SELECTION OF PACU FEMALES TO HORMONAL INDUCTION: EFFECT OF AGE AND EVALUATION METHODS*

Marianne SCHORER[1]; Renata Guimarães MOREIRA[2]; Sergio Ricardo BATLOUNI[3]

ABSTRACT

In this study, we investigated the reproductive cycle and spawning season of old (OFs) and young (YFs) *P. mesopotamicus* females, aiming to improve the process of selection of suitable fish for induced spawning. We also evaluated the accuracy of using external body characteristics in selecting suitable females. To that, 60 OFs and 60 YFs (10 and four years old, respectively) were submitted to seven samplings during two reproductive cycles. In each sampling, females (five to 10 per treatment) were randomly chosen to evaluation of plasma concentrations of gonadal steroids and ovary composition (biopsies and stereology). Females were also submitted to an external evaluation concerning the length of abdominal thickness, the degree of the abdominal rigidity and the aspect of urogenital papilla. In spite of OFs have shown reduced concentrations of 17α-hydroxyprogesterone during the spawning season, we observed that OFs and YFs kept in captivity had similar reproductive cycles and spawning seasons (in December for both groups). We do not recommend the exclusive use of external evaluation for selecting breeders to spawning induction due to a great inaccuracy of data and lack of association with histology and ovarian biopsy, which are more accurate and reliable.

Key words: breeder selection; induced spawning; ovarian biopsies; reproductive cycle; unsuccessful ovulation

SELEÇÃO DE FÊMEAS DE PACU PARA INDUÇÃO HORMONAL: EFEITO DA IDADE E DOS MÉTODOS DE AVALIAÇÃO*

RESUMO

Neste estudo, investigamos o ciclo reprodutivo e a época de desova de fêmeas "velhas" (FVs) e fêmeas "jovens" (FJs) de *P. mesopotamicus*, visando melhorar o processo de seleção de peixes para a indução hormonal. Avaliamos também a acurácia do uso de características corporais externas para a seleção de fêmeas para indução hormonal. Para isso, 60 FVs e 60 FJs (10 e quatro anos, respectivamente) foram submetidas a sete amostragens durante dois ciclos reprodutivos. Em cada amostra, as fêmeas (cinco a 10 por tratamento) foram capturadas para avaliação das concentrações plasmáticas de esteroides gonadais e composição dos ovários (biópsias e estereologia). As fêmeas foram também submetidas à uma avaliação externa relativa à espessura abdominal, o grau de rigidez abdominal e o aspecto da papila urogenital. Exceto pela ocorrência de concentrações reduzidas de 17α-hidroxiprogesterona nas FVs, durante a época de desova, observamos que FVs e FJs, mantidas em cativeiro, apresentam ciclos reprodutivos e épocas de desova similares (em dezembro para ambos os grupos). Não recomendamos o uso exclusivo da avaliação externa para a seleção de reprodutores para indução a desova, devido a uma imprecisão dos dados e falta de associação destes com a histologia e biópsia de ovário, os quais são mais precisos e confiáveis.

Palavras-chave: seleção de matrizes; desova induzida; biópsia ovariana; ciclo reprodutivo; falha na ovulação

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INTRODUCTION

Piaractus mesopotamicus is a large omnivorous fish widely distributed in South America and highly appreciated for aquaculture purposes (BRASIL, 2010). The *P. mesopotamicus* is a total spawned rheophilic fish which spawns during the summer (November to January) (LIMA *et al.,* 1991; BARBIERI and BONDIOLI, 2013; KURADOMI *et al*., 2016). In captivity, their reproduction can only be obtained through a hormonal induction; however, the unpredictability of a successful spawning is one of the main problems related to offspring production (CRISCUOLO-URBINATI *et al*., 2012).

Such reproductive deficiency may be caused by mistakes in determining the spawning season and in selecting breeders for an induced breeding (BOBE and LABBÉ, 2010; MYLONAS *et al.,* 2010; CRISCUOLO-URBINATI *et al.,* 2012). The fact that breeders of different ages may present differences concerning their reproductive cycles and spawning season must also be considered. Moreover, differences in age may have an influence on their reproductive performance, since older fish tend to spawn early in the spawning season, whereas younger ones tend to mature more to the end of the season. Fish of different ages may also have different breeding season periods (TRIPPEL and MORGAN, 1994; WIELAND *et al.,* 2000; FITZHUGH *et al*., 2012). In a natural environment, variations concerning time and fish age may influence the spawning season, especially when the age of the population suffers changes (WIELAND *et al.,* 2000). Therefore, the non-observance of breeders' ages and, particularly, their reproductive cycle may be associated with unsuccessful ovulation and failure in obtaining viable embryos.

Another aspect to be considered is that subjective methods are usually applied for selecting mature females (length of abdominal thickness (LAT), abdominal rigidity, and the aspect of urogenital papilla). The accuracy of these methods is not known and when they are used for *P. mesopotamicus* (CRISCUOLO-URBINATI *et al*., 2012) or other rheophilic South American fish species (HAINFELLNER *et al.,* 2012a, b) part of the selected females do not often ovulate after hormonal treatments.

Thus, the main objective of this study was to investigate possible differences in the reproductive cycle of old (OFs) and young (YFs) *P. mesopotamicus* females which could lead to a mistaken selection of females able to reproduce (by underestimating or overestimating their reproductive status). In addition, we compared the accuracy of the use of subjective methods which consider the external appearance of fish (degree of abdominal distension, degree of dilation of the papilla and abdomen thickness) with objective analyses performed in laboratory, such as plasma concentration of gonadal steroids, ovarian biopsies (egg diameter and position of germinal vesicle), and stereological evaluation of females' ovaries during the breeding season.

METHODS

Animals

For this study, two *P. mesopotamicus* broodstocks groups were used: OFs (10 years old, 3.9 kg ± 0.30 average weight) and YFs (four years old, 2.7 kg ± 0.20 average weight). Both batches were produced by induced spawning. Fish were randomly distributed in three earthen ponds (200 m^2) (0.3 fish m^{-2}), each containing 20 OFs, 20 YFs, 10 old males and 10 young males. Old and young males were as old as OFs and YFs, respectively. Fish were maintained at the "Centro de Aquicultura da Universidade Estadual Paulista (CAUNESP)", Jaboticabal, São Paulo State, Brazil (21°15'17"S 48°19'20"W).

Culture conditions

Fish were fed six days a week, in two portions at 9:00 and 16:00 h, with a commercial extruded diet for omnivores [crude protein (32.0% (maximum), ethereal extract (5.0% (minimum), fibrous matter (7.0% (maximum), ash (7.0% (maximum), calcium (1.2% (maximum), phosphorus (0.6% (minimum)], corresponding to 2.0% of total body weight twice a day. Water parameters were measured weekly: dissolved oxygen (6.34 ± 0.64 mg L^{-1}) (oximeter HI 9146-10 (Hanna instruments), pH (7.58 ± 0.33) (pH meter HI 98172 (Hanna Instruments)), temperature (23.80 ± 0.72 °C), and electrical conductivity of the water (63.24 ± 2.88 µS cm^{-1}) (HI 98311 (Hanna

Instruments)). Total alkalinity (57.12 ± 3.56 mg L^{-1}) (total alkalinity UniKit) and total hardness (61.64 ± 3.84 U mg L^{-1}) (total hardness UniKit) were measured twice in a month.

Sampling

The experimental period lasted 730 days (encompassing two successive breeding seasons (2009/2010 and 2010/2011); during this period, seven consecutive samplings were performed. Samplings were performed during vitellogenic (October), spawning (December) and post-spawning (March) periods for *P. mesopotamicus*, according to LIMA *et al.* (1991). In each sampling, five to 10 females (for each treatment) were randomly selected (December 2009: n = 10 OFs and 10 YFs ; October (2010): n = 6 OFs and 6 YFs ; December (2010): n = 6 OFs and 5 YFs ; October (2011): n = 6 OFs and 6 YFs ; and December (2011): n = 6 OFs and 7 YFs . The number of females varied according to samplings because we opted for a minimal manipulation of individuals when collecting at least five from each group by sampling. This strategy was adopted to standardize the effort to capture between collections and reduce possible changes caused in the concentrations of gonadal steroids. Total length (TL) (from mouth to tail, with an accuracy of 0.01 cm), Length of abdominal Thickness (LAT) (with an accuracy of 0.01 cm), and body weight (BW) (with an accuracy of 0.01 g) were recorded for each animal. In order to consider a possible influence of TL on LAT, we also evaluated the relationship LAT TL^{-1}. The LAT value was regarded as the thickness of the ventral region registered at the urogenital papilla level.

External macroscopic evaluation of reproductive status

Fish abdominal rigidity was determined by gently squeezing it (a method widely used by farmers) and it was then recorded as: soft (SA), intermediate (IA) or hard (HA). Furthermore, we recorded the aspect of urogenital papilla as hemorrhagic (HP), intermediate (IP), or non-hemorrhagic (NHP) (Figure 1). The evaluation was performed by the same person for all parameters. Since there is no standard methodology in the literature for this purpose, the softness of the abdomen was determined considering as "SA" those females with soften abdomen ready to be selected for induced spawning; as "IA" those that still would not be selected, but already showed a slightly soft abdomen; and "HA" those that showed no sign of softness in the abdomen. For this analysis, we considered only females captured in 2010 and 2011, because in those years we could analyze an entire reproductive cycle. These analyzes were performed with the same animals (as well as the same number of replicates) cited in the previous paragraph. Ovarian biopsies and plasma concentration of gonadal steroids.

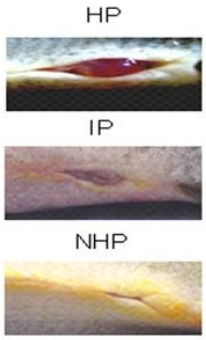

Figure 1. Photographs showing the three different types of urogenital papilla described during the reproductive cycle of Piaractus mesopotamicus. HP: hemorrhagic papilla; IP intermediate papilla and NHP: non-hemorrhagic papilla.

Fish were anaesthetized with a benzocaine solution (2 g ethylaminobenzoate: 150 ml alcohol: 20 L water) and cannulated for collecting 0.5 mL oocytes, which was then divided into two subsamples: the first was used to observe the presence and position of the nucleus using Serra's solution. Oocytes were classified as fully vitellogenic (central or eccentric germinal vesicle) and atretic (germinal vesicle absent); the second sub-sample was used to determine oocytes diameters (200 oocytes $female^{-1}$) using a stereomicroscope LEICA MZ 8 coupled to LEICA DFC 280 plus IM program 50 - LEICA equipment. The average diameters obtained in December samplings were then compared. Subsequently, the obtained diameters were distributed in classes

and plotted on graphs to evaluate, in a descriptive way, the occurrence of trends and compare them between groups. Ovary samples were not collected in March because the urogenital pore of females was closed, preventing the introduction of the cannula.

Afterward, 3 mL of blood were collected from the caudal vein, transferred to Falcon tubes, and then centrifuged for 10 min at 655.2 g. Plasma samples were immediately preserved in liquid nitrogen and stored in a freezer at -80ºC until the time of processing. The gonadal steroids were quantified by enzyme-linked immunosorbent assay (ELISA), using a commercial test (Interteck, Virginia, USA), estradiol (E_2), and 17α - hydroxyprogesterone (17α – OHP) "kits", according to the manufacturer's recommendations. Five samples from four individuals (two OFs and two YFs) for each steroid were validated by intra-assay testing. Intra-assay coefficients variation values ranged from 1.89 to 14.60% for E_2, and 0.67 to 15.70% for 17α - OHP.

Histomorphometric analysis

Only in December (2011) (n = 4 YFs and 5 OFs), extra breeders were randomly captured and euthanized with a benzocaine anesthetic ethylaminobenzoate (10 g L^{-1}): 150 ml alcohol: 20 L water, and then killed by severing the section of the spinal cord next to the operculum. Total length (cm) and body weight (g) values were recorded for each female. Ovarian samples (cranial, medial, and caudal regions) were collected and fixed in Bouin's solution for 24 h.

Stereology

After fixation, ovary samples were embedded in paraplast, cut into 3-5 µm thick sections and subjected to hematoxylin and eosin staining. Histological sections were used to determine the frequency of different oocyte types, considering all oocytes present (pre-vitellogenic, vitellogenic immature (incomplete vitellogenesis), mature (complete vitellogenesis) and atretic). Volume density was determined using light microscopy and a 352-intersection grid. In order to do so, four microscopic fields (5x objective) were evaluated per ovary region (anterior, medial, and caudal), totalizing 12 microscopic fields per randomly selected female, adding a total of 4224 points scored for each animal. For this analysis, we used the same methodology applied by PEREIRA *et al*. (2013, 2016), determining the average percentage of each ovarian component for both groups.

Statistical analyses

Mean values were compared among groups using two-way ANOVA, with standard errors (SE). The level of significance was set at $p \leq 0.05$. Tests of normality and homoscedasticity of variances were performed. Statistical analyses were carried out using the SAS software (SAS INSTITUTE, 1990). Data on the degree of rigidity of the abdomen and urogenital papilla subtypes were not subjected to statistical analysis because many defined subtypes were not found in all periods of analysis, thus preventing the execution of tests.

RESULTS

Samplings

During the experimental period, the survival rate of OFs and YFs was 100%. Throughout the experimental period, the average values for TW were similar between groups (Figure 2), except during breeding seasons (December 2009 and December 2010) when values for OFs were higher ($p \leq 0.05$) (Figure 2). Mean values of LAT and LAT TL^{-1} ratio were similar between groups in the same samplings, except for higher LAT values for OFs in March (2010) (resting) ($p \leq 0.05$) (Table 1). Evaluating data for each group separately, we found that the values of LAT and LAT TL^{-1} for both groups in October (vitellogensis) and December (spawning) were predominantly higher than those in March (resting) of the same year ($p \leq 0.05$) (Table 1).

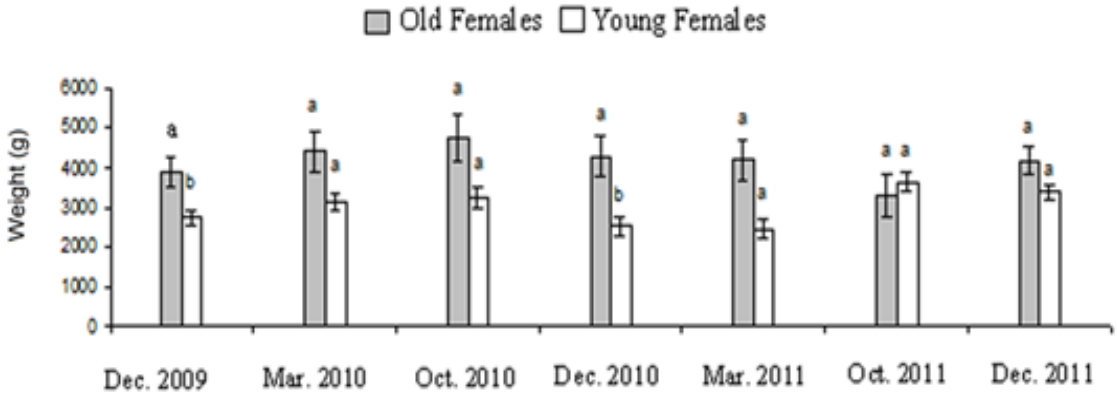

Figure 2. Average values of total body weight of old and young *Piaractus mesopotamicus* females during the reproductive cycle. Means followed by different letters mean statistical differences in the frequency of the same oocyte type between groups ($p \leq 0.05$).

External macroscopic evaluation of reproductive status

In March (resting), 100% of OFs and YFs presented hard abdomen and non-hemorrhagic papilla (data not shown). Data of abdomen rigidity and papilla characteristics for vitellogenic and spawning periods are shown in Table 2. We observed a slightly higher frequency of females with a harder abdomen in October when compared to December, as well as a slightly higher frequency of females with soft abdomen in December compared to October, for both groups. However, we found a great inaccuracy of data and little relation to the other tested variables.

Distribution of oocyte types according to the germinal vesicle

The percentage composition was similar for both OFs and YFs in the same samplings, with a similar pattern of increased percentages of eccentric germinal vesicle oocytes between October and December (Figure 3).

Distribution of oocyte according to the diameter

The average diameter of OFs oocytes (December 2009, 2010, and 2011) was higher than that of YFs ($p \leq 0.05$) (Table 3). In October (2010 and 2011), there was an unimodal distribution (~ 900 µm) for OFs and YFs, which turned into a polimodal one in December (2010 and 2011) (Figure 4). Such pattern of a polimodal distribution also occurred in December (2009). In December (2010 and 2011) the emergence of larger oocytes diameters was more pronounced for OFs (> 900 µm) (Figure 4 c, e).

Plasma concentration of gonadal steroids

E2 plasma levels for OFs increased between March and October (2010 and 2011) ($p \leq 0.05$) and only between March and October (2011) for YFs ($p \leq 0.05$). Comparing plasma concentration of E2 in the same sampling in March (2010 and 2011), values for OFs were lower (Figure 5a) than those for YFs. On the other hand, plasma concentration of E2 for OFs was higher than that for YFs in October (2010). The plasma concentrations of 17α - OHP for OFs were lower than those for YFs ($p \leq 0.05\%$) in December (2010) and October (2011). Analyzing the individual cycle of each group, 17α - OHP plasma concentrations were similar throughout the experimental period (Figure 5b).

Table 1. - Mean values of Length of abdominal thickness and Length of abdominal thickness/ Total Length with SE of old (OF) and young (YF) *Piaractus mesopotamicus* females, during the reproductive cycle. Means followed by different capital letters mean statistical differences ($p \leq 0.05$) in the same group over the period; and means followed by different small letters mean statistical differences ($p \leq 0.05$) in the same period between the groups.

Sampling times	Dec 2009	Mar 2010	Oct 2010	Dec 2010	Mar 2011	Oct 2011	Dec 2011
	Length of abdominal thickness (cm)						
OF	4.86 ± 0.5Ba	3.70 ±0.2Ba	6.40±0.3Aa	6.30±0.3Aa	4.00±0.2Ba	6.30±0.2Aa	5.40±0.2 Aa
YF	4.52 ±0.4ABa	3.13±0.3Bb	5.40±0.3Aa	5.70±0.3Aa	3.90±0.3Ba	5.30±0.2Aa	5.20±0.8Aa
	Length of abdominal thickness / Total length						
OF	0.08±0.006Ba	0.06±0.002Ba	0.11±0.004Aa	0.10±0.005Aa	0.07±0.005Ba	0.10±0.010Aa	0.10±0.005Aa
YF	0.08±0.005Aa	0.06±0.003Ba	0.10±0.004Aa	0.11±0.004Aa	0.08±0.005Aa	0.09±0.010Aa	0.09±0.001Aa

Table 2. Percentage distribution of old (OF) and young (YF) *Piaractus mesopotamicus* females concerning the degree of abdominal rigidity and aspect of the papilla during their reproductive cycle.

Sampling	**Groups**	**Hard** %	**Intermediate** %	**Soft** %	**HP** %	**IP** %	**NHP** %
October 2010	OF	83.3	0	16.7%	16.7	16.7	66.6
	YF	83.3	16.7	0%	0	16.7	83.3
December 2010	OF	50.0	16.7	33.3%	0	50.0	33.3
	YF	0	17.0	83.3%	0	0	33.4
October 2011	OF	50	16.7	33.3%	0	50.0	16.7
	YF	100	0	0%	0	33.4	66.6
December 2011	OF	16.7	33.3	50.0%	83.3	16.7	0
	YF	57.1	28.5	14.2%	14.2	57.1	28.5

Table 3. Average diameter (µm) of *Piaractus mesopotamicus* old (OF) and young female (YF) oocytes obtained by cannulation in December 2009, 2010, and 2011. Averages followed by different letters mean statistical differences ($p \leq 0.05$) in the same period between the groups. "n" refers to the number of fish evaluated. December 2009: n = 10 OF and 10 YF; October 2010: n = 6 OF and 6 YF; December 2010: n = 6 OF and 5 YF; October 2011: n = 6 OF and 6 YF; December 2011: n = 6 OF and 7 YF.

	Oocyte diameter		
	December 2009	December 2010	December 2011
OF	994 ± 124 µm a	987 ±142 µm a	993 ±128 µm a
YF	925 ± 130 µm b	948 ± 116 µm b	928 ± 128 µm b

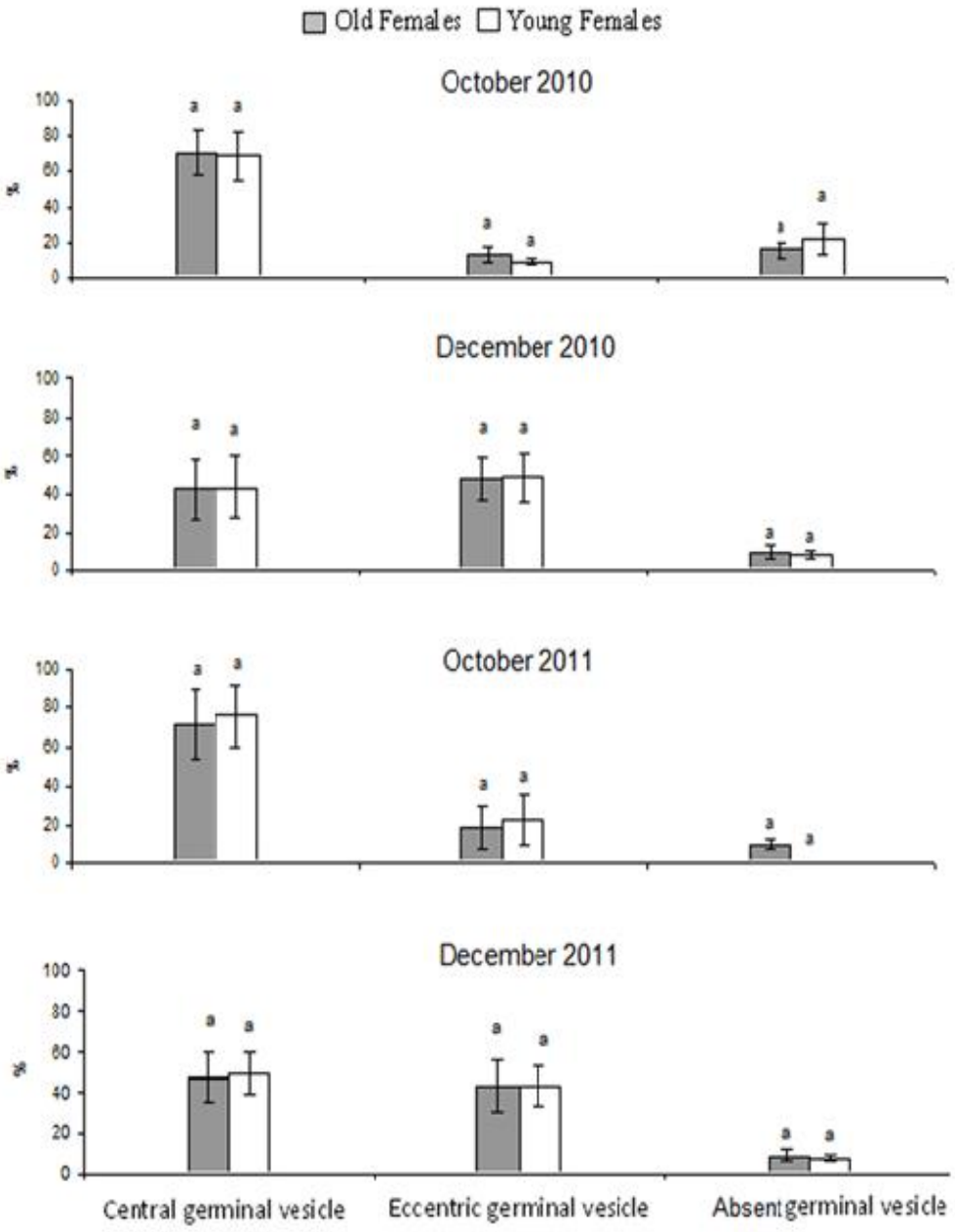

Figure 3. Percentage distribution of oocytes of old and young *Piaractus mesopotamicus* females according to the position of the germinal vesicle. Means followed by different letters mean statistical differences in the frequency of the same oocyte type between groups ($p \leq 0.05$).

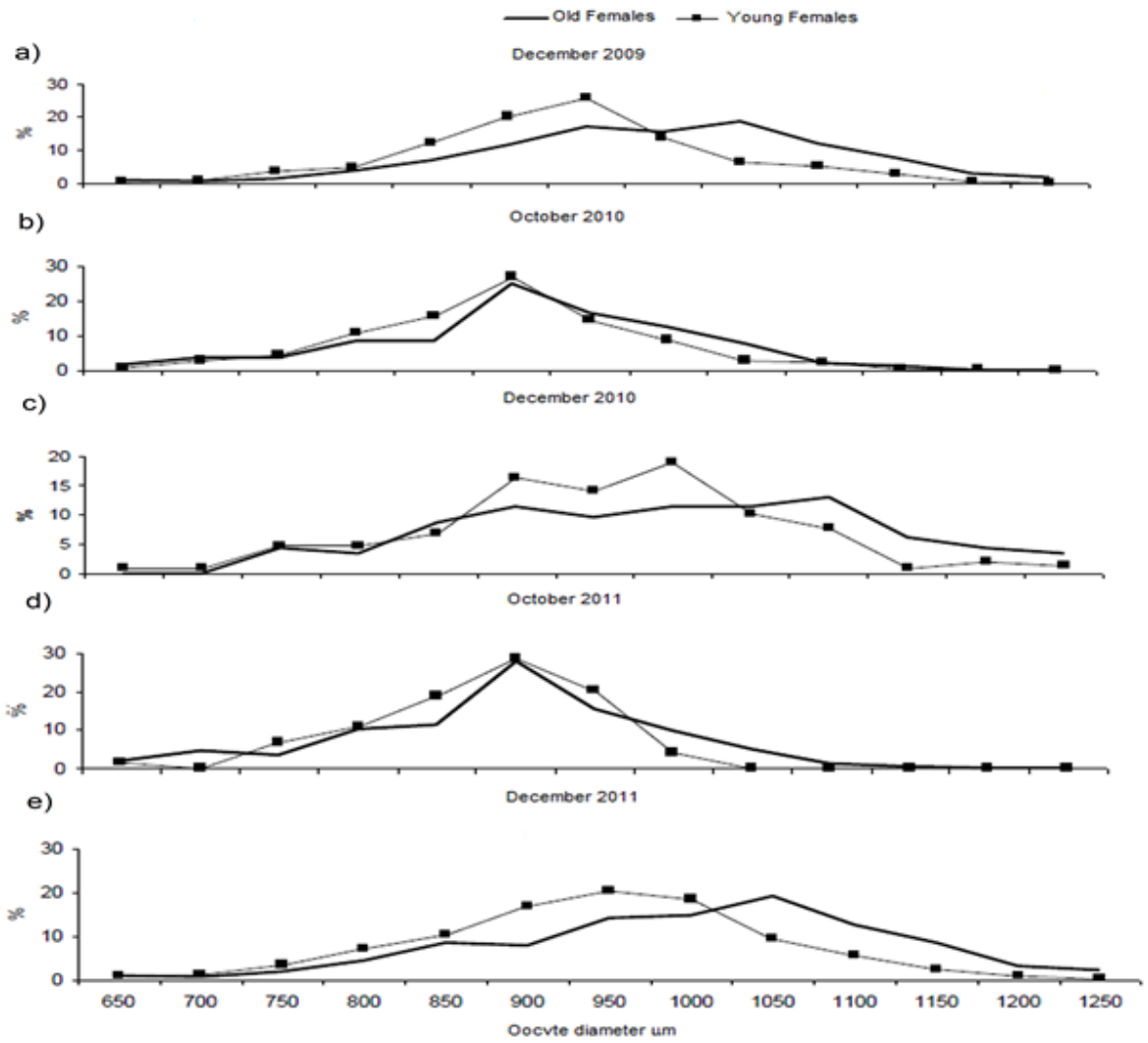

Figure 4. Distribution of oocyte frequencies according to the diameter for old and young *Piaractus mesopotamicus* female.

Volume density of different oocyte types

The average frequencies of the different types of oocytes were similar for both OFs and YFs. The percentage of pre-vitellogenic, immature vitellogenic, mature vitellogenic, and atretic oocytes for YFs and OFs were respectively 37.42 + 8.74% and 38.03 ± 2.89%; 14.90 ± 1.76% and 18.79 + 6.77%; 37.42 ± 8.74% and 38.03± 2.89%; 5.50 ± 0.20% and 6.03 ± 2.64% (Figure 6).

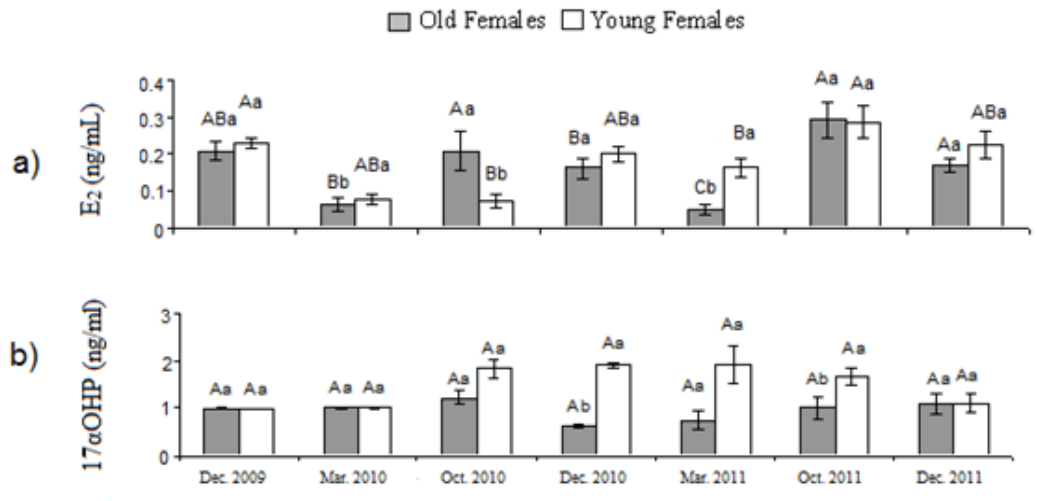

Figure 5. Average values of estradiol (E_2) and 17α - hydroxyprogesterone (17α – OHP) of old and young *Piaractus mesopotamicus* females maintained in the same conditions in captivity during the reproductive cycle. Means followed by different letters mean statistical differences in the frequency of the same oocyte type between groups by the Tukey test ($p \leq 0.05\%$).

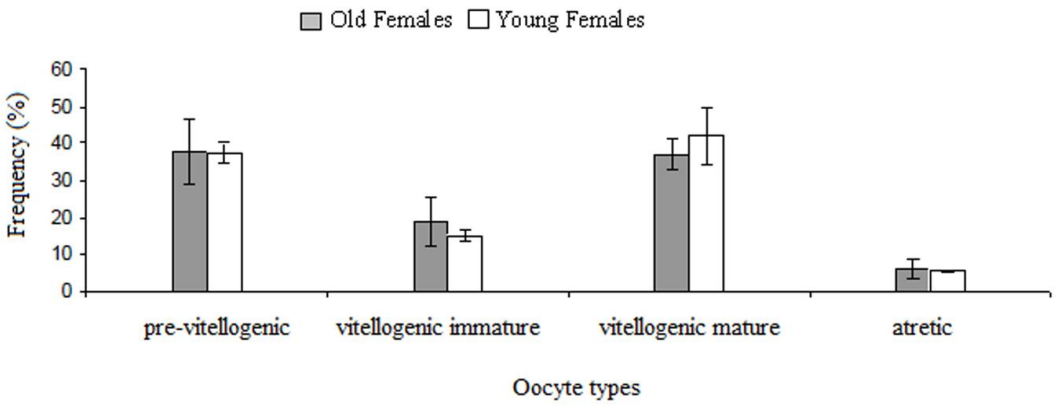

Figure 6. Frequencies of the different oocyte types obtained by volume density evaluation of *Piaractus mespotamicus* old and young females during the breeding season.

DISCUSSION

In this study, vitellogenic and mature ovaries were observed at similar periods for *P. mesopotamicus* OFs and YFs; therefore, with the methodology applied in here, we could not confirm the possibility of different spawning seasons for these groups. On the other hand, in spite of OFs have presented higher percentages of oocytes in classes formed by a larger diameter, the level of 17 - OHP in this group was lower than that of YFs, thus suggesting a potential advantage of the later regarding a better reproductive performance.

Concerning the different methods applied here to describe the reproductive cycle and spawning season, the ovarian biopsy showed

results in accordance with the histological analysis, and is, therefore, a reliable parameter for this species. Concerning the external appearance of breeders (routinely used by fish farmers), the data observed for LAT also pointed to a similar reproductive cycle and breeding season between groups, thus corroborating the results of the ovarian and histological biopsies. For both groups, values of LAT as well as the LAT TL^{-1} ratio were higher in October (vitellogenesis) and December (spawning), compared to March (post-spawning). In this context, the increase in abdominal volume quite surely reflects, an increase in the ovarian volume due to the accumulation of yolk in eggs. Therefore, this parameter seems to be very useful to be used as a supporting tool for selecting suitable *P. mesopotamicus* female breeders for induced breeding.

Still in this context, concerning the external evaluation to select female breeders for spawning induction, we observed that the parameters "rigidity of the abdomen" and "appearance of the urogenital papilla" did not present a logical similarity to the more accurate parameters we used, such as ovarian biopsy and stereological evaluation. In fact, they showed a huge variation, fact that does not seem to be related to the age of females, but to other unknown factors. In this concern, we observed that, for instance, neither a higher percentage of hemorrhagic papillae for OFs in December (2011), nor a higher percentage of soft abdomens for YFs in December (2010) were related to differences in ovary composition or spawning season. Therefore, the appearance of the urogenital papilla and the rigidity of the abdomen may lead to misinterpretations on the reproductive status, since they may also vary according to other unknown factors. We emphasize that "soft abdomen" and "hemorrhagic papillae" were only observed during vitellogenesis (October) or during the breeding season (December), never occurring in March (after the reproductive season), although it does not seem to be a precise and definitive criterion for selecting females suitable for spawning.

On the other hand, ovarian biopsy data were in accordance with the histological analysis; therefore, this is a reliable parameter. Combined, the ovarian biopsy and stereological analysis data allow us to conclude that both OFs and YFs with ≥ 45% of oocytes with eccentric germinal vesicle seem to have mature ovaries and be suitable for induced spawning. Since the ovary biopsy evaluation is available to fish farmers, we recommend that breeders should be selected based mainly on the thickness of the abdomen and ovarian biopsies. With respect to external parameters (simple to measure and used by fish farmers), we concluded that the only ones to effectively represent the ovarian maturation status were LAT and the LAT TL^{-1} ratio, because they showed congruence with the results obtained from the biopsies and ovarian histology analysis of the ovaries.

In this study, OFs presented a higher percentage of oocytes with a diameter greater than 970 µm compared to YFs. Many authors report that the diameter of oocytes has a direct influence and provides greater reserves (BLAXTER and HAMPEL, 1983; KENNEDY *et al*., 2007; STRATMAN and TABORSKY, 2014), besides providing benefits in the hatching process and upon the larval growth process (MARTEINSDOTTIR and STEINARSSON, 1998; KENNEDY *et al*., 2007; ROLLINSON and HUTCHINGS, 2010; STRATMAN and TABORSKY 2014); it also gives rise to larger larvae, which are capable of ingesting a greater range of different sized particles and present greater resistance during the period of fasting (KENNEDY *et al*., 2007; ROLLINSON and HUTCHINGS, 2010; STRATMAN and TABORSKY 2014). However, this theoretical advantage of *P. mesopotamicus* OFs having larger diameter oocytes should be analyzed carefully. Data not shown in this study indicated that OFs do not respond adequately to hormonal treatments and few ovulate after hormonal treatments. Therefore, if on the one hand OFs present larger diameter oocytes with a hypothetical and theoretical advantage, on the other hand, corroborative studies need to evaluate the reproductive performance of these females considering other parameters, such as ovulation and embryonic survival rate. Moreover, according to ROLLINSON and HUTCHINGS (2010), offspring from larger eggs emerged later and at an earlier developmental stage than offspring from smaller eggs, so larvae from larger eggs may not

have a survival advantage over larvae from smaller eggs (KENNEDY *et al*., 2007).

Concerning E_2 plasma concentration, we observed that values were mostly higher during the vitellogenic period (October) compared to the period after spawning (March) for both groups. Increases in E_2 levels are described during the vitellogenic period for several species (PEREIRA *et al*., 2013, 2016), including rheophilic fish (ARANTES *et al.,* 2010; HAINFELLNER *et al*., 2012b). In this study, E_2 elevations during the vitellogenic period (between March and October) were also recorded, but more sharply for OFs. In this concern, significant differences between E_2 plasma concentrations were found between groups throughout their reproductive cycle, including the vitellogenic period (first reproductive cycle); however, these differences did not reflect distinct reproductive cycle patterns, which could be seen through a stereological evaluation or ovarian biopsy. Timing differences in estradiol surges can cause substantial changes in the reproductive period and fecundity (HAINFELLNER *et al.,* 2012b), which were not confirmed in this study.

17α - OHP steroid is the main precursor of 17α, 20 β-dihydroxy-4-prengnen-3-one (DHP) which, in turn, is the most potent inducer of the final maturation process and ovulation in fish (YARON and LEVAVI-SIVAN, 2011). The assessment of DHP levels and their precursors in breeding plasma has shown a positive correlation with successful ovulation, besides quality of fish gametes and larvae (DABROWSKI *et al*., 2003). In this study, we found reduced concentrations of 17α - OHP in both reproductive cycles for OFs, in comparison with YFs during the vitellogenic (October 2011) and spawning season (December 2010). The reduced values of 17α - OHP for OFs were probably related to the low reproductive performance of the same (data not shown in this study). Comparative data of the groups reproduction (not documented here) showed that a very low percentage of OFs spawned after hormonal treatments compared with YFs, thus showing reduced fertility and hatching rates (data not shown). Low concentrations of this steroid in the plasma of OFs may be related to a reduced reproductive performance of this group (data not shown), since it is known that fish age and size interfere with the reproductive performance by several mechanisms (MARTEINSDOTTIR and STEINARSSON, 1998; BERKELEY *et al*., 2004; VLADIĆ and PETERSON, 2015), including the gonadal steroid profile (CIERESZKO *et al.,* 1998). In the wild, older females may arrive at spawning areas earlier and spawn before younger females (MARTEINSDOTTIR and STEINARSSON, 1998; KAMLER, 2008). In some cases, as observed for brook trout (BLANCHFIELD and RIDGWAY, 2005), larger females make use of the best areas, with better food resources to the offspring. However, we did not observe any timing difference in this regard concerning *P. mesopotamicus* OFs and YFs maintained in captivity.

In this study all physical and chemical parameters of water evaluated enable us to classify the quality of water used as adequate for farming tropical rheophilic fish, being similar to that of previous studies concerning breeders management (HAINFELLNER *et al*., 2012b; DE SOUZA *et al*., 2015), thus ensuring reliability to the results obtained and not interfering in the physiological well-being of fish.

CONCLUSIONS

In conclusion, in this study we demonstrated that *P. mesopotamicus* OFs and YFs maintained in captivity under the same conditions presented similar reproductive cycle patterns, concerning the vitellogenic and spawning periods. We observed that the parameters routinely used at fish farms, such as abdominal rigidity and papilla characteristics, are not enough or reliable for a selection of suitable females, which should depend on more accurate techniques. Moreover, the evaluation of abdominal thickness and application of cannulation techniques are reliable, since they are in accordance with each other and with a histological examination of the ovaries (which reflects the ovaries directly, and does not rely on extrapolation or estimation). Old females presented higher frequencies of larger diameter oocytes compared to YFs. On the other hand, OFs showed reduced rates of 17α - OHP during the reproductive phase, which may be associated with a reduction of its steroidogenic capacity. Finally, we conclude that *P. mesopotamicus* females of different ages maintained in captivity had similar

reproductive cycles and spawning seasons in this study; therefore, except for individual particularities, errors in the selection of suitable females for hormonal induction probably happen more due to the use of inaccurate techniques than to fish age.

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REFERENCES

ARANTES, F.P.; SANTOS, H.B.; RIZZO, E.; SATO, Y.; BAZZOLI, N. 2010 Profiles of sex steroids, fecundity, and spawning of the curimata-pacu *Prochilodus argenteus* in the São Francisco River, downstream from the Três Marias Dam, Southeastern Brazil. *Animal Reproduction Science*, *118*: 330-336.

BARBIERI, E. e BONDIOLI, A.C.V. 2013 Acute toxicity of ammonia in Pacu fish (*Piaractus mesopotamicus* , Holmberg, 1887) at different temperatures levels. *Aquaculture Research*, *46*(3): 565-571.

BERKELEY, S.A.; CHAPMAN, C.S.; SOGARD, M. 2004 Maternal age as a determinant of larval growth and survival in marine fish, *Sebastes melanops*. *Ecology*, *85*: 1258-1264.

BLANCHFIELD, P.J. and RIDGWAY M.S. 2005 The relative influence of breeding competition and habitat quality on female reproductive success in lacustrine brook trout (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences, 62*: 2694-2705.

BLAXTER, J.H.S. and HAMPEL, G. 1983 The influence of egg size on Herring larvae (*Clupea harengus* L.). *Journal du Conseil / Conseil Permanent International pour l'Exploration de la Mer, 28*: 211-240.

BOBE, J. and LABBÉ, C. 2010 Egg and sperm quality in fish. *General and Comparative Endocrinology, 165*: 535-548.

BRASIL 2008–2009 Ministério da pesca e Aquicultura. 2010 Sinpesq, Boletim Estatística da Pesca e Aquicultura, Brasília, 129p.

CIERESZKO, R.E.; DABROWSKI, K.; CIERESZKO, A.; OTTOBRE, J.S. 1998 Plasma concentrations of steroid hormones in male yellow perch, *Perca flavescens*: the effect of age and photothermal manipulation. *Environmental Biology of Fishes 51*: 97-105.

CRISCUOLO-URBINATI, E.; KURADOMI, R.Y.; URBINATI, E.C.; BATLOUNI S.R. 2012 The administration of exogenous prostaglandin may improve ovulation in pacu (*Piaractus mesopotamicus*). *Theriogenology, 78*: 2087-2094.

DABROWSKI, K.; RINCHARD, J.J.S.; OTTOBRE F.; ALCANTARA P.P.; CIERESZKO, A. 2003 Effect of oxygen saturation in water on reproductive performances of pacu, *Piaractus brachypomus*. *Journal of the World Aquaculture Society, 34*: 441-449.

DE SOUZA, T.G.; HAINFELLNER, P.; KURADOMI, R.Y.; MUÑOZ, M.E.; HONJI, R.M.; MOREIRA, R.G.; BATLOUNI, S.R. 2015 Inappropriate management conditions, especially for the regressed class, are related to sperm quality in *Prochilodus lineatus*. *Theriogenology, 83*: 797-807.

FITZHUGH, G.R.; SHERTZER, K.W.; KELLISON, G.T.; WYANSKI, D.M. 2012 Review of size-and age-dependence in batch spawning: implications for stock assessment of fish species exhibiting indeterminate fecundity. *Fishery Bulletin, 110*: 413-425

HAINFELLNER, P.; DE SOUZA, T.G.; MUÑOZ, M.E.; FREITAS, G.A.; BATLOUNI, S.R. 2012a Spawning failure in *Brycon amazonicus* may be associated with ovulation and not with final oocyte maturation. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia, 64*: 515-517.

HAINFELLNER, P.; DE SOUZA, T.G.; MOREIRA, R.G.; NAKAGHI, L.S.O.; BATLOUNI, S.R. 2012b Low estradiol levels, delayed vitellogenesis and reduced amounts of yolk are dysfunctions associated with the formation of low quality oocytes in *Prochilodus lineatus* (Teleostei:

Characiformes). *Neotropical Ichthyology, 10*: 601-612.

KAMLER, E. 2008 Parent – egg – progeny relationships in teleost fish: as energetic perspective. *Reviews in Fish Biology and Fisheries, 15*: 399-421.

KENNEDY, J.; GEFFEN, A.J.; NASH R.D.M. 2007 Maternal influence on egg ad larval characteristics of plaice (*Pleuronectes platessa L.*). *Journal of Sea Research, 58*: 65-77.

KURADOMI, R. Y.; DE SOUZA, T. G., FORESTI, F.; SCHULZ, R.W.; BOGERD, J.; MOREIRA, R.G.; FURLAN, L.R.; ALMEIDA, E.A.; MASCHIO, L.R.; BATLOUNI, S.R. 2016 Effects of re-stripping on the seminal characteristics of pacu (*Piaractus mesopotamicus*) during the breeding season. *General and Comparative Endocrinology, 225*: 162-173.

LIMA, R.V.A.; BERNARDINO, G.; VAL-SELLA, M.V. 1991 Tecido germinativo ovariano e ciclo reprodutivo de pacu *(Piaractus mesopotamicus,* Holmberg, 1887) mantidos em cativeiros. *Boletim Técnico do Cepta, 4*: 1-46.

MARTEINSDOTTIR, G. and STEINARSSON, A. 1998 Maternal influence on the size and viability of Iceland *Gadus morhua* eggs and larvae. *Journal of Fish Biology, 52*: 1241-1258.

MYLONAS, C.; FOSTIER, C.A.; ZANUY, S. 2010 Broodstock management and hormonal manipulations of fish reproduction. *General and Comparative Endocrinology, 165:* 516-534.

PEREIRA, T.S.B.; MOREIRA, R.G.; BATLOUNI, S.R. 2013 Dynamics of ovarian maturation during the reproductive cycle of *Metynnis maculatus*, a reservoir invasive fish species (Teleostei: Characiformes). *Neotropical Ichthyology, 11*:821-830.

PEREIRA, T.S.B, BOSCOLO, C.N.P.; FELÍCIO, A.A., BATLOUNI S.R.; SCHLENK, D.E.; ALMEIDA, E.A. 2016 Estrogenic activities of diuron metabolites in female Nile tilapia (*Oreochromis niloticus*). *Chemosphere*, *146*: 497-502.

ROLLINSON, N. and HUTCHINGS, J.A. 2010 Why does egg size increase with maternal size? Effects of egg size and egg density on offspring phenotypes in Atlantic salmon. Evolutionary *Ecology Research, 12*: 949-960.

SAS INSTITUTE. 1990 SAS/STA User's guide: version 6, 4th ed. Cary, NC, SAS Institute.

STRATMAN, A. and TABORSKY, B. 2014 Antipredator defences of young are independently determined by genetic inheritance, maternal effects and own early experience in mouthbrooding cichlids. *Functional Ecology, 28:* 944-953.

TRIPPEL, E.A. and MORGAN M.J. 1994 Age-specific paternal influences on reproductive success in Atlantic cod (*Gadus morhua*) of the Grand Banks, Newfoundland. *ICES Journal of Marine Science, 198*: 414-422.

VLADIĆ, T. and PETERSSON, E. 2015 Relationship between size, age and maturity in atlantic salmon. In: VLADIĆ, T. and PETERSSON, E. Evolutionary Biology of the Atlantic Salmon. CRC Press, Boca Raton. p. 165-184.

WIELAND, K, JARRE-TEICHMANN, A.; HORBOWA, K. 2000 Changes in the timing of spawning of Baltic cod: possible causes and implications for recruitment. *ICES Journal of Marine Science, 57*: 452-464.

YARON, Z. and LEVAVI-SIVAN, B. 2011 Endocrine regulation of fish reproduction. In: FARRELL, A. P. Encyclopedia of fish physiology: from Genome to environment. Academic Press, San Diego. p. 1500-1508.