Arch. Pol. Fish.	Archives of Polish Fisheries	Vol. 16	Fasc. 1	93-100	2008

Short communications

DESCRIPTION OF PIKEPERCH, *SANDER LUCIOPERCA* (L.), SEMEN OBTAINED FROM MALES HELD UNDER DIFFERENT REARING CONDITIONS

Beata I. Cejko, Jan Glogowski*,**, Radosław K. Kowalski*, Dariusz Kucharczyk***, Katarzyna Targońska****

*Department of Molecular Andrology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences in Olsztyn, Poland
**Department of Ichthyology, Faculty of Environmental Sciences and Fisheries, University of Warmia and Mazury in Olsztyn, Poland
***Department of Lake and River Fisheries, Faculty of Environmental Sciences and Fisheries, University of Warmia and Mazury in Olsztyn, Poland

ABSTRACT. The aim of the study was to determine the concentration and motility of sperm and the osmotic pressure and total protein in the seminal plasma of pikeperch, *Sander lucioperca* (L.). The samples investigated were milt from males held under various rearing conditions (ponds, tanks of a closed recirculating systems, cages). The highest percentage of motile sperm was noted in the semen samples from males held in basins, while the lowest percentage of motile sperm and the highest protein content was confirmed in the samples from spawners held in ponds. Sperm motility and the osmolality of the seminal plasma were low in all the spawner groups; this may indicate contamination with urine. As the content of protein rose in the plasma and the sperm concentration increased, so did their motility.

Key words: PIKEPERCH, MILT, HCG, SEMINAL PLASMA, CONTAMINATED SEMEN

The quality of milt is as crucial as that of spawn for the success of both natural and artificial spawning. The basic quality parameters of semen that determine the ability of the sperm to fertilize are motility following activation and concentration (Kruger et al. 1984). Based on the quantity of sperm in a unit measure, conclusions can be drawn regarding testicular development and functioning (Król et al. 2006), among other factors, while the parameters of sperm motility differ among species and individuals

CORRESPONDING AUTHOR: Beata I. Cejko, Instytut Rozrodu Zwierząt i Badań Żywności PAN, Zakład Andrologii Molekularnej, ul. Tuwima 10, 10-747 Olsztyn, Tel. +48 89 5393131, e-mail: bcejko@poczta.onet.pl

(Glogowski et al. 2007). Sperm concentration is not a constant value for this phylum of animal (Ciereszko and Dąbrowski 1993).

The quantity of some of the seminal components are key in predicting the fertilization capability of a semen sample or its suitability for short- or long-term storage. Not all of the seminal components are secreted; some come from damaged sperm or cells from the testes and the vas deferens (Glogowski et al. 2007). Sertoli cells or blood are the source of the protein in seminal plasma (Loir et al. 1990), and the protein content in the seminal plasma is much lower in comparison with the seminal plasma of other vertebrates. The factor that either activates or halts sperm motility in teleost fish is the osmotic pressure in the seminal plasma (Alavi and Cosson 2006).

Among the endemic freshwater fish of Poland, pikeperch, *Sander lucioperca* (L.), is unquestionably a valuable species economically thanks to its rapid growth rate and high-quality meat. As a predator, it also plays an important role in maintaining ecological equilibrium. The progressive eutrophication of waters in Poland is leading to disadvantageous environmental changes, and pikeperch spawners are caught in open waters and then held and spawn artificially under controlled conditions with the application of the appropriate hormonal substances (Demska-Zakęś and Zakęś 2002, Zakęś and Szczepkowski 2004, Zakęś and Demska-Zakęś 2005). The advantages of artificial reproduction under controlled conditions include the possibility of monitoring the course of spawning and choosing gametes of the highest quality. This is reflected in the results obtained and the decision-making process of the use of the sex products obtained. The aim of the current study was to describe the basic parameters of the semen of pikeperch held under various rearing conditions, with a specific focus on the osmotic pressure of the seminal plasma.

Pikeperch spawners were caught in the Martwa Wisła River in spring 2006. In the summer of the same year, they were divided into three rearing groups: I – fish held in ponds with a surface area of 10 are; II – fish held in basins with a volume of 1 m^3 connected to a closed recirculation system; III – fish held in cages with a volume of 8 m^3. The conditions in the two types of ponds were similar with regard to light regime and temperature, while in the basins conditions were created that approximated the natural ones. After about nine months, in spring 2007, when the water temperature in the ponds reached 12°C, all of the fish were moved to basins connected to a recirculating system.

The fish were acclimatized to a temperature of 15°C for several days, then individual fish were examined and stimulated hormonally with human chorionic gonadotropin (HCG) at a dose of 450 IU kg^{-1} (Biogonadyl, Biomed Lublin, Poland). The doses of this preparation were the same for both sexes and were administered once through an injection to the peritoneum. During manipulation, the fish were anesthetized with a dose of 1 ml l^{-1} Propiscin (Inland Fisheries Institute in Olsztyn). The water temperature during manipulation was 15°C, and the period between obtaining the milt and the analyses was three to four hours. Thirty hours after the injection, semen samples were collected with a syringe. The samples were transported on ice (2-4°C) to the Department of Molecular Andrology, PAS in Olsztyn. In the laboratory, the motility of the sperm was determined with the subjective method under magnification of 400 × with 119 mM $NaHCO_3$ + 0.5% albumin or H_2O as the activating agent. After dilution (1000 × in 0.7 % NaCl), the sperm concentration in the semen was determined with the spectrophotometric method as described by Ciereszko and Dąbrowski (1993). The total protein content was determined (Lowry et al. 1951), and the seminal plasma osmotic pressure was determined with a WESCOR® Vapor Pressure osmometer 5520.

Pearson's correlation coefficient was applied to determine the dependencies between the characters of samples from the three groups analyzed (GraphPad Prism 4 Demo, GraphPad Software Inc., USA). The significance of differences of the characters among the three fish groups studied was verified with Tukey's post-hoc test ($P < 0.05$).

A higher percentage of motile sperm was noted in the milt samples that were activated with H_2O than in those activated with 119 mM $NaHCO_3$ with 0.5% albumin (Table 1). The highest percentage of motile sperm was noted in the fish reared in cages (47.50%, N = 4), and the lowest in the fish reared in ponds (33.16%, N = 4). Regardless of the activating fluid applied, sperm motility was positively correlated with concentration (Fig. 1).

Sperm concentrations ranged from 4.28 to 5.26 mld ml^{-1}, depending on which group the fish came from, among which the highest sperm concentration was noted in samples from fish that had been reared in ponds (group I; Table 1). In the samples examined for sperm motility, the mean concentration of sperm was similar for all groups (group I – 7.14; group II – 5.63; group III – 6.64 mld ml^{-1}. In the samples with non-motile sperm, the sperm concentrations were also similar among the groups although decidedly lower (group I – 3.76; group II – 2.03; group III – 2.78 mld ml^{-1}).

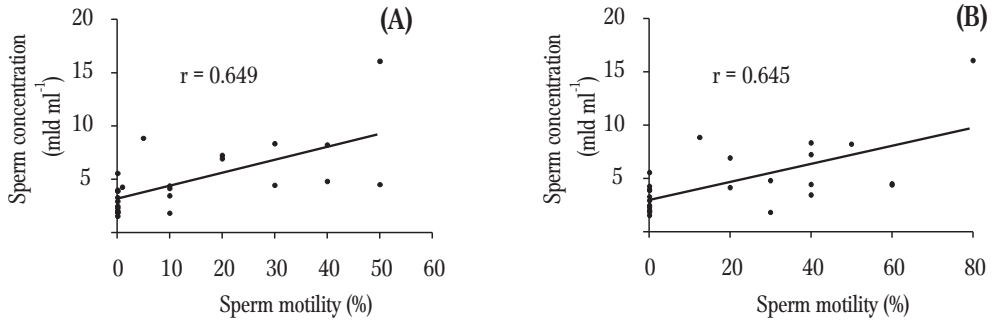

Fig. 1. Dependency between the concentration and motility of sperm activated with 119 mM $NaHCO_3$ + 0.5% albumin (A) or with H_2O (B).

TABLE 1

Basic quality parameters of pikeperch semen obtained from fish reared under different conditions (mean ± SD)

Group	N	Sperm motility (%)		Sperm concentrations (mld ml^{-1})	Osmotic pressure (mOsm kg^{-1})	Concentrations of total protein (mg ml^{-1})
		119 mM $NaHCO_3$	H_2O			
Group I	9	7.22±10,93	14.72±21.81	5.26±2.31	178±74.87	2.43±1.94
Group II	8	17.50±17.52	25.00±21.38	4.28±2.41	227±75.27	1.38±1.24
Group III	8	15.12±21.94	23.75±31.14	4.71±4.74	192±90.03	1.01±0.69

The highest mean value of seminal plasma osmotic pressure was noted in the fish from group II, while the lowest was from group I (Table 1). Samples in which no sperm motility was observed had the lowest values of this parameter (means for groups: I - 123; II - 144; III - 126 mOsm kg^{-1}) in comparison to samples that had a certain percentage of motile sperm (means for groups: I - 247; II - 277; III - 258 mOsm kg^{-1}). In previous studies (Kowalski et al. 2003, Glogowski et at. 2007) higher osmotic pressure values were obtained in European pikeperch (221-287 mOsm kg^{-1}). The seminal plasma protein content ranged from 1.01 to 2.43 mg ml^{-1} (Table 1). It was demonstrated that the concentration of sperm increased along with increasing levels of protein in the plasma ($r = 0.611$, $P < 0.05$). Similarly, with increased seminal plasma osmotic pressure the motility of the sperm increased (Fig. 2).

The lowest percentage of motile sperm and the highest protein contents were noted in the samples obtained from the spawners held in ponds. The motility of sperm as well as the seminal plasma pressure exhibited low values in all fish groups, and this may

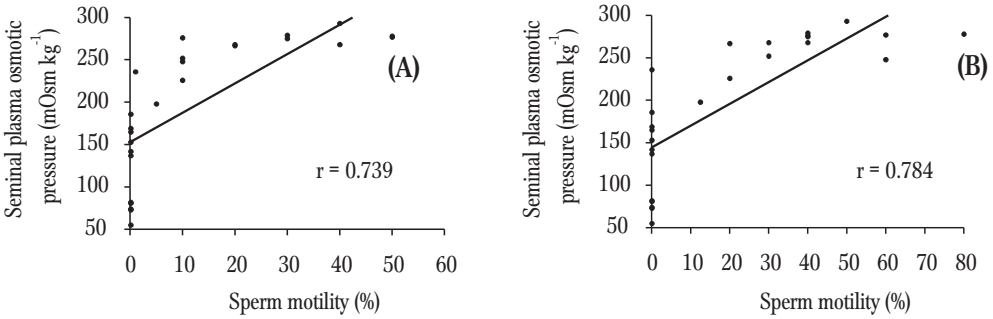

Fig. 2. Dependency between seminal plasma osmotic pressure and sperm motility in pikeperch sperm activated with 119 mM $NaHCO_3$ + 5% albumin (A) or with H_2O (B).

indicate that the milt was contaminated with urine. This problem occurs when stripping milt from many fish species. This can be largely eliminated by collecting semen directly from the vas deferens with a catheter (Glogowski et al. 2000).

The concentration of sperm in the pikeperch groups that were studied was lower than values determined in previous studies of this same species (Kowalski et al. 2003, Glogowski et al. 2007). The concentration of sperm that was determined in European perch, *Perca fluviatilis* L., (Lahnsteiner et al. 1995, Król et al. 2006) and yellow perch, *Perca flavescens* (Mitch.) (Ciereszko and Dąbrowski 1993) also differed and was higher than the values presented herein. In comparison to other semen quality parameters, only the content of total protein in the seminal plasma of group I (mean 2.43 mg ml^{-1}) can be considered as close to the levels reported in the literature for perch (Lahnsteiner et al. 1995) or pikeperch (Kowalski et al. 2003). A positive statistically significant relationship between this parameter and the concentration of sperm may indicate that this protein comes from damaged or dead sperm.

The seminal plasma osmotic pressure in Chondrostei fish, such as the sturgeons, is much lower than that of the Teleostei fish, such as the cyprinids, salmonids, or percids. In addition to variation in genera and species, this parameter can also differ among individuals (Alavi and Cosson 2006). It is worth emphasizing that the value of seminal plasma osmotic pressure also appears to be an indicator, among other things, of semen contamination with urine, which results in its poor quality and the lack of its ability to fertilize or to be stored for short periods or cryopreserved. This situation also occurs when stripping milt under controlled conditions and has been observed in species such as carp, *Cyprinus carpio* L. (Perchec et al. 1995), tench, *Tinca tinca* (L.) (Rodina et al.

2004), European pikeperch (Bokor et al. 2007), and rainbow trout, *Oncorhynchus mykiss* (Walbaum) (Glogowski et al. 2000).

The statistically significant dependency between sperm motility and seminal plasma osmotic pressure confirms that the latter is indeed a milt quality parameter. It appears that urine contamination is responsible for the high variability observed during the present study. Bokor et al. (2007) also noted when collecting milt samples from European pikeperch that it is impossible to avoid entirely the contamination of the milt with urine. In the current study, the milt samples in which the sperm exhibited no motility had seminal plasma osmotic pressure within the range of 55 to 186 mOsm kg^{-1}.

Inducing reproduction with hormonal substances can influence a variety of milt quality parameters including seminal plasma osmotic pressure (Redondo-Muller et al. 1991). It would appear, however, that weak motility or the lack thereof is more likely to result from an inappropriate period between hormonal administration and semen stripping than from the hormone used to stimulate spermiation. HCG is indeed a substance that is both recommended and used widely and one that produces excellent results in the reproduction of this fish species (Demska-Zakęś and Zakęś 2002, Zakęś and Szczepkowski 2004).

From a practical point of view, any contamination of the sex products (water, urine, stool, blood) should be eliminated. One alternative for achieving this is to ensure that the bladder is emptied prior to semen collection as is widely applied when stripping spawners (with abdomen massage) (Perchec et al. 1995). Another method is to use catheters, or solutions to immobilize sperm motility. Using immobilizers leads to the stabilization of the ambient pH, which is crucial when freezing semen (Rodina et al. 2004) or for its short-term storage. Successful sperm revitalization was achieved in carp (Perchec et al. 1995) and other species, and in the case of pikeperch semen, which is often contaminated with urine (during collection), it is recommended to investigate the effectiveness of this procedure in the future.

Studies of the characteristics of semen from various fish species indicate that there is significant variation among individuals in the biochemical parameters of seminal plasma, sperm concentration, and sperm motility. This is impacted by both genetic factors as well as the aging of sperm, seasonality, the type and dose of the hormonal substances administered, and the method of obtaining milt, which could have impacted the results of the current study. Future studies should either address the problem of

semen collection techniques that permit obtaining milt with good motility (in excess of 50%), or those for revitalizing sperm.

REFERENCES

Alavi S.M.H., Cosson J. 2006 – Sperm motility in fishes. (II) Effects of ions and osmolality: A review – Cell Biol. Int. 30: 1-14.

Bokor Z., Müller T., Bercsényi M., Horváth L., Urbányi B., Horváth Á. 2007 – Cryopreservation of sperm of two european percid species, the pikeperch (*Sander lucioperca*) and the Volga pikeperch (*S. volgensis*) – Acta Biol. Hung. 58: 199-207.

Ciereszko A., Dąbrowski K. 1993 – Estimation of sperm concentration of rainbow trout, whitefish and yellow perch using a spectrophotometric technique – Aquaculture 109: 367-373.

Demska-Zakęś K., Zakęś Z. 2002 – Controlled spawning of pikeperch, *Stizostedion lucioperca* (L.), in lake cages – Czech J. Anim. Sci. 6: 230-238.

Glogowski J., Kwaśnik M., Piros B., Dąbrowski K., Goryczko K., Dobosz S., Kuźmiński H., Ciereszko A. 2000 – Characterization of rainbow trout milt collected with a catheter: semen parameters and cryopreservation success – Aquacult. Res. 31: 289-296.

Glogowski J., Kowalski R.K., Ciereszko A. 2007 – Biology and cryopreservation of fish semen - In: The Biology of Animal Reproduction. Biological impact on male reproductive value (Eds.) Strzeżek J. Wyd. UWM, Olsztyn: 265-294 (in Polish).

Kowalski R., Glogowski J., Kucharczyk D., Goryczko K., Dobosz S., Ciereszko A. 2003 – Proteolytic activity and electrophoretic profile of proteases from seminal plasma of teleosts – J. Fish Biol. 63: 1008-1019.

Król J., Glogowski J., Demska-Zakęś K., Hliwa P. 2006 – Quality of semen and histological analysis of testes in Eurasian perch *Perca fluviatilis* L. during a spawning period – Czech J. Anim. Sci. 5: 220-226.

Kruger J.C. De W., Smit G.L., Van Vuren J.H.J., Ferreira J.T. 1984 – Some chemical and physical characteristics of the semen of *Cyprinus carpio* L. and *Oreochromis mossambicus* (Peters) – J. Fish Biol. 24: 263-272.

Lahnsteiner F., Berger B., Weismann T., Patzner R. 1995 – Fine structure and motility of spermatozoa and composition of the seminal plasma in the perch – J. Fish Biol. 47: 492-508.

Loir M., Labbe C., Maisse G., Pinson A., Boulard G., Mourot B., Chambeyron F. 1990 – Proteins of seminal fluid and spermatozoa of rainbow tout (*Oncorhynchus mykiss*): Partial characterization and variations – Fish Physiol. Biochem. 8: 485-495.

Lowry O.H., Rosenbrough N.J., Farr A.R., Randall K.J. 1951 – Protein measurement with Folin phenol-reagent – J. Biol. Chem. 193: 265-275.

Perchec G.P., Cosson J., Andrč F., Billard R. 1995 – Degradation of the quality of carp sperm by urine contamination during stripping – Aquaculture 129: 135-136.

Redondo-Muller C., Cosson M.P., Cosson J., Billard R. 1991 – In vitro maturation of the potential for movement of carp spermatozoa – Mol. Reprod. Dev. 29: 259-270.

Rodina M., Cosson J., Gela D., Linhart O. 2004 – Kurokura solution as immobilizing medium for spermatozoa of tench (*Tinca tinca* L.) – Aquacult. Int. 12: 119-131.

Zakęś Z., Demska-Zakęś K. 2005 – Artificial spawning of pikeperch (*Sander lucioperca* (L.)) stimulated with human chorionic gonadotropin (HCG) and mammalian GnRH analogue with a dopamine inhibitor – Arch. Pol. Fish. 13: 63-75.

Zakęś Z., Szczepkowski M. 2004 – Induction of out-of-season spawning of pikeperch, *Sander lucioperca* (L.) – Aquacult. Int. 12: 11-18.

Received – 03 January 2007

Accepted – 15 February 2007

STRESZCZENIE

CHARAKTERYSTYKA NASIENIA SANDACZA, *SANDER LUCIOPERCA* (L.) POZYSKANEGO OD SAMCÓW PRZETRZYMYWANYCH W RÓŻNYCH WARUNKACH CHOWU

Celem badań było określenie koncentracji i ruchliwości plemników, ciśnienia osmotycznego i białka ogólnego w plazmie nasienia sandacza *Sander lucioperca* (L.). Próby mlecza pozyskano od samców podzielonych na trzy grupy tarlaków, tj. ryby przetrzymywane w stawach, basenach i sadzach, które poddano stymulacji hormonalnej przy użyciu HCG.

Wyniki prezentowanych badań świadczą o istnieniu zmienności osobniczej pod względem badanych parametrów jakości mlecza sandacza. Najwyższym odsetkiem ruchliwych plemników oraz najwyższymi wartościami ciśnienia osmotycznego cechowały się próby mlecza sandaczy, które przetrzymywano w basenach (grupa II; tab. 1). Najwyższą z kolei koncentrację plemników oraz zawartość białka całkowitego w plazmie nasienia stwierdzono w próbach, które pozyskano od samców przetrzymywanych w stawach (grupa I). Najwyższe współczynniki korelacji wyznaczono pomiędzy ruchliwością plemników a ciśnieniem osmotycznym plazmy nasienia i to bez względu na zastosowany roztwór aktywujący ruchliwość plemników (119 mM $NaHCO_3$ + 0,5% albumina lub H_2O), co świadczy o istotnym związku pomiędzy badanymi parametrami. Niski odsetek ruchliwych plemników i znaczna ilość prób z zerową ruchliwością może sugerować zanieczyszczenie mlecza moczem podczas jego pozyskiwania, co uwidocznione jest stosunkowo niskimi wartościami ciśnienia osmotycznego plazmy nasienia.