DOI: 10.5433/1679-0359.2015v36n6p4023

Sperm motility and velocities of Characiformes fishes in different times post-activation

Motilidade e velocidades espermáticas de peixes Characiformes em diferentes tempos pós ativação

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Abstract

The objective of the current study was to observe the performance kinetics (motilities and velocities) of the spermatozoa from *Prochilodus brevis* (curimatã), *Colossoma macropomum* (tambaqui) and *Piaractus brachypomus* (pirapitinga) species in different times post-activation. The sperm of *P. brevis*, *C. macropomum* and *P. brachypomus* species were collected after hormonal induction with carp pituitary extract. The samples with not contamination with water, urine or feces had motility subjective, morphology, osmolality and concentration analyzed. The samples selected were analyzed with Sperm Class Analyzer. Spermatozoa motility and velocities were captured at 10, 30, 60 and 120 s post-activation. No significant differences in total motility of *P. brevis* spermatozoa were observed between 10 s and 30 s post-activation. However, significant reduction was observed in 60 s. This reduction was more accentuated after 120 s. The same pattern of spermatozoa motility decline happened for *C. macropomum* and *P. brachypomus*. Velocities also followed the same pattern for the three species. There was significant reduction in velocities after 30 s; this reduction was more significant after 60 s. There was no significance difference between 60 s and 120 s post-activation. Sperm of *C. macropomum* and *P. brachypomus* show satisfactory sperm quality up to 60 s after activation. On the other hand, sperm of *P. brevis* up to 120 s after activation. These findings show that the rate of sperm motility in different times post activation is change for each species tested.

Key words: *Colossoma macropomum*, *Piaractus brachypomus*, *Prochilodus brevis*, sperm class analyzer

Resumo

O objetivo do presente trabalho foi investigar o desempenho cinético (motilidade e velocidades espermáticas) de *Prochilodus brevis* (curimatã), *Colossoma macropomum* (tambaqui) e *Piaractus brachypomus* (pirapitinga) em diferentes tempos pós ativação. O sêmen de *P. brevis*, *C. macropomum* e *P. brachypomus* foi coletado após indução hormonal com extrato hipofisário de carpa. As amostras não contaminadas com sangue, fezes ou urina tiveram motilidade subjetiva, morfologia, osmolaridade e concentração analisadas. As amostras selecionadas foram analisadas com o Sperm Class Analyzer.

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Recebido para publicação 02/09/14 Aprovado em 01/03/15

A motilidade e velocidade dos espermatozoides foram mensuradas a 10, 30, 60 e 120 s pós ativação. Nenhuma diferença significativa na motilidade total do sêmen de *P. brevis* foi observada entre 10 s e 30 s pós ativação. No entanto, uma redução significativa foi observada aos 60 s. Essa redução foi mais acentuada após 120 s. O mesmo padrão de declínio da motilidade espermática ocorreu para *C. macropomum* e *P. brachypomus*. As velocidades espermáticas seguiram o mesmo padrão para as três espécies. Houve redução significativa nas velocidades após 30 s; essa redução foi mais significativa após 60 s. Não houve diferença significativa entre 60 s e 120 s pós ativação. Os espermatozoides de *C. macropomum* e *P. brachypomus* apresentam satisfatotia qualidade espermática até 60 s pós ativação. Por outro lado, os espermatozoides de *P. brevis* apresentam satisfatória qualidade espermática até 120 s pós ativação. Esses achados mostram que a taxa de motilidade espermática em diferentes tempos pós ativação muda para cada espécie testada.
Palavras-chave: *Colossoma macropomum*, *Piaractus brachypomus*, *Prochilodus brevis,* sperm class analyzer

The *Prochilodus brevis* (curimatã), *Colossoma macropomum* (tambaqui) and *Piaractus brachypomus* (pirapitinga) are Characiformes fish and are native to South America. Characiformes have commercial importance as ornamental species, food in fisheries, aquaculture, or sport fishing (GODINHO; VIVEIROS, 2011; GURGEL et al., 2012; RAMIREZ-MERLANO et al., 2011).

Prior to spermiation, fish sperm are stored in an immotile state in the testes, and the motility of sperm is associated with their sensitivity to osmolality and the ion concentration in the media where the sperm is released (ALAVI; COSSON, 2006). Sperm motility in freshwater fish is best initiated with media that is hypo-osmotic relative to seminal fluid (COSSON, 2010). After initiation of motility, spermatozoa become motile and metabolically active briefly. Thus it is important the knowledge of spermatozoa motility during the period that the sperm is active for each species worked (CARNEIRO, 2007).

Objective analyses can provide precise and accurate information on spermatozoa motility (NASCIMENTO et al., 2010), however, there are few data reported for *P. brevis* and *P. brachypomus*. Furthermore, there are no reports on spermatozoa motility in different periods post-activation for *C. macropomum*, *P. brevis*, and *P. brachypomus*.

The objective of this study was to observe the performance kinetics (motilities and velocities) of the spermatozoa from *C. macropomum*, *P. brevis* and *P. brachypomus* species at different time post-activation of motility.

This work was approved by the ethics committee for animal use with the following protocol numbers 11518754-5/74 (*C. macropomum*), 1278971-1 (*P. brachypomus*) and 12776936-6 (*P. brevis*). *C. macropomum* and *P. brachypomus* males were selected from tanks at the Departamento Nacional de Obras Contra as Secas (DNOCS) in the city of Pentecoste, state of Ceará, Brazil, and *P. brevis* males were selected from tanks at the Laboratório de Biotecnologia da Reprodução de Peixes (LBRP) in the city of Fortaleza, state of Ceará, Brazil. The weight of the *C. macropomum* was 5.88 ± 1.03 kg; the *P. brachypomus* was 3.78 ± 0.9 kg and the *P. brevis* was 0.56 ± 0.1 kg.

Males with detectable sperm under soft abdominal pressure were given a single treatment via the intracelomatic route (2 mg kg^{-1} of body weight) of carp pituitary extract. After 448 hours-degree, animals were anesthetized using a clove oil-based solution (1:10:10000; eugenol:alcohol:water). The urogenital papilla was carefully dried, and sperm were hand stripped directly into graduated tubes. Sperm collection was completed at room temperature (28 ± 1°C), and soon after collection, the animals were returned to the tank. Tubes containing sperm were placed in crushed ice (5 °C). Contamination of sperm with water, urine or feces was carefully avoided.

Immediately after collection, each semen sample was placed on a glass slide and observed under a light microscope at 100 X magnification. Spermatozoa motility (auto-activation) observed was attributed to urine or water contamination and the sample was discarded. Motility was subjectively estimated as the percentage of motile spermatozoa. Selected samples from *C. macropomum* (n = 17), *P. brachypomus* (n=19) and *P. brevis* (n=18) were activated with an activation solution (tank water) at a final dilution ratio of 1:50 (sperm: activation solution; v: v). Samples with at least 80% motile spermatozoa were used in this study.

The analyzed parameters in the sperm samples were volume (mL), directly observed in the graduated tubes; osmolality of seminal plasma (mOsm kg^{-1}), measured with a Peltier digital osmometer (Roebling, Germany); concentration (number of spermatozoa per mL) by diluting samples in 4% formol-citrate solution and analyzing in a Neubauer hemocytometer chamber.

The spermatic kinetic parameters analyzed were: rate of motility (%), curvilinear velocity (VCL, µm s^{-1}), straight line velocity (VSL, µm s^{-1}) and average path velocity (VAP, µm s^{-1}). These parameters were captured until the sperm submit at least 30% motility, observed at 10 s, 30 s, 60 s, 120 s post-activation. The analyze occurred after addition activation solution using 2 mL of each sperm sample diluted in 100 mL of 50 mM NaCl (100 mOsm kg^{-1}) onto a Makler chamber and evaluated under light microscopy (400x) with the Sperm Class Analyzer (SCA; Microptics, Spain). Spermatozoa were considered immotile when the velocity was < 10 µm s^{-1}.

All data were expressed as the mean ± SD. Spermatozoa motility (rates and velocities) were tested for normal distribution. When the data did not fit the normal distribution, an arcsine transformation was performed. All data for spermatozoa motility (rates and velocities) were tested for significant differences using an ANOVA, followed by the Tukey's test when necessary. All data were evaluated using the statistical software SAS v.8 (2000). The level of significance for all statistical tests was $P< 0.05$.

Semen characteristics of *C. macropomum*, *P. brachypomus* and *P. brevis* species after induction with carp pituitary extract were respectively: a) volume (mL): 3.74 ± 1.14, 3.90 ± 1.60 and 1.78 ± 0.62; b) concentration (10^9 mL^{-1}): 5.72 ± 2.82, 4.13 ± 2.12 and 30.82 ± 6.83; c) osmolality (mOsm kg^{-1}): 298.00 ± 48.50, 273.00 ± 52.80 and 292.17 ± 10.81; d) motility subjective (%): 92.10 ± 6.15, 90.00 ± 12.00 and 95.61 ± 3.07.

No significant differences in the motility of the sperm of *P. brevis* were observed between 10 s and 30 s post-activation. However, a significant reduction was observed in 60 s. This reduction was more accentuated after 120 s. The same pattern of sperm motility decline happened in *C. macropomum* and *P. brachypomus*. In these species, total motility at 10 s was similar to 30 s. After 60 s, a decrease in sperm motility ($p<0.05$) in the *C. macropomum* and *P. brachypomus* species was observed, and an important decline also occurred at 120 s (Table 1).

This is the first work that has evaluated spermatozoa motility at different times post-activation for *P. brevis*, *C. macropomum* and *P. brachypomus* species. Moreover, for *P. brevis* and *P. brachypomus*, no previous reports have that evaluated individually the sperm of each male using an automatized analyzer.

Table 1. Percentage of spermatozoa motility at 10s, 30s, 60s, 120s post activation of *C. macropomum* (n = 17), *P. brachypomus* (n=19) and *P. brevis* (n=18) species. Different letters in columns are significantly different at p<0.05.

Times post activation	*C. macropomum*	*P. brachypomus*	*P. brevis*
10 s	84,77 ± 4,11 [a]	82,40 ± 3,63 [a]	96,17 ± 1,09 [a]
30 s	80,43 ± 3,68 [a]	70,69 ± 4,75 [a]	94,40 ± 1,25 [a]
60 s	35,74 ± 3,42 [b]	33,68 ± 3,37 [b]	77,91 ± 3,67 [b]
120 s	9,19 ± 1,27 [c]	9,42 ± 1,65 [c]	34,80 ± 6,53 [c]

Volume and concentration of *C. macropomum* and *P. brachypomus*, in this work, were lower than those found in the literature (RAMIREZ-MERLANO et al., 2011; VARELA JUNIOR et al., 2012). However, these parameters of *P. brevis*, in this work, were higher than those found in the literature (LOPES et al., 2014; SILVA et al., 2014). Reported differences may be related to induction hormonal (MARIA et al., 2010), diet, stage of maturation, environmental conditions (e.g., temperature, salinity, and photoperiod), ATP content, enzymatic activity, hormonal regulation of spermiation, secretory activity of the sperm duct, milt contamination, stress, or a combination of these factors (CIERESZKO, 2007). The osmolality found in this study is within pattern found in the literature for this species (260-313 mOms Kg-1) (LOPES et al., 2014; MARIA et al., 2010; NASCIMENTO et al., 2010).

With the advent of an automated method to evaluate motility (CASA), other aspects of spermatozoa movement have begun to be evaluated, such as curvilinear velocity (VCL), straight line velocity (VSL) e average path velocity (VAP), which are positively correlated with the fertilizing capacity of spermatozoa (VIVEIROS et al., 2010) as well as motility and the duration of motility (RANA, 1996).

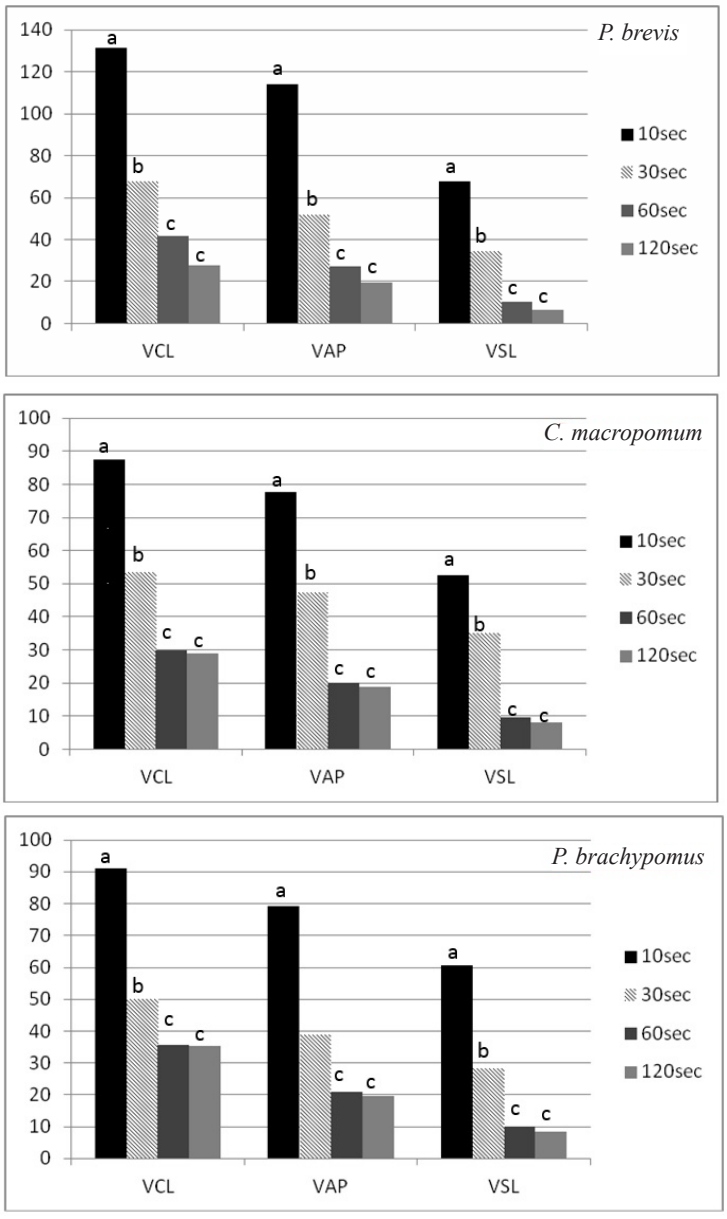
The velocities follow the same pattern for the three species. There was a significant reduction in velocity (VCL, VSL e VAP) at 30 s. This reduction was also significant at 60 s. Thereafter, there was no significant reduction at 120 s post-activation. The *P. brevis* fish had higher velocities post-activation (10 s) with 131.59 ± 6.64 μm s^{-1} (VCL), 113.81 ± 5.97 μm s^{-1} (VAP) and 67.72 ± 3.33 μm s^{-1} (VSL), while the *C. macropomum* fish had the lowest velocities (10 s) with 87.56 ± 10.14 μm s^{-1} (VCL), 77.74 ± 8.97 μm s^{-1} (VAP) and 52.49 ± 6.20 μm s^{-1} (VSL) (Figure 1).

In the present study, the percentage of spermatozoa motility and spermatozoa velocity decreased as a function of time post-activation. The same phenomenon was observed in *Cyprinus carpio* (BORYSHPOLETS et al., 2009). For the three species observed in the present study, the percentage of motile cells remained similar until 30 s; however, until 60 s, there was a significant reduction in motility that was more accentuated at 120 s. In the *P. brevis* fish, despite the significant reduction at 120 s, the motility was at 34.80%, a reasonable value for use in fertilization programs (RAMIREZ-MERLANO et al., 2011; VARELA JUNIOR et al., 2012). Regarding the *C. macropomum* and *P. brachypomus* species, reasonable values are found at 60 s (35.74 and 33.68%, respectively).

The differences found for each species may be related to ionic composition and osmolality of the seminal plasma, time of stripping during the reproductive season, and environmental factors regulating spermiation (i.e., temperature and photoperiod). Activation solution characteristics such as ion concentrations (K^+, Na^+, and Ca^{2+}), osmotic pressure, pH, temperature and dilution rate affect motility (ALAVI; COSSON, 2006), which highlights the importance of the interaction of sperm with the activation solution.

Spermatozoa activation leads to a decrease in the ATP content along with spermatozoa velocity and motility percentage decreases (BORYSHPOLETS et al., 2009). In addition, shorter duration of spermatozoa motility following activation in low hypo-osmolality conditions may be due to damage of the flagellum mediated by alterations in the spermatozoa plasma membrane (ALAVI et al., 2009). Additional studies related to the mechanisms of activation of Characiformes spermatozoa are needed for the understanding of various phenomena related to the quality of motility post-activation in artificial fertilization program.

Figure 1. VCL, VSL and VAP in μm s^{-1} of *P. brevis, C. macropomum* and *P. brachypomus* species at 10, 30, 60 and 120 s post-activation. Velocities followed by different letters are significantly different at $p<0.05$.



Sperm of *C. macropomum* and *P. brachypomus* show satisfactory sperm quality up to 60 s after activation. On the other hand, sperm of *P. brevis* up to 120 s after activation. These findings show that the rate of sperm motility in different times post activation is change for each species tested. Knowledge of seminal behavior is important to assist in the development and use of assisted reproductive protocols.

This study was supported by Departamento Nacional de Obras Contra as Secas (DNOCS) and Laboratório de Biotecnologia da Reprodução de Peixes (LBRP), for providing the facilities and the specimens used in the experiments. The Financiadora de Estudos e Projetos (FINEP) for financial support and Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP) for granting the scholarship.

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