CHAPTER 4

ARTIFICIAL AND SEMI-ARTIFICIAL SPAWNING IN EURASIAN PERCH (*PERCA FLUVIATILIS* L.) FOR MASS EMBRYO PRODUCTION

Policar, T., Alavi, S., Stejskal, V., Křišťan, J., Kouřil, J., 2011. Artificial and semiartificial spawning in Eurasian perch (Perca fluviatilis L.) use for mass embryo production. Edition of Methods, FFPW USB, Vodňany, Czech Republic, No. 117, 24 pp. (in Czech)

It was allowed by the publisher on 19th April, 2012 to include the paper in this Ph.D. thesis.

USE OF ARTIFICIAL AND SEMI-ARTIFICIAL SPAWNING IN EURASIAN PERCH (PERCA FLUVIATILIS L.) FOR MASS EMBRYO PRODUCTION

T. Policar, S.M.H. Alavi, V. Stejskal, **J. Křišťan**, J. Kouřil

University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Research Institute of Fish Culture and Hydrobiology, Zátiší 728/II 389 25 Vodňany, Czech Republic

1. INTRODUCTION

1.1. Current importance of Eurasian perch

Rearing of the Eurasian perch (*Perca fluviatilis* L.) has seen an expansion in European aquaculture in the past two decades (Kestemont and Mélard, 2000). High demand (up to 10 000 tonnes per year) for perch fillets is seen mainly in local markets in Alpine countries: Switzerland, Germany, France, and Austria (Watson, 2008; Policar et al., 2009). Consumers in these countries consider the Eurasian perch a delicacy due to its white, low-fat meat and lack of "Y" bones (Watson, 2008; Stejskal et al., 2010).

The Eurasian perch is also effectively used to reduce excessive populations of small and less valued cyprinids in pond culture (Policar et al., 2009; Stejskal et al., 2010), helping to maintain stable production of valuable fish species in the polyculture fish stocks. In reservoirs, Eurasian perch predation pressure ensures adequate levels of zooplankton for improved water quality (Adámek et al., 2010).

1.2. Production of Eurasian perch in Europe

Four methods of production are currently used for Eurasian perch: extensive, semi-intensive, intensive, and commercial fishing from natural waters (Policar et al., 2009).

Extensive production is based on rearing in ponds in three to four year production cycles. In this method, perch is a complementary species in polyculture with the primary production of common carp (*Cyprinus carpio*) (Bláha, 2006).

Semi-intensive production of perch uses a combination of extensive and intensive production. This method is based on broodstock production in ponds, their artificial or semi-artificial reproduction, artificial incubation of eggs and hatching of larvae, and pond rearing of larvae and juveniles to 30–50 mm total length (TL). After being harvested from the ponds, juveniles are adapted to the recirculation aquaculture system (RAS) and to a dry feed diet, and rearing follows intensive methods in RAS (Policar et al., 2009; Stejskal et al., 2010).

Intensive production of perch uses fully controlled breeding in RAS from reproduction of broodstock to the final production of market-size fish. This method uses domestic broodstock, temperature and light regime stimulation of broodstock

gonadogenesis, semi-artificial and artificial spawning, artificial incubation of eggs, rearing of larvae in controlled conditions using nauplii of *Artemia salina* and starter food mixtures (Biomar, Aller Aqua, and others), and rearing of juveniles in RAS to market size (Fig. 1) or for broodstock (Mélard et al., 1996; Kestemont et al., 2008; Policar et al., 2009). Perch are bred in optimal growing conditions (water temperature 23 °C, favourable water quality parameters and feeding), which considerably reduces the time of production compared to extensive methods. High density of intensively bred perch (up to 60 $kg.m^{-3}$) guarantees high commercial success (Mélard et al., 1996).

The final production method of market-size Eurasian perch is commercial fishing from open waters, e.g. large lakes or rivers, a system primarily used in Scandinavia and the former Soviet countries (Watson, 2008).

Figure 1. *Market-size perch (100–150 g).*

1.3. Current market size perch production in Europe

According to current FAO statistics (2011a,b), market-size perch production in 2009 was 23 524 tonnes. The largest portion (99%) (23 264 tonnes) was from wild harvest in European lakes, rivers, and reservoirs. Most were caught in Finland (10 590 tonnes), Russia (8 785 tonnes), Estonia (1 645 tonnes), Poland (838 tonnes), and Switzerland (342 tonnes) (FAO, 2011a). Total production of farmed Eurasian perch in Europe in 2009 was 260 tonnes (FAO, 2011b). Fourteen countries contributed to this production, most importantly Russia (140 tonnes), Ukraine (25 tonnes), FYROM (29 tonnes), Ireland (24 tonnes), and the Czech Republic (18 tonnes).

1.4. Production of broodstock

The successful intensive production of Eurasian perch is dependent on high quality sexually mature males and females. Currently, two methods of production of perch broodstock are used. The first is classical intensive or semi-intensive

production in ponds in polyculture fish stocks (Policar et al., 2009). In this method of production, broodstock is reared mainly on natural food in the form of small prey fish. Gonadogenesis take place under natural conditions without special intervention with respect to light or temperature regime. This allows well-developed gonads and high-quality gametes, ensuring a high rate of egg fertilization and hatching (Fontaine et al., 2008). However, this method of production has a seasonal character (Kouřil et al., 2001), as the broodstock spawn only during the natural spawning period of Eurasian perch (in Europe mostly March to June) (Ashe, 1997; Rougeot et al., 2008). In the Czech Republic, perch broodstock most often spawn from early to mid-April.

A second method of Eurasian perch broodstock production takes place under consistently controlled conditions in RAS (Policar et al., 2009). Management of controlled production must comprise: (1) optimal maintenance of broodstock, which positively affects the development and quality of gonads and gametes (Abi-Ayad et al., 1995; Fiogbé et al., 1996; Kestemont et al., 1996; Abi-Ayad et al., 1997; Fontaine et al., 1997; Kestemont et al., 2001; Xu et al., 2001; Xu and Kestemont 2002; Fiogbé and Kestemont, 2003; Kestemont et al., 2003; Mathis et al., 2003; Fontaine et al., 2008), and (2) optimal environmental conditions, especially temperature and light regime, ensuring that the broodstock enters the spawning season in optimal condition, with spermatogenesis and oogenesis complete (Fontaine et al., 2008). Detailed description of environmental stimulation of spermiogenesis and oogenesis can be found in Abdulfatah et al. (2008); Fontaine et al. (2008); Jansen and Fontaine, (2008) and Policar et al. (2009). For Eurasian perch broodstock the recommended diet includes artificial feed with adequate highly unsaturated fatty acid (HUFA), namely docosahexaenoic (DHA), eicosapentasneoic (EPA), and arachidonic (ARA) acids (Kestemont et al., 2008) at a ratio of 2DHA:1EPA:1ARA (Fontaine et al., 2008). The optimal Eurasian perch broodstock diet ensures high quality eggs, embryos, and larvae.

1.5. Successful spawning and production of high quality larvae

Successful spawning of broodstock to ensure the mass production of quality larvae is basic to successful breeding and production of market-size perch (Policar et al., 2009). Several methods of perch broodstock spawning have been tested and described, together with methods of stimulation and synchronisation of spawning (West and Leonard, 1978; Kayes and Calbert, 1979; Flajšhans and Göndör, 1989; Dabrowski et al., 1994; Kucharczyk et al., 1996, 1998; Kouřil and Linhart, 1997; Kouřil et al., 1997; Kouřil and Hamáčková, 1999, 2000; Policar et al., 2008a,b,c, 2009). This allows effective reproduction of perch broodstock by artificial, semi-artificial, or natural methods. Artificial reproduction includes hormonal induction of ovulation and spermiation, manual stripping of eggs and sperm, artificial fertilization of eggs, and artificial incubation in controlled conditions. In semi-artificial reproduction, as in artificial reproduction, the broodstock is hormonally stimulated, but spawning and fertilization take place naturally. Fertilized eggs are then collected and incubated artificially under controlled conditions. In natural reproduction, fish are not hormonally stimulated and eggs are fertilized naturally and incubated in controlled or in uncontrolled conditions (Policar et al., 2009).

To stimulate the maturation and release of gametes (eggs and spermatozoa), and to synchronize the spawning of broodstock, hormone injections of carp pituitary,

choriogonadotropins, or the synthetic analogue GnRHa in commercial products, e.g.: Supergestran, Dagin, and Chorulon (Dabrowski et al., 1994; Kucharzcyk et al., 1996, 1998; Kouřil and Linhart, 1997; Kouřil et al., 1997; Kouřil and Hamáčková, 1999, 2000; Kouřil et al., 2001; Policar et al., 2008a,b,c), or controlled temperature regime (Policar et al., 2009) can be used.

We tested methods and verified experimental findings with applied research at the Czech aquaculture facility Rybářství Nové Hrady s.r.o. Eurasian perch larvae mass production was evaluated with artificial and semi-artificial reproduction of broodstock hormonally stimulated with the commercial hormonal treatment Supergestran.

2. AIM OF STUDY

The overall goal of this study was to describe, conduct, and verify in practice technology for mass production of Eurasian perch embryos originating from hormonally stimulated artificial and semi-artificial reproduction using the commercial hormone treatment Supergestran. Through this procedure we described (1) the broodstock rearing method in ponds and (2) the preparation of broodstock for the spawning period. We evaluated (3) injection of broodstock with the hormone treatment Supergestran. During the spawning period, we observed the effectiveness (success rate), latency (the period from the hormone injection of female to spawning), the synchronization of artificial and semi-artificial spawning in females, and the fecundity of females and males. We verified (4) procedures for artificial fertilization of eggs obtained by artificial stripping, (5) artificial incubation of eggs, and (6) hatching of embryos from artificial and semi-artificial spawning, Finally (7), we evaluated the post-spawning mortality of broodstock and the use of broodstock after spawning.

3. STUDY LOCATION

The procedures were verified at the Czech fish company Rybářství Nové Hrady s.r.o. (Fig. 2) in 2009–2011. Most trials concerning broodstock pond culture, artificial and semi-artificial reproduction of broodstock, evaluation of female and male fecundity, artificial incubation of fertilized eggs, and hatching of Eurasian perch embryos were conducted at the experimental facility of the University of South Bohemia, Faculty of Fisheries and Protection of Waters (USB FFPW) in Vodňany (Fig. 3).

Figures 2 and 3. *Hatchery of Rybářství Nové Hrady s.r.o. (left) and the experimental facility of USB FFPW in Vodňany (right).*

4. TECHNOLOGY

4.1. Breeding and acquisition of broodstock for mass reproduction trials

4.1.1. Procedures

In 2009–2011, the broodstock population of Eurasian perch for these trials was bred in polyculture with other fish species, common carp (*Cyprinus carpio*), tench (*Tinca tinca*), grass carp (*Ctenopharyngodon idella*), bighead carp (*Hypophthalmichthys molitrix*), pike (*Esox lucius*), pikeperch (*Sander lucioperca*), and European catfish (*Silurus glanis*) in production ponds: Blatec (48°50′28″N, 14°44′55″E), Nakolický (48°48′21″N, 14°50′5″E), Byňovský (48°49′22″N, 14°48′15″E) and Smutný (48°50′7″N, 14°45′43″E). The ponds also contained less valuable small cyprinids such as roach (*Rutilus rutilus*), rudd (*Scardinius erythrophthalmus*), and invasive topmouth gudgeon (*Pseudorasbora parva*). These species comprised the main diet of the Eurasian perch broodstock. In 2009–2011, during the spring harvesting period, four-year-old Eurasian perch broodstock were captured, and fish in apparently good health and condition were selected and transferred to the hatchery of Rybářství Nové Hrady or to USB FFPW in Vodňany, where they were placed in storage ponds (USB FFPW) or in handling ponds (Nové Hrady) together with the forage fish topmouth gudgeon, at a ratio of 1 kg of Eurasian perch to 2 kg topmouth gudgeon.

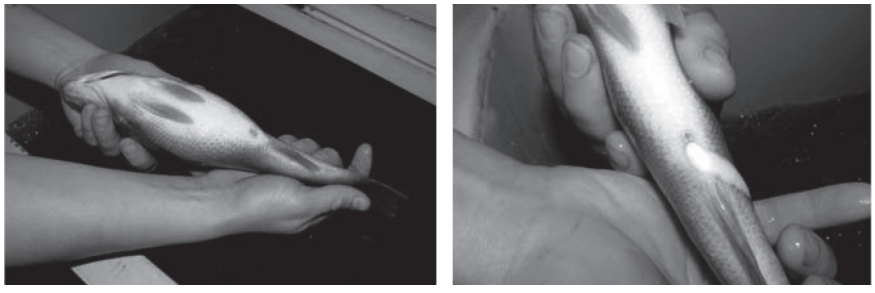
4.1.2. Results

In 2009–2011, 150–160 female broodstock (TL = 215.8 ± 24.5 mm and BW = 187.32 ± 95.0 g) and 150–160 male broodstock (TL = 196.4 ± 18.5 mm and BW = 140.3 ± 76.0 g) were obtained annually for mass artificial and semi-artificial reproduction.

4.2. Manipulation and hormone injection of broodstock

4.2.1. Procedures

After 1–3 weeks of acclimatization, high quality fish with no apparent health problems or skin damage were selected. The selection criterion for females was a full abdomen, characterizing readiness for spawning (Fig. 4). Males were selected that spontaneously released sperm without blood contamination upon abdominal massage (Fig. 5).



Figures 4 and 5. *Suitable female (left) and male (right) broodstock selected for spawning.*

Selected broodstock were divided in two groups of females (n = 60 each) and two groups of males (n = 76 and 60). Spawning took place in controlled conditions at water temperature 15.2 ± 0.2°C and oxygen saturation 8.3 mg $O_2.l^{-1}$ (c. 85% O_2).

For artificial spawning, 60 females were separated into 3 groups of 20 (Fig. 6), and each group was placed in a 0.7 m^3 tank of a semi-recirculating aquaculture system. From the group of 76 males, 60 were randomly designated to fertilize the eggs from artificial spawning and placed in a 6 m^3 tank. The remaining 16, to be used for collection of sperm to determine male fecundity, were placed in a 0.7 m^3 tank. In artificial spawning, males were held separate from female groups.

For semi-artificial spawning, the two remaining groups (n=60 males and 60 females) were separated into 6 groups of 20 broodstock each: 10 females and 10 males. The groups were placed in six 0.7 m^3 tanks.

Figure 6. *Female broodstock separated into groups for artificial spawning.*

For both spawning regimes, fish were acclimatised in tanks over the course of two days, while the water temperature was raised from 11.4 °C (natural temperature) to 15.0–15.5 °C (required temperature). Total length (TL) and body weight (BW) was recorded for all fish. For manipulation, broodstock were anaesthetized 3–4 minutes in a clove oil water bath at 0.03 $ml.l^{-1}$ (Hamáčková et al., 2001; Policar et al., 2009). Fish were tagged with VIE (Visible Implant Elastomer tag, Northwest Marine Technology, Ltd., USA), orange to indicate artificial spawning or red for semi-artificial. All females and 16 randomly selected males intended for collection of sperm to determine male fecundity were tagged for individual identification. The position of the VIE on the head represented the number of the fish within the group, so the fish could be identified during spawning (Fig. 7).

Figure 7. T*agging of broodstock with implanted elastomer tag.*

After tagging, fish were intramuscularly injected under the dorsal fin with Supergestran containing Lecirelin -mGnRHa (D- Tle^{6}, Pro^{9}, Net) containing 25 µg GnRHa.ml^{-1} (Fig. 8). Females were injected with 2 ml kg^{-1} Supergestran, which is equivalent to 50 µg kg^{-1}.GnRHa. All selected males released sperm spontaneously with no hormone stimulation.

Figure 8. *Intramuscular hormone injection.*

4.2.2. Results

Anaesthesia, obtaining biometric measurements of all 256 fish, hormone injection of females, and fish tagging was conducted by four workers over a four hour period.

For artificial and semi-artificial spawning, broodstock of equal size were selected: females TL = 236.4 ± 27.2 mm and BW = 190.2 ± 83.0 g and males TL = 184.2 ± 19.3 mm and BW = 137.6 ± 68.2 g.

No mortality of perch broodstock was observed during the procedure or on the two subsequent days. For hormone stimulation, 22.8 kg of broodstock was injected with a total of 46 ml of Supergestran. Cost of hormone injection was 1 556 CZK (Supergestran 33.82 CZK/1 ml).

4.3. ARTIFICIAL AND SEMI-ARTIFICIAL SPAWNING REGIME

4.3.1. Procedures

4.3.1.1. Control of broodstock before artificial spawning

Twenty-four hours post-injection, examination of broodstock for genital papillae and ovulation was initiated and continued every 6 h. After spawning of the first female, the broodstock were monitored at 3 h intervals. More frequent checking was necessary to prevent spontaneous spawning and release of eggs into the water. Fish were handled carefully to avoid stress and skin damage.

4.3.1.2. Artificial stripping of eggs

Females showing signs of ovulation were transferred to an anaesthesia bath, as during previous manipulation. Before stripping, the abdominal area was dried and eggs were stripped by gentle massage along the abdominal wall. Eggs were stripped into previously weighed and individually tagged dry dishes.

Eggs from each individual were weighed using a Kern PCB 800 balance with accuracy of 0.01 g. Three samples of approximately 1 g each were randomly selected and weighed using a Mettler AE 200 balance with accuracy of 0.001 g. The number of eggs in each sample was counted. To obtain the number of eggs per 1g of egg ribbon, the total number of eggs in the sample was divided by the weight of the sample in grams. To determine the absolute fecundity of a female (the total number of eggs), the number of eggs per 1 g of egg ribbon was multiplied by the total weight of the egg ribbon. The stripping data (date, time, identification number, and absolute fecundity) were recorded. After the spawning period, latency, the period from hormone injection to stripping, expressed in days, in hours, and in degree days, was calculated. The spawning success (percent of stripped females) and synchronization of spawning (number of stripped females per hour) were determined.

The dishes were covered with a damp cloth and placed in a cool area of the hatchery to preserve the eggs for up to 1 h after stripping to allow for the collection of a larger pool of eggs.

4.3.1.3. Sperm Collection

The sperm of 60 selected males was collected into 5–10 ml syringes by gentle massage of the abdomen (Fig. 9). Care was taken to avoid contamination by water, urine, or blood, and sperm was stored at 4–6 ºC. The sperm from a minimum of 3 males was used for artificial insemination of the eggs from each female.

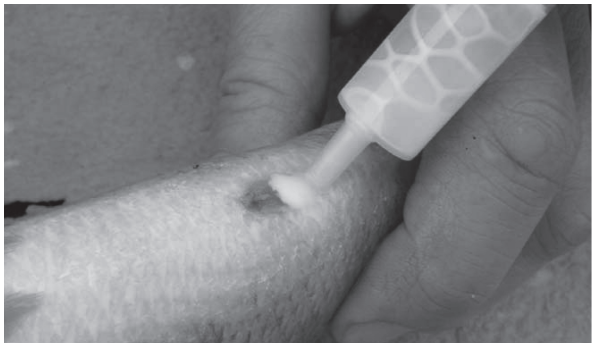
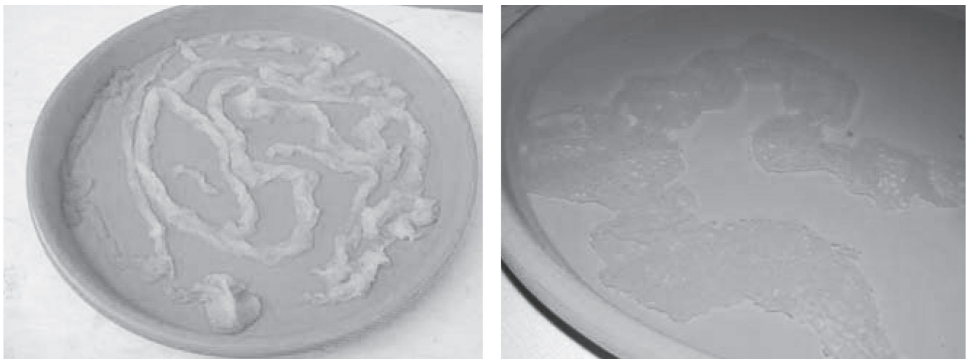


Figure 9. *Collection of sperm.*

The sperm of the 16 males selected in advance and individually tagged was collected to determine male fecundity and sperm characteristics. The volume (in ml) and density (spermatozoa per 1 ml) of collected sperm were determined according to Alavi et al. (2007). The sperm volume was measured in a syringe (accuracy 0.1 ml), and density was determined using a Bürker cell counter. Sperm was diluted 10 000 times with physiological solution (0.7% NaCl), and 10 µl of diluted sperm was put into a haemocytometer. After 10 min sedimentation, the spermatozoa per 16 squares of a haemocytometer were counted. To determine the absolute fecundity (total number of spermatozoa per male), the total volume of sperm was multiplied by the density of spermatozoa per 1 ml. To determine the relative fecundity (the number of spermatozoa per 1 kg of male) we divided the absolute fecundity by weight of each male (in g) and multiplied this number by 1 000.

4.3.1.4. Artificial fertilization of eggs

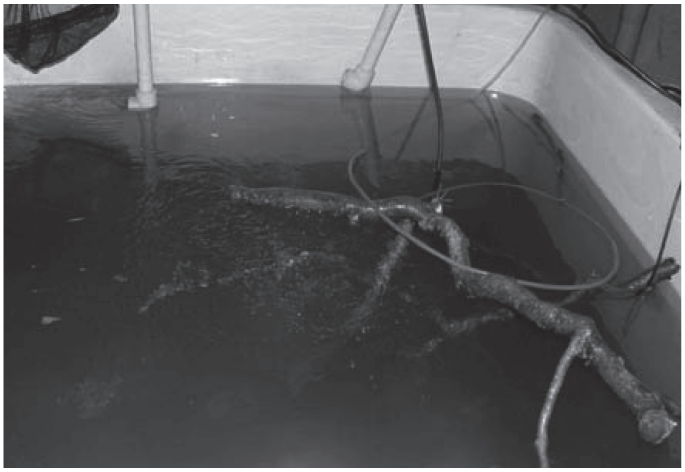
The sperm from three males, collected separately with syringe, was used for fertilization of one egg ribbon (Fig. 10). To 100 g of eggs (approx. 59 200 eggs), 2 ml of sperm (approx. 58 400 000 000 spermatozoa) was used (approx. 1 million spermatozoa/egg). Sperm was added and carefully mixed with eggs, clear hatchery water was added, and gametes were gently mixed and allowed to rest three min (Fig. 11). Eggs were rinsed with clear water and the egg ribbons were placed into plastic incubators (baskets of 300 x 200 x 80 mm) for artificial incubation (Plate 14) in RAS tanks (water temperature 15.5 ± 0.5 ºC).



Figures 10 and 11. *Egg ribbon before (left) and after (right) artificial fertilization.*

4.3.1.5. Semi-artificial spawning

During semi-artificial spawning, neither frequent checking nor the manipulation of broodstock was necessary, as females and males were stocked together. After stocking and female hormone injection, the fish spawned naturally and spontaneously. When spawning began, six dry branches approximately 80–120 cm in length of goat willow, *Salix caprea,* or European black elderberry, *Sambucus nigra*, were placed in each tank to serve as a natural spawning substrate (Fig. 12). The fish swam freely among the branches and used this substrate for fixing the egg ribbons and for the fertilization of eggs. For recording and collection of spawned and fertilized egg ribbons, the broodstock were monitored at 6 h intervals.




Figures 12 and 13. T*anks with spawning substrate for perch broodstock (left) and determination of the egg ribbon volume in semi-artificial spawning (right).*

While collecting egg ribbons, the spawned female was removed and the approximate time of spawning was recorded along with the identification number of the female. The males were kept in tanks until all females had spawned. The volume of a single egg ribbon was calculated using graduated cylinders with water according to Kouřil et al. (1998), Kouřil and Hamáčková (2000), and Kouřil et al. (2001) (Fig. 13).

The number of eggs in 1 ml of egg ribbon (see fertilization of eggs) and the total number of eggs in the egg ribbon were counted. To count the number of eggs in 1 ml of egg ribbon, a sample of approximately 1 ml eggs was taken. The volume of the sample was measured in a graduated cylinder with water. The number of eggs was determined and the number of eggs per 1 ml egg ribbon was calculated as the number of eggs in the sample divided by volume of the sample in millilitres.

The stripping data (date, time of egg collection, female identification number, and number of eggs per egg ribbon) were recorded. After the spawning period, the latency (time from hormone injection to female spawning) and synchronization of the spawning (number of spawned females per hour) was calculated according to the time of spawning. The spawning success (number and percent of spawned females) and the absolute and relative fecundity of females (total number of eggs and number of eggs per 1 kg of female BW) were determined. The egg ribbons were placed in plastic incubator baskets in RAS tanks.

4.3.2. Results

4.3.2.1. Spawning success, latency, and synchronization in artificial and semi-artificial spawning

Results indicated that 83% of females spawned with artificial spawning and 88% of females spawned in semi-artificial spawning (Table 1).

Table 1. *Efficacy, latency, and spawning synchronization of broodstock under artificial and semi-artificial spawning regimes.*

Indicator	Artificial spawning	Semi-artificial spawning
Number of spawned females	50	53
Percent of spawned females	83	88
Number of spawned males	60	Not rated
Percent of spawned males	100	
Latency (days)	3.5 ± 0.8	4.1 ± 0.7
Latency (hours)	84.0 ± 18.3	98.5 ± 17.2
Latency (degree days)	53.2 ± 11.5	62.3 ± 10.6
Synchronization of spawning (percent spawned females/hour)	83/96 h	88/72 h

All 76 males successfully released sperm during artificial spawning; for semi-artificial spawning it was not possible to determine spawning success. No problems with production or release of sperm were observed. For successful artificial spawning, it is essential to provide a sufficient number of quality males. Based on our experience, we recommend 1 : 1 males : females in both artificial and semi-artificial spawning.

Artificial spawning involves a higher demand for labour, time, and staff experience than does semi-artificial spawning, chiefly because of the necessity of more frequent examination of the females to avoid spontaneous spawning and resulting damage to eggs. Under artificial spawning, males must also be stripped of sperm, and the eggs artificially fertilized, which is time consuming and requires high level skills. These activities also significantly influence the quantity and the quality of fertilized eggs and hatched embryos. An advantage of artificial spawning is ease of monitoring production and fertilization of eggs.

The spawning of female broodstock under the semi-artificial regime began spontaneously 14.4 h later than did the artificial spawning. Latency of female perch broodstock was longer in semi-artificial spawning (4.1 ± 0.7 days/ 98.5 ± 17.2 hours/62.3 ± 10.6 °d) than in artificial spawning (3.5 ± 0.8 days/84.0 ± 18.3 hours/ 53.2 ± 11.5 °d) (Table 1).

Spawning was more highly synchronized under the semi-artificial regime, with 88% of fish spawning within a 3 day period (Table 1). Cumulative percent of spawned females under both spawning regimes is shown in Table 2. With artificial spawning, females were stripped over a 4 day period. Artificial spawning began earlier and proceeded more slowly than did semi-artificial spawning (Table 1 and 2).

Table 2. *Cumulative percent of spawned females during artificial and semi-artificial spawning.*

Spawning method	Day post-injection					
	1.	2.	3.	4.	5.	6.
Artificial spawning	0	0	22	67	75	83
Semi-artificial spawning	0	0	0	33	77	88

4.3.2.3. Female fecundity and total egg production

The absolute and relative fecundity of individual females differed considerably with both semi-artificial and artificial spawning. The mean absolute fecundity of artificially spawning females was 30 721 ± 28 796 eggs (from 5 234 to 92 003 eggs). Similar values of absolute fecundity were observed with semi-artificial spawning, with mean absolute fecundity 32 215 ± 30 128 eggs per female (from 4 567 eggs to 153 687 eggs). The average relative fecundity of females was 161 689 eggs kg^{-1} with artificial spawning and 169 552 eggs kg^{-1} with semi-artificial spawning (Table 3). Fecundity was influenced by female body size. An influence of spawning method on female fecundity was not observed.

The number of eggs in 1 g of ribbon was found to average 592.0 ± 162.4 with artificial spawning, and 1 ml of egg ribbon showed at average of 248.0 ± 79.3 eggs in semi-artificial spawning (Table 3).

In total, approximately 1 500 000 eggs were obtained through artificial spawning with 89 000 used for assessment of fertilization and hatching rates. From the 50 artificially spawning females, approximately 1 400 000 eggs were used for incubation.

With semi-artificial spawning, 1 700 000 eggs were obtained from 53 successfully spawning females; 40 000 eggs were used for assessment of fertilization and hatching rates and 1 660 000 eggs for mass incubation.

Table 3. *Absolute and relative fecundity of female broodstock with artificial and semi-artificial spawning.*

Indicator	Artificial spawning	Semi-artificial spawning
Absolute fecundity of female (number of eggs $female^{-1}$)	30 721 ± 28 796	32 215 ± 30 128
Relative fecundity of female (number of eggs kg^{-1} BW)	161 689 ± 149 500	169 552 ± 157 000
Number of eggs in 1 g of ribbon (number of eggs g^{-1} ribbon)	592 ± 162	Not rated
Number of eggs in 1 ml of ribbon (number of eggs ml^{-1} ribbon)	Not rated	248 ± 79

4.3.2.4. Male fecundity in artificial spawning

In artificial spawning, an average volume of 2.8 ± 1.5 ml sperm was collected from each male, ranging from a minimum of 0.55 ml to a maximum 6.7 ml. Average spermatozoan density was high, at $29.2 \times 10^9 \pm 15.3 \times 10^9$ spermatozoa ml^{-1} sperm. The individual minimum sperm density was 3.3×10^9 spermatozoa ml^{-1} and the maximum was 196.8×10^9 spermatozoa ml^{-1} sperm. Males released an average of 81.8×10^9 spermatozoa, or 583.0×10^9 spermatozoa kg^{-1} during artificial spawning. In semi-artificial spawning, fecundity was not evaluated.

4.4. Fertilization and hatching rates in representative samples

4.4.1. Procedures

Three samples of approximately 1 ml from each egg ribbon were taken during mass incubation to determine the fertilization rate 24 h after artificial fertilization

and 24 h after collection of egg ribbons in the semi-artificial spawning regime. Collection was as described for semi-artificial spawning. Samples were incubated in Petri dishes (diameter 120 mm, volume 75 ml) with 70 ml water. Water temperature in dishes was 16.2 ± 0.7 °C, pH = 7.5 ± 0.2, and concentration of dissolved oxygen O_2 = 7.0 ± 0.5 mg.l^{-1}. Parameters were monitored and water changed every 12 h. In each sample, the number of eggs and the number of fertilized eggs was counted. The fertilization rate was calculated as the number of fertilized eggs divided by total number of eggs, multiplied by 100. When hatching was complete, the hatching rate and the period of incubation in days, in hours, and in degree days were determined. The hatching rate was calculated as the number of hatched embryos divided by total number of eggs in the sample, multiplied by 100.

4.4.2. Results

A higher fertilization rate (85.6 ± 8.7%) was observed in samples from semi-artificial spawning than in samples from artificial spawning (67.5 ± 6.5%) (Table 4). An average incubation period of 6.5–6.8 days was observed in both spawning regimes. The period of incubation was related to water temperature. An influence of spawning method on incubation period was not observed.

The fact that the incubation period under semi-artificial spawning was 7h shorter was more likely caused by the fact that the time of semi-artificial spawning could not be accurately determined (error 1–6 h). In artificial spawning the exact time of spawning of each female was recorded (Table 4.). A significantly higher average hatching rate was observed in semi-artificial spawning (72.9 ± 12.3%) than in artificial spawning (58.4 ± 5.2%) (Table 4).

Table 4. *Fertilization rate, incubation period, and hatching rate with artificial and semi-artificial spawning.*

Indicator	Artificial spawning	Semi-artificial spawning
Fertilization rate (%)	67.5 ± 6.5	85.6 ± 8.7
Incubation period (days)	6.8 ±1.5	6.5 ± 1.3
Incubation period (hours)	163.2 ± 36	156.0 ± 30.0
Incubation period (°d)	110.2 ± 24.3	105.3 ± 20.3
Hatching rate (%)	58.4 ± 5.2	72.9 ± 12.3

4.5. Mass artificial incubation

4.5.1. Procedures

After spawning, each egg ribbon was separately incubated in a special basket (Fig. 14). After removing samples for for determination of fertilization and hatching rates, three egg ribbons were incubated together in a single basket in RAS tanks. The average water temperature during mass incubation was 15.8 ± 0.4 °C, pH = 7.4 ± 0.1, and concentration of dissolved oxygen O_2 = 8.0 ± 0.2 mg l^{-1}. The egg ribbons were placed into baskets at time of collection, which later facilitated removal of the baskets and tank maintenance. The water temperature, concentration of dissolved oxygen, and pH during incubation were monitored every 12 h. Embryo development was monitored

at the same interval, which was an important condition for successful incubation; egg ribbons with damaged or dead (white) eggs were removed from baskets to avoid water contamination. At the conclusion of mass incubation, the hatching rate and the incubation period in days, in hours, and in degree days were determined. The hatching rate was calculated as follows: number of hatched embryos divided by total number of eggs in the sample, multiplied by 100.

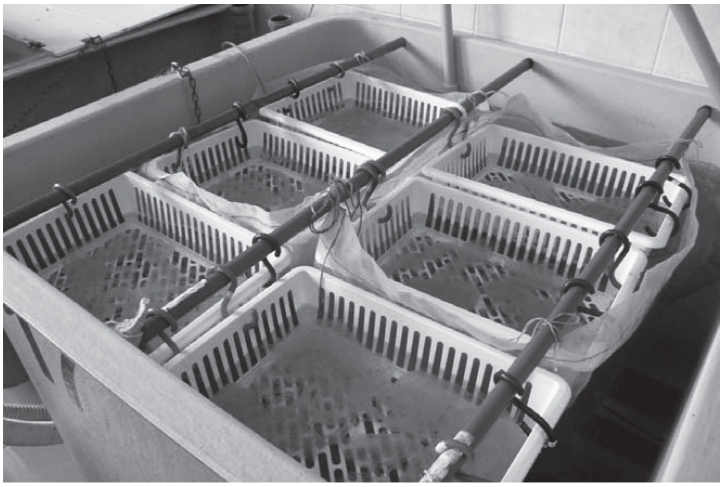


Figure 14. *Mass incubation of egg ribbons in baskets.*

4.5.2. Results

The mass incubation of eggs (Fig. 15) lasted in average 7.5 days in artificial spawning and 7 days in semi-artificial spawning (Table 5). The incubation time was slightly longer than the incubation in samples, because during the mass incubation the eggs were incubated at a water temperature 0.4 °C lower than that in samples.

Higher average hatching rate (68.0 ± 7.5%) was again observed with semi-artificial spawning than in artificial spawning (55.0 ± 9.5%) (Table 5). Hatching rates in mass incubation were lower than in the samples for both regimes, probably due to less hygienic conditions than in the controlled test.

Table 5. *Incubation period and hatching rate in artificial and semi-artificial mass incubation.*

Indicator	Artificial spawning	Semi-artificial spawning
Incubation time (days)	7.5 ± 1.5	7.0 ± 1.0
Incubation time (hours)	180.0 ± 36.0	168.0 ± 24.0
Incubation time (°d)	120.0 ± 24.0	112.0 ± 16.0
Hatching rate of embryos (%)	55.0 ± 9.5	68.0 ± 7.5

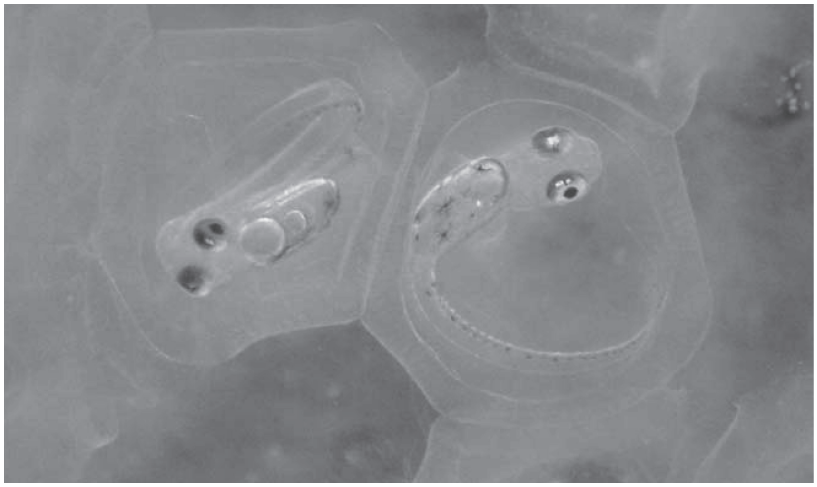


Figure 15. *Embryos of Eurasian perch.*

4.6. Mass hatching and production of embryos for breeding

4.6.1. Procedures

At the conclusion of mass incubation, the baskets were gently shaken to release the embryos. The embryos were moved to tanks with mesh of 300 µm diameter (Fig. 16) and subsequently to a bath with clean hatchery water (Fig. 17). The quantity of hatched eggs was then counted using the following method: The volume of water in the bath was decreased to 10 l, the bath was gently agitated to disperse the embryos, and five samples of 10 ml each were taken. The number of embryos in each sample was counted and average of the five samples calculated. The average number of embryos in 10 ml was multiplied by 1 000 to calculate the number of embryos in 10 l. The overall hatching rate was calculated by dividing the number of embryos by the number of eggs and multiplying by 100.

Figure 16 and 17. *Removal of embryos to tank (left) and transfer to counting bath (right).*

4.6.2. Results

At the conclusion of mass incubation, 1 930 000 embryos were obtained from all females under both spawning regimes. 1 134 000 embryos (59%) were obtained from semi-artificial spawning and 796 000 (41%) from artificial spawning. With respect to total production, semi-artificial spawning was more effective than artificial spawning, due to higher fertilization rate and hatching rate.

4.7. Mortality of broodstock during and after the spawning period

4.7.1. Procedures

The mortality of fish was investigated during the spawning period and on days 7 and 90 post-spawning. The mortality during the spawning period included fish mortality from the time of the hormone injection to spawning. Mortality was evaluated separately for the spawning regimes. After spawning, each fish was treated 5 min in an antifungal bath of potassium permanganate solution at 0.1 $g.l^{-1}$ (Fig. 18) and transferred to a plastic tank, and topmouth gudgeon (*Pseudorasbora parva*) was gradually added at a ratio of 1 kg perch to 2 kg topmouth gudgeon. The broodstock remained in this tank for 7 days after spawning of the final female, at which time mortality was recorded (Fig. 19).

The surviving fish were stocked into a 0.16 ha experimental pond with topmouth gudgeon in the same density as previously stated. Ninety days post-spawning, fish were harvested, the surviving fish were separated into groups according to sex and spawning regime, and mortality per group was determined.

Figures 18 and 19. *Potassium permanganate solution bath (left) and assessing survival of broodstock 7 days after conclusion of the spawning period (right).*

4.7.2. Results

Mortality of broodstock of both sexes during the spawning period and 7 days and 90 days after the conclusion of the spawning period is shown in Table 6. Mortality rate of females during artificial and semi-artificial spawning was 15 and 17%, respectively. No mortality was observed in males during the artificial spawning period; during semi-artificial spawning the mortality of males was 8%.

Higher mortality of broodstock was evident 7 days after the spawning period. 68% of females were lost in the 7 days following both artificial and semi-artificial spawning; 22% of males were lost within 7 days post-artificial spawning and 8% males 7 days post-semi-artificial spawning.

Ninety days after the conclusion of the spawning period, 98% mortality was observed in females, regardless of the spawning regime. High mortality was also observed in males, 92% with artificial spawning and 85% with semi-artificial spawning.

These data shows clearly that most Eurasian perch broodstock die after spawning, whether artificial or semi-artificial. It is not realistic to plan repeat use of either sex of broodstock. According to our experience, it is advantageous to kill the fish immediately or within 7 days of spawning and process it. If the fish is not used in this way, according to our experience, most of the broodstock is lost.

Table 6. *Cumulative mortality of broodstock (%) during the spawning period and 7 and 90 days after artificial and semi-artificial spawning.*

Indicator	Artificial spawning	Semi-artificial spawning
Cumulative mortality of females (%)		
During the spawning period	15	17
7 days post-spawning period	68	68
90 days post-spawning period	98	98
Cumulative mortality of males (%)		
During the spawning period	0	8
7 days post-spawning period	22	8
90 days post-spawning period	92	85

5. ECONOMIC BENEFIT OF THE TECHNOLOGY

The described technology of Eurasian perch embryo mass production will enable the fish company Rybářství Nové Hrady s.r.o. to produce several million embryos annually at minimal cost. These procedures will ensure mass production of perch embryos of similar age and positively influence the rearing of larvae and juveniles in ponds. With a sufficient food supply, fish of the same age should be of similar size, which will minimize cannibalism and increase effectiveness of rearing juveniles and, subsequently, older categories of Eurasian perch.

The aim of these procedures was to produce viable high quality Eurasian perch embryos to be reared in production ponds in a three to four year production cycle. This will increase the production of market-sized perch and, at the same time, limit the presence of less valuable fish species in the ponds to benefit production of the primary market fish, common carp.

It is difficult to calculate precisely the economic benefit of these procedures, but we estimate that, with their full employment, the aquaculture firm Rybářství Nové Hrady s.r.o. could gain several tens of thousands CZK.

6. USE OF THE TECHNOLOGY IN PERCID PRODUCTION

Procedures for mass production of high quality perch embryos described and verified in practice will be used in the aquaculture company Rybářství Nové Hrady s.r.o. In putting these procedures into effect, perch embryos, larvae, juveniles, market fish, and broodstock will be obtained for further fish production or sale.

7. REFERENCES

Abdulfatah, A., Fontaine, P., Marie, M., 2008. Effects of photothermal kinetic, photoperiod amplitude and duration on the rates of out-of-season spawning and egg fertilization in Eurasian perch *Perca fluviatilis*. In: Fontaine, P., Kestemont, P., Teletchea, F., Wang, N. (Eds), Percid Fish Culture – From Research to Production, Proceeding of abstracts and short communications of the workshop, Namur, Belgium, pp. 92–93.

Abi-Ayad, S.M., Mélard, C., Kestemont, P., 1995. Effects of n-3 fatty acid in broodstock diet on survival of European perch (*Perca fluviatilis*) larvae after stress tests: osmotic shock and starving. In: Lavens, P., Jaspers, E., Roelands, I. (Eds), Larvi'95, Fish and Shellfish Larviculture Symposium. EAS Spec. Publ., 24, pp. 12–15.

AbiAyad, S.M., Mélard, C., Kestemont, P., 1997. Effects of n-3 fatty acids in Eurasian perch broodstock diet on egg fatty acid composition and larvae stress resistence. Aquacult. Int. 5, 161–168.

Adámek, Z., Helešic, J., Maršálek, B., Rulík, M., 2010. Applied Hydrobiology (2nd Revised Edition). FFPW USB, Vodňany, Czech Republic, 350 pp.

Alavi, S.M.H., Rodina, M., Policar, T., Kozák, P., Pšenička, M., Linhart, O., 2007. Semen of *Perca fluviatilis* L.: Sperm volume and density, seminal plasma indices and effects of dilution ratio, ions and osmolality on sperm motility. Theriogenology 68, 276–283.

Ashe, D.A., 1997. Cultivating perch. Aquaculture explained. Special publication BIM, No. 20, Dublin, Ireland, 47 pp.

Bláha, M., 2006. Food Feeding biology of Eurasian perch (*Perca fluviatilis* L.) fingerlings in pond culture. Master thesis, AF USB, Ceske Budejovice, 65 pp. (in Czech)

Dabrowski, K., Ciereszko, A., Ramseyer, L., Culver, D., Kestemont, P., 1994. Effect of hormonal treatment on induced spermiation and ovulation in the yellow perch (*Perca flavescens*). Aquaculture 120, 171–180.

FAO, 2011a. Fisheries and Aquaculture Information and Statistics Service – 04/07/2011. http://www.fao.org/fishery/statistics/global-capture-production/query/en.

FAO, 2011b. Fisheries and Aquaculture Information and Statistics Service – 04/07/2011. http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en.

Fiogbe, E.D., Kestemont, P., 2003. Optimum daily ratio for Eurasian perch *Perca fluviatilis* L. reared at its optimum growing temperature. Aquaculture 216, 234–252.

Fiogbé, E.D., Kestemont, P., Mélard, C., Micha, J.C., 1996. The effects of dietary crude protein on growth of Eurasian perch *Perca fluviatilis*. Aquaculture 144, 239–249.

Flajšhans, M., Göndör, R., 1989. Artificial spawning of Eurasian perch (*Perca fluviatilis* L.). Bulletin RIFCH Vodňany 2, 10–13. (in Czech)

Fontaine, P., Gardeur, J.N., Kestemont, P., Georges, A., 1997. Influence of feeding level on growth, intraspecific weight variability and sexual growth dimorphism of Eurasian perch *Perca fuviatilis* L. reared in a recirculation system. Aquaculture 157, 1–9.

Fontaine, P., Kestemont, P., Mélard, C., 2008. Broodstock management. In: Rougeot, C., Torner, D. (Eds), Farming of Eurasian Perch, Special publication BIM, No. 24, Dublin, Ireland, pp. 16–22.

Hamáčková, J., Sedova, M.A., Pjanova, S.V., Lepičová, A., 2001. The effect of 2-Pheoxyethanol, clove oil and Propiscin anaesthetics on perch (*Perca fluviatilis*) in relation to water temperature. Czech J. Anim. Sci. 46, 469–473.

Jansen, H., Fontaine, P., 2008. Recent improvements in the control of the percid reproductive cycle. In: Fontaine, P., Kestemont, P., Teletchea, F., Wang, N. (Eds), Percid Fish Culture – From Research to Production, Proceeding of abstracts and short communications of the workshop, Namur, Belgium, pp. 19–22.

Kayes, T.B., Calbert, H.A., 1979. Effects of photoperiod and temperature on the spawning of yellow perch (*Perca flavescens*). Proceedings of World Mariculture Society 10, pp. 306–316.

Kestemont, P., Mélard, C., 2000. Aquaculture (Chapter 11). In: Craig, J.F. (Ed.), Percids Fishes – Systematic, Ecology and Exploitation. Fish and Aquatic Resources series 3, Blackwell Sciences, pp. 191–224.

Kestemont, P., Mélard, C., Fiogbé, E., Vlavonou, R., Masson, G., 1996. Nutritional and animal husbandry aspects of rearing early life stages of Eurasian perch *Perca fluviatilis*. J. Appl. Ichthyol. 12, 157–166.

Kestemont, P., Vandeloise, E., Mélard, C., Fontaine, P., Brown, P.B., 2001. Growth and nutritional status of Eurasian perch *Perca fluviatilis* fed graded levels of dietary lipids with or without added ethoxyquin. Aquaculture 203, 85–99.

Kestemont, P., Jourdan, S., Houbart, M., Mélard, C., Paspatis, M., Fontaine, P., Cuvier, A., Kentouri, M., Baras, E., 2003. Size heterogeneity, cannibalism and competition in cultured predatory fish larvae: biotic and abiotic influences. Aquaculture 227, 333–356.

Kestemont, P., Rougeot, C., Musil, J., Toner, D., 2008. Larval and Juvenilie Production. In: Rougeot, C., Torner, D. (Eds), Farming of Eurasian Perch. Special publication BIM, No. 24, Dublin, Ireland, pp. 30–41.

Kouřil, J., Linhart, O., 1997. Temperature effect on hormonally induced spawning in perch (*Perca fluviatilis*). Pol. Arch. Hydrobiol. 44, 197–202.

Kouřil, J., Hamáčková, J., 1999. Artificial of perch propagation of European perch (*Perca fluviatilis* L.) by means of a GnRH analogue. Czech J. Anim. Sci. 44, 309–316.

Kouřil, J., Hamáčková, J., 2000. The semiartificial and artificial hormonally induced propagation of European perch (*Perca fluviatilis*). In: Floss, R., Creswell, L. (Eds), Proc. Aqua 2000. Responsible aquaculture in the new millennium, Special Publication, No. 28, E.A. S., Oostende, Belgium, 345 pp.

Kouřil, J., Linhart, O., Relot, P., 1997. Induced spawning of perch, *Perca fluviatilis* L., by means of a GnRH analogue. Aquacult. Int. 5, 375–377.

Kouřil, J., Linhart, O., Hamáčková, J., 1998. Optimization of GnRH analogue and temperature of water during hormonal induction in semi-artificial and artificial spawning of Eurasian perch (*Perca fluviatilis* L.). Bull. RIFCH Vodňany 34, 137–149. (in Czech with English abstract)

Kouřil, J., Hamáčková, J., Lepič, P., Mareš, J., 2001. Semi-artificial and artificial spawning of Eurasian perch. Edition of Methodics (Technological series), RIFCH USB, Vodňany, Czech Republic, No. 68, 11 pp.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Skrzypczak, A., Wyszomirska, E., 1996. Induced spawning in perch, *Perca fluviatilis* L. using carp pituitary extract and HCG. Aquac. Res. 27, 847–852.

Kucharczyk, D., Kujawa, R., Mamcarz, A., Skrzypczak, A., 1998. Induced spawning in perch, *Perca fluviatilis* L., using FSH + LH with pimozide or metoclopramide. Aquac. Res. 29, 131–136.

Mathis, N., Feidt, C., Brun-Bellut, J., 2003. Influence of protein/energy ratio on carcass quality during the growing period of Eurasian perch (*Perca fluviatilis*). Aquaculture 217, 453–464.

Mélard, C., Kestemont, P., Grignard, J.C., 1996. Intensive culture of juvenile and adult Eurasian perch (*Perca fluviatilis*): Effect of major biotic and abiotic factors on growth. J. Appl. Ichthyol. 12, 175–180.

Policar, T., Kouřil, J., Hamáčková, J., 2008a. Induced artificial and semiartificial spawning by Supergestran in perch (*Perca fluviatilis* L.) under different temperature. In: Fontaine, P., Kestemont, P., Teletchea, F., Wang, N. (Eds), Percid Fish Culture – From Research to Production. Namur, Belgium, pp. 124–125.

Policar, T., Kouřil, J., Stejskal, V., Hamáčková, J., 2008b. Induced ovulation of perch (*Perca fluviatilis* L.) by preparations containing GnRHa with and without metoclopramide. Cybium 32, 308.

Policar, T., Toner, D., Alavi, S.M.H., Linhart, O., 2008c. Reproduction and Spawning. In: Rougeot, C., Toner, D. (Eds), Farming of Eurasian Perch, Special publication BIM, No. 24, Dublin, Ireland, pp. 22–29.

Policar, T., Stejskal, V., Bláha, M., Alavi, S.M.H., Kouřil, J., 2009. Technology of intensive culture of Eurasian perch (*Perca fluviatilis* L.). Edition of Methodics (Technological series), RIFCH USB, Vodňany, Czech Republic, No. 89, 51 pp.

Rougeot, C., Fontaine, P., Mandiki, S.M.N., 2008. Perch description and biology. In: Rougeot, C., Toner, D. (Eds), Farming of Eurasian Perch. Special publication BIM, No. 24, Dublin, Ireland, pp. 12–15.

Stejskal, V., Policar, T., Bláha, M., Křišťan, J., 2010. Production of Eurasian perch (*Perca fluviatilis*) using pond and intensive culture. Edition of Methods (Technological series), FFPW USB, Vodňany, Czech Republic, No. 105, 34 pp.

Watson, L., 2008. The European market for perch (*Perca fluviatilis*). In: Fontaine, P., Kestemont, P., Teletchea, F., Wang, N. (Eds), Percid Fish Culture – From Research to Production, Proceeding of abstracts and short communications of the workshop, Namur, Belgium, pp. 10–14.

West, G., Leonard, J., 1978. Culture of yellow perch with emphasis on development of eggs and fry. In: Kendal, R.L. (Ed.), Selected coolwater fishes of North America. American Fisheries Society 11, Washington, D.C., pp. 172–176.

Xu, X., Fontaine, P., Mélard, C., Kestemont, P., 2001. Effects of dietary fat levels on growth, feed efficiency and biochemical compositions of Eurasian perch *Perca fluviatilis*. Aquacult. Int. 9, 437–449.

Xu, X., Kestemont, P., 2002. Lipid metabolism and FA composition in tissues of Eurasian perch *Perca fluviatilis* as influenced by dietary fats. Lipids 37, 297–304.