Oocyte quality of tambaqui (*Colossoma macropomum*) during the reproductive season

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Abstract

The study aimed to analyze the *Colossoma macropomum* reproductive behavior and quality of the female gametes throughout the reproductive season. The experiment was carried out in Pimenta Bueno - Rondônia State (Northern Brazil) during the reproductive season (2010-2011) using 36 females. Each sampling was performed on a 15 ± 5 days interval. Female gametes were collected by stripping and the following analyses were performed: weight of oocytes released (g); productivity index, fertilization and hatching rate. During the sampling period was verified effect ($p < 0.05$) of collecting time into the season for oocytes weight, productivity index and fertilization rate. Although the period 3 (December) did not differ significantly from other periods, it showed better parameters for the quality of *C. macropomum* oocytes.

Keywords: Characidae, fertilization rate, oocytes, reproduction, reproductive period.

Qualidade ovocitária de tambaqui (*Colossoma macropomum*) ao longo da estação reprodutiva

Resumo

O estudo foi conduzido com o objetivo de analisar o comportamento reprodutivo da espécie *Colossoma macropomum*, quanto à qualidade de seus gametas femininos ao longo da estação reprodutiva. O experimento foi executado em Pimenta Bueno-Rondônia durante a estação reprodutiva do *C. macropomum*. Utilizaram 36 fêmeas durante a estação de 2010-2011. Cada coleta apresentou um intervalo de 15±5 dias. Através da extrusão foram coletados os gametas femininos e realizadas as seguintes análises ao longo da estação: peso de oócitos liberados (g); índice de produtividade; taxa de fertilização e eclosão. Durante a estação 2010-2011 foi verificado efeito ($p < 0{,}05$) de período (coleta) dentro da estação para peso de oócitos, índice de produtividade e taxa de fertilização. Apesar do período 3 (coleta – mês de dezembro) não ter diferenciado significativamente de alguns períodos, foi o que apresentou os melhores parâmetros estabelecidos para a qualidade dos oócitos de *C. macropomum*.

Palavras-chave: Characidae, taxa de fertilização, oócitos, reprodução, período reprodutivo.

1. Introduction

From the South American fish species farmed in Brazil *Colossoma macropomum* is characterized as one of the most important, due to its excellent zootechnical characteristics appropriate for farming, and also due to its great acceptance by the Brazilian consumer market (Urbinati and Gonçalves, 2005). It is an important professional and amateur fishing species, with great potential in farming fields and there is several scientific information available in literature. *C. macropomum* became the major species for cultivation in North and Mid-West of Brazil, being farmed in 24 of the 27 Brazilian states (Lopera-Barrero et al., 2011).

Using high quality gametes is extremely important to ensure production of viable descendents for aquaculture (Bromage and Roberts, 1995). The quality of oocytes and production of larvae in captivity are considered very important and limiting factor in fingerlings production (Kjørsvik et al., 1990).

Oocyte quality is closely related to future larvae potential (Kjørsvik et al., 1990) and depends on several factors that can often change during the reproductive cycle. Aspects such as the female endocrine status during oogenesis, quantity and quality of feed during the preparation time, physical and chemical water parameters, stress by handling and others are listed as determinants for success in production of viable larvae (Campbell et al., 1992; Bromage, 1995; Brooks et al., 1997; Christiansen and Torrissen, 1997; Carrillo et al., 2000).

Chambers and Waiwood (1996) studying *Gadus mohua*, reported that the oocytes size reduced during the reproductive season due to the low supply in diet and subsequent maternal sub- nutrition. In reproductive season, oocytes achieve a critical size specific for the species (Romagosa et al., 1988), and larvae size after yolk sac absorption is correlated to the oocytes size.

Studies on fertility are important for fishing research (Almatar and Bailey, 1989). Information regarding eggs number is used to estimate reproductive potential and correlate fish length to fecundity (Laine and Rajasilta, 1998). Differences observed in fish fecundity are assigned to genetic and environmental factors (Baxter, 1959; Burd and Howlett, 1974; Messieh, 1976; Kelly and Stevenson, 1985; Sinclair and Trembley, 1984). Bromage (1995) suggests that female nutritional management influences the production of viable larvae. Furthermore, several authors report that the food level for females affects oocytes numbers (Watanabe, 1985; Horwood et al., 1989; Kamler, 1992; Tyler and Sumpter, 1996) and also variations in egg size (Hettler, 1981; Hay et al., 1988). Winters et al. (1993) reported from the physical environment, the sea water temperature influences the size and average number of eggs produced by fish.

There is no information available regarding the oocytes quality parameters and larvae of native Brazilian fish in captivity and subsequent fertilization and hatching rates. This information becomes important to successful of fish commercial production with constant supply of high quality oocytes.

This study aimed to analyze the breeding behavior of *C. macropomum*, assessing the quality of oocytes during the reproductive season in order to determine the best time to perform reproduction in captivity.

2. Material and Methods

The project was carried out in a commercial fish farm located in Pimenta Bueno, Rondônia, Northern Brazil (11°41'46 0,95"S and 61°13'47.50" W) by the Research Groups: PeixeGen - Maringa State University (UEM) and *Aquam* - Federal University of Rio Grande do Sul (UFRGS), during the *C. macropomum* breeding season, from early November 2010 until the end of March 2011 (150 days).

Data of air temperature and pluviometric index (precipitation) were collected from the meteorological station - CPTEC, in Cacoal city - Rondônia, located near the property which study was conducted. Were used 36 females *C. macropomum* (9.3 ± 2.5 kg and 6 ± 2 years), during the 2010-2011 season, which represented 40% of the breeding herd of the fish farming.

Animals were maintained in six nurseries of 2,000 m^2, with an average water temperature of 28 ± 2°C and 6 mg/L of dissolved oxygen, fed with commercial feed 36% crude protein and 3,400 kcal DE / kg diet. They were fed three times a week with 1% of the biomass from August until the breeding season (October), when it was used the strategy of feeding twice a week with 1% of the biomass. After spawning, animals remained 15 days without food and returned to receive daily feeding with 3.5% biomass.

Animals were identified with transponders. Females were selected by secondary sexual characteristics in migratory fish, such as bulging and smooth abdomen, and reddish urogenital orifice. Afterwards, animals were weighed and submitted to intramuscular hormonal induction (extract of carp pituitary) between the dorsal fin and lateral line, being injected 5.5 mg/kg of body weight, applying 10% at the first administration and the remaining dosage 12 hours later.

Fish were maintained in two management tanks containing 3000 L (maximum of four animals in each one) with 70 cm water column, in constant flux under controlled temperature (28 ± 2 °C) and dissolved oxygen (6 mg/L).

The interval of each sampling was 15 ± 5 days. Being the period (1) - early November, (2) - end of November; (3) - mid December, (4) - early January; (5) - end of January; (6) - early February; (7) - end of February; (8) - early March and (9) - end of March.

Female gametes were collected by stripping (Woynarovich and Horváth, 1980) and the following analyzes were performed along breeding season:

Weight of released oocytes (g) - after each spawning, oocytes were weighed on analytical balance.

Productivity index - quantity of released oocytes (g) divided by animal weight, multiplied by 100 (IP = oocytes (g) / animal weight (g) * 100).

A 60 mL oocytes aliquot from each female was immediately fertilized with 150 µL semen from one male in reproductive period. Afterwards, each aliquot was placed into 60 L incubators to complete the embryonic development. After six hours of incubation (28°C ±1) it was determined the fertilization rate by counting 100 eggs from three aliquots of each incubator. Number of viable embryos and failed eggs was determined.

One hundred embryos were transferred to 3 L incubators, independently for each female. Twelve hours post fertilization (28 ± 1°C) was assessed the hatching rate. Number of hatched larvae and failed eggs was obtained by counting three aliquots of each incubator.

It was used the computer software Statistica© 7.0 to describe the database parameters: oocytes weight, productivity index, fertilization rate and hatching rate. It was also analyzed the assumptions of normality and homogeneity at 5% of waste by the Shapiro-Wilk and Levene test. Then submitted to ANOVA one way which evaluated the effect of reproductive period with 5% significance. In case of significant difference between at least one treatment, means were compared by Tukey test at 5%. It was performed Pearson correlation index between the period and oocytes weight from *C. macropomum* females along the breeding season.

3. Results

Average air temperatures in 2010/2011 were: 26.2, 26.6, 26.2, 26.1 and 25.7 °C in November, December, January, February and March, respectively. Pluviometric indexes in 2010-2011 were 334.3, 229.6, 283.9, 459.1, and 365.4 mm in November, December, January, February and March, respectively (Figure 1).

It was checked effect ($p > 0.05$) for period (samplings) into the season for weight of oocytes released; productivity index and fertilization rate (Table 1). Although period 3 (sampling in December) did not differ significantly ($p > 0.05$) from other periods, it was the best one regarding oocytes quality.

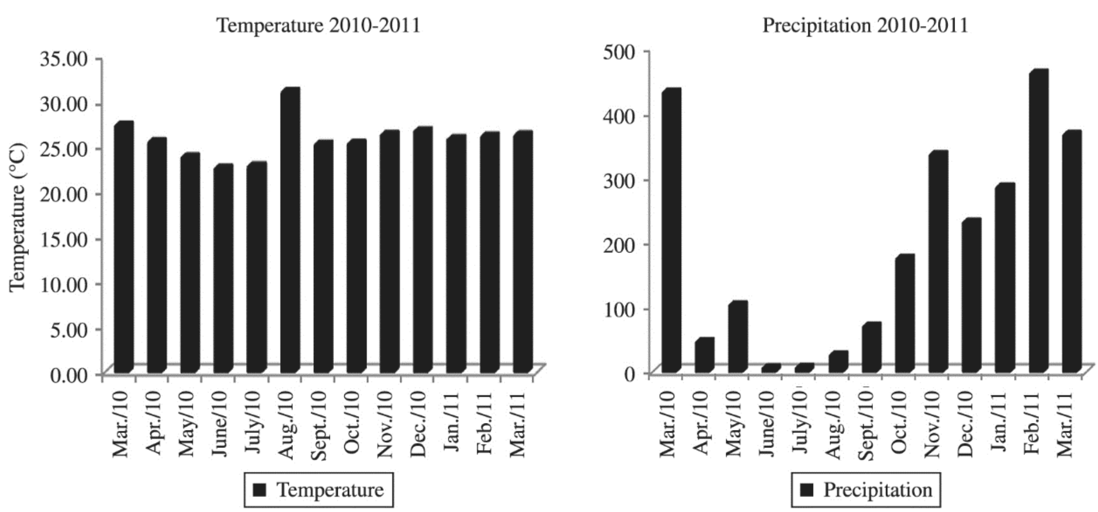

Figure 1. Monthly variation of average air temperature and precipitation in Pimenta Bueno city - Rondônia from March 2010 to March 2011 (AGRITEMPO, 2010)

Table 1. Qualitative parameters of *Colossoma macropomum* oocytes during the breeding season 2010/2011.

Reproductive period (samplings)	Qualitative parameters of oocytes			
	Oocytes weight (g)	Production index (PI)	Fertilization rate (%)	Hatching rate (%)
1	1101.8±124.4ab	10.38±1.41abc	67.44±1.47abc	67.12±14.25a
2	1070.0±204.9ab	13.62±1.19ab	86.30±3.41a	68.57±17.57a
3	1830.0±159.0a	16.90±0.33a	85.75±9.95ab	89.05±4.65a
4	778.00±149.8b	10.34±1.89abc	73.06±12.60abc	76.04±12.76a
5	852.0±225.0ab	10.02±2.65abc	69.00±23.60abc	71.00±21.00ab
6	981.0±276.2ab	13.51±0.85ab	23.25±23.25bc	22.95±22.95bc
7	1113.3±246.7ab	10.01±2.71abc	64.85±8.12abc	54.09±19.54ab
8	894.7±89.8ab	8.19±1.94bc	8.00±6.11c	0.00±0.00c
9	519.7±51.9b	4.98±0.49c	70.32±8.49ab	63.93±13.15a
(F; p)	3.7967; 0.0042	0.36127; 0.9318	4.0668; 0.002778	2.13490; 0.0673

PI = oocytes (g)/body weight (g) * 100.

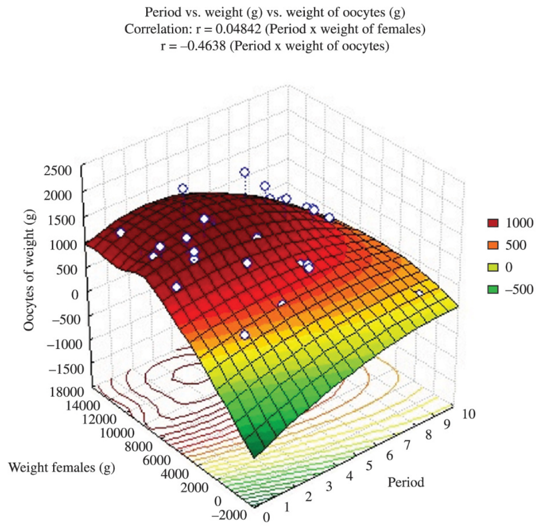

Figure 2. Correlations between the reproductive period, female weight (g) and weight of released oocytes (g).

According to correlations carried out between the reproductive period (samplings) and female weight (r = 0.0484), and between reproductive period (samplings) and weight of released oocytes (r = –0.4638), it was observed females that had greater weight, and higher oocytes weight occurring in the first periods of sampling in November, December and January (Figure 2).

4. Discussion

According to Bobe and Labbe (2010) the oocytes quality in fish can be defined as the ability to be fertilized and consequently develop a normal embryo. In wild conditions or even in captivity, the quality of fish gametes can be highly variable and significantly influenced by several external factors beyond management of breeding and reproductive period (Chambers and Waiwood, 1996), such as environmental factors (Kraus et al., 2000), temperature (Tveiten et al., 2001), the feed level of females (Tyler and Sumpter, 1996) and sex relation of breeders (Pavlidis et al., 2004).

Macchi et al. (2004) studied oocyte production during the reproductive season of *Merluccius hubbsiake* and reported that the monthly production of oocytes varied considerably during the season, with a main peak in January (57% of oocytes produced during the period). In addition, older females (> 5 years) had production in longer period than younger ones. In the present study the female *C. macropomum* spawned from November to March, with a peak of oocytes weight in December. Furthermore, from the correlations between the female's weight, weight and reproductive period of oocytes it was possible to affirm that larger oocytes were released by heavier animals. This occurred in the early periods of sampling, indicating that older females were more precocious. According Signor et al. (2013) in studies with catfish (*Rhamdia voulezi*) observed that the major players have better reproductive capacity, keeping the proportion of eggs in relation to body weight.

Another observation by Macchi et al. (2004) with *Merluccis hubbsi* was that from December to February the number of oocytes produced per unit weight did not change. In March the oocytes production decreased considerably coinciding with increased gonads atresia. This fact was also observed for *C. macropomum*, where the productivity index (number of released oocytes /live weight) did not show differences between November and December, but presented significant difference between March, probably due to increased follicular atresia. The same authors reported that the fecundity of fish group studied was positively correlated with total length, total weight (without ovary) and age of *M. hubbsi* females, it's a range from 100,000 (32 cm total length) to 2.3 million (87 cm total length) of hydrated oocytes.

Related to age, the variation in chemical composition of fish oocytes were found in some studies, but did not seem to be a universal phenomenon. In contrast, the oocyte size can be predicted from the female age. During the breeding season the size of the oocyte may vary between successive batches. The positive relationships between female size and oocyte size with the size of fingerlings and resistance to starvation and predation is a fundamental way to parent-egg-progeny (Kamler, 2005).

The fertilization rate and hatching rate did not differ in the first period of November, December and January, but from February there was a decrease in these rates. The same behavior was observed by Barbieri et al. (2000) with wild *Salminus maxillosus*, reporting the spawning period from November to February. This fact was also observed in this study, as the small variation between the fertilization rates and hatching rates can be explained by abiotic factors such as rainfall.

According to Lowe-McConnell (1975), teleosts of tropical and subtropical regions own a close relationship between the reproductive period and the rainy seasons. For Munro (1990), there is evidence that temperature can be used as a source of information about the advent of appropriate conditions for spawning.

Temperature is an important factor to the reproductive processes in fish occurs regularly. In the overlapping temperature chart study period during the year, two situations bear watching, firstly, in August, when the fish were still under a latency period of embryonic development and second, since reproductive period the average temperature remained stable, with a slight decline in November (2010) to March 2011. Although this appears to be a little variation in temperature it may have been a factor that potentiated stress, after the systematic trawls to capture the animals in storage ponds.

The importance of temperature and photoperiod on reproduction is amply demonstrated in many species of fish (Munro, 1990; Bromage et al., 2001). Papadaki et al. (2008) studied the gametes quality of *Diplodus puntazzo*, and observed occurrence of spawning between the temperatures 19 to 21°C, with an optimum temperature of 21°C. When the temperature began to drop, spawning has also suffered a decrease.

Even in captivity, many species of fish have reproductive behavior similar to nature, abiotic factors such as water temperature and pluviometric rates significantly influence the changes in production of gametes during the reproductive season. According to Winters et al. (1993), from physical environments, seawater temperature influences the average number and size of oocytes produced in fishe. Longer Querol et al. (2002) studied *L. platymetopon* and Melo and Querol (1995) with *L. anus* observed in general, conditions for increasing temperature are linked to the period of greatest reproductive activity, directly affecting gonadal maturation.

Deeper researches in this area and assessment of qualitative and quantitative parameters of *C. macropomum* oocytes in new breeding seasons are required. In addition, observations of new parameters such as oocyte size, amount of fatty acids in the oocytes, number of oocytes/g would help to explain the interaction between the biotic/environmental parameters and oocytes quality. Optimum results were observed during the periods of November, December and January, indicating it as the best time to perform the reproduction of the species.

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