Aquat. Living Resour. 14 (2001) 195–202

S0990744000010998/FLA

Note

Progress in pathological characterization of Syndrome 85 in the black-lip pearl oyster *Pinctada margaritifera*

Michel Comps[a]*, Christian Herbaut[b], Angélique Fougerouse[c], Florent Laporte[a]

[a] *IFREMER, chemin de Maguelonne, 34250 Palavas-les-Flots, France*

[b] *Université de Polynésie française, BP 6570, Faaa aéroport, Tahiti, French Polynesia*

[c] *Service des ressources marines, BP 20-98713 Papeete, Tahiti, French Polynesia*

Received 24 February 2000; accepted 21 July 2000

Abstract − A programme of zoosanitary survey of the populations of black-lip pearl oyster (*P. margaritifera*) has been developed in French Polynesia during 1996 and 1997. Based on histological and ultrastructural examinations, the study of pearl oysters sampled in several pearl farms from the Society Islands, Tuamotu Atolls and Gambier Archipelago, has been particularly turned to enzootic pathological problems. Particular attention has been given to the shell disease and to the necrosis of the adductor muscle, symptoms that had accompanied the mass mortality in 1985.
Virus–like particles, about 40 nm in diameter, were detected in membrane bound vesicles from the necrosized muscle tissue. A new Rickettsia-like organism found in epithelial cells of the digestive tubules was described, while parasites previously reported, such as cestodes and gregarines, were recognized in some animals. Apart from the pathology, a thorough study of 'parasite-like bodies' discovered in the pearl sack epithelium showed dysfunction in the periostracal secretion.

blacklip pearl oyster / shell disease / virus-like particle / rickettsia / sporozoan

Résumé − Nouvelles données pathologiques sur la caractérisation du Syndrome 85 chez l'huître perlière *P. margaritifera*. Un programme de surveillance zoosanitaire de l'huître perlière (*P. margaritifera*) a été développé en Polynésie française en 1996 et 1997. L'étude a été essentiellement basée sur des examens histologiques et ultrastructuraux d'échantillons d'huîtres provenant de fermes des Îles de la Société, des atolls des Tuamotu et de l'archipel des Gambier. Une attention particulière a été portée à l'étude des lésions de la coquille et à la nécrose du muscle adducteur, symptômes antérieurement observés lors de la mortalité de 1985. Des particules d'allure virale, d'environ 40 nm de diamètre environ, ont été mises en évidence au sein de vésicules membranaires dans les tissus nécrosés du muscle adducteur. Par ailleurs une nouvelle rickettsie a été trouvée dans les cellules de l'épithélium digestif et des parasites tels que cestodes et grégarines, déjà signalés chez cette espèce, ont été également détectés. Enfin, en marge de la pathologie, des éclaircissements ont été apportés à un dysfonctionnement dans la sécrétion du périostracum au niveau du sac perlier.

huître perlière / maladie de la coquille / particules d'allure virale / rickettsie / sporozoaire

1. INTRODUCTION

Previous to the development of the pearl industry in French Polynesia from 1975, only few scientists studied lesions of the black-lip pearl oyster *Pinctada margaritifera*. At the beginning, they were especially interested in the possible function of some parasites for inducing pearl formation. As far as we know, little information concerning the pathology in this species has been available and has been restricted to the description of some parasites.

**Correspondence and reprints.*
E-mail address: mcomps@ifremer.fr (M. Comps).

In 1985, a mass mortality occurred, affecting populations of pearl oysters from several islands of the Tuamotu Archipelago. Although a gregarine was observed in the diseased animals (Chagot et al., 1993), no direct evidence existed to implicate a pathogen in this mortality. After many investigations, it was hypothesized that environmental parameters or stress and wounds occasioned by handling and grafting could be the possible causes of the mortality (Grizel, 1986; Cabral, 1990). From 1990, research efforts were directed towards studies of the physiology of *P. margaritifera*, and of the ecological conservation and management aspects of pearl oyster production. These studies were planed as part of the '*Programme général de recherche sur la nacre*' (PGRN), and carried out by biologists from SRM, IFREMER and from several institutes and universities.

We report here the main results obtained during the past three years on the zoosanitary problems of the pearl oyster *P. margaritifera.*

2. BRIEF REVIEW

The production of pearls by the bivalves has for a long time aroused the interest of the biologists due to the parasites associated with pearl oysters. Concerning the bivalves Pteriidae, early investigators have turned their attention to worm parasites. Metacestodes identified as larval stage of the genus *Tylocephalum* have been reported by Seurat (1906) in *Pinctada margaritifera* from Gambier Islands, and later by Southwell (1912) in the Ceylonese pearl oyster. According to Shipley and Hornell (1904), about 5% of pearl oysters from the Gulf of Mannar were parasitized by the nematode *Sulcascaris sulcata*. Other nematodes such as *Cheiracanthus uncinatus* and *Echinocephalus gracilis* were also found in the adductor muscle of these species (Shipley and Hornell, 1904, 1906). Surprisingly, the frequently occurring of trematode stages in the marine bivalves was not reported in *P. margaritifera*, while a bucephalid trematode such as *Bucephalus margaritae* was common in *P. martensii* (Ozaki and Ishibashi, 1934). Among the annelids, two species of polychaetes infesting the shell of the black-lip pearl oyster are known, *Polydora hornelli*, reported by Herdman (1906) and *P. pacifica* described by Takahashi (1937). Mohammad (1972) reported afterwards *P. vulgaris* as a new species infesting the pearl oysters from Arabian Gulf.

The first occurrence of protistan infection in *P. margaritifera* concerned ovoid sporozoan spores encysted in the mantle (Dubois, 1907). This unidentified sporozoan might be a hyperparasite of a worm, probably responsible for pearl formation. On the other hand, the first confirmed sporozoan parasite of these species was a gregarine reported by Chagot et al. (1993). Present especially in the digestive epithelium, forms of this parasite were also found in connective tissue. Its role in the mass mortality of 1985 was not determined. Investigating the cause of abnormal mortality of *P. margaritifera* from Dongonab Bay, Nasr (1982) has described an unidentified microparasite that consisted of spherical bodies invading the digestive cells. Subsequently, Pass and Perkins (1985) reported that similar structures observed in *Pinctada maxima* were not parasites but normal constituents of the digestive cells.

3. MATERIAL AND METHODS

For gross and microscopical examination, samples of *P. margaritifera* were collected in November 1996 from Tuamotu atolls (Takapoto, Manihi and Rangiroa) and from the Society Islands (Raiatea and Tahaa). In October 1997, a new sampling campaign was carried out in Tuamotu atolls (Takapoto and Arutua), the Society Islands (Raiatea and Tahaa) and in the Gambier Archipelago (Mangareva and Aukena). For light microscopy, samples of molluscs were preserved in Davidson's fixative (Shaw and Battle, 1957). Sections were processed according to standard techniques and stained with Mayer's hematoxylin–eosine. For transmission electron microscopy (TEM), tissues were fixed overnight at 4 °C in 2.5% glutaraldehyde buffered with 0.4 M sodium cacodylate (pH 7.2), postfixed at room temperature in 1% OsO_4 and embedded in Epon resin. Sections were contrasted with saturated uranyl acetate in 50% ethanol, followed by lead citrate (Reynolds, 1963). Semi-thin sections stained with buffered toluidine blue (1% toluidine blue in 1% sodium tetraborate) were also used for light microscope examination.

4. RESULTS AND DISCUSSION

4.1. Syndrome 85

The mass mortality of the black-lip pearl oyster *Pinctada margaritifera* that occurred in 1985 in the pearl farms in French polynesia was accompanied by a complex syndrome that we have named 'syndrome 85', and characterized by shell disorders, lesions of the mantle and necrosis of the adductor muscle. While the mortality has abated, these symptoms still persisted in 1996–1997 in some islands at low prevalence. After many investigations, since no information about a possible causative pathogen were available, physical, chemical and biological variables, and various environmental factors were hypothesized as causes of mortality (Grizel, 1986; Cabral, 1990; Dauphin and Cuif, 1990; Cuif and Dauphin, 1996; Dauphin and Denis, 1987). It is also important to mention that Chagot et al. (1993) reported the presence of a gregarine in the diseased animals, but the role of this sporozoan was not confirmed (Fougerouse et al., 1994). In the course of the present work, we report new investigations on syndrome 85.

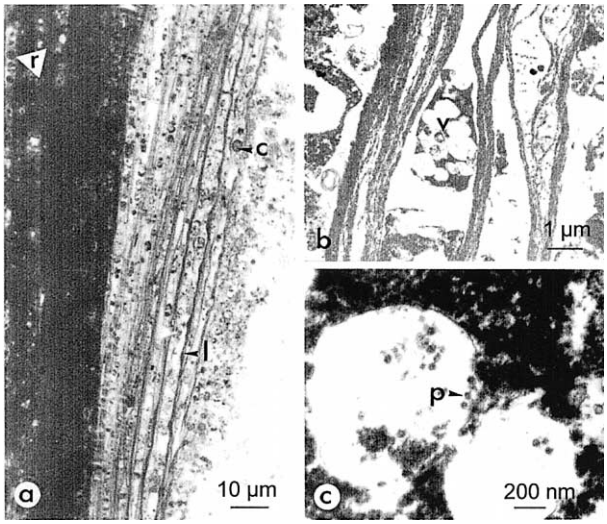

Figure 1. Shell disease: **a.** section through organic deposit from the inner surface of the shell, the part in contact with the mantle consists of thin lamina (l) separated by cellular elements (c), the layer of deposit in contact with the shell appears homogenous with included cellular debris (r) (toluidine blue stain); **b.** detail of the lamellar region: the cells occupying the inter-lamellar spaces are highly vacuolated (v); **c.** electron micrograph showing vacuoles containing electron-dense isometric particles (p).

4.1.1. Shell disease

The name of 'shell disease' refers to the syndrome characterized by a) a deposit of brown organic matter on the inner side of the two valves, and b) a yellowish colour and swelling of the parts of the mantle opposite the organic deposit. This syndrome, previously described on several occasions, has been attributed to 'mechanical trauma' or 'chemical stress' (Grizel, 1986; Fougerouse et al., 1994). In 1996–1997, signs of 'shell disease' persisted in numerous areas, particularly in the lagoons of Tahaa, Raiatea, Takapoto and Arutua. According to the pearl farmers, the prevalence of this syndrome was low in comparison with the prevalence observed in 1985.

Recent studies were centered on the organic deposits. Histological observations of semi-thin sections revealed that this material displayed a complex laminar structure. The organic lamina in contact with the inner surface of the shell consisted of homogeneous periostracum with small lacunas including lysed cells. It is covered by several lamina of periostracum alternating with layers of granulomatous tissue (*figure 1a*). The ultrastructural examination showed that in this tissue, the hemocytes released from the mantle were necrotic and contained karyolytic nucleus and paraspherical vacuoles, 200–500 nm in size (*figure 1b*). Some vacuoles contained aggregated electron-dense grains, membraneous elements and isometric spherical electron-dense particles about 45 nm in diameter (*figure 1c*).

Several cases of 'shell disease' were reported in other marine bivalves. Brown ring disease in the Manila clam, *Ruditapes philippinarum*, characterized by signs close to those observed in *P. margaritifera*, is caused by a bacteria, *Vibrio tapetis* (Borrego et al., 1996) that provokes disorganization of the periostracal lamina and a brown periostracum deposit in the inner surface of the shell (Paillard and Maes, 1990; 1995). Similar symptoms were reported in the scallop *Patinopecten yessoensis* by Mori (1975). Diseased animals exhibited lesions of the shell with a brown substance deposit associated with inflammation in the mantle. Pathological changes that displayed histological similarities with the lesions observed in the pearl oyster were also found in the adductor muscle. No infectious agent was recognized as a possible cause of this disease, which, according to the authors, might result from physiological changes caused by the environmental conditions. Generally speaking, in marine bivalves, organic deposits in the shell are a sign of reaction stimulated by a wound, or by the presence of parasites, debris and hemocytes (Farley, 1968; Aldermann and Gareth-Jones, 1971; Bricelj et al., 1992; Perkins, 1996). According to Grizel (1986), similar process might occurr in *P. margaritifera* as a consequence of culture handling and grafting. Studying the microstructure and the composition of the affected parts of the shell, Marin and Dauphin (1991; 1992) and Cuif and Dauphin (1996) have shown that the malformations in the nacreous layer of *P. margaritifera* were associated with biochemical abnormalities occurring during the biomineralization of the shell. Presently, the observation of electron-dense particles found in the granulomatous tissue contiguous to the organic deposit is not sufficient to suggest the presence of virus-like particles and to hypothesize the role of an infectious agent.

4.1.2. Necrosis of the adductor muscle

Lesions with the appearance of abscess were detected into the adductor muscle of animals exhibiting signs of weakness, associated with abnormal secretion of mucus. From the sampling carried out in 1996 and 1997, the prevalence of these symptoms was the highest in the lagoons of Raiatea, Manihi and Takapoto. Microscopic examinations of sections through the abscess showed focal necrosis of the muscular tissue. At the periphery, the muscle was infiltrated by hemocytes but its structure was preserved, while the central area was occupied by a granulomatous tissue consisting of granulocytes, macrophages and cellular debris. In the intermediate part, the muscle fibres were highly altered with a prominent inflammatory response (*figure 2a,b*). In pearl oysters from Takapoto lagoon, we found internal areas of dense connective tissue including muscle fibre debris, which suggested the occurrence of a healing process (*figure 2c*). Under electron microscopic examination various stages of phagocytosis were seen within the necrotic muscle tissue. Numerous hemocytic cells

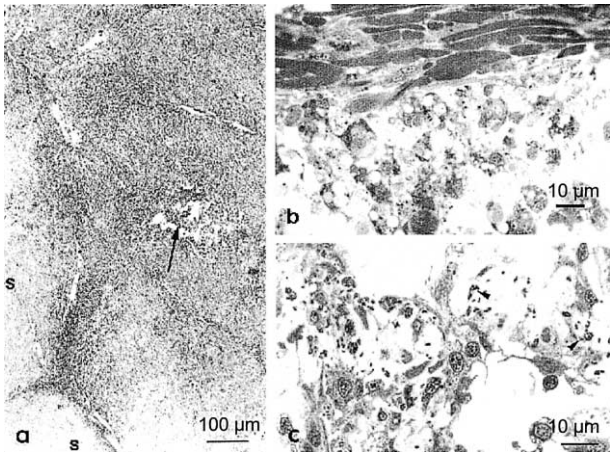

Figure 2. Necrosis of adductor muscle: **a.** section through a focal lesion consisting of granulomatous tissue (arrows) surrounded by partially necrotized and hemocyte infiltrated muscle; sound tissue (s) (hematoxylin-eosinstain); **b.** muscle fibres, in contact with the granulomatous tissue containing vacuolized cells and phagocytes, are fragmented and hemocyte infiltrated (toluidine blue stain); **c.** necrotized muscle partially replaced by fibrous tissue containing numerous basophilic inclusions (arrow heads) (toluidine blue stain).

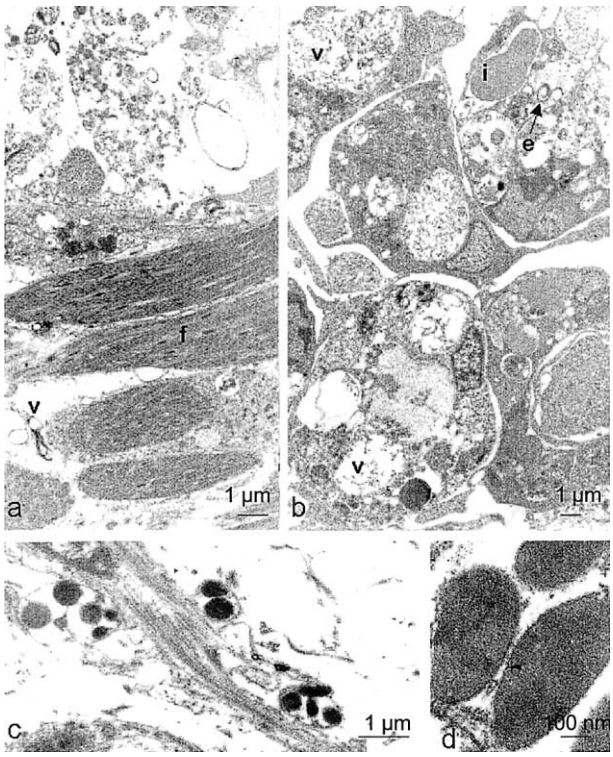

Figure 3. Necrosis of adductor muscle (electron microscopy): **a.** between the disjoined muscle fibres (f), damaged cells display vacuolized cytoplasm (v), electron-dense particles are visible in the sarcoplasm of muscle fibres (arrow); **b.** in the granulomatous tissue, the phagocytary cells exhibit vacuoles containing granular material (v), electron-dense bodies (b) and various membrane-like elements (e); **c.** basophilic inclusions appear as electron-dense granules within membrane-bound vesicles; **d.** electron-dense granules are bound by a membranous element (arrow head).

contained various tissular elements within phagocytic vacuoles resulting presumably from damaged muscle tissue (*figure 3a,b*). Within altered muscle, numerous interstitial or glial cells containing electron-dense and membrane bound ovoid bodies appeared associated with muscle fibres and connective tissue (*figure 3c,d*) The development of such cells could be related to the process of regeneration of muscle tissue (Nicaise, 1973). Surprisingly enough, no bacteria were observed in the muscle lesions, as previously reported by Fougerouse et al. (1994). On other hand, virus-like particles (VLPs) were reported in highly damaged cells from the granulomatous tissue (Comps et al., 1999). With paraspherical or polygonal profile and 40 nm in diameter, VLPs consisted of a membrane-like envelope coating an electron-dense core measuring 35 nm in diameter (*figure 4b*). They spread into the cytoplasm or within membrane bound vesicles (*figure 4a, c, d*). The exact nature and the role of VLPs observed in *Pinctada margaritifera* are still unknown since they have not been purified nor chemically characterized. Similar lesions and associated virus particles were reported by Miyazaki et al. (1999) in the Japanese pearl oyster *Pinctada fucata martensii* during

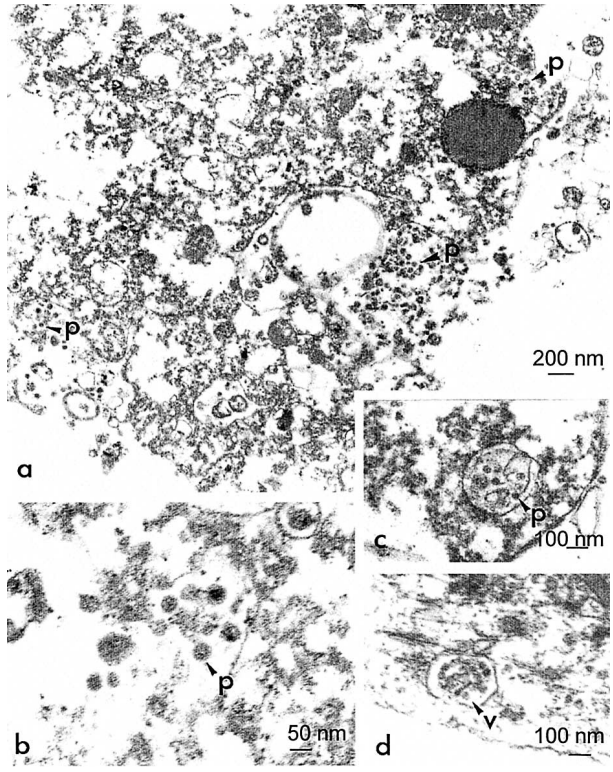

Figure 4. Necrosis of adductor muscle (electron microscopy): **a.** section through a cell containing electron-dense, virus-like particles (p); **b.** morphologically, the virus-like particules (VLPs) consist of a central core (c) coated by a membrane-like structure; **c.** VLPs included within a membrane-bound vacuole; **d.** muscle fibre containing VLPs in a sarcoploasmic vacuole.

a mass mortality which occurred in western regions of Japan in 1996 and 1997. Diseased animals exhibited necrosis, atrophy, swelling and vacuolization of the muscle fibres. In the altered tissues, inclusion bodies contained non-enveloped virions, 25 to 33 nm in size, presumed containing RNA genome on the basis of histochemical data. The pathogen, called akoya-virus, was isolated in EK-1 and EPC fish cell lines. Akoya-pearl oysters experimentally inoculated with isolated virions exhibited the symptoms of the disease. At present, it has not been demonstrated that the akoya-virus also infects *P. margaritifera*. Therefore, except the size of the particles which seems to be different (33 nm for akoya-virus particles vs 40 nm for the particles seen in *P. margaritifera*), the similarities observed suggest that it might be useful to attempt experimental infection of *P. margaritifera* and cross-infections. Previously, Norton et al. (1993) reported the occurrence of viral infection in the gold-lipped pearl oyster *Pinctada maxima*. Provisionally related to the members of the Papovaviridae family, this pathogen infected the epithelium of the labial palps and caused hypertrophy of the nucleus of the infected cell.

4.2. Miscellaneous

4.2.1. Parasite-like bodies of the pearl sack

Abnormal formations which consisted of whitish paraspherical bodies, 0.5 to 1.5 mm in size, were detected during pearl collecting operations in the wall of the pearl sack of pearl oysters from the Takapoto lagoon (*figure 5a,b*). Light and electron microscope examinations have shown that these bodies, initially suspected to be parasites, resulted from a local abnormal secretion of periostracum by the epithelium of the pearl sack (*figure 5c,d*). It was suggested that this phenomenon might be a response to a mechanical wound or to the accidental introduction of a foreign body during grafting (Comps et al., 2000).

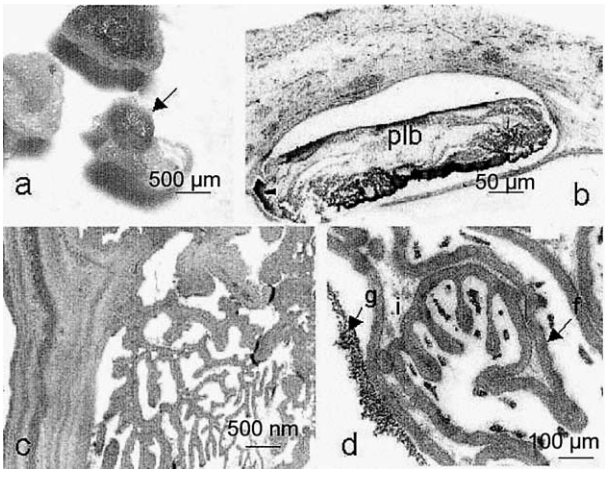

Figure 5. Parasite-like body of the pearl sack: **a.** pieces of pearl bag including some PLBs (arrow); **b.** PLB inserted into a cavity formed in the pearl bag and enclosed by folds of the epithelium (toluidine blue stain); **c.** PLBs consist of a complex arborescent structure resulting from multiple folding of organic lamella; **d.** development of the organic lamella: periostracum granules (g) aggregate to form a thin pellicle (f); fibrous and granulous materiel (i).

4.2.2. Abscess in the pearl sack

Abscesses were sometimes detected within the pearl bag in the course of the grafting. On histological sections, the secretory epithelium of the pearl sack appeared highly damaged. In contact with the nucleus, a layer of granulomatous tissue consisted of hemocytes and cellular debris (*figure 6a*). Intracellular procaryotes were seen within partially lysed cells (*figure 6c–e*). Surrounding tissues were affected by an intense inflammatory reaction (*figure 6b*). Although it would be premature to claim that this necrosis is caused by a procaryotic pathogen, it seems conceivable that microorganisms are introduced in the pearl

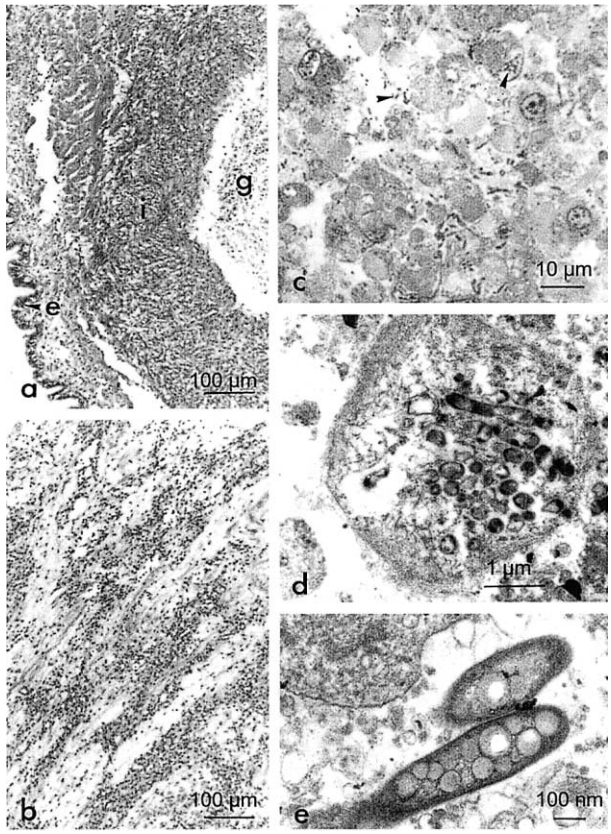

Figure 6. Abscess of the pearl sack: **a.** section through a pearl bag exhibiting an abscess; outer epithelium of the pearl bag (e); the connective tissue (i) is hemocyte infiltrated while the pearl sack epithelium is completely destroyed; the innermost part is occupied by granulomatous tissue (g) (hematoxylin-eosine stain); **b.** connective tissue of the pearl bag displaying host cell response (hematoxylin-eosine stain); **c.** granulomatous area of abscess of the pearl bag: microorganisms (arrow heads) (toluidine blue stain); **d.** intracellular procaryotic microorganisms; **e.** structure of the procaryotes found in the granulomatous tissue.

bag in the course of grafting and become involved in the development of abscesses.

4.2.3. Procaryotic infection

During routine examinations of animals from Manihi and Rangiroa atolls, epithelial cells of the digestive tubules were found to contain microcolonies of bacteria (Comps et al., 1998). The bacteria exhibited characteristics of the members of Rickettsiales. It consisted of reticulate bodies bound by two unit membrane-like elements, ranging from 500 to 800 nm in length and from 200 to 300 nm in diameter. Their cytoplasm contained fibrills and denses granules and, in some micoorganisms, an electron-dense nucleoid-like in the intermediate bodies, which occur in the cycle of development of the Chlamydiales. Other stages of such a cycle, particularly elementary bodies, have not been observed. Lastly, the presence of paracrystalline bodies that resembled the crystals observed in some *Rickettsiella* (Weiss et al., 1984) has been reported. As is generally observed in marine bivalves, the procaryote developed in few host cells and did not induce serious cytopathological changes. No inflammatory response to the infection was observed, but the fibrous layer surrounding some microcolonies of bacteria, presumed to be of host origin, may be a cellular reaction. Similar structures have been reported in *Mercenaria mercenaria* (Fries and Grant, 1992) and in *Donax trunculus* (Comps and Raimbault, 1978).

4.2.4. Sporozoan

The gregarine reported by Chagot et al. (1993) appeared to be a common parasite of the digestive tract of *P. margaritifera*. Still unidentified, this sporozoan does not prevent normal growth of the host (Fougerouse et al., 1994). During the zoosanitary survey in 1996–1997, it was detected in pearl oysters from several lagoons (Gambier, Raiatea and Manihi).

4.2.5. Helminths

A stage of cestode, probably related to the genus *Tylocephalum*, has been observed in the gills of a pearl oyster from Gambier islands. Such worms were previously reported from the same islands by Seurat (1906). In 1997, we found again cestodes in pearl oysters from the lagoon of Raiatea.

5. CONCLUSION

During the severe crisis of 1985 in the pearl oyster industry of French Polynesia, several scientists gave special attention to the pathological manifestations of the disease. Various approaches were considered without resulting in a well-defined etiology. In brief, no pathogen was recognized as a possible cause of the mortality and the authors hypothesized that environmental factors and stresses caused by culture handling and grafting could be responsible for the disease (Cabral, 1990; Grizel, 1986). We have attempted to study the lesions of the disease more thoroughly. Previous studies were based primarily on histological investigation. Therefore, in the current study, particular attention was given to ultrastructural examination. These preliminary results suggesting the presence of a viral disease represent a significant advance in the research of the causes of the persistent syndrome 85. The similarities between the syndromes observed in diseased *P. margaritifera* and in the akoya pearl oyster suggest that it is now important to attempt isolation and characterization of the VLPs present in the muscle lesions in order to evaluate their viral character and to investigate their possible role in the syndrome.

From electron microscope observations, we have also shown that the ‘pearl sack parasite’ was not a parasite, but consisted of lamellar structure that resulted from abnormal secretion of periostracum. The causes of this phenomenon are unknown.

A new rickettsial procaryote, infecting the cells of the digestive epithelium, was found in few pearl oysters. The low prevalence of this microorganism is consistent with the data reported in the majority of Rickettsiales or Chlamydiales infections in marine bivalves. Enzootic parasites, such as cestodes and gregarines, were detected again in *P. margaritifera*.

The results of the investigations reported here show that , without underestating the persistence of a low rate of shell disease, the populations of pearl oyster in French Polynesia during the period 1996–1997 presented a reasonably good zoosanitary condition. Therefore, we have to bear in mind that such a situation is not definitive and that pathogens can be influenced by environmental factors and produce epizootics with resultant mortalities. We think consequently that beside the research on environmental and physiological factors, a sustained effort in blacklip pearl oyster pathology is needed without pending a new crisis.

Acknowledgements. This study was supported by a grant from SRM–IFREMER–CUPF (‘*Contrat de développement État–Territoire*’ no. 32-96, 02/09/96). We thank Dr W. Allen for critical review.

References

Aldermann, D.J., Gareth-Jones, E.B., 1971. Shell disease of oyster. Fish. Invest. 26, 1–19.

Borrego, J.J., Castro, D., Luque, A., Paillard, C., Maes, P., Garcia, M.T., Ventosa, A., 1996. *Vibrio tapetis* sp. nov., the causative agent of the Brown Ring disease affecting clams. Int. J. Syst. Bacteriol. 46, 480–484.

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

Cabral, P., 1990. Some aspects of the abnormal mortalities of the pearl oysters, *Pinctada margaritifera* L. in the Tuamotu Archipelago (French Polynesia). Advances in tropical aquaculture, Aquacop Ifremer, Actes Coll., 9. Ifremer, Plouzané, pp. 217–226.

Chagot, D., Fougerouse, A., Weppe, M., Marquès, A., Bouix, B., 1993. Présence d'une grégarine (Protozoa Sporozoa) parasite de l'huître perlière à lèvres noires *Pinctada margaritifera* (L., 1758) (Mollusca Bivalvia) en Polynésie française. C.R. Acad. Sci. Paris III 316, 239–244.

Comps, M., Fougerouse, A., Buestel, D., 1998. A procaryote infecting the black-lipped pearl oyster *Pinctada margaritifera*. J. Invertebr. Pathol. 72, 87–89.

Comps, M., Herbaut, C., Fougerouse, A., 1999. Virus-like particles in pearl oyster *Pinctada margaritifera*. Bull. Eur. Assoc. Fish Pathol. 19, 85–88.

Comps, M., Herbaut, C.h, Fougerouse, A., 2000. Abnormal periostracum secretion during the mineralization process of the pearl in the black-lipped pearl oyster *Pinctada margaritifera*. Aquat. Living Resour. 13, 49–55.

Comps, M., Raimbault, R., 1978. Infection rickettsienne de la glande digestive de *Donax trunculus* Linné. Sci. Pêche. Bull. Inst. Pêches marit. 281, 11–12.

Cuif, J.P., Dauphin, Y., 1996. Occurrence of mineralization disturbances in nacreous layers of cultivated pearls produced by *Pinctada margaritifera* var. *cumingii* from French Polynesia. Comparison with reported shell alterations. Aquat. Living Resour. 9, 187–193.

Dauphin, Y., Cuif, J.P., 1990. Epizootic disease in the «black lip» pearl oyster *Pinctada margaritifera* in French Polynesia: ultrastructural alterations of the nacreous layer. In: Suga, S., Nakahara, H. (Eds.), Mechanism and phylogeny of mineralization in biological systems. Springer–Verlag, New York, pp. 167–171.

Dauphin, Y., Denis, A., 1987. Altérations microstructurales de la nacre des huîtres perlières (*Pinctada margaritifera*) atteintes par l'épizootie en Polynésie française. C.R. Acad. Sci. Paris III 306, 649–654.

Dubois, R., 1907. Sur un sporozoaire parasite de l'huître perlière, *Margaritifera vulgaris* Jam. Son rôle dans la formation des perles fines. C.R. Scéance Soc. Biol. 62, 310–311.

Farley, C.A., 1968. *Minchinia nelsoni* (Haplosporida) disease syndrome in the American oyster *C. virginica*. J. Protozool. 15, 585–589.

Fougerouse, A., Herbaut, C., Weppe, M., Buestel, D., 1994. Mise en évidence d'une pathologie d'origine infectieuse. Rapport définitif de la Fiche-Programme n°1. Convention particulière n° 09/94 EVAAM-Ifremer.

Fries, C.R., Grant, D.M., 1992. Ehrlichia-like microorganisms in hemocytes in the gills of the marine bivalve, *Mercenaria mercenaria*. J. Invertrebr. Pathol. 59, 210–211.

Grizel, H., 1986. Étude des mortalités de l'huître perlière *Pinctada margaritifera* dans l'archipel des Tuamotu (Polynésie française). Contrat étude EVAAM-Ifremer n° 85/1210177.

Herdman, W.A., 1906. General summary and recommandations. In: Herdman, W.A. (Ed.), Report to the Government of Ceylon on the pearl oyster fisheries of the Gulf of Manaar, part V. Royal Society, London, pp. 109–136.

Marin, F., Dauphin, Y., 1991. Diversité des altérations dans la composition en acides aminés de la phase organique de la nacre des huîtres perlières de Polynésie française (*Pinctada margaritifera*) atteintes par l'épizootie. C.R. Acad Sci. III 312, 483–488.

Marin, F., Dauphin, Y., 1992. Malformations de la couche nacrée de l'huître perlière *Pinctada margaritifera* (L) de la Polynésie française : rapport entre altérations microstructurales et composition en acides aminés. Ann. Sci. Nat. Zool Paris 13, 157–168.

Miyazaki, T., Goto, K., Kobayashi, T., Kageyama, T., Miyata, M., 1999. Mass mortalities associated with virus disease in Japanese pearl oyster *Pinctada fucata martensii*. Dis. Aquat. Org. 37, 1–12.

Mohammad, M.B.M., 1972. Infestation of the pearl oyster *Pinctada margaritifera* (L.) by a new species of *Polydora* in Kuwait, Arabian Gulf. Hydrobiologia 39, 463–477.

Mori, K., 1975. Seasonal variation in physiological activity of scallops,under culture in the coastal waters of Sanriku district, Japan, and a physiological approach of a possible cause of their mass mortality. Bull. Mar. Stat. Asamushi 15, 59–79.

Nasr, D.H., 1982. Observation of the mortality of the pearl oyster, *Pinctada margaritifera*, in Dongonab bay, Red Sea. Aquaculture 28, 271–281.

Nicaise, G., 1973. The gliointerstitial system of molluscs. Int. Rev. Cytol. 34, 251–332.

Norton, J.H., Shepherd, M.A., Prior, H.C., 1993. Papovavirus-like infection of the golden-lipped pearl oyster, *Pinctada maxima*, from the Torres Strait. Australia 62, 198–200.

Ozaki, Y., Ishibashi, C., 1934. Notes on the cercaria of the pearl oyster. Proc. Imp. Acad. Japan 10, 439–441.

Paillard, C., Maes, P., 1990. Etiologie de la maladie de l'anneau brun chez *Tapes philippinarum* : pathogénicité d'un *Vibrio* sp. C.R. Acad.Sci. Paris 310, 15–20.

Paillard, C., Maes, P., 1995. The brown ring disease in the Manila clam *Ruditapes philippinarum*. I. Ultrastructural alterations of the periostracal lamina. II. Microscopic study of the brown ring syndrome. J. Invertebr. Pathol. 65, 91–110.

Pass, D.A., Perkins, F.O., 1985. 'Protistan parasites' or residual bodies in *Pinctada maxima*. J. Invertebr. Pathol. 46, 200–201.

Perkins, F.O., 1996. Shell disease in the gold lip pearl oyster, *Pinctada maxima*, and the Eastern oyster, *Crassostrea virginica*. Aquat. Living Resour. 9, 159–168.

Reynolds, W., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17, 208–212.

Seurat, L.G., 1906. Sur un cestode parasite des huîtres perlières déterminant la production des perles fines aux îles Gambier. C.R. Acad. Sci. Paris (Sér.D) 142, 801–803.

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

Shipley, A.E., Hornell, J., 1904. The parasites of the pearl oyster. In: Herdman, W.A. (Ed.), Report to the Governement of Ceylon on the pearl oyster fisheries of the Gulf of Manaar, part II. Royal Society, London, pp. 77–106.

Shipley, A.E., Hornell, J., 1906. Report on the cestode and nematode parasites from the marine fishes of Ceylon. In: Herdman, W.A. (Ed.), Report to the Governement of Ceylon on the pearl oyster fisheries of the Gulf of Manaar, part V. Royal Society, London, pp. 43–96.

Southwell, T., 1912. The Ceylon pearl inducing worm. Parasitology 5, 27–36.

Takahashi, K., 1937. Notes on the polycheatous annelid, *Polydora pacifica* n.sp., which bores in *Pinctada margaritifera* (L). Palao. Trop. Biol. Stat. Stud. 1, 155–167.

Weiss, E., Dasch, G.A., Chang, K.P., 1984. Bergey's Manual of Systematic Bacteriology, vol. 1. Williams and Wilkins, Baltimore, pp. 713–717.