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NOTE

Characteristics of stripped and testicular Northern pike (*Esox lucius*) sperm: spermatozoa motility and velocity

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Abstract – The effects of osmolality and accidental urine contamination on spermatozoa velocity and motility were studied, combined with an examination of the biological characteristics of stripped and testicular sperm. Analysis of Northern pike sperm showed higher ionic concentrations of Na^+ (123 ± 9 mM), Cl^- (127 ± 7 mM), and K^+ (35 ± 5 mM) in the seminal fluid of testicular sperm (TS), than in that of stripped sperm (SS): Na^+ (116 ± 9 mM), Cl^- (116 ± 7 mM) and K^+ (25 ± 4 mM). Highest osmolality of seminal fluid was observed in TS with a value of 358 ± 77 mOsmol kg^{-1} compared with 273 ± 21 mOsmol kg^{-1} for SS and 68 ± 36 mOsmol kg^{-1} for urine. A significantly higher spermatozoa concentration was observed in TS (34 ± 5 × 10^9 ml^{-1}) than in SS (23 ± 4 × 10^9 ml^{-1}). Spermatozoa concentration per male and per kg body weight was 22 ± 17 × 10^9 for TS and 18 ± 2 × 10^9 for SS, respectively. Both TS and SS showed significantly higher spermatozoa velocities and motilities after dilution in urine than after dillusion in distilled water during the activity period. In conclusion, the results obtained from the present study provide information on northern pike sperm physiology that be used to improve sperm management efficiency for this species.

Key words: Aquaculture / Seminal plasma / Gamete management / Sperm physiology / Artificial reproduction

1 Introduction

The Northern pike *(Esox lucius)* is a socio-economically important freshwater species of the northern hemisphere. Cultivated extensively in Europe and Asia since the middle ages, pike are found in almost every type of freshwater; from cold deep lakes to warm shallow ponds and muddy rivers (Steinberg 1992). Northern pike are considered to be random spawners and not nest builders. Males and females become sexually mature at 2–3 and 3–4 years, respectively (Kouril and Hamackova 1975; Billard et al. 1976), and spawning occurs in the shallows when the water temperature reaches 4–7 °C. The world aquaculture production of Northern pike was 1389 tonnes in 1995, but this decreased to 302 tonnes in 2004 (FAO 2006), while world capture production remained constant between 1995 (24 097 t) and 2004 (24 051 t) (FAO 2006).

Little information is available concerning artificial reproduction of northern pike on fish farms (Lahnstainer et al. 1998). Pike broodstock, mostly based on single individuals, is usually caught in lakes or rivers. These fish are then kept in captivity on fish farms until sexual partners are available, or else they or their gametes have to be transported to other fish farms (Billard et al. 1976). The major problem of artificial fertilization of the Northern pike is that the amounts of sperm which can be stripped are limited and often insufficient (due to premature activation of motility) (Lahnstainer et al. 1998).

Furthermore, few sperm characteristics of Northern pike have been reported, except for colour consistency, volume of first or of subsequent stripping (0.1–1.5 ml kg^{-1} fish weight (BW)), sperm density (14–22 × 10^9 sperm ml^{-1}), and total number of sperm per male BW (10–15 × 10^9 sperm ml^{-1}) (Ginsburg 1968; Montalembert et al. 1980; Koldras and Moczarski 1983; Linhart 1984). Linhart (1984) reported a negative correlation between BW of males and sperm volume. The percentage of motile sperm was 60–80% and their period of motility from 60 to 80 s in hatchery water (Ginsburg 1968; Billard 1978; Koldras and Moczarski 1983; Linhart 1984), but up to 5 min in Ringer's solution (Billard 1978).

Sperm and urine are released through a single urogenital pore in Northern pike, and it has been suggested that variability in sperm volume and the capacity of spermatozoa to move could be strongly affected by urine dilution, which would induce a decline in sperm quality (Billard 1978; Billard and Marcel 1980). Urine has been shown to induce spontaneous movement of spermatozoa in common carp (*Cyprinus carpio*) (Perchec et al. 1995; Poupard et al. 1998), European catfish (*Silurus glanis*) (Linhart et al. 1987; 2004), turbot

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(*Psetta maxima*) (Dreanno et al. 1998), rainbow trout (*Oncorynchus mykiss*) (Glogowski et al. 2000) and tench (*Tinca tinca*) (Linhart et al. 2003; Rodina et al. 2004). Any contamination with urine or external water (more information see Cosson 2004) that could induce premature activation of spermatozoa motility must therefore be avoided.

The aim of the present study was to investigate: (i) the gonadosomatic index, sperm volume and spermatozoa concentration in Northern pike (ii) the ionic composition and osmolality of stripped and testicular sperm and urine, and (iii) the effects of osmolality and urine contamination on spermatozoa motility and velocity.

2 Materials and methods

2.1 Broodstock and sperm and urine collections

During the spawning season, locally from mid-March to mid-May, 37 mature male pike (mean total length 613 mm) were caught in large natural ponds (<20 ha area) in the Hluboká nad Vltavou locality, South Bohemia. They were held in earthen ponds (0.01 ha area) and kept separate from any females. Three days before stripping, the males were transferred to 4 m^3 hatchery tanks with a water flow rate of 0.2 L s^{-1}, water temperature of 8–9 °C and O_2 concentration of 6–7 mg L^{-1} O_2. Standard length (BL), total body length (TL) and weight of each male were recorded before stripping. Spermiation was not hormonally stimulated. Sperm was obtained by abdominal massage (stripped sperm) and collected directly into 5 ml syringes, taking special care to avoid contamination with urine, mucus or faeces. After stripping, males were killed by decapitation, and the testes removed and cleaned. The gonadosomatic index (GSI) was calculated as: gonad weight after stripping/fish weight × 100. The testes were then cut into slices of about 0.5 cm and squeezed through a 150 μm mesh to collect 1.5-2 ml of testicular sperm per individual in Eppendorf tubes. The time lapse from collection of sperm to analyses was 30 min. Both stripped (SS) and testicular sperm (TS) samples were kept on ice until analysis. Urine was collected from the urinary bladder with a Luer syringe fitted with a size 2 needle.

2.2 Evaluation of sperm volume and concentration

Sperm volume and spermatozoa concentration were recorded for all 37 males and expressed in ml and numbers of spermatozoa per ml ($\times 10^9$ spz ml^{-1}), respectively. To determine spermatozoa concentration, samples were fixed in a 2% solution of formaldehyde (diluted in physiological saline) using a sperm:fixative ratio of 1:1000. Spermatozoa concentration was measured, after additional dilution, in a Burker haemocytometer at ×400. The mean number of spermatozoa was counted per 16 squares of the Burker cell, and concentration calculated as follows:

$$\left[\frac{\left(\frac{(1/a^2 \times b)}{N}\right) \times 1000}{10^9}\right] \times \left[\frac{1}{V_1/(V_1+V_2)}\right] \times \left[\frac{1}{V_3/(V_3+V_4)}\right] \times [\Sigma(n \times N)]$$

where a: side of square (mm), b: depth of square (mm), N: number of square, n: number of spermatozoa per square; V_1: volume of sperm (mm^3), V_2: volume of physiological saline solution with formaldehyde (mm^3), V_3: total volume of prediluted sperm (mm^3), V_4: volume of additional diluent (mm^3).

Sperm volume per male and number of spermatozoa per male, volume of sperm per kg of male body weight (BW) and number of spermatozoa per kg of male BW were expressed as billions (10^9) spermatozoa per male and spermatozoa (10^9) per kg of BW respectively, according to methods described by Caille et al. (2006).

2.3 Measurement of osmolality and ionic composition

Ionic composition (Na^+, K^+, Ca^{2+} and Mg^{2+}) and osmolality were measured for both urine and seminal fluid. To separate the seminal fluid, sperm was centrifuged at 2200 g for 5 min at 20 °C and the supernatant carefully removed. Osmolality was measured using a Vapour Pressure Osmometer (Wescor, USA) and expressed in mOsmol kg^{-1}. Ionic compositions were measured with a flame spectrometer SpectrAA 640 (Varian Techtron, Australia).

2.4 Observation and evaluation of spermatozoa motility

To determine the effect of urine on spermatozoa motility, both SS and TS sperm were activated in urine or in distilled water. Sperm (0.5 μl) was mixed on a glass slide with a 49.6 μl drop of either urine or distilled water, to which 0.1% BSA (Bovine Serum Albumin) was added to prevent spermatozoa heads from sticking to the glass slide. Motility was observed and recorded immediately after dilution until 2 min post-activation using a CCD video camera (Sony, SSC-DC50AP) mounted on a dark-field microscope (Olympus BX 50) with the focal plane positioned close to the glass slide surface and illuminated with a stroboscopic lamp (Chadwick-Helmut, 9630, USA) set to a flash frequency of 50 Hz. Video records were made with a S-VHS (Sony, SVO-9500 MDP) video recorder at 25 frames s^{-1}. The positions of the spermatozoa heads were measured from 5 consecutive video frames at 15, 30, 45 and 60 s post-activation and then analyzed using Micro-image software (version 4.0.1. for Windows with a special macro created by Olympus, Czech Republic). The percentage of motile spermatozoa and their velocity were estimated by viewing the position of each spermatozoon head in the five frames using three different colours (frame 1 – blue; frames 2–4 – green; and frame 5 – red), allowing measurement of the distance covered by each head. Thirty to 40 spermatozoa were measured per frame. Any spermatozoa that had moved were visible as a three colour trace, while non-motile spermatozoa appeared as a white spot. The percentage of motile spermatozoa was calculated from the numbers of white (non-motile) and red cells (motile). Spermatozoa velocity was calculated in μm s^{-1} based on the length of the spermatozoa traces from blue to green and red heads, calibrated for the magnification. At least 20 spermatozoa were measured in this way.

Table 1. Descriptive statistics of male Northern pike (n = 37) and summary of all tested parameters. Data are expressed as means; SD: standard deviation; SS: Stripped sperm; TS: testicular sperm; GSI: gonadosomatic index; TL: total length; BL: standard length; BW: fish weight; Min-Max: data range.

Parameters	Abbrev.	Mean	SD	Min-Max
Fish length (mm)	TL	613	53	550–709
	BL	540	39	440–645
Fish weight (g)	BW	1547	467	880–2600
Testis weight (g)		35	5	6.6–61.7
GSI (%)		2.0	0.6	0.6–4.1
Sperm volume (ml)		1.2	0.8	0.1–3.5
Urine volume (ml)		0.5	0.5	0.1–1.6
Spermatozoa	SS	23	4	15.6–29.4
concentration (10^9 ml^{-1})	TS	34	5	21.9–42.7
Osmolality	SS	273	22	204–314
(mOsmol kg^{-1})	TS	358	77	288–596
	Urine	68	36	27–191
Number of Spermatozoa per male (10^9)		22	17	8–26
Number of Spermatozoa (10^9 kg^{-1} BW)		18	2	5–19

2.5 Data analysis

Assessments of percentage spermatozoa motility and velocity were replicated three times per male and time of observation after sperm activation.

Before statistical comparisons were made of the tested parameters, the residuals were checked for normality (Pearson normality test) and homogeneity (Bartlett test), and data were log-transformed when necessary. Data were compared using two-way ANOVAs at each post-activation measurement time, with type of sperm and activation medium as factors. A three factor MANOVA was also used, with time post activation as a third factor.

Finally, a stepwise multiple linear regression model was used to describe relationships between the sperm motility parameters (spermatozoa velocity and percentage motile spermatozoa) as dependent variables and seven independent variables: fish length (BL), spermatozoa concentration, seminal plasma ion concentrations (Na^+, Cl^-, K^+, Ca^{2+}) and osmolality (mOsmol). All analyses were performed at a significance level of 0.05 using STATISTICA 4.0 software for Windows.

3 Results

3.1 Sperm volume and spermatozoa concentration and body parameters

Descriptive data on all measured parameters are presented in Table 1. Large variations were observed in testis weight, GSI, sperm volume, testicular spermatozoa concentration and stripped spermatozoa concentration. Significant correlations found between spermatozoa parameters and body or ionic parameters are shown in Table 2.

3.2 Ionic composition and osmolality of the seminal fluid and urine

Significant differences were found in osmolality and ionic composition of seminal fluid among SS, TS and urine. The Na^+ (123 ± 9 mM), Cl^- (127 ± 7 mM) and K^+ (35 ± 5 mM) concentrations in the seminal fluid of the TS were significantly higher than in the seminal fluid of SS: Na^+ (116 ± 9 mM), Cl^- (116 ± 7 mM) and K^+ (25 ± 4 mM). Both SS and TS showed higher ($p < 0.05$) concentrations than the urine: Na^+ (42 ± 28 mM), Cl^- (35 ± 22 mM) and K^+ (6 ± 4 mM). No significant differences were observed in Ca^{2+}, measured as 1 ± 0.2 mM in SS, 1.1 ± 0.3 mM in TS and 1.6 ± 0.9 mM in urine. Significant differences in mean osmolality were observed between urine and seminal fluid of SS and TS: 68 ± 35, 273 ± 21 and 358 ± 77 mOsmol kg^{-1}, respectively (Table 1).

3.3 Spermatozoa motility and velocity

Motility of stripped spermatozoa ranged from 59% to 91% at 15 s post-activation. Both TS and SS showed a higher percentage of motile spermatozoa during the swimming period after dilution in urine, compared with dilution in distilled water ($p < 0.05$). There was no significant difference in percentage of motile spermatozoa between SS and TS after dilution in the same activation medium (distilled water or urine). Spermatozoa exhibited a longer period of motility after dilution in urine (up to 3 min) than in distilled water (up to 60 s). Less than 10% of spermatozoa were motile at 60 s post-activation ($p < 0.05$) overall, but in TS activated by urine more than 20% of sperm were motile (Fig. 1A).

The mean velocities of SS and TS spermatozoa activated in distilled water were 163 ± 40 and 173 ± 39 μm s^{-1}, respectively, at 15 s post-activation. Both TS and SS showed higher spermatozoa velocity after dilution in urine than in distilled water ($p < 0.05$, Fig. 1B). In both TS and SS, velocity rapidly decreased with time post-activation.

Correlations derived from stepwise multiple regression analysis of spermatozoa velocity and motility with independent variables (BW, testis weight, Na^+ and Cl^-) are presented in Table 2.

4 Discussion

Two methods of sperm collection are used in the artificial reproduction of Northern pike. Most often, males are sacrificed to obtain testicular sperm to inseminate ovulated eggs (Billard et al. 1976; Montalembert et al. 1978a,b; Lahnsteiner et al. 1998). Sacrificing males leads to depletion of captive stock, and wild stock populations have also been reduced in recent years. The other method used is direct stripping of sperm by abdominal massage (Billard et al. 1980). The main disadvantages of this second technique are that only small volumes of sperm can usually be collected and these may be contaminated with urine.

The mean volume of sperm collected per fish was 1.2 ± 0.8 ml, which is higher than that obtained by Linhart (1984). In contrast, the higher standard deviation observed in the present

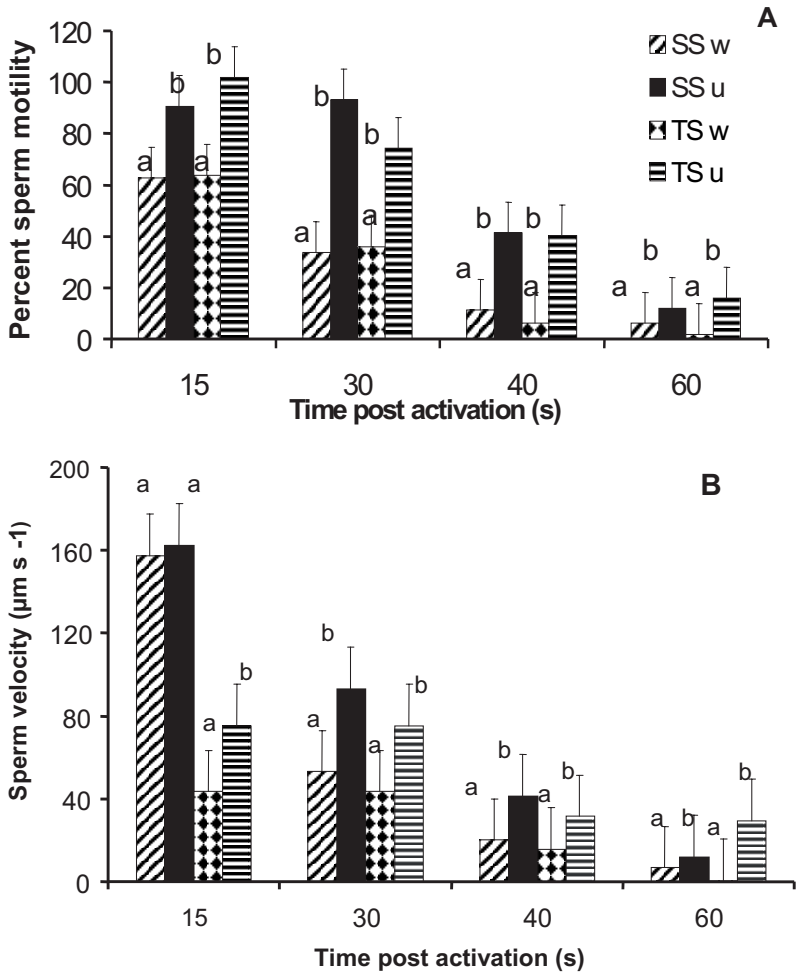

Fig. 1. Effect of two different activation media (*w*: distilled water + BSA and *u*: urine) on duration of sperm motility (A) and on sperm velocity (B) of Northern pike stripped (SS) and testicular (TS) sperm in relation to time post-activation. The different superscripts indicate significant differences ($p < 0.05$) between groups of SS and between groups of TS activated by the different solutions.

Table 2. Significant correlations of spermatozoa parameters with fish size and ionic parameters. BW: fish weight, BL: standard length. * $p < 0.0001$.

Tested parameters	Correlation (r)
Sperm velocity: BW	0.815*
Sperm velocity: Na^+	0.366*
Sperm velocity:Cl^-	0.382*
Sperm motility: BW	0.677*
Sperm motility: Cl^-	0.381*
Testis weight: BW	0.691*
Testis weight: BL	0.691*

study showed that the quantity of stripped sperm that can be collected is very variable in Northern pike, possibly due to accidental dilution by urine. The concentration of stripped sperm was 30–40% lower than that of testicular sperm.

Highly significant differences were observed in Na^+, Cl^- and K^+ concentrations among stripped sperm, testicular sperm and urine. This leads to the conclusion that osmolality is a regulatory factor that triggers pike spermatozoa motility; in a similar way as in many freshwater fish species, but in contrast to sturgeon and salmonids where K^+ concentration plays an important regulatory role (Linhart et al. 1995; Cosson 2004). Furthermore, the ionic composition of the seminal plasma of Northern pike was seen to be different from that of most other teleosts (Linhart et al. 1991; Alavi and Cosson 2006), with proportions of Na^+, K^+, and Ca^{2+} in seminal fluid of SS and TS recorded as 116:35:1 and 122:35:1, respectively. On the other hand, similar concentrations of Na^+ and K^+ ions were reported for muskellunge *Esox masquinongy* (Lin et al. 1996). Finally, significant differences were observed in the level of K^+ in the seminal fluid compared to urine, but multifactorial analysis did not reveal a significant correlation between K^+ concentration and either sperm motility or velocity.

In general, pike spermatozoa (both SS and TS) were activated immediately and 59 to 91% became motile: a result that agrees with previous observations (Billard 1978; Montalembert et al. 1980; Linhart 1984). The velocity of SS and TS activated by distilled water or urine ranged from 163 to

173 ± 39 μm s^{-1} immediately post-activation, which is faster than in carp (Perchec et al. 1995), tench (Rodina et al. 2004) or paddlefish (Linhart et al. 2003).

From the comparison of swimming behaviour of pike spermatozoa in distilled water with that in urine, it appears that sperm are affected mainly by exposure to an osmolality lower than the seminal fluid. Activation in distilled water is less effective than in urine because the osmolality is even lower. After a short period (15 s) in distilled water, blebs are observed along the flagellum, which forms correct and efficient waves that propagate along its length. Later in the motility phase (30 s), the tip of flagellum curls and presents a loop, which shortens the efficient part of the flagellum and restricts the waves to 1/4 or 1/5 of the flagellar length (Linhart unpublished). These effects were not detected in the flagella of sperm induced to swim in urine.

During the earliest post-activation period (15 s), the velocity of pike spermatozoa reached 120–180 μm s^{-1}, but this gradually declined to 20–100 μm s^{-1} by 30 s, effectively limiting efficient progression. Interestingly, the sperm activated by urine occasionally swam for up to 3 min. Similar behaviour was previously noted in studies with common carp (Perchec et al. 1995), bullhead, *Cottus gobio* (Lahnsteiner et al. 1997), perch, *Perca fluviatilis* (Lahnsteiner et al. 1995), European turbot *Psetta maxima* (Dreanno et al. 1998), turbot, *Scophthalmus maximus* (Chauvaud et al. 1995), tilapia, *Oreochromis mossambicus* (Linhart et al. 1999), several sturgeon species and paddlefish *Polyodon spathula* (Cosson et al. 2000; Linhart et al. 2002).

In most fish species, contamination of sperm by urine during stripping is difficult to avoid due to the proximity of the sperm duct and ureter (Dietrich et al. 2005). Sperm contamination by urine was also observed in the present study on Northern pike. The pike urinary bladder presented a volume (0.5 ml) similar to that observed in tench (Linhart et al. 2003) and in tilapia (Linhart et al. 1999). Urine contamination means that sperm samples cannot be stored, even on a short-term basis, except when they are collected in an immobilizing solution.

The problem of sperm contamination by urine has also been addressed in studies with European catfish (Linhart et al. 2004), tench (Rodina et al. 2004), asp (Linhart and Benesovsky 1991) and catfish *Pangasius bocourti* (Cacot et al. 2003). All studies confirmed that the osmolality of the urine triggers the initiation of sperm motility, but that an immobilizing solution with osmolality similar or higher than that of the seminal plasma could protect the sperm from such risks of activation. This implies that the composition of the immobilizing solution needs to be adjusted according to the composition of the seminal plasma (Cacot et al. 2003; Linhart et al. 2004; Rodina et al. 2004). In most freshwater species, where a hypo-osmotic shock induces sperm motility, osmolality of urine lower than that of the seminal plasma is the main explanation for motility triggering of sperm in urine.

The osmolality observed in the present study was similar to that in other freshwater fish species, such as common carp, tilapia, European catfish, *Silurus glanis*, and tench, at 18, 78, 50 and 85 mOsmol kg^{-1}, respectively (Poupard et al. 1998; Linhart et al. 1999, 2003, 2004). The osmolality of urine also shows also some differences among species: 68 in Northern pike, 18 in common carp, 78 in tilapia *Oreochromis mosambicus*, 50 in European catfish and 85 mOsmol kg^{-1} in tench (Poupard et al. 1998; Linhart et al. 1999, 2003, 2004).

5 Conclusion

Based on the present study we can conclude that (1) in Northern pike osmolality is a regulatory factor for triggering spermatozoa motility and a key factor in spermatozoa activation, and (2) the differences in ionic composition may have implications for the development of an immobilization solution for Northern pike sperm and its cryopreservation.

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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.

Billard R., Debruille M., Gérard J.P., Montalembert G., 1976, L'insémination artificielle du brochet. Bull. Fr. Piscic. 262, 30–34.

Billard R., 1978, Changes in structure and fertilizing ability of marine and freshwater fish spermatozoa diluted in media of various salinities. Aquaculture 14, 187–198.

Billard R., Marcel J., 1980, Stimulation of spermiation and induction of ovulation in pike (*Esox lucius*). Aquaculture 21, 181–195.

Cacot P., Eeckhoutte P., Muon D.T., Trieu N.V., Legendre M., Mariojouls C., Lazard J., 2003, Induced spermiation and milt management in *Pangasius bocourti* (Sauvage, 1880). Aquaculture 215, 67–77.

Caille N., Rodina M., Kocour M., Gela D., Flajšhans M., Linhart O., 2006, Quantity, motility and fertility of tench *Tinca tinca* (L.) sperm in relation to LHRH analogue and carp pituitary treatments. Aquac. Int. 14, 75–87.

Chauvaud L., Cosson J., Suquet M., Billard R., 1995, Sperm motility in turbot, (*Scophthalmus maximus)* - initiation of movement and changes with time of swimming characteristics. Environ. Biol. Fishes 43, 341–349.

Cosson J., Linhart O., Mims S.D., Shelton W.L., Rodina M., 2000, Analysis of motility parameters from paddlefish and shovelnose sturgeon spermatozoa. J. Fish Biol. 56, 1348–1367.

Cosson J., 2004, The ionic and osmotic factors controlling motility of fish spermatozoa. Aquac. Int. 12, 69–85.

Dietrich G.J., Kowalski R., Wojtczak M., 2005, Motility parameters of rainbow trout (*Oncorhynchus mykiss*) spermatozoa in relation to sequential collection of milt, time of post-mortem storage and anesthesia. Fish Physiol. Biochem. 31, 1–9.

Dreanno C., Suquet M., Desbruyeres E., 1998, Effect of urine on semen quality in turbot (*Psetta maxima*). Aquaculture 169, 247-262.

Ginsburk A.S., 1968, Fertilization in fishes and the problem of polyspermy. Moskva, Izdatelnaya Nauka.

Glogowski J., Kwasnik M., Piros B., Dabrowski K., Goryczko K., Dobosz S., Kuzminski H., Ciereszko A., 2000, Characterization of rainbow trout milt collected with a catheter: semen parameters and cryopreservation success. Aquac. Res. 31, 289–296.

FAO Fishery Statistics, 2006, available at http://www.fao.org/fi/statist/statist.asp.

Koldras M., Moczarski M., 1983, Properties of pike *Esox lucius* L. milt and its cryopreservation. Pol. Arch. Hydrobiol. 30, 69–78.

Kouril J., Hamackova J., 1975, Fecundity of pond-reared Pike (*Esox lucius* L). Czech J. Anim. Sci. 20, 841–849.

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.

Lahnsteiner F., Berger B., Weismann T., Patzner R.A., 1997, Sperm structure and motility of the freshwater teleost *Cottus gobio*. J. Fish Biol. 50, 564–574.

Lahnsteiner F., Weismann T., Partner R.A., 1998, An efficient method for cryopreservation of testicular sperm from the northern pike, *Esox lucius* L. Aquac. Res. 29, 341–347.

Lin F., Liu L., Dabrowski K., 1996, Characteristics of muskellunge spermatozoa .1. Ultrastructure of spermatozoa and biochemical composition of semen. Trans. Am. Fish. Soc. 125 187–194.

Linhart O., 1984, Evaluation of pike and European catfish sperm. Bull. VURH Vodnany 20, 22–33.

Linhart O., Kouril J., Hamáèkova J., 1987, Increased rate of egg fertilization in artificial propagation of European catfish (*Silurus glanis*) by means of suppressing the movements of spermatozoa with immobilization solution. Aquaculture 65, 353–358.

Linhart O., Benesovsky J., 1991, Artificial insemination in asp (*Aspius aspius* L.). Ziv.Vyr. 36, 973–980.

Linhart O., Proteau J.P., Redondo C., Billard R., 1991, Preservation of gametes in European catfish *(Silurus glanis* L.). 4th Int. Symp. Reproductive Physiology of fish, 7–12 July, 1991, Norwich, 283.

Linhart O., Mims S.D., Shelton W.L., 1995, Motility of spermatozoa from Shovelnose sturgeon and Paddlefish. J. Fish Biol. 47, 902–909.

Linhart O., Walford J., Sivaloganathan B., Lam T.J., 1999, Effects of osmolality and ions on the motility of stripped and testicular sperm of freshwater- and seawater-acclimated tilapia, *Oreochromis mossambicus*. J. Fish Biol.55, 1344–1358.

Linhart O., Cosson J., Mims S.D., Shelton W.L., Rodina M., 2002, Effects of ions on the motility of fresh and demembranated paddlefish (*Polyodon spathula*) spermatozoa Reproduction 124, 713–719.

Linhart O., Mims S.D., Gomelsky B., Hiott A.E., Shelton W.L., Cosson J., Rodina M., Gela D., Bastl J., 2003, Ionic composition and osmolality of paddlefish *Polyodon spathula*, Acipenseriformes) seminal fluid. Aquac. Int. 11, 357–368.

Linhart O., Rodina M., Bastl J., Cosson J., 2003, Urinary bladder, ionic composition of seminal fluid and urine with characterization of sperm motility in tench (*Tinca tinca* L.). J. Appl. Ichthyol. 19, 177–181.

Linhart O., Rodina M., Gela D., Kocour M., 2004, Optimization of artificial propagation in European catfish, *Silurus glanis* L. Aquaculture 235, 619–632.

ontalembert G., Jalabert B., Bry C., 1978a, Precocious induction of maturation and ovulation in northern pike (*Esox lucius*). Ann. Biol. Anim. Biochem. Biophys. 18, 969–975.

Montalembert G., Bry C., Billard R., 1978b, Control of Reproduction in the Northern Pike. American Fisheries Society, Spec. Publ. 11, 217–225.

Montalembert G., Marcel J., Billard R., 1980, La spermiation chez le brochet. 1) Evolution de la quantite de sperme recolte au cours de la saison de reproduction. Bull. Fr. Piscic. 276, 85–103.

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

Poupard G.P., Paxion C., Cosson J., Jeulin C., Fierville F., Billard R., 1998, Initiation of carp spermatozoa motility and early ATP reduction after milt contamination by urine. Aquaculture 160, 317–328.

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

Steinberg D., 1992, Northern Pike and Muskie. CY DeCrosse Inc.