OOGENESIS IN THE WEDGE-SHAPED CLAM, *DONAX VITTATUS* (DA COSTA, 1778) (BIVALVIA: DONACIDAE) INHABITING GAMASA COAST, MEDITERRANEAN SEA, EGYPT

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ABSTRACT

In the present study, ovarian maturation in *Donax vittatus* inhabiting Gamasa sandy beach was followed for about two years (between 1997 and 1998) by means of histological methods. *D. vittatus* is proved to be a gonochoric species lacking hermaphrodite phase. The oogenesis was synchronous between the gonad acini (follicles). Several stages of the oocyte development could be observed in a single gonad and the gonad could be cycled from ripe to partially spawned within a short period. The sex ratio obtained was 1: 1.5, where females represent about 40% of the population. The studied species showed a clear seasonality in its oogenetic cycle, where maturation agreed with spring season (March to June): spawning coinciding with the highest levels of temperature during summer (between late June till September). Spawning activity was decreased by the end of summer (September): the rest period appeared mostly during autumn (from the emission of ova till November) which agreed with the lowest levels of temperature; and recruitment (reactivity) occurred during winter months (December till February). The successive phases of oogenesis have been described in detail. The recognition of these phases were based mainly on cell size, shape, nucleus, nucleolus, cytoplasm granulation and staining characteristics. An additional associated cells of irregular forms, founded in close contact with the advanced phases, have been described for the first time in *Donax* species. The reproductive pattern was discussed in the light of factors controlling oogenesis in other species of *Donax* and some other bivalves.

INTRODUCTION

The individuals of *Donax* species are mostly found in tropical waters and are confined within distinct geographical regions. There are no examples of cosmopolitan distributions [Ansell (1983)]. *Donax vittatus* inhabits sandy bottoms with abundant organic matter [Ansell & Lagardere (1980)]. This species is an Atlantic-Mediterranean warm-temperate species, recorded from the French coast and Scotland [Ansell & Bodoy (1979)] and extending as far north till the coasts of Norway [Ansell & Lagardere (1980)] and from the intertidal sandy beaches of N.W. Africa and Mediterranean Sea [Ansell (1972); Ansell *et al.*, (1980); Abbott & Dance (1982); Neuberger-Cywiak *et al.*, (1990); and Ramón *et al.*, (1995)]. Regarding the geographical occurrence in Egypt, it is highly abundant in Gamasa beach (Dakahlia Province, Mediterranean Sea). *Donax* is known in Egypt by the common name "Om-Olkholool" and its soft parts represent an important sea-food for people inhabiting coastal shores of Egypt. It is an excellent burrower and this makes it well adapted to inhabit the swash zone.

Previous studies on the gonad maturation, life cycle and reproduction of the genus *Donax* were given by [Rao (1967); Wade (1967&1968); Badino & Marchionni (1972); Mouëza & Frenkiel-Renault (1973); De Villiers (1975); Nagabhushanam & Thalikhedkar (1977); Ansell & Bodoy (1979); King (1985); Velez *et al.* (1985); Tirado & Salas (1998); Gaspar *et al.*, (1999) and later by El-Refaay (2000)]. Oogenesis in bivalves has been studied by many authors. Loosanoff (1937) recorded the sexual phases in *Venus*; Cleland (1947) described the oogenesis in the Sydney rock oyster *Ostrea*; Tranter (1958) and Lee (1972) studied the gametogenesis in *Pinctada*; Dinamani (1974) investigated the gonadial changes in *Crassostrea*; and later the gametogenesis of *Scrobicularia* was reported by Rodriguez-Rúa *et al.* (2003). The ultrastructure of the ovary and gametogenesis has been previously studied in *Mytilus* by Herry and Le Pennec (1987), in *Crassostrea* by Eckelbarger and Davis (1996), in *Amiantis* by Al-Mohanna *et al.* (2003), and more recently in *Chamelea* by Erkan (2004). In Egypt, few studies were made on the reproduction in edible bivalves [Eisawy (1974) and El-Refaay (2000 & 2006)].

The determination of the actual breeding time of the studied species is the main target of the present investigation, upon which legal actual regulation of catching could be established. The present study was

planned to give spotlight on the ovarian cyclic stages and oogenesis in such edible bivalve by means of histological techniques. Some of these stages could not be detected by paraffin sections technique. By using semi- thin sections, developing phases of oocyte and cytoplasmic details (granularity and oocyte fragmentation) can be identified. Special interest has been given to the characters of each generative phase of the oogenesis.

MATERIAL AND METHODS

Specimens of *Donax vittatus* (da Costa. 1778) (Mollusca: Bivalvia: Donacidae) were collected monthly from the Mediterranean sandy beach at Gamasa area (Long. 31° 30 ′ N; Lat. 31° 30 ′ E), Dakahlia Province- Egypt. Samples were collected from a depth between 0.5 and 1.5 m by a small framed trawling net (with a mesh size 10 x 10 mm). At least 15 specimens ranging from 2 cm to 3 cm long were collected monthly and transported to the laboratory where they were reared in a well aerated glass aquarium containing filtered and freshly seawater. A piece of the gonadial tissue was taken from the upper region of the foot mass in which it is completely embedded. The tissue was immediately fixed in 10% neutral formalin. dehydrated in ascending series of alcohol, cleared in xylene, embedded in paraffin wax. sectioned at about 5 μm., and double stained with Harriss alum haematoxyline and eosin.

For preparation of semi-thin sections. small fresh pieces of the gonadial tissue were fixed in 2.5% glutraldehyde buffered in 0.1 M sodium cacodylate (pH 7.4) for about 4 hours at 4 °C, washed in the same buffer for about one hour. post-fixed with 1% osmium tetroxide (in sodium cacodylate buffer) at 4 °C for 2 hours and washed again in buffer for one hour. After dehydration in ethanol gradients, specimens were transferred into propylene oxide for 30 minutes to ensure complete dehydration, and finally embedded in LR white Resin and left at room temperature for 16 hours (Sousa and Oliveira. 1994). Semi-thin sections (0.5- 1.0 μm.) were cut using LKB III ultratome. Toluidine blue (1% hot. in 1% borax) was used to stain the sections, then washed in tap water, dried on a hotplate. mounted in Canada balsam and photographed using Dialux 20 EB Leitz photo-research Microscope.

RESULTS

I. Annual ovarian reproductive cycle

Investigation of monthly prepared stained sections of female gonads of *D. vittatus* revealed that the reproductive cycle includes five stages of gonadal development (Fig. 1). These successive stages are:

(i) Early active stage (multiplication stage): It appears at the end of autumn and the beginning of winter (in December and January). where large number of primordial germ cells (c) were observed to aggregate in circles (Fig. 1A).

(ii) Late active stage (growth stage): During winter (in February). the developing oocytes (oc) increase in number, size and arranged in what is called ovarian lobes. alveoli or follicles (of) (Fig. 1B).

(iii) Ripe stage (Maturation): Through spring (from March to June). mature oocytes (mo) were arranged peripherally on the follicular wall (Fig. 1C). Each oocyte was enveloped by eosinophilic coat and attached to the follicular wall by a stalk (st). The other phases could hardly be detected beside the mature oocytes. The percentage of ripening reaches its maximum value during April and June.

(iv) Partially spawned stage (emission state): During summer (from July to September). the ovarian follicles became smaller, interfollicular tissue (ift) was developed and few ova (off) were left free in the lumen (I) of the follicle (Fig. 1D). During August, the frequency of spawning rises to its highest level.

(v) Spent (or rest) stage: During autumn (from September to November), the ovarian follicles (of) were shrunken and the interfollicular tissue (ift) was well developed and conspicuous (Fig. 1E).

II. OOGENESIS

Examination of semi-thin sections. stained with toluidine-blue, through a part of ripe ovary, revealed different generative phases of oocytes fastened closely to the inner wall of each follicle (Fig. 2 and illustrated in Fig. 3). These phases could be differentiated into three main forms:

(a) **The oogonia** (oo): These cells lie at the peripheral region of the follicle in close contact with the basal germinal membrane (Fig. 2A). Each oogonium is oval in shape and measures about 4 x 2 µm.

The nucleus appears as dense chromatin patches or granules, filling most of the cytoplasm and measures about 3 x 1.5 μm. No remarkable inclusions were observed in the cytoplasm, which appears as a clear thin layer.

(b) Auxocytes: These types of cells were defined by Cleland (1947) as oocytes before entry into growth phase. Depending on the cell size, shape. nuclear features, granulation and staining characters of the cytoplasm. Five types of auxocytes were differentiated:

- **Auxocyte 1** (a1): It is slightly rounded, measures 6-8 μm in diameter and contains large spherical nucleus (Figs. 2A & 2B). The nucleoplasm is filled with homogenously distributed densely-stained granules of different sizes, which may represent chromatin patches.

- **Auxocyte 2** (a2): It is nearly oval in shape and measures 9 x 6 μm (Figs. 2A & 2B). The nucleus (n) is rounded and measures about 4 μm in diameter. The nucleoplasm is homogenous and appears to be more densely-stained than the cytoplasm. A small peripheral nucleolus was observed. The area of the cytoplasm is greater than that of a1 and few scattered small granules(g1 & g2) were observed inside it.

- **Auxocyte 3** (a3): This type of cell is oval in shape and greater in diameter than the previous type (a2), where it measures about 11 x 7 μm. It contains a rounded nucleus (measuring about 6 μm in diameter), with a conspicuous peripheral small nucleolus (Fig.2B). Small scattered chromatin patches are obvious in the nucleoplasm. The granules in the cytoplasm become more distinct and can differentiated into two main sizes. small (g1) and large (g2) granules.

- **Auxocyte 4** (a4): This oocyte is nearly spherical in outline and measures about 14 μm (Fig. 2B). The nucleus is nearly rounded (6 μm in diameter), densely-stained by the toluidine blue and lies near the central region of the oocyte. The nucleolus (nu) is nearly rounded. measures about 2 μm in diameter and is more densely stained than the nucleus. The nucleoplasm appears homogeneous, without differentiated chromatin patches, and stains darker than the cytoplasm. The latter contains the two sizes of granules (g1 and g2). which exceeds than those of the previous phase.

- **Auxocyte 5** (a5): This oocyte type is conical in shape and measures about 27 x 24 μm (Fig. 2D). It is attached to the follicular

wall by means of a small stalk (st). It contains a comparatively larger rounded nucleus (11 μm in diameter), which possesses a conspicuous rounded darkly stained nucleolus (6 μm in diameter). The nucleoplasm is composed of homogeneous lightly-stained material in which are embedded few densely-stained fine granules. These granules are aggregated peripherally near the nuclear membrane. The cytoplasm contains scattered amount of small granules (g1) and large granules (g2). Few vacuoles (v) were observed in the cytoplasm, particularly at its peripheral region. At this stage, the oocyte is coated by a thin layer of densely-stained granular material and defined primarily as a chorion (ch), Fig. 2D.

A characteristic feature of this stage (a5), is the presence of small basal follicular cells (bfc) connecting the base of the oocyte to the follicular wall. Each cell has a single spherical nucleus with chromatin patches and its cytoplasm contains fine granules stained slightly with the toluidine blue. Another type of cells was detected in close contact with the lateral regions of the stalk and named as lateral follicular cells (lfc). Each lateral follicular cell has also a single spherical nucleus with chromatin patches, but its cytoplasm is lightly-stained with toluidine blue. The basal and lateral follicular cells were not observed in other regions of the follicular wall.

(c) **Mature oocyte** (mo): This type of oocyte appeared in two forms: most of them are ovale in shape (about 30 μm long) and few are elongated (reach about 45 μm long). The mature oocyte has a prominent stalk (st) at the vegetal pole where the oocyte is presumably attached to the ovarian follicle (Fig. 2E). Later, it becomes free in the lumen of the ovarian follicle.

The oval-shaped oocyte (Fig. 2E) measures 30 x 20 μm and contains a comparatively large nucleus, with lightly stained nucleoplasm and measuring 15 x 11 μm. The nucleus has a nearly rounded conspicuous and homogeneously densely-stained nucleolus (5 μm in diameter). Few densely-stained granules (p) were detected peripherally inside the nucleoplasm (Fig. 2E). The cytoplasm is filled with great number of granules (g1 & g2). Each mature oocyte is enveloped by a gelatinous coat (chorion) consisting of two layers, an outer dense thin layer (dl) and an inner slightly thicker layer of lightly-stained material (bl).

The nurse cells (nc): They are additional type of irregularly-shaped cells, located in the ovarian follicles in close contact with the germinal membrane (Fig. 2C). Each cell measures about 10 μm long and its nucleus is irregular in shape (about 2 μm), and contains a conspicuous nucleolus . The cytoplasm is filled with densely-stained granules of different sizes (g1 & g2). in addition to few small vacuoles (v). The surface of this type of cells appears as a dense layer, from which processes-like structures were detected. In some nurse cells, this coat has been showed ruptured and through which granules appeared to be free into the lumen of the ovarian follicle. Some of these granules are also seen attached to the outer surface of these nurse cells.

The structures and characters of the different phases of oogenesis are illustrated in Figure 3 and Table 1 respectively.

DISCUSSION

Specimens of *Donax vittatus* were difficult to maintain alive in the laboratory except for few days. Therefore, monthly collection is obligatory. El-Refaay (2000) recorded that the studied species at the same area attains its first sexual maturity at age of one year, for individuals of more than 1.4 cm in shell length. Thus the lengths of specimens collected for this study were ranged from 2-3 cm long. These results agree with Alagarswami (1966) who found that *Donax faba* appears to become sexually mature when the clams reach 1.3-1.4 cm within about one year. Mouëza and Frenkiel-Renault (1973) reported the length of the first maturity in *Donax trunculus*, collected from the Algerian coast, as 1.6 cm. Gaspar *et al.* (1999) founded that *D. trunculus* reached maturity during the first year of life in specimens of 1.3 to 2.1 cm shell lengths.

The sex ratio obtained by El-Refaay (2000) in *Donax vittatus* populations from Gamasa coast was found to be about 1: 1.5 (in favour of males) in most of the year. Lucas (1965), Badino and Marchionni (1972), Mouëza and Frenkiel-Renault (1973) mentioned that males of *Donax trunculus* usually show a slightly higher proportion but without any statistically significant difference. Recently, Gaspar *et al.* (1999) attributed the overcoming proportion of males of *D. trunculus* to the differential growth of both sexes and differential mortality probably as a result of spawning effort.

The prolonged breeding habit may characteristic of bivalves that colonize sand beaches in regions where the annual temperature range is small [Redfearn (1974)]. Coe (1955) also recorded that *Donax gouldi* at La Jolla. California (Temp. 17± 4°C) bred from April to November. Alagarswami (1966) recorded that the breeding season for *Donax faba* on the beaches around Mandapam in India (Temp. 28± 2°C) extends over eight months from November to June. Rao (1967), working on *Donax cuneatus* on the Madras coast of India (Temp. 29± 3°C), recorded that the breeding season was for seven months from December to June. Wade (1968) finds that *Donax denticulatus* breeds throughout the year in the West Indies. where the temperature was about 28°C, with peak activity from November to January. Ansell and Bodoy (1979) stated that the Mediterranean species of both *Donax vittatus* and *Donax trunculus* show a longer reproductive period. Gaspar *et al.* (1999) observed that *D. trunculus* (collected from Southern Portugal) exhibited a similar prolonged breeding period. its cycle began in late November and the spawning occurred in summer (between late March and late August).

The sequence of these various stages in the studied species agrees in general lines with that known for other bivalves in the same area. such as *Tellina tenuis* from Italy [Ansell & Trevallion (1967)], *Venerupis aurea* from France [Gallois (1977)], *Venerupis decussata* from Italy [Breber (1980)]. *Perna picta* from the Atlantic coast of Morocco [Shafee (1989)]. and *Scrobicularia plana* from Spain [Rodriguez-Rûa *et al.* (2003)].

The estimated spawning time agrees with the appearance of free mature ova inside the ovarian lumen. The present authors find that D. *vittatus* increases its breeding activity from July to September (summer season). where the water temperature at higher levels. Many authors suggested that the time of spawning is probably take place when the water temperature is on the ascent [Loosanoff (1937); Nayar (1955); Rao (1967) and Dinamani (1974)]. Wilson and Hodgkin (1967) and Ropes (1968) suggested that temperature was not clearly a stimulus to spawning. but other factors must be important. Nayar (1955) finds that the Indian *Donax cuneatus* begins to spawn in January after a sudden drop in salinity as a result of heavy rainfall. The importance of hormonal control has been suggested by Ropes (1968), but reviewed by Giese (1959) and Galtsoff (1961). Later on, Redfearn (1974), Ansell and Bodoy (1979), Hadfield and Anderson (1988) and Shafee (1989) agree that the

reproductive cycle of most bivalves associated with the increase in water temperature and food availability in the form of phytoplankton.

Semi-thin sections through a part of ovary of *Donax vittatus.* in ripe phase using toluidine blue stain, revealed some detail structures of the follicular content:

a. A gelatinous jelly coat encloses either auxocyte 5 or mature oocyte. This coat is distinguished into an outer dense layer of fine granules (Fig. 2E, dl) and a slightly thicker layer of lightly-stained material (Fig. 2E, bl). This coat is similar in structure to that founded in primary oocyte of *Haliotis* [**Young & De Martini (1970)**] and in oocyte of *Tresus* [**Machell & De Martini (1971)**] and named as chorion.

b. Few densely-stained granules were detected peripherally inside the nucleoplasm of the mature oocyte (Fig. 2E, p). Similar observations were recorded by Subramaniam (1937) on *Meretix casia.* who claimed that nucleolus forming buds which attached to the nuclear membrane and diffused out. Reverberi (1971b) defined a large nucleolus forming buds in the latter stages of oocyte development in *Dentalium* but lacking in mature oocyte. The appearance of two parts of nucleolus in some auxoxyte 5 and mature oocytes may presumably to be polar bodies.

c. The mature oocyte was observed in either flattened or in lobed condition. Similar observation was recorded by Young and De Martini (1970) in primary oocyte of *Haliotis*.

d. The present study observed the appearance of small granules in the cytoplasm of auxocyte 2, which become gradually increase in number till become numerous in the mature oocyte. Another larger size of such granules were also detected in the cytoplasm of auxoxyte 3, which multiply to become numerous in the late oocyte. Gallois (1977) detected such granules in his study on the oogenesis of *Venerupis aurea* and declared that young oocytes forming vitellogenesis. Recently, the ultrastructural studies made by Eckelbarger and Davis (1996) on *Crassostrea virginica*, Pal and Hodgson (2002) on *Siphonaria* sp. and later on by Erkan (2004) on *Chamelea gallina* supported vitellogenesis process.

e. Another form of granules were detected, in this study, either on the outer surface area of some generative phases and nurse cells or scattered around them. Such granules, of dense and deeply staining, were named previously by Loosanoff (1937) and Cleland (1947) as

phagocytic cells, and named by Tranter (1958) as degenerating leucocytes. Young and De Martini (1970) expected that these cells probably have a role in necrosis. Machell and De Martini (1971) detected such leucocytes which were common in spent gonad. This allows us to consider them to be associated with cytolysis of the residual gametes.

f.   In this study, small spherical vacuoles were detected in the cytoplasm of auxocytes 5, mature oocytes and nurse cells. Young and De Martini (1970) stated that such vacuoles may possibly containing fatty yolk and increase numerically as the oocyte enlarges. Similar structures were detected by Reverberi (1971a & b) in *Mytilus* and *Dentalium* oocytes. Reverberi considered that these vacuoles may be either lipid drops, mitochondria, pigment granules, yolk granules or vesicular bodies contribute to the constitution of the cytoplasm.

g.   The three types of polygonal cells described in this work, named basal follicular cells, lateral follicular cells and nurse cells, are secondary formed and not observed before the appearance of auxocytes 5. This may detect their role in nourishment and fulfill the ocgenesis process.
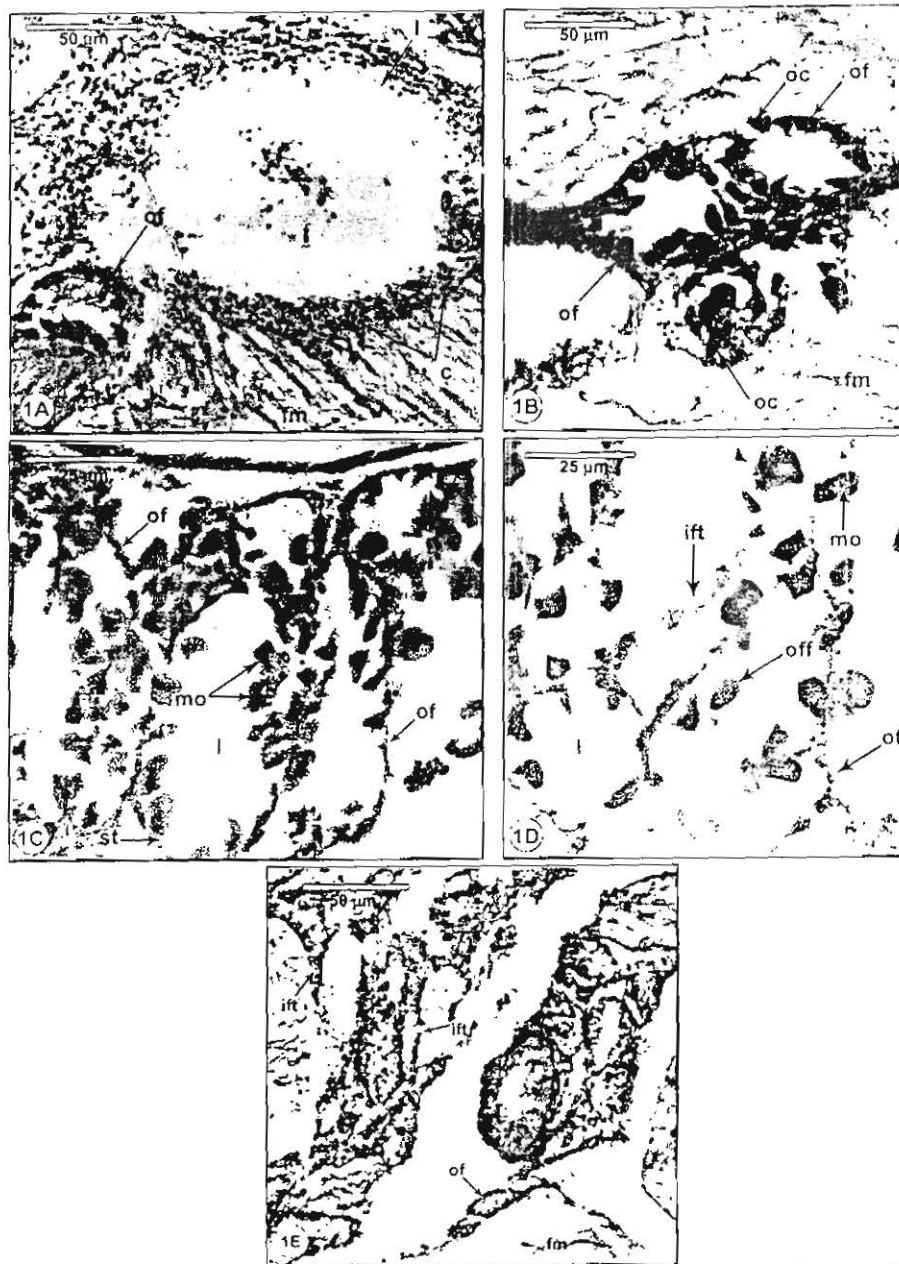
Fig. 1: Photomicrographs of paraffin sections showing the developmental changes in a part of ovary of female specimen of *Donax vittatus.*
(1A): Early active stage; (1B): Late active stage; (1C): Ripe stage; (1D): Partially spawned stage; (1E): Spent stage. Primordial germ cells (c); foot muscles (fm); interfollicular tissue (ift); lumen (l); mature oocytes (mo); oocytes (oc); ovarian follicle (of); free ova (off); stalk (st). Haematoxylin and eosin (double stain).
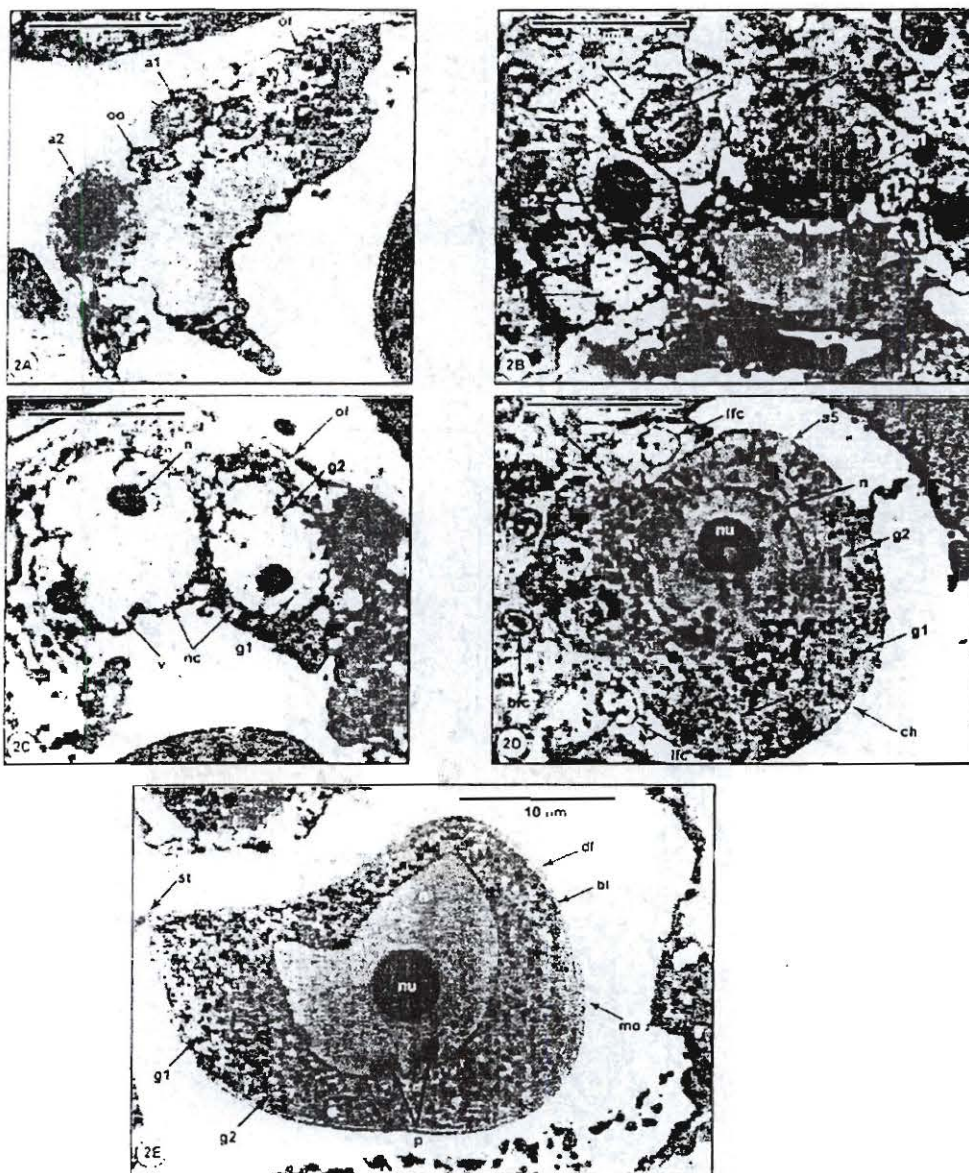
Fig. 2: Photomicrographs of semi-thin sections through a part of ripe ovary, showing the Generative phases of oogenesis in *Donax vittatus*.
(2A): showing oogonia (oo), auxocyte 1 (a1), auxocyte 2 (a2), ovarian follicle (of);
(2B): auxocyte 3 (a3), auxocyte 4 (a4), small granules (g1), large granules (g2), nucleus (n), nucleolus (nu);
(2C): nurce cells (nc); (2D): auxocyte 5 (a5), basal follicular cell (bfc), chorion (ch), lateral follicular cell (lfc), stalk (st), vacuoles (v); (2E): close up on mature oocyte (mo), dense layer (dl), bright layer (bl), polar bodies (p). Toluidine blue stain.
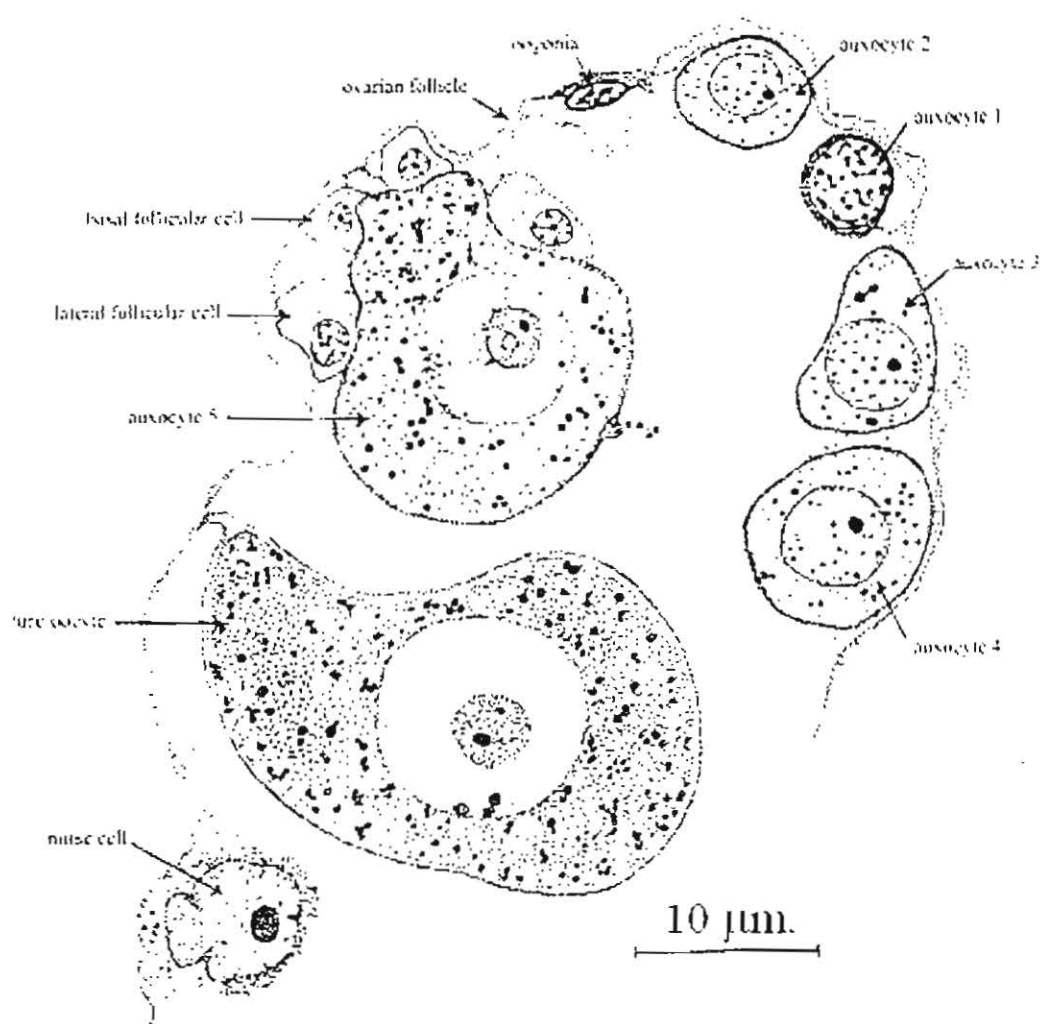
Fig. 3: Schematic diagram showing the different phases of oogenesis inside the ovarian follicle of *Donax vittatus*

Table 1. Characteristics of the different phases of oogenesis in *Donax vittatus*

Phase / Character	Oogonium (oo)	Auxocyte 1 (a1)	Auxocyte 2 (a2)	Auxocyte 3 (a3)	Auxocyte 4 (a4)	Auxocyte 5 (a5)	Mature oocyte (mo)
Shape	Oval	Rounded	Oval	Triangle	Rounded	Conical	Elongated
Size (μm)	4 x 2	6-8	9 x 6	11 x 7	14	27 x 24	35 x 20
Nucleus (n) shape	Elongated	Large-rounded	Medium-rounded	Medium-rounded	Large-rounded	Large-rounded	Large-rounded
Nucleolus (nu) position	—	—	Peripheral	Peripheral	Near the center	Near the center	Near the center
Small granules (g1)	—	—	Few	Few	Plentiful	Numerous	Very numerous
Large granules (g2)	—	—	—	Scanty	Few	Numerous	Very numerous
Stalk (st)	—	—	—	—	—	Small	Small

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التبويض في المحار الأسفيني دوناكس فِيتاتس (داكوستا-١٧٧٨)
(ذوات المصراعين: دوناسيدى) المستوطنة لشاطئ جمصة –
البحر الأبيض المتوسط – مصر

عادل نجيب جرجس – محمد محمد محمد النجار – شريف رمضان الرفاعى

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تم متابعة نضوج المناسل الأنثوية في محار دوناكس فِيتاتس قاطن الشاطئ الرملى لجمصة لمدة عامين (مابين ١٩٩٧ – ١٩٩٨) بالطرق الهستولوجية. وهذا المحار من النوع الذى يمكن التفريق المنسلى فيه ولم يلاحظ له أية حالة خنثوية. وتكوين مراحل البويضات المختلفة من حدوث النضوج وتحرر البويضات تتم في آن واحد وتنتهى في فترة زمنية قصيرة بين الحويصلات المنسلية بحيث يمكن معاينة معظم مراحل نمو الخلية البيضية في مبيض واحد. كانت نسبة الشق الجنسى ١ : ١,٥ ، حيث تمثل الإناث حوالى ٤٠% من الجماعة. ويظهر في دورة تكوين البويضات في هذا الحيوان تمييز موسمى واضح حيث يتفق النضوج مع موسم الربيع (من مارس حتى يونيو) ، وتتحرر البويضات مع إرتفاع درجات الحرارة أثناء الصيف (بين أواخر يونيو وحتى سبتمبر) ، وفترة الراحة غالبا ماتكون في فصل الخريف (منذ تحرر البويضات وحتى نوفمبر) والتى تطابق الانخفاض في درجات الحرارة ، وحدثت فترة الإنتعاش في نشاط المبيض أثناء شهور الشتاء (من أواخر ديسمبر وحتى فبراير). تم إيجاز وصف دقيق لمراحل تكوين البويضات المتتابعة بالإستعانة بطريقة التقطيع نصف الرقيق. وقد اعتمد التمييز بين هذه المراحل على حجم وشكل الخلية البيضية ، شكل النواة والنوية ، تحبب السيتوبلازم وخواص الصبغ. ولأول مرة تم وصف خلايا مرافقة إضافية غير منتظمة الشكل وجدت على إتصال وثيق بالمراحل النهائية للبويضة. وعلى ضوء العوامل التى تتحكم في تكوين البويضات تمت مناقشة هذا النمط من التكاثر مع الأنواع الأخرى من نفس الجنس (دوناكس) ومع بعض ذوات المصراعين الأخرى.