

Comparison of Reproduction Characteristics and Broodstock Mortality in Farmed and Wild Eurasian Perch (*Perca fluviatilis* L.) Females During Spawning Season Under Controlled Conditions

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Abstract

Reproduction characteristics and broodstock mortality were compared in farmed perch (FP) and wild perch (WP) under controlled conditions. Thirty-six farmed females (FF) (92.3%) and 30 wild females (WF) (76.9%) spawned during the reproductive season. Absolute fecundity in FF was 18 660 \pm 6 809 eggs per female compared to 31,081 \pm 3,276 eggs per female in WF. Similar differences were observed in relative fecundity (FF 112,470 \pm 13,370 eggs per kg body weight and WF 137,054 \pm 18,513 eggs per kg body weight). No significant differences in fertilization rate (FR), larval resistance (LR) and larval total length (LTL) were found (FR - 91.9 \pm 6.9% and 90.5 \pm 6%; LR - 51.1 \pm 25.2% and 53.5 \pm 26.2%; LTL - 5.88 \pm 0.55 mm and 5.82 \pm 0.51 mm in FP and WP, respectively). The highest differences were found in hatching rates of 27.9 \pm 9.3% in FP and 62.0 \pm 16.0% in WP. No mortalities were observed during the reproductive season, but post-spawning mortality among WP was 92.5 \pm 2.5% of females compared with 21.5 \pm 12.5% in FP.

Keywords: Wild perch, farmed perch, fecundity, fertilization rate, hatching rate, mortality.

Introduction

The Eurasian perch (Perca fluviatilis L.) is a freshwater fish species highly valued in Europe (Fontaine, 2009). At present, the largest perch producing countries in Europe, where wild perch are mainly caught from local lakes (Öberg, 2008), are Finland, Russia, and Estonia (Watson, 2008). Unfortunately, the perch fisheries are unstable in both quality and quantity of fish produced (Fontaine, 2004) and is in drastic decline due to the decrease of wild stocks and overfishing (Öberg, 2008, FAO, 2009). Consequently, intensive perch culture has been developed in the past two decades, and perch has become important species in European an aquaculture, especially in Ireland, Switzerland, France, Denmark, and Sweden (Fontaine, 2004; Watson, 2008).

The perch market has a local character, but, in spite of this, the current production of market-size perch in Europe is unable to satisfy demand, which is mainly centred in the Alpine region (Watson, 2008; Setälä *et al.*, 2008).

Successful control of reproduction and mass reproduction of perch broodstock are basic prerequisites for profitable intensive perch production (Fontaine *et al.*, 2008). Current reproduction of perch relies mainly on wild broodstock from ponds and other natural waters, captured immediately prior to spawning (Kucharczyk *et al.*, 1998; Kouril and Hamackova, 1999; Policar *et al.*, 2008a). However, an optimal protocol for the induction of reproduction in farmed broodstock, including out-of-season spawning, has been developed under controlled conditions (Migaud *et al.*, 2002, 2004, 2006; Fontaine *et al.*, 2008).

Many studies have found broodstock nutrition to have an effect on the quality of reproduction of farmed fish, including chemical composition of eggs, fertilization and hatching rates, and larval survival (Bell *et al.*, 1997; Izquierdo *et al.*, 2001; Henrotte *et al.*, 2008; Wang *et al.*, 2009; Henrotte *et al.*, 2010). An effect of n-3 highly unsaturated fatty acids (HUFAs), especially eicosapentaenoic acid (20:5 n-3), docosahexaenoic acid (22:6 n-3) and arachidonic acid (20:4 n-6), on the reproduction of perch in captivity has been reported (Henrotte *et al.*, 2010).

Maintaining perch broodstock in captivity requires specialized artificial rearing conditions (light and temperature regimes) and the creation of special artificial diets (Henrotte *et al.*, 2010), which require high initial investment and operating costs to provide continuous production of eggs year-round (Fontaine *et al.*, 2008; Toner and Schram, 2008). On the other

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hand, wild and farmed perch broodstock partially fed on forage fish can be an annual source of eggs and larvae for later use in intensive culture. With annual spawning, requirements for investment and operating cost are much lower (Policar *et al.*, 2008a; Kestemont *et al.*, 2008a, Stejskal *et al.*, 2009).

This study compared the gonadosomatic index, individual reproduction characteristics (female fecundity, spawning rate in female, fertilization rate, hatching rate, larval resistance to the osmotic shock and size of larvae), and post spawning mortality in wild and farmed perch broodstock fed on a combination of commercial salmon food and forage fish during the annual spawning season.

Materials and Methods

Farmed Perch Broodstock management

This study used both farmed and wild populations. Farmed perch broodstock was obtained from a study by Stejskal *et al.* (2009). Perch were reared under controlled conditions in a recirculating aquaculture system (RAS) at the University of South Bohemia, Faculty of Fisheries and Protection of Waters (USB FFPW). In total, 102 three-year-old perch (total length TL = 220.0 ± 20.9 mm and weight W = 146.5 ± 43.9 g) were kept under a natural photoperiod and water temperature regime, in flowthrough systems supplied with water from the River Blanice, at USB FFPW from September 2008 to February 2009. The water temperature and light regime is summarized in Table 1.

Thirty-four fish were held in each of three 480 1 fibreglass tanks (1000 x 800 x 600 mm) and fed daily on a mix of commercial salmon diet (Stejskal al. 2009) and forage fish according to Wang *et al.* (2009). Forage cyprinids *Pseudorasbora parva* (TL = 40-60 mm; W = 0.3-0.4 g), reared at 20°C; with a 16L:8D photoperiod and fed on a dry salmon diet(Stejskal al. 2009), were fed three days a week to perch at a rate of two forage fish each. The dry salmon diet was used on the other four days at a rate of 1% of perch total biomass per tank. The dry diet was distributed by automatic beltfeeders during daylight hours. The ration was adjusted daily according to the quantity of uneaten feed removed from the tanks.

At the beginning of March 2009, all fish were

moved to a controlled environment and placed in three identical tanks in the RAS at USB FFPW. The groups of fish were not mixed and the same feeding regime was maintained. The water temperature was raised by 0.3° C increments per day from 1 March (5.5°C) to 1 April (14.5°C) and kept constant thereafter. A constant light regime (11.5L: 12.5D) was used during this phase of the study.

Wild Perch Broodstock Management

The second group of perch comprised wild perch reared for three years in three experimental ponds (each 0.06 ha). The first two years, culture conditions were similar to studies of Policar et al. (2009) and Kestemont et al. (2008a). In April 2008, a mixed culture of perch and forage cyprinids Pseudorasbora parva, at a biomass rate of 1:5, was stocked in the experimental ponds and held for the winter season of 2008-2009 until March 2009. No additional feeding or manipulation of temperature or lighting was done. All ponds were harvested on 1 March 2009. A total of 102 of the three-year-old wild perch (TL = 174.1 ± 21.9 mm, W = 75.5 ±31.3 g) were moved to the controlled environment as described for the cultivated fish and placed randomly in three similar tanks in the RAS at USB FFPW. Thirty-four fish with a sex ratio of 1:1 were held in each tank. Perch were fed with Pseudorasbora parva obtained from harvested ponds at a rate of two per perch.day⁻¹. The temperature and light regime was the same as for the cultivated perch. Table 1 summarizes average temperature and light regimes for the grow-out season through March 2009.

Morpho-Anatomical Parameters in Perch Broodstocks

On 8 April (7 days after the temperature in the tanks reached its final value of 14.5°C), 8 males and 8 females from each tank (24 females and males from each group) were killed. Total length and standard length (TL, SL \pm 1 mm) was measured and weight (W \pm 0.1 g) of all individuals was recorded. Gonads, liver, and viscera including mesenteric fat were removed and weighed (\pm 0.01 g). The gonadosomatic (GSI), hepatosomatic (HSI), and viscerosomatic (VSI) indices (%) were calculated as follows:

 $GSI = (GW/BW) \ge 100$ $HSI = (LW/BW) \ge 100$

 Table 1 Average water temperature and light regime during out-growing period until spawning period in farmed and wild perch broodstock

Group	Factor	Sep. 08	Oct. 08	Nov. 08	Dec. 08	Jan. 09	Feb. 09	Mar 09	Apr.09
Farmed	Temperature (°C)	14.2 ± 0.4	8.4±0.5	6.2±0.6	3.0.±0.4	2.6±0.3	2.5±0.4	10.0 ± 2.7	14.5±0.3
	Light (h)	12.5±0.5	10.5±0.3	9.3±0.3	8.2±0.3	8.3±0.3	9.3±0.3	11.5 ± 0.0	11.5±0.0
Wild	Temperature (°C)	13.9±1.2	8.0±2.2	5.9±1.9	2.5±1.3	1.8±1.4	1.6±1.1	10.0±2.7	14.5±0.3
	Light (h)	12.5±0.5	10.5±0.3	9.3±0.3	8.2±0.3	8.3±0.3	9.3±0.3	11.5±0.0	11.5±0.0

Data are shown as mean \pm S.D

 $VSI = (VW/BW) \times 100$

Where GW is the weight of gonads (g), LW is the weight of the liver (g), VW is the weight of the viscera (g) and BW is the body weight (g).

Conditions for the Reproductive Season

The 26 remaining perch broodstock: 13 males and 13 females from each tank (78 fish, 39 males and 39 females from each group) were used for comparison of reproductive activity and reproductive characteristics. All remaining perch were measured and weighed (TL and standard length SL±1 mm and W±1 g) on 8 April. Females and males were identified based on characteristics of the dissected perch (mainly according shape of the abdomen). All females were marked with PIT tags and their biometric data and PIT number were recorded. The broodstock was fed on Pseudorasbora parva at a rate of two fish (TL = 40-60 mm) per perch.day⁻¹. The fish were kept at a constant water temperature and light regime (Table 1). Eight dry branches of Sambucus nigra (length = 800 mm) were placed in each tank to provide a spawning substrate according to Policar et al. (2008a). The presence of this spawning substrate stimulated reproduction. No hormonal or other stimulation of perch broodstock was used to induce reproductive activity.

Female Reproductive Activity

The reproductive season was divided into three periods:

- 1- Early (spawning from 8 April to 15 April),
- 2- Middle (spawning from 16 April to 23 April), and
- 3- Late (spawning from 24 April to 1 May).

When the first female began laying egg ribbons, the reproductive activity of both perch broodstock groups was checked at regular 6 h intervals, and egg ribbons were removed from the tanks. The percent of spawned females in each period was recorded and compared between groups, and the total number of spawned females was recorded.

Number of Eggs in Egg Ribbon and Fecundity

After removal, the egg ribbon was associated with a spawned female (empty abdomen) which was caught and removed from the tank. The PIT number was identified with a scanner (I MAX plus, Virbac France SAS). The number of eggs in 1 ml of egg ribbon was counted by the volume method established by Kouril and Hamackova (1999). Four samples of similar size were taken from different parts of each egg ribbon (volume of each sample was approximately 1 ml). The exact volume of the sample was measured with a graduated cylinder±0.05 ml (EV). The number of eggs was determined (EN) and the number of eggs per 1 ml (TEN) egg ribbon was calculated as follows: TEN = EV x EN. The remaining egg volume from each female was measured in a graduated cylinder ± 0.1 ml (RVS). This value was used to calculate absolute fecundity of each female (AF) using the formula: AF = RVS x TEN. Relative fecundity RF (number of eggs. kg⁻¹ of body weight) of each female was calculated according the formula: RF = AF/BW, where BW is body weight (in kg).

Fertilization and Hatching Rate

The four samples with the exact number of eggs of each spawning female recorded were used to assess the fertilization and hatching rate of that female. Egg samples were incubated in two tanks (2600 x 400 x 350 mm) integrated into a recirculation system (total water volume 700 l). Each sample of eggs was separately incubated in a small cage (150 x 100 x 100 mm) for aquarium fish. The water temperature $(14.5\pm0.3^{\circ}\text{C})$ and water flow (0.5 l min^{-1}) in the tanks were kept constant. Twenty-four hours after stocking of eggs into the cages fertilization rate was determined as the percent fertilized eggs of the total number of eggs in each sample. After all larvae in the sample had hatched the hatching rate was determined as the percent of hatched larvae in the stocked eggs. Fertilization and hatching rates were evaluated and compared among the three periods of the reproductive season and between females of the groups.

Larval Total Length and Resistance to Osmotic Stress

Fifty newly hatched larvae from each female were measured under a stereomicroscope Olympus SZ 40 (Olympus, Japan) with a slide micrometer scale to the nearest 0.1 mm.

The resistance of newly hatched larvae to osmotic stress was determined through exposure to a solution of 2% saline, made from hatchery water at 14.5 \pm 0.3°C (Migaud *et al.*, 2001). Thirty-three 24 h post-hatched larvae from each female were placed in 1 l of the saline solution. The trial was performed in triplicate (99 larvae from each female). The survival rate of larvae was determined after 120 min (Migaud *et al.*, 2001).

The larval total length and resistance to osmotic stress was evaluated and compared among the three periods of the reproductive season and between wild and farmed fish.

Broodstock Mortality

The mortality of the perch broodstock (males and females) was observed and compared in both groups during the reproductive season and the following 14 days (to 15 May). At the conclusion of the spawning season (2 May) all broodstock were transferred to the six tanks of the flow-through system that were used for the culture of farmed perch broodstock during the winter period. The water was from the Blanice River (14.0 \pm 0.5°C). *Pseudorasbora parva* (TL = 40-60 mm) were used as feed for all broodstock at a daily rate of two per perch. Mortality during the reproductive season and the following 14 days was compared between groups.

Monitoring of Water Temperature and Quality

The water temperature and the concentration of dissolved oxygen were measured twice daily (08:00 and 16:00), and water pH, NH₃, NO₂, and NO₃ was checked weekly in all ponds and tanks used. All values complied with accepted fish culture requirements.

Statistical Analysis

All determined parameters: size of perch broodstock, anatomical indices, number of eggs in 1 ml egg ribbons, absolute and relative female fecundity, fertilization and hatching rate, larval total length and their resistance to osmotic stress, and survival rate of perch broodstock were recorded and characterized by simple statistics given as means±standard deviation (SD). Two-way analysis of variance ANOVA (P<0.05) was used for comparison of all parameters. The non-parametric Kruskal-Wallis test was used to test for differences in number of eggs in 1 ml of egg ribbons, absolute and relative female fecundity, fertilization and hatching rate, larval resistance to osmotic stress, and survival rate of perch broodstock. Tukey's multiple comparison tests was used for comparison of size of perch broodstock, anatomical indices and larval total length between groups. Statistical assessment of all data was carried out with Statistica 7.0 (StatSoft, Inc., Czech Republic).

Results

Morpho-Anatomical Parameters in Perch Groups

Morpho-anatomical parameters of perch groups are shown Table 2. The highest differences were observed between farmed and wild perch in GSI and VSI.

Female Reproductive Activity

Thirty-six cultivated females (92.3%) and 30 wild females (76.9%) spawned. The first spawning in both groups was recorded on 8 April. The reproductive season extended 19 days in farmed perch and 24 days in wild perch. The highest spawning activity was recorded during the Middle Period in both groups. The lowest spawning activity was found in the Late Period. Table 3 summarises Early, Middle and Late period and are shown number and percentage of spawned farmed and wild perch.

Number of Eggs in Egg Ribbons and Female Fecundity

The mean number of eggs was $156\pm42 \text{ ml}^{-1}$ in farmed perch and $169\pm38 \text{ ml}^{-1}$ in wild perch, which were not significantly different.

Average values of fecundity (both absolute and relative) were significantly different between groups (P<0.05). The mean absolute fecundity of farmed fish was $18,660\pm6,809$ eggs female⁻¹ compared to wild perch with $31,081\pm3,276$ eggs female⁻¹. Similar differences were revealed in relative fecundity (farmed: $112,470\pm13,370$ eggs kg⁻¹ body weight and wild: $317,054\pm18,513$ eggs kg⁻¹ body weight).

Fertilization Rate

No significant difference in average values of

Perch broodstock	TL (mm)	SL (mm)	W (g)	HSI (%)	GSI (%)	VSI (%)
Farmed females	220.4±21.4 ^b	189.8±20.0 ^b	159.4±49.3 ^b	1.8±0.3 ^a	18.5±2.3ª	3.2±1.3 ^b
Wild females	188.8±16.4 ^a	$168.0{\pm}14.8^{a}$	98.9 ± 22.9^{a}	2.8±0.3 ^b	26.9±4.4 ^b	1.9±0.3 ^a
Farmed males	220.4 ± 20.8^{b}	191.2±18.6 ^b	133.5±34.0 ^b	$1.9{\pm}0.5^{a}$	$5.3{\pm}0.9^{a}$	6.0 ± 2.6^{b}
Wild males	159.5±16.2 ^a	139.2±14.3 ^a	52.0±18.2 ^a	2.6 ± 1.9^{b}	8.2 ± 2.6^{b}	3.5 ± 0.8^{a}

Table 2. Morpho-anatomical parameters of perch broodstock at the beginning of the reproductive season

Data are shown as mean \pm S.D. Values within column with different superscripts are significantly different (P < 0.05)

Table 3. The spawning activity during whole reproductive season in wild and farmed Eurasian perch (Perca fluviatilis)

Spawning period	Nummber of spawned FF	Percentage of spawned FF	Nummber of spawned WF	Percentage of spawned WF
Early	12	33	10	33.3
Middle	16	44	17	56.6
Late	8	22.2	3	10

FF – farmed females, WF – wild females

fertilization rates was observed between the two broodstock groups (farmed perch: $91.9\pm4.4\%$ and wild perch: $90.5\pm5.5\%$). Fertilization rates in both groups of females decreased during the season. Eggs from females spawning during the Early Period of the reproductive season achieved significantly (P<0.05) higher fertilization rates (farmed: $97.4\pm3.0\%$; wild: $97.9\pm5.5\%$) compared to females spawning during the Late Period (farmed: $87.1\pm5.6\%$; wild: $84.3\pm1.9\%$). The Middle Period achieved fertilization rates $91.7\pm4.5\%$ in farmed perch and 88.8 ± 3.5 in wild perch and was not significantly different with another period.

Hatching Rate

Highly significant differences were found between hatching rates of the groups. The hatching rates of eggs from the farmed females $(27.9\pm9.3\%)$ were significantly lower than from the wild females $(62.0\pm9.0\%)$. Like the fertilisation rate, the hatching rate was negatively affected by the extended spawning season in both groups. Early spawning farmed females achieved hatching rates of $43.8\pm9.7\%$, compared to $76.6\pm8.5\%$ in wild females. Females spawning later had significantly lowered hatching rates (Late Period: $11.7\pm7.9\%$ and $50.7\pm9.5\%$, farmed and wild perch, respectively). The Middle Period (farmed $28.2\pm12.5\%$ and wild 58.6 ± 13.8) was not significantly different from the Early and Late Periods.

Larval Total Length and Resistance to Osmotic Stress

Significantly different TLs of larvae between groups were not observed. The mean TL of larvae from farmed females was 5.88 ± 0.55 mm compared to 5.82 ± 0.51 mm for larvae from wild fish. The difference in TL of larvae was not substantial with respect to the date of spawning in either group (Early: TL = 5.80-5.84 mm; Middle: TL = 5.80-5.90 mm, and Late period: TL = 5.79-5.92 mm).

No significant differences were found between groups in larval resistance to osmotic stress or of larval survival rate. Larvae from farmed females reached an average survival rate of $51.1\pm25.2\%$ and larvae from wild females achieved a similar average rate of survival ($53.5\pm26.2\%$). Larval survival rate was not associated with the date of spawning. Larvae from early spawning farmed and wild females had average survival rates of $52.4\pm16.3\%$ and $54.1\pm21.7\%$, respectively. Similar average survival rates were found in larvae from Middle and Late Periods, ranging from $48.1\pm18.7\%$ to $50.6\pm18.4\%$.

Broodstock Mortality

During the reproductive season no mortality was observed in females or males of either group.

However, a high mortality rate of wild perch broodstock of both sexes (females $92.5\pm2.5\%$ and males $91.5\pm3\%$) was observed 14 days after the conclusion of reproduction (May 15). Farmed perch broodstock showed significantly lower mortality (females $21.5\%\pm12.5\%$ and males $17.5\pm15\%$).

Discussion

This study showed seasonal spawning of Eurasian perch and compared morpho-anatomical parameters, reproduction characteristics, and mortality of broodstock in farmed and wild perch.

The gonadosomatic index in wild fish $(26.9\pm4.4\%)$ corresponded to the results published by Migaud et al. (2003). These values were significantly higher than in farmed fish (18.5±2.3%), also similar to results in farmed fish recorded by Migaud et al., (2002). Also, HSI and VSI in this study showed significant differences between farmed and wild perch, corresponding to reports by Sulistyo et al. (2000) for wild perch and by Migaud et al. (2002) for farmed perch. Differences between farmed and wild perch have been suggested to be caused mainly by unsuitable feed for the farmed fish (Bell et al., 1997; Kestemont et al., 1999; Izquierdo et al., 2001; Kestemont et al., 2008b; Henrotte et al., 2010), and wild perch showed superior development of gonads under natural conditions (Kouril et al., 1997a). Fontaine et al. (2008) has drawn attention to the lower quality of reproduction in farmed perch bred under controlled conditions. These authors add that, to address lower reproduction in farmed perch, it is necessary to improve the diet by adding prey fish and a larger proportion of natural ingredients to the feed. For improving the reproduction of farmed perch, Kestemont et al. (2008b) recommend the use of feed with higher content of arachidonic acid (C20:4 n6).

According to Craig (2000), temperature increase in spring synchronizes spawning in Eurasian perch. However, in our study, ovarian development was not synchronous among fish, and this could explain that spawning period depended on the high variability steroid concentrations (Ciereszko *et al.*, 1997a, 1997b; Migaud *et al.*, 2003).

The highest spawning activity was recorded in the present study in the Middle Period, although eggs were produced in both the Early and Late Periods. More farmed perch than wild perch spawned. This may indicate that spawning in Eurasian perch, a limiting factor in fish production is affected by stress (Wang *et al.*, 2003). Wang *et al.* (2003) suggested that wild broodstock possessed high cortisol levels, possibly the result of stress due to environmental conditions (handling, storage, etc.) or reproduction. The relative fecundity of wild perch observed in this study (317 054 eggs kg⁻¹) not corresponds to values reported by Policar *et al.* (2008b). Kouril and Hamackova (1999) also reported lower relative fecundity (102 100 eggs kg⁻¹) of wild fish. Fecundity of farmed perch observed here (112 470 eggs kg⁻¹) was significantly lower than for wild perch. Similar results were also observed by Kouril and Hamackova (2000).

The fertilization rate of $91.9\pm4.4\%$ and $90.5\pm5.5\%$ in farmed and wild perch, respectively, was not significantly different and is in agreement with results published by Kouril *et al.* (1997b) and by Migaud *et al.* (2001). Kouril and Hamackova (1999) reported 60-95% fertilization of eggs in perch after artificial stripping. In the present study, the fertilization rate decreased over time which differs from results of Migaud *et al.* (2001).

The highest differences between farmed and wild perch in spawning quality were recorded in hatching rate. A low hatching rate (27.9% in farmed fish) could be due to poor male or female gamete quality of the farmed fish. Changes in hatching rate over time were similar to those in fertilization rate. A gradually decreasing fertilization rate and consequently a lower hatching rate were reported by Kestemont et al. (1999). This author also reported high fluctuations in larval resistance to starvation and osmotic stress. In the present study, fluctuations of larval resistance to starvation and osmotic stress were not observed. No differences in total length of larvae, larval resistance to starvation and osmotic stress were found between farmed and wild perch. Based on these indicators, lower reproductive success of farmed fish was not observed.

In our study, high broodstock mortality was recorded after the spawning season. The post spawning mortality of wild perch (females and males) reached 92% after two weeks and corresponded to results reported by Wang *et al.* (2003). This author reported that high mortality is probably due to elevated levels of cortisol during the spawning period. Mortality of farmed perch was 19.5%, lower than that found by Migaud (2006), who reported 36-72% mortality in farmed perch.

In conclusion, the present study showed the new data about comparison of wild and farmed perch reproduction characteristics. The highest differences between farmed and wild perch were in the morphoanatomical parameters GSI, VSI, HSI, and in the hatching rate. Further studies are required to optimize protocols for using better artificial food in farmed perch and decreased stress level in wild perch.

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