Testicular Maturation of Thin-Lipped Grey Mullet During the Annual Reproductive Cycle in Captivity

Original Article

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ABSTRACT

Introduction: Continuous maintenance of mullet broodstock in captivity and the control of testicular development are important, particularly when linked to mass propagation of juveniles in the hatcheries, however, the complete description of testes maturation still lacking.

Aim of the Work: The main goal of our study to present a complete description for the testes and the obtaining of mature thin-lipped mullet, *Liza ramada*, males suitable for breeding in captivity.

Material and Methods: We performed morphological and histological techniques to describe the testicular cycle of developmental stages of male mullet reared in freshwater and captivity.

Results: In general, the testes of L. ramada exhibit remarkable seasonal variations in shape, size, color and texture. Indeed, five developmental stages were characterized during testicular cycle in L. ramada in freshwater based on the histomorphology and gonadosomatic index. These stages include immature testis, stimulating spermatogenesis, rapid spermatogenesis, mature (ripe) testis and spawning (running) testis. Importantly, the testis development started when the day length and water temperature begun to decrease in September while the gradual decrease in both photoperiod and water temperatures ensure complete gonad maturation (September-December). However, induction of successful spawning in L. ramada occurs when photoperiod and water temperatures decline to their minimum values during December.

Conclusion: The present study provides the morphological and histological description for testes of L. ramada during gonad maturaion in captivity and able to obtain a ripe mullet male suitable for hormonal induced reproduction in these species especially in captivity. In spite of attaining the ripe stage of L. ramada males in freshwater, the spawning can only possible with hormonal and environmental stimulation.

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

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INTRODUCTION

It is well accepted that understanding and manipulating endocrine control of fish reproduction are pivotal to the developed hatchery technology. Accordingly, hypophyseal and gonadal hormones are intimately involved in the regulation of the timing of fish reproduction. Indeed, the successful management of fisheries and fish farming requires an accurate knowledge of the gonadal cycles of these fish and their functional mechanisms. Therefore, the detailed histological structure and seasonal variations of the teleostean gonads have attracted the attention of many investigators[1,2].

The mullets are euryhaline species spawning only in sea water but can also grow in brackish and fresh waters. The culture methods of mullets in Egypt are restricted because the juvenile fish collected, only from the wild, for fish farming. Its production will subject to restriction until the reliable methods of induced spawning techniques are developed. However, mullets do not spawn naturally in captivity and show some degree of reproductive dysfunction[3-5].

Until now, the reproductive cycle of a few grey mullet species in some of the estuaries and lagoons has been investigated[6-9]. It is well know that the reproductive activity of L. ramada started in September, while the highest values of GSI were at November and December[10,11]. Furthermore, the histological and physiological changes during the reproductive cycle of L. ramada were investigated in freshwater fish ponds and during induction of spawning in saline water[12]. Similarly, reproductive biology and the histological assessment of seasonal gonad maturation were investigated in the striped red mullet, Mullus surmuletus[13,14].

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The previous study reported that spawning period of L. ramada in Turkey was occurred between October and December[15]. However, GSI of the male L. parsia was found to be a maximum during the month of February and December[16]. The monthly changes of the gonadosomatic index (GSI) during sexual maturation revealed that L. dumerili reproduces mainly in the rainy season, between April and June[17].

The reproductive biology, spawning and nursery grounds were investigated in four ecologically and economically important Adriatic fish species such as sardine Sardina pilchardus, anchovy Engraulis encrasicolus, European hake Merluccius merluccius, Common pandora, Pagellus erythrinus[18]. Also, Growth and reproduction were investigated in spotted flounder Citharus linguatula[19].

The conclusive informations concerning the reproduction as well as spawning time of mullet is not available. In a more precise manner, the information available on gonad maturation in L. ramada is sparse and sporadic[20,21]. Therefore, the present experiments aimed to follow up the precise morphological and histological description for the maturing of the testes and the acquisition of mature thin-lipped mullet, L. ramada, males suitable for reliable method to induce reproduction in these species especially in captivity.

MATERIAL AND METHODS

Study Site

The experiments of this study were carried out in freshwater fish farm at El-Serw and El-Matareyya Research station during the period extending from 1 January 2019 until 30 December 2020.

Mature mullet breeder's recruitment in captivity

L. ramada fingerlings were originally obtained from hormonal induced spawning at El-Matareyya Research station and then raised in freshwater fish farm at El-Serw for two years. The fingerlings were kept in outdoor 400-m2 earthen ponds at El-Serw (freshwater habitat). The ponds were supplied with running fresh water at an ambient temperature of 15–35 ºC and dissolved oxygen content above 5.0 mg O2 L-1 as well as pH-value ranged from 7.5 to 8.2. The light regime was that of natural photoperiod. Fish were fed diet containing 32% crude protein, 5% lipid, 0.2 % vitamins and 0.2 % minerals with daily rate of 3% of the body weight. Both sexes of L. ramada; male and female, were reached maturation after two years.

Fish Sampling

Two-years-old mature breeders of L. ramada, with 250 to 550 gm average weights and 27 to 35 cm lengths were collected alive at intervals of about one month throughout the year. However, during the prespawning 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.

Spawning or running testis collection

The spawning or running testes were obtained by hormonal induced spawning as follow; priming injection at a dose of 25 mg common carp pituitary glands/kg body weight followed, 24 h later, by a resolving injection of 100 µg triptorelin acetate + 5 mg Metoclopramide/kg body weight of seawater (35‰) acclimated males.

Maturity stages measurements, classification and morphology assessments

Immediately after collection, fishes were transferred alive to the laboratory, then anesthetized in a solution (40 mg/l) of clove oil (Sigma) before handling[22]. In laboratory, fish sex were identified and then weighted in grams as well as standard total lengths were measured. The testis was removed carefully and weighted. After measuring total length and total weight, the gonads 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

To perform histological investigation, a part of testes was removed and fixed in Bouin's fluid for 24 hr at 4◦C. The fixed testes were dehydrated through ascending series of ethyl alcohol and cleared in xylene before finally embedded in paraplast (M.P.: 56- 58◦C).

Consecutive sections of the gonads were cut at 5 µm thickness using microtome. Selected sections were stained with Harris's alum hematoxylin[23] and aqueous solution of eosin as a counter stain.

Statistical analysis

Data were analyzed using SPSS version 11.5 and Excel. Significance between stages was calculated using Student t-test analysis. The multiple range tests of mean differences were applied and was set at $p<0.01$ level.

RESULTS

Testicular cycle in fresh water habitat (captivity)

Morphology

The testes of L. ramada from captivity exhibit paired elongated structures lying ventral to the kidneys and dorsal to the alimentary canal and occupy the posterior region of the abdominal cavity. The testes remain separated from one another for almost their entire length except at the hindmost region, where they unite and become attached together to form a common spermatic duct. However, the testes exhibit remarkable variations in shape, size color and texture during the successive developmental stages (Figures 2,4,6,8,10).

Histology

Each testis of the investigated fish performs kidney-shape in transverse sections as represented in (Figures 1). The testis of L. ramada composed of a large number of coiled seminiferous lobules, which are combined together by inter-lobular septa of connective tissue (Figuress 1,3). These lobules varied in size, but each is bounded by a single layer of fusi-form cells (lobule boundary cells) as represented in (Figure 3).

The bulk of the testicular seminiferous lobules consist of germ cells which remain inactive at first (Immature stage). Then they become very active on the approach of the maturation phase (stimulating spermatogenesis), in which they show various stages of maturation, viz, sperm mother cells, spermatogonia, spermatocytes, spermatids and spermatozoa as illustrated in (Figure 5). Importantly, the seasonal changes in testicular activity (i.e. testicular cycle) of male L. ramada in fresh water can be classified into five stages based on changes in the histomorphology and gonadosomatic index (see Table 1) as described below:

Stage I: Immature stage

This stage extends throughout the year and undepends on both the photoperiod and water temperatures (Table 2). The immature testes are slender, thin, translucent and pale in color (Figure 2) and the gonadosomatic index (GSI) is 0.14 ± 0.05 (Table 1). The testis at this stage contains small-sized lobules and a relatively large interlobular space filled with dense stroma consisting of connective tissue, blood capillaries and occasionally some interstitial cells (see Figure 3). The main components of the testis lobules are sperm mother cells and spermatogonia as demonstrated in (Figure 3).

Stage II: Stimulating spermatogenesis

This period of development last from September to mid-November and accompanied with a gradual decrease in both photoperiod and water temperature (Table 2). During this stage, testes become slightly enlarged, but still translucent (Figure 4) and the gonadosomatic index (GSI) was about 0.3 ± 0.05 (Table 1). The spermatogenic activity was obviously in progress during this stage. Also, the size of the lobules exhibited a gradual increase accompanied by a marked reduction of the interlobular connective tissue as illustrated in (Figure 5).

Moreover, most of the lobules of the testis became filled with cysts of different stages of spermatogenesis as previously demonstrated in figure 5. However, this stage is particularly characterized by the predominance of spermatocytes and appearance of spermatids. However, spermatogenesis does not appear to occur simultaneously in all the seminiferous lobules.

Stage III: Rapid spermatogenesis

This stage covered the period from early October to late November (Table 2). This period is characterized by a gradual decrease of the photoperiod and slight fall in water temperatures. The testes at this stage loses their translucency, exhibits pink coloration (Figure 6) due to their increased vascularity as they also manifested a marked increase of both their weight and volume with GSI 3.36 ± 0.59 (Table 1).

Microscopical investigations revealed that the diameter of the seminiferous lobules apparently increased and the spermatogenic activity reaching its peak. Therefore, the lobules at this stage became filled with cysts of germ cells at all spermatogenic stages, and become separate from each other by a thin interlobular septum (Figure 7). Furthermore, this stage was characterized by the predominance of spermatids and spermatozoa as demonstrated in (Figure 7). Conversely, there was a marked reduction in the number of spermatogonia and spermatocytes.

Stage IV: Mature (Ripe) testis

The ripening period started with the gradual reduction of both the photoperiod and water temperatures extending from early November to late December (Table 2). During this stage, the testes tremendously enlarged in size to the extent that they have almost filled the whole-body cavity of the fish (Figure 8) and GSI become 6.14 ± 0.07 (Table 1). The testis appeared in that stage opaque, milky white, smooth and crumbled in texture (Figure 8). Milt could be easily extruded from the fish by gentle pressure on their bellies.

At this stage, the diameters of the testis's lobules attained their maximum width as represented in (Figure 9). The interlobular spaces of connective tissue appeared to be thinner than the previous stages. Also, these lobules were almost fully packed with mature spermatozoa (see Figure 9).

Stage V: Spawning or running testis

This stage was obtained by hormonal injection in saline water. During this period, both the photoperiod and water temperature have recorded their minimum values during spawning season (Table 2).

During this stage (spawning), the testes filled the whole-body cavity of the fish and become milky and white in color as demonstrated in (Figure 10). Their weights were considerably increased, acquiring a GSI of 12.6 ± 1.55 (Table 1). This period was evidenced by running sperms filled the entire lobule (Figure 11).

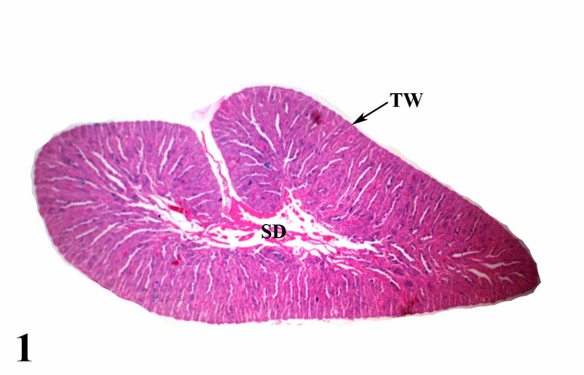

Fig. 1. : Morphology of L. ramada immature testis obtained from fresh water, showing the slender and thin shape, translucent and pale color.

Fig. 2: Transverse section (T.S.) of the testis of L. ramada showing its kidney-shaped appearance, testicular wall (TW) and spermatic duct (SD). X 40.

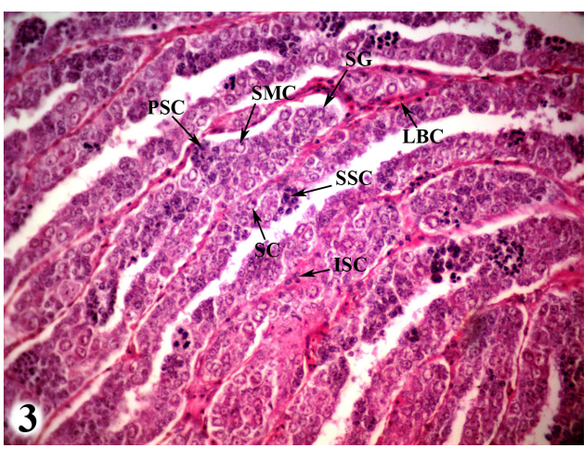

Fig. 3: T.S. in L. ramada immature testis obtained from fresh water designating the seminiferous lobules containing; sperm mother cell (SMC), spermatogonia (SG), primary spermatocytes (PSC), secondary spermatocytes (SSC), and Sertoli cells (SC). Also, interstitial cells (ISC), lobule boundary cells (LBC) and interlobular septum (ILS) are illustrated. X 400.

Fig. 4: Morphology of L. ramada testis obtained from fresh water at stimulating spermatogenesis, showing the testes have become slightly enlarged, but still translucent.

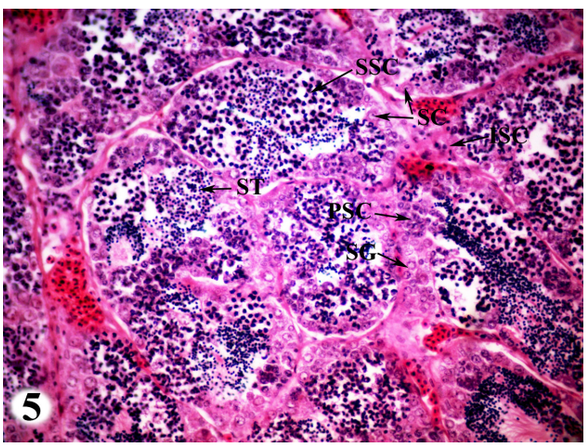

Fig. 5: T.S. in L. ramada testis obtained from fresh water during the period of stimulating spermatogenesis, designating the germ-cells at various stages of maturation during spermatogenesis; spermatogonia (SG), primary spermatocytes (PSC), secondary spermatocytes (SSC), and spermatids (ST). Also, sertoli cells (SC) and interstitial cells (ISC) are present. X 400.

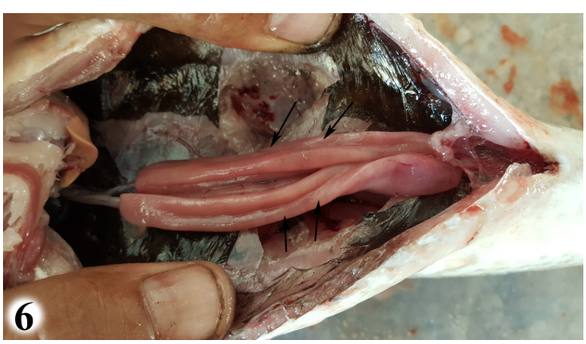

Fig. 6: Morphology of L. ramada testis obtained from fresh water at rapid spermatogenesis, showing the testes have lost their translucency, exhibiting a pink coloration.

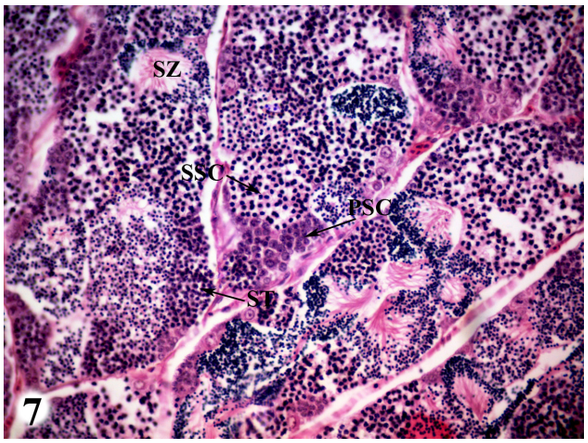

Fig. 7: T. S. of L. ramada testis, obtained from saline water at rapid spermatogensis, showing the seminiferous lobules (SL) which filled with cysts of germ cells at all spermatogenic stages; primary spermatocytes (PSC), secondary spermatcytes (SSC), spermatids (ST) and spermatozoa (SZ). X400.

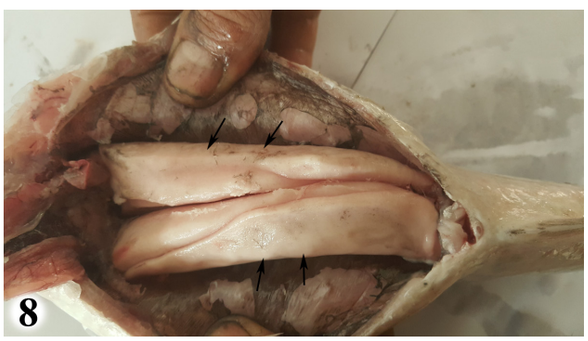

Fig. 8: Morphology of L. ramada ripe testis obtained from fresh water, showing the testes had almost filled the whole body cavity of the fish and appeared opaque, milky white, smooth and crumbled in texture.

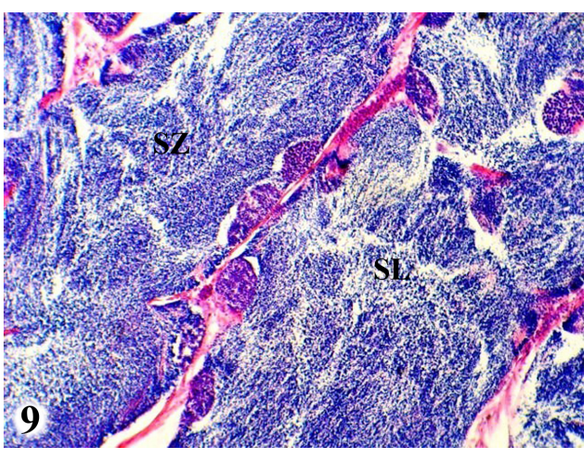

Fig. 9: T. S. of L. ramada ripe testis, obtained from fresh water, showed the seminiferous lobules (SL) which tremendously enlarged in size and fully packed with mature spermatozoa (SZ). X 100.

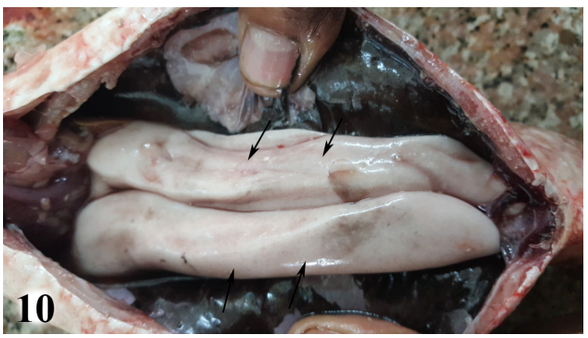

Fig. 10: Morphology of L. ramada spawning testis obtained by hormonal injection in saline water, showing the testes filled the whole body cavity of the fish and appeared milky white, and with running sperms.

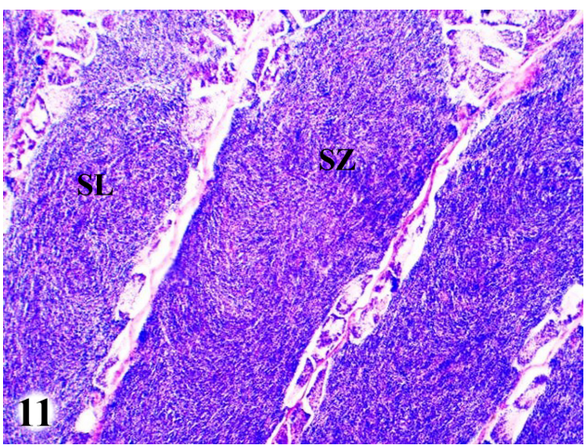

Fig. 11: T. S. of L. ramada spawning testis, obtained by hormonal injection in saline water, showed the seminiferous lobules (SL) fully packed with mature spermatozoa (SZ). X 100.

Table 1: Gonadosomatic index of males *Liza ramada* at different stages of maturation from fresh water (captivity) and during induced spawning in saline water

Testis Stage	Gonadosomatic index (%)	
	No.	Mean ± SD*
I	20	0.14 ± 0.05
II	20	0.3 ± 0.05
III	20	3.65 ± 0.59
IV	20	6.14 ± 0.07
V	20	12.6 ± 1.55

*Means were significantly different ($P < 0.05$).

Table 2: Monthly variations in the frerquency (%) of testicular stages of *Liza ramada* during Testicular cycle in fresh water (captivity)

Month	Water Type	No. of fish	I	II	III	IV
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	60	25	15	
Nov	Fresh	50		40	50	10
Dec	Fresh	50				100

DISCUSSION

I it is well accepted that L. ramada does not exhibit sexual dimorphism. Indeed, males and females look alike except the male appear smaller in size. Therefore, sex identification is not possible externally except during breeding season when female exhibits a distended belly due to the highly swollen ovaries compared with slight pressure on male belly[24,25]. Interestingly, the present study presents a conclusive morphological and histological description for the maturing of the testes of captivated L. ramada. Moreover, we successfully recruited a ripe mullet male suitable for reliable artificially induced reproduction in these species especially in captivity.

The testes in L. ramada are paired, equal in size, but incidence of equality in size is not common. This is ordinary feature with most of the teleosts[3,24,26,27]. The present study revealed seasonal variations in shape, volume and weight of L. ramada testis are obviously correlated with the degree of fish maturity. Similar observations were reported in most fishes such as: Barbus tor[28]; Schizothorax richardsonii[29]; Ictalurus nebulosus[30]; Clarias gariepinus[31]; M. cephalus[3,27] and L. ramada[24].

The present investigation showed that the testis of L. ramada consists of a plentiful number of seminiferous lobules bound together by means of a thin connective tissue, which also covers the whole testis. Such description is also in line with those reported for Barbus tor[28]; Schizothorax richardsonii[29]; Clarias gariepinus[31]; M. cephalus[3] and L. carinata[24,32]. However, the testis of some teleosts such as Ictalurus nebulosus is different and consists of seminiferous tubules[30].

It is well known that the spermatogenesis activity start at different times of the year in various teleosts. Consistently, environmental factors such as photoperiod, temperature and salinity were elucidated to influence reproductive activities in both male and female fish[3,19,24,32-36]. It seems likely that such factors affect spermatogenesis and spawning in L. ramada. Indeed, our investigation demonstrated that spermatogenesis initiated when the day length and water temperature started to decrease, while the gradual decrease in both photoperiod and water temperatures ensure its completion. However, spawning in L. ramada was noticed to occur when photoperiod and water temperatures had declined to their minimum values. In this respect, the reproductive activity of L. ramada inhabiting El-Bardawill lagoon starts in September, while the highest values of GSI were at November and December[10,11,24].

The present studies have also revealed that the captive freshwater L. ramada did not only complete final maturation, but also do not spawn spontaneously when reared in captivity. The failure of captive mullets to undergo final maturation and spawning, without hormonal injection, is thought to be caused by the shortage of gonadotropin synthesis[3-5]. Also, in the present study, injection of pituitary extracts in combination with gonadotropin releasing hormone (triptorelin acetate) and dopamine antagonist (metoclopramide) was effective in inducing the final stages of maturation, and spawning in L. ramada. It appears from the present study that understanding the coordination between environmental factors and the fish internal regulating factors is very important for successful fish reproduction. The act of gonadotropin releasing hormone and gonadotropin act at different endocrine levels to induce gonad maturation and spawning were studied in some fishes[12,37-39].

Collectively, the present study provides the morphological and histological description for testes of L. ramada during gonad maturaion in captivity and able to obtain a ripe mullet male suitable for hormonal induced reproduction in these species especially in captivity.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

نضج الخصية لأسماك الطوبار أثناء دورة التناسل فى الأسر

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

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

المادة والطرق: فى هذا البحث تم إستخدام الطرق المورفولوجية والهستولوجية لوصف دورة الخصية لذكور الطوبار المرباة في المياه العذبة.

النتائج: وجد أن شكل وحجم ولون وقوام خصية سمكة الطوبار عامة يتغير موسيميا. تميزت خمس مراحل، طبقا للصفات المورفوهستولوجية، أثناء تطور الخصية فى المياه العذبة. وهذه المراحل هى: مرحلة الخصية غير الناضجة، مرحلة تحفيز تكوين الحيوانات المنوية، المرحلة السريعة لتكوين الحيوانات المنوية، مرحلة الخصية الناضجة، ومرحلة الخصية أثناء التفريخ. يبدأ تطور الخصية مع بداية تناقص طول اليوم ودرجات الحرارة (شهر سبتمبر) بينما يؤدى الإنخفاض التدريجى لكل من فترة الإضاءة ودرجة حرارة الماء الى إتمام عملية التطور (من شهر سبتمبر الى شهر ديسمبر). ومع ذلك تتم عملية التفريخ لأسماك الطوبار حينما تصل فترة الإضاءة ودرجات الحرارة لمستوياتها الدنيا (شهر ديسمبر).

الخلاصة: على الرغم من وصول ذكور الطوبار إلى مرحلة النضج في المياه العذبة، لا يتم التفريخ إلا من خلال التحفيز الهرموني والبيئي.