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Optimizing culture condition for *Liza ramada* broodstock

Mostafa A. Mousa[1*], Rania O. Barakat[1], Doaa M. El-Sisy[1] and Mosab A. Alzahaby[2]

[1]Aquaculture Division, National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt
[2]Department of Zoology, Section of Marine Biology, Faculty of Science, Al-Azhar University, Assiut Branch, Assiut- 71524, Egypt
[*]**Corresponding Author: mostafa_mousa2002@yahoo.com**

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ABSTRACT

An essential cultural parameter that influences the growth, reproduction, and physiological responses of fish species is stocking density. Thus, the goal of this research was to determine the effects of population density on water quality, growth activity, and reproduction of *L. ramada* in captivity. This investigation examined blood ion levels in *L. ramada* broodstock at varying stocking densities, along with albumin, total protein, and hormones related to gonadal maturation and growth. Ponds with different densities showed no significant differences in water quality. Growth hormone (GH) concentrations increased during gonadal maturity in ponds with low densities, while they decreased in ponds with high densities. Total protein and various serum ion concentrations were similar to GH levels, revealing a notable increase at low density compared to high density, with mature fish exhibiting higher levels. Additionally, the values of albumin and the albumin/globulin ratio changed only slightly across different densities and throughout maturation. High growth rates in total length and weight were observed in *L. ramada* stocked at reduced densities, along with higher values for condition factor and survival rate. Conversely, the food conversion ratio was lower in *L. ramada* raised in low-density ponds. However, broodstock of *L. ramada* reared at minimal density showed an increased sexual activity. Histological examinations of the testes and ovaries revealed that fish stocked at reduced densities had the highest values for the gonadosomatic index (GSI) and hepatosomatic index (HSI). Moreover, a strong correlation was observed between the GSI cycle and the concentrations of estradiol (E2) and testosterone (T). Based on these findings, it was concluded that the reduced population density is beneficial for the growth and gonadal maturation of *L. ramada*.

INTRODUCTION

Aquaculture is very interested in the physiological reactions of farmed fish since it could be possible to optimize growth progress and feed efficiency by knowing these reactions. The producing of healthy, high-quality fish is the main goal of fish farmers; but, it is now acknowledged that these traits are strongly correlated with fish density **(Roy *et al.*, 2021)**. Fish behavior, immunity, growth, survival, and performance are all negatively impacted by stocking densities that are either above or below the suggested

optimal levels **(Mugwanya *et al*., 2022; Kozłowski1 & Piotrowsk, 2024)**. Stocking density in the aquatic habitat is a crucial element that shown to directly affect the growth and physiology of several teleost species **(Montero *et al*., 1999; Ellis *et al*., 2002; North *et al*., 2006; Roy *et al*., 2021)**.

Growth hormone (GH) not only controls animal growth but also has an impact on fish social behavior, development, energy mobilization, and nutritional requirements **(Triantaphyllopoulos *et al*., 2020; Badr El-Bokhty & Amin, 2020; Velez & Unniappan, 2021)**. Numerous behavioral traits, such as predator avoidance, aggression, foraging behavior, and appetite, are impacted by GH and have ecological ramifications **(Yousefian & Shirzad, 2011; Canosa & Bertucci, 2023)**.

The gonads of both sexes synthesize T, which among other things stimulate the pituitary gland to synthesize gonadotrophins. It is also an earlier of E2 and 11-ketotestosterone (11-KT) **(Nagahama, 1994; Lubzens *et al*., 2010; Schulz *et al*., 2010; Farias *et al*., 2021)**. Granulosa cells are stimulated by follicle stimulating hormone (FSH) to produce E2, the primary hormone that causes the production of egg shell proteins and vitellogenin in the liver , which are then integrated into the oocyte in female teleost fish during the process of vitellogenesis **(Lubzens *et al*., 2010; Farias *et al*., 2021)**. FSH induced the secretion of E2 and T and controls gametogenesis; it is primarily found in the blood during early vitellogenesis and spermatogenesis **(Ogino *et al*., 2018; Sang *et al*., 2020; Ojoghoro *et al*., 2021)**.

Thin-lipped mullet, *L. ramada*, is among the most significant species of fish cultivated in Egypt. It grows readily in different densities and aquaculture methods, including mono and polyculture **(Khalil, 2001; Nawareg *et al*., 2020)**. However, knowledge on the impacts of population density on the physiology of growth and reproduction of this species is scarce. Therefore, it's important to know how various densities affect *L. ramada* to choose the right density for raising the species. Thus, the goal of this investigation was to assess how three different stocking densities; one, two, and three fish/m^3 affect the quality of water, *L. ramada* growth and reproduction in captivity. To accomplish this target, serum ion levels (sodium; Na^+, potassium; K^+, calcium; Ca^{2+}, and magnesium; Mg^{2+}), total protein, albumin, and hormones associated with growth and gonad development were examined for *L. ramada* broodstock that was stocked at various densities. In addition, histological examination of the gonads was obtained.

MATERIALS AND METHODS

Broodstock rearing

This work was finished at El-Matareyya and El-Serw Research Stations between January 1, 2021, and January 30, 2023. In freshwater earthen ponds at El-Serw, fingerlings of *L. ramada* were stocked for one year. The fish from the El-Serw ponds were harvested the following year and stocked in cement freshwater ponds at El-Matareyya at three different densities: 1, 2, and 3 fish/m^3. 10% of the ponds' water was

changed each day. Fish were given food twice at 9:00 a.m. and 16:00 pm with an amount of 3% of the average body weight. For six months, from July to December, the stocking density treatments were completed in duplicate. Every month throughout the experiment, live mullet broodstock were sampled to evaluate their growth and reproductive performances. Samples of fish were obtained every 15 days through the season of gonad maturation from November to January to make certain that all maturity stages were obtained.

Physical and chemical properties of the water

A Multi Meter (Model: MM40+ Crison Spain) was employed to measure the salinity, pH and temperature of water. Monthly measurements of the biochemical parameters were created by routine automated biochemistry using the auto analyzer Synchron CX7 clinical system (Bechman Instruments Inc., Fullerton, CA, USA): calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}) and potassium (K^{+}).

Growth performance

Following fish sampling, standard and total lengths were calculated to 0.1cm fraction, and weights were calculated to the closest 0.1g. The condition factor (k) of individual fish (g/cm^3) in each stocking treatment was calculated by applying the subsequent formula, depending on the overall weight and length measurements:

$K = W \times 100 / L^3$ **(Le-Cren, 1951)**, where W = weight (gram) and L = length (cm).

Furthermore, the feed conversion was obtained with the formula:

Conversion of feed = (feed provided per fish) / (weight gain per fish).

Reproductive activity

The liver and gonads were obtained and measured to 0.01 gram fraction. The following formulas were employed in the calculation of the HSI for each maturity stage according to **Sokal and Rohlf (1969)**:

HSI = (Liver weight / Gutted weight) x 100.

But with the following formula, the GSI was calculated for each fish:

GSI = (Gonad weight of / Fish gutted weight) x 100.

Oocytes were preserved in a 0.6% NaCl solution containing 1% formalin to obtain the oocytes diameter. The diameter of the oocytes was then measured using a microscope. The stages of maturity were evaluated, as mentioned by **Mousa (1994)**. The evaluations were derived from GSI and seasonal differences in histomorphology.

Sampling methods and analysis

Ten fish from each density, both immature and mature, were selected for physiological analysis. Moreover, blood was drawn from the caudal vein using heparinized syringes. 40mg/ l of Sigma's clove oil solution was utilized to narcotize the chosen fish while being handled **(Mousa, 2010)**. Following blood collection into centrifuge sampling tubes, the serum was obtained and frozen until examination at -20°C.

The assay of growth hormone, testosterone, and estradiol was measured by the electrochemiluminescence immunoassay (ECLIA) on the Cobas e 601 Immunoassay

Analyzer (Roche Diagnostics, Mannheim, Germany; Elecsys 2010). The following kits were employed in the calculation of the hormones:

1- Elecsys GH (Number of Catalog: 5390125).
2- Elecsys Testosterone Kit (Catalog Number: 05200067190)
3- Elecsys Estradiol Kit (Catalog Number: 06656021190)

Additionally, total protein, albumin, albumin/glubulin ratio, and the different serum ion; calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) levels were measured with the routine automated biochemistry using the autoanalyzer Synchron CX7 clinical system (Beckman Instruments Inc., Fullerton, CA, USA).

Histological technique

According to **Mousa (2010)**, the fish were anesthetized in a solution (20mg/ L) of clove oil (Sigma) prior to handling, and they were then perfused via the ascending aorta with 20mL of normal saline and 50mL of cold Bouin's fluid (4°C). The gonads were immediately postfixed for a full day in Bouin's fluid at 4°C following the dissection. The fixed gonads were then cleared and embedded in paraplast (M.P.: 56–58°C) after being dehydrated using a graded ethanol solution. Gonadal sections were stained with Harris's alum hematoxylin **(Conn, 1953)** and counterstained with an eosin aqueous solution.

Statistical analysis

One-way variance analysis, ANOVA, at $P<0.05$ was utilized to verify that the groups under test and the control exhibit notable differences; the test of Tukey was then appropriate for multiple comparisons.

RESULTS

Physico-chemical characters of water

According to the findings shown in Table (1), the density change in the ponds had no discernible effect on the water's physicochemical properties. There were no noticeable variations in the characteristics of the ponds water.

Growth performance

The findings suggested that the growth hormone levels of *L. ramada* varied with pond density. The mature fish with low density possessed the greatest amounts, while the high density fish were the least (Table 2). Furthermore, Table (2) demonstrates no appreciable changes in the amounts of GH amongst the different densities in immature fish. At the indicated times, the total protein concentrations showed a trend that resembled the GH level (Table 2). However, Table (2) shows that there aren't any notable variations in albumin or albumin/glubulin ratio across the various density treatments.

Fish stocked at varying densities had significant ($P<0.05$) high blood levels of K^+, Na^+, Ca^{2+}, and Mg^{2+} during gonad maturation compared to immature fish (Table 2). Additionally, Table (2) shows that mature fish stocked at low density had noticeably

greater levels of the represented minerals (P<0.05), while fish matured at high density showed lower values (P<0.05).

Stocking density's effects on growth outcome of *L. ramada* broodstock are enumerated in Table (3). Reducing density resulted in an enhanced growth of the mullet broodstock since fish stocked in the one fish per m^3 treatment exhibited high weight and length growth values. The overall weight gains were 240± 2.35, 185± 1.55, and 163± 1.15g/ fish for fish stocked at one, two, and three fish per m^3, respectively (Table 3). As indicated in Table (3), the food conversion ratios for *L. ramada* broodstock stocked at one, two, and three fish per m^3 were 0.51±0.01, 0.58±0.02, and 0.64±0.01, respectively.

L. ramada broodstock raised using treatment of one fish per m^3 had higher condition factor (1.04± 0.03) than that of broodstock stocked in the treatment of two fish per m^3 (0.96±0.02) or at density of three fish per m^3 (0.87±0.03) water, as indicated by the data in Table (3). Reduced stocking density caused a rise in the survival rate of the mullet broodstock since fish housed in ponds with a single fish per m^3 experienced a notable rate of 95% (Table 3).

Reproductive activity

The mullet broodstock demonstrated higher reproductive activity at the low density treatment, according to the findings in Tables (2, 4, 5).

Both testosterone and estradiol had a strong correlation with the GSI cycle, as illustrated in Table (2). In immature testis, the lowest recorded testosterone level was 0.16± 0.01ng/ ml. In male fish stocked at one fish per m^3 in freshwater ponds, the level of testosterone showed a significant (P<0.05) elevation in the ripening testis, peaking at 3.1± 0.11ng/ ml. In an immature female with a previtellogenic ovary, the range of estradiol levels was 0.20± 0.01 to 0.22± 0.01ng/ ml. Estradiol levels in prespawning females had grown dramatically (P<0.05) to reach a peak of 3.5 ± 0.14ng/ ml in the low-density ponds (Table 2).

High levels of HSI and GSI were found at low density, one fish per m^3 (Tables 4 and 5). The HSI peaked during gonad maturation in the various stocking densities, as demonstrated in Tables (4, 5), and it recorded maximum amounts in broodstock stocked in low density. The maximum numbers were noted by ripe males (1.20±0.13) and mature females (1.64±0.05), respectively. However, the HSI values of mature fish in high density were low, ranging from 1.21± 0.09 for males to 1.35± 0.14 for females (Tables 4, 5).

The GSI and HSI possess a strong association. In general, the levels were higher in female mullet than in male mullet. The GSI values of prespawning females and ripe males of mullet broodstock raised in low density during gonad maturation were 15.5± 0.09 and 9.9± 0.25, respectively, higher than those of mature fish raised in high density (8.50± 0.25 in ripe males and 11.5± 0.13 for prespawning females), as demonstrated in Tables (4, 5).

Gonadal histological peculiarities

Testis

The immature testis has small lobules, as shown in Fig, (1a). Spermatogonia and sperm mother cells make up the majority of the immature testis lobules (Fig. 1a). Large-sized seminiferous lobules that seemed to be completely packed with mature spermatozoa were an essential component of the ripe testis in low density ponds during the *L. ramada* spawning season (Fig. 1b). However, the ripe testis exhibited less activity at high densities (two and three fish per m^3), as demonstrated by the existence of an operational spermatogenic process in some lobules (Fig. 1c, d).

Ovary

As shown in Fig. (1a), the previtellogenic ovary displayed primary oocyte stages. The prespawning ovary of *L. ramada* was distinguished during the ovarian maturation period in low density ponds by the presence of maturing tertiary yolk oocytes (550-600µm in diameter), in addition to some primary oocytes (Fig. 2b). Nonetheless, in treatments with high densities, in addition to the tertiary yolk oocytes (diameters of 500–550µm), the prespawning ovaries contained a large number of primary oocytes (Fig. 2c, d).

Table 1. The mean values of water physico-chemistry during rearing of *L. ramada* broodstock at different stocking densities in freshwater ponds (from July to December)

Determinant	Stocking density		
	One fish per m^3	Two fish per m^3	Three fish per m^3
Temp. (ºC)	25-28	25-28	25-28
pH	8.05-8.15	8.10-8.25	8.15-8.3
Salinity (‰)	0.67±0.01	0.73±0.03	0.75±0.02
PO_4^{3-} (mmol l^{-1})	0.046±0.001	0.048±0.0011	0.05±0.0012
Na^+ (mmol l^{-1})	11.0±0.148	11.3±0.137	11.6±0.151
K^+ (mmol l^{-1})	0.41±0.011	0.42±0.012	0.42±0.015
Ca^{2+} (mmol l^{-1})	1.52±0.040	1.46±0.038	1.44±0.035
Mg^{2+} (mmol l^{-1})	1.42±0.016	1.42±0.016	1.42±0.016

Each value represents the Mean ± SD of 10 determinations.
There are no significantly differences (one-way ANOVA followed by a Tukey test, $P<0.05$).

Table 2. Physiological changes in *Iiza ramada* broodstock reared at different stocking densities in freshwater ponds (from July to December)

	Stocking density					
	One fish per m^3		Two fish per m^3		Three fish per m^3	
	Immature	Mature	Immature	Mature	Immature	Mature
GH ng/ml	2.1 ± 0.11	3.6 ± 0.12 [a]	2 ± 0.15	3.1 ± 0.09 [b]	2.1 ± 0.12	2.6 ± 0.11 [c]
Total Protein	2 ± 0.07	3.3 ± 0.11 [a]	2 ± 0.12	2.8 ± 0.09 [b]	1.9 ± 0.11	2.4 ± 0.13 [c]
Albumin	1.7 ± 0.10	1.7 ± 0.11	1.7 ± 0.13	1.6 ± 0.08	1.6 ± 0.12	1.6 ± 0.15
Albumin/Glubulin Ratio	0.7 ± 0.05	0.6 ± 0.06	0.7 ± 0.08	0.6 ± 0.05	0.7 ± 0.06	0.6 ± 0.07
Calcium (Ca)	11.1 ± 0.08	16.1 ± 0.12 [a]	11.3 ± 0.07	14.7 ± 0.06 [b]	11.2 ± 0.09	13.4 ± 0.25 [c]
Magnesium (Mg)	4 ± 0.08	6.5 ± 0.15 [a]	3.8 ± 0.12	5.8 ± 0.13 [b]	3.9 ± 0.11	4.4 ± 0.17 [c]
Potassium (K)	2 ± 0.07	3.8 ± 0.09 [c]	1.9 ± 0.06	3.2 ± 0.07 [b]	2 ± 0.11	2.5 ± 0.05 [a]
Sodium (Na)	160 ± 2.2	190 ± 12.5 [a]	158 ± 3.6	178 ± 6.9 [b]	162 ± 4.5	165 ± 7.5 [c]
Testosterone ng/ml (Males)	0.16 ± 0.01	3.1 ± 0.11[c]	0.17 ± 0.01	2.7 ± 0.09 [b]	0.16 ± 0.01	2.4 ± 0.07 [a]
Estradiol ng/ml (Females)	0.21± 0.01	3.5 ± 0.14 [c]	0.22 ± 0.01	3.15 ± 0.12 [b]	0.20 ± 0.01	2.84 ± 0.06 [a]

Data are reported as means ± SD.
Different letters indicate significantly differences (one-way ANOVA followed by a Tukey test, $P<0.05$).

Table 3. Growth performance of *L. ramada* broodstock reared at different stocking densities in freshwater ponds (from July to December)

Item	Stocking density		
	One fish per m^3	Two fish per m^3	Three fish per m^3
Initial average length (cm/fish)	24±0.2	24.5±0.24	24.3±0.3
Initial average weight (g/fish)	140±1.5	145±1.75	142±1.55
Final average length (cm/fish)	34 ±0.45 [a]	31.5 ±0.35 [b]	30±0.65 [c]
Final average weight (g/fish)	380±2.55 [a]	330±2.15 [b]	305±2.30 [c]
Total gain in weight (g/fish)	240±2.35 [a]	185±1.55 [b]	163±1.15 [c]
Food conversion ratio	0.51±0.01 [a]	0.58±0.02 [b]	0.64±0.01 [c]
Condition factor	1.04±0.03 [a]	0.96±0.02 [b]	0.87±0.03 [c]
Survival rate (%)	95	94	93

Data are reported as means ± SD. Different letters indicate significantly differences (one-way ANOVA followed by a Tukey test, $P<0.05$).

Table 4. Gonadosomatic index (GSI%) and hepatosomatic index (HSI%) of males *L. ramada* at different stages of maturation, reared at different stocking densities in freshwater ponds (from July to December)

Testis Stage	Stocking density					
	One fish per m^3		Two fish per m^3		Three fish per m^3	
	GSI%	HIS%	GSI%	HIS%	GSI%	HIS%
I	0.60±0.11 [c]	1.10±0.11	0.65±0.12 [b]	1.09±0.11	0.55±0.05 [a]	1.10±0.08
II	0.96±0.16 [c]	1.14±0.13	0.85±0.15 [b]	1.13±0.14	0.80±0.12 [a]	1.15±0.09
III	3.35±0.18 [c]	1.18±0.15	2.85±0.21 [b]	1.17±0.15	2.40±0.15 [a]	1.17±0.07
IV	10.9±0.25 [c]	1.23±0.13	9.75±0.35 [b]	1.21±0.15	8.50±0.25 [a]	1.21±0.09

Data are reported as means ± SD.
Different letters indicate significantly differences (one-way ANOVA followed by a Tukey test, $P<0.05$).

Table (5): Gonadosomatic index (GSI%) and hepatosomatic index (HSI%) of females *L. ramada* at different stages of maturation, reared at different stocking densities in freshwater ponds (from July to December)

Ovary Stage	Stocking density					
	One fish per m^3		Two fish per m^3		Three fish per m^3	
	GSI%	HIS%	GSI%	HIS%	GSI%	HIS%
I	0.73±0.05 [c]	1.22±0.04 [c]	0.62±0.06 [b]	1.16±0.03 [b]	0.50±0.08 [a]	0.95±0.04 [a]
II	1.25±0.06 [c]	1.30±0.04 [c]	0.98±0.05 [b]	1.20±0.04 [b]	0.92±0.07 [a]	0.95±0.08 [a]
III	3.60±0.13 [c]	1.35±0.06 [c]	3.25±0.18 [b]	1.25±0.05 [b]	1.80±0.16 [a]	1.15±0.06 [a]
IV	8.5±0.05 [c]	1.40±0.07 [c]	6.55±0.35 [b]	1.30±0.06 [b]	4.5 ±0.22 [a]	1.15±0.13 [a]
V	15.5±0.09 [c]	1.64±0.05 [c]	14.2±0.11 [b]	1.50±0.05 [b]	11.5±0.13[a]	1.35±0.14 [a]

Data are reported as means ± SD.
Different letters indicate significantly differences (one-way ANOVA followed by a Tukey test, $P<0.05$).

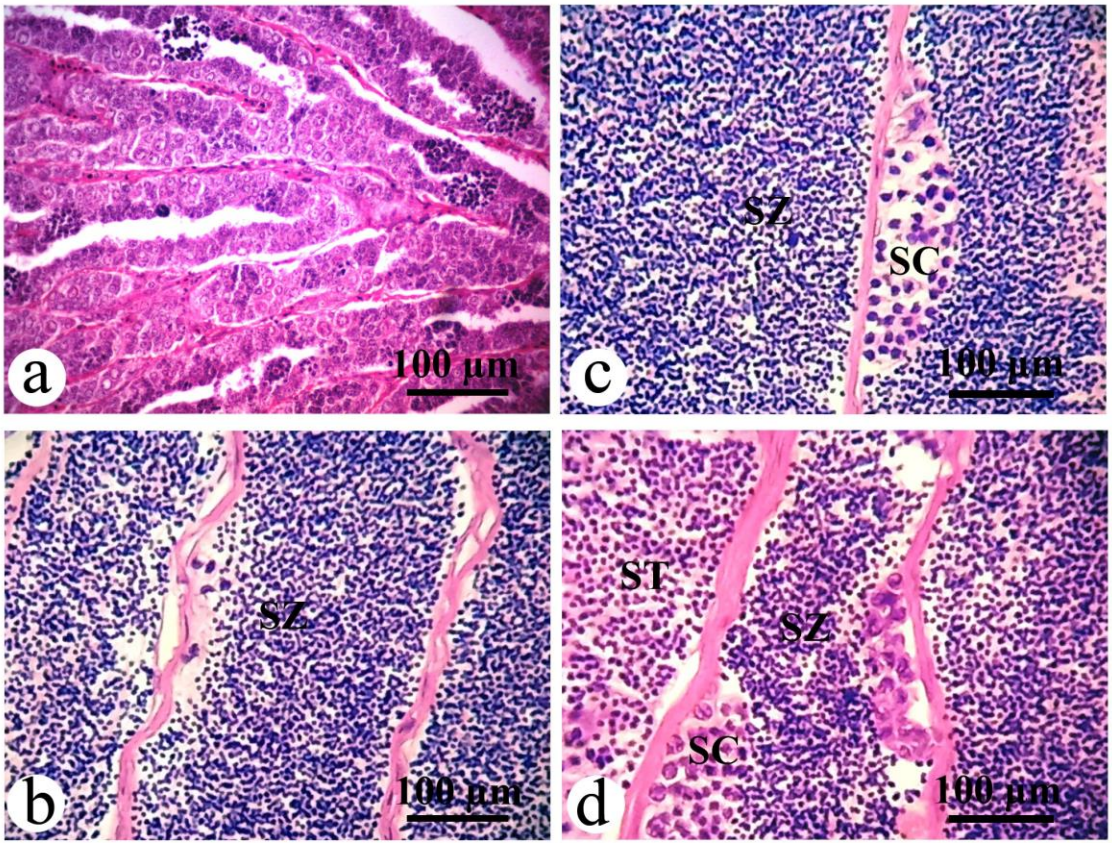

Fig. 1. Transverse section of testis of *L.ramada*, reared at different stocking densities in freshwater ponds, stained with Harris's hematoxylin and eosin: Scale bar: 100µm. **a)** Immature testis at the start of the experiment, designated the seminiferous lobules with germ cells at different stages. **b)** Ripe testis of males stocked at one fish per m^3 showing the seminiferous lobules which tremendously enlarged in size and fully packed with mature spermatozoa (SZ). **c)** Ripe testis of males stocked at two fish per m^3 showing the seminiferous lobules as **(b)** beside spermatocytes (SC). d) Ripe testis of males stocked at three fish per m^3 showed the seminiferous lobules as **(b)** beside spermatocytes **(SC)** and spermatids **(ST)**

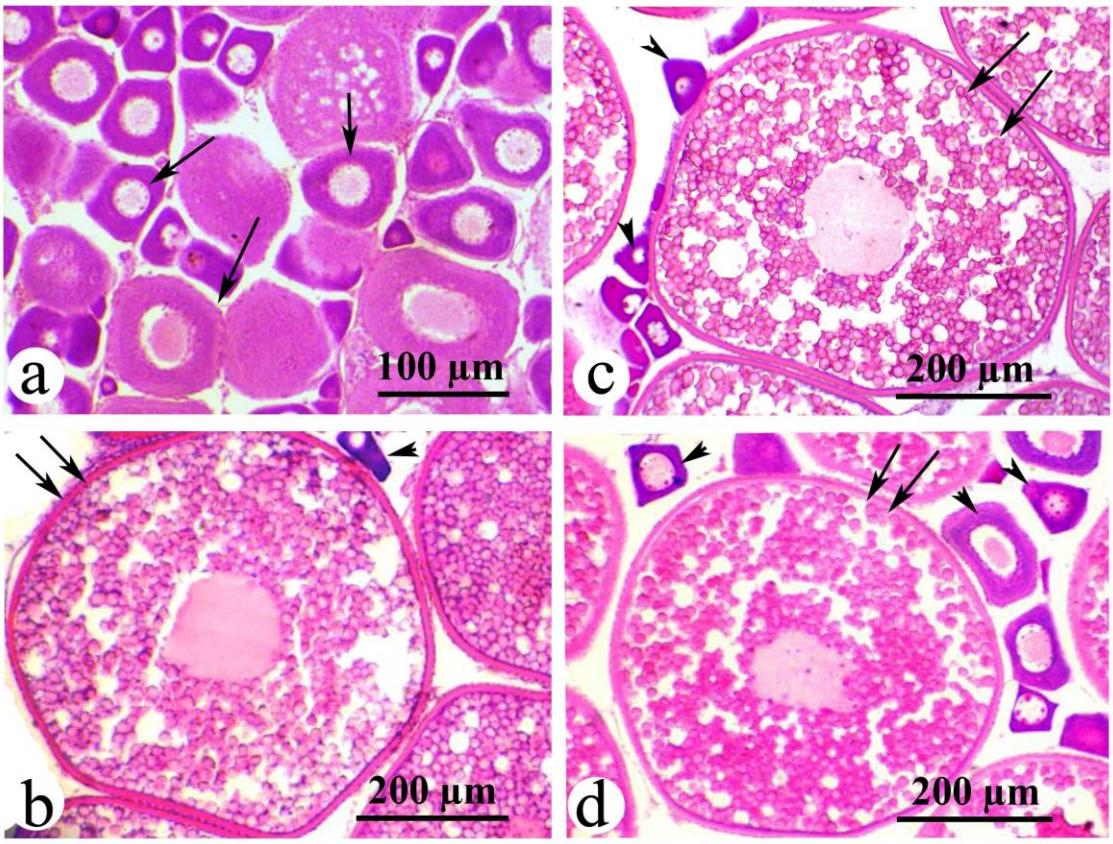

Fig. 2. Part of prespawning ovary of *L.ramada*, reared at different stocking densities in freshwater ponds, stained with Harris's hematoxylin and eosin: **a)** Previtellogenic ovary at the start of the experiment, indicated primary oocytes; PO (arrows). Scale bar: 100µm. **b)** Prespawning ovary of females stocked at one fish per m^3 exhibited tertiary yolk oocyte (arrows), in addition to some of primary oocytes (arrow head). **c)** Prespawning ovary of females stocked at two fish per m^3 exhibited tertiary yolk oocyte (arrows), beside many primary oocytes (arrow heads). **d)** Prespawning ovary of females stocked at three fish per m^3 had tertiary yolk oocyte (arrows), beside multi primary oocytes (arrow heads). Scale bar (b-d): 200µm

DISCUSSION

Stocking density is a crucial culture parameter that affects the reproduction and development, in addition to the physiological reactions of fish species. The study's results show that stocking density is a significant environmental component that influences *L. ramada* growth, reproduction, and physiological responses. Density affected blood hormones related to reproduction and growth additionally to ion levels in *L. ramada* broodstock raised in freshwater ponds of varying densities. Conversely, no discernible variations in the water's quality were observed in ponds with different densities. Mullet are

well known for being effective grazers that can eat enormous quantities of organic matter, including bacteria linked with detritus **(Erler *et al.* 2004*;* Hoang *et al.*, 2018)**.

Considering the presented data, *L. ramada* broodstock with low density possessed the greatest concentrations of growth hormone, and those with high stocking densities had the lowest. Additionally, GH increased in all densities during gonad maturation. GH influences various biological functions, such as gonadal development, appetite, and social behavior **(Munro & Lam, 1996; Canosa *et al.*, 2007)**. However, in contrast, the total protein pattern and the serum ion concentrations exhibited a notable increase in low density relative to high density, much like the growth hormone pattern. Mature fish exhibited increased values for them. Furthermore, during maturation, albumin values and the albumin/glubulin ratio varied slightly depending on density. Our findings confirmed the theory that, to keep healthy physiological responses and allow for an optimal growth, a reduced number of fish must be cultivated **(Roy *et al.*, 2021)**.

As stated by the current findings, the mullet broodstock's reproductive activity was elevated in low density ponds. One possible explanation for this could be the water's high ion concentration which stimulates the secretion of calcitonin that has a beneficial effect on reproduction **(Evans *et al.*, 2005; Mohammadi *et al.*, 2011)**. The GSI and HSI values of fish stocked at a reduced density were discovered to be high, according to the histological observation of the testes and ovaries. However, as noted, in fish species, there is a decline in the reproductive activity as stocking density increases **(Tahoun *et al.*, 2008; Shubha & Reddy, 2011)**. Fish metabolic activity and energy status are frequently estimated using the HSI **(Janssens & Waterman, 1988; Frisso *et al.*, 2020)**, and it was indicated that there was a greater need for these energy reserves at a lower density here. Additionally, a strong correlation was discovered among the concentrations of the sex steroids testosterone and estradiol and the GSI cycle. Similar observation was obtained for *Scophthalmus rhombus* and *Aphanopus carbo* **(Hachero-Cruzado *et al.*, 2007; Farias *et al.*, 2021)**. The observed sex steroid levels were suitable for *L. ramada*'s acquisition and stimulation of reproduction in captivity. Increases in the aforementioned hormones enabled the mullet to go through the physiological changes required to finish developing its gonads **(Martemyanov, 2015; Birnie-Gauvin *et al.*, 2023)**.

Fish well-being is measured in units of g/cm^3 using the condition factor (K). Increases in condition factor values, survival rate, and length and weight growth were observed in *L. ramada* stocked in freshwater ponds with low density. Likewise, reducing the number of stockings maximizes the productivity and welfare of *Colossoma macropomu* **(Frisso *et al.*, 2020)**. Space constraints are a determining factor for fish development, so the amount of space available in fish production systems is critical for growth performance **(Frisso *et al.*, 2020)**. **Watanabe *et al.* (2010)** stated that because animals in intensive systems are subjected to densification, confinement, a restricted amount of space, and potentially detrimental effects on feeding, these environments can operate as stressors **(Nash *et al.*, 2000; Rahman *et al.*, 2006; Shubha & Reddy, 2011;**

Demétrio *et al*., 2012). Profitability of fish farms is known to increase when space is used optimally for maximum production in intensive fish culture techniques **(Chakraborthy *et al*., 2010; Shubha & Reddy, 2011)**. Although it was observed that the survival rate in this research varied with density, it generally stayed high. This high survival rate (93–95%) could be made clearer by an adequate food supply, frequent water changes, and ongoing water aeration **(Al-Harbi & Siddiqui, 2000; Shubha & Reddy, 2011)**. Furthermore, *L. ramada* broodstock grown in freshwater ponds with lower densities had a lower ratio of food conversion. Moreover, broodstock of *L. ramada* showed strong reproductive activity at lower densities. Low density ponds had high percentages of mature oocytes additionally to high GSI and HSI values.

Thus, understanding the impacts of different densities on *L. ramada* provides scientists using the information required to ascertain the ideal stocking density for raising it. The findings of the present investigation allow us to conclude that *L. ramada* in captivity grows and reaches sexual maturity best in lower densities freshwater ponds.

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Conflict of Interest

The authors state that there are no conflicts of interest.

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