

Full Length Research Paper

The reproductive biology and the histological and ultrastructural characteristics in ovaries of the female gadidae fish *Merluccius merluccius* from the Egyptian Mediterranean water

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Monthly analysis of the maturity stages distribution revealed that the gadidae fish *Merluccius merluccius* has a long spawning period extending from early January to early June. All the females over 34.4 cm in body length are mature. The peak value of gonadosomatic index (GSI) was attained in January and continued to May and thereafter decreased gradually from June to August. The analysis of ova diameter revealed that there are eight ova groups in each ripe and spawning ovary; the first four groups are small and transparent and the remaining four groups are yolky. Fecundity shows a wide range for a given length, the absolute fecundity has a linear relationship with length groups and gutted weight. Relative fecundity ranged from 743 to 1699 egg per 1 cm and varied from 92 to 148 eggs per 1 g. The general pattern of the histological development of the ovaries includes six periods: 1. Immaturity period characterized by "small spherical cells each with a large nucleus". 2. The maturation period characterized by "appearance of isolated follicular epithelial cells around the oocyte and the formation of yolk nuclei". 3. Vacuolization period characterized by appearance of "marginal vacuoles; the oocyte wall consists of zona radiata coated with a follicular epithelial layer". 4. The yolk deposition period characterized by "the presence of yolk granules in the cytoplasm". 5. Ripening period characterized by "migration of the nucleus to the animal pole". 6. Spawning-spent period characterized by presence of "empty follicles, different stages of cytoplasmic growth". The ultrastructure of the vacuolized and ripe oocyte wall showing the presence of five different layers, in which the outer most layers, is theca layer; the second layer is the follicular epithelial layer and then the third and fourth layers are zona radiata externa and zona radiata interna, respectively. The fifth layer is known as "cortical alveoli".

Key words: *Merluccius merluccius*, reproductive biology, oogenesis- histology, ultrastructure.

INTRODUCTION

Merluccius merluccius is considered as a common and commercially important gadidae fish in Mediterranean Sea. Murua and Motos (2004) studied reproductive biology and histological examination of European hake (*Merluccius merluccius*) in the Bay of Biscay. Gonadal maturation and oocyte development for *Merluccius hubbsi* have been analyzed by Louge (1996). Muru et al. (2004) studied the temporal and spatial variation in batch fecundity of the European hake, *M. merluccius* and indicated that this species have protracted spawning

period, with individuals in spawning states all year around, but with a well-defined spawning peak from January to April. The reproductive biology of other species were studied by many authors: EL-Gharabawy (1996) for *Lithognathus mormyrus*; Zaki et al. (1998) for *Sparus aurata*; Assem (2000 and 2003) for *Caranx crysos* and *Pagellus erythrinus*; Honji et al. (2006) for *Merluccius hubbsi* and Garcia-Diaz et al. (2006) for *Serranus atricauda*. Oogenesis has been studied in many teleost fish and illustrated by several authors. This included Portela et al. (1994) for *Loligo*, *Illex argentinus* and *Merluccius hubbsi*; Louge (1996) and Honji et al. (2006) for *Merluccius hubbsi*. Ultrastructural studies have been done on many teleost fishes by a number of authors

Abbreviations: GSI, Gonadosomatic index; EM, electron microscope.

as *Centropomus undecimalis* by Grier (2000); *M. hubbsi* by Cornejo (1998) and Honji (2006); *Caranx crysos* and *Pagellus erythrinus* by Assem (2000 and 2003). This study has sought to characterize the reproductive biology, histology and electron microscopy of the ovary in female *M. merluccius* in the Mediterranean Sea.

MATERIALS AND METHODS

Specimens of the fish *M. merluccius* were collected three times, a month intervals, during the period from September 2005 to October 2007. 320 specimens from trammel and purse seine catch of the Egyptian Mediterranean Sea landed at Alexandria coast ranging in total length from 25 to 43 cm and varied in total weight from 110 to 629 g. For each fish, the date of capture, total length to nearest mm, total weight and gutted weight to nearest gram were recorded. The fish was dissected to determine sex and maturity stages in agreement with gonadosomatic index were computed as a percentage weight of the ovary to the total weight of the fish. The paired ovaries were weighed to the nearest milligram. 0.1 g of each ripe and spawning ovary was preserved in 4% neutral formalin, these samples of ovaries were counted for estimating fecundity and the diameter of all the eggs was measured. The egg diameters was divided into eight groups: the first four (0.08, 0.16, 0.24 and 0.32) are small, transparent and hexagonal in shape while the remaining ova ranging between 0.4 and 0.64 mm in diameter are yolky. The fixed ovary were washed in 70% ethyl alcohol for two days prior dehydration, then cleared and embedded in paraffin wax. Sections of 3 - 6 μ m thick were stained with Eirlich hematoxylin. Four small blocks (2 x 2 mm) of ovary specimens were fixed overnight at 4°C in 4% buffered glutaraldehyde and then in 1% osmium tetroxide for one hour at room temperature, rinsed twice in cacodylate buffer, dehydrated through a graded ethanol series, cleared in propylene oxide and embedded in polarbed 812 (polaron) epoxy resin. Ultra-thin sections of one micron thick were prepared using glass and diamond knives and stained with uranyl acetate and lead citrate. Sections were examined using a transmission electron microscope.

RESULTS

The Biological Studies

The maturity stages

In the present study, a scale for maturity stages was given, taking into account the scales of both Zaki et al. (1995) and Assem (2003) as follows:

- I. The immature stage: Ovaries are almost cylindrical with two tapering ends, the length of the ovary is almost one third of the body cavity and is present along the whole year.
- II. The maturation stage: Ovaries are increased in size, pinkish in colour and occupy nearly half of the body cavity. This stage is detected during the whole year.
- III. The nearly ripe stage: The nearly ripe ovaries are yellowish in colour; their size reaches about two thirds of the body cavity. The eggs are distinguishable with the naked eye. This stage is detected from late November to early April.

IV. The ripe stage: At this stage the ovaries show the maximum development in thickness and width and occupy the entire length of the body cavity. Ovaries are orange yellow in colour. The belly of the female seems swollen and eggs can be released out by a slight pressing on the belly. This stage is detected throughout four months of the year from late January to early April.

V. The spawning stage: Spawning takes place at intervals. The discharge of a considerable amount of ripe ova, during the course of spawning causes a decrease in the weight of the ovary, after which the ovaries increase again in weight but such increase, is less than before. This stage is detected from early April until late June.

VI. The spent stage: The ovaries are severely shrunken, flaccid, collapsed and reddish yellow in colour. They are highly vascularized and much reduced in size. Ovaries at this stage have a large number of surface blood vessels. This stage is detected from late May and continues to August.

Monthly distribution of maturity stages

Monthly variation of the maturity stages in female *M. merluccius* throughout the period from September 2005 to October 2007 is shown in Figure 1. The immature and maturing females are present during the whole year and fluctuates from one month to another to reach a maximum percentage in September (44%) for immature and (58%) in October for mature female. The period of ripening starts at late January with a peak value attained in February and March (54 and 61%, respectively). In April and May the period of spawning reaches its maximum percentage (57.1 and 50%, respectively).

The length at first sexual maturity

The percentage distribution of immature and mature female for each length group was used to determine the size at the onset of sexual maturity. Figure 2 shows that female *M. merlucciu* smaller than 30.5 cm in length are all immature fish. The mature individuals appear with a percentage 7.7% at length of 30.5 cm. The percentage of mature female increase to 50% at length of 32.5 cm. All females larger than 34.4 cm in length are mature.

The gonadosomatic index (GSI)

The monthly variations of the GSI values are shown in Figure 3. In November, the ovaries started to increase in weight and GSI increased gradually with an average which varied between 0.91 ± 1.03 (in November) and 1.53 ± 1.23 (in December) during the pre-spawning period. The GSI reached to peak value in February (7.71 ± 4.77) and March (8.11 ± 2.4). The spawning period continued to June (3.44 ± 3.3). From early July the GSI

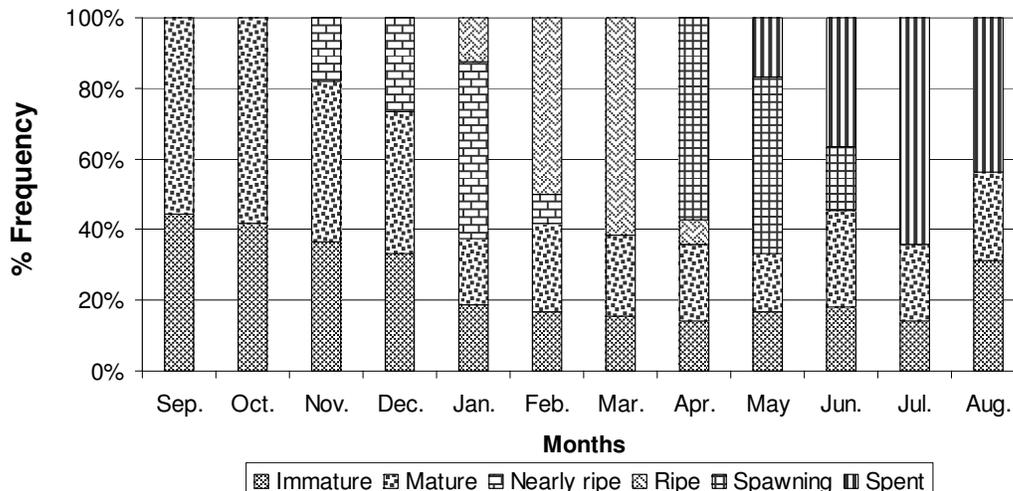


Figure 1. Monthly distribution of different maturity stages in female *M. merluccius* throughout the period from September 2005 to October 2007.

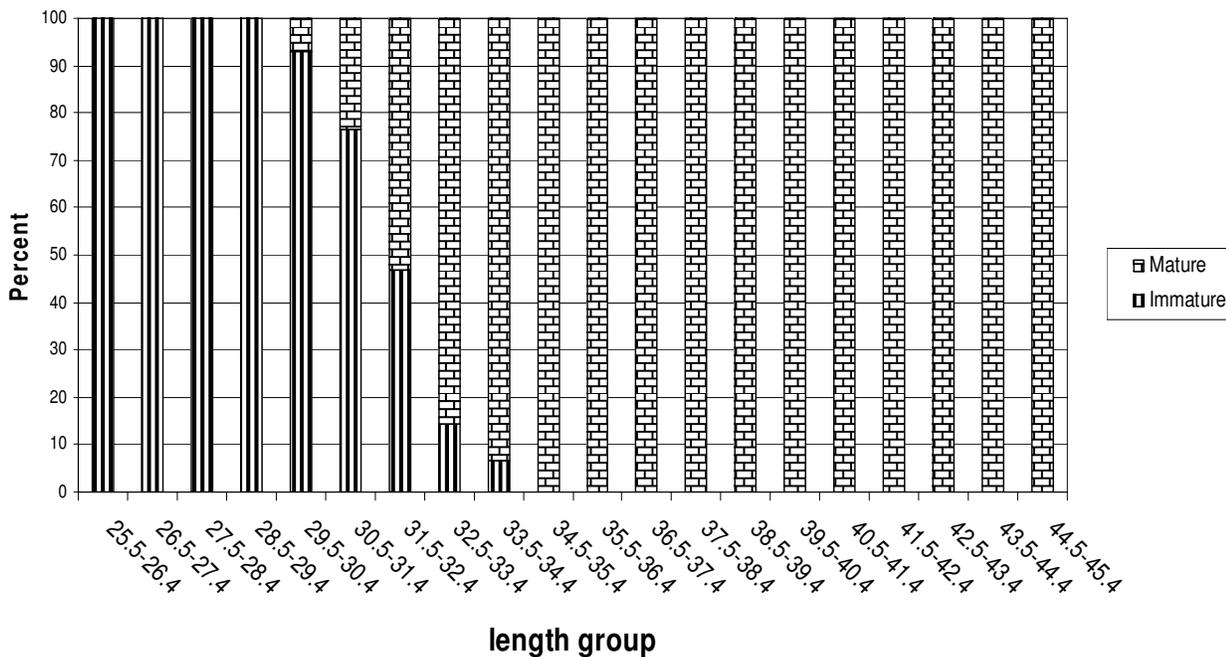


Figure 2. The percentage distribution of mature and immature female individuals of *M. merluccius* throughout the period from September 2005 to October 2007.

started to decrease from 0.48 ± 0.06 in July and continued until August (0.27 ± 0.03) during the spent period. The minimum GSI values were recorded in August and September (0.27 ± 0.03 and 0.39 ± 0.01 , respectively).

Egg diameter and spawning

At late January (Figure 4a), the GSI value is 4.1; the

ovary of this fish have small ova whose count varies between 5 and 20% and a number of yolky eggs ranging from zero to 31%. At February and March (Figure 4b, c and d), the GSI value varies between 7.22 and 9.57; the ovaries of these fish have transparent ova whose count varies between 1 and 22% and a number of yolky eggs count between 5 and 25%. At April (Figure 5a and b), the GSI value varies between 6.1 and 7.3; the ovaries of these fish have transparent ova whose count varies between 4 and 20% and a number of yolky eggs vary

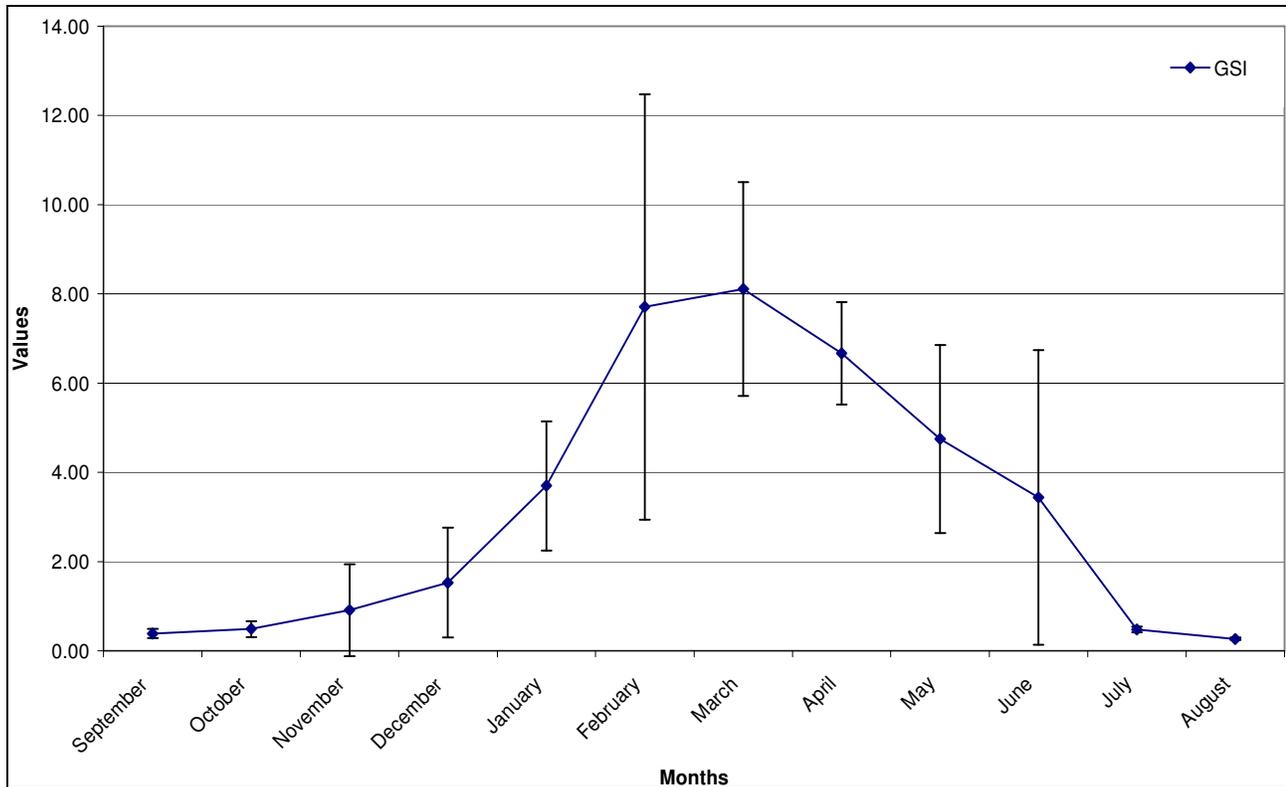


Figure 3. Monthly variation of gonadosomatic index (GSI) in female *M. merluccius* throughout the period from September 2005 to October 2007.

between 11 and 23%. At May (Figure 5c and d), the GSI value varies between 4.33 and 5.67; the ovaries of these fish have transparent ova whose count varies between 5 and 13% and a number of yolky eggs vary between 5 and 28%. At June (Figure 5e), the GSI value is 3.91; the ovary of this fish have small ova whose count vary between 16 and 22% and a number of yolky ova ranging from 12 to 15%. The frequency distribution of ova diameters for the fish under study through six months from January to June indicates that the fish discharges its ripe ova in batches during spawning period and the withdrawal of the eggs from the egg stock to undergo a continuous maturation process successively. Moreover, the presence of more than two modes of sizes of ova indicates the fractional spawning characteristics and long spawning seasons.

Fecundity

In the study of fecundity two terms are generally used; these are the absolute fecundity and relative fecundity.

Analysis of fecundity length relationship

There are considerable variations in fecundity at any

length group (TL); the regression equation is:

$$Fa = -131258 + 4707.3 TL$$

Where (Fa) is the absolute fecundity. This equation is to calculate individual regression fecundity at each length group in Table 1; the interpretation of different forms in this equation indicates 0.9391 correlation coefficient, while the relationship between absolute fecundity and total length indicates satisfactory agreement at each group length. The relative fecundity against total length was estimated in the way as the absolute fecundity. The equation can be expressed as follows:

$$Fr = -2033 + 92.808 TL$$

where (Fr) is the relative fecundity. The correlation coefficient was found to be 0.927. The observed and calculated relative fecundity related to each group length were shown in Table 1, a satisfactory agreement at each group length is confirmed.

Analysis of fecundity - weight relationship

The average absolute fecundity related to each gutted weight are shown in Table 2, the regression equation can

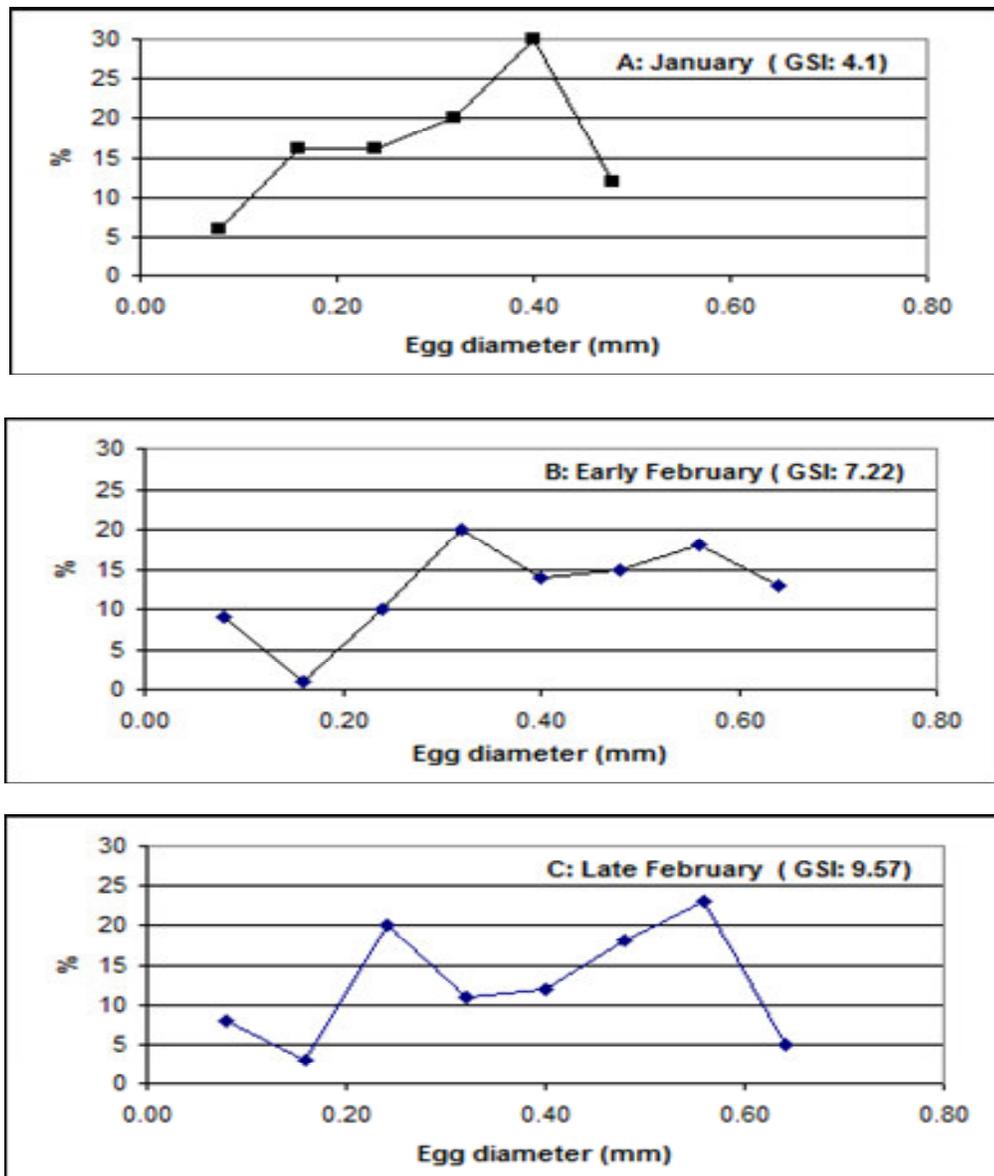


Figure 4. Frequency distribution percent of ova diameter in *M. merluccius* at January, February and March.

be expressed as follows $F_a = 3035.1 + 115.18 W$ where (W) is the gutted weight in grams with correlation coefficient of 0.8347. This shows a satisfactory agreement of both observed and calculated absolute fecundity for gutted weight from 200 to 629 g. There is a weak relationship between relative fecundity and gutted weight, which can be expressed by the regression equation $F_r = 127 - 0.0102 W$. The correlation coefficient was found to be 0.008 as shown in Table 2.

Histological and fine structure characteristics in ovaries of *Merluccius merluccius*

The course of development of the egg is divided into

stages or periods in order to differentiate the gradual changes in their conditions. Oocyte development in *M. merluccius* could be divided into six periods as follows:

Immaturation period

Oogonia of this period were very small spherical cells, each with thin indistinct peripheral cytoplasm and a large pale nucleus which ranged in diameter between 11-13 μ m. Oogonia or presynaptic oocytes were not conspicuous and were present solitary as shown in Figure 6a.

The primary oocyte was mostly polygonal or hexagonal, and varied in diameter between 25 and 87 μ m. These were characterized by large spherical nucleus which varied in

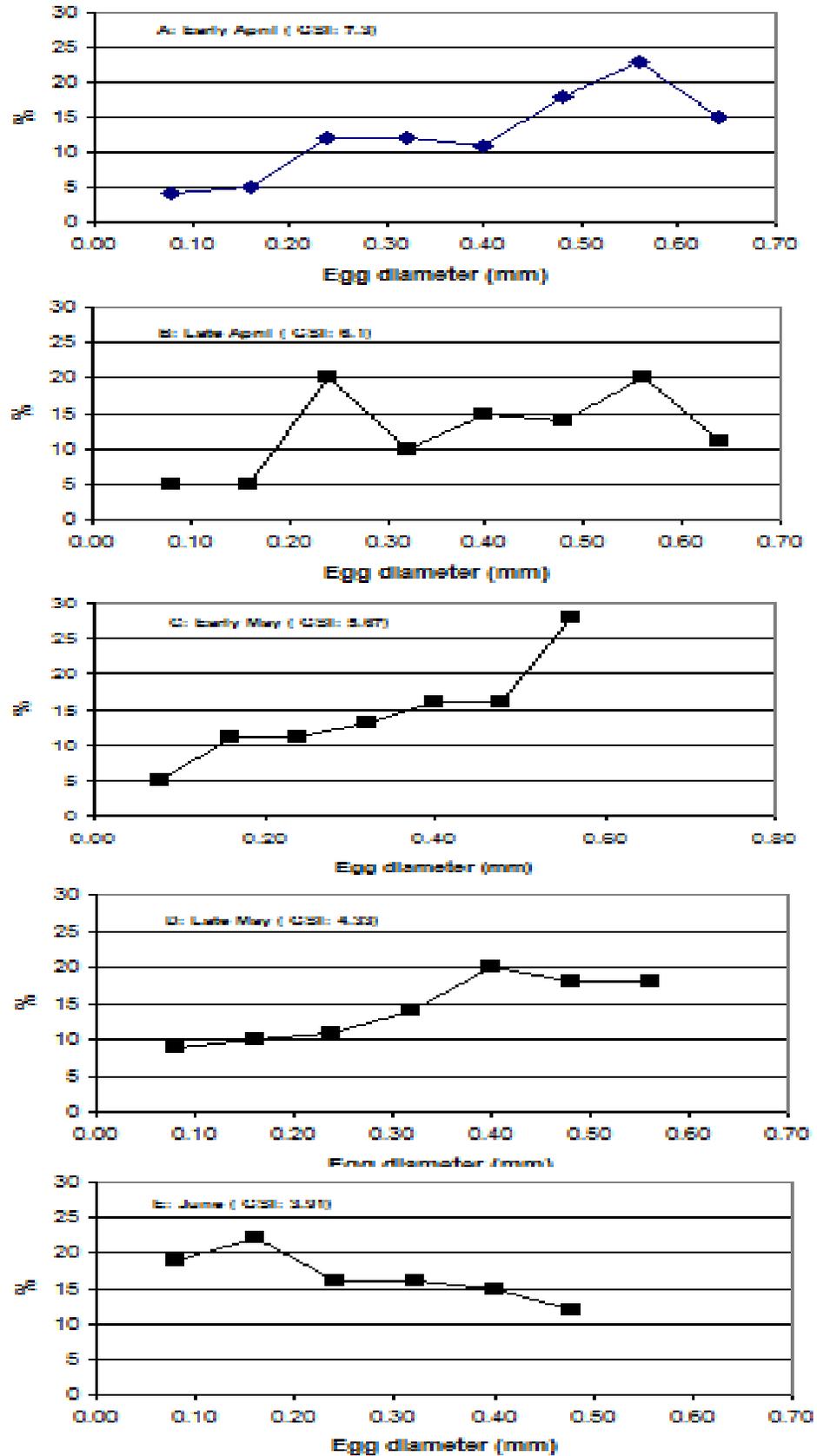


Figure 5. Frequency distribution percent of ova diameter in *Merluccius merluccius* at April, May and June.



Figure 6a. photo-micrograph of a cross section in semithin of immature ovary showing (a) pre-synaptic cells (toluidine blue) x1000.

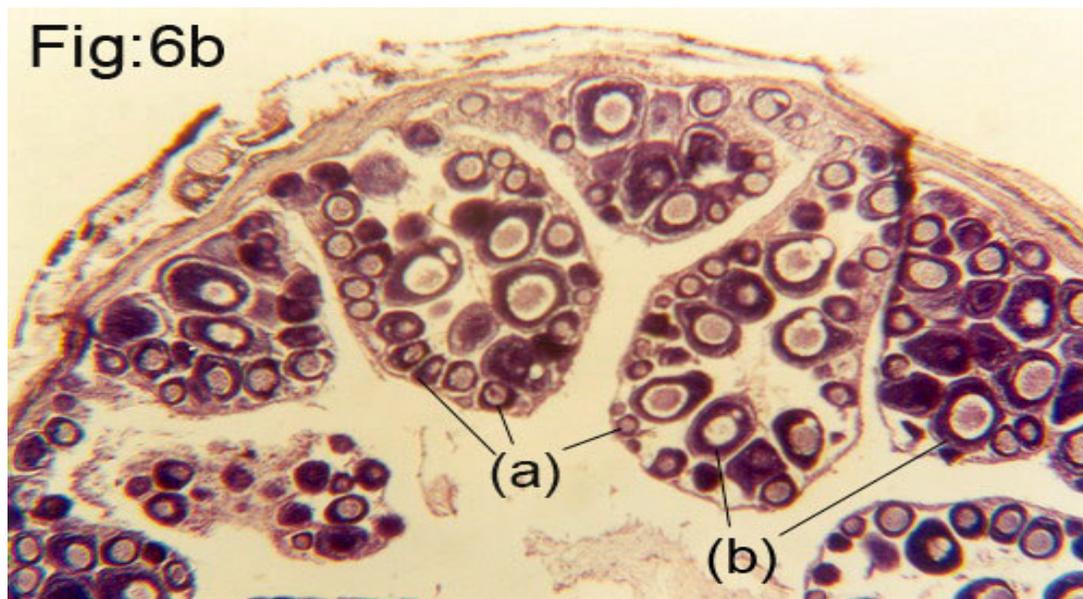


Figure 6b. photo-micrograph of a cross section of immature ovary showing (a) primary oocyte (b) more advanced primary oocyte (H&E). x 100.

diameter between 16 and 44 μm (Figure 6b). The nucleoli varied in number between 2 and 9 and their diameter varied from 1 to 3 μm .

The electron microscope examination of presynaptic oocyte revealed the nucleus characterized by dense chromatin material; there are a large number of mitochondria, endoplasmic reticulum and Golgi bodies in the

cytoplasm of the presynaptic oocyte (Figure 6c).

Maturation period

The maturation period is characterized by the appearance of isolated follicular epithelial cells around the oocyte

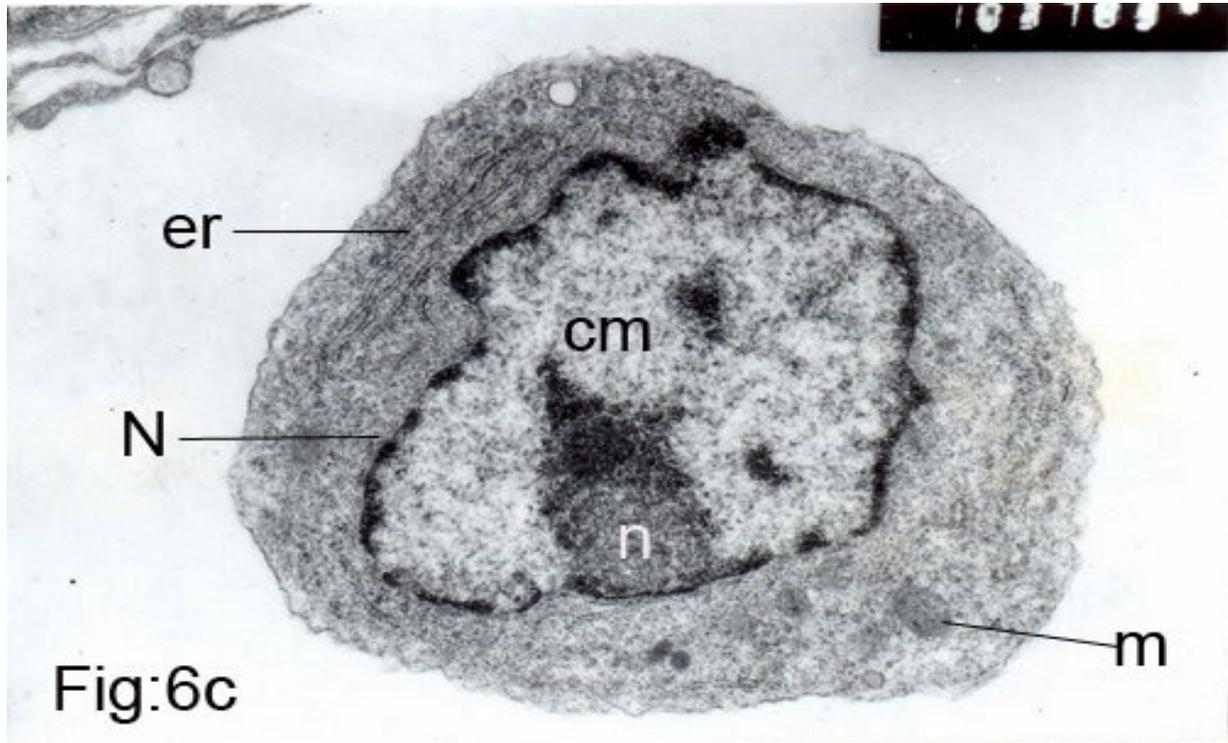


Figure 6c. Photo-electron-micrograph of a cross section in immature ovary showing pre-synaptic cells with nucleus (N), chromatin material (cm), mitochondria (m), endoplasmic reticulum (er) and nucleoli (n). (Uranyl acetate and Lead citrate) x 10000.

(Figure 7a). The oocyte reached 110 μm in diameter. The cytoplasm is faintly stained and is characterized by the appearance of bright corpuscle (yolk nucleus). There were 25 nucleoli arranged at the periphery of the nucleus and varied in diameter from 2 to 5 μm . Figure 7b is an electron micrograph of two primary oocytes at the maturation period, showing the yolky nucleus and the follicular epithelial layer between the primary oocytes. The walls of secondary oocyte were characterized by presence of elongated or oval nucleus of an interstitial cell (Figure 7c). These cells play an important role in secreting of steroid hormones.

Vacuolization period

The oocytes of this period are characterized by the appearance of cytoplasmic vacuoles (Figure 8a). The vacuoles were at first few in number, small in size and appeared at the periphery of the cytoplasm. In *M. merluccius* the oocyte ranged in diameter between 160 and 220 μm with a nucleus that varied in diameter between 80 and 100 μm . The vacuoles ranged in diameter between 9 to 20 μm . The nucleoli were arranged at the periphery of the nucleus, varied in number from 18 to 25 and ranged in diameter from 3 to 6 μm . The oocyte wall consisted of zona radiata of about 4 μm in thickness coated with a follicular epithelial layer of about 5 μm thick.

The ultrastructure of vacuolized oocyte wall showed the presence of five different layers, in which the outermost layer is theca. The second layer is the follicular epithelial layer and then the third and fourth layers are zona radiata externa and interna. The fifth layer is cortical alveoli (Figure 8b). Basement membrane separate between the outer theca layer and follicular epithelial layer.

Yolk deposition period

Oocytes of the primary yolk stage are generally characterized by the appearance of yolk granules at the periphery of the cytoplasm. In *M. merluccius*, the rounded and elongated oocytes ranged in diameter between 300 and 390 μm with a nucleus varying in diameter from 80 to 100 μm . The vacuoles ranged in diameter from 6 to 20 μm . The nucleoli were arranged at the periphery of the nucleus varying in number from 13 to 22 and ranged in diameter from 3 to 6 μm . The yolk granules were scattered in the cytoplasm and varied in diameter from 1 to 7 μm . The oocyte wall consisted of zona radiata of about 10 μm in thickness and coated with a follicular epithelial layer of about 5 μm in thickness (Figure 9a).

The secondary yolk stage oocyte increased in diameter and reached 580 x 510 μm across its long and short axis respectively with a nucleus whose dimension reached about 130 x 90 μm . The vacuoles ranged in diameter

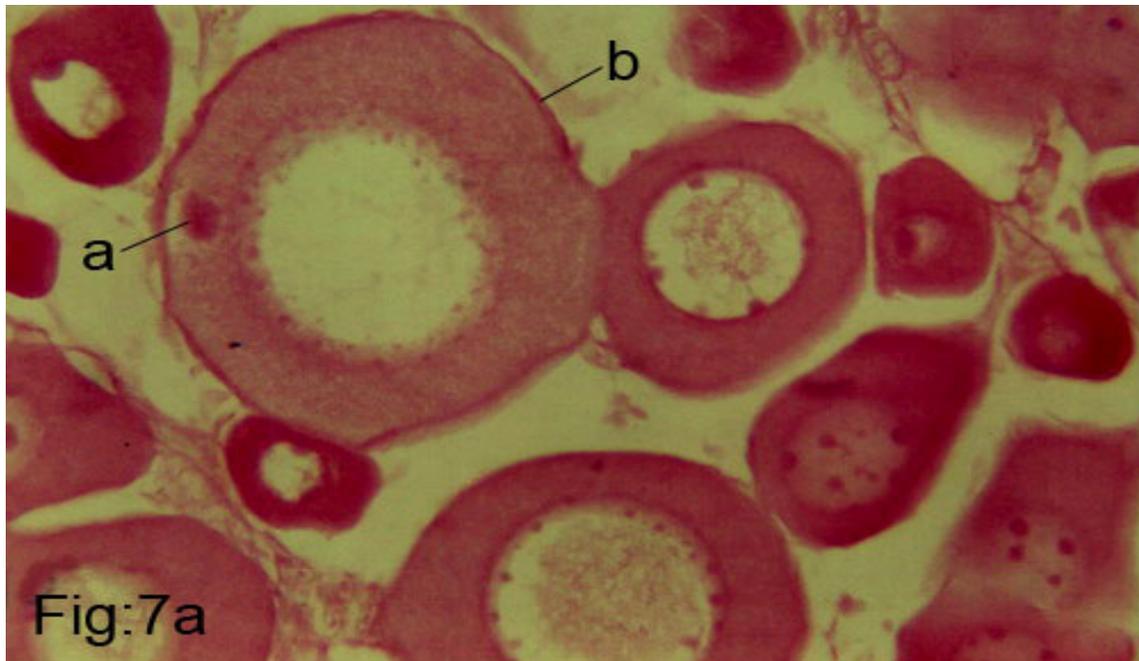


Figure 7a. Photomicrograph of a cross section in maturing ovary showing (a) yolk nucleus and (b) follicular epithelium (H&E). x 500.

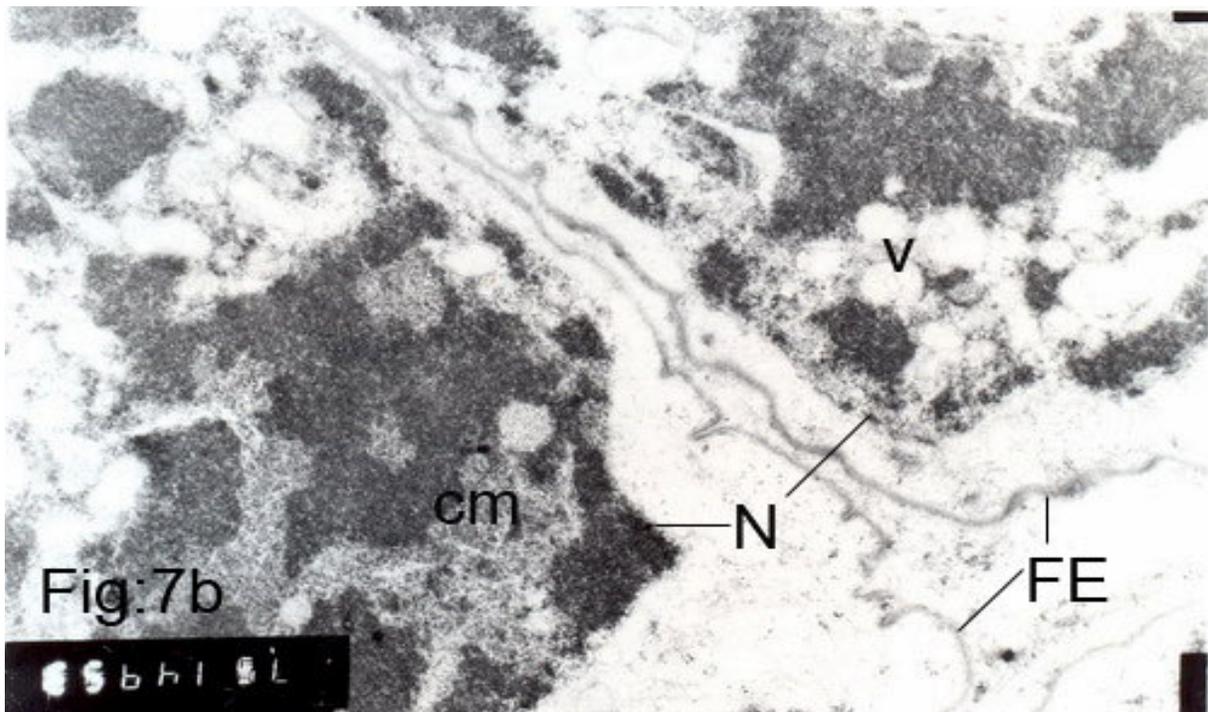


Figure 7b. Photo-electron-micrograph of cross section at maturing ovary showing follicular epithelial layer (FE) with their nuclei (N), chromatin material (cm) and vacuoles (v). x 4000.

from 4 to 63 μm . The yolk granules were scattered in the cytoplasm and varied in diameter from 3 to 14 μm (Figure 9b). The oocyte wall consisted of zona radiata about 11

μm in thickness and coated with follicular epithelial layer of about 5 μm in thickness. At this stage zona radiata layer reached its maximum thickness throughout the

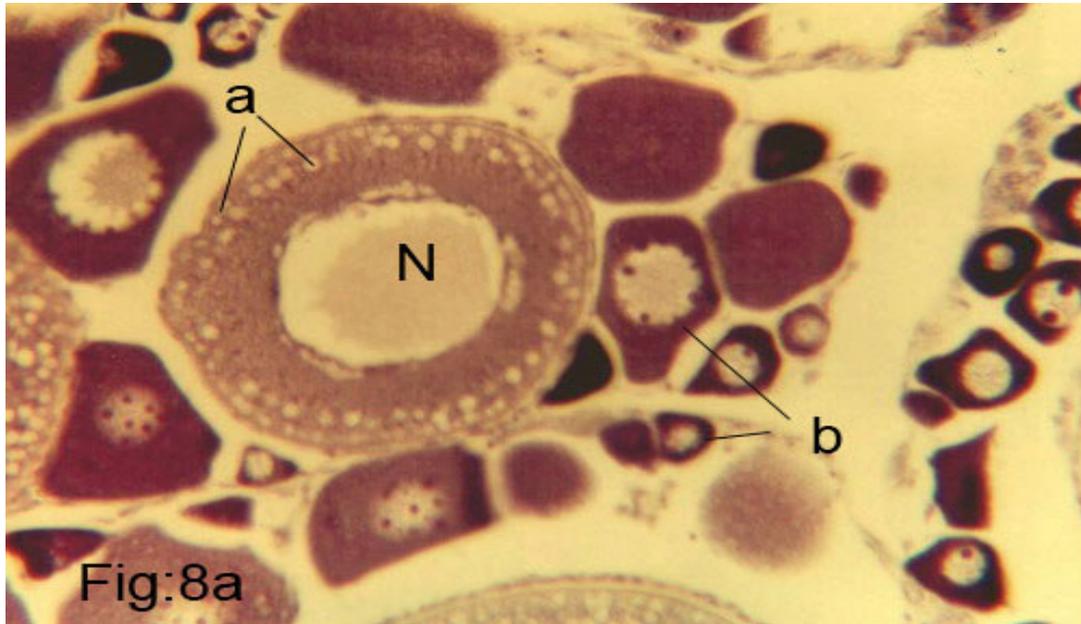


Figure 8a. Photo-micrograph of cross section in vacuolization stage showing two rows of vacuoles (a) with nucleus and cytoplasmic growth (b). (H&E) x 40.

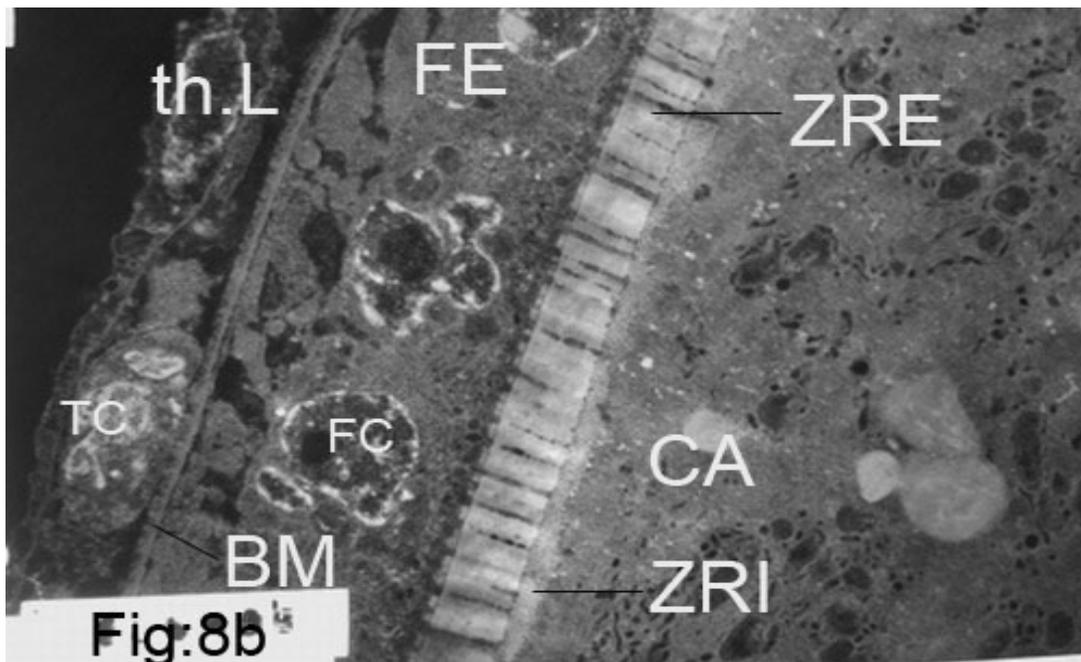


Figure 8b. Photo-electron-micrograph of vacuolized cell showing, theca layer (th.L), thecal cell (TC), basement membrane (BM) follicular epithelium layer (FE), follicle cell (FC), Zona radiata externa (ZRE), Zona radiata interna (ZRI) and cortical alveoli (CA). x4000.

whole year. The ultrastructure of the cell wall in oocyte at the yolk stage (Figure 10a) showed the presence of an outer theca layer which included special theca cells followed by a basement membrane, follicular epithelial layer, zona radiata, which consisted of zona radiata

externa and zona radiata interna and then the layer known as cortical alveoli.

Magnification of the outermost layer of the oocyte indicated the presence of elongated thecal cells; each thecal cell had a large elongated nucleus with dense



Figure 9a. Photo-micrograph of cross section in yolk deposition period at primary yolk stage showing (a) yolk granules (b) vacuoles (c) zona radiata (d) follicular epithelium (e) nucleus (H&E) x 250.

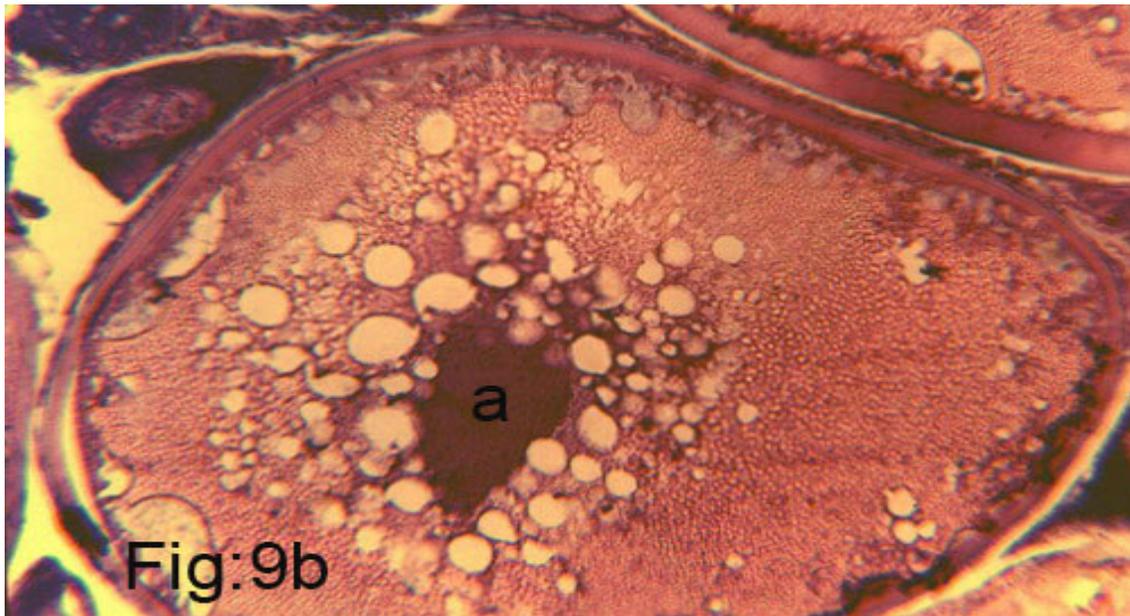


Figure 9b. Photo-micrograph of cross section in secondary yolk stage oocyte showing, decrease in diameter of nucleus (a) (H&E) x250.

chromatin material. The follicular epithelial layer includes large number of irregular nucleus with undifferentiated cytoplasm. The cytoplasm embodies Golgi bodies and mitochondria (Figure 10b).

Magnification of zona radiata layer by the electron microscope resolved two cell layers with canals in-between (Figure 10c).

Ripening period

This period is characterized chiefly by the migration of the nucleus towards the animal pole. The oocyte was increased in its diameters that reached $710 \times 780 \mu\text{m}$ across its short and long axis, respectively. The nucleus decreased in size and reached $90 \mu\text{m}$ in diameter (Figure

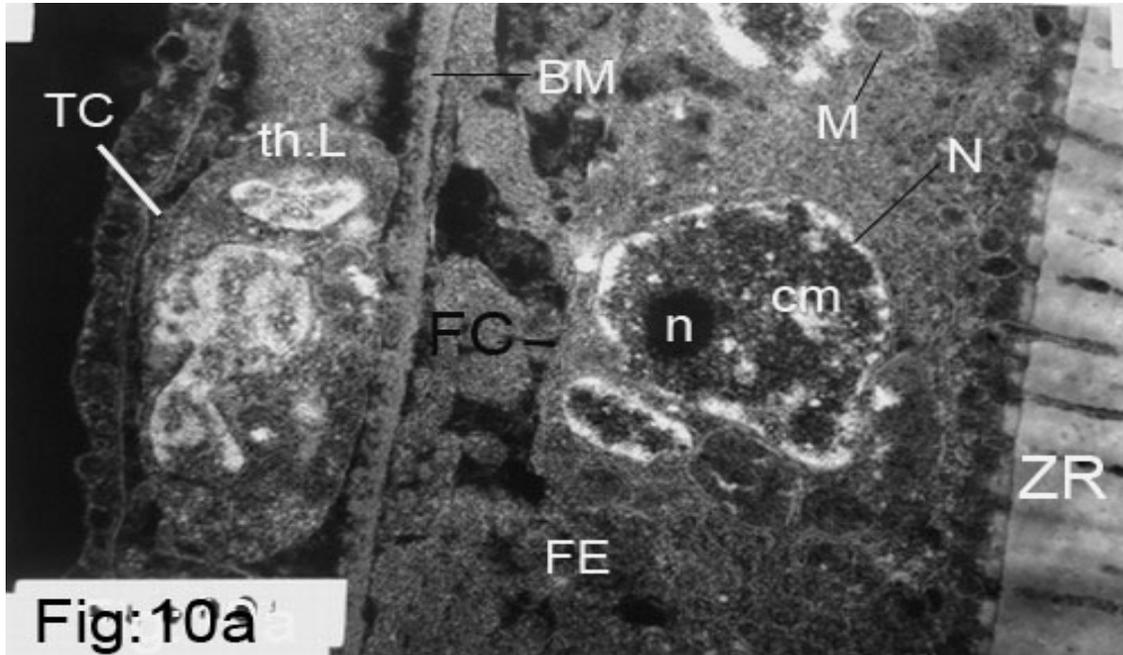


Figure 10a. Photo-electron-micrograph of cross section in wall of secondary yolk stage showing theca layer (th.L), thecal cell (TC), basement membrane (BM), follicular epithelial layer (FE), follicle cell (FC) showing chromatin material (cm), mitochondria (M), nucleus (N) and nucleolus (n) AND Zona radiata (ZR).x75000.

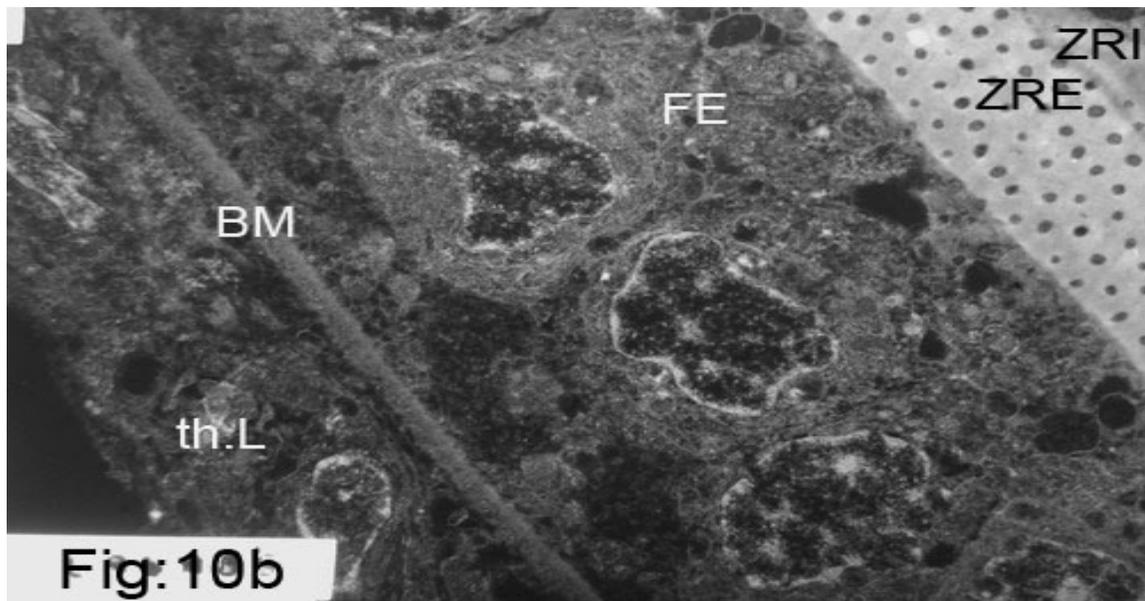


Figure 10b. Magnification of theca layer (th.L), basement membrane (BM) and follicular epithelium layer (FE) Zona radiata externa (ZRE) and Zona radiata interna (ZRI), x 10000.

11). The nucleoli were scattered in the nucleus and reached 14 in number, each ranged in diameter from 2 to 6 μm . The vacuoles increased in size and intermixed with the yolk granules. The oocyte wall consisted of zona radiata about 14 μm in thickness and coated with a follicular epithelial layer of about 5 μm in thickness.

Spawning - spent period

The ovary displayed various peculiarities at this stage of the spawning season. At the onset of spawning it closely resembled the ripe ova in having a large number of empty follicles, atretic oocyte and different stages of cells.

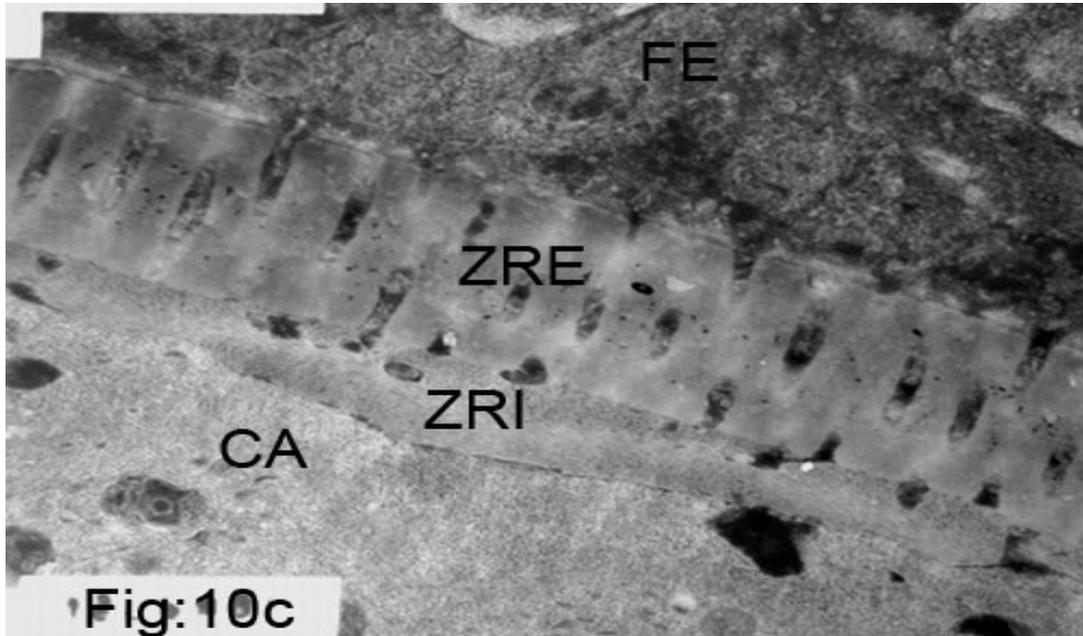


Figure 10c. Magnification of wall in ripe oocyte showing, follicular epithelial layer (FE) Zona radiata externa (ZRE), Zona radiata interna (ZRI) and cortical alveoli (CA). x10000.

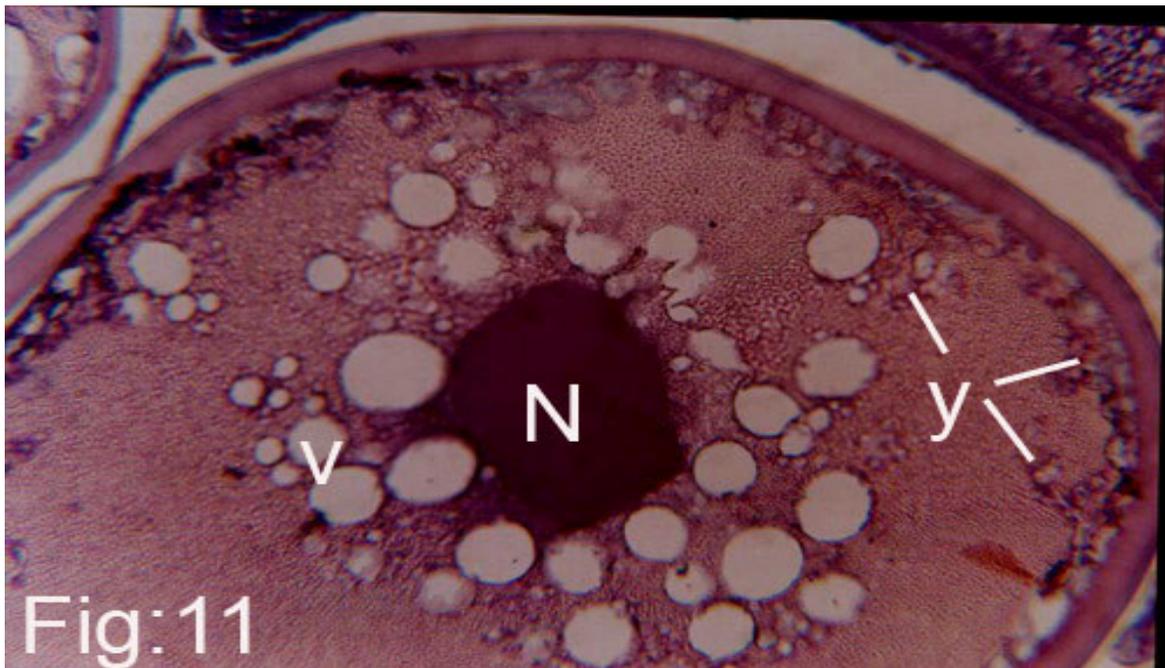


Figure 11. Ripening period showing migration of the nucleus (N), vacuoles (v) and yolk granules (y). (H&E) x 250.

The discharge of a considerable quantity of ripe ova during the spawning season was accompanied by a decrease in the number of the ripe ova. At the end of this period, spent stage was noticed by appearance of empty follicles and new generation of small oocytes as shown in Figure 12.

Notes on the seasonal variation in the ovary during the annual reproductive cycle: From July to August, multiple spawners are in the spent stage. Sections in ovaries during these months showed that the wall of the ovary was very thick and reached 100 μm in thickness. The ovary consisted mainly of atretic oocytes, empty follicle

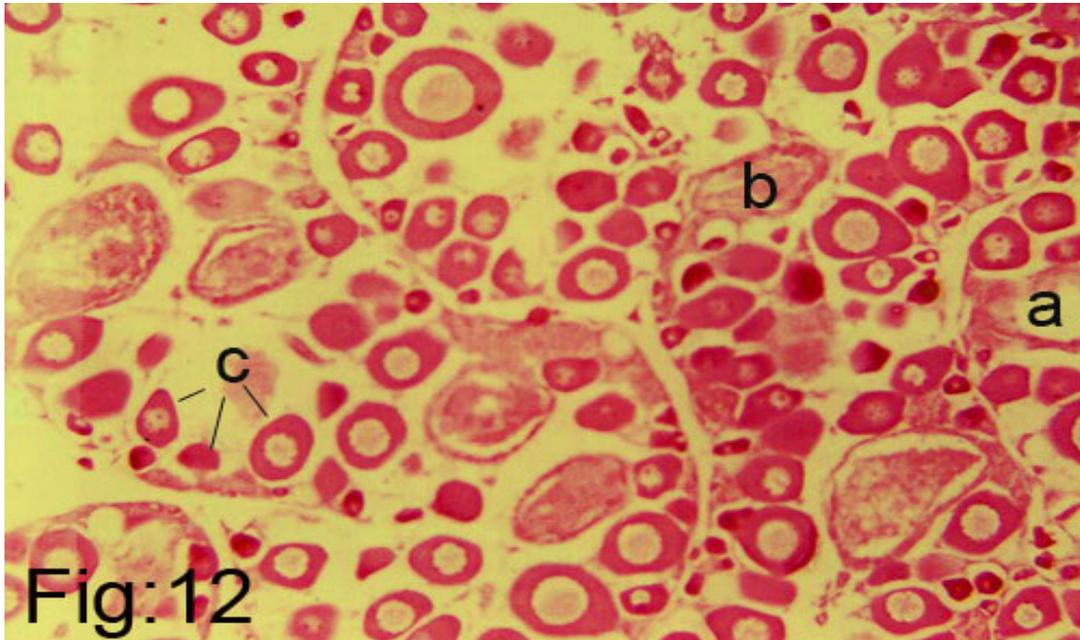


Figure 12. Photomicrograph of cross section in spent stage showing (a) empty follicle (b) atretic oocytes (c) different stages of cytoplasmic growth (H&E) x 100.

and new generation of small oocytes. Throughout this period, the gonadal index varied from 0.25 minimally to 0.53 maximally. Throughout the period from September to October, the ovaries displayed more advanced cytoplasmic growth of oocytes in addition to the premature and mature oocytes (Figures 6a, b, c, 7a, b and c). The gonadal index varied from 0.39 to 0.49. In November and December, the vacuolized stage appeared in many fish (Figures 8a and b). The gonadal index was about 1.53 on the average. Ripe and spawning females increased in number throughout the period from January to May. At this period, the gonadal index varied from 3.44 in July minimally to 8.11 in March maximally on average value.

DISCUSSION

In the present study on the fish *Merluccius merluccius*, 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 gonads of *M. merluccius* conforms with that of most teleosts (Zaki et al., 1995; Assem, 2000 and 2003) with slight modification as follows: immature, maturing, nearly ripe, ripe, spawning and spent.

Monthly analysis of the distribution of the maturity stages revealed that *M. merluccius* has a long spawning period extending from January to early June. In the present study, monthly analysis of various maturity stages detected the minimum size attained by the ripe

fish, thus all females whose body length is over 34.4 cm are mature.

Millan (1999) stated that maturity of female anchovy *Engraulis encrasicolus* L.C from the bay (Cadiz SW Spain) is attained at a total length 11.2 cm while Assem (2000) found that the female of *Caranx crysos* attains 50% maturity at a total length 17 cm. Assem (2003) revealed 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.

As a whole, the present results obtained from the monthly fluctuations in the gonadosomatic index (GSI) agree (to a great extent) with the finding obtained from the monthly variations of the maturity stages. Thus, the GSI increased progressively with increased percentage of the ripe individuals towards the spawning seasons.

The peak value was attained in January and continued to early June, then the GSI decreased gradually from July until August. The most common practice for the determination of a species spawning season is the establishment of its gonadosomatic index and the histological examination of the gonads (El-Greisy 2000; Assem, 2000, 2003; Honji et al., 2006).

The analysis of ova diameter for the species under study revealed that there are eight groups of ova in different samples; the first four diameter groups are small and transparent; the other four diameter- groups are yolky. The present results support the opinion of Zaki et al. (1995) who postulated that the presence of two or more ova sizes in the ovary shortly before the spawning

indicates the long spawning season and the fractional spawning characteristic and stated that the analysis of ova diameter of *Oblada melanura* revealed that there were nine diameter-groups of ova in different samples. Massut and Nin (1997) pointed out that the size distribution of oocytes with at least two groups of oocytes in the ovaries suggests that *Coryphaena hippurus* is a multiple spawner, with an extended spawning season in the study area. El-Greisy (2000) indicated that the analysis of frequency distribution of egg diameter of *Diplodus sargus* during the breeding season revealed the presence of several modes of ova sizes. In *M. merluccius*, fecundity shows a wide range for a given length but in general, the absolute fecundity has a linear relationship with length groups and gutted weight. 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). These authors concluded the same idea for Atlantic cod (*Gadus morhua*) where large number of small cod can be trapped there, as growth and fecundity increased by feeding in captivity. In *M. merluccius* the relation between absolute fecundity and total length was highly expressive, whereas the relation between absolute fecundity and gutted weight was weak. This is in agreement with Zaki et al. (1995) for *Oblada melanura*; Allam (1996) for *Trachinotus ovatus*, Assem (2000) for *Caranx crysos*, Murua et al. (2004) for *M. merluccius* and Garcia-Diaz et al. (2006) for blacktail comber *Serranus atricauda*.

The present results showed that the relative fecundity ranged from 743 to 1699 egg per 1 cm and varied from 92 to 148 egg for each 1 g. Murua et al. (2004) stated that two levels of relative batch fecundity were found for European hake, *Merluccius merluccius*: a) related to the main spawning period months, from January to April, of 149 egg g⁻¹ in average (SE = 3.9); and b) for the period outside of the peak spawning period (116 eggs g⁻¹ SE = 2.9). Assem (2003) pointed that the relative fecundity of *Pagellus erythrinus* ranged from 902.5 to 1519.7 egg per 1 cm and varied from 177.2 to 291.17 egg for each 1 g.

The seasonal ovarian cycle in *M. merluccius* was divided into six periods as revealed by the histological studies of the ovaries. The general pattern of histological development of the ovaries of the present study conforms to that of most teleosts (EL-Gharabawy, 1996; Assem 2000 and 2003) with slight modification. These are as follows: 1- Immaturation; 2- Maturation; 3- Vacuolization; 4- Yolk deposition; 5- Ripening and 6- Spawning and spent period. In the present observations, 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 *Mugil capito* and Assem (2000) for *Caranx crysos* and chromatin nucleolus stage of Abdo (1996) for *Dicentrarchus labrax*. In *M. merluccius*, 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). In the present study, pre-synaptic oocyte appeared in EM micrograph provided with Golgi apparatus, mitochondria and irregular vacuoles. These observations are in agreement with Gaber (2000) who indicated that the chromatin material was clearly shown in the nucleus of two oocyte maturation (chromatin nucleolus and perinucleolus stage).

The present results showed that the maturation period in *M. merluccius* was characterized by appearance of isolated follicular epithelial cells around the oocyte and formation of yolk nuclei. The yolk nucleus appears first as a small spherical corpuscle in close adherence to one side of the nucleus and then migrates to the periphery of the cytoplasm, where it finally disintegrates and disappears. Herrera et al. (1988) pointed out that the follicular epithelial cells are considered as a good proof for synthesis of sexual steroids.

The vacuolization period in *M. merluccius* was characterized by the presence of marginal vacuoles and by the fact that the oocyte wall consisted of zona radiata coated with follicular epithelial layer. The ultrastructure of the vacuolized and ripe oocyte wall shows the presence of five different layers, in which the outermost layer is theca layer. The second layer is the follicular epithelial layer, then the third and fourth layers are zona radiata externa and zona radiata interna. The fifth layer is the cortical alveoli. Grant (1990) characterized the vacuolization stage by cortical alveoli formation. In agreement with the present study, York et al. (1993) indicated that the follicle cell layer generally consists of an inner sub-layer which was separated by a basement membrane. Between the surface of the oocyte and the granulosa layer, there is a cellular layer zona radiata. The structure of oocyte covering layer in *Pagellus erythrinus* appears to be similar to that of Atlantic croaker (York et al., 1993). In *M. merluccius*, the yolk deposition period was characterized by the presence of yolk granules in the periphery of the oocyte cytoplasm. The yolk granules were spread centripetally into the whole central cytoplasm. The yolk deposition in the oocyte of the present species showed the same picture described by many authors for some other fishes such as: Salem et al. (1994) for *Mugil seheli*; EL-Gharabawy (1996) for *Lithognathus mormyrus* and Assem (2000 and 2003) for *Caranx crysos* and *Pagellus erythrinus*. At the yolk deposition and ripening period in *M. merluccius*, theca cells do not show any structural modification. Theca cells constitute the outer epithelial layer surrounding the oocyte, separated from the follicle cells by the basement membrane. They are flattened with an elongated nucleus and have faintly stained cytoplasmic organelles. Observations of the present work are in agreement with the investigations of Assem (2003) for *Pagellus erythrinus*. In *M. merluccius*, oocytes at ripening period continue the process of accumulation of the yolk material.

Most of the cytoplasm is filled with yolk granules of

various sizes, in between fat vacuoles. The nucleus occupies a central position, the number of nucleoli decrease due to the assembly of the small nucleoli into large ones. The same observation was recorded in *Caranx crysos* and *Pagellus erythrinus* (Assem, 2000 and 2003). Examination of the ovaries of *M. merluccius* throughout the annual reproductive cycle indicates that the presence of oocytes at different stages of development belongs to the fish with prolonged and fractional spawning season. Therefore, the fish may spawn more than once along the spawning period, as indicated by Salem et al. (1994) for *Mugil seheli*, by El-Greisy (2000) for *Diplodus sargus*, by Honji et al. (2006) for *Merluccius hubbsi*; and by Garcia Diaz et al., 2006 for blacktail comber *Serranus atricauda*.

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