

Quality of semen and histological analysis of testes in Eurasian perch *Perca fluviatilis* L. during a spawning period

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ABSTRACT: A qualitative assessment of the Eurasian perch *Perca fluviatilis* semen describing the basic parameters of seminal plasma was completed. The histological methods used in this study showed changes in perch gonads during a spawning and post-spawning period. At the late period of the spawning season, the structure of testes was clearly loosened and spermatozoa did not fill uniformly all the ampullae of the testes, leaving free spaces at their banks and no spermatids were observed. The results confirmed that there was an additional period after spawning in the annual reproductive cycle of the male Eurasian perch. In both years of investigations (2000–2001), an essential decline in sperm motility at the late period of the spawning season was observed, from more than 85% to 56% in 2000. The sperm motility was not influenced by sperm concentrations because throughout the spawning time no changes in the sperm concentration were observed, 32.4 and 32.6 mld/ml at beginning and at the late period of spawning period, respectively. In contrast to sperm concentrations, protein concentrations in seminal plasma increased in the late period of spawning season, from 3.95 to 5.16 g/l in 2001. This study confirmed that protein concentrations in the seminal plasma of most fish are much lower than in the other vertebrates. Among the fish examined, perch is characterized by one of the highest values of protein concentrations in plasma. Our observations confirmed that a high water temperature influenced anatomical and functional parameters of the reproductive system in male perch.

Keywords: perch; gonads; semen; spawning time

In the majority of teleost fish, fertilization occurs externally when in the act of spawning, eggs and sperm are discharged in close proximity. Fish sperm consists of spermatozoa which are produced during spermatogenesis and seminal plasma produced by the gonads and spermatid ducts. The parameters which determine the fertilization capability of spermatozoa are their motility and concentration. The movement of fish spermatozoa for external fertilization begins at the moment of their contact with water. Based on the sperm concentration, we can indirectly determine the correct or irregular functioning of male gonads.

Fish seminal plasma contains mineral components and smaller quantities of proteins, carbohydrates, organic phosphorus, lactic acid and lipids. The seminal plasma of teleost fish is characterized by a very low concentration of proteins in comparison with mammalian semen (Mann and Lutwak-Mann, 1981). Generally in fish, the protein concentration in seminal plasma does not exceed 2 g/l. Currently, only two protein groups were identified in fish seminal plasma: lipoproteins (Loir et al., 1990) and anti-proteinases (Ciereszko et al., 2000). Besides that, some proteolytic activity was found in fish seminal plasma (Lahnsteiner et al.,

Supported by UWM Olsztyn, Poland (Project No. 080302.0214).

1995, 1997; Kowalski et al., 2004), but in most cases the physiological role of seminal plasma proteases is not well understood yet.

The annual reproductive cycles of gonads in both genders of teleost fish are different depending on the spawning time and length of spawning period. Fish species are essentially different in spermatogenesis during the annual cycle, e.g. spermatozoa in the male gonads of carp, *Cyprinus carpio*, are present throughout the year (Koldras et al., 1990), contrary to trout, *Salmo trutta* m. *fario*, where spermatozoa are present only during the reproductive period, which is in autumn or winter (Billard et al., 1982).

At present, there is a lot of data concerning spermatogenesis of teleost fish – especially in *Salmonidae* and *Cyprinidae* – but this process is not clear in *Percidae*. Investigations into oogenesis and spermatogenesis of Eurasian perch were conducted by Sulistyó et al. (1998, 2000). Studies on sperm motility and concentration, and on the levels of steroid hormones in the spawning time in perch have already been carried out for many years but not in relation to spermatogenesis in the testis.

The aim of our study was to evaluate the qualitative determinants of Eurasian perch semen and to characterize the basic parameters of seminal plasma relative to changes in perch testes during the spawning period. We found that the combination of anatomical and biochemical methods for investigations of the physiological processes in the reproductive system of male perch could result in valuable information. The comparison of data on semen quality between the beginning and late period of the spawning season in relation to

histological analysis of the gonads, and the effect of increasing temperature on semen parameters and spermatogenesis could show changes in the reproductive system of Eurasian perch during the spawning period.

MATERIAL AND METHODS

Eurasian perch were collected in 2000 and 2001 from Drwęckie Lake located in Northwestern Poland using gill nets. After catching, the fish were transported to a laboratory at the Department of Ichthyology, University of Warmia and Mazury in Olsztyn. In both years, fish were kept under natural thermal conditions at $10^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ (Table 1) for three days. After this time in 2000, fish were divided into two experimental groups in two different tanks from which sperm was taken at different times: beginning (P1) and late period (P2) of the spawning season. In 2001, the two experimental variants were repeated for groups P3 and P4. In order to shorten the period of spawning readiness other two experimental groups (P5 and P6) were placed in another two tanks. In group P5 the water temperature was gradually increased for the next two days to $15^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and in group P6 for the next one week to $17^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. In both groups water temperatures were kept at these levels until the end of the study. In group P5 and P6, sperm from males was collected in five and ten days after the temperature was elevated to 15°C and 17°C , respectively (Table 1). For each individual the volume of collected milt was estimated.

Before the semen collection, fish were anaesthetized in 2-phenoxyethanol solution (0.3 ml/l).

Table 1. Number of males, mean values \pm standard deviations for total body length and weight of fish from experimental groups with water temperature and time of material collection

Experimental groups	Number of males	Date of material collection	Water temperature at the time of material collection	Body length (l.t.) (cm)	Body weight (g)
P1	15	12. 04. 2000	10°C	16.22 ± 2.58	49.19 ± 33.40
P2	13	10. 05. 2000	10°C	14.31 ± 2.70	33.14 ± 23.36
P3	15	15. 04. 2001	10°C	16.03 ± 2.63	54.70 ± 47.07
P4	16	09. 05. 2001	10°C	16.53 ± 2.36	53.64 ± 23.62
P5	19	24. 04. 2001	15°C	15.76 ± 1.87	48.51 ± 22.63
P6	5	09. 05. 2001	17°C	15.13 ± 0.75	34.28 ± 7.30
P7	10	October 2001	*	16.37 ± 0.83	49.71 ± 16.62

*for a histological study fish were taken directly from natural environment

Milt samples were collected by stripping, avoiding the water, urine, and faeces contamination, and transported on ice (2°C) to the Institute of Animal Reproduction and Food Research, Polish Academy of Sciences in Olsztyn. Seminal plasma was obtained by centrifugation (8 000 g, 10 min) within 12 hours after sampling and stored at –70°C until used.

Sperm motility (in 0.5% NaCl activating solution) was estimated using a light microscope (400×) and expressed as a percentage of motile spermatozoa. The dilution of sperm/solution ratio was approximately 1:6 (5 µl of milt and 30 µl of NaCl). Only the progressive straight-line movement of spermatozoa was noted. Sperm concentration was estimated using a spectrophotometric method described by Ciereszko and Dabrowski (1993).

Protein concentration was measured using the Lowry et al. (1951) method.

Additionally in both years, at the same time when the semen was collected, 5 fishes from each group were sacrificed, testes were removed and fixed in Bouin's solution for a histological study. A separate group (P7) consisted of fish caught outside the spawning season (October 2001), which was used only for a histological analysis. Gonads were then dehydrated in ethyl alcohol solutions, kept in xylene and embedded in paraffin. Histological sections were obtained by cutting the blocks with a rotational microtome RM 2155 (Leica, Germany) into 5 µm thick sections. The sections were stained with haematoxylin and eosin (Pearse, 1985) and examined by light microscopy. The serial cross-sections were analyzed for the shape, size and type of germ cells present in gonads.

Sperm concentration and motility, and protein concentration of seminal plasma were statistical-

ly analyzed using a one-way analysis of variance (ANOVA). The Fisher test was used for post-hoc comparisons at a significance level $P < 0.05$. Percentage data of sperm motility were arcsine-transformed before analysis. Analyses were performed using Statistica™ software.

Fish were measured (body length – longitudo totalis – l.t.) to the nearest 5 mm and weighed (body mass) to the nearest 0.1 g (Table 1).

RESULTS

Quality and biochemical parameters of perch semen

In both years at the beginning of spawning season (P1 and P3), sperm motility was comparatively high and amounted to more than 85% (Table 2). At the late period of spawning season in both years, an essential decline in sperm motility was observed for P2 and P4, but a statistically significant difference was observed only in 2000 (Table 2). A more distinct difference in sperm motility at the late period to the beginning of spawning season was observed in fish that were kept at a higher temperature of water (P5 and P6). The variability of sperm motility in each group at the late period of spawning time was very high, which was documented by high values of standard deviations (Table 2).

Sperm concentration ranged from 21.71 to 32.60 mld/ml (Table 2). No significant difference in sperm concentration was observed between the beginning and the late period of the spawning season in both years, when fish were kept at 10°C. Sperm concentration decreased at the late period in relation to the beginning of spawning season only in groups

Table 2. Mean values ± standard deviations for qualitative determinants of semen and protein concentration in the seminal plasma of perch

Experimental groups	Sperm motility (%)	Sperm concentration (mld/ml)	Protein concentration (g/l)
P1	86.71 ^a ± 6.17	32.44 ^a ± 1.96	4.82 ^a ± 1.38
P2	56.92 ^b ± 23.23	32.60 ^a ± 6.64	6.14 ^b ± 2.16
P3	86.00 ^a ± 6.32	29.27 ^a ± 4.04	3.95 ^a ± 1.23
P4	70.83 ^a ± 19.29	25.46 ^a ± 1.89	5.16 ^c ± 1.36
P5	82.63 ^a ± 7.33	29.13 ^a ± 3.22	4.32 ^a ± 0.96
P6	27.5 ^c ± 21.23	21.71 ^b ± 6.51	6.72 ^b ± 0.40

significant differences ($P < 0.05$) are indicated by different letters

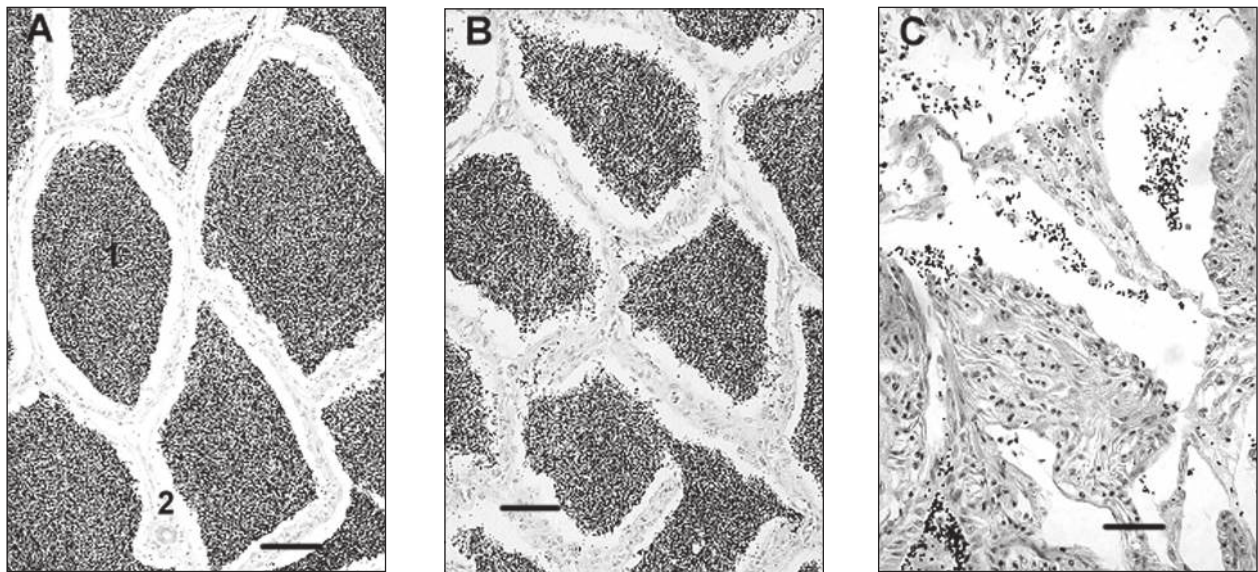


Figure 1. Cross-section of perch testis. A: at the beginning of spawning season (group P3); 1 – seminal vesicles with spermatozoa, 2 – connective tissue containing single spermatogonia. B: at the late period of spawning season (group P4). C: at the late period of spawning season (group P6)

Bar = 100 μ m

of fish that were kept at a high water temperature (Table 2). The volume of collected sperm differed between males from 0.5 to 4.8 ml and no significant effect of time on the sperm volume was found. No correlation between body weight or body length and the volume of collected milt was observed.

Protein concentrations in seminal plasma increased at the late period of spawning season in all experimental groups. Protein concentrations in groups ranged from 3.95 to 6.72 g/l (Table 2).

Histological analysis of perch gonads

At the beginning of spawning season, the ampullae of the testes were fully filled with spermatozoa and separated by a thin layer of connective tissue with single spermatogonium cells (Figure 1a). At the late period of spawning season, the structure of testes was clearly loosened and spermatozoa did not fill uniformly all the ampullae of the testes, leaving free spaces at their banks. The connective

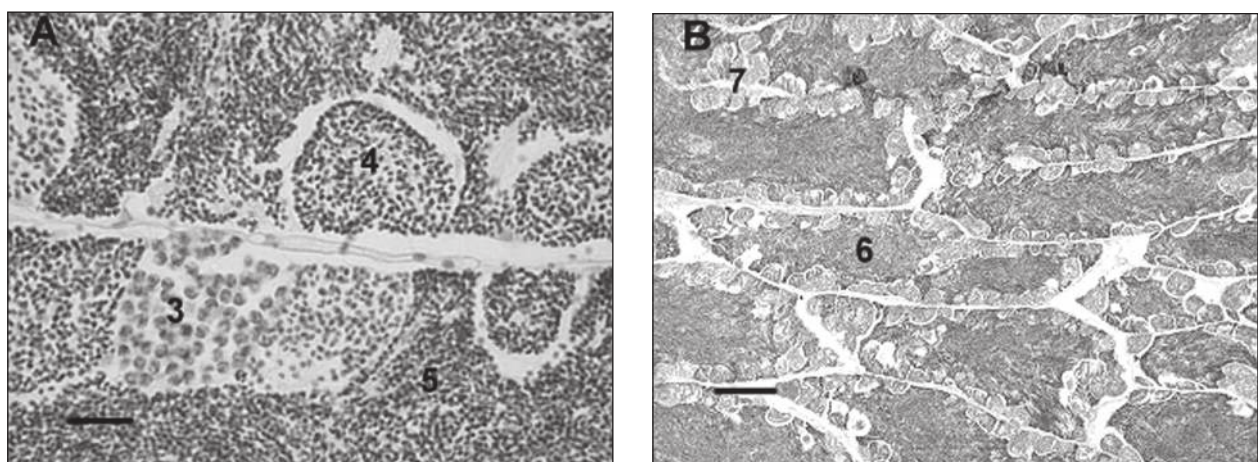


Figure 2. Cross-section of perch testis (collected in October, group P7). A: seminal vesicles with: 3 – spermatocytes, 4 – spermatids, 5 – spermatozoa. B: seminal vesicles with: 6 – spermatozoa and spermatids, 7 – spermatogonia and spermatocytes

Bar = 40 μ m for Figure 2a; Bar = 200 μ m for Figure 2b

tissue was enlarged and numerous spermatogonia were observed (Figure 1b). In either of the periods no spermatids were observed. In the testes of fish that were stimulated by the temperature (Figure 1c) numerous blood vessels and spermatogonia cells were clearly observed. In the testes of fish collected in October, all phases of germ cells were observed (Figure 2a). Spermatozoa and spermatids were observed inside the testis ampullae, whereas spermatogonia and spermatocytes were observed in the periphery of the tissue wall (Figure 2b).

DISCUSSION

Researches on the anatomy and physiology of the male reproductive system are important to better understand the biology of animal reproduction, including fish. Sperm quality is a very important factor conditioning the correct process of natural or artificial spawning. The present methods of artificial reproduction permit to use the sperm that is stored outside the male organism for a longer time. In genomic manipulations (androgenesis, gynogenesis, polyploidy), parameters of sexual products can decide on reproduction success. In this context, researches on the biology of semen are very important, especially in the sphere of fish reproduction. Sperm motility and concentration are parameters which determine the capacity of spermatozoa to fertilize. A more distinct difference in sperm motility at the late period to the beginning of spawning season observed in fish that were stimulated by the temperature may be related to the fact that at this moment of the spawning period of perch processes preparing the testes for subsequent spermatogenesis already take place. A higher temperature of the environment probably stimulates and/or accelerates occurrences leading to a decline in sperm motility. Besides, Munkittrick and Moccia (1987) suggested that at the end of spawning season in rainbow trout *Oncorhynchus mykiss*, together with a decline in sperm motility their ability to fertilization dropped.

Based on the sperm concentration we can indirectly infer about the correct or irregular function of males gonads. Sperm concentration was estimated for several teleosts: e.g. pike *Esox lucius*, (Linhart, 1984) and bream *Abramis brama*, (Glogowski et al., 1999a). In yellow perch, *Perca flavescens*, this parameter was estimated to amount

to 41.58 mld/ml (Ciereszko and Dabrowski, 1993), and even to 48.5 mld/ml (Glogowski et al., 1999b). In this study sperm concentrations in each group were higher than in perch obtained by Lahnsteiner et al. (1995). The sperm concentration decreased at the late period of reproduction season in perch only in the group of fish that were kept at a high water temperature, which suggested that the higher temperature of environment negatively affected the quality of sperm, and it could also influence the shortening of the spawning period of perch.

The seminal plasma of teleost fish contains a very low protein concentration in comparison with the other vertebrates, probably because in fish gonads there are no additional sexual glands that produce organic components, e.g. proteins (Billard, 1986). However, despite the lack of coagulations and semen liquefaction in teleosts, a proteolytic activity was found in seminal plasma (Lahnsteiner et al., 1995, 1997), but physiological functions of these proteases in semen or testes are still unknown. Among the proteins that were identified in the seminal plasma of teleosts, the proposed role of lipoproteins may be related to their interaction with sperm plasma membranes (Loir et al., 1990), whereas that of the anti-proteinase inhibitors may be related to the protection of spermatozoa from a proteolytic attack (Ciereszko et al., 2000). Possible sources of proteins present in the fish seminal plasma are Sertoli cells, spermatozoa remaining after the spawning season or blood (Loir et al., 1990). The protein concentration in seminal plasma increased at the late period of spawning season in all experimental groups, which may be a result of beginning degradation process of spermatozoa and/or a result of disturbing the barrier blood – gonads and penetration of this protein from blood to the reproductive system. Protein concentrations in the seminal plasma of carp (Billard and Cosson, 1989), bream (Glogowski et al., 1999a), rainbow trout (Scott and Baynes, 1980) and pike (Glogowski et al., 1997) were much lower than in perch in this study. A similar protein concentration in the seminal plasma of perch was reported by Kowalski et al. (2003). Our study confirmed that protein concentrations in seminal plasma were much lower in fish than in the other vertebrates. Among the fish that were examined, perch is characterized by one of the highest values of protein concentrations in plasma.

The annual reproductive cycle of testes in teleost fish is dependent on environmental conditions

such as temperature that determines the time and duration of spawning period. Annual reproductive cycles of testes can be divided into spring, spring-summer or autumn ones. Spermatogenesis in the annual cycle of some species is essentially different, e.g. spermatozoa in the male gonads of carp are present throughout the year (Koldras et al., 1990), while in trout spermatozoa are present only in the reproductive period which occurs in autumn or in winter (Billard, 1986). In some species there is an additional period after spawning in the annual reproductive cycle lasting 1–2 months. These observations were described by Sulistyó et al. (2000) in Eurasian perch testes and were confirmed in the present study. The lack of spermatids in the late part of spawning period in the testes of perch suggests a pause in the spermatogenesis process after the end of the reproductive season. The occurrence of an additional period in the annual cycle of testes was observed in yellow perch (Dabrowski et al., 1994) and in the testes of rainbow trout a set-back of spermatogenesis was ascertained in the post-spawning period (Billard and Takashima, 1983). In the testes of fish collected in October, all phases of germ cells were observed. Similar histological observations of perch testes were reported by Sulistyó et al. (2000).

The water temperature has an influence on the spermatogenesis process in teleosts and accelerates and/or changes the time of spawning. In thermally polluted lakes, a shift of the time of spawning of perch from April to March was observed by Długosz (1983). In this case the post-spawning period was observed in most males in April, when in normal thermal conditions the testes are in the phase of spawning readiness (Sulistyó et al., 2000; this study).

Our results confirmed that the temperature has an influence on anatomical and functional changes of reproductive system and quality of sperm. The worst semen quality at the end of reproduction season could cause worse effects of artificial reproduction in Eurasian perch.

Acknowledgements

We thank H. Karol, D. Kubiak and E. Ziomek for excellent technical assistance and J. Rinchar from Ohio State University for comments and correction of the English text.

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Received: 2005–05–30

Accepted after corrections: 2006–01–30

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