3.2 The Cook Islands experience: pearl oyster health investigations

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ABSTRACT

A survey of the health of black-lip pearl oysters (*Pinctada margaritifera*) and other bivalves in Manihiki Lagoon, Rakahanga Lagoon and Penrhyn Atoll, Cook Islands, was conducted in May 1998. That study revealed the presence of a few trivial infections (e.g. gregarines, ciliates, trematode metacercariae) and three potentially pathogenic groups, boring clionid sponges, fungal infection of drill holes caused by an *Ostracoblabe implexa*-like fungus and a species of *Perkinsus* which occurred at low intensities in kuku (*Arca ventricosa*). The *Perkinsus* sp. was most probably *P. olseni* which has been previously reported from the region, however it appeared that pearl oysters were less susceptible than many other hosts and therefore perkinsosis may not be a great threat to pearl farming in the Cook Islands.

Subsequent to the original survey, a disease outbreak was reported in black-lip pearl oysters cultured in Manihiki Lagoon in November 2000. The disease was characterized by retraction of the mantle and deposition of a broad, brown conchiolin deposit on the nacre inside the pallial line. Overall, 64.7 percent of oysters examined showed signs of the disease at all sites examined within the lagoon, with the prevalence of the lesions ranging from 3.3 percent in wild oysters from Site 3, to 100 percent in cultured oysters from Site 2. There was no evidence of widespread infection of any viral, protozoan or metazoan pathogens in the diseased pearl oysters examined. A variety of bacteria were isolated from the hemolymph and adductor muscle of the oysters sampled for bacteriology, most notably *Vibrio harveyi.* However, there was little evidence to suggest one pathogenic strain of bacteria was present in diseased oysters and there was no significant correlation between the severity

of conchiolin deposits and the severity of bacterial infections. A follow-up survey of all three sites in December 2003 showed that a high percentage of the oysters in Manihiki Lagoon exhibited shell scarring as the brown lesions were overlaid with new nacre, while oysters sampled from Penrhyn Atoll were also affected with similar gross signs to those observed in Manihiki Lagoon in November 2000. These data suggest that the November 2000 disease outbreak in Manihiki Lagoon was an unprecedented event. It appears that the disease outbreak in November 2000 was associated with infection of pearl oysters by opportunistic vibrios following a transient reduction in lagoon water quality due to a long period of unusually calm, dry weather. The situation may have been exacerbated by high oyster stocking densities and occurred at a time of the year when oysters may have been further stressed after spawning. Management of the problem may, therefore, be based on controlling the stocking density of oysters in lagoons and modifying husbandry practices to reduce stress on oysters when they are spawning. Ongoing monitoring of key water quality parameters in the lagoons would also be useful so that the epidemiology of disease outbreaks which may occur in the future might be better understood.

INTRODUCTION

Culture of black-lip pearl oysters (*Pinctada margaritifera*) in the Cook Islands (Sims, 1994) has increased dramatically in recent years and production of black pearls now rivals tourism as the major source of foreign exchange (Rowntree, 1993). Because of their importance to the Cook Islands economy, a survey of the health of *P. margaritifera* and other bivalves was done in May 1998, funded by the Asian Development Bank (Hine, 1998). The survey was carried out to determine the health status of farmed and wild pearl oysters in Manihiki lagoon. Oysters were also examined from Rakahanga, a nearby island, Penrhyn Atoll, another site of black pearl culture and Aitutaki. *Perkinsus olseni*, an internationally notifiable organism, infects pearl oysters (Norton *et al.*, 1993; Hine and Thorne, 2000), but it occurs more commonly in members of the families Tridacnidae (Goggin, 1996), Arcidae and Isognomonidae (Goggin and Lester, 1987; Hine and Thorne, 2000). Therefore members of those families were also sampled to determine whether *Perkinsus* occurs in the lagoon. Subsequent to that survey, a disease outbreak was reported in black lip pearl oysters cultured in Manihiki Lagoon in November 2000 (Diggles and Hine, 2001). This paper presents some of the results of investigations conducted by the National Institute of Water and Atmospheric Research (NIWA) to determine the identity of the agents and environmental conditions associated with diseases of black-lip pearl oysters in the Cook Islands.

PEARL OYSTER HEALTH INVESTIGATIONS

Materials and Methods

In the May 1998 health survey, 547 pearl oysters were sampled from 11 sites within Manihiki lagoon. At one of these sites, 40 wild virgin shells and 20 drilled and hung virgin shells were sampled. At another site, a sample was collected of 50 oysters fouling the farm site. Also, adult pearl oysters were sampled from the nearby island of Rakahanga (n = 58) and from Penrhyn Atoll (n = 49) and spat from Penrhyn Atoll (n = 16) and Aitutaki (n = 12). In addition, 42 pipis (*Pinctada maculata*), 77 razor shells (*Isognomon isognomum, Isognomon perua*), 50 kuku (*Arca ventricosa*) and 50 paua (*Tridacna maxima*) from Manihiki were sampled. The shells of all animals sampled were measured and examined externally and internally for pests. A standard section was cut through the digestive gland, gonad, mantle, gills, adductor muscle, heart, kidney and foot and fixed in 10 percent formalin made up with seawater for histology. The remaining tissues of 50 *P. margaritifera,* 21 *I. isognomum,* 25 *A. ventricosa* and 3 *P. maculata*, were incubated in Ray's fluid thioglycollate medium (RFTM) (Ray, 1966; Bushek and Allen, 1996) for the detection of *Perkinsus.*

During the disease outbreak in November 2000, black lip pearl oysters (n = 300) and water were sampled from 10 sites within Manahiki lagoon for histopathology, bacteriology and toxicology. Examination began by noting the presence of grossly evident infections with metazoan parasites and fouling organisms (mudworms, fungi, boring sponges, etc.). The dorso-ventral measurement (DVM) of each oyster was recorded to the nearest mm before the oyster was opened by severing the adductor muscle. The nacre of each shell valve was examined for abnormal conchiolin deposits and the severity of conchiolin lesions was assessed using a six-grade qualitative scoring system (Table 3.2.1).

The first 10 oysters examined from each site were subjected to bacteriological analysis prior to being processed for histopathology and RFTM incubation. Samples of 0.1 ml of hemolymph were obtained from the pericardium with a sterile 1 ml syringe and 25 G hypodermic needle and inoculated onto one half of plates containing thiosulphate citrate bile salt sucrose agar (TCBS) and for oysters from sites 1 to 6, Tryptone Soya Agar (TSA) with 2 percent NaCl added (TSA+2). The other half of each plate was streaked with a flamed wire loop which had swabbed the surface of the adductor muscle which had been cut with a sterile scalpel to provide an uncontaminated surface. Sub-samples of 0.1 ml of seawater samples obtained for plankton analysis from the top and the bottom of the water column from each site were also examined for waterborne bacteria in a similar manner. The bacteriology plates were incubated at room temperature (30 °C) and examined for bacteria after 18 hrs and again after 36 hrs. Samples of different colony types observed were sub-cultured to ensure purity before being stored in long term preservation medium (Beuchat, 1974) for up to 2 weeks prior to identification.

Biochemical characterization of selected isolates was undertaken by subjecting them to 52 phenotypic tests using methods described by Baumann, Baumann and Mandel (1971), Furniss, Lee and Donovan (1978) and West and Colwell (1984). Phenotypic data were compared to a probabilistic data matrix for *Vibrio* species (Bryant and Smith, 1986) using a regularly updated version of the Bacterial Identifier program (Bryant and Smith, 1991). An acceptable identification was reached when the identification score equaled or exceeded 0.98.

The first 10 oysters examined from each site were also examined for the presence of *Perkinsus* sp. by placing excised samples of mantle, digestive gland, gills, foot and kidney into RFTM. Tissues were incubated in RFTM in the dark between 7 and 14 days then removed, blotted to remove excess RFTM and placed in dilute Lugol's iodine before examination under a dissecting microscope at 40x magnification for stained enlarged hypnospores (Ray, 1966; Bushek and Allen, 1996).

All 30 oysters from each site were sampled for histopathology. An oblique transverse section, approximately 5-7 mm thick, was cut from each oyster using a scalpel. The section was oriented to include mantle, gonad, digestive gland, gills, foot and sometimes kidney. The tissues were fixed in 10 percent formalin in filtered seawater for at least 96 hrs before being embedded in paraffin on the cut surface. One section 6 µm thick was cut and stained with hematoxylin and eosin (H&E) using standard histological techniques before being examined under a compound microscope.

TABLE 3.2.1
The qualitative scoring system used to grade the severity of abnormal conchiolin deposits in oysters from Manihiki Lagoon

Grade	Abnormal conchiolin lesion severity
0	apparently healthy, no lesions evident
1	1 or 2 focal lesions
2	<25% of shell valve perimeter affected
3	25 to 50% of shell valve perimeter affected
4	50 to 75% of shell valve perimeter affected
5	>75% of shell valve perimeter affected

Samples of pearl oysters were also collected from two sites in Manihiki Lagoon and a control site in Penrhyn Lagoon for toxicological analysis. The two samples from Manihiki lagoon included a sample of relatively healthy wild oysters (n = 4, lesion grade 0 to 1) and a sample of diseased cultured oysters (n = 5, lesion grade 3 to 5). The samples from Penrhyn Lagoon consisted of two sub-samples of 6 oysters without lesions. One sample consisted of 6 wild oysters and the other of 6 cultured oysters from the same locality. All samples were analysed for selected heavy metals (zinc, copper, arsenic, mercury chromium, cadmium, nickel and lead) using nitric/hydrochloric acid digestion followed by inductively coupled plasma mass spectrometry (ICP-MS) determination methods and total hydrocarbons by accelerated solvent extraction (ASE) or sonication extraction followed by gas chromatography/flame ionization detection (GC-FID) quantification.

A follow-up survey conducted in December 2003 saw a total of 654 pearl oysters sampled for pathological and microbiological analysis from 12 sites within Manihiki Lagoon (n = 357 oysters), 6 sites from Rakahanga Lagoon (n = 120 oysters) and 6 sites from Penrhyn Atoll (n = 177 oysters). The histological and microbiological methods used to examine oysters in the 2003 survey were identical to those used during the original disease outbreak in November 2000.

RESULTS

May 1998

Gross observations: *Pinctada margaritifera*

Three types of shell infection could be macroscopically identified; these are boring sponges, mudworm tunnels and fungal-like infestations derived from holes drilled in the shell for suspension-hanging.

Boring sponges appeared as orange inclusions, 1.0-5.0 mm across, underlying the nacre. Two types were apparent. One comprised of equally spaced inclusions of 1.0-3.0 mm (but usually 1.6-2.1 mm) diameter. Inhalent-exhalent holes 0.7-1.1 mm in diameter could be seen on the outer surface of infected valves. The second type of boring sponge was only observed in two wild oysters from one site. It appeared as orange inclusions of unequal size, 3.5-5.0 mm in diameter, angular to ovoid in shape, that were widely distributed throughout the shell, including nacre underlying the adductor muscle attachment. Holes on the outer surface of the shell were 1.0-1.4 mm in diameter, but erosion of the shell between holes were <5 mm in diameter. These infestations appeared severe enough to cause mortalities, either by causing detachment of the adductor muscle, or disintegration of the shell.

Mudworm tunnels were only seen in 5 oysters. They appeared as 1-3 mm wide dark brown to black straight tunnels extending up to 14 mm from the edge of the nacre toward the centre of the shell. The corresponding outer surface of the shell had a shallow indentation into the surface between the layers of shell. These infestations appeared to be trivial and unlikely to cause any adverse effect on oyster health.

Infections associated with holes drilled in oyster shell for suspension-hanging occurred at the site of the drilled hole, but progressed as a greenish brown or yellow discolouration of the inner shell extending toward the centre of the shell. The overlying nacre was thin and can easily be broken by slight pressure. The infections appeared to develop in oysters with drill holes made too far from the edge of the shell, causing damage to the underlying soft-tissues of the oyster (presumably the mantle).

Histopathology: *Pinctada margaritifera*

In 18-68 percent of *P. margaritifera* from different sites in Manihiki lagoon, a gregarine-like apicomplexan occurred between the epithelial cells, or underlying the basement membrane of the posterior gut. Occasionally low density infections occurred in connective tissue throughout the oyster. Gregarines in the connective tissues were

usually larger than those located between epithelial cells. Although there was no host inflammatory reaction to connective tissue infections, gregarines in the gut epithelium were often partially engulfed by brown cells. Prevalence was highest in wild shell (63-68 percent). Gregarines were less prevalent (10 percent) in Rakahanga oysters and absent from Penrhyn oysters and spat transferred from Penrhyn to Aitutaki.

A few ciliates with a distinctive pellicle occurred in the gut of 3–14 percent of pearl oysters from Manihiki. These ciliates were never numerous and there was no apparent tissue damage associated with their presence. Rakahanga oysters had similar levels of ciliate infection (3 percent). Infection levels were much higher in Penrhyn adults (39 percent) and Aitutaki spat derived from Penrhyn (42 percent), but were absent from Penrhyn spat. Although quantification was not possible, infected Penrhyn oysters appeared to have elevated levels of degeneration of the digestive diverticula epithelium.

Trematode metacercariae were encysted in the connective tissue of the mantle of five oysters. Infections were light and none of the encysted helminths caused a host cellular response.

Examinations for the presence of Perkinsiid protozoan parasites using RFTM incubation gave negative results.

Histopathology: *Pinctada maculata*

The epithelium and underlying connective tissue of the posterior gut was infected with gregarine-like apicomplexans that were morphologically indistinguishable from those at the same site in *P. margaritifera.* The prevalence (29 percent) was also similar, but the intensity of infection was higher, with many gregarines crowding the gut epithelium. Despite this, there was no evidence of a cellular response by the host, suggesting that these parasites do not cause disease. Ciliates resembling those in *P. margaritifera* were also present. An un-identified thick-walled protozoan cyst, 22 µm in diameter, occurred in the connective tissue of one pipi. It contained a few basophilic refractile reniform spore-like bodies 4 x 8 µm in diameter. RFTM incubation tests gave negative results.

Histopathology: *Isognomon* spp. and *T. maxima*

No infections or abnormalities were observed and incubation of tissue samples in RTFM gave negative results.

Histopathology: *A. ventricosa*

Gregarines, indistinguishable from those seen in other hosts, occurred in the digestive epithelium of the posterior gut of one kuku. Coccidian sporozoites infected the connective tissues of the digestive gland of four *A. ventricosa.* There appeared to be eight sporozoites per sporocyst and two sporocysts per oocyst, tentatively placing this organism within the eimeriine genus *Dorisiella.* Ciliates were common in the gut and digestive diverticulae and, in one animal, infected the adductor muscle, causing dissociation of the muscle fibres.

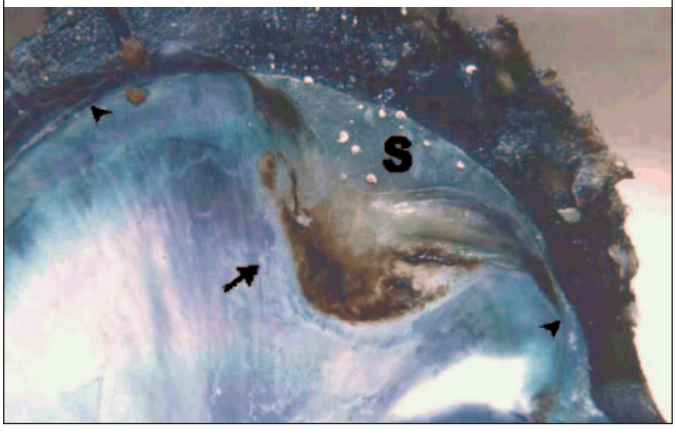
Groups of *Perkinsus* schizonts, 12–18 µm across, comprising individual schizonts 4-5 µm long, occurred in the connective tissue between the digestive diverticula and the gonad follicles of three (6 percent) and in the heart of one (2 percent) of the kuku examined. There was no apparent cellular response to these light infections. RFTM incubation gave two positives, being two of the four kuku in which *Perkinsus* was detected by histology.

November 2000

Gross observations

A total of 194 of the 300 oysters examined (64.7 percent) in November 2000 exhibited varying degrees of a broad, brown conchiolin deposit on the nacre inside the pallial

FIGURE 3.2.1
A focal area of broad based conchiolin deposition in *P. margaritifera* from Manihiki lagoon. The pallial line (arrowhead) indicates the original position of the mantle, while the conchiolin deposit (arrows) lies outside a portion of the retracted mantle. Note sediment (S) in areas of nacre exposed by the retracted mantle



line (Figure 3.2.1). The inner edge of the abnormal conchiolin deposits approximated the general position of the outer surface of the retracted mantle. Abnormal conchiolin deposits were found in oysters from all sites examined. Prevalence of the lesions ranged from 3.3 percent in wild oysters from site 3, to 100 percent in cultured oysters from site 2 (Table 3.2.2). The mean severity of the lesions ranged from 1 in wild oysters at site 3, to 3.3 in cultured oysters at site 9 (Table 3.2.3).

Microbiology

A variety of bacteria were isolated from the hemolymph and adductor muscle of the oysters sampled for bacteriology. Most oysters had mixed bacterial infections and there was little evidence to suggest one pathogenic strain of bacteria was present in diseased oysters. Bacteria were not always isolated from oysters exhibiting abnormal conchiolin deposits, while

TABLE 3.2.2
Prevalence and mean score of abnormal conchiolin deposit lesions on nacre inside shell valves

Site examined	Lesion prevalence	Mean lesion score	Range	Standard deviation
Site 1	93.3 %	2.9	0 – 5	1.44
Site 2	100 %	2.7	1 – 5	1.32
Site 3	3.3 %	1	0 – 1	–
Site 4	60 %	1.2	0 – 3	0.51
Site 5	73.3 %	1.7	0 – 4	1.08
Site 6	53.3 %	2.1	0 – 4	1.31
Site 7	70 %	2	0 – 5	1.3
Site 8	70 %	2.9	0 – 5	1.6
Site 9	56.7 %	3.1	0 – 5	1.5
Site 10	66.7 %	2	0 – 5	1.38
All sites	64.7%	2.33	0 – 5	1.42

TABLE 3.2.3
Comparison of the mean severity of abnormal conchiolin deposits and the mean severity of bacterial infections of the adductor muscle and hemolymph as detected by plate culture using the following grading system: 0 = no bacteria, 1 = 1 to 10 colonies isolated, 2 = 10 to 100 colonies isolated, 3 = > 100 colonies isolated. A total of 10 oysters and 2 water samples (0.1 ml) were examined by bacteriology from each site. Nd = not done

Site	Prevalence of conchiolin lesions	Mean lesion intensity	Prevalence of bacterial infections by plate culture	Mean bacterial infection severity (TCBS)	Mean bacterial infection severity (TSA+2)	Mean severity of bacteria in water, Top	Bottom
1	80%	1.3	90%	1.1	1.4	1	1
2	100%	2.4	90%	1.5	1.6	1.5	1.5
3	0%	–	30%	1	1.3	0	1.5
4	40%	1	50%	1.5	1.5	1	1.5
5	90%	2.1	70%	2.3	2	1.5	1
6	80%	2.5	40%	1	3	0	1
7	50%	1.8	0%	–	Nd	1	0
8	60%	1.8	10%	1	Nd	1	1
9	80%	3.3	0%	–	Nd	1	2
10	80%	1.3	50%	2.2	Nd	0	1

TABLE 3.2.4
Bacteriology results for selected isolates obtained from pearl oysters and water during field sampling

Isolate number	Sample source	Identification
1	Site 1, bottom water, yellow on TCBS	*Vibrio* sp. not identifiable
2	Site 1, oyster D hemolymph, yellow on TCBS	Vibrio tubiashii
3	Site 2, oyster A adductor muscle, yellow on TCBS	*Vibrio harveyi*
4	Site 2, oyster F, hemolymph, yellow on TCBS	*Vibrio harveyi*
5	Site 5, oyster E adductor muscle, yellow on TCBS	*Vibrio pelagius biovar II*
6	Site 1, oyster F adductor muscle, green on TCBS	*Vibrio harveyi*
7	Site 2, oyster D adductor muscle, green on TCBS	*Vibrio harveyi*
8	Site 5, oyster H small green colonies on TCBS	*Staphylococcus-Micrococcus*-like sp.
9	Site 2, bottom water, cream on TSA+2	*Vibrio* sp. not identifiable
10	Site 2, bottom water, clear on TSA+2	Not recovered on subculture
11	Site 2, oyster F, hemolymph, clear on TSA+2	*Vibrio mediterranei*
12	Site 1, oyster C, hemolymph, cream on TSA+2	*Vibrio tubiashii*
13	Site 1, bottom water, spreading on TSA+2	*Vibrio* sp. not identifiable
14	Site 2, bottom water, spreading on TSA+2	*Vibrio harveyi*
15a	Site 2, oyster F, hemolymph, spreading on TSA+2	*Vibrio* sp. not identifiable
15b	Site 2, oyster F, hemolymph, spreading on TSA+2	*Vibrio* sp. not identifiable
16	Site 1, oyster P, from brown stain on nacre	*Vibrio* sp. not identifiable
17	Site 4, oyster F, adductor muscle, very small colonies	*Acinetobacter*-like

some oysters without obvious conchiolin deposits had bacterial infections (Table 3.2.3). There was no significant correlation between the severity of conchiolin deposits and the severity of bacterial infections. Only light to moderate numbers of bacteria were isolated from water samples, with more bacteria being isolated from bottom samples. The identifications for 17 representative isolates of bacteria obtained from pearl oysters and from water collected during field sampling are summarised in Table 3.2.4. Isolates of *Vibrio harveyi* predominated in samples from adductor muscle, hemolymph and water. All isolates of *V. harveyi* examined had different biochemical phenotypes. Undescribed species of *Vibrio* were also common in water, hemolymph and from the surface of conchiolin deposits. Less common isolates included *V. tubiashii* from hemolymph, *V. mediterranei* from hemolymph and *V. pelagius* biovar II from adductor muscle.

Histopathology

Most bacteria occurred in the mantle epithelium and were associated with necrosis and sloughing of epithelial and sub-epithelial cells (Figure 3.2.2). Other sites of infection included the gonad and digestive gland tubule epithelium. Oysters with higher conchiolin scores (2 or more) tended to have depleted or resorbing gonads (Figure 3.2.3). Infections with gregarine protozoans were noted principally in the epithelium of the mid and hind gut (Figure 3.2.4), but also in the sub-epithelial connective tissue surrounding the gut. There was no significant correlation between the intensity of gregarine infection and conchiolin lesion severity.

Other notable pathological lesions in the oysters examined by histopathology included breakdown, sloughing and focal necrosis of the digestive gland epithelium

FIGURE 3.2.2
A necrotic lesion in the mantle associated with bacterial infection

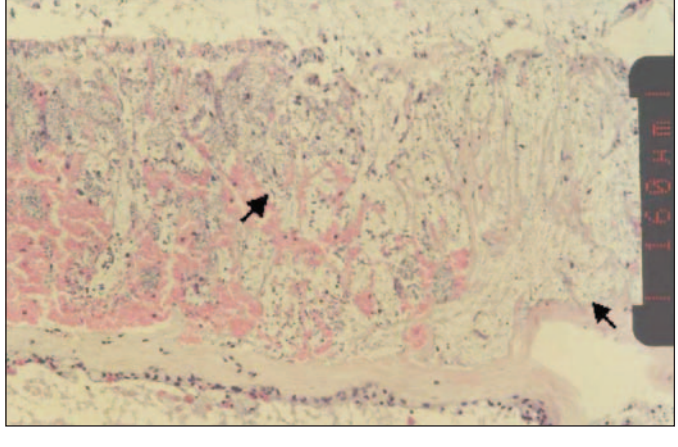


FIGURE 3.2.3
Comparison of the spawning stage of oysters with bacterial infections compared to their conchiolin deposit score. A relationship between spawning stage and conchiolin deposition was evident

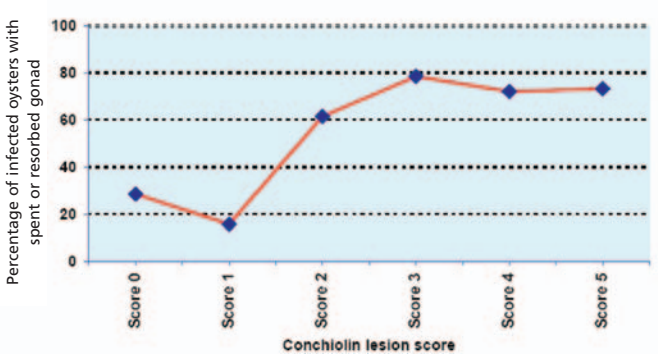


FIGURE 3.2.4
Gregarine protozoa (arrows) in the epithelium of the gut

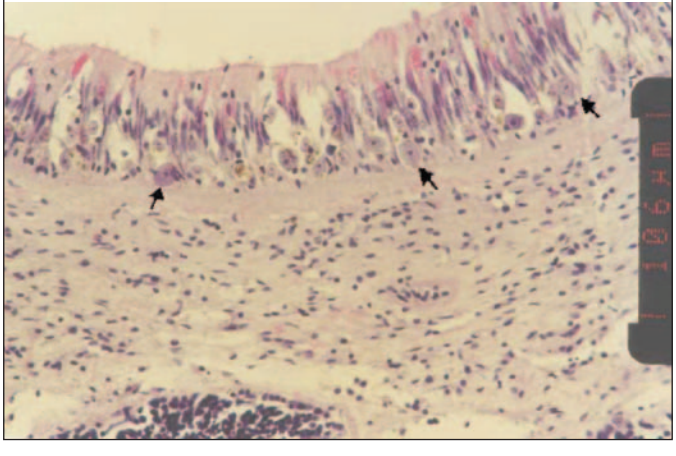
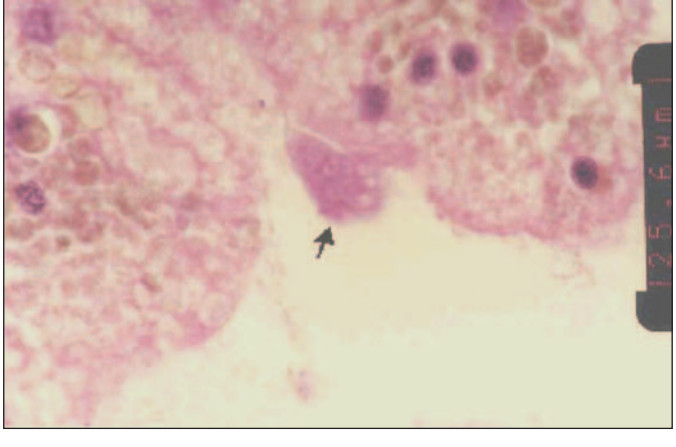


FIGURE 3.2.5
An unidentified protozoan (arrow) attached to the digestive tubule epithelium



(32 percent of oysters), atrophy of digestive gland tubules (5 percent of oysters), and abnormal kidney pathology (necrosis or hyperplasia of the kidney epithelium, 2.7 percent of oysters). Parasitic infections also occurred at low prevalence, including trematodes in the foot and mantle (4 percent of oysters), copepods in the gut and digestive tubules (6.6 percent of oysters) and an unidentified protozoan found attached to the digestive tubule epithelium with a stalk-like process in two oysters (0.7 percent prevalence, Figure 3.2.5). Groups of heavily basophilic prokaryote-like organisms were also present in the periphery of the mantle of all oysters examined (Figure 3.2.6). These may have been harmless symbiotic organisms as their presence appeared to bear no relationship with oyster health.

Thioglycollate incubation

None of the 100 oysters examined were positive for *Perkinsus* by incubation of tissue samples in RTFM.

Plankton analysis

None of the plankton species found in the water samples were known to be toxic, hence the possibility of a bloom of a toxic plankton species appeared highly unlikely.

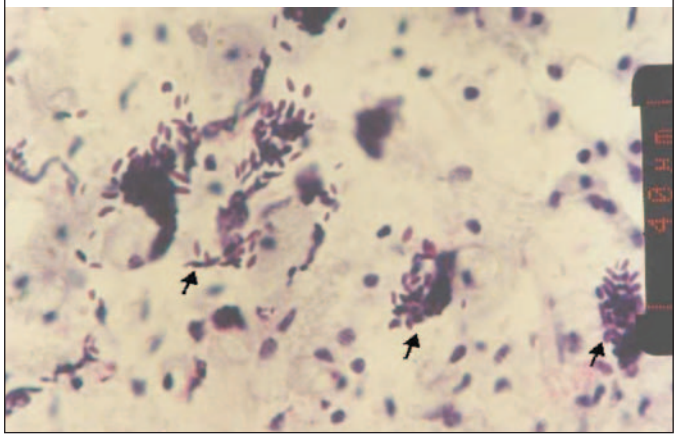
Toxicology

Analysis of samples of oysters taken from Manihiki Lagoon showed that lead levels were elevated in cultured oysters with grade 3–5 conchiolin lesions compared to wild oysters with grade 0 and 1 lesion (Table 3.2.5). The levels of lead in the affected oysters were higher than levels of concern recommended by the Food and Drug Administration (FDA) of the United States of America (Table 3.2.5). Healthy oysters from Penrhyn lagoon had very low levels of lead compared to the oysters from Manihiki lagoon, but had elevated arsenic, though these were below levels of concern listed by the FDA. Levels of zinc were elevated in oysters from Manihiki lagoon, while hydrocarbons were higher in oysters from Penrhyn.

December 2003

A significant proportion of both wild and cultured oysters from Manihiki and Penrhyn lagoons displayed brown coloured conchiolin deposits in the nacre of one or both shell valves (Diggles and Maas, 2004). The oysters from Penrhyn were the worst affected, while this lesion was absent from oysters sampled from Rakahanga Lagoon. The prevalence and intensity of this lesion in oysters from Manihiki Lagoon was much lower than recorded in November 2000, but its persistence suggests that the oysters in Manihiki remained affected by environmental stress in at least some parts of the lagoon. The emergence of the conchiolin lesions in oysters from Penrhyn lagoon may be related to stress from poor water quality due to low levels of dissolved oxygen recorded by water quality monitoring buoys in that lagoon.

FIGURE 3.2.6
Groups of deeply basophilic prokaryote-like organisms (arrows) in the mantle



Signs of recovery from the previous outbreak of bacterial disease in November 2000 were observed in both wild and cultured oysters from Manihiki Lagoon. In these oysters the conchiolin lesions remained visible but had been overlaid by newly deposited nacre (Figure 3.2.7). This permanent scarring of the nacre of the shell was also associated with development of a prominent check around the periphery of the shell (Figure 3.2.8), indicating that shell growth ceased for a significant period of time subsequent to November 2000. Recovering oysters were not observed in any of the earlier samples of oysters from Manihiki Lagoon from May 1998 or November 2000, suggesting that the November 2000 disease episode was unprecedented and had significant long term effects on the health of surviving oysters in Manihiki Lagoon.

TABLE 3.2.5
Comparison of toxicology tests for oysters from Manihiki and Penrhyn lagoons. Results are for 4 to 6 pooled oysters from each site. Notable differences between lagoons are in bold

Sample name	Manihiki Lagoon, cultured oysters	Manihiki Lagoon, wild oysters	Penrhyn Lagoon, cultured oysters	Penrhyn Lagoon, wild oysters	USA FDA levels of concern
Range of conchiolin lesion severity	Grade 3 to 5	Grade 0 to 1	Grade 0, healthy	Grade 0, healthy	
Zinc (mg/kg)	52	80.3	13.6	6.35	n/a
Copper (mg/kg)	0.33	0.53	0.38	0.48	n/a
Arsenic (mg/kg)	7.27	5.79	13.1	10.5	86
Cadmium (mg/kg)	1.02	0.85	1.63	1.61	3.7
Mercury (mg/kg)	<0.005	0.008	0.004	0.004	1
Chromium (mg/kg)	0.13	0.16	0.08	0.11	13
Nickel (mg/kg)	0.1	0.19	0.1	0.16	80
Lead (mg/kg)	2.7	0.08	0.007	0.009	1.7
Hydrocarbons					
C7-C9 (mg/kg)	<10	<10	<4	<4	-
C10-C14 (mg/kg)	<20	<20	<8	<7	-
C15-C36 (mg/kg)	50	100	160	183	-
TOTAL	<80	100	160	180	-

FIGURE 3.2.7
This photo of an oyster taken from Manihiki lagoon in December 2003, around 3 years after the November 2000 disease event shows conspicuous regrowth of nacre over conchiolin deposits (arrows) laid down during the disease event

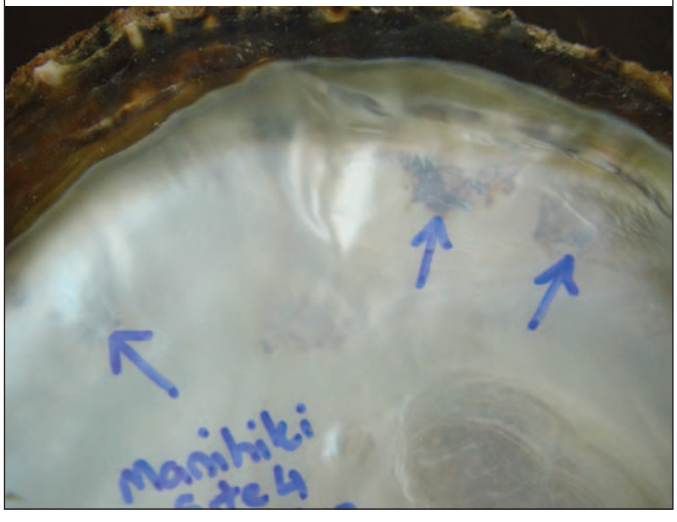
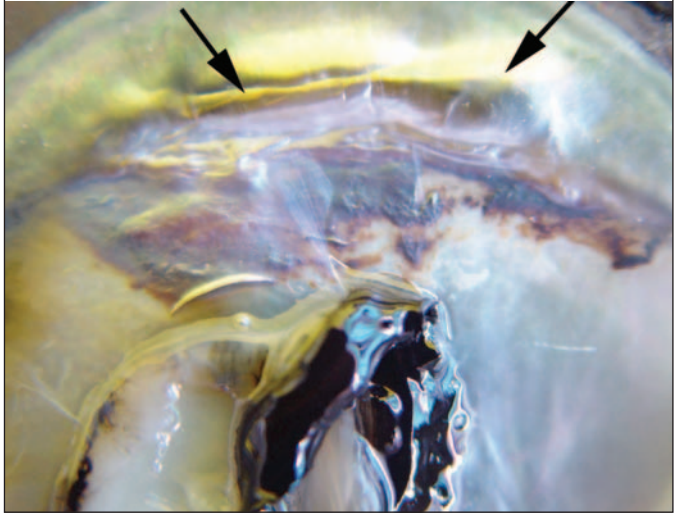


FIGURE 3.2.8
Regrowth of nacre over conchiolin deposits immediately proximal to a prominent check in shell growth (arrows) in an oyster taken from Manihiki lagoon 3 years after the November 2000 disease event



DISCUSSION

The study of May 1998 revealed the presence of a few trivial infections (e.g. gregarines, ciliates, trematode metacercariae) and three potentially pathogenic groups, boring sponges, the fungus from infected drill holes and *Perkinsus.* The identity of the orange sponges that bore into Manihiki and Rakahanga oyster shell is currently unclear, as is the taxonomy of clionid sponges. The descriptions of many species differ from the descriptions of the same species by other authors. The more common orange sponge resembles *Cliona celata*, but the description by Thomas (1979) is too vague to be of use and *C. celata* has been reported from cold temperate waters (Baxter, 1984). The other, less common, boring sponge resembles *Cliona vastifica*, which infects *P. margaritifera* in French Polynesia (Mao Che *et al.*, 1996), but *C. vastifica* ranges from dark green to yellowish grey in colour and the dimensions of the sponge differ from those given by Thomas (1979). *Cliona vastifica* and *C. celata* are the most widespread clionid infestations in pearl shells (Thomas, 1979; Mao Che *et al.*, 1996).

The organism associated with the infection of drilled holes appeared hyphae-like and most closely resembles the fungus *Ostracoblabe implexa* from *P. margaritifera* in French Polynesia (Mao Che *et al.*, 1996). *Ostracoblabe implexa* is primarily a fungal pathogen in shells of bivalves in temperate waters (Li *et al.*, 1983), however the site and growth of the organism reported here appears to be the same as for *O implexa.* The burrowing fungus appears to gain entrance through drill holes and obtains its nourishment from the breakdown of the proteinaceous shell matrix. However, when it reaches the inner shell surface it sets up an irritation that changes the cellular structure of the mantle, resulting in deposition of conchiolin-rich nacre over the fungus. This results in the thin nacre, lacking refringence and lustre that overlays the infection and which can be readily broken by light pressure. Fortunately it appears that infection is rare, except through holes drilled in the shell for suspension hanging and improvements in technique will overcome the problem.

Perkinsus is potentially the most serious pathogen. The species in Manihiki lagoon is probably *P. olseni*, as this species is known to occur around northern New Zealand (Hine and Diggles, 2002), Australia (Lester and Davis, 1981; Hine and Thorne, 2000),

Korea (Choi and Park, 1997; Park, Choi and Choi, 1999; Lee *et al.*, 2001) and Japan (Hamaguchi *et al.*, 1998). This suggests that it is an Indo-Pacific species and that it is likely therefore that the Manihiki *Perkinsus* is *P. olseni.* It has been shown that another *Perkinsus, P. atlanticus*, which occurs in clams in Spain, is actually *P. olseni* (Robledo *et al.,* 2000), that was probably introduced into Spain in Manila clams (*Ruditapes decussatus*) from Southeast Asia.

Perkinsus olseni was originally described in association with mass mortalities among abalone (*Haliotis* spp.) on the coast of South Australia (Lester and Davis, 1981). It was subsequently shown that it infects many families and species of molluscs (Goggin and Lester, 1987; Hine and Thorne, 2000) and that isolates from one species can infect many other species (Goggin *et al.*, 1989). Any one isolate has developmental stages of sizes that vary from host to host and therefore size does not distinguish species. In the great majority of infections, the parasite occurs as spherical clusters of schizonts without eliciting a host response (Hine and Thorne, 2000), as was observed here. When *P. olseni* causes disease, the other histozoic developmental stages (merozoites which develop to meronts and then to schizonts) are present. What triggers the parasite to change from a benign parasite into a proliferating pathogen is unknown. It does appear, however, that pearl oysters are less susceptible than many other hosts and therefore perkinsosis may not be a great threat to pearl farming.

The study in November 2000 suggested that the outbreak of disease associated with brown conchiolin deposits on the nacre had a bacterial aetiology. There was no evidence of widespread infection of any viral, protozoan or metazoan pathogens in the diseased pearl oysters examined. The presence of the gregarine protozoans in the gut epithelium was not correlated with the severity of the conchiolin lesions. The 1998 survey found that these protozoans also occur in apparently healthy pearl oysters, as also found by Humphrey *et al.* (1998). We consider the association of gregarines with diseased *P. margaritifera* both here and previously in French Polynesia (Chagot *et al.*, 1993) as likely to be incidental and they are considered unlikely to cause disease under normal circumstances (Humphrey *et al.*, 1998). Their presence in *P. maculata* and *A. ventricosa* indicate that both other pteriid species and other bivalve families are infected by these apparently benign parasites. The basophilic prokaryote infections of the mantle seen in the November 2000 survey do not appear to have previously been reported, although similar inclusions have been reported from the digestive epithelia of *P. margaritifera* from French Polynesia (Comps *et al.*, 1998).

The predominance of the bacterium *V. harveyi* and other vibrios in cultures taken from affected oysters, together with the occurrence of pathological lesions consistent with bacterial infection in many affected oysters, indicates that infection by *V. harveyi* and other opportunistic vibrios, was associated with this disease syndrome. There was no evidence to suggest that the disease syndrome was primarily caused by a single pathogenic strain of *V. harveyi*. This is a fundamental difference between the disease syndrome of *P. margaritifera* in Manihiki Lagoon and that of Brown Ring Disease (BRD). BRD is also characterised by abnormal brown conchiolin deposits adhering between the pallial line and the edge of the shell (Paillard and Maes, 1994). However, BRD is caused only by strains of a pathogenic bacterium, *Vibrio tapetis* (formerly known as *Vibrio* P1) (Borrego *et al.*, 1992; Paillard, Maes and Oubella, 1994; Novoa *et al.*, 1998) and the disease can be reproduced by experimental exposure to these strains.

The anomalous conchiolin deposition in BRD occurs as a definite thin brown ring on the nacre around the perimeter of the shell (Paillard and Maes, 1994). In contrast, most of the oysters sampled from Manihiki Lagoon displayed a broad conchiolin deposition resulting from retraction of the mantle, with the deposits lying outside the edge of the mantle. This condition was virtually identical to that described for *Pinctada maxima* from Western Australia associated with the presence of *Vibrio harveyi* (see Dybdahl and Pass, 1985; Pass, Dybdahl and Mannion, 1987; Perkins, 1996).

Bacterial diseases in aquaculture are often associated with opportunistic bacteria which invade hosts which are stressed due to unfavorable conditions such as overcrowding, abnormally high or low water temperatures and/or poor water quality. Pass, Dybdahl and Mannion (1987) found that *V. harveyi* infection in *P. maxima* was associated with poor water quality conditions, low water temperatures and overcrowding during transport of oysters to lease sites. Figueras *et al.* (1996) found the highest prevalence of BRD in storage areas where clams were kept at high population densities for at least 1 month. In Manihiki Lagoon, one potential stressor which may have been related to the onset of mortalities was the high stocking density. Prior to the disease outbreak the number of oysters cultured in Manihiki Lagoon was reportedly at an all time high (B. Ponia, MMR, pers. comm.).

There is also evidence to suggest that a decline in water quality preceded the disease outbreak. Water exchange in the semi-enclosed Manihiki lagoon is poor, at around one water exchange every 2 months (Anderson, 1998). A long period of unusually calm, dry weather was reported prior to the disease outbreak. At the time oysters were first sampled in late November 2000, water temperatures in Manihiki Lagoon were normal (29 °C), but dissolved oxygen (DO) levels were low, ranging between 1 and 3 mg/l (S. Sharma and G. Frost, SOPAC, pers. comm.). However, during the one week period in which field sampling was conducted, the mean DO of the lagoon increased to over 6 mg/l, probably due to high wind conditions which would have helped with aeration and water exchange in the lagoon (S. Sharma and G. Frost, SOPAC, pers. comm.). These water quality data, albeit limited, suggest that the disease outbreak followed a transient period of poor water quality in Manihiki lagoon.

High stocking densities reduce the food supply available to each oyster (Anderson, 1998). Starvation may have been indicated in some of the diseased oysters by pathologies such as atrophy of digestive gland tubules. Reduced food supply can also be an important stressor in bivalve molluscs (Tomaru *et al.*, 2001) and can cause mortality (Numaguchi, 1995). Spawning is another potential stressor which may have predisposed oysters to disease. We found that oysters with bacteria present in sections and with high lesion scores (2 or more) tended to have depleted or resorbing gonads.

Anecdotal evidence supplied by oyster farmers suggest the performance of pearl oysters in Manihiki Lagoon in the years following the November 2000 disease outbreak has been reduced compared to pre-outbreak times. Certainly many of the oysters which survived were still showing signs from the disease outbreak in a follow up survey of oyster health done 3 years later in December 2003 (Diggles and Maas, 2004). These signs included permanent scarring of the nacre of the shell and development of a prominent check around the periphery of the shell, indicating that shell growth ceased for a significant period of time subsequent to November 2000. These signs of disease had never been previously seen by pearl oyster farmers throughout the production history of the lagoon, indicating that the November 2000 disease outbreak in Manihiki Lagoon was unprecedented and had significant long term deleterious effects on oyster health in that lagoon.

In conclusion, our data suggest that an unprecedented disease outbreak in *P. margaritifera* in Manihiki lagoon in November 2000 was associated with vibriosis caused by *V. harveyi* and other opportunistic vibrios. The disease outbreak appeared to follow a transient reduction in lagoon water quality associated with a period of calm weather and was probably exacerbated by high oyster stocking densities. Furthermore, the mortalities occurred at a time of the year when oysters may have been further stressed after spawning. Management of the disease could, therefore, be based on controlling the stocking density of oysters in the lagoon, and modifying husbandry practices to reduce stress on oysters when they are spawning. Ongoing monitoring of key water quality parameters such as water temperature, DO, chlorophyll α, and both sediment oxygen demand (SOD) (Anderson, 1998) and biological oxygen demand

(BOD) may also be useful so that the epidemiology of disease outbreaks which may occur in the future might be better understood.

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