Full Length Research Paper

Biological, histological and ultra-structural studies of female mullet, *Mugil cephalus*, ovaries collected from different habitats during annual reproductive cycle

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This study investigates some biological characters (oocyte diameter, fecundity, histological and ultra structural features) of female *Mugil cephalus* ovaries collected from three different natural habitats: marine (MW), brackish (BW) and fresh (FW) water. Monthly gonadosomatic index (GSI) values clearly showed that the time period of reproductive activity in female *M. cephalus* from marine and brackish water habitats was from early September to late November. No peak value of GSI in females collected from freshwater was observed throughout the year. Analysis of ovum diameter for *M. cephalus* in the two habitats revealed that, there are small diameter ova (less than 0.3 mm) and large ova (larger than 0.35 mm). The percentage of small ova diameter was 5±1% in marine habitat, while 27±3% for brackish water habitat. The mean oocyte diameters in fresh water fish were less than 350 μm. The oocytes did not develop enough to be differentiated into small and large diameter ova. The total number of ripe ova in marine fish varied between 0.84 ± 0.05 to 4.14 ± 1.01 x10^6 for a total length ranging between 35 and 52 cm, respectively; whereas, the total number of ripe ova in brackish water fish ranged from 0.57 ± 0.14 to 3.81 ± 0.59 x10^6 for the same length groups. There was highly significant correlation ($p>0.01$) between the number and length of ripe ova in 37 and 50 cm length group from the two habitats. Yolky nucleus or Balbiani's body and interstitial epithelial cells are a characteristic feature of oocytes at maturing stage, which is clearly detected in marine water fish with isolated follicular and active organelles. In brackish and fresh water fish ovary, the cytoplasm was compacted without accumulation of active organelles. Ultra structurally vacuolized oocyte wall in marine fish showed the presence of the fifth layer (cortical alveoli) while no cortical alveoli formation was observed in oocyte of brackish or fresh water females. The percentage of atretic oocytes in late vitellogenic ovary of marine water fish was about 2.5%, while in brackish water fish it was about 92±2%. In both brackish and fresh water fishes the initial stage of oocytes atresia degeneration was observed. In conclusion, the comparative study shows that ovary of marine and brackish *M. cephalus* morphologically overlaps from ripening to re-sorption stages. With the histological and fine structure characteristics, it was possible to understand the functional relationship between oocyte size and stage of fish maturation. This knowledge is of huge importance in establishing the reproductive status of the fish which is related to the functional expression of the folliculogenesis in female individuals.

Key words: Ova, *Mugil cephalus*, marine, brackish and fresh water fish.

INTRODUCTION

Mugilids are widely distributed in coastal and brackish waters of all tropical and temperate regions of the world. In many countries, mugilids are targeted by commercial fisheries (Ibanez-Gallardo-Cabello, 2004) and have also

been widely cultured (Lee and Ostrowski, 2001). In Egypt, this species has been used for traditional aquaculture and culture-based fisheries since the late 1920s and is still of major importance today in other Mediterranean

countries and Taiwan Province of China (Saleh and Salem, 2005; Basurco and Lovatelli, 2003). Based on the statistics published by the Food and Agriculture Organization of the United Nations (FAO), the world total catch of mullet in 2004 was about 261,000 tones, representing only 0.3 percent of the world fish catch (FAO, 2004). Most mullet aquaculture activities rely on the use of wild seed, for example Egypt (Saleh, 1991). Commercial hatchery production of mullet seed is carried out in some countries. Induced spawning and production of fry has been achieved on an experimental and semi-commercial basis in the United States of America and Taiwan Province of China. The production of mullet fry on a limited scale for aquaculture has been reported in Italy, Israel and Egypt (Saleh, 2006). It is of vital importance to understand the reproductive biology of this species and start a national program for hatchery production of fry.

Mullets are usually grown in extensive, semi-intensive ponds and netted enclosures in shallow coastal waters. Mullet can be polycultured successfully with many other fish, including common carp, grass carp, silver carp, Nile tilapia and milkfish and can be reared in fresh, brackish and marine waters.

The reproductive biology of Mugilids was studied by many authors (McDonough et al., 2003; Ibanez and Gallardo, 2004; Kendall and Gray, 2008; Assem et al., 2008; Albieri and Araujo 2010). Teleost oocytes as in *Mugil cephalus* are surrounded by two major cell layers as an outer thecal layer and inner granulsa. In the present work, a comparative study was carried out between steroid hormone producing cells (thecal and follicular cells) and cortical alveoli layer in the ovaries of female caught in three different environments. Ovarian follicles have the ability to synthesize estrogen, androgens and corticosteroids. Matsuyama et al. (1991) indicated that the thecal cells during vitellogenesis and oocyte maturation possess organelles, characteristic of steroid-producing cells.

Therefore, this study investigates and clarifies some biological, histological and ultrastructural characters differences of ovaries of *M. cephalus* from three different habitats- marine, brackish and fresh water.

MATERIALS AND METHODS

Fish sampling biometric measurements

Specimens of *Mugil cephalus* were collected three times a month; from May 2012 to January 2013. Four hundred (400) specimens from three areas were studied. The first main natural marine habitat area was Bardawil Lagoon (El-Areesh governorate), the second brackish area was El-Deepa triangle (Port Said governorate) and the third fresh water area was Kafer El-Sheik fish farms (Bohera governorate). *M. cephalus*' total length is from 35 to 52 cm. The

length is well characterized at first maturity (Assem et al., 2008), having total weight of 400 to 1380 g. For each fish, the date of capture, total length to nearest mm, total weight to nearest mg were recorded. The fish was dissected to determine the maturity stage, according to Assem et al. (2008). Ovaries at all stages of maturity were fixed in 10% formal saline solution until used for histological studies. The gonadosomatic index was calculated (ovarian percentage weight to the total weight of the fish). Paired lobes of ovaries were weighted; 0.1 g of ripe and spawning specimens was fixed in 4% neutral formalin. These samples of ovaries were counted for estimating fecundity; also, diameter of all eggs was measured. The total number of ripe ova in the ovary is known as absolute fecundity, whereas relative fecundity is the number of ripe ova per unit weight or length. The egg diameter is divided into fourteen groups; the first six groups (0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 mm) were small, transparent and hexagonal in shape. While, the remaining ova group ranging between 0.35 mm and 0.7 mm in diameter was yolky eggs.

Histological and fine structure examination

Fixed ovaries were washed in 70% ethyl alcohol prior to dehydration. Then they were cleaned and embedded in paraffin wax. Sections from 5-7 µm thick were stained with Eirlich Hematoxylin and eosin. Four small blocks of ovary specimens were fixed overnight in 4% buffered glutaradialdehde and then in 1% osmium tetraoxide for 1 h at room temperature; they were rinsed twice in cacodylate buffer (pH 7.2), dehydrated through a graded ethanol series, cleared in propylene oxide and embedded in polarbed 812 (polaron) epoxyresin. Ultra-thin sections of 1 micron thick were prepared using glass and diamond knives, and stained with uranyl acetate and lead citrate. Sections were examined using transmission electron microscope.

Statistical analysis

Significant differences in GSI and fecundity were tested by Microsoft Excel 2003 one way ANOVA, followed by LSD test. For all the procedures, level of significance recorded was 0.05 and 0.01.

RESULTS

The biological studies

The gonadosomatic index (GSI)

Monthly variation of GSI values is the ovarian percentage weight to the total weight of the fish. In early September, the ovaries started to increase in weight and then GSI value increased gradually. In August and September, the GSI values of brackish and marine water female were increased to reach the peak value in October and November, and then decreased throughout December. Slight increase in GSI values in fresh water female were noticed in August, September and December, but they were still at immature stage. Highly significant correlation

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Table 1. Monthly variation in average gonadosomatic indices values (GSI) of female *Mugil cephalus* at three different habitats, natural marine, brackish and fresh water throughout the period from May 2012 to January 2013.

Month	GSI±SE		
	Marine	**Brackish**	**Fresh**
May-12	0.29±0.12	0.25±0.11	0.18±0.05
Jun	0.31±0.09	0.28±0.02	0.16±0.06
Jul**	0.86±0.06^a	0.55±0.04^b	0.31±0.05^c
Aug	3.96±1.73	1.70±0.76	1.10±o.71
Sep*	6.89±1.43^a	5.91±1.15^a	2.34±0.38^b
Oct**	22.55±2.62^a	24.79±1.53^b	0.25±0.07^b
Nov**	18.4±11.97^a	19.83±2.98^b	0.43±0.07^b
Dec**	0.72±0.20^b	1.98±0.37^a	0.69±0.04^b
Jan. 2013	0.56±0.11	1.11±0.37	0.37±0.17

Values represent mean± (SE), means in same row not shearing the same superscript are ** highly significantly different (p<0.01) and * significant (P<0.05).

was detected in GSI values at p<0.01 throughout July, October, November and December. Furthermore, significant correlation at p<0.05 was noticed in GSI value throughout September as indicated in Table 1.

Fecundity

In the study of fecundity, two terms are generally used: absolute and relative fecundity.

Analysis of fecundity length relationship: Regression equation of marine females is: Fa = 211789.2 TL – 7145156.7; while for brackish water is Fa= 196203.58 TL-6634.885 (where, Fa is the absolute fecundity, and (TL) is total length (Table 2). The correlation coefficient was 0.923 marine and 0.9474 for brackish females. Highly significant correlation (p>0.01) in 37 and 50 cm length group was detected in the two habitats. The equation of relative fecundity for marine was Fr = 3742 TL – 117807.7, where, Fr is the relative fecundity hile, in brackish female was: Fr = 3515 TL – 111635.05. The correlation coefficient was found to be 0.9142 for marine and 0.9615 for brackish water.

Analysis of fecundity- weight relationship: The equation of average absolute fecundity related to each gutted weight is Fa = 3411.506 W – 619539 for marine female, while for brackish is: Fa = 3164.4 W – 677131, where, W is gutted weight in grams. Correlation coefficient recorded was 0.6370 for marine and 0.7090 for brackish female with highly significant correlation in the two habitats at p> 0.01, in all the absolute fecundity for gutted weight groups except for 800 and 1100 g.

Egg diameter determination and spawning

Marine and brackish water: At the beginning of spawning season of *M. cephalus* (early September) (Figure 1a and b), the ovaries of both habitats had large percentage of small diameter ova and small percentage of yolky eggs. In late September (Figure 2a), for marine female, there was small percentage of transparent ova compared to those of brackish water diameter groups (Figure 2b). At the peak of spawning season of *M. cephalus* (October and November), in general all the ovaries of ripe marine female were in ripe process (Figure 3a and b). While ovaries of ripe female (Figure 4a and b) have a large percentage of small diameter.

Fresh water: All ovaries of fresh water females contained small ova diameters only.

Histology and ultrastructure studies of ovaries of female *Mugil cephalus* collected from three different habitats

Pervitellogenic (immaturation) stage

At this stage, the early mother cells or persynaptic in the three habitats (marine, brackish and fresh water) are similar. Ultra structure examination of presynaptic oocyte showed that the nucleus is characterized by dense chromatin material; the presynaptic group of cells was surrounded by large number of active organelles (mitochondria and endoplasmic reticulum) in marine females, whereas in brackish and fresh water females, the mother cells had poor organelles (Figure 5a, b and c).

Early-vitellogenic (maturing) stage

Marine habitat: The maturation period is characterized by the appearance of isolated follicular epithelial cells around the oocyte. The percentage of normal oocytes ranged between 88 and 95%. The normal oocytes reached 85 µm in diameter. The cytoplasm is faintly stained and is characterized by the appearance of bright corpuscle (yolk nucleus) as shown in Figure 6, a and b. Figure 7 is an electron micrograph of two primary oocytes at maturation period, showing the interstitial follicular epithelial layer; the cytoplasm is provided with mitochondria and endoplasmic reticulum.

Brackish habitat: The wall of the ovary thickly varied in diameter between 65 and 80 µm. The percentage of normal oocytes was about 47%. All the abnormal oocytes were atretic (Figure 8a and b), then they shrank gradually till degeneration and resorption. In electron micrograph, the cytoplasm of primary oocyte was compacted without accumulation of active organelles as shown in Figure 8c.

Table 2. Average absolute fecundity, relative fecundity for each length and gutted weight groups for ripe female *Mugil cephalus* of two different habitats (marine and brackishwaters).

| Measurement | Fecundity±SE | | | |
| | Absolute | | Relative | |
	Brackish	Marine	Brackish	Marine
Total length (cm)				
35	574584±147680	841898±56232	16416	24054
37**	777952±47880[a]	912396±24792[b]	21025	24659
40	958484±33629	971098±25305	23962	24277
42	1390366±96765	1311370±158282	33103	31223
44	1991538±351597	1730123±271943	45262	39320
46	2277693±209216	2889315±528795	49515	62811
48	2612319±495065	3077791±40864	54423	64120
50**	2049975±465093[a]	3696710±27667[b]	40999	73934
52	3816669±598870	4146903±101636	73397	79748
Gutted weight (g)				
400**	698964±24768[a]	776719±18374[b]	1747	1941
500**	794073±39247[a]	1858017±88954[b]	1588	3716
600**	1792598±158051[a]	2433067±125630[b]	2987	4055
700**	1908180±138979[a]	958104±10612[b]	2725	1368
800	1266650±130945	971331±11549	1583	1214
900**	1045338±85144[a]	1380922±80223[b]	1161	1534
1000**	2689165±178407[a]	3446815±54696[b]	2689	3446
1100	2864960±128309	2959513±110131	2604	2690
1200**	3685244±115472	4272235±102279	3071	3560
1300**	3685244±109416[a]	4272235±106011[b]	2834	3286

Values represent mean± (SE), means in same row not shearing the same superscript are **highly significantly different ($p<0.01$) and *significant ($P<0.05$).
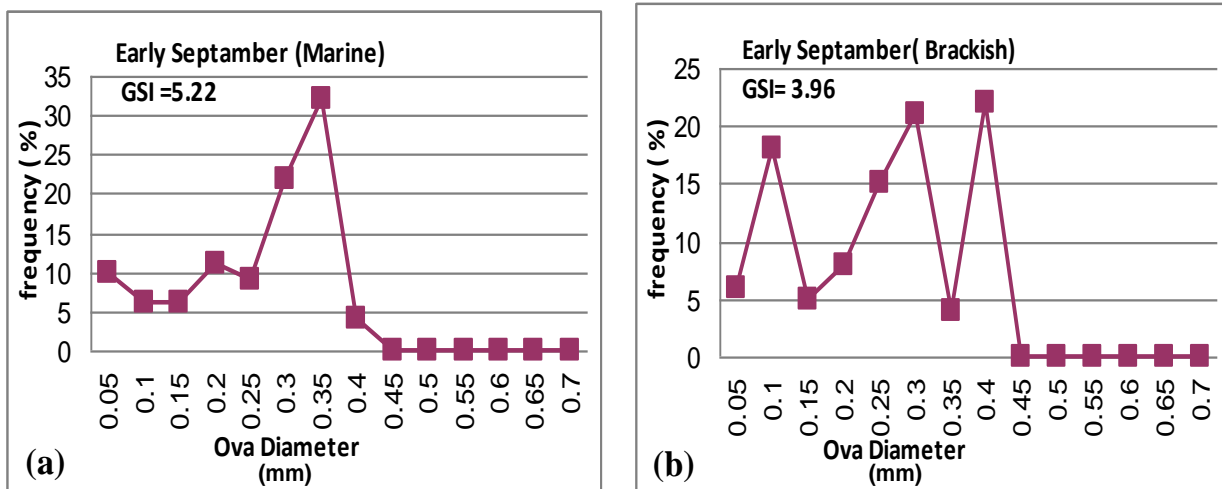

Figure 1. Ova diameter frequency distribution for female *Mugil cephalus* throughout the period from early September, 2013 caught from two different habitats (marine and brackish water).

Fresh habitat: The wall of ovary is moderate in thickness than that of marine and brackish ovaries varied in diameter between 45-50 µm. Also, the wall was characterized by rich innervations of blood capillaries.
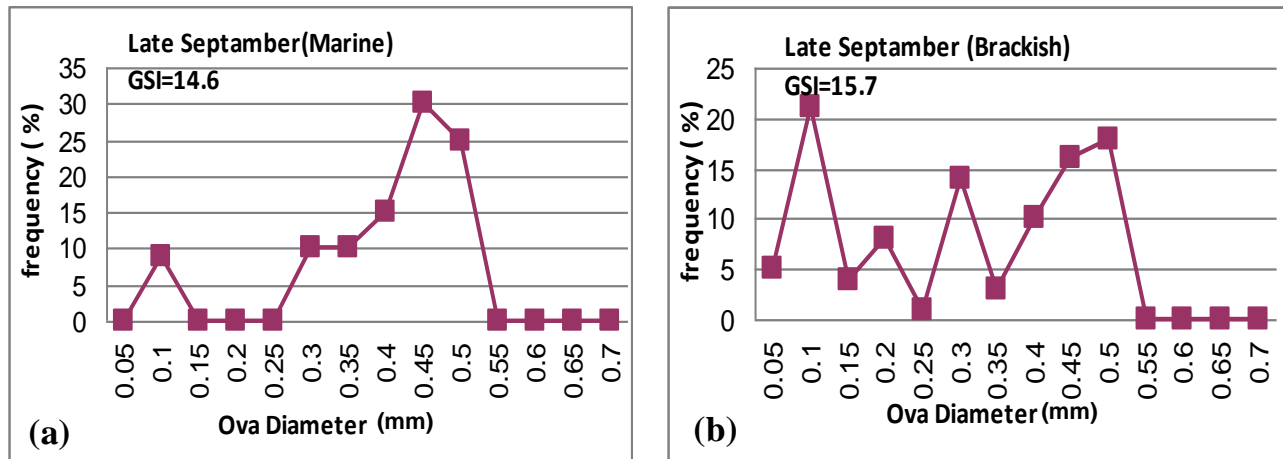
Figure 2. Ova diameter frequency distribution for female *Mugil cephalus* throughout the period from late September, 2013 caught from two different habitats (marine and brackish water).
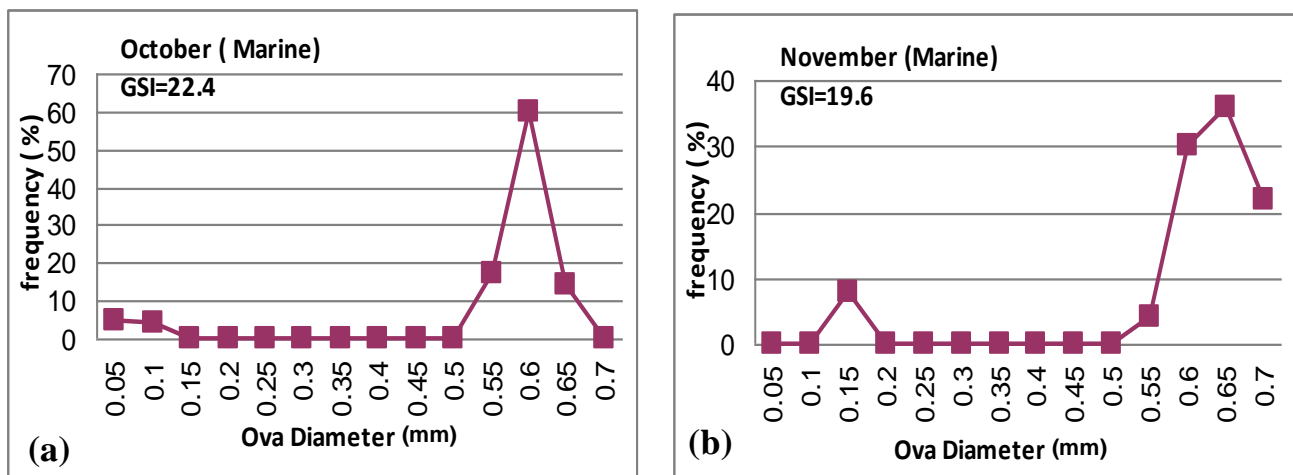

Figure 3. Ova diameter frequency distribution for female *Mugil cephalus* throughout the period from October and November, 2013 caught from marine water.
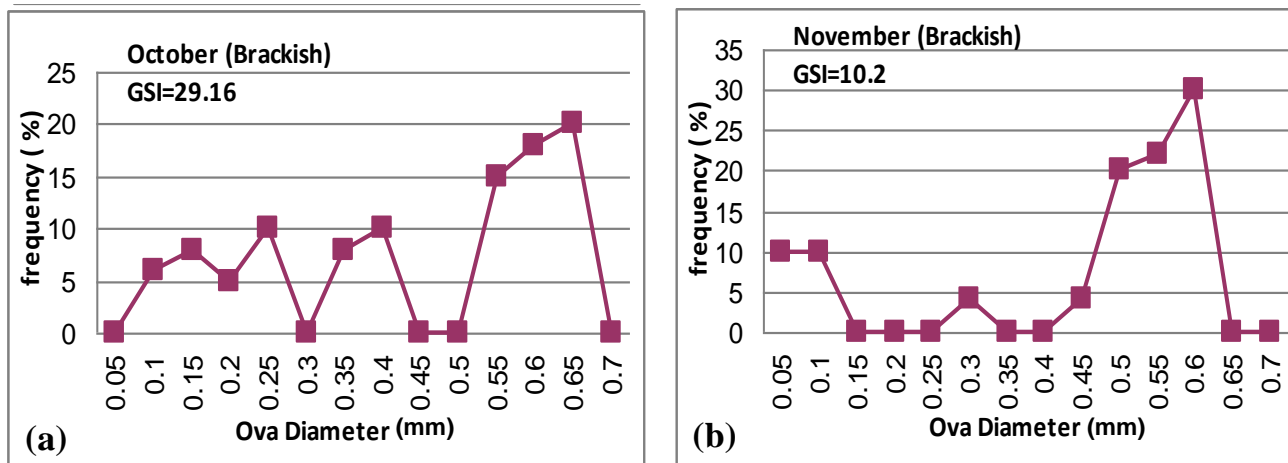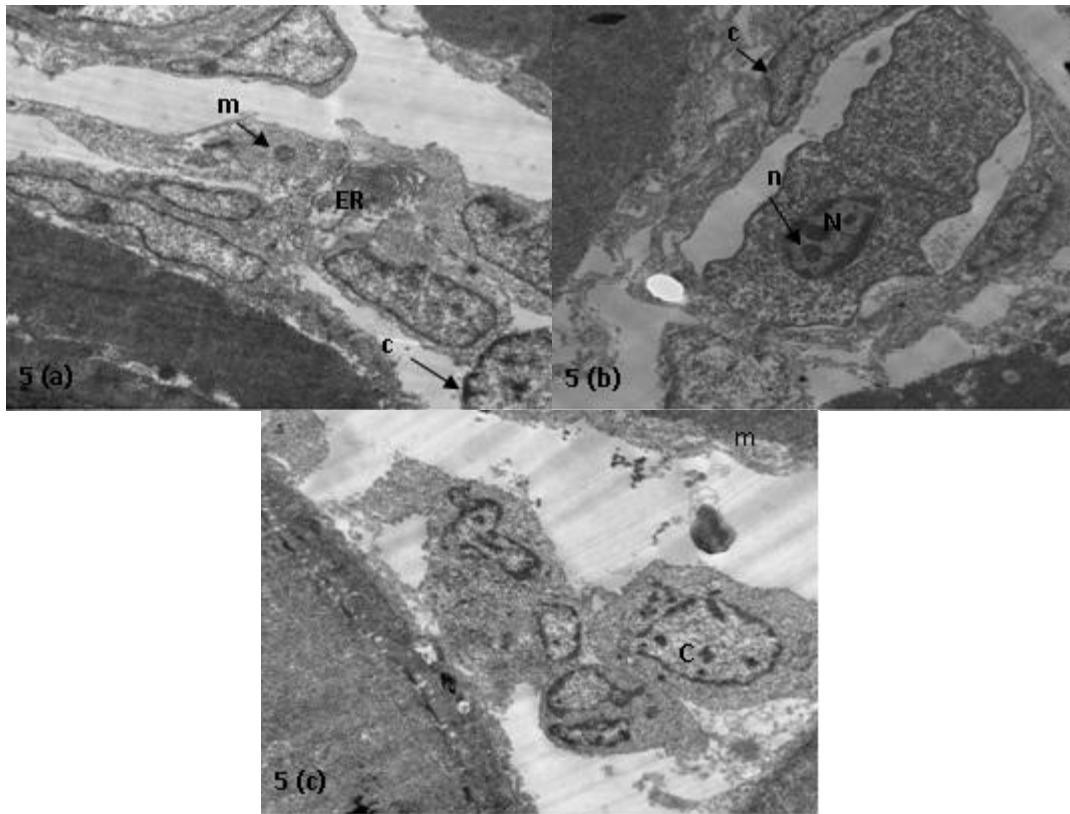

Figure 4. Ova diameter frequency distribution for female *Mugil cephalus* throughout the period from October and November, 2013 caught from brackish water.

Figure 5. Photo-electron-micrography of cross section (cs) in immature ovary *of Mugil cephalus* showing **(a**) group of presynaptic mother cell in marine habitat, (ER) endoplasmic reticulum (m) mitochondria, (c) chromatin material. **(b)** Presynaptic cells in brackish habitat, (N) large nucleus with **(c)** dense chromatin and (n) nucleolus. (5c) Group of presynaptic mother cells in fresh water habitat with minute mitochondria (m) and faint chromatin (c). X 3000 stained with uranyl acetate (UA) and lead citrate (LC).
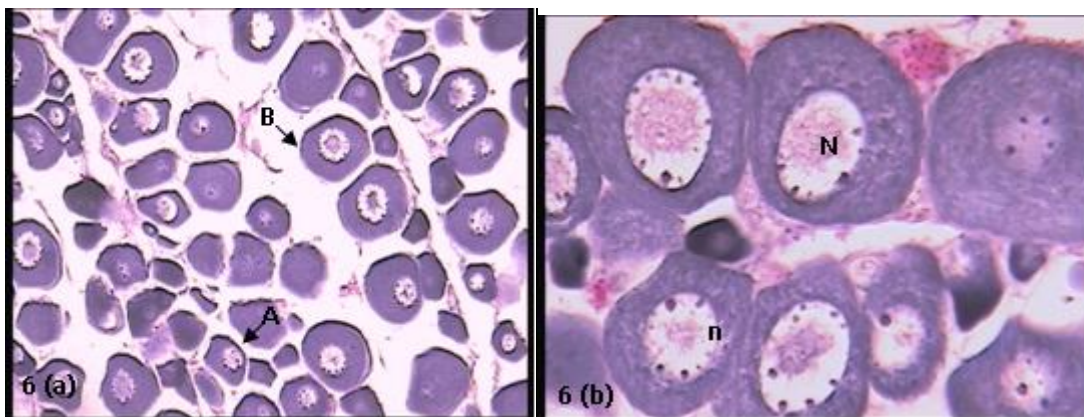
Figure 6. photo-micro and electron graph of CS in maturing ovary of marine habitat showing **(a)** primary (A) and more advanced oocyte (B) X100 Hematoxylin and Eosin (H and E). **(b)** Magnification of (a) showing follicular epithelial layer (FE), nucleus (N), nucleolus (n), yolky nucleus (Yn).

The ovary has about 19% of the total oocytes in normal shape, as shown in Figure 9a and b. The electron micrograph showed that interstitial follicular epithelial cells existed in the primary oocyte wall at maturation
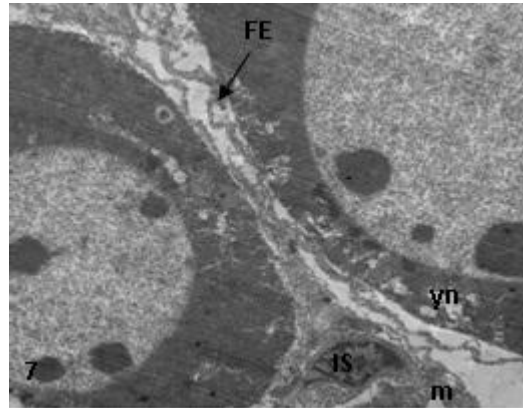
Figure 7. Two cytoplasmic growth cells with isolated follicular epithelial layer (FE) with embedded interstitial cells (IS) and yolky nucleus (Yn) rich in mitochondria (m). X2500.
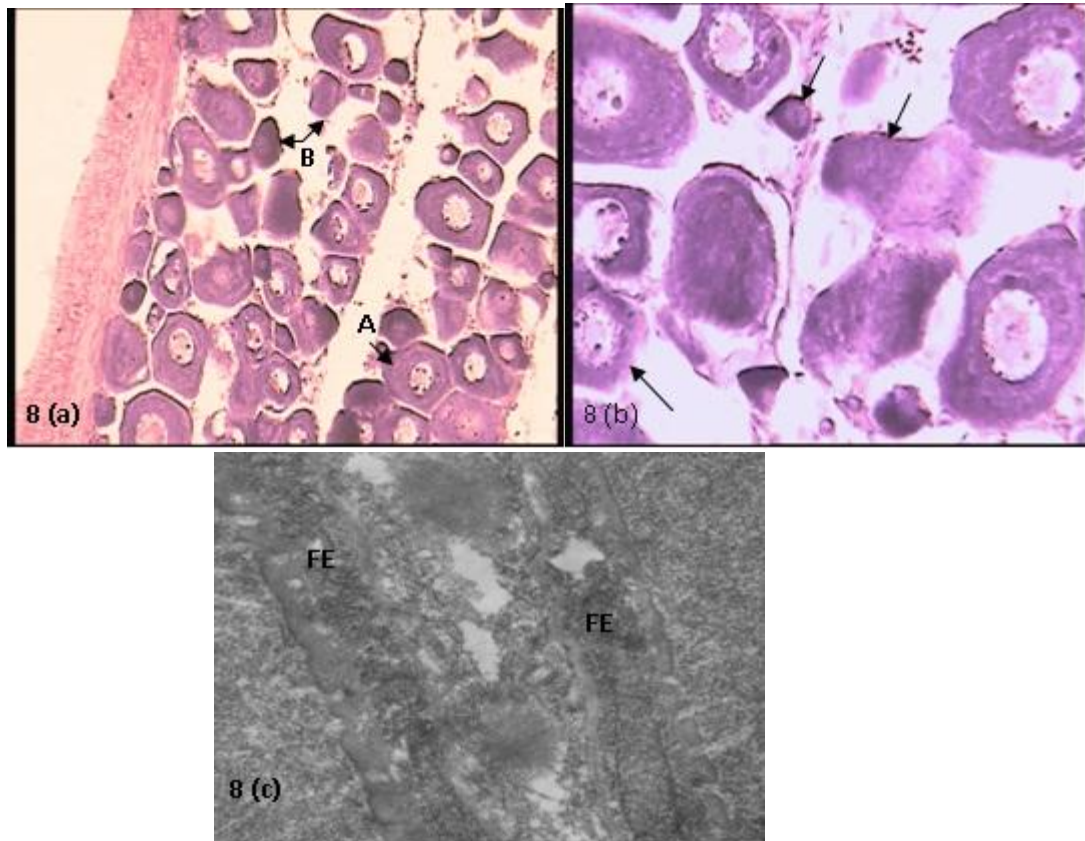

Figure 8. photo-micro and electron graph of CS in maturing brackish ovary showing, **(a)** cytoplasmic growth oocytes in normal (A) and deformed (b) shape (x 100, H and E). **(b)** Magnification of cytoplasmic growth showing shrank degeneration and resorption (arrows) of atretic cells. (X250 H and E). **(c)** Two cytoplasmic growth cells with isolated follicular epithelial layer (FE) notice no any activity in the cytoplasm (X3000).

period. However, the cytoplasm of primary oocyte was   not provided by active organelles (Figure 9c).
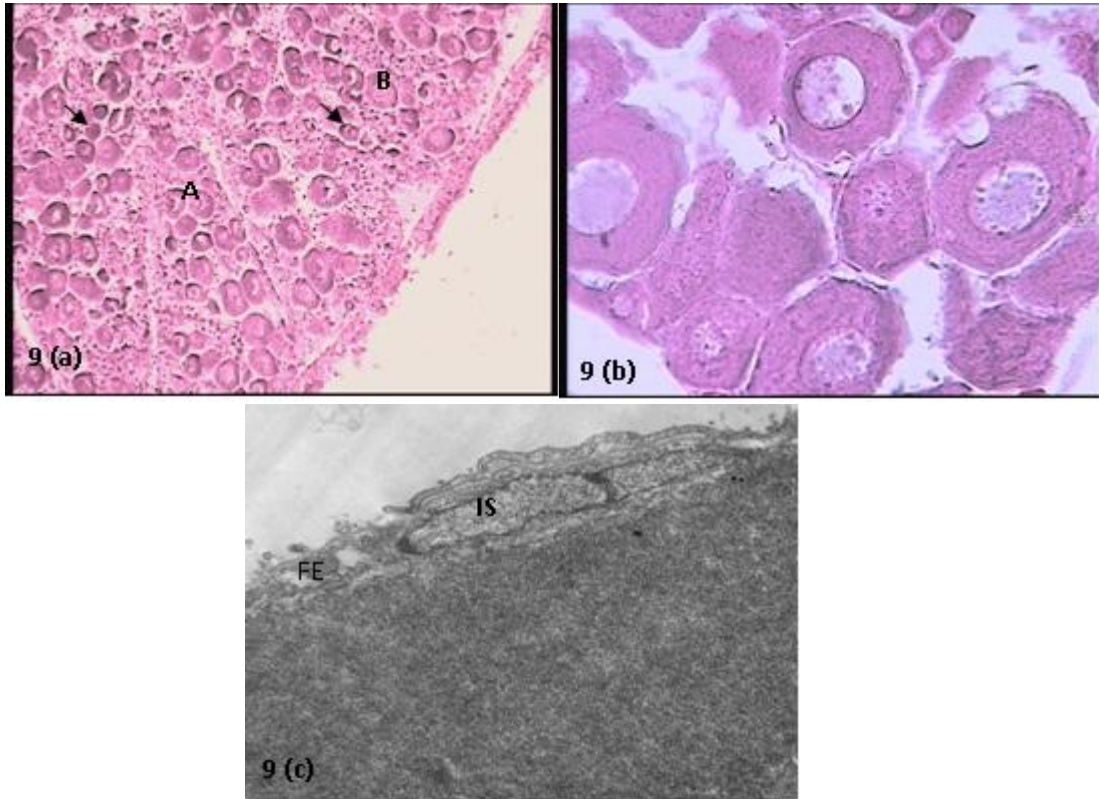
Figure 9. Photo-micro-electron graph of CS in maturing fresh water habitat ovary showing **(a and b)** cytoplasmic oocyte with magnification in normal (A) and deformed (B) shape note degeneration of atretic cells (arrows). **(c)** Cytoplasmic growth cells with interstitial cells (IS) embedded in follicular epithelial layer (FE). Note no any activity in the cytoplasm. X 5000.
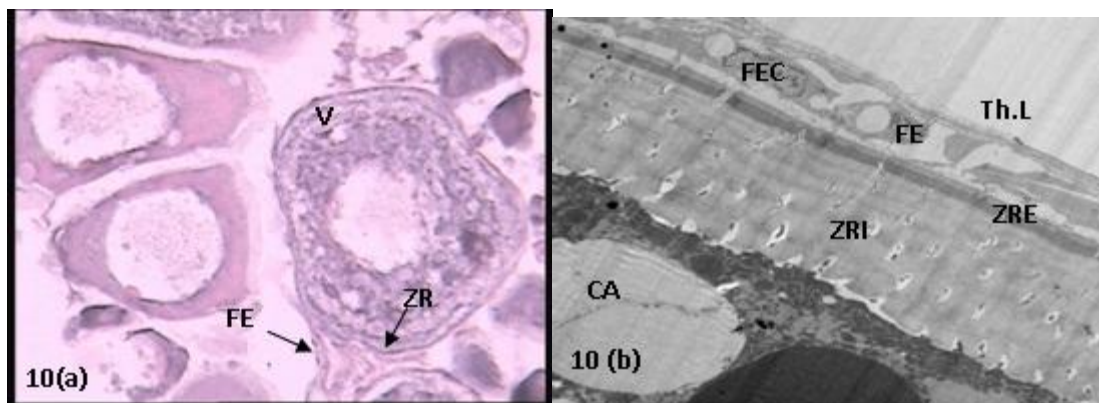

Figure 10. Photo-micro and electron graph of CS in vacuolized ovary of marine habitate showing, (X250), **(a)** one row of vacuoles (V) outer isolated follicular epithelial (FE) and inner zona radiata (ZR), **(b)** theca layer (Th.L), follicular epithelial layer (FE) with follicular epithelial cells (FEC), zona radiata externa (ZE) and zona radiata interna (ZI) and cortical alveoli (CA). X1500.

Mid-vitellogenic (nearly ripe) stage

Marine habitat: Ovaries at this stage have about 2% atretic oocytes. However, normal oocytes are characterized by the appearance of cytoplasmic vacuoles (Figure 10a). The oocyte diameter ranged from 160 to 210 µm. The oocyte wall consisted of zona radiate of about 5 µm in thickness coated with follicular epithelial layer of 6 µm
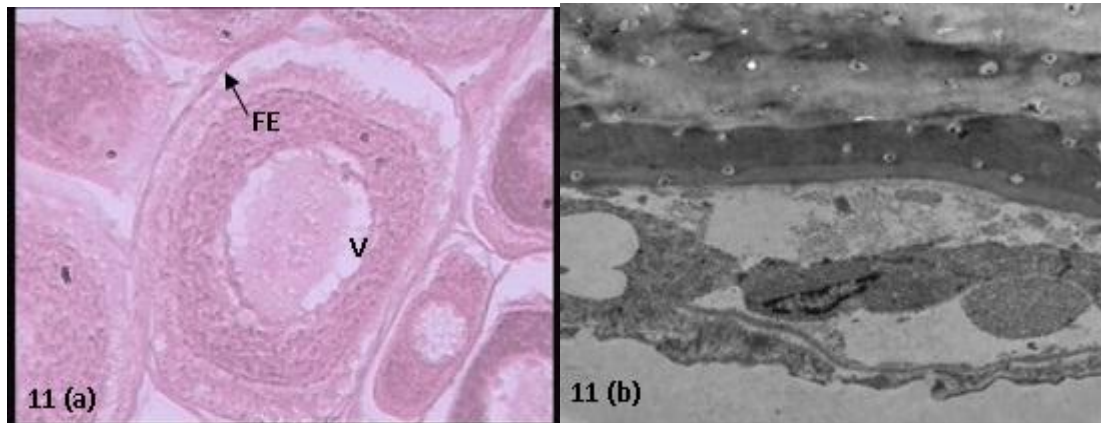
Figure 11. (a) CS in vacuolized ovary of captive brackish water (X 250) showing one row of vacuoles (V), thick follicular epithelial layer (FE) and inner zona radiata (ZR). **(b)** Absence of cortical alveoli layer. X 3000.

thick. The ultra structure of vacuolized oocyte wall showed the presence of five different layers in which the outer most layers is the theca layer. Then the follicular epithelial layer, the third and fourth layers are zona radiate externa and interna. The fifth layer is cortical alveoli (Figure 10b). Large number of mitochondria, ribosomes, endoplasmic reticulum and follicular epithelial cells were detected in the theca layer and follicular layer.

Brackish habitat: Ovaries have about 86% atretic oocytes (Figure 11a). The ultrastructure of vacuolized oocyte wall showed the presence of four layer only, the follicular epithelial layer (with few number of follicular epithelial cells) and absence of cortical alveoli as shown in Figure 11b. No organelles, mitochondria, endoplasmic reticulum and ribosomes were detected in the theca layer.

Fresh habitat: Ovaries have about 83% atretic oocytes, which ranged in diameters from 100 to 280 μm (Figure 12a). Ultrastructure of oocyte wall consists of thin zona radiata (about 3 μm) as shown in Figure 12b. No steroid producing tissue or active organelles or cortical alveoli layer was detected in the oocyte wall.

Late – Vitellogenic (Ripe) stage

Marine habitat: Ovaries of marine female have about 2-3% atretic oocyte. The wall of the normal oocytes consisted of zona radiata (about 11±2 μm in thickness), coated with follicular epithelial layer (about 5±1 μm) as shown in Figure 13a and b. The ultra structure of cell wall at ripe stage revealed the presence of five different layers (Figure 14a). Magnification of the outer most layer of the oocyte indicated the presence of elongated thecal cells

and follicular epithelial cells, with dense chromatin material, mitochondria, ribosomes and endoplasmic reticulum. Zona radiata layers contained pore canals and cortical alveoli contained cortical granules (Figure 14b and c).

Brackish habitat: *M. cephalus* ovaries have about 92±2 abnormal and atretic oocytes. The atretic oocytes have disorganized nucleus and cytoplasm, distorted and hypertrophied follicle, liquified yolk globules, disintegrated and fragmented zona radiate and follicle epithelium layer, engulfed zona radiata and phagocyted yolk, as indicated in Figure 15 a and b.

The ultrastructure of the oocyte wall at this stage showed the presence of outer theca layer without special theca cells followed by basement membrane, follicular epithelial layer "without follicular epithelial cells", then zona radiata externa and zona radiata interna. No cortical alveoli layer was detected in brackish female ova as shown in Figure 15b and c.

Spawning – resorbed and spent stage

Marine habitat: The ovary displayed all the peculiarities of spawning at this period by having a large number of empty follicles due to egg production. Few atretic oocytes were also detected. At the end of spent period, ovaries were characterized by appearance of empty follicles and new generation of small oocytes as shown in Figure 16.

Brackish habitat: The ovary was characterized by presence of different stages of atretic oocytes. The wall of the oocyte consisted of thin follicular epithelial layer of about 3 μm and thick zona radiata of about 14 μm. At the end of atretic oocyte stage, the yolk granules completely resorbed, leading to the formation of lipofusin granules.
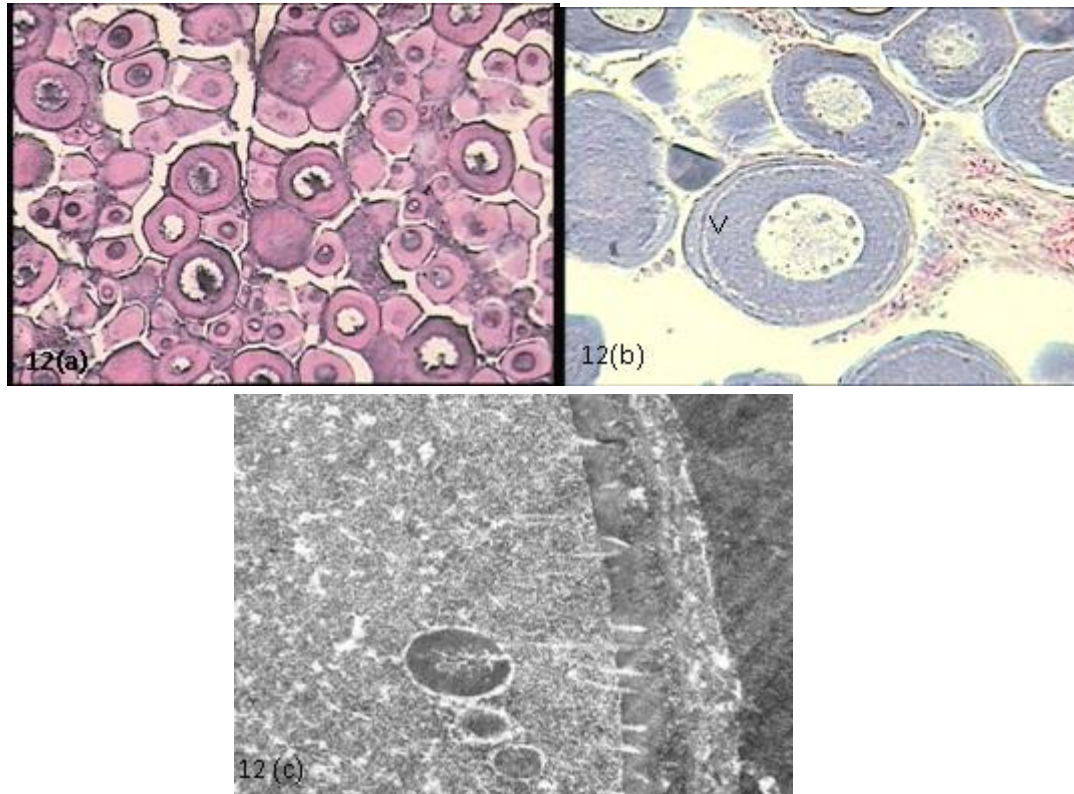
Figure 12. Vacuolized ovary of fresh water habitat ovary showing **(a & b)** X100 and X250 different diameters of cytoplasmic growth. **(c)** Fine structure of fresh water habitat wall of oocyte (X5000), note absence of cortical alveoli, follicular epithelial cells and theca cells.
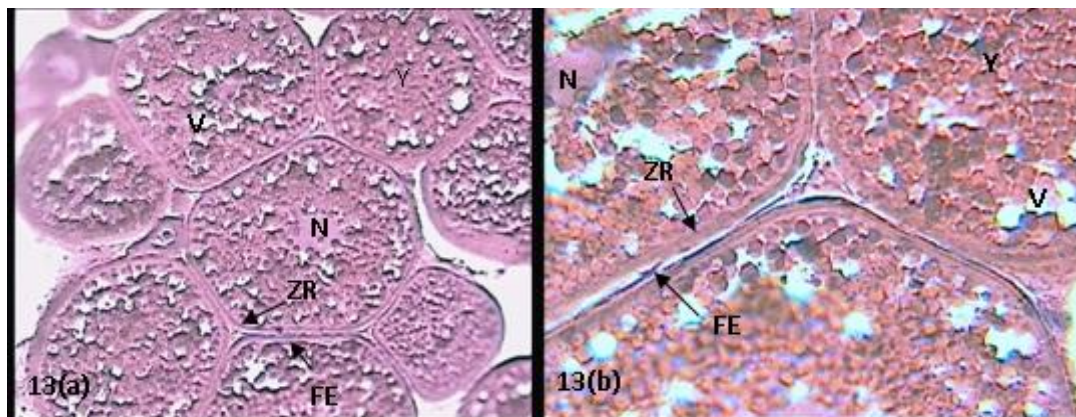

Figure 13. CS in ripe ovary of marine habitat showing ripe ova with nucleus (N), follicular epithelial layer (FE), zona radiata (ZR), yolk granules (Y) and vacuoles (V). (a- X 100 & b- X250).

Convoluted and fragmented basal membrane persisted and then the granulocytes appeared close to the atretic follicles (Figure 17).

DISCUSSION

The biological feature of *M. cephalus* has been well documented, but much less information is available on the biological aspects of reproduction in the wild (Render et al., 1995; McDonough et al., 2003). Oocytes of *M. cephalus* investigated developed on a group as synchronous pattern (McDonough et al., 2003). This is typical for many other mugilids, including Klun Zinger's mullet (*Liza Klun Zingeri)* (Day, 1888; Abou Seedo and Dadzie, 2004), large scale mullet (*Liza macrolepis)*
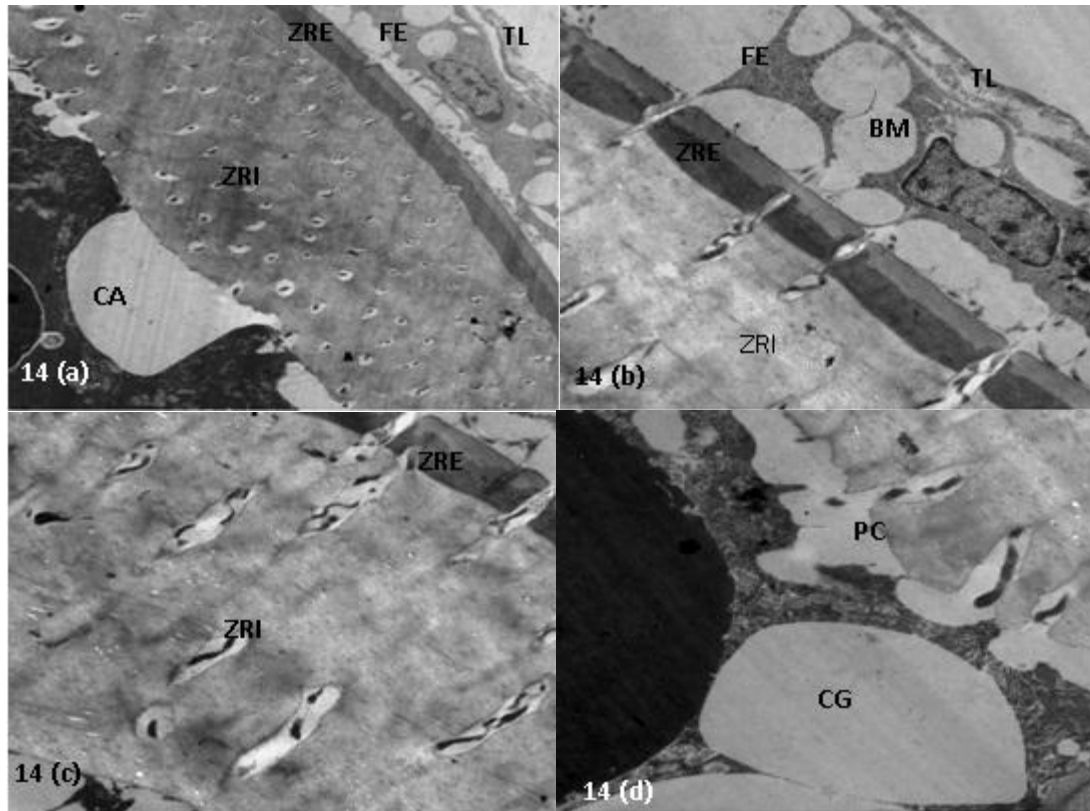
Figure 14a, b, c and d. Photo-electron graph of wall of marine oocyte, which consisted of outermost theca layer (TL) basement membrane (bm), follicular epithelial layer (FE) with follicular epithelial cells (FC), zona radiata externa (ZRE), zona radiata interna (ZRI) with pore canal (PC), then the fifth layer the cortical alveoli (CA) with cortical granules (CG).

(Smith, 1846; Chen et al., 1999), green back mullet (*Liza subviridis*) (Valenciennes, 1836; Chan and Chua, 1980) and striped mullet (*Mugil platanus)* (Gunther, 1880; Romagosa et al., 1988).

Due to the world wide distribution of *M. cephalus,* some comparison with other areas is possible. The GSI data for *M. cephalus* from Mediterranean Sea varied between 0.39 minimally and 27.22 maximally (Mourad, 2009). In estuaries in South Carolina (USA), the GSI for fecund fish ranged from 7.7 to 27.7 (McDonough et al., 2003). In another study on Egyptian gray mullet in Lake Temsah female, GSI observed was 12.4 (El-Halfawy et al., 2007). Significantly higher GSI values were also reported for Turkish waters. Ergene (2000) reported a value of 16.67 for grey mullet females. The highest GSI value, close to 40, was obtained for *M. cephalus* in the waters of North-west Gulf of Mexico (Ibanez and Gallardo Cabello, 2004). In contrast, in the waters of Korea the highest female GSI of only 5.32 was found at the peak of the reproductive season (Kim et al., 2004). These spatial differences in GSI among the same species as already discussed for *M. cephalus* could be caused by differences in food resources, living temperature or the evolutionary adaptation of different population to the specific ecological properties

of specific ecosystems.

In present study, the analysis of ova diameter for *M. cephalus* marine, brackish and freshwater habitat revealed that, there are small diameter ova (less than 0.3 mm) and large ova over 0.35. All oocytes in the ovary of female in marine habitat were in vitellogenic growth phase, the oocyte size coincides with the highest GSI values and the females clearly migrate to open waters for spawning. In parallel with the growth of oocytes that will be released, the ongoing recruitment of new oocytes is apparent in the gonads. This new batch of oocytes grows during the next season as indicated by Bartulović et al. (2011). In the present study, the oocyte diameter did not change with fish size, fecundity or GSI as reported by Albieri et al. (2010) who found that oocyte diameter did not change with fish size. Also in the present study, all ovaries of fresh water female used throughout the year were not used for fecundity or oocyte diameter count. All samples had less-developed ovaries; with low GSI value and mean oocyte diameters less than 350 μm. the oocytes did not develop enough to be separable from the smaller oocytes.

The absolute and relative fecundity in marine habitat is larger than those of brackish habitat. There is a
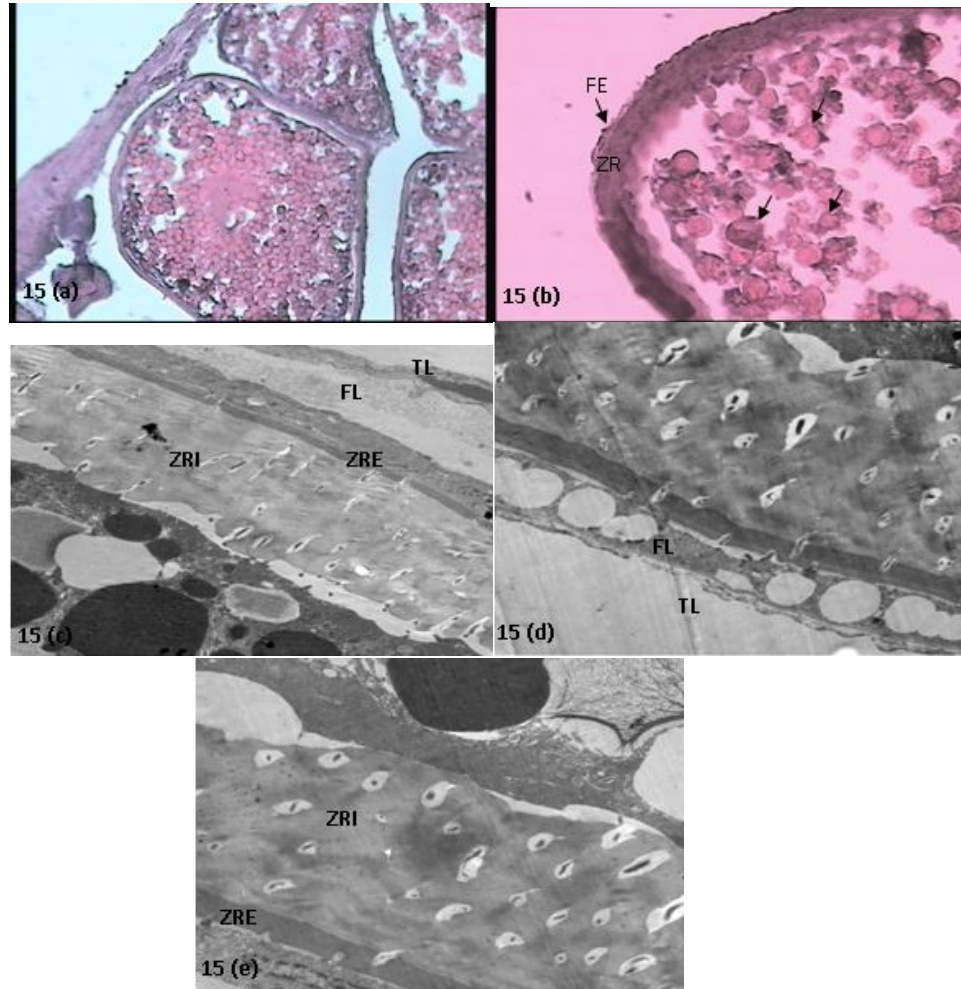
Figure 15. CS in ripe oocyte of brackish habitat **(a)** X100 normal oocyte **(b)** atretic oocyte characterized by hypertrophy of follicle layer and zona radiata (ZR) with liquification of the yolky globules (arrows).   **c, d and e.** Electron- graph of the wall of brackish habitat oocyte; (c) hypertrophy of theca layer (TL) and follicular layer (FL) without theca cells or follicle cells, then zona radiata externa (ZRE) and interna (ZRI). X1500. (d & e) Magnification of outer theca (TL) and follicle layer (FL) X2500.
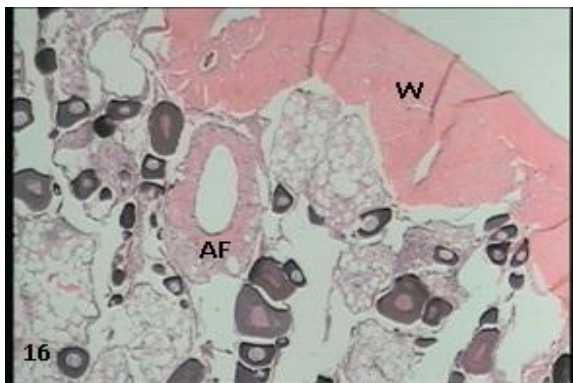

Figure 16. Light micro graph of CS in early spent ovary at marine habitat showing theca wall (W) of ovary new generation of cytoplasmic growth (arrows), atretic follicle (AF). X100.

relationship between the number of oocytes and their diameters. McDonough et al. (2003) demonstrate that there is an inverse relationship between oocyte density and oocyte diameter. Fecundity in *M. cephalus* highly correlated with length and weight. Fecundity may vary due to different adaptations to environmental habitats (Witthames et al., 1995). In female gametogenesis, the young meiotic oocyte originates from the oogonia, primary oocyte growth giving rise to the previtellogenic oocyte, secondary growth or vitellogenesis, and the formation of the cortical alveoli and yolk granules (Selman and Wallace, 1989). In *M. cephalus*, oogonia or presynaptic cells are the smallest germ cells found in the gonads, as in other Teleost species, showing a common morphologic pattern (Grier, 2000). In the present study, the fine structure of presynaptic group of cell in marine habitat was surrounded by large number of active
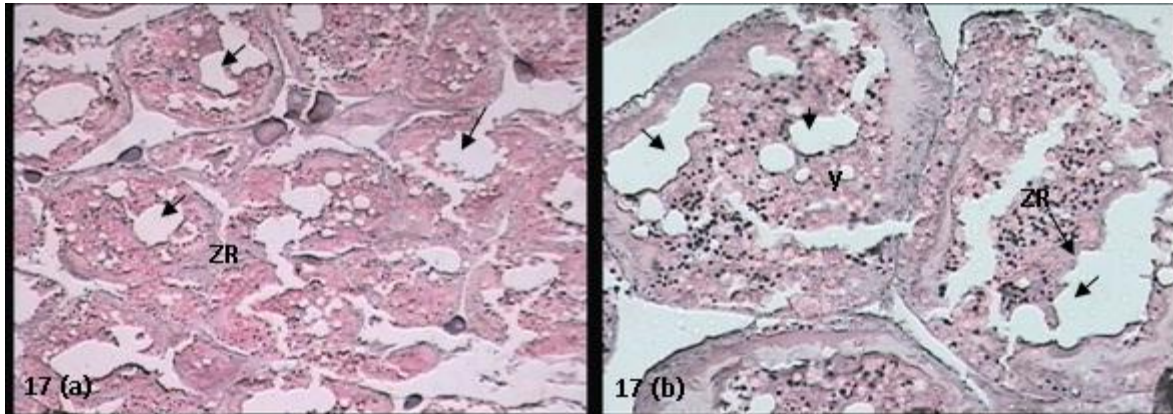
Figure 17. CS in brackish habitat resorbed ovary showing advanced atresia, liquification of yolk (Y), convoluted zona radiata (ZR), vacuolated area in the ooplasm (arrows) during the phagocytosis process. (a - X100 and b-X250).

organelles (mitochondria and endoplasmic reticulum), whereas in brackish and fresh water habitat, the presynaptic cells were poor in organelles. The presence of these cells in nests with the ovigerous lamellae also occurs in other species (Guimaraes and Quagi Gorassiotto, 2001; Spadella et al., 2005).

Oogonia can be found all through life and continue mitotic division maintaining permanent oogonia production (Grier, 2000). In present study, yolky nucleus or Balbiani's body and interstitial epithelial cells are characteristic of oocytes at maturing stage, which was clearly detected in marine habitat with isolated follicular and active organelles. In brackish and fresh habitat ovary, the cytoplasm was compacted without accumulation of active organelles.

The fine structure of atresia occurred at early vitellogenic maturing stage of female from marine habitat. Miranda et al. (1999) support the present result that revealed the changes in the ooplasm including the disintegration of the cytoplasm organelles. The annulated lamellae lost their organization, thus showing a disintegration process in the atretic oocytes of *L. reinhardti.* Selman and Wallace (1989) suggested that the Balbiani's body is involved in the intense organelle production which is frequent during this development stage of the oocyte. Other authors say that this structure is of great importance in vitellogenesis (Dettlaf and Vassetzky, 1988). All abnormal oocyte was detected in follicular atresia. Atresia is more frequent in vitellogenic oocytes, although in previtellogenic oocytes as observed in brackish ovaries in present study as well as by Rizzo and Bazzoli (1995). The first signs of atresia observed in *M. cephalus* were the disintegration in the oocyte nucleus, followed by the fragmentation and hypertrophy of the follicle cells, as also described by Mylonas et al. (1997). Miranda et al. (1999) described two types of oocyte nuclear disintegration. In the first type, in the intracellular nuclear disintegrations, the nuclear content

disappears rapidly in the ooplasm following lysis of the nuclear envelope. In the second type, the oocyte nucleus together with part of the ooplasm is eliminated through an opening in the follicle wall into the ovarian lumen, where it breaks up into various fragments. These two types of atresia were observed in brackish and fresh water ovary.

The present results reveal that there was no cortical alveoli formation in oocyte of captive brackish or fresh water females. Grant (1990) characterized the vacuolization stage by the cortical alveoli layer formation. Spadella et al. (2005) indicated that: alveoli formation starts with secondary growth and at the end of this stage these vesicles are in their highest concentration in the same region. According to Patino and Sullivan (2002) and Arocha (2002), cortical alveoli are necessary during fertilization because it releases its contents into the perivitelline space, to be structurally and functionally transformed to vitelline envelope, which blocks polyspermia. In agreement with the present results, Assem 2003, indicating that the follicle cell layer generally consists of inner sub-layers which are separated by a basement membrane. In present study, the percentage of atretic oocytes in late vitellogenic ovary of marine water was about 2.5%, while in brackish water it was about 92± 2%.

The process that controls atresia in fish ovary is not well known. It is very difficult to establish the time necessary for oocytes absorption under natural conditions, but under captivity, it can be estimated (Romagosa et al., 1988). In the initial stage of atresia in *M. cephalus* degeneration in the present studies was observed; it was characterized by necrosis such as dissolution and disappearance of the nucleus and changes in the organelles, suggesting that the death of the oocyte could be involved in this process. Some studies have show apoptosis, a type of physiological cell death. Miranda et al. (1999) indicate occurrence of apoptotic cell death during regression of the atretic follicles in two teleost

species. The resorbed ovary in brackish habitat of *M. cephalus* was characterized by large number of blood cells; Ferreira (1993) mentioned the presence of blood cells derived from the theca in atretic fish oocytes which are involved in their resorption. According to Palmer et al. (1995), the cell derived from the ovarian stroma and /or the theca may act together with the follicle cells in the resorption of the atretic follicles.

Ultrastructure of vitellogenic oocyte in marine habitat showed the presence of five layers: the outer most theca layer which included special theca cell followed by basement membrane, follicular epithelial layer including follicular epithelial cells then third and fourth layer zona radita externa and internal, the cortical alveoli. Ultrastructure of vitellogenic oocyte in brackish habitat was characterized by absence of special theca cells, follicular epithelial cells and cortical alveoli layer. Dettlaf and Vassetzky, 1988 indicated that follicle and thecal cells could be involved in r RNA synthesis for the oocyte and may participate in vitellogenesis and hormone secretion. Unal et al. (2005) revealed that special theca cells possess specific organelles, which are characteristic of steroid-producing cells and suggest that during vitellogenesis the special theca cells are the sites of steroid synthesis in *Calcalburnus trichi* ovary.

In conclusion, the morphological comparative study between ovary of marine and brackish water *M. cephalus* showed an overlap between ripening and resorption stages; it was possible to understand the functional relationship between oocyte size, oocyte frequency and stage of maturity due to histological and fine structure characteristics. This knowledge is of highly importance in understanding the reproductive status of the fish which is related to the oogenesis in the Mugillidae.

Conflict of interests

The author(s) did not declare any conflict of interest.

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