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Storage tissue and reproductive strategy in *Lucina pectinata* (Gmelin), a tropical lucinid bivalve adapted to a reducing sulfur-rich, mangrove environment

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Summary

The large Lucinidae *Lucina pectinata* (Gmelin) is a dominant species in the most confined areas of mangrove swamps where it lives deeply burrowed in reducing mud. In most female individuals, the gonads of this protandric species are in an apparently permanent state of maturity, having a various proportion of small previtellogenic oocytes together with larger oocytes undergoing vitellogenesis and full-grown oocytes which are characterized by their thick jelly coat. Oocyte resorption is also frequent in these gonads. Such a maturation schedule results in a continuous reproductive competence. Resorption of spermatozoa and simultaneous oogonia multiplication take place in spent male gonads allowing for a progressive sex change. In spent and developing stages, gonad-wall cells constitute a thick pseudo-stratified epithelium which gets progressively thinner with gonad maturation and thickens again soon after spawning. The complex cycle of these follicular cells is the result of two complementary functions: storage of lipid, protein and carbohydrate compounds ready for transfer to maturing oocytes, and resorption of degenerating oocytes through a peculiar lysosomal activity, demonstrated by cyto-enzymological identification. Having permanently mature gonads ready for spawning is a reproductive strategy of *L. pectinata* which, in addition to gill sulfur-oxidizing bacterial endosymbionts and high bacteriocyte hemoglobin concentration, is adapted to the high-stress environment of mangrove swamps. Resorption of oocytes and recovery of metabolites through the follicular cell lysosomal function appears to be the most efficient means to minimize the metabolic cost of maintaining a permanent state of maturity.

Key words: Mangrove, tropical bivalve, reproductive biology, histology, ultrastructure, cyto-enzymology

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Introduction

Most studies of reproductive activity of bivalves concern temperate species of economical value, mainly oysters and mussels (for a review, see Sastry, 1979). These species have a seasonal reproductive strategy dependent on the development of specific storage cells. More recently species from various environments such as tropical and deep-sea areas have been studied. Their reproductive strategies and storage patterns are more diversified than expected (Lubet and Mann, 1987). According to Mathieu and Lubet (1993), many types of somatic cells are involved in the storage and mobilization of nutrients. The main types are specific storage cells as in various mussels and oysters, muscular cells as in pectinid clams, and intragonadal epithelial cells as in some venerid clams. Storage tissue has been considered to be an essential feature for marine bivalves to overcome variable environmental conditions as regards temperature fluctuations, variations in food availability, or competitive and predatory pressure. This storage tissue is generally associated with a seasonal reproductive strategy. However, many bivalve species live in areas with very little temperature fluctuation or variation in food resources. They usually possess a continuous reproduction strategy whose storage requirements are quite different from those species with a seasonal reproduction. Among those bivalves which rely on symbiotic bacteria for a large part of their nutritional requirement, very little is known of their reproductive strategy. The temperate Lucinidae, *Loripes lucinalis*, living in shallow water, has seasonal reproduction (Ollivier, 1972; Johnson and Le Pennec, 1994). Information on the reproduction of such bivalves in the deep sea is scarce (Berg, 1985). Recent studies have dealt with *Solemya reidi* which has continuous reproduction (Gustafson et al., 1987) whereas species of *Calyptogena* undergo some variations (Lisin et al., this issue).

Most information concerning tropical Lucinidae deals with a large Caribbean species living in sea-grass beds, *Codakia orbicularis*, which has a seasonal reproduction (Berg and Alatalo, 1984; Frenkiel and Mouëza, 1988). *Lucina pectinata* is another large species of the family Lucinidae ranging from the Caribbean area to Brazil (Warmke and Abbott, 1962; Abbott, 1974) that lives deeply burrowed in black reducing mud. In Guadeloupe, it is a dominant species in the most confined areas of mangrove swamps. As emphasized by Read (1964) and Jackson (1973), *L. pectinata* is one of the most resistant species to confinement, in which there is a simultaneous lack of oxygen and a high concentration of hydrogen sulfide. It has been considered by Guelorget et al. (1990) as a typical "paralic" species which lives only in restricted areas. Similar to other Lucinidae, *L. pectinata* harbors endosymbiotic sulfur-oxidizing bacteria in gill cells termed bacteriocytes. A particularly high concentration of hemoglobin in its gill cells (Read, 1962; Wittenberg, 1985; Kraus and Wittenberg, 1990), combined with such a symbiosis, represents an essential metabolic factor that allows *L. pectinata* to resist those conditions which would be lethal for other bivalve species (Frenkiel et al., 1996). In support of this hypothesis, *C. orbicularis*, which hosts similar sulfur-oxidizing bacteria, is not resistant to hypoxic conditions or to sustained emersion (Frenkiel and Mouëza, 1995) and the hemoglobin supply in *C. orbicularis* is some 30-fold lower than in *L. pectinata*. Another noteworthy difference between these species is related to their reproductive patterns. *C. orbicularis* has a clear-cut seasonal maturation (Frenkiel and Mouëza, 1988); all the gonads are spent from January to April and most individuals are fully mature from June to November. Conversely, in *L. pectinata* a large proportion of the population is apparently mature all the year round. Frenkiel and Mouëza (1985) found that *L. pectinata* is a protandric species with up to 90% of the population being males in the smaller size classes and the proportion of females increasing with size until it reaches approximately 50% in the population larger than 50 mm.

The aim of the present study is to investigate the gonad structural and metabolic turn-over in the female stage of *L. pectinata*, a species adapted to a reducing sulfur-rich environment where it has few apparent competitors. The ultrastructural study is limited to the follicular wall as it appears to be a key factor in the peculiar storage and maturation schedule of this species. The reproductive strategy of *L. pectinata* will be tentatively deduced from the histological characterization of its female gonad cycle.

Material and Methods

Sampling

More than 100 adults (30 mm to the largest available size, 50–80 mm) were collected bimonthly during 1985 in a micro-lagoon situated in the mangrove complex that borders the Grand-Cul-de Sac Marin of Guadeloupe. The macroscopic examination of the visceral mass allows a male status to be distinguished by a creamy white gonad and a female status by a brown gonad in most of the population. The sex status of the population was determined by sampling 2650 individuals through extemporaneous

biopsies examined with a light microscope (Frenkiel and Mouëza, 1985). Four to 10 females were fixed monthly for histological study. Four individuals were representative of the current status of the population when the sample appeared homogeneous and more when it appeared to be variable. Specimens were also fixed to ascertain the sex status of unidentified individuals and to clarify any ambiguous status of unmature individuals. We use the term "unmature" to indicate animals that have reached sexual maturity but do not possess gametes, in distinction from "immature", which indicates animals that have not reached reproductive size. Then, over a period of three more years, a small sample of female individuals was fixed monthly. The present work is based on the examination of various histological aspects of the female gonads during the whole sampling period.

Histological and histochemical methods

Whole visceral masses were fixed in Bouin-Hollande's fluid for 48 h, then washed and bisected before dehydration and embedding in paraplast. Serial transverse sections, 7 μm thick, were cut and slides with four sections each were prepared for further processing at regular intervals of 50 sections for a total of up to 10 slides to ensure the sampling of a large part of the gonad. Sections were stained in Goldner trichrome (Gabe, 1968) for morphological observations. Some additional slides were stained with Alcian blue at pH 1 and pH 3 to discriminate the type of mucosubstances stainable by FCF Green, and with Periodic Acid, Schiff reagent (PAS) with and without salivary amylase pretreatment to determine the presence of glycoproteins and glycogen. Additional histochemical techniques were performed on tissues fixed by formaldehyde to detect protein compounds according to Danielli's tetrazoreaction (in Gabe, 1968). Schmorl's and Perls' reactions (in Pearse, 1985) were used to characterize the pigment granules identified in the gonad wall. Light microscopy (LM) observations were performed using a Leica Orthoplan microscope fitted with a Vario-orthomat camera.

Ultrastructural analysis

Female gonad tissues identified as being maturing, mature, or spent were prefixed for 2 h in a standard fixative composed of 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2 adjusted to 1000 m OsM with NaCl. To improve membrane preservation, 1 mM $CaCl_2$ was added to the buffer. After a brief rinse, the gonad tissues were fixed for 1 h at 4°C in 1% osmium tetroxide in the same buffer, then rinsed in distilled water before dehydration in an ethanol series and propylene oxide and embedding in Epon-Araldite mixture prepared according to Mollenhauer (in Glauert, 1974). Some pieces were post-fixed for one hour at room temperature in 2% uranyl acetate in distilled water before dehydration. Semi-thin and thin sections were cut using an Ultracut E Leica microtome. Semi-thin sections (0.5 μm thick) were stained by 0.5% toluidin blue in 1% borax buffer. Thin sections (70 nm thick), collected on collodion-coated Cu-Rh 100-mesh grids, were contrasted with 2% uranyl acetate in distilled water and 0.1% lead citrate before examination in a Hitachi H8000 TEM at 100 kV accelerating voltage.

Cytochemical characterization of lysosomal activity

Acid phosphatase activity was detected by the β-glycerophosphate method of Barka and Anderson (in Pottu-Boumendil, 1990) in Tris-maleate buffer adjusted to pH 5.2, and modified for marine invertebrates by addition of NaCl in the incubation mixtures. Arylsulphatase activity was detected by the P-nitrocatechol and barium salt technic in acetate buffer adjusted to pH 5 of Hopsu-Havu et al. (in Lewis and Knight, 1992). For both procedures, incubation was maintained at 37°C for 0.25 h to 1 h and a control without substrate was performed for each incubation time. Thin sections were observed with or without a short lead citrate contrast.

Results

Histology of the female gonad

Gonads in a developing stage limited to previtellogenic oocytes represented less than 3% of the population at any time of the year, except in the months of May to July when they attain 20% to 24%. These few unmatured gonads, found in animals of any size between 30 and 50 mm, were most often in the process of sex change. Some individuals retained parts of gonad follicles clearly identified as testis, whereas small oogonia were already developing in other parts of the same gonad (Fig. 1); a small number of spermatozoa may be present in these female developing parts of gonads. Invading amoebocytes characterized by an acidophilic cytoplasm stained orange in Goldner trichrome are involved in the resorption of all these residual spermatozoa. Developing stages, characterized by the sole presence of small previtellogenic oocytes attached to a thick pseudostratified follicular wall, are infrequent (Fig. 2) and the occasional presence of amoebocytes inside

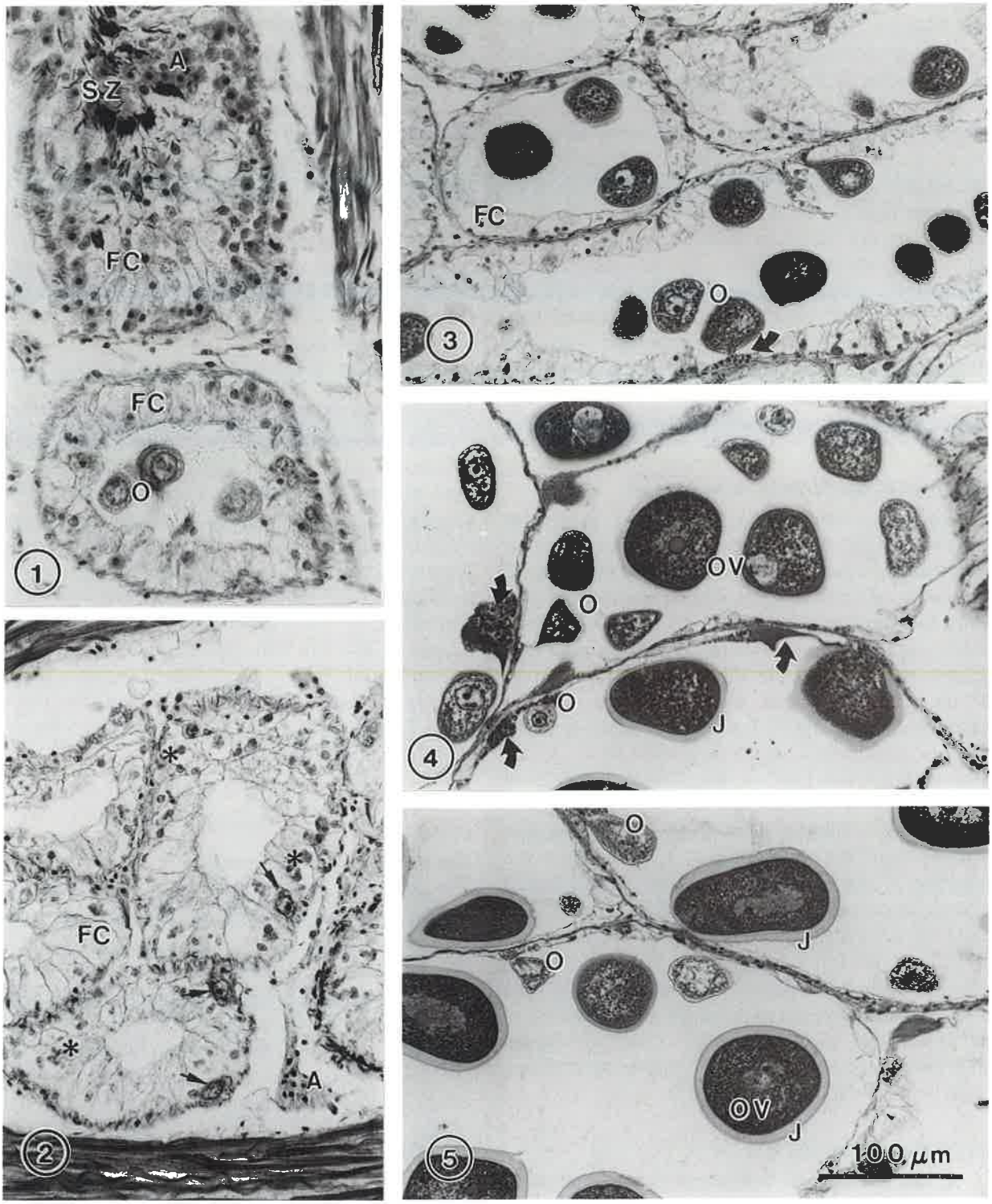


Fig. 1–5. *Lucina pectinata*, developing stages of the female gonad; (light microscopy) all the pictures at the same magnification. Fig. 1. Sex change from male to female stage in a spent gonad. The acini are much reduced in size; the follicular wall is thick with a pseudostratified aspect (FC). One follicle contains residual spermatozoa (SZ) and small cells with a very acidophilic cytoplasm stained orange in Goldner trichrome; these cells are invading macrophagic amoebocytes (A) which are lysing residual spermatozoa. The other follicle contains some small oocytes (O) but no amoebocytes. Fig. 2. Developing female stage with few oogonia (arrow) inserted between the follicular cells (FC); some lipofuscin granules are

such female follicles appears to be indicative of the end of a male phase before oogenesis takes place.

In maturing gonads, the large follicular wall decreases in thickness (Fig. 3). Most gonads contain various stages of oocyte growth from small previtellogenic oocytes with a diameter smaller than 60 μm to large pedunculate and detached oocytes having stored a large supply of vitelline platelets which attain a diameter of more than 100 μm when they are full grown (Fig. 4). Simultaneously to the accumulation of vitellus, these oocytes progressively develop a jelly coat — up to 15 μm thick — stained by PAS (Fig. 7), by Danielli's tetrazoreaction and by Alcian blue. The combined Alcian blue-PAS gives it a lilac color which indicates that this coat is composed of a mixture of glycoproteins and acid mucosubstances. During vitellogenesis, the jelly coat gets thicker giving reliable assessment of oocyte growth independently of section orientation. Previtellogenic and vitellogenic oocyte populations are identified in most gonads at any time of the year (Figs. 4 and 5) and the proportion of oocytes with a conspicuous jelly coat never drops under 50% at any time of the year while some full grown oocytes undergo degeneration and resorption in various proportions at all times. Oocyte resorption is not limited to post-mature unspawned oocytes but constitutes an apparently normal part of the gonad cycle. Fragments of oocytes at various stages of deterioration frequently adhere to the follicular wall (Figs. 4 and 9), but no macrophagic hemocytes are associated to these degenerating oocytes. The most abundant spawning occurs generally in May and spent gonads remain quite abundant until July; however, partial spawning may occur all the year round as a large proportion of the population possess full-grown oocytes.

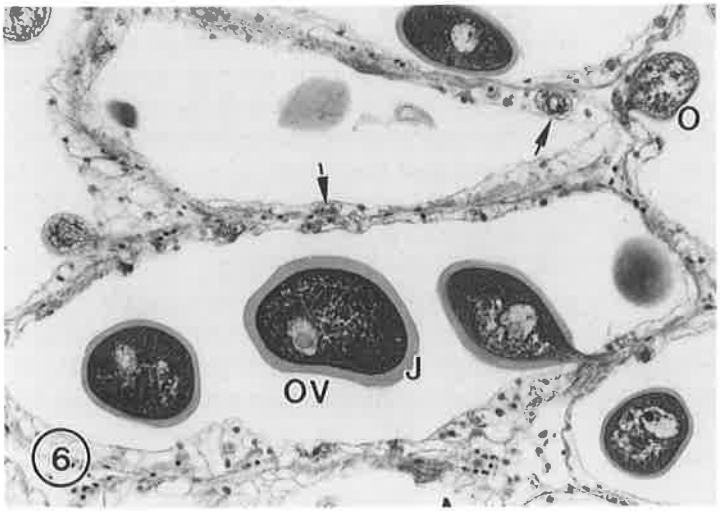
(continued from previous page)

identified inside the follicular cells (asterisk). Amoebocytes (A) are located in the connective tissue surrounding the gonad follicles. Fig. 3. The follicular wall (FC) is decreasing in thickness while small oocytes (O) are increasing in size and begin vitellogenesis. A small oocyte has an enlarged stalk full of vitelline platelets (curved arrow). It is very similar to the oocyte observed in TEM on Fig. 15. Fig. 4. A maturing gonad with various oocyte stages inside enlarged follicles with a thin wall. Simultaneously with vitellogenesis, the larger oocytes (OV) develop a jelly coat (J) which increases in thickness until the oocytes are full grown. Some degenerating oocytes are adjacent to the thin follicular wall (arrows). Fig. 5. A mature stage, characterized by large rounded, full-grown oocytes (OV) with a thick jelly coat (J), together with small previtellogenic oocytes (O) which will develop to prepare the next spawn.

The follicular wall structure is most variable according to the developmental stage of the gonads. Follicular epithelial cells undergo a complex cycle emphasized by histochemical characterization and by ultrastructural observations. In unmatured ovaries, the thick follicular wall (Fig. 2) is made up of large vesicular cells surrounding a core made up of long muscle bundles and connective tissue. These follicular cells frequently contain large yellow lipofuscin granules as typified by their morphological aspect and color, and by Schmorl's and Perls' positive reactions. Their height decreases with oogenesis (Fig. 3). In mature gonads, the follicular wall is thin (Fig. 4) and delimits large follicular spaces which are occupied by numerous full-grown oocytes ready to be spawned mixed with various previtellogenic oocytes. In a spawning gonad (Fig. 5), the follicular wall is thin but its thickness increases soon after the gonad is spent (Fig. 6). During oogenesis, glycogen, as identified by PAS reaction and amylase digestion, is located in the muscular and connective core of the follicular wall but not in the epithelial follicular cells. Conversely, spent gonads store glycogen in the epithelial follicular cells (Fig. 7) while large lipofuscin granules become abundant. In spent gonads, the follicular cells fill the lumen of the ovarian acini and unspawned oocytes are scarce (Fig. 8). Some large inclusions, similar to degenerating oocyte fragments are occasionally identified inside the follicular cells (Fig. 9). Such a conspicuous phagocytosis of unspawned oocytes is not usually so readily observed. Nevertheless large lipofuscin granules, which are likely to constitute a more advanced phase of oocyte resorption (Fig. 10), are much more frequent. No macrophagic amoebocytes have been observed inside the gonad during the female phase, even though they do exist in the connective tissue of the visceral mass outside the gonad wall (Fig. 9).

Ultrastructural analysis of the follicular wall

The core of the follicular wall is a connective tissue associated with muscular fibers (Figs. 11 and 13) in contact with haemolymph lacuna. This connective tissue adheres to the large follicular cells through a convoluted basal lamina (Figs. 11 and 12). Whatever their size and shape, these follicular cells are almost devoid of the regular complement of cellular organelles. Their small nuclei are located near the basal membrane (Figs. 13 and 16) and some mitochondria are found along the lateral cell membranes which are more or less intricate, depending on their storage status (Figs. 16 and 17).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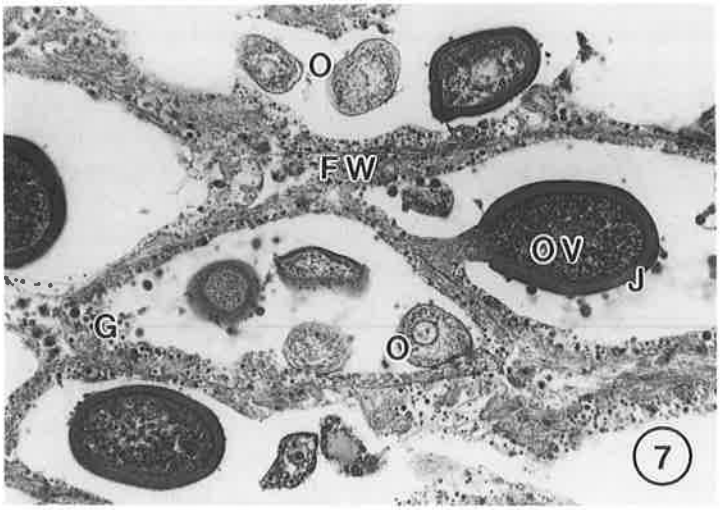
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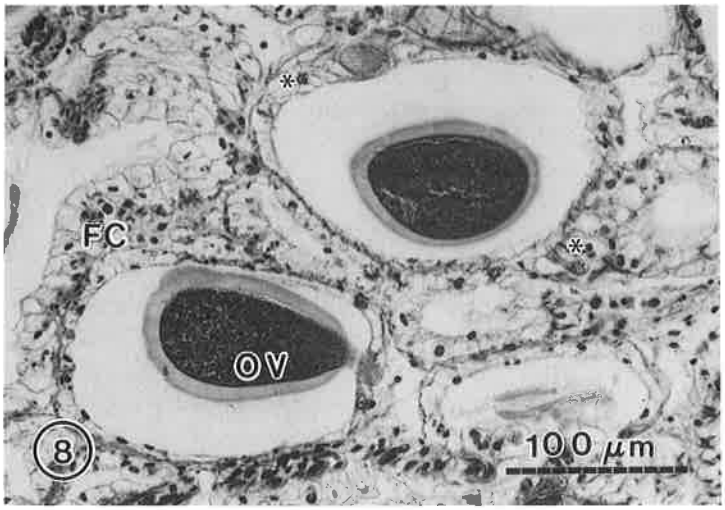
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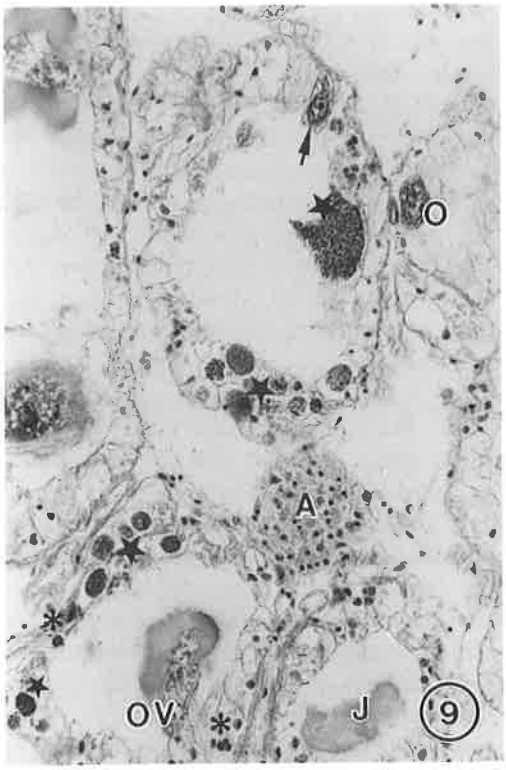
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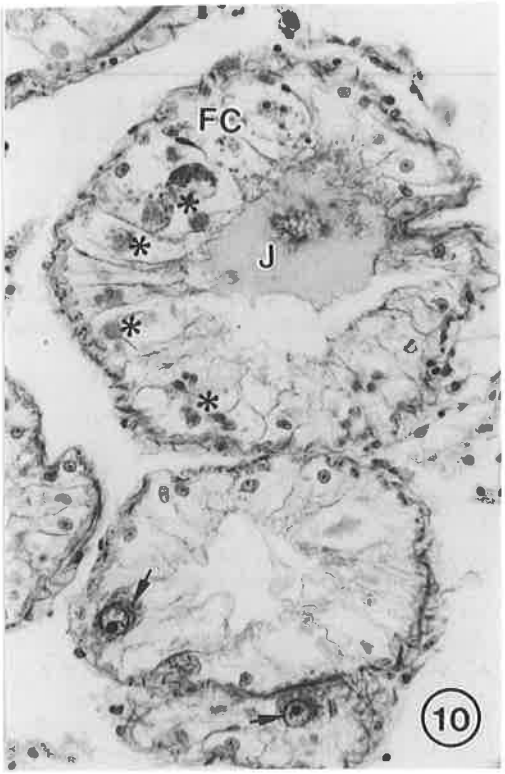
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In their flat configuration, these follicular cells contain mostly glycogen β-granules (Figs. 11, 12 and 14). Some lipid non-membrane bound patches become progressively abundant (Figs. 11, 14 and 15) and, when in the high configuration of the follicular cells, the main storage form is lipid (Fig. 13) though glycogen is still present as scarce cytoplasmic β-granules. During oogenesis, some follicular cells differentiate also numerous membrane-bound inclusions — approximately 0.6 μm in diameter — with an apparently protein content (Figs. 16 and 17) which will have to be more precisely identified through cytochemical methods.

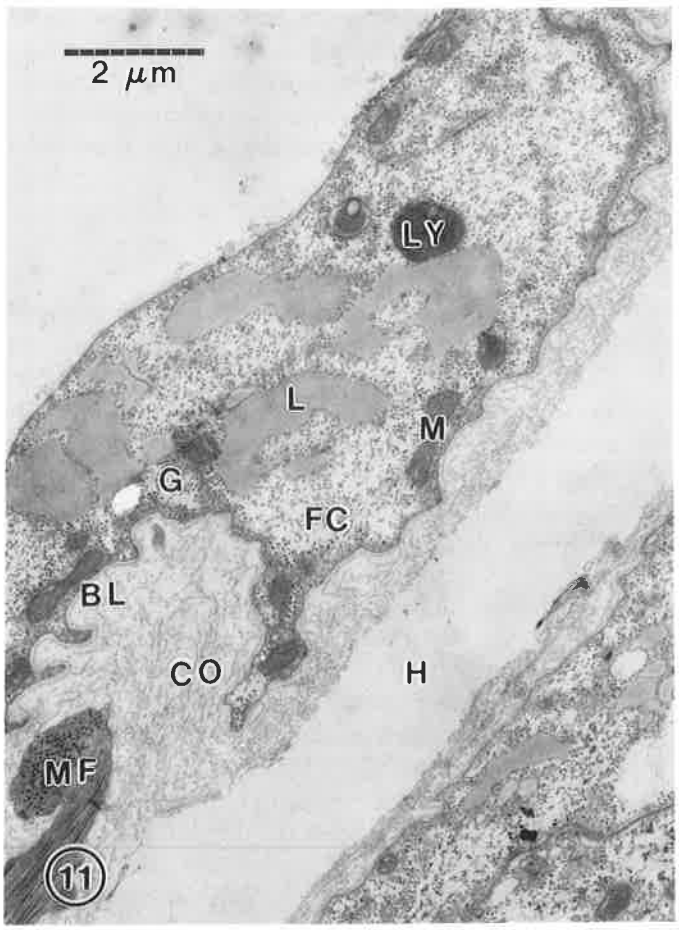
Besides their storage function, the same follicular cells have an active function of resorption of oocyte components. The cyto-enzymological detection of acid phosphatase and arylsulphatase, which are reliable lysosomal markers, corroborate that the numerous large membranaceous inclusions in the follicular cells (Fig. 16) are lysosomal residual bodies. In fact, these lysosomal structures are permanent whatever the follicular cell stage. They constitute small inconspicuous inclusions in the flat follicular cells of mature gonads (Figs. 14 and 15) whereas they become large in spent gonads and correspond to the lipofuscin granules observed with the light microscope. Moreover, extracellular arylsulphatase and acid phosphatase are in contact with degenerating oocyte fragments which adhere to the follicular cells (Fig. 14). Therefore, preliminary fragmentation and lysosomal extracellular lysis of degenerating oocytes appear to be more usual than phagocytosis.

Figs. 6–10. *Lucina pectinata*, female gonad from spawning to resorption; (light microscopy) all the pictures at the same magnification. Fig. 6. Spawning stage characterized by large oocytes (OV) with typical jelly coat (J). Small oogonia, included in the thickened follicular wall, (arrows) and previtellogenic oocytes (O), implanted in the follicular wall through their stalk, prepare a new batch of oocytes. Fig. 7. Spent stage with thick follicular wall (FW); the oocyte jelly coat (J) stained red by PAS appears dark; previtellogenic oocytes (O) are the most numerous; glycogen stained red by PAS appears as dark granules (G). Fig. 8. Another aspect of a spent gonad with few residual oocytes (OV) in much reduced follicular cavities. The large follicular cells (FC) contain some yellow lipofuscin granules (asterisk); no oogonia or small oocytes are visible in this area. Fig. 9. Resorption of degenerating oocytes by phagocytosis of large fragments of mature oocytes identified by stars outside and inside the follicular cells. Inclusions in the follicular wall are more often smaller lipofuscin granules (asterisks). Residual mature oocyte (OV) and pieces of jelly coat (J) are readily identified in the follicle cavity. Oogonia (arrow) and previtellogenic oocytes (O) are ready for the next developing stage. Macrophagic amoebocytes (A) are located in the connective tissue but do not enter in the follicular cavities. 10. Completely spent female gonads have high follicular cells (FC) with lipofuscin granules (asterisks), remnants of jelly coat (J) and some oogonia (arrow) developing between the follicular cells.

Discussion

As oogenesis is a process with very high energy requirements, seasonal reproduction is considered by Olive (1992) as a dominant adaptative characteristic of major groups of marine invertebrates that allows for the allocation of limited resources to reproduction. Such a synchronization at the most convenient period is considered by Olive (1992) either to maximize offspring survival — the optimum larval survival strategy — or to maximize fitness — the optimum fertilization strategy. Both strategies suppose costs associated with energy storage that must be compensated by the selective advantage accruing from delayed deployment of resources. Seasonal reproduction is frequent in marine bivalve molluscs which live in littoral areas where they are subject to variable environments (Mathieu and Lubet, 1993). This instability includes temperature fluctuations, variation in food availability, and competitive and predatory pressures. In temperate areas, most marine bivalves have an annual reproductive cycle with precise periods of gametogenesis and spawning, these being determined, mostly in females, by storage, as controlled by the variation either of temperature or photoperiod (Paulet and Bouchet, 1991). Conversely, in the tropics, the permanently high temperature allows oocyte growth all year round so that many bivalves reproduce continuously (Sastry, 1979). Likewise, in the deep-sea which constitutes a stable environment with constant temperature and nutritional conditions (Tyler and Young, 1992), most species reproduce year round whilst a limited number of species reproduce on a seasonal basis.

However, the occurrence of seasonal and non-seasonal breeding is not just a latitudinal or depth phenomenon. Continuous breeding has also been reported for temperate-zone and continental-shelf bivalves. For example, asynchronous gametogenesis and spawning, with the possibility for spawning year round, was described by Morvan (1987) in *Glycymeris glycymeris* which is a common bivalve in French Brittany. The symbiotic bacteria-bearing protobranch bivalve *Solemya reidi* living in a cold-temperate area at a depth of 40 m, has a continuous reproduction pattern (Gustafson et al., 1987), whereas the shallow-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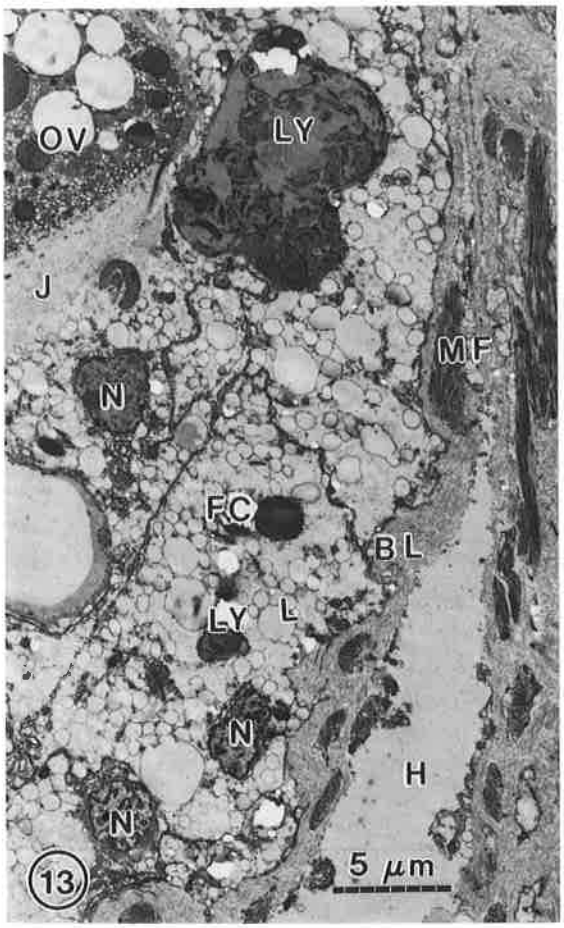
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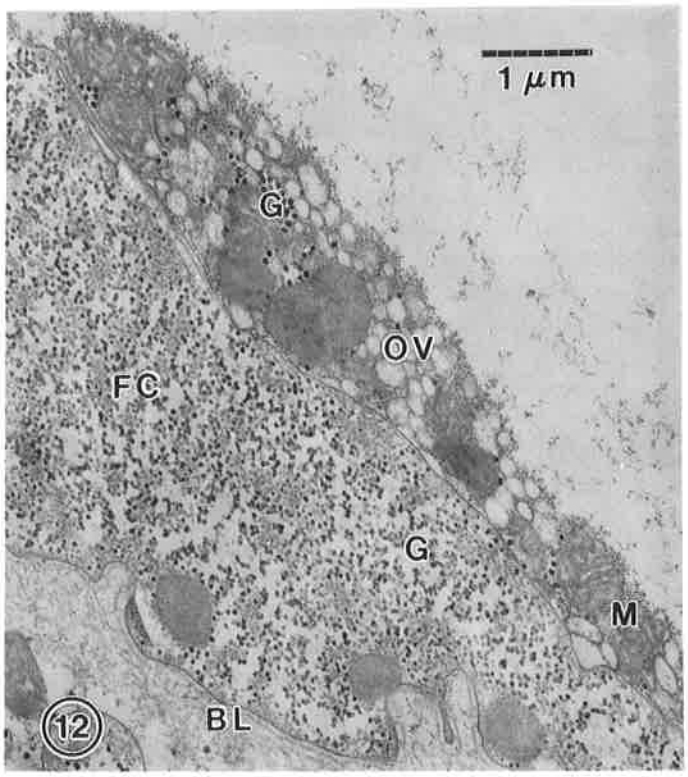
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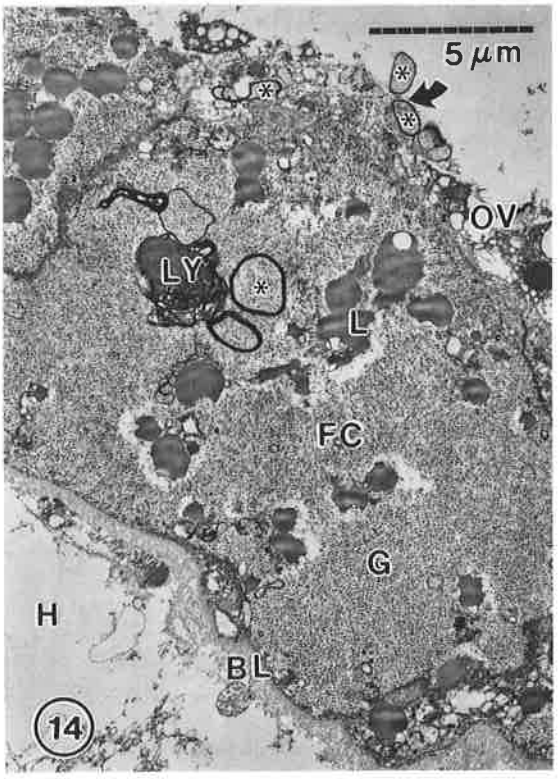
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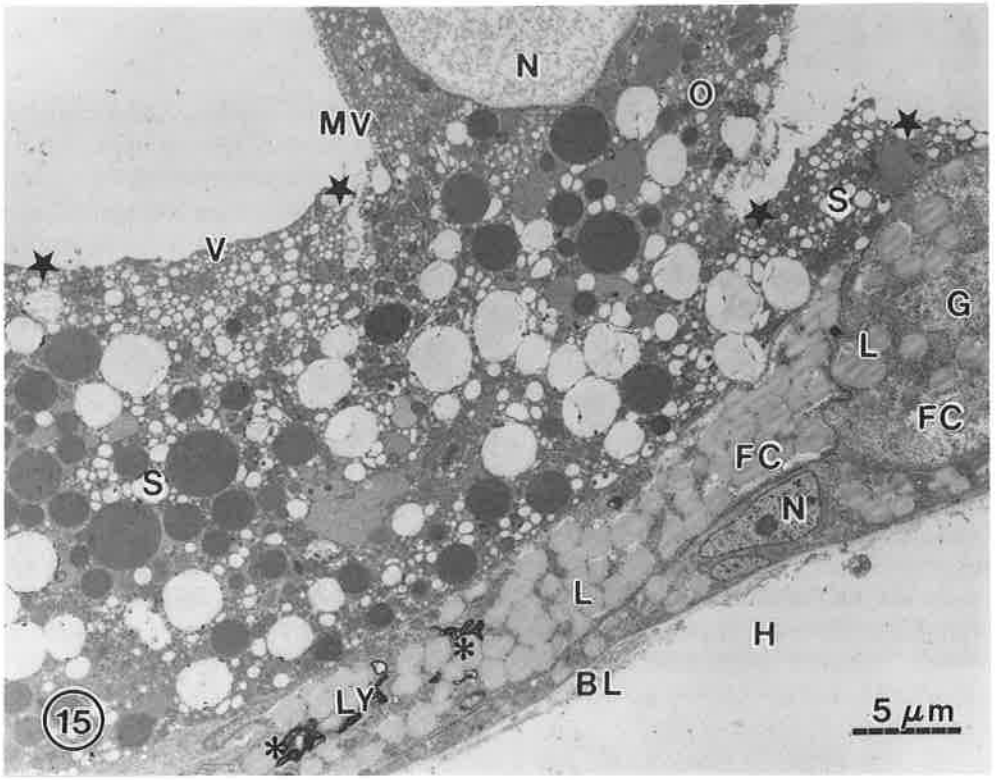
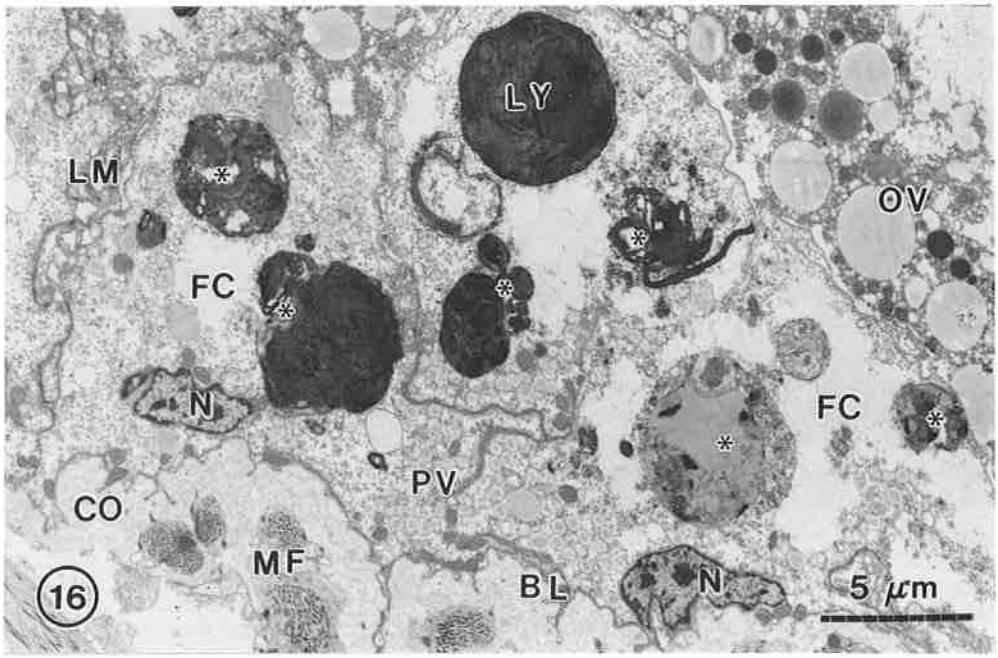
water related species, *Solemya velum*, spawns seasonally. Among the Lucinacea, *Thyasira gouldi* (Blacknell and Ansell, 1974) and *Lucinoma borealis* (Tunberg, 1984) are non-seasonal spawners. Conversely, *Loripes lucinalis* has a seasonal reproduction cycle with a spawning period from March to July (Ollivier, 1972). The reproduction patterns of the two large tropical Lucinidae under study in Guadeloupe are equally distinct. *C. orbicularis* living in sea-grass beds has a clear-cut seasonal reproduction cycle (Berg, 1984; Frenkiel and Mouëza, 1988), whereas *L. pectinata* living in mangrove muddy swamps has a continuous reproduction pattern (Frenkiel and Mouëza, 1985; further personal observations).

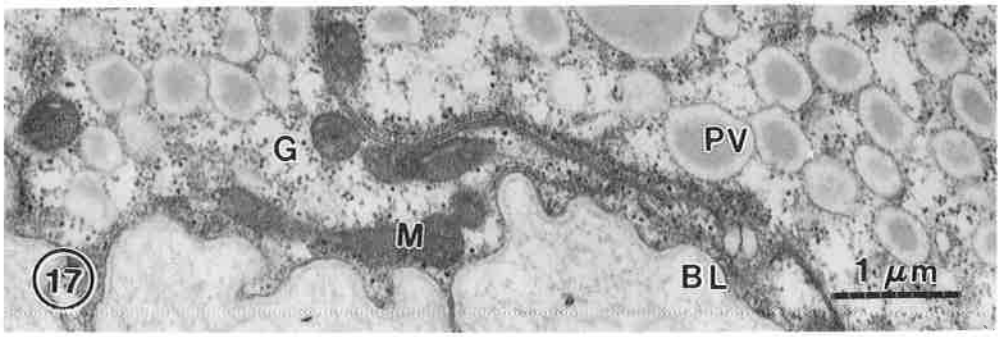
In temperate environments, increasing temperature and photoperiod are the main environmental variations which trigger gametogenesis and spawning processes (Lubet, 1976; 1986). The main impact of the tropical climate on shallow-water marine species are neither temperature nor photoperiod, therefore these environmental factors do not trigger gametogenesis and spawning events either in sea-grass beds or in mangrove muddy bottoms. In these environments, the most important fluctuating environmental factor is rainfall which has a seasonal component with a dry season from January to May when rainfalls are lower than 100 mm monthly and a rainy season from July to the end of the year with monthly rainfalls which vary from 180 mm to more than 350 mm (Guelorget et al., 1990). *C. orbicularis* spawns during the rainy season. Therefore, the gonad maturity necessary to respond to spawning stimuli is likely to be determined during the dry season. The situation is quite different for *L. pectinata*. In microlagoons, where the salinity stratification is such that the bottom water fluctuates less according to rainfall and evaporation than the upper layer, the occurrence of exceptional rainfalls would be the only major influence on the bottom interstitial water salinity (Louis, 1983). Although exceptional salinity variations triggered spawning of the small Veneridae, *Anomalocardia brasiliana*, and caused the death of most of the adult individuals through an acute lack of oxygen (Monti et al., 1991), neither the drop in salinity nor the lack of oxygen affected the reproduction and survival of *L. pectinata*, which lives deeply burrowed in reducing mud. The increase in the proportion of spent gonads observed during the month of May in three successive years (pers. obser.) may have been due to a major spawning. However the triggering factor in spawning *L. pectinata* is still unknown. The general aspect of most individuals in May and June is suggestive of nutritional deprivation which may impair a fair recovery after spawning; therefore, a study of the digestive gland turn-over, together with a simultaneous study of the status of the gill bacteriocytes before and after a similar stress period, would be necessary to put forward an interpretation of these phenomena.

According to Mathieu and Lubet (1993), the vitellogenesis and spawning of any species is dependent on its storage schedule and nutrient mobilization. Among Lucinidae, the storage structure is very similar in the temperate species, *Loripes lucinalis* (Johnson and Le Pennec, 1994) and in the tropical species *C. orbicularis* (pers. obser.), both of which have seasonal reproduction. In *Lucina pectinata* which may spawn year round, the storage schedule pattern is modified. In these three species, large follicular cells, almost devoid of the regular complement of cellular organelles but extremely rich in glycogen and lipid droplets, form the storage tissue. Vacuoles which may be pinocytotic are also abundant. However, in *L. pectinata*, we have not observed glycogen particles, lipid-like droplets, and bacteria in the hemolymphatic spaces, such as described by Johnson and Le Pennec (1994) in the gonad of *L. lucinalis*. The gonad-wall cells are so modified as to store either lipid or protein components or glycogen

Figs. 11–14. *Lucina pectinata*, follicular cells with glycogen and lipid storage (transmission electron microscopy). Fig. 11. The follicular wall is made up of muscular fibers (MF) and collagen (CO) in contact with hemolymph (H) and with the follicular epithelial cells (FC) through a convoluted basal lamina (BL). The follicular cells are almost deprived of organelles, only some mitochondria (M) being located along the cell membrane. At this stage, follicular cells are a few microns thick and contain glycogen granules (G) and lipid patches (L) as storage. LY, lysosomal structure. Fig. 12. A fragment of degenerating oocyte (OV) in a mature gonad is adhering to a flat follicular cell (FC) crowded with glycogen granules (G). Notice that there are no microvilli and that the oocyte fragment appears as deprived of cell membrane on either side; some mitochondria (M) are still identified. BL, basal lamina. Fig. 13. The thick follicular wall of the developing gonad is made up of large follicular cells (FC) which appear vesicular as they have accumulated lipid droplets (L). Nuclei (N) are located near the basal lamina (BL) and a large lysosomal residual body (LY) is in the apical part of the cell. An oocyte (OV) is in contact with the follicular wall through its jelly coat (J) in the upper left corner. H, hemolymph; MF, muscular fibers. Fig. 14. Cytochemical detection of arylsulphatase in a follicular cell (FC) at the glycogen (G) and lipid (L) storage phase. Lysosomal structures are located inside the cell (LY) and outside (curved arrow) in contact with a degenerating oocyte (OV). Several annular lysosomal structures are identified (asterisk). Basal lamina (BL) is in contact with hemolymph (H).


N
O
MV
V
S
G
L
FC
FC
S
N
L
H
BL
LY
15
5 μm


LY
LM
OV
FC
FC
N
PV
CO
MF
BL
N
16
5 μm


G
PV
M
BL
17
1 μm

according to the reproductive stage of the gonad. This follicular-cell storage may be adapted to seasonal maturation and spawning or to permanent oogenesis and discontinuous spawning which may be triggered by unknown environmental stress. Unlike the follicular cells of *L. lucinalis* (Johnson and Le Pennec, 1994) and of *C. orbicularis* (personal observation) which have only a storage function, those of *L. pectinata* are dedicated to the double function of storage of reserves and resorption of unspawned oocytes, which allows for the recovery of unused metabolites. The storage function involves a cytoplasmic turn-over. The lipid storage phase takes place when the high follicular cells of spent gonads are organized as a pseudo-stratified epithelium, whereas the glycogen storage is greatest in the thin epithelium configuration which is prevalent in ready-to-spawn gonads; the period of protein storage has not been clearly defined as yet, but it may be associated with intermediate maturing phases. However, we agree with Johnson and Le Pennec (1994) in concluding that, in the various species so far examined, the lack of most organelles in these follicular cells indicates that the metabolites accumulated within the follicular cells are not produced by the cells themselves.

Figs. 15-17. *Lucina pectinata*, follicular cells with protein inclusions and lysosomal resorption (Transmission electron microscopy). Fig. 15. A small oocyte (O) in an early vitellogenic phase — with a vitellin coat made of microvilli (MV) and glycocalyx but no jelly coat — is adhering to the follicular cell wall which appears flat at this place, due to the anchorage of the pedunculate oocyte. A basal lamina (BL) separates the follicular cells from hemolymph (H). These follicular cells are in a glycogen (G) and lipid storage phase (L) and the epithelium is in the thick configuration. Small lysosomal structures are identified by arylsulphatase reaction (LY and asterisk). The anchoring stalk (S) of the oocyte appears to have a large number of small superficial vesicles (V) which may be pinocytotic vesicles. This surface of the enlarged stalk (stars) is not covered by microvilli which are differenciated along the oocyte surface proper (MV). Nucleus of oocyte and of follicular cell (N). Fig. 16. Large follicular cells with basal nuclei (N) and interpenetrating lateral membranes (LM). The upper right corner is occupied by a degenerating oocyte (OV) without cell membrane and the lower left corner by the muscle fibers (MF) and collagen (CO) of the gonad wall core separated from the follicular cells by a basal lamina (BL). Between large lysosomal residual bodies (LY and asterisks), membrane bound vesicles (PV) are considered to be a form of protein storage. Fig. 17. An enlargement of a part of Fig. 16 shows mitochondria (M) along the cell membranes, the vesicles considered to have a protein content (PV) and scarce glycogen granules (G). The basal lamina (BL) is convoluted.

In *L. pectinata*, resorption of unspawned oocytes is fulfilled by follicular cells. It is most conspicuous after a spawning phase when large parts of the follicular epithelium are crowded with lipofuscin granules. Notwithstanding the fact that typical phagocytosis is not observed, acid phosphatase and arylsulphatase activities demonstrate a permanent lysosomal activity which takes the form of an extracellular lysis of degenerating oocytes before these oocytes undergo intracellular resorption. Such a resorption process is different from those described which involve macrophagic amoebocytes (Lubet et al., 1987; Dorange and Le Pennec, 1989). In *L. pectinata*, a macrophagic amoebocyte activity is restricted to spermatozoa resorption, whereas it is active for oocyte resorption in *C. orbicularis* (pers. obser.).

Conclusion

The ovary of *Lucina pectinata* has follicular cells similar to other Lucinidae but with a storage and resorption schedule adapted to continuous reproduction. The resorption function necessary to recycle the components of numerous unspawned oocytes, fulfilled by the follicular cells, is enhanced by the development of a lysosomal extracellular activity. In the high-stress environment of the most confined areas of mangrove swamps where *L. pectinata* reproduces, the permanent occurence of full-grown ovocytes ready for spawning constitute a successful reproductive strategy allowing for opportunistic spawning. The frequent resorption of oocytes through an intense lysosomal activity of the follicular cells is a most efficient way to minimize the metabolic cost of such a permanent state of maturity. However the stimulus which triggers spawning, in this species remains unknown.

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