Ovarian Maturation of Liza Ramada in Captivity

Original Article

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ABSTRACT

Introduction: Continuous maintenance of broodstock in the captivity and the control of ovarian development are crucial for fish reprodcution, particularly when linked to mass propagation of juveniles in the hatcheries.

Aim of the Work: The present work was to perform a conclusive description of the maturing ovaries and recruit mature females of thin-lipped grey mullet, Liza ramada suitable for relaiable breeding in captivity.

Material and Methods: We used the morphological and the histological methods to describe the ovarian cycle of female mullets in captivity. Then, we will use hormonal administration to obtain the mature mullet females in captivity.

Results: The ovaries of the fish cultured in freshwater, exhibit marked variation in size, weight and color accompanying the stage of maturity. During ovarian development, only one clutch of oocytes undergoes maturation. Also, six stages of oocyte development could be identified, namely primary oocytes stages, vesicles stage, primary yolk stage, secondary yolk stage, tertiary yolk stage and spawning (ripe) stage. Yolk deposition occurre during vesicles stage, primary yolk stage, secondary yolk stage and tertiary yolk stage. The ovarian development started as early-vitellogenesis when the day length (photoperiod) and water temperature began to decrease. Whereas the gradual decline of both the photoperiod and water temperatures ensure completion of ovary development. However, the ripe stage was absent in captivity, except by hormonal injection. Importantly, the injection of carp hypophyseal homogenate in combination with gonadotropin releasing hormone (triptorelin acetate) was powerful in trigger reproductive activities in mature spawners of mullet.

Conclusion: Ripe oocytes are known to be prevailing in the ovaries occur immediately before ovulation. However, ripe oocytes were observed only in the females, acclimated to saline water and stimulated by hormones.

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Key Words: Freshwater; L. ramada; maturation; ovaries; reproductive cycle.

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INTRODUCTION

L. ramada is a euryhaline marine teleost. It is well know that this species is widely cultured in brackish and freshwater semi-intensive fish ponds due to their herbivorous, fast-growing, and disease resistance. However, mullet broodstock do not breed spontaneously in captivity[1-5]. Unfortunately, mullets is widely accepted as commercially important fishes but the ovarian ripening and the breeding activities are inhibited in captivity[1,2,5-7]. Indeed, despite the extensive importance of the mullet, the information concerning its gonadal maturation, reproduction and spawning is very limited. For instance, little attention has been paid to the dynamics of oocyte development and ovarian recrudescence in natural or captivated striped mullet populations. In a more precise manner, the conclusive description of gonad maturation in L. ramada is sparse and sporadic[8,9].

Liza parsia was found to be spawn for several months with a peak extended from November to December[10]. The GSI data and histological and ultrastructural observations revealed that the spawning season of Liza parsia is from December to February[11]. However, the highest values of the gonadosomatic index in the white mullet Mugil curema, occurred in April, August and November. Also, reproductive biology was studied in Liza falcipinnis[12], the striped red mullet, Mullus surmuletus[13] and the red mullet, Mullus barbatus[14].

It is well know that the hormonal therapy is essential for induction of fish breeding in hatcheries[5,15-17]. Consistently, the females of many species fail to produce their gametes naturally under the conditions of captivity in hatcheries. Indeed, the most effective method to produce mature gametes is successfully achieved by hormonal induction for mature breeders. Moreover, to stimulate the reproduction in fish, reproductive hormones were injected. Two main hormones are most applicated to trigger the reproductive activities in teleosts; gonadotropin (pituitary gland (PG), human chorionic gonadotropin (HCG)) and gonadotropin-releasing hormone (GnRHa)[5,15,16,18-21]. Up till now, further investigation is needed to determine the optimal protocols required for each species individually.

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Therfore, this work was designed to investigate the morphological and histological description for the maturing of the ovaries and the acquisition of mature L. ramada females suitable for breeding in captivity.

MATERIAL AND METHODS

Search location

The experiments of the present study were done at the Research station of El-Matareyya and El-Serw Fish Research Station in the period lasting from 1 January 2019 until 30 December 2020.

Obtaining mature mullet breeders in captivity

L. ramada fingerlings were originally obtained from spawning at El-Matareyya Research station and raised in El-Serw fish farm for two years. The fingerlings were reared in fresh water ponds of 400-m2. A balanced diet, containing 32% protein, was used for broodstock feeding at a rate of 3% of their weight. The females of L.ramada have reached maturation after the second year.

Fish sampling

Mature females of L. ramada, at least two years old, with average weights ranged from 500 to 750 gm and lengths ranged from 32 to 40 cm, were collected monthly alive throughout the year. However, during the pre spawning and spawning season from (September to January), fish were collected at intervals of about 15 days to ensure that all stages of gonad maturation were included.

Obtaining of spawning or ripe ovary

The spawning of ripe ovaries was obtained by hormonal induction using a first injection of 25 mg PG and a second injection, 24 h later, of GnRH-a (triptorelin acetate) at a dose of 100 µg in combination with 5 mg dopamine antagonist (Metoclopramide)/kg body weight of the pre-spawning females acclimated in seawater (35‰).

Measurements, classification and morphology of maturity stages

The mullet females were anesthetized in clove oil (Sigma) before handling as previously described[22]. Then, both standard and total lengths as well as weighted of fishes were measured. After measuring the total length and total weight the ovaries were dissected out and photographed for morphology.

The gonadosomatic index (GSI) was calculated for each fish according to the following formula:

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

Histological method

In order to perform histochemical analysis, parts of ovaries were fixed at 4°C for 72 hr in Bouin's fixative. Then, the fixed ovaries were dehydrated through ascending series of ethyl alcohol and cleared in xylene before finally infiltrated and inserted in paraplast wax (M.P.: 56- 58°C).

Ovaries were cut with rotator Microtome in consecutive sections at a thickness of 5 µm. Selected ovary sections were stained with Harris's alum hematoxylin[23] and counter stained with eosin aqueous solution.

Statistical analysis

Data were analyzed using SPSS version 11.5 and Excel. Analysis of data: A student t-test was applied to calculate significance. The multiple range tests of mean differences were applied and were set at $P<0.01$ level.

RESULTS

Ovarian cycle in freshwater habitat (captivity)

Morphology

The paired ovaries of L .ramada are elongated structures and fused only at their hind ends. They lie in the abdominal cavity, being firmly attached to the dorsal peritoneum by a membranous tissue. The morphological features indicated that the size, weight, and color of the ovaries vary greatly with the stages of ovarian maturation (Figures 2,4,6,8,10,12).

Histology

The cross-sections of ovary of L .ramada appears elliptical or circular in shape. The eggs in the ovary of L. ramada are arranged in a bead-like structure in contratst to the random arrangement exist in the ovaries of other animals. (Figure 1). The oogonia exhibited successive stages of development during the ovarian cycle and the production of the ripe ova. During the ovarian cycle, the oocyte development stages generally pass through six stages; primary oocyte stages (chromatin–nucleolus and peri-nucleolus stages), vesicles stage, primary yolk stage, secondary yolk stage, tertiary yolk stage, and ripe stage.

According to the morphological and histological characteristics available for the ovaries, the incidence rates and the duration of the different oocyte maturational stages, are represented in (Table 1), while the corresponding values for gonadosomatic indices (GSI) are illustrated in (Table 2). The sexual maturity of L.ramada females in freshwater needs at least two years; and during the ovarian cycle, five stages of ovarian development have been identified. These stages include the following: previtellogenesis, early-vitellogenesis, mid-vitellogenesis, late-vitellogenesis, and prespawning. In addition, the spawning (ripe) stage was observed only in the females, acclimated to saline water and stimulated by hormones.

Stage I: Previtellogenesis

This stage of development represents the period of oocyte growth extending throughout the year with different appearance rates and overlapping with the other stages as specified in Table 1. During this stage, the ovaries appeared

small, transparent, and slightly fleshy in color. The ovaries occupy a small area of the body cavity during this stage of development (Figure 2). These ovaries contain the primary oocyte stages (Figure 3). The mean gonadosomatic index (GSI) value of the females at this stage of development was 0.5 ± 0.05 (Table 2).

Stage II: Early vitellogenesis

The present developmental stage was obtained during the period from early September to early November (Table 1). During this period, the ovaries increased in size compared to that of the previous stage. Consistently, the ovaries occupied nearly half of the body cavity and their color was changing from red to yellow (Figure 4). Moreover, the diameter of oocytes in the early vitellogenic ovary ranged from 20 to 80 µm and the average GSI for females in this period was 0.9 ± 0.15 (Table 2). The current stage of ovarian development marks the onset of yolk deposition, which was marked by the appearance of fine spherical yolk granules in the outer layer of the cytoplasm of the bitinctorial oocytes, in conjunction with the spread of a vesicular-lipid ring around the nucleus (Figure 5). Most of the oocytes in the early vitellogenic ovary are considered to represent the vesicles stage (or primary yolk stages) owing to the presence of small cytoplasmic vesicles.

Stage III: Mid-vitellogenesis

This developmental stage of ovaries extended from early October to late November (Table 1). The ovaries acquired a yellowish color at this stage and occupy more than half of the body cavity (Figure 6). Most of the oocytes in the mid-vitellogic ovary were seen in the primary yolk stages (Figure 7). In general, their diameters varied from 200 to 350 µm. GSI of the females at this stage was 6.15 ± 0.6 (Table 2).

Stage IV: Late vitellogenesis

The late-vitellogenic stage extended from early October to mid-December as shown in (Table 1). At this stage of ovarian maturation, the ovaries occupy almost the entire body cavity, acquiring a yellowish color, with a more or less swollen structure (Figure 8). The oocytes of the late-vitellogenic ovaries were belonging to the secondary yolk stage (Figure 9). The diameter of the large oocytes in these ovaries ranged form 350 to 480 µm and the mean GSI at this stage was 8.4 ± 0.67 (Table 2).

Stage V: pre spawning

The pre spawning females were obtained during the period extending from early November to late December as presented in (Table 1). In these females, the ovaries were full, thus occupying almost the entire body cavity, acquiring a transparent golden appearance (Figure 10). Most of the oocytes in these ovaries mainly belong to the tertiary yolk stage (Figure 11). Their diameters ranged between 480 and 600 µm. As marked in the (Table 2), the GSI of the females at this stage was 18.57 ± 0.8.

Stage VI: Spawning

No spawning ovaries could be found in the fish collected from freshwater. But, this stage was noticed to occur during induced spawning. The spawning stage was experimentally simulated by injecting hormones into acclimatized females in seawater. The ovaries obtained from this stage appeared completely swollen, reached the maximum size, and thus almost completely occupied the body cavity, acquiring a pronounced golden appearance (Figure 12). Most of the oocytes within the obtained ripe ovaries belong to the ripe oocytes (Figure 13). Their diameters ranged between 900 and 1000 µm. The mean GSI at this stage was 35.4 ± 2.55 (Table 2). These oocytes are characterized by the enormous size of the oil droplets, which were coalescence and usually forming one large droplet located in the central part of the oocyte. Besides, the yolk globules appeared to be fused (Figure 13).

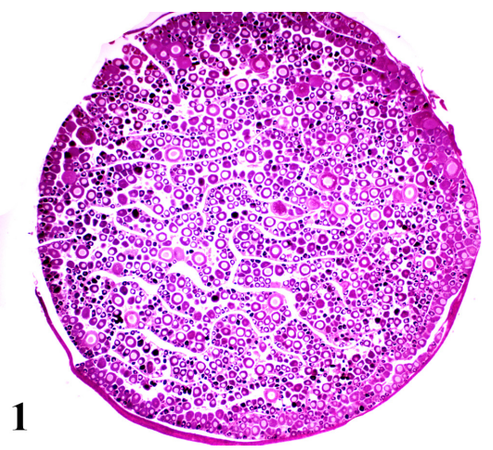

Fig. 1: Transverse section (T.S.) of previtellogenic ovary of L.ramada, showing the bead-like arrangement of oocytes. X40.

Fig. 2: Morphology of the previtellogenic ovarian stage, showing ovaries (arrows) appeared small in size, translucent and slightly fleshy in colour. They occupied a small area of the body cavity. X 100.

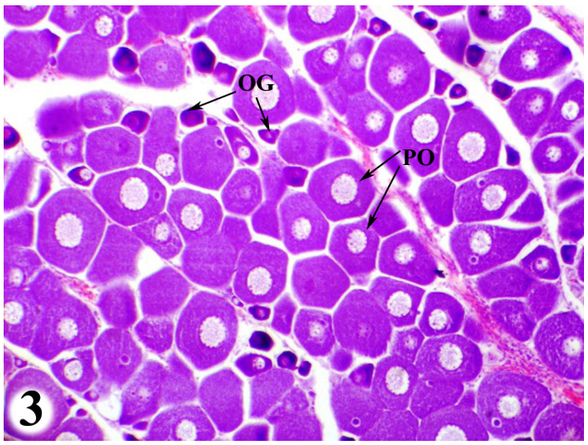

Fig. 3: A magnified portion of the previtellogenic ovary T.S., showing Oogonia (OG) and primary oocytes (PO). X 100.

Fig. 4: Morphology of early-vitellogenic ovarian stage, showing ovaries (arrows) appeared increased in volume than the preceding stage, occupying nearly half of the body cavity and their colour was in transition from reddish to yellowish. X 100.

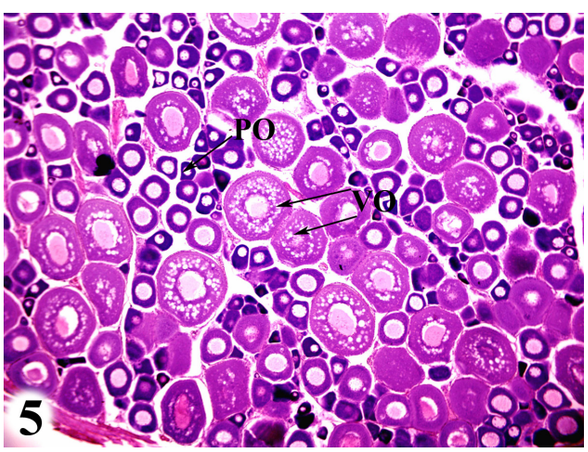

Fig. 5: Transverse section (T.S.) of early-vitellogenic ovary of L.ramada, showing the vesicles oocytes (VO) and the primary oocytes (PO) stages .X 40.

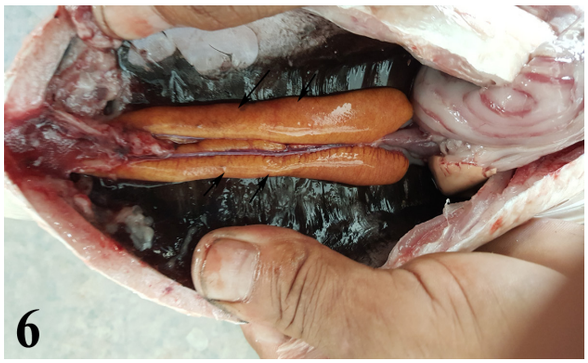

Fig. 6: Morphology of mid-vitellogenic ovarian stage, showing ovaries (arrows) acquired a yellowish coloration, occupying more than half of the body cavity. X 100.

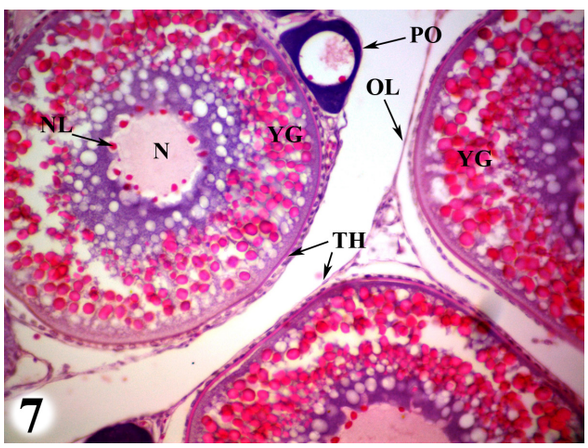

Fig. 7: Transverse section (T.S.) of mid-vitellogenic ovary of L.ramada, showing the primary yolk oocytes having the minute yolk globules (YG), beside the theca layer (TH) which originating from the ovigerous lamellae (OL) and covering the oocyte. Nucleus (N), nucleoli (NL) and primary oocyte (PO) are also illustrated. X 100.

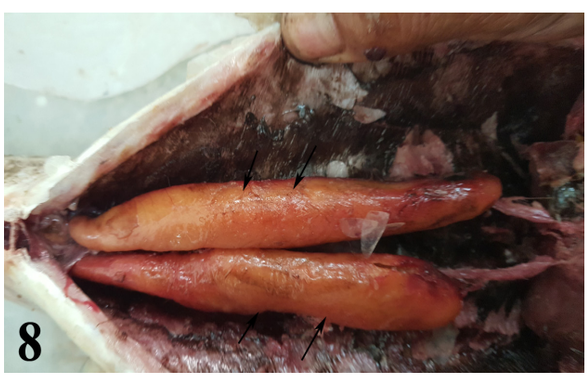

Fig. 8: Morphology of late-vitellogenic ovarian stage, showing ovaries (arrows) occupied nearly the entire length of the body cavity, exhibiting a yellow colour, with more or less turgid structure. X 100.

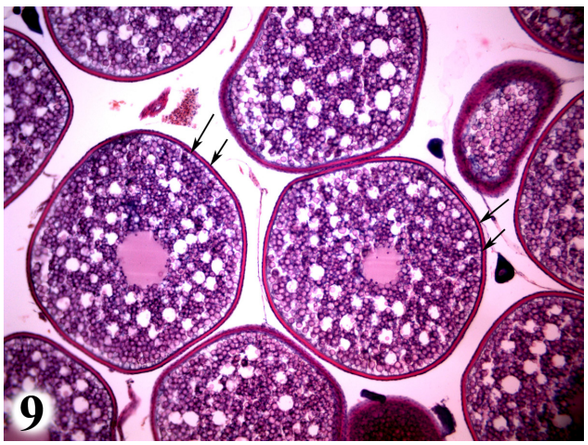

Fig. 9: Transverse section (T.S.) of late-vitellogenic ovary of L.ramada, showing the secondary yolk oocyte (arrows). X40.

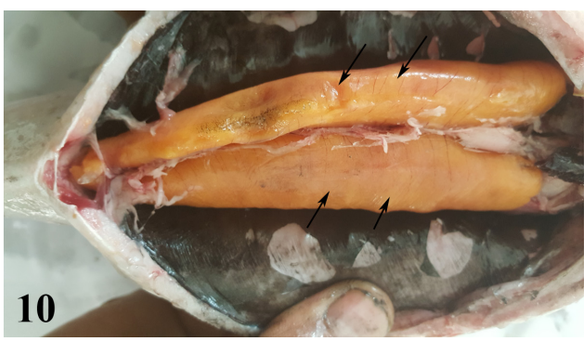

Fig. 10: Morphology of prespawning ovarian stage, showing ovaries (arrows) were fully distended, occupying nearly the entire body cavity, and exhibiting a translucent golden appearance. X 100.

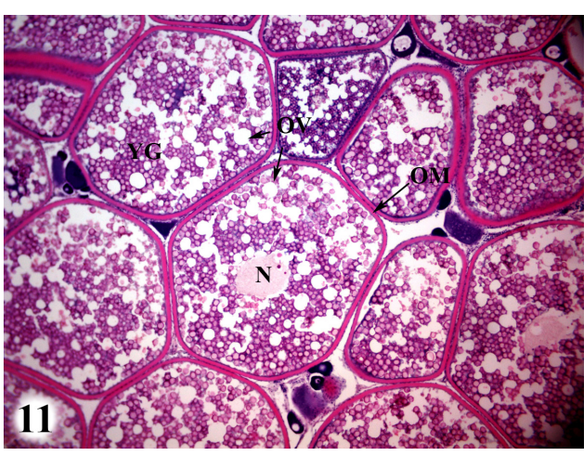

Fig. 11: Part of prespawning ovary of L.ramada, showing the coalescence of both yolk globules (YG) and oil vesicles (OV) in the tertiary yolk oocyte, beside the disapearance of nuclear membrane. Oocyte membrane (OM) and nucleus (N) are also designated. X100.

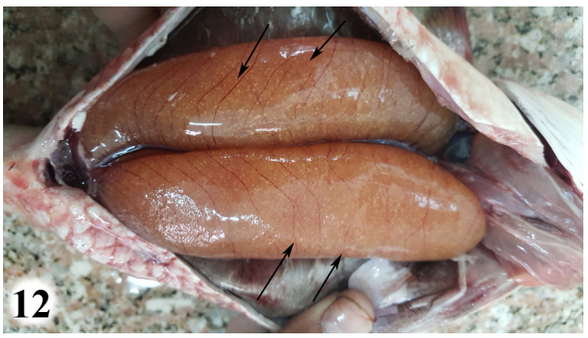

Fig. 12: Morphology of induced-spawning ovarian stage, showing ovaries (arrows) were fully distended, attaining the maximum volume, occupying the entire body cavity and exhibiting a clear golden appearance. X 100.

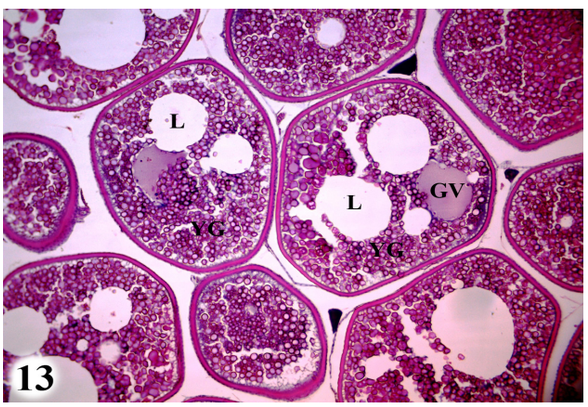

Fig. 13: Transverse section of ovary from injected female L.ramada showing advanced yolk globules (YG) coalescence and breakdown of germinal vesicle (GV). Also, complete coalesced lipid (L) is represented. X100.

Table 1: Monthly variations in the frerquency (%) of ovarian stages of Liza ramada during ovarian cycle in fresh water (EL-Serw fish farm)

Month	Water Type	No. of fish	I	II	III	IV	V
Jan	Fresh	50					
Feb	Fresh	50	100				
Mar	Fresh	50	100				
Apr	Fresh	50	100				
May	Fresh	50	100				
Jun	Fresh	50	100				
Jul	Fresh	50	100				
Aug	Fresh	50	100				
Sep	Fresh	50	80	20			
Oct	Fresh	50	64	16	10	10	
Nov	Fresh	50		30	25	25	20
Dec	Fresh	50				20	80

*VI: Spawning (Ripe) stage not shown

Table 2: Gonadosomatic index of females L. ramada at different stages of maturation, from fresh water (El-Serw fish farm), and during induced spawning in saline water

Ovary Stage	Gonadosomatic index (%) Fresh water	
	No.	Mean ± SD*
I	20	0.50 ± 0.05
II	20	0.90 ± 0.15
III	20	6.15 ± 0.60
IV	20	8.40 ± 0.67
V	20	18.57± 0.80
VI	20	35.4 ± 2.55

*Means were significantly different ($P < 0.0005$)

DISCUSSION

The ovaries of L.ramada are paired elongated structures connected by a thin connective tissue sheet. This anatomical feature also occurs in most teleostean species, such as Euculia inconstans[24]; Schizothorax richardsonii[25]; Lethrinus nebulosus[26]; M.cephalus[6] and O.niloticus[27]. However, some fishes possess only a single bilobed ovary, such as Fundulus heteroclitus[28] and Fundulus grandis[29].

Several criteria have been used to identify the different stages of oogenesis including size, amount, and distribution of various cell inclusions, especially yolk granules together with the appearance of the nuclei and nucleoli. The present study showed that the process of oogenesis in L.ramada comprise six successive stages, namely: primary oocyte stage (chromatin – nucleolus and peri-nucleoli stages), vesicles stage, primary yolk stage, secondary yolk stage, tertiary yolk stage and spawning (Ripe) stage. The yolk deposition is present in four of vitellogenic stages; vesicles stage, primary yolk stage, secondary yolk stage and tertiary yolk stage. Similar stages were also recognized in rainbow trout[30] and M.cephalus[1].

The present morphological and histological observations obtained during the reproductive cycle of L.ramada indicate that the ovaries pass through successive stages, or periods of maturation. Seven ovary maturational stages were recognized. Similar findings were obtained in M.cephalus inhabiting the coastal waters of northeast Florida[31]. Also, similar description was obtained in M.cephalus[6,32].

The present morphological and histological observations obtained during the reproductive cycle of L.ramada indicate that the ovaries pass through successive stages or periods of maturation. Seven stages of ovarian development were recognized. These findings are similar to the previous observations for M.cephalus inhabiting the coastal waters of northeast Florida[31]. Also, similar descriptions were obtained in M. cephalus[6,32]. The ovarian stage I (previtellogenesis) of L.ramada was characterized by the presence of previtellogenic oocytes. This stage of egg growth was not dependent upon the environmental factors (temperature; photoperiod), as it had extended throughout the whole year in fresh water with different frequencies overlapping with the other stages.

The vitellogenesis (yolk deposition) occurred in freshwater, as the ovarian stage II (early- vitellogensis) had commenced concomitant with the decrease in both photoperiod and water temperatures from September to November. Most of the oocytes at this period belong to the primary yolk stage and/or vesicles stage, which were characterized by the appearance of yolk granules (proteid yolk). The vitellogenic stages III, IV and V (mid-; late -vitellogenesis; pre-spawning) commenced concomitant with a gradual decrease of both photoperiod and water temperatures. The negative correlation of L.ramada egg growth with temperature and day length is consistent with previous reports for M. cephalus in Hawaiian waters[33,34]. It is generally accepted that photoperiod and temperature are the two major environmental cues that mediate reproductive activities in fishes[35-38]. In this respect, the highest values of the gonadosomatic index of Mugil curema occurred in April, August and November[39].

The present studies have also revealed that the captive freshwater fish did not complete final maturation; a feature that was also observed in different teleost fishes by the previous studies[1,2,40-42]. The present investigation showed that L. ramada does not spawn spontaneously when reared in captivity. The failure of captive mullets to undergo final oocyte maturation, without hormonal injection, is thought to be caused by the shortage of gonadotropin synthesis[1,2,6].

Also, in our study, injection of PG and HCG in combination with GnRHa and dopamine antagonist was potent in trigger the final reproductive activities of L. ramada. In fish, understanding the arrangement between environmental cues and the internal regulatory factors is determinant for reproduction success. The used reproductive hormones act at different endocrine levels and trigger reproductive activities[20,43-47].

Combined treatment with GnRHa and a dopamine antagonist has been a powerful agent for reproductive success and obtaining good gametes in different species of teleosts reared in captivity[17,48-55]. In addition, PG, HCG, combination of PG+HCG and GnRHa with metoclopramide were essential for final ovarian activities in salinity acclimatised Liza parsia[21].

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

نضج المبيض لأسماك الطوبار فى الأسر

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المقدمة: الرعاية المستمرة لأمهات البوري في الأسر والتحكم في تطور المبيض مهمة، خاصة عندما تكون مرتبطة بالانتاج المكثف للزريعة في المفرخات.

الهدف من الدراسة: تم التخطيط للدراسة الحالية لإجراء وصف لنضج المبيض والحصول على إناث الطوبار الناضجة المناسبة للتفريخ في الأسر.

المادة والطرق: فى هذا البحث تم إستخدام الطرق المورفولوجية والهستولوجية لوصف دورة المبيض لإناث الطوبارالمرباة في المياه العذبة.

النتائج: وجد أن شكل وحجم ولون وقوام مبيض سمكة الطوبار عامة يتغير موسيميا. أثناء تطور المبيض، مجموعة واحدة فقط من البويضات تنضج سنويًا. تميزت ستة مراحل، طبقا للصفات المورفوهستولوجية، أثناء تطور المبيض فى المياه العذبة. وهذه المراحل هى: مرحلة البويضات الإبتدائية، مرحلة الفجوات، مرحلة المح الأولى، مرحلة المح الثانية، مرحلة المح الثالثة، مرحلة النضج (التفريخ). يبدأ تطور المبيض مع بداية إنخفاض فترة الإضاءة ودرجات الحرارة، بينما يؤدى الإنخفاض التدريجى لكل من فترة الإضاءة ودرجة حرارة الماء الى إتمام عملية تطور المبيض. فى حين أن مرحلة النضج (التفريخ) لم يتم مشاهدتها ولم يتم العثور عليها أثناء هذه الدراسة ولكن تم الحصول على هذه المرحلة تجريبيا بالحقن بالهرمونات. أوضحت النتائج أن إستخدام الغدة النخامية لأسماك المبروك كجرعة تحفيزية متبوعة بهرمون التريبتوريليين كجرعة متممة للتفريخ كان فعالا فى تحفيز النضج النهائي للبويضات، التبويض والتفريخ فى أسماك الطوبار الناضجة.

الخلاصة: من المعروف أن البويضات الناضجة تظهر في المبيض فقط مباشرة قبل التبويض. وقد وجدت البويضات الناضجة فقط في الإناث المحفزة بالهرمونات والمؤقلمة للمياه المالحة.