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RESEARCH ARTICLE

# Preliminary characteristics of lake minnow, *Eupallasella percnurus* (Pall.), semen

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**Abstract**. The objectives of this study were to determine the basic parameters of lake minnow, *Eupallasella percnurus* (Pall.), semen in terms of sperm concentration, seminal plasma composition, and sperm motility. Although *E. percnurus* milt characteristics are similar in many respects to those of common carp, *Cyprinus carpio* L., distinct species-specific differences were noted. The semen was characterized by its minute volume (~ 9 µl), and one of the highest sperm concentrations ($21 \times 10^9$ ml$^{-1}$) among teleosts. Osmolality was relatively low (~ 235 mOsm), perhaps due to urine contamination. The anti-trypsin activity (~ 64 U l$^{-1}$) and protein concentration (~ 0.5 mg ml$^{-1}$) was one of the lowest noted among fish. *E. percnurus* semen was characterized by a high proportion of motile sperm (~ 89%) and relatively high straight-line velocity (93 µm s$^{-1}$). The parameters of sperm trajectory were within the range typical for cyprinid sperm. A characteristic feature of the semen was its stickiness, which manifested in agglutination during storage. These preliminary results are the first to describe the basic parameters of *E. percnurus* semen. This information will be useful in work to develop a methodology for the short and long-term storage of semen in the active protection of this endangered species.

**Keywords**: lake minnow, sperm quality, CASA

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## Introduction

The lake minnow, *Eupalasella percnurus* (Pall.), is a fish species from the family Cyprinidae, which has been under strict protection since 1975, and is entered in all issues of Polish red books and red lists of animals or ichthyofauna, as an endangered (EN) or critically endangered (CR) species (Wolnicki and Radtke 2009). This species is in the exclusive group of animals with the highest priority of importance in the European Ecological Natura 2000 Network, and is included in the group of species requiring the application of active protection plans in Poland. Therefore, it is evident that the practical application of *E. percnurus* active protection measures is an urgent task of great environmental importance.

Methods for short and long-term semen storage without any loss of fertilization properties significantly facilitates the artificial reproduction of rare and protected animal species and the preservation of high genetic diversity (Glogowski and Ciereszko 2008). The methodology of such measures, however, must be adapted individually to the specific natures of each species. Therefore, the development of

methods for the storage of *E. percnurus* semen and its application in artificial reproduction requires detailed studies to be conducted to describe the species' semen in terms of its basic physicochemical and biochemical sperm characteristics as well as sperm motility parameters.

Currently, no information on the features of *E. percnurus* sperm is available. Such information is necessary to understand the species specificity of reproduction, and conditions for the regulation of sperm functioning depending on environmental conditions. The objectives of this study were to determine the basic parameters of *E. percnurus* semen in terms of sperm concentration, seminal plasma composition, and sperm motility.

## Materials and methods

### Source of fish

The experiment was carried out on *E. percnurus* (mean total length of 5.3 cm ± 0.6) which originated from a body of water in Zielonka in Mazowieckie Voivodeship in central Poland. The fish were caught in May 2009 (water temperature was 17°C) using traps. They were then transported to the laboratory at the Pond Fishery Department in Żabieniec, Inland Fisheries Institute where they were acclimatized for two weeks to the following conditions: water temperature 15°C, pH=7.9, oxygen saturation 89%. This species does not lose its ability to produce milt when kept in captivity at 15°C for at least one month.

### Collection of milt and seminal plasma

Milt was obtained by applying gentle abdominal pressure to force the milt out of the spermatic ducts. Flowing milt was extracted from the genital pore with a micropipette and stored in a small test-tube (1.5 mL capacity) on crushed ice (4°C). Sperm movement was recorded on videotape within 1 hour after collection. Because of the small volume of *E. percnurus* milt, seminal plasma was obtained for the five groups (n=5) from pooled milt by centrifugation (10000 ×g, 10 min) within 1 h after sampling. All measurements of seminal plasma characteristics were made at least in duplicate for each n.

### Analytical methods

Sperm concentration was measured according to the method described by Ciereszko and Dabrowski (1993). Protein concentration was measured using the Lowry et al. (1951) method. Osmolality of seminal plasma was measured with a Vapor Pressure Osmometer 5520 (WESCOR). Anti-proteinase activity of seminal plasma was evaluated by the inhibition of cod trypsin amidase activity (Ciereszko et al. 1998).

### Description of basic sperm motility characteristics of *E. percnurus*

Basic sperm motility characteristics were measured using a two-step procedure that was developed previously for fish sperm (Dietrich et al. 2010). In the first step, sperm was diluted 50-fold in an immobilizing buffer (94 mM NaCl, 27 mM KCl, 50 mM glycine, 15 mM Tris–HCl, pH 7.5; Volckaert et al. 1994) in a small polyethylene tube. Next, 1 μL of this solution was mixed with 19 μL of distilled water (final semen dilution 1:1000) and 0.7 μL of activated sperm was immediately placed into the well of a 12-well multi-test glass slide (ICN Bio-medicals Inc., OH, USA) and covered with a cover-slip and videotaped. The distilled water used for activation contained 1% bovine serum albumin to prevent the sperm sticking to the glass slides. Motile spermatozoa were recorded using a microscope with a 10× negative phase objective and a Sony CCD black and white video camera (SPT-M108CE). Video recordings were analyzed using a Hobson Sperm Tracker (Hobson Vision Ltd, Baslow UK) with settings as reported in Wojtczak et al. (2007) and modified for *E. percnurus*. Sperm motility parameters were measured over a 15 s period and between 5-20 s post activation time. From fifteen motility parameters, straight line velocity (VSL),

curvilinear velocity (VCL), average path velocity (VAP), linearity (LIN=100× VSL/VCL), beat cross frequency (BCF), amplitude of lateral head displacement (ALH), and percentage of motile sperm (MOT) were chosen for further analysis. All measurements of motility were made at least in duplicate for each of three individual sperm samples.

## Results and Discussion

The results presented in the current study provide, for the first time, data on the basic characteristics of *E. percnurus* milt as well as sperm motility characteristics as determined by the computer-assisted sperm motility analysis (CASA) system. Although *E. percnurus* milt characteristics (Table 1) were similar in many regards to *C. carpio* milt, distinct species-specific differences were noted.

**Table 1**

Characteristics of *E. percnurus* milt (n=5 samples of from pooled milt; only in case of milt volume n=5 individual sperm samples)

| Parameter | Mean ± SD | Range |
|---|---|---|
| Milt volume (µl) | 8.8 ± 7.1 | 2.8-17 |
| Sperm concentration (x $10^9$ $ml^{-1}$) | 20.8 ± 9.4 | 11.3-32.6 |
| Osmolality (mOsm $kg^{-1}$) | 235 ± 6 | 226-241 |
| Protein concentration (mg $ml^{-1}$) | 0.51 ± 0.08 | 0.44-0.63 |
| Anti-trypsin activity (U $l^{-1}$) | 63.9 ± 6.5 | 55.9-73.6 |

The semen was characterized by its minute volume (~ 9 µl), and one of the highest sperm concentrations among teleosts (Suquet et al. 1994). Osmolality was relatively low (~ 235 mOsm) compared to *C. carpio* (~ 264 mOsm) perhaps due to contamination by urine. The anti-trypsin activity (~ 64 U $l^{-1}$) and protein concentration (~ 0.5 mg $ml^{-1}$) was one of the lowest among fish (Ciereszko 2008). For example, in *C. carpio* the values of anti-trypsin activity and protein concentration are about 300 U $l^{-1}$ and 1.7 mg $ml^{-1}$ respectively. A characteristic feature of the semen was its stickiness which manifested in agglutination during storage. Further studies are required to determine if the stickiness of *E. percnurus* semen is an important element of reproduction.

CASA systems allow an objective quantification of sperm motility parameters, such as sperm velocities and trajectory changes, that cannot be measured using subjective methods. Moreover, positive correlations have been found between fish sperm velocities and fertilization rates (Dietrich et al. 2010); thus, not only the percentage of motile sperm but also the velocity of the spermatozoa is regarded as a determinant for the successful fertilization of eggs. In the present work, we have successfully adapted the CASA procedure for *E. percnurus* sperm by adjusting parameters set previously for *C. carpio* (Wojtczak et al. 2007), which suggests the high similarity of activation conditions for both species. *E. percnurus* semen was characterized by a high proportion of motile sperm (~ 89%; Table 2) and a relatively high straight-line velocity (~ 93 µm $s^{-1}$).

**Table 2**

Sperm motility characteristics of *E. percnurus* milt (n=3 individual sperm samples; percentage of motile sperm (MOT), curvilinear velocity (VCL), average path velocity (VAP), straight line velocity (VSL), linearity (LIN=100× VSL/VCL), beat cross frequency (BCF) and amplitude of lateral head displacement (ALH))

| Parameter | Mean ± SD | Range |
|---|---|---|
| MOT (%) | 89.0 ± 6.0 | 82.0-96.0 |
| VCL (µm $s^{-1}$) | 142.0 ± 30.9 | 108.5-169.3 |
| VAP (µm $s^{-1}$) | 124.3 ± 33.1 | 88.7-154.2 |
| VSL (µm $s^{-1}$) | 91.7 ± 32.9 | 59.3-125.1 |
| LIN (%) | 60.4 ± 8.8 | 52.8-70.1 |
| BCF (Hz) | 4.3 ± 1.4 | 3.5-5.9 |
| ALH (µm) | 8.2 ± 0.6 | 7.7-8.8 |

The parameters of sperm trajectory (LIN, BCF, ALH) were within a range typical for cyprinid sperm (Wojtczak et al. 2007). Further studies need to be done to determine detailed species-specific sperm activation conditions, particularly how pH and cations might modify the motility characteristics of *E. percnurus* sperm.

These preliminary results are the first to describe the basic parameters of *E. percnurus* semen. This information will be useful in work related to development of methodology for the short and long-term

storage of *E. percnurus* semen for the active protection of this endangered species.

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## Streszczenie

### Wstępna charakterystyka nasienia strzebli błotnej *Eupallasella percnurus* (Pall.)

Celem niniejszej pracy było określenie podstawowych parametrów nasienia strzebli błotnej w zakresie koncentracji plemników, składu plazmy nasienia i parametrów ruchliwości plemników. Choć pod wieloma względami nasienie strzebli błotnej przypomina mlecz karpia *Cyprinus carpio* L. to niektóre z parametrów wydają się specyficzne dla tego gatunku. Mlecz strzebli błotnej charakteryzowała niska objętość (~ 9 μL) i jedno z najwyższych koncentracji plemników ($21 \times 10^9$ ml$^{-1}$) wśród kostnoszkieletowych. Osmolalność była stosunkowo niska (~ 235 mOsm), być może ze względu na zanieczyszczenie moczem. Aktywność anty-proteolityczna (~ 64 U l$^{-1}$) oraz stężenie białka (~ 0,5 mg ml$^{-1}$) był jednymi z najniższych obserwowanych wśród ryb. Nasienie strzebli błotnej charakteryzował wysoki odsetek ruchliwych plemników (~ 89%) i stosunkowo wysoka prędkość prostoliniowa (93 μm s$^{-1}$). Parametry trajektorii plemników mieściły się w zakresie typowym dla ryb karpiowatych. Cechą charakterystyczną nasienia była jego kleistość, która przejawiała się postępującą aglutynacją plemników podczas przechowywania. Nasze wstępne wyniki są pierwszymi opisującymi podstawowe parametry plemników strzebli błotnej. Te informacje będą przydatne w pracy związanej z rozwojem metodologii krótko- i długoterminowego przechowywania nasienia strzebli błotnej dla potrzeb czynnej ochrony tego zagrożonego gatunku.