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Reproductive biology (histological & ultrastructure) and biochemical studies in ovaries of *Mugil cephalus* from Mediterranean water

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ABSTRACT

Mugil cephalus has a short spawning period extending from early August to late October. All the females over 41 cm in body length are mature. The peak value of gonadosomatic index (GSI) was attained in September and continued to October. The sequence of the egg diameters were divided into two groups (transparent and yolky). Fecundity shows a wide range for a given length, the absolute fecundity has a linear relationship with length groups and gutted weight. The general pattern of the histological development of the ovaries includes six periods: 1-Immaturation period characterized by “small spherical cells”. 2-The maturation period characterized by “appearance of isolated follicular epithelial cells around the oocyte”. 3-Vacuolization period characterized by appearance of “marginal vacuoles; the oocyte wall consists of two layers”. 4- The yolk deposition period characterized by “the presence of yolk granules”. 5- Ripening period characterized by “migration of the nucleus to the animal pole”. 6-Spawning period characterized by presence of “empty follicles, and cytoplasmic growth”. The ultrastructure of the vacuolized and ripe oocyte wall showing the presence of five different layers, (theca, follicular epithelial, zona radiata externa and zona radiata interna, then fifth layer are known as “cortical alveoli". Total lipid and fatty acids content of ripe ovary were discussed. Many differences were recorded in concentration of fatty acids content of ovary.

Keywords: Reproductive biology, histology, ultrastructure *Mugil cephalus.*

INTRODUCTION

Mugil cephalus L. is one of the most common fish species in the Mediterranean. An increasing attention has been given to its propagation and productivity facing the marked deficiency of its fries (El-Gharabawy *et al*., 2006). It is distributed circumglobally in tropical and semitropical waters (Rossi *et al*., 1998), it is a euryhaline and can be found throughout the full range of estuarine salinities in the southeastern United States (El-Dahhar, 2006).The biological feature of striped mullet has been well documented (Chubb *et al.*, 1981), but much less information is available on the biological aspects of reproduction in the wild (Greeley *et al*., 1987; Mc-Donough *et al*., 2003).

Histological, ultrastructural and seasonal variation of the teleostean gonads, have attracted the attention of many investigators (Zaki *et al*., 1998) for *Sparus aurata*; Assem (2000, 2003) for *Caranx crysos* and *Pagellus erythrinus*. Fecundity and spawning season of *Mugil cephalus*, in South Carolina estuaries were studied by Mc-Donough *et al*. (2003). Hotos *et al*. (2000), studied the reproductive biology of *Liza aurata* in the lagoon of Klisova. Ilkyaz *et al*. (2006), studied age, growth, and sex ratio of golden grey mullet, *Liza aurata*. Ogata *et al*., 2004, studied the fatty acid composition of five candidate aquaculture species in central Philippine and indicated that, broodstock management and larviculture technologies based on the nutritional traits.

Dietary lipids are important sources of energy and essential fatty acids (EFA) for fish (NRC, 1993). Most marine fish require n-3 HUFA such as EPA and DHA for normal growth and development (Izquierdo *et al*., 1989). Therefore, it is important to develop feed with appropriate class and levels of EFA to meet nutritional requirements of the fish (Kim *et al*., 2002).

Ghioni *et al*., 1999 stated that: specific fatty acid requirements of eggs and newly hatched larvae must be provided via maternal inputs and subsequently through larval diets at the onset of exogenous feeding. The present work aims to investigate the important aspects of the reproductive biology, histology and ultrastructure of *Mugil cephalus* ovaries in the Mediterranean Sea. Determine the concentration of total lipid and fatty acids in ripe ovary of wild broad stock to clarify the requirement of dietary offspring.

MATERIALS AND METHODS

Biological studies: 85 female *Mugil cephalus* were collected from the Egyptian Mediterranean Sea landed at Alexandria coast throughout the period from January to December 2007 ranging in total length and total weight from 30.5 to 49.5 cm and 550 to 1300 g. For each, the date of capture, total length to nearest mm and total weight to nearest g were recorded. The fish were dissected to determine sex and maturity stages in agreement with gonadosomatic index (percentage weight of the gonad to the gutted weight of the fish) as indicated in Table (1). To study the egg diameter in the fully matured ovaries a known portion by weight was preserved in 10% formalin. For fecundity study

Table 1: Morphology and duration for ovarian cycle of *Mugil cephalus* according to gonadosomatic indices (GSI).

Stages of maturity	Morphology and Duration	Average GSI
Immature	Thin cylindrical, nearly occupy one third of the body cavity, detected throughout the year.	0.51±0.11
Mature	Ovary increase in size; pinkish in color, detected throughout the year.	1.11±0.61
Nearly ripe	Ovaries are yellowish in color; eggs are distinguishable with the naked eye and blood vessels can be seen within the ovary detected, from July until September.	8.97±2.30
Ripe and Spawning	Ovaries reach its maximum development. Ovaries, orange yellow in color, from August to October.	25.19±3.40
Spent	Ovaries reduced in size, reddish yellow in color, have a large number of blood vessels externally, from November to January.	1.86±0.30

known weight of ovary were counted then the number of eggs in the paired lobes of ovary for each female was calculated as absolute fecundity, whereas the relative fecundity is the number of eggs per unit weight or length.

For histological changes, ovaries were treated passing through fixation, dehydration, clearing, embedding and staining with Haematoxylin and Eosin. To study the ultrastructure four small pieces of ovary were fixed in 4% glutaraldehyed, washing, post fixation , dehydrated ; ultrathin sections were cut with a glass knife then stained with uranyl acetate and lead citrate.

Biochemical studies

Samples were dried at 60 ºC overnight. Moisture content was calculated the samples were kept frozen until further analysis (AOAC, 1984).

Total lipid and fatty acid extraction: Lipid was extracted by homogenizing sample in chloroform: methanol in ratio 1:2 (v: v) (Folch *et al*. 1957). Fatty acid methyl esters were analyzed according to Radwan (1978).

RESULTS

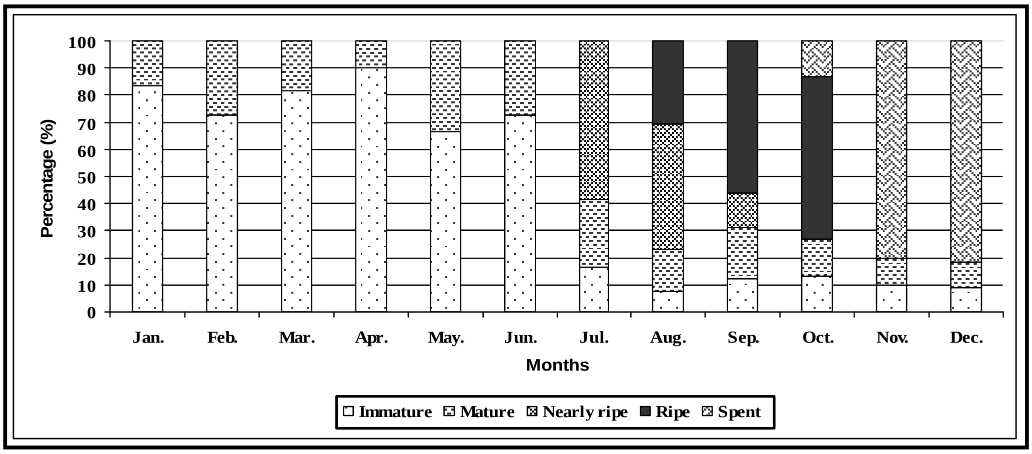
Maturity stages, monthly distribution of maturity stages, length at first sexual maturity, gonadosomatic index, egg diameter and fecundity of mullet *Mugil cephalus* were applied.

Monthly distribution of maturity stages of Mugil cephalus

The monthly variation of the maturity stages in females (*Mugil cephalus*) throughout the period from January to December 2007 is shown in Figure (1). The immature and maturation recorded during the whole year and fluctuates from one month to another to reach a maximum percentage (90%) in April for immature and (33.3%) in May for mature. The nearly ripe stage was recorded in July, August and September with 58.3 %, 46.2% and 12.5%, respectively. The period of spawning started at early August with a percentage of 30.8% and peak value attained in October to reach 60.0%. The spent appeared in October and continued until December.

Figure 1: ***Monthly distribution of maturity stages in striped mullet female Mugil***

***cephalus* throughout the period from January to December (2007).**



Length at first sexual maturity of Mugil cephalus:

From Figure (2) it is clear that all female (*Mugil cephalus*) smaller than 35 cm are immature. The mature females appear with small percentage 16.7% at length 36 cm. The percentage of mature female increases to 91.7% at length 40 cm. All female larger than 41 cm is mature female.

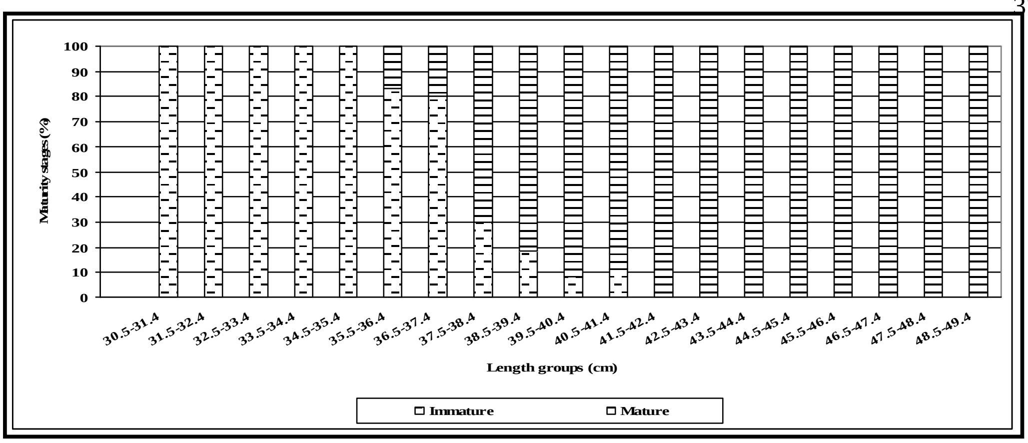
Gonadosomatic index of Mugil cephalus

The average GSI value was 0.51±0.11 during the immature stage, while in the mature stage (1.11±0.61), then increased significantly at ($p<0.05$) to reach 8.97±2.3 during the nearly ripe stage, then reached its maximum values in ripe stage (25.19±3.4) in October. The minimum GSI values were recorded in spent stage (1.86±0.3) (Fig.3).

Egg diameter and spawning of Mugil cephalus:

In order to determine the spawning frequency and the duration of spawning period, ova diameter were analyzed. The analysis of ova diameter for *Mugil cephalus* revealed that, there were six diameter groups of ova in different samples. The sequence of the egg diameters were divided into two groups (transparent and

Figure 2: ***The percentage distribution of the immature and mature striped***



mullet female in Mugil cephalus throughout the period from January to December (2007).

yolky). Egg diameter of the first two groups ranged from 0.16 to 0.24 mm, they were transparent. The second four groups were yolky, their diameter ranged between 0.32 and 0.56mm. All the diameter groups were present with variable percentages during the spawning time. At August (Fig.4 A, B & C), the fish with GSI varied between 8.15 and 18.6 have transparent ova varies between 1% as a minimally and 29% as a maximally and yolky egg appear 9.8% as a minimally and 85.2% as a maximally.

At September (Fig. 4 D, E & F), the fish with average GSI 23.9 have transparent ova ranging from 1% minimally to 5 % maximally, and yolky egg varies between 2.7% as minimally and 72.12% as average maximally. At October (Fig. 4 G), the fish with GSI 25.3 have transparent ova varies between 5% as a minimally and 6% as a maximally, and yolky egg varies between 15% as average minimally and 25% as average maximally. At early November (Fig.4 H), the fish with GSI 8.15 have transparent ova varies between 10% as a minimally and 65% as a maximally, and yolky egg about 25% as average.

Analysis of absolute fecundity-length relationship of Mugil cephalus

The fecundity - length relationship was described by the following liner equation:

$$Fa = 393056.45\ TL + 924551$$

Where (Fa) is the absolute fecundity and (TL) is the total length group. The equation indicates (0.9648) correlation coefficient.

The relationship between the relative fecundity and total length was estimated by liner equation:

$$Fr = 7481.9\ TL + 28381$$

Where (Fr) is the relative fecundity and (TL) is the total length group. The correlation coefficient was found to be (0.9451).

Analysis of absolute fecundity-weight relationship of Mugil

cephalus:

The average absolute fecundity of *Mugil cephalus* in relation to each gutted weight can express as:

$$Fa = 4764.41 W + 730882$$

Where (W) is the gutted weight, with correlation coefficient of 0.9083.

The observed and calculated relative fecundity in related to each gutted weight can express as:

$$Fr = 170.49 W + 2509.7$$

Where Fr is the relative fecundity and the correlation coefficient = 0.5311. Table 2 indicates the average absolute and relative fecundity related to each total length and gutted weight.

Figure 3*: Gonadosomatic indices (GSI) values of sexually mature striped mullet female Mugil cephalus at different stags of maturation.*

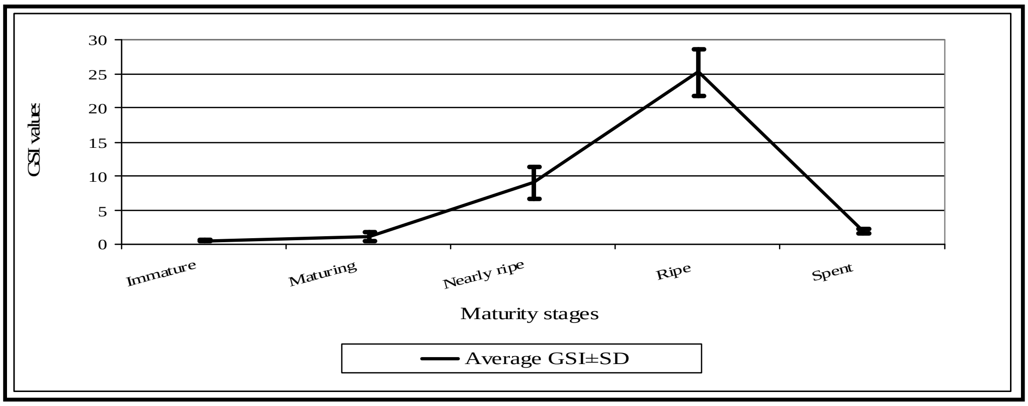
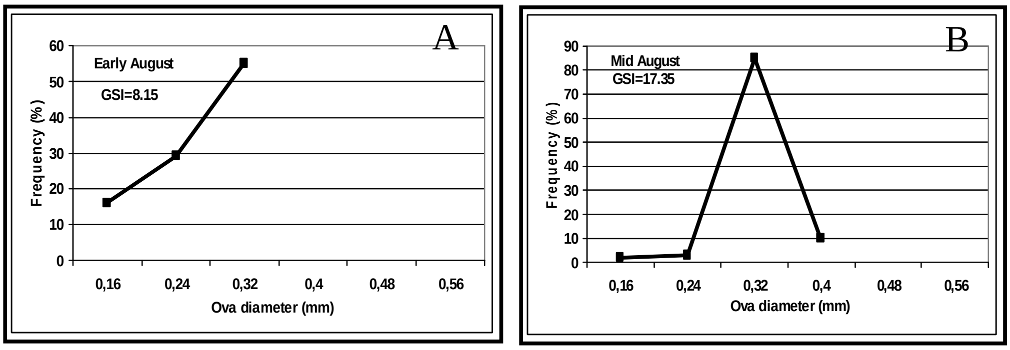
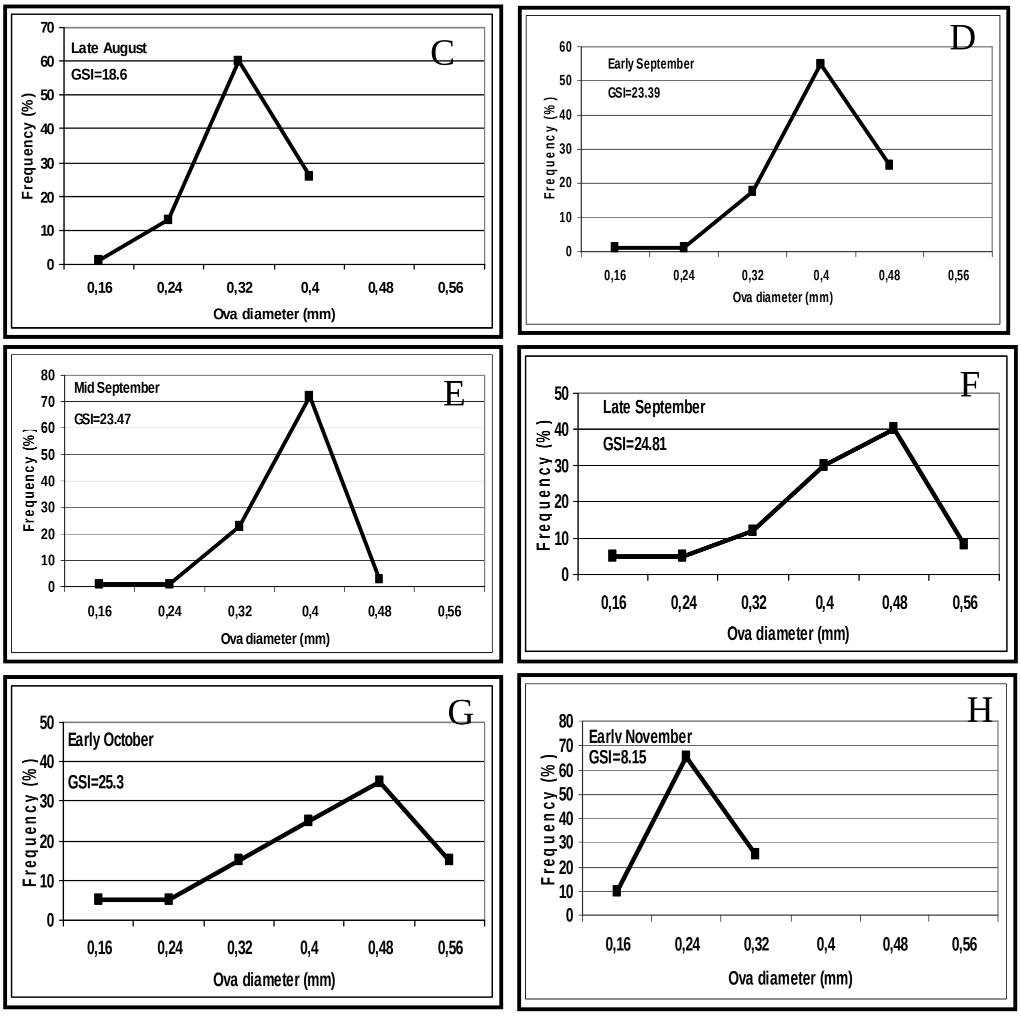


Figure 4: ***Frequency distribution profile of egg diameter for striped mullet Mugil cephalus throughout August, September, October and November (2007).***





Histological and ultra structure characteristics in ovaries of Mugil cephalus:

Developmental stages of oocyte were applied according to Zaki and El-Gharabawy (1991) and Assem (2003) description with slight modifications as follows.

Immaturation period

The oogonia at this period were very small spherical cells with a diameter ranged from 12 to15 µ, having a thin peripheral zone of faint staining transparent cytoplasm and a large pale nucleus. The oogonia or presynaptic oocyte were not conspicuous and were present solitary or in clusters of two cell. The ultrastructure of presynaptic was shown in Figure (5). Their cytoplasm was provided with Golgi body, mitochondria and irregular vacuoles, surrounded by thin plasma membrane. Their nucleus was provided with scatter dense darkly stained chromatin material and nucleoli mixed with nuclear content. At late immaturation period the primary oocyte increase in diameter ranged between 35 to 70 µ with a nucleus, which varied in diameter from 10 to 35 µ. The nucleoli reached 10 in number; they ranged in diameter from 2 to 4 µ (Fig. 6).

Maturation period

The maturation period was characterized by the appearance of isolated follicular epithelium cells around the primary oocyte forming follicular layer. The primary oocyte was provided with large nucleus and scattered nucleoli. Electron microscope studies of two adjacent cytoplasmic growth cells indicate the presence of follicular epithelial layer Figure (7). Nucleus of interstitial cells was large irregularly elongated. At the end of maturation stage, the oocyte increased in size reached about 132 µ. Nucleus was varied in diameter between 35 to 53 µ; there were 11 nucleoli, which varied in diameter from 2 to 3 µ.

Vacuolization period

The oocyte of this period was characterized by the appearance of marginal vacuoles. The vacuoles were at first few in number, small in size and appeared scattered in the cytoplasm, while follicular layer become more conspicuous

Table 2: The average values of absolute and relative fecundity in striped mullet *Mugil cephalus* related to each total length and gutted weight throughout the period from January to December (2007).

Total length (cm)	No. of fish	Fecundity		Gutted weight (g)	No. of fish	Fecundity	
		Absolute	Relative			Absolute	Relative
39.5-40.4	6	1345843	34509	550-600	6	1475524	2951

40.5-41.4	6	1667553	41689	650-700	6	1538871	2565
41.5-42.4	7	2186844	53338	750-800	7	2186844	3124
42.5-43.4	6	2388448	56868	850-900	8	2505231	3132
43.5-44.4	5	2513778	58460	950-1000	8	2448564	2721
44.5-45.4	5	3387447	76987				
45.5-46.4	6	4089713	90883	1050-1100	6	4194684	4195
46.5-47.4	6	4276452	92966	1150-1200	6	4233642	3849
47.5-48.4	5	4426773	94187	1250-1300	10	4415573	3680
48.5-49.4	5	4580762	95433				

and additive layer of zona radiata was formed Fig. (8). The oocyte ranged in diameter between 120 to 210 µ. The nucleus varied in diameter from 40 to 53 µ. The vacuoles ranged in diameter from 3 to 5 µ. The nucleoli were arranged in periphery of nucleus and varied in number from 6 to 14. They ranged in diameter from 2 to 5 µ. The oocyte membrane consisted of zona radiata of about 3 µ in thickness coated with a follicular layer of about 1 µ Fig. (9).

Ultra structure of vacuolized oocyte cell membrane showing the presence of five different layers, the outer most layer (theca layer) indicate the presence of small rounded thecal cells having large elongated nucleus with dense chromatin, the second layer is basement membrane, while the next layer was follicular layer, which is characterized by having a large number of follicular cells. Magnification of the zona radiata shows the presence of two different layers: zona radiata externa and zona radiata interna, the fifth layer was cortical alveoli Fig. (9).

Yolk deposition period

The oocyte of this period is gradually characterized by the appearance of yolk granules in the periphery of the cytoplasm. The oocyte at primary yolk stage ranged in diameter between 250 to 360 µ with a nucleus varied in diameter from 40 to 60 µ. The nucleoli were arranged in the periphery of the nucleus. The yolk granules were scattered in the cytoplasm and varied in diameter from 4 to 6 µ. The oocyte membrane consisted of zona radiata of about 5 µ in thickness, coated with a follicular layer of about 2 µ Fig. (10). The oocyte at secondary yolk stage characterized by vacules fusion to each other, oocyte at this stage ranged in diameter between 350 to 400 µ with a nucleus which varied in diameter from 48 to 56 µ.

The oocyte at tertiary yolk stage characterized by formation of amoeboid nucleus intermingled with cell's content.Ultra structure of yolk stage oocyte cell wall showing the presence of outer theca layer which include special theca cell, then followed by basement membrane, follicular layer, zona radiata and cortical alveoli and then ooplasm Fig. (11).

Ripening and Spawning period

This period is characterized mainly by migration of the nucleus oocyte towards the animal pole and also by the egg rupture from the follicle. The oocyte increased in diameter and reaches 560 x 610 µ across its long and short axis respectively. The nucleus appeared intermingled with the cytoplasm. The nucleoli were scattered in the nucleus, reached the diameter of 30 µ. The vacuoles intermixed with the yolk which appeared as a liquid. The oocyte membrane consisted of zona radiata of about 7

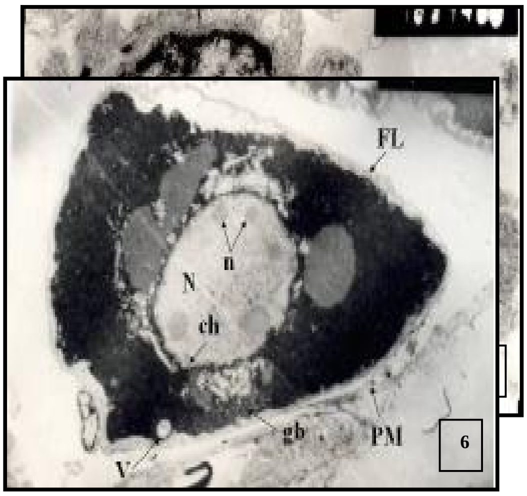
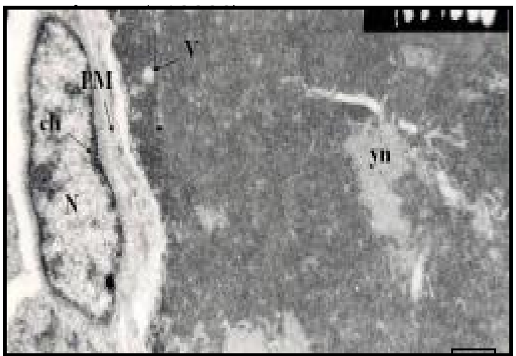
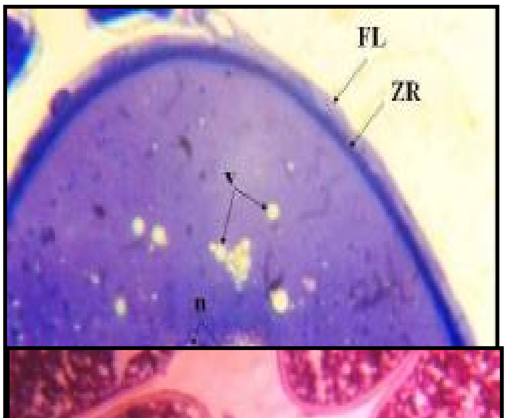


Fig. 5: Electronmicrograph of cross section (cs) in ovary of Mugil cephalus at immaturation stage showing: presynaptic cells. Note Golgi bodies (gb), mitochondria (m), dens chromatin material (ch) scattered in the nucleus (N) and nucleoli (n) mixed with nuclear contents, plasma membrane (PM). Stained with uranyl acetate and lead

Fig. 6: Electronmicrograph of (cs) in advanced primary oocyte (a) with follicular layer (fl), Golgi bodies (gb), irregular vacuoles (V), dens chromatin material (ch) nucleus (N) and nucleoli (n) (x10000).





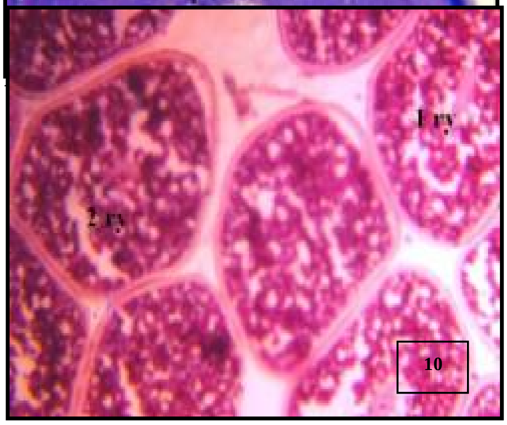


***Fig. 9:** Electronmicrograph of (cs) in vacuolized oocyte at showing: outer theca layer (th.L), with theca cells (Thc), basement membrane (BM),follicular layer (FL) with follicular cell (FC), middle layer of zona radiata externa (ZRE) and zona radiata interna (ZRI) with pore canals (PC) and inner layer of cortical alveoli (CA) (x10000).*

***Fig. 10:** photomicrograph of (cs) at ripe stage showing, primary yolk (1ry) and secondary yolk (2ry) stages. Stained with haematoxylin-eosin (H & E) (x100).*

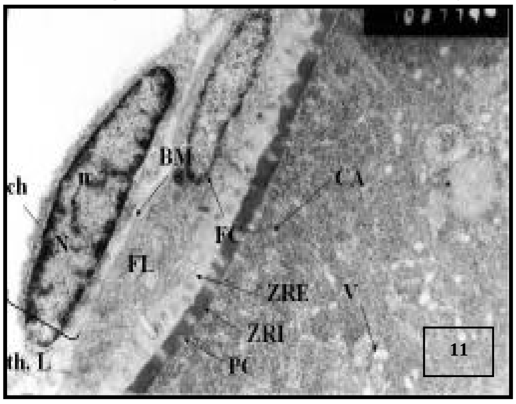
µ, coated with a follicular layer of about 3 µ in thickness Fig. (12). At this stage, the ovary displayed various peculiarities having a large number of empty follicles and different stages of yolk deposition oocyte, early stages of cytoplasmic growth were appeared as a new generation for the next breeding season.

Spent period

At this period the unovaluated (residual) eggs undergo atresia at the end of breeding season. The ovary decreased in size and accompanied by a decrease in the number of ripe ova with the appearance of empty follicle Fig. (13). At the end of atresia ooplasm and its yolky content were completely disappeared.

Biochemical analysis in ovary of Mugil cephalus:
Total lipid and fatty acids content in ripe ovary of Mugil cephalus

Lipids are important constituents of cell protoplasm of living individuals. Chemically,



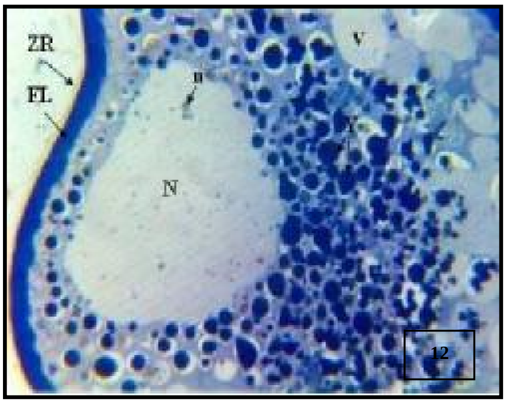


Fig. 11: Electronmicrograph of (cs) in wall of ripe oocyte showing: outer theca layer (th.L), with theca cells (Thc), basement membrane (BM), irregular vacuoles (V), dens chromatin material (ch) scattered in the nucleus (N) and nucleoli (n) mixed with nuclear contents, follicular epithelial layer (FE) with follicular cells (FC), zona radiata externa (ZRE) and zona radiata interna (ZRI) with pore canals (PC) and inner layer of cortical alveoli (CA). Stained with uranyl acetate and lead citrate (x10000).

Fig. 12: Photomicrograph of semithin (cs) at ripe period showing: vacuoles (V), yolk deposition (Y), nucleus (N) and nucleoli (n) mixed with nuclear contents, follicular layer (FL), zona radiata (ZR). Stained with toludine blue (x250).

lipids are esters of fatty acids or capable of forming esters. Total lipid content of ripe ovary in *Mugil cephalus* reach to 27.735% dry ovary weight and moisture 51.379% of ovary weight.

Fatty acids with high concentration present in ripe ovary were palmitoleic acid (16:1), oleic acid (18:1), palmitic acid (16:0) and Linoleic acid (18:2) Table (3). Sum of saturated fatty acid considered as 29.5% from total content, the lowest concentration was Myristic acid (14:0).

The n-3 fatty acid detected as 6.1% of total fatty acid while n-6 fatty acid detected as 21.4% of total fatty acid. The highly unsaturated fatty acids detected as 9.1% of total fatty acid with high concentration in Arachidonic acid (ARA) 2.4%, Docosahexaenois acid (DHA) 4.7% and Eicosapentaenoic acid (EPA) 1.3%.

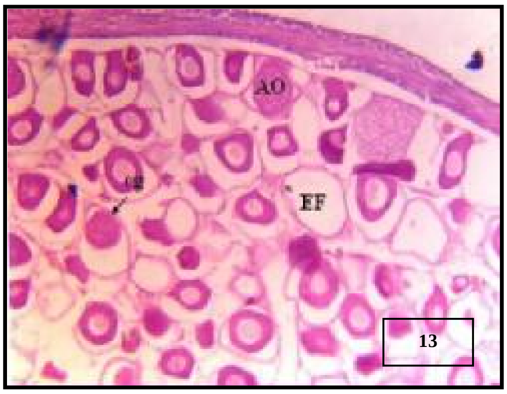


Fig. 13: ***photomicrograph of (cs) at spent stage showing: atretica oocyte (AO), empty follicle (EF) and new generation of***

cytoplasmic growth oocytes (cg). (H & E) (x100).

DISCUSSION

Maturity stages, length at the onset sexual maturity, gonadosomatic index, egg diameter, fecundity and oogenesis were discussed in order to clarify some characteristics related to reproduction. The general pattern of developmental stages of the ovary conforms to that of most teleosts (Zaki *et al*., 1995 and Assem, 2000 &2003) with slight modification.

In the present study, immature and maturing were found throughout the whole year. Similar results were reported by McDonough and Wenner (2003) on *Mugil cephalus*.

Mugil cephalus is considered isochronal spawning fish (Greeley *et al*., 1987; Render *et al*., 1995; McDonough and Wenner, 2003) (i.e. they have synchronous gamete development and spawn all their reproductive material at once or in batches over a very short period of time). All females whose body length is over 41 cm are mature female *M.cephalus* attains 70% maturity at total length of 38 cm. Assem (2000) found that the female of *Caranx crysos* attains 50% maturity at a total length 17cm. Assem (2003) stated that the female *Pagellus erythrinus* attains 40% at total length of 16 cm. Garcia-Diaz *et al* (2006) reported that individual blacktail comber (*Serranus atricauda*) reached 50% maturity at 19.8 cm total length , while reached 95% at 33.1 cm total length.

In the present study, the peak value of GSI for female *Mugil cephalus* was attained in mid August and continued to early October, and then decreased gradually from November until August, there is one peak for many species during the spawning season as previously recorded by Zaki *et al*. (1995) on *Oblada melanura*; Millan (1999) on *Engraulis encrasicolus*; Hotos *et al*. (2000)

Table 3: ***Fatty acid composition (% total fatty acids) for lipid include in ripe ovary of Mugil cephalus.***

Fatty acids	Percentage (%)
C6:0 (Caproic Acid)	0.14

C8:0 (Caprylic Acid)	0.18
C10:0 (Capric Acid)	0.17
C11:0 (Undecanoic acid)	0.04
C12:0 (Lauric Acid)	0.05
C13:0 (Tridecanoic acid)	0.43
C14:0 (Myristic acid)	1.20
C15:0 (Pentadecanoic acid)	0.10
C16:0 (Palmitic acid)	16.30
C17:0 (Heptadecanoic acid)	0.30
C18:0 (Stearic acid)	3.50
C20:0 (arachidic acid)	0.30
C21:0 (Heneicosanoic acid)	6.60
C23:0 (Tricosanoic acid)	0.20
∑ Saturated	29.50
C14:1(Myristoleic acid) n-5	0.30
C15:1(cis10-pentadecenoic) n-5	0.10
C16:1(Palmitoleic acid) n-7	19.10
C17:1(heptadecenoic acid) n-7	0.50
C18:1(Oleic acid) n-9	17.50
C20:1(eicosenoic acid) n-9	0.70
C22:1(erucic acid) n-9	0.10
∑Mono unsaturated	38.30
C18:2 (Linoleic acid)	14.7
C20:2 (Eicosadienoic acid)	1.40
C20:3 (mead acid)	0.70
C20:4 (Arachidonic acid) ARA	2.40
C22:2 (Docosadienoic acid)	2.30
∑ n-6 HUFAs	21.40
C18:3 (Alpha-linolenic acid)	4.90
C20:5 (Eicosapentaenoic acid) EPA	1.30
C22:6 (Docosahexaenoic acid) DHA	4.70
∑ n-3 HUFAs	6.10
∑ HUFAs*	9.10
∑ Unsaturated	65.70
n-6/n-3	3.50
DHA/EPA	3.50
EPA/DHA	0.30

HUFAs = *Highly unsaturated fatty acids; defined as fatty acids with 20 or more carbon atoms and 2 or more double bonds

on *Liza aurata*; Ergene (2000) on *Liza ramada*; and McDonough *et al*. (2003) and El-Gharabawey *et al*. (2006) on *Mugil cephalus*.

In the present study, analysis of ova diameter revealed that there are six groups of ova in different samples. Fecundity shows a wide range for a given length but in general, there is a difference in number of ova for the same length group; this may be related to environmental factors as indicated by Wroblewski *et al.* (1999). The absolute fecundity of *Mugi cephalus* was highly correlated with fish length and weight, similar result was obtained by many authors including Zaki *et al*. (1995), Allam (1996) and McDonough *et al*., (2003).

The seasonal ovarian cycle in *Mugil cephalus* was divided into six periods as revealed by the histological studies of the ovaries. The immaturation period was characterized by small spherical cells with large nuclei (pre-synaptic oocyte). This period is similar to the pre-maturation period of Zaki and EL-Gharabawy (1991) for *Liza ramada*. In *Mugil cephalus* as the oocyte grows, the number of nucleoli increased, these additional nucleoli are believed to be formed by division or fragmentation of the original nucleoli as described by Assem (2003).

The present results showed that the maturation period was characterized by appearance of isolated follicular epithelial cells, marginal vacuoles appeared in the vacuolization period, and the oocyte wall consisted of zona radiata coated with follicular epithelial layer. The ultrastructure of the vacuolized oocyte wall shows the presence of five different layers. Similar results were indicated by Grant (1990) and York *et al*. (1993). At the yolk deposition period the yolk granules were spread centripetally into the whole central cytoplasm, similar results were recorded by EL-Gharabawy (1996) for *Lithognathus mormyrus* and Assem (2000 & 2003) for *Caranx crysos* and *Pagellus erythrinus*.

Mugil cephalus spawn once along the spawning period, similar results were indicated by Salem *et al*. *(*1994) for *Mugil seheli* by McDonough *et al*. (2003) for *Mugil cephalus* and McDonough and Wenner (2003) for *Liza ramada*.

Takaaki *et al*. (2001) stated that with growth of the oocyte, the zona radiata, particularly zona radiata interna, increase in thickness and is transverse perpendicularly by pore canals containing processes from both granulosa layer (theca and follicular layer) develop microvillar processes; these penetrate the zona radiata to a variable distance depending on the stage of oocyte growth. These ultrastructure changes are believed to increase the surface area for the absorption of substances as indicated by Maksimova (1991).

Lipids are needed as a source of energy and to maintain the structure and function of cell membranes. They also play an important role in buoyancy control in some fishes.

The total lipid content in ripe ovary Mugil *cephalus* was 27.74% dry weight. Abdel-Aziz *et al*. (1993) found that the gonadal lipid content of mature *Rhinobatos rhinobatos* ranged from 1.53 to 15.81% in females.

Assem (2004) stated that seasonal variation in lipid content can be directly related to availability of food. In the present study, many differences were detected in ovary fatty acid concentration; may be related to physiological requirement of fish. Many authors observed the relation between major biochemical components and gonadal maturation of fish (Craige, *et al*. 2000).

Corraze and Kaushik (1999) stated that; fish are characterized by higher proportion of n-3 PUEAs, which had direct implication on fatty acids requirements.

In this study, palmitic acid (16:0) is considering the highest value among the saturated fatty acids, similar results were detected by Şengör *et al*. (2003).

In the present study, major fatty acids in ovary were palmitic (16:0), palmitoleic (C16:1n-7), oleic (C18:1n-9), and linoleic (C18:2n-6) acids. These results agree with who's reported by Şengör *et al*. (2003). They reported that the Major fatty acids of raw and beeswaxed caviar oils were C16:0, C16:1, C16:2, C18:1 and C18:4. Other unsaturated fatty acid contents in the present study, such as eicosadienoic (20:2n-6), ARA

(C20:4n-6), docosadienoic (C22:2n-6), linolenic (C18:3n-3), EPA (C20:5n-3) and DHA (C22.6n-3) acids were significantly high. The essentiality of EPA and DHA for reproduction and larvae growth has been extensively noted for cold water/temperate water species (Sargent *et al*., 1999a, b; Izquierdo *et al*., 2001).

In the present study, ovaries of *Mugil cephalus* had 2.4% ARA, 1.3% EPA, and 4.7% DHA in the total fatty acids, respectively, which supports the result of Groff *et al*. (1995) who reported that, in many animals ARA is an important precursor of prostaglandins which have important regulatory roles in the body including vasdilation, platelet aggregation and perhaps osmoregulation. ARA also is an important constitute of phospholipids including phosphotidylinositol-biphosphate which is a plasma membrane constituent that plays a major role in cellular responses to hormones and monoamine neurotransmitters, thereby controls the opening of membrane calcium channels and also the release of calcium from the intercellular stores as indicted by Gallagher *et al*. (1998).

In the present study, ovaries of *Mugil cephalus* had ARA/EPA/DHA ratios of 2.4:1.3:4.7 for total fatty acids. In wild the ovaries of White Sea bream broodstock showed ARA/EPA/DHA ratios of 0.7:1.0:4.7 for total lipid (Cejas *et al*., 2003). By contrast, as a general characteristic, gonads and eggs of high and temperate latitude species in the northern hemisphere have high levels of EPA and DHA, and consequently most of these species show low ARA/EPA ratios (Takama *et al*., 1994; Bell *et al*., 1996).

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دراسات بيولوجية (هستولوجية , التركيب الدقيق) و بيوكيميائية علي مبيض اسماك البورى *Mugil cephalus* في مياه البحر المتوسط.

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***المعهد القومي لعلوم البحار والمصايد-قايتباي-الإسكندرية.**

تعتبر أسماك العائلة البورية ذات القيمة الاقتصادية العالية حيث أنها تتحمل مدى واسع من الملوحة, وعلى أساس ما سبق كان أتجاه الدراسة فى إطار إجراء دراسات بيولوجية و بيوكيميائية على مناسل الأمهات. وقد تمت الدراسة الحالية في المفرخ البحري بالمعهد القومي لعلوم البحار والمصايد- قايتباى- الإسكندرية.

دل التوزيع الشهري لمراحل النضوج على إن أسماك البوري لها موسم تبويض قصير يمتد من أوائل أغسطس إلى أواخر أثبتت الدراسة أن جميع الإناث الأطوال من41 سم بالغة جنسيا. كذلك حقق دليل المناسل أقصى قيمة له فى شهر سبتمبر و أكتوبر. أتضح من تحليل اقطار البويضات أن هناك نوعين من أقطار البويضات، النوع الأول يتميز بصغر الحجم و الشفافية، بينما النوع الثاني تميز بكبر الحجم و احتوائه على المح. لوحظ وجود علاقة خطية قوية و متنوعة بين الخصوبة المطلقة و كلا من الطول الكلى و الوزن المنزوع الأحشاء.

يمكن تقسيم التغيرات الموسمية فى نسيج المبيض إلى ستة في ضوء الدراسات الهستولوجية كما يلي: 1- مرحلة ما قبل البلوغ؛ تتميز بوجود خلايا صغيرة ذات أنوية كبيرة. 2- مرحلة البلوغ؛ تتميز بوجود أغلفة من خلايا طلائية تحيط بالبويضات. 3- مرحلة تكوين حبيبات الدهون؛ تتميز بوجود قطيرات دهنية بالسيتوبلازم. 4- مرحلة ترسيب حبيبات المح؛ تتميز بوجود حبيبات المح بالسيتوبلازم. 5- مرحلة النضوج الجنسي؛ تتميز بهجرة النواه إلي احد قطبي الخلية 6- مرحلة النضوج و إطلاق الأمشاج؛ تتميز بوجود مساحات بين الخلايا مع وجود خلايا صغيرة.

من مرحلة الفقاعات الدهنية إلى مرحلة النضوج الجنسي باستخدام الميكروسكوب الإلكتونى يمكن تمييز خمسة طبقات مختلفة التركيب وهى كالآتى: الطبقة الخارجية وهى السيكا ثم الخلايا الطلائية ثم الزونا رادياتا الخارجية والداخلية أخيرا الطبقة الداخلية الخامسة وهى كورتيكل ألفيولاى

من التحليل الكيميائي لأمهات البوري لوحظ وجود اختلافات قوية في تركيزات الأحماض الدهنية في المبيض خلال مرحلة النضوج الجنسي.