CPE], human chorionic gonadotropin [hCG]) directly affecting Leydig cells in the testis or follicle cells in ovaries. This results in production of maturation inducing steroid (MIS) promoting final maturation of gametes and, in consequence, spermiation or ovulation (for more details see Sects. 4.2 and 4.3).

In the case of wild females, the necessity of hormonal stimulation during the spawning season mainly depends on the maturity stage of females (Kucharczyk et al. 2007). Fish at the latest maturation stage VI (after the GVBD; according to the classification of Żarski et al. 2011a, 2012a) would most likely ovulate without the hormonal treatment. However, the moment of ovulation is then very hard to predict. Fish at less advanced maturational stages very often may not ovulate (Kucharczyk et al. 2001; Żarski et al. 2011a, 2013a) or ovulation is extended up to few weeks and its effectiveness is usually very low (Dąbrowski et al. 1994; Kucharczyk et al. 1996, 1998; Ronyai and Lengyel 2010). Reproductive effectiveness in such cases may also depend on other factors such as temperature (Dąbrowski et al. 1994) or possible pheromonal stimulation which was proven to promote GVBD in pikeperch (Barry et al. 1995) or FOM in Eurasian perch (Żarski 2012). Such differences may also stem from handling stress affecting reproductive effectiveness negatively (Schreck et al. 2001; Wang et al. 2006). It is then almost not possible to predict the timing of ovulation and therefore perform *in vitro* fertilization. Also, the effectiveness of artificial reproduction of wild fish may be dependent on the time of fish capture. Kestemont et al. (1999) reported lower egg quality in late spawning season (when fish are the most advanced in maturation and hormonal treatment may be not necessary) compared with those obtained earlier during the spawning period. That is why many authors have focused on the development of hormonal treatment protocols allowing higher synchronization and predictability of ovulation in females of percids.

In percids the most commonly applied hormonal preparations are human Chorionic Gonadotropin (hCG) and GnRH analogues (GnRHa) while carp pituitary extract (CPH) was used less frequently (see Tables 4.1, 4.2, 4.3 and 4.4). Although, application of GnRHa in the case of percids was reported to be effective without dopamine antagonist (DA) injection, contrarily to some other fish species such as

the cyprinids (Kucharczyk et al. 2005, 2008), in many cases administration of DA was reported (see Tables 4.1, 4.2, 4.3 and 4.4). Interestingly, in some cases such preparations (GnRHa+DA) were reported to affect ovulation rate and egg quality negatively (Zakęś and Demska-Zakęś 2009), whereas GnRHa administered alone revealed usually satisfied results (Kouril et al. 1997; Schlumberger and Proteau 1996; Ronyai and Lengyel 2010). However, it was reported in Walleye (Barry et al. 1995; Malison et al. 1998) and pikeperch (Żarski et al. 2013a) that the effectiveness of GnRHa could be also dependent on the maturity stage of the females where reproductive success was higher when more advanced in maturation fish were spawned.

Induction of ovulation is very often dependent on the dose of the hormone applied. In percids the most commonly applied preparation, which is hCG, was proved to be effective within a relatively wide range of doses, from 200 to 5700 IU per kg of females body weight. The most commonly applied dose ranged between 400 and 500 IU kg^{-1} (Tables 4.1, 4.2, 4.3 and 4.4).

The most effective doses of GnRHa (administered with 10 mg kg^{-1} of pimozide which is a DA) in yellow perch ranged between 110 (administered in two doses of 10 and 100 μg kg^{-1} respectively; pimozide was administered with the first dose) and 300 μg kg^{-1} (Dąbrowski et al. 1994). Similarly, injection of Eurasian perch with 125 μg kg^{-1} resulted in higher spawning and fertilization rate as compared to groups treated with 25 μg kg^{-1} (Kouril et al. 1997). In walleye a single GnRHa dose of 100 μg kg^{-1} was effective to cause GVBD (Barry et al. 1995). The effective dose of mammalian GnRHa (administered together with DA – metoclopramide) in Eurasian perch was 22 μg kg^{-1} (Kucharczyk et al. 2001) and in pikeperch 40 μg kg^{-1} (Żarski et al. 2013a). Malison et al. (1998) reported that doubled injection of GnRHa (35 and 100 μg kg^{-1}) caused ovulation only in walleye females with already migrating GV, as compared to the fish with oocyte exhibiting GV in the central position (less advanced in maturation). Such differences in effectiveness of GnRHa application could stem from difference in both fish maturation stages (Żarski et al. 2013a) and activeness of GnRHa forms (amino acid sequence), as already reported in other fish species (Podhorec and Kouril 2009; Targońska et al. 2010).

Application of CPH was very often performed in two doses, with the first one (priming dose) constituting 10–50 % of the second (resolving) one. In general, the average applied total doses ranged between 3 and 5 mg kg^{-1} (Tables 4.1, 4.2, 4.3 and 4.4). However, in pikeperch (Müller et al. 2004) and walleye up to 15 mg kg^{-1} and 26.8 mg kg^{-1} (in two equal doses) (Lessman 1978) were used, respectively. Even such high doses seemed to be not harmful to the fish. Nevertheless, the data on the application of this spawning agent are quite limited, as compared to the other hormonal preparations.

As mentioned above, when considering reproduction of wild females of percids, the procedures which may be undertaken (photothermal manipulation, hormonal and/or pheromonal stimulation) are strictly dependent on the maturational stages of

females. And the biggest problem is that wild females during the spawning season, even caught from the same population and at the same time, are usually at different maturity stages (from I to VI as described by Żarski et al. 2011a, 2012a). It is worth mentioning that stimulation with hCG at a maturation stage I and II (according to the classification given by Żarski et al. 2011a, 2012b, c) resulted in lower ovulation and fertilization rates compared with the fish hormonally stimulated at stages III–VI. Thus, it may be recommended to reproduce wild percids females at a stage III or later.

4.4.2.5 Gamete Collection

Collection of gametes in percids does not create any difficulties as it is in sturgeon females where special surgical techniques are necessary for obtaining the eggs from the genital pore (Mims et al. 2004; Pourasadi et al. 2009) or in catfish males where testes have to be dissected to extract a sufficient amount of sperm (Legendre et al. 1996; Bokor et al. 2010). For the artificial spawning purposes collection of sperm and eggs in percids is proceeded through simple stripping (gentle massaging of the abdomen parts of the fish body) (Rinchard et al. 2011; Kucharczyk et al. 2007; Zakęś and Demska-Zakęś 2009; Żarski et al. 2011a, 2012a).

In males there is a need for taking care to avoid urine contamination of stripped semen when the sperm is collected into the syringes (or any other container) because spermatozoa might have been activated by the urine (Bokor et al. 2007). This can lead to low motility rate of spermatozoa, even after short period of storage (Satterfield and Flickinger 1995a) and, consequently, affect fertilization rate negatively. A possible method is to use a catheter for sperm collection (Bokor et al. 2007; Grozea et al. 2008; Korbuly et al. 2010). It was previously reported that such method allows avoiding urine contamination in salmonids (Glogowski et al. 2000). Another way is to strip the sperm shortly before using it for fertilization (Kucharczyk et al. 2007). More details are provided in the Chap. 5.

Collection of eggs is very simple when fish is taken for stripping shortly after ovulation (Fig. 4.6). However, it exist huge differences among percids regarding the type of ovulated eggs. Eggs of pikeperch and walleye are released as a batch of separated eggs, whereas Eurasian and yellow perch eggs are included within a large, cylindrical gelatinous strand called ribbon (Probst et al. 2009; Formicki et al. 2009, see also Chap. 2). This creates some differences in the in vitro fertilization procedures. Eggs obtained from few pikeperch or walleye females are usually mixed in one bowl and are fertilized according to the "dry" method which involves mixing of eggs and sperm before activation with activating solution. In the case of both perch species it is recommended to fertilize the eggs from each female separately in order to avoid covering one egg ribbon by another, as already reported in Eurasian perch by Żarski et al. (2012d).

One of the biggest obstacles in percids reproduction is to get the 'dry' eggs. This stems from the fact that females are able to release eggs in the tank spontaneously (Żarski et al. 2011a, 2012a). In order to get the dry eggs a frequent control of ovulation, sometimes even every 1 h (Křišťan et al. 2013), is necessary. This, in

Fig. 4.6 Eggs stripping from Eurasian perch (on the *left*) and pikeperch (on the *right*). In the case of Eurasian perch eggs in the form of 'ribbon' may be noticed (Photo: S. Krejszeff)

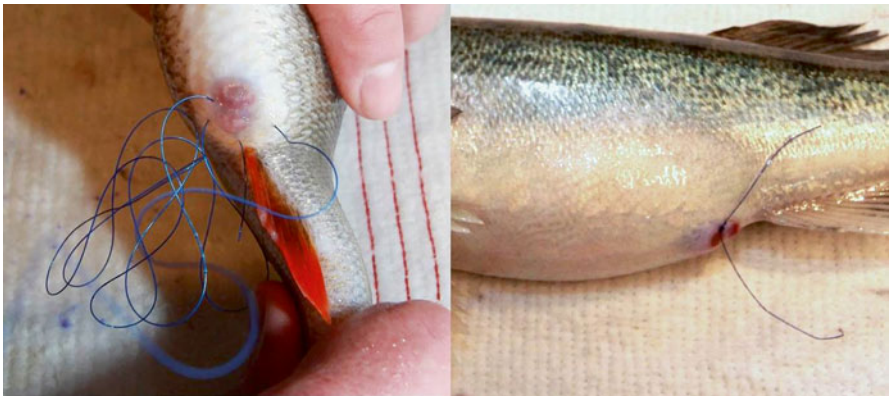

Fig. 4.7 A genital pore of Eurasian perch female being sewn up with the sterile surgical thread (on the *left*) and female pikeperch with sewn up genital pore (on the *right*) (Photo: S. Krejszeff)

turn, causes huge stress to the fish what can presumably affect the egg quality and health status of the female (e.g. Lessman 1978). Therefore, in some cases in Eurasian perch the genital pore of the fish was sewn up to prevent the spontaneous release of eggs in the tank (Kucharczyk et al. 1996, 1998, 2001). This method, with the high attention paid to the sterility of the procedure performed, was also successfully applied to pikeperch (Żarski, unpublished data, Fig. 4.7). Recently, it was found that the eggs of pikeperch may be 'stored' in the ovaries of the female for at least 12 h following ovulation at a temperature of 15 °C without negative effect on eggs viability (Mohagheghi Samarin, Blecha, Policar, unpublished data). Although, the 'operation' of the sewing up the genital pore of the fish may be quite laborious, this method is very promising alternative when the 'dry' eggs are absolutely essential from the breeding point of view.

4.4.2.6 *In Vitro* Fertilization

The *in vitro* fertilization is very important part of finfish controlled reproduction. This process involves exposure of the gametes (eggs and sperm) in a medium (so called 'activating solution' [AS]) which is able to activate sperm and eggs at the same time (Żarski et al. 2015b). After the contact with water (or any other activating solution) eggs acquiring the ability to be fertilized (Coward et al. 2002; Minin and Ozerova 2008). This ability retains for a certain period, which for many fish species is unknown. Żarski et al. (2012d) found, that eggs of Eurasian perch eggs remain active up to 2.5 min post-activation with hatchery water. However, the sperm after activation was motile only up to 37 s. And the spermatozoa motility rate was highly decreasing in time. Alavi et al. (2007) reported that only 7 % of spermatozoa are motile after 45 s post activation, whereas almost 92 % motility is recorded after 15 s post activation. Taking into account that eggs of cyprinids (Żarski et al. 2015b, 2014) and pikeperch (Żarski et al. 2013b) remain active for about 1 min following activation, eggs of Eurasian perch retain the fertilizability for a relatively long period of time. Interestingly, it was reported, that application of the 'dry method' for fertilization in this species (eggs and sperm mixed first and then exposed to AS; e.g. Dąbrowski et al. 1994) affected lower fertilization rate in comparison to the method where eggs were activated first and after 15 s sperm was added (Żarski et al. 2012d). This phenomenon was the most probably related with the fact that eggs within the ribbon upon activation are covered one by another. This enables to penetrate all the eggs by spermatozoa, which very suddenly lose their motility (see Chap. 5) and ability to successfully fertilize the eggs. Therefore for procedure of *in vitro* fertilization in Eurasian perch it may be recommended to activate the eggs first and next, after 15–30 s, add the sufficient amount of sperm (Fig. 4.8). This should allow spreading the whole ribbon within the activating solution and exposing all the eggs to the active spermatozoa, when added. Moore (2003) also reported that repeated addition of sperm to the eggs (at 0, 30 and 60 s post-activation of eggs) improved fertilization effectiveness in walleye, although mechanism is unclear since there is no data on the duration of eggs and sperm activation. Considering the fact that eggs of pikeperch lose their fertilizability very suddenly (within first minute following activation; Żarski et al. 2013b), it may be suggested that the observations made by Moore (2003) in walleye stemmed from other reasons. Nevertheless, in the view of the recently published data and considering the high variability in the fertilization rate usually observed in percid fishes there is still need for development of novel techniques of eggs fertilization (Satterfield and Flickinger 1995b; Rinchard et al. 2005; Zakęś and Demska-Zakęś 2009; Żarski et al. 2011a, 2012d).

For the purposes of *in vitro* fertilization as an activating solution (AS) the most commonly 'hatchery water' was used. However, composition of such AS is not standardized and parameters (e.g. osmolality, ionic composition) are different for different hatcheries. That is why more often other AS-s are used instead of hatchery water, which are very often precisely formulated and standardized. Different

Fig. 4.8 A procedure of *in vitro* fertilization of eggs of Eurasian perch (according to Żarski et al. 2012d). Eggs are first activated with 'hatchery water' in order to spread the egg-ribbon in the water (in order to avoid of covering the eggs one by another) (on the *left*) and after 15–30 s sperm is added (on the *right*) (Photo: S. Krejszeff)

activating solutions have been tested only in Eurasian perch so far. It was proven that application of the Woynarovich solution (3 g of urea and 4 g of NaCl in 1 L of distilled water; Woynarovich and Woynarovich 1980) may improve the fertilization as compared to hatchery water. It stems from the fact that in Woynarovich solution eggs retain the ability to be fertilized for over 3 min while motility of spermatozoa was observed up to 89 s post activation (Żarski et al. 2012d). Prolonged motility of percids spermatozoa after the application of specifically formulated AS-s was reported earlier (for details see Chap. 5). However, the possibility of application of those AS-s for *in vitro* fertilization remains unknown. Especially, when it was already reported that AS which successfully activated sperm motility was not suitable for fertilization (Saad and Billard 1987; Żarski et al. 2014). Considering the fact that application of AS-s other than hatchery water (such as Woynarovich solution in Eurasian perch) may bring huge benefits for the fish farmers increasing fertilization effectiveness, more work is needed aiming at the development of the activating solutions specifically dedicated to the particular species.

One of the most important aspect of *in vitro* fertilization is to use proper amount of sperm per particular amount of eggs, what is called in the literature as a "sperm:egg ratio" (e.g. Linhart et al. 2006). Rinchard et al. (2005) reported, that maximization of fertilizing ability in walleye may be secured with the minimal sperm:egg ratio of 25,000: 1. On the other hand, Casselman et al. (2006) reported that only 5000 of spermatozoa per egg affected very high (over 90 %) survival rate of embryos in this species. Such contrary results may stem from the fact that different methods for estimation of sperm concentration were used (spermatocrit method by Casselman et al. (2006) and counting chambers method by Rinchard et al. (2005)). Most recently, Křišťan et al. (2012b) found that 100,000 spermatozoa per egg may be

considered as a secure sperm:egg ratio in pikeperch. Mean pikeperch sperm concentration may be considered at a level of 20×10^9 spermatozoa per mL (see e.g., Zakęś and Demska-Zakęś 2009; Bokor et al. 2008). Whereas, mean number of eggs may be considered at a level of 1000 eggs g^{-1}, because recorded number of "dry eggs" (before activation) per gram ranged approximately between 800 and 1300 (e.g., Bokor et al. 2008, Żarski, unpublished data). Considering the above mentioned it may be recommended to use 0.5 mL of very good quality sperm for each 100 g of eggs in pikeperch and in walleye. Until now, no published data about the effect of sperm:egg ratio on fertilization effectiveness in Eurasian and yellow perch are available.

4.4.2.7 Eggs Treatment, Incubation and Hatching

After *in vitro* fertilization eggs need to be incubated under optimal conditions. In Eurasian and yellow perch incubation of eggs is usually performed without any problem as far as oxygenated water flow is provided washing all the eggs within the whole ribbon. For that purpose small floating cages or flow-through chambers have been successfully used (Kucharczyk et al. 1996; Żarski et al. 2011b, Fig. 4.9). However, pikeperch and walleye eggs are getting to be sticky after their contact with water. Thus, prior to incubation in regular hatchery devices (such as Weiss or Zuger jars), an unsticking procedure must be applied. For that purpose talc with sodium chloride (Schlumpberger and Schmidt 1980; Steffens et al. 1996; Kucharczyk et al. 2007), enzymes (Krise et al. 1986, 1988; Zakęś and Demska-Zakęś 2009) and

Fig. 4.9 An incubation of the Eurasian perch egg-ribbons on the wooden frame covered with a net (mesh size 0.5 mm) from the bottom (on the *left*) and in the floating plastic 'cages' where bottom was replaced with the net (on the *right*) (Photo: S. Krejszeff)

tannic acid (sometimes preceded by immersion in Woynarovich solution; Bokor et al. 2008) have been used (Demska-Zakęś et al. 2005; Rinchard et al. 2005, 2011; Kucharczyk et al. 2007). In percid fishes the latter compound is the most commonly used method, due to, among others, the low cost of its application. However, opinions about its application for commercial purposes were very often inconsistent (Demska-Zakęś et al. 2005; Kucharczyk et al. 2007; Zakęś and Demska-Zakęś 2009). A procedure involving 2–5 min baths in a tannic acid solution a few min following fertilization, with concentrations ranging between 0.4 and 1.5 g L^{-1}, was usually applied (Czesny et al. 2005; Demska-Zakęś et al. 2005; Rinchard et al. 2005, 2011; Kucharczyk et al. 2007). Demska-Zakęś et al. (2005) suggested that this procedure affect low survival rate of embryos or eggs did not lose its adhesive properties. However, the most recent studies indicated that the problem of low effectiveness of this method was related with the time of application of tannic acid following eggs activation (Żarski et al. 2015a). It was proven that in the eggs of pikeperch following activation an extreme chorion deformation, caused by the cortical reaction, occurred (see also Chap. 6). This deformation lasted up to 10 min post activation (Żarski et al. 2012b), whereas tannic acid was usually applied shortly after activation (up to 5 min post activation) (Czesny and Dąbrowski 1998; Czesny et al. 2005; Demska-Zakęś et al. 2005; Rinchard et al. 2005, 2011; Kucharczyk et al. 2007). According to the findings of Żarski et al. 2015a the best moment of application of tannic acid (at a dose of 0.75 g L^{-1}) was 30 min post fertilization coinciding with the end of the egg swelling process (Żarski et al. 2012b, 2015a). Such procedure allowed applying tannic acid for removing the pikeperch egg stickiness in commercial hatcheries successfully (Żarski, unpublished data, Fig. 4.10).

Generally, all percids have similar thermal requirements as considering egg incubation. Median thermal tolerance limits (on the base of normal hatched larvae following incubation at a constant temperature from fertilization to hatching) for walleye, Eurasian and yellow perch is actually almost the same and ranging from 6.0–6.8 °C to 19.2–20.9 °C. Only pikeperch eggs require slightly higher thermal regimes ranging from 9.0–10.0 °C to 21.5–24.0 °C (after Hokanson 1977). Koenst and Smith (1976) reported that optimal temperature for incubation of walleye eggs ranged between 9 and 15 °C. For pikeperch eggs incubation temperatures between 12 and 16 °C were recommended (Kokurewicz 1969; Kucharczyk et al. 2007; Zakęś and Demska-Zakęś 2009). Saat and Veersalu (1996) reported that optimal temperatures for Eurasian perch ranged between 8 and 18 °C, while for yellow perch Hinshaw (2006) suggested temperatures from 5 to 15 °C. However, on the base of the literature data Teletchea et al. (2009b) indicated that for walleye, Eurasian and yellow perch thermal regime for incubation should be fixed at 12.5 °C, and 14 °C for pikeperch. At such temperatures incubation of Eurasian and yellow perch lasted 165 degree-days, while eggs of walleye and pikeperch had to be incubated for 140 and 115 degree-days, respectively (Teletchea et al. 2009b).

Hatching of percids larvae is usually an asynchronous event. In some cases it may last even several days among the same egg batch or egg-ribbon. It is important to emphasize that even very low quality larvae (highly malformed) were observed to hatch spontaneously (Żarski et al. 2011b, see also Chap. 6 for more details). It

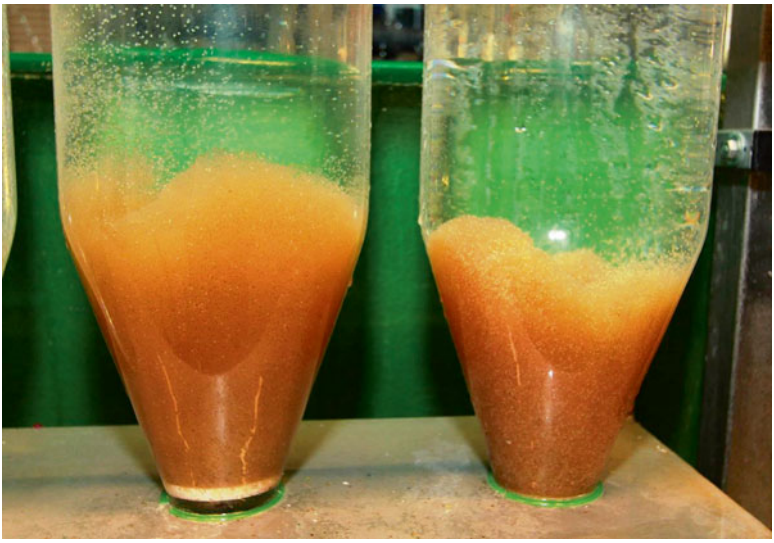

Fig. 4.10 A commercial-size incubation of eggs treated with tannic acid (0.75 g L^{-1}, 30 min following eggs activation) according to the method described by Żarski et al. 2015a (Photo: D. Żarski)

was already reported that early hatched Eurasian perch larvae were characterized by a higher survival rate than those hatched 2 days later. Also, it was proven that joint rearing of larvae hatched at different times may affect survival and growth rate of the larvae significantly (Kestemont et al. 2003, see also Chaps. 9, 10 and 11). Although it is not clear whether the larvae hatching earlier or later are of different biological properties, a special attention must be given to the extension of hatching time, and possible separation of early and late hatched larvae can be recommended before the initial larval rearing stage.

4.5 Conclusions

Based on published data, it may be concluded that percids represent a quite unique group of fish exhibiting specific traits from the perspective of artificial reproduction. For example, one of the most characteristic features is that in this group of fish, hormonal stimulation does not have to be supported by the anti-dopaminergic treatment, which is necessary in other freshwater fishes (cyprinids, catfishes and salmonids). Despite the efforts undertaken in the development of protocols allowing full control over reproduction (mostly synchronization of ovulation and hormonal therapy) in percids, there are still many aspects to be investigated. There is a considerable lack of data regarding hormonal regulation of the final gamete maturation process as well as stress-related effects on these processes. This knowledge may

help to understand the variability of gamete quality, still widely observed in these species. Also, there are many ambiguities related to gamete management, both prior to and following fertilization (e.g. short-term storage, removing the adhesiveness of eggs), which may facilitate and thus improve this important step of artificial reproduction. Therefore, future research on the artificial reproduction of percids should employ modern scientific tools (e.g. proteomics, transcriptomics, etc.) possibly allowing to answer many unanswered questions.

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