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Gonadal aspects, testosterone and estradiol profiles of the Lebranche mullet *Mugil liza* subjected to different temperatures during the larviculture phase

Aspectos gonadais, perfil de testosterona e estradiol da tainha Lebranche *Mugil liza* submetida a diferentes temperaturas durante a fase de larvicultura

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Abstract: In Brazil, the Lebranche mullet is the most important Mugilidae species for the fishing industry. However, recently, the species has been recommended for classification as near threatened. In this sense, aquaculture has emerged as a powerful tool for natural resource conservation and development. The present study evaluated the influence of different temperatures during *Mugil liza* larviculture and their late effects on gonadal development and hormonal quantification after 24 months. The fertilized eggs (45 eggs L^{-1}) were initially kept in a 60 L circular tank until hatching. After hatching, the larvae were subjected to four treatments in triplicate at different temperatures (21, 24, 27 and 30 °C) for 35 days. The juveniles were subsequently transferred to growth units where they remained identified, according to the treatments, for 24 months. The weight and length of the fish were significantly different in the larviculture phase. Histological analyses revealed immature, maturing, and mature male gonads. The estradiol levels in the fish were low, regardless of the maturation stage. For testosterone, the highest values were observed in mature males (1.29 ± 0.07 ng mL^{-1}). No significant differences were observed ($p > 0.05$) in the concentrations of estradiol and testosterone in relation to temperature. However, there were significant differences ($p < 0.05$) in testosterone concentration depending on sexual maturation. The results suggest that temperature exerts a masculinizing effect on *M. liza*. Furthermore, temperature directly affected larval growth. In summary, the results provide evidence that temperature may play a crucial role in sex determination in *M. liza*.

Keywords: marine fish farming; Mugilidae; hormonal quantification; gonadosomatic index.

Resumo: No Brasil, a tainha Lebranche é a espécie Mugilidae mais importante para a indústria pesqueira. Contudo, recentemente a espécie foi recomendada para classificação como *quase ameaçada*. Neste sentido, a aquicultura surge como uma ferramenta poderosa para a conservação e desenvolvimento dos recursos naturais. O presente estudo avaliou a influência de diferentes temperaturas durante a larvicultura de *Mugil liza* e seus efeitos tardios no desenvolvimento gonadal

e na quantificação hormonal após 24 meses. Inicialmente, os ovos fecundados (45 ovos L^{-1}) foram mantidos em tanque circular de 60 L até a eclosão. Após a eclosão, as larvas foram submetidas a quatro tratamentos em triplicata em diferentes temperaturas (21, 24, 27 e 30 °C) durante 35 dias. Em seguida, os juvenis foram transferidos para unidades de crescimento onde permaneceram identificados, conforme os tratamentos, durante 24 meses. O peso e o comprimento dos peixes foram significativamente diferentes na fase de larvicultura. As análises histológicas mostraram gônadas masculinas imaturas, em maturação e maduras. Os níveis de estradiol nos peixes foram baixos, independente do estágio de maturação. Para a testosterona os maiores valores foram observados em machos maduros (1,29 ± 0,07 ng mL^{-1}). Não foram observadas diferenças significativas ($p > 0,05$) nas concentrações de estradiol e testosterona em relação à temperatura. Porém, houve diferenças significativas ($p < 0,05$) na concentração de testosterona dependendo da maturação sexual. Os resultados sugerem que a temperatura exerce um efeito masculinizante na tainha Lebranche. Além disso, a temperatura afetou diretamente o crescimento larval. Em suma, os resultados fornecem evidências de que a temperatura pode desempenhar um papel fundamental na determinação do sexo em *M. liza.*

Palavras-chave: piscicultura marinha; Mugilidae; quantificação hormonal; índice gonadossomático.

1. Introduction

The Lebranche mullet *Mugil liza* is a pelagic fish from the Mugilidae family that is widely distributed on the Atlantic coast of South America, from the Caribbean to Argentina [1, 2]. With respect to reproductive biology, it is a dioecious species with migratory habits that results in the formation of large schools [3]. The reproductive migration of mullet, which is commercially exploited between the states of São Paulo and Rio Grande do Sul, begins in April and lasts until July, with spawning peaks occurring in June between the states of Santa Catarina (northern region) and Paraná [4].

In Brazil, the Lebranche mullet is the most important Mugilidae species for the fishing industry [5], where the annual production of the states of Santa Catarina and São Paulo is close to 8,069 and 1,304 tons, respectively [6]. However, recently, the species has been recommended for classification as near threatened [1, 3, 7]. In this sense, aquaculture has emerged as a powerful tool for natural resource conservation and development. In recent years, Lebranche mullet has stood out in several studies in Brazil, demonstrating the ecological relevance of this species, as well as its enormous aquaculture potential in experimental fish farming [8, 9, 10, 11, 12, 13]. However, a fundamental requirement for the domestication of potentially promising species is understanding and controlling the reproductive processes of fish in captivity [14].

According to Baroiller and D'Cotta [15], temperature is the environmental factor that most affects phenotypic sex determination in fish. Temperature interferes with gonadal steroidogenesis, modulating the expression of the aromatase gene and other genes, enzymes, and nonsteroidal reproductive hormones produced in the brain and pituitary gland [16]. Therefore, it is expected that temperature-based treatments may influence specimens during the labile period [17], a phase that characterizes the beginning of the gonadal differentiation process, and that the animals are susceptible to sex definition [18, 19].

Some physiological characteristics that emerge later in other developmental stages of fish can be influenced by temperature during larval development, including growth rate, reproductive distribution and migration, and sex determination [20]. The influence of temperature on the phenotypic modulation of mullets for sexual determination is not clear; however, it is known that environmental factors such as decreasing temperature and increasing salinity are stimuli for school migration and reproductive aggregation [21, 22].

On the other hand, under controlled conditions in aquaculture, with different temperatures (17, 20, 23, 26 and 29 °C), the highest hatching rate of Lebranche mullet *M. liza* eggs occurred at 23.2 °C, with the highest larval survival occurring at 23.9 °C. At 16.5 °C, 70% mortality occurs [13]. Perhaps manipulating the temperature during the larval stage, from hatching to the juvenile stage, can influence the gonadal aspects and sex hormone profile of the mullet, as observed in many species, where greater numbers of females occur at lower temperatures, whereas more males occur at higher temperatures [23]. Therefore, the present study aimed to evaluate the influence of four different temperatures (21, 24, 27 and 30 °C) on the early stages of larviculture, as well as to verify the late effects of temperature variation on the gonadal aspects, testosterone, and estradiol profiles of the Lebranche mullet *M. liza* two years postlarviculture.

2. Material and methods

All procedures were approved by the Ethical Committee on the Animal Use of Federal University of Santa Catarina (UFSC) (CEUA protocol number 3102220419).

2.1. Experimental location and origin of the fish

The experiment was conducted at the marine fish culture laboratory (LAPMAR), Elpídio Beltrame Mariculture Station (EMEB) of UFSC, Barra da Lagoa, Florianópolis, Santa Catarina, Brazil (27° 34' 02" S, 48° 25' 44" W). Lebranche mullet eggs were obtained from induced spawning of breeding stock already acclimatized at LAPMAR, following procedures described by Cerqueira et al. [24] and Magnotti et al. [25]. The breeders were kept in circular tanks (3.2 m diameter and 1.0 m depth) with 8,000 L capacity and a continuous flow-through system. The salinity was 35‰, the oxygen content was approximately 6 mg L^{-1}, the temperature ranged from 18 °C (winter) to 28 °C (summer), and the natural photoperiod was maintained.

2.2. Experimental design and specific procedures

Once spawning at 23.9 °C, fertilized eggs (45 L^{-1}) were transferred to 12 experimental units (60-L circular tanks). After hatching, the larvae were maintained for 35 days at 21, 24, 27 and 30 °C (in triplicate), the ideal temperatures for larval embryogenesis, survival, and initial development, according to previous studies [13]. The experimental tanks contained static "green water" using the microalga *Nannochloropsis oculata* at 300,000–500,000 cells mL^{-1} for 18 days, followed by a continuous flow-through system, initially with 10% water renewal daily to 100% at 35 days [24, 26]. The experimental room was air-conditioned at a constant

temperature of 21 °C, while the temperatures in the treatments were controlled with 500 W thermostat heaters (accuracy of ± 1 °C).

Until the 15th day, daily the larvae were fed the rotifer *Brachionus rotundiformis* (2 to 30 mL^{-1}). The nauplii (0.5 to 1.0 mL^{-1}) and *Artemia* sp. metanauplii (1.0 to 5.0 mL^{-1} three times daily 8 am, 12 pm and 4 pm), which were subsequently enriched for 24 h with fatty acids (red pepper, Bernaqua, Bel) according to larval development. Finally, the transition from live feed to an inert diet was performed via a commercial dry diet (Sano S-Pak 5/8, Inve, USA), offered in six daily portions (8 am, 10 am, 12 pm, 2 pm, 4 pm and 6 pm).

After total feed transition (at the 35th day), a sample of 30 fish from each treatment was weighed (total mass) and measured (total length). Then, 120 animals from each treatment were transferred to 500-L experimental units (three for each temperature), with continuous flow-through (100% daily) and a water temperature ranging from 20 to 24 °C. The fish were fed a 0.8 mm commercial diet (Nutripiscis Starter, Presence, Brazil; 45% crude protein, 9% lipid) five times daily to apparent satiety [12] until the fish reached approximately 5.0 g.

After exposure to different temperatures, the fish were transferred and maintained separately (according to thermal treatments) in experimental units measuring 3.2 m in diameter × 1.0 m in height, with a capacity of 8,000 L with continuous water flow, pumped directly from the ocean at Mozambique beach, Florianópolis, Brazil (27° 34'02''S, 48°25'44''W), with 200--300% water renewal daily. The water temperature ranged from 18 °C (August) to 27 °C (January). The juvenile mullets were initially fed 1.3 mm commercial feed (NutriSCIS Starter, Presence, Brazil, 45% crude protein) until they reached 100 g. The fish were subsequently fed commercial 2--3 mm feed (Nutripiscis AL45, Presença, Brazil, 45% crude protein) four times daily until apparent satiety.

After 12 months, sampling was performed to assess sexual maturation. Twenty individuals from each experimental unit were euthanized with benzocaine (200 mg L^{-1}), and gonads were extracted through a ventral incision for macroscopic evaluation [27]. Gonads were weighed to determine the gonadosomatic index (GSI) [28]. As no evidence of sexual maturation was observed at this time, 50 individuals from each treatment were maintained under the same conditions for an additional year.

After 24 months, when the water temperature was approximately 18 °C, sampling was performed to assess sexual maturation. Twenty fish from each treatment were euthanized with benzocaine (200 mg L^{-1}), and gonads were extracted through a ventral incision for macroscopic evaluation [27]. Gonads were weighed to determine the gonadosomatic index (GSI) [28], fixed in Davidson solution for 24 h, and transferred to 70% ethyl alcohol for conservation until histological slide preparation.

Blood samples from 20 fish per treatment were collected via puncture of the caudal vessel with insulin syringes coated with the anticoagulant solution HEMSTB (EDTA K2 15 gd L^{-1}) and kept in microtubes under refrigeration. The blood samples were subsequently centrifuged at 2,000 × *g* for 10 min at 4 °C to obtain plasma, which was stored at -20 °C until quantification of the hormones testosterone (T) and estradiol (E2).

2.3. Histological analysis and classification of gonadal development

Histological analyses of the fish gonads were performed at the Aquatic Organism Health Laboratory AQUOS/UFSC. The samples were dehydrated in an increasing series of ethyl alcohol, clarified in xylol, embedded in paraffin at 60 °C, cut into 4 µm sections (Microtome LUPETEC MRP09, Lupetec®, Brazil) and stained with hematoxylin–eosin (H&E). The slides were subsequently prepared in Entellan® media and analysed. The development stages of the germ epithelium were identified via light microscopy (Leica, ICC50 HD, Germany) and classified according to the degree of gonadal development proposed for *M. liza* via methodology adapted from Albieri and Araújo [27] and Lemos et al. [4]. For gonadal development, the histological characteristics included the presence of spermatogonia, the largest cells of the spermatogenic lineage. For maturation, all developmental stages present, after mitotic divisions of spermatogonia (spermatids, spermatocytes and spermatozoa), sperm cells clustered in nests. For functional maturation, tubules full of spermatozoa begin to accumulate in the ductus deferens (sperm duct). Spermatids are most visible near the walls of tubules, but all cell types are present.

2.4. Hormone quantification

Hormone quantification was performed at the Laboratory of Biomarkers of Aquatic Contamination and Immunochemistry LABCAI/UFSC. Testosterone and estradiol levels were quantified via commercial kits (testosterone EIA-1559, estradiol EIA-2693; DRG Instruments GMBH, Germany), with which the solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding was performed, where endogenous Testosterone of fish sample competes with a Testosterone horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is reverse proportional to the concentration of Testosterone in the sample. After addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of Testosterone in sample. Steroids were extracted from 400 µl of blood plasma with 2.0 mL dichloromethane solvent for each sample. After solvent evaporation via liquid nitrogen, the samples were reconstituted in 100 µl of standard solution zero from the DRG kit containing zero ng ml of estradiol and testosterone. Analysis was performed according to the manufacturer's guidelines. The samples were analysed with a spectrophotometer (SpectraMax 5, Molecular Devices, United States) at 450 nm, and data analysis and graphing were performed using GraphPad Prism 8 software. Mullet plasma presented recovery (technique "spike and recovery"): 113.8% testosterone and 106.0% estradiol; the coefficients of intra-assay variation were 5.57 for testosterone and 7.74 for estradiol; and the coefficients of interassay variation were 6.19 for testosterone and 8.67 for estradiol.

2.5 Statistical analysis

The data were subjected to the Shapiro–Wilk and Levene tests to verify normality and homoscedasticity of variance, respectively. Data that met the prerequisites were subjected to analysis of variance (ANOVA), and when a significant difference was found, they were

subjected to the Tukey test via the statistical program STATISTICA 7. A regression analysis model was applied for the final weight and length of juvenile Lebranche mullet (*Mugil liza*) on the 35th day of larviculture at different temperatures. All the statistical analyses were performed with a significance level of 5%.

3. Results and discussion

3.1 Growth indices

In the present study, larval development in the first 35 days was directly related to temperature. Significant differences (p <0.05) were observed in the weight and length of the fish due to water temperature. During larviculture, a linear increase in weight and length was observed, according to the increased temperature (Figure 1). However, after 24 months, significant differences (p < 0.05) were observed only for length. Compared with the fish subjected to the 30 °C treatment, those subjected to the 21 °C treatment during larviculture reached greater lengths after two years. On the other hand, weight did not significantly differ (p > 0.05) between the treatments (Table 1).

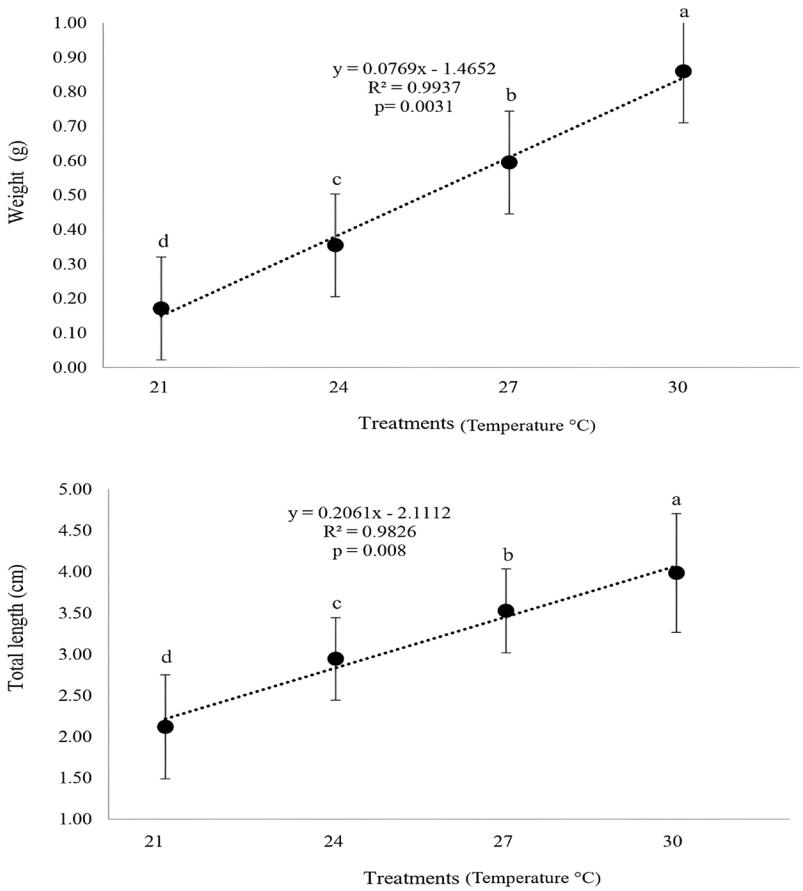

Figure 1. Regression analysis of the weight and total length of Lebranche mullet (*Mugil liza*) on the 35th day of larviculture at different temperatures (21, 24, 27 and 30 °C). The regression analysis results were significant at the 5% level. Different lowercase letters indicate significant difference between treatments. Data were collected from 30 fish from each treatment.

Table 1. Total weight, total length, and gonadosomatic index (GSI) of Lebranche mullet (*Mugil liza*). The data are related to the 2nd year after the larviculture phase at different temperatures of 21, 24, 27 and 30 °C. The values are presented as the means and standard deviations (±SDs). Different lowercase letters in the same column indicate significant difference between treatments. Samples were collected from 20 fish from each treatment.

Treatments (°C)	Weight (g)	Length (cm)	GSI (%)
21	466.56 ± 15.67	35.27 ± 0.39^{a}	0.56 ± 0.671^{a}
24	463.80 ± 11.24	34.55 ± 0.26^{ab}	0.030 ± 0.002^{b}
27	465.61 ± 18.59	34.77 ± 0.43^{ab}	0.32 ± 0.542^{a}
30	419.91 ± 15.40	33.42 ± 0.41^{b}	0.53 ± 0.708^{a}
ANOVA (*p* value)	0.0991	0.0077	0.0136

**GSI = Gonadosomatic index.*

These results were expected for a tropical/subtropical species whose temperature for best growth was determined by Okamoto et al. [8]. Researchers have tested temperatures of 20, 25 and 30 °C in the cultivation of juvenile mullet weighing 0.87 ± 0.25 g and observed that the growth of the fish was optimized at a temperature of 30 °C. However, these mullets were captured in the wild and transferred for fattening in captivity. During the fattening period, we can highlight the growth recovery capacity of *M. liza*, even after impairments caused by low temperatures during the larval period. At the end of the two-year period, growth was compatible with data already observed for the same species (in which fish of 11 months of age had length of 25.7 ± 0.4 cm and weight of 205.7 ± 11.5 g) [25] and was greater than the gray mullet, which at two years of age had an average weight of 210.3 ± 20.04 [29].

3.2. Gonadal histological analysis

Twenty-four months postlarviculture, it was possible to identify only male gonads in the specimens, which were classified into three stages of testicular development: A- Immature, B- Maturation and C- Functional maturity (Table 2). Female or feminized gonads, i.e., those containing testicular tissue composed exclusively of female germline cells, were not observed. In the immature testicles, only spermatogonia, the largest sperm germline cells, could be observed (Figure 2a). In the maturing phase, all stages of cell development were observed: the cells were grouped into nests of spermatogonia, spermatocytes and spermatids. Some fish presented spermatozoa in the lumen of the seminiferous tubules and spermatic ducts (Figure 2b and c). Males with functional maturity had spermatozoa accumulated in the tubules and sperm ducts in large numbers (Figure 2d), and spermatids were more visible near the walls of the tubules, although all types of cells were present (spermatogonia, spermatocytes and spermatids).

Complete gonadal maturation in male *M. liza*, with semen classified as suitable for spawning, was verified at 11 months of age [30], and the males were able to reproduce with 25.7 ± 0.4 cm in length and 205.7 ± 11.5 g in weight [11]. The GSIs of the fish subjected to different temperatures during the larviculture phase significantly differed ($p < 0.05$) among

the treatments after 24 months. Compared with the other treatments, the 24 °C treatment presented a lower GSI (Table 1). In the present study, GSI data, macroscopic observations and histological analyses of the gonads revealed the differentiation and sexual maturation of the fish, confirming that we found only male *M. liza* in the second year, regardless of the temperature treatment. This is an unusual finding, considering that in previous works on artificial propagation of this species, there were no similar reports of monosexual batches [24,25, 31]. Similarly, we have no information that this could occur in natural populations of the genus *Mugil* or other mugilids [4, 32, 33]. However, this is a very relevant question that allows for the construction of many hypotheses. It is suspected that the aforementioned issues may be differentiated by population, which until recently were considered two distinct species (*M. platanus* and *M. liza*), which even had distinct morphological characteristics and, perhaps, even a different life cycle. Therefore, we believe that new studies should be conducted with the M. liza population in the southern region of Brazil.

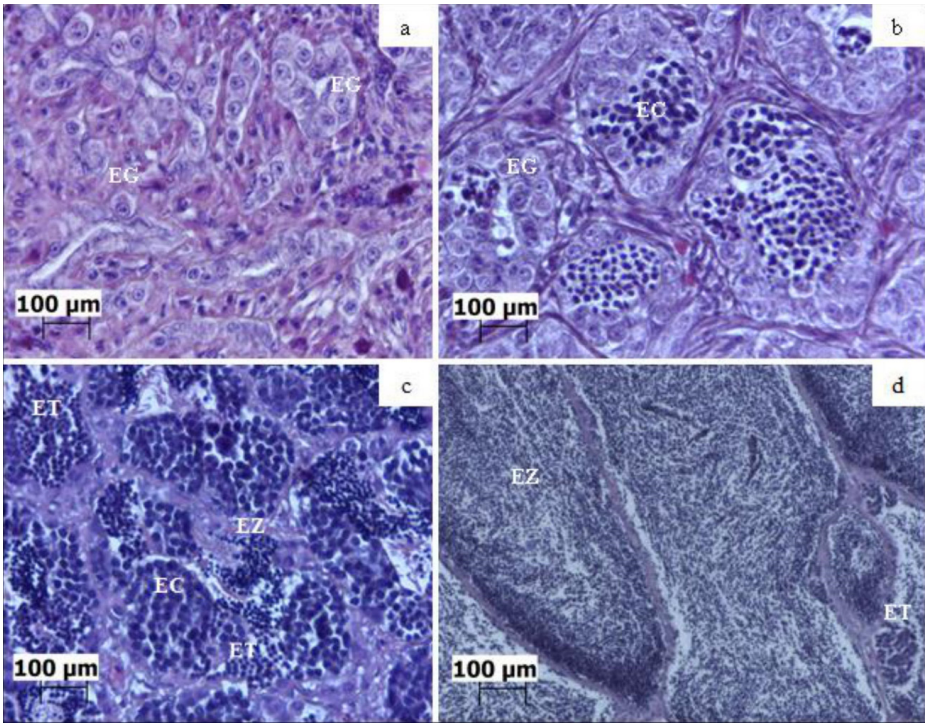

Figure 2. Gonadal histological analysis of Lebranche mullet (*Mugil liza*). The data are related to the second year after the larviculture phase at different temperatures of 21, 24, 27 and 30 °C. Longitudinal sections of testicles at 24 months of age. (a) Immature testicles: cords of spermatogonia (EG). (b) maturing testicles: presence of spermatogonia (EG) and spermatocytes (EC). (c) Spermatocytes (EC) and spermatids (ET) were grouped into spermatocytes, in addition to the presence of spermatozoa (EZ). (d) Functional maturity testicles: a large number of spermatozoa are present in the lumen of the tubules; spermatids (ET) are more visible close to the tubule walls. Hematoxylin–eosin (H&E) staining. Samples were collected from 20 fish from each treatment.

For some species, such as European seabass [34, 35] and the marine silverside [36], sex ratios are female biased at low temperatures, whereas for others, such as the Patagonian silverside, sex seems to be genetically determined [36]. Additionally, the effects of relatedness and inbreeding can cause depression in sexual variables [37, 38]. The results of the present study suggest that temperature manipulation during the larval stage, from hatching to

transformation into juvenile, could influence the sex determination of Lebranche mullet. However, more experimental data are needed to confirm this hypothesis. Furthermore, temperature-dependent sex determination in fish has been investigated in recent years since the occurrence of a unique sex ratio pattern in some species may be influenced by the possible effects of climate change [23, 39].

Table 2. Testicular development of two-year-old Lebranche mullet (*Mugil liza*) postlarviculture at different temperatures of 21, 24, 27 and 30 °C. The data show the number of individuals classified within each phase of testicular development.

Testicular development	Treatments (°C)			
	30	27	24	21
Immature	8	11	10	9
Maturing	5	6	6	4
Functional maturity	7	3	4	7
Total of males	20	20	20	20

3.3 Hormonal analysis

The values of estradiol in male fish were low, regardless of the maturing stage. For testosterone, the highest value was observed in mature males (1.29 ± 0.07 ng mL^{-1}). No significant differences were detected ($p > 0.05$) in the concentrations of estradiol and testosterone related to temperature. However, there were significant differences ($p < 0.05$) in testosterone concentration depending on sexual maturation (Table 3).

Early sexual development in commercially important species is desirable in aquaculture. The ability to control the sexual plasticity of fish is an important factor that can influence both the efficient reproduction and commercialization of fish because it influences growth and product quality [40]. In the present study, at the end of the second year, it was possible to macroscopically identify sexually mature or maturing males, indicating that early exposure to different temperatures did not affect gonadal development. Even without the observation of female samples, our data confirm the potential of *M. liza* as a suitable species for aquaculture, since in wild male Lebranche mullet, GSI values greater than 4.2% were observed for fish with a length of 41.76 cm [4], whereas in captivity, Castro et al. [11] reported GSI values of up to 1.8 ± 0.4% for mature males aged 1 year and 25.7 ± 0.4 cm. Smaller mature fish can likely facilitate brood stock management in the aquaculture industry.

Table 3. Hormonal quantification in the blood plasma of Lebranche mullet (*Mugil liza*) subjected to different temperatures of 21, 24, 27 and 30 °C. The data are related to the second year postlarviculture at different temperatures and are presented as the means (± SDs) and the maximum and minimum values of testosterone and estradiol in fish blood plasma. Different lowercase letters in the same column indicate significant difference between testicular development. Total males = *n*25; Mature = *n*15; Maturing = *n*4; Immature = *n*6.

Treatments (°C)	Testosterone (ng mL^{-1})			Estradiol (pg mL^{-1})		
	Mean	Maximum	Minimum	Mean	Maximum	Minimum
21	0.56 ± 0.46	1.29	0.116	29.69 ± 11.00	42.29	18.62
24	0.23 ± 0.13	0.45	0.103	32.12 ± 7.37	41.09	22.26
27	0.30 ± 0.36	0.85	0.050	36.58 ± 15.67	64.86	21.51
30	0.37 ± 0.40	0.99	0.041	34.77 ± 10.82	44.63	19.44
p value	0.4122			*p* value		0.4483
Testicular development	Mean	Maximum	Minimum	Mean	Maximum	Minimum
Mature	0.51 ± 0.36^{a}	1.29	0.07	30.44 ± 9.06	43.14	18.62
Maturing	0.16 ± 0.17^{b}	0.45	0.05	31.51 ± 8.73	41.64	21.12
Immature	0.09 ± 0.03^{c}	0.11	0.04	36.22 ± 8.77	44.63	21.51
p value			0.0001	*p* value		0.5810

In wild *Mugil* populations, the length at first maturity for females is 450.60 mm, whereas for males, it is 436.30 mm [41]. In captivity, the first maturation occurs in animals less than 380 mm in length for females and 330 mm in length for males [24]. Although adult female mullets are generally larger than males, the species does not exhibit external sexual dimorphism; therefore, visually, it is not possible to distinguish sex [33]. The gonads of fish smaller than 5.0 cm in total length did not present any male or female characteristics. Furthermore, although 0.2% of the 15 to 20 cm long fish and 37.3% of the male fish began sexual differentiation, the majority (62.5%) of the fish remained undifferentiated, and only those larger than 20 cm were sexually differentiated (83%). Nevertheless, spermatogenesis begins in fish larger than 25 cm, whereas oogenesis begins in fish larger than 29 cm [33, 42]. We herein estimate the size of the first sexual maturity of male *M. liza* at approximately 33--35 cm at two years of age, as previously, it was not possible to visualize the initial cells, i.e., there were no male or female characteristics, corroborating the information mentioned above.

Testosterone affects spermatogenesis in male fish, influencing the multiplication of spermatogonia and sperm formation [43, 44]. This information corroborates the findings of the present study and explains the hormonal profile observed in the blood plasma of fish, where testosterone values in mature males were greater than those in immature males (0.507 and 0.091 ng mL^{-1}), whereas estradiol values were not related to testicular development (30.44–36.22 pg mL^{-1}). Our findings establish, in an unprecedented way, the first data on the hormonal profile of the male Lebranche mullet *M. liza* of aquaculture origin. On the other hand, unlike our findings, in mature wild *M. cephalus*, the mean testosterone value was 182 ng mL^{-1}, whereas the estradiol value was 0.9 pg mL^{-1} [45], indicating a different pattern between the wild and cultivated environments.

Our current projects are aligned with these concerns and aim to elucidate the conditions and factors that may influence the sexual determination of Lebranche mullet. We have received a significant amount of information from the fishing industry in diverse regions of Brazil at different latitudes, which will aid us in comprehending why we encounter masculinized batches of fish in captivity at LAPMAR. Does our geographical position influence this? Does the medium and long-term farming system favor this condition? Perhaps the nutritional issue, since we provide commercial feeds with proteins of vegetable origin, influences gonadal maturation and some hormonal issues? Therefore, numerous hypotheses arise, and the present study becomes a starting point for new investigations involving the sexual determination of *M. liza*.

4. Conclusions

The results provide evidence that temperature may play a crucial role in sex determination in *M. liza*. The temperature range used during the larval stage suggested masculinization of the mullet and directly affected larval growth. Therefore, it is recommended that future studies explore temperatures below 21°C to deepen the understanding of the mechanisms involved in sex determination in *M. liza*, providing essential information for management and conservation strategies.

Conflict of interest statement

The authors declare that there are no competing interests.

Data availability statement

The data will be provided upon request.

Author contributions

Conceptualization: V. M. Rocha, C. S. Silva, M. C. Santos, M. R. Pilotto, F. S. Pedrotti, and M. S. Owatari. Data curation: V. M. Rocha, C. S. Silva, M. C. Santos, M. R. Pilotto, F. S. Pedrotti, and M. S. Owatari. Formal analysis: V. M. Rocha, C. S. Silva, M. C. Santos, M. R. Pilotto, F. S. Pedrotti, and M. S. Owatari. Investigation: V. M. Rocha, C. S. Silva, M. C. Santos, M. R. Pilotto, F. S. Pedrotti, and M. S. Owatari. Project management: C. Magnotti, M. L. Martins and V. R. Cerqueira. Supervision: C. Magnotti, M. L. Martins and V. R. Cerqueira. Visualization: C. Magnotti, M. L. Martins and V. R. Cerqueira. Writing (original draft): V. M. Rocha, C. S. Silva, M. C. Santos, M. R. Pilotto, F. S. Pedrotti, and M. S. Owatari. Writing (proofreading and editing): C. Magnotti, M. L. Martins and V. R. Cerqueira.

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