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Quality of semen in the reproductive cycle of Cachara (*Pseudoplatystoma fasciatum*) raised in captivity

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ABSTRACT. In this study, we evaluated the semen quality of Cachara (*Pseudoplatystoma fasciatum*) raised in captivity during its reproductive period. For the evaluation of qualitative parameters, we analyzed: sperm morphology, sperm motility, and sperm vigor; while for the quantitative parameter, we analyzed sperm concentration. In general, the main significant difference was found in March, with a motility rate of 40% and a duration of 39 seconds. The highest mean concentration ($1.2x10^8$) was observed in February, as well as the highest percentage of defects in the intermediate part of the spermatozoa (59%). The morphological analysis of spermatozoa showed an average of 24.2% abnormalities in the head and 22.7% in the intermediate part of spermatozoa of *P. fasciatum* for the four months studied. We did not find any evident relationship between the climate changes evaluated and the qualitative and quantitative parameters. The greatest difference between the percentage of normal spermatozoa and the sharp increase of defects in the intermediate piece, observed in February, can be explained by the sudden climate change, with a significant decrease in temperature and relative humidity, and increase in precipitation. These findings demonstrate the importance of maintaining an optimum climate interval during the breeding season for *P. fasciatum*.

Keywords: Semen; Cachara; *Pseudoplatystoma*; Spermatozoa.

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Introduction

Brazil stands out as one of the countries with the greatest potential for aquaculture expansion, following the increasing world demand for food of aquatic origin, not only due to population expansion but also by the growing awareness of the population regarding the origin of ingested protein (Navarro et al., 2012). Brazil still has the richest freshwater fish fauna in the world, with about 2,587 species described, and there are still many unknown species (Buckup et al., 2007). However, despite the rapid growth of the Brazilian aquaculture industry in recent years, the rational and optimized use of aquaculture potential in the country is still incipient (Lopes et al., 2016).

The species *P. reticulatum*, also popularly known in Brazil as cachara, surubim and surubim-cachara, had its name changed to *Pseudoplatystoma fasciatum* after the reorganization of the genus *Pseudoplatystoma* (Silva, Lima, & Lundstedt, 2015), which comprises the 130 largest fish of the family *Pimelodidae, Siluriformes* (Romagosa, Paiva, Andrade-Tamelli, & Godinho, 2003). This species inhabits exclusively freshwater ecosystems, and have a wide geographic distribution, being found in the main South American river basins, such as the Amazon and the Prata (Romagosa et al., 2003; Biutrago-Suárez, 2007). It can measure more than 126 cm, having a fusiform body, without scales, covered only by a thick skin, or covered, partially or totally, by bony plates. The color of the dorsum is gray, ventrally white, with peculiar morphological characteristics of the species, light and dark transverse bands perpendicular to the body, spaced apart. It has three pairs of barbells, one of which is black in the jaw, and the other two are white in the chin. The mouth is large, with villiform teeth.

In the Central-West region, the cultivation of native species is remarkable, with the surubim-cachara (*Pseudoplatystoma fasciatum*) being extensively farmed. The significant increase in production of this species occurred between 2010 and 2011. According to Silva, Lima, and Lundstedt (2015) this production increased by 80% in 2013. Several companies in the region have invested in the cultivation of this species,

with satisfactory results due to the optimization of the aquaculture systems, as well as the technological advance based on increasing knowledge of the species biology.

Meat characteristics and zootechnical performance make the cachara an attractive species for production, however, technological assemblages for commercial production still require adjustments, especially regarding the reproductive aspect (Crepaldi et al., 2008). According to Soli-Murgas, Felizardo, Ferreira, Andrade and Veras (2011), the major problem of captive fish breeders is the lack of gonadal development and maturation. As cachara is a rheophilic fish and its production depends on environmental stimuli, thus the evaluation of this captive fish semen is of great importance for the establishment of artificial fertilization. Therefore, in this study we aim to asses the semen quality of captive cacharas during their reproductive period, aiming to improve the reproductive efficiency of this species.

Material and methods

The experiment was carried out in the Companhia do Peixe (Cia do peixe), a company located in the rural area of the municipality of Cidade Ocidental, State of Goiás, Brazil, which is specialized in fish production. Samples were collected once a month between December 2015 and March 2016. Five different males (*Pseudoplatystoma fasciatum*) were analyzed every month, totalizing 20 specimens analyzed over the studied period. The fish average weight was 1.69 ± 1.17 kg, and length was 61.40 ± 11.61 cm.

Collection of semen

A fishing net was used to capture the animals, which were contained with a dry cotton towel as soon as they were removed from the water. The individual ejaculation samples were collected from the males as follows: after capture, the eyes were blindfolded with a towel and the urogenital papilla was cleaned and completely dried with paper towel. Manual compression of the celomatous wall was performed following the craniocaudal direction. The ejaculate was collected in sterile microcentrifuge tubes and then conditioned, under the light, for subsequent laboratory analysis. The semen was checked for contamination or activation immediately after collection. If early spermatozoa activation was verified, the semen was discarded.

Analysis of semen *in natura*

The analysis of the semen quality *in natura* from the studied specimens was performed using a 10 µL aliquot of semen deposited on a microscope slide and observed under a light microscope at 400x magnification. The semen was activated by adding water from the fish tank in the proportion of 1:4 (semen:water) to evaluate its quality.

All the ejaculates collected were analyzed by measuring the rate (%) and duration (seconds) of sperm motility, with one person observing the semen. Motility rate was measured subjectively by observation under light microscopy, as the percentage of spermatozoa showed progressive motility. The duration of motility was evaluated under the same conditions, in which a timer was started at the time of activation and stopped when 10% of sperm were still moving (Miliorini et al., 2011).

For the spermatozoa morphology assessment, semen samples of all analyzed animals were used *in natura*. An aliquot of 10 µl of semen was diluted in 990 µl of 4% formalin-saline solution. Subsequently, a 10 µl fraction of the fixed sample was deposited on a histological slide, stained with 10 µl of rose bengal dye and covered with a coverslip before submitted to examination under a fluorescence microscope using episcopic illumination (Nikon, model OPTIPHOT-2) at 1000x magnification. Immersion oil was used to increase the optical resolving power of the microscope. To quantify the morphological disturbances, 100 spermatozoa were randomly assessed for each sample. Semen morphology assessment was performed under an increase of 1,000 diopters. The pathologies of the head, the intermediate part and the remaining tail (main and terminal parts) were investigated, and then, the semen was stored in a refrigerator for further analysis. The morphological analyses of the semen *in natura* were performed in the Aquaculture Laboratory (FAV) of the *Universidade de Brasilia* (UnB), Brazil.

For the sperm concentration measurement, the diluted sample (10 µL of semen + 990 µL of formalin-saline solution) was placed on a Neubauer chamber and counted under optical microscope at 100x magnification (Navarro et al., 2014) The spermatozoa found inside the reticles of the chamber were counted using the following formula:

$$\frac{A}{\frac{1}{B} * \frac{n}{25} * \frac{1}{10}}$$

where: A = mean sperm count in the two reticles; B = sample dilution; n = number of squares counted; $\frac{1}{10}$ = fixed value.

Statistical analyses

The data was analyzed using a PROC ANOVA (one-way Analysis of Variance) in SAS (Statistical Analysis System) software version 9.4, to test for significant differences in the parameters of semen quality throughout the breeding season. Means were compared using the Tukey test using a significance level of 0.05.

Results

Mean temperature, relative humidity, and precipitation of each month of collection are shown in Table 1. The mean relative humidity and precipitation were observed between December (2015) and March (2016). We found a significant difference ($p < 0.05$) for relative humidity between all months, except when comparing December with February. Precipitation, although higher in January compared to December and February, did not present a significant difference between the months studied ($p > 0.05$).

Table 1. Values of temperature (ºC), relative humidity (%) and precipitation (mm) during the months of December 2015 to March 2016, for the Federal District. (collected data Automatic Weather Station Database Fazenda Água Limpa – FAL/UnB.

	December	January	February	March
Temperature (ºC)	22.5	21.6	22.2	21.7
Relative humidity (%)	76.8	87.7	74.0	82.6
Precipitation (mm)	4.7	8.5	4.9	7.4

The motility rate was statistically significant in December, January, and February in comparison to March. The same result was observed for motility duration. The quantitative and qualitative parameters of the surubim-cachara (*Pseudoplatystoma fasciatum*) semen is presented in Table 2. The variation in sperm cell concentration in semen was high for this study. February showed the highest concentration (125,299,700 cells mm^{-3}) and January presented the lowest (114,5250 cells mm^{-3}). December was significantly different from February and March.

Table 2. Characteristics of cachara semen (*Pseudoplatystoma fasciatum*).

Items		December	January	February	March
Spermatozoa					
	Motility rate (%)	84±23.02[a]	99±2.2[a]	100[a]	40±21.6[b]
	Duration (seconds)	98.8±28.3[a]	108.8±22.7[a]	132.4±38.9[a]	39.8±16.8[b]
	Strenght	4[ab]	5[a]	5[a]	3[b]
	Concentration (cells mm^{-3})	108,047,400[a]	114,525000[ab]	125,299700[b]	100,310000[b]
Spermatozoa morphology	Normal	47.6±26.25[a]	10.2±11.75[b]	8.8±6.30[b]	19.6±27.86[ab]
	Degenerated head	4.6±2.19	22.2±18.13	7.8±13.93	2.6±6.5
Head	Isolated head	3.4±4.21	9.4±3.84	2.0±2.34	41.6±46.32
	Macrocephaly	2.2±2.04	2.2±3.19	4.6±4.27	0.25±0.5
	Microcephaly	1.0±2.23	0	0	0
Intermediate piece	PID	6.4±4.97	20.2±9.78	59±18.42	4.6±7.58
	Fractured tail	5.4±4.09	1.4±3.13	0.2±0.44	1.2±3
Tail	Folded tail	13±5.04	9.8±2.28	3.6±5.85	2±5
	Degenerated tail	2±2.34	2±1.58	0	4.2±7.88
	Coiled tail	14.4±14.67	22.6±2.96	13.4±13.55	4±4.08

CV: coefficient of variation; Numbers for each month are given as mean ± standard deviation.

The number of normal sperms decreased by 47.6% in December in comparison to March (19.6%). For semen abnormalities, we found no significant difference between the months studied. Some examples of abnormalities found in the surubim-cachara (*Pseudoplatystoma fasciatum*) spermatozoa are shown in Figure 1. The abnormalities of the heads were: degenerate head, which was observed in 22% of spermatozoa

collected in January; isolated head, which was observed in 41% of the spermatozoa collected in March; macrocephaly, that had a coefficient of variation of 1.7% for the months studied; and microcephaly, which was observed only in spermatozoa collected in December (Figure 1). The defect in intermediate parts varied from 59 to 4.6% between February and March, respectively.

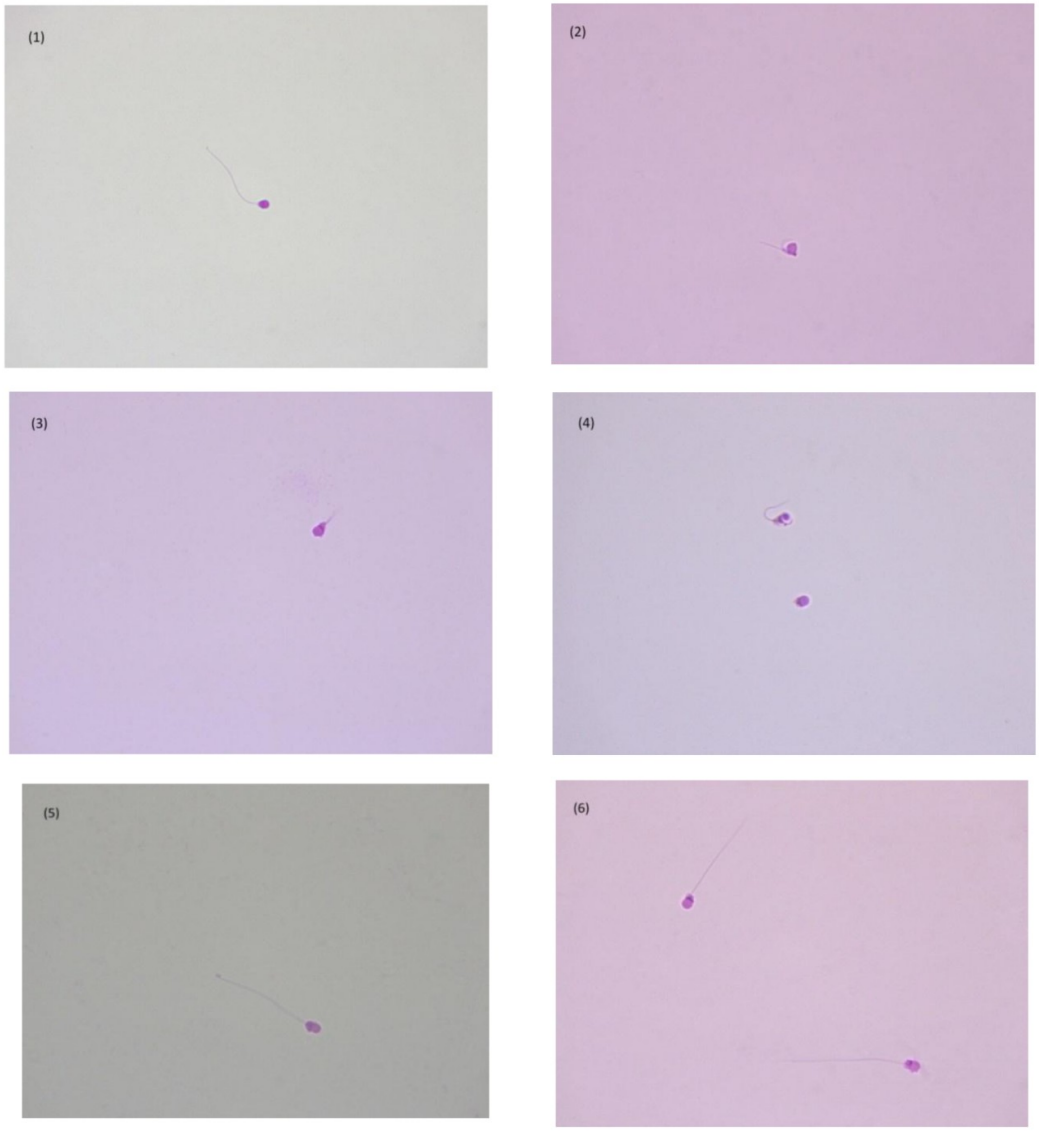

Figure 1. Images made in an optical microscope with objective of 100x showing normal spermatozoa. with primary and secondary defect. (1) Normal sperm; (2) Tail strongly wrapped around head and strongly bent; (3) Spermatozoon with degenerate tail; (4) Defect in spermiogenesis indicated by arrow and loose normal head; (5) Spermatozoa with tail curled at the tip and elongated head; (6) Degenerate head.

Defects in the intermediate piece had a coefficient of variation of 25.27%. and was the highest obtained for abnormalities in this experiment.

Discussion

Sperm concentration is a highly variable trait in freshwater fish and may be influenced by individual variation, weight, and size of specimens. It is interesting to note the variation of sperm concentration obtained from *P. fasciatum* on farm environment and natural environments in the period of piracema (fish breeding). Streit Jr. et al. (2012) observed a concentration 20x lower for cacharas cultivated in the municipality of Maringá, State of Paraná. Some authors (Miranda, Strüssmann, & Somoza, 2009; Ribeiro & Moreira, 2012) have observed that temperature exerts an effect on gametogenesis and that temperature increase can lead to sperm maturation.

The motility rate was high for *P. fasciatum* from December to February, always above 80%. According to Resende et al. (1996), the reproduction of *P. fasciatum* follows the precipitation regime which would explain the increase in motility in January. Yet, we found a noticeable decrease in motility in March, despite the increased precipitation in March in comparison to February. This sharp decrease could be explained by the end of the natural reproductive cycle. The cachara species has a relatively short breeding season, from November to February. Romagosa et al. (2003), studying *P. fasciatum* raised in captivity, showed a decrease in the gonadosomatic index in March, which explains the decline in the quality of gametes in this period for cultivated specimens. Therefore, we conclude that the collection performed in March does not match a suitable season for high sperm motility.

The duration of sperm motility ranged from 39.8 seconds in March to 132.4 seconds in February. The decrease in sperm movement capacity in March occurred along with the end of the reproductive cycle. As a consequence of the end of the cycle, the hormonal cascade of GtH I and GtH II, which promote spermatogenesis and spermiation, is inhibited, influencing the semen quality and the amount of energy available for motility. The results obtained in this study are within the normal motility time for freshwater fish species, which can vary strongly between species, with some showing a motility of only 30 seconds (Billard, Cosson, Perchec, & Linhart, 1995), while others have an extensive motility extending up to 480 seconds (Maria et al., 2004). The motility duration did not present a direct correlation with the climatic parameters studied. However, the variation of temperature and relative humidity were found following the natural habitat of the animal, in the Amazon, Paraná, and Orinoco river basins, which explains the non-interference of climate in sperm motility.

The morphological abnormalities could be found in 91.2% of the analyzed spermatozoa. Defects in the spermatozoa head were the main abnormalities observed for *P. fasciatum* in this study, followed by abnormalities in the tail and intermediate part, respectively. Defects found in the spermatozoa, regardless of the location, may lead to failure at the time of fertilization, caused by a deficiency in their genetic material or on their locomotion (Kavamoto, Barnabe, Campos, & Andrade-Talmelli., 1998).

In the present study, 47% of surubim-cachara spermatozoa were normal in December and only 8% in February. The decrease in the normality rate in February was likely caused by excessive stress in the animals due to food handling and management at the time of collection and abrupt change of climatic parameters during this month. According to Soli-Murgas et al. (2011), fish semen can be compromised by reproductive diseases, poor feeding management or stress. January and February also showed a sharp increase in deformed intermediate pieces, which corroborates with the premise of high stress at the time of the collection.

We found no direct correlation between temperature, relative humidity, and precipitation with abnormalities rate of the intermediate pieces in January and February. However, we found a significant difference in December and March when compared to January and February. Although no variable separately support the higher PID incidence in February, the analyses of the three climate parameters together indicated a relationship between the temperature increase, followed by an decrease in humidity and precipitation rate, and the higher rate of abnormalities in the intermediate part of spermatozoa (Ribeiro & Moreira. 2012). We also found no statistically significant difference in tail abnormalities between the months studied, although observed defects decreased more than 50% in March compared to January.

Defects in spermatozoa head were not statistically significant between the months studied. However, we found a sharp increase in morphological defects in January and March. The increase in cases of head abnormalities follows changes in the climate: January and March had a decrease in temperature, as well as an increase in precipitation and humidity. It is known that for many animal species that abrupt temperature changes may increase the chances of a cell injury in the acrosome (Heise, Puntarulo, Nikinmaa, Abele, & Pörtner, 2006). Hitherto, no relationship has been reported between humidity and precipitation and increased abnormalities in spermatozoa head, yet fish habitat may affect its internal osmolarity, including osmolarity of the seminal fluid. Moreover, rainwater tends to be acidic, which increases the amount of H^+ in water, decreasing pH and altering osmolarity. Both changes in pH and osmolarity may contribute to increasing sperm defects (Pinheiro et al., 2016; Rurangwa et al., 2004).

Conclusion

The quality of cachara semen showed higher motility rates in December, January, and February, indicating that the reproduction of this species can be extended under captive conditions, without losses to

the reproductive quality of the animals. This information may guide new experiments, improve cultivation conditions, value the activity economically, as well as increase the number of fingerlings intended for aquaculture.

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