Sperm motility of *Prochilodus lineatus* in relation to dilution rate and temperature of the activating medium

By E. Romagosa¹, B. E. Souza², E. A. Sanches², D. M. Baggio³ and R. A. Bombardelli³

¹Instituto de Pesca, APTA, SAA, São Paulo, Brazil; ²CAUNESP, Jaboticabal, São Paulo, Brazil; ³Universidade do Oeste do Paraná – UNIOESTE, Toledo, Paraná, Brazil

Summary

The objective of this study was to assess the effects of an activating solution on the sperm motility duration (SMD) of *curimbatá*, *Prochilodus lineatus* through the definition of qualitative and quantitative parameters of the semen pool used in the experiment; evaluation of the effects of different ratios of semen dilution corresponding to 1 : 1, 1 : 2, 1 : 20, 1 : 200, 1 : 2000, 1 : 20 000 and 1 : 100 000 semen:dilute solution on the SMD and, assessment of the effects of different temperatures of the activating solution (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50°C) on the SMD. The results of SMD were directly proportional to the dilution (P < 0.05), starting from the dilution of 1 : 2 (semen:water), with 23.04 s. Were used three replicates of the semen pool for each test. Two-year-old brookstock were maintained in ponds culture conditions. In November–December 2006, twelve mature males broodfish were selected (mean weight and length of 405.8 ± 134.2 g and 25.6 ± 3.1 cm, respectively). The males released that semen under slight pressure of the urogenital papilla were selected for the experiment. The SMD increased proportionally to the increase in dilution, until it reached a maximum of 28.83 s for the ratio 1 : 100 000 semen: dilute solution. The results of SMD in relation to the temperature of the activating solution exhibited a quadratic behavior (P < 0.05) with a maximum theoretical performance in terms of sperm motility duration of 21.36 s at a temperature of 17.3°C. Thus, for the species considered, the increase in the dilution ratio proved favorable for the rise in motility duration until the maximum value studied of 1 : 100 000 semen:dilute solution. As for the temperature of the activating solution, the best results of SMD were obtained at the temperature of 17.3°C. At higher temperatures used in the experiment (25, 30, 35, 40, 45 and 50°C), a decrease in motility duration.

Introduction

*Prochilodus lineatus*, commonly known as ‘curimbatá’ or ‘curimbá’, belongs to the genus *Prochilodus* and is widely distributed throughout South America. In Brazil, it is present in the main hydrographic basins as a species of migratory fish (Galdioli et al., 2002). It has great social (Barbieri et al., 2004), ecological (Lizama, 2000), fishing and aquicolous (IBAMA, 2007) importance.

One of the main aspects for the intensification of fish production is the use of artificial propagation or induced reproduction (Leonardo et al., 2005). However, for most species of freshwater fishes, the process for the massive production of juveniles is not totally mastered yet; therefore more studies to improve the efficiency of this process are needed (Romagosa and Andrade, 2008). Honji et al. (2009) claimed that the offer of fish produced in fish farms in the local markets decreases the pressure on the native population to go fishing, thereby providing maintenance of the natural stocks. It has become imperative to develop technology for the large-scale production of continental water fishes in different culture systems (Babin et al., 2007).

The low viability and poor quality of zygotes of several fish species when reared in captivity is on critical impediment for the development of aquaculture using new candidate species (Nordeide, 2007). Sperm motility is of the most widely used parameters for the evaluation of the quality of the spermatozoa (Alavi et al., 2008; Cosson et al., 2008). The simple microscopic viability estimate of the fresh semen is used for an initial evaluation of the quality of sperm (Kavamoto and Fogli da Silveira, 1986). Many factors influence the sperm motility duration, such as activation, volume, pH and temperature of the activating solution (Billard et al., 1995), as well as dilution (Billard and Cosson, 1992). Egg morphological features, hatching rate, biochemical composition, and levels of specific nutrients are generally considered to be good indicators of egg quality (Babin et al., 2007).

In order to assess the effect of the activating solution on the sperm motility duration (SMD) of *Prochilodus lineatus*, this study (i) defined the qualitative and quantitative parameters of the semen pool; (ii) evaluated the effects of different ratios of semen dilution or volume of semen to volume of water ratios on the SMD, and (iii) verified the relationship between SMD and different temperatures of the activating solution.

Materials and methods

Two-year-old (*Prochilodus lineatus*) broodfish were maintained in pond culture conditions until used for experiments. Twelve mature males broodfish were selected in November–December 2006 (mean weight = 405.8 ± 134.2 g and mean length = 25.6 ± 3.1 cm) and kept in a circular tank (1500 L) equipped with aeration and constant water renewal (750 L h⁻¹). The males that released semen under slight pressure of the urogenital papilla were selected for the experiment (Zaniboni Filho and Weingartner, 2007). Three replicates of the semen pool were used for each test.

These males were injected intraperitoneally with crude carp pituitary extract (CPE) at a dose of 0.5 mg kg⁻¹ and then 5.0 mg kg⁻¹ 12 h before stripping. The water temperature was registered.
Spermatozoa were collected 6 h after hormonal induced when the water temperature was 27.0°C. The fishes were dried (by cloth and paper towel), and a massage in the ventral region was applied individually along the encephalo-caudal axis. The first drop of semen was discarded to avoid possible contamination, and the remainder was collected with a disposable syringe (5.0 ml) for the measurement of the volume of released semen. The spermatozoa concentration of the sperm pools was measured and the sample of 5 μl of semen was collected and diluted (5 ml) buffered formol saline resulting in a dilution of 1 : 1000. The sperm cell count was performed in a Neubauer hemocytometric chamber according to the methodology recommended by Wirz and Steinmann (2006). The SMD of the pool was measured, by mixing 5 μl of semen with 200 μl of water. Simultaneously, the time count was initiated by the use of a chronometer; 5 μl withdrawn from the mix was examined under a light microscope (40x). The estimate of the percentage of moving cells was based on an arbitrary scale of 0–100%. When 50% of the cells lost their motility, the chronometer was stopped and the motility duration was measured (Sanches et al., 2009). The evaluation of the sperm survival rate was carried out using and eosin-nigrosin staining (Blom, 1950; Murgas et al., 2003), using 30 μl of semen (immediately after collection) and 90 μl of each stain. Afterwards, the smear was obtained and analyzed under a light microscope (40x), and 400 spermatozoa were counted. The unstained spermatozoa were considered to be alive while the stained (pink) ones were considered to be dead.

For verified the effect of the ratio volume of semen:volume of water on the SMD was used fully randomized experimental design, with seven treatments and three repetitions was used. The treatments consisted of the ratios: 1 : 1, 1 : 2, 1 : 20, 1 : 200, 1 : 2000, 1 : 100 000 semen:dilute solution. A plastic recipient (200 ml) was considered as an experimental unit containing semen activated by the different volumes of activating solution. The measurement of the SMD was performed simultaneously to with the homogenization of the semen and activating solution, as previously described. The activating solution used in the process of sperm activation was water obtained from an artesian spring (pH = 6.80; dissolved oxygen = 4.26 mg L−1; alkalinity = 996.30 μEq. L−1; hardness = 43.96 mg L−1; ammonia = 0.126 mg L−1; nitrite = 0.0038 mg L−1; nitrate = 0.29 mg L−1).

The effect of the temperature of the activating solution on the SMD was verified to used a fully randomized experimental design, with seven treatments and three repetitions was used. The treatments consisted of the following temperatures of activating solution: 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50°C in the process of sperm activation. SMD was evaluated as previously described.

The results obtained were submitted to a regression analysis with a level of significance of 5%. The software used for the statistical analyses was Statistica® (Statsoft, 2005).

Results

Table 1 presents the mean values of semen volume, sperm concentration, motility, survival rate, and relative semen volume of Prochilodus lineatus, and Fig. 1 shows that the SMD was directly proportional to the dilution rate with the medium (P < 0.05).

The SMD was directly proportional (P < 0.05) to the increase in semen dilution ratio (Fig. 1). The linear relationship indicates an almost 30 s of the motility time can be observed at a dilution of 1 : 100 000.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Means ± standard deviation</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>Semen volume (ml)</td>
<td>0.51 ± 0.35</td>
<td>12</td>
</tr>
<tr>
<td>Relative semen</td>
<td>1.3 ± 0.7</td>
<td>12</td>
</tr>
<tr>
<td>volume obtained (ml kg−1)</td>
<td>2.95 × 1010</td>
<td>1</td>
</tr>
<tr>
<td>Sperm concentration (spermatozoa.ml−1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm motility duration (s)</td>
<td>23.47 ± 0.86</td>
<td>3</td>
</tr>
<tr>
<td>Sperm survival rate (eosin-nigrosin) (%)</td>
<td>97.5</td>
<td>1</td>
</tr>
</tbody>
</table>

Fig. 1. Relationship between sperm motility duration (SMD) and different ratios semen:activating solution (water) employed during the process of sperm activation in Prochilodus lineatus

Figure 2 shows the values of SMD using water as an activating solution at different temperatures for the activation of Prochilodus lineatus spermatozoa. The SMD in relation to the temperature of the activating solution (water) exhibited a quadratic behavior (P < 0.05), with a maximum theoretical performance in terms of sperm motility duration of 21.36 s at a temperature of 17.3°C.

Fig. 2. Relationship between sperm motility duration (SMD) and different temperatures of the activating solution (water) employed in the process of sperm activation in Prochilodus lineatus
Discussion

In the lowest dilution, 1 : 1 μl semen:water, it was not possible to measure the sperm motility duration due to the incomplete activation of the spermatozoa. According to Billard and Cosson (1992), a relatively high dilution (over 1 : 1000) is necessary for the synchronized activation of the spermatozoa.

The spermatozoa are not activated at low dilutions, and the activation occurs progressively for some minutes after the dilution. This fact, together with the short period of time when the spermatozoa remain active (Billard and Cosson, 1992), makes it difficult to measure the sperm motility duration correctly, and might explain the discrepancies found in literature (Billard et al., 1995).

The SMD of *Prochilodus lineatus* reached a time of 23.04 s at a dilution of 1 : 2 (semen:water). Simultaneously, the increase in SMD and in dilution reached the maximum value of 28.83 s for the ratio 1 : 100 000. However, whether such a high dilutions rate support a good fertilization rate must be tested in the future.

Alavi et al. (2007) evaluated the dilutions 1 : 25, 1 : 50 and 1 : 100 μl semen:diluent for *Perca fluviatilis*, and determined that the best results were the ones obtained with a dilution of 1 : 50 μl semen:diluent. Alavi and Cosson (2005) evaluated the dilutions 1 : 10, 1 : 50 and 1 : 200 μl semen:diluent for *Acipenser persicus* and showed that the dilutions 1 : 50 and 1 : 200 μl semen:diluent were the most efficient ones. Gallis et al. (1991) also studied the effect of dilution on sperm motility and observed an increase in motility and dilution from 1 : 6 to 1 : 100 for *Acipenser baerii*.

Alavi et al. (2004) affirmed that the fish species mentioned above, when compared to tropical species such as *Prochilodus lineatus*, present motility at low dilution and concentration of inorganic cation in the semen.

In this experiment a temperature of 17.3°C promoted a sperm motility duration of 21.36 s. Such behavior of the motility duration may be inversely proportional to the increase in temperature, as described by Billard and Cosson (1992) for the rainbow-trout, Vladic and Jarvi (1997) for the brown trout and Atlantic salmon, Jezierska and Witeska (1999) for the common and grass carp, and Williot et al. (2000) for the Siberian sturgeon.

Billard and Cosson (1992) claimed that the increase or decrease in the temperature of the activating solution has a direct influence on sperm motility duration. It is mainly due to the fact that the energy stock of the fish spermatozoa is limited, and consequently, the increase in sperm cell activity caused by the rise in the temperature of the activating solution induces the reduction of sperm motility duration. However, that fall in the temperature of the activating solution might result in the increase in sperm motility duration due to the reduction in the cellular metabolism of the spermatozoa (Alavi and Cosson, 2005). There are other factors that might influence sperm motility duration, such as the quantity of gametes, volume and pH of the activating solution (Billard et al., 1995).

Thus, for the studied species, the growth in the dilution ratio proved favorable for an increase in motility duration until the maximum value studied of 1 : 100 000 μl semen:water. As for the temperature of the activating solution, the optimum temperature for each fish species must be observed since a decrease in motility duration can be detected at higher temperatures, due to the increase in the metabolism and consequently, in the consumption of the energy stocks of the spermatozoa (Alavi and Cosson, 2005).

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Author’s address: E. Romagosa, Avenida Francisco Matarazzo, 455 – Água Branca, SP, Brazil.
E-mail: eromagosa@pesca.sp.gov.br