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ABSTRACT

Reproductive characteristics of *Liza ramada* in Timsah Lake showed that the male reaches first sexual maturity smaller than the female at a total body length of 14 cm. and 16 cm., respectively. Overall sex ratio is 1: 1.7 for males to females. The gonado-somatic index of males was lower than that of females. Where, the maximum GSI values were recorded in November 5.03 % and 12.4 % for males and females, respectively. The maturity stages of male and female *Liza ramada* are morphologically separated according to the changes in shape, size and colour of the gonad in different successive stages. These stages are virgin, maturing virgin, developing, developed, gravid and spent stages. Histologically, the oocyte maturation showed the following phases; first growth phase which includes chromatin stage, early and late perinucleolus stages. Second growth phase includes vacuolization stage, yolk stages (primary, secondary and tertiary), mature stage and ripe stage. The testes maturation classified into spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. Different points of view which take place in this work show that of the spawning season of *Liza ramada* in Timsah lake which may be extending from November to January.

1. INTRODUCTION

Suez Canal is considered as a passage way to the immigrant fauna either from the Red Sea or the Mediterranean. It is also suitable for these fauna to live in it. When it was opened in 1869, the Suez Canal connected two different environments, that of the tropical Red Sea (an area extremely rich in species) and that of the sub-tropical eastern Mediterranean (an area with rather low number of species). Therefore, it seemed justified to expect that the canal might cause a mixing of great zoogeographic importance (Thorson, 1968). Many researches have been carried on the commercial fish species in Lake Timsah (El-Etreby, 1986) studied the species composition of the juvenile

commercial fish species. Sharaf (1987) studied the biology of Dicentrarchus labrax and concluded that Lake Timsah is an excellent nursery and feeding grounds to this species. Fouda (1993) gave a list of fish species in Timsah Lake and stated that the most dominant species are euryhaline and hence could be acclimatized to different salinities. Family Muglidae are of great economic importance both to Egypt as a whole and to the Suez Canal region in particular. They represent a great part of the commercial catch and great potential in aquaculture in Suez Canal region. Liza ramada have great nutritious value and are an important alternative to other food sources (Ergene, 2000). Many authors studied the production of Liza ramada such as (Brusle,

1981; Salem and Mohammed, 1982; Yerli, 1991; Zaki and EL-Gharabawy, 1991, EL-Halfawy, 2004 and Ibrahim, 2004). The present study deals with the reproductive biology and histology of gonad of *Liza ramada* in Lake Timsah

2. MATERIALS AND METHODS

Monthly samples of Liza ramada were collected during the period from October 2002 to September 2003 from Ismailia fishing center, which lies on the Suez Canal at 80 km south to Port Said (Fig.1). Length measurements of specimen were taken in centimeters and weight was determined in grams. According to Pitt (1970) the length at which 50% of fish reach sexual maturity (L_{50}) is considered to be the length of onset of its sexual maturity. To determine sexual maturity, gonads from males and females were weighed and morphologically examined according to Hajort scale (1910) in order to determine time of reproduction, gonadosomatic index values for each month, were used according to the following formula :

GSI % = gonad weight (g) X 100 /Body weight (g)

A piece of male and female gonad were fixed in Bouin solution, dehydrated in alcohol, embedded in paraffin and sections, 5-7 μ thick, were prepared. Sections were stained with haematoxylin and eosin. Slides were examined by Nikon microscope.

3. RESULTS

Length at first sexual maturity

The length of male and female *Liza* ramada at first sexual maturity was determined by the percentage distribution of mature and immature fishes for each length group. Figs. 2, 3 demonstrate that the

smallest ripe male was 14 cm with percentage 9 %, while the smallest ripe female was 16 cm with percentage 5 %. The percentage increased to reach 50 % at total length 18.6 cm for male and at total length 19.8 cm for female. All males larger than 22 cm. and females larger than 24 cm. are sexually mature.

Sex ratio

For *Liza ramada*, in lake Timsah, the sex ratio of the collected sample (238 fish) was 1:1.7 male to female, respectively. This means that females predominate males. The sex ratio is not constant throughout the different months; males outnumber the females in October (53.1 %), February (81.3 %), May (54.5 %) and June (60 %) while the females predominated in the remaining months. The lowest percentage of males is recorded in April (13.6%), whereas the females have the highest percentage of 82.0 % in December (Fig. 4).

Gonadosomatic index (Reproductive period)

Monthly variations in GSI of both sexes were quite apparent (Fig. 5). Maximum values were recorded in November (5.03 & 12.40 for males and females, respectively). A slight decrease occurred in December for both sexes followed by a considerable drop in January (0.88 % & 0.53 for males & females, respectively) and reach minimum levels in May (0.07 & 0.20 for males and females, respectively). Thereafter, the indices remained almost constant till September (0.05 & 0.21 for males & females, respectively). From October onwards the values increased, reaching their maximum level in November. These cyclic changes in GSI indices are considered as further a proof that the spawning season is short, lasting from November to January.



Fig. (1): Map of Lake Timsah.



Fig. (2): Length at first sexual maturity for male *Liza* ramada from Lake Timsah during the period from October 2002 to September 2003.



Fig. (3): Length at first sexual maturity for female *Liza* ramada from Lake Timsah during the period from October 2002 to September 2003.



Fig. (4): Monthly variations in sex ratio of *Liza ramada* from Lake Timsah Suez Canal from October 2002 to September 2003.



Fig. (5): Monthly variaton of the average Gonado-Somatic Index of Liza ramada from Lake Timsah, Suez Canal from October 2002 to September 2003.

3.1. Histological characteristics of ovaries.

3.1.1. Immaturation period (First growth phase):

3.1.1.1. Oogonia

Oogonia were small, round cells with relatively clear zone of cytoplasm. They occurred either solitary or in small nests, cells diameter ranged from 10 to 15 μ . It was weakly basophilic more than the nucleus in stain. The nucleus diameter varying from 8 to 13 μ (Fig. 6).

3.1.1.2. Early perinucleolus stage:

The oocytes of this stage were mostly polygonal and varied in diameter from 17 μ to 38 μ . The nucleus ranged from 12 μ to 32 μ in diameter. The cytoplasm was homogeneous and strongly basophilic. Oocyte membrane was not yet differentiated. The nuclei were arranged in the periphery of the nucleus and varying in number from 2 to 5 (Fig. 7).

3.1.1.3. Late perinucleolus stage:

This stage was the final stage in the immaturation period, the oocyte reached to 150 μ in diameter. The nucleus increased in size and reached to 50 μ in the average with a numerous number of nucleolei about 7 to 11. The nucleolei arranged in the periphery of the nucleus (Fig. 7).

3.1.2. Maturation period (Second growth phase):-

3.1.2.1. Vaculization stage:

This stage was characterized by the appearance of yolk vesicles in the cytoplasm.

These vesicles structure appeared empty. The oocytes reached to 150 μ in average. Nuclear diameter ranged from 60 to 90 μ . At the end of this stage the yolk vesicles reach to 23 μ in diameter. They were few in number and appeared in the periphery of the cytoplasm. The oocyte was surrounded by zona radiata of about 3 μ in thickness coated with a follicular epithelial layer of 1 μ (Figs. 8, 9).

3.1.2.2. Yolk granule stage:

This stage demonstrated by the appearance of yolk globules in the periphery of the cytoplasm. The oocyte ranged in diameter from 250 to 295 µ. The nucleus appeared granulated with irregular boundary and its diameter ranged from 80 to 110 μ . The nucleoli were still arranged on the periphery of the nucleus, they ranged from 7 to 11μ in diameter each. The yolk vesicles were distributed at the outer border of the cytoplasm. The thickness of the zona radiata measured about 7 μ on the average while the follicular epithelium became 2 µ thick (Fig. 10).

3.1.2.3. Vitellogenic stage:

During this stage yolk accumulation proceeded rapidly and obviously increased the oocyte diameter. The diameter of the oocyte increased to 400 μ and the nucleus appeared with diameter 98 μ in average. Yolk granules were densely packed and occupied mostly the total volume of the cytoplasm of oocytes. The zona radiata increased in thickness varying from 4 to 9 μ and follicular epithelium was about 4.5 μ in thickness. The vacuoles ranged in diameter from 2 μ to 10 μ (Fig. 11).



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3.1.2.4. Germinal vesicle migration stage:

Oocyte diameter ranged from 400 to 550 μ , nucleus diameter ranged from 80 to 100 μ (Fig. 12). At this stage the nucleus was migrating towards the periphery of the oocyte and contained many nucleoli. The vegetative pole was relatively thick than the animal pole. At the end of this stage, the nucleus became amoeboid in shape. The nucleoli were scattered in the nucleus and reached to 16 in number and ranged in diameter from 2 to 4 μ . The wall of oocyte composed of zona radiata that reach to about 11 μ in diameter and follicular epithelium recorded about 4 μ in average.

3.1.2.5. Mature yolk stage:

At this stage, the oocyte diameter varied from 500 to 700 μ in diameter. This representes the final stage of oocyte maturation. At this stage the zona radiata became well differentiated to reach about 32 μ . The nucleus could not be seen and lost in the cytoplasmic mass beneath the animal pole. The nuclear membrane disappeared and the nucleus lost its shape and became as nuclear material. The vacuoles increase in diameter and coalesce more than the preceding stage to form large vacuoles, which was the characteristic feature for the pelagic ova (Figs. 13, 14).

Atretic oocyte

Due to the activity of ovary, some oocytes fail to be ovulated. In *Liza ramada* there are two types of atresia.

Non bursting atresia:

The oocytes lose its identity and shape as follows:

• Cystic type where the follicular epithelium thickened and the nucleus dissolved in the oocyte material (Fig. 15 A).

• Phagocystic type where the epithelial cells in the ovarian follicle

became phagocytes and devour the oocyte material (Figs. 15 B, 15 C).

3.2. Histological characteristics of testes:

The process of spermatogenesis occurred progressively during the annual reproductive cycle. The present study showed five spermatogenic cells.

3.2.1. Spermatogonia:

It is the first spermatogenic stage in the testis. Spermatogonia were often observed as nests, each with a diameter ranging from 15 to 23 μ . The formed spermatogonium is rounded, the nucleus of which had a much greater affinity for haematoxylin staining (Fig. 22).

3.2.2. Primary spermatocytes:

The primary spermatocytes are formed by mitotic division of spermatogonia which appear in nests. Its diameter is about 9 μ . The nuclei are densely packed with chromatin material at one pole of the nucleus (Figs. 23, 24).

3.2.3. Secondary spermatocytes:

The secondary spermatocyte has a diameter ranging from 5 to 6 μ . These cells are formed by meiotic division and characterized by homogeneously stained nuclei. They are found in large nests extending into the lobular lumen (Fig. 24).

3.2.4. Spermatids:

It was formed from the subsequent maturation of secondary spermatocytes (mitotic division). Spermatids are slightly smaller in diameter than secondary spermatocytes 2 μ . The first existence of sperms arranged in a parachute shape (Fig. 24).







3.2.5. Spermatozoa:

Figure 25 showed spermatids with certain metamorphosis to form spermatozoa. Later,

the sperms 1 μ acquired the ability to be mobile in the seminal fluid. This is the final product of the process of spermatogenesis.

Macroscopic and Microscopic structure of maturity stages

A) Females

Maturity	Macroscopic	Microscopic
stages General structure	The ovary is a hollow, paired organ, consisting of two ovarian lobes that are separated by a septum. Both lobes joined near the urirnogenital pore.	Numerous ovigerous folds projected into the ovarian cavity. The lamellae consisted of connective tissue lined by germinal epithelium which contained cell nests of oogonia. Ovarian follicles developed along the lamellae and the mature oocytes are ovulated into the ovarian cavity.
Stage I (Virgin stage)	The ovaries are narrow tube like bands and transparent in colour, and present from March to May (Fig. 28).	Wall spaced ovigerous folds oriented towards the centre of the ovary; containing both oogonia (15-20 μ m) and perinucleolus stage (early perinucleolus 36 μ m, late perinucleolus 72 μ m). Oogonia generally occur in nests (Fig. 16).
Stage II (Maturin g virgin	The ovary is small, pink in colour and translucent. The eggs became visible, and present in October and from March to September (Fig. 28).	Oogonia still present, spaces between the ovigerous folds are closer; primary oocytes at all stages present especially late perinucleolus (77 μ m) (Fig. 17).
Stage III (Develop ing stage)	Ovary become larger than above stages and occupied about 1/2 of the body cavity, reddish-yellow in colour and eggs are visible (slight granular appearance. This stage appeare in October, December and from June to September (Fig. 28).	Ovigerous folds fill the ovarian cavity; majority of cortical alveoli oocytes and yolk granules occur. The cytoplasm shows small lipid vesicles. Oocytes diameters range from122-142 µm. (Fig.18).
Stage IV (Develop ed stage)	The ovaries occupy 2/3 of body cavity, swell, reddish yellow in colour, eggs visible and numerous. This stage occurs from October to December (Fig. 28).	Oocytes increase in diameter to reach 384 µm in average. The maturation period is characterized by the appearance of oocytes in yolk granule stage (primary, secondary and tertiary) and vitellogenic stages besides to few numbers of cortical alveoli and germinal vesicles migration. The wall of oocyte increase in thickness (Fig.19)
Stage V (Gravid stage)	Ovary is long and broad, filling all the body cavity and yellow in colour. This stage record in December (Fig. 28).	Oocytes at the germinal vesicles migration stage predominated and presence of mature yolky stage (512 µm in average) (Fig. 20).
Stage VI (Spent stage)	Ovary is flaccid but not fully empty, deeper in colour. This stage appears from December to March (Fig. 28).	Irregular convoluted ovigerous folds containing large atretic follicles and net-shape connective tissue besides few numbers of perinucleolus stage (early and late) (Fig. 21).

B) Male

Maturity stages	Macroscopic	Microscopic
General structure	The testes were elongated paired organs attached to the dorsal body wall.	The testes are kidney bean shape compose of numerous lobules which are separated from each other by a thin layer of fibrous connective tissue within the lobules, spermatogonia divide to produce primary and secondary spermatocytes and spermatides.
Stage I (Virgin stage)	The testes are narrow ribbon like and transparent in colour. This stage appear from March to May (Fig. 29).	The testes are small in size and contain spermatogonia which are the only cellular components and kidney shape. (Fig.22)
Stage II (Maturing virgin	The testes were slightly thickened and occupy 1/3 of the body cavity with developing white colour. This stage appears from March to October (Fig. 29).	The testes exhibit active spermatogenesis more than in the preceding stage (spermatogonia and primary spermatocytes) (Fig. 23).
Stage III (Developing stage)	The testes are white and oval like. They are broader, thicker and more softy. This stage appear from October to December and return to appear from June to September (Fig. 29).	In this stage the cells of all stages of development could be seen spermatogonia, spermatides and sperms in a parachute shape (Fig. 24).
Stage IV (Developed stage)	The testes are creamy and broadens, milt extrude by pressure on the belly. This stage illustrate from October to December and return to appear in September (Fig. 29).	This stage illustrate parachute shape in a large number beside all other developmental cells (Fig. 25).
Stage V (Gravid stage)	The testes approximately are filling the body cavity. The milt discharges by a gentle pressing on the abdomen. This stage appears in November and December (Fig. 29).	In this stage the parachute shape is broken and the testes appear filled with sperms (Fig. 26).
Stage VI (Spent stage)	The testes are bloody in colour and flaccid. This stage appears from December to March (Fig. 29).	Residual spermatozoa and spermatogonia present towards the spermatic duct (Fig. 27).



4. DISCUSSION

Generally, in the natural mullets community the males mature before the females (Broadhead, 1958; Stenger, 1959; Bralhet, 1975; Salem and Mohammed, 1982 and El-Halfawy, 2004). The present study showed that the smallest male attained first maturity at 14 cm. total length and the smallest female at 16 cm total length. This means that the male reach first sexual maturity at a slightly smaller length by two cm in total length than females. Also, the percentage of sexually mature fish gradually increase with the increase of fish length. EL-Maghraby et al. (1973) stated that in Borullus lake, females and males Liza ramada reached to sexual maturity at total length of 17 cm and 13 cm., respectively. Quignard and Farrugio (1981) determined sexual maturity of male and female Liza ramada at length 23 cm. and 25 cm., respectively. On other hand, Ergene (2000), obtained that values of sexual maturity of Liza ramada at Akgöl-Paradeniz at length 26.9 and 27.01 cm. for female and male, respectively. Generally, most of the above results indicate that L₅₀ prevailed at 18.6 cm for males and at 19.8 cm for females..

In this study the sex ratio of Liza ramada was 1:1.7 with percentage 37 % & 63 % for male and female, respectively. At all lengths females constitute the majority. EL-Maghraby et al. (1973) in Borullus Lake found that the ratio of females was 62 % while that of males were 38 %. The results of the study of Liza ramada in Broulls lake are similar to the results of this study. While Brusle (1981) recorded the proportion of Liza ramada to be 84 % for females and the ratio of males was 16 %. The present results coincide with those of Salem and Mohammed (1982), Yerli (1991) and Ergene (2000). The above mentioned results show that every male needs two females as an application in the fish farms.

Spawning season of Liza ramada in Timsah Lake extended from November to January on the basis of GSI values. Sagi and Abraham et al. (1985) reported that Liza ramada has maximum GSI values during period of migration to the sea. Salem & Mohammed (1982) in Lake Timsah found that the GSI values of Liza ramada in October, November and December were 2.2, 4.3 and 2.2, respectively. Slastenenko (1956) mentioned that Liza ramada reproduction continues in the Black sea during July and September. Yerli (1991) found that the values of GSI was 18.96 at November and 16.17 at December. Also, Katavic (1980) evaluating the time of emergence of fingerling Liza ramada and suggested that spawning occurs between late December, January and February. It was found that spawning period as reported for Eisawy et al. (1974); Salem & Mohammed (1982): Yerli (1991): Zaki et al. (1994) and Ergene (2000) are all in agreement with each other and with the present study.

In *Liza ramada* the gravid ovaries appeared in low percentages in spawning season. This phenomenon may be due to the migration of ripe females to the Mediterranean Sea with some ripe males and this explained the presence of higher percentage of males than females. These results coincide with results obtained by Salem and Mohammed (1982).

The histological studies demonstrated that there are two developmental phases of the oocyte namely, the primary growth phase and secondary growth phase. Also, these two phases were investigated by many authors (Latif and saddy, 1973; Guraya *et al.*, 1975; Ramadan *et al.*, 1978; EL-Garabawy and Abdel-Aziz, 1988 and EL-Gharabawy, 1996). The role of the follicle cells in the oocytes is to form an active part in the transfer of proteins and other nutrients from the blood to the developing egg as reported by Norrevang (1968); Zaki, (1989) and El-Gamal (2003). The vacuolization period is characterized by

the appearance of vacuoles at the periphery of the cytoplasm with increase in number and size at this stage. The wall of this stage is formed of zona radiata and follicular epithelium.

The beginning of yolk deposition is an indication of the phase of vitellogenesis (Rastogi, 1968; EL-Garabawy, 1996 and El-Gamal, 2003), the trophoplasmic growth period (Latif and Saady, 1973), the primary, secondary and tertiary yolk stage (Dixit and Agrawala, 1974) and the yolk formation stage (Ramadan et al., 1978; Zaki and EL-Garabawy, 1991). The yolk depositions first appeared in the peripheral cytoplasm, thereafter scattered towards the center of oocvte as described by Zaki et al. (1986); Zaki & EL-Gharabawy (1991); EL Gamal (1997 and 2003) and Mousa (2002). At the ripening of oocyte in Liza ramada, the migration of the nucleus to animal pole occurs. The yolk deposition in the oocytes of the present species shows the same feature as described by other authors (Zaki et al., 1986; Zaki, 1989; Zaki and EL-Garabawy, 1991; Assem, 1992, 1995 and Aly, 1995). When the females are fully ripe, they swarm and migrate from lake to the sea (Hashem et al., 1973).

Oocytes which do not succeed to be spawned are reabsorbed and become atretic. Some oocytes became cystic where the follicular epithelium thickened and the nucleus dissolved in the oocytes material and other oocytes transformed into phagocytic cells that attack the oocytes and dissolve it, these oocytes belong to non-burasting atresia. Various authors attributed the follicular atresia to environmental stress (Ball, 1960; Kamel, 1990; EL-Halfawy, 1995; Ramadan, 1995 and El-Gamal, 2003).

In *Liza ramada*, the testes of different individuals caught during the same period were exhibiting different spermatogenic activities. Spermatogenesis takes place progressively during the annual reproductive cycle and the spermatozoa were discharged gradually from the seminiferous lobules. The present results, therefore, support the findings of Zaki et al. (1994) for Mugil seheli.

6. CONCLUSION

From the reproductive and the histological studies, it can be concluded that the histological results supports the findings from the biological results which elucidated the spawning season of *Liza ramada* to extend from October to December forming a peak in November.

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- Figure 6. Cross Section in ovary of *Liza ramada* showing (A) chromatin nucleolus oocyte. (Eosin and haematoxylin X100).
- Figure 7. Cross section in ovary of *Liza ramada* showing (A) early perinucleolus, (B) late perinucleolus, (N) nucleus and (n) nucleolus. (Eosin and haematoxylin X 40)
- Figure 8. Cross section in ovary of *Liza ramada* demonstrating beginning of vacuolization stage (arrow) (Eosin and haematoxylin X 40).
- Figure 9. Cross section in ovary of *Liza ramada* illustrating vaculization stage. (N) nucleus, (n) nucleolus and (V) vacuoles. (Eosin and haematoxylin X 20).
- Figure 10. Cross section in ovary of *Liza ramada* demonstrating oocyte in primary yolk stage (N) nucleus, (n) nucleolus, (V) vacuoles (Eosin and haematoxylin X 20).
- Figure 11. Cross section in ovary of *Liza ramada* showing oocyte in secondary yolk stage (YV) yolk vesicles, (YG) yolk granules, (N) nucleus, (n) nucleolus, (ZR) zona radiata and (EF) epithelial follicle (Eosin and haematoxylin X 20).
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- Figure 16. Cross section in ovary of Liza ramada at stage I. (Eosin and haematoxylin X 50).
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- Figure 22. Cross section in testis of *Liza ramada* at stage I showing (A) spermatogonia cells. (Eosin and haematoxylin X 200).
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- Figure 26. Cross section in testisof Liza ramada at stage V. (Eosin and haematoxyline X 200).
- Figure 27. Cross section in testisof *Liza ramada* at stage VI demonstrating residual of spawning (Eosin and haematoxyline X 200).