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The Effects of Salinity on the Stress Response, Osmoregulation, Growth and Reproduction of *Liza ramada* in Captivity

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

Salinity is a crucial environmental factor that influences the growth, reproduction, and physiological responses of fish species. Understanding how different salinities affect *Liza ramada* is essential for selecting optimal conditions for aquaculture. This study aimed to assess the effects of salinity on the growth, osmoregulation, stress response, and maturation of *L. ramada* in captivity. In this investigation, blood levels of hormones related to growth, adaptation, and stress response were measured, along with glucose levels in *L. ramada* broodstock raised in the waters of varying salinities. Results indicated that prolactin and growth hormone levels were higher in freshwater and lower in saline conditions. Additionally, these hormone levels increased during gonad maturation across all salinities. In contrast, thyroxine concentrations exhibited a pattern similar to cortisol, with significantly higher levels in saline water compared to freshwater. Mature fish also displayed elevated hormone levels. Glucose concentrations in the fish raised in different salinities followed a similar trend to cortisol levels. Furthermore, triiodothyronine hormone levels varied slightly during maturation and across different salinities. The *L. ramada* stock in freshwater demonstrated superior growth rates in both length and weight, alongside higher condition factor and survival rates. However, broodstock grown in freshwater had a lower food conversion ratio. In saline environments, *L. ramada* exhibited increased sexual activity, with a higher frequency of mature gonad stages and elevated values of the gonadosomatic index (GSI) and hepatosomatic index (HSI). In light of these findings, we conclude that freshwater is conducive to the growth of *L. ramada*, while saline water is essential for achieving sexual maturity.

INTRODUCTION

An essential environmental component that affects fish species' growth, survival, and physiological responses is salinity. The gonads maturation, adaptation to seawater, and spawning induction can all cause stress in mature *L. ramada* fish **(Mousa & Mousa, 2006)**. Stress negatively affects fish growth, osmoregulation and reproduction

(Wendelaar Bonga, 1997; Flik & Wendelaar Bonga, 2001; Schreck *et al*., 2001). Research on the physiological alterations induced by salinity variations in aquatic environments has predominantly focused on commercially raised species **(Cataldi *et al*., 2005; Farshadian *et al*., 2019)**. Many key hormones, such as thyroxine (T4), triiodothyronine (T3), prolactin (PRL), cortisol, and growth hormone (GH), regulate the ionosomotic balance in fish **(Sakamato & McCormick, 2006; Yeşilbaş & Oğuz, 2022)**.

Following a salinity challenge, altered blood parameters, such as glucose or cortisol levels, are referred to as stress indicators **(Cataldi *et al*., 2005; Tsui *et al*., 2012; Farshadian *et al*., 2019)**. Enough energy provision is a crucial component of stress management, and numerous studies have assessed the alterations in blood metabolites that take place throughout a salinity challenge **(Arjona *et al*., 2009; Salati *et al*., 2011; Anni *et al*., 2016)**. The primary function of cortisol is to facilitate the metabolism of proteins, fats, and carbohydrates **(Wang *et al*., 2022)**. Gluconeogenesis, which produces glucose molecules mainly from the liver's synthesis of fat and protein, is provoked by glucocorticoids and causes a rise in blood glucose levels **(Han *et al*., 2022)**. Growth hormone (GH) affects fish social behavior, development, energy mobilization, and nutritional needs in addition to regulating animal growth **(Triantaphyllopoulos *et al*., 2020; Velez & Unniappan, 2021)**. GH affects a variety of behavioral traits with ecological consequences, including predator avoidance, aggression, foraging behavior, and appetite **(Yousefian & Shirzad, 2011; Canosa & Bertucci, 2023)**.

Thyroid hormone is well known to control fish development, differentiation, osmoregulatory effects, and regulation **(Peter *et al*., 2000; Movahedinia *et al*., 2009; Deal & Volkoff, 2020; Seale *et al*., 2021)**. The two thyroid hormones whose secretion is impacted by activity levels are triiodothyronine (T3) and thyroxine (T4). Increased production of metabolic heat and accelerated glucose oxidation are the main mechanisms by which T4 and T3 enhance metabolism **(Zwahlen *et al*., 2024)**. In addition, thyroid hormones promote the growth of bones, tissues, and the nervous system **(Zwahlen *et al*., 2024)**.

To determine possible causes of fish death prior to oviposition and to develop an efficient hatchery technology, it is imperative to know the physiological makeup of fish during the cycle of reproduction and acclimation to seawater. In this study, blood glucose levels and hormones linked to growth, adaptation, and stress response were assessed in *L. ramada* broodstock raised in waters with different salinities. Understanding the hormone levels described above will give biologists and other researchers a starting point for monitoring and assessing the general health and metabolic status of the mullet, whether they are from the wild environment or kept in captivity.

MATERIALS AND METHODS

Rearing of broodstock

Between January 1, 2022 and January 30, 2024, this research was completed at El-Matareyya and El-Serw Research Stations. Fingerlings of *L. ramada* were stocked for a year in freshwater earthen ponds at El-Serw. The following year, fish from the El-Serw ponds were harvested and put into cement ponds at El-Matareyya that had varying salinities: 0‰ for freshwater, 15‰ for brackish water, and 30‰ for saline water. In 35m^3 cement ponds, fish were stocked at a density of two fish per m^3, and fed twice a day at 9.00 a.m. and 16.00 pm 3% of their live body weight, on average. The six-month trial treatments were conducted in triplicate from July to December. During the experiments, live mullet broodstock were collected every month to examine their reproductive as well as growth outcomes. To ensure that all maturing phases were met, fish were obtained half monthly from November to January during the spawning season.

Growth performance

Following fish collection, weights were calculated to the closest 0.1gm and total and standard lengths were determined to the nearest 0.1cm.

Condition factor

Using the following equation, the condition factor (k) for each fish (g/cm^3) was calculated:

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

Feed conversion

The following was employed in the calculation of the conversion of feed:

Feed conversion = (feed given per fish) / (gain in weight per fish).

Reproductive activity

After being removed from the body cavity, the gonads and liver were weighed to the closest 0.01 gram.

Hepatosomatic index (HSI)

In accordance with **Sokal and Rohlf (1969)**, the HSI was determined for each maturity stage using the following equations:

HSI = (Weight of the liver/ Gutted weight) x 100.

Gonadosomatic index (GSI)

Based on the subsequent formula, the GSI was found out for every fish:

GSI = (Weight of gonad / Weight of gutted fish) x 100.

To measure the oocyte diameter, the oocytes were kept in a 0.6% solution of NaCl comprising 1% formalin. Following that, they were placed on a slide of glass and measured using an ocular micrometer. According to **Mousa (1994)**, the phases of maturity were evaluated. GSI and seasonal variations in histomorphology formed the basis for the assessments. For both sexes, the monthly percentage of each maturity stage was computed and documented.

Sampling and analytical techniques

For physiological analysis, ten fish at the immature and mature stages were taken from each treatment. Heparinized syringes were used to draw blood from the caudal vein. During handling, the selected fish were narcotized using 40mg/ l clove oil solution (Sigma) **(Mousa, 2010)**. After drawing blood into centrifuge sampling tubes, the serum was separated using the centrifuge and stored freezing at -20°C until analysis.

The electrochemiluminescence immunoassay (ECLIA) on the Cobas e 601 Immunoassay Analyzer (Roche Diagnostics, Mannheim, Germany; Elecsys 2010) was employed to get the assay of cortisol, T4, T3, GH, and PRL. The hormones were calculated utilizing the kits listed below:

1- Elecsys Cortisol Kit (Catalog Number: 06687733190).
2- Elecsys T4 Kit (Catalog Number: 09007784190).
3- Elecsys T3 Kit (Catalog Number: 09007733190).
4- Elecsys Prolactin (Catalog Number: 03203093190).
5- Elecsys GH (Number of Catalog: 5390125).

Additionally, the auto analyzer Synchron CX7 clinical system (Bechman Instruments Inco. USA) was employed to assess serum glucose levels.

Statistical analysis

To confirm significant differences between the tested and control groups, the one-way analysis of variance (ANOVA) was performed at a significance level of $P<0.05$ for multiple comparisons. Following this, Tukey's post-hoc test was applied to determine which specific groups showed significant differences from one another.

RESULTS

The current findings showed physiological changes in *L. ramada* broodstock raised in varying salinities. The measured levels of glucose and the hormones responsible for growth, adaptation, and response to stress in immature and mature *L. ramada* are displayed in Table (1).

Growth hormone

Overall, the findings demonstrated that *L. ramada* growth hormone levels varied according to the water's salinity, with the highest levels found in freshwater fish and the lowest in saline water fish (Table 1). Additionally, as Table (1) shows, mature fish had higher concentrations of GH than immature fish.

Stress-response and acclimatization hormones

Table (1) shows that the levels of cortisol hormone were higher in saline water than in fresh water fish. Additionally, cortisol levels rose dramatically during sexual maturity, giving higher levels in mature fish in waters with different salinities (Table 1). For the fish raised in the various salinities, glucose concentrations showed a pattern comparable to that of cortisol, as indicated from data presented in Table (1).

The acclimation hormone, prolactin, exhibited lower levels in brackish and saline waters compared to freshwater environments. However, it was observed that prolactin

levels increased during maturation across all water types, suggesting its crucial role in the physiological adaptation of *Liza ramada* to varying salinities as well as in the reproductive process (Table 1).

Thyroid hormones

The observed thyroxine concentration revealed a related pattern to cortisol, showing a notable rise in saline water compared to fresh water (Table 1). The highest levels were obtained in mature fish. However, the values of triiodothyronine hormone had slight differences in different waters and during maturation, as obtained in Table (1).

Growth performance

Table (2) provides a summary for the impacts of water salinity regarding the growth outcome of *L. ramada* broodstock. Lowering the concentration of water salinity resulted in better mullet broodstock growth, as fish stocked in freshwater showed high growth values in both length and weight. For fish raised in freshwater (0°), brackish (15°), saline (30°), and the overall weight gains were 245±2.25, 170±2.15, and 150±1.15g/ fish, respectively (Table 2).

The food conversion ratios for *L. ramada* broodstock raised in freshwater (0°), brackish (15°) and saline (30°) waters were 0.50±0.01, 0.57±0.02, and 0.65±0.01, respectively, as shown in Table (2).

The condition factor (K) is a metric used to quantify fish well-being (g/cm^3). According to the data in Table (2), the condition factor of *L. ramada* broodstock raised in fresh water was higher (1.05±0.04) than that of broodstock raised in brackish (0.95±0.02) or saline (0.85±0.02) water.

The survival rate of mullet broodstock increased with reduced salinity in the water since a remarkable 100% survival rate was noted for fish kept in freshwater ponds (Table 2).

Reproductive activity

The results obtained and presented in Tables (3- 6) show that the mullet broodstock exhibited an increased reproductive activity in saline water. In saline water, high values of HSI and GSI were found, along with a high percentage of frequency for mature gonad stages (Tables 3- 6).

As seen in Tables (4, 6), the hepatosomatic index rose gradually throughout the gonad maturation in the various waters and recorded high values in the mullet broodstock raised in saline water. Ripe males and prespawning females gave the highest values, 1.25±0.02 and 1.65±0.07, respectively. On the other hand, mature fish in freshwater had low HSI values, with 1.22±0.13 in males and 1.40±0.15 in females (Tables 4, 6).

Hepatosomatic index (HSI) and gonadosomatic index (GSI) are strongly correlated. They generally indicated higher levels in the mullet females than in males. As shown in Tables (4, 6), the GSI values for the mullet broodstock raised in saline water during gonad maturation were higher (11.0±0.21 for ripe males and 16.0±0.08 for

prespawning females) than those of mature fish raised in fresh water (7.50±0.35 for males and 12.0±0.12 for females).

Table 1. Physiological changes in *Iiza ramada* broodstock reared in waters with different salinities for six months

	Freshwater (0‰)		Brackish water (15‰)		Saline water (30‰)	
	Immature	Mature	Immature	Mature	Immature	Mature
GH ng/ml	2 ± 0.10 [a]	3.5 ± 0.12 [b]	1.8 ± 0.22 [c]	2.8 ± 0.06 [d]	1.5 ± 0.12 [e]	2.4 ± 0.25 [f]
Cortisol ng/ml	130 ± 3.50 [a]	170 ± 3.4 [b]	140 ± 2.7 [c]	190 ± 2.9 [d]	202 ± 3.8 [e]	325 ± 6.2 [f]
Glucose mg/ml	0.80 ± 0.03 [a]	0.94 ± 0.04 [b]	1.01 ± 0.06 [c]	1.07 ± 0.03 [d]	1.15 ± 0.03 [e]	1.29 ± 0.04 [f]
Prolactin ng/ml	1.9 ± 0.12 [a]	3 ± 0.14 [b]	1.3 ± 0.21 [c]	2.6 ± 0.05 [d]	1.1 ± 0.11 [e]	2.2 ± 0.31 [f]
T_3 ng/ml	5.51 ± 0.20 [a]	4.9 ± 0.13 [b]	6 ± 0.15 [c]	4.2 ± 0.17 [d]	5.5 ± 0.20 [e]	6.6 ± 0.21 [e]
T_4 ng/ml	18 ± 1.1 [a]	38 ± 1.3 [b]	22 ± 1.2 [c]	66 ± 0.55 [d]	30 ± 2.1 [e]	85 ± 3.2 [f]

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

Table 2. Growth performance of *L. ramada* broodstock reared in waters with different salinities for six months

Item	Freshwater (0‰)	Brackish water (15‰)	Saline water (30‰)
Initial average length (cm/fish)	25.5±0.12	26.0±0.14	25.5±0.25
Initial average weight (g/fish)	145±2.15	150±2.75	140±2.55
Survival rate (%)	95	90	90
Final average length (cm/fish)	35 ±0.65	32 ±0.45	30±0.55
Final average weight (g/fish)	390±3.75	320±2.95	290±3.35
Total gain in weight (g/fish)	245±2.25	170±2.15	150±1.15
Food conversion ratio	0.50±0.01	0.57±0.02	0.65±0.01
Condition factor	1.05±0.04	0.95±0.02	0.85±0.02

Table 3. Monthly variations in the frequency (%) of testicular stages of *L. ramada* during testicular cycle in waters with different salinities for six months

Month	Water salinity	No. of fish	I	II	III	IV
Jul	Freshwater (0‰)	10	100			
	Brackish water (15‰)	10	100			
	Saline water (30‰)	10	100			
Aug	Freshwater (0‰)	10	100			
	Brackish water (15‰)	10	100			
	Saline water (30‰)	10	100			
Sep	Freshwater (0‰)	10	70	30		
	Brackish water (15‰)	10	50	50		
	Saline water (30‰)	10	35	65		
Oct	Freshwater (0‰)	10	40	40	20	
	Brackish water (15‰)	10	40	30	30	
	Saline water (30‰)	10	20	30	50	
Nov	Freshwater (0‰)	10	10	40	30	20
	Brackish water (15‰)	10		20	40	40
	Saline water (30‰)	10		20	30	50
Dec	Freshwater (0‰)	10		10	20	70
	Brackish water (15‰)	10			20	80
	Saline water (30‰)	10				100

Table 4. Gonadosomatic index (GSI%) and hepatosomatic index (HSI%) of males *L. ramada* at different stages of maturation reared in waters with different salinities for six months

Testis Stage	Water salinity					
	Freshwater (0‰)		Brackish water (15‰)		Saline water (30‰)	
	GSI%	HIS%	GSI%	HIS%	GSI%	HIS%
I	0.45±0.07 [a]	1.20±0.12 [a]	0.50±0.15 [b]	1.08±0.215 [b]	0.60±0.11 [c]	1.10±0.02 [b]
II	0.70±0.11 [a]	1.13±0.21 [a]	0.80±0.25 [b]	1.14±0.144 [a]	0.90±0.15 [c]	1.16±0.03 [a]
III	2.20±0.25 [a]	1.20±0.15 [a]	2.90±0.35 [b]	1.17±0.254 [a]	3.20±0.17 [c]	1.19±0.05 [a]
IV	7.50±0.35 [a]	1.22±0.13 [a]	9.50±0.45 [b]	1.21±0.15 [a]	11.0±0.21 [c]	1.25±0.02 [a]

Data are reported as means ± SD.

Different letters indicate significantly differences (one-way ANOVA followed by a Tukey test, $P<0.05$).

Table 5. Monthly variations in the frequency (%) of ovarian stages of *L. ramada* during ovarian cycle in waters with different salinities for six months

Month	Water Type	No. of fish	I	II	III	IV	V
Jul	Freshwater (0‰)	10	100				
	Brackish water (15‰)	10	100				
	Saline water (30‰)	10	100				
Aug	Freshwater (0‰)	10	100				
	Brackish water (15‰)	10	100				
	Saline water (30‰)	10	100				
Sep	Freshwater (0‰)	10	80	20			
	Brackish water (15‰)	10	70	30			
	Saline water (30‰)	10	60	40			
Oct	Freshwater (0‰)	10	60	20	10	10	
	Brackish water (15‰)	10	50	20	20	10	
	Saline water (30‰)	10	30	30	20	20	
Nov	Freshwater (0‰)	10		40	30	20	10
	Brackish water (15‰)	10		20	30	30	20
	Saline water (30‰)	10		10	20	40	30
Dec	Freshwater (0‰)	10			10	20	70
	Brackish water (15‰)	10				20	80
	Saline water (30‰)	10					100

Table 6. Gonadosomatic index (GSI%) and hepatosomatic index (HSI%) of females *L. ramada* at different stages of maturation reared in waters with different salinities for six months

Ovary stage	Water salinity					
	Freshwater (0‰)		Brackish water (15‰)		Saline water (30‰)	
	GSI%	HIS%	GSI%	HIS%	GSI%	HIS%
I	0.55±0.11 ^{a}	0.94±0.05 ^{a}	0.60±0.04 ^{b}	1.15±0.05 ^{b}	0.70±0.05 ^{c}	1.25±0.04 ^{c}
II	0.90±0.09 ^{a}	0.95±0.08 ^{a}	0.95±0.07 ^{b}	1.30±0.04 ^{b}	1.20±0.07 ^{c}	1.30±0.05 ^{c}
III	1.90±0.15 ^{a}	1.15±0.07 ^{a}	3.20±0.15 ^{b}	1.30±0.05 ^{b}	3.50±0.12 ^{c}	1.40±0.06 ^{c}
IV	5.0 ±0.25 ^{a}	1.15±0.12 ^{a}	7.50±0.55 ^{b}	1.35±0.08 ^{b}	9.0±0.06 ^{c}	1.45±0.05 ^{c}
V	12.0±0.12 ^{a}	1.40±0.15 ^{a}	14.0±0.12 ^{b}	1.50±0.07 ^{b}	16.0±0.08 ^{c}	1.65±0.07 ^{c}

Data are reported as means ± SD.

Different letters indicate significantly differences (one-way ANOVA followed by a Tukey test, $p<0.05$).

DISCUSSION

Based on the findings of this investigation, the salinity of the water is a significant environmental component that affects the growth, reproduction, and physiological responses of *L. ramada*. When *L. ramada* broodstock was raised in waters with different salinities, the salinity had an impact on glucose levels as well as hormones linked to growth, adaptation, and stress response in the blood. Based on the aforementioned hormones' trends, water salinity may serve as an environmental cue for the regulation of hormone activity **(Costa *et al*., 2019; Sánchez-Vázquez *et al*., 2019; Zhang *et al*., 2024)**.

In light of the information received, fresh water had the highest levels of growth and prolactin hormones in *L. ramada* broodstock, while saline water had the lowest levels. Moreover, during gonad maturation, they increased in all waters. Growth hormone (GH) affects many biological processes, such as appetite, social behavior, growth, and gonadal development **(Munro & Lam, 1996; Canosa *et al*., 2007)**. Similar to prolactin, growth hormone aids in the acclimation of several teleost fish to seawater **(Sakamoto & McCormick, 2006; Ferreira-Martins *et al*., 2023)**. Reports state that cortisol and prolactin aid in the chloride cells development and differentiation in freshwater, but cortisol and GH control the differentiation and growth of chloride cells of the seawater kind and branchial epithelia **(Sakamoto & McCormick, 2006)**. In fish, prolactin serves a variety of physiological purposes. Fish prolactin affects reproduction, migration, the behavior of caring for offspring, pregnancy, and the provision of nutrients to juveniles in various ways **(Freeman *et al*., 2000; Saha *et al*., 2021)**. Water and ion flow are controlled by the PRL **(Anderson & Itallie, 2009; Saha *et al*., 2021)**. PRL also promotes the growth of fish sexual organs and aids in the reproductive cycle **(Saha *et al*., 2021)**.

The pattern of thyroxine concentration, however, was comparable to that of cortisol and indicated a notable rise in saltwater fish in contrast with freshwater fish. The highest values of them were discovered in mature fish. Additionally, the glucose levels of the fish raised in various salinities showed a cortisol pattern similarity. Additionally, the amounts of triiodothyronine hormone varied slightly in different waters and throughout maturation. Numerous fish studies have demonstrated that the thyroid axis's constituent parts respond to external cues **(Grau, 1988; Cowan *et al*., 2017)**. THs have a variety of roles in teleosts, including growth and development, feeding and nutrition metabolism, metamorphosis, and reproduction **(Mousa *et al*., 2018; Deal & Volkoff, 2020; Seale *et al*., 2021; Prazdnikov & Shkil, 2023)**. THs accelerate the rate at which glucose is oxidized, increasing the quantity of metabolic heat generated **(Oki & Atkinson, 2004; Deal & Volkoff, 2020)**. The induction of spawning in mullet required a higher degree of homeostasis due to the stressful environment, which required a continuous production of cortisol. Under stress, cortisol is the main hormone released by fish. While, the primary role of cortisol, in helping animals metabolically withstand stressor insult, is energy repartitioning during stress **(Milla *et al*., 2009; Pankhurst, 2016)**.

The levels that were noted were appropriate for the acquisition and stimulation the reproduction of *L. ramada* in captivity. Elevations in the previously mentioned hormones allowed the mullet to experience the physiological adjustments necessary for acclimatization to the spawning environment, stress reaction, and complete development **(Martemyanov, 2015; Birnie-Gauvin *et al*., 2023)**. *L. ramada* stocked in freshwater ponds showed greater condition factor values, survival rate, and growth in both length and weight. Moreover, the ratio of food conversion of *L. ramada* broodstock raised in freshwater ponds was lower. Fish acclimating to their new environment experienced changes in metabolic and growth parameters, which likely inhibited their potential for growth due to potential energy costs or osmoregulation stress **(Hayashi *et al*., 2021)**. Conversely, *L. ramada* broodstock exhibited a high reproductive activity in saline water. In addition to the high gonadosomatic index (GSI) and hepatosomatic index (HSI) values, a high percentage of mature gonad stages were seen in the saline water.

Consequently, knowing how various salinities impact *L. ramada* gives scientists the data required to ascertain the best habitat for raising it. We can conclude based on the findings of this investigation that fresh water is good for the growth of *L. ramada* and saline water is necessary for sexual maturity.

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Conflict of Interest

As stated by the authors, there aren't any conflicts of interest.

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