Diseases of Cultured Paua (*Haliotis iris*) in New Zealand

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ABSTRACT

Mortalities of cultured paua (*Haliotis iris*) in New Zealand have been associated with a variety of disease agents. During the summers of 1999/2000 and 2000/2001, mortalities of between 82.5 and 90% of juvenile paua were reported in one commercial culture facility. Histology of moribund paua showed heavy systemic infections of a novel haplosporidian. The epidemiology of the haplosporidian disease remains poorly understood, and a national survey was conducted during the summer of 2001/2002 to determine whether the haplosporidian occurred in cultured paua in other farms throughout the country. The survey examined 1094 paua collected from 5 spat producing farms and 3 grow out farms, but did not detect the haplosporidian. A number of other potential disease agents and syndromes were recorded, however, including rickettsial inclusions in the gut, foci of intense inflammation, granuloma-like lesions in internal organs, unidentified protozoa in the epithelium of the foot mantle and epipodium, and erosion of external epithelia associated with bacterial infection and ectocommensal ciliates. Other disease agents found in cultured paua in followup work done after the survey included a coccidian-like protozoan which infected the epithelium of the eosophageal pouch, a mycosis of the inside of the shell and infestation by the mudworm *Polydora hoplura*. Substantial stock losses due to toxic algal blooms have also been recorded in some farms. The possible effects of these disease agents on paua farming in New Zealand are discussed.

INTRODUCTION

The farming of paua (*Haliotis iris*) in New Zealand is a relatively new industry and until recently there has been little information available about diseases of cultured paua. Besides occasional large scale mortalities associated with algal blooms (Chang, 1999; Chang *et al*., 2001; B. K. Diggles, unpublished), reports of disease outbreaks in paua farms have been limited to an outbreak of haplosporidosis in juvenile paua in one commercial culture facility in the North Island during the summers of 1999/2000 and 2000/2001. This disease, caused by a novel haplosporidian (Hine *et al*., 2002; Reece and Stokes, 2003) was associated with heavy systemic infections of uni-to multi-nucleate plasmodia in all major organs (Figure 1) and mortality rates of up to 90% (Diggles *et al*., 2002). The presence of the haplosporidian in paua from the affected facility was associated with chronic mortalities mainly during the summer months when water temperatures increased to 20°C and above (Diggles *et al*., 2002). The Haplosporidia are a protistan group (Perkins, 1989) containing a number of internationally notifiable pathogens of molluscs including *Haplosporidium nelsoni* and *H. costale*, both of which cause disease in oysters in the northern hemisphere (OIE, 2000).

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Laboratory infection trials were attempted but haplosporidian infections were not transferred horizontally by cohabiting heavily infected paua with uninfected paua for 3 months in aquaria, or by injecting healthy paua with hemolymph containing haplosporidian plasmodia (Diggles *et al*. 2002). In light of the complete lack of information about transmission of the disease agent, and considering that the source of infection at the affected farm was unknown, the possibility that the haplosporidian was introduced via spat or juveniles purchased from industry suppliers was investigated. In New Zealand a number of commercial spat production facilities breed from adult broodstock and rear the resulting larvae through to settlement. The spat are then grown to around 8 to 10 mm shell length before selling them to grow out farms which rear the juveniles to marketable size. A national survey which included 5 prominent paua spat production facilities, and 3 growout facilities situated in areas where water temperatures exceeded 20°C during the summer months, was undertaken in the summer of 2001/2002 mainly to investigate the extent of haplosporidian infections in cultured paua, but also to obtain baseline data on the health of cultured paua throughout the country.

MATERIALS AND METHODS

Samples of 150 paua from 5 spat producing farms in both the North and South Islands were examined for disease by histopathology. Samples of 60 paua from an additional 3 grow out farms in the North Island which experienced summer water temperatures above 20 °C (including the farm originally affected by the haplosporidian disease outbreak) were also examined. Details of the farms sampled, sampling date and the number and size of paua examined are included in Table 1. The sampling targeted freshly dead or overtly diseased juvenile paua, then runt paua and apparently healthy paua until the statistically determined sample numbers were achieved. Sample sizes were chosen to achieve 95% confidence of detecting at least 1 infected paua based on an assumed disease prevalence of 2% in spat production facilities and 5% in grow out farms (OIE 2000). It should be noted that the 95% confidence interval assumes that histology was 100% sensitive for detecting disease agents if they were present.

Table 1. Farms sampled, sampling dates and details of *Haliotis iris* examined during this survey.

Spat producing farms	Location	Date sampled	Water temperature	Number of paua	Age (months)	Mean shell length (mm)	Range	Std. Dev.
Farm 1	North Island	May 2001	12°C	150	5	7.4	5-11	1.46
Farm 1	North Island	10 Jan 2002	18°C	160	6	10.1	6-16	1.98
Farm 2	South Island	29 Jan 2002	18°C	150	5-7	8.0	4-15	2.23
Farm 3	South Island	4 Feb 2002	18°C	151	20	14.0	9-20	2.4
Farm 4	South Island	5 Feb 2002	14°C	151	6	14.0	9-21	2.5
Farm 5.	North Island	21 Feb 2002	19.5°C	152	6-8	14.0	8-24	3.45
Grow out farms								
Farm 6	North Island	20 Feb 2002	20°C	60	12	15.75	11-23	2.81
Farm 7	North Island	25 Feb 2002	20.8°C	60	18-24	35.2	23-39	3.16
Farm 8*	North Island	26 Feb 2002	21.2°C	60	18-24	23.5	12-37	7.3
TOTAL	-	-	-	1094	5-24	14.5	4-39	7.24

* denotes farm with previous history of haplosporidian infection.

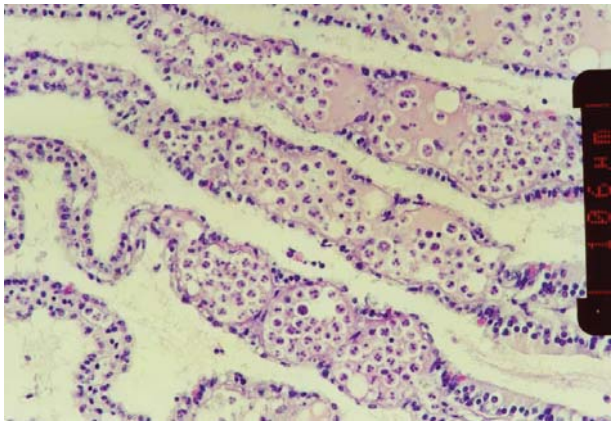

Figure 1. Numerous multinucleate plasmodia in the hemal sinuses of the gills of *H. iris* heavily infected with a novel haplosporidian. Scale bar = 106 mm.

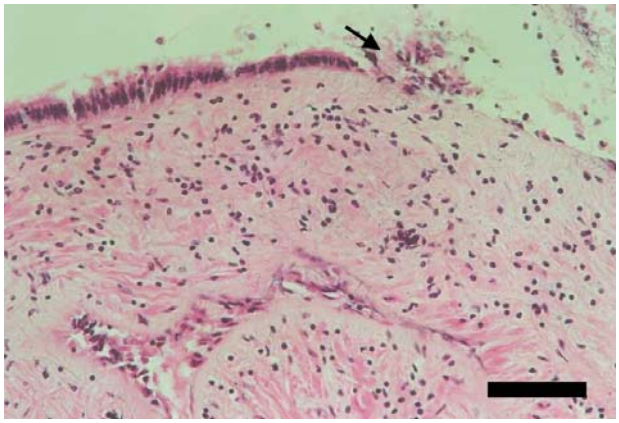

Figure 2. Erosion and exfoliation of the epithelium of the foot (arrow) of *H. iris.* In this case the lesion is associated with a bacterial infection as indicated by the presence of Flexibacter/ Cytophaga-like and Vibrio-like bacteria in the sloughed epithelium and also in underlying muscle. Scale bar = 55 mm.

Pieces of foot, mantle, epipodium and internal organs from each paua sampled were excised with a scalpel and fixed in 10% formalin in filtered seawater, embedded for wax histopathology using routine methods, and two 5 µm sections taken at different levels in the block were stained with hematoxylin and eosin (H&E). The slides were examined specifically for the haplosporidian parasite using light microscopy, but other disease agents of lesser regulatory significance were also recorded. Sampling was performed mainly between January and March 2002 to increase the chances of detecting the haplosporidian parasite, due to its apparent association with warmer water temperatures. The timing of the sampling was also expected to increase the chances of detecting other opportunistic microbial and protozoan disease agents which may multiply more rapidly during peak summer water temperatures.

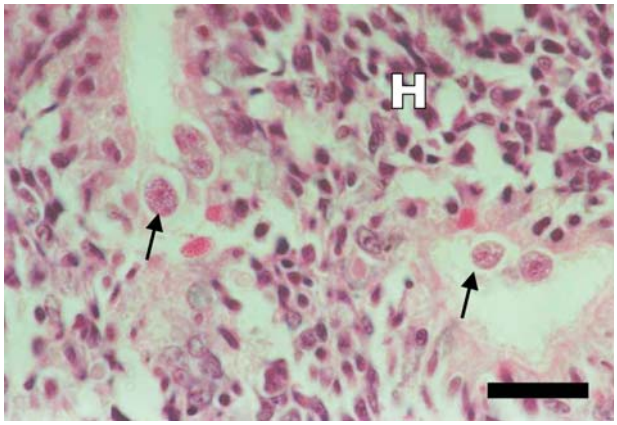

Figure 3. An unidentified protozoan (arrows), possibly an apicomplexan, in tunnels within the epithelium of the foot of *H. iris.* The presence of these was usually associated with hyperplasia (H) of the epithelial cells. Scale bar = 16 mm.

RESULTS

The haplosporidian parasite was not detected in any of the 1094 paua examined during the present study (Table 2), however a number of other pathological conditions and lesions were observed (Table 2). The most common lesion observed was erosion and exfoliation of the epithelium of the foot and epipodium (Figure 2). This lesion was recorded in paua from all farms, reaching its highest prevalence (45%) in systems where paua were held at high densities, and in these cases was often associated with bacterial infection of the epithelium by long *Flexibacter/Cytophaga* -like rods, and short *Vibrio* - like rod shaped bacteria (Figure 2). Oval shaped sporozoite-like stages of an unidentified protozoan (Figure 3) 4 to 6 µm long were evident at low prevalence in the epithelium of the foot, epipodium and mantle of paua from Farm 4. The presence of these protozoans was associated with hyperplasia of epithelial cells. Rickettsial inclusions (Figure 4) up to 17 µm in diameter were evident at low prevalence in the epithelium of the gut of paua from two spat producing farms and one grow out farm (Table 2). Colonisation of external surfaces of the foot and epipodium by *Scyphidia* -like ectocommensal ciliates (Figure 5), and of the gills by *Sphenophrya*-like ciliates (Figure 6), was observed in 4 out of 5 spat producing farms and all grow out farms (Table 2). Ectocommensal ciliates were particularly common (up to 78% prevalence) in farms which had larger, older paua.

Bacterial infections were evident at low prevalence in 4 out of 5 spat producing farms and 2 of 3 grow out farms. Infections varied from rare, pustule-like lesions with a central area of necrotic cells, to more common external infections associated with erosion and exfoliation of the epithelium of the foot and epipodium (Figure 2), and occasionally extensive post mortem infections in the small number of dead paua sampled. Focal areas of non-specific necrosis were found in paua from all farms. Granuloma-like lesions (Figure 7) up to 90 µm diameter of unknown aetiology were observed in the foot and internal organs of paua from 2 spat producing farms and 2 grow out farms.

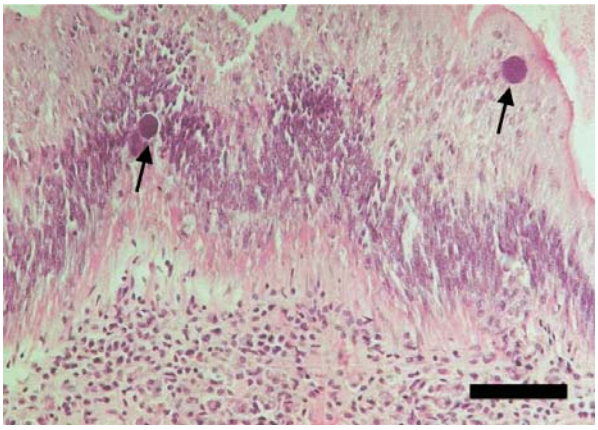

Figure 4. Rickettsial inclusions (arrows) in the epithelium of the gut of *H. iris*. Scale bar = 71 mm.

Table 2. Prevalence (%) of histopathological lesions observed in *Haliotis iris* sampled during this survey.

Spat producing farms	Haplos poridian	Epithelial erosion	Rickettsial inclusions in gut	Protozoa in foot epithelium	External ciliates	Bacterial infection	Non-specific necrosis	Granuloma-like lesions	Haemocytic neoplasia-like inflammation	Gregarines Nematopsis/ unidentified
Farm 1 (May 2001)	0	na[#]	0	0	0	na	na	0	0	0/0
Farm 1 (January 2002)	0	0.6	0	0	0	3.7	8.7	0	0	0/0
Farm 2	0	2.7	0	0	2.7	5.3	6.7	0	0	0/0
Farm 3	0	34.4	2.6	0	2.6	0	1.3	0	6.6	0/0
Farm 4	0	45[#]	0.7	6.6	2	0.7	2	4.6	1.3	0/0
Farm 5	0	27	0	0	16	3.9	13.8	0.7	3.3	0.7 / 0.7
Grow out farms										
Farm 6	0	31.7	0	0	8.3	8.3	20	3.3	1.7	1.7 / 0
Farm 7	0	13.3	3.3	0	78.3	0	6.7	1.7	61.7	0 / 1.7
Farm 8*	0	15	0	0	23.3	5	20	0	23.3	3.3 / 1.7

[#] Not available.
[##] Highest prevalences in bold.
* Denotes farm with previous history of haplosporidian infection.

A focal inflammatory lesion suggestive of haemocytic neoplasia, but lacking the distinctive cell hypertrophy of that lesion, was observed at low prevalences in the epipodium and muscle adjacent to the branchial sinus of paua from 3 spat producing farms, and more often in paua from grow out farms. The prevalence of the syndrome appeared to increase with the size and age of the paua, and reached over 60% at farm 7, which had the largest paua sampled. The syndrome was characterised by focal areas of degraded muscle with prominent voids containing large numbers of haemocytes (Figure 8), many of which exhibited irregular shaped nuclei (Figure 9). Between the haemocytes were very small (1-2 µm diameter) particles which may have been cellular debris (Figure 9). Oocysts of the gregarine *Nematopsis* sp. were observed in the foot muscle and viscera of paua from one spat producing farm and 2 grow out farms.

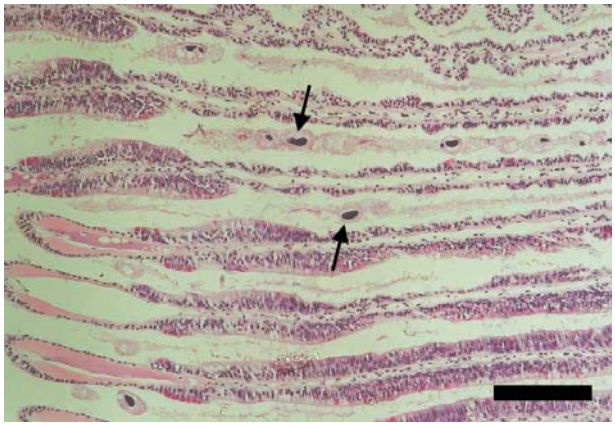

Figure 6. Ectocommensal Sphenophrya-like ciliates (arrows) in the gills. Scale bar = 145 mm.

In follow up work conducted at farm 4 on a cohort of larger paua [37 - 60 (mean 47) mm shell length] after completion of the main survey, moderate to heavy infections of the epithelium of the eosophageal pouch by an intracellular coccidian- like protozoan parasite (Figure 10) were recorded at a prevalence of 15%. Cells infected by the protozoan contained relatively large (8-12 x 6-8 μm) round to oval shaped meront-like stages with a strongly eosinophillic nucleus, or up to 6 smaller (5-8 x 2.4-3 μm), elongate merozoite-like stages (Figure 10). Also recorded in a subsample of 7 underweight paua from this cohort were mild cases of brown conchiolin deposition near the apex region of the interior of the shell caused by a fungus (1/7 paua, 14.3% prevalence), and moderate to heavy infections (2 to 7 blisters/paua) of the inside (in the mantle cavity) and rear of the shells with mudworms identified as *Polydora hoplura* (6/7 paua, prevalence 85.7%).

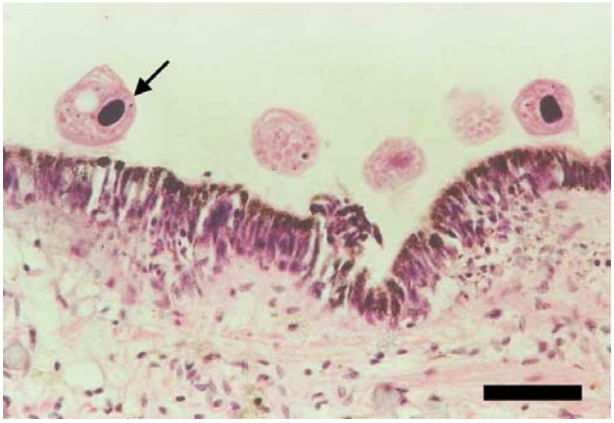

Figure 5. Ectocommensal *Scyphidia*-like sessile ciliates (arrow) with conspicuous adhesive disc (scopula). These were commonly found on the external surfaces of the foot, epipodium and mantle. Scale bar = 45 mm.

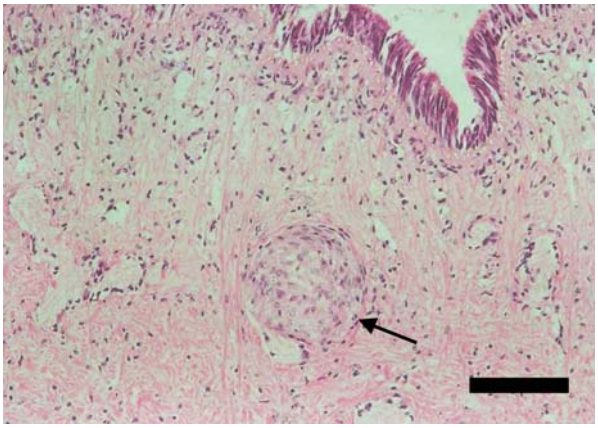

Figure 7. Granuloma -like lesion (arrow) adjacent to nerves in the foot. Scale bar = 90 mm.

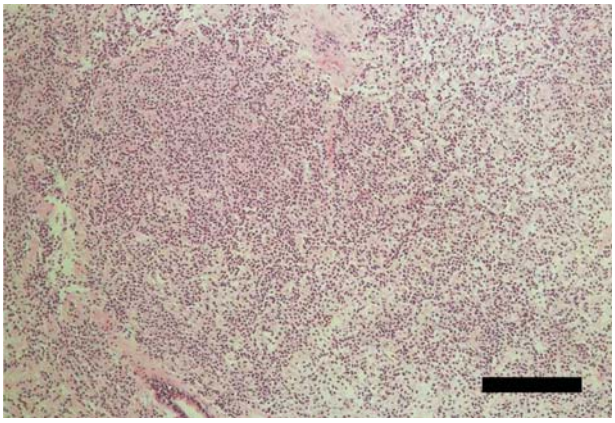

Figure 8. Low power view of an inflammatory lesion with large numbers of haemocytes within voids in the muscle adjacent to the branchial sinus. Scale bar = 180 mm.

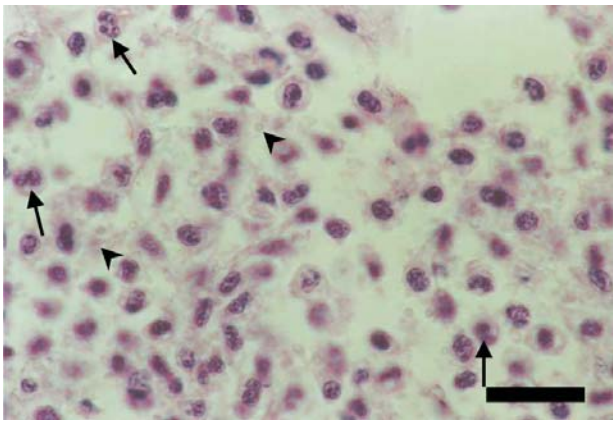

Figure 9. High power view of the inflammatory lesion showing haemocytes with irregularly shaped nuclei (arrows). Also evident are very small particles (arrowheads) which appear to be cellular debris. Scale bar = 17 mm.

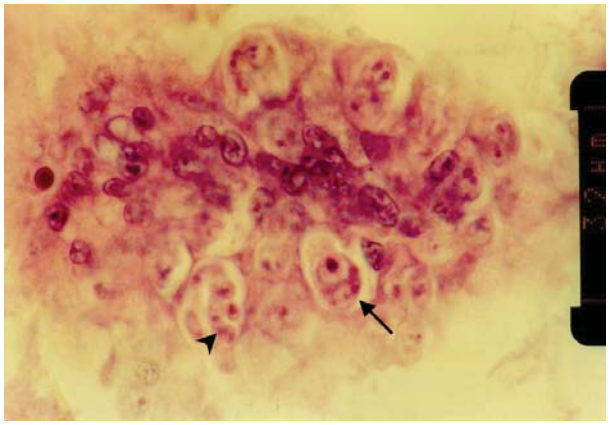

Figure 10. Meront-like (arrow) and merozoite-like (arrowhead) intracellular stages of a coccidian-like protozoan infecting the epithelium of the oesophageal pouch. Scale bar = 32 mm.

Figure 11. Juvenile *H. iris* with bacterial disease of the epithelium of the mantle, epipodium and foot (specimens = 8 and 10 mm shell length). Bacteriology of eroded mantle epithelium isolated *Cellulophaga* lytica and numerous isolates of *Vibrio* sp.

An outbreak of bacterial disease of the epithelium of the mantle, epipodium and foot (Figure 11) was also reported in a cohort of smaller paua (8 - 15 mm shell length) from the same system in farm 4 from which the sporozoite-like protozoan (Figure 3) was found during the disease survey. These small paua were in a different system to those infected with the mudworm and coccidian-like protozoan described above. The bacterial disease outbreak resulted in substantial mortalities of over 1000 paua per day in affected raceways. Bacteriology of eroded epithelium produced numerous isolates of *Vibrio* sp. on TCBS cholera medium, while analysis of 16S sequence data obtained from one isolate of Flexibacter/ *Cytophaga* -like rods obtained on marine agar (2216) found 99% sequence similarity to *Cellulophaga lytica* (bacteriology data not shown).

DISCUSSION

The paua examined from the 5 spat producing farms were found to be free of infection by the haplosporidian parasite. Failure to detect the haplosporidian parasite in the samples examined gives 95% confidence that, if the haplosporidian was present in the remaining paua in those farms, it was present at a prevalence of less than 2% (assuming histology is 100% sensitive for detecting the haplosporidian). Water temperatures did not exceed 20°C at any of the spat producing farm sites. As the haplosporidian appeared to cause disease only at 20°C or above, it appears unlikely that the original source of infection at farm 8 was spat purchased from industry suppliers. Another factor supporting this observation is that water temperatures adjacent to at least 2 other grow out farms in the North Island also exceed 20°C for some period during summer. These 2 farms also sourced their spat from the same suppliers utilised by farm 8 for at least 2 years, but no haplosporidians or unusual mortalities have ever been reported from these other 2 sites. This evidence appears to suggest that the source of the haplosporidian at the affected farm was local and not from spat produced by commercial suppliers.

Sampling of the three grow out farms failed to detect infection by the haplosporidian parasite during this survey. Unlike the previous two summers, unusual mortalities were not recorded at farm 8 over the summer of 2001/2002. During the previous disease outbreaks at farm 8 in 1999/2000 and 2000/2001 the prevalence of the haplosporidian parasite ranged between 50 and 87%, as determined by histological analysis of small samples (< 20 paua) from affected raceways (Diggles *et al*., 2002). During the present survey no haplosporidians were detected in a sample of 60 paua, suggesting that in 2001/2002 the haplosporidian was either absent in these grow out farms, or present at very low prevalence (< 5%) and not associated with mortalities. After the original disease outbreaks, the farm 8 raceways were decontaminated with chlorine, fallowed for a short period and new stock was introduced. The only notable changes in husbandry procedures were: (1) inclusion of filter bags upstream of the raceways to filter the incoming water down to around 25 μm, and (2) separation of adult, wild caught broodstock paua from raceways and water supplies which feed into raceways containing juveniles. The apparent absence of the haplosporidian during the 2001/2002 summer could therefore be due to coarse filtration of the incoming water, and/or physical separation of broodstock paua from juveniles. This suggests that the source of the original infection may have been infective stages carried into the facility either by adult, wild caught broodstock paua, or via the water supply. If the water supply was the source of infection, it is considered unlikely that the infective stage of the haplosporidian was greater than 25 μm in size, and hence was removed directly by filtration. Instead, it may be possible that filtration excluded an intermediate host greater than 25 μm in size which carried infective stages.

A number of other pathological conditions and lesions were observed during this study. While some were associated with mortalities and hence may be significant to aquaculturists, none appear likely to be of regulatory significance. Some, such as bacterial infections of external epithelia, are likely to be precipitated by overstocking and/or mechanical damage during occasional movement of paua between tanks. Others, such as infections of ectocommensal ciliates and gregarine oocysts, are likely to be relatively benign infections which also occur in wild paua (B. Diggles, unpublished data). However, the following appear worthy of further discussion.

The detection of rickettsial inclusions in the gut epithelium at low prevalences in three farms was notable because one of the most prominent diseases of abalone in the Northern Hemisphere, withering syndrome (WS), is caused by a rickettsial organism which infects the same organ system (Gardener *et al*., 1995; Friedman *et al*., 1997; 2000). The emergence of WS in wild and cultured abalone along the west coast of the United States has been linked with climate change, particularly increased water temperatures associated with *El Nino* events (Moore *et al*., 2000). The rickettsial inclusions in *H. iris* were much smaller and rarer than those associated with withering syndrome, and their presence in paua wasn't associated with disease. However, with the global warming process, it remains to be seen whether rickettsial disease could emerge in both wild and cultured paua in northern parts of New Zealand.

The detection of granuloma-like lesions at low prevalence in paua from 4 farms was notable because another disease of Northern Hemisphere abalone, namely amyotrophia of black abalone (*Nordotis discus discus*) in Japan, is characterised by superficially similar, tumor-like lesions adjacent to nerves in the foot (Nakatsugawa *et al*., 1999). This disease causes epizootics associated with wasting of the foot muscle. The cause of amyotrophia is unconfirmed, but a virus has been implicated (Nakatsugawa *et al*., 1999). In New Zealand the presence of the granuloma-like lesions has not been associated with wasting of the foot muscle or mortalities in juvenile paua. Similar, but more extensive granuloma-like lesions have been observed in wild caught adult paua in New Zealand (Diggles, unpublished data) but in those cases they affected various organs and were not limited to areas adjacent to the nerves of the foot. In the cases examined to date acid fast bacilli have not been detected in these lesions (Diggles, unpublished data). The rarity of this lesion has precluded more detailed study of its aetiology at this time.

An inflammatory lesion suggestive of haemocytic neoplasia, but lacking the characteristic cell hypertrophy observed in that lesion, was particularly prevalent in older paua sampled from grow out farms in the North Island. Haemocytic, or disseminated neoplasias, are pathological conditions which have been reported in at least 15 species of molluscs, mainly oysters, mussels and clams, worldwide (Elston *et al*., 1992; McGladdery *et al*., 1993). Those conditions are commonly associated with morbidity and mortality, and their aetiology appears multifactorial. Increased prevalence of haemocytic neoplasia has been associated with environmental perturbations such as warmer water temperatures, exposure to pollution and carcinogens, physiological condition of the host, and genetic factors such as inbreeding (Elston *et al*., 1992). The presence of these intense inflammatory lesions could, therefore, reflect exposure of paua to an undetermined disease agent, and/or unfavourable environmental conditions in culture. They may also be a metabolic phenomenon, possibly associated with paua utilizing muscle as an energy source during starvation, or could result from reduced genetic diversity in hatchery produced paua (Smith and Conroy, 1992). However, since the inflammatory lesions were not associated with overt disease, investigation into their cause was not undertaken and hence their aetiology and relevance remains undetermined at this time.

Subsequent to the survey infection by the mudworm *Polydora hoplura*, a fungus and a coccidian-like protozoan were detected in samples of larger paua from farm 4. The paua infected with *P. hoplura* harboured up to 7 mud worm blisters either inside the shell in the mantle cavity or at the rear of the shell. All affected paua were markedly underweight and in all instances the presence of mudworms on the inside of the shell was associated with brown conchiolin deposits, suggesting that their presence was damaging the paua. Reduction in weight of abalone infected with mudworm has been previously reported (Kojima and Imajima, 1982; Lleonart *et al*., 2003). Infections by spionid polychaetes have previously been recorded in wild paua in New Zealand (Sinclair, 1963), and have been associated with mortalities of cultured *Haliotis* spp. in Australia (Lleonart *et al*., 2003).

The fungal infections were caused by the same agent described by Grindley *et al*. (1998), but were not well advanced, and did not interfere with the attachment of the foot to the shell. However the presence of the fungus in cultured paua remains significant due to the chronic progressive nature of the mycosis, which means the disease may become problematic if paua are reared to larger sizes. The intracellular coccidian-like protozoan in the epithelium of the eospohageal pouch had not been recorded previously in New Zealand paua. The rarity of this parasite has frustrated attempts to gather material for study by TEM and molecular methods to confirm its identity. However the morphology of the various developmental stages observed by histology was similar to meronts and merozoites described by Friedman *et al*. (1995) for a renal coccidian in *Haliotis* spp. from California. Coccidian infections in abalone are generally non-pathogenic (Friedman *et al*., 1997), however the presence of heavy coccidian-like protozoan infections in some of the paua which were also heavily infected by mudworm could suggest that moribund or compromised paua are more susceptible to infection, as found by Caceres-Martinez and Tinoco-orta (2001) for coccidian infections in red abalone in Mexico.

The outbreak of bacterial disease of the epithelium of the mantle, epipodium and foot of paua from farm 4 subsequent to the survey was initially thought to be suspicious, as the presence of the sporozoite-like protozoans in external epithelia (which were found only at farm 4 during the survey), could have predisposed the affected paua at that farm to bacterial disease of that organ. However, further investigations revealed that the protozoan infections had remained at low prevalence and intensity throughout, and thus were considered unlikely to have directly precipitated the disease outbreak. On the balance of the information available it appears the outbreak of bacterial disease was more likely due to overstocking, or other husbandry or water quality related factors.

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