Does semen quality of *Colossoma macropomum* change the productivity of larvae during the reproductive period?

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Abstract: This study aimed to analyze the sperm quality of *Colossoma macropomum*, during the reproductive period. A total of 23 males of *C. macropomum* in the breeding season were used. Male gametes were collected after the hormone induction protocol using volumetric syringes for quantitative and qualitative analyses throughout the reproductive season, the fish were captured every 15 days. The following parameters were evaluated: volume, motility rate, motility time, sperm concentration, sperm morphology, fertilization rate, and hatching rate. There was no effect of period within season for semen volume, motility time and sperm concentration. For motility rate a quadratic effect was observed between collection periods. As for sperm morphology, there were differences ($p< 0.05$) in the probability of occurrences of normal spermatozoa, primary and secondary abnormalities as a function of collection period. For the fertilization rate a quadratic effect was verified and the hatching rate declined linearly throughout the reproductive period. Changes in the qualitative parameters of *C. macropomum* semen during the reproductive period were observed. In terms of the sperm quality of *C. macropomum*, the aging process of the spermatozoa is evident and consequently interferes in the fertilization and hatching rates, being more accentuated in the last month of the reproductive season.

1. Introduction

Among the groups of species cultivated in Brazil, the Characiformes stand out because of the texture and flavor of their meat and the good carcass yield. The tambaqui *Colossoma macropomum* is the most cultivated endemic species in Brazil (Lima *et al*., 2020). Today the species is the main one in commercial importance in the Amazon state (PeixeBR, 2021). The fingerlings are obtained through artificial reproduction by hormonal application, characteristic of rheophilic fish with reproduction occurring in the months (From September to March) between spring and summer (Vieira *et al*., 1999).

The use of gametes with a good sperm quality-quantitative index of fish broodstock is strategic to ensure the production of "quality" fingerlings for aquaculture (Bromage and Roberts, 1995). According to Beirão *et al*. (2009) sperm quality may be related to inefficient reproduction and propagation in some fish species. In commercial culture, there is much doubt regarding the quality and quantity of semen, which may be interfering with the fertilizing capacity in the process of artificial reproduction, consequently impacting the production of fish fingerlings (Rurangwa *et al*., 2004).

Studies have shown that there may be changes in gamete quality of fish in captivity (Murgas *et al.*, 2012). Streit Jr. et al. (2008) found high rates of sperm pathology in gilthead sea bream (*Salminus maxillosus*) semen quality parameters in captivity. Egger *et al.* (2021) evaluating seminal parameters of *Prochilodus lineatus* between the November and March, observed higher sperm concentration in November, better motility rate in November, January and February, and longer duration of motility time in January. However, changes in semen quality characteristics may occur during the reproductive period (Fauvel *et al.*, 1999). Stress in captive fish can have a negative effect on reproductive function and gamete quality (Papadaki *et al*., 2008), as has been demonstrated for *Hippoglossus hippoglossus* (Babiak *et al*., 2006) and *Mastacembelus mastacembelus* (Sahinoz *et al*., 2007). Therefore, the objective of the study was to identify the best reproductive period of captive-bred *C. macropomum* through semen quality analysis.

2. Materials and methods

2.1 Place of experiment

The study was conducted in a commercial fish farm located in Rondônia, Brazil (11°41'46 .95 "S and 61°13'47 .50 "O). Air temperature and rainfall index data were collected from the Cacoal-CPTEC weather station (latitude -11.48 and longitude -61.37).

The average, maximum and minimum temperature and rainfall index data collected during the year during the breeding season are shown in Figure 1.

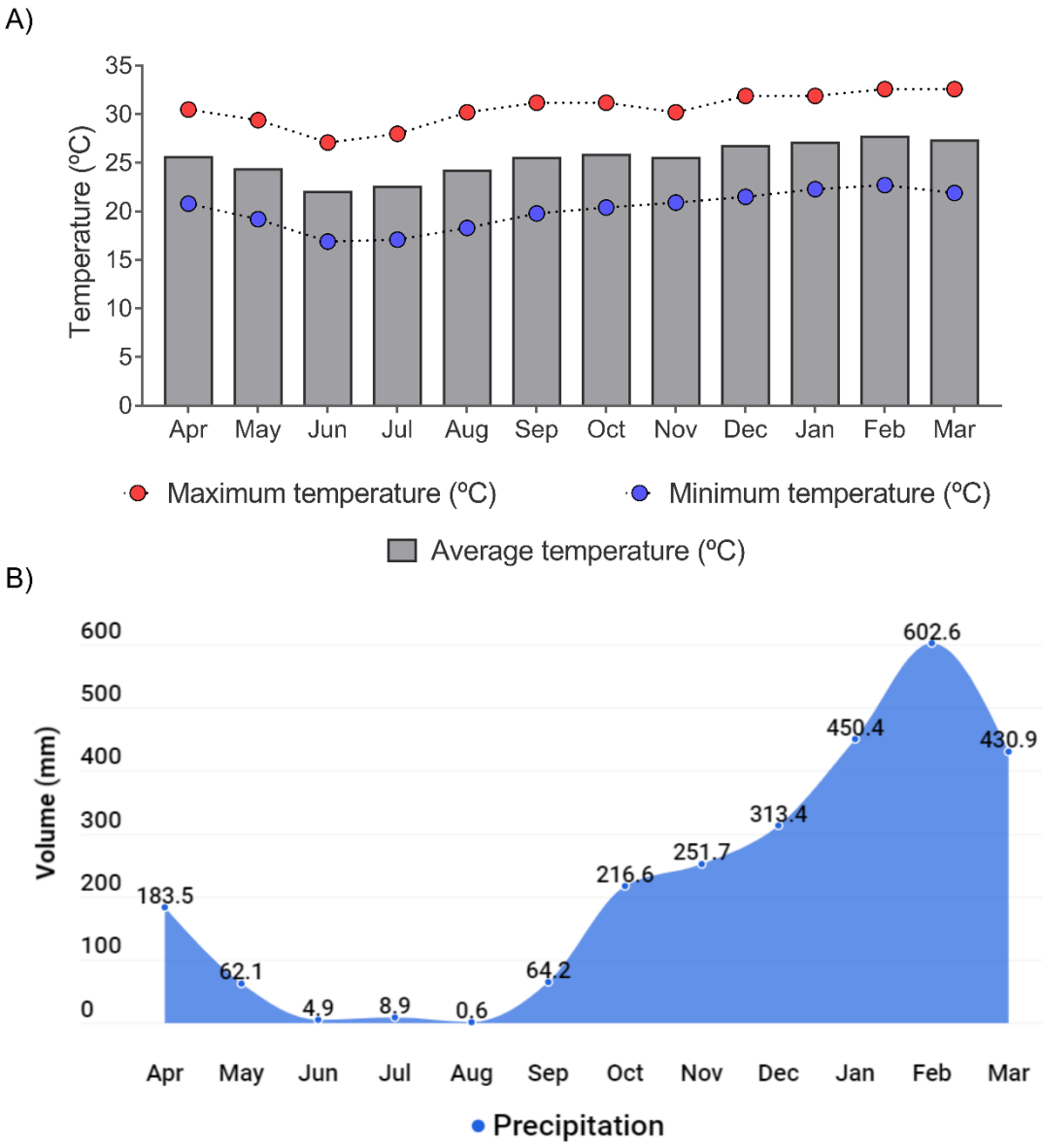

Figure 1. Monthly variation of precipitation (mm) and air temperature (ºC) obtained from the Cacoal-CPTEC weather station (latitude -11.48 and longitude -61.37 (Source: http://www.agritempo.gov.br/agroclima/pesquisaWeb).

2.2 Animals and experimental design

The experiment was conducted, from a population of 80 broodstock, aged five years and with an average weight of (7.4 ±1.5 kg). The broodstock were stocked in two earthen ponds of 2,000m^2 (forty broodstock in each pond. The fish were fed once a day throughout the year (adjusted to 1% of body weight each month) a commercial diet with

36% crude protein and 2900 kcal of digestible energy. The water quality parameters, average temperature (28±1ºC) and dissolved oxygen (6 mg/L), were measured daily. The average monthly rainfall was 338.5 mm during the experimental period (90 days), The average photoperiod at the experiment site was in November 13.1, December 13.6, and January 13.2 hours of daylight (Source: https://pt.climate-data.org/america-do-sul/brasil/rondonia/cacoal-31797/#climate-table).

During the historical reproductive period of the species in the study region (early November to late January), a total of 23 males were selected from five samples with an interval of fifteen days between them. The selected animals had secondary reproductive characteristics of migratory fish, a semen release with a slight compression on the abdomen in the craniocaudal direction. All broodstock selected had microchips (AnimalTAG®) inserted below the dorsal fin, thus allowing individualized monitoring throughout the experiment. For sampling, four animals were selected in the first (7.8 ±1.3 kg), seven in the second (7.2 ±1.5 kg), four in the third (6.3 ±0.1 kg), four in the fourth (8.9 ±2.5 kg) and finally four animals in the fifth and last sampling (6.6 ±0.5 kg). After each sampling, the reproducers were submitted to hormonal induction with 2.5 mg/Kg of body weight, in a single dose of carp pituitary extract (Zaniboni-Filho and Weingartne, 2007). Semen was collected from each animal after 215 hours/degrees 28 ± 1°C (Souza et al., 2018).

2.3 Evaluation of fresh sperm

After abdominal massage, the semen released from each individual fish was collected in 10 mL syringes by suction near the urogenital orifice (Billard et al., 1995) and the Seminal Volume (ml) was measured. Seminal samples were analyzed according to the quali-quantitative parameters described below.

Immediately after collection, each semen sample was subjectively evaluated to check a possible previous activation of the spermatozoa by contaminants or water. A sample of activated sperm (2 µL) was obtained by diluting 20 µL of sperm in 400 µL of distilled water and placed on an optical microscope slide. It was then covered with a cover slip and immediately evaluated for sperm motility rate under a light microscope (Nikon® E200, Tokyo, Japan) at X 400 amplification, and scored from 0 to 100%. For motility time (seconds), a stopwatch was started when sperm motility started, marking the time elapsed until the last sperm stopped moving in the optical field at X 400 amplification.

For the sperm concentration and morphology, the samples of each male were fixed in 10% buffered formaldehyde solution at a 1:1000 dilution (1 µL sperm: 999 µL formaldehyde solution). An aliquot (10 µL) of diluted sperm was pipetted into each counting field of a *Neubauer* hemocytometer chamber (Olen®, Kasvi, São José dos Pinhais, Brazil) covered by a coverslip, a waiting period of 15 min for cells to stabilize. Using a microscope at X 400 amplification (Nikon® E200, Tokyo, Japan) and a manual counter, the gametes were quantified by counting 10 squares. After cell counting, the sperm concentration was calculated using the following equation (Sanches *et al.*, 2011):

$$Spermatozoa\ mL^{-1} = \left(\frac{\sum SPTZ}{10\ s.c}\right) x \left(\frac{25\ t.s\ x\ dilution\ x\ 1000}{chamber\ depth\ (mm)}\right)$$

Spermatozoa mL^{-1}: Number of spermatozoa per milliliter of sperm

∑SPTZ: Total number of spermatozoa counted

10 s.c: Squares counted

25 t.s: Total squares

Dilution: Factor of dilution of the sperm by the fixative.

Chamber depth: Normality 0.1 mm

For evaluation of sperm morphology the samples previously were diluted in Bengal Rose dye (4%) (Merk®, Darmstadt, Germany) at a dilution of 1:10 in a plastic tube (1.5 mL). Smears made with 20 µL of stained sperm were evaluated under an optical microscope X 1000 amplification (Nikon® E200, Tokyo, Japan) (Streit Jr. *et al.*, 2004). Spermatozoa (n=200) were evaluated in each sample and the number of normal and abnormal cells was expressed as a percentage. Sperm morphologies were classified according to Bloom (1973) and Barth and Oko (1989) in Primaries: degenerate head, broken tail, curled and degenerate; and Secondary: folded tail, microcephaly and macrocephaly, cytoplasmic drops, in addition to a loose head and tail.

2.4 Fertilization and Hatch Rate

To evaluate the fecundation capacity of spermatozoa during the reproductive season, three females of *C. macropomum* were induced in each of the five male samplings performed. Thus, three females were matched with 4; 7; 4; 4 and 4 males (1; 2; 3; 4and 5 weeks, respectively). The females were induced intramuscularly with carp pituitary extract, 5.5 mg CPE/Kg of body weight divided into two fractions, 10% of the total dosage at the first application and the remainder (90%) 12 hours after the initial application (Souza *et al*., 2018). Oocyte extrusion occurred after 9 hours (28 ± 1°C) and a six gram sample of the oocyte mass from each animal was fertilized by each male semen

sample, a volume of 0.1 mL of semen from each animal was standardized. Then the eggs were laid in 60-liter incubators (individualized for the eggs from each male). After the closure of the blastula (six hours of incubation at 28 ± 1°C), the fertilization rate was estimated from three counts of three samples of 100 eggs, evaluating the viable and non-viable eggs. As for the hatching rate, after counting the fertilization rate, 100 viable embryos were transferred from each of the 60-liter incubators to smaller 3-liter incubators. After five hours, in these 3 L incubators (28 ± 1°C), all hatched larvae from the initial sample of 100 embryos were counted. Then, the difference of hatched (after 14 degree-hours of incubation hatching occurred) and unhatched larvae resulted in the Hatching Rate and then the larvae were classified as: normal (regular movement); defective (no vigorous movement when moving or notochord deformity) and dead (larvae hatched but dead at the time of counting).

2.5 Scanning Electron Microscopy

For scanning electron microscopy used the technique performed by Moitra *et al.*, (1987), samples with a volume of 10 µL of "in natura" semen were fixed in 990 µL in 2.5% Glutaraldehyde solution, with 0.1M cacodylate buffer at pH 7.2 and refrigerated at 5ºC until the moment of dehydration (sample processing). The samples were then centrifuged at 10,000 rpm/3 minutes and washed with cacodylate buffer three times. Dehydration occurred in increasing alcohol series, going through concentrations of 50, 70, 80, 90, and 95%/10 minutes in each step and three baths in 100% alcohol/10 minutes in each exposure. The samples were fixed with L-polysin on coverslips and drying was achieved in a BAL-TEC CPD 030 Critical Point Dryer using liquid CO^2. The fragments containing the semen samples were mounted on aluminum metal bases (stubs) and then metallized with gold-palladium ions in Shimadzu IC-50 Ion Coater. In the electron microscopy procedures, the material was examined and photographed with a Superscan - Scanning Electron Microscope (Shimadzu SS-550)

2.6 Statistical Analysis

The data on seminal quality, fertilization and hatching, as well as the evaluation of the hatched larvae, were submitted to homogeneity (Levene's test) and normality (Shapiro Wilk and Kolmogorov Smirnov) analysis. For data that showed normal distribution, a one-way analysis of variance was applied, followed by Tukey's test. For the non-parametric data, a Kruskal Wallis analysis was applied, followed by Dunn's test.

Regression curves were fitted to describe the behavior of the variables as a function of the months. Before estimating the regression equations, data normality was tested. Thus, regression models were applied to the variables of seminal quality, fertilization evaluation and also on larval quality. Variables adjusted to the simple linear regression model and variables adjusted to the polynomial regression model (quadratic) were identified. The variables that will fit a regression model significantly ($p<0.05$) are shown in the results.

3. Results

3.1 Qualitative characteristics of C. macropomum semen throughout the reproductive season

No significant difference was observed between reproductive periods in the parameters of seminal volume ($p = 0.2177$) and sperm concentration ($p = 0.4936$) (Figure 2A and B). Motility rate was different ($p = 0.0165$) between reproductive periods, with the highest value observed in the second collection (95.71±3.45%), differing from the first collection (77.5 ± 6.5%) (Figure 2C). Similarly, motility time was different ($p = 0.0457$) between reproductive periods, with the longest time observed also in the second collection (64.86 ± 9.60s), which differed from the time observed in the first collection (49.75 ± 6.13s) (Figure 2D).

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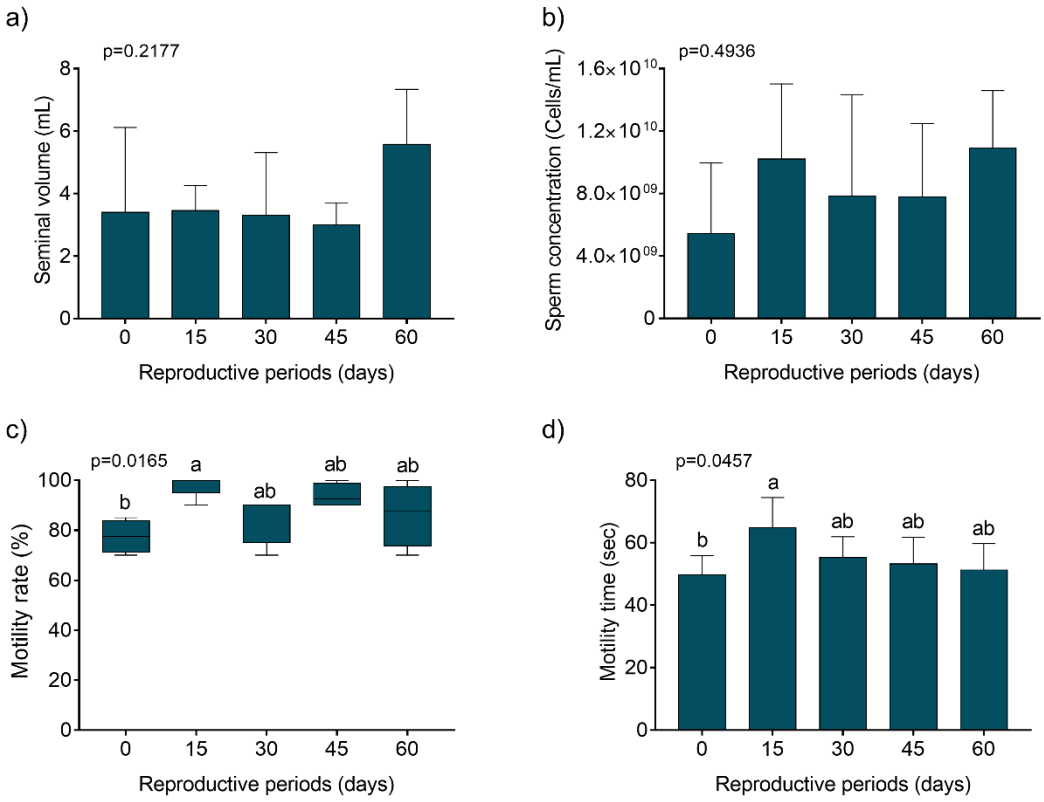
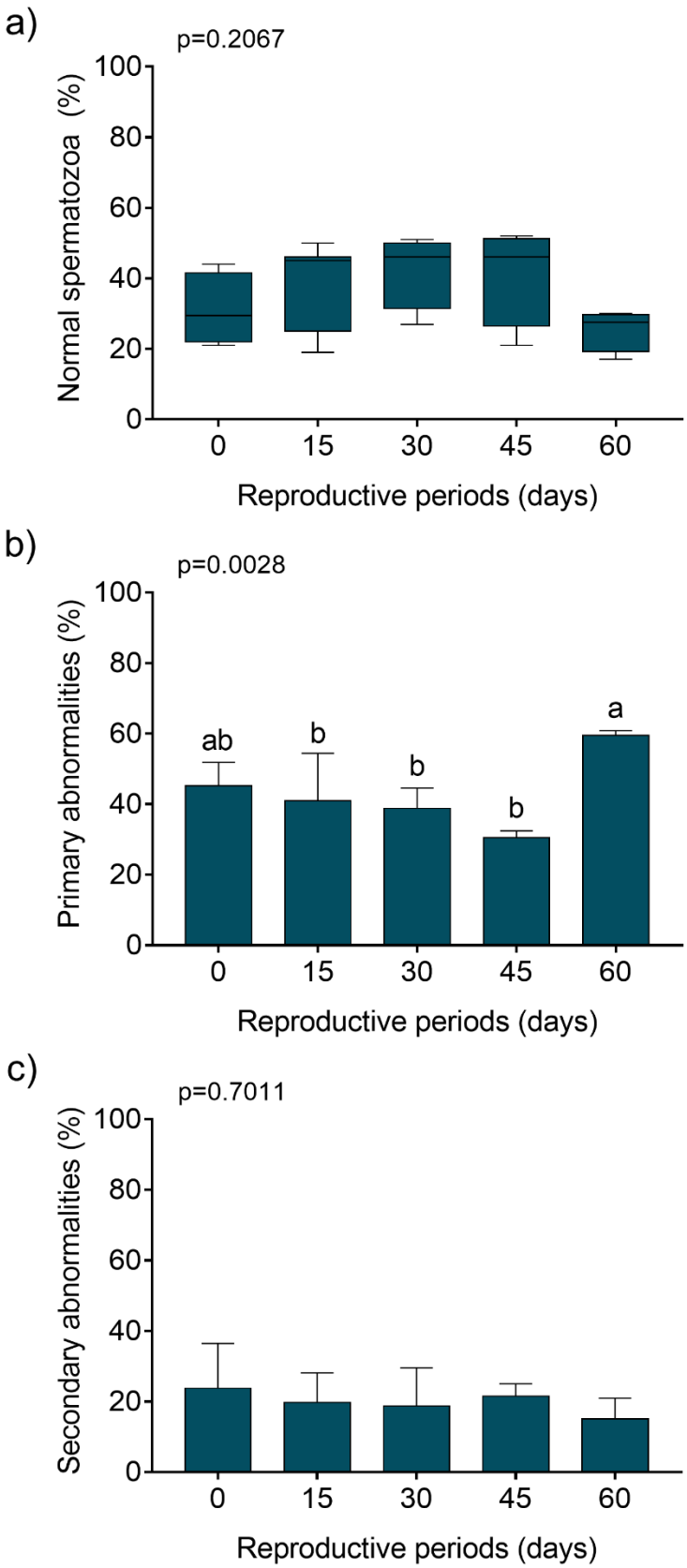


198 Figure 2. Sperm characteristics of *Colossoma macropomum* throughout the reproductive
199 period. a) seminal volume (mL); b) sperm concentration (Cells/mL); c) motility rate (%);
200 d) motility time (seconds). Different letters indicate significant difference by Dunn's test
201 (motility rate), and by Tukey's test (motility time).
202

203 The sperm morphology parameters of *C. macropomum* throughout the
204 reproductive period can be observed in Figure 3. The percentage of sperm with normal
205 morphology was not different between the reproductive periods (p = 0.2067), as well as
206 the percentage of secondary sperm abnormalities (p = 0.7011). The primary sperm
207 abnormalities, were different (p = 0.0028) between the reproductive periods, being the
208 last of the five collections the highest value observed (59.5 ± 1.29%), which was
209 statistically different from the second (40.86 ± 13.58%), third (38.75 ± 5.85%) and fourth
210 (30.50 ± 1.92%) collections.



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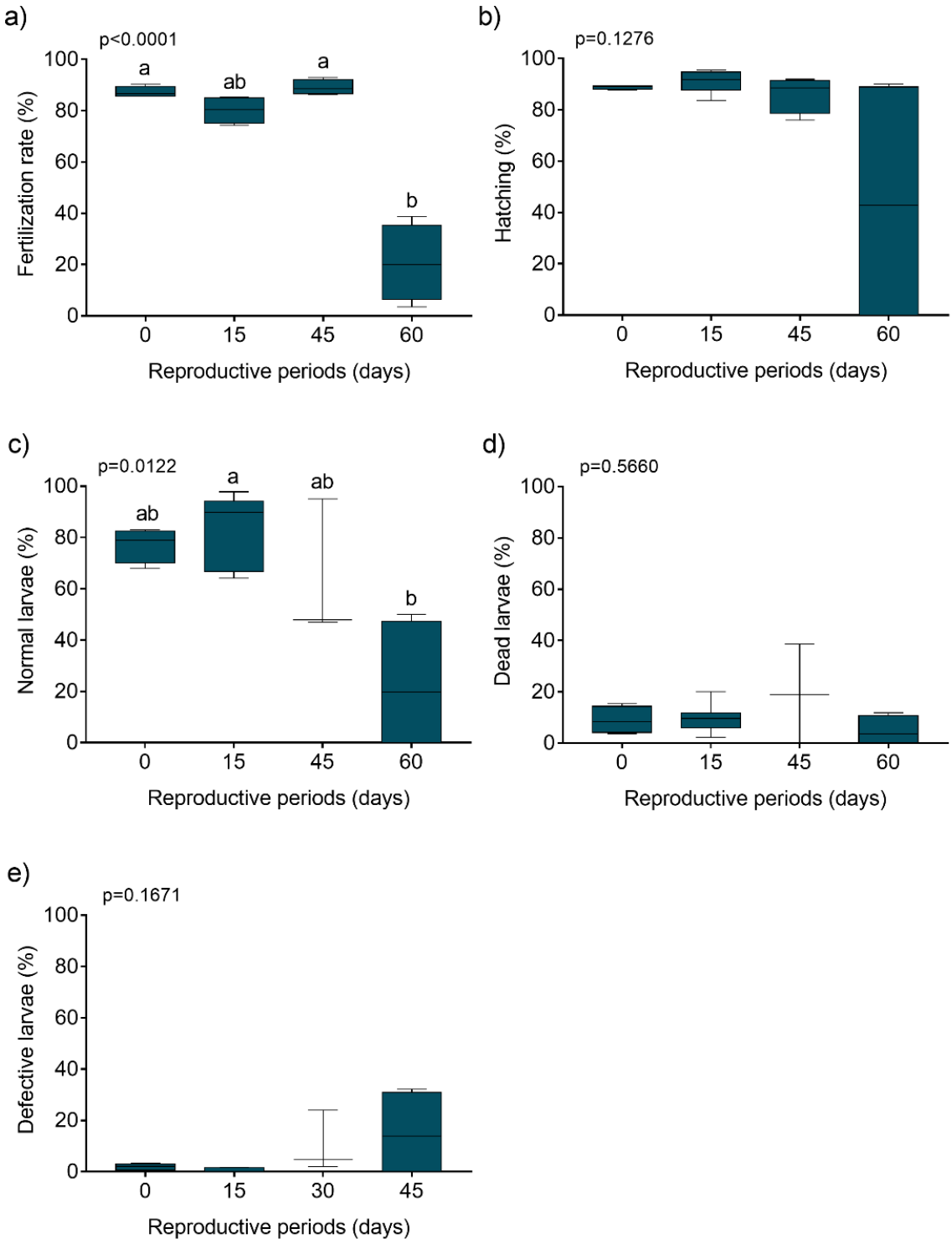
212 Figure 3. Sperm morphology of *Colossoma macropomum* throughout the reproductive
213 period. a) Sperm with normal morphology (%); b) Primary abnormalities (%); c)

Secondary abnormalities (%). Different letters indicate significant difference by Tukey test.

The results obtained for the reproductive parameters and larval quality can be seen in Figure 4. There was a difference between the reproductive periods for the variables fertilization rate ($p < 0.0001$) and larvae with normal morphology ($p = 0.0122$). In the fertilization rate there was a decrease in the last collection performed throughout the reproductive period (Figure 4A). Similarly, the percentage of larvae with normal morphology was also lower in the last reproduction performed during the reproductive period but differing only from the second reproduction (Figure 4C). There was no difference between the reproductive periods for the hatching rate ($p = 0.1276$), the percentage of dead larvae ($p = 0.5660$) and the percentage of defective larvae ($p = 0.1671$).



227 Figure 4. Reproductive parameters of *Colossoma macropomum* throughout the
228 reproductive period. a) fertilization rate (%); b) hatching rate (%); c) percentage of larvae
229 with normal morphology (%); d) percentage of dead larvae (%); e) percentage of defective
230 larvae (%). Different letters indicate significant difference by Dunn's test.

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3.2 Sperm Morphology

Spermatozoa of *C. macropomum* with morphological alterations coiled tail; folded, broken and loose head and tail were identified by scanning electron microscopy analysis during the reproductive period of the species (Figure 5).

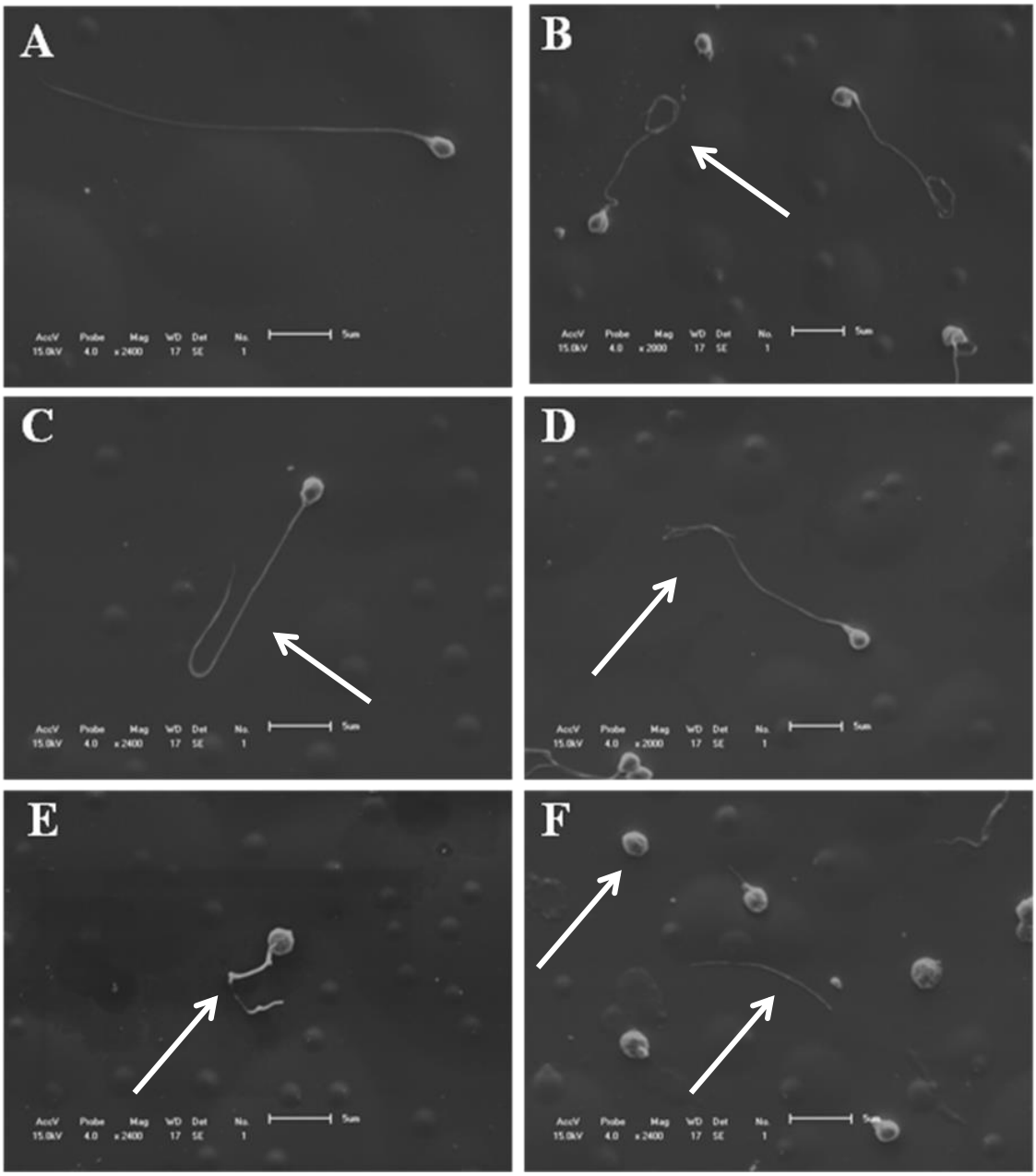

Figure 5. Illustrations of morphologies obtained by scanning electron microscopy of *Colossoma macropomum* spermatozoa throughout the reproductive season. (A) normal; (B) tail curled at the end; (C) tail folded; (D) tail folded at the end; (E) tail broken; (F) tail and head loose.

3.3. Regression models are fitted to describe the behavior of the variables as a function of the reproductive period

The regression models significantly adjusted the variables studied are shown in Figure 6. We observed a quadratic behavior of the motility rate variables (Figure 6a; r2 = 0.21; p = 0.0402), spermatozoa with normal morphology (Figure 6b; r2 = 0.27; p = 0.0161), spermatozoa with primary abnormalities (Figure 6c; r2 = 0.36; p = 0.0049), fertilization rate (Figure 6d; r2 = 0.56; p = 0.0240) and larvae with normal morphology (Figure 6f; r2 = 0.68; p = 0.0176). A negative linear behavior was observed for the hatching rate throughout the reproductive period (Figure 6e; r2 = 0.28; p = 0.0209), and for the percentage of defective larvae a positive linear behavior was observed (Figure 6g; r2 = 0.31; p = 0.0159).

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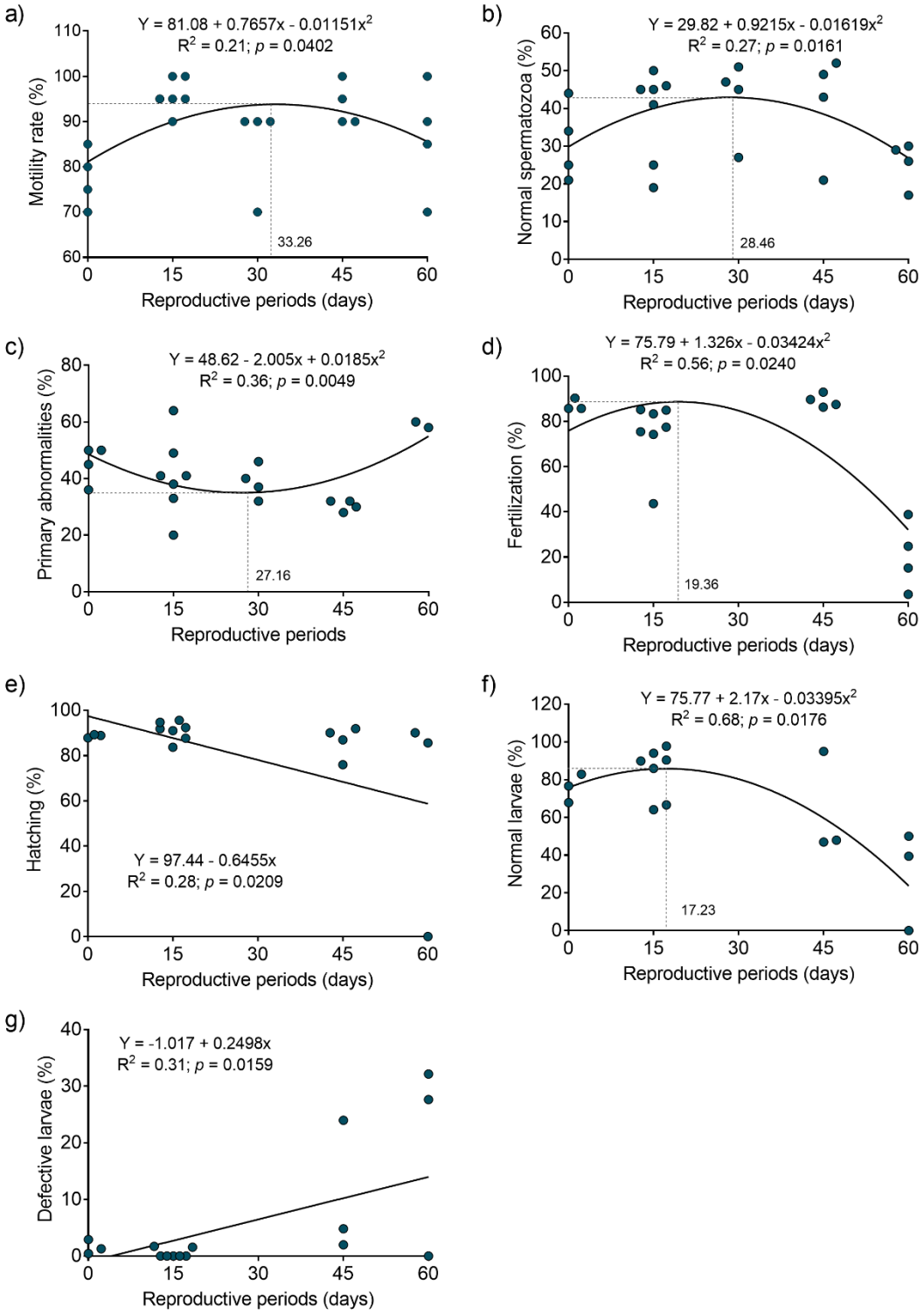

259 Figure. 6. Behavior of variables studied throughout the reproductive period of *Colossoma*
260 *macropomum*, adjusted by regression models.

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3.4. Pearson's correlation coefficients between the reproductive variables of Colossoma macropomum throughout the reproductive period

For sperm quality variables, we observed a positive correlation between motility rate and motility time (Figure 7a, r=0.50; p=0.0080); motility rate and sperm concentration (Figure 7b, r=0.37; p=0.0420); and the volume and presence of spermatozoa with primary abnormalities (Figure 7c, r=0.45; p=0.0150). On the other hand, we observed a negative correlation between the percentage of normal sperm and sperm with primary abnormalities (Figure 7d, r=-0.58; p=0.0020); the percentage of normal sperm and sperm with secondary abnormalities (Figure 7e, r=-0.42; p=0.0220); sperm concentration and sperm with secondary abnormalities (Figure 7f, r=-0.39; p=0.0300); and between spermatozoa with primary and secondary abnormalities (Figure 7g, r=-0.39; p=0.0350).

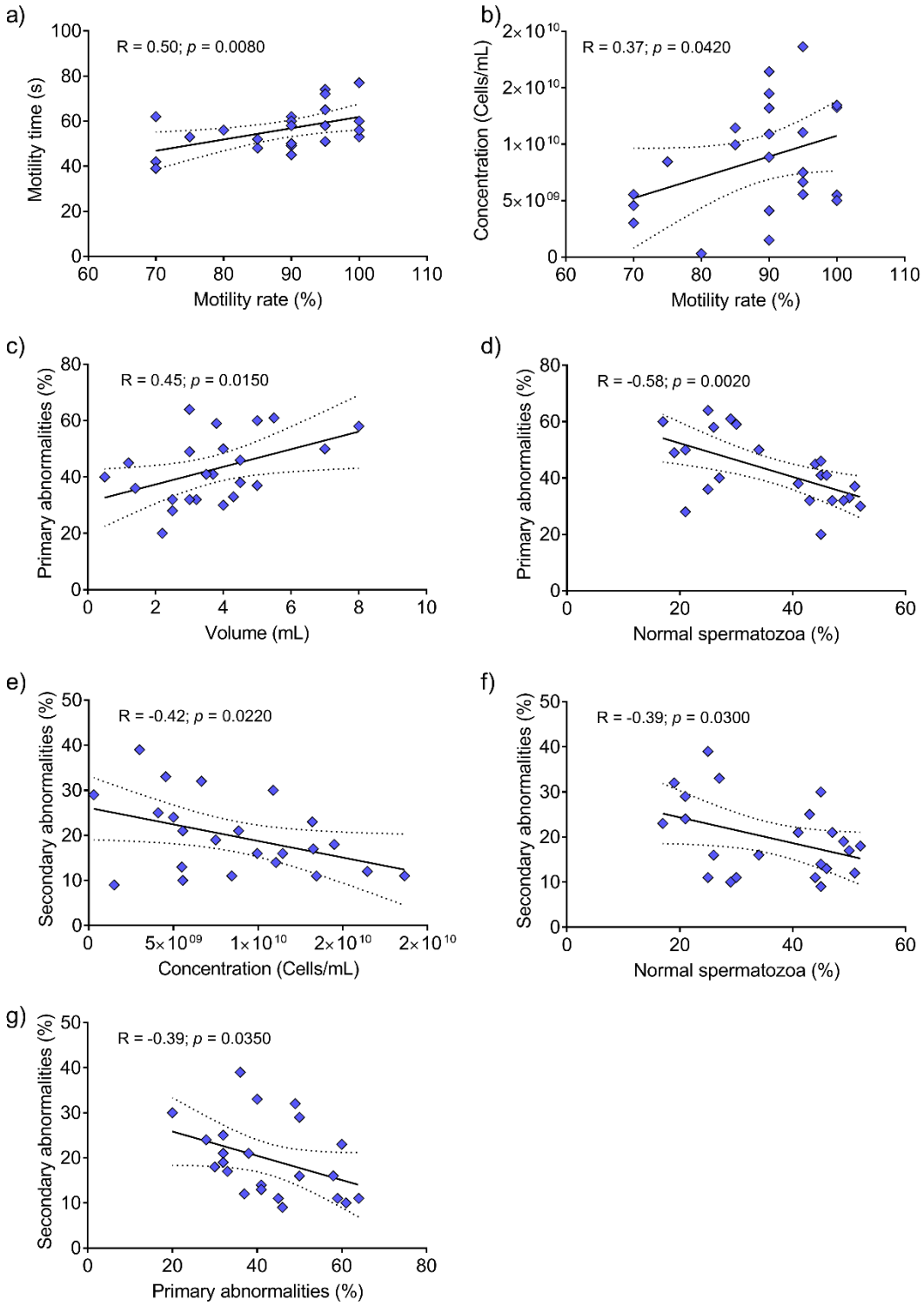

Figure 7. Significant Pearson's correlation coefficient between sperm quality variables of *Colossoma macropomum*.

When we evaluated the correlation between sperm quality variables and fertilization capacity/larvae quality (Figure 8), we observed a positive correlation only

between percentage of normal sperm and larval mortality (r=0.50; *p*=0.0160). Meanwhile, we observed a negative correlation between seminal volume and fertilization rate (r=-0.58; *p*=0.0040); the percentage of spermatozoa with primary abnormalities and fertilization rate (r=-0.65; *p*=0.0010); the percentage of spermatozoa with primary abnormalities and the hatching rate (r=-0.45; *p*=0.0270); seminal volume and percentage of larvae with normal morphology (r=-0.41; *p*=0.0470); and the percentage of spermatozoa with primary abnormalities and percentage of larvae with normal morphology (r=-0.44; *p*=0.0330).

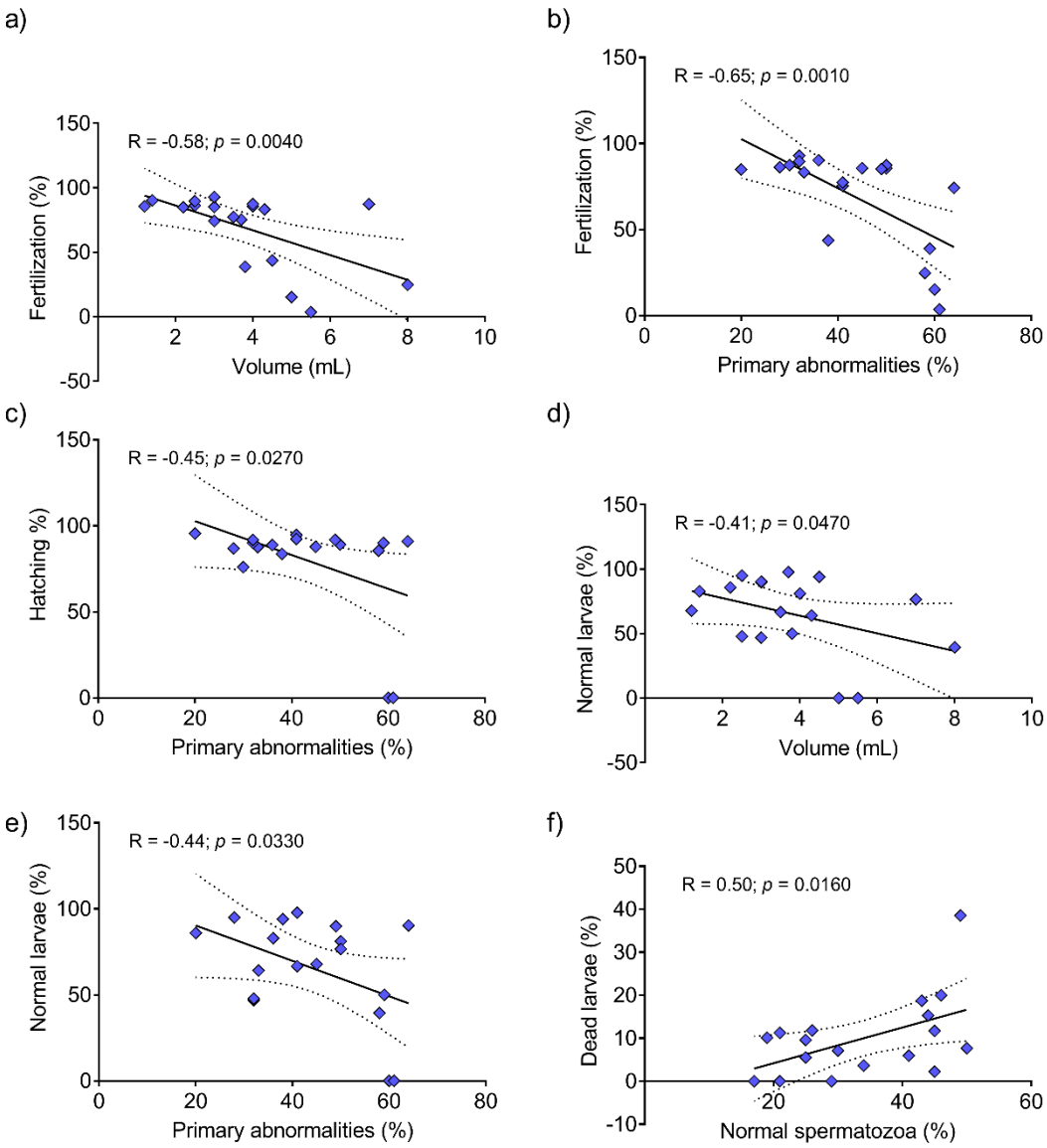

Figure 8. Significant Pearson's correlation coefficient between sperm quality variables, fertilization variables and larvae quality of *Colossoma macropomum*.

When we evaluated the correlation between variables related to fertilization capacity and larval quality (Figure 9), only significant positive correlations were observed. There was a positive correlation between fertilization and hatching rates (r=0.76; *p*=0.0001); fertilization rate and larvae with normal morphology (r=0.70; *p*=0.0010); hatching rate and larvae with normal morphology (r=0.80; *p*<0.0001); fertilization rate and percentage of dead larvae (r=0.42; *p*=0.0410); and hatching rate and percentage of dead larvae (r=0.40; *p*=0.0470).

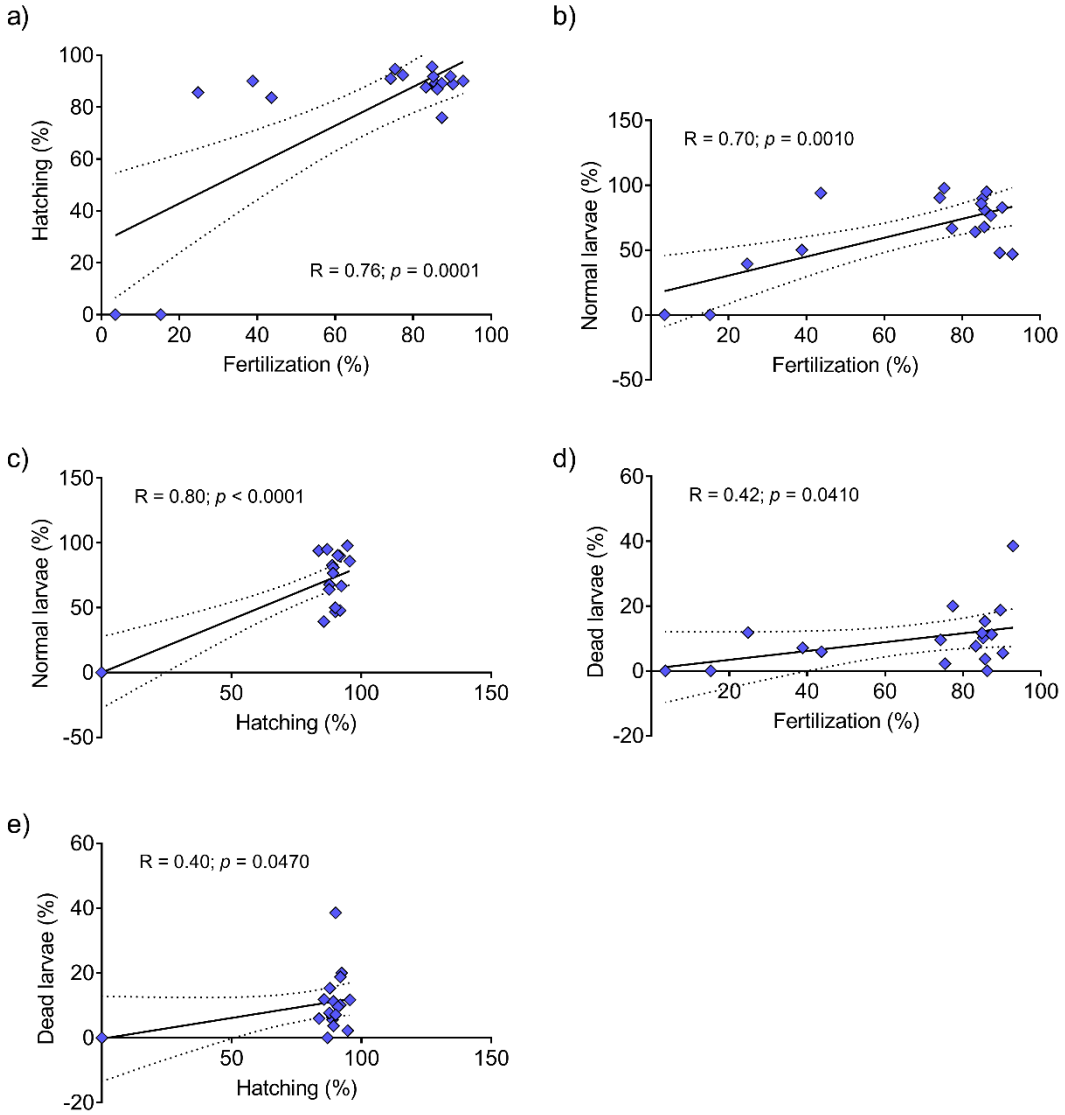

Figure 9. Significant Pearson's correlation coefficient between fertilization variables and larvae quality of *Colossoma macropomum*.

4. Discussion

Low viability of larvae at the end of the reproductive period of *C. macropomum*

was observed, this result may be more linked to the females that are normally in atresia at this stage, in the present study the previous evaluation of the germinal vesicle in the females was not carried out to verify that they had not started the regression process, however, the fact that a motility rate above 90% was observed in males, the low productivity of larvae at the end of the reproductive period must be more linked to the females. Thus, we cannot make a direct relationship between the reduction in the percentage of normal spermatozoa at the same time as the increase in sperm with primary pathologies and the decrease in fertilization and hatching rates, as well as in the production of normal larvae that was observed in the study, for this point of discussion it is suggested that further investigations evaluating the quality of the germinal vesicle of the females be carried out, to prove that there is no regression process. The variation in the values of qualitative and quantitative seminal parameters of *C. macropomum*, was expected, and with some exceptions at the beginning of the reproductive period, the best seminal quality was obtained in the middle of the reproductive period as it is possible to observe in the simulation of Figure 10, based on the hypothetical representation proposed by Babiak *et al.* (2006).

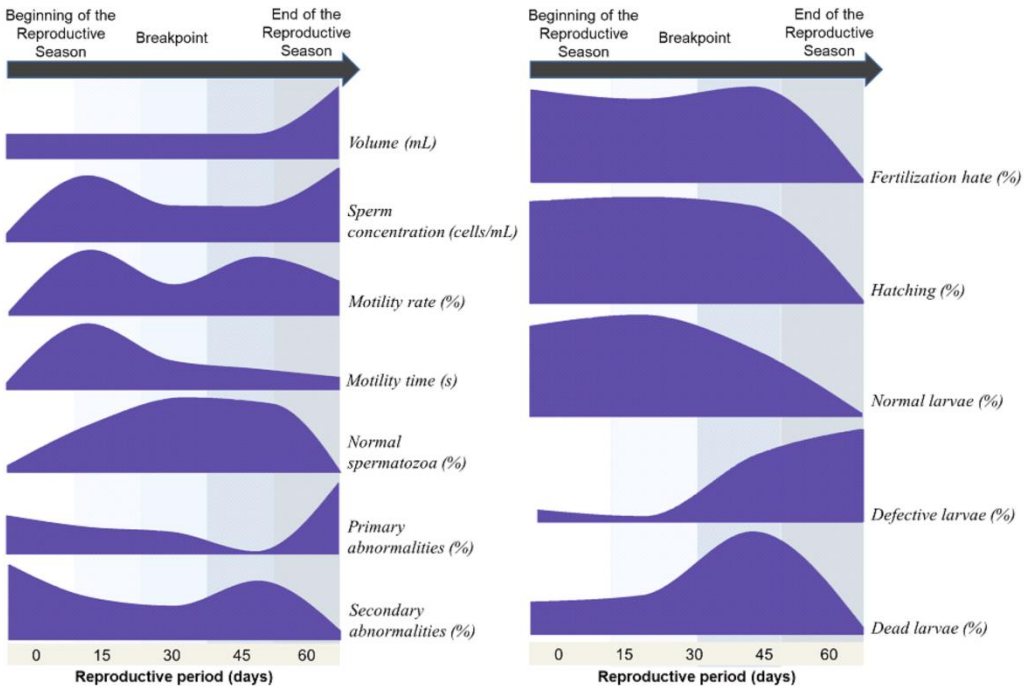

Figure. 10. Hypothetical representation, from the results obtained, of the variation in the qualitative and quantitative characteristics of *Colossoma macropomum* semen during the reproductive period of the species from November to January. Adapted from Babiak *et al*. (2006) with data obtained with *Colossoma macropomum* in this study.

4.1 Qualitative and quantitative semen parameters, fertilization rate, hatching and morphology of the larvae

In a classical model expected for the behavior of sperm motility rate during the reproductive cycle, by absolute values increase until a certain period and subsequently decreases, as observed Bondarenko *et al.* (2018) in *Esox lucius* L, and Nynca *et al.* (2012) in *Oncorhynchus mykiss*. The negative quadratic behavior was also observed in the present work, reaching its maximum point in the final third of the period, according to the suggested equation. If, on the one hand, at the end of the reproductive period, the progressive decrease in motility rate may be related to sperm deterioration, the lower rate at the beginning of the period is not clear. It may be related to the low concentration of circulating hormone for sperm maturation or even the low quantity of primary and secondary spermatids that give rise to spermatozoa. On the other hand, this fact was not clear in this study, because there was no variation in sperm concentration throughout the reproductive period.

In general, in fish, germ cells pass rapidly through spermiogenesis and meiotic phases, making the time required to produce a spermatozoon very fast (Nóbrega *et al*., 2009). Thus, in migratory species like *C. macropomum*, sperm remain for long periods stored inside the gonads, since they start being produced in the winter months (Grier and Taylor, 1998; Brown-Peterson *et al*., 2002; Batlouni *et al*., 2006) to be released only in the short spawning period. From this context, it is reasonable to assume that regardless of the management and the abiotic factors in which the animals are susceptible in fact, that sperm quality decreases mainly with respect to sperm stored longer in the gonads (Beirão *et al*., 2019). From the concept of germ cell recruitment, formation, storage and subsequent release of spermatozoa, the phenomena observed in the seminal parameters of *C. macropomum*, can be explained with greater logic. For, the coincidence of the negative quadratic behavior of sperm motility rate and the percentage of normal spermatozoa, contrary to the incidence of primary abnormalities (positive quadratic), there is a clear indication of sperm aging at the end of the reproductive season. This observation is documented by numerous authors, (Billard *et al*., 1997; Dreanno *et al*., 1999) for *D. labrax*; Mylonas *et al*. (2003) for *Pagrus pagrus* and Babiak *et al*., (2006) for *H. hippoglossus*, who further relate the morphological changes to biochemical changes that result in poor sperm quality in terms of quantitative parameters and fertilization capacity. The observation of Suquet *et al*. (1998) reinforces as to the result

of the aging process, which leads to deterioration in sperm morphology, including in the midpiece region, between the sperm head and tail.

Although motility rate has always been related as a preponderant factor for seminal quality, the results obtained with *C. macropomum*, reaffirm the idea of the prevalence of morphological changes as decisive, due to the "aging" of the spermatozoon and, therefore, will result in low motility rate. In this sense it is pertinent the observation of Babiak *et al.* (2006) about the aging of spermatozoa from *H. hippoglossus*, resulting in physical decomposition, observing loose tails, and destroyed heads, at the end of the reproductive season. The head region of *Scophthalmus maximus* spermatozoa also underwent the most morphological changes in aged spermatozoa, including chromatin condensation (Suquet *et al*., 1998). The same authors cite that the changes observed in semen quantitative parameters during the reproductive season may reflect the intensive outcome of the three processes involved within semen formation: spermiogenesis, hydration and cell decomposition. In addition, embryo survival reduces significantly, indicating that the aging processes of the sperm reduce the capacity for zygote formation. This fact was evidenced in this study, which observed a reduction in fertilization rate, hatching and percentage of normal larvae, along with an increase in deformed larvae.

The influence of seminal parameters, especially sperm morphology on fertilization rate was evidenced in this study, as well as observed by Galo *et al.*, (2019) for *P. mesopotamicus*. Although the seminal parameters did not show influence on the hatching rate, a strong correlation ($r2 = 0.5906$; $p = 0.0001$) between the rates (fertilization and hatching) was noted. A fact that confirms the observation of Varela Junior et al., (2012), that the fertilization and hatching rates of *C. macropomum* semen were highly correlated ($r = 0.87$; $p < 0.01$) in his study. The same author cites that to estimate the in vivo sperm quality of *C. macropomum* after thawing only one of these assessments is necessary. Rizzo *et al*. (2003) observed in *Prochilodus marggravii*, a negative correlation ($r = -0.82$) between fertilization rate and the percentage of deformed larvae, occurring during storage "in situ" ($26^{o}C$). Springate *et al*. (1984) observed a high correlation between fertility rate and the percentage of deformed larvae, which according to these authors may be sufficient to indicate the performance of embryos and larvae through the fertilization rate.

4.3 Influence of abiotic factors on the reproductive period

The reproductive process in fish depends on the interaction of endogenous (hormones) and exogenous factors, such as temperature, precipitation, photoperiod, water column level, among others (Rotili *et al*., 2021). Hardly a single abiotic factor influences

the reproductive physiology of fish due to the complexity of the reproductive process (Barbieri *et al.*, 2000). In King *et al*. (2016) study with different wild fish species, it was proven that it is necessary to consider that multiple abiotic factors in the process of gamete release during the reproductive period. The role of environmental factors in the synchronization of reproductive cycles, emphasizing the influence of photoperiod and water temperature in this process, was discussed by Bye, (1984). According to this author, fish living outside the tropics, present cycles, such that larvae and young are produced when environmental conditions are favorable for survival.

In this study, sperm motility rate behaved in correlation with temperature (ºC) and rainfall (mm) oscillations during the breeding season. According to Akhter *et al.* (2020) spawning seasons are variable and species-specific and could be related to variations in the water temperature, photoperiod, spawning grounds and water currents. This conjunction of abiotic factors is confirmed by Lopes *et al.* (2018) who attributed trigger for the initiation of the reproductive process in *Prochilodus costatus*, the rainfall, hydrological fluctuations, and lunar phase. Another species of Characidae, *Prochilodus argentus*, Boncompagni-Júnior *et al.* (2012) identified a positive correlation for males and females of the species between gonadossomatic indices with rainfall, turbidity, and water temperature. The same behavior was observed by Vazzoler *et al*. (1997) for dominant fishes in the upper Paraná River floodplain. According to Lowe-McConnell, (1975) teleosts from tropical and subtropical regions have a close relationship between reproductive period and rainy seasons, this was confirmed in the study by Zaniboni Filho *et al.* (2017) with *Salminus brasiliensis* in the Uruguay River basin.

Querol *et al.* (2004), investigating abiotic factors in the reproductive dynamics of Loricariichthys platymetopon, found a higher gonadosomatic index for males in the months of November and December, coinciding with the period of temperature elevation. In the present study, the gonadosomatic index was not evaluated, because the reproducers were from a private property, and sacrificing them was not possible. However, the qualitative and quantitative semen parameters were analyzed, observing better sperm motility rate and percentage of normal spermatozoa during the period of increasing temperature and rainfall index (December and January), coinciding with the same period of the best gonadossomatic index of L. platymetopon (Querol *et al.*, 2004) and S. maxillosus (Barbieri *et al.*, 2000). Querol *et al.* (2002), studying L. platymetopon and

Melo *et al.* (1995) with L. anus observed that, in general, the increasing temperature conditions are linked to the period of greater reproductive activity, directly influencing gonadal maturation. According to Barbieri *et al.* (2000) there is a huge and complex interaction between biological events among themselves and between these and environmental events, and there is a need for further research in this area of study.

5. Conclusion

The qualitative and quantitative parameters of the semen of *C. macropomum* suffer alterations during the reproductive period, which may influence the fertilization rates and consequently the hatching rates. The best rates of sperm parameters were observed in the first two months of the reproductive period, consequently the best time to perform the reproduction of the species.

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