Arab Journal of Scientific Research, 18 (1), pp 23-31 (2000)

ORIGINAL PAPER

S. Dadzie, F. Abou-Seedo and T. Al-Shallal

Histological and Histochemical Study of Oocyte Development in the Silver Pomfret, *Pampus argentus* (Euphrasen), in Kuwait Waters

Abstract: Histological and histochemical studies of oocyte development in the silver pomfret, *Pampus argenteus* (Euphrasen), collected from the Kuwait waters of the Arabian Gulf, showed that the process of development is divided into four phases: (i) primary growth phase, (ii) secondary growth phase, (iii) oocyte maturation phase and (iv) oocyte atresia phase. The first two phases break into a series of sub-phases referred to as stages. Germ cells (oogonia) proliferate through mitotic divisions and are transformed into oocytes. Growth of the oocytes as a result of synthesis of vitelline substances eventually results in maturation. Lipid inclusions which appear in the primary growth phase oocytes are probably transient since they disappear before the oocytes enter the secondary growth phase. Three types of inclusions are formed during vitellogenesis. Lipid yolk accumulates first in the secondary oocytes as lipid vesicles, followed by protein yolk in the form of discrete protein yolk granules. The third type of inclusion is carbohydrate which is present in the *zona radiata*. While the protein yolk granules maintain their structural integrity through to maturation and coalesce only thereafter, the lipid yolk vesicles continually coalesce, forming a large lipid globule which migrates centripetally.

دراسة الأنسجة وكيمياء الأنسجة لتطور بويضات
سمكة الزبيدي *Pampus argenteus* (Euphrasen)

المستخلص: أظهرت دراسة الأنسجة وكيمياء الأنسجة أثناء تطور بويضات سمكة الزبيدي أن عملية تطورها تنقسم إلى أربعة أطوار : طور النمو الأولي ، طور النمو الثانوي ، طور نضج البويضات و طور الحوصلة المتحللة. وينقسم الطور الأولي وطور النمو الثانوي إلى سلسة من تحت أطوار يشار إلى كل منها كمرحلة. تتكاثر الخلايا الجرثومية (أمهات البويضات) من خلال الانقسام الميتوزي (الانقسام غير المباشر) وتتحول إلى بويضات. ونتيجة لتكون مادة المح في نهاية مرحلة النضج تنمو البويضات . والتجمعات الدهنية التي تظهر خلال طور النمو الأولي قد تكون مؤقتة نظراً لاختفائها قبل دخول البويضات الى طور النمو الثانوي. ويتكون ثلاثة أنواع من المحتويات خلال عملية تكوين المح. ويتجمع المح الدهني أولاً في خلايا البويضة الثانوية على شكل حويصلات دهنية. ويتبع ذلك ظهور المح البروتيني على شكل حبيبات منفصلة. وتمثل الكربوهيدرات النوع الثالث من المحتويات وهي تتواجد في المنطقة الشعاعية. وعلى حين أن حبيبات المح البروتيني تحافظ على سلامة تركيبها طوال فترة النضج ثم تندمج بعد ذلك فإن حويصلات المح الدهني تندمج باستمرار مكونة كريات دهنية كبيرة تهاجر في النهاية الى القطب المركزي للخلية البويضية.

Introduction

The silver pomfret, *Pampus argenteus* (Euphrasen), locally called zobaidy, is a member of the family Stromateidae. This typical marine fish is widely distributed in the tropical coasts, from the Arabian Gulf (Mohamed and Ali, 1992; Ali and Mahmood, 1993), through the Bay of Bengal to Japan Sea (Mito and Senta 1967) and East China Sea (Higashikawa and Masumitsu 1976). It accounts for about 15% of the commercial fish landings in Kuwait (Morgan 1985) and is the most highly priced and the most sought-after fish in the country (Al-Shallal 1998). *P. argenteus* also plays an important role in the fisheries of the other countries bordering the Arabian Gulf (Mohamed and Ali 1992, Ali and Mahmood 1993), Indonesia (Dwiponggo 1984), China (Luo *et al.* 1993), Korea (Kim and Lee 1992) and Bangladesh (White 1985). The species has also been reported in the North Sea (Davis and Wheeler 1985).

Despite its economic importance, only a few studies have been undertaken on aspects of the reproductive biology of *P. argenteus* in Kuwait waters and, indeed, in the Arabian Gulf as a whole (Hussain and Abdullah 1977, Abu-Hakima *et al.* 1983, Dadzie *et al.* 1998). Furthermore, the species is a desirable candidate for aquaculture in Kuwait

S. Dadzie, F Abou-Seedo and T Al-Shallal*
Department of Biological Sciences
Kuwait University,
P.O. Box 5960, Safat 13060
Kuwait
Tel: (965) 4811188 Ext. 7131
Fax: (965) 4847054
E-mail: stevedadzie@kuc01.kuniv.edu.kw
** Author to whom correspondence should be addressed*

and neighbouring countries. This study was therefore undertaken to describe the histological and histochemical changes in the ovaries, and also to delineate the periods of occurrence of the different developmental phases during the reproductive cycle of the fish in Kuwait waters. This research will provide data useful for aquaculture and management of the species in Kuwait waters of the Arabian Gulf.

Materials and Methods

Fresh samples of *P. argenteus* were obtained from commercial gill-net catches from the northern part of the Kuwait waters of the Arabian Gulf (Fig. 1). The nets were 1,000-2,500 m long, with a 13.8 cm mesh size, and were laid at depths ranging between 7 and 15 m. They were set at dawn, between 0300 and 0500 h and were examined and fish collected between 1300 h and 1400 h. The vessels with *P. argenteus* catches docked by 1530 h and samples for the study were obtained between 1530 and 1700 h. Monthly sampling lasted for 24 months, from March 1996 to February 1998. Since pomfret fishing was banned during April-May 1997, samples for those months were obtained under a special permit using a private fishing vessel (courtesy of Kuwait Public Authority for Agriculture Affairs and Fish Resources), and keeping to the same fishing method and maintaining the same sample size.

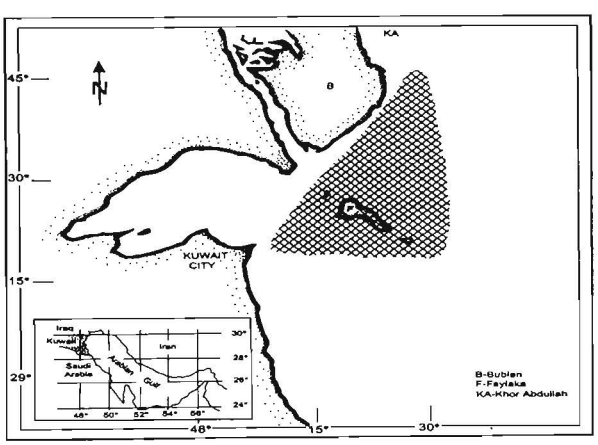


Fig. 1. Map of Kuwait waters of the Arabian Gulf, showing the fishing area (hatched)

The total length (TL, cm), standard length (SL, cm) and body weight (g) of each sampled fish were recorded upon arrival in the laboratory. The fish were then dissected, sexed, and the gonads removed, weighed (mg), and the parts for routine histological processing fixed in Bouin's fixative for a minimum of 24 hrs. They were then dehydrated in alcohol, cleared in toluene, infiltrated with parafin wax and embedded also in paraffin wax. 5-10 μm sections were stained in haematoxylin and counterstained with eosin to study the histological changes during development

Pieces of the gonads from the central part of the organ were preserved in appropriate fixatives and processed accordingly for histochemical studies, according to Drury and Wallington (1980), with modifications where necessary, as follows.

Demonstration of Lipids

Thin pieces (2-3 mm) of ovaries were fixed in 4% formalin for 24-36 hrs. They were then washed in running tap water for 12-16 hrs and post-fixed in 2% osmium tetroxide for 24 hrs, dehydrated, cleared in toluene and embedded in paraffin wax. 5-7 μm sections were examined with the light microscope.

Demonstration of Proteins

Thin pieces (2-3 mm) of ovarian tissues were fixed in absolute alcohol for 4-5 hrs, cleared in toluene and embedded in paraffin wax. 5_m sections were dehydrated and immersed in preheated Ninhydrin at 37°C for 16-20 hrs. After washing in running tap water, the sections were immersed in Schiff's reagent for 30 mins and washed again for 3 mins. They were then counterstained with Coomassie-brilliant blue, instead of haematoxylin, as per Drury and Wallington (1980), and washed, dehydrated, cleared in xylene and mounted in balsam, ready for examination.

Demonstration of Carbohydrates

Thin pieces (2-3 mm) of ovarian tissues were fixed in 10% formalin for 24-36 hrs, washed in running tap water overnight, cleared in toluene and embedded in paraffin wax. 5 μm sections were immersed in tap water for 3-5 mins, then in periodic acid for 7 mins. After rinsing in distilled water, the sections were immersed in Schiff's reagent for 20 mins, then, in contrast to Drury and Wallington (1980), treated in three solutions of sulphurous acid for 2 mins each, before being washed in running tap water for 5 mins and counterstained with Harris haematoxylin. The sections were then dehydrated, cleared in xylene and mounted in balsam.

Oocyte Measurement

Cell and nuclear sizes were measured using an ocular micrometer fitted in Zeiss 010516 microscope at x10, x40 and x100 objectives. For each development phase or subphase, ovaries of five

fish were examined and, in each ovary, oocyte and nuclear diameters were measured for 50 randomly-selected cells and the means determined. Only those oogonia and oocytes which had been sectioned through the nucleus were measured. This procedure has been shown to be representative of the true oocyte diameter (Foucher and Beamish 1980).

Results

Histology of Oocyte Development

The germ cells of the ovary of *P. argenteus* undergo a series of morphological changes during development and, for descriptive purposes, can be divided into various stages on the basis of: (i) cell and nuclear sizes, (ii) staining affinity, (iii) structure of the nucleus and nucleoplasm, (iv) number and location of the nucleoli within the nucleus, (v) the appearance and structure of the chorion and (vi) the appearance, location and organization of different inclusions (yolk) in the oocyte. Based on these features, the process of development of the ovaries of *P. argenteus* will be divided into the following four phases: (a) primary growth phase, (b) secondary growth phase, (c) oocyte maturation phase and (d) oocyte atresia phase. The first two phases break into a series of sub-phases referred to as stages.

Primary Growth Phase

Oogonia stage (Fig. 2a)

The oogonia are the primary germ cells of the ovary. They are small, spherical cells (12±2μm) with large nuclei (8±1 μm) relative to the size of the oogonia. The centrally-located nuclei have conspicuous nuclear membranes. The oogonia occur either singly, within cysts of developing germ cells, or in groups, usually at the edge of ovigerous folds. These cells proliferate through mitotic divisions, giving rise to oogonia of subsequent generations. Oogonia are present in the ovaries of *P. argenteus* throughout the year, but in Kuwait waters, the peak period of oogonial proliferation is from May to August, following immediately the first spawning peak, since nests of oogonia were present in greatest abundance in recovering spent ovaries, apart from immature virgins.

Chromatin nucleolus stage (Fig. 2b)

Following the last mitotic division of the oogonia, the last daughter oogonial cells enter a short period of growth and transform into primary oocytes. Such cells are characterized by a narrow zone of cytoplasm and a small number of strongly basophilic nucleoli, while the nucleus is slightly less basophilic.

Early perinucleolar stage (Fig. 2c)

The appearance of the early perinucleolar stage oocytes marks the beginning of the primary growth phase proper. These oocytes are recognized, among other characteristics, by their size. They have a minimum size of 21 μm but the overall cell size is 43±14 μm and 19±6 μm for the nucleus. The nuclei undergo reorganization in connection with meiosis as evidenced by their slight increase in size and the conspicuousness of the chromosomes which shorten, thicken and form loose threads scattered in the granular nucleoplasm - a characteristic of the diplotene stage of meiotic prophase. Early perinucleolar oocytes are also characterized by the number of nucleoli they bear, one in the younger ones and 2-3 in the slightly advanced ones. The cytoplasm and nucleoli are strongly basophilic. During the early perinucleolar stage, tiny vesicles appear in the cytoplasm and persist into the late perinucleolar stage. They increase in number and size as the oocyte grows and are randomly distributed throughout the cytoplasm. Towards the end of the primary growth phase they seem to disappear.

Late perinucleolar stage (Fig. 2d)

The oocytes are characterized by greater sizes, the smallest measuring 74±9 μm. The large nucleus (35±5 μm) contains numerous small, round peripheral nucleoli. The cytoplasm is less basophilic as compared to that of the early perinucleolar oocytes.

During the primary growth phase, each ovary becomes enveloped by a thin, single layer of connective tissue with dark, spindle-shaped nuclei, forming the theca (Fig. 2e). Also during the same growth phase, a conspicuous cytoplasmic structure, the yolk nucleus, appears in the cytoplasm of the developing oocyte as a round or oval basophilic body near the nuclear membrane. It migrates to the periphery of the oocyte where it breaks down and completely disperses prior to the next growth phase.

By the end of the late perinucleolar stage the oocyte increases in size up to 102±2 μm, while the nucleus measures 45±13 μm. Females with perinucleolar stage oocytes, characteristics of maturing virgins or recovering spent, are common from June to February in Kuwait waters, with a clear peak from September to January.

Secondary Growth Phase

A number of larger primary growth phase oocytes undergo recruitment into the secondary growth phase. The latter are characterized by the presence of yolk inclusions, thus marking the beginning of vitellogenesis.

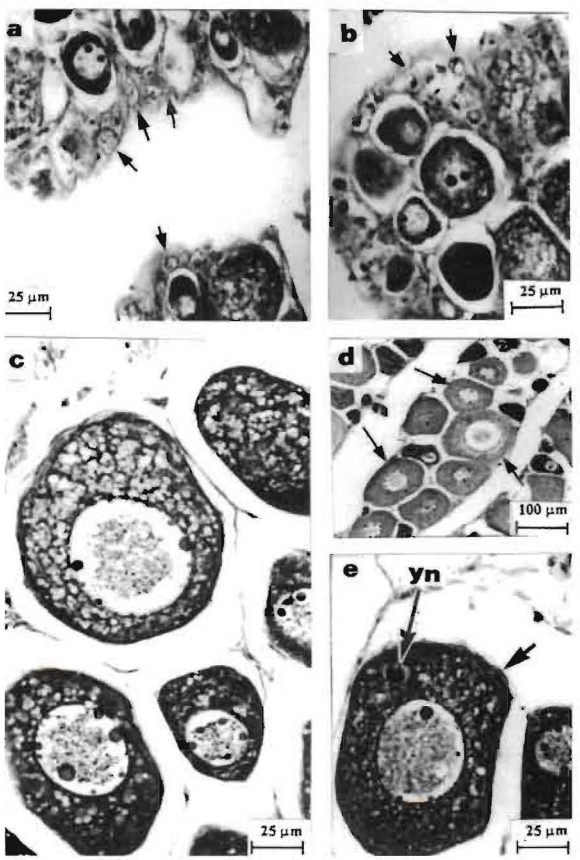


Fig. 2. Histological appearance of primary growth phase oocytes in *P. argenteus*.(a) Oogonia(arrowed). (b) Chromatin nucleolus stage (arrowed). (c) Early perinucleolar stage oocytes exhibiting numerous vesicles in the cytoplasm. (d) Late perinucleolar stage oocytes exhibiting reduced basophilia in the cytoplasm (arrowed). (e) Early perinucleolar stage oocyte exhibiting a layer of connective tissue (arrowed) and a yolk nucleus (yn).

Lipid vesicle stage I (Fig. 3a)

Oocytes in this stage measure 120±22 µm for the cell and 45±13µm for the nucleus. Lipid vesicles occur in a discrete zone in the mid cortex without much evidence of the randomly-distributed lipid vesicles which appeared in the primary growth phase oocytes. By the end of the stage, lipid vesicles increase in number and size. Granulosa cells appear between the theca and the plasma membrane and the nuclear membrane loses its smooth contour and becomes convoluted (Fig. 3b).

Lipid vesicle stage II (Fig. 3c)

As the oocyte further increases in size (145±29 µm for the cell and 56±14 µm for the nucleus), the lipid vesicles also increase in size and number and now appear distributed throughout the cytoplasm. A thin acellular membrane, the *zona radiata*, becomes visible around the periphery of the oocyte, occupying the area earlier taken by the layer of granulosa cells, thus pushing the latter behind. In Kuwait waters, *P. argenteus* with oocytes in the lipid vesicle stage, characteristic of developing ovary, are present throughout the year but dominate from February to April.

Primary yolk granule stage (Fig. 3d)

Oocytes in this stage measure 274±31 µm and the nucleus, 81±17 µm, and are characterized by the appearance of numerous small granules (5 µm) in the outer cortex. By this time the lipid vesicles have become larger (15±2µm) and the *zona radiata* more prominent. *P. argenteus* with oocytes in this stage, characteristics of maturing, are observed in Kuwait waters from February till October.

Secondary yolk granule stage (Fig. 3e)

Oocytes in this stage increase in size to 304±78 µm and the nucleus, 83±19 µm. The large, centrally-located nucleus is now very convoluted. The yolk granules have increased in number and size (9±2 µm) and are coalescing. The two trophic substances do not occupy discrete zones but are interspersed. The *zona radiata* increases in thickness (14±2 µm) and becomes differentiated into an outer eosinophilic layer, the *zona radiata externa* and an inner basophilic layer, the *zona radiata interna*.

Tertiary yolk granule stage

Oocytes in this stage measure 405±56 µm and 72±18 µm for the nucleus. There is an increase in the coalescence of the lipid droplets which have now enlarged to 65±12 µm, while the protein yolk granules also increase in size (32±5 µm). This marks the end of the period of growth. Females with yolk granule stage oocytes, characteristic of the mature condition, are present in Kuwait waters in April-August with peaks in May and August.

Migratory nucleus stage (Fig. 3f)

At the attainment of the maximum size of the oocyte, the nucleus migrates centrifugally to one of the poles (nuclear polarization) which delineates the animal pole of the oocyte. This is followed by a further and final coalescence of the lipid droplets

into a large lipid globule measuring 140±79 µm. The oocytes are now termed "postvitellogenic oocytes".

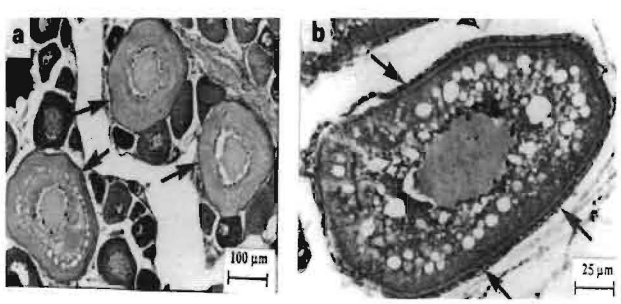
Oocyte Maturation Phase

Oocyte maturation begins with the peripheral migration of the nucleus and the resumption of meiosis which had been arrested at the diplotene stage of meiotic prophase during the primary growth phase. Nuclear migration is followed by the dissolution of the nucleus, referred to as the germinal vesicle breakdown. With the latter process, coupled with the coalescence of the protein yolk granules and lipid yolk globules, the oocyte rapidly increases in volume due to hydration, causing the follicle to stretch and thus become thin. During these changes, the *zona radiata,* which is now called chorion, remains prominent. The stage after the completion of maturation is referred to as the egg stage and the oocyte is now referred to as an egg. The contents of the egg now appear homogeneous and translucent. This marks the end of development.

Oocyte Atresia Phase

During development a number of oocytes suddenly cease to develop and, instead, undergo degenerative changes leading to their resorption from the oocyte by the process of atresia. Although atretic oocytes are found in *P. argenteus* females throughout the year, they are commonly found during and after the spawning season. Indeed, after spawning, all vitellogenic oocytes which remain in the ovary become atretic.

The first signs of atresia in vitellogenic oocytes in *P. argenteus* is detected when the *zona radiata* becomes convoluted and starts to break up. Concomitantly, the granulosa cells proliferate and hypertrophy and invade the oocyte, ingesting the yolk by phagocytosis (Fig. 3g). Finally, the phagocytic granulosa cells, in turn, also degenerate, leaving behind a lightly staining fibrous mass surrounded by connective tissue elements.



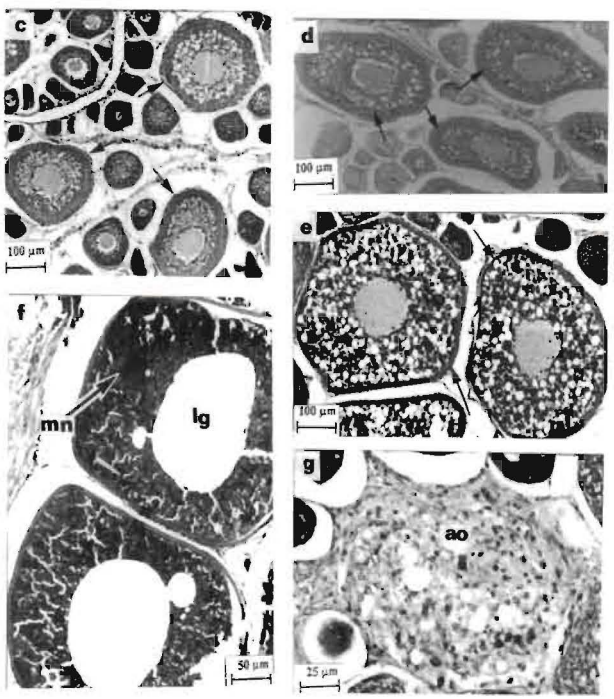


Fig. 3. Histological appearance of secondary growth and atretic phase oocytes. (a) Lipid vesicle stage I oocytes (arrowed). (b) Lipid vesicle stage I oocyte exhibiting granulosa cells (arrowed). (c) Lipid vesicle stage II oocytes (arrowed). (d) Primary yolk grannule stage oocytes (arrowed). (e) Secondary yolk grannule stage oocytes (arrowed). (f) Migratory nucleus (mn) stage oocytes, exhibiting coalesced lipid globule (lg). (g) Atretic oocyte (ao)

Histochemistry of Oocyte Development

Lipids

Both the primary and secondary growth phase oocytes revealed positive reactions with osmium tetroxide. The tiny vesicles and the slightly larger ones observed in the early and the late perinucleolar stage oocytes respectively appeared as black spots on histochemical preparations (Fig. 4a), indicating the contents to be lipids. The lipid vesicle stages of the secondary growth phase oocytes are also characterized by the appearance of lipid vesicles in the cytoplasm, first as individual vesicles but later coalesce. Osmium tetroxide-positive reactions were detected in the vesicles of all the oocytes tested histochemically (Fig. 4a).

Proteins

The small granules that appear, in histological slides, in the outer cortex of the primary yolk stage oocytes during the secondary growth phase, gave a positive reaction (pinkish-red) with the Ninhydrin-Schiff reagent, a specific test for protein (Fig. 4b).

As the oocyte grows and transforms into the subsequent stages, the protein yolk granules increase in size and number and are distributed throughout the cytoplasm.

Carbohydrates

Periodic-Acid-Schiff-positive reaction, a specific test for carbohydrates, was detected positively only in the *zona radiata* as magenta-red granules (Fig. 4c).

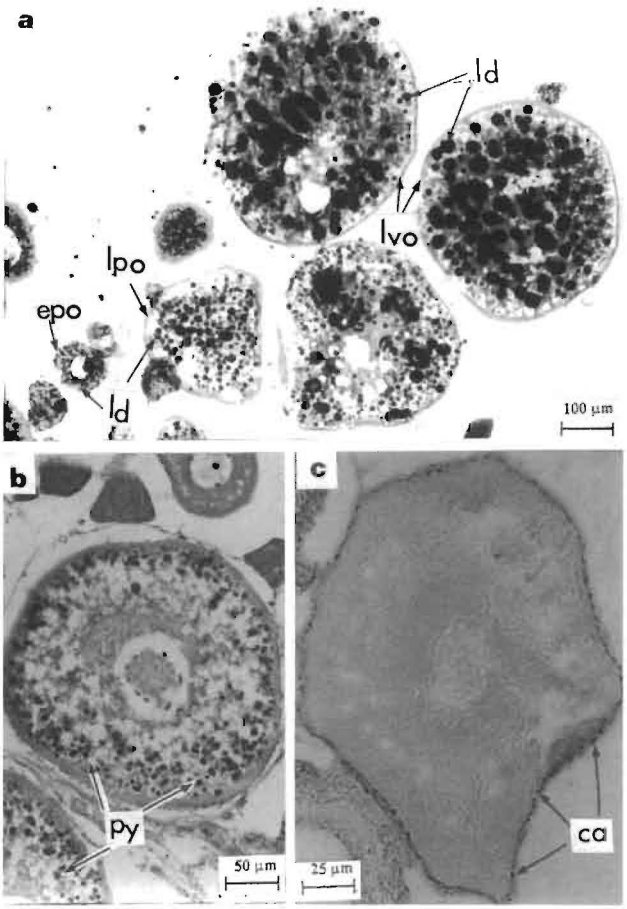


Fig. 4. Histochemistry of oocyte development. (a) Section stained for lipids with osmium tetroxide. Lipids are stained black in early perinucleolar stage oocyte (epo), late perinucleolar stage oocyte (lpo) and lipid vesicle stage oocytes (lvo). (b) Section stained for protein with Coomasie-brilliant blue. (c) Section stained for carbohydrates with PAS. ld, lipid droplet; py, protein yolk grannule; ca, carbohydrate

Discussion

The process of development of the oocytes of *P. argenteus* follows the same basic progression as that described in other teleostean species (Yamamoto 1956, Dadzie 1974, Forberg 1982, Mayer *et al.* 1988, Abou-Seedo and Al-Khatib 1995, Coward and Bromage 1998, Dadzie and Owiti 1998, Maddock and Burton 1999). Oogonia proliferate and grow into immature, previtellogenic oocytes which characterize the immature ovary. Transformation of the oocyte into the maturing phase, characterized by the appearance of yolk inclusions, marks the beginning of vitellogenesis, followed by oocyte growth and maturation and, finally, nuclear polarization and ovulation.

Controversial reports exist on the onset of spawning in *P. argenteus* in Kuwait waters. While Hussain and Abdullah (1977) and Abu-Hakima *et al.* (1983) observed spawning beginning in March and April respectively, Dadzie *et al.* (1998) provided evidence that indicates a delay in spawning until May, with the first spawning peak in May, and the second in August.

As proposed by Dadzie *et al.* (1998), a number of environmental cues are thought to stimulate spawning activity in fully mature fish (Summers 1996). For instance, an increase in stream discharge (Webb and Hawkins 1989) and spate conditions (Owiti and Dadzie 1989) initiated spawning in a number of species. For the enhancement of gametogenesis, leading to maturity and spawning, the role of temperature is undisputably recognized (Ahsan 1966, Asahina and Hanyu 1983, Summers 1996). In the Northern Arabian Gulf, low salinities, caused by discharge from the Euphrates river, are generally believed to trigger spawning in *P. argenteus*. In the recent past, the entire discharge of fresh water from the Euphrates has been diverted in the Northern Arabian Gulf as a result of economic and social changes. It is probable that persistent high salinities, coupled with temperature changes, are responsible for the late maturity and delay observed in the spawning of the silver pomfret. These findings support the banning of the species from May and not March, as previously practised, in Kuwait waters.

With the change in the onset of spawning, a change in the annual variations in the maturity stages (Abu-Hakima *et al.* 1983) has also been observed. In their recent study, Dadzie *et al.* (1998) delineated a seven-stage scale of maturity of the ovaries but did not indicate the times of the year when females in the different stages dominate the pomfret population. This missing information is now provided in this study and, interpreting it together with the data on maturity stages (Dadzie, *et al.* 1998), the following holistic summary may be made (Fig. 5): Stage I (immature) females are common from May to February, Stage II (maturing

virgin or recovering spent) females dominate from February to April, Stage III (developing) are commonest from February to October, Stages IV and V (maturing and mature) females appear from April to August with peaks in May and August, Stage VI (running) fish occur from May till August, while Stage VII (spent) females show up soon after spawning in May and persist till October.

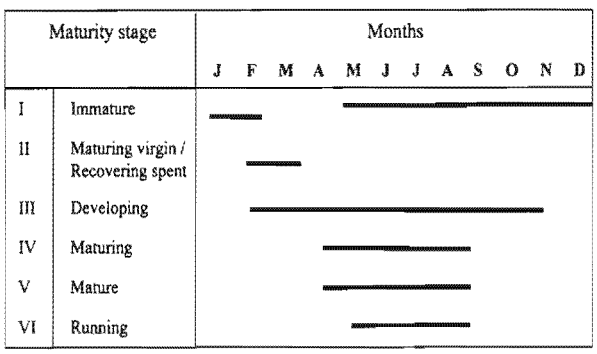


Fig. 5. Relationship between maturity stages and season in P. argenteus in Kuwait waters.

Histochemical studies of fish ovaries have, in most cases, targeted vitellogenic or secondary growth phase oocytes. However, during oogenesis, lipids are sometimes formed in perinucleolar or primary growth phase oocytes as confirmed through histochemical analysis (Dadzie 1974). Such lipid inclusions, however, tend to disappear from the oocytes towards the end of the primary growth phase as osmium tetroxide test proves negative. Similar observations were made in the present study. Dadzie (1974) postulated that such lipids may not be considered as contributing to the lipid yolk and that they are probably used by the young oocytes for their growth; hence their disappearance from the oocyte before the vitellogenic phase. Their presence may therefore be considered as transient.

Two types of yolk inclusions, *viz.* yolk vesicles and yolk granules, have been described in the vitellogenic oocytes of *Liopsetta obscura* and *Clupea pallasii* (Yamamoto 1956) and *Carassius auratus* (Khoo 1979). In *Brachydanio rerio* these inclusions were designated as travesicular and intravesicular (Malone and Hisoaka 1963). However, the occurrence of two types of yolk is not universal among teleosts. In some species, three types of yolk have been observed. Yamamoto (1956) described yolk vesicles, yolk globules and lipid globules in *Hypomesus japonicus*, Guraya (1965) noted three types of yolk in *Channa marulius*, while Mayer *et al.* (1988) described lipid yolk, protein yolk and carbohydrate yolk in *Dicentrarchus labrax*. Similar to the observations of Mayer *et al.* (1988), three types of inclusions are formed during vitellogenesis in *P. argenteus*, *viz.* lipid yolk droplets, protein yolk granules and carbohydrate yolk. These inclusions differ distinctly in their morphology, tinctorial affinities and rhythm of deposition.

In *P. argenteus*, lipid yolk, in the form of distinct lipid droplets, is the first type of yolk inclusion to accumulate in vitellogenic oocytes. The appearance of these endogenous lipid droplets has been considered to mark the start of vitellogenesis (Shackley and King 1977). The accumulation of lipid yolk prior to that of protein yolk is common in most teleosts studied (see review by Wiegand 1982).

Protein yolk accumulation occurs after lipid yolk accumulation in *P. argenteus*. Protein yolk, exogenous in origin, is sequestered in the form of discrete granules and become the predominant yolk inclusion. While the protein yolk maintain their structural integrity at maturation, the lipid droplets, on the other hand, coalesce and migrate centripetally. The protein yolk granules actively coalesce only after maturation. Similar observations, about the fate of the lipid yolk droplets and protein yolk granules, were reported by Mayer *et al.* (1988).

The typically small vesicles (cortical alveoli), termed carbohydrate yolk (Raven 1961), close to the oocyte periphery as reported by Dadzie (1974), Bromage and Cumaranatunga (1988) and Coward and Bromage (1998) were not revealed by the PAS-test in the oocytes of *P. argenteus*. PAS-positive reaction, indicating the presence of carbohydrate, was detected only in the *zona radiata*, similar to the findings of Shackley and King (1977) in *Blennius pholis* and Mayer *et al.* (1988) in *Dicentrarchus labrax*.

Acknowledgements

We are very grateful to Kuwait University for funding this project under Research Grant No. SZ041. Mrs. Jalaja V. Sukumaran provided excellent technical assistance. Mr. Maher A.R. Mohammed assisted with sample collection, while Dr. Majed Alnaqeeb kindly provided photomicrography facilities.

References:

Abou-Seedo, F. and Al-Khatib, H. (1995) A histological and macroscopic study of ovarian development in the grey mullet, *Liza carinata* (Valenciennes 1836). *Journal of the University of Kuwait* (Science), **22:** 239-254.

Abu-Hakima, R., Al-Abdul-Elah, K.M., El-Zahar, C. and Akatsu, S. (1983) The reproductive biology of *Pampus argenteus* (Euphrasen) (Family Stromateidae) in Kuwait waters. Technical Report MB25, Food Resources/ Mariculture and Fisheries Department, Kuwait Institute for Scientific Research, 31 pp.

Ahsan, S.N. (1966). Effects of temperature and light on the cyclical changes in the spermatogenetic activity of the lake chub, *Couesius plumbeus* (Agassiz). *Canadian J. Zool.*, **44:** 161-171.

Ali, T.S. and Mahmood, A.R. (1993) Growth, mortality and stock assessment of silver pomfret *Pampus argenteus* (Euphrasen) in north western Arabian Gulf. *Ipa Journal of Agricultural Research*, **3:** 18-32.

Al-Shallal, T. (1998) Histological changes during the reproductive cycle and the biology of reproduction in silver pomfret, *Pampus argenteus* (Euphrasen) in Kuwait waters. M.Sc. Thesis, Univ. of Kuwait, 194 pp. (Unpublished).

Asahina, K. and Hanyu, I. (1983). Role of temperature and photoperiod in the annual reproductive cycle of the rose bitterling, *Rhodeus ocellatus ocellatus. Bulletin of Japanese Society of Scientific Fisheries*, **49:** 61-67.

Bromage, N.R. and Cumaranatunga, P.R.T. (1988) Egg production in the rainbow trout. In *Recent Advances in Aquaculture* (Muir, J.F. and Roberts, R.J., eds). pp. 65-138. London: Croom Helm.

Coward, K. and Bromage, N.R. (1998) Histological classification of oocyte growth and the dynamics of ovarian recrudescence in *Tilapia zillii J. Fish Biol.* **53:** 285-302.

Dadzie, S. (1974) Oogenesis and the stages of maturation in the female cichlid fish, *Tilapia mossambica. Ghana J. Sci.* **14:** 23-21.

Dadzie, S., Abou-Seedo, S. and Al-Shallal, T. (1998) The onset of spawning in the silver pomfret, *Pampus argenteus* in Kuwait waters and its implications for management. *Fisheries Management and Ecology.* **5:** 501-510.

Dadzie, S. and Owiti, D.O. (1998) Histological changes in the ovaries associated with the reproductive cycle of the catfish, *Clarias mossambicus* (Peters). *Kenya J. Sci. Techn.* **12:** 69-82.

Davis, P. and Wheeler, A. (1985) The occurrence of *Pampus argenteus* (Euphrasen 1788), (Osteichthyes, Perciformes, Stromateoidei, Stromateidae) in the North Sea. *J. Fish Biol.* **26:** 105-109.

Drury, R.A.B. and Wallington, E.A. (1980). Carleton's Histological Technique. Fifth edition. Oxford University Press.

Dwiponggo, A. (1984) Notes on the demersal fishery of the Java Sea and population parameters of some demersal species. *FAO Fisheries Report.* **318:** 58-71.

Forberg, K.G. (1982) A histological study of development of oocytes in capelin, *Mallotus villosus* (Muller). *J. Fish Biol.* **20:** 143-154.

Foucher, R.P. and Beamish, R.J. (1980) Production of nonviable oocyte by Pacific hake (*Merluccius productus*). *Can. J. Fish. Aquat. Sci.* 37: 41-48.

Guraya, S.S. (1965) A comparative histochemical study of fish (*Channa maruleus*) and amphibian (*Bufo stomaticus*) oogenesis. *Z. Zellforsch.* **65:** 662-700.

Higashikawa, S. and Masumitsu, S. (1976) On the white pomfret of the East China Sea II. Relation between the oceanographical condition and distribution of the white pomfret in the south-west region of the East China Sea. *Memoirs of the Faculty of Fisheries, Kagoshima University, Kagoshima.* **25:** 181-191.

Hussain, N.A. and Abdullah, M.A.S. (1977) The length-weight relationship, spawning season and food habits of six commercial fishes in Kuwait waters. *Indian J. Fish.* **24:** 181-194.

Khoo, K.H. (1979) The histochemistry and endocrine control of vitellogenesis in gold fish ovaries. *Can. J. Zool.* **57:** 617-626.

Kim, Y.M. and Lee, D.W. (1992) Stock assessment of silver pomfret, *Pampus argenteus* (Euphrasen), in Korean waters. *Bulletin of National Fisheries Research and Development Agency (Korea).* **46:** 41-51.

Luo, B., Lu, J., Lan, Y., Huang, S., Zhu, D and Kong, X. (1993) Population dynamics and life history patterns or main marine fishes in the coastal waters of China. *Studia Marina Sinica/Haiyang Kexue Jikan. Qingdao.* **34:** 123-138.

Maddock, D.M. and Burton, M.P.M. (1999) Gross and histological observations of ovarian development and related condition changes in American plaice. *J. Fish Biol.* **53:** 928-944.

Malone, T.E. and Hisaoka, K.K. (1963) A histochemical study of the formation of deutoplasmic components in developing oocytes of the zebrafish, *Brachydanis rerio J. Morphol.* **112:** 61-76.

Mayer, I., Shackley, S.E. and Ryland, J.S. (1988) Aspects of the reproductive biology of the bass, *Dicentrarchus labrax* L. I. An histological and histochemical study of oocyte development. *J. Fish Biol.* **33:** 609-622.

Mito, S. and Senta, T. (1967) On the egg development and prelarval stages of silver pomfret with reference to its spawning in the Seto Inland Sea. *Bulletin of the Japanese Society of Scientific Fisheries.* **33:** 948-951.

Mohamed, A.M. and Ali, T.S. (1992) Fishery and ecology of silver pomfret, *Pampus argenteus* (Euphrasen) in the north-western Arabian Gulf. *Ipa Journal of Agricultural Research.* **2:** 271-283.

Morgan, G.R. (1985) Stock assessment of the

pomfret (*Pampus argenteus*) in Kuwait waters. *J. Cons. Int. Explor. Mer.* **42:** 3-10.

Owiti, D.O. and Dadzie, S. (1989). Maturity, fecundity and the effect of reduced rainfall on the spawning rhythm of a siluroid cat fish, *Clarias mossambicus* (Peters). *Aquaculture and Fisheries Management.* **20:** 355-368.

Raven, C.P. (1961) *Oogenesis: The Storage of Development Information.* London: Pergamon Press.

Shackley, S.E. and King, P.E. (1977) Oogenesis in a marine teleost, *Blennius pholis* L. *Cell Tissue Res.* **181:** 105-128.

Summers, D.W. (1996). Environmental influences on the timing of Atlantic salmon, *Salmo Solar* L., in the River North Esk. *Fisheries Management and Ecology*, **3:** 281-283.

Webb, J. and Hawkins, A.D. (1989). The movements and spawning behaviour of adult salmon in the Girnock Burn, a tributary of the Aberdeenshire Dee. *Scottish Fisheries Research Report*, **40:** 1- 41.

White, T.P. (1985) Marine fisheries resources survey demersal trawling. Survey Cruise Report No. **5.** 1984. FAO/UNDP. Chittagong (Bangladesh). 62 pp.

Wiegand, M.D. (1982) Vitellogenesis in fishes. In *Proceedings of International Symposium on Reproductive Physiology of Fish.* (Richter, C.J.J. and Goos, J.J. Th., eds). pp 136-146. Wageningen: Pudoc.

Yamamoto, K. (1956) Studies on the formation of fish eggs. I. Annual cycle in the development of ovarian eggs in the flounder, *Liopsetta obscura. J. Fac. Sci. Hokkaido Univ.* **12:** 362-373.

(Received 29/05/1999 In revised form 04/11/2000)