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Original article

Mobilization of energetic substrates in the endangered catfish *Steindachneridion parahybae* (Siluriformes: Pimelodidae): changes in annual reproductive cycle in captivity

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This study aimed at analyzing the energetic substrate (ES) in the main storage tissues of *Steindachneridion parahybae,* throughout the reproductive cycle in captivity. Differently from wild, in captivity, feeding is not interrupted during the reproductive period, the females do not spawn spontaneously, and they are sedentary. Adult females were sampled monthly and based on their histology and gonadosomatic index (GSI), ovaries were classified into: previtellogenic (PRV), vitellogenic (VTG), and regression (REG) stages. Ovaries at the VTG stage showed higher protein and lipids levels than at the PRV stage with a positive correlation between these substrates and the GSI. Muscle was the main source of proteins transferred to the ovaries, according to the negative correlation between these organs. Lipids remained unchanged in the liver, which is an important supplier in vitellogenesis, a pattern that probably occurs due to the continuous feeding. Muscular glycogen levels were higher at the VTG and REG than at the PRV stages. Plasma triglycerides were also higher during REG, while glucose levels were more elevated during the VTG stage. These results suggest that with constant food supply, the pattern of deposition of ES in *S. parahybae* is different from that described for other wild potamodromous species.

Keywords: Glycogen, Lipids, Metabolic substrate, Protein, Reproductive cycle.

O objetivo deste estudo foi analisar a composição do substrato energético (SE) nos principais tecidos de armazenamento de *Steindachneridion parahybae,* durante todo o ciclo reprodutivo em cativeiro. Diferentemente do ambiente natural, em cativeiro, a alimentação desses animais não é interrompida durante o período reprodutivo, as fêmeas não desovam espontaneamente, e são sedentárias. Fêmeas adultas foram amostradas mensalmente e baseada na histologia e no índice gonadossomático (IGS), os ovários foram classificados: estádios pré-vitelogênico (PRV), vitelogênico (VTG) e regressão (REG). Os ovários no estádio VTG apresentaram uma maior concentração de lipídeos e proteínas em relação ao estágio PRV. Esses substratos correlacionaram-se positivamente com o IGS. O músculo foi a principal fonte de proteína transferida aos ovários, como foi confirmado pela análise de correlação negativa entre esses órgãos. Os lipídeos mantiveram-se inalterados no fígado, considerado um importante órgão fornecedor de lipídeos para a vitelogênese, padrão que possivelmente ocorreu devido à contínua alimentação. A concentração do glicogênio muscular foi mais elevada durante os estágios VTG e REG em relação ao PRV. A concentração de triglicerídeos plasmática apresentou maiores valores no estádio REG enquanto a concentração de glicose no plasma foi maior durante os estádios VTG. Esses resultados sugerem que com alimentação constante, as fêmeas de *S. parahybae* apresentam um distinto padrão de mobilização dos substratos energéticos em relação ao que já foi descrito para outras espécies potamódromas de ambiente natural.

Palavras-chaves: Ciclo reprodutivo, Glicogênio, Lipídeos, Proteínas, Substrato metabólico.

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Introduction

Several studies have used energetic substrates in teleosts as a model to evaluate storage dynamics and mobilization in fish life cycle under different conditions. These studies evaluated, for instance: (1) the reproductive cycle or reproductive performance of fish in captivity (Izquierdo *et al.,* 2001); (2) larval, juvenile, and adult nutrition (Sargent *et al*., 1995, 1999; Izquierdo *et al*., 2001; Andrade *et al*., 2010; Araújo *et al*., 2012); (3) smoltification (Björnsson *et al*., 2011); (4) trophic interrelationship (Lowe-McConnell, 1999; Bittar *et al.,* 2012); and (5) effects of pollutants on energetic substrates (Lima *et al.,* 2011; Vieira *et al*., 2013). Besides, in several fish species that are candidates for aquaculture, deposition of adequate energetic substrates in the tissues, as liver and ovaries, are essential for a good reproductive performance, successful egg mass, and fingerling production (Fernández-Palacios *et al*., 2011).

Studies on the improvement of broodstock nutrition show that protein and lipid (including fatty acid) mobilization significantly improves not only egg and sperm quality but also larvae production (Fernández-Palacios *et al*., 2011; Araújo *et al*., 2012). Mobilization patterns include the preferential accumulation of energetic substrates among different teleost species. These mobilization patterns change according to reproductive conditions and strategies of oocyte development (Soengas *et al*., 1993; Sheridan, 1994; Cerdá *et al*., 1995; Schreck *et al*., 2001; Jerez *et al*., 2006). Throughout the reproductive cycle, there are seasonal changes in the biochemical composition (water, lipids, glycogen, and proteins) of fish tissues, especially in females, in which the hepatic metabolism is stimulated during vitellogenesis (Lubzens *et al*., 2010).

In fish, changes in tissue biochemical composition have been related to gonadal weight, which could reach 15 to 45% of body mass in females and 3.7% in males (Tyler, Sumpter, 1996). This increase in gonadal mass observed in females is primarily due to vitellogenesis, triggered by the vitellogenin synthesis (Jalabert, 2005; Reading, Sullivan, 2011). Vitellogenin is produced in the liver under the regulation of the gonadal steroid 17β-estradiol, and incorporated to the oocytes as yolk proteins, providing the main nutrients required for larval development (Reading, Sullivan, 2011). During this process, there is usually a decrease in lipids from the liver and adipose tissue (Moreira *et al*., 2002; Blanchard *et al*., 2005; Jalabert, 2005) and proteins, which are mobilized to the ovaries development. According to theoretical studies, the energetic need during vitellogenesis corresponds to two-thirds of the energy spent during spawning (Pecquerie *et al*., 2009), which shows the high metabolic cost of this process. Additionally, in migratory fish, such as salmonids, migration is accompanied by a hypophagic state, *i.e*, the animals do not eat (Hochachka, Somero, 2002), and energetic substrates (initially lipids, then proteins, and at last carbohydrates) are mobilized to sustain migration (Mommsen *et al*., 1980). However, when there is no longer a migratory behavior, as in captivity, migratory fish especially potamodromous species, do not stop eating before the reproductive season, and commonly the females do not spawn (Honji *et al*., 2009).

Steindachneridion parahybae (Steindachner, 1877), locally known as *surubim-do-Paraíba*, is an example of potamodromous teleost. It is a Neotropical catfish (gonochoristic, medium-sized), endemic to the Paraíba do Sul River Basin, critically endangered in the Brazilian red list, and considered regionally extinct in the state of São Paulo (Garavello, 2005; Caneppele *et al*., 2009; Honji *et al*., 2009, 2012, 2013, 2015, 2016, 2017). Besides this critical situation, the maintenance of *S. parahybae* female broodstock in fish farms is not entirely successful, as females do not reproduce when reared in captivity, *i.e,* females fail to ovulate or spawn naturally (Honji *et al*., 2015), despite their continuous feeding. Since 2003, the energy company *Companhia Energética de São Paulo* (CESP) has funded studies on the biology of *S. parahybae*. These studies include the maintenance and management of broodstocks in captivity, egg and sperm quality, and successful larval rearing (Honji *et al*., 2009, 2012, 2013, 2015, 2016, 2017; Sanches *et al*., 2013, 2014, 2015). They aim at ensuring successful and controlled breeding programs, involving conservation actions and a fish restocking program.

In the last few years, our research group and collaborators have advanced the knowledge on fundamental aspects of the reproductive biology of *S. parahybae* in captivity (Caneppele *et al*., 2009, 2015; Honji *et al*., 2012, 2013, 2015, 2016, 2017; Sanches *et al*., 2013, 2014, 2015; Lopes *et al.,* 2015). Knowledge of mobilization and deposition of energetic substrates on different tissues linked to their function in the reproductive cycle is required to understand the energetic requirement of *S. parahybae*, support further studies on broodstock nutrition, and contribute to the conservation of the species. Hence, the objective of the present study was to describe the energetic substrate deposition in the main reserve tissues in adult female *S. parahybae* in captivity throughout the reproductive cycle. Our hypothesis is that the absence of a hypophagic state in captivity changes the pattern of energetic substrate mobilization observed in wild migratory species.

Material and Methods

Experimental design and collection of animals. The experiment was carried out at the *Unidade de Hidrobiologia e Aquicultura, Companhia Energética de São Paulo* (CESP) located in Paraibuna, state of São Paulo, southeastern Brazil. Adult specimens of *S. parahybae* (F1) used in our study came from artificially induced reproduction made with wild broodstock (F0) at the same unit (Caneppele *et al*., 2009). In December 2007, one hundred *S. parahybae* females born and raised in CESP were selected on the basis of the typical morphological characteristics of sexual ripeness, according to the principles previously established for this species (Caneppele *et al*., 2009). Briefly, the females were selected

by the external characteristics, as the hyperemic genital pore and swollen abdomen. Males were chosen according to the presence of running sperm when the abdominal region was gently massaged. Then, they were randomly divided into two ponds (200 m^2) (duplicate) with concrete walls and a sandy bottom. During the experiment, the broodstocks were fed with commercial extruded feed for carnivorous fish containing 40% of crude protein at a rate of 2% biomass/day, offered twice a day (according to CESP fish farm routine), at 08:00 and 16:00h, following previously studies (Honji, 2011; Honji *et al*., 2012, 2013, 2016). During the experiment, the water temperature was 21.10 ± 0.14 ºC and the dissolved oxygen was 7.58 ± 0.36 mg/L, without significant variation between tanks. The concentration of these two parameters was monitored with an oximeter, Horiba-ModU10.

Monthly, from January 2008 to March 2009 (except June, July and August, winter months), four animals were randomly picked from the ponds (four animals/pond, eight in total, in each sampling event), and transported to the CESP laboratory. We chose not to handle the animals in the fish ponds during these months to avoid the stress and emergence of diseases, such as the one caused by the ciliate *Ichthyophthirius multifiliis*, avoiding the known risks of mortality during these months (Hurst, 2007). Fish were anesthetized with 0.1% benzocaine (ethyl-p-aminobenzoate) following the literature (Gilderhus, 1990; Morato-Fernandes *et al.,* 2013); and each animal had the total length (cm) and total body weight (g) recorded. Then, fish were killed by decapitation at the level of the operculum and dissected. After that, ovaries and liver were quickly removed and weighed. Gonadosomatic (GSI) and hepatosomatic (HSI) index was estimated for each individual. GSI is expressed as the percentage of body weight related to ovaries [GSI = (gonad weight/ total weight) x 100], whereas HSI is the percentage of body weight represented by the liver [HSI = (liver weight/ total weight) X 100] (Vazzoler, 1981, 1996). Plasma, ovaries, liver, epaxial muscle samples were collected and stored at -80ºC until the analysis of energetic substrates. The present study was conducted in compliance with the Animal Ethics Committee of the Institute of sciences, Biosciences, University of São Paulo (Protocol 072/2008). Additionally, some examined specimens were deposited in the collection of the Laboratory of Metabolism and Reproduction of Aquatic Organisms (*Laboratório de Metabolismo e Reprodução de Organismos Aquáticos* - LAMEROA) of the Institute of Biosciences of University of São Paulo (IBUSP) (number 01, 02), and animals from the same production batch were collected and stored in the fish collection of the Zoological Museum of São Paulo University - MZUSP, with catalog number 100672, 108433, 122965, and 122966.

To determinate gonads maturity stage, GSI and histological analyzes of the ovaries were used. From the GSI analysis three stages of gonadal development could be observed as described by Honji (2011): previtellogenic (PRV), vitellogenic (VTG), and regression (REG). The pattern of adipose tissue deposition was observed macroscopically; however it was not possible to perform a quantitative analysis.

Energetic substrates. Total proteins from the tissues (epaxial muscle, ovaries, and liver) were extracted with perchloric acid 6% (precipitation) and potassium hydroxide 2.5% (solubilization), following Milligan, Girard (1993). Tissue and plasma proteins levels were determined by the colorimetric method of Lowry *et al*. (1951), using bovine serum albumin as standard (Bovine serum albumin, Sigma Diagnostics, St. Louis, MO, USA), measured at 660 nm, and expressed as mg/g.

Total lipids from the epaxial muscle, ovaries and liver were extracted with a mixture of chloroform, methanol, and water (2:1:0.5), following Folch *et al.* (1957) modified by Parrish (1999) for aquatic organisms. Next, the total lipid content in tissues and plasma was quantified by the enzyme-colorimetric method, following Frings *et al*. (1972), using cod liver oil (Cod liver oil fatty acid methyl esters, Sigma Diagnostics, St. Louis, MO, USA) to yield the standard curve, measured at 540nm, and expressed as mg/g.

Glycogen level from the epaxial muscle and liver was extracted with potassium hydroxide (1mL; 6N), following Bidinotto *et al*. (1997). Glycogen levels were quantified following DuBois *et al*. (1956), with the hydrolytic method using D-glucose (Sigma Diagnostics INS) as the standard curve at 480nm and expressed in µmols glucose/g.

Plasma glucose level was measured using a Glucose Monoreagent kit (Bioclin®) with colorimetric enzymatic reaction, following the manufacturer's guidelines. The reading was performed on a microplate reader (Spectra Max 250, Molecular Devices) at a wavelength of 505 nm. The plasma cholesterol and triglycerides level were analyzed with a Cholesterol and Triglycerides Monoreagent kit (Bioclin®) also with colorimetric enzymatic reaction, following the manufacturer's guidelines. The reading was performed on a microplate reader (Spectra Max 250, Molecular Devices) at a wavelength of 500 nm for both.

Statistical analysis. Energetic substrates (lipids, protein, glycogen, glucose, triglycerides and cholesterol) from each tissue analyzed (liver, muscle and ovaries) and plasma were compared according to the maturation stage (PRV, VTG, and REG), using one-way analysis of variance (ANOVA), followed by Bonferroni Test. The statistical difference was significant when $P < 0.05$. The analyses were made in the statistical software SigmaStat for Windows (Advisory Statistics for Scientists, Systat Software Inc, Copyright, version 3.10). The energetic substrates concentration from tissues, the values of GSI and HSI were transformed to log10 to normalize the data and the Pearson Correlation Test was applied (BioEstat Statistical Program, version 8.0). Data were expressed as mean ± standard error (M± SEM).

Results

Tab. 1 presents the biometrical parameters (total length and total body mass), HSI, and GSI index; tissues and plasma energetic substrate levels during annual reproductive cycle (PRV, VTG, and REG stages). Females consumed all the feed given (2% of the biomass, daily) during the reproductive cycle, without any hypophagic period. In addition, all females maintained a high deposition of adipose tissue along the digestive tract, throughout the year. The GSI increased from the PRV to the VTG stage ($P < 0.01$) and decreased at the REG stage ($P < 0.01$). The HSI did not presented significant statistical difference during the reproductive cycle ($P = 0.349$).

Tab 1. *Steindachneridion parahybae*: Biometrical parameters (total length and total body mass); hepatosomatic (HSI) and gonadosomatic (GSI) index; tissues and plasma energetic substrate levels during annual reproductive cycle (PRV: previtellogenic; VTG: vitellogenic; and REG: regression stages). Data are presented as the mean ± standard error of the mean. *n* total number of individuals analyzed; TL - total length; TW - total body mass; GSI - gonadosomatic index; HSI - Hepatosomatic index. [ab] Statistical difference among reproductive stages ($P < 0.05$).

	Parameters/Tissues	PRV (*n*=20)	VTG (*n*=12)	REG (*n*=8)
Biometric parameters	TL (cm)	41.30 ± 0.48	40.04 ± 0.75	38.09 ± 0.72
	TW (g)	698.58 ± 30.02	692.46 ± 40.17	534.45 ± 28.37
	GSI (%)	0.52 ± 0.07[a]	1.68 ± 0.36[b]	0.90 ± 0.10[a]
	HSI (%)	0.70 ± 0.04	0.78 ± 0.05	0.76 ± 0.05
Proteins	Muscle (mg/g)	327.36 ± 31.13[a]	184.89 ± 25.94[b]	269.82 ± 15.90[ab]
	Ovaries (mg/g)	209.66 ± 8.74[a]	294.89 ± 18.45[b]	281.33 ± 11.72[b]
	Liver (mg/g)	118.74 ± 5.09	112.65 ± 10.69	135.69 ± 11.61
Lipids	Muscle (mg/g)	5.33 ± 0.97	4.41 ± 0.79	5.58 ± 0.85
	Ovaries (mg/g)	17.58 ± 2.44[a]	33.52 ± 2.73[b]	24.18 ± 3.01[ab]
	Liver (mg/g)	14.75 ± 2.23	15.66 ± 2.55	15.00 ± 1.07
Glycogen	Muscle (m mols glucose/g)	3.23 ± 0.48[a]	6.63 ± 0.73[b]	10.86 ± 1.52[b]
	Liver (m mols glucose/g)	409.60 ± 58.08	448.88 ± 68.94	385.04 ± 41.91
Plasma	Lipids (mg/mL)	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00
	Proteins (mg/mL)	49.67 ± 1.14	48.55 ± 1.37	47.36 ± 1.60
	Triglycerides (mg/dL)	178.69 ± 8.53[a]	169.99 ± 14.89[a]	249.43 ± 43.96[b]
	Glucose (mg/dL)	49.39 ± 2.23[a]	69.75 ± 6.21[b]	66.50 ± 2.46[b]
	Cholesterol (mg/dL)	195.45 ± 11.00	190.15 ± 7.91	215.88 ± 15.78

Energetic substrates. Females at VTG and REG stages showed higher total protein concentration in ovaries than at PRV stage ($P < 0.001$). In the epaxial muscle, higher protein concentration was observed at PRV stage, decreased at VTG stage and remained unchanged during the REG stage ($P = 0.012$). Hepatic protein levels did not change during the annual reproductive cycle, as well as its total protein plasma level. Similarly to protein levels, females at VTG stage showed higher total lipids levels in ovaries than at PRV stage ($P < 0.001$). The lipid content remained unchanged during REG stage. Hepatic and muscular lipid levels did not change during the annual reproductive cycle, as well as its plasma level. Glycogen concentration was higher in *S. parahybae* muscle at VTG and REG stages than at PRV stage ($P < 0.001$). However, this substrate did not change in the hepatic tissue during the reproductive cycle. The results of plasma substrate levels showed that glucose was lower ($P < 0.001$) in PRV than VTG and REG stages. However, triglycerides levels in REG was higher ($P = 0.027$) than in other stages. Cholesterol did not show change throughout the reproductive cycle.

In the correlation analysis, ovarian proteins and lipids showed a positive correlation with GSI, whereas muscle protein showed a negative correlation with ovarian protein (Tab. 2). The HSI showed a positive correlation with hepatic glycogen, a negative correlation with hepatic protein and no correlation with GSI. Ovarian lipids showed a positive correlation with hepatic lipids (Tab. 2).

Tab. 2. *Steindachneridion parahybae*: Pearson correlation among metabolic parameters (total lipids, protein and glycogen) and somatic indexes (gonadosomatic (GSI) and hepatosomatic (HSI) indexes) and tissues (epaxial muscle, ovarian and liver) sampled throughout the reproductive period. Values that indicate correlations are presented ($P < 0.05$).

Lipids	Ovaries	Liver	GSI
Muscle	-	-	
Liver	0.67	-	
HSI	-	-	
GSI	0.54	-	
	Protein		
Muscle	-0.35	-	
HSI	-	-0.55	
GSI	0.81	-	
	Glycogen		
HSI	-	0.34	-

Discussion

In this study, we described the deposition of energetic substrates in the main reserve tissues throughout the reproductive cycle. We highlight that the absence of both, migration and the hypophagic phase, affected the pattern of substrate mobilization, especially regarding lipids.

Morphological indices, such as GSI and HSI, have been used to express the dynamics of energetic substrates in gonads and liver, respectively (Collins, Anderson, 1995). These indices, together with the gonadal histology (Vazzoler, 1996) and biochemical composition analyses can be quantitative indicators of the reproductive period and energetic investment in reproduction (Marcano *et al.,* 2007). GSI was higher in *S. parahybae* females with fully developed ovaries (VTG stage), whereas the lowest GSI value occurred at the PRV stage, which evidenced that ovaries performed vitellogenesis, even in captivity. The ovarian histological analyses in these animals confirmed this fact (Honji, 2011). In contrast, HSI remained unchanged throughout the reproductive cycle and did not correlate with GSI. In some wild fish species carnivorous an inverse correlation between GSI and HSI have been observed such as *Ctenolabrus rupestris* (Sayer *et al*., 1995), *Perca fluviatilis* (Blanchard *et al.*, 2005) and *Curimatella lepidura* (Alvarenga *et al*., 2006). It suggests an intense mobilization of energetic substrates from the liver to ovaries during vitellogenesis, when the feeding activity is reduced or interrupted (Bennemann *et al*., 1996). In captivity, *S. parahybae* was fed daily during the reproductive cycle. Therefore, this nutritional status can abolish the need for depletion of liver substrates, maintaining unchanged HSI values throughout the year, even with an intense increase in GSI.

Lipids and protein are the most important substrates involved in fish reproduction due to their role as structural and energetic source to the embryo development (Yaron, Sivan, 2006). The lipid content in ovaries, its positive correlation with the GSI, and histological findings (Honji, 2011) suggest the incorporation of this substrate in oocytes, through of vitellogenin or other lipoproteins of the yolk (Lubzens *et al.,* 2010). *Perca fluviatilis*, a carnivorous fish (Blanchard *et al*., 2005) and *Percophis brasiliensis*, an omnivorous species (Rodrigues *et al.*, 2013), importance of hepatic lipids in the process of vitellogenesis (Lubzens *et al.*, 2010), this energetic substrate did not change in *S. parahybae* females during the reproductive cycle, as also observed in the HSI and muscle lipids. These data suggest that lipids from liver and muscle could be transferred to oocytes, but due to the constant feeding, the content of this energetic substrate is rapidly recovered as described in compensatory growth (Ali *et al.*, 2003). Conversely, in *Diplodus sargus* (Pérez *et al.,* 2007) and *Merluccius merluccius* (Domínguez-Petit *et al.*, 2010) lipids increased in liver and muscle during the reproductive period, which was not characterized by reduced or interrupted feeding. So, in stable environments, dietary intake can be directly responsible for lipid accumulation in ovaries (Pérez *et al.,* 2007).

Similarly to lipids, protein content in ovaries was higher in females at the VTG stage, as a result of the yolk incorporation in oocytes (Jalabert, 2005). Besides, protein content was higher than lipids, as the vitellogenin incorporated in the oocytes shows a composition of 19% of lipids and 79% of proteins (Yaron, Sivan, 2006). Additionally, the positive correlation between the GSI and ovarian proteins and lipids evidenced the contribution of these substrates to development oocytes. In females at the VTG stage, muscle protein levels decreased and it was observed a negative correlation between muscle and ovarian protein suggesting a possible mobilization this substrate between these organs. Besides that, it is likely that proteins derived directly from the diet also contributed to the vitellogenin synthesis, even considering that the animals did not stop eating.

Hepatic substrates and the HSI did not change during the annual cycle. The variation of liver protein is not expected, because the liver has a major role in the synthesis, metabolism, and transport of this substrate but no a storage function for protein (Bruslé, Anadon, 1996). In *Salminus brasiliensis*, another tropical potamodromous species, the levels of hepatic protein did not change during the reproductive cycle in the wild (Moreira *et al.,* 2002). Lipids are considered the primary source for reproduction, especially in migratory fish. These highly energetic molecules, mainly in the form of triacylglycerol, are mobilized from the adipose tissue, muscle, and liver to be used in processes of high energetic demand, such as reproduction (Tocher, 2003). Lipids were incorporated in *S. parahybae* oocytes; however, the hepatic and muscular deposit did not change during the reproductive cycle, which suggests that the adipose tissue also was the major source of the lipids used in vitellogenesis even its feeding condition of the animals, a situation that maintained the adipose tissue deposits during the reproductive cycle. In the wild, reophilic fish increased the energy demand and showed a sharp reduction in food frequency during reproductive stages (Jonsson, Jonsson, 1993). Such situation did not occur in the present study; here, females maintained the same feeding activity (2% of body mass daily) throughout the year, and, consequently their adipose tissue stocks, reducing the role of liver and muscle as lipids sources for vitellogenesis.

The glycogen provides the energy necessary for a short-time rather than a long-time demand. The amount of glycogen stored in the liver depends on physical, chemical, and biological factors faced by the fish (Coban, Sen, 2011). These same authors also mention that the rapid movement, stress, and environmental hypoxia decrease carbohydrate reserves (first glycogen in the liver and muscle) but glycogen plays a minor role in oocyte development. In carnivorous species such as Gadus *morhua* (Lambert, Dutil, 1997), *Onchorhynchus mykiss* (Barciela *et al.,* 1993) and *Capoeta umbla* (Coban, Sen, 2011), liver glycogen and condition factor were changed during the reproductive season. These

results did not corroborate with the present study, where *S. parahybae* did not change hepatic glycogen throughout the reproductive cycle, probably due to the daily intake of food in captivity. However, this carbohydrate is accumulated in muscle during the REG stage.

Higher plasma levels of glucose were found in VTG and REG stages. In *Oncorhynchus mykiss*, also from captivity, the peak of plasma glucose was observed during maturation stage (Kocaman *et al.,* 2005), demonstrating that glucose seems to be important to vitellogenesis process; however there is little information about this substrate during the reproduction. Plasma triglycerides levels were also higher values in REG stage, different from *O. mykiss*, where the higher triglyceride values were found during pre-maturation and maturation stages (Kocaman *et al.,* 2005). These distinct results emphasize that the absence of spawning changed the substrates mobilization/deposition process, reflecting in a possible reabsorption process of triglycerides to the others tissues during REG stage.

In conclusion, in *Steindachneridion parahybae* the muscle is the main source of ovarian protein while hepatic energetic substrates remain unchanged. In the plasm the elevated level of glucose in VTG and REG seems to be important to vitellogenesis process and the higher triglycerides concentration in REG stage can be explained for absence of spawning changed the substrates mobilization/deposition process, reflecting in a possible reabsorption process of triglycerides to the others tissues. The process of mobilization of energetic substrates during the reproductive cycle in *S. parahybae* females, in captivity and with constant food supply, occurs at different way than described for other potamodromous fish, in the wild, corroborating our hypothesis. Muscle is the main source of ovarian protein while hepatic energetic substrates remain unchanged in *S. parahybae*.

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