Aquat. Living Resour., 1996, **9**, 159-168

Shell disease in the gold lip pearl oyster, *Pinctada maxima* and the Eastern oyster, *Crassostrea virginica*

Frank O. Perkins

School of Marine Science, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062, U.S.A.

Present address: Departments of Zoology and Microbiology, Pathology and Parasitology, North Carolina State University, Raleigh, NC 27695, U.S.A.

Received July 17, 1995; accepted March 19, 1996.

Perkins F. O. *Aquat. Living Resour.*, 1996, **9**, 159-168.

Abstract

A description is provided of the anomalous conchiolin deposits which are formed by *Pinctada maxima* and which are associated with unusual mortalities. Comparisons are made with brown ring disease found in *Ruditapes philippinarum* and juvenile *Crassostrea virginica*. In *P. maxima*, the deposits are not organized into a ring but rather are broad-based and result in retraction of the mantle with the deposits lying outside the edge of the mantle. *Vibrio* sp. have been implicated in causing the diseases of *P. maxima* and *R. philippinarum* whereas the etiological agent of the disease in *C. virginica* is unknown. It is suggested that the coccoid bodies formed in the mantle of *C. virginica*, but not in affected *P. maxima* and *R. philippinarum*, are sequestered portions of cytoplasm formed as a result autophagocytosis. Stimuli which could be responsible for inducing sequestration are discussed. The ultrastructure of the presumptive autophagosomes is described and compared to similar bodies found in *C. gigas* infected with a herpes-like virus.

Keywords: *Pinctada maxima*, *Crassostrea virginica*, shell disease, conchiolin, ultrastructure, light microscopy.

Maladie de la coquille chez les huîtres Pinctada maxima *et* Crassostrea virginica.

Résumé

Les anomalies de dépôt de conchioline secrétée par *Pinctada maxima*, associées avec des mortalités inhabituelles, sont décrites ici. Des comparaisons sont faites entre la maladie de l'anneau brun trouvé chez *Ruditapes philippinarum* et chez des jeunes *Crassostrea virginica*. Chez *P. maxima*, les dépôts ne sont pas organisés en anneau mais plutôt à partir d'une base assez large et résulte d'une rétraction du manteau avec des dépôts à l'extérieur du bord du manteau. Les *Vibrio* sp. ont été impliqués dans les maladies de *P. maxima* et *R. philippinarum* tandis que l'agent étiologique de la maladie est inconnu chez *C. virginica*. Les corps de forme cocoïde formés dans le manteau de *C. virginica*, mais n'affectant pas *P. maxima* ni *R. philippinarum* seraient des fractions de cytoplasme isolées, « sequestrées », résultant d'une autophagocytose. Les stimulus qui pourraient être responsables de ces fractionnements sont passés en revue. L'ultrastructure des autophagosomes présumés est décrite et comparée à des corps similaires trouvés chez *C. gigas* infecté par un virus de type herpès.

Mots-clés : *Pinctada maxima, Crassostrea virginica*, maladie de la coquille, conchioline, ultrastructure, microscopie optique.

INTRODUCTION

Anomalous deposits of conchiolin on the nacre have been described in a diversity of species of marine bivalve molluscs as a result of irritation of the mantle by a variety of different microbes and metazoan parasites and commensals (literature reviewed by Paillard *et al.*, 1994). Of particular interest have been those cases where there are high mortalities of the bivalves involved. The most extensively studied is brown ring disease of the Indo-Pacific clam (*Ruditapes philippinarum*) in the coastal waters of France and Spain. In that host the causative agent is a species of *Vibrio*, termed *Vibrio* P1 or VP1 which expresses itself between 7 and 21°C (Paillard and Maes, 1990; Paillard *et al.*, 1994).

Another shell disease is associated with juvenile oyster mortalities during the summer months (22-25°C) in juvenile *Crassostrea virginica* of 15 to 24 mm (mean shell heights) from hatcheries in the northeastern United States, primarily along the coast of Long Island Sound (Bricelj *et al.*, 1992). As in *R. philippinarum*, the conchiolin deposits are most pronounced in the form of a brown ring on the nacre, around the perimeter of the shell. Another characteristic in some individuals is a pronounced cupping and overgrowth of the left valve over the level of the right valve.

In the search for the causative agent of juvenile oyster mortalities, a number of hypotheses have emerged; however, the agent has not been conclusively identified. The search for the causes(s) has centered around the possibility that a protist or bacterium initially causes retraction of the mantle, followed by abnormal conchiolin deposition. Further progression of the disease, resulting in death, involves bleeding from the mantle epithelium and detachment of the adductor muscle when conchiolin is deposited between the adductor and the shell. Secondary invasion of microorganisms into the lesions is believed to exacerbate the problem. It has also been suggested that toxic dinoflagellate blooms or some other toxic phytoplankton species or some chemical contaminant could be responsible for the pathological condition (Bricelj *et al.*, 1992). Other papers concerning juvenile oyster disease can be found in the present issue of this journal.

Anomalous deposits of brown conchiolin have been reported on the inside surface of the shells of pearl oysters, *Pinctada maxima*, involved in high mortalities which occurred as part of cultured pearl operations on the western coast of Australia (Pass *et al.*, 1987; Perkins, 1993). Pass *et al.* (1987) tentatively concluded that *Vibrio harveyi* was responsible, at least in part, for the mortalities when the oysters were subjected to stressful conditions during handling and shipment.

With the recent increased interest in shell disease of bivalve molluscs as evidenced by the convening of the International Workshop on Shell Disease in Marine Invertebrates: Environment-Host-Pathogen Interactions in Brest, France during March 29-31, 1995, it appeared particularly appropriate to reexamine the morphology of shell disease in *Pinctada maxima* and juvenile *Crassostrea virginica* in light of recent findings concerning other bivalve shell diseases. The purpose of this paper is to present a more complete description of the anomalous conchiolin deposits found on *Pinctada maxima* shells than is currently available in the literature. There has been only one photograph published thus far (Perkins, 1993). The syndrome is compared to that of *Crassostrea virginica* affected by juvenile oyster disease.

MATERIALS AND METHODS

Pinctada maxima was sampled (N = 89) from populations involved in the series of stages from 1) harvesting off Eighty-Mile Beach south of Broome, Western Australia to 2) placement in carrier boats at Broome to 3) transport over a 36 to 40 hour time period to Kuri Bay, located inside Augustus Island (15° 27′ to 15° 30′ S Lat., 124° 30′ to 124° 40′ E Long.) where they were hung under rafts (operations described in greater detail in Dybdahl and Pass, 1985; Pass *et al.*, 1987). Location diagrams have been provided in Pass *et al.* (1987). Sample sizes were as follows: a) harvested and held in carrier boat for one day at Broome, N = 10; b) in carrier boat at Broome for 2 days, N = 10; c) in carrier boat at Broome for 4 days, N = 10; d) under rafts in Kuri Bay for 1 to 2 months (N = 59). None of the oysters had been implanted with spherical seed for pearl production. All above samples were obtained in the time period of June 22 to 26, 1979.

Other samples consisted of oysters harvested off Eighty Mile Beach and fixed immediately (N = 5); oysters harvested off Eighty Mile Beach and held in Roebuck Bay (a few kilometers south of Broome) for 2 months (N = 12); oysters involved in hemispherical (blister or half) pearl production which were harvested off Eighty Mile Beach and a) placed in Roebuck Bay for 3 weeks then beads attached followed by 2 days in Roebuck Bay before fixation (N = 10), b) beads added and held in Roebuck Bay for 2 months before fixation (N = 12) and one month before fixation (N = 2), and c) beads added and oysters held in Roebuck Bay for 3 days before fixation (N = 1). Samples were obtained June 28 and 29, 1979.

Oysters from the above sites with no clinical signs of the disease and those with various degrees of anomalous conchiolin deposits on the inner surface of the shell, both gaping and non-gaping were sampled as well as those which showed signs of recovery in that calcarious deposits were formed on the anomalous conchiolin deposits. Those with anomalous conchiolin deposits were from populations which were experiencing mortalities. Organ samples were obtained from 131 oysters for fixation in Davidson's fixative

(Shaw and Battle, 1957) and 10% phosphate buffered formalin. The organs sampled were digestive gland, gill, heart, mantle, adductor muscle, rectum, gonad and mid-gut. Histological sections were obtained from paraffin-embedded organs and stained in hematoxylin and eosin. In addition, samples of digestive gland, gill and mantle were obtained from 70 oysters held in the carrier boats for 4 days and under the rafts at Kuri Bay for 16 to 61 days. These samples were placed in fluid thioglycollate medium and assayed for the presence of *Perkinsus* sp. using the Ray technique (Ray, 1954). Sizes of the oysters ranged from 110 to 220 mm shell height (dorsoventral axis) (mean = 145 mm; SD = 15.4; N = 122).

In the present study of juvenile *C. virginica* disease, ten to 18 mm long oysters were havested on July 21, 1992, shipped to the Virginia Institute of Marine Science and fixed on July 22, 1992. The oysters were from a population suffering from mortalities and demonstrating brown ring deposition. They were obtained from a growout raft facility maintained by Frank M. Flower and Sons, Inc. at Oyster Bay, NY. Small (ca. 1 mm^3) pieces of mantle overlying or juxtaposed to brown ring deposits of conchiolin from six of the oysters were fixed in glutaraldehyde and osmium tetroxide as already described (Bricelj *et al.*, 1992). Likewise mantle from samples from one oyster which had no anomalous conchiolin deposits were fixed for ultrastructural observations. The rest of the oysters' tissues were fixed in Davidson's fixative and histological sections cut from paraffin-embeddings then stained in hematoxylin and eosin or stained using the Feulgen technique to reveal DNA (Humason, 1962; light green used instead of fast green).

RESULTS

Pinctada maxima

The shells of *P. maxima* with no signs of anomalous conchiolin deposition (ACD) had pearly nacre inside a peripheral zone of brown conchiolin (*fig.* 1). The interface between the two is sharply defined and is the pallial line (Takemura and Kafuku, 1957). In the earliest or in mild cases of ACD, a wedge-shaped or a broad zone of thin deposits of conchiolin were found on the nacre, extending from the pallial line toward the center of the shell with the broad base of the wedge on the pallial line (*fig.* 2). It appeared that if the pathological condition was not too severe then the depositions were covered with a wrinkled layer of nacre (*fig.* 3). In the process of deposition the sharp interface between the nacre and peripheral conchiolin was blurred. In more severe cases of ACD, the conchiolin was formed in a broad zone and consisted of a thin parchment-like layer (*fig.* 4) which, through further deposition, became thicker, more brittle, darker brown and often multilayered (*fig.* 5). The occasional presence of small barnacles on the anomalous deposits indicated that the pathological condition could persist without causing death of the oyster. Also indicative of tolerance was the deposition of nacre on the ACD (*fig.* 5).

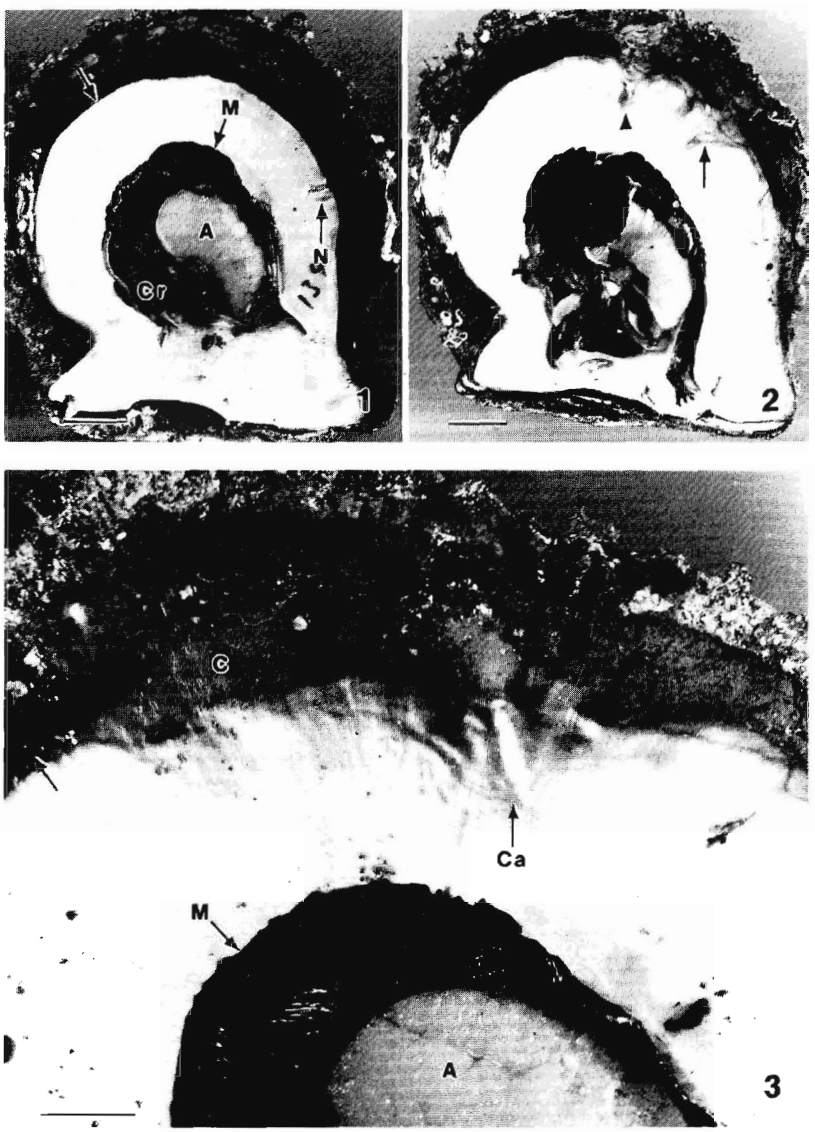
Most often the ACD was found along the ventral margin of the shell with an affected arc of 130 to 170° not being unusual. However, less pronounced deposits were found in other regions such as near the ligament on the posterior and anterior "ears" (terminology of Takemura and Kafuku, 1957). Associated with ACD was a retraction of the mantle from the affected area at least until deposition had advanced to the point where further retraction was not possible. This retraction resulted in sediment accumulation (*fig.* 6) and, as noted above, some biofouling of the nacre and ACD.

When hemispherical beads were attached to the nacre of oysters for blister pearl formation and there was an onset of ACD, often the encroachment of the ACD led directly to the beads (*figs.* 6 and 7). Conchiolin deposits were then formed on and around the beads sometimes followed by wrinkled nacre deposition on tthe conchiolin depending on the severity and stage of the condition (*figs.* 7 and 8).

Histological sections of *P. maxima* which exhibited no signs of ACD and those in various stages of ACD did not reveal any parasite which could be correlated with ACD. In addition, the organ samples examined after fluid thioglycollate medium treatment contained no *Perkinsus* sp. A description of the parasites observed is found in Dybdahl and Pass (1985). In an ultrastructural study Pass and Perkins (1988) found virus-like particles and intranuclear inclusions resembling virogenic stromata in cells of the digestive gland. However, no correlation with ACD or mortalities were found.

Since the organ most closely associated with the ACD is the mantle, histological sections of that organ were most closely examined in the present study. Evidence was sought for sloughing of the epithelia along both the inner and outer surface and the outer, middle and inner lobs of the mantle edge. In addition, cellular lysis and lesion formation in specific loci associated with exudates containing hemocytes were sought. The mantle region sampled was that in close juxtaposition to the adductor muscle. The dissections contained part of the adductor muscle, a cross section through the rectum and the attached mantle out to the distal edge. Most often the sample was about 20 to 30° from the medial dorsoventral axis.

Surprisingly the presence of lesions were not correlated with presence or absence of ACD. In the mantle samples examined, 57 were from oysters with ACD and 39 from oysters without ACD. Lesions and epithelial sloughing were found in 68% of the former and 72% of the latter. Most often hemocytic exudates were not observed, and when observed (*figs.* 9 and 10) there was no correlation between their presence and presence of ACD. Bacteria were not observed in the sections (all were hematoxylin and eosin stained) nor were intracellular, spheroidal bodies observed, as

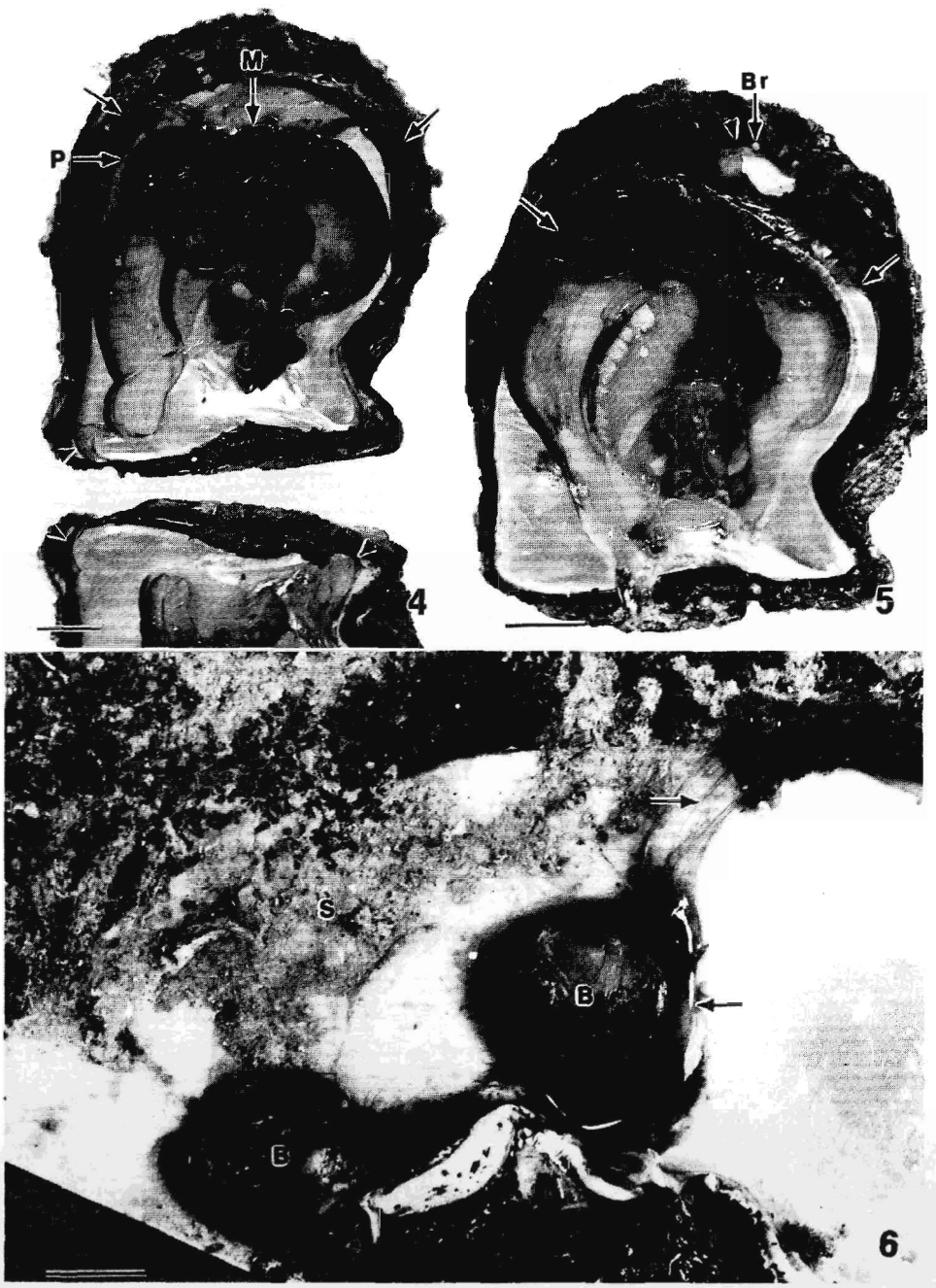


Figures 1 to 3. – *Pinctada maxima.* **1** Apparently healthy oyster, examined a few days after harvesting from a wild population. The well-defined pallial line and interface between the nacre and conchiolin is visible (arrow). Visceral mass has been removed to expose most of nacre. Adductor muscle (A); pinnotherid crab (Cr); mantle edge (M); nacre overlying *Polydora* tunnel (N); Bar = 20 mm. **2-3** Oysters harvested off Eighty-Mile Beach and held in Roebuck Bay for two months. **2** Early or mild case of anomalous conchiolin deposition (ACD) disorder in which wedge-shaped intrusions of ACD are found on the nacre (arrow). On one instrusion a calcareous deposit has been formed over the conchiolin (arrow head), Bar = 20 mm. **3** More advanced case of ACD in which mantle has retracted from the original pallial line (arrow) and in the process has deposited conchiolin (C) on the nacre. A layer of calcareous material has been formed over some of the ACD (Ca). Mantle edge (M); adductor muscle (A). Bar = 10 mm.

described in Bricelj *et al.* (1992) and in the following section on juvenile oyster disease. Convoluting and whirling of the conchiolin as it emerged from the periostracal groove was observed in mantle sections from oysters with ACD (*fig.* 11).

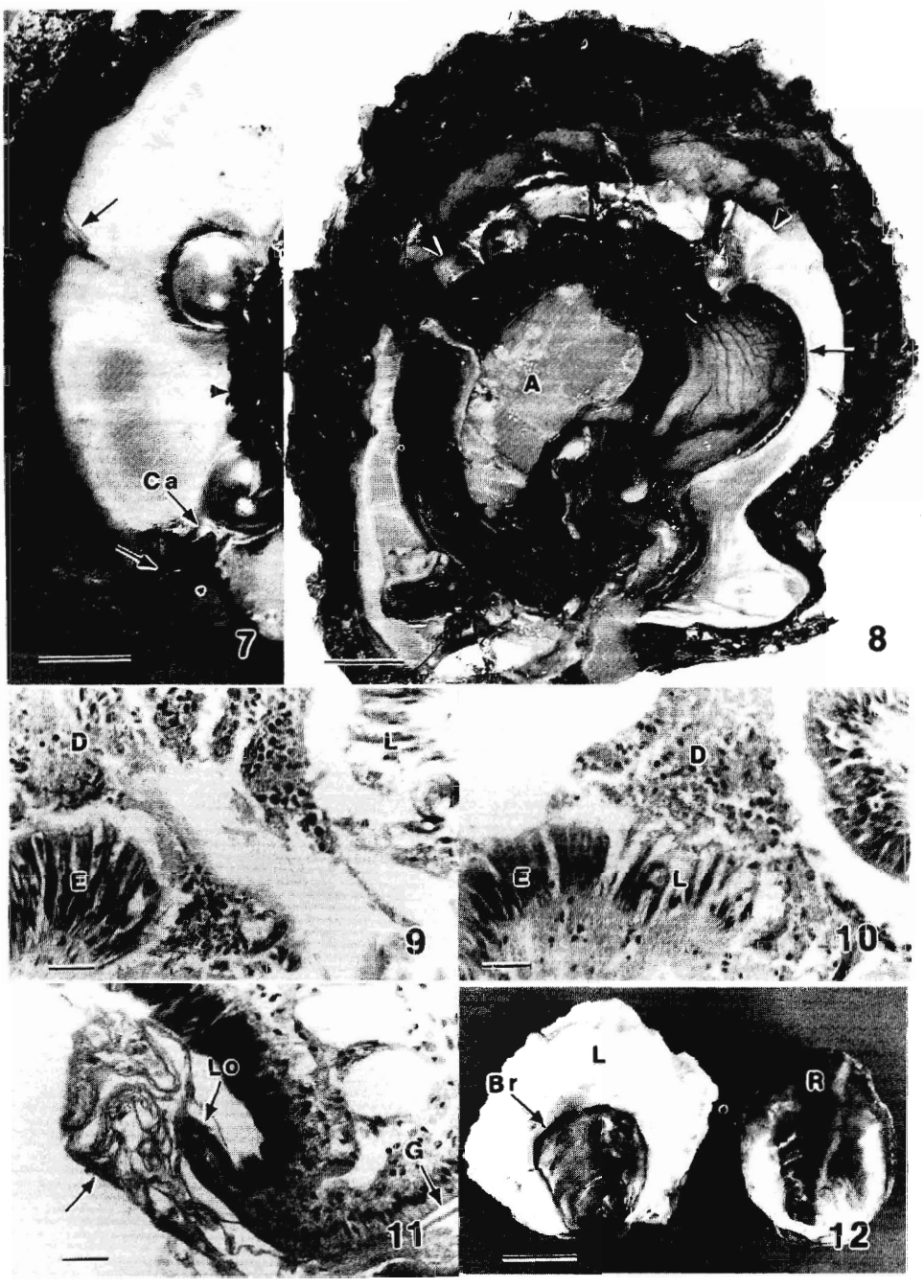
Crassostrea virginica

In juvenile oyster disease (JOD) a well-defined brown ring of ACD was often deposited inside the shell margin on one or both valves (*fig.* 12) with a thin layer of ACD within the ring. In the absence of a ring, thin layers of ACD could be found in various places on the nacre of affected individuals. Since the morphological aspects of the condition have been described in some detail in Bricelj *et al.* (1992), they will not be repeated herein, with the following exception. Due to the ultrastructural similarity of unusual intracellular bodies found in both juvenile *Crassostrea gigas* infected by a herpes-like virus (Renault *et al.*, 1994*a*; 1994*b*) and juvenile *C. virginica* affected by JOD, it appeared appropriate to reexamine the ultrastructure of the bodies in *C. virginica*.



Figures 4 to 6. – *Pinctada maxima.* **4** Early stage in a severe case of ACD in which a broad zone of parchment-like chonchiolin (arrows) has been deposited along the ventral region of the shell and around the hinge region (arrow heads). The mantle (M) has retracted from the region of the ventral deposition. Pallial line (P), Bar = 20 mm. **5** Advanced stage of ACD in which thickened, laminae of conchiolin have been formed within the pallial line along the ventral arc of the shell (between arrows). Conchiolin was removed from one area to show original pallial line (arrow head). Barnacle (Br), Bar = 20 mm. **6** *P. maxima* affected by ACD. Hemispherical beads (B) had been attached on the shell for 2 months. The mantle had retracted behind the level of the beads and conchiolin (arrows) was deposited on and around the beads. Sediment (S) accumulation on the nacre, Bar = 5 mm.

In epithelial and connective tissue cells of the mantle of *C. virginica* affected by JOD, unit membrane-bound, sequestered units of cytoplasm (SU) were observed, They were located in cytoplasmic vacuoles or cell-free in lesions (*fig.* 13). In the most complex form, they consisted of rough endoplasmic reticulum often in flexed parallel arrays, numerous mitochondria, lipoid bodies, a finely granular and electron-dense body, myelin whorls, numerous small vesicles, a large electron-lucent region, and membrane-free ribosomes (*figs.* 14-17). Less complex forms lacked one or more of the characters listed. The SU were not



Figures 7 to 11. – *Pinctada maxima*. **7** Hemispherical beads attached on shell for one month. Nacre either formed on upper bead before onset of ACD (upper arrow) or ACD formation was inhibited in its advancement. Encroachment of ACD (lower arrow) onto the lower bead did occur. Mantle edge (arrow head); anomalous nacre deposit (Ca), Bar = 10 mm. **8** Shell with 3 hemispherical beads. Affected mantle retracted and deposited ACD to level indicated by arrow heads and formed layer of anomalous (wrinkled) nacre on beads. Adductor muscle (A); mantle edge (arrow), Bar = 20 mm. **9** and **10** Lesions in mantle epithelium facing shell. Hemocytes and cell debris (D); relatively intact epihelium (E); lysed epithelium (L), Bar = 25 μm. **11** Periostracal groove (G) and outer lobe (Lo) of affected mantle. Conchiolin secretions are convoluted and multilayered (arrow). No lesions are visible, Bar = 25 μm.

Figure 12. – *Crassostrea virginica*. Left (L) and right (R) valves of oyster shell affected by juvenile oyster disease (JOD). Brown ring (Br) of conchiolin within which have been formed deposits of conchiolin on the nacre, Bar = 5 mm.

observed in the mantle of oysters lacking anomalous conchiolin deposits. In histological sections the SU appeared as spheroidal bodies of < 1 to 6 μm diameter often with usually one, sometimes 2 or 3 internal basophilic, punctate regions of various sizes (*fig.* 13) (Bricelj *et al.*, 1992). Those punctate regions were

Figure 13. – *Crassostrea virginica.* Section of mantle epithelium in which are visible sequestered, spheroidal units of epithelial cell cytoplasm (arrows) in clear, nonstaining regons within and between the cells. Some of the units have a punctate, basophilic region (left arrow), Bar = 25 μm.

also weakly Feulgen-positive indicating the presence of DNA. The smaller SU generally lacked visible basophilic inclusions. The electron-dense, granular regions, not delimited by unit membranes (*figs.* 16 and 17) probably correspond to the basophilic inclusions.

There is no reason to suggest that the SU are protists since no nucleus was observed. It appeared that the oyster cells had subdivided into units containing many of the cytoplasmic organelles of the cell of origin. The mitochondria had mostly shelf-like cristae like those of oysters (*figs.* 14 and 15) but a few had cristae which were more tubular. The rough endoplasmic reticulum was most often a dominant feature of the SU being found at the perimeter of the SU in an arc which extended around as much as 180° of the SU (*figs.* 14, 15 and 17). It was not unusual to find a high degree of organization in that the membranes were spaced equidistant and folded in stacks of saccules with the ribosomes attached in rows on the outside of each saccule. In some SU the rough E.R. was not as organized and could be found in various regions of the cytoplasm of the SU.

Also in the ground cytoplasm of the SU were membrane-free ribosomes (*fig.* 17). In some SU there were numerous free vesicles and multivesicular bodies (*figs.* 15 and 16) scattered throughout the cytoplasm. In many SU the cytoplasm contained an electron-lucent region centrally or eccentrically located and not membrane-bound (*figs.* 14 and 15). Lipoid bodies were often found in and around the perimeter of the lucent region (*figs.* 14 and 15).

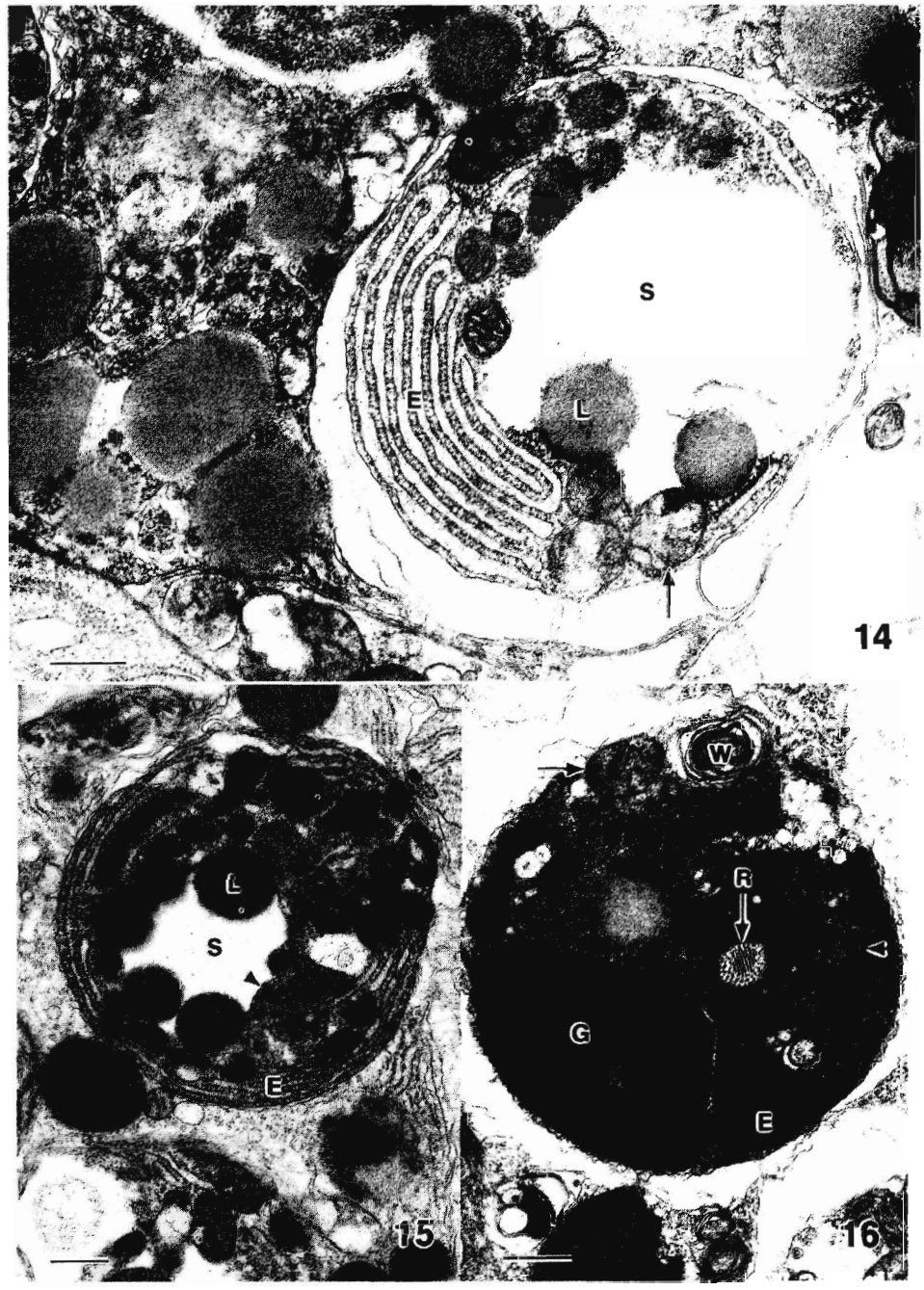
Myelin whorls were found in a few of the SU, usually in the cortex (*fig.* 16). The whorls had the same structure one would expect to see in degenerating cells. A final structure observed were bodies in the ground cytoplasm which consisted of groups of electron-dense filaments in parallel arrays sometimes with two groups organized at right angles to each other (*fig.* 16).

Unlike the SU observed by Renault *et al.* (1994; 1994) in *C. gigas*, herpes-like viruses were not found to be associated with the SU's in juvenile *C. virginica*. In addition, no other virus-like particles were observed with the possible exception of one SU where 89 to 100 nm diameter particles of unknown identity were found near the center of the SU and a smaller class of particles 67-95 nm diameter found peripherally in the SU (*fig.* 17). The absence of the particles from the many other SU's examined and from the associated oyster cells, leads one to conclude at this point that no viral agent is responsible for formation of the SU. The centrally-located particles in *figure* 17 resemble nuclear pores. However, it is unlikely that they are such pores because neither nuclei nor membrane-bound nuclear fragments were observed.

DISCUSSION

Differences in shell disease between that found in *P. maxima* and that found in *R. philippinarum* and *C. virginica* consists of the lack of a ring of anomalous conchiolin deposition (ACD) around the periphery of the inner surface of the shell (*i.e.* brown ring) in the former species and the presence of a ring of conchiolin in the other two species. A similarity involves higher than normal mortalities associated with the ACD. Mantle lesions were noted for affected *C. virginica* and *R. philippinarum* with unaffected individuals lacking such lesions (Bricelj *et al.*, 1992; Paillard *et al.*, 1994). However, in *P. maxima* there was not a clear difference in expression of lesions between unaffected and affected individuals. In fact, two of four freshly harvested and seemingly healthy pearl oysters had lesions in the mangle epithelium. This apparent lack of a distinction between affected and unaffected oysters may have been due to an inability to distinguish between artifacts or mechanical damage in handling the mantle samples and lesions associated with the ACD syndrome.

Although bacteria were found in the affected mantle of *P. maxima* (Pass *et al.*, 1987) and in the mantle and associated ACD of *C. virginica* (Bricelj *et al.*, 1992) and *R. philippinarum* (Paillard *et al.*, 1994), the numbers seen in histological sections were conspicuously small, there being no extensive invasion of the mantle except in moribund individuals. In sections of *C. virginica*-affected mantle less than 30% of the oysters were found to have bacteria associated with the mantle. It appears that the near lack of bacteria as seen in histological sections of tissues is a characteristic which is common to all three species of affected molluscs. As Paillard *et al.* (1994) have suggested for *R. philippinarum*, it may be that bacteria are generally not invasive and proliferate on the surface of the mantle epithelium, secreting toxic products which induce ACD. Formation of ACD represents a defense mechanism which serves to entrap bacteria between lamellae of the ACD and with the syntthesis of melanin in the conchiolin some degree of bacteriocidal protection is provided (Paillard *et al.*, 1994). However, only in *R. philippinarum*



Figures 14 to 16. – *Crassostrea virginica.* Sequestered units of mantle epithelial cell cytoplasm containing parallel arrays of rough endoplasmic reticulum (E), mitochondria (arrow), lipoid droplets (L), multivesicular body (arrow head) and an electron-lucent space (S) which is not membrane-bound. The units are membrane-bound and contained in a vacuole, Bar = 0.1 μm. **16.** – Sequestered unit containing an electron-dense, finely granular body (G) which is believed to be comparable to the basophilic, punctate bodies seen in some sequestered units in histological sections. Myelin whorl (W); mitochondrion (arrow); rough endoplasmic reticulum (E); multivesicular body (arrow head); body with parallel arrays of electron-dense rods (R), Bar = 0.1 μm.

has evidence of melanin been found. Tests for its presence in *C. virginica* and *P. maxima* have not been conducted.

Bacteria, and more specifically *Vibrio* sp., may be involved in inducing ACD formation by *C. virginica*; however, efforts to find a bacterial causative agent have not been conclusive (Bricelj *et al.*, 1992; see also other papers in this journal issue). The suggestion that the sequestered units (SU) with basophilic centers which are found in the mantle are protists is rejected since nuclei are not present (Bricelj *et al.*, 1992).

They are not believed to be prokaryotes for a number of reasons, among which are the presence of mitochondria and the absence of a nucleoid.

There are striking similarities between the SU of *C. gigas* suffering from herpes-like infections (Renault, Le Duff, Cochennec and Maffart, 1994, *fig.* 7) and the SU of *C. virginica* mantle cells affected by juvenile oyster disease (*figs.* 14, 15 and 17 herein and Bricelj *et al.*, 1992, *fig.* 11). In both oysters the rough endoplasmic reticulum is often arranged in parallel arrays located around the perimeter of the body in which mitochondria are found as well as a finely granular electron-dense region with a rounded profile and without a delimiting membane. Whether these similarities indicate that a virus is the causative agent of ACD in *C. virginica* was considered. However, no conclusive evidence of viruses being associated with the SU was found. It could be that the formation of SU can result from a diversity of stresses; however, the entities are unique enough to have not been reported in oysters prior to the papers by Bricelj *et al.* (1992) and Renault, Le Duff, Cochennec and Maffart (1994). Other than the present paper, they have not been reported since then.

Bricelj *et al.* (1992) suggested that the "bodies are secondary lysosomes resulting from an extreme example of autophagy" as described by Constantinides (1984). This suggestion may be valid since ultrastructurally similar bodies are found as a result of cellular sequestration (autophagocytosis) in a diversity of other species such as in rat pancreatic acinar cells (Swift and Hruban, 1964). The SU in *C. virginica* are ultrastructurally similar to those in the acinar cells in that parallel arrays of rough endoplasmic reticulum and mitochondria are present. The difference is that basophilic bodies have not been reported in the examples of autophagocytosis, found in the literature. This is not surprising since nuclear material is not sequestered during autophagocytosis in the cells previously described (Hirsimäke *et al.*, 1983). It should be noted that not all of the bodies in *C. virginica* were found to have basophilic regions. Since they are Feulgen-positive, the basophilic regions in the oyster SU may be chromatin, derived from degraded oyster nuclei. A cytochemical test for acid phosphatase must be conducted to help substantiate whether the bodies are a result of autophagocytosis.

Constantinides (1984) stated that there are 5 situations in which "autophagocytosis with subsequent enzymatic lysis of the cell's own organelles is initiated". These situations include starvation, involution (degradation) of tissues, change of cell programming as during differentiation or dedifferentiation, sublethal injury of cells as in anoxia, and action of certain chemicals such as glucagon and phlorizin. Viruses were not cited as stimulatory factors for inducing sequestration.

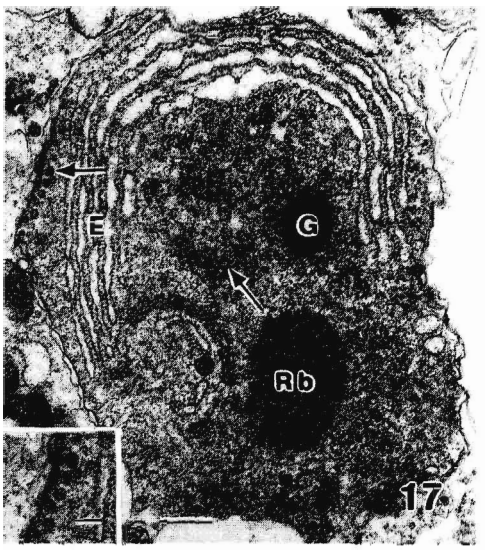


Figure 17. – *Crassostrea virginica*. Sequestered unit in which are visible two types of particles (arrows); parallel array of rough endoplasmic reticulum (E); aggregate of ribosome-like particles (Rb); granular body (G) as in *figure* 16. Inset is higher magnification of one set of particles, Bars = 100 nm and 30 nm.

In the absence of any ultrastructurally detectable agent of SU formation, it appears reasonable to consider the situations listed by Constantinides (1984). The action of a toxin or toxins warrants investigation. Such chemicals could be formed by microbes on the surface of the mantle epithelium or be present in the ambient water, either anthropogenically formed or formed by phytoplankton as suggested by Bricelj *et al.* (1992). Since the oysters used in this study were apparently not starved or exposed to anoxic conditions, those conditions are unlikely to have induced SU formation. Upon occasion when oysters were suffering high mortalities and were being held in high density in trays, lowered oxygen conditions probably prevailed for some of them (Bricelj *et al.*, 1992); however, this was not the case for all oysters examined where SU were seen in histological sections.

Obviously more work must be conducted to determine the causative agent or agents or juvenile oyster disease.

Acknowledgements

I am grateful for the expert assistance provided by Dr. D. A. Pass in whose laboratory photographs of the pearl oysters were obtained. I am also indebted to Dr. Trevor White who was Project Officer of the Fishing and Allied Industries Committee at

the time of the study and who organized for the pearl oyster study reported herein; Mr. J. Looby, Fisheries Officer in Broome for making oysters available; members of the pearl industry for their cooperation and assistance; and Dr. Bernie K. Bowen, Director of the Department of Fisheries and Wildlife for financial assistance. Mr. David Relyea, Frank M. Flower and Sons, Inc., is thanked for providing juvenile *C. virginica* used in the study of juvenile oyster disease. This is Contribution No. 2009, accepted from the Virginia Institute of Marine Science, College of William and Mary, VA, U.S.A.

REFERENCES

Bricelj V. M., S. Ford, F. Borrero, F. O. Perkins, G. Rivera, R. Hillman, R. A. Elston 1992. Unexplained mortalities of hatchery reared, juvenile oysters, *Crassostrea virginica* (Gmelin, 1791). *J. Shellfish Res.* **11**, 331-347.

Constantinides P. 1984. Ultrastructural Pathobiology. Elsevier, Amsterdam, 611 p.

Dybdahl R., D. Pass 1985. An investigation of mortality of the pearl oyster, *Pinctada maxima*, in Western Australia. Rep. 71, Fisheries Department, Perth, Western Australia, 78 p.

Hirsimäki P., A. U. Arstila, B. F. Trump, L. Marzella 1983. Autophagocytosis. In: Pathobiology of Cell Membranes, vol. III, Chap. VIII. B. F. Trump, A. U. Arstila eds. Academic Press, New York, 201-236.

Humason G. L. 1962. Animal Tissue Techniques. W. H. Freeman and Co., San Francisco, 468 p.

Paillard C., P. Maes 1990. Étiologie de la maladie de l'anneau brun chez *Tapes philippinarum*: pathogenicité d'un *Vibrio* sp. *C. R. Acad. Sci. Paris, Sér. III* **310**, 15-20.

Paillard C., P. Maes, R. Oubella 1994. Brown ring disease in clams. *Ann. Rev. Fish Dis.* **4**, 219-240.

Pass D. A., R. Dybdahl, M. M. Mannion 1987. Investigations into the causes of mortality of the pearl oyster, *Pinctada maxima* (Jamson), in Western Australia. *Aquaculture* **65**, 149-169.

Pass D. A., F. O. Perkins 1988. Viruslike particles in the digestive gland of the pearl oyster (*Pinctada maxima*). *J. Invert. Pathol.* **51**, 166-167.

Perkins F. O. 1993. Infectious diseases of molluscs. In: Pathology of marine and estuarine organisms. J. A. Couch, J. W. Fournie eds. CRC Press, Boca Raton, Florida, 255-287.

Ray S. M. 1954. Biological studies of *Dermocystidium marinum*, a fungus parasite of oysters. Rice Institute Pamphlet, Spec. Issue. The Rice Institute, Houston, Texas, 114 p.

Renault T., N. Cochennec, R. M. Le Deuff, B. Chollet 1994*a*. Herpes-like virus infecting Japanese oyster (*Crassostrea gigas*) spat. *Bull. Eur. Assoc. Fish Pathol.* **14**, 64-66.

Renault T., R. M. Le Deuff, N. Cochennec, P. Maffart 1994*b*. Herpesviruses associated with mortalities among Pacific oyster, *Crassostrea gigas*, in France-Comparative study. *Rev. Méd. Vét.* **145**, 735-742.

Shaw B. L., H. I. Battle 1957. The gross and microscopic anatomy of tthe digestive tract of the oyster *Crassostrea virginica* (Gmelin). *Can. J. Zool.* **35**, 325-347.

Swift H., Z. Hruban 1964. Focal degradation as a biological process. *Federat. Proc.* **23**, 1026-1037.

Takemura Y., T. Kafuku 1957. Anatomy of the silver-lip pearl oyster *Pinctada maxima* (Jameson). Bull. Tokai Regional Fisheries Research Laboratory, Tokyo, Japan. No. 16, April 1957, 39-40 (+11 plates).