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ZOOLOGY

Seminal features and reproductive index of Thai tilapia (*Oreochromis* sp.) on different reproductive periods

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ABSTRACT. Learning about the biology of the species is essential to the success of intensive farming. This study aimed to evaluate the semen of Thai tilapia during the four seasons of the year and thereby analyze their reproductive indices. Thus, 60 breeding males of Tilapia were used and were randomly divided into four water tanks and fed with isoproteic and isocaloric feed. The experiment lasted 12 months, starting from October 2014 and ending on September 2015. Thus, it was possible to collect sperm material of animals during the four seasons, twice a month, as well as to evaluate the water quality parameters in the tanks (temperature, pH and dissolved oxygen). The semen was evaluated from a light microscope in an increase of 100 x, then was activated with water. Motility was measured subjectively in the light microscope, as well as the percentage of sperm showing progressive motility. The duration was evaluated with the addition of a timer. For analysis of the morphology of the semen, the test consisted of morphopathology observation of 100 sperm focused in various fields throughout the slide in the light microscope. Once obtained, these data were analyzed through ANOVA and Tukey test as post-hoc analysis, with the help of the software R Statistics. Water quality factors (temperature, pH and$_2$ dissolved) were acceptable and during the 12 month period the sperm of tilapia (*Oreochromis* sp.) had a good ability for fertilization, seen that it performed below the average of the percentage of critical abnormalities, and quality was perceived by the parameters that also influence fertilization (motility rate, duration of motility and vigor).

Keywords: semen; aquaculture; sperm; reproduction.

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Introduction

The knowledge of the biology of the species, especially in relation to reproductive aspects, is essential when you want to perform the intensive rearing of fish (Andrade & Yasui, 2003; Godinho, 2007).

The knowledge of the reproductive performance of these species throughout the breeding period is scarce, proving the need for reflection on the obtaining of such information. It must be considered that climate change is becoming evident every year, and seasons of the year are losing their outstanding characteristics, due to the uncontrolled actions of man over nature, such as the intensification of the greenhouse effect, the deforestation and the destruction of the ozone layer (Walther et al. 2002; Pereira, Andrade, Costa, Vidal Jr, & Yasui, 2007).

Consequently, these climatological changes can result in advance or delay in the final maturation or in spawning of these fish in different periods of reproduction. One of the most exotic fish species used in breeding and rearing is tilapia, which offers great advantages to breeding due to the rusticity and the high growth rate (Navarro, Navarro, Felizardo, Murgas, & Pereira, 2014).

The knowledge of the interaction of behavioral aspects influenced by the environment, which results in physiological responses, is a useful tool for the improvement of techniques used in fish farms. This project aims to evaluate the quality of semen of Tilapia over a year.

Material and methods

The experiment was accomplished with mature fish of Thai tilapia (*Oreochromis* sp.) at the Fish company Cia do Peixe, located in the city named Cidade Ocidental in Goiás state, Brazil.

Sixty breeding males of Thai Tilapia were used, from the same fish farm, with an average weight of 788.31 ± 119, 11 g and lengths 35.96 ± 2.06 cm. Specimens were distributed in four water tanks of $4m^3$ (2 x 2 four treatments and four replicates x 1 m) inside of a bigger tank (20 x 10 x 1 m) with approximately 200000 L water, in an entirely random design. The fish were tagged and distributed in the water tanks and fed on a ration of isoproteic 32% PB and 3300 kcal of ED isocaloric kg^{-1}.

During a period of 12 months, between the years 2014 and 2015, we collected the semen '*in natura*' of males of Tilapia, identified by a microchip, twice a month. The project was analyzed by an ethics committee with the protocol UnBDoc number 155393/2014.

A gill net was used for the capture of specimen followed by a dry cotton towel for containment. After the capture, the eyes of the specimen were blindfolded and the urogenital papilla cleaned and dried with paper towels. Manual compressions were held the celomatic wall, towards skull-caudal, with the care that no contamination occurs or neither the activation of the semen. Those activated prematurely were discarded. The ejaculates were collected in sterile test tubes, being packed and immersed in ice, in the dark, for subsequent laboratory analysis.

For the analysis of semen quality, semen *in natura* (and other subsequent analysis) of each male used, it took an aliquot of 10 μL of semen deposited on a slide of microscopy and observed under a light microscope, previously focused on the increase of 100 x. The semen was activated by the addition of water in the ratio 1:4 (semen: water) to your quality assessment.

All ejaculations collected were analyzed and subsequently the rate (%) and the duration (seconds) of sperm motility were measured. Motility was measured subjectively in the light microscope, as well as the percentage of sperm showing progressive motility. The duration of motility was evaluated under the same conditions, being a timer fired at the time of adding the Activation Agent and stopped when 10% of the sperm were still moving, and presented in second(s) (Miliorini et al., 2011).

For analysis of semen morphology, semen samples '*in natura*' of 60 animals were used. An aliquot of 10 μL of fresh semen was diluted in 990 μL of the solution of formaldehyde-citrate. After, a fraction of 10 μL of the sample set was deposited in an histological slide and covered with a coverslip. The examination consisted of morphopatology observation of 100 sperm focused in various fields throughout the slide. The morphological analysis of semen '*in natura*' were held in an optical microscope, a compound with episcopic fluorescent lighting (Nikon OPTIPHOT-2 model), in the laboratory of Animal Reproduction.

For the analysis of water quality parameters, the dissolved oxygen was measured from a digital Pulse Oximeter (YSI Bernauer, Blumenau, Brazil) twice a day during 7:00 and 5:00 pm. The pH and temperature were also measured, but with the help of a multi-parameter query digital apparatus for portable pH meter (Bernauer F-1002, Blumenau, Brazil), with the same frequency as the oxygen.

Statistical analysis was used to evaluate the parameters related to semen motility, including *repeated measures* ANOVA and Tukey test (alpha = 0.05) as post-hoc analysis, with the help of the software R Statistics version 4.0.0 'Arbor day' with the packages 'R Base' and 'Agricolae', respectively, to compare the values. While the mean and standard deviation of the data related to semen morphopathology were calculated using Microsoft Excel 2010.

Results

Water quality evaluated from the mean values of temperature, pH and O_2 dissolved during 12 months are shown in Table 1. There are greater temperature average values in the months of August and September, reaching 27°C and the lowest average in June, reaching 19.3°C. The pH was found higher in October, with an average of 9.4 and bottom in December, with an average of 4.5. As for the dissolved O_2, October features a discrepant average value compared to other months, reaching 21.5 mg L^{-1}.

Table 2 shows the means and standard deviations (x ± DP) of each variable of tilapia semen sperm morphopathology over 12 months. There was no statistically significant difference in the data obtained from normal sperm. Nevertheless, the highest average of normal sperm was in February (46.5%) and the lowest average in September (15%).

The spermatic abnormalities were divided into the strongly coiled tail, strongly folded tail, dislocated tail, isolated head, degenerate head, giant head and degenerate intermediate part. Regarding the degenerated head abnormality, May presented a statistically significant difference compared to the other months, presenting the highest average (26%). In August there was the largest presence of isolated heads (9.8%), while in November it had the lowest average (1.5%). For sperm with a giant head, July had the highest average (7.8%) while June and February had the lowest averages (0%).

Table 1. Mean values of temperature, pH and dissolved O_2 during 12 months.

Month / Year	Temperature (°C)	pH	Dissolved O_2 (mg L^{-1})
Oct/14	25	9.4	21.5
Nov/14	23.4	8.9	9.5
Dec/14	25	4.5	7.8
Jan/15	23.5	...	7.0
Feb/15	20.5	7.6	7.5
Mar/15	26.1	6.9	7.3
Apr/15	26	7.6	7
May/15	24	6.6	7.1
Jun/15	19.3	6.6	...
Jul/15	26	6.3	...
Aug/15	27	6.1	...
Sep/15	27	7	...

Table 2. Sperm morphopathology of Thai Tilapia (*Oreochromis* sp.) Semen. The values of deformities are expressed as a percentage.

Month / Year	Normal	Tightly coiled tail	Strongly bent tail	Offset tail	Isolated head	Degenerated head	Giant head	Intermediate part degenerated
Oct/14	35.32±21.54	37.50±21.55	9.83±3.98	1.64±2.39	3.64±3.49	1.82±1.37	2.50±2.61	4.00±3.37
Nov/14	45.60±17.54	13.20±14.25	16.90±8.07	1.90±3.07	1.50±2.84	2.50±3.57	2.80±3.12	2.80±3.12
Dec/14	43.32±6.25	12.66±7.51	3.32±2.69	6.82±5.60	8.00±5.79	6.14±2.89	3.32±3.25	5.50±1.81
Jan/15	28.00±9.48	13.60±9.39	7.72±3.66	9.50±5.09	5.00±1.94	5.22±3.51	1.22±0.73	7.72±2.88
Feb/15	46.50±4.95	4.50±1.18	5.50±0.24	8.00±2.83	5.00±1.41	4.50±1.65	0.00±0.00	16.50±0.24
Mar/15	25.00±4.55	16.00±10.47	5.00±3.89	2.00±0.82	5.25±2.18	6.72±3.57	1.00±0.47	36.72±11.49
Apr/15	34.00±0.47	30.00±0.47	6.00±0.47	4.00±0.47	5.00±0.94	3.50±0.71	0.00±0.00	18.50±0.71
May/15	24.31±4.63	16.33±5.17	5.62±0.72	4.00±0.94	3.62±1.36	26.00±1.89	3.00±1.70	3.00±0.00
Jun/15	30.00±0.00	13.00±0.00	25.00±0.00	1.00±0.00	3.00±0.00	4.00±0.00	0.00±0.00	17.00±0.00
Jul/15	30.80±3.81	3.40±1.53	16.80±6.49	1.60±0.89	8.00±2.87	7.20±0.56	7.80±2.64	20.60±4.96
Aug/15	16.80±3.38	3.00±1.83	13.40±5.00	0.20±0.30	9.80±2.64	5.20±2.18	6.60±2.34	44.40±9.61
Sep/15	15.00±3.13	5.00±3.56	16.25±6.02	0.00±0.00	9.50±1.67	20±1.63	4.50±1.11	46.72±2.18

As for tail abnormalities, October obtained the highest average (37.5%) of strongly curled tail and August obtained the lowest average (3%). In November there was a higher average (16.9%) of the bent tail, while the lowest average was in December (3.32%). Displaced tail pathology was highlighted in January (9.5%) and presented the lowest average in September (0%).

Regarding the abnormality in the intermediate piece, its degeneration was more frequent in December, reaching an average of 46.72% and less frequent in November, reaching an average of 2.8% (Table 2).

In Figure 1 are represented images obtained by an optical microscope with 100x objective of some of the strains found in the sperm of Tilapia.

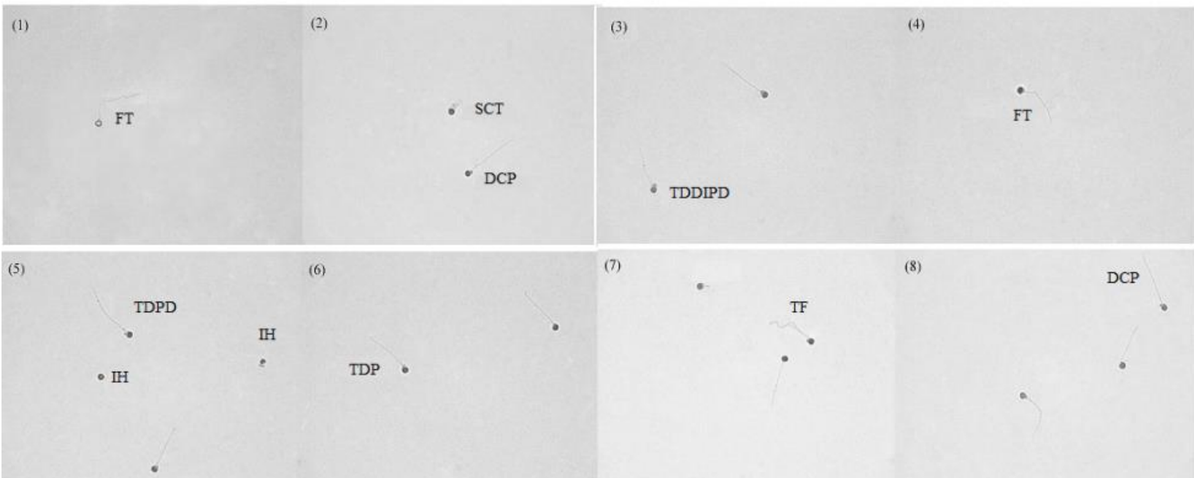

Figure 1. Tilapia semen observed under an optical microscope with a 100X objective showing major and minor pathologies.(1) Sperm with fractured tail (FT); (2) strongly coiled Tail (SCT) and degenerated connecting piece (DCP); (3) with Tail drop distal and intermediate piece degenerate (TDDIPD); (4) Fractured Tail (FT); (5) with Tail drop proximal and distal (TDPD) and isolated head (IH); (6) with Tail drop proximal (TDP); (7) the tail folded (TF); (8) degenerated connecting piece (DCP).

Table 3 presents the sperm characteristics in the course of 12 months. In relation to motility rate, there was no significant difference between the months. As the duration of motility, November was statistically superior to other months, reaching an average of 300 seconds. The vigor also did not showed a significant difference.

Table 3. Mean values of sperm characteristics of Thai tilapia (*Oreochromis* sp.)

Month / Year	Motility Rate (%)	Motility Duration (s)	Vigor (s)
Oct/14	68.75±14.06 [a]	233.29±79.75 [abc]	3.62±0.47 [b]
Nov/14	72.27±12.48 [a]	303.82±47.80 [a]	3.63±0.46 [b]
Dec/14	59.30±17.96 [a]	273.57±98.65 [abc]	4.00±0.41 [b]
Jan/15	66.25±3.75 [a]	301.25±61.87 [ab]	2.75±0.37 [b]
Feb/15	65.00±5.00 [a]	8.00±2.00 [c]	3.00±0.00 [b]
Mar/15	70.00±15.00 [a]	285.00±75.00 [abc]	3.50±0.50 [b]
Apr/15	66.42±13.06 [a]	237.14±67.80 [abc]	3.28±0.41 [b]
May/15	67.14±8.98 [a]	233.86±32.73 [abc]	3.14±0.24 [b]
Jun/15	80.00±0.00 [a]	300.00±0.00 [abc]	3.00±0.00 [b]
Jul/15	72.50±9.17 [a]	142.00±68.00 [bc]	3.33±0.44 [b]
Aug/15	70.00±10.00 [a]	103.67±47.22 [c]	3.16±0.55 [b]
Sep/15	69.19±61.11 [a]	90.50±29,50 [c]	3.50±0.50 [b]

Discussion

The motility rate in this study showed effective results. Kime et al. (2001) state that to predict the effectiveness of sperm quality it is necessary to evaluate parameters related to fertilization, as well as motility rate, motility duration and vigor. The distance to be traveled by the sperm to reach the female's micropyle depends on the vigor and duration of motility (Suquet, Billard, Cosson, Normant, & Fauvel 1995), as the opening of the micropile lasts only 10 to 20 seconds (Cosson, 2004). The data found in all seasons presented values that emphasize the good capacity of fertilization, since they were longer than the time verified for the micropile opening. Joining with vigor values it guarantees the fertilizations success. Navarro, Lemos, and Ribeiro (2019) observed that the quality of cachara semen showed higher motility rates in December, January, and February.

According to Rebouças, Lima, Dias, and Barbosa Filho (2014), water quality is one of the most important factors in fish farming and determines the success or failure of a farm.

Fish are pecilothermals animals, therefore, the temperature of the environment has great influence in the body of these animals. For tilapias, the temperature is the abiotic factor more impactful in sexual maturation (Baldisserotto, 2002). Changes in the temperature of the environment may speed up or slow down the maturation of gametes due to alteration of the normal functioning of the endocrine function (Pankhurst & King, 2010). In addition, critical values of the temperature can change the effectiveness of the fish, for example, in winter, hunger imposes constraints on the body size (Fry, 1971). The tilapias feature thermal comfort between 28 to 31°C (Baldisserotto, 2002). However, there was no correlation between temperatures below 27°C in reducing growth and appetites of tilapias. The lowest temperature was during the month of June, with 19.3°C and the average length and weight of the tilapias were, respectively,788.31 g and 35.96 cm.

The pH measures the acidity of water and is associated with an equilibrium related to the concentrations of carbon dioxide (fish respiration product), carbonate and bicarbonate dissolved in water (Baldisserotto, 2002). According to Egna and Boyd (1997), the ideal range of fish is between 7.0 and 8.5, but satisfactory development in ranges between 6.5 and 9.5 is also allowed. In this context, it is noted that the pH value during these 12 months was acceptable, except for December, which obtained the value of 4.5. Values below 5.0 are fatal to most fish (Egna & Boyd, 1997).

Oxygen is one of the essential factors for aquaculture, as it often has low water solubility and can become a limiting factor for aquatic life (Lourenço et al., 1999). For Ostrensky and Boeger (1998), in relation to dissolved oxygen, the ideal condition for most fish species grown in Brazil is 4 to 6 mg of oxygen per liter of water, while values between 0 and 1 mg are lethal.

The integrity of sperm structures (head, intermediate part and tail) are essential for good sperm performance (Kavamoto, Barnabe, Campos, & Andrade-Talmelli,), as morphological abnormalities in semen may reduce fertilization (Cosson, Dreanno, Billard, Suquet, & Cibert, 1999). Milliorini et al., (2011) believes that the critical percentage of types of sperm abnormalities of external fertilization fish, as well as tilapia, should be around 50%.

Regarding head abnormality, including all types, May was the month that presented a higher average of the degenerated head (26%). As for tail abnormalities, October was the highest percentage (37.5%), with sperm with a tightly coiled tail. Regarding abnormalities in intermediation, May presented a higher average (46.72%). Morphological abnormalities of the intermediate part and tail cause changes in motility, increasing the number of sperm with circular or circular motions and, consequently, decreasing the fertilization rate (Kavamoto et al., 1999). However, all these values are below the average oscillation of the critical percentage of morphological abnormalities observed by Miliorini et al. (2011), that is, they have a good fertile capacity.

Conclusion

Even in different seasons, in the Brazilian Cerrado, the environmental variables did not show great influences on semen quality of Tilapia, since, during the 12 months the sperm of tilapia (*Oreochromis* sp.) have a good ability to fertilization, performing below the average of the percentage of critical abnormalities and acceptable values of motility rate, duration of motility and vigor.

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