DEVELOPMENT OF AN INTEGRATED MANAGEMENT PROGRAM FOR THE CONTROL OF SPIONID MUDWORMS IN CULTURED ABALONE

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OBJECTIVES:

1. In general, to develop methods for the control of mud worms in farmed abalone, based on the principles of sustainable aquaculture.

2. To thoroughly investigate the ecology and reproductive biology of spionid mud worms and their interaction with abalone, through monitoring, field and laboratory experiments.

3. To gather long term data on the efficacy of the chemical treatment(s) (including antifoulants) throughout the production cycle.

4. To develop a protocol of chemical treatment within the production cycle to optimise the efficiency of chemical control in relation to abalone survival, growth and marketability, cost and responsible chemical use.

5. To gather information on the epidemiology of mud worm infestation in relation to ecological and hydrodynamic characteristics of sites, cage design and deployment and stock husbandry.

6. To use information collected to refine culture methods so as to minimise the level and consequences of mud worm infestation, preferably without the use of chemical treatments.

NON TECHNICAL SUMMARY:

BACKGROUND AND NEED

Early sea based abalone grow out facilities in Tasmania were severely compromised by stock mortality in the mid 1990's. Affected stock were found to have large shell blisters caused by marine worms known as spionid polychaetes or mud worms. Two major species were identified and one of these, *Boccardia knoxi*, was especially common at the farm with the greatest stock losses. As a result of the mud worm infestations many marine farms abandoned attempts at commercial abalone culture in the sea. The overall objective of this project was therefore to devise strategies by which mud worm infection could be minimised.

To achieve this objective several research avenues were explored, including:

- Study of mud worm reproductive cycles and strategies with an emphasis on the *Boccardia knoxi* species. By this means it was hoped that any seasonal variation in mud worm reproduction could be identified and exploited to avoid initial infection.
- Ways by which mud worm infection might be treated. This involved testing many anti-parasitic drugs commonly used in aquaculture and agriculture. Environmental treatments such as fresh water soaking and air exposure were also tested as a preferred treatment option.
- Other aspects of the research involved examination of stock and environmental characteristics that might contribute to mud worm infestation. These included stock size and species, fouling organisms present on abalone, rearing cage design and depth in the water.
- Finally the effect of mud worm infection on abalone health was examined at regular sampling periods. Mortality rates, growth, flesh weight and blister characteristics were measured as were changes in abalone physiology. From this work we sought to define indicators of abalone health that would be useful as diagnostic tools in abalone farming generally.

AVOIDANCE STRATEGIES

Study of mud worm reproduction showed that the *Boccardia knoxi* species produced larvae only in the spring months. Larvae were released into the plankton at approximately 0.5 mm and after a few weeks settled on abalone and burrowed in the shell. Therefore a simple avoidance strategy for this mud worm species was devised. Abalone placed in the sea in December or later remained free of this mud worm species for approximately 9 months – allowing a good start to the grow out phase.

Another species of mud worm, *Polydora hoplura*, was found to settle on abalone in the spring and summer and infestations could be substantially avoided by December to January stocking of culture systems. Control of *P. hoplura* infestations older than 6-9 months is difficult, as local settlement occurs in all months. Thus it is critical for farms husbandry to ensure stocks are monitored and treated early.

TREATMENTS

Testing of mud worm treatments found that several chemicals and drugs were capable of killing mud worms removed from the abalone shell. However, mud worms present in their shell burrows were well protected from drug treatments given as a bath. Such treatments had a minimal effect on mud worms in burrows or were harmful to abalone at doses high enough to kill the worms. The best treatment for mud worm in abalone was found to be simple air-drying of stock, as has been traditionally used by oyster growers to fight mud worms. Antifoulant paints supplied by the CRC for biofouling were tested but as they lacked a suitable application method for abalone no long term efficacy study of these was conducted. As air drying of abalone was found to be a superior treatment option to chemical immersion, long term efficacy studies focused on this treatment.

Two to four hours out of water at about 15 to 20 °C was found to reduce mud worm numbers by up to 90% or more. Treatment was especially effective if abalone had been infected for less than six months. The older the infections, the longer the exposure time required to kill a significant proportion of the mud worms. Appropriate conditions to ensure effective drying are considered to be

temperatures >15°C and humidity less than approximately 63%. These conditions are not uncommon on sunny days in Tasmania outside the period late autumn to early spring. Where shells did not fully dry treatment was ineffective. Lack of drying was associated with high humidity, larger stock and severe shell fouling. The difficulty of treating heavily infected stock also emphasises the need to treat early. Such treatment was considered safe for abalone but had potential to reduce the growth rate of stock and should therefore be used as a second line of defence rather than the main management tool.

STOCK HUSBANDRY

It is recommended that in mud worm susceptible areas abalone be transferred to leases after December then checked for mud worm infection about a year later and treated then if necessary. A single treatment would then give the best part of a year's relief from infection, generally allowing enough time for abalone to reach market size.

Study of mud worm settlement patterns (to achieve objective 5) showed that the mud worm larvae, especially those of *B. knoxi,* were attracted to certain fouling organisms sometimes present on abalone shells. The main risk enhancers were tube building polychaete species such as spirorbids that did not themselves burrow through the abalone shell but simply colonised the surface. Heavy fouling with such tube building polychaetes could enhance mud worm settlement by 500%. Transfer of abalone to sea leases post spring would avoid substantial natural spirorbid settlement.

Stock placed at < 35 mm remained substantially mud worm free even as they grew to approximately 60 mm, whereas stock placed at about 50 mm became relatively heavily infested.

Thus if there is a requirement to transfer stocks to spionid susceptible sea based sites during or soon before mud worm dispersal periods the use of smaller and relatively spirorbid free animals would minimize risk. It would be prudent to place larger abalone after the completion of the spring settlement period. Heavily fouled hatchery stock should be avoided by farms in mud worm susceptible areas, but if they are to be used, they should also be placed after the spring settlement period. While this would minimise the mud worm infection in the first summer, such animals would provide some enhancement of *P. hoplura* infestation and would still attract larger numbers of *B. knoxi* as well as further tube-building polychaetes in the subsequent spring. Unless animals are close to harvest after their second summer in the sea, treatment of this infection could well be required, but could be of limited effectiveness given the larger size and heavy fouling of these animals. Thus stock growth rates and cost structures for sea based sites will determine which hatchery stock are acceptable and whether individual farms use these sites for all or part of the grow-out phase.

REARING SYSTEM DESIGN

Rearing container design with respect to mesh area for water exchange had an effect of mud worm settlement and the progression of the infestation. Cage design should take into account the need for containment, adequate water exchange (especially in summer) and ease of removal of dead abalone to maintain water quality, and suitability for air-drying *in situ* for practicality of treatment. Restricted water flow restricted mud worm settlement from the spring larval dispersal, but favoured local spread of *P. hoplura* at other times. Removal of fouling only after this period but prior to summer has some advantages with regard to this. Lidded trays provide good drying exposure. Abalone reared near the bottom (1 m clearance) were found to have greater infection with the *P. hoplura* mud worm but not *B. knoxi* than stock reared higher in the water column (4 m). Thus mud worm infestation can be minimised by taking into account these risk factors.

ABALONE HEALTH

In relation to abalone health, mud worm infestation was shown to reduce the growth rate and abalone with larger mud worm blisters tended to be smaller than less affected stock. Mud worm affected abalone had less energy reserves than non infected stock. This was consistent with the observed transfer of effort into shell healing.

Lack of energy reserves was indicated by histological changes to the right kidney and digestive tubules, particularly pigment accumulation consistent with increased cell turnover; with depletion of glycogen and protein tissue reserves; reduction in haemolymph glucose and with

increased oxygen consumption. Such changes were likely the result of mobilization and direction of host resources to shell repair, at least in part, though more direct effects from surface tissue damage and possibly reduced appetite have not been excluded. Low energy reserves may have been manifested as reduced growth, and in some instances death. Spionid infestation was also associated with reduced capacity to regulate potassium and in severely impacted abalone with significantly lowered circulating haemocyte counts. Similar low levels of haemocyte counts were seen in "runted" stocks and animals starved experimentally for 3 months, suggesting lack of haemocyte production may contribute to this. Haemocyte counts are considered potentially useful as a general indicator of health status.

The widespread mortality seen in the mid 1990's was not repeated in field studies from August 1998 to January 2001. Mud worm settlement levels during field studies from 1998-2001 were generally very low compared to those observed in abalone surviving the original outbreaks of 1995 and 1996. This suggests mud worm infection of farmed abalone may not be a consistent problem for farmers in the future but that monitoring of stocks will be required. The research has shown that mud worm infestation can be managed by avoidance strategies in the first instance and treatment if required.

OUTCOMES ACHIEVED

The major knowledge outputs are that mud worm infestations can be substantially avoided in the first instance and treated if necessary. The major project outcome from this knowledge and a new sense of perspective regarding the risk of severe infestation has been a rise in investor confidence leading to renewed interest in sea based abalone farming. This has contributed to the development of at least three new sea based abalone farms in Tasmania. Project findings have been made available directly to industry and specific discussions held on risk minimisation with some individual farms.

KEYWORDS: abalone, mud worm, spionid, aquaculture, parasitism, treatment.

ACKNOWLEDGMENTS

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A considerable proportion of this work comprised the PhD studies of Mark Lleonart, whose thesis has been submitted and accepted.

1. BACKGROUND

1.1 General Introduction

Abalone culture in Australia commenced in the mid 1980's with land based farms in Tasmania and South Australia. The primary culture species were the blacklip abalone *Haliotis rubra* Leach and the greenlip abalone *H. laevigata* Donovan. In the mid 1990's there were five sea based experimental or pilot scale farms in southern Tasmania. By 1996 these farms had experienced stock mortality levels in excess of 50% which were associated with blisters to the inside of the abalone shell. Both greenlip and blacklip abalone were affected as were hybrids of the two species. Preliminary investigation showed that two-spionid polychaete "mud worm" species were present within the blisters of infested abalone. Mud worms are noted pests of farmed bivalve molluscs and consequently considerable research exists on their impact on this group (Blake and Evans 1972, Lauckner 1983).

The spionid species present in the shells of live and deceased abalone were identified as *Polydora hoplura* Claparede and *Boccardia knoxi* Rainer. The former species was previously recorded from Tasmanian farmed and wild oysters (Wilson et al., 1993) but *B. knoxi* was only previously known from New Zealand (Rainer 1973**,** Read 1975). This species was the dominant spionid at the culture facility with the greatest number of infested stock and there was some concern that *B. knoxi* may have been an introduced and particularly destructive mud worm.

By November 1997 when this study commenced, some marine farms in southern Tasmania had abandoned abalone culture trials or relocated facilities and all farms had discontinued transfers of new stock to their leases. It was apparent that a loss of confidence in sea based abalone farming had occurred and strategies to minimize the economic impact of spionid infestation would be required before further investment could occur.

1.2 General background: spionid biology and impacts

Mud worms belong to the family Spionidae, one of the largest families of marine annelid worms in the class Polychaeta. The family is very common in all marine environments and its members include free-living forms in sand and mud as well as species that build permanent burrows in soft substrates (Fauchald 1977). Several genera of spionids, including *Boccardia* and *Polydora*, are capable of boring in calcareous substrates including the shells of molluscs. Boring is achieved by the production of acid secretions (Zottoli and Carriker 1974, Almeida et al. 1996). The name “mud worm” may derive from the often-muddy appearance of blisters formed in molluscs as a result of burrowing activity, though large blisters in these abalone were often filled with clear fluid.

Because of the economic importance of oyster cultivation world-wide, there have been many studies on spionid impacts (see reviews by Blake and Evans 1972, Skeel 1979, Lauckner 1983, Handley 1997). In Australia, oyster culture industries have been severely impacted in the past. Early mud worm infestations were investigated by Whitelegge (1890) and from about 1870 in NSW were so severe that the industry changed from sub-tidal dredging to the present intertidal culture system (Smith 1984, Nell and Smith 1988). By about 1900 mud worm infestation in Queensland had devastated sub-tidal production in that state (Smith 1982, Nell and Smith 1988). Potential treatment of spionid infestation in oysters has been previously investigated (Whitelegge 1890, Korringa 1952, Mackenzie and Shearer 1959, Bailey-Brock and Ringwood 1982, Nel et al. 1996). The species most often associated with damage to commercially important bivalve species are *P. ciliata* Johnston, *P. hoplura* and *P. websteri* Hartman (Blake and Evans, 1972).

There is considerably less literature on the incidence and effects of spionids in abalone. Mud worms have been reported from wild haliotid species by: Sinclair (1963), Shepherd (1973), Clavier (1989), Horne (1996) and Grindley et al. (1998). In Japan, Kojima and Imajima (1982) found that more than 10 *Polydora* per shell significantly decreased the flesh weight of wild *H. diversicolor* Reeve. References to mud worm incidence in cultured abalone have been fewer. Ruck and Cook (1999) note that *Polydora* is a potential problem in *H. midae* and McCormick (1999 pers. comm.) described incidents of severe mud worm infestation in *H. discus hannai*.

In addition to impacts on molluscs there have been many studies on the reproduction of spionids (Wilson 1928, Hopkins 1958, Dorsett 1961, Blake 1969, Anger et al., 1986). A dichotomy in larval feeding and dispersal modes have been described (Radashevsky 1994) where spionids either obtain nutrition from yolk supplies (lecithotrophy) growing to a relatively large size with reduced dispersal capabilities or feed in the plankton (planktotrophy) and potentially disperse widely. These variations in spionid reproductive strategies may in part explain how different spionids impact on molluscs to differing levels.

1.3 The original Tasmanian abalone mortality episode

During the period 1995-1997 various abalone culture facilities within Tasmania contacted the State Government Fish Health Laboratory to report cumulative mortality levels of up to 50% or more associated with mud worm blisters. Heavily blistered abalone were examined from four sea based grow out farms in the south of the state and similarly infested scallops from a further farm. Mortality at one of the southern sea farms was in excess of 95%, involving the loss of more than 30 000 animals by late 1997 (O'Brien 2001, pers. comm.). Hindrum (1996) reported approximate 40% mortality between November 1995 and April 1996 at one site. Infested abalone at the southern farms, mainly located in the D'Entrecasteaux Channel, showed the presence of *B. knoxi* and *P. hoplura* but no attempt was made at the time to quantify spionid numbers until late 1997. Stock from three of the southern farms were made available for study. Two of the farms (Huon Aquaculture Company Pty Ltd, and Aquatas Pty Ltd) subsequently became study sites for this research. A small number of remnant stock from Tasmanian Tiger Abalone were made available for study. Mud worm associated mortality was also reported from a sea-based farm outside the south of the state and the same two-spionid species recovered from blisters. A further history of mud worm associated mortality was reported in remnant 5-6 year old, long term infested stocks at an east coast land based farm. Samples from this source revealed *P. hoplura* but not *B. knoxi* was present in severe blisters. Samples obtained from a land-based farm in South Australia also showed solely *P. hoplura* infestation in blistered older stock with a history of mortality.

Some mortality records for the period 1994 to April 1996 were available for Huon Aquaculture Company stock (Appendix 3, 1A) and showed a high death rate during the austral summer 1995/1996. Infested stock from this source examined in 1996 had extensive blister damage that was associated with almost exclusively *B. knoxi* infestation. The ventral surface of many shells was characterized by thick, soft, blisters devoid of nacre and apparently failing to heal. By 1997 approximately 1000 shells of dead and mud worm infested live stock remained and these were made available for experiments on spionid treatment options (Sections 5.2 and 5.3). A subjective shell damage assessment was made on a sample of these abalone (Appendix 3, 1B) using the method described in Section 4.9. An estimate of spionid infestation made using the method developed by late 1997 (Section 4.6) found a mean *B. knoxi* count of 36.5 (SD=19, n=40). Mean percentage flesh weight of remnant heavily blistered stock from Tasmanian Tiger Abalone was 52.6% (SD = 3.1%, n=10).

1.4 Previous and preliminary data on spionids in Tasmanian molluscs

In addition to abalone, mortality episodes involving spionid blisters in farmed scallops and Pacific oysters *Crassostrea gigas* Thunberg were also reported to Tasmanian aquaculture health authorities in the mid 1990's. Annual data on blister prevalence in farmed oysters was collected by the Fish Health Unit as part of research and surveillance programs (Appendix 3, 1C). These data indicate that blister rates for oysters may vary considerably year to year. Additionally, the surveillance program showed the southern region of the state where affected abalone farms were also located had consistently higher levels of oyster blistering than other regions of the state (Unpublished DPIWE Fish Health Unit data). Wilson et al. (1993) found 3 species of spionids in Tasmanian oysters. In order of prevalence these were *P. websteri*, *P. hoplura* and *B. chilensis* Blake and Woodwick. Although *B. knoxi* was not found in the survey by Wilson et al. (1993) its presence at that time cannot be ruled out since most oysters surveyed were intertidal and *B. knoxi* appears to have a sub-tidal distribution in New Zealand (Handley 1997).

Following the identification of *B. knoxi* in cultured abalone stock Tasmanian aquaculture health authorities commenced sampling of wild abalone populations. Surveys showed the presence of *B. knoxi* in wild stocks from the south, south-east, and east coasts but not in a small number of samples from the north-west (DPIWE unpublished data). No mud worm associated mortality from wild stocks has been reported. Re-examination of shells from wild abalone collected by abalone fishery research staff at DPIWE Taroona Laboratories over a number of years showed that shell damage in wild stocks was common (Appendix 3, 1D). Most of the damage was attributed to spionid polychaetes but boring sponge damage was also present in many shells. Anecdotal evidence from abalone divers and processors suggests that there are populations of stunted abalone with shells considered too damaged to be used for the jewelry trade. These are typically located in very sheltered area such as the lees of islands and peninsulas. Analysis of samples from such areas present in the shell collection at the Taroona Laboratories indicated they had higher rates of shell damage than larger abalone (Appendix 3, 1E).

1.5 Background: Project development

The Tasmanian abalone farming industry, through the growers association (TAGA) sought support for research into mud worm problems as a result of the initial outbreaks described above. In the first instance TAGA, in collaboration with the Tasmanian Department of Primary Industries, Water and Environment (DPIWE) commenced a program to identify mud worm species present and their distribution in farmed and wild stocks around the state.

The Cooperative Research Centre (CRC) for Aquaculture provided funding for a 12 month study into development of chemical control methods for spionids in farmed abalone. This project (CRC project number A.2. 6) commenced in November 1997. The FRDC funded component of the research (98/307) commenced November 1998 with the aim of producing an integrated approach to spionid management. Thus the biology, including reproduction of the mud worms was studied, as was the epidemiology of infestation and the effects on abalone health.

2. NEED

Abalone culture is an expanding industry with commercial farming under way in all the southern states. To date most farms are land based facilities but sea based culture offers

lower capital investment and provides a broader range of industry options. There are currently sea based farms in Tasmania and Victoria. Although land based farms are not immune from mud worm infestation the most serious problems have occurred in sea based facilities in southern Tasmania. Advances in mud worm management strategies are essential if sea based farming in Tasmania is to be viable and will benefit the abalone farming sector generally.

3. OBJECTIVES

1. In general, to develop methods for the control of mud worms in farmed abalone, based on the principles of sustainable aquaculture.

2. To thoroughly investigate the ecology and reproductive biology of spionid mud worms and their interaction with abalone, through monitoring, field and laboratory experiments.

3. To gather long term data on the efficacy of the chemical treatment(s) (including antifoulants) throughout the production cycle.

Note: that air drying of abalone was found to be a superior treatment option to chemical immersion so long term efficacy focused on this. Antifoulant paints supplied by the CRC for biofouling were tested but lacked a suitable application method for abalone. Thus long term testing was not conducted.

4. To develop a protocol of chemical treatment within the production cycle to optimise the efficiency of chemical control in relation to abalone survival, growth and marketability, cost and responsible chemical use.

Note: as above, air drying rather than a chemical treatment was used.

5. To gather information on the epidemiology of mud worm infestation in relation to ecological and hydrodynamic characteristics of sites, cage design and deployment and stock husbandry.

6. To use information collected to refine culture methods so as to minimise the level and consequences of mud worm infestation, preferably without the use of chemical treatments.

4. METHODS

4.1 Experimental Animals

The majority of abalone used in the research were blacklip (*H. rubra*) stock from a land based culture facility located on the east coast of Tasmania (farm 1). Many abalone used in experiments were from a year class spawned in the summer of 1997/1998. These were approximately 20 mm shell length when first transfers to study sites were made in spring 1998, growing to 40 mm by late 1999 when the last intake of this age cohort was used. The subsequent year class (summer 1998/1999 spawning) was used in December 1999 and 2000. No evidence of any mud worm species was seen in these year class stocks during the research.

Another important experimental group from this farm was a remnant population approximately three years' old in mid 1998. These abalone had low incidence (< 5% of population) along with a low severity (generally < 3 worms) of *B. knoxi* infection. This group was used in some treatment experiments as described in Sections 4 and 5 using the presence of characteristic *B. knoxi* chimneys to select for infested animals. Further abalone from the group were used in some transfers to study sites selecting *B. knoxi* negative animals on the basis of lack of chimneys and drying them to kill any mud worms present (Section 5.3).

Where abalone other than blacklip stock were used in specific experiments, or animals were obtained from culture facilities other than farm 1, this is noted in the appropriate section of the report.

4.2 Study Sites

A field study site was located in North West Bay at Simmonds Point (DPIWE lease number 154) and owned by Aquatas P/L, (Figures 4.1 and 4.2). The site is 17-18 m deep, with sediments described as ranging from very fine dark brown sand to muddy sands dark grey in colour. The current speed is 20 $cm.s^{-1}$ and the tidal range 1.2 m (from D'Entrecasteaux Channel Marine Farming Development Plans for Tasmania, February 1997). Aquatas was predominantly involved in salmon farming during the course of the research but had investigated sea based abalone farming in the early and mid 1990's. This previous history of mud worm infestation prompted the use of the site for the current research. Abalone were originally housed in culture vessels hung in 3-4 m of water from a barge on the lease but later moved to a long line 200 m north of the barge.

A second southern Tasmanian study site was located in the Huon River at Hideaway Bay (DPIWE Farm Number 93) and belonged to the Huon Aquaculture Company P/L (hereafter referred to as Huon Aquaculture). (Figures 2.1 and 2.2). The environment is estuarine with a pH range of 7 - 8 and variable salinity. Average monthly temperature range is 11-19 °C. The study site was located in that part of the lease (MF 93) where the depth ranges from 8-12 m and current speed ranges from 1-20 $cm.s^{-1}$. The sediments are fine sands (DPIWE Lease Monitoring Baseline data provided by Huon Aquaculture Company P/L). During the research this was predominantly a salmon farming company but with an interest in shellfish including Pacific oysters and abalone. The site was chosen because of its past history of mud worm infestation in abalone. Abalone were housed in culture vessels suspended at a depth of 3-4 m from the collars of an empty salmon cage moored in 10-12m of water.

A third sea-based study site was located on the east coast of Tasmania (Figure 4.1). This is referred to as Site 3, or Site 3 (East Coast). Stock were reared on a commercial scale at this site, suspended from long-lines in 20 m of water.

4.3 Holding Conditions

Field Trials

Various types of rearing vessels were used to contain abalone during the research. When mud worm infestations were originally recorded, abalone at several culture facilities were held in vessels constructed from 250 mm PVC pipe fitted with 6 mm mesh ends. These were referred to as "tubes" (Figure 4.3A) and measured 1500 mm in length. Monthly intakes of "clean" abalone were housed in tubes from August 1998 to November 1998. Most abalone were eventually removed from tubes and housed in other types of rearing containers described below. This was because there were insufficient tubes to house all the abalone required for the research and because tubes were relatively expensive to manufacture and heavy to handle.

Nearly all intakes of abalone subsequent to summer 1998/99 were housed in "basket type" culture vessels. These were modified polyethylene laundry baskets fitted with oyster mesh and plastic inserts to increase mesh free substrate area (Figure 4.3B). The baskets were approximately cylindrical, 800 mm high with a diameter of 400 mm. A third type of rearing vessel was the Aquatek Aquatray® (Figure 4.3C) measuring 900 mm on each side and 10 mm deep. Bases of these were solid with a 12 mm mesh lid and sides. Abalone maintained in the long term were transferred to these from tubes and baskets in late summer 1999 and 2000 respectively. This was to group abalone with similar levels of mud worm infection together in a smaller number of rearing vessels to reduce maintenance time.

Abalone held at the southern study sites were generally treated in the same way as when the original mud worm problems arose in the mid to late 1990's. Feeding was weekly or bimonthly using Adam and Amos (Mt. Barker, S. Australia) sea cage formulated abalone food.

Laboratory Holding Conditions

Abalone were moved from field sites to the Animal Health Laboratory (Mt. Pleasant Laboratories, Department of Primary Industry Water and Environment, Launceston, Tasmania) for analysis. Transportation was in water to minimise stress to abalone and polychaetes. Abalone were held for no more than two weeks in a 650 l recirculating system. This consisted of a fibre glass tank with a conical base and a 400 l sump containing 50 l of "bioballs" as a biological filter medium. The system was aerated and temperature control maintained by use of a 250 W aquarium heater and/or reverse cycle air conditioning. Water temperature was maintained at that of the study sites (±2 °C). Ammonia and nitrite measurements were made on occasion using Aquasonic (Ingleburn, NSW) aquarium test kits and pH was measured using Sigma (range 4.5-10) indicator paper. Salinity was measured using a Shibuya S-10 refractometer. Ten to twenty percent of tank volume was exchanged on a weekly basis. Abalone samples were separated within the system by placement in mesh bags or plastic aquaria with mesh lids through which air stones were fitted.

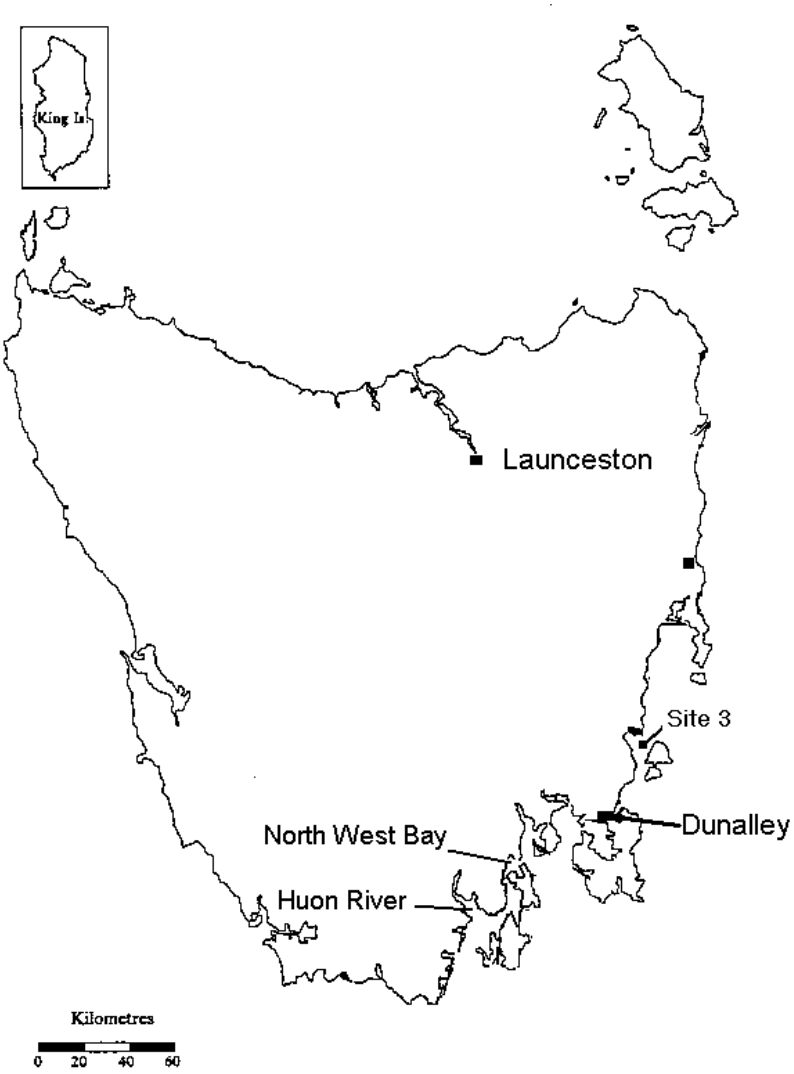


Figure 4.1 Map of Tasmania showing study sites and source farms

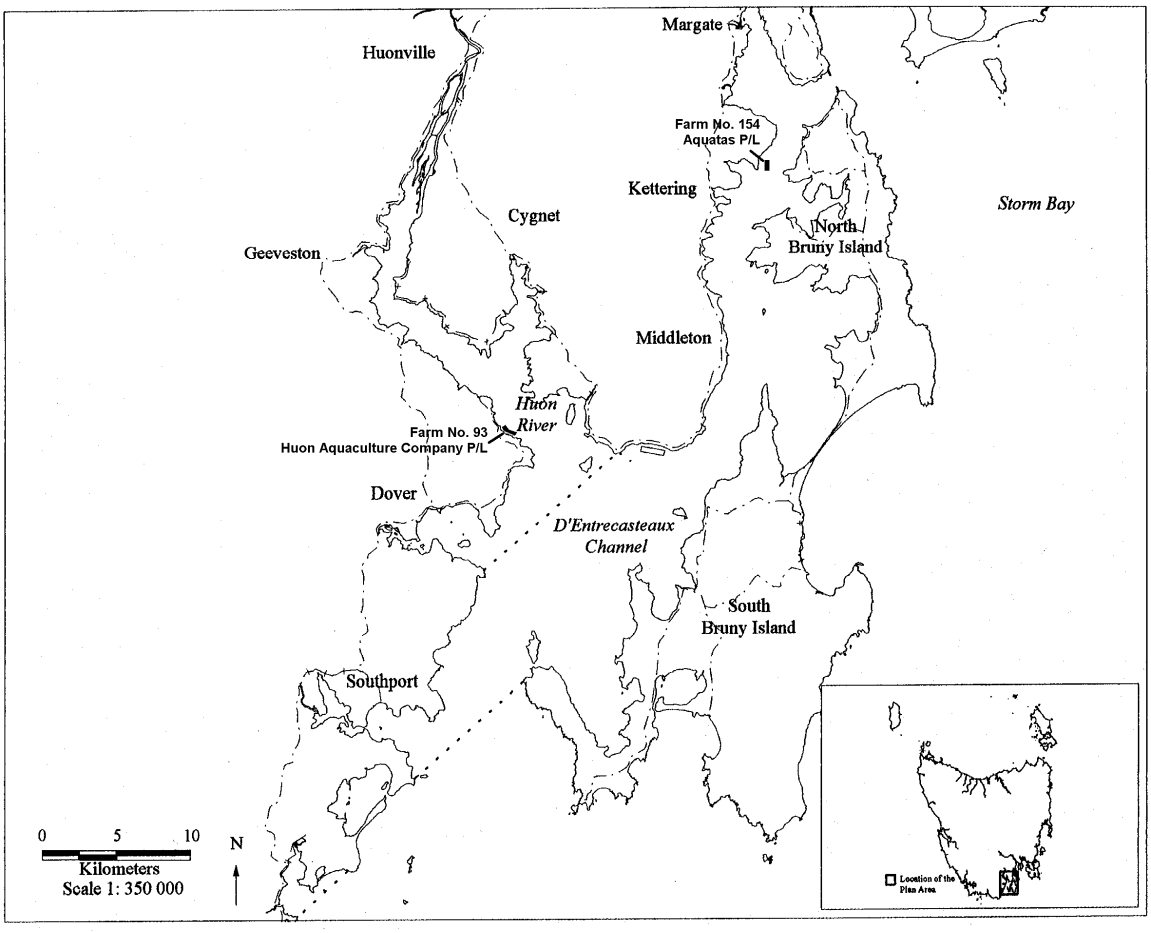
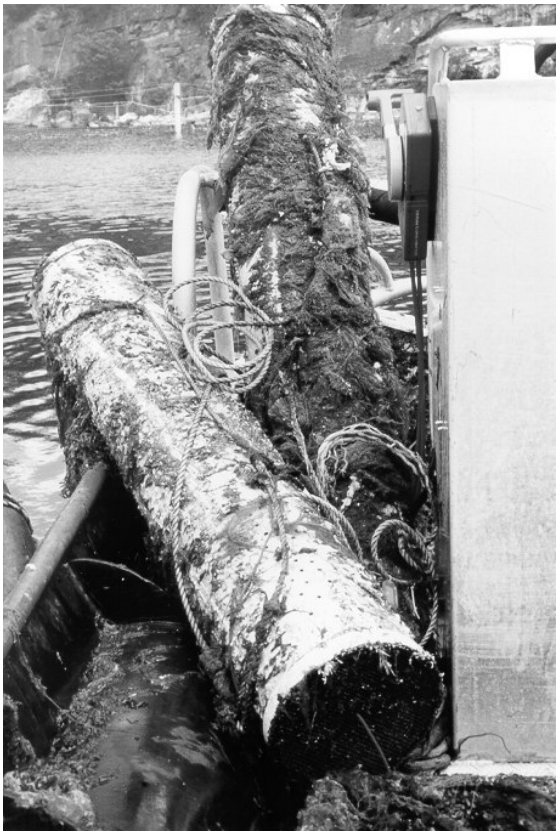


Figure 4.2 Map of D'Entrecasteaux Channel, southern Tasmania showing study sites at Huon Aquaculture Company P/L and Aquatas P/L.



A. Tube

B. Basket

C. Tray

Figure 4.3 Abalone containment vessels

4.4 Speciation of polychaetes

Spionid species were identified on the basis of fifth setiger, prostomium, and pygidium morphology, gill distribution, eyespots, colour and size. Descriptions of species encountered in the research are given in Rainer 1973, Read 1975, Blake and Kudenov 1978. Presumptive identifications of *B. knoxi* and *P. hoplura* were confirmed by Dr. Rainer who first described *B. knoxi* (Rainer1973). Initial identification of *Boccardia proboscidea* Hartman was made by Dr. Geoff Read. Non-spionid polychaetes found on or within the shell were not speciated but were included in total polychaete counts where appropriate. Calcareous tube building polychaetes, such as spirorbids and *Pomatoceros* sp. (Figure 4.4), both usually grouped in the family Serpulidae (Fauchald, 1977) were not included in polychaete counts.

4.5 Quantification by *Boccardia knoxi* chimney count

Preliminary investigation of mud worm infestation showed that *B. knoxi* had a distinctive transparent tube or chimney at the burrow entrance (Figure 4.5). Such chimneys were typically 2-10 mm in length and produced approximately a month after *B. knoxi* settlement.

While other Tasmanian mud worm species at times produced a burrow entrance structure, the *B. knoxi* chimney was considered distinctive enough to be a useful diagnostic tool. Chimneys were found to survive the death of the worm by many months in the field, and for at least eight months in a laboratory test (M. Lleonart unpublished data). Remnant, heavily infested abalone from mortality episodes in 1997 were found to have a 90% occupancy rate of live *B. knoxi* on the basis of chimney counts (mean *B. knoxi* count 36.5, SD=19, n=40 shells). This fell to 55% for shells of abalone dead for several months, with a mean live *B. knoxi* count of 23 (SD=15.5, n=40). Thus chimney counts allowed potential estimation of present and/or past *B. knoxi* numbers. Severe shell fouling interfered with accurate assessment of chimneys. Caceres- Martinez (1999) also found that spionid polychaetes survived the death of the molluscan host.

4.6 Quantification through expulsion of spionids from shells

Mud worm infestation levels were quantified by the use of chemical vermifuges to expel polychaetes from shell burrows or the surface of the shell. This was a mixture of 100 PPM 0-dichlorobenzene and 500 PPM phenol in seawater and was based on the methods of Mackenzie and Shearer (1959) and Handley (1997)**.** Abalone were shucked and shells placed in either 50 ml or 400 ml pots depending on size. Pots were at least 60% full of vermifuge solution. When mud worm colonization was considered relatively recent, shells were immersed in vermifuge in the morning and removed in the late afternoon. Longer time periods tended to kill small polychaetes and post larvae rendering identification difficult. Shells with obvious signs of mud worm infestation were exposed to vermifuge solution overnight at a minimum temperature of 15° C. After vermifuge exposure the contents of pots were drained through 90 µm sieves and worms rinsed to petri dishes for examination with a dissecting microscope. In addition, the surface of the abalone shell was examined under low power microscopy to include any polychaetes only partly expelled from burrows. On occasion mud worm blisters were dissected to check for the presence of polychaete eggs and retained worms.

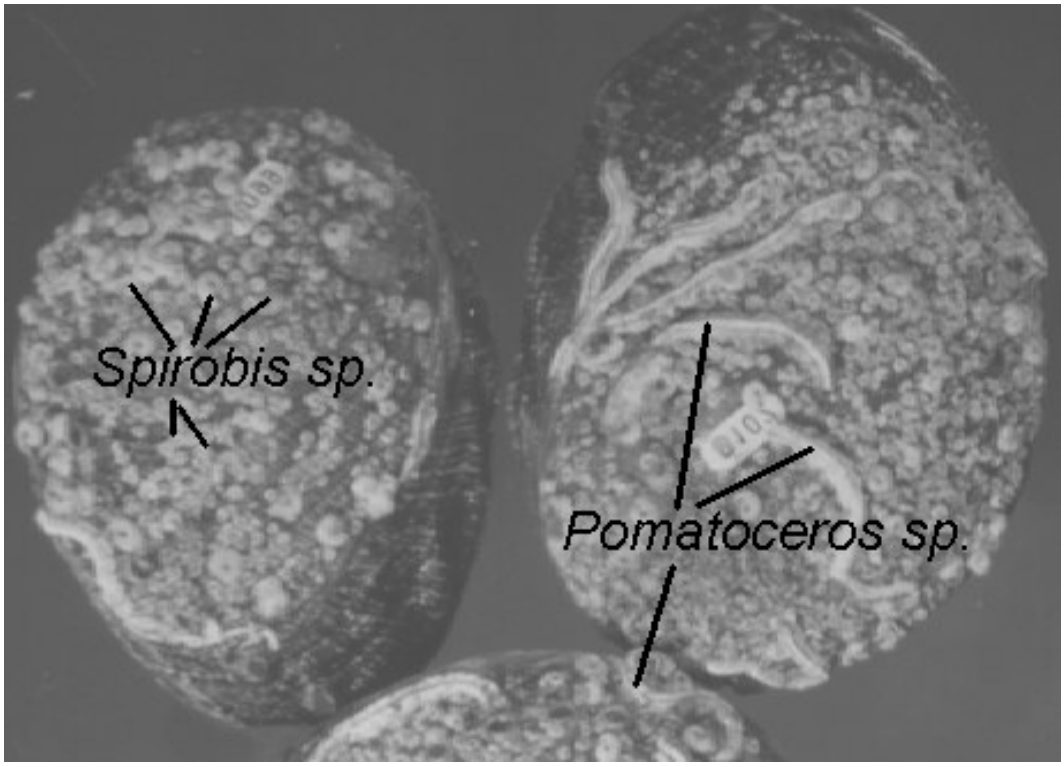


Figure 4.4 Calcareous tube building polychaetes fouling abalone shells

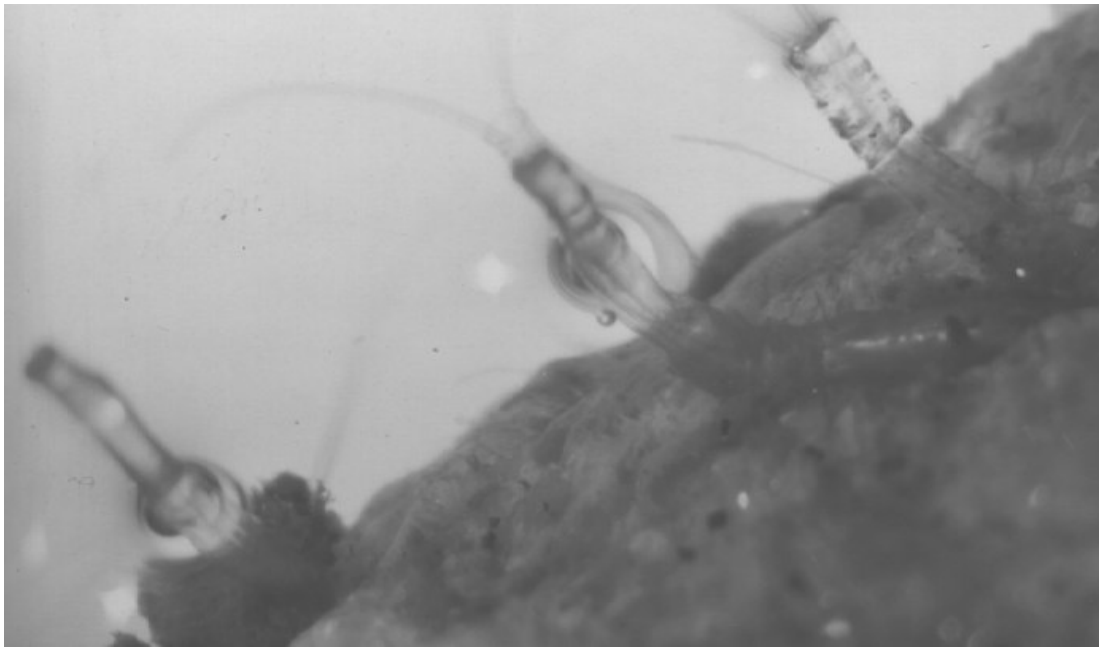


Figure 4.5 *Boccardia knoxi* worms inside characteristic chimney tubes

4.7 Estimation of spionid kill efficacy

The distinctive *B. knoxi* chimney allowed an estimate of treatment efficacy by comparison of chimney counts to worm numbers post treatment. This was termed the estimated individual percentage (EI%) kill and calculated:

$$\text{EI\%Kill} = \frac{\text{No. chimneys - No. live } \textit{B. knoxi}}{\text{No. chimneys}} \bullet 100$$

Comparison of surviving worms of other species was calculated by comparison of treatment and control group means. This is referred to as the group mean comparison (GMC) percentage kill. Kill data for *B. knoxi* was calculated by the latter method when excessive fouling rendered chimney counts difficult and when untreated control group data indicated many *B. knoxi* burrows no longer contained live worms.

4.8 Growth

Length of abalone was measured to the nearest 0.1 mm using callipers and weight measured after towelling dry to the nearest 0.1 g. Where abalone were tagged this was done with soft plastic tags (Hallprint, South Australia) fixed to dried shells with fast drying adhesive. This allowed mean individual growth to be calculated. Specific Growth Rate (SGR) which gives a measure of growth independent on abalone size was calculated as the difference between the natural logarithms of the initial and final measures (in mm or g), divided by time and multiplied by 100.

4.9 Blister Assessment

The extent of mud worm blistering was assessed by two methods: a subjective score and an estimate of percentage blister cover. The subjective score was based on approximate area of blister damage, depth and degree of apparent shell healing. Scores of 0,1,2 and 3 were possible and the system referred to as the subjective shell damage rating (SSDR). Scores of "0" indicated an absence of shell blistering. Scores of "1" indicated light damage, typically blistering to less than 10% of the shell area with blisters characteristically flat and yellow or alternatively well healed with a thick coating of shell nacre over them. An SSDR of "2" indicated damage to 10-25% of the shell area with blister characteristics like that of rating "1" near the upper end of the affected area range, or alternatively raised brown to black blisters over a lesser area of the shell. Shells with raised brown to black blisters occupying at least 25-30% of shell area often with deformity around the apex were assigned the maximum SSDR of "3" This rating was based on shells observed in the original mortality episodes that lead to the research. Examples of shell damage ratings are given in Figure 4.6.

Assessment of percentage blister coverage was made by tracing blisters and shell perimeter on flexible plastic with a printed grid. Areas of blisters and total shell were then calculated by counting squares and percentage blister coverage calculated. Blister coverage was further assessed by classification into "active" and "healed" blisters (Figure 4. 6C). Healed blisters were described as those blisters with a substantial amount of shell nacre deposited over them and which in time could become virtually indistinguishable from the normal shell. Active blisters included those displaying the yellow colour of early conchiolin deposition through to brown and black blisters as a result of the "mud" deposits showing through a relatively thin

surface layer. This distinction between blister types was intended to help quantify the age of blisters and the host response to spionid infestation.

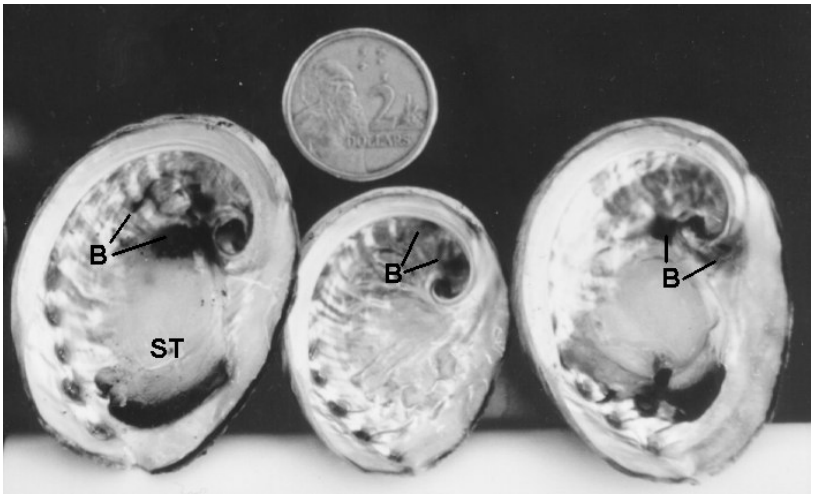


Figure 4.6A SSDR "1" B = Blisters, ST= soft tissue remnant

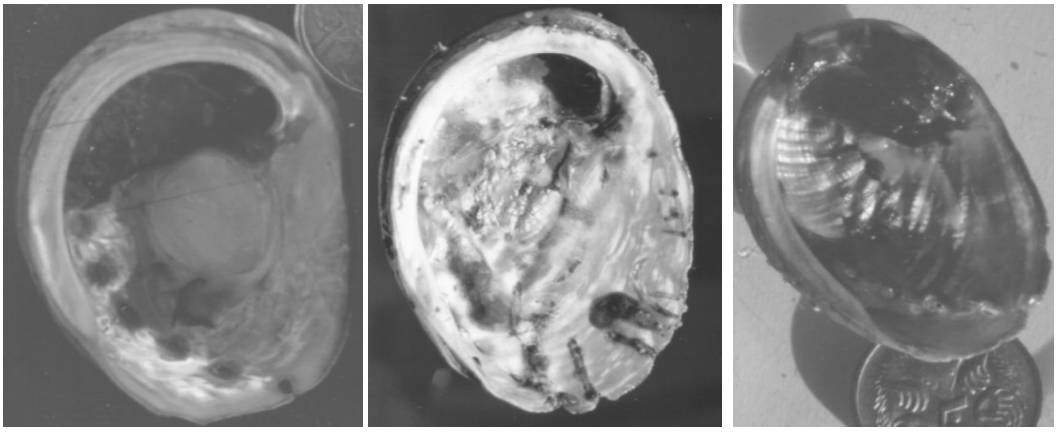


Figure 4.6B Three shells with SSDR of "2"

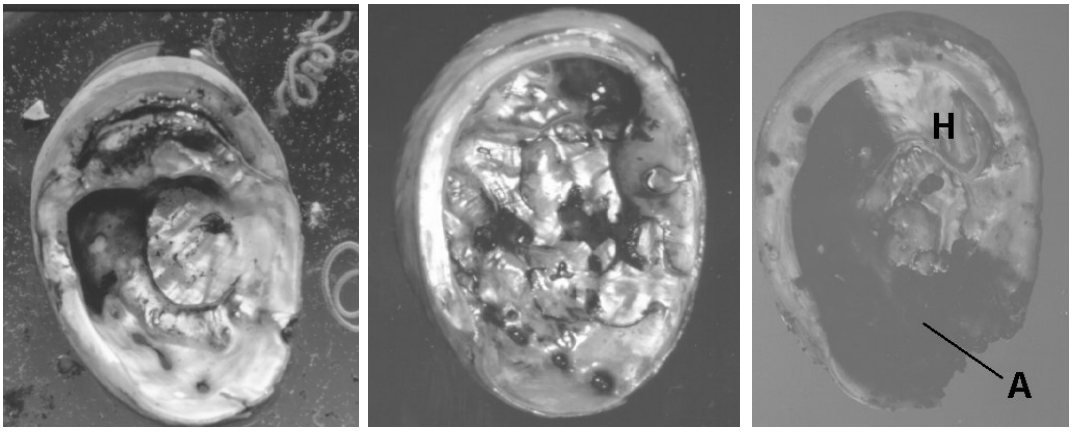


Figure 4.6C Three shells with SSDR of "3"A = Active blister, H = Healed Blister

4.10 General Statistical Methods

Statistical analysis was conducted using Genstat 5 software (©1998 Lawes Agricultural Trust, Rothamsted Experimental Station). For temporal change experiments data were subjected to one or two way ANOVA with time, and time and treatment regime as factors respectively. Assumptions of normality and homogeneity of variance were checked by examination of the residual plots option in Genstat. REML (Residual Maximum Likelihood technique) was used instead of ANOVA for unbalanced data sets (where sample numbers varied between times). Where individual data sets were transformed this is noted in the methods section of the appropriate section. Separation of means was performed by the use of the LSD (Least Significant Difference) function in Genstat.

Spionid count data when the values were low tended to be non- normally distributed and the non-parametric Mann-Whitney U Test, suitable for such data, (Sokal and Rohlf, 1995) was used. Non-parametric methods were also used for analysis of subjective SSDR ordinal data.

The non-parametric or distribution free Kruskall-Wallis test (Zar, 1984) was preferred to ANOVA when data sets were small and the nature of the underlying distribution was unknown. Mean separation was performed by the method of Zar (1984) relating to this test.

4.11 Spionid reproductive biology and settlement

4.11.1 Descriptive methods, including larval rearing and spionid reproductive histology

Mud worm egg strings were dissected from blisters in cultured abalone and examined using Olympus dissecting and stage microscopes with camera adapters. Where required, specimens were relaxed with 10% magnesium chloride.

Spionids were cultured by releasing larvae from capsules at approximately the 5-setiger stage and rearing in 1-l plastic aquaria. Water was filtered to 10 µm and supplied with cultured microalgae (School of Aquaculture, University of Tasmania – Launceston) including *Isochyrsis galbana*, *Pavlova lutheri* and *Tetraselmis* sp. Ambient water temperature was 13-16 °C and larvae were captured on 90 µm sieves each 3-4 d and relocated to clean aquaria. For reproductive studies presumptive adult (>10 mm) *B. knoxi* were relaxed in 10% magnesium chloride, fixed in 10% seawater formalin and histological sections prepared (section 4.12). Worm sections were examined at a magnification factor of 100 for the presence of eggs. Eggs were measured with an eye piece graticule and the presence of mature eggs, up to 95 µm in diameter (Read, 1975) recorded.

4.11.2 Timing of spionid settlement

To investigate the annual pattern of spionid settlement, non- mud worm infected stock from farm 1 was transferred to the sea based study sites at Aquatas and the Huon Aquaculture Company (section 4.2). This was done monthly: August-January 1998, June- December 1999 and July-December 2000 with 100 abalone in each of 2 replicates per study site. Generally 10 abalone were sampled per replicate the following month with further samplings every 2-4 months subsequently. Additional stock present at farms prior to August 1998 was also examined periodically. Abalone

were housed in tube or basket type rearing vessels and maintained as described in Section 4.3. Abalone at Site 3 (East Coast) (Section 4.2) was surveyed April 1999 and 6 times between April and November 2000. Abalone were transferred to the site January-February of the sampled year.

Samples were relocated to the Fish Health Laboratory, Launceston in water and mud worms detached from shells using the chemical vermifuge method (Section 4.6). Examination of shells exposed for a month showed that spionids present were < 3mm in length and thus worms of this size present in shells with prior mud worm exposure were also considered to provide evidence of recent larval settlement. Mud worm blisters were investigated for the presence of adult worms and their reproductive state assessed. Spionids were considered to have mature eggs within the body if these ruptured easily and were released during examination. The presence of extruded egg strings was recorded and they were examined for the presence of active larvae within the capsules. As not all samples contained blisters within a given month the absence of body egg and extruded shell egg/larvae data in results Tables 5.1 and 5.2 does not confirm the absence of these stages of reproductive development.

4.12 Chemical treatments

4.12.1 Experimental animals and rationale

When these experiments commenced fewer than 1000 heavily infested abalone remained from the original mid 1990's spionid outbreak. These were made available for treatment research and continued to die during 1998 as experiments proceeded. Consequently, initial experimental emphasis was on performance of screening trials to identify promising treatments while experimental animals remained. Later it was found that infested shells retained viable mudworms for months after death of abalone and such "empty shells" were used in many experiments. As infested abalone shells of any type became scarce further experiments were conducted *in vitro* using spionid mudworms, primarily *B. knoxi*, extracted from infected Pacific oysters. Experiments on toxicity of potential treatment agents to abalone were conducted on non-infected healthy stock in the absence of infected stock. Follow up work for the most promising treatment was conducted on a limited supply of lightly infested stock considered still curable.

4.12.2 *In vitro* trials of toxicity to spionids

Spionids were sourced from known *B. knoxi* infected Pacific oysters resident at the Huon Aquaculture Company. Shells containing presumptive *B. knoxi* were determined by the presence of the distinctive transparent "chimney" (section 4.5). The majority of spionids selected for use were *B. knoxi* but there was no attempt to exclude worms of other species from closely related genera.

Mud worms were removed from shells by mechanical destruction with bone forceps or by the use of chemical vermifuges (section 4.6). Where chemical extraction was used harvested worms were placed in several changes of fresh seawater then left for 1-2 d in aerated water. Worms were sorted using a dissecting microscope selecting substantially intact specimens with normal colour.

During toxicity trials mud worms were housed in 200 ml specimen jars for initial exposure to treatment chemicals and the duration of the recovery period. No aeration was supplied and water changes were performed daily. Toxicity trial recovery periods were up to 3 weeks and worms were not fed. Spionids were

examined grossly at water changes and microscopically once or twice per week. Specimens were considered dead if no movement could be detected and decomposition had begun. Generally, chemical treatments were tested at 3 or more different concentrations with one specimen jar of 5-10 worms used for each concentration. Exposure times were three hours unless otherwise stated. Ambient temperature ranged from 15-18 °C.

4.12.3 Spionids in situ toxicity trials

Abalone used in these trials were remnant severely *B. knoxi* infested animals from Huon Aquaculture. On going stock deaths indicated the health of experimental animals was severely compromised. Stock was 40 - 80 mm in length and 4-5 years old. In excess of 10 distinctive *B. knoxi* chimneys were present on stock selected for experiment*s.* Experiments used live infested abalone, infested "empty shells" or a combination of both as indicated.

Potential treatments were generally tested at 3 different levels of chemical concentration or exposure times with an untreated control. Ten abalone were used per treatment level with individual tagging using "supa glue" (Selleys, Padstow, NSW) and plastic tags (Hallprint, South Australia). During treatment abalone and "empty shells" were removed to 5-l aquaria outside the recirculating system (section 4.3), and returned to it after chemical exposure and thorough rinsing.

Aeration was supplied during experimental exposure and afterwards in the holding tank. Exposure times were generally 3 h and water temperature was 15-18 °C. Abalone and shells were maintained post treatment for approximately 1 week. This allowed sufficient time for mud worms and abalone to recover from treatment if they were capable of doing so. It also allowed sufficient time for dead mud worms to decompose, assisting in the assessment of surviving worms. Abalone mortality was recorded and dead animals removed, shucked and the shells replaced in the aquaria. At the termination of the recovery period surviving mud worms were driven from their shells by chemical vermifuges (section 4.6) and quantified.

Treatment efficacy was estimated by comparison of group means between control and treatment groups (section 4.7). Alternatively, where appropriate the individual kill rate for each shell was calculated by use of the EI%Kill (section 4.7).

4.12.4 Abalone Toxicity Trials

Live infected abalone were not always available for *in situ* efficacy testing of potential mud worm treatments. Where such animals were available their health was considered poor due to the severe extent of mud worm infestation. Thus healthy abalone were used for toxicity work on promising chemical treatments. This stock was sourced from farm 1 (section 4.1). Most of the abalone was 40-50 mm, 12-25 g blacklip between 2 and 3 years old. A second population 18-20 mm, 0.7-1.1 g blacklip of 10 to 11 months of age was also used on occasion. Toxicity trials were conducted in the aquaria described previously. Generally 5-10 abalone were used for each treatment level tested. A reserve population of at least 20 abalone in the recirculating system served as a control. Immersion exposure times were 3 h at 14-16 ° C unless otherwise stated. After experimental exposure abalone were returned to the recirculating system until any mortality had apparently ceased. Where no mortality occurred animals were maintained for 1-3 weeks with formulated commercial abalone feed provided.

4.12.5 Selection of treatment agents

The experiments conducted on spionid mud worms *in vitro*, *in situ* in abalone (including "empty shells) and toxicity trials conducted on abalone were considered screening trials. Their intention was to test a wide range of treatment agents so that a suitable treatment could be found while infected stock remained.

Freshwater exposure has a history of use in oyster culture for spionid treatment and avoidance and has been assessed by Korringa (1952), Bailey-Brock and Ringwood (1982) and Tonkin (1997). Death occurs through osmotic shock. As abalone and mudworms (annelids) are closely related, chemotherapeutic agents chosen sought to exploit the size differential between mud worms and host.

Potassium permanganate is effective against ectoparasites and external fungal infections in fish (Cross and Needham, 1988). These authors cite concentration and exposure times of 10 PPM for 30-60 min, 25 PPM for 15 min or 4 PPM permanently. Kirby and Baker (1995) note that potassium permanganate has been used in the past to expel earthworms (which like polychaetes are annelids) from grass sporting surfaces. Accordingly mud worm toxicity trials *in vitro* and *in situ*, and abalone toxicity experiments were conducted in the range between 2 and 50 $mg.l^{-1}$.

Gentian violet is listed by the Merck Index as a vermicide. Toxicity trials on mud worms *in vitro* were initially conducted at between 10 and 100 $mg.l^{-1}$ to find the effective range. Two representatives from the benzimidazole class of drugs were tested, these being mebendazole and fenbendazole. Such drugs are commonly used in terrestrial animal production against nematodes, flukes and tapeworms. Cross and Needham (1988) indicate fenbendazole will kill nematodes in fish when added to feed. The two drugs tested did not dissolve effectively in water and treatment concentrations may be considered suspensions rather than solutions. Concentration ranges of between 25 and 500 $mg.l^{-1}$ were used for mud worm toxicity trials *in vitro* and *in situ*, and abalone toxicity experiments.

Levamisole belongs to the imidazothiazole class of drugs used in various forms in terrestrial animal production against nematodes in particular. Its use here against a parasite of an aquatic animal is perhaps novel but was considered worth investigating. This drug was tested in the range 0.32 to 640 $mg.l^{-1}$ on mud worms and abalone.

Malachite green has been used to treat fungal and protozoan infection in fin fish at 2 PPM for 30-60 minutes (Cross and Needham, 1988) and filamentous bacteria in prawns at 10 $mg.l^{-1}$ (Owens et al., 1988). It is widely used in aquarium fish medications. Concentration ranges of 1, 5, 10 and 20 $mg.l^{-1}$ were used for toxicity trials on mud worms and/or abalone.

Trichlorofon is an organophosphate, a class of drugs used against nematodes and tapeworms in terrestrial production animals. Neguvon, the active ingredient of which is trichlorofon, used at 300 PPM for 15-60 min will kill salmon lice in farmed salmon (Brandal and Egidius, 1979). Langdon (1990) states that trichlorofon is effective against skin and gill flukes, *Argulus* sp. and copepods at 0.5 $mg.l^{-1}$ in sea water. The drug was used in the range 0.1 to 1000 $mg.l^{-1}$ for *in vitro* mud worm toxicity trials and 10-500 $mg.l^{-1}$ in abalone toxicity trials. Praziquantel concentration of 10 $mg.l^{-1}$ for 3 h is effective against monogenean flukes in fish (Cross and Needham 1988, Langdon 1990). Lester (1988) lists it as an effective agent against tapeworms when used in food. A range of doses up to 100 $mg.l^{-1}$ was tested against mud worms *in vitro.* Hydrogen peroxide is an oxidising agent used in aquaculture for disinfection purposes. Exposure ranges of 50 to 1000 PPM were used for toxicity trials with mud worms *in vitro* and/or abalone.

Formalin is widely used in aquarium fish medications, often in combination with malachite green. Cross and Needham (1988) list concentration rates of 167-250 PPM (60 min) as suitable for treating parasites of fin fish under certain conditions. Concentrations of 50-200 mg.l^{-1} were used for toxicity trials against mud worms and abalone.

Ivermectin belongs to the macrolide endectocide class of drugs and is used in terrestrial animal production against nematodes and arthropods. Wislocki et al. (1989) present toxicity data for the related drug abamectin, stating 96 h LC50 values of 430,153 and 3.2 PPB for Eastern oysters (*Crassostrea virginica),* blue crabs (*Callinectes sapidus* Rathbun) and rainbow trout (*Oncorhynchus mykiss*) respectively. As with levamisole the use of ivermectin against an aquatic parasite is some what novel. Ivermectin was tested against mud worms *in vitro* and/or abalone at between 0.004 and 0.4 mg.l^{-1}.

It was an aim of the screening process to test representatives of various drug classes. Accordingly, Exelpet®, "All-Wormer for Dogs" (Wyong, NSW, 2259) tablets containing febantel, pyrantel embonate and praziquantel were tested. This combination is intended to treat a variety of nematodes and tapeworms found in dogs. Febantel and pyrantel embonate belong to the probenzimidazole and pyrimidine groups of drugs respectively, which had not been previously tested. Praziquantel, also present, had been tested previously and found ineffective. Interaction effects between the three drugs would require investigation if early results were promising. The concentration range was 25, 14.4 and 5 mg.l^{-1} respectively of each drug through to five times these doses for mud worms *in vitro*. Abalone mortality was assessed at the higher level only.

Metronidazole is effective against external protozoa (Cross and Needham, 1988) at 25 mg.l^{-1} in fish. This and the related drug dimetronidazole were tested in the range five to 200 mg.l^{-1} and 20 to 500 mg.l^{-1} respectively against spionids *in vitro.*

Methylene Blue is widely used in over the counter medicines used for the aquarium fish trade. It is effective against protozoan and fungal infections in fish at 3 PPM (Cross and Needham, 1988) and against protozoans (ciliates) in prawns at 8 PPM (Owens et al., 1988). Mud worm toxicity experiments were conducted *in situ* at 1, 5 and 10 mg.l^{-1} and *in vitro* at 20 – 200 mg.l^{-1}.

4.12.6 Follow up experiments performed on lightly infected stock

Further experiments using lightly infected stock considered still treatable, were performed for treatment options showing most promise from the experiments conducted *in vitro*, *in situ* with severely infected stock, and from abalone toxicity trials. Experimental animals were chosen from a small pool of lightly infected, *B. knoxi* positive, 40-50 mm abalone on the basis of visible chimney structures. Animals were individually tagged as described previously.

A chemotherapeutic bath experiment consisted of four treatment groups, including an untreated control, each with two replicates of 10 abalone. The treatments were 3.5 h immersions at 15 °C in one of the three following chemicals: gentian violet 5 mg.l^{-1}, mebendazole 200 mg.l^{-1} or potassium permanganate 15 mg.l^{-1}. The untreated control group was maintained in a recirculating system at 15 °C.

Following treatment abalone were maintained in the recirculating system for 1 week at 15°C with out food and shells processed as described previously to quantify polychaete infestation. Data were analysed using chi-square analysis. Chi-square analysis was performed by comparing the number of presumed dead and live worms

recovered at the end of the trial. Dead worms were calculated as number of chimneys minus number of live worms recovered for each shell.

4.13 Air drying treatment

4.13.1 General experimental protocol: air drying trials

Experimental animals were obtained from 1 of 4 commercial abalone culture facilities. Where treatment experiments were conducted in the laboratory stock was transported in water as were animals treated on site then assessed in the laboratory. Treatment group abalone were removed from culture vessels and exposed for air drying on a suitable substrate such as plastic sheet. Where replication was used separate substrates were provided for each group. Temperature was measured at half-hour intervals as was humidity using a wet and dry bulb hygrometer (Masons type, Zeal, London). In some experiments abalone were individually tagged (section 4.8). Untreated control animals were returned to water as soon as possible after tagging. Replicates were kept in separate mesh bags or plastic aquaria.

Following treatment, air exposed and control abalone were maintained in the recirculating system (section 4.3) for one week at 15-16°C with out food. This allowed time for dead mud worms to decompose clarifying assessment of surviving mud worms. Abalone were subsequently shucked and shells placed individually in a mixture of 500 PPM phenol and 100 PPM 0-dichlorobenzene (vermifuge solution) in seawater over night to expel worms from shells (section 4.6). All spionid mud worms were speciated but other polychaetes were grouped as "others" and may contribute to total polychaete data in some experiments. Fouling polychaetes such as *Pomatoceros* sp. and Spirorbids were excluded from counts. Treatment efficacy data was calculated by the estimated individual percentage kill (EI%Kill), (section 4.7) where possible and otherwise by group mean comparison (GMC).

4.13.2 Stock history and method specifics

Trial 1. Treatment of remnant severely infested abalone

Blacklip abalone present at Huon Aquaculture since 1994 or 1995 and severely *B. knoxi* infested were assigned to treatment groups of 3, 5 and 8 h air exposure. Abalone were 50-70 mm in length with SSDR scores of 2-3 (section 4.9) and blister coverage to > 25 % of shell area. Ten shells were assigned to each air exposure time, the majority of which were live abalone. Five shells including one live abalone were used for an untreated control.

Abalone were placed in plastic aquaria and located in light shade outside at the Fish Health Laboratory. Air temperature inside aquaria ranged from 21-24° C during exposure which was staggered so that all aquaria were returned to water at the same time. The trial was conducted in late November 1997.

Trial 2. Treatment of stock infested with B. knoxi ≤ eight months, four hours at 24 °C

Blacklip abalone approximately 3 years old and positive for *B. knoxi* chimneys were selected from available stocks at farm 1. Forty animals were chosen a sample of which measured 44.6 mm (SD=3.6 mm, n=15). The infection level was considered

low with generally 1-3 chimneys per abalone. The infestation period was estimated at approximately eight months.

Twenty abalone were assigned to each of treatment and control groups and tagged. The treatment group was exposed to air for 4 h at 24 ± 1 °C using reverse cycle air conditioning. The humidity was measured by later recreation of the conditions at 46%.

Trial 3. Treatment of B. knoxi infected stock at different temperatures

A total of 105 *B. knoxi* positive blacklip abalone, mean length 47.1 mm (*SD* = 3.9 mm, n = 40) were obtained from farm 1. This group was considered lightly infected with typically 1-3 chimneys seen by stereo microscopy. Larval settlement was believed to have occurred about 8 months previously. Abalone were individually tagged.

Twenty-one abalone in each of 4 treatment groups were exposed to air for 4 h. Air temperatures were: 15, 18, 21 and 24 °C and were controlled using reverse cycle air conditioning or column heating. Humidity values were: 60, 62, 53 and 71% in ascending temperature order. An untreated control group was maintained in the recirculating system at 15°C.

Trial 4. Treatment of B. knoxi infected abalone at different exposure times

Eighty *B. knoxi* positive abalone were selected from the same source as Trials 2 and 3 above and tagged. There were 5 treatment groups, including an untreated control each consisting of 2 replicates of 8 abalone. The treatment air exposure times were 1, 2, 3 and 4 h. Air temperature was 21 ± 0.5 °C and humidity was 60%. The experiment was conducted in the laboratory using reverse cycle air conditioning. The untreated control group was maintained in the recirculating system at 15°C. In this trial the recovery time was 3 rather than 7 d before processing.

Trial 5. Air drying of old, heavily, polychaete infested greenlip abalone

A group of 60- 85 mm greenlip abalone was obtained from Tasmanian Tiger Abalone, Dunalley (section 4.2). The stock was 5-6 years old and had become spionid infected at least 3 years previously. Previously the abalone were cultured in a sea based grow out system but had since been transferred to a land based system with filtered water. The animals were survivors of a group with mortality linked to mud worm infestation commencing 3-4 years previously. The health of the stock was considered poor with some of the animals apparently under weight for their shell length. The shells were eroded on the dorsal surface and had blistering to 20-30% of the ventral shell area.

From the pool of stock 20 *B. knoxi* positive abalone were chosen for the experiment and tagged. There were two experimental groups: an untreated control and a group treated by air exposure. Ten animals were assigned to each group with 2 replicates of 5 per group. The treatment conditions were 4 h air exposure at 21 ± 0.5 °C. Treatment was performed in the laboratory using reverse cycle air conditioning and humidity was 60 %. The untreated control group was maintained in the holding system at 15 °C.

Trial 6. Air drying of recently infected stock under field conditions

This experiment was performed at Huon Aquaculture on December 9 1998. Stock were blacklip abalone approximately 3 years old when placed on the farm in August 1998. Length was 46.5 mm ± 2.9 mm (mean ± SD, n=40). Settlement of *B. knoxi* and *P. hoplura* spionids was known to have occurred in the interval between placement and treatment. The exposure time was 3.5 h and ambient temperature and humidity were 16-18°C and 49-62% respectively during the time period 12:30 h to 16:00 h. Abalone were removed from each of 2 replicate culture vessels and placed on plastic sheet in direct but weak sun light for 5 min and then in shade for the remaining time. Thirty abalone were assigned to control and treatment groups from each replicate. Stock was not inspected to exclude not infected abalone. The control group was returned to water as soon as possible after selection.

From the pool of stock, 20 treated and 20 control abalone (in 2 replicates of 10) were taken to the laboratory for efficacy analysis. The remainder stayed at the site to provide data on long-term treatment benefits (section 5.5).

Trial 7. Air drying of heavily fouled, P. hoplura infected stock under field conditions

This trial was performed at Aquatas on January 20 1999. Experimental animals had been transferred to the farm in December 1997 at approximately 2 years of age. Stock at treatment were 30-50 mm in length and had acquired various degrees of fouling with *Pomatoceros* sp*., Spirorbis* sp. and Pacific oysters. Previous examination of the stock indicated *P. hoplura* was the most common spionid present.

Exposure time was 4 h between 10:30 h and 14:30 h. Treatment group animals were placed in direct sunlight for the first 10 min of air exposure and in shade on plastic sheet for the remaining time. Air temperature ranged between 17.5 and 22 °C and air humidity between 43 and 65%. Twenty animals were assigned at random to control and treatment groups without inspection to exclude any abalone not infected. The control group was returned to water as soon as possible after selection.

Trial 8. Two and half hour drying of recently B. knoxi infected stock

Blacklip abalone, mean length 42.3 mm (SE =0.6 mm, n=80), were collected from Huon Aquaculture and treated on site 12 November 1999. Stock had been on site for 12-14 months but the infection rate from the preceding spring 1998 *B. knoxi* settlement period was considered low. Stock was treated towards the end of the presumptive 1999 *B. knoxi* settlement period. The maximum recent *B. knoxi* infection period was approximately 3 months.

Forty animals were assigned at random to control and treatment groups with out inspection to exclude not infected stock. The treatment group was placed on plastic sheet outside in the shade. The day was sunny and air temperature ranged from 16 to 17 °C during the 2.5 h exposure. Humidity was 50 to 54%.

Trial 9. Drying of 14 month mud worm infested blacklip abalone

Blacklip abalone, mean length 52.6 mm (SE = 1.0 mm, n=20), were collected from Huon Aquaculture and treated outside at the laboratory 25 October 1999. Stock was approximately 4 years old and had been on site for 14 months, acquiring shell fouling in addition to mud worm infestation.

Ten abalone from each of 2 rearing vessels were assigned to a treatment or control group giving 2 replicates of 5 animals per treatment. Control group animals were tagged and returned to water as soon as possible. Treatment group abalone were exposed to air for 3.5 h. Air temperature ranged from 16 to 20 °C (but was generally in the range 16 – 17 °C), humidity ranged from 48 to 59%. Post treatment abalone mortality data was not collected in this trial

Trial 10. Repeat drying of abalone previously treated one year earlier

Blacklip previously treated in December 1998 were re-treated December 13 1999. Stock were 60.9 mm (SE = 0.8, n = 19) and drawn from the same population as in Trial 6 above. Abalone were dried at Huon Aquaculture for 4 h. Temperature ranged from 15-18°C and humidity from 46 and 58% after an early reading of 80%.

4.13.3 Drying and mortality

In a preliminary drying mortality trial 140 blacklip abalone 15-20 mm were obtained from farm 1 (section 4.2). There were 6 exposure times (1-6 h) and an untreated control with 20 animals in each treatment. Abalone were dried using reverse cycle air conditioning at 21°C ± 0.5°C. Post treatment mortality was recorded for 17 d while animals were held in small plastic aquaria within the recirculating system (section 4.3).

A second drying mortality experiment used 156 blacklip abalone (41.5 ± 4.1 mm, mean ± SD) obtained from Huon Aquaculture. Mud worm infection was minimal in the group and the animals had been tagged previously. After 5 d to acclimatize, without feeding, abalone were randomly assigned to 12 baskets with 13 abalone per basket. Experimental treatment consisted of 3 exposure times: 5, 8 and 11 h and an untreated control. There were three replicates for each treatment. Cages were 200 mm by 200 mm and 250 mm high with 6 mm mesh bases and plastic sides. Cages were semi-randomized in the recirculating holding tank in block design.

Abalone were dried in their cages outside on a cool sunny day April 5 2000. The temperature varied between 15 and 20 °C and humidity between 40 and 73% (and was less than 65% for all except the initial reading). Following air exposure abalone in cages were returned to water and fed to slight excess for 54 days. Water temperature was measure daily and ammonia and nitrite were measured 2-3 times per week using appropriate aquarium test kits (Australian Pet Supplies, Smithfield NSW). Growth comparisons between treatments were made by one way ANOVA on the calculation of SGR length and weight (section 4.8) for individual animals. A further treatment with an exposure time of 15 h was run concurrently but with an abbreviated recovery period. Two replicates of 15 abalone were exposed over night using reverse cycle air conditioning. Temperature range was 17-21 °C and humidity was 65%. Abalone were then returned to water and housed as described above for 2 weeks.

4.13.4 Drying and long term growth

Two hundred blacklip abalone, mean length 39.3 mm (SD = 4.7 mm) and weight 8.9 g (SD = 3.3 g) were obtained from an east coast land based farm (referred to hence as farm 2). All the animals were tagged and 100 (in 2 replicates of 50) assigned to each of an untreated control and drying treatment group. Air dried abalone were placed in shade on a sunny day for 4 h; the temperature range was 16-20.5°C

and the humidity 57-65% except for a short interval at 69%. Replicates were dried on separate sheets of plastic in the same general location and assigned to separate "hides" in a commercial scale grow-out tank. Control animals were returned to water immediately after tagging with each replicate placed in a separate hide. As many tagged experimental abalone as possible were recaptured and re-measured 168 d following treatment.

A similar experiment was conducted 6 October 1999 at Tasmanian Tiger Abalone, Dunalley (section 4.2) using greenlip × blacklip hybrids. A total of 200 abalone, mean length 30.4 mm (SD = 5.6 mm) and mean weight 3.8 g (SD = 1.7 g), were tagged for treatment with replication as above. Air exposure time was 3 h with a temperature range of 15-17°C and a humidity range of 60-84%. Abalone were placed in direct sunlight for up to 10 min at a time to ensure thorough drying. Treatment and control groups were returned to a commercial scale grow-out tank with 50 treated and 50 untreated animals assigned to each of 2 replicate hides. After a 214 d growth period experimental abalone were recaptured and re-measured.

Experiment 3 commenced at Huon Aquaculture 15 February 2000. The abalone used had been present on the farm for 6 months and were 2 years old. Mean length and weight (± SD, n=146) were 37.4 ± 4.1 mm and 7.3 ± 2.2 g respectively with approximately equal numbers in a treated group and a control. Air exposure was 2.5 h with a temperature and humidity range of 20-21 °C and 56-64% respectively. The on growing period was 210 d during which abalone were grown in Aquatech© Trays (section 4.3) with a similar number of treated and control animals in each of 2 trays. The null hypothesis in long term growth trials was that there would be no difference in growth between air dried and control stocks. Statistical analysis for the three growth comparison trials consisted of Specific Growth Rate comparisons for weight and length between treatments.

4.14 Epidemiology/Risk factor methods

4.14.1 Experimental animals and location

Abalone used in the experiments were *H. rubra* free of spionid infestation and sourced from a land based farm outside the study area in southern Tasmania. Experimental work was conducted at Huon Aquaculture and Aquatas in southern Tasmania using the husbandry methods described previously (section 4.3).

4.14.2 Influence of size experiment

Three abalone size cohorts of approximately 6 months, 18 months and 3 years of age were selected. Samples of stock were 15.0 ± 0.4 mm (n=23), 34.3 ± 0.5 mm (n=100) and 50.9 ± 0.7 mm (n=20), ($\overline{X}$ ± SE). These size groups hence forth referred to as "small", "medium" and 'large" were considered to represent the extremes and middle of the range at which sea based grow out farms are likely to purchase hatchery stock.

Fifty stock of each size class was placed in 4 culture vessels. Two such cages were hung within 2 m of each other at each of the two study sites. Experiments began in the Austral winter and were concluded in the late summer exposing abalone to presumptive spring/summer spionid settlement periods.

Mud worm infestation levels were quantified by exposure to vermifuge solution (section 4.17.2). Preliminary investigation showed low rates of mud worm settlement

so abalone were shucked and shells exposed to vermifuge solution in replicates of 5. Counts of characteristic *B. knoxi* chimneys, expelled *B. knoxi* worms and *P. hoplura* worms were performed. Shell damage was assessed by the % blister and SSDR methods as described above. Basket replicates for each study site were assessed for each size class and measured variable by Mann-Whitney U Test. Where replicate variables were not significantly different (P>0.05) they were combined for further assessment. Size class data were compared by Kruskal-Wallis test for each of: *B. knoxi* chimneys, *B. knoxi* worm counts, *P. hoplura* worm counts, SSDR, and % blister coverage. Mean separations for significant Kruskal-Wallis tests were performed by the method of Zar (1984).

4.14.3 Fouling organism survey method

A survey of *B. knoxi* chimney location was conducted on 80 abalone 20- 40 mm in length and infested for less than 6 months. Shells were examined with low power stereo microscopy. Preliminary examination suggested the use of common chimney location categories. All chimneys were assigned to such a category, these being: on spirobids, on other shell fouling organism, within the apical groove of the shell and elsewhere on the shell.

Nine abalone fouled with the red bryozoan were collected during October 1998 from Huon Aquaculture. These animals were remnant stock from a cohort that had suffered considerable mud worm mortality in 1996 and 1997. The abalone had a mean length of 56 mm (SE=3 mm) and were at least 4 years old at the time of collection. *B. knoxi* chimney counts were compared to those of 10 animals from the same intake time and culture vessel with out the bryozoan (length 64 ± 2 mm, $\overline{X}$ ± SE). Chimney counts of bryozoan and non-bryozoan fouled stock were compared using Mann-Whitney U Test.

4.14.4 Method for assessment of spirorbid fouling effect

Four hundred abalone, a sample of which measured 34.3 ± 0.7 mm ($\overline{X}$ ± SE, n=200) were selected on the basis they had little obvious spirorbid fouling. Subsequent microscopic examination showed mean spirorbid infestation per abalone in these "clean" stock was 7.8 ± 0.7, n=100). Relatively heavily spirorbid infested stock from the same size, age and tank cohort had a mean count of 62.0 ± 4.0 (n=32).

One hundred "clean" stock were placed in each of 4 rearing vessels, two at each study site. Additionally, 23 and 11 spirorbid infested stock were assigned to each basket at Huon Aquaculture and Aquatas respectively. The exposure period was the same as that for the size effect experiment described above. Some nominally clean stock were removed before completion to provide data for other studies on timing of mud worm settlement. Counts were performed as described above in replicates of 5 shells for expelled *B. knoxi* and *P. hoplura* worms and *B. knoxi* chimney counts were performed on individual shells. Shells were assessed for mud worm shell damage by Subjective Shell Damage Rating (SSDR) described above (section 4.9). Basket replicates in the spirorbid fouling experiment were compared using the Kolmogorov-Smirnov Two-sample test for data collected in replicates of 5. The Mann-Whitney U Test was used for data collected from individual shells. Where replicates were not significantly different (P>0.05) they were combined and further tests as appropriate conducted between nominally clean and spirorbid fouled stock for each of: *B. knoxi* chimneys counts, *B. knoxi* and *P. hoplura* worm counts and SSDR. Where basket

replicates could not be combined the 2 statistical tests described were performed separately as appropriate between “clean” and fouled stock. Tables in the results section indicate whether basket replicates have been combined or not.

4.14.5 Comparison with long term spionid affected stock

Further stocks transferred to Huon aquaculture in August and September 1998 and reared for in excess of 2 years were available for comparative purposes. Samples of animals transferred in August had mean length 46.9 mm (SE=0.3, n=100) and in September 18.8 mm (SE=0.1, n=150). The former stock had some spirobid fouling (generally < 20 per abalone) whereas the latter animals were essentially spirorbid free.

4.14.6 Rearing vessel comparison

This comparison used similar abalone stocks being held in different rearing vessels in the winter/spring of 1999 at the 2 study sites. Experimental animals had been placed on the study sites August 1998, promptly becoming infected. By the winter/spring of 1999 abalone were between 50 and 65 mm in length and approximately 4 years old.

Abalone rearing vessels were of two designs. "Tube type" vessels were in widespread usage when stock mortality was originally recorded in 1995-1996. These were 1500 mm lengths of 250 mm diameter PVC pipe fitted with 6 mm mesh ends. “Basket type” culture vessels were modified polyethylene laundry baskets fitted with 6 mm oyster mesh over existing mesh sides and plastic inserts to increase mesh free substrate area. The "baskets" were approximately cylindrical, 800 mm high with a diameter of 400 mm and had considerably more meshed area than "tubes".

Spionid counts were performed before and after the known settlement period for the pest species *B. knoxi*. At Huon Aquaculture a pair of “basket” reared stock were compared to a pair of “tube” reared stock. Additionally, another pair of basket reared stock with known lesser levels of spionid infestation were available

At Aquatas one pair of “basket type” and a single “tube type” rearing vessel stocked with similarly infested abalone were available for comparison purposes. Polychaete counts before and after the *B. knoxi* settlement season and between rearing container types were compared using the Mann-Whitney U Test.

4.14.7 Position in water column

One hundred abalone were placed in duplicate at each of 3, 6 and 9 m from the surface in 10 m of water at Huon Aquaculture. The 40-45 mm animals were transferred in August 2000 and exposed to spionid settlement until January 2001. Following exposure to chemical vermifuges spionids and *B. knoxi* chimneys were recorded for replicates of 10 abalone. A SSDR was assigned to each shell as an indicator of spionid impact.

4.14.8 Species comparison

One hundred *H. laevigata* 26.7 ± 3.2 mm and 100 *H. rubra* 27.3 ± 2.8 mm ($\overline{X}$ ± SD, n=100) were placed in each of two "basket type" rearing vessels at Huon Aquaculture. Stock were transferred in August 2000 and assessed January 2001, allowing exposure to the presumptive 2000 *B. knoxi* settlement period. Abalone were

shucked and exposed to chemical vermifuge solutions in replicates of ten to expel mud worms. Spionid counts between abalone species were compared by Mann-Whitney U-test.

4.15 Abalone health I: mortality, growth and condition

4.15.1 Blister morphology & location

The size and severity of mud worm blisters was assessed by the blister tracing and subjective shell damage rating methods as described previously. The location of blisters was quantified by assigning blisters to a type locality map as depicted in Figure 4.7. Six hundred blistered shells were assessed and separate location records kept for those shells infected by a single species of spionid.

The volumes of a sample of the largest mud worm blisters encountered were measured by allowing shells to dry, puncturing the blister and then submerging shells in water. The water was then removed from the blister with a 21 gauge needle and 1-5 ml syringe as appropriate and the volume recorded. For comparison purposes a sample of the largest blisters formed in Pacific oysters by *B. knoxi* at Huon Aquaculture were assessed. Histological examination of blisters was performed on seawater formalin fixed, decalcified shells.

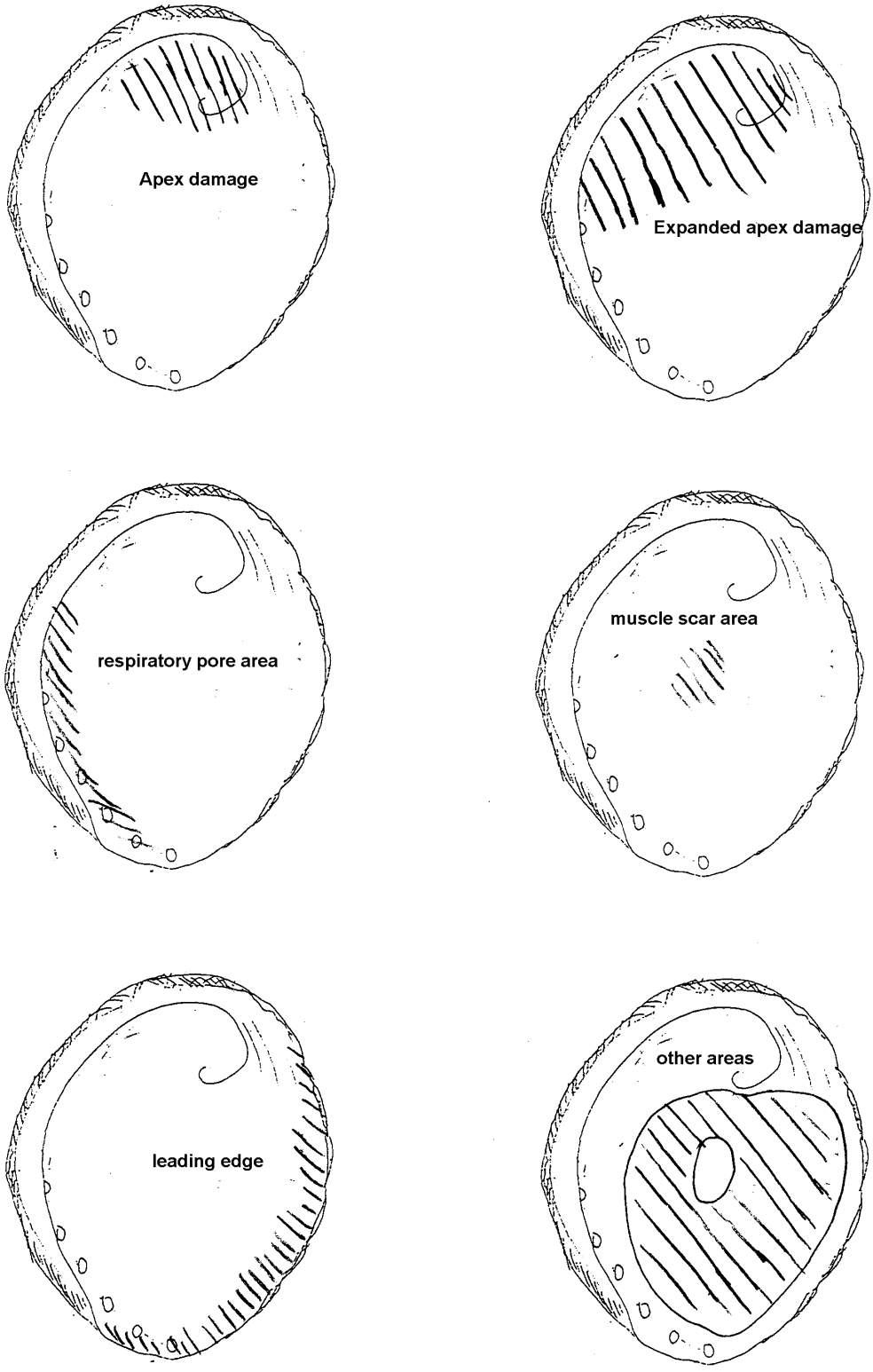


Figure 4.7 Common mud worm blister locations in abalone

4.15.2 Measures of abalone condition

The condition, or relative "fleshiness" of abalone was assessed by calculating the percentage flesh weight. This is simply the shucked flesh weight divided by the whole live weight and multiplied by 100. An advantageous of this method was that after measurement the flesh could be fixed in formalin for histology or frozen for later chemical testing. Dry weight indices were calculated by separating meat and shells and drying in an incubator at 70° C for 48 h until constant weight was achieved.

The dry weight index CI_{LENGTH} was calculated as:

$$CI_{LENGTH} = \frac{\text{Dry flesh weight (g)} \times 100}{\text{Shell length (mm)}}$$

The dry weight index CI_{WEIGHT} as recommended by Lucas and Beninger (1985) was calculated as:

$$CI_{WEIGHT} = \frac{\text{Dry flesh weight (g)} \times 10}{\text{Dry shell weight (g)}}$$

The dry to wet flesh weight ratio as also recommended by Lucas and Beninger (1985) for use in aquaculture studies was also calculated. The various indices were compared using non infested abalone to find the most suitable measure for long-term health assessment.

4.15.3 Experimental design: growth, mortality, long term treatment efficacy

Placement of non mud worm infected stock at two sea lease sites with a history of severe mud worm infestation was intended to recreate the conditions which led to stock mortality in the mid to late 1990's. To this end the same hatchery and feed sources were used as were the initial containment vessels. Regular sampling to determine spionid infestation levels was performed and the consequences of this in terms of stock mortality and health indicators such as growth, condition and shell blistering was quantified.

By allowing a stock cohort to become mud worm infested and then treating a portion of that stock to eliminate or reduce spionid infestation, the affects of such infestation could be compared for otherwise matched groups. The null hypothesis was that differential spionid infestation levels would have no affect on abalone health measures including mortality rate, growth, shell blistering and condition indices. This experiment was conducted using August 1998 intake abalone at Huon Aquaculture and stock assigned to this experiment are referred to as "cohort 1" in the results and discussion sections.

To control mud worm infestation levels in one group of stock relative to the other air drying of half the animals was conducted in December 1998 and 1999. Details of the drying conditions and efficacy are given in section 4.13.2 (Trials 6 and 10). December was chosen as the drying period as initial data suggested that larval settlement of *B. knoxi*, the major focus of the research, had concluded by then (section 5.1). For the experiment comparing health of matched infested and non-infested stock to succeed, long-term suppression of spionid infestation in one of the groups would be necessary. This was an experiment in itself, with the null hypothesis that annual post *B. knoxi* settlement season air drying treatment would severely reduce spionid infestation by this species.

The recommendation from section 5.1 that *B. knoxi* infestation could be avoided by placement of stock post November was tested by comparison of stocks placed in September 1999 and December 1999 at both southern study sites. The null hypothesis was that there would be no difference in *B. knoxi* counts and shell blistering between the two intake time cohorts.

Long-term treatment trial data (Huon Aquaculture August 1998 cohort 1) were analysed using ANOVA or REML in situations where sample sizes varied giving an unbalanced non-orthogonal design. Thus spionid count data was analysed using two-way ANOVA, after log transformation of data and blister coverage data was analysed using REML. Percentage flesh weight data was arcsine transformed using then analysed using 2 way ANOVA. The non-parametric Mann-Whitney U Test was used to analyse SSDR data. Linear Regression data were analysed using the simple linear regression option in Genstat. Residual plots were examined to check for major departures from the assumptions of normal distribution and uniform variance.

4.16 Abalone health II: physiology and histology

4.16.1 Clinical Pathology

Haemolymph samples were drawn from a foot incision as described for haemocyte counts or from the cephalic sinus (Jorgensen et al. 1984). This site is located 2-3 mm deep on the midline between the mouth and the anterior portion of the foot. Samples were taken by use of a 1 ml syringe and 27 gauge needle. At least 0.3 ml was drawn from the appropriate sample site and centrifuged at 3000 rpm for 3 minutes to remove haemocytes. Samples not analysed immediately were frozen at -20°C for subsequent analysis.

Measurement of protein, glucose and all ions except copper was performed on a Roche Cobas-MIRA automatic analyser. Samples were diluted 1:4 for sodium, potassium, chloride and calcium and 1:40 for magnesium. Sodium and potassium were measured using ion-selective electrodes. Chloride was assayed spectrophotometrically using the thiocyanate method (Cobas-MIRA, 1987). Calcium and magnesium ion concentration was determined spectrophotometrically using the arsenazo method (Cobas-MIRA, 1987). Glucose and protein were undiluted and determined spectrophotometrically using the hexokinase and biuret methods respectively (Cobas-MIRA, 1987). Copper concentration was determined by adding 0.1 ml sample to 1 ml of 20% trichloroacetic acid and reading at 324.8 nm on a spectrophotometer.

Background data on normal levels for non-spionid infested abalone was established by sampling groups of 5-10 presumptive healthy animals acquired in the period 1998-2000. Such animals were growing well at the time and had no obvious health problems. The abalone were obtained from farm 1 or Site 3 on the east coast. These were compared to mud worm infected stocks from Huon Aquaculture, Aquatas and Tasmanian Tiger Abalone. Samples assigned to the mud worm affected group were collected 1998-2000 and comprised moderately to severely infested animals.

The August 1998 intakes of abalone to Huon Aquaculture and Aquatas were sampled at regular intervals until mid- late 2000. These groups were subsequently shown to be the most severely mud worm infested (section 5.5) and thus of most interest for clinical pathology investigations.

Statistical methods for comparison of data varied with the size and nature of data sets. The non-parametric Mann-Whitney U Test was used to compare haemolymph ions, copper, protein and glucose drawn from cephalic sinus haemolymph between

presumptive normal and mud worm infested stock. As a larger data set was available for haemolymph drawn from the foot, distributions approached the normal distribution and the more powerful t-test was used. Similarly, the distribution free Kruskal-Wallis test was used to assess Na^+/K^+ ratio data from the relatively small data set of starved animals (section 5.6.1) whereas one way ANOVA was used to assess the larger temporal variation data sets from the two study sites.

4.16.2 Haemocyte counts

Haemocyte counts were carried out to help define the physiology with regard to stock stunting. In order to interpret these findings, additional tests on the effect of temperature and copper exposure on haemocyte counts were carried out as an indication of haemocyte sensitivity and the practicality of haemocyte counts as a general health indicator in abalone.

Total haemocyte counts were performed with a haemocytometer (Assistent, Germany) on samples taken from an incision in the foot, as many animals were too small for reliable collection from haemolymph sinuses. A small cube of flesh was removed with a scalpel blade, severing haemocyte channels, and approximately 0.2 ml of haemolymph/tissue fluid transferred from the wound to the haemocytometer with a syringe. Counts were performed immediately at ×200 magnification by light microscopy to avoid aggregation of haemocytes. Five of 25 small squares were counted on each side of the haemocytometer (total volume 0.2 mm^2 area x 0.1 mm depth) and a mean calculated for each sample (Count data were converted to $cells.ml^{-1}$ by multiplying by 50 000).

A normal range was established by collecting data from presumptive healthy, mud worm free abalone in the range 30-65 mm. Samples generally consisted of at least 3 abalone acquired from land-based farm 1, sea-based Site 3 (East Coast) or from Huon Aquaculture within 3 months of transfer and in the absence of shell blistering. Simple linear regression analysis of size (length) versus count data was performed using Genstat.

From 1999 regular samples were taken from the August 1998 intakes at Huon Aquaculture and Aquatas as these were the most heavily mud worm infested groups. Overall mean counts from the 2 sites were compared using Mann-Whitney U-test.

Abalone from 3 age classes were collected from farm 1, September 9 2000 to examine variations in haemocyte counts between stunted and fast growing stock of the same age and from the same tank. Three year old animals were assigned to 1 of 2 size classes. These being “runts” (mean shell length 36.9 mm, SD=2.5, n=10) and larger animals (mean shell length 53.3 mm, SD=1.9, n=10). Ten, 2 year old animals were assigned to each of three relative size classes these being “runts”, medium and large stock. Mean length of 2 year old abalone size classes were 27.4 mm (SD=2.6), 44.0 mm (SD=2.7) and 54.6 mm (SD=2.9). A sample of larger abalone only, less than 6 months old, were also collected: mean length (26.1 mm, SD=1.7, n=10).

Further data from one and two year old stock was collected from farm 1 February 9 2001. Stocks were selected to fit 1of 3 relative size classes these being “runts”, medium and large stock. Ten animals were bled for each age and size class. Mean length of 2 year old abalone size classes were 34.9 mm (SD=4.0), 45.1 mm (SD=2.9) and 58.2 mm (SD=3.9) and of one year abalone: 15.5 mm (SD=1.6), 24.6 mm (SD=1.4) and 30.0 mm (SD=1.6). Data were tested using ANOVA for differences due to size and age class. .

For experiments using temperature stress to assess the suitability of haemocyte counts for evaluation of short term environmental stress, thirty abalone of mean length

60.4 mm (SD=4.0) were obtained from farm 1. These animals had been exposed, on site, to relatively warm water (22-23 °C) for 4-8 weeks and were removed to water at 16 ± 0.5°C on February 9 2001 (recirculation system- Fish Health Laboratory). Haemocyte counts were made 4, 7 and 14 days later and were compared using ANOVA.

A further investigation commenced March 21 2001 where abalone were removed from tanks at 14-15°C to tanks at either 16.5 ± 0.5°C or 21 ± 0.5°C for a period of 6 d.. This experiment was conducted in 40 l aquaria with complete water changes daily. Mean length of abalone was 53.9 mm (SD=4.4, n=50) and the animals were not fed during the trial. Twenty abalone were assigned to each temperature treatment in 2 replicates of 10 animals. Haemocyte counts at higher and lower temperature were compared using Mann-Whitney U Test..

For examination of the short-term effects of the known toxicant copper (Mance 1987) on abalone haemocyte counts, abalone, mean length 51.3 mm (SD = 4.1, n=18) were assigned to either an untreated control group or 0.2 $mg.l^{-1}$ copper sulphate exposure for 9 d at 15.5 ± 0.5 °C. Animals were housed in 40 l aquaria without feeding. Haemocyte counts of copper exposed and control groups were compared using Mann-Whitney U test.

4.16.3 Histology

Abalone tissues were fixed in 10% seawater formalin. Routine processing was performed using a Shandon Hypercenter XP for dehydration through ethanol to xylene then paraffin embedding on a Tissue tek work centre and sectioning on a Microm HM 340 microtone at 4 µm. Routine Harris's Haemotoxylin and Eosin (H & E) staining was performed using a Leica Jung Autostainer XL for automatic staining. Sections were mounted in DPX and examined and photographed through a light microscope**.**

Tissues routinely examined included right kidney, digestive gland, intestine, gills and foot. Some comparisons were made with Martius Scarlet Blue (MSB) (Ellis, 1992) stained sections to examine foot muscle depletion in spionid infested abalone. Sections of severely mud worm infested abalone were stained using the Periodic Acid Schiff reaction (PAS) (Ellis, 1992) to check for fungi and tissue glycogen levels. Unaffected control animal material was also PAS stained for comparison.

Remnant severely mud worm infested abalone stocked at southern sea farms in the mid 1990's were sampled for histological examination in the period 1998-99. These changes were compared to changes that occurred when mud worm free stock cohorts were transferred to the same areas in August 1998 and September-November 1998. A subjective score was developed to assess the extent of brown pigment granules some times present in the right kidney and digestive tubules of abalone sampled. A zero score indicated no pigment and a score of 3 indicated maximum pigment levels seen in worst affected animals.

4.16.4 Blister environment and microflora

The pH of a sample of 10 large blisters was measured by drawing at least 0.2 ml of blister fluid with a syringe and needle and wetting the tip of pH test strip (range 4.5 -10.0, Sigma). Blisters tested came from remnant stock surviving the initial reported outbreaks in the mid 1990's until sampling in 1998-99.

Similarly, samples of blister fluid from some of the most mud worm damaged shells available were cultured for bacteria on TCBS, Johnson's Marine Agar and Blood Agar plates and incubated at 20 °C (AHL Fish Health Methods Manual, 2001). Abalone shells and adjacent tissue were processed for histology and stained using Periodic Acid Schiff reaction (PAS) (Ellis, 1992) to test for the presence of fungi. Shells were decalcified using RDO rapid bone decalcifier (Phoenix Scientific, Vic., Australia).

4.16.5 Tissue chemistry

Foot tissue was frozen and stored at –20°C and soluble protein content measured using a Sigma Diagnostics protein assay kit (Procedure No. P5656) based on the Lowry method. Sample preparation involved placement of 0.05-0.15 g frozen tissue in cold SEI buffer (250 mM sucrose, 5 mM EDTA in 0.1 M imidazole buffer). Tissue was then homogenized with a teflon rod while kept on ice. Homogenate was centrifuged for 7 min at 3500 rpm and the supernatant discarded. The remaining pellets were resuspended in 1 ml SEID solution (consisting of SEI plus 0.1 g sodium deoxycholate per 100 ml SEI) re-homogenized and left on ice for 15 min. The homogenate was then centrifuged for 6 minutes at 5000 rpm and a 0.2 ml sample of supernatant diluted to 1 ml as per protein test kit method. Absorbency was measured at 700 nm with a light spectrophotometer and the protein concentration determined from a standard curve. Data were calculated as mg protein per gram tissue as expressed at % protein wet weight foot tissue.

Statistical analysis of tissue protein data were performed using the nonparametric Kruskal-Wallis test in conjunction with non-parametric Tukey-type multiple comparisons to separate means (Zar, 1984).

4.16.6 Respirometry

Respirometry trials to assess the effect of mud worm infestation on metabolic rate, were performed at University of Tasmania, Launceston in March 1999 and May 2000. In the first trial chronically mud worm infested stock (SSDR 1-3) were sourced from Huon Aquaculture and compared to mud worm free stock of similar age from farm 1. Mean shell lengths were 57.8 mm (SD = 2.7, n=8) and 58.1 mm (SD=7.4, n=4) respectively. Abalone selected for the second experiment were part of the long term treatment trial at Huon Aquaculture. Five mud worm infested animals with spionid counts of 62.8 ± 24.4 and blister coverage of 18.2% ± 9.8% ($\overline{X}$ ± SD) were compared to 5 lesser infested stock (previously air dried December 1998 and 1999) with spionid counts of 18.4 ± 5.8 and blister coverage 12.9% ± 9.0 ($\overline{X}$ ± SD). Mean lengths of the untreated and less infested groups were 61.9 ± 3.6 mm and 64.9 ± 5.1 mm respectively.

Abalone were held for 1-2 weeks without food, after selection and transport and were transferred to respirometry chambers attached to petri dish substrates to minimize handling. One experimental animal was assigned to each plastic 1.5 l respirometry chamber for data runs consisted of 5 abalone plus 1 blank. One ml water samples were taken at regular intervals over a period of 2-4 h or until the water oxygen tension dropped to approximately 100 mmHg partial pressure of oxygen. Oxygen consumption was measured using an E101 oxygen electrode in conjunction with a Radiometer BMS Mk 2 blood gas analyzer maintained within 0.5 °C of ambient respirometry chamber water, and connected to a PHM 71 Acid-Base

Analyzer (Radiometer, Copenhagen). The oxygen meter was calibrated using 2% sodium sulphite (zero PO_2) and air saturated seawater set to a PO_2 of 159 mmHg. Sample readings were taken after 3 min equilibration.

Trial 1 was conducted at 20 °C and trial 2 at 16 °C. Oxygen consumption was measured as reduction in mmHg PO_2, converted to µmol O_2 (Cameron 1986) and expressed as µmol $O_2.g^{-1}$soft body weight.h^{-1}. Statistical comparison was made using Mann-Whitney U Test as data sets were small and underlying distributions unknown.

4.16.7 Ammonia excretion

Ammonia excretion rates were compared using the long term spionid treatment cohort (August 1998 intake 1- section 4.15.3) at Huon Aquaculture, sampled in October 2000. Ten heavily mud worm infested animals were compared to 10 less infested animals air dried 10 months previously.

Mean length and weight of abalone were 63.6 ± 4.4 mm and 38.3 ± 7.1 g respectively. Mean blister coverage was 28.6% (SD= 8.8) for highly infested stock compared to 10.9% (SD=7.9) for the less infested group. These differences, which could not be assessed until the completion of ammonia excretion measurements were significant by Mann-Whitney U Test ($U=9.0$, $P<0.01$). Abalone were placed in 2 l aquaria, with aeration at 13 °C for 6 h and 50 ml samples taken and frozen at –20 °C. Ammonia was measured using the salicylate-hypochlorite assay (Verdouw et al., 1978) against a range of standards read at 650 nm on a light spectrophotometer. Ammonia excretion was expressed as µmole NH_4-$N.g^{-1}.h^{-1}$ and statistical comparison made by Mann-Whitney U Test.

4.16.8 Starvation comparison

One cohort of abalone with severe mud worm infestation began to die after approximately 18 months exposure (section 5.5.1). As underfeeding was also considered a factor in this outcome a 3 month starvation trial was conducted in an attempt to differentiate between the effects of mud worm infestation and starvation.

A pool of 40 abalone, mean length 44.6 ± 4.2 mm, mean weight 12.7 ± 4.2 g was obtained from farm 1 in early February 2001. Abalone were tagged, their length and weight recorded and housed in plastic aquaria within the Fish Health Unit recirculating system. At the beginning of the time sequence and at 4 week intervals thereafter for 12 weeks 5 animals were sampled and haemocyte counts and Na^+/K^+ ratio determined. Material was also fixed for histology.

5. RESULTS & DISCUSSION

5.1 Reproductive biology and settlement of spionids

Descriptive studies

Five species of spionids were found in experimentally exposed abalone, these being *B. knoxi*, *B. chilensis*, *B. proboscidea, Polydora hoplura* and *P. armata*. Of these *P. hoplura* and *B. knoxi* were overwhelmingly the most common and were therefore described in detail.

Most mature specimens of *B. knoxi* were 10-20 mm in length with the occasional specimen up to 35 mm recorded. The mean of a sample of fixed specimens was 16.3 mm (SD=16.1 mm, n=15) for a mean setiger count of 98.8 (SD=95.5, n=13). Specimens of *P. hoplura* were generally 20-30 mm in length with larger specimens of 50 mm (>200 setigers) recorded. Specimens confirmed to line drawings and technical descriptions of these species by Blake and Kudenov (1978), Rainer (1973) and Read (1975).

B. knoxi egg strings had a mean of 21.8 capsules per string (SD=8.6, n=9) and capsules contained a mean of 32.8 larvae (SD=8.7,n=46) for a mean brood yield of 715 larvae. Egg strings often exhibited a greenish tinge and all eggs developed into larvae, there was no evidence of nurse eggs. Asetigerous embryos were approximately 240 µm long, growing to approximately 350 µm at 5 setigers when they were capable of surviving outside the capsule. Larvae of this size were strongly phototaxic and their translucent bodies had a green tinge. Larvae released from egg capsules at a mean size of 369 µm and cultured reached 670 µm 5 d later and 900-1200 µm for 16-17 setigers about 2 weeks after release. By this time larger larvae were commencing metamorphosis with the beginning of crawling behaviour, and showed the presence of fifth setiger spines and palps. A small number of larvae were maintained for a further week after this but failed to grow any larger.

Histology of adult *B. knoxi* showed that parapodial cavities were densely packed with oocytes of 90-110 µm diameter in May. Sections of worms sampled June to August showed essentially empty body cavities – corresponding with extrusion of eggs from the body to the shell burrow. Immature oocytes could be seen developing in the ovary by November/December and by January to March had been released once more into the parapodial cavities to mature.

Egg strings of *P. hoplura* tended to be deep yellow in colour and were often 20-30 mm in length for > 30 capsules. Nurse eggs were always observed and capsules generally contained 40-50 such eggs and 4-6 larvae of about 500 µm early in the development sequence. Larvae as large as 1900 µm were observed within capsules near the exhaustion of nurse egg supplies. Small worms 2000-2500 µm were sometimes observed excavating burrows adjoining the maternal burrow having never apparently left the host shell. No direct evidence of planktotrophic larval development of *P. hoplura* was observed in infested abalone.

B. proboscidea was seen sufficiently frequently to allow some assessment of its reproductive cycle. Egg strings, with distinctive oval capsules characteristic of the species (Woodwick 1977, Blake and Kudenov 1981) were seen once in each of December 1998 and September 1999. Lecithotrophic larvae 1.4 mm long were observed within capsules October 1999. Adults with well developed eggs within their bodies were seen between September 1998 and January 1999.

Timing of settlement

Settlement of *B. knoxi* larvae was confined to the months of the Austral spring in the period 1998-2000 at both study sites (Tables 5.1 and 5.2). Mature body eggs were observed between April and August and extruded eggs in June and September. Viable unhatched larvae were present in most August samples at both study sites with the earliest observation June 1999 and the latest January 2001 (Tables 5.1 and 5.2).

Post-larvae of *P. hoplura* and adults with mature body eggs were observed in every calendar month at one time or another during the study period (Tables 5.1 and 5.2). The majority of post larval worms were considered to be the lecithotrophic offspring of worms already present in blisters. At times post larval *P. hoplura* were found inhabiting otherwise spionid free shells and it was considered possible such specimens had undergone planktonic development. Most of such specimens were found in the spring and summer (Tables 5.1 and 5.2).

Settlement intensity was low compared to data for infestations during 1995-1997. Abalone transferred to study site 1 in September 1998 had acquired a mean spionid infestation of 1.2 (SE=0.2, n=64) worms per shell by January 2001.

Discussion

The findings above relate to the specific project objective No. 2 on investigation of spionid reproductive biology. Field studies between 1998 and early 2001 and the laboratory rearing trial ensured this objective was achieved. Knowledge of spionid settlement patterns contributed to mud worm control strategies as given in objectives 1 and 6 (page 9) and discussed below.

Of the 5 mud worm species found infesting stock, only *B. knoxi* and *P. hoplura* were present in sufficient numbers to be considered pest species. Of the 3 minor species *P. armata* has been previously recorded in *H. rubra* from Victoria and in *H. roei* from South Australia (Blake and Kudenov 1978). *B. chilensis* is quite common in Tasmanian oysters, but neither *B. proboscidea* nor *P. armata* were previously known from this source (Wilson et al. 1993). *P. hoplura* had previously been seen in Tasmanian abalone and oysters (Wilson et al. 1993) and the species has often been associated with damage to commercially important bivalve species world-wide (Blake and Evans, 1972).

The *B. knoxi* species appears to be somewhat larger in Tasmanian abalone than described in New Zealand (Rainer 1973, Read 1975). There was no evidence of brooded *B. knoxi* production as reported by Read (1975). Rather, the lack of nurse eggs, release of larvae from capsules at the 4-5 setiger stage and relatively large clutch size indicate planktonic larval production as described by Handley (1997, 2000). The estimated clutch size of > 700 larvae for Tasmanian *B. knoxi* is similar to that of other spionids with planktotrophic development including *P. websteri* (500-550, Blake 1969), *B. chilensis* (460±160, Skeel 1979), *P. ciliata* (225-400, Wilson 1928; 200-1100 Dorsett 1961) and *P. haswelli* (1200-2400, Skeel 1979).

By contrast, *P. hoplura*, although physically larger than *B. knoxi*, was less fecund consistent with the observed lecithotrophic development. Maximum fecundity would be less than 150-180 larvae, consistent with estimates for the species by Skeel (1979) Radashevsky (1994), and Gromadzki (1994). There was no direct evidence of planktonic larval production within *P. hoplura* blisters in cultured abalone. However, the presence of *P. hoplura* larvae in otherwise spionid free stock at some distance

from the nearest known source of infection may suggest a swimming ability not normally associated with large lecithotrophic larvae.

It would not be without precedent if *P. hoplura* were capable of producing two types of larvae to maximize its reproductive potential. Wilson (1928) noted that if larval *P. hoplura* within capsules containing brood eggs were removed and fed on micro-algae they developed normally and were indistinguishable from larvae allowed to complete lecithotrophic development. As noted previously *B. knoxi* apparently produces both brooded and planktonic larvae in New Zealand.

The ability of *P. hoplura* to produce lecithotrophic larvae at most times of the year once established in the abalone may render control difficult. Fortunately the presence of *P. hoplura* post larvae on clean stock was mainly confined to spring and summer (Tables 5.1 and 5.2). Likewise, *B. knoxi* settlement data, and other observations of the reproductive cycle (including histology) strongly suggest that if stock are placed post November they will remain substantially spionid free until the following spring. Current sea based abalone grow-out operations generally place seed stock at 20-30 mm and on grow to at least 60 mm. Placement of seed stock in susceptible areas December to February should allow approximately a years growth before the impact of any spionid settlement is felt. This may be sufficient to allow grow out to marketable size. Where on growing to larger sizes is required treatment options (sections 5.2 and 5.3) might be required during the second year. Infestation rates for *B. knoxi* were low compared to available previous data suggesting recruitment rates may vary considerably year to year.

Table 5.1.Presence-Absence of *B. knoxi* and *P. hoplura* life history stages at Aquatas

	Sep 98	Oct 98	Nov 98	Dec 98	Jan 99	Feb 99	Mar 99	Apr 99	May 99	Jun 99	Jul 99	Aug 99
Mature body eggs										+•	•	•
Extruded egg strings										+•		•
Unhatched larvae												+
Settled post-larvae	+•	+•	+•		••#		••#				•	•

+ = *B. knoxi*; • = *P. hoplura*; •# = suspected planktonic *P. hoplura* post larvae

	Sep 99	Oct 99	Nov 99	Dec 99	Jan 00	Feb 00	Mar 00	Apr 00	May 00	Jun 00	Jul 00	Aug 00
Mature body eggs	•		•		•		•	•		•		
Extruded egg strings	•		•				•		•	•		
Unhatched larvae	•	+	•									
Settled post-larvae	+ •	+ •	+••#		•		••#	•		•		

	Sep 00	Oct 00	Nov 00	Dec 00
Mature body eggs	•			
Extruded egg strings	•			
Unhatched larvae				
Settled post-larvae	+••#	+ •	+ •	••#

Table 5.2 Presence-Absence of *B. knoxi* and *P. hoplura* life history stages at Huon Aquaculture

	Sep 98	Oct 98	Nov 98	Dec 98	Jan 99	Feb 99	Mar 99	Apr 99	May 99	Jun 99	Jul 99	Aug 99
Mature body eggs		•		•		•		+•	•		•	•
Extruded egg strings	+	•									•	•
Unhatched larvae										+		+•
Settled post-larvae		+••#	+•		••#	•	••#	•	•	+••#		•

+ = *B. knoxi*; • = *P. hoplura*; •# = suspected planktonic *P. hoplura* post larvae

	Sep 99	Oct 99	Nov 99	Dec 99	Jan 00	Feb 00	Mar 00	Apr 00	May 00	Jun 00	Jul 00	Aug 00
Mature body eggs	•			•	•		•				•	+•
Extruded egg strings	•	•	•	•	•		•					•
Unhatched larvae	+•	+•		•	•		•					+
Settled post-larvae	+••#	+••#	+••#	••#	•	•	••#					•

	Sep 00	Oct 00	Nov 00	Dec 00	Jan 01
Mature body eggs			•		
Extruded egg strings			•		
Unhatched larvae			•		+**
Settled post-larvae		+•	+•		••#

+** = record consists of 2 worms

5.2 Chemical treatments

5.2.1 Screening trials

Major findings for the considerable body of screening trial data are described below, more detail is presented in Appendix 3, section 5, as indicated. Fresh water immersion of spionids *in vitro* proved 100% fatal after 10 min exposure. Exposure of spionids *in situ* proved ineffective for 1 h immersion, rising to 40% GMC (Group Mean Comparison) kill at 2 h, at the cost of 80% abalone mortality (Appendix 3,5A).

Potassium permanganate exposure appeared to have some potential against spionids *in vitro* at 15-20 $mg.l^{-1}$, and 100% mortality was seen 7 d post exposure at 50 $mg.l^{-1}$. Potential for abalone mortality was seen at 20 $mg.l^{-1}$ and above with 80% mortality seen 16 d post treatment at 20 $mg.l^{-1}$, 4 h immersion. Three-hour treatment of spionids *in situ* at 25 $mg.l^{-1}$ $KMnO_4$ resulted in 60% abalone mortality and 11% GMC spionid kill (Appendix 3, 5B).

Gentian violet exposure was found to be 100% lethal to mud worms *in vitro* at 10 $mg.l^{-1}$ 4 d post treatment. Some abalone mortality was seen at 10 and 20 $mg.l^{-1}$ but not at 5 $mg.l^{-1}$, 17 d post treatment. Four hour exposure of mudworms *in situ* to 7.5 $mg.l^{-1}$ gentian violet resulted in 23% GMC kill (Appendix 3, 5C).

Spionid mortality *in vitro* approached 100% 9-10 d post treatment with mebendazole and fenbendazole in the range 50-500 $mg.l^{-1}$. No abalone mortality was seen following 50 $mg.l^{-1}$ exposure to mebendazole for 9 h and 200 $mg.l^{-1}$ exposure for 3 h. Lethality to spionids *in situ* was estimated at 53% by GMC kill. Similarly, no fenbendazole toxicity to abalone was seen at 250 $mg.l^{-1}$, and GMC kill was 41% for spionids tested *in situ* empty shells (refer to Appendices 5D and 5E)

Levamisole immersion of spionids *in situ* resulted in GMC kills of 42 and 66% respectively at 64 and 640 $mg.l^{-1}$, resulting in abalone mortality of 50 and 100%. Follow up abalone toxicity trials using healthy 20 mm stock showed no mortality 8 d post exposure to 64 $mg.l^{-1}$ levamisole, rising to 33% at 320 $mg.l^{-1}$ and 100% mortality at 500 $mg.l^{-1}$. Larger healthy stock (40-50 mm) showed no mortality at 320 $mg.l^{-1}$ 18 d post exposure (Appendix 3, 5F).

Malachite green exposure to mud worms *in vitro* was 100% lethal at 5 and 10 $mg.l^{-1}$ but ineffective at 1 $mg.l^{-1}$. Concurrent exposure to spionids *in situ* (empty shells) gave GMC kill data of 38 and 60% at 5 and 10 $mg.l^{-1}$ respectively. Toxicity trials on healthy abalone (20 mm) showed 10% mortality at 5 $mg.l^{-1}$ and approached 100% at 10 $mg.l^{-1}$ (Appendix 3, 5G).

Spionids *in vitro* showed limited mortality 8 d post exposure to trichlorofon at 500 and 1000 $mg.l^{-1}$. Healthy 40-50 mm abalone were not killed by exposures of up to 500 $mg.l^{-1}$ 11 d post treatment (Appendix 3, 5H). Similarly, praziquantel at up to 100 $mg.l^{-1}$ produced limited mortality to spionids *in vitro* 20 d post treatment (Appendix 3, 5I). Hydrogen peroxide in the range 200-1000 PPM was lethal to 60% of spionids *in vitro*. No abalone mortality was seen at in healthy stock at 200 PPM (Appendix 3, 5J).

Formalin produced 30% mortality at 100 PPM for spionids *in vitro*, dropping to 22% (GMC kill) for spionids *in situ* empty shells. At 200 PPM *in vitro* spionid mortality exceeded 80%, dropping to 49% for concurrent estimate *in situ*. Follow up trials on healthy abalone showed no mortality a week post treatment at 100 PPM but 90% mortality at 200 PPM (Appendix 3, 5K).

Mud worm toxicity *in situ* was zero at 0.004 and 0.04 $mg.l^{-1}$ ivermectin rising to 57% (GMC kill) at 0.4 $mg.l^{-1}$ with mortality of 85% for infested abalone at the same concentration. Follow up work in the range 0.1-0.3 $mg.l^{-1}$ showed ivermectin was

ineffective against spionids *in vitro* and 100% lethal to healthy 20 mm abalone above 0.2 $mg.l^{-1}$ (Appendix 3, 5L).

A commercial product, Exelpet®, "All-Wormer for Dogs" containing febantel, pyrantel embonate and praziquantel (at 125, 72 and 25 $mg.l^{-1}$ respectively in solution) was ineffective against mud worm *in vitro* and non lethal to healthy abalone at twice the concentration (Appendix 3, 5M).

Metronidazole at 200 $mg.l^{-1}$ was non lethal to spionids *in vitro* and similarly dimetronidazole produced limited mortality at 500 $mg.l^{-1}$(Appendix 3, 5N).

Methylene blue was found non toxic to spionids *in situ* empty shells and healthy abalone at up to 10 $mg.l^{-1}$. Further trials on spionids *in vitro* showed no mortality at up to 200 $mg.l^{-1}$ (Appendix 3, 5O).

5.2.2 Follow up chemical treatment trial on lightly spionid infested abalone

Gentian violet at 5 $mg.l^{-1}$, mebendazole at 200 $mg.l^{-1}$ and potassium permanganate at 15 $mg.l^{-1}$ were chosen on the basis of screening trial data for follow up bath trials on spionids *in situ* lightly infested abalone. Spionid killing efficacy data were of marginal statistical significance (P=0.057, Pearson Chi-square value 7.5, 3 df). Mebendazole was the most effective of the bath treatments with a mean *B. knoxi* estimated individual percentage kill of 32.5% (Table 5.3). Gentian violet treatment resulted in the death of 1 in 20 experimental animals.

Table 5.3 Treatment of lightly infected abalone by one of three chemical baths (means ± SD).

	Control	Gentian violet 5 $mg.l^{-1}$	mebendazole 200 $mg.l^{-1}$	$KMnO_4$ 15 $mg.l^{-1}$
Mean *B. knoxi* chimneys	1.5 ± 0.9	1.5 ± 0.9	1.6 ± 0.8	1.6 ± 0.8
Mean surviving *B. knoxi*	1.4 ± 0.7	1.1 ± 0.6	1.1 ± 0.8	1.3 ± 0.6
Mean EI% *B. knoxi* Kill	3.8 ±11.9	18.8 ± 32.5	32.5 ± 43.0	13.5 ± 26.5
Abalone Mortality (%)	0	5	0	0

n=20 each treatment

Discussion

The testing of chemical treatments was commenced as part of the preceding CRC funded project and continued as part of objectives 3 and 4 in this FRDC project. As indicated by the data above (and Appendix 3, section 5), short term chemical treatments were not highly promising and once this was apparent greater research effort was directed towards an alternative treatment (section 5.3).

Sixteen potential chemotherapeutic agents and fresh water immersion were tested. Available literature on effective concentrations for bath treatment of fish and shellfish parasites was used as a starting point. It was concluded that trichlorofon, praziquantel, hydrogen peroxide, febantel, pyrantel embonate, metronidazole, dimetronidazole and methylene blue were not effective against spionid polychaetes at doses which were high relative to available literature.

Immersion in freshwater, potassium permanganate, gentian violet, levamisole, malachite green, formalin and ivermectin was highly effective against mudworms *in vitro*. However, the protection conferred by the burrows of spionids *in situ* rendered effective concentrations unsafe for abalone. The benzimidazoles (mebendazole and

fenbendazole) showed the largest differential between the level toxic to mud worms *in vitro* (100% mortality at 50 $mg.l^{-1}$) and level toxic to stock (0% mortality at 200 $mg.l^{-1}$ or greater). However, as for other chemotherapeutic agents, the protection conferred by spionid burrows rendered the drugs largely ineffective for bath treatment. This may be compounded by their poor solubility in water.

The benzimidazole class of drugs may have some potential for microencapsulation as treatment for various polychaetes, (including families other than the Spionidae such as Serpulids and Sabellids), with infest abalone. This approach has been explored previously by Shields et al. (1997) using copper sulphate as the effective agent to treat a sabellid polychaete. More recently Overweter (2000) used microencapsulated albendazole with success against the calcareous tube building polychaete *Galeolaria* sp. Further work in this area was not considered a high priority because the alternative approach of air drying set out in the next section, appeared more promising.

5.3 Air drying treatment

5.3.1 Treatment efficacy of air drying.

Trial 1. Treatment of remnant severely infested abalone by shell drying.

Results for both efficacy and safely in severely affected abalone are presented in Table 5.4. ANOVA (arcsine transformed data) of estimated % kill data was highly significant (F=19.2, df 3,34 P<0.001) with a mean 90% kill rate seen for the 8 h exposure. Abalone mortality occurred at 5 and 8 h exposure times.

Table 5.4 Treatment of severe *B. knoxi* infestation by drying: 3-8 h, 24 °C

	Air Exposure Time (Hours)			
	Control	3	5	8
B. knoxi chimneys ($\overline{X} \pm$ SE)	53.8 ± 7.8	28.1 ± 3.2	45.8 ± 7.7	38.8 ± 7.8
Surviving *B. knoxi* ($\overline{X} \pm$ SE)	49.4 ± 4.6	11.1 ± 1.7	15.4 ± 4.0	3.9 ± 1.4
EI% Kill ($\overline{X} \pm$ SE)	8.9^{A} ± 5.1	62.6^{B} ± 4.1	68.4^{B} ± 4.1	90.2^{C} ± 2.6
Abalone mortality	0/1	0/8	1/7	3/9

n=10 infested shells all treatments except control where n=5
EI%Kill means with shared superscripts are not significantly different (P>0.05)

Trial 2. Treatment of stock recently infested with B. knoxi (< 6-8 months) by drying for four hours at 24 °C

Treatment caused considerable reductions in total and mean recovered *B. knoxi* and the estimated percentage kill was 96.8% (Table 5.5). The treatment effect was statistically significant (Pearson Chi-square value 31.97, 1 df P<0.001). Of the 20 treated abalone live *B. knoxi* were recovered from 2 shells. One of these has 10 *B. knoxi* chimneys from which 3 live worms were recovered. Most of the shells from each group had small shell blisters (< 5% shell coverage) in the apex region. There was no abalone mortality.

Table 5.5 Treatment of *B. knoxi* infestation by drying: 4 h, 24 °C

	Total Chimneys	Total Recovered *B. knoxi*	Mean ± SE Recovered *B. knoxi*	Mean ± SE EI%K *B. knoxi*	Abalone Mortality
Control	31	25	1.25 ± 0.19	16.0 ± 6.2	0/20
Treated	35	4	0.20 ± 0.15	96.8 ± 2.1	0/20

n=20 each group, Pearson Chi-square value 31.97, 1 df P<0.001

Trial 3. Treatment of B. knoxi infected stock by drying at variable temperatures

Comparison by Chi-square analysis of dead and live worms after 4 hours of drying at different temperatures indicated a significant treatment effect (Pearson chi-square value 48.26, 4 df, P<0.001). The most effective treatment at 21°C reduced the mean *B. knoxi* count to less than 10% of the control count (Table 5.6). Estimates of percentage worm kills were approximately 70% or greater for all treatment except that at 24°C. The shells of stock exposed at 24 °C did not appear dry at the conclusion of the exposure time. This was associated with the high humidity (71%) and did not result in a treatment effect (Table 5.6). There was no abalone mortality during treatment or in the week post air exposure. Shell blistering was minimal and generally less than 5% when present.

Table 5.6 Treatment of *B. knoxi* infestation at variable temperatures

Temperature (°C)	Total Chimneys	Total Recovered *B. knoxi*	Mean ± SE Recovered *B. knoxi*	Mean ± SE EI%K *B. knoxi*	Abalone Mortality
Control	32	26	1.24 ± 0.18	19.0 ± 7.9	0/21
15	30	11	0.52 ± 0.17	72.2 ± 9.2	0/21
18	31	10	0.48 ± 0.14	69.4 ± 9.3	0/21
21	35	3	0.14 ± 0.08	91.3 ± 5.2	0/21
24	35	25	1.19 ± 0.20	29.0 ± 8.5	0/21

n=21 all treatments, Pearson chi-square value 48.26, 4 df, P<0.001

Trial 4. Treatment of B. knoxi infected abalone by drying, variable exposure times

Chi-square analysis showed a significant treatment effect (Pearson Chi-square value 30.27, 4 df P<0.001). Trends for recovered worm and estimated kill data showed a potential treatment effect at 2 h and increasing with exposure time (Table 5.7). Only one *B. knoxi* in total survived the most effective 4 h exposure. The relatively high value (33.3%) of the control EI%K indicates derelict *B. knoxi* burrows were common. Mud worm blisters were small when present, covering less than 5% of shell area. There was no abalone mortality during desiccation or in the 3 d post air exposure.

Table 5.7 Treatment of *B. knoxi* infestation at 21°C and variable exposure times

Exposure Time (h)	Total Chimneys	Total Recovered *B. knoxi*	Mean ± SE Recovered *B. knoxi*	Mean ± SE EI%K *B. knoxi*	Abalone mortality
Control	17	12	0.75 ± 0.14	33.3 ± 11.8	0/16
1	20	14	0.88 ± 0.18	29.2 ± 11.0	0/16
2	21	8	0.50 ± 0.25	68.8 ± 11.6	0/16
3	23	4	0.25 ± 0.11	81.3 ± 8.7	0/16
4	21	1	0.06 ± 0.06	93.8 ± 6.1	0/16

n=16 all treatments, Pearson Chi-square value 30.27, 4 df P<0.001

Trial 5. Air drying of old, heavily polychaete infested greenlip abalone

Despite the exposure time, temperature and humidity conditions (4 hours, 21 ± 0.5 °C, 60% humidity), the shells did not appear dry at the conclusion of treatment. There was no statistically significant reduction in numbers of *B. knoxi* (Pearson Chi-square value 1.27, df 1, P=0.260) and clearly no reduction in total polychaetes (Table 5.8). Control EI%K data indicated derelict *B. knoxi* burrows were common. There was 1 abalone mortality in the treated group during the recovery period. Polychaete worms other than mud worms were very common, with 400 recorded in one treated shell. The majority of the shells were seriously blistered in the range 10-50% blister coverage, frequently with large walled off chambers.

Table 5.8 Efficacy of air drying for old heavily polychaete infested greenlip abalone

	Control	Treated
B. knoxi chimneys ($\overline{X}$ ± SE)	10.5 ± 1.7	12.4 ± 2.4
Surviving *B. knoxi* ($\overline{X}$ ± SE)	6.2 ± 1.3	6.4 ± 1.5
EI% *B. knoxi* Kill ($\overline{X}$ ± SE)	38.1 ± 9.0	52.2 ± 7.8
total worms ($\overline{X}$ ± SE)	19.5 ± 5.6	71.6 ± 38.0
Abalone Mortality	0/10	1/10

n= 10 each group, Pearson Chi-square value 1.27, df 1, P=0.260 for *B. knoxi*.

Trial 6. Air drying of recently infected stock under field conditions

Drying treatment resulted in a mean reduction of *B. knoxi* from 3.3 to 0.4 per shell under field conditions (16-18ºC, 49-62% humidity) (Table 5.9). The EI% Kill value was very high at 87.5%. Analysis of surviving *B. knoxi* data by Chi square analysis was significant (Pearson Chi-square value 64.2, df 1, P<0.001). Small numbers of *P. hoplura* mud worms were present in the control but not in the treatment group. This treatment effect was also statistically significant (Mann-Whitney U Test, U = 120.0, P <0.01). There was no abalone mortality. Mean blister coverage was 4.3% (SD = 7.2%, n = 20)

Table 5.9 Field trial treatment of recent (< 3 month) mud worm infestation

	Total Chimneys	Total *B. knoxi*	Mean ± SE *B. knoxi*	Mean ± SE EI%K *B. knoxi*	Mean ± SE *P. hoplura*
Control	77	65	3.3 ± 0.5	18.0 ± 6.0	1.0^{A} ± 0.4
Treated	53	7	0.4 ± 0.2	87.5 ± 21.6	0.0^{B} ± 0.0

n=20 for both groups, Pearson Chi-square value 64.2, df 1, P<0.001 for *B. knoxi*
Column means with shared superscripts are not significantly different (P>0.05)

Trial 7. Air drying of heavily fouled, P. hoplura infected stock under field conditions

Due to excess surface fouling the shells of many treated abalone did not appear completely dry at the conclusion of air exposure (17.5-22ºC, 43-65% humidity). Analysis of counts data by Mann-Whitney U Test showed the reductions in *B. knoxi* and large *P. hoplura* between control and treatment groups (Table 5.10) were not statistically significant (P>0.05). However, the reductions in small *P. hoplura* and non-spionid polychaetes were statistically significant (U = 53.0, P=0.03 and U =14.5, P<0.01 respectively). Non-spionid polychaetes were common and many appeared to live on the surface of the shell rather than within it.

Abalone mortality data could not be assessed reliably in this trial because after transport to the laboratory for mud worm quantification one control animal and several in the treatment group died or were moribund. It was believed this was due to transport stress and poor water quality in the holding tank. Additionally 10 of 20 abalone in the control group escaped from their holding cage after treatment but before transport. Of the remainder, one abalone was excluded from statistical analysis of count data as it contained > 100 *P. hoplura* (mostly post larvae), a level more than twenty times the mean for the rest of the group. Mean blister coverage for the control group was 20.4% (SD = 22.6, n=10).

Table 5.10 Field trial treatment of heavily fouled stock with 13 month exposure to mud worm settlement

	Mean ± SE *B. knoxi*	Mean ± SE Small *P .hoplura*	Mean ± SE Large *P. hoplura*	Mean ± SE non-spionid polychaetes
Control	1.1^{A} ± 0.2	1.4^{A} ± 0.2	3.0^{A} ± 0.4	12.0^{A} ± 0.8
Treated	0.5^{A} ± 0.02	0.3^{B} ± 0.02	1.2^{A} ± 0.09	2.0^{B} ± 0.13

n=20 treatment group. n=9 control group .
Column means with shared superscripts are not significantly different (P>0.05)

Trial 8. Two and half hour drying of recently B. knoxi infected stock

Although 40 abalone were assigned to each treatment group subsequent analysis showed that less than half the animals had any *B. knoxi* infestation on the basis of chimney counts. Table 5.11 displays data for the *B. knoxi* positive abalone only. The 2.5 h air exposure(16-17ºC, 50-54% humidity) was highly significant in reducing infestation (Pearson Chi-square value 30.22 df 1, P<0.001). Shell blistering was rare and generally less than 5% coverage when present. There was no mortality in either group (total n=80) in the week following treatment.

Table 5.11 Short air exposure treatment of three month *B. knoxi* infected stock.

Treatment	Statistic	*B. knoxi* Chimneys	Small *B. knoxi*	large *B. knoxi*
Control	Sum	22	18	5
	Mean ± SE	2.00 ± 1.61	1.64 ± 1.21	0.45 ± 0.69
air dried	Sum	18	1	2
	Mean ± SE	1.38 ± 0.65	0.08 ± 0.28	0.15 ± 0.38

n=11 and 13 for control and treated groups respectively
Contingency table analysis: control group 23:0 (live worms: dead worms) and treatment group 3:15 (live worms: dead worms) Pearson Chi-square value 30.22 df 1, P<0.001.

Trial 9 Drying of 14 month mud worm infested blacklip abalone

Mud worm counts were reduced in air dried abalone compared to the control animals for all worm categories. However, none of the reductions (Table 5.12) were statistically significant (P>0.05, Mann–Whitney U Test) except for the category "total small mud worms" (U = 30.0, P = 0.03)**.**

Table 5.12 Treatment of 14 month mud worm infested abalone

Worm count ($\overline{X}$ ± SE)	Control	Air Dried
Small *B. knoxi*	$0.2^{A} \pm 0.2$	$0.0^{A} \pm 0.0$
Large *B. knoxi*	$2.1^{A} \pm 0.6$	$1.7^{A} \pm 0.7$
Total *B. knoxi*	$2.3^{A} \pm 0.7$	$1.7^{A} \pm 0.7$
Small *P. hoplura*	$0.3^{A} \pm 0.2$	$0.0^{A} \pm 0.0$
Large *P. hoplura*	$7.9^{A} \pm 0.9$	$5.9^{A} \pm 1.2$
Total *P. hoplura*	$8.2^{A} \pm 0.9$	$5.9^{A} \pm 1.2$
Total small mud worms	$0.5^{A} \pm 0.2$	$0.0^{B} \pm 0.0$
Non-spionid mud worms	$5.4^{A} \pm 5.0$	$0.8^{A} \pm 0.5$
Total polychaetes	$16.3^{A} \pm 5.4$	$8.9^{A} \pm 2.0$

n = 10 both groups
Row means with shared superscripts are not significantly different (P>0.05)

Trial 10. Repeat drying of abalone previously treated one year earlier

Drying resulted in lower counts for all mud worm categories (Table 5.13). These were statistically significant by Mann-Whitney U Test for: total polychaetes (P=0.01), large *P. hoplura* (P=0.01), small *P. hoplura* (P=0.03) and total *P. hoplura* (P=0.01). Reductions were not statistically significant for large and total *B. knoxi* respectively (P=0.10,and P=0.06). Insufficient small *B. knoxi* were present for a sensible test. Mean shell blister coverage was 9.6% (SD=10.0%, n=19). One of the control group animals was lost from the experiment before transport to the laboratory for assessment.

Table 5.13 Summer 1999 re-treatment of previously treated stock

Worm count ($\overline{X}$ ± SE)	Control	Air Dried
Small *B. knoxi*	0.3 ± 0.3	0.0 ± 0.0
Large *B. knoxi*	3.7^{A} ± 0.8	2.1^{A} ± 0.9
Total *B. knoxi*	4.0^{A} ± 0.8	2.1^{A} ± 0.9
Small *P. hoplura*	0.9^{A} ± 0.4	0.1^{B} ± 0.1
Large *P. hoplura*	3.9^{A} ± 0.8	1.0^{B} ± 0.5
Total *P. hoplura*	4.8^{A} ± 1.0	1.1^{B} ± 0.6
Non-spionid mud worms	1.2^{A} ± 1.2	0.3^{A} ± 0.2
Total polychaetes	10.2^{A} ± 1.5	3.5^{B} ± 1.4

n = 9 control group, n=10 treated group
Row means with shared superscripts are not significantly different (P>0.05)

Table 5.14 summarises the results of treatment trials reported above. The efficacy of treatment column classes treatment as effective only if reductions in worms were statistically significant

Table 5.14. Summary of trial effectiveness

Trial No.	Infection (months)	% Shell Blisters	Treatment Time (h)	Efficacy of treatment
1	> 24	>25%	3,5,8	Effective for *B. knoxi*
2	8	<5	4	Effective for *B. knoxi*
3	8-10	< 5	4	Effective for *B. knoxi* 15-21 °C
4	8-10	< 5	1-4	Effective for *B. knoxi* 2-4 hours
5	>36	10-50	4	Ineffective for *B. knoxi* and non-spionids
6	< 3	4.3	3.5	Effective for *B. knoxi* and *P. hoplura*
7	13	20.4	4	Effective: *B. knoxi*, non-spionids, small *P. hoplura* Ineffective: large *P. hoplura*
8	3	< 5	2.5	Effective for small *B. knoxi*
9	14	17.2	3.5	*Effective for small mud worms only*
10	16	9.6	4	Effective: for *P. hoplura* all sizes Ineffective: for large *B. knoxi*

Effective treatment defined as statistically significant spionid reduction (P<0.05)

5.3.2 Drying and abalone mortality

Mortality of 15-20 mm stock dried at 21°C was minimal with no deaths for control, 1, 2, 3 and 5 h air exposure treatments (Table 5.15). In the second experiment there was no mortality in the 8 week observation period following air exposure for 11 h (Table 5.16). Growth was poor in all treatment groups including the non dried control (0.89 mm ± 0.76 mm, 1.69 g ± 1.21 g; $\overline{X}$ ± SD, n=146). There was no significant difference between SGR of treatments for length (F = 1.40, df 3,140, P= 0.246, ANOVA) or for weight (F =1.39, df 3,140, P = 0.249, ANOVA).

Tank conditions were not ideal during the recovery period. Temperature extremes of 10-20 °C were recorded with a mean daily reading of 15.0°C (SD = 2.1°C). Un-ionised ammonia readings of 0.25 $mg.l^{-1}$ were recorded which might have contributed to suppressed growth. One dead abalone was found to have a discoloured

lesion on the foot believed to have been a knife wound. There were a few escapes from the experimental cages and/or holding tank. Feed consumption was approximately 2% body weight.d^{-1}. Of 30 abalone air dried for 15 h on 4-5 April 2000, most appeared moribund at completion of drying but subsequently recovered with only 1 mortality in the 2 week recovery period.

Table 5.15. Mortality data: 1- 6 h exposure, 15-20 mm abalone

Cumulative mortality	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
Initial	0/20	0/20	0/20	0/20	0/20	0/20
17 days	0/20	0/20	0/20	1/20	0/20	2/20

Table 5. 16. Mortality data: 5-11 h exposure, 42 mm abalone

Cumulative mortality	Control	5 hours	8 hours	11 hours
Initial	0/39	0/39	0/39	0/39
54 days	1/39	2/37	1/39	0/39

5.3.3 Drying and long term growth

Forty-one tagged blacklip abalone (11 control, 30 air dried were recovered from the grow out tank at farm 2. A mortality episode over the summer 1999/2000 reduced abalone numbers generally and affected recovery of experimental abalone. There were also 5 deaths in the air dried group in the week following treatment. There was no significant difference between the groups in SGR for length (t=-0.69, df 39, p=0.497 t -Test) nor for SGR weight (t=-0.43, df 39, p=0.669 t -Test). Growth in length (pooled treatments) was 13.1 ± 3.4 mm and weight increment was 15.0 ± 4.9 g ($\overline{X}$ ± SD, n = 41 both data sets).

At Tasmanian Tiger Abalone 62 of the hybrid experimental abalone (42 control, 20 air dried) were recovered 214 d after commencement of the trial. As at Marine Shellfish Hatcheries an unrelated mortality episode at the farm significantly reduced stock numbers in some tanks, including the tank housing the trial stock. There was no significant difference in SGR of air dried and control stock for length (t=-0.77, df 59, p=0.447 t-test) nor for SGR weight (t=-0.25, df 60, p=0.804 Unpaired t-test). Growth in length (pooled treatments) was 9.9 ± 2.5 mm (X ± SD) and growth in weight was 5.3 ± 2.6 g (X ± SD).

At Huon Aquaculture 78 abalone (42 control, 35 dried) were recovered 210 d post drying. Treatment group animals appeared stressed immediately after air exposure, with limited clinging and turn over ability. However, there were no dead animals in the weeks immediately following drying treatment. After 210 d there were 5 dead abalone in the control treatment and 3 in the air dried group. Mean length and weight of control animals increased 4.0 mm (SD= 2.0, n=42) and 3.4 g (SD=1.5, n=42) respectively. Mean length and weight increment for dried abalone were 3.2 mm (SD=1.5, n=35) and 2.6 g (SD=1.8, n=35). This was a reduction of approximately 20 and 24 % for length and weight respectively compared to the control group. Comparison of SGR by unpaired t–test found significant differences for length (t=2.02, df 76, p=0.047) and weight (t=4.55, df = 75, p<0.001).

Discussion

The results presented above relate to the original project objective No. 4 on protocols for treatment within the production cycle. As noted previously the treatment focus was shifted from chemical to air drying treatment when the former proved to be ineffective. For this treatment, the objectives relating to abalone survival, growth, cost, marketability have been met. The integration of the treatment regime into an overall method to minimise mud worm infestation in line with objectives 1 and 6 (page 9) is outlined below.

The body of experimental work demonstrates that treatment of small *B. knoxi* (and small *P. hoplura*) can be highly effective. In fact all treatment trials except No. 5 showed a reduction in numbers for all classes of worms between control and air dried groups. These reductions were not necessarily statistically significant as summarised in Table 5.14. Larger mud worms (> 5 mm and typically 10-25 mm) may be susceptible to drying treatment depending on a host of factors. These appeared to include: infestation time, blister severity, stock size and stock fouling. Increasing infection period allowed mud worms time to become established in burrows deep within the shell. Where larger chambers occurred within blisters survival appeared especially likely. Non-spionid polychaetes were highly susceptible to drying treatment in most trials where they were present. This may be because they tend to occur on the surface of the shell rather than within it.

Appropriate conditions to ensure effective drying are considered to be temperatures >15°C and humidity less than approximately 63%. These conditions are not uncommon on sunny days in Tasmania outside the period late autumn to early spring. Where shells did not fully dry (Trials 5, 7 and one treatment in Trial 3) treatment was ineffective. Lack of drying was associated with high humidity, larger stock and severe shell fouling. The effect of humidity is clearly demonstrated by comparison of results for *B. knoxi* survival dried at 24°C in trials 2 and 3. The treatment was highly effective in the former at low humidity and ineffective in the latter at higher humidity.

Previous work on treating mud worms in shellfish has focused on oysters. Environmental treatments have included: fresh water soaking (Bailey-Brock and Ringwood 1982, Nel et al., 1996 and Tonkin 1997), heated seawater (Bailey-Brock and Ringwood 1982, Nel et al., 1996) and soaking in hyper saline water (Mackenzie and Shearer 1959, Bailey-Brock and Ringwood 1982, Tonkin, 1997). The latter method generally requires heating to dissolve sufficient salts. Leighton (1998) successfully used elevated temperature to treat sabellid polychaete infection in two tropical abalone species but this approach was unsuitable for the more commercially valuable temperate species *H. rufescens*. Quantified studies on the efficacy of air drying appear to be lacking although there are references to its use in oyster growing. Nell and Smith (1988) note that 10-14 d out of water is a traditional treatment for mud worms in Sydney rock oysters. This appears to be based on the work of Whitelegge (1890). Fortuitously, desiccation is effective as a treatment for infected abalone at much lower exposure times. In relation to this, observed blister morphology differences between abalone and mud worm infested bivalve molluscs in Tasmania are of interest. In bivalves *B. knoxi* is observed to inhabit very large water filled blisters whereas in abalone blisters rarely have a significant volume. These differences are quantified in section 5.5.3 (shell blistering section). Thus, blisters typical of *B. knoxi* and to a lesser extent *P. hoplura* in abalone may provide relatively poor protection against desiccation strategies.

Air drying treatment trials showed that mud worm infestation rates for lightly infested abalone could be considerably reduced by air drying for 2-4 h. Post drying recovery times of a week or so failed to show any abalone mortality in lightly infected stocks. Longer air exposure and recovery times were used in follow up experiments with the intention of defining a safe period for treatment air exposure. Abalone proved surprisingly tolerant of air drying. There was no mortality of 15-20 mm stock dried for up to 3 h and of 40 mm animals dried for 11 h, providing ample scope for effective spionid treatment. This contrasts with poor survival for smaller abalone (5-10 mm) air dried in shade at 24°C (Whang and Chung 1977). Wells and Baldwin (1995) found that larger abalone of two New Zealand species were "less susceptible to anaerobic stress than smaller animals". The larger abalone conserved relatively more adenylate energy charge and had lower concentrations of the glycolytic end products lactate and tauropine. It was considered beyond the scope of the present research to establish the point at which air drying abalone results in significant deaths of animals.

There was no difference in growth of control and air dried (5, 8, 11 h) abalone 54 d post treatment. However, growth was poor in the control group and temperature fluctuation was a problem. Ammonia levels were potentially high enough to suppress growth according to data of Harris et al. (1998). Thus the effect on growth of air drying may have been masked by other factors. Certainly it would not be unexpected if air drying resulted in a short term growth decrease. Edwards et al. (2000) has shown that removal of abalone from the substrate with a knife is enough to depress the growth rate over a two month period.

The series of three experiments on medium-long term post drying growth effects produced conflicting results. Two trials showed no growth depression but the third trial demonstrated growth depression of 20-25%. It may be significant that stock in the trial showing reduced performance were growing slowly before the trial began and untreated control animals continued to perform relatively poorly. The recorded growth depression was similar to that reported by Shepherd and Hearn (1983) for wild juvenile abalone handled in hot weather.

Given the potential for air drying treatment to suppress growth of stock this management option should be used prudently with the emphasis on avoidance of infestation. Where treatment is required, stress to abalone will likely be minimised by early intervention, reduced drying time, reduced air temperature and reduced differential between sea and air temperatures. Additionally, Watanabe et al. (1994) found that starved abalone survived air exposure at considerably greater levels than fed animals.

The use of air exposure as a mud worm treatment is considered a very favourable outcome. The treatment is environmentally benign, uncontroversial compared to potential chemotherapeutic treatments, and requires no withholding period. Compared to any potential chemical treatments it minimises handling and thus labour costs since it is difficult to conceive of a treatment protocol that does not require removal of abalone cages from the water as an initial step. Additionally, there are no consumable input costs associated with the treatment. Thus a potential treatment option exists should farmed stock become infected. In susceptible areas the treatment option should be secondary to and compliment avoidance strategies (section 5.1). Treatment if required should be exercised earlier rather than later to maximise mud worm kill rates. Summer would appear the most appropriate time for treatment as spionid settlement is largely completed and weather conditions are at their most suitable for effective drying. Thus stock placed post November-January to avoid spionid infestation can be treated if required approximately a year later if required and

should then remain mud worm free for the best part of another year before probable sale.

5.4 Epidemiology/Risk factors

5.4.1 Effect of stock size

At Aquatas mud worm settlement as measured by the presence of *B. knoxi* chimneys, *B. knoxi* worms and *P. hoplura* worms increased generally with increasing stock size class (Table 5.17). The large size class was significantly different ($P<0.05$, Kruskall-Wallis test) to the other size classes. Similarly, at Aquatas the largest stock size acquired significantly more mud worm settlement by all measures than medium and small stock size categories ($P<0.05$, Kruskall-Wallis Test). Table 5.18 displays this data with replicates separate as *P. hoplura* data could not be grouped in the medium size class ($P<0.01$ $U = 0.0$, Mann-Whitney U Test) and because the small size class category was deleted from replicate 2 due to poor recovery of animals.

Settlement of *B. knoxi* on experimental stock at both sites was considered low with a maximum of approximately 8-12 chimneys per replicate of 5 large size class abalone. Survival of *B. knoxi* worms until assessment in February – March was even lower at about half chimney count levels (Tables 5.17 and 5.18).

Shell damage attributable to mud worms was significantly higher ($P<0.05$, Kruskall-Wallis test) in the larger stock size class at both sites as measured by both percentage blister cover and the SSDR methods (Table 5.19).

Table 5.17. Mud worm settlement on three size classes of abalone at Aquatas. Means with standard error and n in parentheses.

	Small	Medium	Large
B. knoxi chimneys	0.0^{A} (0.0,14)	0.5^{A} (0.3, 17)	8.6^{B} (0.7, 21)
B. knoxi	0.0^{A} (0.0, 14)	0.4^{A} (0.1, 17)	4.2^{B} (0.8, 21)
P. hoplura	0.9^{A} (0.2, 14)	1.7^{A} (0.5, 17)	12.8^{B} (2.4, 21)

Row means with shared superscripts are not significantly different ($P>0.05$) means are replicates of 5 abalone

Table 5.18. Mud worm settlement on three size classes of abalone at Huon Aquaculture. Means with standard error and n in parentheses.

	Replicate	Small	Medium	Large
B. knoxi chimneys	1	0.0^{A} (0.0, 3)	0.8^{A} (0.7, 6)	7.9^{B} (1.0, 9)
B. knoxi		0.3^{A} (0.6, 3)	0.8^{A} (0.7, 6)	5.1^{B} (0.5, 9)
P. hoplura		1.7^{A} (0.7, 3)	5.8^{A} (1.1, 6)	39.0^{B} (6.4, 9)
B. knoxi chimneys	2	-	1.9^{A} (0.4, 7)	12.2^{B} (1.4, 10)
B. knoxi		-	0.9^{A} (0.5, 7)	4.4^{B} (0.6, 10)
P. hoplura		-	1.0^{A} (0.2, 7)	21.3^{B} (7.1, 10)

Row means with shared superscripts are not significantly different ($P>0.05$)
means are replicates of 5 abalone

Table 5.19. Shell damage assessment following spionid infestation for 3 size classes of abalone (means with SE and n values in parentheses)

		Small	Medium	Large
Huon Aquaculture	SSDR	0.28^{A} (0.11, 10)	0.54^{A} (0.06, 25)	1.29^{B} (0.06, 25)
	% blister	0.5^{A} (0.5,10)	1.2^{A} (0.4,25)	13.3^{B} (1.3, 25)
Aquatas	SSDR	0.1^{A} (0.04,20)	0.2^{A} (0.1, 20)	1.1^{B} (0.1, 20)
	% blister	0.7^{A} (0.7, 20)	0.3^{A} (0.2, 20)	9.0^{B} (1.7, 20)

Row means with shared superscripts are not significantly different (P>0.05)

5.4.2 Spionid settlement and fouling organisms

Examination of recently formed *B. knoxi* chimneys showed that settlement did not occur evenly over the shell surface but rather favoured certain sites. Although the number of spirorbids and the surface area they occupied on abalone shells was relatively small in the examined stock, 38% of *B. knoxi* chimneys occurred on or in these fouling polychaetes (Table 5.20). Dead spirorbids were especially favoured with the chimney often projecting from the tube opening. The most commonly settled part of the abalone shell itself was the groove running around the base of the shell apex (34% of chimneys). Chimneys were also found in shell irregularities such as shell fractures, growth ridges and within respiratory pores, especially closed ones. Observation in the period 1998-2000 showed spirorbid settlement occurred in the spring and early summer in the south of the state.

Table 5.20 Distribution of *B. knoxi* chimneys on abalone shells

Location	%
Apex groove	34
Spirorbid	38
Other fouling structure	7
Other shell location	21

n= 178 chimneys

As suspected bryozoan coated abalone had significantly fewer *B. knoxi* chimneys than other abalone (P<0.01 U = 4.5, Mann-Whitney U test), 1.3 ± 0.5 compared to 14.2 ± 3.1 respectively ($\overline{X}$ ± SE). Bryozoans living on shucked abalone shells in a recirculating holding system remained alive for over 6 months but did not appear to spread nor did they colonise other nearby shells.

The quantitative spirorbid fouling experiment showed settlement of *B. knoxi* was considerably higher on spirorbid fouled stock than on "clean" stock. This was true for chimney count (P≤ 0.01, Mann - Whitney U Test) and surviving worm data (P < 0.05, Kolmogorov –Smirnov Two-sample Test) where levels were 6-10 times higher on spirorbid fouled stock at both study sites (Tables 5.21 and 5.22). Note that basket replicates were not combined for Huon Aquaculture, as there were significant differences (P<0.05, Mann-Whitney U Test) between *B. knoxi* chimney data, *B. knoxi* worm data and SSDR for the "clean" group.

Settlement of *P. hoplura* was not as high as that of *B. knoxi* and there was no significant difference (P>0.05, Kolmogorov-Smirnov Two-sample test) between

P. hoplura settlement on fouled and nominally clean abalone at Aquatas and the second of the Huon Aquaculture replicates.

The differences in mud worm settlement (chiefly *B. knoxi*) between spirorbid fouled and "clean" stock, led to higher levels of shell damage in the fouled stock as described by the SSDR. There was an approximate 10 fold increase in mean SSDR for fouled stock at Aquatas ($P<0.01$, $U=125.5$ Mann -Whitney U Test) and a 2-6 fold increase at Huon Aquaculture ($P \leq 0.02$, Mann -Whitney U Test, both replicates) compared to nominally clean stocks (Table 5.23).

Table 5.21 Spionid settlement indicators for "clean" and spirorbid fouled abalone stock at Aquatas. Means* with SE and n in parentheses

	Clean	Spirorbid fouled
B. knoxi chimneys	0.3^{A} (0.1, 70)	3.2^{B} (0.5, 20)
B. knoxi	0.9^{A} (0.2,14)	9.0^{B} (1.6, 4)
P. hoplura	0.1^{A} (0.1, 14)	0.0^{A} (0.0, 4)

* means are replicates of 5 for *B. knoxi* and *P. hoplura* worm data
Row means with shared superscripts are not significantly different (P>0.05)

Table 5.22 Spionid settlement indicators for "clean" and spirorbid fouled abalone stock at Huon Aquaculture. Means* with SE and n in parentheses

	Replicate	Clean	Spirorbid fouled
B. knoxi chimneys	1	0.3^{A} (0.1, 50)	3.1^{B} (0.5, 20)
B. knoxi		1.2^{A} (0.6, 10)	9.5^{B} (1.6,4)
P. hoplura		0.1^{A} (0.1,10)	2.3^{B} (1.0, 4)
B. knoxi chimneys	2	0.8^{A} (0.2,50)	4.9^{B} (0.9, 12)
B. knoxi		3.0^{A} (0.5, 10)	19.0^{B} (3.0, 2)
P. hoplura		1.1^{A} (0.6, 10)	4.5^{A} (3.5, 2)

* means are replicates 5 for B. knoxi and P. hoplura worm data
Row means with shared superscripts are not significantly different (P>0.05)

Table 5.23 Subjective shell damage ratings for clean and spirorbid fouled abalone at 2 study sites Means with SE and n in parentheses

	Clean	Spirorbid fouled
Huon Aquaculture, Rep. 1	0.2^{A} (0.1, 50)	1.3^{B} (0.1, 20)
Huon Aquaculture, Rep. 2	0.6^{A} (0.1, 50)	1.1^{B} (0.2, 12)
Aquatas	0.1^{A} (0.1, 70)	1.1^{B} (0.1, 20)

Row means with shared superscripts are not significantly different (P>0.05)

The differences in shell damage between spirorbid fouled and "clean" stock prompted the examination of growth between the 2 groups. Comparison of "clean" and spirorbid fouled stock growth rates was performed separately for each replicate (Table 5.24). Nominally clean stock grew significantly faster in replicate 1 for both SGR length ($t=2.49$ df 41, $P<0.01$, one tailed t-test) and weight ($t=2.73$ df 23, $p<0.01$, one tailed t-test) and for replicate 2, SGR weight ($t = 1.80$ df 36, $P=0.04$) but not for SGR length replicate 2 ($t = 1.21$ df 40, $P=0.12$). As the value of n was low for a reliable t-test in replicate 1 SGR weight comparison this was repeated as a Mann-Whitney U Test ($U = 12$, $P=0.01$). Mean growth depression as a result of increased shell blistering in spirorbid fouled stock was 28% (SD=8%, n=2) for length and 39% (SD=19%, n=2) for weight.

Table 5.24 Comparison of growth (SGR) between "clean" and spirorbid fouled stock. Means ± SD, n in parenthesis.

	Stock status	Replicate 1	Replicate 2
SGR length	"clean"	$0.054^{A} \pm 0.020$ (24)	$0.032^{A} \pm 0.016$ (28)
	spirorbid fouled	$0.036^{B} \pm 0.027$ (19)	$0.025^{A} \pm 0.015$ (10)
SGR weight	"clean"	$0.226^{A} \pm 0.062$ (5)	$0.133^{A} \pm 0.049$ (28)
	spirorbid fouled	$0.108^{B} \pm 0.091$ (20)	$0.100^{B} \pm 0.055$ (10)

Data pairs by replicate with shared superscripts are not significantly different (P>0.05)

5.4.3 Long term spionid infestation and growth comparison

Abalone transferred August 1998 grew from 46.9 mm (SE=0.3, n=100) to 61.8 mm (SE=1.1, n=23) and had acquired a mean spionid count of 54.1 (SE=5.9, n=23) by October 2000. By contrast, smaller abalone transferred September 1998 grew from 18.8 mm (SE=0.1, n=150) to 57.6 mm (SE=0.62, n=94) by January 2001 acquiring a considerably smaller mean spionid count of 1.2 (SE=0.2, n=64). Mean percentage shell blistering was 28.7 (SE=2.9, n=23) and 1.9 (SE=0.5, n=64) in August and September intake groups respectively.

5.4.4 Rearing vessel comparison

Comparison of worm numbers infesting Huon Aquaculture basket reared stock sampled before and after the *B. knoxi* settlement season showed significant increases for *B. knoxi* chimneys (P<0.01 U=0.0, Mann-Whitney U Test) and *B. knoxi* worms (P<0.01 U=6.5), (Table 5.25). Count data for *P. hoplura* also increased significantly between the sample times (P<0.01 U=7.0). By contrast, worm numbers infesting tube reared stock at the site showed no significant increase for *B. knoxi* chimneys (P>0.05 U=39.5) or worms (P>0.05 U=39), (Table 5.26). Count data for *P. hoplura* increased significantly between the two sample times (P<0.01 U=8.5).

Before the beginning of the *B. knoxi* settlement season at Huon Aquaculture there was no significant difference between numbers of *B. knoxi* chimneys (P>0.05 U=30), *B. knoxi* worms (P>0.05 U=42.5), or *P. hoplura* (P>0.05 U=36) on abalone in tubes and baskets (Tables 5.25 and 5.26). By completion of the *B. knoxi* settlement period (sampled December for baskets and January for tubes) there was a significant difference for *B. knoxi* chimneys and worms (P<0.01, U=0.5, 9.5 respectively) between baskets and tubes. In the baskets the number of *B. knoxi* as indicated by chimney counts and recovered worms increased approximately 5-6 fold during the exposure period, where as in the tubes there was no increase in *B. knoxi* numbers. There was no significant difference for *P. hoplura* (P>0.05 U=36.0, 41.0) between baskets and tubes after the settlement season. Increase in numbers of this species were greater than 10 fold in both baskets and tubes.

In the less infested basket reared set at Huon Aquaculture the trend was the same as for the more infested basket reared stock at the site. There were significant increases in counts of: *B. knoxi* chimneys, *B. knoxi* worms and *P. hoplura* worms (P<0.01 for all; U=2.0, 5.5, 7.0 respectively), (Table 5.27)

As for data from the tubes at Huon Aquaculture there was no significant increase in *B. knoxi* chimneys (P>0.05 U=45.5) or worms (P>0.05 U=49.5) for tube

reared stock at Aquatas (Table 5.28). There was a significant increase in *P. hoplura* ($P<0.01$ $U=16.5$) as for tube reared stock at Huon Aquaculture.

Stock in the basket type rearing vessels at Aquatas had suffered heavy mortality by early summer 2000 when they were resampled. Only *B. knoxi* chimney count data was considered reliable as previously spionids numbers had been seen to decline several months following the death of the host. Chimney structures were known to survive the death of the worm by several months (section 4.5) and counts increased from 1.7 ± 1.9 (mean ± SD, n=10) in September to 3.1 ± 0.4 (mean ± SD, n=39) in January/February. This result was borderline statistically significant ($P=0.05$ $U=117.5$).

Table 5.25 Mud worm progression for Huon Aquaculture abalone reared in baskets: June 1999 - December 1999. Means ± SE

Time	*B. knoxi* Chimneys	*B. knoxi*	*P. hoplura*
Jun 99	$3.2^{A} \pm 0.5$	$1.7^{A} \pm .5$	$1.4^{A} \pm 0.35$
Dec 99	$20.9^{B} \pm 2.4$	$10.5^{B} \pm 2.0$	$16.8^{B} \pm 4.6$

Column means with shared superscripts are not significantly different ($P>0.05$)
n= 10 both data sets

Table 5.26 Mud worm progression for Huon Aquaculture abalone reared in tubes: August 1999 - January 2000. Means ± SE

Time	*B. knoxi* Chimneys	*B. knoxi*	*P. hoplura*
Aug 99	$5.0^{A} \pm 1.0$	$2.0^{A} \pm 0.7$	$2.3^{A} \pm 0.8$
Jan 00	$4.6^{A} \pm 1.2$	$2.3^{A} \pm 0.5$	$29.8^{B} \pm 7.5$

Column means with shared superscripts are not significantly different ($P>0.05$)
n= 9 Aug 99, n = 10 January 2000 samples

Table 5.27 Mud worm progression for Huon Aquaculture less infested baskets: June 1999- December 1999. Means ± SE

Time	*B. knoxi* Chimneys	*B. knoxi*	*P. hoplura*
Jun 99	$0.2^{A} \pm 0.1$	$0.1^{A} \pm 0.1$	$0.4^{A} \pm 0.2$
Dec 99	$4.6^{B} \pm 1.1$	$3.7^{B} \pm 0.7$	$4.8^{B} \pm 1.0$

Column means with shared superscripts are not significantly different ($P>0.05$)
n= 9 Jun 99, n = 10 Dec 99 sample

Table 5.28 Mud worm progression tube reared stock at Aquatas: August 1999-January 2000. Means ± SE

Time	*B. knoxi* Chimneys	*B. knoxi*	*P. hoplura*
Aug 99	$1.4^{A} \pm 0.4$	$1.0^{A} \pm 0.5$	$0.4^{A} \pm 0.2$
Jan 00	$1.7^{A} \pm 0.8$	$1.1^{A} \pm 0.6$	$3.1^{B} \pm 1.0$

Column means with shared superscripts are not significantly different (P>0.05) n=10 both groups

5.4.5 Position in water column

There was no significant difference between the 6 and 9 m depth abalone for *B. knoxi* chimney and worm counts (P>0.05, Mann-Whitney U-test). Counts of *P. hoplura*, total spionids and subjective shell damage rating (SSDR) were significantly different (P<0.05, U =117, 124 and 129 respectively, Mann-Whitney U-test) between the 6 and 9 m treatments (Table 5.29). Spionid settlement was very low overall but approximately 4 times higher for *P. hoplura* at 9 m (representing a minimum 1 m off the sea floor) compared to 6 m. The 3 m depth baskets were lost before completion and Table 5.29 provides an estimate based on the sum of 1 month samplings during the same time period used for determining settlement timing (section 5.1).

Table 5.29 Comparison of spionid settlement and impact at two positions in the water column. Means ± SE

Depth (m)	No. Chimneys	No. *B. knoxi*	No. *P. hoplura*	Total spionids	SSDR
3*	0.2	0.2	0.35	0.55	_
6	$0.20^{A} \pm 0.12$	$0.15^{A} \pm 0.11$	$0.20^{A} \pm 0.12$	$0.35^{A} \pm 0.21$	$0.45^{A} \pm 0.21$
9	$0.35^{A} \pm 0.15$	$0.20^{A} \pm 0.12$	$0.95^{B} \pm 0.26$	$1.15^{B} \pm 0.30$	$1.05^{B} \pm 0.22$

Means in columns with shared superscripts are not significantly different (P>0.05)
n=10 samples for all means, each consisting of a replicate of 10 abalone
* data presented as mean of replicate of 10 abalone for comparison purposes no statistical variation data available.

5.4.6 Comparison of abalone species

Spionid settlement was very low with a maximum mean of 0.2 (SE = 0.09, n=20) *P. hoplura* per replicate of 10 abalone for *H. laevigata*. This was not significantly different (P>0.05, U=170 Mann-Whitney U-test) to data for *H. rubra* (0.05 ± 0.05, $\overline{X} \pm$ SE, n=20). No *B. knoxi* settlement was detected in either group.

Discussion

Data presented above and discussed below relate specifically to project objective No. 5 on epidemiology of mud worm infestation and in part to objective 3 on interactions between mud worm reproductive behaviour and abalone. These findings contribute to the minimisation of mud worm risk as per objectives 1 and 6 (page 9).

Examination of *B. knoxi* chimney locations revealed that settlement was not random on the surface of the abalone shell but favoured certain areas. The apical groove was a favoured site and this was consistent with the observation that *B. knoxi* shell blisters were common in the ventral shell apex region. Fouling organisms with calcareous shells such as spirorbids and to a lesser extend *Pomatoceros* sp. and Pacific oysters were also targeted by settling *B. knoxi* larvae. It is unclear whether larvae target these areas because they are relatively easy to penetrate or because they provide shelter like the apex groove and other "shell irregularities". Zottoli and Carriker (1974) noted that *P. websteri* larvae settle in crevices present in oyster shells. Certainly the tubes of dead spirorbids would appear to provide an instant shelter for settling *B. knoxi* larvae.

The presence of *B. knoxi* has previously been recorded in some of these fouling organisms in New Zealand. Read (1975) noted the presence of the species in "serpulid tube masses" and Handley (1997) recorded the presence of *B. knoxi* in *Pomatoceros* sp and Pacific oysters.

The higher rate of settlement on the spirorbid fouled, relative to the "clean" group of experimental animals has implications for abalone culture in *B. knoxi* susceptible areas. Although the extent of fouling in the spirorbid positive group was high for animals in land based systems during the study period it was not remarkably for abalone transferred to sea based study sites for a period of time. As spirorbid settlement was observed mainly in the spring months, post spring abalone transfer should result in relief from the spionid enhancement effect associated with spirorbids.

Simultaneous transfer of 3 different sized stock groups resulted in significantly higher spionid infestation of the largest group (mean length 51 mm) following a single spring/summer spionid settlement season. Interestingly, abalone transferred at less than 20 mm attracted little spionid settlement initially and subsequently for two years while growing to in excess of 50 mm. By contrast, abalone initially stocked at 47 mm and spirorbid positive acquired greater than 50 spionids per animal after two years in the sea. The shell location settlement data and this size/growth data suggest that spionid settlement is not simply passive and could be influenced by spionid behaviour.

The significance of stock size on mud worm settlement has rarely been mentioned in cultured mollusc mud worm studies. One exception is Handley (1997) who suggested that small oyster stock were not attractive for settlement as a possible explanation for their failure to become infected with *B. knoxi*. Studies of wild abalone and mud worm have also shown increasing infestation with increasing size (Kojima and Imajima 1982, Clavier 1989). The former authors found a minimum shell length of 29 mm before *H. diversicolor* became infested with *Polydora* sp. The incidence and severity of infection increased with increased abalone shell length.

The pattern of mud worm infestation over a *B. knoxi* settlement period differed between rearing container types at both study sites. Infestation levels of the mud worm species *P. hoplura* increased in both tube and basket type rearing vessels by a factor of approximately 10. In contrast, *B. knoxi* chimney and/or worm counts increased significantly in basket type but not in tube type rearing vessels at both sites.

Dissection of spionid blisters showed that *P. hoplura* produced brooded (lecithotrophic) larvae from nurse eggs (section 5.1). Such larvae leave the maternal burrow at a relatively large size and may omit a planktonic swimming stage (refer to reviews by Woodwick 1977, Skeel 1979, Radashevsky 1994). On several occasions *P. hoplura* larvae were observed to form new burrows adjacent to the maternal burrow. It is plausible therefore that the increase in *P. hoplura* numbers in all rearing container types was substantially driven by reproduction of worms present in infested

stocks. In contrast to the strategy of *P. hoplura*, *B. knoxi* in Tasmania appears to produce only planktonic larvae as previously reported by Handley (1997, 2000) in New Zealand. Such larvae spend 2-3 weeks in the plankton after release from capsules at a relatively small size (~ 500 µm). Therefore, by the time such larvae are ready to settle (1500-2000 µm) they may have been moved considerable distances by currents and wave action.

The smaller mesh area on tube type rearing containers may reduce the probability of *B. knoxi* larvae from the outside gaining access to the stock, relative to the more open basket type culture vessels. Thus *B. knoxi* numbers increased in the more easily accessed stocks and remained static in the more sheltered stocks. Escape of hatching planktonic larvae from rearing containers may be explained as an active process since such larvae were observed to be strongly phototaxic as previously noted by Handley (1997). They would therefore be expected to swarm towards the light emitting mesh areas of the tube type rearing containers and swim out and up towards the surface.

Thus mud worm settlement on abalone may depend on the interactions between reproductive strategy of the polychaete species and the effective water exchange of the containment vessel. Whether restriction of water flow in abalone rearing containers as a means of risk reduction for *B. knoxi* infection is a worthwhile strategy is unclear. There must be a point where restriction of water flow is detrimental to abalone health. However, with respect to cleaning of rearing vessels or transfer of stock from fouled to clean containers it may be worth considering the timing of such events in relation to *B. knoxi* settlement.

Spionid settlement data showed *P. hoplura* infestation was greatest 9 m from the surface, or more importantly 1 m from the bottom. Greater sediment can be expected near the bottom and this has been considered in the past to have a role in spionid infestation of oysters. Nell and Smith (1988) state that some oyster growers in NSW spray mud from oysters as this is considered to reduce the risk of mud worm infestation. Following experiments on intertidal oyster culture and mud worms, Handley and Bergquist (1997) recommended that oysters be grown at least 50 cm above the bottom to optimize spionid avoidance. Likewise Caceres-Martinez et al. (1998) found placement of oysters away from the bottom may reduce the prevalence of *Polydora* sp. Pregenzer (1983) found that spionid infestation (which included *P. hoplura*) of mussels was greatest on the bottom and in silty areas.

At some culture facilities empty shells and feral Pacific oysters may be found on the bottom and could serve as hosts for *P. hoplura.* The limited dispersal ability of *P. hoplura* may therefore increase the risk of colonisation in near bottom reared stocks at some locations. By contrast, *B. knoxi* with a long planktonic larval phase has greater scope for dispersion. As in the present study, Handley (1997) found no difference in *B. knoxi* settlement rates in oysters at two depth treatments.

Differences in respiratory pore size and shell sculpturing did not result in differential spionid settlement between *H. rubra* and *H. laevigata*. This was possibly due to the very low rate of spionid settlement generally in the experiment.

In summary, Larger stock (~50 mm) attracted greater mud worm settlement and subsequent shell damage than stock of ~35 mm or smaller. Likewise spirorbid fouling was found to enhance spionid settlement, especially that of *B. knoxi*. Thus if there is a requirement to transfer stocks to spionid susceptible sea based sites during or soon before mud worm dispersal periods the use of smaller and relatively spirorbid free animals would minimize risk. It would be prudent to place larger abalone after the completion of the spring settlement period. Stock growth rates and cost structures for sea based sites will determine whether individual farms use these sites for all or part of the grow-out phase. The settlement intensity of *B. knoxi* was seen to vary

considerably depending on the design of the abalone containment vessel. Settlement of *P. hoplura* but not *B. knoxi* was greater on abalone reared near the bottom than higher in the water column. Thus the design of rearing vessels, especially with respect to water flow and their placement in the water column has implications for farming of abalone in mud worm susceptible areas.

5.5 Abalone health I: mortality, growth & condition

5.5.1 Mortality

Generally, there was little mortality that could be attributed to spionid mud worm infestation during field studies 1998-2001. Approximately 9000 abalone were transferred to the study sites for experimental work detailed in sections 5.1-5.4 and the present section. Of these only a few hundred animals became heavily mud worm infested. The two most heavily infested cohorts provided a contrast in mortality data.

At Aquatas the heavily infested August 1998 intake group experienced mortality of 48 of 61 (79%) remaining abalone between November 1999 and June 2000. Whereas, in the similarly infested, same intake time cohort at Huon Aquaculture there were 3 (from initial pool of 200) abalone deaths between May 1999 and Feb 2000 featuring severe blistering. In the minimally mud worm infected spring 1998 intake at Huon Aquaculture there was a 7% mortality rate over the length of the study.

5.5.2 Growth

Growth was generally poor by commercial abalone culture standards as shown in Table 5.30. The August 1998 intake at Aquatas that experienced considerable mortality showed a mean reduction in weight over the course of the study. Growth data is presented in more detail below for those groups where there was a significant degree of mud worm infestation.

August 1998 Cohort 1 Huon Aquaculture (long term treatment comparison)

Growth comparison between mud worm infested stock and the matched group treated by drying to reduce infestation levels is shown in Figure 5.1. At the time of treatment (December 1998) there was no significant difference in length of the 2 groups but by July 1999 the air dried group were significantly longer ($P<0.01$, $t=-2.79$, df 152, two-tailed t-test). Based on group mean comparisons treated stock grew at 57 microns.day $^{-1}$ compared to 44 microns.day $^{-1}$ for untreated stock. This is a growth depression of approximately 20% attributable to the level of mud worm infection present in the time period . As seen in Figure 5.1 growth of both treated and untreated abalone was poor in this intake cohort after July 1999. Between July 1999 and October 2000 both groups grew only another 3 mm corresponding with a reduction in feeding frequency from approximately weekly to twice monthly.

August 1998 Cohort 2 Huon Aquaculture.

This group was reared in “tube type” vessels throughout the length of the study. As seen later, this group was subject to very high levels of mud worm infestation including shell blistering and grew from approximately 46 to 67 mm in over 2 years (Table 5.30).

Table 5.30 Size and growth data for abalone at three farm sites, mean ± SD (n)

	Huon Aquaculture Company					
cohort	Aug 1998 (1)	Aug 1998 (2)	Spring 1998	Spring 1999	Dec 1999	Apr 2000
Date in	11/8/98	11/8/98	9/9/98	23/9/99	13/12/99	11/4/00
Date out	10/10/00	21/11/00	9/1/01	11/11/00	9/1/01	9/1/01
Time (d)	791	833	843	473	392	274
Initial length (mm)	46.9 ± 2.6(100)	46.2 ± 3.1 (100)	18.8 ± 1.4 (150)	42.0 ± 6.1 (100)	28.2 ± 3.2 (100)	31.5 ± 4.3 (172)
Initial weight (g)	15.4 ± 2.8 (100)	14.7 ± 2.9 (100)	1.0 ± 0.2 (150)	10.7 ± 4.5 (100)	2.9 ± 1.0 (100)	4.2 ± 1.8 (172)
Final length (mm)	61.8 ± 5.5 (23)	66.9 ± 7.1 (49)	57.6 ± 6.0 (94)	43.4 ± 4.7 (10)	42.6 ± 5.3 (10)	44.7 ± 5.0 (10)
Final weight (g)	36.0 ± 10.1 (23)	46.7 ± 13.0 (49)	25.0 ± 11.4 (88)	12.2 ± 3.9 (10)	11.9 ± 5.4 (10)	13.4 ± 3.9 (10)
Growth: length ($\mu.d^{-1}$)	18.8	24.9	46.0	3.2	36.7	48.3
Growth:weight ($mg.d^{-1}$)	26.1	38.4	28.5	3.5	22.9	33.7
SGR length	0.035	0.045	0.133	0.008	0.105	0.128
SGR weight	0.108	0.139	0.383	0.031	0.361	0.427

Huon Aquaculture August 1998 intakes: (1) = control group from long term mud worm treatment experiment, (2) = stock reared in "tube" type containers

	Aquatas				Site 3 (East Coast)
cohort	Aug 1998	Spring 1998	Spring 1999	Dec 1999	April 2000
Date in	11/8/98	9/9/98	23/9/99	13/12/99	11/4/00
Date out	13/6/00	12/11/00	10/10/00	11/4/00	29/11/00
Time (d)	671	793	382	119	278
Initial length (mm)	46.9 ± 2.7 (200)	20.9 ± 2.1 (50)	39.1 ± 4.3 (100)	26.1 ± 3.6 (100)	33.8 ± 4.6 (10)
Initial weight (g)	15.7 ± 2.8 (200)	1.2 ± 0.4 (50)	8.5 ± 2.7 (100)	2.4 ± 1.0 (100)	9.5 ± 3.7 (10) *
Final length (mm)	47.6 ± 2.5 (10)	34.5 ± 4.4 (18)	40.3 ± 3.6 (10)	27.5 ± 3.7 (20)	50.4 ± 6.8 (10)
Final weight (g)	12.8 ± 2.7 (10)	5.5 ± 1.7 (18)	9.2 ± 2.9 (10)	2.7 ± 1.1 (20)	20.5 ± 8.1 (10)
Growth: length ($\mu.d^{-1}$)	1.0	17.1	3.0	12.0	71.6
Growth:weight ($mg.d^{-1}$)	-4.4	5.3	1.8	3.1	62.3*
SGR length	0.002	0.063	0.008	0.045	0.172
SGR weight	-0.031	0.186	0.020	0.122	0.435*

* = First Site 3 weight data taken June 6 2000, later than first length data.

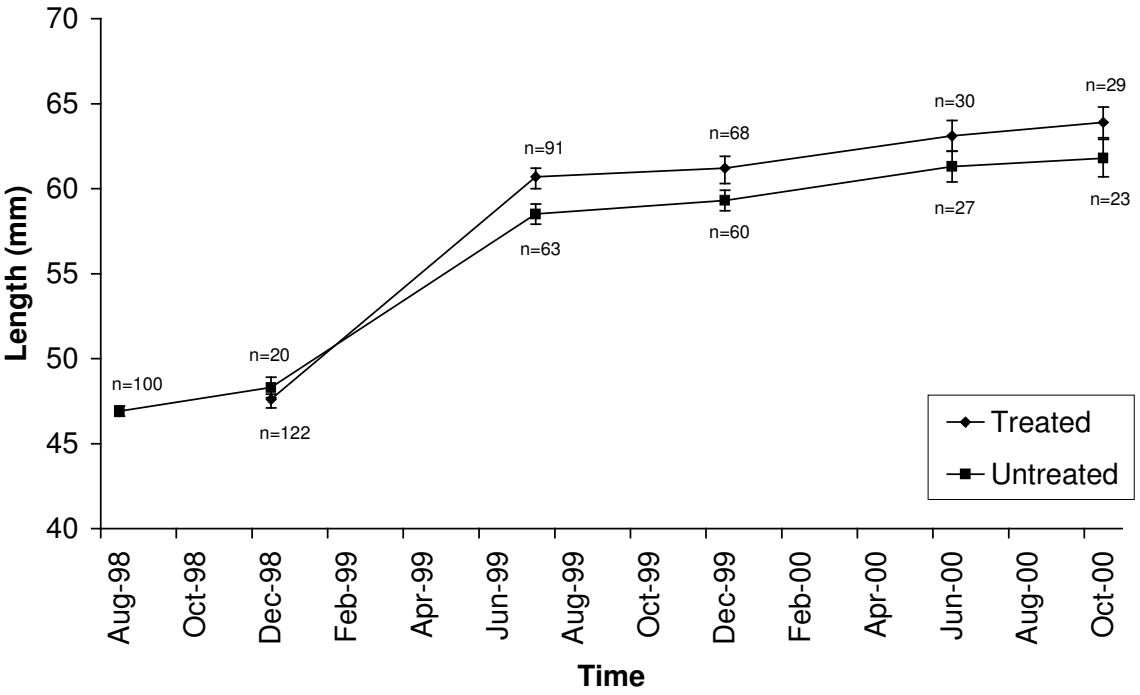


Figure 5.1 Length comparison for mud worm treated and untreated abalone. Huon Aquaculture August 1998 intake (1). Means ± SE.

Growth of other abalone intake time cohorts

The spring 1998 cohort at Huon Aquaculture Company was stocked at approximately 20 mm or less and approached market size (~60 mm) about 2 years later in early January 2001 (Table 5.30). Specific growth rates (Table 5.30) were similar for the spring 1998, December 1999 and April 2000 cohorts at Huon Aquaculture.

Poor growth of stock at Aquatas generally was linked to increasingly infrequent feeding in the second half of the project. Stock sampled at Site 3 (East Coast) grew faster than stocks at the other study sites (Table 5.30). All 3 sites used the same sources for spat and feed. Aquatech® trays were used to grow the stock at Site 3 and for some intake cohorts at Aquatas and Huon Aquaculture.

5.5.3 Shell blistering and spionid counts

Shell blistering was most common in the apex area of the shell, with blisters in the vicinity of the respiratory pores the second most common. (Table 5.31). Blisters caused by *B. knoxi* worms were located overwhelmingly in the shell apex area (63%) contrasting with *P. hoplura* blisters that tended to be more evenly spread around the host shell (Table 5.31).

Table 5.31 Blister location by spionid species

Blister location	Mixed spionids (n=600 shells)	*B. knoxi* (n=81 shells)	*P. hoplura* (n=70)
Apex	31%	63%	31%
Expanded apex	9%	5%	0%
Respiratory pores	26%	19%	33%
Muscle scar	2%	3%	1%
Leading edge	10%	3%	11%
Other areas	22%	7%	24%

Early mud worm blisters were flat and yellow in colour, a result of initial conchiolin deposition (Blake and Evans, 1972). Actively growing *B. knoxi* blisters tended to be amber to light brown in colour and were frequently located in the shell apex as indicated by Table 5.31. Eventually such blisters usually developed a thick coat of nacre over them contrasting with the soft, non-healed blisters associated with the initial mortality reports.

There was considerably variability of blister location with *P. hoplura* infestation including damage to the leading edge, respiratory pores, apex and other areas. With time *P. hoplura* blisters became dark brown/ black in appearance unless substantial shell nacre was deposited over them. Older blisters of both spionid species were raised up to 5 mm from the base of the shell. Long-term infested abalone usually had a mixture of spionid species and corresponding damage to the shell. A range of shell blistering is presented in Figure 4.6 (methods section).

The volumes of 10 representative large blisters from the apex area of the shell were measured. These blisters, caused by *B. knoxi* and had volume range of 0.1 to 0.6 ml. By contrast the volume of a sample of large *B. knoxi* blisters measured in Pacific oysters was an order of magnitude greater, with one oyster recording a volume of 10 ml. Histological cross sections of shell, including blisters, mud worms and blister detritus are shown in Figures 5.2 and 5.3.

August 1998 cohorts

In cohort 1 at Huon Aquaculture shell blistering was detected September 1998, one month after transferto the site (Figure 5.4). "Active" category blisters (section 4.9) dominated samples for the first few months but by 1999 blisters assigned to the "healed" category were common, indicating nacre was being deposited. By October 2000 shell blistering in the untreated control stock had reached approximately 30% of shell area (Figure 5.4). This was comparable blister coverage to that associated with the initial abalone mortality episodes of the mid 1990's (introduction). Comparison of % total blister coverage in treated stock (air dried in December 1998 and 1999) and control untreated abalone is shown in Figure 5.5. REML analysis with time and treatment as factors was highly significant ($P<0.001$) for both factors. Untreated stock were significantly more blistered than treated stock from the same time sample in March and October 2000 (Figure 5.5).

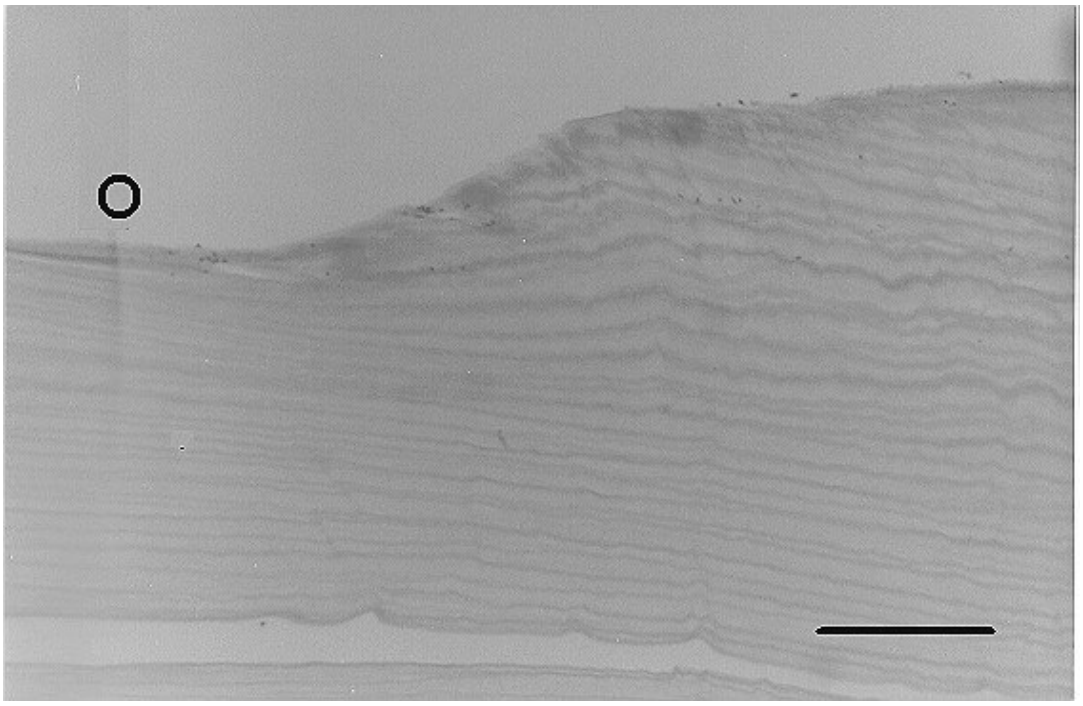


Figure 5.2 Normal abalone shell structure, O = outside of shell, Bar = 100 μm

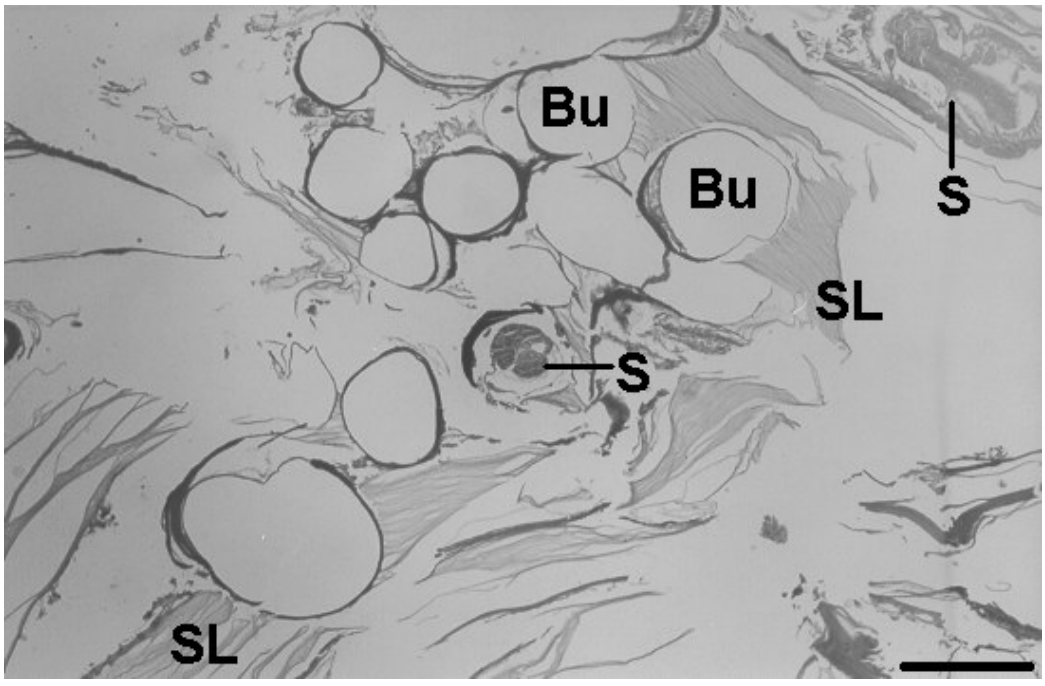


Figure 5.3 Cross-section of abalone shell honey combed with spionid burrows. SL = disrupted shell layers, BU = spionid burrows, S = spionid in cross-section. Bar = 500 μm

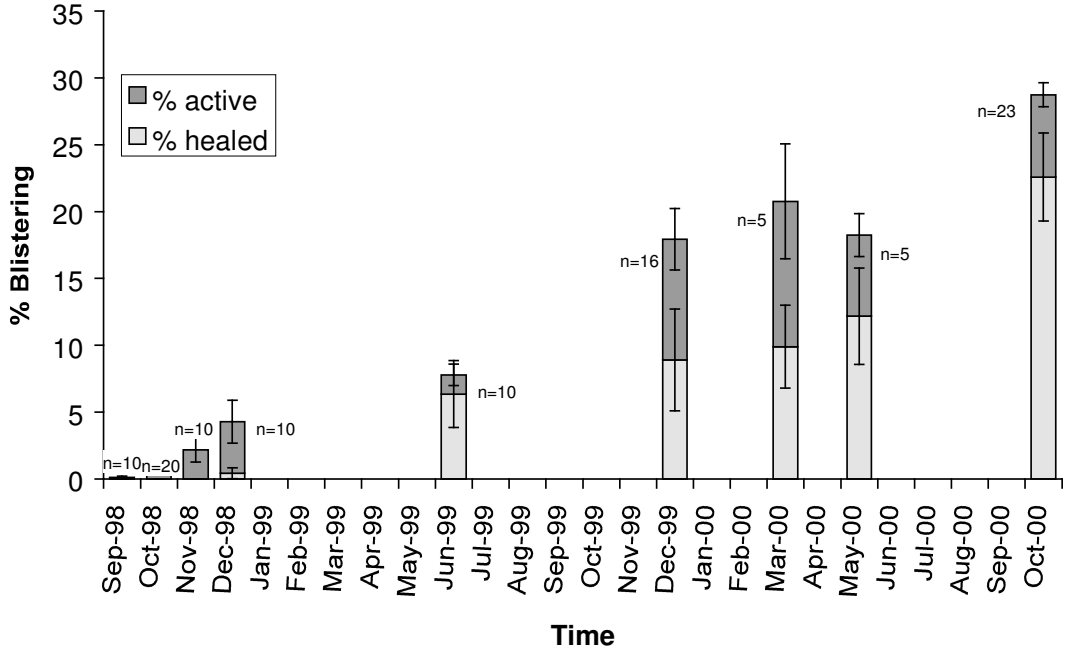


Figure 5.4 Progression and nature of shell blistering Huon Aquaculture August Intake cohort 1 (untreated control abalone) means ± SE

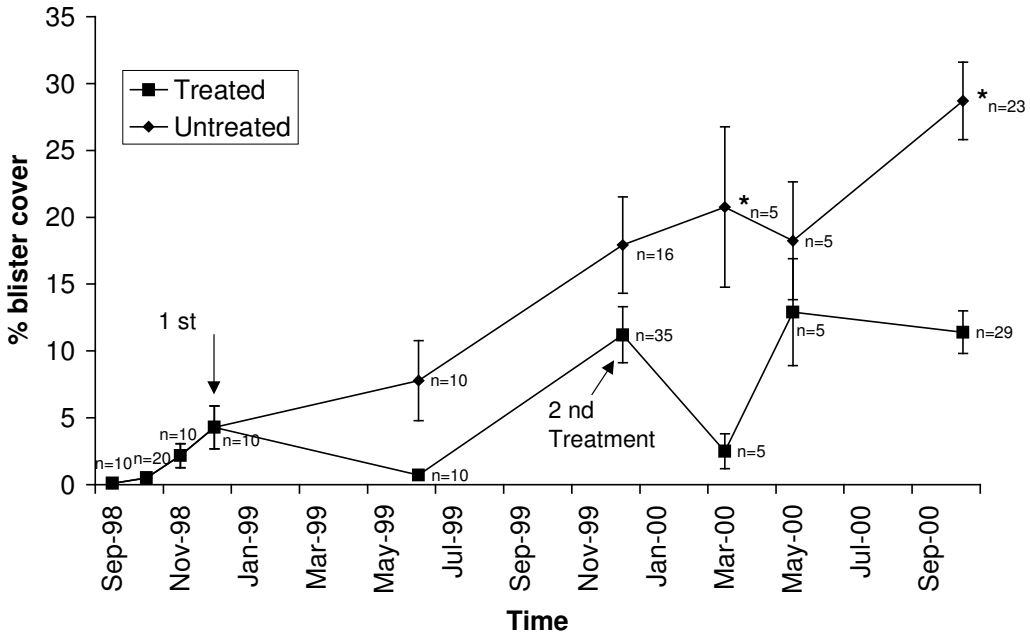


Figure 5.5 Mud worm blister comparison for treated and untreated abalone Huon aquaculture August 1998 intake (means ± SE). * denotes significantly different data Pairs (P<0.05).

Subjective blister data showed the maximum SSDR value of "3" was recorded in samples from untreated stock in 2000 but not in previous years . The treated abalone recorded no Grade 3 SSDR scores and contrasting with the untreated stock recorded some zero SSDR scores during 2000. Mann-Whitney U Test analysis of SSDR data for October 2000 (final sample) showed a significant difference between the treated and untreated stock ($P<0.01$, $U=196$).

Spionids were present in abalone shells within a month of transfer and mean counts were approximately 50 per shell by May 2000 (Figure 5.6). This level was previously associated with stock mortality (background, section 1.3). However, a dominance of *B. knoxi* worms were previously recorded in the shells of dead and dying abalone at the site, contrasting with this study where *P. hoplura* dominated numerically (Figure 5.6). Air drying treatment (December 1998 and 1999) significantly reduced mud worm counts over time (Figure 5.6). Two-way ANOVA performed on log transformed data with time and treatment as factors was highly significant ($P<0.001$ for both factors and for both spionid species). Counts of *B. knoxi* in untreated stock were approximately five times higher than in air dried stock over the duration of the study. This difference was statistically significant at every time sample ($P<0.05$). Similarly, counts of *P. hoplura* were significantly ($P<0.05$) higher in untreated stock at all sample times except June1999. Spionids of both species increased markedly in number in untreated abalone post June 1999 after the second spring/summer mud worm reproductive period. Treatment efficacy data for the 2 drying periods are reported in section 5.3.1 (Trials 6 and 10).

In the second August 1998 intake cohort at Huon Aquaculture low level shell blistering was also detected a month after transfer to the site. Blisters categorised as "active" dominated during the first spring until autumn the following year (1999). Mean total blister coverage reached approximately 35% by January 2000 and remained at this level until final sampling in November 2000 (Table 5.32). There was a significant inverse relationship between % total blister and both final length (Figure 5.7) and whole weight of abalone (df 1 47, $P<0.001$ for both). Full regression analysis summaries including estimates of parameters and significance of r values are shown in Appendices 5P and 5Q.

The pattern of spionid infestation in cohort 2 at Huon Aquaculture was similar to that of August 1998 cohort 1 untreated control stock until early 2000. By November 2000, however, total spionid numbers had increased in cohort 2 from approximately 50 to 160 worms per abalone (Table 5.32). Approximately one third of *P. hoplura* were juveniles no more than 5 mm long and considered less than 3 months in age. Count data showed that *P. hoplura* was more common than *B. knoxi* in both August 1998 cohorts at this site, but more so in relative and absolute terms in cohort 2 compared to cohort 1 (Table 5.32). This was attributed to an interaction between spionid reproductive strategy and rearing container type (section 5.4.4).

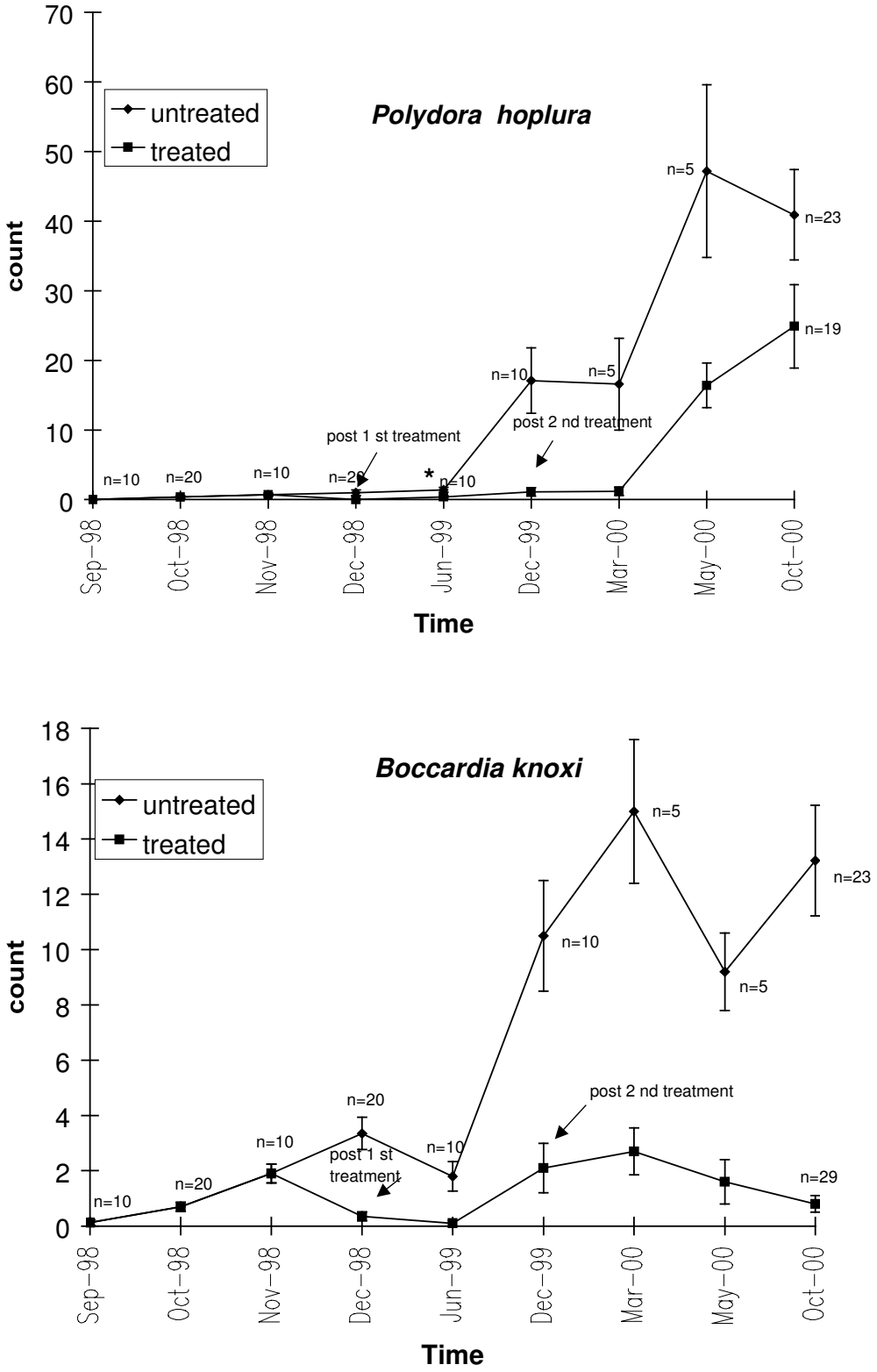


Figure 5.6 Mud worm counts for Huon Aquaculture August 1998 intake (cohort 1) treated and untreated abalone (means ± SE, n values equivalent for each data pair unless indicated Sample time data pairs marked with an * are not significantly different (P>0.05).

Table 7.2 Final % blister coverage and spionid count data for abalone at three farm sites, mean ± SE (n)

	Huon Aquaculture Company					
cohort	Aug 1998 (1)	Aug 1998 (2)	Spring 1998	Spring 1999	Dec 1999	Apr 2000
Date in	11/8/98	11/8/98	9/9/98	23/9/99	13/12/99	11/4/00
Date out	10/10/00	21/11/00	9/1/01	11/11/00	9/1/01	9/1/01
Time (d)	791	833	843	473	392	274
Shell blistering	28.7 ± 2.9 (23)	35.2 ± 1.8 (49)	1.9 ± 0.5 (64)	2.8 ± 1.5 (10)	0.2 ± 0.2 (10)	0.3 ± 0.3 (10)
B. knoxi	13.2 ± 2.0 (23)	2.1 ± 1.0 (49)	0.4 ± 0.1 (64)	***0.1 ±0.1 (10)***	0 ± 0 (10)	0 ± 0 (10)
P. hoplura	40.9 ± 6.5 (23)	163.5 ± 10.0 (49)	0.8 ± 0.2 (64)	5.2* ± 1.7 (10)	0.1 ± 0.1 (10)	0 ± 0 (10)
Total spionids	54.1 ± 5.9 (23)	165.6 ± 8.3 (49)	1.2 ± 0.2 (64)	5.3 ± 1.7 (10)	0.1 ± 0.1 (10)	0 ± 0 (10)

Huon Aquaculture August 1998 intakes: (1) = control group from long term mud worm treatment experiment, (2) = stock reared in "tube" type containers
* most worms newly settled < 3 mm

			Aquatas		Site 3 (East Coast)
cohort	Aug 1998	Spring 1998	Spring 1999	Dec 1999	April 2000
Date in	11/8/98	9/9/98	23/9/99	13/12/99	11/4/00
Date out	13/6/00	11/11/00	10/10/00	11/4/00	29/11/00
Time (d)	671	793	382	119	278
Shell blistering	17.5 ± 4.0 (10)	8.0 ± 2.0 (18)	3.1 ± 1.4 (10)	0.1 ± 0.1 (20)	0 ± 0 (10)
B. knoxi	3.1 ± 1.0	0.7 ± 0.2 (19)	2.3 ± 0.7 (10)	0 ± 0 (10)	0 ± 0 (10)
P. hoplura	3.4 ± 0.8	3.7 ± 0.7 (19)	0.1 ± 0.1 (10)	0 ± 0 (10)	0 ± 0 (10)
Total spionids	6.5 ± 1.3	4.4 ± 0.6 (19)	2.4 ± 0.7 (10)	0.1 ± 0.1 (10)	0 ± 0 (10)

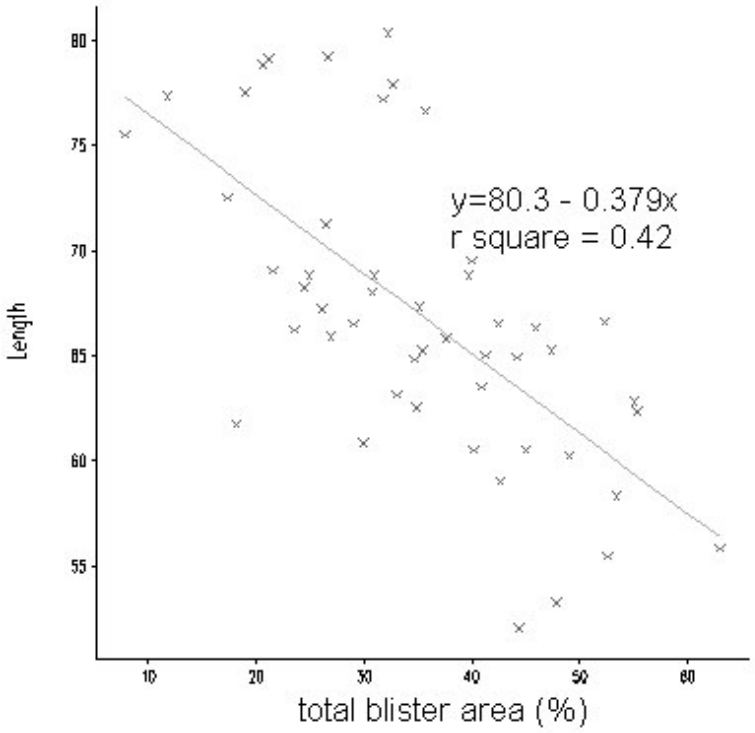


Figure 5.7 Total blister coverage versus length for Huon Aquaculture August 1998 (intake 2) abalone

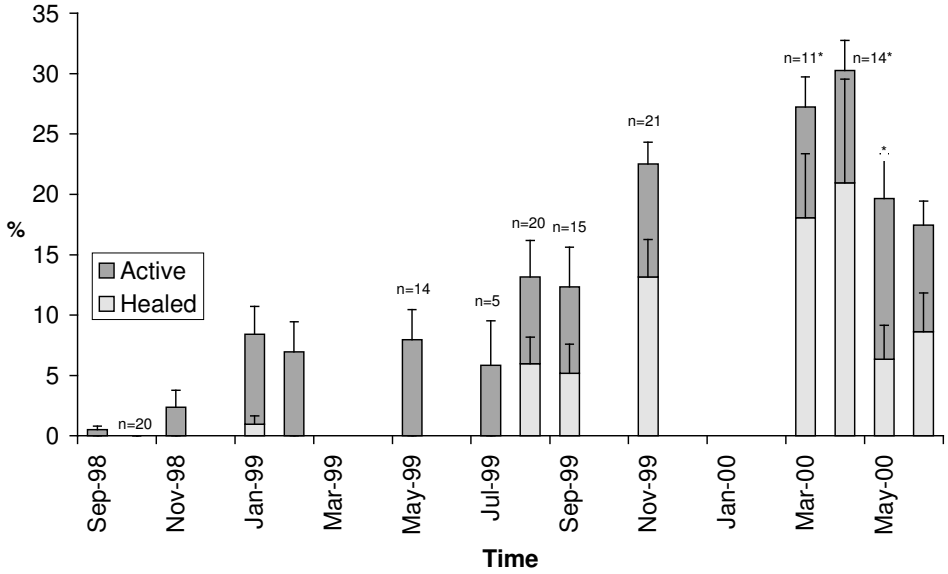


Figure 5.8 Progression and nature of shell blistering Aquatas August 1998 intake. Means ± SE, n=10 unless otherwise specified. * = sample of "dead" shells

At the Aquatas site, blistering of the August 1998 intake abalone increased steadily with little nacre present on blisters in the first year. Maximum blister coverage exceeded 25% in March and April 2000 as stock mortality was recorded (Figure 5.8). Maximum recorded blister levels were not significantly different (U=116, P>0.05, Mann-Whitney U-test) between similarly reared August 1998 intake cohorts at Aquatas (30.3% ± 9.0, 14) and Huon Aquaculture (28.7% ± 2.4, 23), ($\overline{X}$ ± SE, n). Final samples from Aquatas had a low proportion of healed blisters (<50%, Figure 5.8) compared to the comparable Huon Aquaculture samples (Figure 5.4). The subjective shell damage rating system indicated a rapid decline in the proportion of zero rated (blister free) shells in the first 6 months after transfer. Rating 2 (moderately blistered) shells dominated by the late spring 1999 and a small proportion of severe blister ratings (SSDR = 3) occurred in shell samples post November 1999.

Spionid counts were significantly lower (U=0.0, P<0.01 Mann-Whitney U-test) for the last Aquatas sample in June 2000 (6.5 ± 1.3, n=10) compared to a similar (May 2000) Huon Aquaculture sample (56.4 ± 8.6, n=5), ($\overline{X}$ ± SE). Spionid counts were generally <10 per sample for this intake at Aquatas (Figure 5.9), although as previously indicated maximum blister levels were similar to more infested stock at Huon Aquaculture.

Data for other intake times

Spionid infestation and subsequent shell blistering was minimal in stocks transferred to Huon Aquaculture and Aquatas in the spring months of 1998, 1999 and December 1999 (Table 5.32). After exposure to 3 successive spring/summer spionid dispersal periods the Huon Aquaculture spring 1998 intake had <2 spionids per abalone by January 2001 (Table 5.32). Significantly less, (U=0.0, P<0.01 Mann Whitney U-test) than the lesser infested of the 2 August 1998 intakes (Table 5.32). Mean blister coverage was similarly low at <2% by January 2001. This group grew from 18 to 58 mm during the study period (Table 5.30) but spionid infestation did not increase significantly with size. Placement of abalone at study sites in December 1999 to avoid presumptive *B. knoxi* settlement (section 5.1) was successful at Huon Aquaculture with no worm of this species recorded by January 2001. Similarly no *B. knoxi* was recorded at Aquatas up to and including April 2000, following which time the baskets were lost (Table 7.2).

Stock present at Site 3 on the east coast of Tasmania were sampled 7 times (n=10 per sample) between April 2000 and January 2001. Both *B. knoxi* and *P. hoplura* were present in the cumulative total of 4 adult spionids and 10 recently settled larvae. Only 1 mud worm blister, covering 10% of the shell was detected.

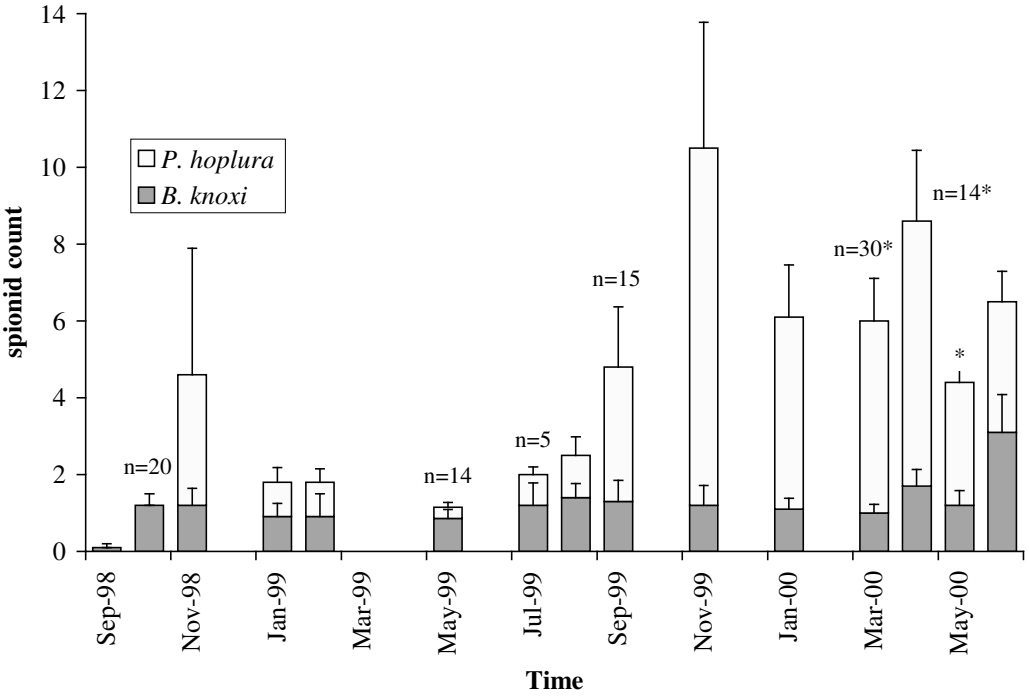


Figure 5.9 Temporal change in spionid count and species mix in August 1998 abalone intake at Aquatas. Means + SE, n=10 unless otherwise shown.

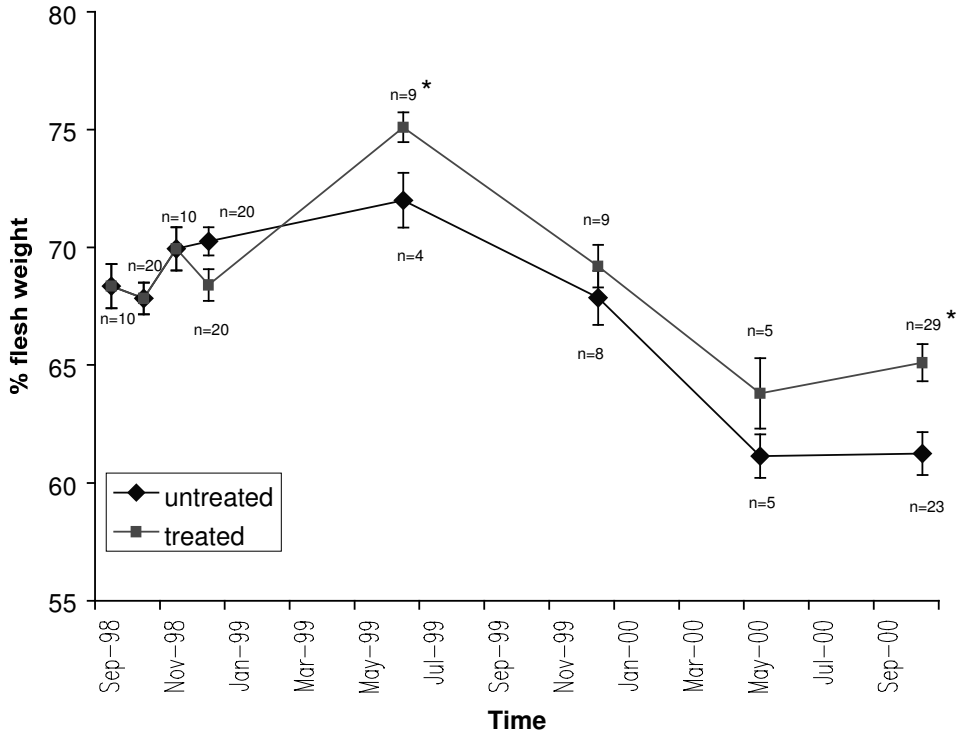


Figure 5.10 Percentage flesh weight comparison: Treated and untreated abalone, August 1998 intakes at Huon Aquaculture (means ± SE, n values shown.) Data pairs denoted with an * are significantly different (P<0.05).

5.5.4 Measures of abalone condition

Preliminary assessment of various condition indices was made to ascertain the most suitable for long-term health monitoring. Regression analysis showed there was a highly significant relationship between weight and CI_{LENGTH} (df 1 41, P<0.001) with the condition index increasing with weight of stock. Further, the dry weight index CI_{LENGTH} increased as a function of shell length (df 1 41, P<0.001).Thus, as CI_{LENGTH} increased with stock size it was unsuitable for long term-term studies where growth was expected. This was also true of the relationship between percentage dry flesh weight data and length (df 1 41, P=0.006) and weight (df 1 41 P=0.039).

By contrast, there was no significant relationship between length and percentage wet flesh weight (df 1 41, P=0.401) and weight and percentage flesh weight (df 1 41 P=0.361), indicating % flesh weight was independent of these measures of animal size. In both regressions the residual variance exceeded the variance of the response variance (percentage flesh weight) and no r-squared value could be calculated.

Regression analysis of shell length and CI_{WEIGHT} was also not statistically significant (df 1 41, P=0.073) and nor was that between whole wet weight and CI_{WEIGHT} (df 1 41, p=0.107). Although the probability values for the likely hood of a significant relationship between CI_{WEIGHT} and the two measures of abalone size were >0.05 they were much closer to being statistically significant than those of percentage flesh weight and abalone size, measured by weight and length. Thus percentage flesh weight was felt to provide a better measure of relative "fleshyness" or condition than either dry weight condition index.

A normal range for percentage flesh weight data was established by calculating means for 24 samples of 10 abalone between 20 and 70 mm in length. Mean percentage flesh weight was 71.1% (SD=2.1%, n=24). By contrast, an assessment of % flesh weight on remnant stock infested with mud worm in the mid 1990's and held at Tasmanian Tiger Abalone showed mean % flesh weight of 52.6 (SD=3.1%, n=10). These abalone had blistering to approximately 25% of their shells with approximately one third having the maximum SSDR of 3. Data for abalone transferred and spionid infested during 1998-2001 is presented below.

Huon Aquaculture August 1998 cohort 1

Two-way ANOVA after arcsine transformation of % flesh weight data found P values of <0.001 and 0.007 for time and treatment as factors respectively. Air dried stock samples were approximately 3 percentage points higher than those of non-treated control stocks following the initial December 1998 treatment episode (Figure 5.10). This differential was maintained throughout the length of the study. Percentage flesh weight of treated and untreated stock fell from peak values in May 1999 (Figure 5.10). This coincided with decrease in growth and increases in blistering and mud worm counts for both air dried and untreated stock. The final sample value for the untreated control stock was 61.2% (SD=4.3, n=23), significantly lower (P<0.01, U=18.0, Mann-Whitney U Test) than the mean for the established normal range of 71.1% (SD=2.1%, n=24).

Discussion

Data on spionid impacts affecting abalone health relate to project objective 2 on interactions between mud worms and abalone and in part to objective 3 on long term efficacy of drying treatment.

Early pilot scale, sea based abalone grow out ventures in southern Tasmania were severely compromised by stock mortality associated with high spionid mud worm counts and shell blistering. With one exception, in the present study, there was little evidence of mud worm related mortality in stock transferred to 2 sites with a previous history of severe infestation. Some stock were exposed to mud worms for up to 27 months, acquired in excess of 50-150 worms and blistering to approximately one third of the shell area yet did not suffer the mortality episodes previously seen at the site.

Stock mortality occurred in one intake time group (August 1998) at one site but not the other. Shell blister damage levels were similar in this intake time group at both sites but there was steady, albeit, slow growth of the stock at one site but not the other. At the latter site stock mortality began approximately 18 months after initial stocking and subsequent spionid colonisation. Growth data indicated suppressed growth at the Aquatas site generally and this was considered to be largely due to relatively infrequent feeding. However, relatively poor nutrition alone, in the absence of significant spionid infestation did not result in mass stock mortality. Thus it appeared that high levels of shell blistering in addition to poor nutrition resulted in stock mortality. Whereas, high levels of spionids and shell damage, in the presence of adequate nutrition did not result in significant mortality at Huon Aquaculture.

The effect of spionid infestation on growth rate of abalone has not been addressed in previous studies on wild stocks but is of considerable interest to the grower. The comparison between spionid treated (air dried) and infected control abalone (August 1998, Huon Aquaculture cohort 1) showed that the air dried stock grew significantly faster in the 6 months post treatment (to June 1999). The suppression of the growth rate in the untreated infected stock was estimated at 20 percent. Maximum spionid infestation associated with this growth depression was 4 worms per abalone corresponding to blister coverage of 7%. Growth depression was also seen in section 5.4 where spionid infestation with 3-5 worms and subsequent blistering in spirorbid fouled stock caused a mean 28% reduction in shell growth compared to minimally infested control stock. Regression analysis on heavily infested abalone (Huon Aquaculture, August 1998 intake cohort 2) showed that size of abalone defined as both length and weight declined with increased blister cover.

Of the many stock cohorts placed at the study sites for temporal health studies (reported in this section), risk factor analysis, or larval dispersal experiments only the August 1998 intakes became heavily blistered. Blister coverage of approximately 30% was seen in the present study as in the previous abalone mortality episodes that led to this research. However, while blister coverage levels were similar, SSDR data from remnant stock surviving the original mortality episode until 1997-1998 (Appendix 3,1B) suggested the original shell damage episodes were more severe. Those previously seen blisters were often very soft consisting of 5 mm or more of apparent conchiolin material over the ventral surface of the shell. Blisters of this nature were absent during the course of the present study and the maximum SSDR score was rarely used. Large blisters in the present study were usually covered with a hard layer of pearly nacre. Possibly factors such as nutrition could account for some of the differences between blisters in past and present episodes. Certainly, in Australia

considerable research and development effort has gone into the area of formulated abalone diets since the original spionid outbreaks.

Blisters formed by *B. knoxi* worms were frequently located in the shell apex. This was consistent with the finding (section 5.4.2) that chimney structures created by this species were often located in the groove under the shell apex. It would therefore appear that *B. knoxi* is capable of burrowing directly through the shell of an abalone. Blake and Evans (1972) in reviewing mode of spionid blistering noted that two routes of host invasion were reported. In the first the larvae swim into the mantle cavity or burrow between the shell edge and the mantle. In the second spionid larvae settle on the outside and penetrate directly. This latter method is undoubtedly favoured by *B. knoxi* with initial settlement of the apical groove consistent with the report of Zottoli and Carriker (1974) that *P. websteri* larvae settle wherever there are crevices in oyster shells. The respiratory pores of abalone appear to be another favoured site for spionid settlement. Whether this is solely because they present a shell irregularity and thus a degree of protection or because spionid larvae actually enter the mantle cavity by this means is unclear. There is no doubt *P. hoplura* larvae can enter the abalone shell through the leading edge but the diversity of blister locations suggests they may also be capable of burrowing directly through the shell. Some second generation larvae appear to never leave the shell (section 5.1) creating new burrows adjacent to the maternal blister.

As noted earlier, the majority of the different intake time cohorts suffered negligible spionid infestation. The spring 1998 intake at Huon Aquaculture, stocked at 18 mm was exposed to 3 full or partial spring/summer mud worm settlement seasons and acquired an average of only 1 spionid per abalone. Interestingly, this group was minimally infested after the first spring exposure and remained that way despite subsequent growth to 58 mm (Table 5.30). The only stocks to become heavily spionid infested were the August 1998 intakes at both study sites. These animals have since been shown to be at higher risk due to some initial fouling with spirorbids, shell irregularities and greater stocking size. These data may give industry some confidence that the events of the mid 1990's were not typical.

Of the five mud worm species found to infest stock in the present study only *B. knoxi* and *P. hoplura* were present in sufficient numbers to be considered pest species. Interestingly, *P. hoplura* was far more numerous than *B. knoxi* in this study. This is the reverse of the pattern seen in the remnant stock from Huon Aquaculture that was used in the treatment options studies (sections 5.2 and 5.3).

Clavier (1989), by the use of a condition index found no physiological effect of mud worm infestation on abalone. However, the author was of the opinion that extreme infestations (not considered in the random sampling method used) were certainly detrimental to *H. tuberculata*. Kojima and Imajima (1982) found that 10 spionids per abalone reduced the flesh weight in a statistically significant way. In the present study percentage flesh weight declined significantly with time (as blistering increased) and for untreated infected stock compared to less infected air dried stock. The severe declines in percentage flesh weight seen previously (to 53% in remnant stock from original mortality reports) were not repeated in this study, with the exception of some cohorts that were underfed rather than heavily infested.

Considerable variability exists in the literature regarding lethal and sublethal effects of spionid mud worms in molluscs. Whitelegge (1890) investigated mass mortality in Sydney rock oysters *Saccostrea commercialis* Iredale and Roughley associated with *P. ciliata*, but later considered to be *P. websteri* (Blake and Evans,

1972). Whitelegge recommended changes to culture techniques including lifting oysters off the substrate and air exposure at low tide. These were highly effective and are still used today. Smith (1982, 1984) reviews the collapse of the New South Wales and Queensland oyster industries in the late 1880's and early 1900's and attributes a great part of the blame to mortality caused by spionid mud worms. Korringa (1952) reviews spionid infestation in oysters from the 1940's and early 1950's. In one case involving high levels of infestation widespread mortality occurred but generally the affects were more minor from a host health perspective.

Korringa (1952) states that when oysters contained over 25 *P. ciliata* or over 5 *P. hoplura* they showed poor growth and were often leaner than non infested oysters. The *P. hoplura* species is among the largest of the spionids that infest shellfish and usually considerably larger than *B. knoxi*. Therefore, it is true that larger worms cause greater damage to the host, then infestation by ~35 *B. knoxi* (as seen in remnant stock from the original outbreak examined in 1997**-** section 1.3) should be less serious than infestation by 150 *P. hoplura* (as seen in this study). Yet as this was not apparently the case it may be suspected other factors are involved.

The work of Owen (1957) contrasts with reports attributing oyster mortality to high levels of mud worm infestation. In this study 100% of the oysters studied were infested and the spionid *(P. websteri*) numbers exceeded 100 per host at some study sites. The author concluded that though in some areas a positive correlation was found between oyster mortality and degree of infestation this was not confirmed in controlled laboratory experiments. He concluded that *P. websteri* does not cause oyster mortality per se but contributes towards the formation of a poor environment.

Sub-lethal impacts of spionids on bivalve molluscs have reported by several authors. Kent (1979) found that heavy infestations of *P. ciliata* were associated with reduced flesh content in mussels. Wargo and Ford (1993) found a significant negative correlation between % blister coverage and condition index in the oyster species *Crassostrea virginica*. When half of the shell was blistered the condition index of oysters was reduced by up to one third. This study concluded that spionid infestation reduced the ability of the host to accumulate nutritional reserves. Handley (1997) found that *B. knoxi* produced a statistically significant negative impact on condition of sub-tidally cultured oysters but that the effect was too weak to have any biological significance. Cacerez-Marinez et al. (1999) found that heavy infestation of the black clam *Chione fluctifraga* Showerby with *Polydora* sp. could break the shell leading to increased predation.

In further studies Caceres-Martinez et al. (1998) found no correlation between *Polydora* sp. numbers and blister area with condition of the oyster species *C. gigas*. However, infestation levels were light with generally 1-3 worms recorded per shell. Similarly, low level of infestation (generally < 5 worms per shell) in intertidally cultured *C. gigas* was not considered to have any significant biological impact by Handley and Bergquist (1997). Low level infestation by *P. ciliata* in the Indian oyster *Crassostrea madrasensis* Preston was not reported to seriously injure the host (Stephen, 1978). Heavy infestations of a mixture of spionids were reported in the Japanese scallop *Patinopecten yessoensis* by Sato-Okoshi and Nomura (1990) but health effects if any were not mentioned.

Contrasting conclusions in previous studies as to the health impacts of spionids on molluscs may be due in large part to differences in the severity of infestation (measured by spionid count or blister damage), the size of the host, host species and other environmental factors such as food availability.

The long term treatment trial (objective 3, page 9) involving air drying in December 1998 and 1999 was highly effective in reducing infestation by *B. knoxi*.

Unfortunately *P. hoplura* rather than *B. knoxi* was the more numerous polychaete in this study - reversing the pattern seen during the original mortality episodes. Some post treatment infestation by *P. hoplura* meant that stock did not remain essentially mud worm free between treatments. Even so, in the long term the treatment regime resulted in lower infestation with *P. hoplura*, less blister damage and higher percentage flesh weight in treated as opposed to untreated control stock. The treated air dried stock also grew faster in the initial 6 months post treatment.

In summary, the majority of abalone intake time cohorts transferred to the 2 southern Tasmanian study sites failed to become significantly mud worm infested. This was despite some abalone being exposed to up to 3 successive annual spring/summer spionid larval dispersal periods. This is a positive finding for industry indicating as it does that spionid infestation of stock may be a comparatively rare event. Where abalone stocks did become heavily infested they appeared, in retrospective, to have been at increased risk of infestation in terms of size and shell fouling. None the less these stocks were able to sustain very high mud worm numbers and associated shell blister coverage for up to two years without significant mortality. Where stock mortality did occur abalone were exposed to poor husbandry conditions in addition to high levels of shell blistering. Spionid infestation was shown to potentially cause growth suppression and reduced flesh yield. A post larval settlement treatment regime based on air drying reduced spionid numbers and associated blistering. Monitoring of mud worm levels in future farmed stock from susceptible areas would be prudent so as decisions can be made as to whether treatment is appropriate.

5.6 Abalone health II: Physiology and histology

5.6.1 Clinical pathology data

Testing of apparently healthy abalone populations was conducted to ascertain a normal range for farmed abalone and allow comparison with moderately and severely mud worm infested stocks (Table 5.33). Only potassium ($P<0.05$, $t=2.98$, 18 df) and the Na^+/K^+ ratio ($P<0.001$, $t=-5.41$, 22 df) were significantly different (by 2 tailed t test) between spionid infested and control stocks for foot bleed data (Appendices 5R and 5S). Statistical analysis of the smaller data group for the cephalic sinus bleed site showed no significant differences between mud worm infested and normal abalone ($p>0.05$ Mann-Whitney U Test) for any of the variables. As for foot bleed data there was a rise in the Na^+/K^+ ratio in spionid infested compared to normal abalone for the cephalic sinus data (Table 5.33), but this was not significant. Testing of water samples from the abalone culture sites found a mean Na^+/K^+ ratio of 44.4 (SD=0.5, n=5).

Sufficient sample for full testing of electrolytes could not be consistently drawn from the cephalic sinus in abalone under 50 mm in length. Sampling haemolymph from the foot of abalone less than 20 mm length was also difficult. Samples taken from 15 mm abalone (including composite samples from 2 or more animals) had a Na/K ratio of 18.3 (SD=1.7, n=3), rising to 30.0 (SD=2.7, n=7) for 19 mm abalone and 32.2 (SD=2.7, n=5) for 28 mm abalone.

Sodium/potassium ratio values changed significantly with time at both Huon Aquaculture (df 8 69, $P<0.001$) and Aquatas (df 7 67, $P<0.001$). The initial control sample for both sites (August 1998) had a value of 34.0 (SE=0.65, n=20) while the final sample taken Oct 2000 at Huon Aquaculture had risen to 36.6 (SE=0.5, n=10)(Figure 5.11). This was a lower rise than that of the equivalent cohort at Aquatas where the final two Na^+/K^+ ratio samples in March and June 2000 exceeded

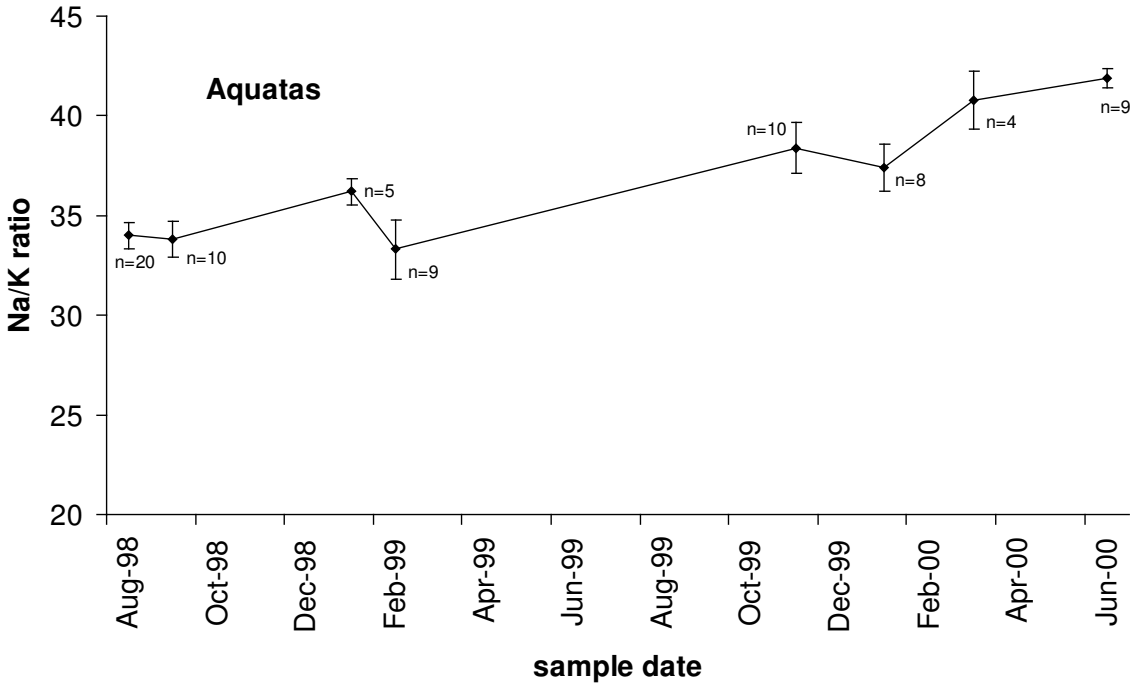
40 (Figure 5.11). Aquatas stock of this cohort had begun to die in early 2000 (section 5.5).

There was no significant difference by Kruskal-Wallis test ($H_c = 6.154 < \chi^2_{0.05, 3} = 7.815$) between Na^+/K^+ ratio data for monthly samples (Feb, Mar, Apr, May 2001) from experimentally starved abalone. The grand mean was 37.2 (SD=3.4, n=19).

Table 5.33 Haemolymph parameters from two bleed sites for normal and mud worm infested abalone. Means with SD and n values in parenthesis.

	normal abalone		**spionid infested abalone**	
Units	foot	Cephalic sinus	foot	cephalic sinus
Cu^{2+} ($\mu mol.l^{-1}$)	209.2 (27.5, 10)	213.7 (29.8, 6)	235.1 (100.5, 10)	337.0 (21.0, 5)
Cl^- ($mmol.l^{-1}$)	499.2 (30.7, 9)	508.4 (22.2, 6)	490.7 (44.6, 11)	503.2 (18.5, 5)
K^+ ($mmol.l^{-1}$)	13.5* (1.5, 11)	11.6 (0.6, 6)	11.9* (1.2, 13)	11.1 (0.6, 5)
Na^+ ($mmol.l^{-1}$)	463.5 (46.7, 11)	469.1 (31.0, 6)	465.0 (53.7, 13)	465.1 (19.7, 5)
Na^+/K^+ ratio	34.6* (2.1, 11)	40.6 (1.2, 6)	39.2* (2.0, 13)	41.8 (0.9, 5)
Ca^{2+} ($mmol.l^{-1}$)	10.6 (1.1, 8)	10.6 (0.9, 6)	10.7 (0.8,10)	11.0 (0.7, 5)
Mg^{2+} ($mmol.l^{-1}$)	49.8 (4.3, 8)	50.9 (4.0, 6)	47.4 (2.9, 10)	47.6 (5.7, 5)
Glucose ($mmol.l^1$)	0.4 (0.2, 4)		0.2 (0.2, 6)	
Protein ($g.l^{-1}$)	9.8 (0.4, 4)	9.3 (0.6, 2)	9.5 (1.8, 6)	11.1 (0.6, 3)

* site location pairs for row means are significantly different (P<0.05)



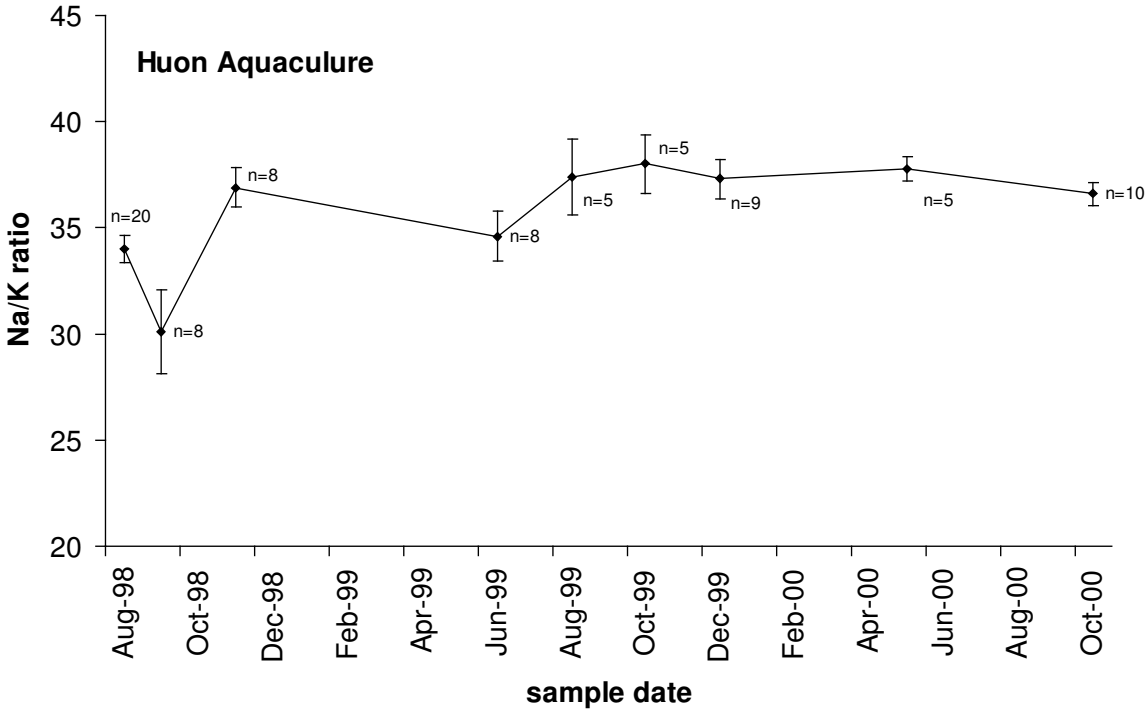


Figure 5.11 Temporal change in haemolymph sodium/potassium ratio for mud worm infested abalone at two study sites (means ± SE)

5.6.2 Haemocyte Data

A normal range for farmed abalone haemocyte counts was established by taking a mean of 16 sample means from presumed healthy stock. These abalone between 30 and 65 mm in length had a mean haemocyte count of 6.1×10^6 cells.ml^{-1} (SD=1.9×10^6 n=16). Individual sample means are shown in Figure 5.12, regression analysis found no significant relationship between shell length and haemocyte count (df 1 14 P=0.793).

Temporal data for the August 1998 intake groups showed significantly higher haemocyte counts (P<0.05, U=11.0, Mann-Whitney U test) were maintained in mud worm infested abalone at Huon Aquaculture ($5.41\times10^6 \pm 3.94\times10^5$, $\overline{X} \pm$ SE, n=9) than at Aquatas ($3.41\times10^6 \pm 6.47\times10^5$, $\overline{X} \pm$ SE, n=7) (Figure 5.13). There was only one sample from the Aquatas sample sequence with a count greater than 4×10^6 cells.ml^{-1} and therefore approaching the normal range (Figure 5.12). By contrast, all except one sample from the Huon Aquaculture stock had a count of approximately 4×10^6 cells.ml^{-1} or greater.

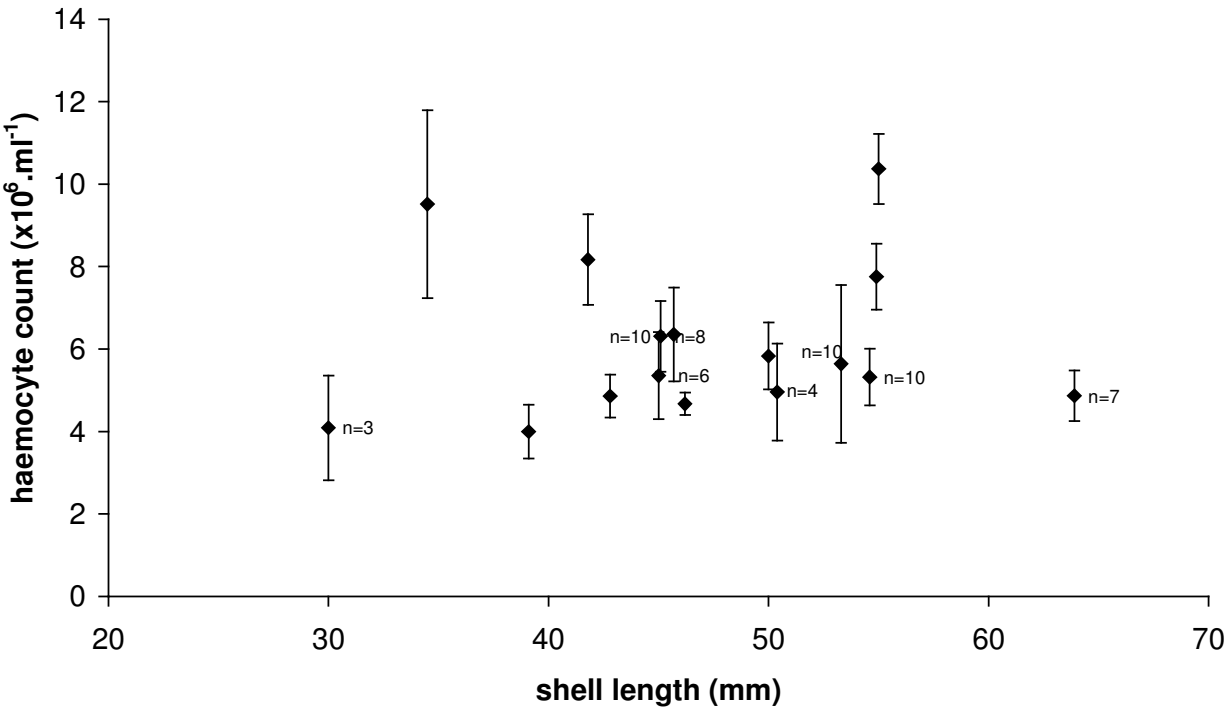


Figure 5.12 Haemocyte count data for 16 populations of presumptive healthy abalone (means ±SE, n=5 unless otherwise indicated).

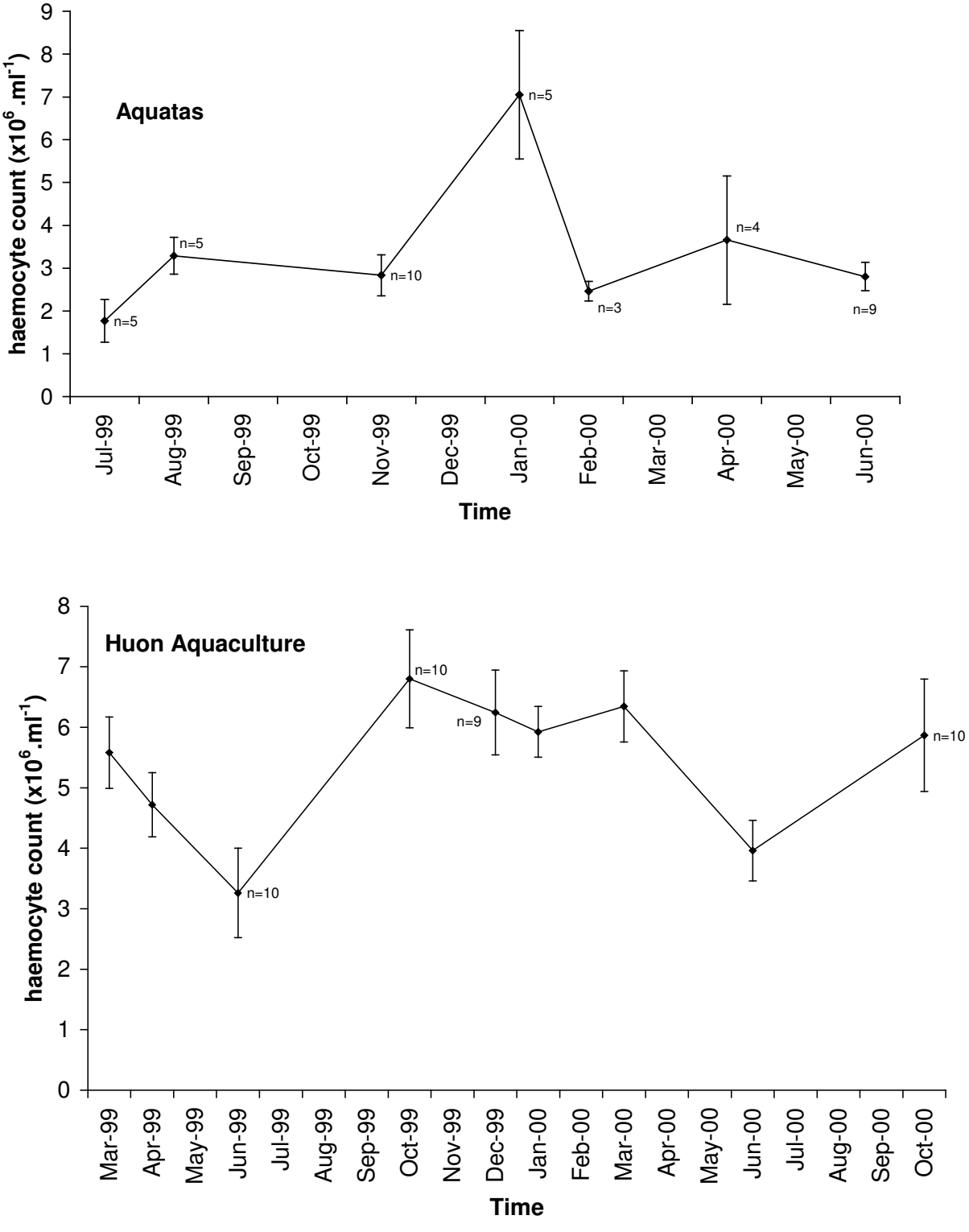


Figure 5.13 Temporal change in abalone haemocyte counts for August 1998 intake stock at two study sites (means±SE, n=5 unless otherwise indicated)

Comparison of haemocyte counts in non-spionid infested, fast growing and stunted abalone of the same age showed a trend for decreased haemocytes with increasing degree of stunting (measured by size and relative to age)(Figures 5.14 and 5.15). This contrasts with data for presumptive normal abalone (Figure 5.12) where haemocyte counts were independent of size (and age)

The "runts" of 2 year classes examined in 2001 had haemocyte counts greater than 1 standard deviation below the normal range as previously established above and comparable to spionid infested stock at Aquatas (Figure 5.13). Two way ANOVA (size and age as factors) showed the biggest, fastest growing of the 1 year old stock had significantly higher haemocyte counts ($P<0.05$) than the runts of the 2 year age class (Appendix 3, 5T for ANOVA table), although the latter abalone were larger (Figure 5.14). Likewise, the runts of 2 year classes examined in 2000 had significantly lower haemocyte counts (df 5 52, $P<0.05$) by ANOVA than other abalone in their age group. Fast growing stock less than 1 year old had a higher mean haemocyte count than larger, older 2 and 3 year old runts (Figure 5.15) but this was not statistically significant (Appendix 3, 5U).

The value of haemocyte variation as an indicator of health was further explored in relation to temperature stress. The first trial used 2 year old stock from farm 1 that had been exposed to water at 22-23 °C for 4-8 weeks. These animals showed a significant increase in haemocyte counts by ANOVA (df 2 27, $P<0.05$) at 3 sample times after transfer to water at 16 °C (Table 5.34). The mean count of 8.02×10^6 cells.ml^{-1} 14 d post transfer was approximately 1 standard deviation above the previously established normal mean. Count data from 2 abalone 14 d post transfer were excluded from statistical analysis as they were much lower than other data and 1 of the animals was moribund. The mean count for these animals was 1.4×10^5 cells.ml^{-1} (SD= 4.2×10^4), more than an order of magnitude lower than count data for the other abalone (Table 5.34).

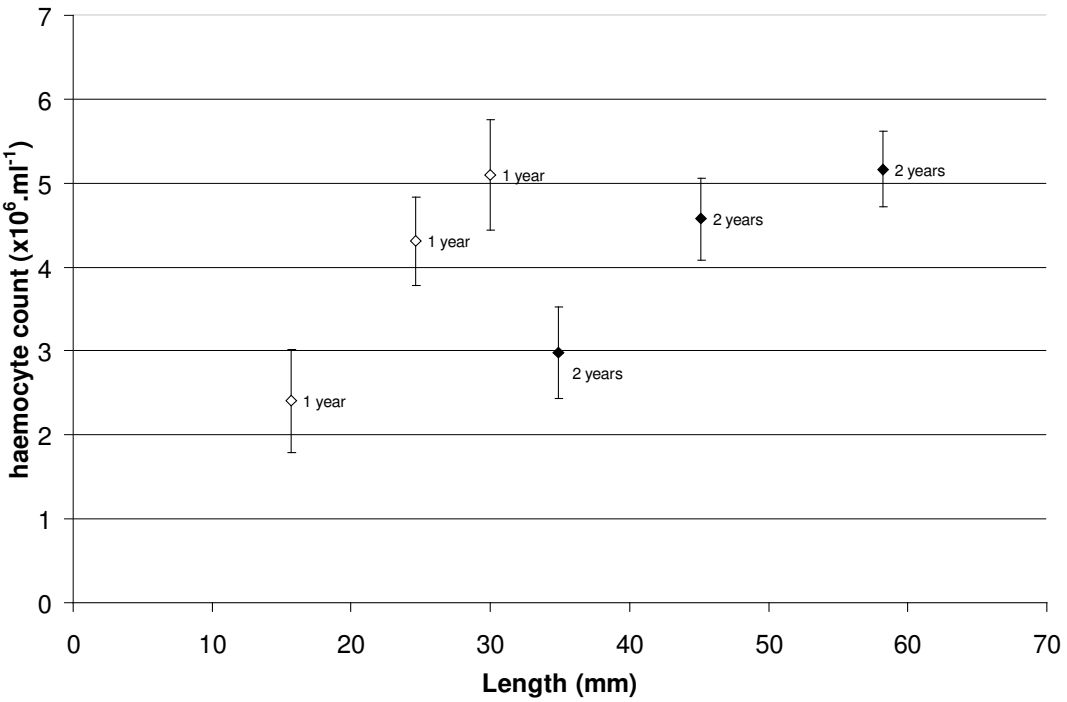


Figure 5.14 Haemocyte data 2001. Two year classes, three size classes. Means ± SE

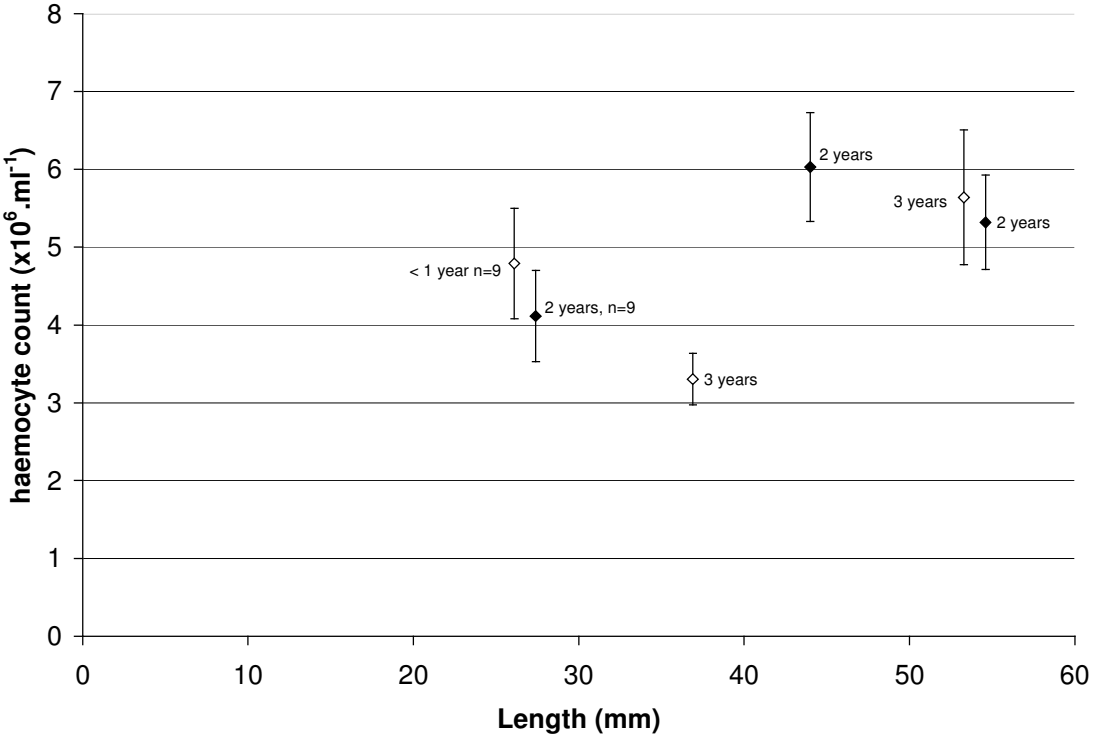


Figure 5.15 Haemocyte count data 2000 with samples from three age classes. Means ± SE, n=10 unless otherwise specified.

Table 5.34 Haemocyte counts following exposure to temperature reduction from 21-22 °C to 16 °C, mean ± SD (cells. $ml^{-1} \times 10^6$)

	4 days	7 days	14 days
Mean ± SD	$5.17^{A} \pm 1.42$	$6.21^{AB} \pm 2.81$	$8.02^{B} \pm 2.57$

n=10, Means with shared superscripts are not significantly different (P>0.05)

Table 5.35 Haemocyte counts following exposure to elevated temperature, mean ± SD (cells. $ml^{-1} \times 10^6$)

	control (14-15 °C)	6 days at 16 °C	6 days at 21 °C
Mean ±SD (n)	$6.65^{A} \pm 3.1$ (n=10)	$8.11^{A} \pm 5.50$ (n=16)	$7.27^{A} \pm 2.46$ (n=9)

Means with shared superscripts are not significantly different (P>0.05)

Table 5.36 Variation in haemocyte counts with experimental starvation, mean ± SD (cells. $ml^{-1} \times 10^6$)

	Initial count	1 month	2 months	3 months
Mean ±SD	$4.58^{AB} \pm 1.54$	$5.63^{AB} \pm 1.29$	$7.61^{A} \pm 1.96$	$3.41^{B} \pm 1.84$

Means with shared superscripts are not significantly different (P>0.05)
n = 10 initial count, n=5 remaining data

In a second temperature trial abalone that had been maintained at 14-15°C for 4 weeks were transferred to water at either 16 or 21°C. This resulted in cumulative mortality after 6 d of 55 and 10% respectively. Haemocyte counts in the treatment groups were elevated compared to the initial measure (Table 5.35), but not significantly so (df 2 27, P>0.05, ANOVA). Three moribund abalone had a mean haemocyte count of 4.89×10^6 cells.ml^{-1} (SD=1.11×10^6), lower than other groups in the trial but with insufficient data for an appropriate statistical test.

Nine days exposure to 0.2 mg.l^{-1} copper sulphate resulted in an elevated mean haemocyte count of 7.83×10^6 cells.ml^{-1} (SD=1.95×10^6, n=9) compared to the mean control count of 5.79×10^6 cells.ml^{-1} (SD=2.18×10^6, n=9). There was no mortality in either group. The statistical significance of this small data set was borderline by t-test (df 16, t=2.09, P=0.053) and Mann-Whitney U-test (U=19.5, P=0.06).

Kruskal-Wallis testing of haemocyte count data from abalone starved for three months found significant ($H_c = 10.2991 > \chi^2_{0.05, 3} = 7.815$) variation between monthly sample means (Table 5.36) as a result of the considerable decline in counts for the 3 month starved sample.

5.6.3 Histology

Abalone infested in 1994-1996

All heavily mudworm infested stock showed elevated levels of brown staining pigment granules in the right kidney tubules (Figure 5.16). Low levels of fine pigment were sometimes present in non mudworm affected presumptive healthy control animals. Severely spionid infested abalone also showed reduced right kidney definition (Figure 5.16) and the lumen was often enlarged compared to controls (Figure 5.17). Brown pigmented granules were typically located in the tubules of the digestive gland or digestive diverticulum (Figure 5.18) and not seen in control animals (Figure 5.19). Enlargement of the digestive tubule lumen and decrease in interstitial tissue were also seen in severely mud worm infested abalone. Epithelial cells of the stomach and intestine also showed the presence of brown pigment, more so than normal controls.

There appeared no consistent changes in gill structure of mud worm infested abalone but ciliates were not uncommon. Sections of foot tissue stained with PAS showed reduced PAS positive glycogen tissue (Figure 5.20) contrasting with normal abalone (Figure 5.21). Glycogen tissue was also depleted in the digestive tubules of mud worm infested stock compared to healthy abalone. There was no evidence of increased haemocyte activity or lesions in the shell muscle adjacent to blistered shell. Nor was there obvious or consistent depletion of muscle mass seen by H&E or specific Martius Scarlet Blue stain for muscle fibres.

Histology: August 1998 Intake

Animals in this time cohort were at least 3 years old in mid 1998 and were considered relatively slowly growing. Histology of abalone collected May 1998 before transfer of animals to the sea showed no pigment in the right kidney. Further histology on abalone collected August 1998 at transfer to the sea based farms showed the presence of grade 1 right kidney pigment in 3/5 animals and grade 1 pigment in the digestive tubules of one specimen. Two animals also had some pigment in the epithelial cells of the gut. In abalone from Huon Aquaculture, moderate to heavy (grades 2 and 3 respectively) quantities of brown pigment in the right kidney became progressively more common in the 2 years following transferral to this site (Figure 5.22). Conversely the final sample taken October 2000 showed a return to the minimal kidney pigmentation observed at the beginning of the time sequence. The extreme levels of pigment seen previously in abalone surviving the first reported mud worm outbreaks were not recorded. Reduction in right kidney definition and enlargement of the lumen were rarely seen.

Brown pigment in the digestive tubules was absent during the first year of sampling, with some scores of “1” and “2” recorded post October 1999. Moderate reduction in digestive tubule height and interstitial tissue was associated with light to moderate digestive tubule pigmentation in abalone sampled December 1999 and later. Pigmentation in the gut epithelium was widespread in all abalone sampled June 2000. Final samples taken October 2000 showed the presence of gill ciliates in 4 of 7 abalone. Gonad development was observed in samples taken summer 1999 and 2000. There was insufficient material to determine whether the heavy mud worm infestation present by late 2000 suppressed sexual maturity.

The equivalent August 1998 stock transfer to Aquatas developed grade “2” pigment in the right kidney by September 1999 as well as some digestive tubule pigment. By March 2000 when many of the animals had died, scores of 2 and 3 were recorded in the right kidney of remaining specimens. The last sample taken in June 2000 was characterized by poor right kidney definition including the presence of vacuoles and an increase in the lumen area of many tubules. Fine brown pigment was widespread but only rated “1” in 4 abalone sectioned. There was a reduction of interstitial tissue between digestive tubules in all samples, and a decrease in digestive tubule height in 3 of 4 samples. Pigment in the digestive gland was rated 1-2. Other than spionid infestation there was no evidence of significant pathology of abalone tissue or shells.

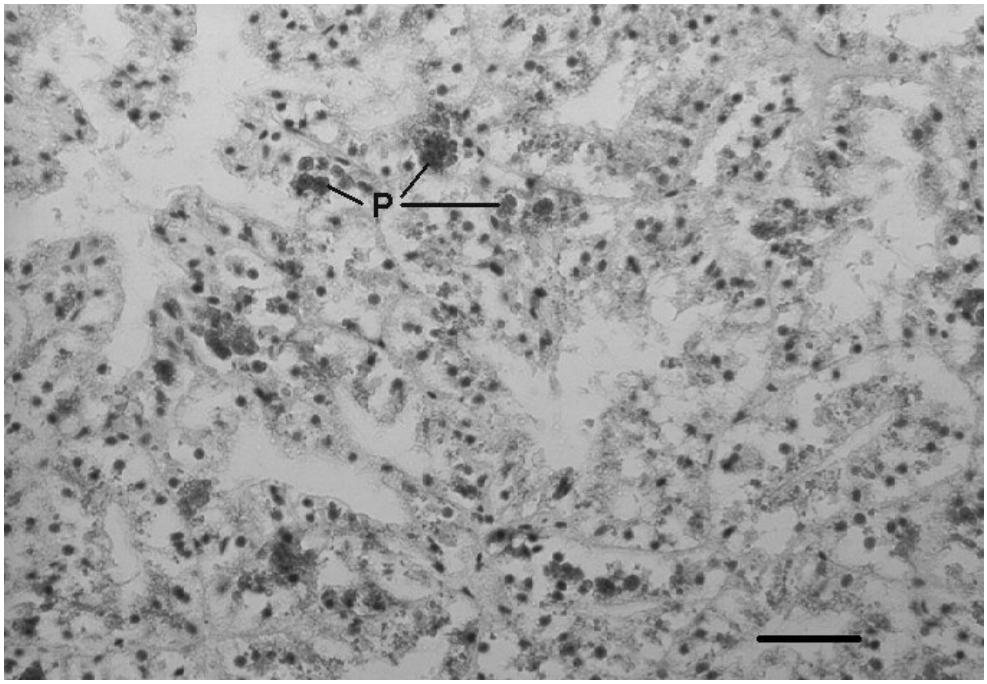


Figure 5.16 Right kidney in abalone with severe mud worm infestation. P = large pigment aggregations, bar = 100 μm

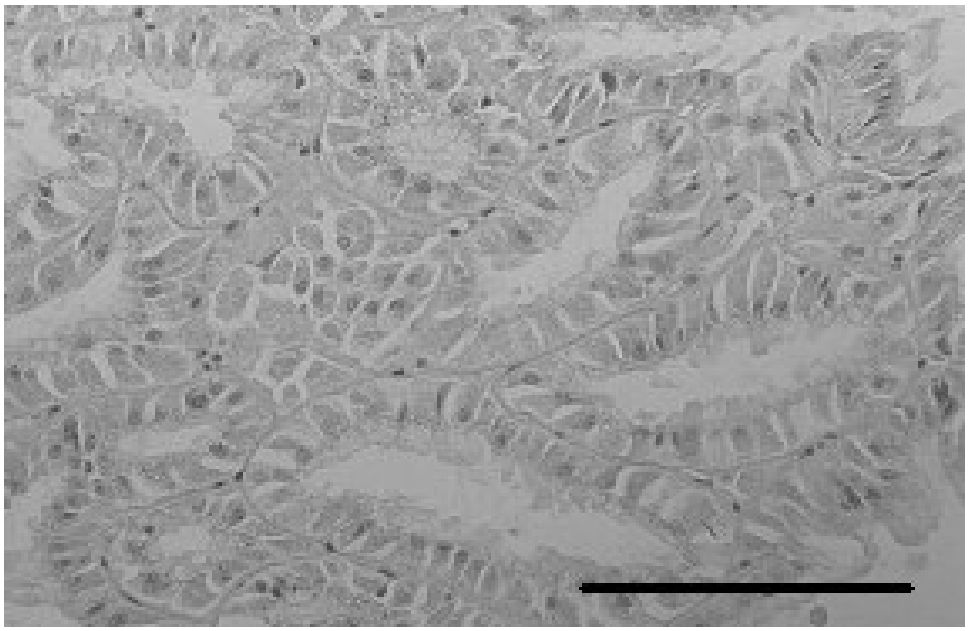


Figure 5.17 Right kidney structure in control, non-mud worm infested abalone, bar = 100 μm

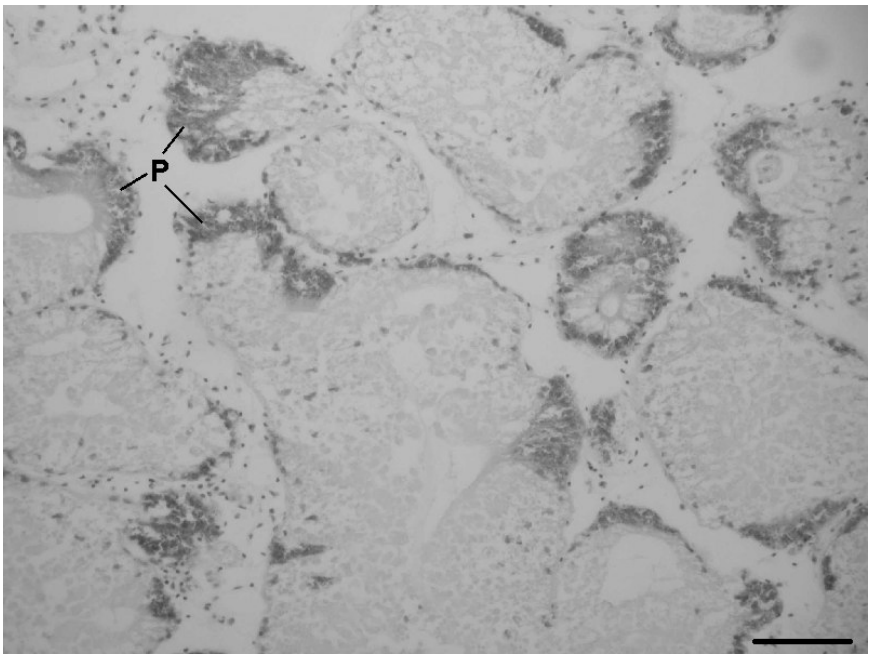


Figure 5.18 Digestive gland in severely mud worm infested abalone. P = pigment deposits, bar = 100 μm

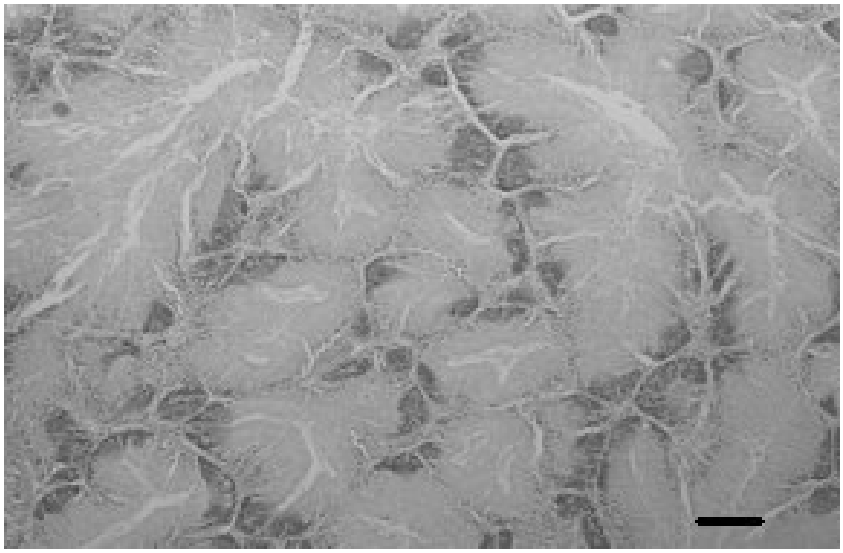


Figure 5.19 Normal digestive gland structure, bar = 100 μm

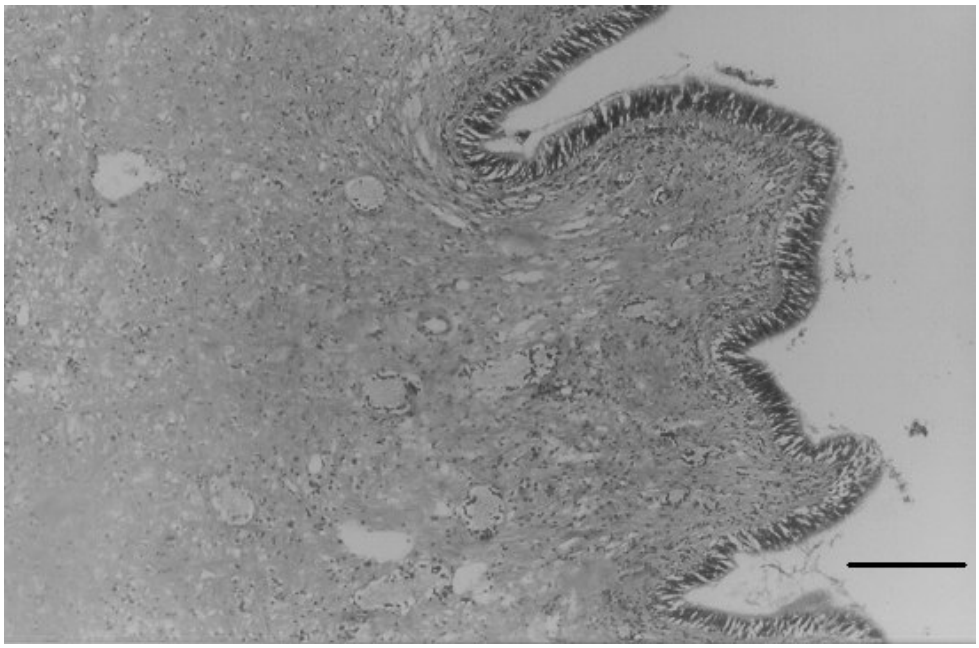


Figure 5.20 Foot tissue, spionid infested abalone showing reduced PAS +ve (red staining) glycogen tissue, bar = 100 µm PAS stain.

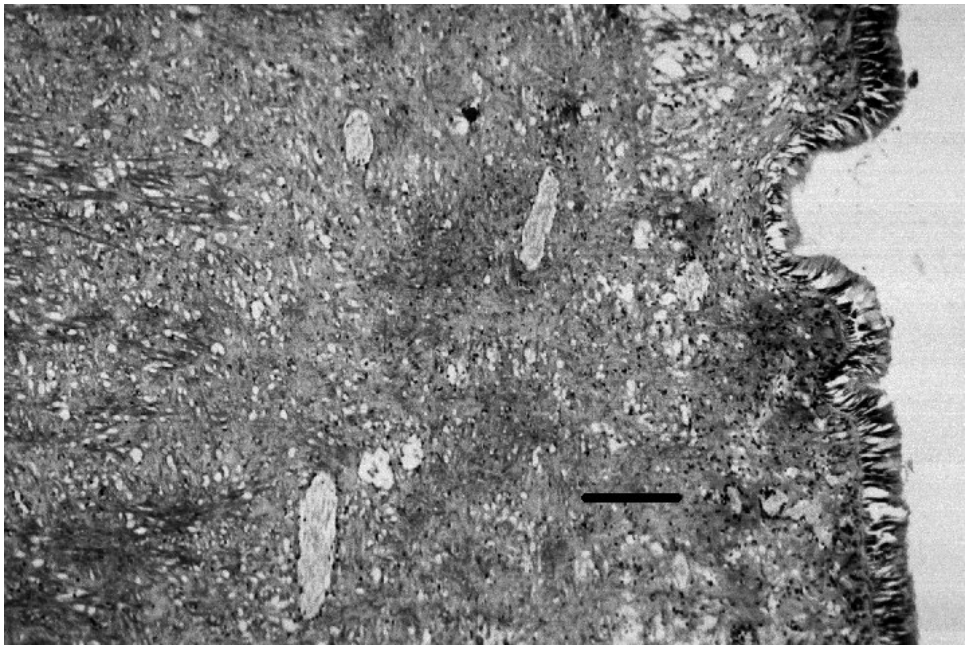


Figure 5.21 Foot tissue, normal abalone showing glycogen (red staining) tissue, bar = 100 µm PAS stain.

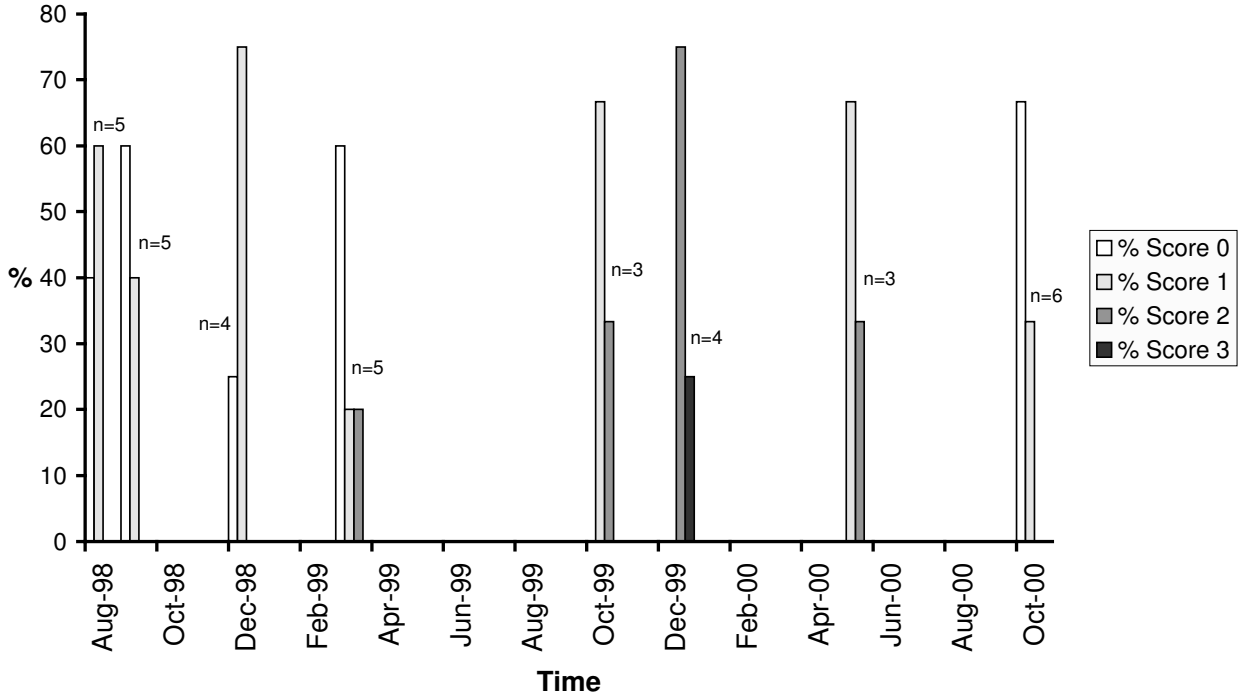


Figure 5.22 Temporal change in subjective right kidney pigment score for August 1998 intake abalone at Huon Aquaculture

5.6.4 Blister environment and microflora

All 10 blisters tested had pH in the range 7.5-8.0 as indicated by test strips (test strip range 4.5-10.0). Water drawn from blister cavities and cultured on standard media (Fish Health Unit, Launceston) showed no known pathogens and bacteria were not considered present in large quantities by subjective assessment (Wagner 1999 pers. Comm.). Mixed *Vibrio* species were present and 2 of these were identified as *V. splendicus* and *V. aestuarianus.*

Histological sections of severely mud worm infested shells from a variety of sites showed no evidence of fungal hyphae in PAS and H&E stained sections. Similarly, no evidence of fungi was observed in soft tissues adjacent to blisters or elsewhere in infested abalone.

5.6.5 Tissue chemistry

Initial mean protein level in foot tissue was 2.98% (SE=0.53, n=5) wet weight in September 1998, 1 month post transfer. In animals from Huon Aquaculture protein levels decreased significantly with time, ($H_c = 20.731 > \chi^2_{0.05, 5} = 9.488$, Kruskal-Wallis test) concurrent with increasing shell blistering and recovering to initial levels in the final October 2000 sample (Table 5.37). In terms of shell blistering the October 2000 sample had the greatest blister coverage but also the highest proportion of substantially nacre covered blisters (Figure 5.4). In abalone from Aquatas foot protein levels declined significantly ($H_c = 13.45 > \chi^2_{0.05, 3} = 7.815$, Kruskal-Wallis test) during the study period to a final value of 1.25% (SE=1.25, n=5) (Table 5.37).

Table 5.37 Temporal variations in foot tissue protein content with mud worm infestation at two study sites (means ± SE).

Huon Aquaculture		Aquatas	
Date	% protein	Date	% protein
Sep 1998	$2.98^{AB} \pm 0.53$, n=5	Sep 1998	$2.98^{A} \pm 0.53$, n=5
Dec 1998	$1.81^{BCD} \pm 0.01$, n=4	Feb 1999	$1.01^{AB} \pm 0.03$, n=5
Dec 1999	$1.68^{BC} \pm 0.14$, n=7	Nov 1999	$0.79^{B} \pm 0.08$, n=5
May 2000	$1.59^{C} \pm 0.29$, n=4	Jun 2000	$1.25^{AB} \pm 0.21$, n=5
Oct 2000	$2.58^{AD} \pm 0.20$ n=13		

Column means with shared superscripts are not significantly different (P>0.05)

5.6.6 Respiration rate

Mean oxygen consumption was 1.50 ± 0.19 μmol.g^{-1}.h^{-1} ($\overline{X}$ ± SE, n=8) in chronically mud worm infested stock at 20 °C, significantly higher (U=3.0, P=0.01 Mann-Whitney U Test), than that of non mud worm infested control abalone 0.81 ± 0.15 μmol.g^{-1}.h^{-1} ($\overline{X}$ ± SE, n=4). Dissection of animals revealed that percentage flesh weight of mud worm infested stock was 60.3% ± 2.3% compared to 82.3% ± 4.4% for non -infested stock ($\overline{X}$ ± SE, n=8 and 4 respectively) a statistically significant difference (U=0, P<0.01 Mann-Whitney U Test).

In a second experiment at 16 °C mean oxygen consumption was 0.96 ±0.05 μmol.g^{-1}.h^{-1} ($\overline{X}$ ± SE, n=5) in heavily spionid infested abalone, significantly higher (U=0.0, P=0.01 Mann-Whitney U Test) than that of lesser (methods: section 4.16.6) mud worm affected animals 0.68 ±0.04 μmol.g^{-1}.h^{-1} ($\overline{X}$ ± SE, n=5).

5.6.7 Ammonia excretion

Ammonia excretion in heavily mud worm blistered abalone was 0.29 ± 0.08 μmol.g^{-1}.h^{-1} ($\overline{X}$ ± SE=, n=10), not significantly higher (U=44, P>0.05, Mann-Whitney U Test) than that of lesser affected animals 0.21 ± 0.04 μmol.g^{-1}.h^{-1} ($\overline{X}$ ± SE, n=10). The less blistered abalone were intended to be a non-spionid infested control but post experiment dissection revealed blister coverage of 10.9% compared to 28.6% for the highly infested animals.

5.6.8 Starvation Trial

Sixteen of 40 abalone died in the week following transport and the commencement of the trial period. Clearly these deaths could not be assigned to starvation but appeared to be due to transport stress. A further abalone died 8 weeks following the commencement of the trial. Following 12 weeks starvation abalone had recorded a mean weight loss of 11.9% (SD=6.5%, n=6). Histological and clinical pathology findings are discussed in the appropriate sections above.

Discussion

As for the preceding health section the data above relate to objective 2 on investigation of interactions between spionids and abalone. The haemolymph of marine molluscs, which corresponds to the blood and interstitial fluid of higher animals, is very similar in ionic composition to the surrounding seawater (Burton, 1983). Haemolymph solute concentrations were similar between this study and that of greenlip abalone (Harris, 1999) for potassium, sodium and chloride. Calcium and magnesium levels are somewhat higher in this study than that of Harris (1999) and both sodium and chloride levels are about 50 $mmol.l^{-1}$ higher than those reported from greenlip abalone by Boarder (1997). However this is likely to reflect slight differences in salinity of the surrounding sea-water. These studies in total comprise a useful data base of blood parameter regulation that may be useful in determining health status of abalone generally.

While there is little if any regulation of sodium, chloride and magnesium in marine molluscs, potassium concentration in haemolymph may exceed that of seawater by a factor of 1.1-1.5 (Burton, 1983). Potassium levels in normal abalone from this study averaged 13.5 and 11.6 $mmol^{-1}$ for foot and cephalic sinus sample sites respectively (Table 5.33, ionic composition). These are elevated compared to mean potassium concentration of 9.7 $mmol^{-1}$ from southern marine farms (Appendix 3, 5V) and thus consistent with Burton (1983). Interestingly, while potassium levels in healthy abalone were regulated at higher than seawater concentrations these levels fell, significantly so, for samples drawn from the foot of mud worm infested animals (Table 5.33). As noted above, potassium haemolymph levels were greater in the foot than cephalic sinus of abalone as was the magnitude of the decrease in moderately to severely mud worm infested animals. In part this may be a consequence of potassium leakage from cells damaged as a result of the foot sample method. Russell and Evans (1989) report that several authors describe means by which the foot can be isolated from the rest of the abalone cardiovascular system. If this is true it may be that mud worm stressed abalone attempt to maintain osmoregulation in the visceral mass at the expense of the foot, leading to a greater drop in haemolymph potassium from this site relative to the cephalic sinus. Whatever the cause of this variation between sampling site, haemolymph potassium levels are regulated in abalone, with less effective regulation in mud worm infested animals. As the magnitude of potassium variation was greatest in samples from the foot this site may provide a more sensitive measure of stress than the cephalic sinus. Potentially, testing of haemolymph potassium levels may have application as a general stress test in abalone, provided this is coupled with external salinity and / or haemolymph Na / Cl levels to establish relative unregulated levels.

Testing of haemolymph copper found no significant differences between moderately to severely mud worm infested abalone and normal animals from either bleed site. The foot bleed data set for worm infested stock was highly variable (Table 5.33). Such variation was noted in experimental control abalone by Harris (1999) and in field studies by Ainslie (1980). Therefore assessment of respiratory pigment level by indirect copper measurement appears of little value as a health indicator. Possibly, direct measurement of the abalone respiratory pigment haemocyanin is worth investigating.

Haemolymph glucose levels in mud worm infested abalone were approximately half that in normal abalone (Table 5.33). However, this difference was not statistically significant due to the small size of these data sets (Appendix 3,5S). A reduction in

glucose levels in spionid infested stock would not be unexpected given the respiration data (section 5.6.6) indicated an increase in metabolic rate for these abalone. Cheng and Lee (1971) and Livingstone and De Zwaan (1983) reported significantly lower glucose levels in the haemolymph of parasitized molluscs. The mean level of 0.2 $mmol^{-1}$ glucose in the present study mud worm affected stock was very similar to the levels reported for reduced salinity stressed abalone by Boarder (1997). Normal levels of haemolymph glucose were higher in the study by Boarder (1997) compared to the present study (0.75 compared to 0.40). Sample collection trips were conducted over 2-3 days in the present study followed by relocation to the laboratory before processing. Abalone were not fed in this time which would be sufficient, based on data presented by Carefoot et al., (1993), for glucose levels to drop markedly. For this reason haemolymph glucose sampling was not considered a high priority in this study. It may, however, be worthy of further investigation as an indicator of abalone health, especially where stock are still feeding.

There were no significant differences between haemolymph protein levels in mud worm infested and normal stock. This is consistent with the copper data as most protein in the haemolymph would be expected to be associated with the copper in the haemocyanin respiratory pigment. Lee and Cheng (1972) as cited by Malek and Cheng (1974) found that haemolymph protein reduction in the planorbid snail *Biomphalaria glabrata* as a result of parasitism with *Schistosoma mansoni* was largely due to reduction in the respiratory pigment (haemoglobin) concentration.

A normal haemocyte range was established for cultured blacklip abalone that was found to be well above that for long term mudworm infested abalone in poor health at Aquatas. Malek and Cheng (1974) note that there is no total agreement on types of haemolymph cells that occur in molluscs – except that all species possess leukocytes. Various classification systems are discussed by these authors and by Simkiss and Mason (1983) and Sahaphong et al. (2001). Bevelander (1988) states that only one leukocyte type is found in the haemolymph of abalone with other cell types present in connective tissue, although this does not appear true of all abalone species (Sahaphong et al, 2001). Haemocyte counts have previously been found useful in abalone disease investigations by Shields et al. (1996). These authors found significant differences in subclasses of leukocytes between healthy *H. cracherodii* and those with wasting or withering syndrome. Friedman et al. (2000) in a study of the same disease in the same host abalone species found that the haemocytes of diseased abalone were compromised in their immune functions and these may serve as early indicators of the disease. Friedman et al. (1997) found the New Zealand abalone *H. australis* had a mean circulating haemocyte count of 6.72×10^6 which is within the normal range established here for *H. rubra* and close to the mean of 6.1×10^6. Furthermore, these authors found that haemocytes were significantly elevated in *H. australis* affected by fungal lesions of the shell (to 1.19×10^7). By contrast, there was no difference between fungal infected and control abalone of a further species, *H. iris*. Changes in gastropod haemocyte numbers as a result of parasitism have also been reported by Stumpf and Gilbertson (1980, as cited by Bayne 1983) and as a result of temperature variation (Pauley and Krassner 1971).

Haemocyte count variation was also seen in the present study in relation to temperature stress and also to sub lethal toxin exposure (copper). These changes occurred relatively rapidly (1-2 weeks) and therefore have potential as a general stress indicator. The three-month starvation trial resulted in an initial rise in haemocyte count followed by a significant decline in the last sample. By contrast there were no significant changes in Na^+/K^+ ratio during this time.

Histological examination was performed on abalone surviving from the initial mud worm outbreaks in the mid 1990's and on a sequence of increasingly infested stock placed at the study sites in 1998. Major findings involved changes to the right kidney and digestive tubules, including substantial deposition of brown pigment granules. Reduction in right kidney definition and enlarged lumen associated here with moderate and severe mud worm infestation has been reported by Harris et al. (1998) in relation to ammonia exposure. Brown pigment granules were described in the cytoplasm of normal abalone right kidney cells (Bevelander, 1988) and the elevated quantities associated with moderate and severe mud worm infestation have been previously reported by Harris et al (1998) in relation to elevated nitrite exposure. These authors suggested increased pigment might be a reflection of increased kidney protein and hence cell turnover. Malek and Cheng (1974) note that yellow/brown "excretory or ferment globules" are a feature of parasitized molluscan digestive glands and especially so when the host parasite interaction is of long duration. These authors state that the globules represent accumulated metabolic wastes in the digestive gland. The pigment granules in the two tissue types therefore indicate a long term stress consistent with length and severity of mud worm infestation in the present study.

Mud worm infestation apparently leads to depletion of host energy reserves. During starvation of abalone glycogen, triglyceride and protein reserves are utilized consecutively (Takami et al., 1995). In withering foot syndrome of *H. cracherodii* (caused by a rickettsia), where the digestive diverticula or tubules are directly damaged by infection and carbohydrate storage is reduced in non-infected tubules of infected animals (Gardner et al., 1995), the resulting prolonged starvation leads to foot muscle atrophy including severe depletion of muscle fibres. PAS staining of severely mud worm infested abalone showed reduced carbohydrate stores in the digestive gland and routinely stained sections showed increased tubule lumen size, also indicative of poor nutrition. However while the foot of severely mud worm infested abalone was sometimes completing lacking in glycogen tissue, depletion of muscle fibres was not seen, confirming that the degree of foot atrophy was less than with severe withering foot syndrome. Histological examination of severely spionid infested abalone was negative for the characteristic rickettsia seen in abalone with withering foot syndrome.

Normal levels of soluble protein in the foot were similar to data given by Takami et al. (1995) for *H. discus* and the reduction in values to less than about 1.5% wet weight in mud worm infested stock was similar to levels in 30 d starved abalone (Takami et al., 1995). Extreme low levels reached in some infested stock from Aquatas (Table 5.37) were like those of 70 d starved stock reported by Takami et al. (1995). Interestingly, foot protein levels in mud worm infested stock at Huon Aquaculture recovered from low values to close to the normal level in the final October 2000 sample (Table 5.37). This foot protein recovery coincided with an absence of moderate pigment deposits in the right kidney. These latter, had been present in most samples taken in the previous year and the finding supports the notion that the right kidney pigment was the result of catabolism of foot protein.

This is interesting in relation to temporal shell blistering for the cohort, in that, blisters present were progressively better healed with the maximum value (80% healed – derived from Figure 5.4, Section 5) occurring in the October 2000 sample. By contrast, in the comparable Aquatas cohort, blisters assigned to the "healed" category constituted less than half of blister area in the last two samples (Figure 5.8, Section 5). Thus the assignment as "active" blisters on the basis of their conspicuous conchiolin protein colour was consistent with continued low foot protein values.

Respiration rates in this study are in line with previous studies on abalone by Uki and Kikuchi (1975), Barkai and Griffiths (1987), Nimura and Yamakawa (1989), Segawa (1991), Carefoot et al., (1993), Paul and Paul (1998) and Harris (1999). Studies on heart rate and respiration of the gastropod *B. glabrata* parasitized by the larval trematode *S. mansoni* have generally showed an increase in infected stock relative to controls (Malek and Cheng, 1974). This was consistent with the two respiration trials in the present study but contrasted with oxygen consumption data for abalone with withering foot disease where consumption decreased (Kismohandaka et al., 1993) or remained unchanged (Kismohandaka et al., 1995) relative to unaffected animals. Possibly these differences reflect interaction between the disease and extent of host starvation. It has been shown by Segawa (1991) and Gaty and Wilson (1986) that oxygen consumption in abalone decreased in starved animals.

There was no significant difference between ammonia excretion for greater and lesser mud worm infected stock. Excretion rates were similar to those of abalone of comparable size reported by Segawa (1991) for *H. diversicolor* but higher than excretion rates for *H. midae* (Barkai and Griffiths, 1987). There is little literature relating ammonia excretion in abalone or other molluscs to disease. Kismohandaka et al. (1993) found that abalone with withering syndrome excreted 3.8 times more ammonia than did healthy animals. The contrast between this study and the mud worm infestation data may be due to the lack of a suitable mud worm free control in the latter case. As for oxygen consumption data the extent of starvation associated with the disease condition might also influence the outcome. Segawa (1991) found that ammonia excretion at first increased in starved abalone then decreased after 17 d.

The changes that occurred in abalone that contracted mud worm infestation as the result of experimental placement in 1998 were not as great as those that occurred when abalone were first farmed in susceptible areas. Stock first infected in 1994-96 and examined 1996-1999 had greater subjective shell damage ratings, decreased percentage flesh weight, increased *B. knoxi* counts and more extreme histological change compared to abalone placed subsequently to replicate the pattern for study purposes. These changes manifested themselves in high mortality rates at a number of farms in the mid to late 1990's. Possible reasons for different levels of infestation and spionid impacts between past and present are examined in the general discussion.

In summary, moderate to severe mud worm infestation lead to depletion of abalone energy reserves. This was indicated by histological changes to the right kidney and digestive tubules consistent with increased cell turnover; with depletion of glycogen and protein tissue reserves; reduction in haemolymph glucose and with increased oxygen consumption. Such changes were likely the result of mobilization and direction of host resources to shell repair, that may have manifested itself as reduced growth, and in some instances death. Spionid infestation was also associated with reduced capacity to regulate potassium and in severely impacted abalone with significantly lowered circulating haemocyte counts. Similar low levels of haemocyte counts were seen in "runted" stocks and 3 month experimentally starved animals. Haemocyte counts are considered potentially useful as a general indicator of health status.

5.7 General discussion

Background and Need

High levels of mortality of sea-farmed abalone in southern Tasmania during the mid 1990's, which led to this research, was linked to spionid mud worm infestation after extensive laboratory examination showed this to be the only consistent finding (Handlinger, unpublished). These mortalities progressed slowly over many months in most groups on several farms in the sea based farms in southern Tasmania and involved the mortality of > 30 000 animals, comprising a very high percentage of total stock. It should be noted, however, that mud worm problems were not and are not confined to southern Tasmanian off shore sites. Severe infestations, including mortality, have also been found in abalone reared in land based systems in Tasmania, South Australia and Chile, and in sea based systems in West Australia and New Zealand (reviewed in Sections 1 and 2). There was considerable data on spionid infestation of mollusc populations, including abalone, but until the development of abalone farming, the perception of negative impacts was largely restricted to oysters, for which shell treatment by air drying or other osmotic insults had long been developed and were readily used with minimal impact on oyster health. As univalves, abalone are unable to avoid these osmotic effects through shell closure, and a more comprehensive approach to spionid control was needed. The spionid species *B. knoxi,* previously unknown in Australia, appeared to be the major cause of the losses, being present on all affected farms and numerically dominant at the farm with the greatest stock losses. As this species was not previously recorded in Tasmania (Wilson et al., 1993) it was speculated that it may have been a recent and relatively harmful spionid introduction from New Zealand. The research was therefore intended to focus on the spionid species *B. knoxi*, though unexpectedly, *P. hoplura* was the most numerous species present in abalone during this field research and was therefore also the focus of study.

A multi-pronged approach was devised with the aim of producing an integrated package of measures to minimize the impact of spionids on abalone culture in these susceptible locations. The first control strategy investigated was chemical and physical treatments. Once treatment options were found to be limited and stressful to stock, broader studies were undertaken to allow parasite management through understanding and manipulating the host-parasite interaction. The final recommendations involve a mixture of parasite avoidance through minimising opportunities for spionid settlement on stock, particularly timed placement of stock, and strategic treatment.

Developing a treatment of mud worm infected abalone

Chemical treatment

The testing of chemical treatments was undertaken as part of the preliminary CRC funded project. Sixteen potential chemotherapeutic agents and fresh water immersion were tested, using available literature on effective concentrations for bath treatment of fish and shellfish parasites as a starting point. It was concluded that the anti-parasitic compounds trichlorofon, praziquantel, febantel, pyrantel embonate,

metronidazole, dimetronidazole, and the surface treatment agents hydrogen peroxide and methylene blue were not effective against spionid polychaetes at doses which were high relative to dosage quoted in available literature. Immersion in freshwater, formalin, the topical treatment dyes potassium permanganate, gentian violet, malachite green, and the levamisole, ivermectin, and the benzimidazoles mebendazole and fenbendazole was highly effective against mud worms *in vitro*. However, the protection conferred by the burrows of spionids *in situ* rendered effective concentrations unsafe for abalone. The most promise *in vitro* treatment was shown by the benzimidazole class of drugs. These may have some potential for improvement as a treatment for various polychaetes by using microencapsulation of the drug as burrow protection in these trials is likely to have been compounded by poor solubility in water. Further work in this direction was abandoned in favour of the promising results from preliminary air drying experiments.

Treatment by air drying

Treatment by air drying not only showed the most promising results from the preliminary treatment experiments, but had the advantages of practicality, safety to operators, and lack of residue concerns. Like most traditional mollusc treatments for spionids, it utilises osmotic challenge with relative host protection provided on the basis of size, rather than metabolic differences which the above results indicate are generally slight between these two marine invertebrate groups. While abalone have poor tolerance to osmotic changes when emersed, they are moderately well protected by mucus against short-term air exposure, which is a natural experience of abalone in some parts of their range. Air drying of stock has a history of use in oyster culture (Smith 1984, Nell and Smith 1988) and is the basis of the success of intertidal oyster culture which sacrifices feeding opportunities (when immersed) for the treatment benefits when exposed.

Treatment duration - time for effective treatment: All spionids were shown to be highly susceptible to drying *in vitro,* but to be protected to a greater or lesser extent from effective drying *in vivo* by their location within the shell. The body of experimental work demonstrates that treatment of small *B. knoxi* (and small *P. hoplura*) can be highly effective, with reduction in numbers for all classes of worm in almost all experiments using treatment times of 4 hours. Larger mud worms (> 5 mm and typically 10-25 mm) may be susceptible to drying treatment depending on a host of factors. These appeared to include: infestation time, blister severity, stock size and stock fouling. Increasing infection period allowed mud worms time to become established in burrows deep within the shell. Where larger chambers occurred within blisters survival appeared especially likely.

In relation to this, observed differences in blister morphology between mud worm infested abalone and bivalve molluscs in Tasmania are of interest. In bivalves *B. knoxi* is observed to inhabit very large water filled blisters whereas in abalone blisters rarely have a significant volume. Traditionally treatment for mud worms in Sydney rock oysters utilizes much longer drying times, such the 10-14 day emersion time noted by Nell and Smith (1988), which appears to be based on the work of Whitelegge (1890). While this may be excessive for shells relatively clear of mud, there has been little effort to define a minimum effective time as Sydney rock oysters have a high tolerance to both air exposure, and to freshwater as an alternate treatment. Recent trials on treatment of large Pacific oysters heavily infested with *B. knoxi* suggested freshwater treatment of at least 24 hours duration was required to kill the

majority of the mud worms protected by large blisters (Lleonart and Handlinger, unpublished). Such treatment is certainly beyond the physiological limits of abalone. In view of the lower resistance of abalone to drying it is fortuitously that blisters typical of *B. knoxi* and to a lesser extent *P. hoplura* in abalone may provide relatively poor protection against desiccation strategies.

Treatment duration - safe levels: Abalone of 15-20 mm or greater proved surprisingly tolerant of air drying. Spionid treatment data (section 5.3) showed that abalone consistently survived 2-4 h air drying, sufficient to significantly reduce mud worm levels. There was no mortality of stock of this size dried for up to 3 h and of 40 mm animals dried for 11 h water under conditions appropriate for elimination of spionids. This proves ample scope for effective treatment. This contrasts with poor survival reported for smaller abalone (5-10 mm) air dried in shade at 24°C (Whang and Chung 1977). These longer exposure data relate mainly to blacklip abalone. There may be differential drying tolerances between haliotid species requiring investigation in the future. However while it was shown that several hours of air-drying rarely resulted in immediate post-treatment mortality, there was the potential for reduced growth over the following months in at least some groups. As heavy spionid burdens also reduce growth (at levels below overt mortality), current recommendations would be to avoid longer air drying by treating while shorter exposure times (2-4 hours) are likely to be effective, and to practice avoidance strategies to minimize the number of treatments required.

Temperature and humidity: Treatment for this duration (2-4 hours) was more effective at 21 °C (up to 90% reduction under trial conditions), than at temperatures of 15 – 18 °C (approximately 70% reduction). Treatment at 24 °C failed due to higher associated humidity which resulted in incomplete drying of shells. However other factors (such as duration and severity of infection, and the degree of treatment induced stress) had a greater influence on the optimum economic time for treatment. Avoiding extremes and minimising the differential between air and sea temperature were considered more important than timing treatments to achieve an optimum treatment temperature.

Methods for mud worm control - Recommended protocol for air-drying

The following protocol summarises the more detailed treatment protocol given in the attached Farm Manual (Appendix 4). Short term treatment air drying may potentially, but not necessarily, reduce the growth rate of stock. To minimize stock stress it is suggested that the differential between air and sea temperature be minimized, temperatures > 24 °C or < 15 °C be avoided, and stock be starved for several days before treatment in line with the findings of Watanabe et al. (1994). Air humidity should be less than 64%, to ensure that the shell of the abalone dries sufficiently. If shells of stock are not apparently drying within ~30 minutes of air exposure the conditions are probably unsuitable. Typically, days with minimal cloud cover will be sufficiently dry for treatment in southern Australia. Abalone should be dried in shade, not direct sunlight.

Methods for control of mud worm – avoidance strategy

SETTING UP THE FARM

Assessing overall level of mud worm risk

Assessment of the risk of significant mud worm infection is an important component of site selection and design, but must be balanced against other considerations. The economically significance will depends both on the value of lost production and the cost of treatment or risk amelioration.

To define factors affecting this risk, the spionid life cycles and host-parasite interaction was studied by replicating as far as possible the original culture situation between mid 1998 and early 2001, using in excess of 9000 abalone placed at two locations with a previous history of severe spionid infestations. Interestingly only 200-300 of this stock became infested to the levels previously associated with abalone mortality. This indicates that the risk of severe mud worm problems in these locations are not as great as previously feared by growers on the basis of events in 1995-1996.

The mortality rate in this study was low and confined to mud worm infested underfed stock at one site. This contrasts markedly with events in the mid 1990's where considerable mortality occurred at several locations. Explanations for the difference in mortality rates between past events and the present study are of considerable interest to growers. There appear to be two major possibilities: that either, during the past mortality episodes there were other important stress factors operating in addition to mud worm infestation, or that infestation levels were higher and more severe in the past due to a different infestation pattern at that time. Factors affecting the level of infection and the of severity of consequences are discussed below, as both are relevant to management decisions regarding utilisation and management of mud worm susceptible sites.

Factors affecting the level of infection.

Spionid species: In trying to reconstruct factors that may have lead to the mass mortality of the mid 1990's the role of spionid species must be examined. Although *B. knoxi* was implicated as the most significant spionid species in the original outbreaks, *P. hoplura* was unexpectedly found to be the most numerous species present in abalone during this field research, outnumbering *B. knoxi* by 5-10 to 1 in heavily infested stock. *B. knoxi* was the dominant species present at the Huon Aquaculture Company during the original infestations, contrasting with its representation at both study sites during field trials. It should be recalled, however, that infested abalone at sea based operations other than Huon Aquaculture showed the presence of both *B. knoxi* and *P. hoplura*. Furthermore in the early days of land based abalone farming one such farm in each of Tasmania and South Australia suffered stock mortality associated with purely *P. hoplura* infestation. Since that time verbal reports of abalone mortality associated with spionids other than *B. knoxi* have originated from Australia and overseas. Thus it is difficult to make a convincing case that *B. knoxi* is an unusually destructive spionid species. However different life-cycle strategies may affect the level of infection.

Spionid life cycle and larval dispersal pattern: In the present research, the reproduction of the two major spionids, *B. knoxi* and *P. hoplura*, was investigated

with an emphasis on the possibility of avoiding larval settlement. *B. knoxi* was found to have exclusively planktotrophic larval development confined to the Austral spring, consistent with the findings of Handley (2000) for *B. knoxi* in New Zealand. The reproductive strategy behind planktotrophy is one of high larval production (estimated at ~700 larvae/brood – section 5.1) to account for a high attrition rate. There is also considerable scope for dispersion, due to the length of time in the plankton. This "boom or bust" capacity for larval production may account for both the original mortality episodes as described above and the annual variation and relative scarcity of the species 1998-2001. Thus *B. knoxi* (and other planktotrophic spionids) may have the potential to cause significant harm to mollusc culture enterprises when environmental conditions favour high larval survival. The consistency of *B. knoxi* seasonal reproduction fortuitously allows a simple avoidance strategy to be practiced in susceptible areas. Clearly, transfer of stock to susceptible sites post November allows approximately 10 months growth before potential infestation in the following September.

Larval settlement data for *P. hoplura* (section 5.1) suggested that initial colonization with this species could also substantially be avoided by placement of stock post spring and preferably after mid summer. Once established in abalone, however, the species showed the potential for year round lecithotrophic reproduction (crawling not swimming larvae, leading to a local spread). Thus numbers of this species have the potential to rise steadily over time and this needs to be taken into account and monitored by growers – especially if the grow out period extends over 2-3 years in the sea.

Variations in annual spionid settlement rates: Anecdotal evidence from Tasmanian oyster farmers that "there are good and bad mud worm years" is supported by Pacific oyster blister data collected by the Fish Health Laboratory (Appendix 3, 1C). This data gives the percentage of blister positive oysters in farmed populations but not the extent of blistering or the spionid species responsible. None the less it is interesting that on the basis of this data 1995 recorded the highest blistering levels. Available abalone mortality data shows death rates of 12% and 32% between December 1995 and April 1996 for animals stocked January and August 1995 respectively. Stock placed June 1994 suffered little mortality until December 1995, with an overall death rate by April 1996 of 31% (Appendix 3,1A- Figure 1). These farm mortality data are consistent with the growth trial studies of Hindrum (1996) which reported 40% abalone mortality between November 1995 and April 1996. These records support the possibility of heavy *B. knoxi* settlement at this site in the spring of 1995, and the likelihood of future annual variations of risk. Thus data over several years may be required to fully assess the mud worm risk of a site.

Most of the abalone for which temporal mortality data are available were placed at 20-30 mm. This contrasts with the present study, where stock placed at between 20-40 mm were minimally infected, in line with experiments on stock size and mud worm settlement (section 5.4.1). In combination these also suggests relatively high numbers of larvae in the water spring 1995. The rainfall data for Dover (Appendix 3**,** 5W) near Huon Aquaculture are of interest in relation to factors that might contribute to spionid infestation. July and August 1995, just before the presumptive *B. knoxi* larval dispersion phase of 1995 received approximately twice the 100 year average for these months. Calculation showed that rainfall for July and August of that year, plus the spring months when the *B. knoxi* is in the water column was the third highest in 100 years (Appendix 3, 5W). Runoff from heavy rain could

benefit spionids by putting more nutrients in rivers and the sea leading to enhanced production of spionid larval food sources. This is consistent with references to increased spionid populations near drains and other sources of organic pollution (Woodwick 1964, Anger 1977). This has predictive value, identifying wet springs as years requiring vigilance, and should also be taken into consideration is assessing mud worm risk data from a limited number of seasons.

The rate of mud worm recruitment rather than the numbers per se may be important determinants of host impact, with a likely interaction with host size. Abalone from Huon Aquaculture, placed 1994-1995 and surviving until 1997 had up to 40-50 *B. knoxi* chimneys (section 4.5). It is plausible therefore that stock deceased before this time had even higher levels of infestation. Total spionid numbers in the August 1998 Huon Aquaculture experimental intake abalone eventually reached levels seen in the 1997 survivors but this took approximately 2 years (section 5.5). If, by contrast, in spring 1995 the Huon Aquaculture Company abalone experienced settlement of, for example, >30 *B. knoxi* the effects might have been sufficiently devastating to explain the onset of mortality in December 1995 - April 1996.

The effect of shell fouling on spionid settlement: In addition to avoidance strategies based on spionid reproduction and settlement, the research identified other factors of importance in minimizing spionid impacts. Tube building polychaetes, especially Spirorbis sp. when present on abalone were found to enhance settlement of B. knoxi. Culture facilities in susceptible areas would thus be advised to source spat without significant quantities of these fouling organisms. Fortunately, in southern Tasmania the settlement season for spirorbids is similar to that of spionids (section 5.4.2). Therefore stocking regimes designed to avoid mud worm infestation in the first year will result in minimal spirorbid fouling and thus relief from the B. knoxi enhancement effect for a substantial time. Control of spirorbids in land based farms may be an area requiring further research, and the level of spiorbids another factor in assessing site risks.

Effect of abalone size on settlement rates: When stock of several size classes were exposed to spionids at the same time, larger stock became significantly more infected. By contrast, as small minimally affected abalone grew from approximately 20 to 60 mm over 2 years spionid infestation remained at very low levels.

Effect of height in water column on settlement rates: Abalone reared closer to the bottom were found to recruit higher numbers of *P. hoplura*. Thus it may be prudent to raise culture vessels from the bottom as has been recommended previously for oyster culture (section 5.4.4).

Factors affecting severity of consequences.

Additional stress – underfeeding: As noted previously, there was mortality of the underfed spionid infested stock relative to the better managed cohorts in the study. This suggests the potential for adverse conditions to magnify the impact of spionid infestation. However, at least one grower has indicated that stocks were properly maintained with regard to feeding before and during the original mud worm outbreaks. Further, Hindrum (1996) reported growth rates of 80-100 $\mu m.day^{-1}$ (not typical of underfeeding) in sea farmed abalone that later experienced heavy mortality rates.

Additional stress – water quality factors: Retrospective shell grading of abalone submitted from Huon Aquaculture in 1997 and used for treatment trial experiments (sections 5.2 & 5.3) showed that 16% had slight or no mud worm infestation (SSDR grades 0-1, Appendix 3,1B). Shells examined from this source in 1997 sometimes included high proportions of shells from dead abalone and at least some of these shells had little or no mud worm infestation. It is possible, therefore, that the most severely affected abalone, (grade 3 rated shells account for 36% of the 1997 sample-Appendix 3,1B), died largely as a result of their spionid infestation. Subsequently, the resulting poor water quality, in combination with some degree of mud worm infestation may have caused the deaths of further stock.

Available farm mortality data (Appendix 3, 1A) for 1994-96 shows a high death rate in the Austral summer for 1995/1996. Another factor of potential importance in these deaths was the widespread use in the mid 1990's of "tube" type rearing vessels. These had relatively restricted water flow, especially once fouled with seaweed after some time in the water (sections 4.3 & 5.4.3). Thus the combination of mud worm infestation, summer water temperature and the presence of dead stock in a small, relatively sheltered containment vessel could potentially magnify the impact of severe mud worm infestation.

Physiological consequences of infestation: As discussed above, where experimentally exposed abalone became heavily spionid infested, mortality could be attributed to the combination of severe shell blistering and underfeeding but not to significant infestation as a sole known stress factor. Spionid infestation led to reduced growth and a decrease in percentage flesh weight. These gross changes were manifestations of disruption to normal physiology and biochemistry, including increase in oxygen consumption and utilization and reduction of tissue glycogen and protein stores. These latter were reflected in histological changes to the right kidney and digestive gland. Thus the health of abalone was compromised, possibly because resources were diverted from normal functions to repair of the shell. Severely spionid compromised abalone also showed a reduced capacity to regulate potassium and had lower circulating haemocyte counts than normal animals. These changes, especially the latter may have potential as general indicators of chronic stress in abalone. Similar low levels of haemocyte counts were seen in "runted" stocks and 3 month experimentally starved animals. For effective interpretation of such changes, however, knowledge is also required of the responses and variability of haemocyte responses to short term stresses. For this, repeat experiments need to be performed using larger numbers of abalone and a variety of challenges such as other infectious agents and adverse water conditions such as sub optimal ammonia, nitrite, pH and dissolved oxygen levels. If the response patterns are sufficiently predictable, long term monitoring of haemocyte counts in stocks before and during summer could be a useful addition to health studies on so called "summer stress syndrome". This has emerged as a serious problem for land based abalone farms in parts of Australia and is linked to elevated water temperature and bacterial infection, but there are reports that mud worm infestation may also sometimes be a factor.

Thus there is ample evidence, despite the low levels of mortality during these experiments, that spionid mud worm shell infestation does significantly compromise production and health. As treatment may also compromise health, the general principles of mud worm control should be aimed at optimizing growing conditions, minimizing spionid settlement through timing of stock deployment and selection of

appropriate sites and farming systems to ensure adequate water quality and flow, plus treatment by air drying when necessary. Though primarily for marine farming, this is applicable to abalone farming using other culture technology and in different locations. As abalone farming expands into new locations and production increases it is likely that severe spionid infestations will be encountered from time to time. Vigilance will be required!

Strategies for avoidance

Farm site selection: Ideally this should be based on data on levels of mud worm infection from pilot studies or other abalone sites in the area, but the findings above suggest this may be confounded unless considerable data is available. Review of general principles regarding spionid abundance suggest that suitable sites for abalone culture should exclude very sheltered, static sites with muddy sediments near estuaries and sources of organic pollution (Medcof 1945, Korringa 1951, Woodwick 1964, Anger 1977, Pregenzer 1983, Baxter 1984, Smyth 1990). While sites with better water flow will favour spionid settlement less, the broad dispersal pattern of planktotrophic larvae means that sea based farms without these characteristics may require deployment of stock to restricted to limited periods. Stock growth rates and cost structures for sea based sites will determine whether individual farms use these sites for all or part of the grow-out phase.

Cage design: The settlement intensity and impact of *B. knoxi* were seen to vary considerably depending on the design of the abalone containment vessel, with restricted flow at the time of settlement apparently restricting entry of planktotrophic larvae, but favouring the local spread of *P. hoplura* via lecithotrophic larvae. Restricted flow and possible secondary reduction in water quality can provide an additional stress on infected abalone, increasing the detrimental effect on health. Settlement of *P. hoplura* but not *B. knoxi* was greater on abalone reared near the bottom than higher in the water column. Thus the design of rearing vessels, especially with respect to water flow and their placement in the water column has implications for farming of abalone in mud worm susceptible areas. Generally containers favouring good water flow are preferable, but it has been suggested that cleaning of rearing vessels could be timed to allow fouling to remain during the spring *B. knoxi* settlement period, while ensuring maximum water flow over the warmer summer months. This could be useful during the year following initial deployment. Containers which restrict water flow favour local spread of species such as *P. hoplura* throughout the year.

Cage design may also affect whether abalone can be appropriately air dried *in situ* within rearing vessels. This will depend largely on the degree of fouling acquired by such vessels and their degree of enclosure. The Aquatek© trays (section 4.3) with lids removed allow appropriate drying of stock compared to more enclosed designs such as tubes (section 4.3). Abalone culturists may find that air drying treatment is best combined with other handling activity such as grading or thinning out of rearing vessels. In such situations sheet plastic may serve as an appropriate substrate during drying.

Cage position Vertical position of containers appears more critical for *P. hoplura* than *B. knoxi,* with a general recommendation of good clearance from the sea floor which may harbour wild reservoirs of infected shells, in a variety of mollusc species.

Clearance of 1 m was shown to be insufficient, as enhanced *P. hoplura* levels were seen at this level, compared to 4 m clearance.

MANAGEMENT FOR AVOIDANCE

Timing of deployment of stock: The consistency of *B. knoxi* seasonal reproduction fortuitously allows a simple avoidance strategy to be practiced in areas susceptible to this species, by transfer of stock to susceptible sites post November. This allows approximately 10 months growth before potential infestation in the following September. Management strategies will necessarily be site specific and depend on the stocking size, growth rate and marketed size of stock. Placement of stock at approximately 25 mm in November/December may allow growth under ideal conditions to approximately 55-60 mm during the next year. This represents the probable minimum market size for abalone and stock grown under this regime would thus be subjected to little *B. knoxi* influence. Where there is a requirement to grow stock to larger sizes e.g. 80-100 mm, potential exposure to *B. knoxi* settlement in the second year may necessitate treatment.

Larval settlement data for *P. hoplura* (section 5.1) suggested that initial colonization with this species could substantially be avoided by placement of stock post spring and preferably after mid summer. Once established in abalone, however, the species showed the potential for year round lecithotrophic reproduction. Thus numbers of this species have the potential to rise steadily over time and this needs to be taken into account and monitored by growers – especially if the grow out period extends over 2-3 years in the sea.

Monitoring of spionid levels and the effect on abalone health: Because exposure may vary from year to year, and treatment constitutes an additional stress, monitoring of mud worm levels and species and of the general condition of the animals, is essential in areas with significant mud worm risk. This is to ensure treatment is utilised only where warranted, but before the stress of heavy infection and of rising water temperatures increases the treatment associated risks. As the effect of stressors on energy metabolism is essentially additive, such monitoring will highlight situations where intervention may be required earlier than schedules. The research findings suggest in particular an interaction of mud worm associated mortality with underfeeding, which may not otherwise be apparent..

Methods for mud worm control – treatment schedule

Treatment on needs basis:

Given the potential for air drying treatment to suppress growth of stock this management option should be used prudently with the emphasis on avoidance of infestation. Where treatment is required, stress to abalone will likely be minimised by early intervention, reduced drying time, reduced air temperature and reduced differential between sea and air temperatures. Additionally, Watanabe et al. (1994) found that starved abalone survived air exposure at considerably greater levels than fed animals.

As infestation with approximately 5 spionids may reduce growth by about 25% (sections 5.4.2 & 5.5.2) as may, potentially, air drying treatment (section 5.3) infestation at this level or above is considered worth treating. In practice this decision

will be based on the duration of time remaining before stock can be sold and on the spionid species involved. For instance, it may be counterproductive to treat recent infestations if stock can be sold within a few months. Growers should also consider that *B. knoxi* numbers will not increase in stock until the following spring dispersal period whereas *P. hoplura* counts have the potential to rise steadily from a low level of infestation to >50 per abalone in a 2 year period (section 5.5). It is recommended that abalone infested with greater than 10 - 20 spionids early in the grow out phase be treated by air drying as on balance the risks associated with not doing so appear greater than the potential consequences of treatment side-effects.

Treatment by schedule:

Although the need for treatment should be monitored, and treatment brought forward where necessary to avoid severe mud worm stress, practicalities of potentially treating large numbers of tanks require a planned treatment schedule based on predicted risk.

For *B. knoxi* susceptible sites, transfer of stock should be planned for post November, which allows approximately 10 months growth before potential infestation in the following September. To be fully effective, treatment is recommended within 6 months of infestation, but avoiding either low or high temperature extremes (> 15 ^{o}C but > 24^{o}C) and excessive humidity should be avoided if possible, as outlined in the treatment protocol. Therefore the first treatment should be scheduled for the early or late summer of second summer at sea, with the actual month of treatment likely to vary between locations. Stock placed at other times will also require treatment within 6 months of larval dispersal around September.

Where *P. hoplura* is the major problem, placement of stock post spring and preferably after mid summer will minimise initial colonisation, but as this species has the potential to rise steadily over time, predicting the time when treatment is needed is less certain. Monitoring of this species is particularly important, especially if the grow out period extends over 2-3 years in the sea.

6. BENEFITS

As identified in the original application, the sea cage sector of the abalone culture industry is the major beneficiary of the research. This sector previously appeared non-viable and affected the confidence of the abalone culture sector in Tasmania generally. All the original sea based farms most affected by mud worm in the mid 1990's had ceased commercial abalone culture attempts by this method within a year or two. The loss of confidence associated with the early mortality episodes has at least been partly alleviated as a result of this project. A new and relatively large sea based farm has been established since the project began and consulted with project staff on mud worm risk assessment during the development phase. In the last year or two further experimental or pilot scale sea based farms have begun to re-emerge in Tasmania and new land based farms have been established.

The treatment regimes are also applicable to abalone in other farming sytems, though these have greater options for avoidance of spionid infestations. Techniques and data generated by the project have also been utilised by the oyster industry in relation to mud worm assessment and risk management in relation to export of spat

from Tasmania. The data on health testing has contributed to the body of knowledge on abalone health in the culture situation and will assist in future abalone health investigations such as that concerning summer stress syndrome.

7. FURTHER DEVELOPMENT

The results of the research have been distributed directly to local industry via regular attendance at Tasmanian Abalone Growers Association meetings and to the wider abalone culture industry through the annual FRDC subprogram workshops.

Project results indicated severe mud worm infestations may not be common and could possibly occur only once or twice a decade. Consequently, corporate memory of the potential for spionid stock damage may be lost. This has in part been addressed by production of a small handbook suitable for growers, which includes a pictorial guide to early signs and progression of infestation, a species identification guide, notes on spionid reproduction and a protocol for treatment (Lleonart, 2001). The farm guide has been submitted to FRDC Abalone Aquaculture Subprogram to be supplied to all existing farms and distributed by the state bodies to new grower members.

8. PLANNED OUTCOMES

The major project outputs are that mud worm infestations can be substantially avoided in the first instance and treated if necessary. The major project outcome this knowledge and a new sense of perspective regarding the risk of severe infestation has produced has been a rise in investor confidence leading to renewed interest in sea based abalone farming. This has contributed to the development of at least three new sea based abalone farms in Tasmania.

In so far as this project incorporated a Ph.D. studentship, a further outcome is the completion and submission of a Ph.D. Thesis based on this work (Lleonart, 2002), and awarding of Doctor of Philosophy to Mark Lleonart.

9. CONCLUSION

Widespread stock mortality in Tasmanian sea cage farmed abalone prompted this research project. The general objective of developing a sustainable means to control mud worms in farmed abalone was met. Of the specific project objectives the objective of investigation of the ecology and reproductive biology of spionids found that the major focus of the project, *Boccardia knoxi*, had a strictly seasonal reproductive cycle with larval settlement in the spring months. Thus post spring placement of stock in susceptible areas allows up to a year's relief from the impacts of infestation by this species.

Likewise, settlement of a further common spionid species *Polydora hoplura* was mainly confined to the spring and summer allowing for an avoidance strategy. Unlike *B. knoxi*, *P. hopl*ura was found to be capable of near year round reproduction of larvae once established in the shell. Thus, the numbers of this species infecting abalone have

the potential to rise steadily with grow out time whereas *B. knoxi* numbers only increase in the spring. Three other spionid species were found in abalone, two of which had not previously been reported from Tasmania. These species were present at very low levels.

The original objectives relating to chemical treatments and their application within the production cycle were altered to reflect the use of a non-chemical treatment option. In addition, an original objective to examine the efficacy of antifoulant paints could not be satisfactorily met as no application method suitable for abalone was found. The use of air drying to kill mud worms in abalone rather than chemicals or drugs was considered a very favourable outcome in terms of health and safety issues, withholding periods, licensing issues and public perception.

Air exposure for 2-4 hours in shade at 15-21 °C and humidity less than approximately 63% are the recommended treatment conditions. In laboratory and field trials such treatment eliminated >90 % of infesting spionids present for less than 6 months. Spionid reduction in long term severely infested stock is possible but varies with severity of blisters, size of stock and degree of fouling organisms present on abalone shells. It is suggested growers in mud worm susceptible areas monitor stock in mid summer to determine whether treatment is warranted. As there was some evidence that drying treatment could depress growth in the medium-long term avoidance is preferred in the first instance, followed by treatment, if required after potential spionid exposure the following spring/summer. Treatment could be combined with other handling activities such as grading, stock transfer or cage cleaning.

A further specific project objective related to gathering of epidemiological information on culture environment and stock characteristics. Experiments conducted on stock characteristics showed that larger stock attracted more spionid settlement at initial placement. Fouling organisms such as Pacific oysters and calcareous tube building worms especially spirorbids substantially increased the risk of settlement by *B. knoxi* mud worms. Abalone were found to be at increased risk of *P. hoplura* infestation near the bottom than higher in the water column and rearing vessels with reduced mesh area excluded planktonic *B. knoxi* larvae. A survey of the literature with regard to spionid infestation in commercial bivalves suggests that abalone farms should avoid areas with muddy or silty sediments, estuaries and locations susceptible to organic pollution.

In conclusion the abalone mud worm project has shown that severe spionid infestation is not as common as first feared following the mortality events of the mid 1990's; that infestation can be substantially avoided by appropriate management of stock transfer times and stock selection; and that mud worm infestation can be successfully treated if required. These findings have contributed to renewed confidence in abalone farming in Tasmania, including the re-establishment of some sea based farms. The knowledge that spionid infestation can be managed should contribute to the abalone industry generally in the future as such outbreaks are likely to occur again from time to time.

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APPENDIX 1: Intellectual Property

There are no intellectual property issues associated with this project.

APPENDIX 2: Staff

Handlinger, Judith – Principal Investigator, TAFI Fish Health Unit
Powell, Mark - Academic supervisor, TAFI, University of Tasmania
Lleonart, Mark - PhD student Uni of Tasmania
O'Brien, Dominic – Huon Aquaculture Company P/L
Lee, Peter - Aquatas P/L
Cieura, Joe - Aquatas P/L
Finnigan ,Greg - Technical Officer, TAFI

APPENDIX 3 (Raw data & statistics)

Section 1: Pre-project data for Huon Aquaculture Company

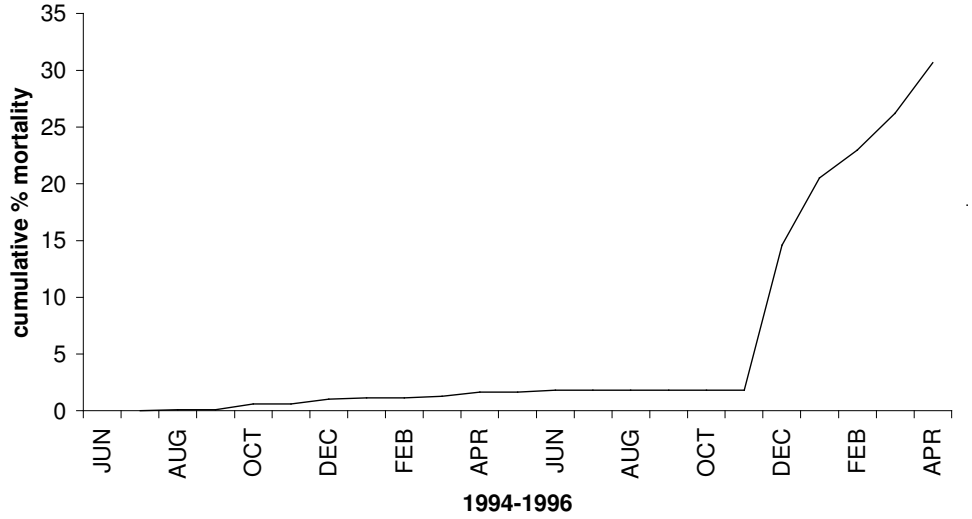
1A: Mortality data for Huon Aquaculture Company, 1994-1996.

	Initial	**Intake**	MORTALITY DATA						
Species	No.	**Date**	1995 DEC	1996 JAN	1996 FEB	1996 MAR	1996 APR	TOTALS	%kill
H. rubra	220	14.06.94	74	13	2	7	13	109	50
H. rubra	220	14.06.94	48	25	8	13	6	100	45
H. rubra	220	14.06.94	12	10	0	5	4	31	14
H. laevigata	129	22.06.94	11	7	4	2	12	36	28
H. rubra	220	14.06.94	36	7	4	6	7	60	27
H. rubra	220	14.06.94	13	10	9	16	6	54	25
H. laevigata	129	22.06.94	24	19	3	3	7	56	43
H. rubra	220	14.06.94	7	5	7	6	9	34	15
H. rubra	220	14.06.94	5	15	5	1	9	35	16
H. rubra	220	14.06.94	28	8	7	7	17	67	30
Totals:	2018		258	119	49	66	90	582	29%

	Initial	**Intake**	MORTALITY DATA						
Species	No.	**Date**	1995 DEC	1996 JAN	1996 FEB	1996 MAR	1996 APR	TOTALS	%kill
H .laevigata	120	14.08.95	15	6	4	4	3	32	26.7
H .laevigata	120	14.08.95	24	14	4	10	6	58	48.3
H .laevigata	120	14.08.95	31	5	4	5	1	46	38.3
Hybrid	60	14.08.95	6	0	0	5	0	11	18.3
Hybrid	60	14.08.95	3	0	3	3	1	10	16.7
Hybrid	60	14.08.95	5	1	4	3	2	15	25
Totals:	540		84	26	19	30	13	172	32%

	Initial	**Intake**	MORTALITY DATA						
Species	No.	**Date**	1995 DEC	1996 JAN	1996 FEB	1996 MAR	1996 APR	TOTALS	%kill
H. rubra	200	05.01.95	2	0	67	3	0	72	36
H. rubra	200	05.01.95	0	1	0	11	12	24	12
H. rubra	200	05.01.95	0	0	6	2	1	9	4.5
H. rubra	100	05.01.95	0	0	0	5	0	5	5
H. rubra	100	05.01.95	0	0	6	2	1	9	9
H. rubra	150	05.01.95	1	0	4	4	4	13	8.7
H. rubra	150	05.01.95	0	0	0	0	2	2	1.3
H. rubra	200	05.01.95	2	2	4	0	2	10	5
H. rubra	200	05.01.95	2	6	11	4	3	26	13
H. rubra	200	05.01.95	0	0	2	2	7	11	5.5
H. rubra	200	05.01.95	0	0	9	2	3	14	7
H. rubra	200	05.01.95	2	0	25	15	10	52	26
H. rubra	200	05.01.95	4	9	6	10	1	30	15
H. rubra	200	05.01.95	3	3	3	12	10	31	15.5
	2500		16	21	143	72	56	308	12.3%

1A Figure 1. Cumulative mortality for June 1994 abalone intake at Huon Aquaculture Company.



1B Subjective shell damage ratings (SSDR) for remnant Huon Aquaculture Company abalone sampled 1997-1998.

SSDR	0	1	2	3
% each grade	1	15	52	36

n = 149, shell kept and assessed retrospectively May 2001

1C Annual Pacific Oyster blister frequency data

Year	% stock with shell blisters	No. oysters
1999	6.7	1600
1998	8.6	1890
1996	5.4[#]	1247
1995	10.2[#]	684
1994	6.9[#]	1316
1990-92	4.0*	>5000

[#] Data from Ryan. (2000)
* Data from Wilson et al. (1993)
Other data from DPIWE Fish Health Lab records.

1D SSDR scores for wild abalone from Tasmania (Taroona collection)

(% values in parentheses)

SSDR score	0	1	2	3
Greenlip	45(64.3)	11(15.7)	9(12.9)	5(7.1)
Blacklip	101(24.0)	166(39.5)	143(34.0)	10(2.4)

n=490, some shell damage may be due to boring sponge
Chi-square analysis found significant difference between species (P<0.001)

1E Comparison of SSDR scores between stunted and normal population samples (Taroona collection)

(% values in parentheses)

SSDR score	0	1	2	3
Normal	143(31.1)	166(36.1)	137(29.8)	14(3.0)
"stunted"	3 (10)	11(36.7)	15(50)	1(3.3)

Chi-square analysis found significant difference between size groups ($P<0.05$)

Section 5: Chemical Immersion treatment screening data]

5A Fresh water

Spionids *in vitro*	Time (min)			
Survival	10	15	30	45
48 h post treatment	0/5	0/5	0/5	0/5

Temperature 18 °C

Spionids *in situ*, $\overline{X}$ (SD)	Fresh water Immersion Time (min.)			
	Control	30	60	120
Mean chimneys	21.6(8.1)	30.4(14.8)	26.2(11.8)	18.3(8.3)
Mean surviving *B. knoxi*	26.5(10.1)	32.8(13.7)	30.6(15.2)	16.0(13.5)
Mean *B. knoxi* (EI)% Kill	0.3(0.9)	8.6(14.7)	13.7(26.4)	30.2(33.3)
(GMC)% Kill –*B. knoxi*	-	0	0	39.6
Mean survival – Total worms	27.0(10.0)	32.9(13.7)	31.1(15.4)	16.3(13.6)
(GMC)%Kill – Total worms	-	0	0	39.6
% Abalone mortality	0	10	50	80

n=10 all treatments (5/10 live in control group)

5B Potassium permanganate

Spionids *in vitro*	Concentration KMnO4 ($mg.l^{-1}$)					
Survival	2	4	8	15	20	50
7 d post treatment	1/5	5/5	4/5	3/5	2/5	0/5

Temperature 18 °C

Spionids *in situ,* $\overline{X}$ (SD)	Concentration KMnO4 (mg.l^{-1})	
	Control	25
Mean surviving *B. knoxi*	15.9(11.3)	20.6(26.9)
(GMC)% Kill –*B. knoxi*		0
Mean survival – Total worms	33.2(23.9)	29.5(40.6)
(GMC)%Kill – Total worms		11.2
% Abalone mortality	0	60

n=19 each group

$KMnO_4$ toxicity to abalone. Combined data for 3 exposure time and concentration experiments.

40-50 mm abalone	5 mg.l^{-1}	10 mg.l^{-1}	15 mg.l^{-1}	20 mg.l^{-1}	25 mg.l^{-1}	30 mg.l^{-1}	50 mg.l^{-1}
Mortality 14 d post treatment (3 h)			0/5		0/5		1/5
Mortality 16 d post treatment (4 h)		0/5		4/5		4/5	
Mortality 8 d post treatment (9 h)	0/10						

5C Gentian violet

Spionids *in vitro*	Concentration of Gentian Violet (mg.l^{-1})				
Survival	control	10	20	50	100
Immediate post treatment	10/10	3/5	3/5	2/5	0/5
4 d post treatment	9/10	0/5	0/5	0/5	0/5

Temperature 15°C

Spionids *in situ*, $\overline{X}$ (SD) Concentration gentian violet (mg.l^{-1})		
	Control	7.5
Mean surviving *B. knoxi*	11.5(7.1)	10.0(7.8)
(GMC)% Kill -*B. knoxi*		13.0
Mean survival – Total worms	18.4(10.6)	14.1(9.0)
(GMC)%Kill -Total worms		23.4

n=18 each group

Gentian violet toxicity to abalone. Data for 2 exposure time and concentration experiments

40-50 mm abalone	3 mg.l^{-1}	5 mg.l^{-1}	10 mg.l^{-1}	20 mg.l^{-1}
Mortality after 17 d (3 h exposure)		0/5	2/5	3/5
Mortality after 11 d (9 h exposure)	1/10			

5D Mebendazole

Spionids *in situ*	Concentration of mebendazole (mg.l^{-1})				
Survival	control	50	100	200	500
Immediate post treatment	5/5	5/5	5/5	5/5	5/5
9 days post treatment	5/5	0/5	0/5	1/5	0/5

Temperature 16 °C

Spionids *in situ*, $\overline{X}$ (SD)	Concentration of Mebendazole (mg.l^{-1})	
	Control	200
mean surviving *B. knoxi*	4.9(4.1)	2.0(1.7)
(GMC)% Kill -*B. knoxi*		59.2
mean survival – Total worms	8.8(9.6)	4.1(3.8)
(GMC)%Kill -Total worms		53.4

n=20 each group

Mebendazole toxicity to abalone. Combined data one week post exposure for three exposure time and concentration experiments

40-50 mm abalone	25 mg.l^{-1}	50 mg.l^{-1}	200 mg.l^{-1}
3 hour exposure		0/5	0/5
9 hour exposure		0/10	
17 hour exposure	0/10		

5E Fenbendazole

Spionids *in vitro*	Concentration of fenbendazole (mg.l^{-1})				
Survival	control	50	100	200	500
Immediate post treatment	10/10	5/5	5/5	5/5	4/5
10 Days post treatment	9/10	0/5	0/5	1/5	0/5

Temperature 15 °C

Spionids *in situ*, $\overline{X}$ (SD)	Concentration of fenbendazole (mg.l^{-1})	
	Control	250
Mean surviving *B. knoxi*	10.9(6.5)	7.6(7.6)
(GMC)% Kill -*B. knoxi*		30.3
Mean survival – Total worms	14.0(6.3)	8.3(7.7)
(GMC)%Kill -Total worms		40.7

n=20 each group

Fenbendazole toxicity to abalone (3 h exposure)

40-50 mm abalone	100 mg.l-1	250 mg.l-1
Mortality 19 d post treatment	0/4	0/6

5F Levamisole

Spionids *in vitro*. Combined trial data.			Concentration levamisole (mg.l^{-1})			
Survival	Control	0.32	3.2	32	128	320
Trial 1 Immediate post treatment	6/6	6/6	6/6	6/6		6/6
8 d post treatment	6/6	4/6	1/6	5/6		4/6
Trial 2 Immediate post treatment	10/10		5/5	4/5	4/5	4/5
8 d post treatment	9/10		4/5	0/5	1/5	3/5

Temperature 15 -16°C

Spionids *in situ*. $\overline{X}$ (SD)		Concentration of levamisole (mg.l^{-1})			
	Control	6.4	64	640	2 h dry & 64 mg.l^{-1}
mean chimneys	42.6(23.9)	40.1(13.5)	30.6(14.1)	38.0(16.9)	41.0(12.9)
mean surviving *B. knoxi*	28.0(14.7)	19.3(9.5)	16.1(6.1)	8.1(6.0)	22.8(8.42)
(GMC)% Kill -*B. knoxi*		31.1	42.5	71.1	18.6
mean survival - Total worms	30.6(14.5)	27.9(19.8)	17.9(5.6)	10.5(4.4)	25.6(8.2)
(GMC)%Kill - Total worms		8.8	41.5	65.7	16.4
% Abalone mortality	0	16.7	50	100	60

n=10 infested shells, n=5-6 abalone mortality data

Levamisole exposure data at 320 mg.l^{-1}, mudworms *in situ*, $\overline{X}$ (SD), n=20

	Control	levamisole
Mean surviving *B. knoxi*	4.9(4.1)	3.2(2.5)
(GMC)% Kill -*B. knoxi*		34.7
Mean survival - Total worms	8.8(9.6)	4.9(3.4)
(GMC)%Kill –Total worms		44.3

Levamisole toxicity to abalone (exposure 3 h). Combined data for two stock sizes

40-50 mm abalone	0.32 mg.l^{-1}	3.2 mg.l^{-1}	32.0 mg.l^{-1}	320 mg.l^{-1}
Mortality 18 d post treatment	0/5	0/5	1/5	0/5

18-20 mm abalone	64 mg.l-1	320 mg.l-1	512 mg.l-1
Mortality 8 d post treatment	0/15	5/15	15/15

5G Malachite green

Malachite green toxicity to abalone data (exposure time 3 h)

18-20 mm abalone	5 mg.l-1	10 mg.l-1	20 mg.l-1
Mortality 8 days post treatment	1/10	11/12	12/12

Spionids *in situ* and *in vitro*, $\overline{X}$ (SD)	Concentration malachite green (mg.l^{-1})			
	control	1	5	10
Mean surviving *B. knoxi*	35.1(10.9)	28.4(21.5)	20.0(13.8)	17.2(10.8)
(GMC)% Kill -*B. knoxi*		19.1	43.0	51.0
Mean survival – Total worms	58.7(16.6)	51.5(38.3)	36.6(15.0)	23.7(14.6)
(GMC)%Kill – Total worms		12.3	37.6	59.6
Spionid survival immed. post treat.	22/22	12/12	17/17	31/31
Spionid survival 7 post treat. n=10 each abalone treatment	22/22	12/12	0/17	0/31

5H Trichlorofon

Spionids *in vitro*	Concentration of trichlorofon (mg.l^{-1})						
Survival	control	0.1	1	10	100	500	1000
Immediate post treatment	5/5	5/5	5/5	5/5	5/5	5/5	5/5
8 d post treatment	4/5	5/5	5/5	5/5	5/5	4/5	4/5

Temperature 13 °C

Trichlorofon toxicity to abalone (exposure time 3 h)			
40-50 mm abalone	10 mg.l^{-1}	100 mg.l^{-1}	500 mg.l^{-1}
Mortality 11 d post treatment	0/5	0/5	0/5

5I Praziquantel

Spionids *in vitro*	Concentration of Praziquantel (mg.l^{-1})						
Survival	control	0.5	1	5	10	50	100
Immediate post treatment	6/6	6/6	6/6	6/6	6/6	6/6	6/6
20 d post treatment	5/6	6/6	6/6	6/6	4/6	6/6	5/6

Temperature 14 °C

5J Hydrogen peroxide

Spionids *in vitro*	Concentration of Hydrogen Peroxide (ppm)					
Survival	control	50	100	200	500	1000
Immediate post treatment	5/5	5/5	5/5	5/5	5/5	5/5
9 d post treatment	4/5	5/5	5/5	3/5	3/5	3/5

Temperature 15 °C

Hydrogen peroxide toxicity to abalone (exposure time 3 h)		
40-50 mm abalone	50 mg.$^{l-1}$	200 mg.$^{l-1}$
Mortality 14 d post treatment	0/5	0/5

5K Formalin

Formalin toxicity to abalone (3h exposure)

18-20 mm abalone	50 mg.l-1	100 mg.l-1	200 mg.l-1
Mortality after 7 d	0/10	0/10	9/10

Spionids *in vitro* and *in situ*, $\overline{X}$ (SD). Concentration of Formalin (ppm)

	control	50	100	200
mean surviving *B. knoxi*	28.7(14.8)	22.9(10.0)	25.5(16.0)	18.3(6.1)
(GMC)% Kill -*B. knoxi*		20.2	11.1	36.2
mean survival - Total worms	39.1(21.9)	37.2(14.6)	30.3(16.5)	19.9(7.2)
(GMC)%Kill - Total worms		4.9	22.5	49.1
spionid survival immed. post treat.	5/5	15/15	10/10	6/6
Spionid survival 8 d post treat.	4/5	14/15	7/10	1/6

5L Ivermectin

Spionids *in situ*, $\overline{X}$ (SD). Concentration Ivermectin ($mg.l^{-1}$)

	control	0.004	0.04	0.4
Mean surviving *B. knoxi*	20.9(11.6)	30.1(18.5)	28.7(18.1)	8.3(3.7)
(GMC)% Kill –*B. knoxi*		0	0	60.3
Mean survival – Total worms	25.5(11.2)	37.5(30.6)	32.1(22.2)	14.5(7.3)
(GMC)%Kill – Total worms		0	0	56.9
% Abalone mortality	33.3	23.1	46.2	84.6

n=10 infected abalone, n=13 for mortality data

Spionids *in vitro* Concentration of Ivermectin ($mg.l^{-1}$)

Survival	control	0.05	0.1	0.2	0.3
Immediate post treatment	5/5	5/5	5/5	5/5	5/5
7 d post treatment	5/5	5/5	2/5	5/5	5/5

Temperature 16°C

Ivermectin toxicity to abalone data (3 h exposure)

40-50 mm abalone	0.05 $mg.l^{-1}$	0.1 $mg.l^{-1}$	0.2 $mg.l^{-1}$	0.3 $mg.l^{-1}$
Mortality after 18 d	0/4	1/4	4/4	4/4

5M Exposure to febantel, pyrantel embonate and praziquantel in combination.

Spionids *in vitro*	Concentration of Febantel and Pyrantel Embonate (mg.l^{-1}) *				
Survival	control	25 & 14.4	50 & 28.8	125 & 72	250 & 144
Immediate post treatment	5/5	5/5	5/5	5/5	5/5
8 d post treatment	0/5	2/5	4/5	4/5	lost

***** Febantel concentration shown first in column headings, praziquantel not shown as previously shown ineffective. Temperature 15°C

Abalone mortality data. Exposure to febantel, pyrantel embonateand praziquantel in combination	
40-50 mm abalone	250mg/ l & 144 mg.l^{-1} *
Mortality after 15 d	0/5

***** Febantel concentration shown first, praziquantel not shown as above.

5N Metronidazole & Dimetronidazole

Spionids *in vitro*	Concentration metronidazole (mg.l^{-1})					
Survival	5	10	20	50	100	200
48 hr post treatment	10/10	10/10	7/10	9/10	7/10	10/10

Temperature 18 °C

Spionids *in vitro*	Concentration dimetronidazole (mg.l^{-1})					
Survival	Control	20	50	100	200	500
Immediate post treatment	5/5	5/5	5/5	5/5	5/5	5/5
8 d post treatment	2/5	0/5	3/5	1/5	3/5	4/5

Temperature 16 °C (but with large fluctuation)

5O Methylene blue

Spionids *in situ*, $\overline{X}$ (SD).	Concentration of methylene Blue (mg.l^{-1})			
	Control	1	5	10
Mean surviving *B. knoxi*	20.6(6.3)	32.7(10.9)	23.0(10.4)	23.1(10.8)
(GMC)% Kill -*B. knoxi*		0	0	0
mean survival – Total worms	30.9(7.2)	39.3(12.7)	30.5(11.8)	35.2(12.1)
(GMC)%Kill – Total worms		0	1.3	0
% Abalone mortality	0	0	0	0

n=10 infected shells, n=3 mortality data

Spionids *in vitro*	Concentration methylene Blue (mg.l^{-1})				
Survival	20	30	50	100	200
Immediate post treatment	5/5	5/5	5/5	5/5	5/5
7 d post treatment	4/5	2/5	4/5	5/5	5/5

Temperature 18 °C

5P [Section 5.6 Health (1)]

Regression analysis Total blister Vs shell length

	df	Ss	ms	vr	F pr.
Regression	1	1033	1033.03	35.28	<0.001
Residual	47	1376	29.28		
Total	48	2409	50.19		

Estimates of parameters

	estimate	Se	t(47)	t pr.
Constant	80.26	2.37	33.80	<0.001
Total blisters	-0.3793	0.0639	-5.94	<0.001

Significance of r value: $r_{0.05, 47} = 0.288$, $r_{critical}$ 0.288 < 0.648 ($r_{observed}$), so is significant.

5Q [Section 5.6 Health (1)]

Regression analysis Total blister Vs whole weight

	df	Ss	Ms	vr	F pr.
Regression	1	2744	2744.1	23.94	<0.001
Residual	47	5387	114.6		
Total	48	8131	169.4		

Estimates of parameters

	estimate	Se	t(47)	t pr.
Constant	68.40	4.70	14.56	<0.001
Total blisters	-0.618	0.126	-4.89	<0.001

Significance of r value: $r_{0.05, 47} = 0.288$, $r_{critical}$ 0.288 < 0.566 ($r_{observed}$), so is significant

5R [Section 5.6 Health (2)]

t statistics for comparison of spionid infested &healthy abalone stocks.

Foot bleed data for clin. path.

	t value	Df	p value
Cl	0.47	18	0.642
K	2.98	22	0.007
Na	-0.07	22	0.944
Na/K ratio	-5.41	22	<0.001
Ca	-0.29	16	0.772
Mg	1.37	16	0.190

Na/K ratio data arcsine transformed

Mean, SD and n values in Table 5.33

5S [Section 5.6 Health (2)**]**

Mann-Whitney U Test statistics for comparison of mud worm infested and healthy abalone stocks.

Foot bleed data for clinical pathology

	U value	P value
Cu	47.0	0.82
Glucose	6	0.13
Protein	17.0	0.66

Mean, SD and n values in Table 5.33

5T [Section 5.6 Health (2)]

Haemocyte counts 2000. Three size classes and 2 year classes. 2 way ANOVA

Source of variation	Df	Ss	Ms	vr	F prob.
Size	2	6.317E+13	3.158E+13	10.32	<0.001
Age	1	1.398E+12	1.398E+12	0.46	0.502
Size.age	2	6.397E+11	3.199E+11	0.10	0.901
Residual	54	1.652E+14	3.059E+12		
Total	59	2.304E+14			

Comparison of means for above ANOVA table. Haemocytes/ml

Size	1 year old	2 years old
Large	5092500[A]	5167500[A]
Medium	4307500[A]	4572500[A]
Runts	2404000[B]	2980000[B]

Means with shared superscripts are not significantly different (5% level)

5U [Section 5.6 Health (2)]

ANOVA 1999 Haemocyte data for 6 groups of abalone

Source of variation	Df	Ss	Ms	vr	F prob.
Size/age group	5	5.116E+13	1.023E+13	2.43	0.047
Residual	52	2.193E+14	4.216E+12		
Total	57	2.704E+14			

Comparison of means for above ANOVA table

Size&age group	Haemocytes/ml
3 year – big	5640000[AB]
3 year – runts	3302500[C]
2 year – big	5317500[AB]
2 year – medium	6030000[A]
2 year – runt	4113889[BC]
< 1 year – big	4788889[ABC]

Means with shared superscripts are not significantly different (5% level)

5V [Section 5.6 Health (2)]

Seawater profile from southern study sites ($mmol.l^{-1}$)

	Ca	Mg	Na	K	Na/K ratio	Cl
	10.53	49.72	475	10.6	45	524
	10.24	53.54	451	10.4	44	534
	10.01	46.41	427	9.6	44	487
	10.2		400	8.8	45	460
	10.08	46.00	412	9.2	45	480
Mean	10.21	48.92	433.0	9.7	44.6	497.0
SD	0.20	3.50	30.2	0.8	0.5	31.0

5W [Section 5.6 Health (2)]

Monthly rainfall data for Dover weather station 1901-2000

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1901			127.3	185.2	80.5	77.5	64.3	49.3	95	165.9	75.7	24.4
1902	113.5	132.8	27.2	45.5	37.1	65.5	27.2	60.2	112.5	32.5	55.4	76.7
1903	42.2	94.5	74.9	70.4	65	173.5	127.8	69.6	77.2	50.3	73.2	97.8
1904	60.7	97.8	166.1	20.6	146.3	143.8	135.6	153.7	177.3			
1905	119.6	55.4	68.3	78.7	183.1	76.2	85.6	48.3	154.2	61.2	63	43.9
1906	16.5	15	75.2	94.5	58.7	117.1	94.2	56.1	22.4	188.2	69.9	19.6
1907	82	45.5	68.1	61.2	72.1	47.5	89.2	61.5	147.1	154.4	40.6	166.4
1908	31	22.1	84.6	37.3	55.6	74.4	49	50.5	61.5	126	54.4	28.2
1909	42.7	29.2	98.8	180.1	82.3	134.4	69.9	90.9	48.3	81.5	50.5	88.6
1910	87.1	23.4	48.3	88.4	72.9	127.3	70.4	74.9	128.5	75.7	49	112.8
1911	18.5	79.2	118.9	75.9	160.3	95.8	9.1	43.9	37.6	109.2	50.5	122.7
1912		2.8	48.8					84.3	133.9	97.8		64
1913	79.5	27.4	72.9	9.9	27.9	40.9	53.1	78.7	82.8	73.4	109.5	45.5
1914	7.1	17.8	38.6	115.8	10.7	42.7	60.2	63.8	52.3			
1924					46	175.1		49.8	44		84.6	
1925		37.3	87.3	126.3	83.6				48.6	86.6	22.5	
1926		46.9	28.4	95.9	66.5	83.3	110.5	114.5	72.9	133.3	79.1	
1927		76.4	37.8	16.1	87.1	141						
1945			44.2	16.9	15.6	36.3	50.3	86	115.2	49.8	29.2	27.3
1946	31.3	100.2	295.4	64.6	69.4	128.8	168.2	225.7	102.6	91.9	95.8	42
1947	31.6	41.2	104.1	26.2	98.3	173.5	138.5	98.7	73.6	250.3	34.5	96.9
1948	26.2	43.9	68.2	62.8	79.5	56.9	56.9	47.8	90.7	126	119.1	96.9
1949	104.6	104.4									118.9	
1956	69.5	53.1	64.3	66.3	147.1	180.4	70.9	104	32.4	206.7	122.3	76.6
1957	41.1	37.1	62.8	100	81	21.8	36.3	38.5	160.3	68	92.8	82.3
1958	17.1	58	90.4	47.3	243.6	88.1	84	193.9	36.9	109.4	71.7	112.8
1959	43.2	45.8	26.1	55.3	33	55	72.5	72.6	89.5	34.5	23.3	128.8
1960	32.7	28	26.4	262	133.7	34.8	66.9	55.1	72.7	74.4	69.7	8.7
1961	36.6	47.6	31.1	49.3	46.5	116.7	114.2	67.2	69.4	58.2	41.7	58.9
1962	54.5	47.7	46.5	50.7	99.2	114.7	86.1	134.2	152.8	95.3	43.8	42.1
1963	35.3	29.5	24.8	48	34.8	41.3	113.8	50.9	66.9	15.3	52.4	27.2
1964	51.7	163.4	48	34	56.9	83.8	125.2	112.9	51.6	51	89.7	134.1
1965	58.2	33.1	74.8	92.5	83.3	45.5	36.7	56.1	67.4	52.9	92	50.6
1966	20.3	24.9	58.4	101.2	60.5	18.9	115.7	54.8	90.7	97	58	27.5
1967	24.7	22	32.9	30.3	34.9	24.8	171.1	72.5	55.9	66.6	114.2	68.1
1968	16	74.9	47.5	55.5	105.7	117.3	56.2	101	103.6	89.9	157.2	43.4
1969	37.1	112.3	52.5	74.4	79.1	89.4	66.6	76.3	39.9	30.2	108.8	153.3
1970	174.4	17.4	50.9	40.8	47.9	42.3	171.6	120.2	73.4	130.9	67.8	210.3
1971	90	95.9	35.3	49.3	114.7	81.3	63.5	86	88.5	114.8	72.9	60.5
1972	34.4	52.5	29.7	73.5	29.4	60.9	152	54.5	82.6	46.6	40.1	65.8
1973	36.2	50.9	56.4	106	98.4	96.4	31.9	51.5	65	104	57.8	72.6
1974	10	45.4	55.6	73.7	77.2	94.2	187.1	68	92.4	53.2	75.6	122.4
1975	81.6	18	110.8	51.8	138.2	50.2	116.6	193.8	65.2	89.9	79	20.2
1976	117.6	11.6	55.4	41.8	60.2	58.4	57.6	142.4	84.8	68.2	118.2	133.6
1977	56.8	41.4	78.2	56	66.8	68.4	145.4	46.6	51.6	48.8	104.2	73
1978	31.2	85.6	20.4	56.4	70.2	75.6	114.4	158.4	29.8	54.6	88.4	75.4

5W Continued….

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1979	32.2	45.8	33.2	78.4	48.4	8.2	41	91	106.6	71	40.4	78.2
1980	60	59	92.6	73.8	60.2	51.4	51	115.6	105.2	80.6	57.2	47.6
1981	25.6	13.2	108.4	113.6	43.2	121	104.6	131.8	83.8	82	34.4	70.2
1982	43.8	49	72.2	26.4	91.6	50.4	69.2	42	112.4	46.4	55	38.2
1983	48	10.4	159.6	57.6	49.6	180.2	65.6	63	167.8	58.6	143.8	22.6
1984	24.2	36.2	44.6	51.2	45.6	64.4	70.8	181.2	150.6	93.4	77.8	137
1985	84.2	57	52.4	75.8	42.8	79.6	118.8	65.4	63.6	91.8	62.2	184.6
1986	116.8	63.4	39.4	112	120.8	87.4	94.2	39.6	52.2	104.6	37.2	108.8
1987	118	44.6	77.8	25.4	70.8	44.6	57.4	54	70.2	66	67	
1988	28	10.6	17.2	21.8	75.8	88.6	99.4	75.6	82.2	182	86.4	46.4
1989	80.8	22	61	40	47.4	80.8	84.4	49.6	38	116.4	29	41.4
1990	41	61.6	39.8	26.8	72.2	48.6	204	86	31	55.6	56.4	79.8
1991	56.4	9	55.8	55.6	19.4	51.2	70.6	164	58.6	69.2	72	111.4
1992	78.6	40.8	15	48.8	55.8	41.8	125.8	83.6	87.8	49.4	90.6	53.6
1993	39	61.2	41.6	33.2	60.7	87.6	29.2	80.6	41.6	81.8	116.4	149.8
1994	57	18.4	20.2	57.2	107.4	47	75.6	118.8	111	54	112.2	10.4
1995	**72.6**	**22.8**	**75.8**	**103.4**	**36.2**	**73.2**	**139.2**	**161.8**	**69.2**	**97**	**113.4**	**96.2**
1996	100.4	152.4	98.6	179.8	11.2	55	60.4	80	104.4	79.2	101.4	49
1997	143.2	77	118.2	44.8	36	21.2	59.8	118	58.6	95.8	55.4	49.8
1998	17.2	82	21.4	51	67.6	95.8	43.8	50.2	71.8	90.4	86.8	81.8
1999	8.8	163.6	72.6	55	38.6	19.6	89.6	27.2	29.6	53.4	67.8	36.8
2000	42.8	31.4	38.4	30	84.4	49.6	108.4	82.6	86.8	114.2	28.4	100.8
2001	11.4	15.6	80.6	85.2	52.8	123						
Highest	174.4	163.6	295.4	262	243.6	180.4	204	225.7	177.3	250.3	157.2	210.3
Lowest	7.1	2.8	15	9.9	10.7	8.2	9.1	27.2	22.4	15.3	22.5	8.7
Mean	**54.7**	**52.1**	**65.7**	**68.7**	**72.8**	**79.4**	**88.6**	**87.4**	**81.6**	**88.1**	**73.6**	**76.6**
Median	42.8	45.5	55.8	56.2	66.8	75	75.6	75.6	73.5	81.5	70.8	71.4
Number	62	66	67	66	67	66	63	65	66	63	64	60