PART 3

EXPERIENCES IN DEALING WITH PEARL OYSTER MORTALITIES

3.1 Review of pearl oyster mortalities and disease problems

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Jones, J.B. 2007. Review of pearl oyster mortalities and disease problems, pp. 61–70. In: M.G. Bondad-Reantaso, S.E. McGladdery and F.C.J. Berthe. Pearl oyster health management: a manual. *FAO Fisheries Technical Paper.* No. 503. Rome, FAO. 2007. 120p.

ABSTRACT

The aquaculture of pearl oysters is an expanding multimillion dollar industry in the tropical marine environment of many countries, including Australia, French Polynesia, the Middle East, China, Southeast Asia and Japan. Despite the size and extent of the industry there is remarkably little known about the diseases and parasites of the genus *Pinctada*. There is a growing awareness among the industry that, as with other molluscs under cultivation, disease can be an important constraint and that translocation of shellfish poses a serious risk. This paper will review the known diseases caused by pathogenic agents as well as those with a non-infectious aetiology. Management techniques, which can be used to minimize the impact of disease, will also be discussed.

INTRODUCTION

The aquaculture of pearl oysters of the genus *Pinctada* is an expanding industry in the tropical or sub-tropical marine environment of many countries including the Middle East, China, Japan, South-East Asia including Australia and French Polynesia. Contemporary farming practice requires a supply of oysters to replenish oysters harvested for their shell, meat and pearls. These activities, in turn, require introductions of seed stocks from hatcheries and the wild-harvest sector and movements of oysters around the coast. The movement and introduction of marine molluscs to new areas has a long history of initiating major disease impacts on aquaculture industries (Grizel *et al.*, 1986). Recent severe mortalities in Japan, Australia and French Polynesia suggest that *Pinctada* spp. are no exception (Chagot *et al.*, 1993; Miyazaki *et al.*, 1999; Hirano, Sugishita and Mobin, 2005). This paper reviews the major diseases of *Pinctada* spp. and management practices that reduce their effect on the industry.

INFECTIOUS DISEASES AND PARASITES

As with other mollusc species, techniques for the routine culture of virus, fungi and protozoans affecting pearl oysters are not generally available and both fungi and protozoa, with the exception of *Perkinsus* spp. are still routinely identified by

histopathology. Though molecular techniques such as Polymerase Chain Reaction (PCR) are useful for identifying specific pathogens, histology is still preferable as it is capable of detecting multiple infections and other physiological problems such as starvation or loss of condition following spawning. In pearl oysters, the most common response to an insult is an inflammatory reaction. Cellular elements that participate in the inflammatory response and wound repair include agranular amoebocytes, basophilic semi-granulocytes and eosinophilic granulocytes. A typical response of aggregation, phagocytosis, hyperplasia and encapsulation has been described (Feng, 1988; Suzuki, 1992). The haemocyte accumulations, infiltrations and granuloma formations seen in the absence of aetiological agents therefore represent a spectrum of past or existing inflammatory response to a variety of antigens, including soluble antigens, not visible histologically.

Viruses

Pass, Perkins and Dybdahl (1988) described intranuclear viral inclusion bodies present in the digestive gland epithelium of oysters (*Pinctada maxima*) from Australia. These inclusion bodies have been demonstrated to consist of large, icosahedral, enveloped virus particles (Humphrey *et al.*, 1998).

Norton, Shepherd and Prior (1993a) reported that papova-like viral inclusions occurred commonly in the epithelium of the palp in both wild caught and farmed oysters (*P. maxima*) in Queensland, Australia. Mild to moderate digestive gland hyperplasia and degeneration are associated with heavy infections suggesting that the causative agents may potentially be pathogenic (Humphrey *et al.*, 1998) and as a result movement of live infected oysters between Queensland Northern Territory and Western Australia is currently prohibited.

Suzuki, Kamakura and Kusuda (1998) and Kitamura *et al.* (2000) reported the presence of a birnavirus in the haemocytes and digestive gland tubules of *Pinctada fucata martensii* in Japan. The pathological significance of the birnavirus is uncertain. It should be noted that pearl oysters, as with other oyster species, probably sequester a wide variety of viruses from the environment - not all of which will be pathogenic to oysters and some, such as hepatitis and Norwalk virus, would be pathogenic to humans.

In 1995, there was a mass mortality of *Pinctada margaritifera* in pearl farms in several Atolls of the Gambier Archipelago, French Polynesia (Comps, Herbaut and Fougerous, 1999; 2001). On-going research has identified a 40 nm virus associated with necrosis of the adductor muscle, the histopathology of which Comps, Herbaut and Fougerous (2001) suggested was similar to that described in Akoya oysters by Miyazaki *et al.* (1999). Severe mortalities of *P. maxima* in Exmouth Gulf, Western Australia during 2006 are also believed to have a viral aetiology, though no infectious agent has yet been identified.

"Akoya virus"

Mass mortalities of Japanese oyster (*P. fucata martensii*) were first noticed in Yusu Bay and Uchiumi Bay, in Ehime Prefecture, Japan during 1994 and have subsequently occurred annually both at these sites and other bays in western Japan. Mortalities include juvenile, adult and seeded oysters. In 1996 and 1997, losses were reported to be 50 percent of all oysters in production in western Japan (Miyazaki *et al.*, 1998; 1999) though local losses of about 80 percent have been recorded (Tomaru, Kawabata and Nakano, 2001). The mortalities have been the subject of intensive study within Japan including a workshop in September 1999 to overview research on the Japanese pearl oyster mortalities (Muroga, Inui and Matsusato, 1999).

Gross pathology associated with the syndrome includes poor growth of shell on valves, sluggish closing of valves, atrophy and a distinctive yellowish to red-brown colour of adductor muscle and watery appearance due to lack of nutrient storage

(Miyazaki *et al.*, 1998). The histopathology includes necrosis, haemocyte infiltration, atrophy, swelling and vacuolization of adductor and foot muscle fibers. Digestive diverticulae with large lumen and greatly reduced vacuolation and lysosome activity by epithelial cells are consistent with starvation (Shinomiya *et al.*, 1997; Miyazaki *et al.*, 1998). Kurokawa *et al.* (1999) showed that the mortalities were associated with an infectious agent causing the distinctive colour change as well as the histopathological changes to the digestive gland. Miyazaki *et al.* (1999) independently described a small (25–33 nm) virus (Akoya virus) which affected all smooth muscle fibers, not just adductor muscle, affecting feeding, respiration and cardiac function. Miyazaki *et al.* (2000) showed that the effects of the virus could be reduced by injections of a recombinant feline interferon-w.

The Japanese mortalities were associated with illegal imports of Chinese pearl oysters (Wada, 1997) and subsequently large numbers of hybrid akoya/Chinese pearl oysters have been produced in Japan because the hybrid is resistant to the disease, although producing pearls of low quality (Miyazaki *et al.*, 2000). The disease may kill other molluscs, including *Chlamys nobilis* and *Crassostrea gigas* (Miyazaki *et al.*, 1998).

Tomaru *et al.* (2001) suggested, based on sampling in Uchiumi and Yusu Bays, that there was a relationship between water temperature and mortality in autumn (September to November) and that these mortalities were not due to toxic algae. They hypothesized that, when *Nitzschia* spp. (which is inedible to oysters) dominated the culture area, the health of the oyster deteriorated due to food limitation and high water temperature and that subsequently, the infective agent affected the digestive glands causing death by starvation. This hypothesis may explain the results obtained by Hirano, Kanai and Yoshikoshi (2002) who demonstrated, using contact infection trials with diseased oysters in pocket-type cages in the sea, that infection (as expressed by mortality) was not transmitted. Further analysis of these mass mortality events by Hirano, Sugishita and Mobin (2005) concluded that the disease is not due to viral, bacterial, fungal or parasitic organisms but might be due to organic pollution effects from neighbouring fish farms, a view challenged by Nagai *et al.* (2000) who again emphasized the infective nature of the disease and its dependence on high water temperature.

Rickettsiales

Polymorphic rickettsiales have been described from *P. maxima* from the South China Sea (Wu and Pan, 1999) and from *P. margaritifera* in the Pacific (Comps, Fougerouse and Buestel, 1998). These appear, by light microscopy, to be identical to the rickettsiales also seen in Western Australia in *P. maxima*. Rickettsiales have also been reported from *P. maxima* in Queensland and the Northern Territory in Australia (Humphrey *et al.*, 1998). Worldwide, rickettsiales are recognized as asymptomatic infections in a range of molluscs including *Crassostrea gigas*, *C. virginica* and *Mya arenaria* and have been associated with mass mortalities of the scallop *Placopecten magellanicus* (Sparks, 1985).

Bacteria

Bacterial culture under tropical conditions, where water temperatures are around 29 °C to 33 °C, frequently results in the isolation of large numbers of commensal or contaminant organisms, which cannot be readily distinguished from potential pathogens. However, a number of studies have identified bacteria, particularly species of the genus *Vibrio*, as pathogens of oysters, often associated with stress induced by poor management practices or environmental perturbations. Bacterial infections usually incite haemocytic inflammatory lesions (Pass, Dybdahl and Mannion, 1987; Suzuki, 1995). The formation of conspicuous deposits of brown-black conchiolin on the inner nacreous surface of the shell of *P. maxima,* described by Perkins (1996),

was observed in 12–14 percent of shell by Humphrey *et al.* (1998) and has also been reported in *P. margaratifera* in French Polynesia (Marin and Dauphin, 1991; Cuif and Dauphin, 1996). Though common in many bivalves and attributed to bacterial irritation (Paillard, Maes and Oubella, 1994), the aetiology is still uncertain.

Haplosporidium

An unnamed species of *Haplosporidium* was found in a batch of *P. maxima* spat from the Carnarvon hatchery, Western Australia, in 1993 (Hine and Thorne, 1998). The same haplosporidian was subsequently found in *P. maxima* spat from a marine farm site at King Sound in the Kimberley region of Western Australia in 1995 and again in spat at a marine farm site in Broome in 2000 (Jones and Creeper, 2006). The 1995 incident involved spat that had been free of infection on leaving the hatchery and 6 weeks later were found to have a prevalence of infection of 4.6 percent. On re-testing 15 days later, the prevalence had increased to just over 10 percent, at which point the spat were destroyed. Electron microscope examination confirmed that the parasite was the same parasite as that reported by Hine and Thorne (1998). The relationship between this haplosporidian and one occurring in *Saccostrea cucculata* in the same area is currently under investigation (Bearham *et al.*, 2007).

Perkinsus

Perkinsus sp. is routinely diagnosed by thioglycollate culture rather than histology. It has been commonly isolated by culture from tropical Australian molluscs, including *Pinctada* spp. in the absence of any pathology (Goggin and Lester, 1987, 1995). *Perkinsus*-like protozoa were described by Norton *et al.* (1993b) in focal granulomatous lesions in adult *P. maxima* from a population undergoing high mortality in Torres Strait, Australia, though a causative relationship could not be established. Park *et. al.* (2001) were unable to demonstrate the presence of *Perkinsus* in *P. fucata martensii* from Korea either by thioglycollate culture or histology.

Other agents

A number of infectious organisms do not appear to cause significant tissue damage or inflammatory response from their host. These include turbellarians and Ancistracomid-like ciliates. While turbellarians caused no apparent damage or host response in the study by Humphrey *et al.* (1998), northern hemisphere studies on the turbellarian *Urastoma cyprinae* have shown that it is attracted to oyster gill mucous (Brun, Boghen and Allard, 1999) and causes biochemical changes to gill mucous of infected *Crassostrea virginica* (Brun, Ross and Boghen, 2000). Therefore, absence of histopathological change does not mean that there is no effect on pearl oysters. In 2001, an intracellular rhynchodid-like ciliate was found in oysters (*P. maxima*) from the Exmouth Gulf and Montibello Islands in Western Australia. The ciliate appears similar to those described from mussels (*Mytilus* spp.) in Europe (McGladdery and Bower 2002; Jones and Creeper, 2006). Sampling has shown that the ciliate occurs in high prevalence and abundance in juvenile oysters, disappearing from oysters over about 90 mm diameter.

Gregarines occur in *P. maxima* in Australia and appear not to cause significant damage (Humphrey *et al.*, 1998). However, an unidentified intracellular gregarine parasite, described from the gut of *P. margaritifera* from atolls in the Tuamotu Archipelago, French Polynesia, from the Fiji Islands and from the Red Sea (Gulf of Aqaba), causes local or complete destruction of rectal epithelial cells and may be associated with mortalities in French Polynesia (Chagot *et al.*, 1993).

Metacercariae of a bucephalid trematode occur in the gonad of *P. radiata* in the Persian Gulf (Khamdan, 1998). Third and fourth stage larvae of the ascariid nematode has been reported to occur in the adductor muscle, digestive gland and gonads of

Pinctada spp. The adult nematode occurs in loggerhead turtles, *Caretta caretta* (Berry and Cannon, 1981).

Larval cestodes of the family Lecanicaphalidae which include the genera *Tylocephalum* and *Polypocephalus* are commonly associated with discrete focal or multifocal granuloma in interstitial tissues. Larval *Tylocephalum* sp. have been reported in most bivalves examined in northern Australia (Wolf, 1976, 1977; Hine and Thorne, 2000). Larval *Tylocephalum* sp. are not considered host specific and may occur at high prevalence and intensity in oysters where they may reduce the condition of their host (Sindermann, 1990).

The copepod *Anthessius pinctadae* was described from *P. mararitifera* from the Torres Strait (Humes, 1973). The copepod also occurs in *P. maxima* throughout northern Australia (Humphrey *et al.*, 1998). In moderate numbers, this copepod causes erosion of the oesophageal epithelium and entrapment in the digestive gland resulted in encapsulation and a haemocytic response. Thus, under certain conditions, the parasite may be potentially pathogenic or predispose the oyster to infection.

Fouling organisms

Boring molluscs *Lithophaga* spp. are common in Australia and produce large holes of 1-2 cm sometimes disrupting or breaching the nacreous layer. Polychaete worms invading the shell nacre are also common, resulting in "mud blisters" (Humphrey *et al.*, 1998). Boring sponges, family Clionidae, including the bright red coloured *Cliona* sp., are a major problem for the industry in Australia, leading to severe erosion of the shell matrix and premature removal of shells from pearl production (Vblayudhan, 1983; Taylor, Southgate and Rose, 1997). Treatment and prevention involves regular scrubbing of shell, either manually or by high-pressure hose. There has also been some success with freshwater baths for 30–60 min, while trials are underway testing anti-fouling paints.

Commensal animals

Pea crabs and shrimps are common, occurring in up to 85 percent and 72 percent of Australian *P. maxima* oyster populations respectively (Humphrey *et al.*, 1998). Apart from local oedema in the mantle and depressions in both the mantle and gill tissues caused by the pea crabs, no pathology is associated with these commensal organisms (Dix, 1973).

DISEASES WITH NON-INFECTIOUS AETIOLOGY

Temperature

There is little published information on the histopathology associated with temperature, but it has a marked effect on the oysters. Tomaru *et al.* (2002) showed that growth in height, length and thickness of the shells of *P. fucata martensii* was limited by water temperatures less that 20 °C. Pouvreau and Prasil (2001) showed that temperatures over 30 °C had a negative impact on growth of *P. margaratifera*. Likewise, Mills (2002) showed that survival of *P. maxima* spat was greatest between 23 °C and 32 °C, with 35 °C resulting in high mortalities.

Toxic algae

During a red tide event in Ago Bay, Japan in 1992, the maximum cell density of *Heterocapsa circularisquama* reached over 85 000 cells/ml and there was a concurrent mass mortality of pearl oysters. Subsequent trials (Nagai *et al.*, 1996; Nagai *et al.*, 2000) showed that mortality of two-month-old pearl oysters (*P. fucata*) was closely correlated with the cell density of *H. circularisquama* and that, on exposure, oysters rapidly contracted their mantles and closed their shell valves. Negri *et al.* (2003) showed that *Trichodesmium* blooms also caused poor condition in pearl oysters.

Coral spawning

Mortalities caused by low oxygen levels associated with coral spawning are not uncommon, though not reported in the literature.

Starvation

Degenerative changes, not associated with causative agents, are relatively common and include oedema, increased pigmentation in macrophages in interstitial tissues and kidney and mineralisation. Oedema is believed by Humphrey *et al.* (1998) to be a degenerative response in a physiologically compromised oyster and is also seen commonly in oysters removed from the water column and held at high ambient air temperatures. The significance of increased brown pigmentation in cells (so-called brown cells) is unclear but is believed to be associated with prior cellular breakdown and detoxification (Zaroogian and Yevich, 1994) though it should be noted that brown pigmentation of the heart and epithelium of the mantle tissue is normal. Lamellar mineralization is associated with pearl formation, however, mineralization may occur without nacre formation (Comps, Herbaut and Fougerouse, 2000).

Miscellaneous

Tearing of the adductor muscle through the practice of wedging open oysters for seeding operations also results in a recognizable wound healing response in the affected muscle (Norton, 2000). A non-specific inflammatory haemocytic infiltration in the adductor muscle is typical of this change (Humphrey *et al*., 1998).

Slightly refractile ovoid brown pigmented "protistan parasites" have been described in cytoplasm of digestive gland epithelium of *P. maxima* in Australia (Wolf and Sprague, 1978) and in *P. margaritifera* from the Red Sea (Nasr, 1982). These bodies occur commonly in both healthy and diseased oysters. They are "residual bodies", storage or secretory products which are ultimately released from the cell (Pass and Perkins, 1985) and are not of pathological significance.

Though neoplasia are rare, neurofibromatous tumours in Australian *P. maxima* were recorded by Humphrey *et al*. (1998). Two polypous mesenchymal tumours in *P. margaritifera* have also been described from Australia (Dix, 1972).

MANAGEMENT OPTIONS

There are two factors that together make pearl farming less susceptible to disease-induced mass mortalities than other molluscs. The first is the panel based culture system, which apart from providing a degree of predator protection, makes monitoring of individual shells possible. The second is the requirement to lift and clean biofouling off the panels every four weeks or so, which means that shell is regularly inspected for mortality.

The majority of the internationally notifiable diseases of molluscs have been spread by human activities, usually associated with aquaculture. Movement controls to prevent the indiscriminate movement of shell between areas and between countries is thus the first step in disease management. Within Australia, regional differences in distribution of several agents including Papovavirus-like inclusion bodies were identified by Humphrey *et al.* (1998) and form the basis for regional control measures including batch testing of animals for disease status prior to authorizing movements. In Western Australia, the use of quarantine sites and the provision of a mandatory five nautical mile exclusion zone for pearl oyster farming activities around farms serves, in part, as a barrier to prevent the spread of disease from farm to farm.

CONCLUSIONS

Pearl oysters are relatively free of serious pathogens – so far. However, there has been little study of causes of mortalities of pearl oysters in regional areas where farming is

now spreading. For that reason, both government agencies and industry members need to be careful about the source of their stocks and the movement of oysters between regions if the disasters that have befallen other shellfish industries are to be avoided.

ACKNOWLEDGEMENTS

A wide range of people have assisted and contributed to my understanding of pearl oysters in Australia and I am grateful for the help and advice from Dr John Humphrey (Northern Territory). Members of the pearling industry and company staff have also gone out of their way to provide helpful material and logistic support.

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