BOLETIM DO INSTITUTO DE PESCA
Scientific Article

# Steroid profile and ovarian gene expression in *Genyatremus luteus* (Teleostei, Perciformes: Haemulidae) during sexual maturation

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## ABSTRACT

The aim of the present study was to assess histological features, sex steroid profile, and ovarian gene expression throughout the ovarian maturation of *Genyatremus luteus* in Brazilian Coastal Equatorial Amazon. Specimens were categorized into different gonadal maturation stages–immature (n = 7), maturing (n = 7), mature (n = 7), and spawned (n = 7)–based on histological features of ovarian follicles and on oocyte development. Plasma E2, 11-KT and 17α-OHP levels were measured through enzyme-linked immunosorbent assays. The gene expression of estrogen (*er*) and LH (*lhr*) receptors in the ovary was analyzed through real-time polymerase chain reaction. The highest plasma E2 concentration was found in mature specimens ($P < 0.05$). Plasma 17α-OHP concentrations have significantly increased from the immature to the final maturation stage ($P < 0.05$). Maturational reproductive stage did not have any effect on plasma 11-KT concentrations. *Er* expression in ovaries has significantly increased throughout ovarian maturation ($P < 0.05$), and it remained high at mature and spawned stages. *Lhr* expression showed similar trend throughout the maturation process, although its peak was observed at spawned stage ($P < 0.05$). In conclusion, the gene expression profile of *er* and *lhr* and plasma E2 and 17α-OHP concentrations are proportional to oocyte development; they play important role in regulating ovarian development and ovulation in *G. luteus*.

**Keywords:** Gonadotropin receptor; Estradiol; Gonads; Reproduction; Grunts.

## Perfil de esteroides e expressão gênica ovariana em *Genyatremus luteus* (Teleostei, Perciformes: Haemulidae) durante a maturação sexual

## RESUMO

O objetivo deste estudo foi avaliar as características histológicas, o perfil de esteroides sexuais e a expressão gênica ovariana ao longo do desenvolvimento da maturação ovariana de *Genyatremus luteus* na Amazônia equatorial costeira brasileira. Os espécimes foram categorizados em diferentes estágios de maturação gonadal – imaturo (n = 7), em maturação (n = 7), maduro (n = 7) e desovado (n = 7) –, com base nas características histológicas dos folículos ovarianos e no desenvolvimento do oócito. Os níveis plasmáticos de E2, 11-KT e 17α-OHP foram medidos por meio de ensaio de imunoabsorção enzimática. A expressão gênica dos receptores de estrogênio (*er*) e LH (*lhr*) no ovário foi analisada por reação em cadeia da polimerase em tempo real. A maior concentração plasmática de E2 foi encontrada em espécimes maduros ($P < 0,05$). As concentrações plasmáticas de 17α-OHP aumentaram significativamente desde o estágio imaturo até o estágio final de maturação ($P < 0,05$). O estágio reprodutivo maturacional não teve nenhum efeito nas concentrações plasmáticas de 11-KT. A expressão de *er* nos ovários aumentou significativamente ao longo da maturação ovariana ($P < 0,05$) e permaneceu alta nos estágios de maturação e desova. A expressão de *lhr* apresentou tendência semelhante ao longo do processo de maturação, embora tenha atingido seu pico na fase de desova ($P < 0,05$). Em conclusão, o perfil de expressão gênica de *er* e *lhr* e as concentrações plasmáticas de E2 e 17α-OHP são proporcionais ao desenvolvimento oocitário e desempenham papel importante na regulação do desenvolvimento ovariano e ovulação em *G. luteus*.

**Palavras-chave:** Receptor de gonadotrofina; Estradiol; Gônadas; Reprodução; Roncador.

**Received:** January 26, 2022 | **Approved:** August 28, 2023

## INTRODUCTION

Estuarine and marine teleost fish are important natural resources, mainly for the subsistence of riverside populations. However, these resources' sustainability is threatened by natural populations living in Coastal Equatorial Amazon due to overfishing (Santos and Santos, 2005). Thus, it is necessary conducting studies focused on investigating the reproductive biology of these fish species, in association with socio-environmental studies, in order to better substantiate future management and conservation actions to be taken to help maintaining aquatic ecosystems (Cochrane, 2002).

Fish belonging to family Haemulidae, as well as to order Perciformes, are distributed in this scenario. The family comprises approximately 145 species, which are distributed into 17 genera. Among them, one finds *Anisotremus*, *Conodon*, *Orthopristis*, *Pomadasys*, *Genyatremus* and *Boridae*, which are already listed for the Atlantic Ocean (Johnson, 1980; Nelson, 2006; Tavera et al., 2012).

Oocyte maturation and ovulation processes in teleost fish are entangled, similar to those of other vertebrates. These processes are primarily triggered by an endocrine signal featured by increased follicle-stimulating hormone (FSH) level (Lubzens et al., 2010). FSH acts in theca cells to convert cholesterol into testosterone. Then, testosterone is carried to follicular cells and aromatized in 17β-estradiol (E2) through the action of the aromatase cytochrome P450 enzyme, due to the action of FSH (Levavi-Sivan et al., 2010). E2 accounts for inducing vitellogenin and choreogenin production by hepatocytes (Senthilkumaran et al., 2004). Vitellogenin is carried through the bloodstream to the ovary, in which it is incorporated to oocytes during vitellogenesis (Matsubara and Sawano, 1995).

The steroidogenic pathway changes E2 into 17α-hydroxyprogesterone at the end of the maturation stage (17α-OHP). 17a-OHP production by theca cells is induced by FSH decrease; it is followed by LH increase and converted into maturation-inducing hormone, 17α-20β-dihydroxy-4-pregnen-3-one (DHP) or 17α-20β-trihydroxy-4-pregnen-3-one by 20β-hydroxysteroid-dehydrogenase enzyme (Nagahama and Yamashita, 2008; Lubzens et al., 2010). DHP enables the final oocyte maturation, which plays an essential role in ovulation and spawning (García-López et al., 2007; Nagahama and Yamashita, 2008; Honji and Moreira, 2017).

Achieving maturation and ovulatory competences in ovarian follicles of teleost fish requires orchestrated gene expression at the post-vitellogenic period, increase or decrease in the number of steroidogenic enzymes during estrogen transition to progestin (Nagahama and Yamashita, 2008), as well as changes in gonadotropin receptors, paracrine regulators, proteases (Bobe et al., 2004), and inflammation-related genes (Bobe et al., 2006; 2009). However, gene products associated with maturation and ovulatory competences can be also synthesized at early developmental stage as vitellogenic follicles sensitive to exogenous gonadotropin stimulation in vivo, as well as induced to final oocyte maturation and ovulation (Zohar and Mylonas, 2001).

Although few *G. luteus* (Bloch, 1790) biology aspects have already been investigated, studies about hormonal and molecular features associated with ovarian maturation development in this species remain scarce. Gene expression of estradiol (*er*) and LH (*lhr*) receptors in the ovary were analyzed through SYBR real-time polymerase chain reaction (PCR) assays. Plasma estradiol (E2), 11-ketotestosterone (11-KT) and 17α-hydroxyprogesterone (17α-OHP) levels were measured in enzyme-linked immunosorbent assays (ELISA) to feature endocrine changes during gonadal development in female *G. luteus* individuals. The analysis of ovarian stages development in female *G. luteus* specimens was based on macroscopic and histological features, according to Vazzoler (1996).

## MATERIALS AND METHODS

### Fish and sample collection

The present research was approved by the Ethics Committee on Animal Use of Universidade Federal do Maranhão, under protocol number 23115.004707/2017-50, as well as authorized by the Biodiversity Authorization and Information System, of the Instituto Chico Mendes de Conservação da Biodiversidade, under protocol number 66551.

Female *G. luteus* individuals were captured through artisanal fishing with hand line with two hooks, based on validated methodologies (Almeida et al., 2005; Marques et al., 2016). Sampling took place in São José Bay (02°43'03"S, 44°12'03" W), Maranhão State Coast, Amazon Equatorial Coast, Brazil, from June to September 2019.

Blood samples were collected by puncturing the fish gills with heparinized syringe (Liquemine, Roche®), right after their capture; they were centrifuged at 1,500 g for 10 min, and plasma was aliquoted and immediately frozen at -80°C, until hormonal analysis time. Subsequently, specimens were euthanized by exposing them to freezing cold water and subjected to laparotomy, right away, to enable collecting gonad fragments (5 × 5 mm) that were stored in RNAlater (Ambion®), in separate, and kept frozen at -20°C, for RNA extraction.

Fish were measured with ichthyometer at 1-mm measurement scale. The following biometric parameters were measured: total length (TL), which corresponded to the distance (in cm) from the tip of the cranial end to the end of the caudal fin; and partial length (PL), which corresponded to the distance from the tip of the cranial end to the last vertebra. Subsequently, total weight (TW) was measured in precision scale (accuracy = 0.01 g).

Gonads were analyzed based on macroscopic aspects, such as size, color, and vascularity; they were also excised and weighed to calculate the gonadosomatic index (GSI), by taking into consideration variables like body weight rate and total weight of ovaries, according to Eq. 1 (Vazzoler, 1996):

$$[GSI = (\text{gonad weight} / \text{body weight}) \times 100] \quad (1)$$

In addition, median region fragments of fish ovaries were collected for histological analysis, after fixation, in Bouin's solution and processing, based on routine histological methods (Yoshida, 1964). Females were categorized based on the macroscopic aspects of their gonads and on the histological configuration of ovarian follicles and oocytes; it was done by following the gonadal maturation scale developed for Hamulids, according to Shinozaki-Mendes et al. (2013), and adapted to four maturation stages, namely: immature (n = 7), maturing (n = 7), mature (n = 7), and spawned (n = 7).

## Steroid analyses

Plasma 17β-estradiol (E2), 17-α-hydroxy-progesterone (17α-OHP), and 11-ketotestosterone (11-KT) levels were determined through enzyme-linked immunoasorbent assays (ELISA) (IBL International, Hamburg, Germany, for E2 and 17α-OHP; and Cayman Chemicals Company, Michigan, United States of America, for 11-KT). Tests were carried out based on manufacturers' recommendations.

Pilot trials were initially conducted by using five dilutions (1:1, 1:2, 1:4, 1:8, and 1:16) in two samples, at different maturation stages (immature and mature); it was done to establish appropriate dilutions to detect assay limitations–1:2 dilution was established for assays comprising all three hormones. In addition, plasma samples were analyzed in duplicate, whereas test kits were validated by calculating the intra and inter-assay coefficients of variation (% CV). Assays' detection limits were 10.6 pg/mL for E2, 30 pg/mL for 17α-OHP, and 1.3 pg/mL for 11-KT. Absorbance measurements were performed in microplate reader (Spectra MAX 250). Plasma E2 and 17-OHP concentrations were determined at wavelength of 450 nm, and 11-KT concentrations were determined at wavelength of 405 nm. E2, T and 11-KT (pg/mL) sample concentrations were calculated according to a standard curve by applying a logit-log curve fit in the Eq. 2, as recommended by the manufacturer:

$$\text{LogitDO: log [DO / (100- DO)]} \quad (2)$$

## Total RNA isolation and reverse transcription

Total RNA was extracted from individual ovary samples in 1 mL of trizol (Invitrogen; Carlsbad, CA, United States of America) for *er* and *lhr* expression analysis, according to manufacturer's instructions. RNA pellet was dissolved in DEPC-treated water and quantified based on its absorbance at OD260/OD280. RNA integrity was assessed in 1.5% agarose gel. All RNA samples were treated with DNase (DNase I, Applied Biosystems, Washington, United Kingdom); cDNA was synthesized in 1.5-µg RNA by using the commercial kit SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, United States of America), according to the manufacturer's instructions, at final volume of 20 µL per reaction. Primers in this rapid reverse transcriptase kit for reverse transcription were Oligo-dT primers. cDNA was diluted 3-fold and stored at -20°C.

## Quantitative real-time polymerase chain reaction

Primers used for *lhr* and β-actin were described by Rhody et al. (2015) and Wang et al. (2009), respectively. Primers used for estrogen receptor were designed in Primer 3 software, based on the AB007453.1 sequence available in the National Center for Biotechnology Information database, as described in Table 1.

Duplicates of each cDNA sample were amplified by SYBR quantitative real-time PCR (qPCR) and followed by melting curve analysis to investigate whether each PCR product had only a unimodal dissociation curve. The qPCR was performed by using 2.5-µL cDNA, 10-µM specific primer pair for each gene and 12.5-µL SYBR Green qPCR SuperMix (Thermofisher, Carlsbad, CA, United States of America), at final volume of 25 µL per reaction.

The qPCR efficiency of each primer pair was assessed through standard curves from a graded series of diluted cDNA (3-fold; 9-fold; 27-fold; 81-fold; 243-fold) to ensure that the PCR efficiency of the selected primer pair ranged from 90 to 100% (R2 > 0.99).

The qPCR parameters were 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 1 min, in ABI 7500 Real-time PCR System (Applied Biosystems, United States of America). Data were analyzed based on the comparative Ct method (Livak and Schmittgen, 2001). Ct values were normalized based on β-actin expression (Wang et al., 2009).

**Table 1.** List of genes and primers used in quantitative polymerase chain reaction.

| Gene | Primers | Genbank Accession No. | Annealing Temperature (°C) |
|---|---|---|---|
| *lhr*-F | TCCTCCTGGTGTGGACCCAGTT | KF314819 | 62 |
| *lhr*-R | TCGGGTTGCAGGCTCTCAAAGG | | |
| *er*-F | ACATGTACCCCGAAGACAGC | AB007453.1 | 60 |
| *er*-R | CAGGGGTGGAGTGGCTATAA | | |
| β-actin-F | AATCGCCGCACTGGTTGTTG | AY190686 | 57 |
| β-actin-R | ACGATACCAGTGGTACGACC | | |

The cDNAs from *Genyatremus luteus* were subjected to conventional PCR to assess primers' specificities. Specific fragments were purified in Wizard SV Gel and sequenced in BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, United States of America), in ABI PRISM 3100 Genetic Analyzer. The *lhr* and *er* mRNA sequences obtained for *G. luteus* were confirmed by alignment with previously deposited sequences at GenBank using the BLASTN interface.

### Statistical analysis

Frequencies of each gonadal maturation stage were analyzed through Fisher's exact test, according to Sampaio (2002), in GraphPad Instat® software (GraphPad Software, San Diego, CA, United States of America).

The remaining data analyses were performed in Analysis System for Windows SAS® software (versão 9.4. SAS Inst. Inc., Cary, NY, United States of America). Shapiro-Wilk test (SAS Proc Univariate application) was used to investigate the residue normality of each variable. Data transformation was carried out, whenever necessary (logarithm to basis 10 – Log10 X), to meet analysis of variance assumptions.

Continuous dependent variables of normal distribution (ichthyological data) were expressed as mean and standard error of the mean (mean ± SEM). They were subjected to analysis of variance; means recorded for each gonadal maturation stage were compared to each other through Duncan's test (PROC GLM of SAS). E2, 17-OHP, and 11-KT concentrations, as well as differential cDNA expression for estrogen (*er*) and LH (*lhr*) receptors, presented non-normal distribution. Thus, the model was adjusted for Poisson distribution, and its associations with animals' maturational reproduction status were subjected to Poisson regression analysis (PROC GLIMMIX of SAS), according to Wang et al. (2014) and Svensson et al. (2019).

Association between variables was investigated based on principal component analysis (PCA) method, in Statistica 7.1 software, according to which two graphically produced axes represented the strongest data pattern. In other words, it explains the important role played by the two main components in total data variation. Significance level of 5% was adopted to reject H0 (null hypothesis), i.e., significance level lower than 0.05 has evidenced the effect of both classificatory variables and interactions among them.

## RESULTS

### Reproductive maturational stages and gonadosomatic index analyses

Macroscopic analysis has shown a pair of tubular gonads covered by fibrous connective tissue capsule (tunica albuginea), which defined the intraovarian limit typical of cystovarian species, whose oocyte visualization, in separate, was only possible at the most advanced maturity stages. It was possible observing different size, symmetry, color, consistency, and vascularity patterns in the ovaries of *G. luteus*, depending on their maturation status.

It was also possible microscopically identifying the investigated species as gonochoric due to evidence of unisexual gonads in all specimens, regardless of their reproductive maturation stage. Based on the morphology of ovigerous lamellae and oocytes, *G. luteus* specimens were classified into four maturation stages, namely: immature, maturing, mature, and spawned (Figs. 1a–1d). They were also featured as multiple-batch group synchronous species, since they simultaneously presented gonads with follicles and oocytes at different development stages (Fig. 2).

Total (TL), furcal (FL), and partial (PL) lengths of immature females were significantly shorter ($P < 0.05$) than those of maturing, mature and spawned females ($P > 0.05$) (Table 2). Spawned females have shown longer lengths, as well as higher total and eviscerated weight ($P < 0.05$).

Mature females presented gonadal weight higher than that of immature females ($P < 0.05$); these variables did not show statistically significant difference between animals at the mature and spawned stages. Immature females recorded GSI value 3.2 and 4 times lower than those of maturing and mature adult females, respectively ($P < 0.05$). Mature and maturing females did not differ from each other in any of the investigated ichthyometric variables ($P > 0.05$) (Table 2).

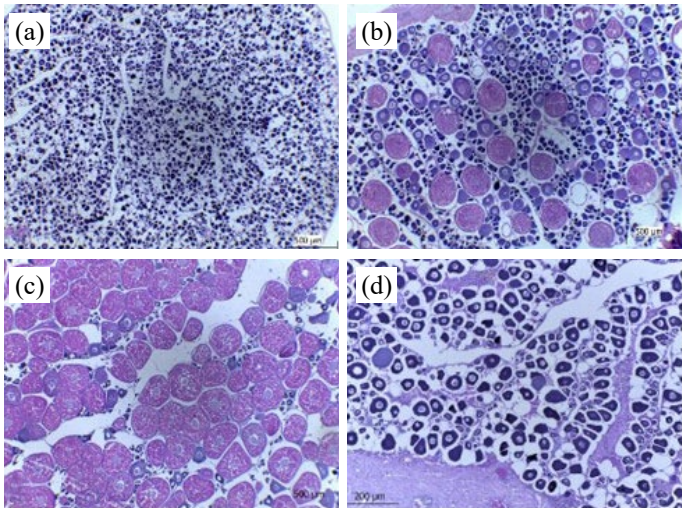



**Figure 1.** Histological sections of ovarian maturation stages in *Genyatremus luteus*. (a) immature; (b) maturing; (c) mature; (d) spawned. Hematoxylin and eosin 4x; 10x.

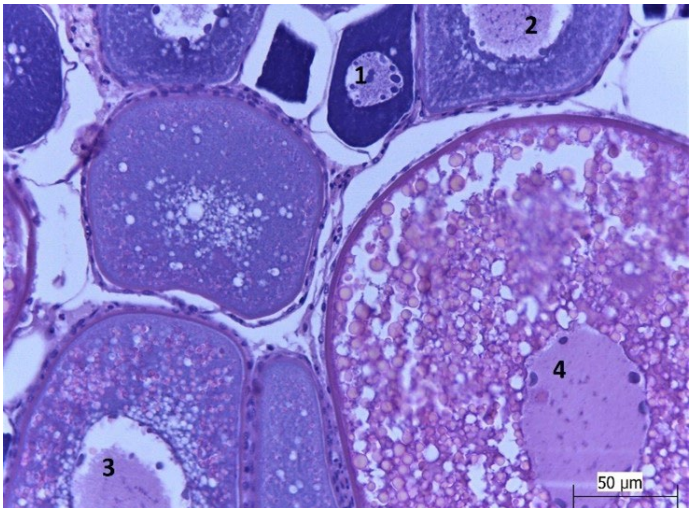



**Figure 2.** Histological sections of different oocyte developmental stages in *Genyatremus luteus*: (1) previtellogenic, perinucleolar; (2) previtellogenic, cortical alveoli formation onset; (3) early vitellogenic; and (4) mid-vitellogenic, found in a single ovary.

## Plasma steroids

The highest plasma E2 concentrations were found in mature specimens, whereas the lowest concentrations of it were found in spawned specimens ($P < 0.05$). On the other hand, reproductive maturation transitional process did not mean increased E2 concentrations in comparison to immature status ($P > 0.05$) (Fig. 3).

Plasma 17α-OHP concentrations have significantly increased from the immature to the final maturation stage, although they returned to the lowest concentrations in spawned females ($P < 0.05$) afterwards. There was no effect of reproductive maturation stage on plasma 11-KT concentrations in female *G. luteus* individuals.

## Gene expression profiles recorded for er and lhr

The *er* mRNA expression has significantly increased during ovarian maturation development ($P < 0.05$), and it remained high and stable in animals at mature and spawned stages, as shown in Fig. 4a. The LH receptor expression has shown similar trend throughout the maturation process. However, there was also significant increase in it from the mature stage onwards; it reached its peak at spawned stage ($P < 0.05$) (Fig. 4b).

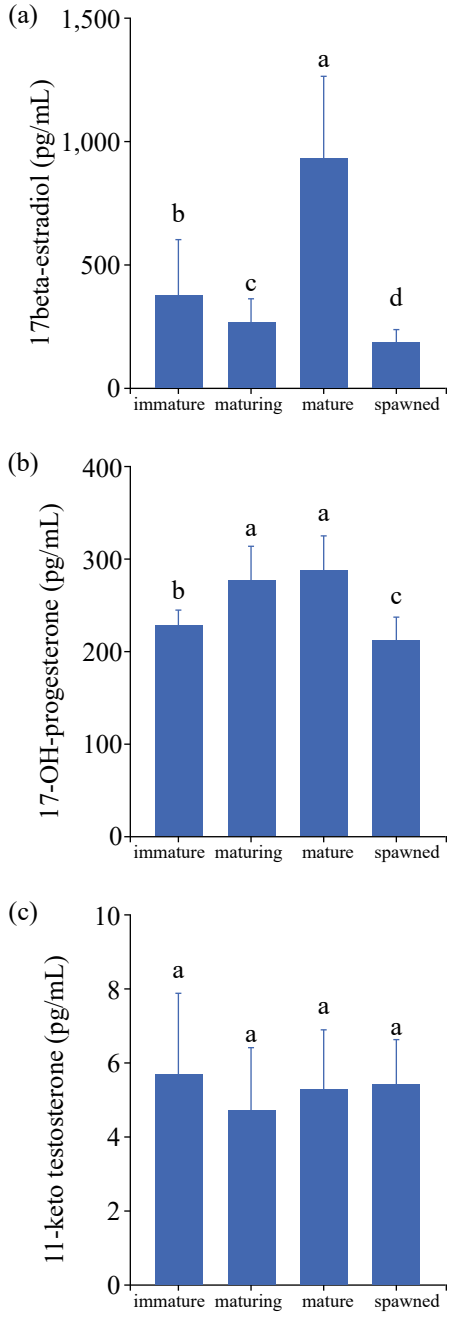
## Association among ichthyometric variables, gonadosomatic index, hormonal profile, and gene expression

Association among ichthyological parameters, gonadosomatic index, hormonal profile, and ovarian gene expression of *lhr* and *er* in female *G. luteus* individuals was analyzed through PCA (Fig. 5). Results have showed that the two main components, altogether, explained 65.7% total data variation, with emphasis on component 1, according to which ichthyometric variables were more representative due to longer vectors closer to component 1 axis.

**Table 2.** Ichthiometric and gonadosomatic index (GSI) data recorded throughout the reproductive cycle of female *Genyatremus luteus* individuals. Data were expressed as mean ± standard error of the mean*.

| Variable | Reproductive maturation stages | | | |
|---|---|---|---|---|
| | Imature | Maturing | Mature | Spawned |
| Total length (cm) | 18.35 ± 1.22$^{c}$ | 23.14 ± 1.73$^{b}$ | 24.07 ± 1.83$^{b}$ | 29.19 ± 0.24$^{a}$ |
| Furcal length (cm) | 17.21 ± 1.06$^{c}$ | 21.57 ± 1.50$^{b}$ | 22.57 ± 1.67$^{b}$ | 27.44 ± 0.23$^{a}$ |
| Partial length (cm) | 15.21 ± 1.06$^{c}$ | 19.28 ± 1.45$^{b}$ | 20.14 ± 1.54$^{b}$ | 24.11 ± 0.36$^{a}$ |
| Total weight (g) | 130.57 ± 36.63$^{c}$ | 238.57 ± 40.90$^{bc}$ | 267.14 ± 58.78$^{b}$ | 414 ± 14.11$^{a}$ |
| Eviscerated weight (g) | 118.42 ± 35.78$^{c}$ | 216.64 ± 37.00$^{bc}$ | 240.00 ± 50.28$^{b}$ | 379 ± 10.92$^{a}$ |
| Gonadal weight (g) | 1.07 ± 0.75$^{b}$ | 3.98 ± 1.60$^{ab}$ | 6.41± 2.75$^{a}$ | 3.44 ± 0.32$^{ab}$ |
| GSI (%) | 0.48 ± 0.19$^{b}$ | 1.54 ± 0.55$^{a}$ | 1.92 ± 0.46$^{a}$ | 0.84 ± 0.09$^{ab}$ |

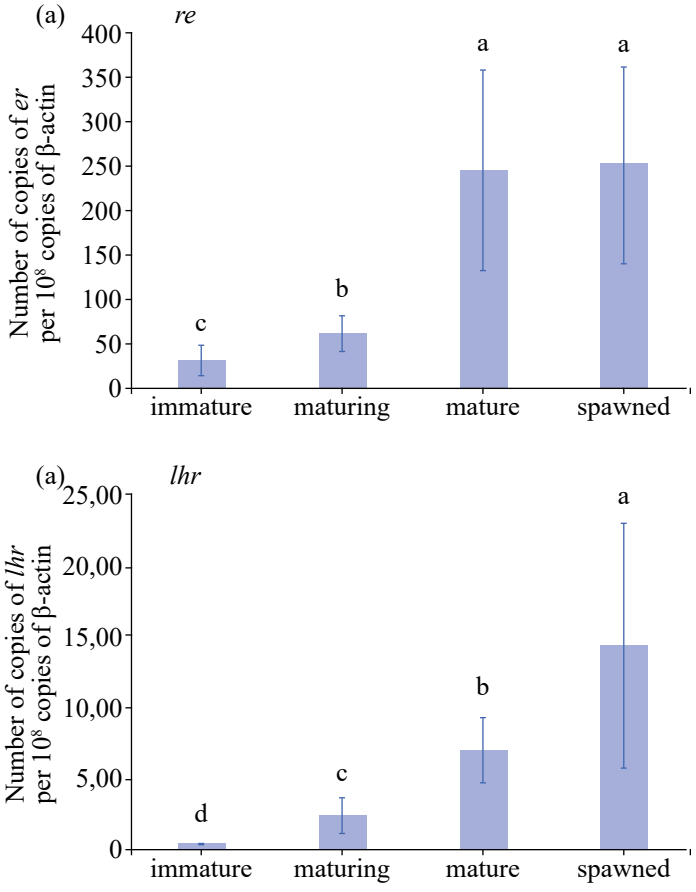
*Different letters on the same line indicate significant difference between reproductive stages ($P < 0.05$).




*Different letters indicate statistically significant difference ($P < 0.05$).

**Figure 3.** Plasma steroid concentrations throughout the reproductive cycle of female *Genyatremus luteus* individuals. (a) Plasma 17β-estradiol concentration. (b) Plasma 11-ketotestosterone concentration. (c) Plasma 17 α-hidroxyprogesterone concentration. Data were expressed as mean ± standard error of the mean.

Plasma E2, GW, and GSI concentrations were highly associated with each other, since they formed acute angles between their respective vectors, which were determined by the high representativeness of the mature stage for these parameters. Similarly, *er*, TW and TL expressions were also closely associated with each other, as well as positively correlated to *lhr* expression–the spawning stage was the most representative of these vectors. Based on Fig. 5, 17OHP and 11-KT concentrations were the variables that mostly contributed to component 2; they were inversely correlated to each other and did not present significant association with other variables.

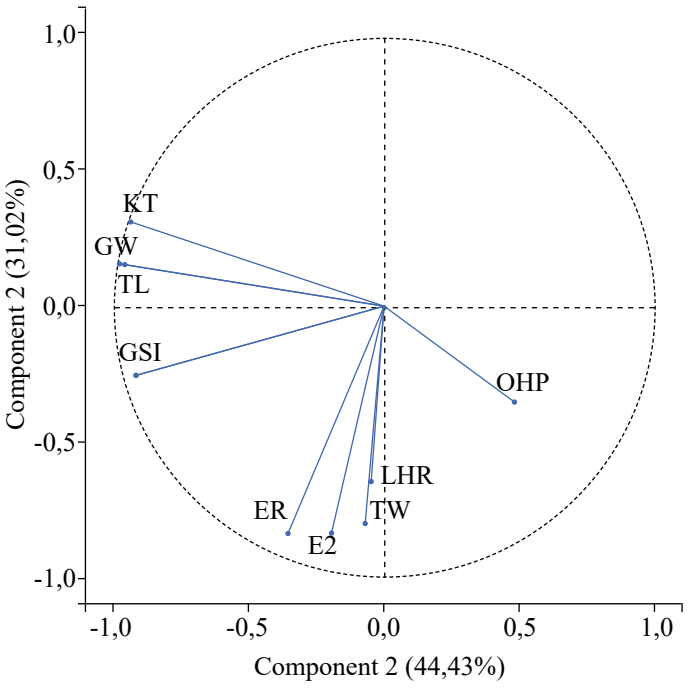



*Different letters indicate statistically significant difference ($P < 0.05$).

**Figure 4.** Gene in the gonad of *Genyatremus luteus* females at different maturation stages. (a) *er* expression. (b) *lhr* expression. Columns represent arithmetic mean (n = 7) and standard error*.

## DISCUSSION

Macroscopic assessment of *G. luteus* ovaries has shown that this organ has the same basic anatomical pattern as that of most teleosts. This finding met data about this species available in the literature (Noleto-Filho et al., 2012). Its featuring as gonochoric and cystovarian species with group-synchronous oocyte development and multiple-spawning–as evidenced, for the first




LHR: LH receptor; ER: estrogen receptor; KT: 11-ketotestosterone; E2: 17β-estradiol; OHP: 17-α-hydroxyprogesterone; TW: total weight; TL: total length; GW: gonad weight; GSI: gonadosomatic index.

**Figure 5.** Principal component analysis applied to classify the influence of *lhr* and *er* gene expression, ichthyological parameters, GSI, and maturation stage on variables assessed for *Genyatremus luteus* females.

time, in the current manuscript, according to findings detailed by Wallace and Selman (1981), Tyler and Sumpter (1996), and Blazer (2002)–is similar to another "grunt" species belonging to family Haemulidae, namely: *Haemulon plumierii* (Shinozaki-Mendes et al., 2013).

Sex differentiation based on the macroscopic features of developed gonads was evident in *G. luteus*; however, this visual classification did not apply to immature individuals due to their similarity to each other (small, translucent gonad with no apparent vascularization). Thus, the histological analysis enabled performing accurate assessment to differentiate animals' sexes and maturation stages to avoid identification-accuracy issues. *G. luteus* oocytes' development did not differ from that of most multiple-spawning teleost species (Wallace and Selman, 1981; Godinho et al., 2005; Amaral et al., 2019).

It was possible identifying oogonies and oocytes at all gonad maturation stages, including at the spawned stage, and it suggests that the investigated species is continuously available for vitellogenesis and reproductive maturation resumption. Cortical alveolar oocytes were identified at the subsequent oocyte development stage due to the incidence of spherical vacuoles that looked like empty structures–this stage is commonly called endogenous vitellogenesis.

Vitellogenic oocytes are featured by significant increase in size due to the incorporation of highly acidophilic granules, which accumulate inside the cytoplasm vesicles, through a process known as exogenous vitellogenesis (Peter and Yu, 1997; Yaron and Silvan, 2006). This oocyte development stage was essential to classify *G. luteus* gonadal stage as mature. The incidence of post-ovulatory follicles (POF) in the ovaries of *G. luteus* individuals was observed in spawned females; they are featured as structures formed after ovulation that comprise follicular cell layers that, after the expulsion of mature oocytes, keep on presenting broken and deflated appearance inside the ovaries after spawning.

As expected, ichthyometric and GSI values increased as ovarian development progressed. The lowest values were recorded for animals at immature stage, whereas the highest values were recorded for animals at mature stage, which showed normal gonadal development. According to Vazzoler (1996), ichthyometric values associated with GSI are a good reference for the reproductive activity of fish; they can be used to determine gonadal maturation stages according to Le Cren (1951), since the maturation of germ cells happens at the same time female gonad weight increases.

High plasma E2 levels were found in female *G. luteus* individuals during oocyte development; they reached their peak in animals at mature stage and decreased in animals at spawned stage ($p < 0.05$). Studies have shown that E2 acts directly on germ cell progression throughout early oogenesis stage; plasma E2 levels of 0.4 ng/mL were recorded during mitotic egg proliferation in Japanese huchen (*Hucho perryi*) and common carp (*Cyprinus carpio*) (Miura et al., 2007; Kazeto et al., 2011). This finding evidenced the fundamental role played by E2 in germ cell proliferation since the immature stage, which was also observed in the present study. In addition, E2 plays an essential role in vitellogenesis, since it stimulates hepatic vitellogenin synthesis, which promotes oocyte growth and yolk incorporation to it (Honji and Moreira, 2017; Reading et al., 2017). Increased E2 levels associated with high GSI levels in mature *G. luteus* individuals suggests the role played by this steroid in inducing hepatic vitellogenin synthesis. Variations in plasma E2 levels at different maturation stages were also reported in other multiple spawning teleost species, before and during spawning (Rinchard et al., 1997; Rahman et al., 2000; Amaral et al., 2019).

The present study has found increased plasma 17α-OHP levels in *G. luteus* from the immature to the mature stage; these levels have decreased in spawned females. Studies conducted in vitro have shown that 17α-OHP was converted into 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-DP) by 20β-hydroxysteroid dehydrogenase (20β-HSD) enzymatic action during the final oocyte maturation (FOM) stage (Matsuyama et al., 1998; Ohta et al., 2002; Matsuyama et al., 2005)–this outcome explains the decreased plasma 17α-OHP levels observed in animals at spawned stage. The 17α, 20β-DP is the main steroid acting in the maturation and final ovulation of oocytes in most teleosts; it is commonly known as maturation-inducing steroid (MIS) (Lubzens et al., 2010). In addition, 17α-OHP alternatively participates as precursor for its conversion into 17α, 20β, 21-trihydroxy-4-pregnen-3-one (20β-S) in teleost species, such as Atlantic croaker (*Micropogonias undulatus*), spotted seatrout (*Cynoscion nebulosus*) and bambooleaf wrasse (*Pseudolabrus sieboldi*) (Trant et al., 1986; Thomas and Trant, 1989; Matsuyama et al., 1998; Tokarz et al., 2015; Ogino et al., 2016).

Plasma 11-KT levels in the current study were low at all maturation stages. The highest levels (5.7 pg/mL) were found in immature specimens, and this finding suggests that pre-vitellogenic and vitellogenic oocyte development in *G. luteus* may not require high 11-KT concentrations. Thus, further studies focused on addressing some issues associated with the role played by androgens in the sexual development of females belonging to this species should be conducted.

Although 11-KT is considered androgen, some studies have shown that it is involved in controlling the growth of pre-vitellogenic oocytes in some teleost species (Lokman et al., 2007; Endo et al., 2008; Tosaka et al., 2010; Hermelink et al., 2011; Wang et al., 2020). Amaral et al. (2019) reported lower 11-KT levels in female *A. gigas* individuals than in other species, although these levels increased and reached their peak in animals at mature stage (71.54 pg/mL). The aforementioned authors have suggested that this hormone is not limited to vitellogenesis. It also acts in the final maturation/ovulation stages in this species.

Treatments conducted in vitro and in vivo with 11-KT in Atlantic cod (*Gadus morhua*) have accelerated the growth and development of pre-vitellogenic oocytes. This outcome has suggested the role played by this androgen in early oocyte growth in controlled environments (Kortner et al., 2009a; 2009b). On the other hand, Kohn et al. (2013) did not observe the effects of treatment with 11-KT on both ovarian development and total lipid concentrations in the liver of female *Polyprion oxygeneios* individuals. These controversial results were reported in different studies, according to which the role played by 11-KT in ovarian development is likely species-dependent.

The current results have shown gradual increase in *er* mRNA expression during gonadal development. Similar results were reported by Zapater et al. (2018) in studies conducted with *Dicentrarchus labrax*, which evidenced low ovarian *er* transcription during pre-vitellogenesis, although it increased during vitellogenesis and reached peak expression at mature stage. The regulation of *er* transcript levels may be associated with increased circulatory estradiol-17β (E2) levels during oocyte maturation stages (Silva et al., 2016). It happens because the activation of estrogen receptors depends on cell ligands (E2) (Edwards 2005; Thomas et al., 2006; Levin et al., 2009; Nagler et al., 2010). Thus, if one takes into consideration that 17β-estradiol (E2) is one of the main ligands for estrogen receptor, its greater expression indicates higher levels of this steroid in gonads. Kang et al. (2015) reported increased plasma E2 levels in female *Hapalogenys nitens* individuals, based on oocyte development and on changes in GSI. This factor induced vitellogenin production increase at the mature stage, maintained high levels of it during spawning and, consequently, reduced levels of it at post-spawning stage, as also observed in the current study. ERs can transduce E2 signals in the ovary to initiate or maintain ovarian development. Study conducted by Hou et al. (2018) with female *Oncorhynchus mykiss* individuals has shown positive correlation between increased serum E2 levels and *er* expression during ovarian maturation. The *er* expression level significantly affects the action of E2, since its high expression sensitizes and increases cell response to this hormone (Nelson and Habibi, 2010).

However, estrogens in teleosts and mammals have pleiotropic functions, which are not just limited to the reproductive axis, since they are found in several cell types and tissues, such as those in the immune system (Burgos-Aceves et al., 2016; Amenyogbe et al., 2020). Assumingly, most estrogenic actions are mediated by estrogen receptors (er) that belong to the family of steroid hormone receptors and that have different isoforms (ERα and ERβ) that are differentially regulated based on cell type (Nelson and Habibi, 2013). Casanova-Nakayama et al. (2018) have evaluated the gene expression of four *er* isoforms in immune system organs, liver, and ovaries during the reproductive cycle of female rainbow trouts; they reported differential transcription profile for each isoform, as well as changes in the number of transcripts in immune cells and organs, although they differed from those found in animals' liver and gonads. In addition, they found moderate-to-low correlation between the number of *er* gene transcripts and serum E2 concentrations. This outcome suggested that the low responsiveness of estrogens may encompass other

mechanisms, such as non-genomic pathways or indirect effects. Similarly, the current study has only observed this low correlation at the spawned stage in animals who presented decreased serum E2 levels, despite the high values recorded for *er* transcripts.

A previous study aimed at clarifying *er* function in the teleost model (*Oryzias latipes*), based on knockout technology. It has shown that *er2a* -/- females had atresic oviduct, in contrast to *er2a* +/- females that spawned every day and presented oviduct with full opening. This outcome confirmed *er* participation in the development of female genital morphology, mainly of the oviduct (Kayo et al., 2019).

The *lhr* expression profile started increasing at immature stage, kept on increasing through maturational development and reached its peak during spawning. Previous studies with female salmonid individuals have suggested that LH plays important role in regulating the final maturation and ovulation of semelparous individuals (Bobe et al., 2004; Yaron and Sivan, 2006; Sambroni et al., 2007). On the other hand, *lhr* synthesis in fish multiple-batch group synchronous reproduction presenting varying ovarian development levels (Wallace and Selman, 1981; Brown-Peterson et al., 2011) is likely required for different vitellogenesis stages, as well as for final oocyte maturation, which happens simultaneously. These previous studies have contributed to justify the result found in the current study, according to which there was increased *lhr* expression in female *G. luteus* individuals at the final maturation stage.

The *Lhr* expression in fish species presenting multiple spawning, such as *Dicentrarchus labrax* (García-López et al., 2011) and *Seriola dumerili* (Nyuji et al., 2016), increases at the same time oocytes develop to reach peak spawning. This fact was also observed in the current study, since *G. luteus* has synchronous spawning in multiple batches and presented residual oocytes at the final maturation stage, during the spawning period. *Oncorhynchus mykiss* recorded remarkable increase in *lhr* expression at the final vitellogenesis stage, and it persisted during oocyte maturation and ovulation. This outcome corroborates the interpretation that both LH and its receptor act in the final maturation of ovarian follicles and oocytes (Gomez et al., 1999). Studies have reported high plasma LH levels during the post-spawning period in rainbow trouts and *S. dumerili*, although their role remains unclear (Breton et al., 1998; Gomez et al., 1999; Nyuji et al., 2016), but it is assumingly associated with findings in the current study, according to which *lhr* expression in spawned females was also significantly high.

Significant association among ichthyometric parameters, plasma E2 and *er*/*lhr* expression has indicated regulation common to cell, endocrine and molecule features, as well as led to sexual maturation process in *G. luteus*. Liu et al. (2017) have shown that E2 stimulates *lhr* expression through *er* in *Danio rerio*'s ovary. Based on results in the current study, estrogen and LH receptors act together in the gonadal development of female *G. luteus* individuals, mainly at spawning stage, whose success depends on specific stimuli aimed at oocyte vitellogenesis and final maturation.

## CONCLUSION

The current study was pioneer in featuring different reproductive maturation stages in female *G. luteus* based on cellular, endocrine, and molecular aspects. It has shown that the gene expression profile of estrogen and LH receptors, as well as plasma E2 and 17α-OHP concentrations, are related to oocyte development, and that they play important role in regulating ovarian development and ovulation in *G. luteus.*

## CONFLICT OF INTEREST

Nothing to declare.

## DATA AVAILABILITY STATEMENT

All dataset were generated/analysed in the current study.

## AUTHORS' CONTRIBUTIONS

**Conceptualization:** Ribeiro DLS, Ribeiro LSS; **Methodology:** Ribeiro DLS, Ribeiro LSS, Silva JM, Noleto KS, Bezerra NPC; **Investigation:** Ribeiro LSS, Carvalho-Neta AV; **Resources:** Carvalho-Neta AV, Torres Junior JRS; **Data Curation:** Ribeiro DLS, Noleto KS, Bezerra NPC, Souza FA, Torres Junior JRS; **Formal Analysis:** Ribeiro DLS, Bezerra NPC, Souza FA; **Validation:** Bezerra NPC, Souza FA; **Supervision:** Carvalho-Neta AV; **Project Administration:** Torres Junior JRS; **Funding Acquisition:** Torres Junior JRS; **Writing – Original Draft:** Ribeiro DLS; **Writing – Review & Editing:** Ribeiro LSS, Bezerra NPC, Torres Junior JRS; **Final approval:** Torres Junior JRS.

## FUNDING

Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão

Grant No: 00640/17

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Procad/Amazônia

## ACKNOWLEDGEMENT

Not applicable.

## REFERENCES

Almeida, Z.S.; Nunes, J.L.S.; Alves, M.G.F.S. 2005. Dieta alimentar de *Genyatremus luteus* (Bloch, 1790) - (Teleostei, Perciformes: Haemulidae) na Baía de São José, Maranhão, Brasil. *Atlântica Rio Grande*, 27(1): 39-47. Available at: https://labaqua.com.br/wp-content/uploads/2020/01/Dieta-de-Genyatremus-luteus.pdf. Acessed on 10 Jan. 2022.

Amaral, J.S.; Venturieri, R.L.; Moreira, R.G. 2019. Gonadal steroids and energy availability during ovarian maturation stages of the Amazonian pirarucu *Arapaima gigas* (Teleostei: Osteoglossidae) in the wild. *Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 230: 106-114. https://doi.org/10.1016/j.cbpa.2019.01.008

Amenyogbe, E.; Chen, G.; Wang, Z.; Lu, X.; Lin, M.; Lin, A.Y. 2020. A Review on Sex Steroid Hormone Estrogen Receptors in Mammals and Fish. *International Journal of Endocrinology*, 2020: 5386193. https://doi.org/10.1155/2020/5386193

Blazer, V. 2002. Histopathological assessment of gonadal tissue in wild fishes. Fish *Physiology and Biochemistry*, 26: 85-101. https://doi.org/10.1023/A:1023332216713

Bobe, J.; Montfort, J.; Nguyen, T.; Fostier, A. 2006. Identification of new participants in the rainbow trout (*Oncorhynchus mykiss*) oocyte maturation and ovulation processes using cDNA microarrays. *Reproductive Biology and Endocrinology*, 4: 39. https://doi.org/10.1186/1477-7827-4-39

Bobe, J.; Nguyen, T.; Fostier, A. 2009. Ovarian function of the trout preovulatory ovary: new insights from recent gene expression studies. *Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 153(1): 63-68. https://doi.org/10.1016/j.cbpa.2008.10.021

Bobe, J.; Nguyen, T.; Jalabert, B. 2004. Targeted gene expression profiling in the rainbow trout (*Oncorhynchus mykiss*) ovary during maturational competence acquisition and oocyte maturation. *Biology of Reproduction*, 71(1): 73-82. https://doi.org/10.1095/biolreprod.103.025205

Breton, B.; Govoroun, M.; Mikolajczyk, T. 1998. GTH I and GTH II secretion profiles during the reproductive cycle in female rainbow trout: relationship with pituitary responsiveness to GnRH-A stimulation. *General and Comparative Endocrinology*, 111(1): 38-50. https://doi.org/10.1006/gcen.1998.7088

Brown-Peterson, N.J.; Wyanski, D.M.; Saborido-Rey, F.; Macewicz, B.J.; Lowerre-Barbieri, S.K. 2011. A standardized terminology for describing reproductive development in fishes. *Marine and Coastal Fisheries*, 3(1): 52-70. https://doi.org/10.1080/19425120.2011.555724

Burgos-Aceves, M.A.; Cohen, A.; Smith, Y.; Faggio, C. 2016. Estrogen regulation of gene expression in the teleost fish immune system. *Fish and Shellfish Immunology*, 58: 42-49. https://doi.org/10.1016/j.fsi.2016.09.006

Casanova-Nakayama, A.; Wernicke von Siebenthal, E.; Kropf, C.; Oldenberg, E.; Segner, H. 2018. Immune-Specific Expression and Estrogenic Regulation of the Four Estrogen Receptor Isoforms in Female Rainbow Trout (*Oncorhynchus mykiss*). *International Journal of Molecular Sciences*, 19(4): 932. https://doi.org/10.3390/ijms19040932

Cochrane, K.L. 2002. *A fishery manager's guide book: Management measures and their application*. Fisheries technical paper FAO. Rome: Food and Agriculture Organization of the United Nations. 236 p.

Edwards, D.P. 2005. Regulation of signal transduction pathways by estrogen and progesterone. *Annual Review of Physiology*, 67: 335-376. https://doi.org/10.1146/annurev.physiol.67.040403.120151

Endo, T.; Todo, T.; Lokman, P.M.; Ijiri, S.; Adachi, S.; Yamauchi, K. 2008. In vitro induction of oil droplet accumulation into previtellogenic oocytes of Japanese eel, *Anguilla japonica*. *Cybium*, 32(2 Suppl.): 239-240. https://doi.org/10.26028/cybium/2008-322SP-118

García-López, Á.; Couto, E.; Canario, A.V.; Sarasquete, C.; Martínez-Rodríguez, G. 2007. Ovarian development and plasma sex steroid levels in cultured female *Senegalese sole Solea senegalensis*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 146(3): 342-354. https://doi.org/10.1016/j.cbpa.2006.11.014

García-López, Á.; Sánchez-Amaya, M.; Prat, F. 2011. Targeted gene expression profiling in European sea bass (*Dicentrarchus labrax*, L.) follicles from primary growth to late vitellogenesis. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 160(3): 374-380. https://doi.org/10.1016/j.cbpa.2011.07.006

Godinho, H.P.; Santos, J.E.; Formagio, P.S.; Guimarães-Cruz, R.J. 2005. Gonadal morphology and reproductive traits of the Amazonian fish *Arapaima gigas*. *Acta Zoologica*, 86(4): 289-294. https://doi.org/10.1111/j.1463-6395.2005.00213.x

Gomez, J.M.; Weil, C.; Ollitrault, M.; Le Bail, P.Y.; Breton, B.; Le Gac, F. 1999. Growth hormone (GH) and gonadotropin subunit gene expression and pituitary and plasma changes during spermatogenesis and oogenesis in rainbow trout (*Oncorhynchus mykiss*). *General and Comparative Endocrinology*, 113(3): 413-428. https://doi.org/10.1006/gcen.1998.7222

Hermelink, B.; Wuertz, S.; Trubiroha, A.; Rennert, B.; Kloas, W.; Schulz, C. 2011. Influence of temperature on puberty and maturation of pikeperch, *Sander lucioperca*. *General and Comparative Endocrinology*, 172(2): 282-292. https://doi.org/10.1016/j.ygcen.2011.03.013

Honji, R.M.; Moreira, R.G. 2017. Controle neuroendócrino da ovogênese em peixes teleósteos. *Revista Brasileira de Reprodução Animal*, 41(1): 86-93.

Hou, Z.S.; Wen, H.S.; Li, J.F.; He, F.; Li, Y.; Tao, Y.X. 2018. Expression of estrogen receptors in female rainbow trout (*Oncorhynchus mykiss*) during first ovarian development and under dense rearing condition. *General and Comparative Endocrinology*, 259: 1-11. https://doi.org/10.1016/j.ygcen.2017.10.001

Johnson, G.D. 1980. *The limits and relationships of the Lutjanidae and associated families*. California: University of California Press, v. 2.

Kang, H.W.; Cho, J.K.; Son, M.H.; Park, J.Y.; Hong, C.G.; Chung, J.S.; Chung, E.Y. 2015. Gonadal development, spawning and plasma sex steroid levels of the indoor cultured grunt, *Hapalogenys nitens*. *Development & Reproduction*, 19(1): 33-41. https://doi.org/10.12717%2FDR.2015.19.1.033

Kayo, D.; Zempo, B.; Tomihara, S.; Oka, Y.; Kanda, S. 2019. Gene knockout analysis reveals essentiality of estrogen receptor β1 (Esr2a) for female reproduction in medaka. *Scientific Reports*, 9(1): 8868. https://doi.org/10.1038/s41598-019-45373-y

Kazeto, Y.; Tosaka, R.; Matsubara, H.; Ijiri, S.; Adachi, S. 2011. Ovarian steroidogenesis and the role of sex steroid hormones on ovarian growth and maturation of the Japanese eel. *Journal of Steroid Biochemistry and Molecular Biology*, 127(3-5): 149-154. https://doi.org/10.1016/j.jsbmb.2011.03.013

Kohn, Y.Y.; Symonds, J.E.; Lokman, P.M. 2013. The effects of 11-ketotestosterone on ovarian physiology of previtellogenic captive hapuku (*Polyprion oxygeneios*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 166(3): 496-502. https://doi.org/10.1016/j.cbpa.2013.07.032

Kortner, T.M.; Rocha, E.; Arukwe, A. 2009a. Androgenic modulation of early growth of Atlantic cod (*Gadus morhua* L.) previtellogenic oocytes and zona radiata-related genes. *Journal of Toxicology and Environmental Health, Part A*, 72(3-4): 184-195 https://doi.org/10.1080/15287390802539020

Kortner, T.M.; Rocha, E.; Arukwe, A. 2009b. Previtellogenic oocyte growth and transcriptional changes of steroidogenic enzyme genes in immature female Atlantic cod (*Gadus morhua* L.) after exposure to the androgens 11-ketotestosterone and testosterone. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 152(3): 304-313. https://doi.org/10.1016/j.cbpa.2008.11.001

Le Cren, E.D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *Journal of Animal Ecology*, 20(2): 201-219. https://doi.org/10.2307/1540

Levavi-Sivan, B.; Bogerd, J.; Mañanós, E.L.; Gómez, A.; Lareyre, J.J. 2010. Perspectives on fish gonadotropins and their receptors. *General and Comparative Endocrinology*, 165(3): 412-437. https://doi.org/10.1016/j.ygcen.2009.07.019

Levin, E.R. 2009. Plasma membrane estrogen receptors. *Trends in Endocrinology & Metabolism*, 20(10): 477-482. https://doi.org/10.1016/j.tem.2009.06.009

Liu, K.C.; Lau, S.W.; Ge, W. 2017. Spatiotemporal expression analysis of nuclear estrogen receptors in the zebrafish ovary and their regulation in vitro by endocrine hormones and paracrine factors. *General and Comparative Endocrinology*, 246: 218-225. https://doi.org/10.1016/j.ygcen.2016.12.011

Livak, K.J.; Schmittgen, T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods*, 25(4): 402-408. https://doi.org/10.1006/meth.2001.1262

Lokman, P.M.; George, K.A.N.; Divers, S.L.; Algie, M.; Young, G. 2007. 11-ketotestosterone and IGF-I increse the size of previttelogenic oocytes from shortfinnedeel, *Anguilla australis*, in vitro. *Reproduction*, 133(5): 955-967. https://doi.org/10.1530/REP-06-0229

Lubzens, E.; Young, G.; Bobe, J.; Cerdà, J. 2010. Oogenesis in teleosts: how fish eggs are formed. *General and Comparative Endocrinology*, 165(3): 367-389. https://doi.org/10.1016/j.ygcen.2009.05.022

Marques, N.E.F.; Sampaio, J.A.A.; Bezerra, S.F.B.; Yauri, W.M.; Santos, R.L. 2016. Indução hormonal à desova do peixe pedra *Genyatremus luteus* (Bloch, 1790) (Teleostei: Haemulidae). *Boletim do Laboratório de Hidrobiologia*, 26(1): 32-35.

Matsubara, T.; Sawano, K. 1995. Proteolytic cleavage of vitellogenin and yolk proteins during vitellogenin uptake and oocyte maturation in barfin flounder (*Verasper moseri*). *Journal of Experimental Zoology Part A*, 272(1): 34-45. https://doi.org/10.1002/jez.1402720105

Matsuyama, M.; Ohta, K.; Morita, S.; Hoque, M.M.; Kagawa, H.; Kambegawa, A. 1998. Circulating levels and in vitro production of two maturation-inducing hormones in teleost: 17α, 20β-dihydroxy-4-pregnen-3-one and 17α, 20β, 21-trihydroxy-4-pregnen-3-one, in a daily spawning wrasse, *Pseudolabrus japonicus*. *Fish Physiology and Biochemistry*, 19(1): 1-11. https://doi.org/10.1023/A:1007761729290

Matsuyama, M.; Shiraishi, T.; Sundaray, J.K.; Rahman, M.; Ohta, K.; Yamaguchi, A. 2005. Steroidogenesis in ovarian follicles of chub mackerel, *Scomber japonicus*. *Zoological Science*, 22(1): 101-110. https://doi.org/10.2108/zsj.22.101

Miura, C.; Higashino, T.; Miura, T. 2007. A progestin and an estrogen regulate early stages of oogenesis in fish. *Biology of Reproduction*, 77(5): 822-828. https://doi.org/10.1095/biolreprod.107.061408

Nagahama, Y.; Yamashita, M. 2008. Regulation of oocyte maturation in fish. *Development Growth & Differentiation*, 50(Suppl. 1): S195-S219. https://doi.org/10.1111/j.1440-169X.2008.01019.x

Nagler, J.J.; Davis, T.L.; Modi, N.; Vijayan, M.M.; Schultz, I. 2010. Intracellular, not membrane, estrogen receptors control vitellogenin synthesis in the rainbow trout. *General and Comparative Endocrinology*, 167(2): 326-330. https://doi.org/10.1016/j.ygcen.2010.03.022

Nelson, E.R.; Habibi, H.R. 2010. Functional significance of nuclear estrogen receptor subtypes in the liver of goldfish. *Endocrinology*, 151(4): 1668-1676. https://doi.org/10.1210/en.2009-1447

Nelson, E.R.; Habibi, H.R. 2013. Estrogen receptor function and regulation in fish and other vertebrates. *General and Comparative Endocrinology*, 192: 15-24. https://doi.org/10.1016/j.ygcen.2013.03.032

Nelson, J.S. 2006. *Fishes of the World*. Hoboken: John Wiley and Sons.

Noleto-Filho, E.M.; Yauri, W.L.M.; Luvizotto-Santos, R. 2012. Captura de reprodutores de peixe-pedra *Genyatremus luteus* (Block, 1797) (Pisces: Haemulidae) e manutenção em sistema fechado. *Boletim do laboratório de Hidrobiologia*, 25(1): 55-60. Available at: https://periodicoseletronicos.ufma.br/index.php/blabohidro/article/view/1948/104. Acessed on 10 Jan. 2022.

Nyuji, M.; Kazeto, Y.; Izumida, D.; Tani, K.; Suzuki, H.; Hamada, K.; Okuzawa, K. 2016. Greater amberjack Fsh, Lh, and their receptors: plasma and mRNA profiles during ovarian development. *General and Comparative Endocrinology*, 225: 224-234. https://doi.org/10.1016/j.ygcen.2015.10.008

Ogino, Y.; Miyagawa, S.; Iguchi, T. 2016. Subchapter 94C—17,20β,21-Trihydroxy-4-pregnen-3-one. In: Takei, Y.; Ando, H.; Tsutsui, K. (eds.). *Handbook of hormones: Comparative endocrinology for basic and clinical research*. Academic Press, p. 511-512. https://doi.org/10.1016/B978-0-12-801028-0.00222-1

Ohta, K.; Yamaguchi, S.; Yamaguchi, A.; Gen, K.; Okuzawa, K.; Kagawa, H.; Matsuyama, M. 2002. Biosynthesis of steroids in ovarian follicles of red seabream, *Pagrus major* (Sparidae, Teleostei) during final oocyte maturation and the relative effectiveness of steroid metabolites for germinal vesicle breakdown in vitro. *Comparative Biochemistry and Physiology Part B*, 133(1): 45-54. https://doi.org/10.1016/s1096-4959(02)00106-9

Peter, R.E.; Yu, K.L. 1997. Neuroendocrine regulation of ovulation in fishes: basics applied aspects. *Reviews in Fish Biology and Fisheries*, 7: 173-197. https://doi.org/10.1023/A:1018431610220

Rahman, M.S.; Takemura, A.; Takano, K. 2000. Correlation between plasma steroid hormones and vitellogenin profiles and lunar periodicity in the female golden rabbitfish, *Siganus guttatus* (Bloch). *Comparative Biochemistry and Physiology Part B*, 127(1): 113-122. https://doi.org/10.1016/S0305-0491(00)00240-6

Reading, B.J.; Sullivan, C.V.; Schilling, J. 2017. Vitellogenesis in fishes. *Reference Module in Life Sciences*, 1-14. https://doi.org/10.1016/B978-0-12-809633-8.03076-4

Rhody, N.R.; Davie, A.; Zmora, N.; Zohar, Y.; Main, K.L.; Migaud, H. 2015. Influence of tidal cycles on the endocrine control of reproductive activity in common snook (*Centropomus undecimalis)*. *General and Comparative Endocrinology*, 224: 247-259. https://doi.org/10.1016/j.ygcen.2015.08.007

Rinchard, J.; Kestemont, P.; Heine, R. 1997. Comparative study of reproductive biology in single and multiple- spawner cyprinid fish.2. Sex steroid and plasma protein phosphorus concentrations. *Journal of Fish Biology*, 50(1): 169-180. https://doi.org/10.1111/j.1095-8649.1997.tb01349.x

Sambroni, E.; Le Gac, F.; Breton, B.; Lareyre, J.J. 2007. Functional specificity of the rainbow trout (*Oncorhynchus mykiss*) gonadotropin receptors as assayed in a mammalian cell line. *Journal of Endocrinology*, 195(2): 213-228. https://doi.org/10.1677/JOE-06-0122

Sampaio, I.B.M. 2002. *Estatística aplicada à experimentação animal*. 3rd ed. Belo Horizonte: FEPMVZ, 264 p.

Santos, G.M.; Santos, A.C.M. 2005. Sustentabilidade da pesca na Amazônia. *Estudos Avançados*, 19(54): 165-182. https://doi.org/10.1590/S0103-40142005000200010

Senthilkumaran, B.; Yoshikuni, M.; Nagahama, Y. 2004. A shift in steroidogenesis occurring in ovarian follicles prior to oocyte maturation. *Molecular and Cellular Endocrinology*, 215(1-2): 11-18. https://doi.org/10.1016/j.mce.2003.11.012

Shinozaki-Mendes, R.A.; Santander-Neto, J.; Silva, J.R.F.; Hazin, F.H.V. 2013. Gonad maturation of *Haemulon plumieri* (Teleostei: Haemulidae) in Ceará state, northeastern Brazil. *Brazilian Journal of Biology*, 73(2): 383-390. https://doi.org/10.1590/S1519-69842013000200019

Silva, F.F.G.; Støttrup, J.G.; Kjørsvik, E.; Tveiten, H.; Tomkiewicz, J. 2016. Interactive effects of dietary composition and hormonal treatment on reproductive development of cultured females eel, *Anguilla anguilla*. *Animal Reproduction Science*, 171, 17-26. https://doi.org/10.1016/j.anireprosci.2016.05.007

Svensson, K.; Just, A.C.; Fleisch, A.F.; Sanders, A.P.; Tamayo-Ortiz, M.; Baccarelli, A.A.; Burris, H.H. 2019. Prenatal salivary sex hormone levels and birth-weight-for-gestational age. *Journal of Perinatology*, 39(7): 941-948. https://doi.org/10.1038/s41372-019-0385-y

Tavera, J.J.; Acero, A.; Balart, E.F.; Bernardi, G. 2012. Molecular phylogeny of grunts (Teleostei, Haemulidae), with an emphasis on the ecology, evolution, and speciation history of New World species. *BMC Ecology and Evolution*, 12: 57. https://doi.org/10.1186/1471-2148-12-57

Thomas, P.; Dressing, G.; Pang, Y.; Berg, H.; Tubbs, C.; Benninghoff, A.; Doughty, K. 2006. Progestin, estrogen and androgen G-protein coupled receptors in fish gonads. *Steroids*, 71(4): 310-316. https://doi.org/10.1016/j.steroids.2005.09.015

Thomas, P.; Trant, J.M. 1989. Evidence that 17α, 20β, 21-trihydroxy-4-pregnen-3-one is a maturation-inducing steroid in spotted seatrout. *Fish Physiology and Biochemistry*, 7: 185-191. https://doi.org/10.1007/BF00004706

Tokarz, J.; Möller, G.; de Angelis, M.H.; Adamski, J. 2015. Steroids in teleost fishes: A functional point of view. *Steroids*, 103: 123-144. https://doi.org/10.1016/j.steroids.2015.06.011

Tosaka, R.; Todo, T.; Kazeto, Y.; Mark Lokman, P.; Ijiri, S.; Adachi, S.; Yamauchi, K. 2010. Expression of androgen receptor mRNA in the ovary of Japanese eel, *Anguilla japonica*, during artificially induced ovarian development. *General and Comparative Endocrinology*, 168(3): 424-430. https://doi.org/10.1016/j.ygcen.2010.05.005

Trant, J.M.; Thomas, P.; Shackleton, C.H. 1986. Identification of 17α, 20β, 21-trihydroxy-4-pregnen-3-one as the major ovarian steroid produced by the teleost Micropogonias undulatus during final oocyte maturation. *Steroids*, 47(2-3): 89-99. https://doi.org/10.1016/0039-128X(86)90081-4

Tyler, C.R.; Sumpter, J.P. 1996. Oocyte growth and development in teleosts. *Reviews in Fish Biology and Fisheries*, 6: 287-318. https://doi.org/10.1007/BF00122584

Vazzoler, A.E.A.M. 1996. *Biologia da reprodução de peixes teleósteos: teoria e prática*. Maringá: Eduem.

Wallace, R.A.; Selman, K. 1981. Cellular and dynamic aspects of Oocyte growth in Teleosts. *Integrative and Comparative Biology*, 21(2): 325-343. https://doi.org/10.1093/icb/21.2.325

Wang, L.; Liang, X.F.; Zhang, W.B.; Mai, K.S.; Huang, Y.; Shen, D. 2009. Amnesic shellfish poisoning toxin stimulates the transcription of CYP1A possibly through AHR and ARNT in the liver of red sea bream *Pagrus major*. *Marine Pollution Bulletin*, 58(11): 1643-1648. https://doi.org/10.1016/j.marpolbul.2009.07.004

Wang, N.; Wang, Y.; Hao, H.; Wang, L.; Wang, Z.; Wang, J.; Wu, R. 2014. A bi-Poisson model for clustering gene expression profiles by RNA-seq. *Briefings in Bioinformatics*, 15(4): 534-541. https://doi.org/10.1093/bib/bbt029

Wang, W.; Zhu, H.; Tian, Z.; Sun, A.; Dong, Y.; Dong, T.; Hu, H. 2020. Effects of 11-Ketotestosterone on Development of the Previtellogenic Ovary in the Sterlet, *Acipenser ruthenus. Frontiers in Endocrinology*, 11: 115. https://doi.org/10.3389/fendo.2020.00115

Yaron, Z.; Silvan, B. 2006. Reproduction. In: Evans, D.H.; Claiborne, J.B. (eds.). *The Physiology of Fish*. Boca Raton: CRC Press.

Yoshida, H.O. 1964. Skipjack tuna spawning in the Marquesas Islands and Tuamotu Archipelago. *Fish Bulletin*, 65(2): 479-488.

Zapater, C.; Molés, G.; Muñoz, I.; Pinto, P.I.; Canario, A.V.; Gómez, A. 2018. Differential involvement of the three nuclear estrogen receptors during oogenesis in European sea bass (*Dicentrarchus labrax*). *Biology of Reproduction*, 100(3): 757-772. https://doi.org/10.1093/biolre/ioy227

Zohar, Y.; Mylonas, C.C. 2001. Endocrine manipulations of spawning in cultured fish: from hormones to genes. *Aquaculture*, 197(1-4): 99-136. https://doi.org/10.1016/S0044-8486(01)00584-1