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Effect of urine contamination on semen quality variables in Eurasian perch *Perca fluviatilis* L.

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ABSTRACT

The objectives of the present study were to determine values for semen quality variables in the Eurasian perch (i.e., osmolality of seminal plasma as well as sperm motility characteristics analyzed with CASA system) in response to (1) the method of milt collection (stripping or catheterization) and (2) experimental contamination of catheterized semen with urine (0%, 5%, 10%, 20%, 30% and 50% of contamination). Additionally, the effect of short-term chilled storage of experimentally contaminated semen (during the 24 h post semen collection period) on motility characteristics was investigated. Use of a typical stripping procedure resulted in about 5%–10% contamination of semen with urine, what is much less compared with other species. Markedly lesser values of straight line velocity (VSL) and consequently less linearity of spermatozoa movement (LIN) in perch semen, however, occurred as a result of stripping (46 ± 4 µm/s and 38 ± 4% for VSL and LIN, respectively), when compared to sperm collected by catheterization (87 ± 5 µm/s and 77 ± 2% for VSL and LIN, respectively), indicate that even a 10% contamination of semen with urine may have negative effects on quality. Exposure of semen to urine resulted in a significant dose-dependent decrease in the percentage of motile spermatozoa (MOT) and both velocity variables (VSL and VCL). Amount of urine contamination also affected MOT, VCL, VSL and LIN value during short-term storage. In conclusion, it is important to avoid semen contamination by urine when using the stripping procedure in the Eurasian perch, either for controlled reproduction or sperm preservation.

1. Introduction

Eurasian perch, *Perca fluviatilis*, is a strong candidate for the diversification of European intensive freshwater aquaculture (Toner, 2015). In the last decade, interest in the development of the methods of perch aquaculture has been increasing substantially (Fontaine et al., 2015; Żarski et al., 2015). Even with the considerable efforts, however, further development of commercial production of this species is restricted due to the lack of a standardized method for greater control of the reproduction procedure (Fontaine et al., 2016), which is an important aspect of the effectiveness of the entire intensive production process (Żarski et al., 2017b). One of the greatest limitations hindering rapid development of aquaculture of this species and other percids is significant variability in gamete quality

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(Schaerlinger and Żarski, 2015; Żarski et al., 2017a).

Gamete quality can be defined as the capacity to fertilize (sperm) or to be fertilized (eggs), and subsequently develop into a normal embryo (Bobe and Labbe, 2010). In this context, successful fish production principally depends on the efficiency of controlled reproduction methods and thus on the quality of male and female gametes, especially in species emerging for aquaculture such as the Eurasian perch (Migaud et al., 2013; Fontaine et al., 2015). In Eurasian perch, previous results indicate that the observed intraspecific variations of semen quality depend on numerous factors such as: in-season or out-of-season spawning, photothermal condition, nutrition, status and age of males and the type of hormonal stimulation applied (Krol et al., 2006; Migaud et al., 2006; Wirtz and Steinmann, 2006; Wang et al., 2008; Alavi et al., 2010; Żarski et al., 2017a). In percids, as in other freshwater fish species, however, there is another very important factor affecting sperm quality, which is the method of semen collection. A typical hatchery practice involves collection of semen prior to use for fertilization by stripping directly into a dry container (syringe or Eppendorf tube). This method, however, has the significant risk of contamination of the semen with urine, which may negatively affect sperm quality (Perchec et al., 1995; Linhart et al., 2003; Cejko et al., 2008). Practically, in many fish species – including percids – contamination is inevitable when sperm is stripped in a traditional way, due to the proximity of sperm and urinary ducts through a single urogenital pore (Linhart et al., 2003; Nynca et al., 2012). The contamination of milt with urine can be avoided by the application of a specific technique, where semen is collected with a catheter (Glogowski et al., 2000; Sarosiek et al., 2016). There is no published information on the usefulness of this method in Eurasian perch and the evaluation of sperm-quality-related indices from the perspective of urine contamination of semen in this species.

Preservation of semen (short- or long-term storage) is a highly useful procedure in aquaculture allowing for the overcoming of problems such as synchronization of gamete production, gamete transfer and storage for fertilization, and simplification of broodstock management (Cabrita et al., 2010). Contamination of sperm with urine was reported to be a primary problem in the success of sperm cryopreservation of percid species (Bokor et al., 2007). Although it has been reported that the sperm of Eurasian perch diluted and chilled in a specific medium can effectively be stored for more than 2 weeks (Sarosiek et al., 2014) or successfully cryopreserved (Bernáth et al., 2015), it should be emphasized that for both preservation techniques, semen was collected with the use of catheterization. There, however, are no data regarding the comparative analysis of perch semen collected with either syringe or with a catheter. Furthermore, there is no information on the consequences of dilution of sperm with urine from the perspective of its quality as well as the effectiveness of short-term storage of perch semen.

In the present study, semen quality variables were investigated in response to the method of sperm collection or increasing the proportion of urine in the catheterized semen diluted *in vitro* in controlled conditions. Furthermore, the effects of experimental urine addition on perch semen quality were investigated in terms of CASA variables after chilled short-term storage for a duration of 24 h.

2. Material and methods

The study has been conducted in accordance with regulations of the European and national legislation for fish welfare and approved by the Local Animal Ethics Committee in Olsztyn, Poland.

2.1. Fish origin and sampling method

Adult Eurasian perch ($n = 10$), with an average body weight of 145 ± 78 g, were captured from wild stock in Sasek Wielki lake (NW Poland) at the peak of the spawning season, and were transported to the laboratories of the University of Warmia and Mazury, Olsztyn, Poland. The fish were placed in 800 l tanks connected to a recirculating aquaculture system (RAS) and maintained at 12.0 ± 0.5 °C for 3 days. After the acclimating period, five fish were anesthetized in a solution of MS-222 (Argent, USA) at a dose of 150 mg/l. The genital pore of each fish was subsequently wiped dry and first sperm from each male was stripped using a flexible catheter (40 mm long cannula, with external dimeter of about 1.3 mm was inserted 10 to 20 mm into the sperm duct) directly into a dry 1.5 ml Eppendorf tube, which was then immediately placed on melting ice (4 °C) prior to further analyses. After semen collection, urine was collected directly into a dry Eppendorf tube from the same perch males using a similar catheter. Collected urine was also immediately placed on melting ice and the urine samples were subsequently pooled prior to use. Semen samples collected from each individual male with catheter were mixed with either 5%, 10%, 20%, 30% or 50% of urine (constituting separate urine-contaminated treatments) and stored at 4 °C prior to further procedures. Urine-free semen samples were collected from each individual without the addition of urine. The final volume of mixed or urine-free samples was 300 µl (refer semen sample from each male made in duplicate). Additionally, semen from another five perch males were collected in a traditional way, i.e. by striping directly into syringe (without catheter). Semen collection by catheterization and hand stripping were performed separately on different fish due to amount of milt collected from one male.

2.2. Semen analysis

Each semen sample collected by catheterization was divided into three sub-samples and each stripped semen sample was divided into two sub-samples. The first sub-sample (100 µl) was centrifuged at 10,000 *g* for 10 min to obtain seminal plasma. The osmolality of seminal plasma was subsequently determined using a Vapor Pressure Osmometer 5600 (Wescor, Logan, Utah, USA). The assay was performed in triplicate, using 10 µl of seminal plasma each time.

A second sub-sample (50 µl) was used for the evaluation of sperm motility variables. Six motility variables were measured using the CASA system (Sperm Class Analyzer v. 4.0.0. by Microptic S.L., Barcelona, Spain): spermatozoa motility (MOT, %), curvilinear