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Experimental Studies on the Reproduction of the Thin-Lipped Mullet, *Liza ramada*

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ABSTRACT

Like females of many commercially important fishes, *Liza ramada* fail to complete ovarian development and do not undergo final maturation (FOM), ovulation or spawning when reared in captivity. The aim of the present work was to investigate the histological and physiological changes during the reproductive cycle of *Liza ramada* reared in freshwater fish ponds and during induction of spawning in saline water.

In the present study, the levels of total thyroxine (T4), triiodothyronine (T3) and cortisol in the plasma of *Liza ramada* in a complete reproductive cycle were measured in correlation with the seasonal histological changes in gonads. During the reproductive cycle of females, serum triiodothyronine (T_3), thyroxine (T_4) and cortisol decreased during ovarian early-vitellogenesis and increased during mid-vitellogenesis to reach a peak for both T_4 and cortisol. Then, these hormones declined to low levels during late-vitellogenesis. At the prespawning stage, all mentioned hormones re-increased to high levels and finally declined during induction of spawning. There was a decrease in serum levels of thyroid and cortisol hormones coincided with an increase in testicular activity of the fish. T_3 and T_4 increased during testis ripening to reach a peak during spawning, while cortisol reached a peak during ripe stage and decreased to low levels during spawning.

In conclusion, the seasonal changes in thyroid hormones and cortisol concomitant with gonadal maturation and spawning of *Liza ramada* support role for these hormones in reproduction and stress response of this fish.

INTRODUCTION

In teleosts, thyroid hormones (THs) have been found to be involved in a variety of physiological processes. Among their many possible functions, these hormones are thought to influence seasonal adaptations and annual events such as osmoregulation and reproduction (Biswas *et al*., 2006; Arjona *et al*., 2008; Nelson *et al.*, 2011; Habibi *et al.*, 2012). Although reproductive hormones and environmental factors are primarily responsible for the regulation of the seasonal gonadal cycle (Das, 2011), the influence of other endocrine factors such as thyroid and adrenal are poorly known in teleosts. Thyroid hormone elevations occurring during ovarian maturation may provide a source of thyroxine and triiodothyronine for deposition in eggs, and later embryonic or larval metabolism (Norberg *et al*., 1989).

Other reports have ascribed such peaks to direct or indirect thyroid involvement in gonadal maturation (Parhar *et al*., 1994; Björnsson *et al*., 1998). There is a general inverse relationship between thyroid hormones (T3, T4) and advanced maturity stage in several freshwater species: the brook trout, *Salvelinus fontinalis* (White and Henderson, 1977); rainbow trout, *Oncorhynchus mykiss* (Pavlidis *et al.*, 1991; Eales and Brown, 1993); Atlantic salmon, *Oncorhynchus nerka* (Biddiscombe and Idler, 1983); and Pacific salmon, *Oncorhynchus keta* (Ueda *et al.*, 1984). However, there were no significant differences in serum T3 and T4 levels among the maturity stages of *Dentex dentex*. Thyroid hormones may enhance early ovarian development and stimulate vitellogenesis in female *Dentex dentex* (Pavlidis *et al*., 2000). Furthermore, Das *et al*. (2013) suggested that T3 and T4 are involved probably to trigger oocyte growth and vitellogenesis, whereas, cortisol, epinephrine, norepinephrine and insulin synergistically help to induce final maturation in the spawning phase of *Mugil cephalus*.

Cortisol, secreted by the interrenal cells of the head kidney, is a potent gluco- and mineralocorticoid in teleostean fish. It plays a pivotal role in the stress response and in osmoregulatory processes (Wendelaar Bonga, 1997; McCormick, 2001; Flik *et al*., 2006; Arjona *et al*., 2008). Suchiang and Gupta (2011) have also reported interrelationship of Peak T3 level with peak testicular activity, confirmed by Gonadosomatic index (GSI) and mature spermatozoa in male catfish. But, cortisol levels are higher in spermiating males and ovulated females than in prespawning white suckers (*Catostomus commersoni*) when gonadotropin (GtH) and estradiol-17β (E2), testosterone (T), 11-keto testosterone (11-kt), 17α-hydroxyprogesterone (17-P). 17α-hydroxy-20β-dihydroxyprogesterone (17, 20P) and androstenedione (A) remain low (Scott *et al.* 1984).

The mullets are euryhaline species spawning only in salty water but can also grow in brackish and fresh waters. Like females of many commercially important fishes, mullets fail to complete ovarian development and do not undergo final oocyte maturation (FOM), ovulation or spawning when reared in captivity (Mousa, 1994; Mousa and Mousa, 1997; Mousa and El-Gamal, 1999). Reproduction in fish is regulated by external environmental factors that trigger internal mechanisms into action (Rottmann *et al*., 1991). The reproductive cycle can be controlled by either placing the fish in an appropriate environment or by changing the fish internal regulating factors with injected hormones or other substances (Das, 2011; Das *et al*., 2013). Most marine fish eggs are pelagic (Blaxter, 1969), but in salinities below a certain threshold these eggs sink. The salinity threshold for buoyancy is important when the lower salinity tolerance of pelagic fish eggs is being considered, because sinking eggs will encounter different environmental conditions, which may or may not be conducive to embryonic development (May, 1974).

Little information is available on the histological and physiological changes during complete reproductive cycle of *Liza ramada*. Therefore, the aim of the present work was to investigate the levels of extra-gonadal hormones; total thyroxine (T4), triiodothyronine (T3) and cortisol in the plasma of *Liza ramada* reared in freshwater fish ponds and during induction of spawning in saline water.

MATERIALS AND METHODS

The experimental design and treatments:

L. ramada fingerlings were originally obtained from spawning at El-Matareyya Research station and raised in El-Serw Fish Research Station for two

years. Fingerlings were stocked in earthen ponds at a density of 1 fish/ 4 m^3. They were fed daily with 35% protein diet at a rate of 1.5 % of their biomass. Water quality in the ponds was maintained by partial water exchange (10%) daily. After two years *L.ramada* reached maturation.

Mature *L.ramada,* with standard length larger than 23cm, were collected alive at intervals of about one month throughout the year. However, during the prespawning and spawning season from (November to January), fish were collected at intervals of about 15 days to ensure that all stages of gonad maturation were included.

Ripe ovary stage was obtained according to the method of Mousa (1999). In breif, the prespawning females were acclimated to saline water (32 ‰) and maintained in 2000-l circular fiberglass tanks (15 fish / Tank) equipped with seawater and constant aeration. Final oocyte maturation was achieved utilizing the pregnyl (HCG) as a priming injection at a dose of 15,000 IU/kg body weight followed, 24hs. later by resolving injection of 30,000 IU HCG in combination with 200 μg LHRH–a/kg body weight .

Blood sampling and hormones determination:

Immediately after catch, the fish were anesthetized in a solution of clove oil (20 mg l^{-1}) (Sigma) before handling according to the previous studies by Mousa (2004). Blood samples of 15 ml were taken by caudal severance into micro centrifuge tubes and centrifuged. Plasma was separated and stored frozen at –20° C until required.

Plasma total thyroxine (T4) (Schurrs and van Weeman 1977), total triiodothyronine (T3) (Walker 1977) and cortisol (Barry *et al*., 1993) were measured using an enzyme-linked immunoassay (ELISA).

Measurements and classification of maturity stages:

After the collection of fishes, their total and standard lengths were measured to the nearest 0.1 Cm.

The gonads were extirpated from the body cavity, weighed to the nearest 0.01 gm. The gonadosomatic index (GSI) was calculated for each fish according to the following formula:

$$GSI = \frac{\text{Weight of the gonad}}{\text{Gutted weight}} \times 100$$

For the measurements of the oocyte diameter, the oocytes were preserved in a solution of 1% formalin in 0.6% NaCl. They were then placed on a glass slide and measured with an ocular micrometer.

Based on the gonads morphology and their gonadosomatic index (GSI), five sexual maturity stages were signified as adult males; stage I, stage II, stage III, stage IV (Ripe) and stage V (Spent or postspawning).

For females, six ovarian stages were distinguished according to morphological and microscopical appearance, egg diameter and GSI data: stage I, stage II, stage III, stage IV, stage V, stage VI (Ripe stage).

Tissue processing and histology:

The fishes were anesthetized in a solution (20 mg/l) of clove oil (Sigma) before handling according to Mousa (2004) and then perfused via the ascending aorta with 20 ml of normal saline, followed by 50 ml of Bouin's fluid at 4°C. The gonads were removed and post fixed in Bouin's fluid for 24 h at 4°C. The fixed gonads were thereafter dehydrated through graded ethanol solution, cleared and embedded in

paraplast (M.P.: 56–58 °C). Consecutive transverse sections of the gonads were made at 4 μm thickness. Sections of gonads were stained with Harris's alum hematoxylin (Conn, 1953) and aqueous solution of eosin as a counter stain.

Statistical Analysis

Data were analyzed by ANOVA using a randomized block design with the experiment as blocking factor. Post hoc comparisons are based on Tukey's honestly significant difference (HSD) test. All statistical tests were performed with SPSS (Statistical Package for the Social Sciences, IBM version 22). Statistical significance was accepted at $P < 0.05$.

RESULTS

Gonadal cycle:

Testicular cycle:

On the basis of seasonal changes encountered in the histomorphology and gonadosomatic index (Table 1), the seasonal changes in testicular activity of male *L.ramada* in fresh water can be classified into five stages. Stage I consisted of fish having immature testis with small-sized lobules. The main components of the lobules are sperm mother cells and spermatogonia (Fig. 1A). The gonadosomatic index (GSI) is about 0.16±0.06. Fish in stage II were in stimulating spermatogenic stage which charecterized by the predominance of spermatocyte and appearance of spermatids as well as small clusters of spermatozoa are seen (Fig.1B). The GSI of fish in stage II was 0.29±0.04. Stage III consisted of fish with rapid spermatogenic testis. At this stage, the spermatogenic activity was at the peak and the lobules become filled with cysts of germ cells in the different stages of maturation (Fig. 1C). Further, this stage is characterized by the predominance of spermatids and spermatozoa. In such cases, GSI was 3.55±0.60. Male in stage IV having ripe (mature) testis with seminiferous lobules fully packed with mature spermatozoa (Fig. 1D). GSI was 6.24±0.06. Fish in stage V having spent testis which marked by almost total cessation of the spermatogenic activity; by increase in the thickness of the interlobular septa; and by the presence of spermatozoa in the lumen of some seminiferous lobules after spermiation (Fig. 1E). The GSI of fish in stage (V) was 1.75±0.65.

Ovarian cycle:

On the basis of seasonal changes encountered in the histomorphology and Gonadosomatic index (Table 1). The ovarian cycle of female *L.ramada* can be classified into six stages. Stage I consisted of fish with previtellogenic ovaries which had a GSI of 0.48±0.04. In this stage, the primary oocytes dominate the ovarian components (Fig. 2A). Fish in stage II had early vitellogenic ovaries with GSI of 0.76±0.06. Most of the oocytes at this stage were noticed to belong to the vesicle stage and or primary stages (Fig. 2B). Stage III consisted of fish with mid-vitellogenic ovaries which had a GSI of 2.82±0.05. Most of the oocytes were in the primary and secondary yolk stages (Fig. 2C). These oocytes are characterized by yolk globules deposition. Fish in stage IV had late-vitellogenic ovaries with GSI of 6.25±0.85. Most of the oocytes were noticed to belong to the secondary yolk stage (Fig. 2D). Stage V consisted of fish with prespawning ovaries which had a GSI of 14.5±0.65. Most of the oocytes in the prespawning ovaries belong mainly to the tertiary yolk stage (Fig. 2E). The ripe females had a GSI of 33.55±0.69. Most of the oocytes from the obtained ripe ovaries belong mainly to the ripe oocyte (Fig. 2F).

Hormonal cycle:

Plasma thyroid hormones (T_4 and T_3):

There was a decrease in plasma levels of thyroid hormones (T_4 and T_3) coincided with an increase in testicular activity of the fish (Table 1, Figs. 3A and 3B). T_3 and T_4 increased (223±4.6 and 4.7±0.25 ng/ml for T_4 and T_3 respectively) during testis ripening to reach a peak (394±5.1 and 5.9±0.32 ng/ml for T_4 and T_3 respectively) during spawning as illustrated in table (1) and Figures (3A and 3B).

During the reproductive cycle of females, plasma T_4, T_3 decreased during ovarian early-vitellogenesis and increased during mid-vitellogenesis (1260±20.9 and 17.91±0.32 ng/ml for T_4 and T_3 respectively) to reach a peak for T_4. Then, these hormones declined to low levels during late-vitellogenesis (28.2±2.2 and 4.62±0.15 ng/ml for T_4 and T_3 respectively) as represented in table (1) and Figures (4A and 4B). At prespawning stage, T_4 and T_3 re-increased to high levels (1184±19.5 and 29.1±1.45 ng/ml for T_4 and T_3 respectively) and finally declined during induction of spawning (248.6±5.2 and 5.21±0.40 ng/ml for T_4 and T_3 respectively) (Table 1, Figs. 4A and 4B).

Plasma cortisol:

There was low levels in plasma cortisol coincided with an increase in testicular activity of the fish (Table 1 and Fig. 3C). During testis ripening, plasma cortisol reached a peak (28.04±1.58 ng/ml) during ripe stage and decreased to low level (16.78±0.85 ng/ml) during spawning as represented in table (1) and Figure (3C).

During the reproductive cycle of females, plasma cortisol decreased during ovarian early-vitellogenesis (4.7±0.5 ng/ml) and increased during mid-vitellogenesis to reach a peak (907.25±15.6 ng/ml) as illustrated in table (1) and Figure (4C). Then, cortisol declined to low level (272.25±4.9 ng/ml) during late-vitellogenesis. At prespawning stage, cortisol re-increased to high level (544.24±7.2 ng/ml) and finally declined (37.03±1.32 ng/ml) during induction of spawning (Table 1 and Fig. 4C).

Table 1: Seasonal changes of hormonal content; T_4, T_3, cortisol, and gonadosomatic index of *Liza ramada* at the different stages of gonads maturity during the reproductive cycle.

Maturity Stage	**GSI (%)**	**T_4 (ng/ml)**	**T_3 (ng/ml)**	**Cortisol (ng/ml)**
Male:				
Stage I	0.16 ± 0.06 [a]	163 ± 3.7 [a]	1.7 ± 0.09 [a]	0.9 ± 0.03 [a]
Stage II	0.29 ± 0.04 [b]	47 ± 1.6 [b]	1.64 ±0.05 [a]	0.54 ± 0.02 [b]
Stage III	3.55 ± 0.60 [c]	47 ± 2.1 [b]	1.3 ± 0.03 [b]	0.71 ± 0.03 [c]
Stage IV	6.24 ± 0.06 [d]	223 ± 4.6 [c]	4.7 ± 0.25 [c]	28.04 ± 1.58 [d]
Stage V	1.75 ± 0.65 [e]	394 ± 5.1 [d]	5.9 ± 0.32 [d]	16.78 ± 0.85 [e]
Female:				
Stage I	0.48 ± 0.04 [a]	38.6 ± 2.1 [a]	20.04 ± 1.05 [a]	35.9 ± 1.55 [a]
Stage II	0.76 ± 0.06 [b]	35.8 ± 1.8 [b]	8.43 ± 0.18 [b]	4.7 ± 0.5 [b]
Stage III	2.82 ± 0.05 [c]	1260 ± 20.9 [c]	17.91 ± 0.32 [c]	907.25 ± 15.6 [c]
Stage IV	6.25 ± 0.85 [d]	28.2 ± 2.2 [d]	4.62 ± 0.15 [d]	272.25 ± 4.9 [d]
Stage V	14.5 ± 0.65 [e]	1184 ± 19.5 [e]	29.1 ± 1.45 [e]	544.24 ± 7.2 [e]
Stage VI	33.55 ± 0.69 [f]	248.6 ±5.2 [f]	5.21 ± 0.40 [f]	37.03 ± 1.32 [f]

Data are reported as means ± SD.

Significantly different means ($P < 0.05$) are indicated by different letters (Tukey test).

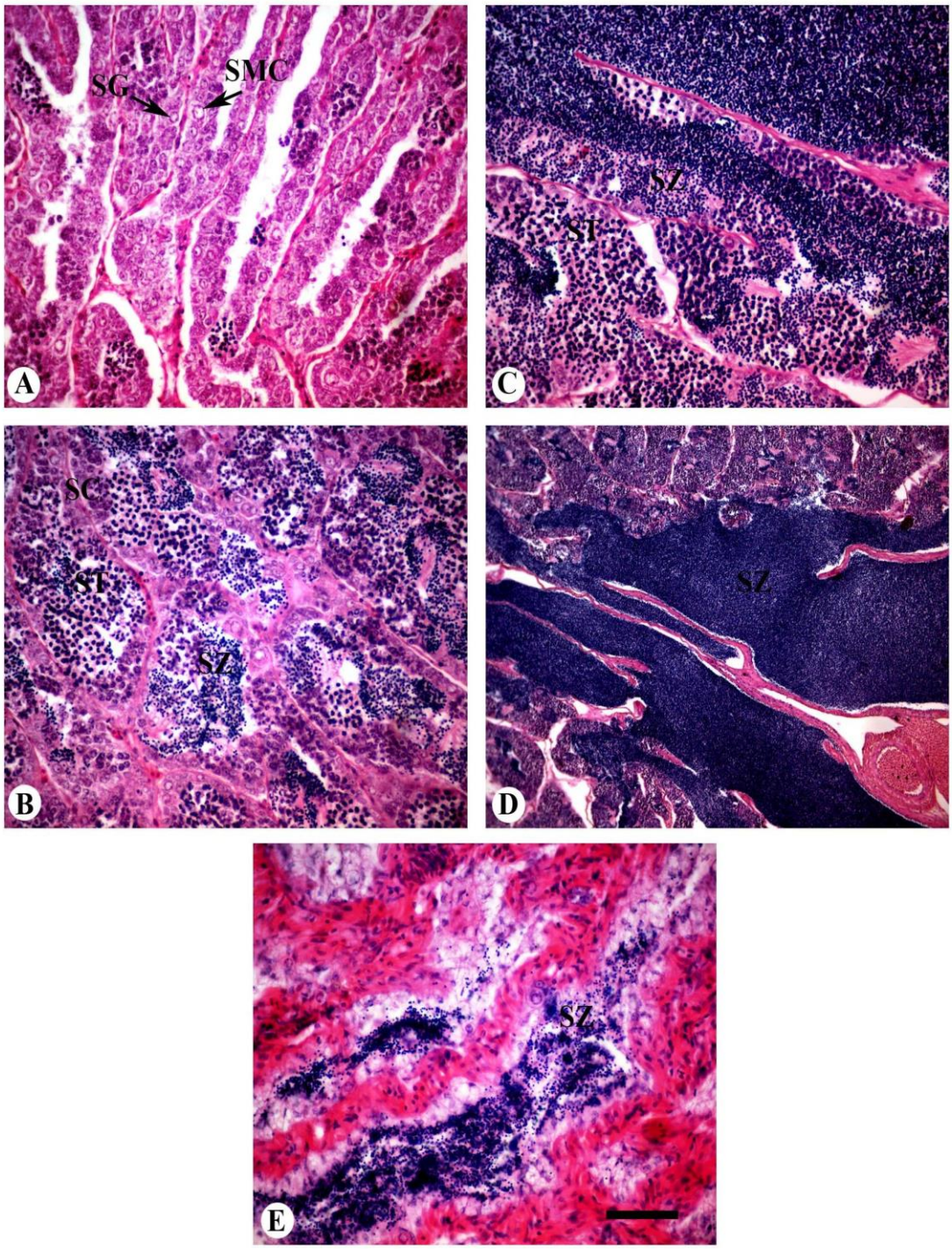

Fig. 1: Sections of L. ramada testis in different stages of development, stained with hematoxylin and eosin. A) Immature testis, showing seminiferous lobules containing sperm mother cells (SMC) and spermatogonia (SG). B) Testis of fish, obtained during the period of stimulating spermatogenesis, showing the germ-cells at various stages of maturation; spermatocytes (SC), spermatids (ST) and spermatozoa (SZ). C) Testis of fish, obtained during the period of rapid spermatogenesis, showing the predominance of spermatids (ST) and spermatozoa (SZ) in the seminiferous lobules. D) Ripe testis, showing seminiferous lobules filled with spermatozoa (SZ). E) Spent testis, showing the thick interlobular septa (ILS) and the presence of spermatozoa (SZ) in the lumen of some seminiferous lobules. Scale bar = 25 µm.

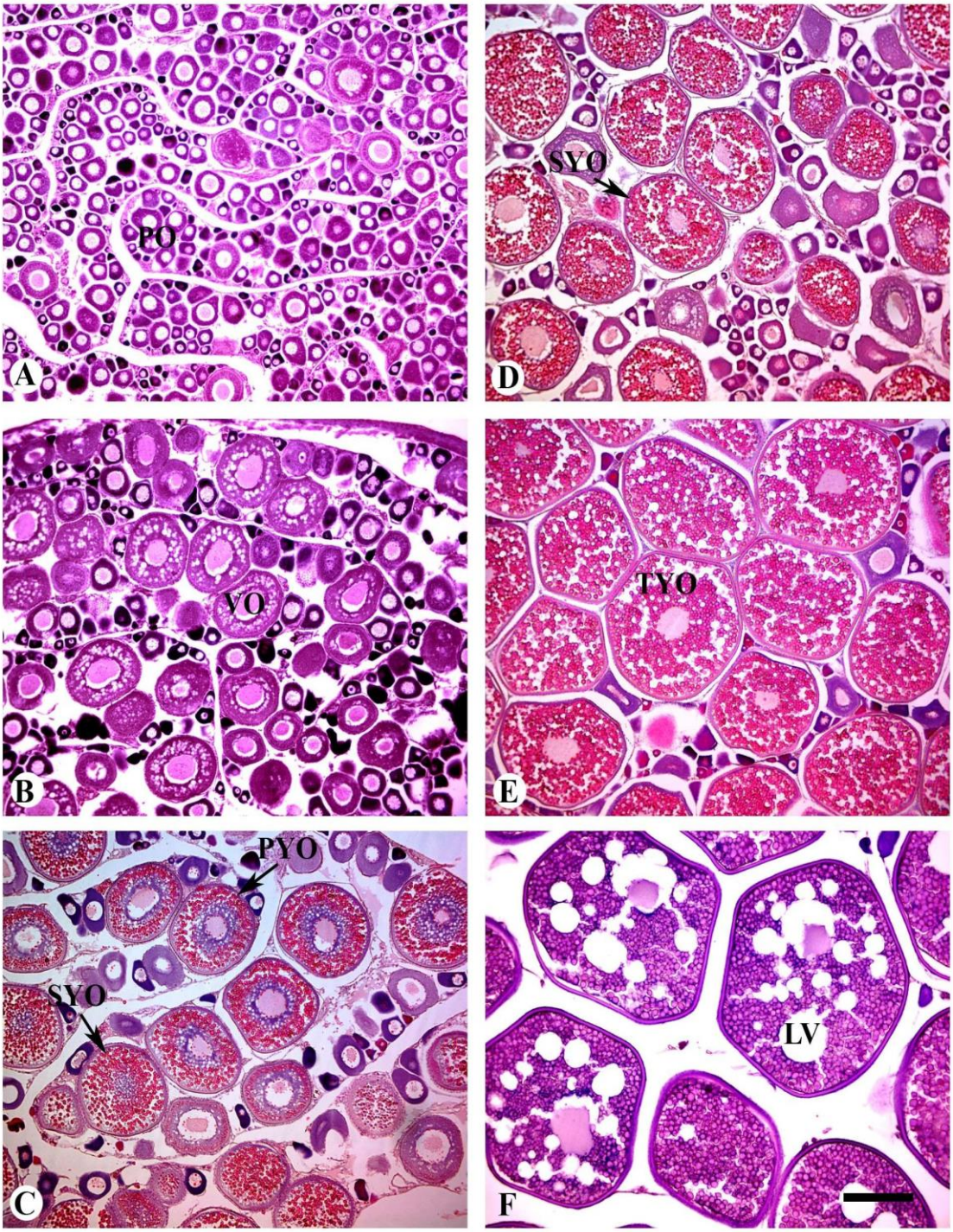

Fig. 2: Sections of L. ramada ovary in different stages of development, stained with hematoxylin and eosin. A) Previtellogenic ovary containing only the primary oocytes (PO). B) Early vitellogenic ovary containing the vesicles oocytes (VO). C) Ovary of female, obtained during the period of mid-vitellogenesis, showing the primary (PYO) and secondary yolk oocytes (SYO). D) Late-vitellogenic ovary, containing oocytes in the secondary yolk stage (SYS). E) Prespawning ovary, containing the tertiary yolk oocytes (TYO). F) Spawning (Ripe) ovary, induced experimentally by injection of hormones, showing the ripe oocytes having large lipid vesicles (LV). Scale bar = 250 µm.

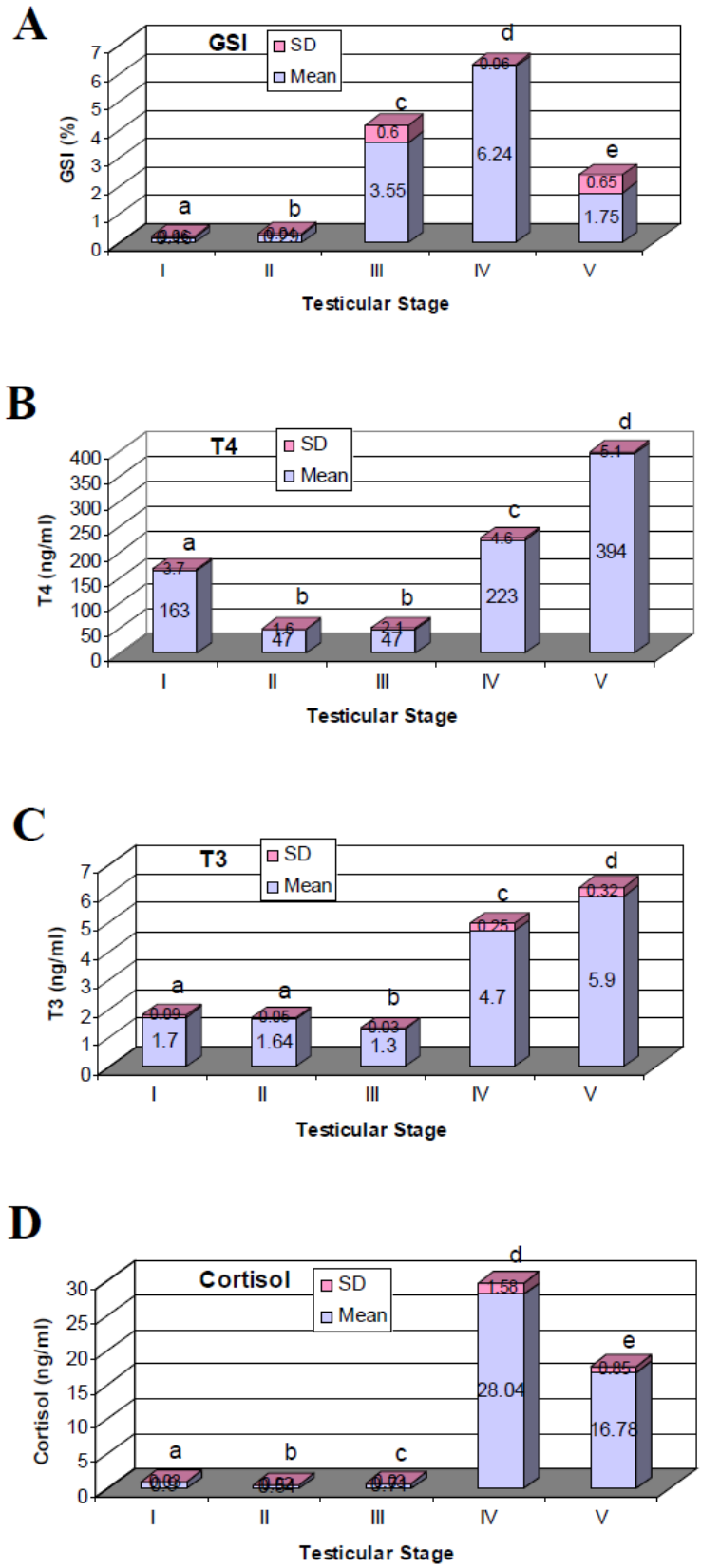

Fig. 3: Gonadosomatic index; GSI (%) (A), Plasma T4 (B), Plasma T3 (C) and Plasma cortisol levels (D) of male L. ramada at different maturity stages. Data are reported as means ± SD. Significantly different means (P < 0.05) are indicated by different letters (Tukey test).

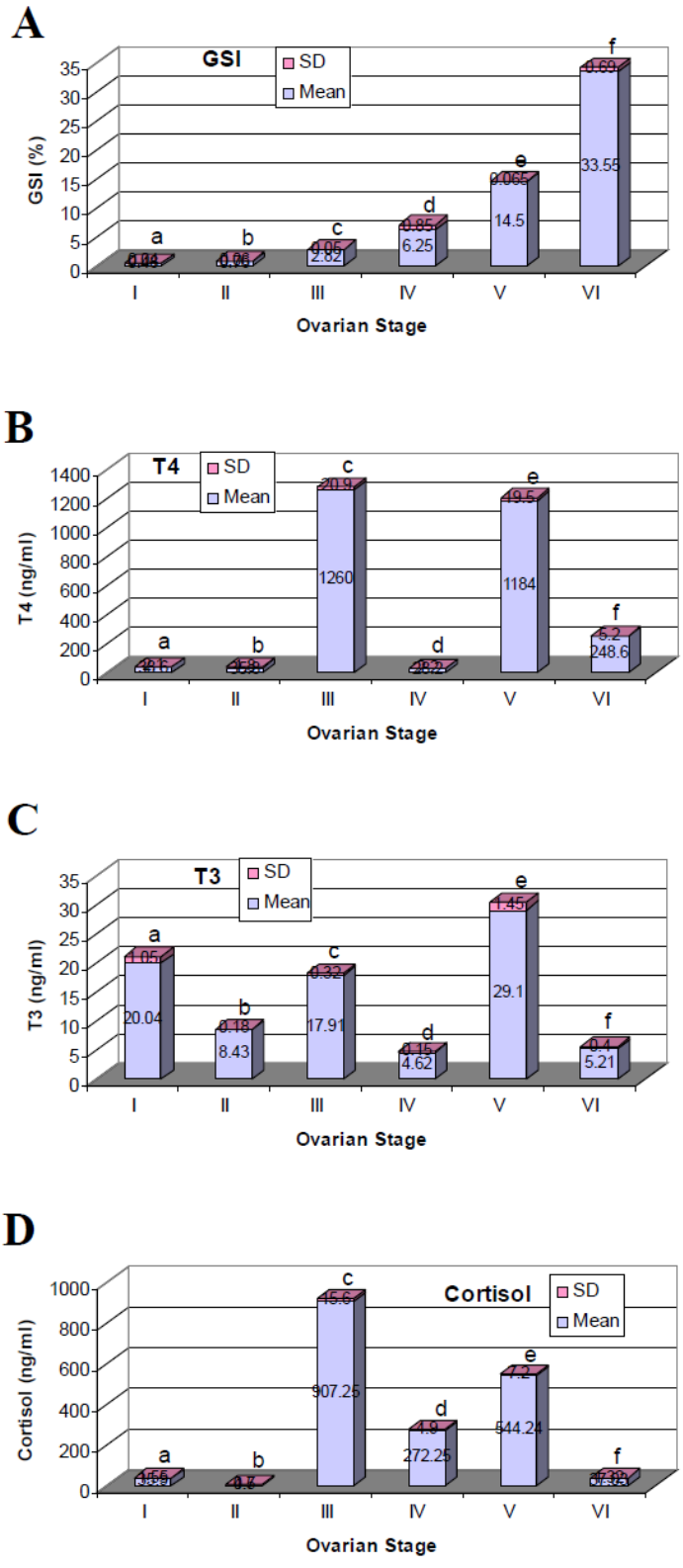

Fig. 4: Gonadosomatic index; GSI (%) (A), Plasma T4 (B), Plasma T3 (C) and Plasma cortisol levels (D) of female L. ramada at different maturity stages and during induced spawning. Data are reported as means ± SD. Significantly different means ($P < 0.05$) are indicated by different letters (Tukey test).

DISCUSSION

The present results indicated that *L. ramada* attained prespawning stage and do not undergo final oocyte maturation (FOM), ovulation or spawning in captivity. Without exogenous hormone stimulation, ova will not advance to final maturation and ovulation, but will undergo atresia and degenerate (Mousa, 1994; Mousa and Mousa, 1997; Mousa, 2010). The failure of captive mullets to undergo FOM, without hormonal injection, is thought to be caused by the shortage of gonadotropin synthesis (Mousa, 1994; Mousa and Mousa, 1997; Mousa and El-Gamal, 1999). To complete the reproductive cycle of *L. ramada* final oocyte maturation was achieved experimentally by utilizing the pregnyl (HCG) as a priming injection at a dose of 15,000 IU/kg body weight followed, 24 h later by resolving injection of 30,000 IU HCG in combination with 200 µg LHRH– a/kg body weight.

Both gonadal and extragonadal hormones are rather equally essential for the induction of circadian ovarian cycle of the grey mullet (Das *et al*., 2013). In our study, we have shown that thyroid hormones (T_4 and T_3) and cortisol profiles were significantly altered with the reproductive cycle in *L. ramada*. There was a decrease in serum levels of thyroid and cortisol hormones coincided with an increase in testicular activity of the fish. T_4 and T_3 increased during testis ripening to reach a peak during spawning, while cortisol reached a peak during ripe stage and decreased to low levels during spawning. During the reproductive cycle of females, T_3, thyroxine T_4 and cortisol decreased during ovarian early-vitellogenesis and increased during mid-vitellogenesis to reach a peak for both T_4 and cortisol. Then, these hormones declined to low levels during late-vitellogenesis. At prespawning stage, all mentioned hormones re-increased to high levels and finally declined during induction of spawning. Extra-gonadal hormones are known to contribute to oocyte growth (Peyon *et al*., 1996). Cortisol is the principal adrenocortical hormone of the interregnal gland in teleosts (McCormick, 2001; Reinecke *et al*., 2006), and it may have some role in vitellogenesis, because first and second phases of cortisol secretion or its peak coincide with vitellogenesis and spawning respectively in female catfish, *Heteropneutus fossilis* (Lamba *et al*., 1983). In the mullet, cortisol may also be involved in vitellogenesis and maturation of oocytes, because cortisol level was elevated in the breeding phase (Das *et al*., 2013). There is a steady rise in plasma cortisol associated with maturation and spawning in salmonid (Onuma *et al*., 2003). Cortisol may also play some metabolic role in respect of energy production by stimulating glucose formation through gluconeogenesis from amino acid and fatty acids (Bloom *et al*., 2000). In the rainbow trout, cortisol affects gluconeogenic enzymes suggesting a gluconeogenic role of cortisol in fish (Freeman and Idler 1973). Cortisol also elicits hyperglycemia in wide varieties of fish (Vijayan *et al*., 1997; Zena *et al*., 2018).

The present results indicated that thyroid status is increased during early oogenesis or spermatogenesis. Similar thyroid status is correlated with various stages of vitellogenesis and oocyte development in viviparous rock fish (Kwon *et al*., 1999). Also, thyroid hormones level is increased during vitellogenesis in some iteroparous fishes (Eales, 2006). In addition thyroid hormones level is increased in spawning and remains universally low after spawning in *L. ramada*. Thyroid hormones level is associated with the increase of testosterone level, and androgens can increase T3 production and plasma T3 level (Cyr and Eales, 1996). Thyroid hormone has a permissive role to facilitate GtH action. Additionally, thyroid hormones may also

participate in energy supply which is required during gametogenesis (Wiens and Eales, 2005; Reinecke *et al*., 2006). In general a positive correlation has been shown between thyroid hormones and fish reproductive status; where thyroid hormones are associated with testicular development, growth and maturation (Duarte-Guterman, *et al*., 2014; Tovo-Neto *et al*., 2018). In *M. cephalus*, both T3 and T4 and testosterone levels were increased in the pre-breeding phase ((Das *et al*., 2013). Thus, the involvement of T3 and T4 with testosterone production cannot be ignored in the mullet. Thyroid hormones may also participate in energy production in the mullet, because thyroid hormone levels were high when blood glucose level was elevated in pre-breeding mullets (Das *et al*., 2013). Das *et al*. (2013) reported that the thyroid hormones may play a critical permissive signal in timing of final gonadal development, by facilitating GtH action, testosterone production, vitellogenesis and oocyte growth in Indian grey mullets.

In essence, thyroid hormones (T_3 and T_4) are probably involved in oocyte growth including vitellogenesis; whereas cortisol may be involved in final oocytes growth and oocyte maturation to meet high energy requirement in the breeding phase. Thus, the role of extra-gonadal hormones is no way less important than gonadal hormones. Both gonadal and extra-gonadal hormones are rather equally essential for the induction of maturation and spawning of *L. ramada*.

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ARABIC SUMMARY

دراسات تجريبية على تكاثر أسماك الطوبار *Liza ramada*

مصطفى موسى - منصور إبراهيم* - محمد قورة - مصطفى زيادة
معمل تناسل وتفريخ الأسماك - المعهد القومى لعلوم البحار والمصايد – مصر.
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مثل العديد من أمهات الأسماك ذات القيمة الاقتصادية فإن أسماك الطوبار تفشل فى الوصول إلى النضج الكامل للمبيض وبالتالى لايتم النضج النهائى والتبويض أو التفريخ عند إستزراعها فى المزارع السمكية. كان الهدف من هذه الدراسة هو فحص التغيرات الهستولوجية والفسيولوجية أثناء دورة التكاثر لأسماك الطوبار المستزرعة فى أحواض أسماك المياه العذبة وأثناء تحفيز التفريخ فى المياه المالحة.

فى هذه الدراسة تم قياس مستويات كل من هرمون الثيروكسين وهرمون الترای أيودوثيرونين وهرمون الكورتيزول فى البلازما لأسماك الطوبار فى علاقة مع التغيرات النسيجية الموسمية للمناسل أثناء دورة التناسل. إنخفض مستوى هرمون الترای أيودوثيرونين وهرمون الثيروكسين وهرمون الكورتيزول أثناء المرحلة المبكرة لترسيب المح لدورة تكاثر الإناث، ثم ازداد أثناء المرحلة المتوسطة لترسيب المح ليصل لأعلى مستوى لكل من هرمون الثيروكسين وهرمون الكورتيزول. بعد ذلك إنخفض مستوى تلك الهرمونات لأقل معدل أثناء المرحلة المتأخرة لترسيب المح. عاودت تلك الهرمونات الارتفاع لمستويات مرتفعة فى مرحلة ماقبل التفريخ ثم إنخفضت أثناء تحفيز التفريخ. أثناء إزدياد نشاط الخصية وجد إنخفاض فى مستوى المصل من هرمون الترای أيودوثيرونين وهرمون الثيروكسين وهرمون الكورتيزول. عند نضج الخصية إزداد معدل هرمونى الترای أيودوثيرونين والثيروكسين ليصل لأعلى مستوى أثناء التفريخ، بينما وصل هرمون الكورتيزول لأعلى مستوى فى مرحلة النضج للخصية، ثم إنخفض لمستويات أقل عند التفريخ.

مما سبق يمكن التوصية بأن التغيرات الموسمية لهرمون الترای أيودوثيرونين وهرمون الثيروكسين وهرمون الكورتيزول المصاحبة لتطور المناسل والتفريخ لأسماك الطوبار تدعم دور تلك الهرمونات فى تكاثر وإستجابة أسماك الطوبار للإجهاد.