ABALONE AQUACULTURE SUBPROGRAM: A NATIONAL SURVEY OF DISEASES OF COMMERCIALLY EXPLOITED ABALONE SPECIES TO SUPPORT TRADE AND TRANSLOCATION ISSUES AND THE DEVELOPMENT OF HEALTH SURVEILLANCE PROGRAMS.

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AQUACULTURE
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Tasmanian Aquaculture
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TABLE OF CONTENTS

NON TECHNICAL SUMMARY

2002/201 Abalone Aquaculture Subprogram: a national survey of diseases of commercially exploited abalone species to support trade and translocation issues and the development of health surveillance programs.

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OBJECTIVES:

1. To undertake, over approximately one year, a single-round health survey of abalone from representative wild groups of commercial abalone species throughout their range in five States, using statistically relevant samples appropriate to maximize the chances of detection of serious diseases and define the disease agents present.
2. To similarly examine equivalent samples from all abalone farms and reseeding operations in these States.
3. From these to develop a database of abalone disease, their location and apparent prevalence (with confidence limits), then to present these findings to the wild and aquaculture industries and State and National government agencies, and to record them pictorially in accessible electronic format.
4. To expand the pool of abalone health expertise by holding an initial training workshop for collaborating pathologists to facilitate the survey, and a national abalone health meeting to present disease and pathology findings to all relevant pathologists and health service providers
5. Ensure the resulting information and skills are fully utilised by assisting in the development of cost-effective on-going health surveillance programs through collaboration with the abalone aquaculture industry and State authorities.

NON TECHNICAL SUMMARY:

Concerns regarding abalone diseases are increasing worldwide with the emergence of abalone aquaculture, and the recent emergence of several significant new abalone diseases (some of which have been translocated) and recognition that disease has played a part in the decline of some wild abalone populations. That relatively few abalone diseases are known worldwide has been recognised to be a result of the lack of examination (absence of proof, rather than proof of absence) and that more diseases were likely to emerge with the rapid development of abalone aquaculture. Intensive abalone aquaculture is developing in Australia, mainly as land-based farms involving intensive stock management and use of manufactured feeds, and the likelihood of disease is high, particularly in the presence of excessive stress and potential pathogens. Management of disease and stress levels is an essential on-going requirement of all intensive farming systems to maximise profits and provide appropriate levels of animal welfare. Long term profitability also requires the ability to access to source new stock off-farm to maintain genetic diversity, access genetically improved stock or by consolidating hatchery production, a process that may carry a risk of disease translocation. To address these needs, each aquaculture sector need access to laboratory and field expertise with knowledge of the abalone diseases likely to be present, and an understanding of their pathology and impact.

Disease investigations of wild Australian stock had previously been largely restricted to *Perkinsus* investigations, usually with tests targeted only to this agent. Health surveillance of farmed stock has been largely passive and opportunistic, with the majority of investigations undertaken in Tasmania.

This survey was developed after discussions with both the aquaculture and wild harvest sectors of the industry. Abalone Growers Associations in Victoria, Tasmania and South Australia gave their support for the establishment of health monitoring programs of their farm's stock, to prevent disease in their stock and to meet future market expectations and translocation requirement. They are willing to contribute financially for establishing these and for ongoing monitoring. This project was seen as essential to provide both the knowledge of disease and disease processes, and to broaden the expertise base essential for such programs. Similarly the wild harvest abalone industry recognised vulnerability from a lack of knowledge of the occurrence and distribution of diseases in Australian wild stocks, and also sought assurances on the health of farmed stock and of stock used for reseeding operations.

Methods

The health survey included the abalone aquaculture industry and the wild fishery in South Australia (SA), Victoria, Tasmania, New South Wales NSW) and Western Australia (WA), and was undertaken by the major aquatic animal diagnostic laboratory in each of these States. The sampling strategy was designed to maximise the detection of serious disease, while providing useful information on disease status each sample site, and for each State, as well as Australia as a whole. Serious disease was defined as that capable of infecting at least 10 % of a population, and resulting in significant pathology in infected individuals. Wild samples were collected in a random manner within each site, the sites being selected on a systematic basis as far as possible, given the nature of available resources for collection, and covered the whole of the geographic range of each commercially harvested species. Samples from farms include each species present, and were biased towards rather than away from groups with a history of disease. Examination was primarily by gross and histological examination for the presence of disease agents and pathology, using sample sizes of 30 animals per source. Additional tests for *Perkinsus* infection were undertaken in each State, plus additional tests such as bacterial culture on animals with suggestive gross lesions or history.

Objective 1 and 2, to undertake a one-round disease survey in wild and cultured abalone.

The one-round survey was undertaken over a somewhat longer period than originally anticipated, but meeting the objective of sampling wild commercial abalone throughout their natural range and an almost complete census of farms and reseeding operations. The time frame largely reflecting the low initial level of abalone health resources and framework, which resulted in delays in organizing wild samples and samples from some farms. All abalone farms with commercial quantities of abalone were sampled with the exception of 2 farms in South Australia that did not submit samples. Approximately 3160 abalone were examined from the systematic sampling program, comprising 1841 wild and 1322 cultured abalone. Additional animals were examined from groups subsequently showing pathology that was not seen in that area during the survey.

No viruses were detected (though two virus-like changes were investigated to eliminate virus involvement). There was no evidence of pathology suggestive of the rickettsial disease, withering syndrome, though low number of similar intracellular bacteria were seen in several States, with no associated pathology.

Agents of little concern common to other molluscs were generally found throughout the survey, and included surface and epithelial ciliates, gregarines, trematodes, non-pathogenic rickettsia-like agents, and one animal with kidney coccidia. The latter is considered likely to be an aberrant infection. The only finding of note is the relatively high level of metazoan infection (mainly trematodes) from the Western Australian population. No reason for this was found, but the only pathology was inflammation in migratory tracts, which sometimes included the nerve sheaths.

Protozoan-like parasites apparently specific to abalone, but with little pathogenic potential, include a cryptosporidia-like parasite adhered to the hind-gut, an intracellular parasite of the digestive gland tubules and a parasite of the oesophageal parasite, none of which have been readily classified by light or electron microscopy. The latter had not previously been seen in Australia, partly because the oesophageal pouch is rarely examined in detail. This was seen mainly from Tasmanian wild stock, though occasional similar parasites were suspected in Victoria. This is apparently the same as a parasite previously reported from New Zealand. The only pathology was localised replacement of normal pouch epithelial cells by parasitised patches. The other two parasites were seen in at least two States (the hind-gut parasite in Tasmania and Western Australia, the digestive gland parasite in Tasmania, Western Australia, and South Australia). Both have been seen in South African farms, with minimal effect but some long-term concerns. The hind-gut parasite has also been seen in red abalone in California and Chile.

Ubiquitous agents with at least secondary pathogen potential probably included the majority of the spionid mudworm found, sponges, most *Vibrio* species and *Flavobacterium*-like bacteria. The distribution of most of these bacterial species was not established. Only sporadic cases of the *Flavobacterium*-like infections were seen in this survey, conforming to the two patterns of pathology that have been described previously from Tasmania. No pathology other than apparently terminal effects was seen in abalone from with *Vibrio* species were isolated, other than as described below.

Mudworm infections were seen in all States, with some heavy infections in both farmed and wild stock. Mild tissue changes as described previously for mudworm infection appeared common to a number of conditions, and have not been fully correlated for all mudworm affected populations, but overall the effects rarely appeared to be as severe as those recorded from the previous mudworm studies. As compromised nutrition was a factor in the previous study cases, this may suggest most abalone are better fed and be less likely to undergo significant mudworm related mortality, but mudworm infections would still appear likely to

compromise productivity. Heavy sponge infection appears to have a similar effect on health and productivity. However this survey did not have the expertise or techniques available to quantify or speciate sponge infestations, or indeed those of most other shell invaders.

Pathogens of undoubted disease potential and probably limited distribution include *Perkinsus olseni,* pathogenic strains of *V. harveyi* and of *V. splendidus* I, and possibly the spionid *Boccardia knoxi.* The latter was seen in Tasmania and New South Wales, but not in South Australia. There is no information on species involved in mudworm infections Western Australia or Victoria. The pathology of a *V. harveyi* outbreak studied in Tasmania was as described previously, though in the outbreak studied blacklip-greenlip hybrid abalone survived better than blacklips, but retained high numbers of the bacteria to pose a high risk of further outbreaks and disease spread. In this survey, *V. splendidus* infections were mostly incidental, though occasional outbreaks have been seen previously. Both of these infections appear to be due to specific strains of the bacteria, though this needs to be confirmed by comparison of strains from outbreaks in all States.

One new parasite meeting the survey criteria for apparent pathogenic potential is a haemocyte parasite from wild populations in Bass Strait, which was shown to be capable of infecting at least 50% of animals in the population, with an apparently persistent infection. Historical and clinical evidence suggests this is associated with poor growth, poor quality meat and poor survival following movement stress, but mortality appears low as large stocks of stunted animals are still present in infected areas. The overall effect on productivity appears to be marked, with minimal harvest from the most affected area over a prolonged period, although it is uncertain if this is due only to this parasite. The geographic distribution of the infected area is unknown, but it may be of significance that the legal harvest size for *H. rubra* is not only decreased in the recognised stunted Bass Strait zone, but also in the adjacent Northern Zone, where the parasite has been confirmed at the zone margins. Given the prevalence in infected areas, this parasite should be considered as a potential translocation risk from these areas unless proved otherwise. Currently the life cycle of this parasite is unknown, and this could affect the translocation risk. Low levels of the parasite may be very difficult to detect, and the effect of the infection not immediately obvious, so translocated infection is likely to be difficult to detect until high levels of infection have been established. Further studies of this parasite are indicated, particularly with regard to direct mortality, life cycle and seasonality of infection level, to define the distribution, the translocation risk, and appropriate periods for tests for this parasite. Further studies of other stunted stock are also recommended.

Perkinsus infection was seen only in the known infected area of New South Wales, although it is known also to occur in areas of South Australia which are not harvested, and were not included in the survey. There was one equivocal finding from Western Australia where gill tissue gave a positive Ray's test (where the organism is induced to enlarge to a from that can be seen under a dissection microscope after staining with iodine), but no organisms were seen on histopathology. No organisms were seen by either test on re-sampling. The possibility of a related organism being stained by the original Ray's test cannot be excluded, though *Perkinsus* organisms have occasionally been seen previously in Western Australia. Nevertheless, the *Perkinsus* organism is regarded as widespread round mainland Australia, but disease is abalone is very restricted. The reason for this is unknown.

Perkinsus was the only pathogen identified by the NSW study that appeared to be responsible for significant damage to the host, and is considered a significant factor in the decline in abalone populations between Port Stephens and Jervis Bay since 1992. The pathology appeared different to that reported in South Australia, with the NSW blacklips apparently unable to wall off and contain the parasites, dying without the abscesses seen in SA. This pattern of mortality is not one that has been matched in other infected abalone populations where *Perkinsus* is known to be present. This suggests that either the NSW host is more susceptible or the pathogen is more virulent, or both. Survey results suggest the highest levels

of infection are likely to be at the margins of the infected zone. Thus the epidemiology is consistent with a propagating epizootic, entering a naïve population of stock. Further work on the epidemiology and pathogenesis of perkinsosus in blacklip abalone in NSW, to adequately explain the losses in wild NSW blacklip abalone fisheries is recommended. Increased environmental temperatures have been suggested, but appear not be involved, as these have not risen overall over this decade and would not explain the apparent northward and southward spread. Other environmental factors, the *Perkinsus* species involved, and / or strain differences between this and the SA *P. olseni* should be further investigated for the risks of this infection to the wider industry to be assessed.

Objective 3. To develop a database of abalone disease, their location and apparent prevalence, present these findings to the wild and aquaculture industries and government agencies, and to record them pictorially in accessible electronic format.

The findings from each State disease survey have been consolidated and tabulated (Chapter 5.7). The summarised results, including photographs of the major diseases and new parasitic findings, have been presented to the 12th Abalone Subprogram annual workshop in 2005 and are available in the workshop proceedings. Several Subprogram workshop presentations made of specific disease findings, and findings have been presented to a national workshop on abalone translocation. Results have also been presented to the broader Australian abalone industry through the Australasian Aquaculture 2004 Conference, the 3rd National Abalone Convention held in Hobart in May 2005, and internationally through the 6th International Abalone Convention in Chile, February 2006. Electronic appendices will include the primary databases from each State, color pages of photographs and detailed maps from the full report, and additional representative photograph collections from each State. Additional primary data will be retained by the laboratories for use on an as-needs basis, according to agreed guidelines. Slide collections will also be held, and representative reference slides held in each State.

A more extensive photographic record in a teaching format is being developed as an electronic atlas of abalone diseases and pathology. This will include diseases from other countries that may present an exotic disease risk or diagnostic conundrum to Australian diagnosticians, and will therefore be a valuable resource world-wide.

Objective 4. To expand the pool of abalone health expertise

This objective was achieved, with direct participation in the project of considerably more than the expected number of pathologists and other abalone health service providers in the survey (13 veterinary pathologist and one mollusc pathologist plus 4 other health experts). The project framework fostered development of an inter-laboratory network that is on-going. This network was further expanded to include other health service providers by the Abalone Specialist Health Workshop held in Sydney on September 30-October 31, 2004. This was attended by 34 people, including eight of the survey pathologist and 2 pathologists involved with abalone disease surveys in other countries (Dr Anna Mouton from South Africa, and Dr Ben Diggles who undertook the recent New Zealand abalone disease survey), plus field-based veterinarians, regulatory health specialists, and other diagnostic and abalone health researchers.

Maintaining an on-going pool of expertise will be facilitated by circulation of an electronic Proceedings from this workshop to participants, and the electronic *Atlas of Diseases and Pathology of Abalone: A guide to diagnosis from an Australian Perspective*, developed within a framework provided by collaboration with TAFE Tasmania MultiMedia students.

Objective 5. To ensure the resulting information and skills are fully utilised by assisting in the development of cost-effective on-going health surveillance programs.

Advice on a framework for health surveillance programs for the abalone industries, and steps

to develop this, have been provided in the Abalone Aquaculture Subprogram "Ab-brief" Newsletter, and in the Workshop Proceedings for 2005, together with the results of this survey of diseases present within Australia, and their distribution. State Abalone Growers Associations are working towards such programs for the abalone aquaculture industries. Such a framework is being pursued by the recently formed Australian Abalone Growers Association, and will form a component of the Environmental Management System (EMS) under development for this industry. The need for health surveillance of the wild fisheries sector, to confirm Australia's early warning capability for abalone disease incursions as well as for the direct protection of the industry, has been highlighted, and presented to the wild fisheries sector and managers through forums outlined above.

The overall outcome from this project has been a considerable expansion of knowledge on abalone diseases within Australia, knowledge of abalone pathology, a considerable expansion of the diagnostic resources available to the Australian abalone industries, and heightened awareness of both industry sectors. This provides many of the resources needed to maintain the competitiveness of Australia's abalone industries, though there is still a need to develop the on-going health surveillance programs, the availability of other health service providers, particularly for field disease investigations, to ensure health surveillance capability for the wild sector, and to maintain awareness of disease issues among both industry and regulatory sectors.

OUTCOMES ACHIEVED

The major outcome achieved is a greatly expanded knowledge of the diseases present in Australian commercially exploited abalone species, together with knowledge of their distribution and pathology.

This has been achieved with the involvement of diagnostic laboratories in five States, which has greatly expanded the number of laboratories and diagnosticians with experience in abalone diseases. This increased resource will be maintained with teaching material prepared from the survey findings.

The information from this survey has been widely distributed to industry, researchers and regulatory authorities, together with advice to assist in the establishment of on-going abalone health surveillance programs.

KEY WORDS: abalone, disease, Perkinsus, bacteria, parasite, haemocyte

Acknowledgments

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1 BACKGROUND

1.1 General Background

With the development of intensive abalone aquaculture in Australia, mainly as land-based farms involving intensive stock management and use of manufactured feeds, the likelihood of disease is high, particularly in the presence of excessive stress and potential pathogens. Management of disease and stress levels is an essential on-going requirement of all intensive farming systems to maximise profits and provide appropriate levels of animal welfare. This requires knowledge of environmental requirements of abalone, knowledge of the disease agents present and an understanding of the processes that lead to disease and recovery. Maximising profitability also requires the ability to source new stock off-farm, to maintain genetic diversity, access genetically improved stock or by consolidating hatchery production. This requires knowledge of risk of introduction of diseases not already on farm.

Added to this is an increasing awareness of trade access health issues and an increased opportunity for the spread of disease through the international trade in live abalone. Australia is one of the world's largest wild harvest abalone producers, of which much is exported live, as well as having a growing abalone aquaculture sector. At the time this survey was planned, Australia also had the only internationally notifiable abalone disease, but like most of the world had little data on other abalone diseases.

Concerns regarding abalone diseases are increasing worldwide with the emergence of abalone aquaculture, and the recent emergence of several significant new abalone diseases (some of which have been translocated) and recognition that disease has played a part in the decline of some wild abalone populations. The most notable example is California where a combination of a withering syndrome recognised since 1985 and a previously unrecognised small shell-dwelling sabellid polychaete (that is now known to have been introduced) limited the species farmed and contributed substantially to the closure of a previously significant wild industry and the decline of the Black abalone species to threatened status. Neither disease was recognised prior to translocation from South Africa of the stock suspected to have introduced one of these diseases. The OIE Fish Disease Commission has recently (Sept. 2001) considered withering syndrome as an emerging disease, and has now listed it as a reportable disease of international concern.

That relatively few abalone diseases are known worldwide has been recognised to be a result of the lack of examination (absence of proof, rather than proof of absence) and that more diseases were likely to emerge with the rapid development of abalone aquaculture. Consequently, comprehensive abalone disease surveys were carried out in recent years in a number of countries, including Mexico and Chile. (Caceres-Martinez and Tinoco-Orta, 2001, Godoy and Munoz, 2003). None had previously been undertaken in Australia, despite the importance of the wild abalone harvest sector and the potential of abalone farming, which is currently undertaken in Tasmania, South Australia (SA) and Victoria and is commencing in Western Australia (WA). The aquaculture sector is estimated by the Abalone Aquaculture Subprogram to have produced approximately 300 tonnes in 2003/4, and expected to almost double that by 2007/8, and by the Aquaculture Industry Action Agenda, June 2001, to have a potential annual value by 2010 of $150 million. Wild reseeding with hatchery reared stock is undertaken experimentally in New South Wales (NSW) and is likely to increase in other States during the next decade.

Disease investigations of wild Australian stock have been largely restricted *to Perkinsus olseni* investigations, usually with tests targeted only to this agent. Health surveillance of farmed stock has been largely passive and opportunistic, with the majority of investigations undertaken in Tasmania where the Department of Primary Industry, Water and Environment have long supported passive health surveillance for new aquaculture industries. *Perkinsus olseni* infection (the only abalone disease notifiable to OIE at the time this project commenced) is present in Australia, but there little data on its distribution in wild stock outside two known areas. Without further information on general disease status and the distribution of this disease, Australia could have potentially lost access to international markets for abalone products, especially live product. Under the SPS agreement on international trade (Anon, 1995), setting out sanitary and phytosanitary conditions, unless Australia is able to demonstrate a level of biosecurity regarding translocation of serious disease within the country comparable to the level of protection it regards as appropriate for quarantine measures from other countries, Australia could be unable to justify a high level of quarantine protection for this industry any external risks other than those currently recognised as an international risk and therefore listed by OIE as reportable. Given that the quarantine conditions for aquatic animal products are in general scheduled for review under the aquatic animal quarantine review program, Australia needs information on the extent and distribution of endemic abalone diseases.

2 NEED

Abalone Growers Associations in Victoria, Tasmania and South Australia gave their support for the establishment of health monitoring programs of their farm's stock, and are willing to contribute financially for establishing these and for ongoing monitoring. They see such programs as putting them in a unique position to enable them to prevent disease in their stock and of meeting future market expectations in relation to health accreditation. This process is threatened by inadequate data on diseases in wild stock, and in some States by limited experience in abalone diagnosis.

Similarly the wild harvest abalone industry recognised vulnerability from a lack of knowledge of the occurrence and distribution of diseases in Australian wild stocks, in a climate where inter-State translocation and international trade access and quality issues are increasing. They also seek assurances on the health of farmed stock and of stock used for reseeding operations. As a result, the wild abalone sector and fisheries and animal health authorities in these States also strongly supported a baseline survey of diseases present in the exploited abalone species, and development of improved surveillance capability.

This project was established to acquire background data on abalone disease, by a health survey covering the natural range of the exploited temperate abalone species. It includes the abalone aquaculture industry and the wild fishery in SA, Victoria, Tasmania, NSW and WA. To utilise and retain this data, the project incorporated an initial cross-training workshop for participating laboratories, plus presentation of collated results, a review of other known abalone diseases in an accessible electronic format, and an abalone disease symposium to discuss the findings and develop a net-work of State-based resources for on-going diagnosis, health certification and other shellfish health related functions at reasonable cost to the industry.

Development of the on-going State-based surveillance programs for aquaculture is now progressing, in conjunction with this project but beyond the scope of this report. This will also involve consultation with wild fisheries industries and managers, who will also benefit from improved capability for diagnostic and surveillance services.

2.1 Project background and development

The background to this support is the long-term support that the Abalone Growers Associations in Victoria, Tasmania and South Australia have given to the establishment of health monitoring program. Development has been under way for several years. Growers are taking a nationally coordinated approach and are willing to contribute financially to ongoing monitoring. They recognise that lack of knowledge on abalone diseases in Australia as an impediment to this process, as is the limited opportunity for most aquatic animal pathologists to develop experience in this area, as diseases and responses of abalone differ significantly from more commonly studied filter feeding mollusc. As a first step towards setting in place the appropriate resources and response strategies in the event of a diseases outbreak, the Victorian Abalone Growers Association had already sought funds from the Marine and Freshwater Resources Institute and produced a CD containing the histology of the major organs of the greenlip and blacklip abalone (Handlinger, 2000.)

Both government and abalone growers across the southern States supported a proposal for an initial national health survey of wild and cultured abalone to address the lack of knowledge on abalone diseases in Australia and to define future health and surveillance needs. Health issues are also a concern of the wild fisheries sector and as an initial step in developing a joint project, Dr Handlinger was invited to discuss disease issues and their impacts (including this proposal) at the 2002 National Abalone Convention. A high level of support for this proposal was expressed, including suggested extension to hatchery reared stock for reseeding.

3 OBJECTIVES

1. To undertake, over approximately one year, a single-round health survey of abalone from representative wild groups of commercial abalone species throughout their range in 5 States, using statistically relevant samples appropriate to maximize the chances of detection of serious diseases and define the disease agents present.
2. To similarly examine equivalent samples from all abalone farms and reseeding operations in these States.
3. From these to develop a database of abalone disease, their location and apparent prevalence (with confidence limits), then to present these findings to the wild and aquaculture industries and State and National government agencies, and to record them pictorially in accessible electronic format.
4. To expand the pool of abalone health expertise by holding an initial training workshop for collaborating pathologists to facilitate the survey, and a national abalone health meeting to present disease and pathology findings to all relevant pathologists and health service providers
5. Ensure the resulting information and skills are fully utilised by assisting in the development of cost-effective on-going health surveillance programs through collaboration with the abalone aquaculture industry and State authorities.

4 GENERAL METHODS

The primary methodology was gross and histological examination, recommended by the OIE (Anon, 2003) as the standard screening method as it provides a large amount of information. Macroscopic examination provides only limited pathognomic signs, and reliance entirely on more specific tests will not determine the cause of mortality due to several disease agents or physiological problems acting in combination, which is a common occurrence in molluscs.

In order to provide a background for this survey and assist in expansion of the diagnostic expertise available to the industry, the existing literature on diseases of abalone, both within Australia and in other populations, was reviewed and the pathology of these diseases presented at an initial training workshop.

4.1 Survey strategy:

The major emphasis was to define the disease agents present, especially those capable of causing serious disease, and to expand the experience of diagnostic laboratories by inclusion of laboratories in NSW, Victoria, Tasmania, SA and WA. Fisheries agency or industry assistance was provided for sample collection, minimising the cost to the project of acquiring samples. The predominant method of disease detection by gross and histological examination is a broad scale method appropriate for new or currently unrecognised diseases as well as all known significant abalone diseases. Additional diagnostic tests were applied to individual animals, as appropriate, to reach a diagnosis where disease was detected grossly, or to sample sites where disease was detected histologically. Additional tests were also applied to a proportion of samples from Tasmania, to increase background knowledge of circulating haemocyte variations and levels of bacterial carriage.

4.2 Sampling strategy:

The primary aim was to maximise the likelihood of detection of serious pathogens, defined as those capable of achieving a biologically significant prevalence (defined for this survey as 10%). The proposed survey design was one sample, generally of 30 animals, from each of multiple sites in each State, and both industry sectors. Where separate identifiable populations were present, such as separate species or different age groups with differing history, these were generally regarded as separate populations and sampled separately. Otherwise the aim was for the sample to include representatives from each major sub-group, with a bias towards animals with disease or a history of disease.

A random sample of 30 animals would be sufficient to detect one diseased individual (with 95% confidence) where a disease had a prevalence of 10%, provided the sample was truly random. In fact true random sampling is seldom achievable, especially for aquatic animals whose location is governed by complex spatio-temporal processes resulting in autocorrelation (Simrad et al., 1992). The much larger sampling programs of this survey assumed there may be sub-populations, and the sample strategy would accommodate these.

4.2.1 Sampling bias:

As the aim was to maximise the chance of disease detection, rather than primarily to determine prevalence, the above strategy for sampling and site selection was varied to include

bias towards diseased individuals and populations, including some samples with suspected disease where possible (but still covering all areas). While this will confound the calculation of prevalence statistics (and may result in an over estimate of total prevalence), it increases confidence in negative results.

Where possible, sampling was spread throughout the year, with a bias towards summer (when disease is more likely to occur), and all available age groups were included.

Sampling from the aquaculture sector was to comprise a census of farms (which meet the criteria of individual populations). Bias was towards sick animals rather than attempting true random sampling across farm populations, for the same reasons as described above. Each species on all commercial farms and reseeding operations, plus additional nursery juveniles were sampled.

Industry and fisheries authorities in participating States generally provided the resources to contribute (as in-kind contributions) appropriate whole animal samples as above. Where these were collected in conjunction with existing collection programs, inherent bias away from areas of concern by commercial harvesters was avoided, but this was unavoidable where commercial harvesters were utilised for sample collection. Available resources for the laboratory tests dictated that between 20 to 30 samples of 30 animals were collected per State, giving a survey total in excess of 3000 abalone. The proportions of wild stocks varied between States but approximate at least half of the samples in all States.

4.3 Examination methods:

4.3.1 Gross examination

Gross examination included evaluation of general condition and the level of shell damage from known endemic infestations such as spionid "mudworms" and sponges, plus examination for shell pathogens considered exotic. The latter include shell fungal disease known from New Zealand and the sabellid shellworm (*Terebrasabella heterouncinata)*, that results in limited production efficiency and survival of cultured abalone in South Africa and California. Shells were examined for spionid "chimneys" which are an indication of possible infection by the spionid *Boccardia knoxi.* This spionid was specifically targeted as it was thought to have limited Australian distribution and has therefore become a translocation issue. Further examinations were undertaken where these diseases are suspected, though these varied with specific State concerns.

Methods for follow-up investigations where illness is suspected included haemolymph collection, cell counts and cell morphology examinations, the use and interpretation of clinical biochemistry, methods for shell-worm examination and for primary bacteriology. Reference laboratory support for bacterial pathogen identification was provided by Dr J. Carson (FHU, DPIWE, Tasmania), and bacterial identification cross-referenced with FRDC Project 2001/628 (Aquatic Animal Health Subprogram: vibrios of aquatic animals: development of a national standard diagnostic technology). Results between States were compared during the project, to ensure uniformity of assessment.

4.3.2 General histological methods

Seawater formalin (10% formalin in seawater) was chosen as the fixative of choice for routine examination as this had proved to be satisfactory, was low cost, was a practical fixative for large volume fixation. It also stored well and could be prepared readily in remote locations, and provided adequate fixation for electron microscopy where retrospective examination of survey samples was required. Small animals were fixed whole, larger animals could also be initially fixed whole if necessary (for example, for collection from remote locations). However shell removal, followed by trimming of excess tissue was preferred, retaining sufficient tissue to maintain organ shape during initial fixation. After initial fixation and tissue hardening, a further period of fixation was allowed after trimming to the tissues for examination (for example, within the processing cassette), to maximise the penetration of fixative. After at least 24 hours fixation, samples were processed through alcohol, embedded in paraffin blocks, and sectioned and stained with haematoxylin and eosin by standard laboratory procedures.

4.3.3 General histological examination

Preparation and examination of histological sections was the major cost for the project, dictating the number of animals that could be examined, using an estimated average of two slides per animal. To maximise the number of tissues that could be examined, three standardised partial cross sections were taken from animals too large for whole body or complete cross sections, with additional slides where there is gross evidence of pathology. The aim was that histological screening should include the foot, epipodium, oesophagus, digestive gland and hind-gut, left and right kidneys, heart, gills and gonad from each animal. Thus each organ was examined in situ, preserving the relationship to surrounding organs. It was recognised that small organs such as the smaller left kidney would sometimes be missed in section, as this may be difficult to discern in heavily pigmented blacklip abalone. Standardisation of section site was attempted and a standard method manual for abalone pathological examination was provided following the initial training/standardisation meeting. Distortions during fixation, plus individual laboratory variations, added to the general difficulties of obtaining the same section level in different sized animals where the fine anatomy was not always appreciable in fixed gross material. This resulted in variation in the smaller non-target organs (such as the oesophageal pouch or salivary glands) that were included. Tissues examined and all parasites, abnormalities and potential pathogens were recorded from all animals, together with size and location details.

For diseases of significance, prevalence data was to be compared with existing site data from on-going population surveys.

4.3.4 Examination for specific targeted diseases

For *Perkinsus olseni* infections, Ray's fluid thioglycollate culture (Ray, 1954, 1966) was included in all States, but not necessarily on all or the same samples as resource levels and collection conditions preclude this as a universal test. Though this test is currently recommended for *Perkinus* species detection, its limitations are recognised (Anon, 2003). Its inclusion in this survey was partly to increase the confidence of surveillance for this disease and partly to allow a comparison of sensitivity of this test with histopathology, which will detect well-developed infections and is also recommended. Within NSW, thioglycollate culture was undertaken on all animals as histological samples were collected in collaboration

with existing *Perkinsus* thioglycollate surveys, which would be significantly augmented by this correlation.

As well as defining the distribution of *Perkinsus olseni* infections (of which clinical disease was then recognised in limited areas of SA and NSW), this examination could detect or exclude the following significant, presumed exotic abalone diseases:

1) Withering syndrome due to intracellular rickettsia-like bacteria of gut cells.

2) Nerve tumours such as those of the Japanese abalone disease amyotrophia, that are suspected to be virally induced.

3) Haplosporidia infection, as detected in New Zealand.

Additional diagnostic tests included bacterial culture and clinical pathology tests if indicated by gross examination.

4.3.5 Expanding the pool of expertise and ensuring adoption of results by diagnostic services

A workshop was required initially to ensure all participants had current knowledge of abalone diseases and to coordinate diagnostic methods for the survey. A broader workshop, targeting all abalone health providers, was held at the completion of the survey to ensure wider adoption of findings and to achieve the aim of developing a network of Australian abalone disease expertise.

The initial workshop involved at least one experienced pathologist from each collaborating laboratory and covered revision of abalone histology, and interpretation of normal variations reflecting differing physiological states, review of known abalone diseases, including multi-header examination of reference slides from the principal investigator's reference collection and material from other States, and discussion of abalone pathological responses. At this workshop, methods for follow-up investigations where illness is suspected were standardised as much as possible. The workshop was held in conjunction with an international mollusc training workshop associated with the 5th Symposium on Diseases of Asian Aquaculture. This workshop provided additional value as it offered an opportunity for general revision of mollusc disease and extension of the abalone teaching material to mollusc from a wide range of abalone growing Asian countries. The international mollusc training program was a response to the view that the general lack of molluscan diagnostic knowledge in many Asian countries increased the risk of disease spread within the region (potentially including increased risks to Australian industries. Holding the initial project training workshop in conjunction with this was an initial step in reducing this risk for abalone industries across the region.

A final resource building 2-day workshop for participating pathologists in the survey was held in Sydney in 2004. The purpose of the workshop was to review all the factors affecting abalone health (including but not limited to the disease of abalone found in Australia during the current project) as a background for developing a comprehensive diagnostic capability for the industry. Thirty-four people attended, providing a wide cross-section of expertise on abalone and an excellent forum for such a review. Participants included seven of the 11 abalone pathologists involved with the Australian survey, plus the South African abalone pathologist, Dr Anna Mouton, and Dr Ben Diggles who has carried out New Zealand abalone disease surveys and now services both Australia and New Zealand from Queensland, plus three field-based veterinarians, three regulatory health specialists, two diagnostic research specialists (in microbiology and molecular test development), six scientists who have or are

working in abalone health related research, four abalone fisheries staff, five abalone farmers, one abalone ecologist and one mollusc immunologist. Regarding the latter, we were particularly fortunate to have the participation of Dr David Raftos, as he presented an exciting perspective on recent research on mollusc immune systems, which harbours well for the ability to manipulate levels of abalone immunity as a means of disease control in the future. The first day was spent reviewing the known diseases of abalone (infectious and non-infectious), the second on a review of the physiology involved, the pathology and epidemiology of abalone diseases, and how this influences disease control. The comprehensive nature of this workshop consolidated our understanding of these interacting impacts on health. Participants agreed to have all presentations (with additional text where appropriate) compiled onto a CD for distribution to participants.

To ensure the on-going availability of suitable abalone diagnostic expertise, suggested standardised examination methods are included in the electronic Abalone Pathology Atlas currently being prepared. This atlas will incorporate the abalone histology atlas prepared previously. It will capture the survey findings and review other abalone diseases in a largely pictorial format, providing a permanent electronic reference source for abalone diseases, especially those from Australia. The electronic platform for this resource was prepared by a TAFE Tasmania Multi-Media student class project, in conjunction with IT@Work.

4.3.6 Facilitation of utilisation of results by industry and development of health surveillance programs

In developing this project, the abalone aquaculture industry made a commitment to utilise the results of this survey and the increased diagnostic capabilities for the establishment of an on-going health surveillance program for abalone farms. Development of a suitable framework protocol during the project was seen as an in-kind commitment to the overall project. Project staff have provided assistance to industry, animal health authorities and other service providers in developing this framework. Preliminary results have been presented at Subprogram Workshops (Handlinger et al., 2003, Handlinger et al., 2004a) as a basis for surveillance and translocation protocol development. Advise on surveillance program development has been provided in the Subprogram Newsletter and at the Subprogram Workshops (Handlinger, 2005).

5 RESULTS AND DISCUSSION

5.1 REVIEW OF KNOWN ABALONE DISEASES

Currently, two diseases of abalone are internationally listed as notifiable to the OIE: perkinsosis due to *Perkinsus olseni* and withering syndrome of abalone due to *Candidatus* Xenohaliotis californiensis.

A summary of these conditions is available on the OIE Diagnostic Manual for Aquatic Animals (Anon, 2003). A more general summary of diseases of abalone (though lacking some literature from Asia) is available from Bower and McGladdery, 2001 and 2004. Diseases of abalone are herein briefly reviewed on a systematic and regional basis.

5.1.1 Known diseases and parasites of abalone in Australia

Diseases of Australian abalone have been reviewed and described by Handlinger (2001), and Handlinger et al. (2002, 2005).

Prior to this survey commencing, no viral agents had been identified in abalone in Australia. Of bacterial diseases, vibriosis was recognised as a variable problem in some abalone hatcheries, and at least two *Vibrio* species and two forms of Flavobacterial infection were recognised as implicated in outbreaks of disease in grow-out stock, although a wider variety of *Vibrio* species have been sporadically isolated from, and possibly implicated in, some disease outbreaks. (Handlinger et al., 2002, 2005). Overall, vibriosis in larval abalone is expected to be somewhat less of an issue than for other cultured shellfish as the abalone do not feed throughout the larval stage.

The most problematic bacterial infection has been due to *Vibrio harveyi,* which has been recognised for a number of years as a problem in some abalone farms in both Tasmania and South Australia (Reuter and McOrist, 1999, Handlinger et al., 2005b). This causes abscess-like formations, mainly within the foot, as well as septicaemia. This problem has been of significant magnitude to result in antibiotic use and sufficient concern to initiate a study of the potential to control outbreaks using antibiotics (Handlinger et al., 2005, 2006). *Vibrio harveyi* is now regarded as synonymous with *Vibrio carchariae* which has been identified as causing a similar problem in abalone in Japan (Nishimori et al., 1998) and France (Nicolas et al., 2002). *Vibrio splendidus* I has also been recognised as causing outbreaks of disease on farms, although abscesses and prolonged retention in the population are not a feature (Handlinger et al., 2002, 2005b). The identity of both of these bacteria has recently been reviewed and subjected to further study (Carson, 2004). A variety of other *Vibrio* species have on occasion been isolated from sick abalone in Australia but have not been implicated as significant pathogens.

Of the Flavobacterial diseases, most isolates have not been identified to species level, although one isolate was identified as *Tenacibaculum maritimum* (previously *Flexibacter maritimus*).

Nevertheless, two distinct syndromes of Flavobacterial disease have been seen. The most common syndrome presented as a loss of pigmentation followed by shallow epithelial erosions in which mats of debris containing slender filamentous *Flavobacterium*-like bacteria were seen underrunning and replacing the epithelium with very limited superficial penetration and minimal host response. Such lesions were generally seen during the cooler months of May to October and often in conjunction with stress, or particularly, traumatic stress. The second Flavobacterial syndrome presented as a more aggressive invasion during summer months when temperatures were close to or outside the species' optimum, with a significant outbreak being found in wild, greenlip abalone held in high-stocking densities in tanks. This syndrome has been seen as sporadic findings in other species and other situations.

One fungal disease of abalone has been reported, although other sporadic fungal lesions have been seen. Outbreaks of the disease described tubercle mycosis have been seen in Australia in several States, in which thin-walled fungi of variable diameter have been identified. Similar lesions have been seen in Japan with two fungi being implicated *Atkinsiella awabbi* (Kitancharoen et al., 1994) and *Haliphthoros milfordensis* (Hatai, 1982). In Australia, one isolate from a typical outbreak has been cultured and identified as *Atkinsiella awabbi*. Tubercle mycosis is generally seen as a sporadic disease of wild stocks, although these may be readily transmitted in holding systems and similarly have been brought into farms with wild stock.

Of the protozoan and protozoan-like parasites known from Australia, the *Perkinsus olseni* is by far the most important. This was originally described by Lester and Davis (1981), from blacklip, *Haliotis rubra.* Although other abalone species, such as greenlip abalone *H. laevigata* and *H. scalaris* are also known to be affected, and significant mortality and economic effects have occurred in *H. laevigata*. Nevertheless, this infection remains a more severe disease of blacklip abalone and is currently known from the western region of South Australia and from New South Wales where disease is of more recent origin. The study of this disease outbreak in New South Wales is the major focus of the New South Wales study. The study of *P. olseni* outbreaks is complicated by the uncertainties of the parasite identity. *Perkinsus* organisms have been identified in Australia in at least eight mollusc species and many of them outside the known areas of abalone disease (Goggin et al., 1989).

Such parasites had low host specificity in the laboratory. Despite this, *Perkinsus* infection in *H. rubra* was shown to be maintained in this species with negligible contributions from other susceptible abalone species or other molluscs (Lester et al., 2001). Recent genetic analysis of two nucleoside sequences have confirmed that *Perkinsus olseni* from abalone in Australia is conspecific with *Perkinsus atlanticus* which is a recognised pathogen of clams in Portugal and in molluscs in other areas. Isolates from one clam species in which infection has been found in Australia, *Anadara trapezia* have been shown also to be of this species. Nevertheless, the OIE recognises that conspecificity is not proved for many of the tropical isolates from Australia and elsewhere that resemble *P. olseni*. It is also demonstrated by the fact that polyclonal antibodies raised against *Perkinsus marinus* bound to some but not all Perkinsus species isolated from various species of Australian molluscs (Bower 2004a).

Thus, the epidemiology of this disease is somewhat complex. The pathology may also vary as *P. olseni* may frequently be located in connective tissues although, particularly in *H. rubra*, may be sequestrated into larger abscesses or pustules up to 8mm (Anon 2003). Despite this, the morphology of life stages is similar for all the *Perkinsus* species and all stages are characterised by a large vacuole and a displaced nucleus that generally allows the organism to be readily detected in routine histological examinations.

An additional test available for this organism is the Rays fluid thioglycollate medium in which small pieces of tissue are incubated for between 4-7 days in the dark in a medium that provides an anaerobic environment. This encourages enlargement of the trophozoite states of the parasite and development into the much larger hypnospore stage. Hypnospores (but not undeveloped trophozoites), stain black with Lugol's iodine solution sufficient to detect the spherical bodies in wet preparations (Ray, 1952). Hypnospores also develop within abscesses (where the dead material may provide a similarly anaerobic environment) and may already be present in the tissue when fixed. These will stain with iodine even after being killed by formalin fixation. Killed trophozoite will not enlarge with thioglycollate media into hypnospores (Moore et al, 2002).

Perkinsosis is a recognised disease in wild abalone in limited locations and so broodstock is unlikely to be sourced from these locations. However, there are two documented occasions when this disease has been brought into farms and resulted in 30-40 percent mortality among greenlip (*H. laevigata)* grow-out stock (3-4cm). In both cases, blacklip abalone had been taken from an infected site and introduced to the facility (Goggin and Lester, 1995). Both outbreaks were controlled and eliminated from the farms.

No other protozoan diseases of significance had been seen in abalone prior to this survey, although several protozoan parasites had been detected. Sessile single celled coccidia-like parasites had been seen attached to gut epithelial cells on at least two occasions in Tasmania (once from a sea-farm, once from an early farm with wild caught broodstock feeding on macroalgae). Similar parasites have been seen more frequently in South Africa, more so in

stock fed macroalgae collected from natural beds than in artificially fed animals (Mouton, 2000, 2006).

An unidentified intracellular spore-forming parasite had been seen in the digestive glands of wild abalone on one occasion (Handlinger, unpublished). Similar parasites have also been seen in South Africa (Mouton, 2003).

There has been one incidental finding of coccidia-like organisms in one left kidney from a wild animal (Handlinger, unpublished) that resembled *Pseudoklossia* parasites reported in California and other areas (Freidman, 1991, Freidman et al, 1995). The rarity of this finding in Australia has led to a suspicion of a likely cross-species infection.

Of the larger parasites, fluke larvae with a gonad and other tissue invasive pattern resembling that described for Bucephalid flukes had been seen sporadically in gonad, kidneys and other organs of wild abalone (Handlinger, unpublished), sometimes to levels visible grossly as organ discolouration (Harrison and Grant, 1971, from Tasmania, Shepherd and Breen, 1992, from other areas of Australia).

Other metazoan parasites had been sporadically seen in foot tissue (Handlinger and others, unpublished). However, the most significant metazoan effects on abalone health hitherto reported from Australia were those of spionid polychaetes invading the shell. Lleonart has characterised the severity and nature of the impact of spionid mudworm infestations on abalone health (Lleonart, 2002, Handlinger et al., 2004). Although this study was primarily directed at *Boccardia knoxi*, he demonstrated that other spionids that penetrated the shell had a similar physiological effect and *Polydora hoplura* was often numerically and clinically as important or more important than *B. knoxi* (Lleonart et al., 2003b).

Other organisms that on occasions cause significant damage in molluscs are boring sponges (well recognised in oysters and mussels) where the sponge tunnels may become inhabited by other organisms such as polychaete worms. This results in chronic soft-tissue irritation and secondary bacterial or fungal infections, especially in scallops, where it was found that shell damage could be most easily prevented by growing the scallops off-bottom. There appears to be no systematic study of sponge damage in Australian abalone, although such lesions are well recognised by the industry.

5.1.2 Abalone diseases and parasites presumed to be exotic to Australia

Viral diseases

At the commencement of this project the only available description of virus associated disease in abalone was the disease amyotrophia in the Japanese abalone *Nordotis discus discus*. Serious viral disease has since emerged in China although, until recently, much of the information was available only in Chinese. More recently a herpes-like viral disease of the nervous system has been described in Taiwan, though the relationship to the above viruses, if any, is unknown.

Amyotrophia of Japanese abalone is seen grossly as atrophy of the foot musculature that is due to lack of innovation related to tumours on the nerve trunks of the pedal ganglia (Nakatsugawa, et al, 1998). The characteristic histological finding in this disease is the tumours of the pedal ganglia and nerve trunks. This disease was suspected to be viral in origin (Nakatsugawa, 1990) and has been transmitted after filtration with a membrane filter of 220nm but not when a filter of 100nm was used (summarised by Otsu and Sasaki, 1997, Nakatsugawa et al., 1999). A virus, suspected to be a retrovirus, has been isolated from juvenile Japanese abalone with this disease (Nakatsugawa et al., 1999). The virus in primary

haemocyte cell culture was icosahedral, 120 nm in diameter, observed just outside the cell or in the budding stage, and was not inhibited by the IudR, which inhibits herpes viruses. The cultured virus was not confirmed as the infectious agent by transmission trials, possibly due to too small an inoculum. In general, there are no known treatments for viral diseases, particularly for invertebrates. However, recent findings suggest that control of this disease through genetic selection is possible (Hara et al., 2004).

Information has since emerged of two serious virus-related diseases in abalone culture in China (summarised by Zhang et al, 2004). Wang et al., (2004) describe an acute, serious disease in *Haliotis diversicolor* that has devastated farms culturing this species in the south of China and spread since 1999. Further information on this virus and of a second viral disease in *Haliotis hannai* called cracked shell disease has since been summarised by Shi and Handlinger, (2004).

The cracked shell disease in *H. hannai hannai,* first described by Wang et al, 1997, is relatively slowly progressing, showing low activity, loss of appetite resulting in a thin shell, decreased growth rate and up to 50 percent mortality over one to several months. The reported histological signs are necrosis and disorder of connective tissues of all organs.

The disease in *H. diversicolor* is more acute with rapid mortality, all stages of abalone being affected and up to 100 percent mortality within several days. The reported histological signs again are disorder and disruption, particularly of epithelial cells and up to three types of virus have been associated with this condition. Thus, despite the summary of available information, both conditions lack clear case definition, clearly identified pathological agent and a clear description of pathology.

In contrast the recent description of an acute herpes-like virus in Taiwan is well described by Chang et al, 2005, with regard to both the nature of the virus, and the pathology affecting the cephalic and surrounding nerve trunks.

Bacterial diseases

Of the exotic bacterial diseases of abalone, by far the most important is withering syndrome. This disease is due to an intracellular rickettsia-like bacterium not yet definitively named (*Candidatus* Xenohaliotis californiensis). Information on this disease has been summarised by Bower, 2004b. It was first detected in the central Channel Islands area of California in 1985 and was associated with population crashes on six of the eight Channel Islands by 1992 where 95-100 percent of the *H. caracherodii* (black abalone) were lost. There was also a decline in red abalone *H. rufescens*. The disease has since been translocated with infected stock to Chile and probably Japan, Israel and other countries (Anon, 2003).

Rickettsia are intracellular bacteria that can be readily detected in routine histological sections but as they grow only intracellularly, they cannot be cultured. Bacteria with this appearance are common in molluscs and are seldom associated with disease.

The particular pathology of this disease is the morphological changes induced in the digestive gland by the infection, resulting in starvation, which ultimately results in withering of the muscle as energy reserves and muscle tissue are metabolised. These changes were first reported by Gardner et al, in 1995, and their role in the disease later confirmed by Moore et al (2000). Most rickettsia-like bacteria in molluscs cause no alteration of the tissue. This disease is characterised by degeneration of the tubules and particularly by metaplasia of the digestive tubules. The metaplasia (or change in the tissue type) involves the replacement of secretary and absorptive acinar (tubule) epithelium with duct-type epithelium, which is not functional, and hence the animal starves. The OIE case definition of this disease includes the presence of both the presumptive agents and significant gut pathology. The OIE (Anon, 2003)

acknowledges PCR and similar tests are available for this disease but also recognises diagnosis by histological examination where both the bacteria and gut pathology are present. Diagnosis must include the presence of the bacterium and the morphological changes to the digestive gland and may include foot withering. The recent listing of this disease as reportable to the OIE makes this an important assessment of this survey.

Of the other bacterial diseases, only *Vibrio fluvialus* II infection appears likely to be an exotic primary pathogen. *V. fluvialus* is in fact widespread in environments throughout the world. However, abalone disease has only been recorded in the Dalian area of China in *H. discus hannai.* The described pathology for this disease, seen grossly as blister disease, is of connective tissue and muscle fibre dissolution and intense inflammatory response in the centre of the lesions very similar to our findings in *V. harveyi*. The pathology, distribution and control of this disease have been described in a series of Chinese papers by Li et al., (1996, 1997ab, 1998), and Liu et al. (1995). Antibiotic usage for control of this disease appears to have been initially attempted, although later studies suggested several strains had resistance to 18 different antibiotics. Subsequent efforts of control have included exposure of abalone to a formalin treated culture of the organism and the use of a phage (virus of the bacteria) inoculated into the foot and pustules of abalone. Both increased survival rates of abalone by up to 50 percent (Tai-wu et al., 2000).

The presence of disease due to *Vibrio carchariae,* which is a synonym of *V. harveyi,* in Japan and France has already been mentioned. The disease in Japan is associated with white spots, white necrotic muscle fibres and bacteria in the foot. No histological studies were undertaken of the disease in France, although the organism was isolated from land-based abalone farms and associated with spread in the natural environment.

There are no other recorded cases of consistent involvement of bacteria in repeated disease outbreaks, though a wide variety of other bacteria have been isolated sporadically from abalone, and implicated in limited disease outbreaks, particularly in juveniles, since the first (1983) description by Elston and Lockwood of the pathology of juvenile abalone vibriosis. This includes *Chlostridium lituseberense* and *V. alginolyticus* in South Africa (Dixon et al, 1991), and *Vibrio parahaemolyticus* which has been described as a pathogen of small abalone in Taiwan, as well as causing gastroenteritis in humans (Liu et al, 2000, Huang et al, 2001). In general the pathology of these infections has not been described, or they have apparently been associated with septicaemia with little specific pathology. As terminal septicaemia is often a secondary event, this makes assessment of their role as primary pathogens difficult. However, Liu et al (2001), described abscesses and ulcers in the mantle of small *Haliotis diversicolor supertexta* abalone in Taiwan with *Vibrio alginolyticus* infection.

Fungal diseases

Of the fungal disease, the presence of tubercle disease in Japan has been mentioned. A second fungal disease has been seen as a shell mycosis in New Zealand. Fungal lesions in shells from New Zealand abalone have been described by Grindley at al. (1998). In general, the blisters of conchiolin and nacreous would appear to be a common finding, but the extent of these lesions was unusual and they were associated with the interior, rather than the exterior, of the shell. The brown jelly-like material on the inside of the shell resulted in loss of value of the shells. This condition was observed to be fatal to animals kept in captivity, particularly if the attachment to the muscle was involved.

Protistan and protistan like parasites

One of the first protozoan-like diseases of abalone to be described (other than *Perkinsus olseni* infection) was a *Labyrinthuloides* parasite of small juvenile abalone in a British Colombian facility (Bower, 1987a, b and c and Bower, 1989). While this parasite has been very well described and summarised by Bower and Meyer, (2003) and was associated with up to 100 percent mortality in young juveniles, it has not been seen since and is not regarded as a current risk. This thraustachritid like parasite elicited no immune response in the very young juvenile age group affected. Older animals that mounted a response did not succumb. Perhaps the most interesting aspect of this study is the implications for age at which abalone may become competent against certain parasites.

Although other protozoa have been seen in association with abalone, only one other parasite has been clearly associated with disease outbreaks, a Haplosporidian parasite seen in New Zealand (Diggles et al, 2002). This parasite was seen as the systemic infection with uni- to multi-nucleate haplosporidia-like plasmodia in juvenile *Haliotis iris* from one commercial culture facility. It was seen in two summers, 1999-2000 and the following summer it was associated with chronic mortalities of up to 90 percent of juveniles. Animals exhibited lethargy, lack of adhesion and runty and mortality when water temperatures exceeded 20^{o}C. The organism was not found in the following summers and there is no information on its distribution in wild stock. Histological examination showed massive systemic haplosporidian infection throughout all major organs including muscle, connective tissue, nervous tissue and haemolymph. Therefore, heavy infections would be readily detected. The parasite was not transmitted between *H. Iris* during cohabitation for three months nor injection (Diggles et al., 2002). The parasite shows similarities to other haplosporidia but with some ultra structural differences. (Diggles et al., 2002; Reece and Stokes, 2003). The role, if any, of a rickettsial organism within the haplosporidia is not known (Hine et al., 2002). The parasite shows some affinity to *Bonamia* as shown by moderate/strong reactions with in-situ hybridisation probes for *Bonamia ostria* of flat oysters, and is regarded as belonging to a primitive Haplosporidia family, possibly ancestral to other groups such as *Bonamia.*

Diggles et al., 2002, also described a coccidian-like infection, sometimes heavy, in the epithelium of the oesophageal pouch of paua from one farm. He suspected the severity of infection indicated stress. Metacercarial infections have been reported previously from Australia and South Africa but are regarded as having probably a global distribution, although each species may be confined geographically. Similar infections are reported in other molluscs and sea urchins. There is little effect on survival, heavy infections in the gonad may occasionally cause castration but high levels have sometimes been reported from South Africa, including gill discolouration to orange due to large numbers insisted within the gill (Botes et al., 1999). They have previously been reported from Tasmania (Harrison and Grant, 1971) and in other areas of Australia (Shepherd and Breen, 1992).

Pseudoklossia-type coccidia have been detected in the kidneys of red abalone in California, Mexico, Chile, and in *H. midae* in South Africa (Friedmam et al., 1995; Caceres-Martinez and Tinoco-Orta, 2001, Godoy and Munoz, 2003, and Mouton, 2000). High levels were initially seen in some animals with withering syndrome and this parasite was investigated in that context. However, the role of the coccidian was ultimately regarded as, at most, an additional stress factor (Friedman et al., 1997).

Other protozoa reported from abalone without ill effect include a variety of mantoscyphidian-like ciliates attached to the gills, mantle cavity and oesophageal pouch epithelium or attached to or cover without obvious host response or pathology (summarised by Bower, 2004c)

Metazoan parasites

Metazoan parasites described for abalone include nematodes and trematode metacercaria. *Echinocephalus* species nematodes are reported to be associated with some degree of muscle weakening in heavy infections in pink abalone in Southern California, USA and the Gulf of California, Mexico in the 1950s and early 1960s (summarised by Kine, 1980).

Shell parasites

Spionid mudworm infestations are widely distributed with some species being well studied in Australia (Handlinger et al., 2004).

A sabellid polychaete *Terebrasabella heterouncinata* was found as a cause of poor productivity in Californian and Mexican (Baja California) abalone and it is believed this was translocated to the Americas from South Africa in the late 1980s. This sabellid is a major problem in South African abalone farms among *Haliotis midae,* held at high stocking densities, although management efforts directed at this parasite have resulted in a considerable improvement. This parasite is a functional and simultaneous hermaphrodite with a short generation time at high temperatures. Therefore, it is readily susceptible to translocation, even when present in low numbers. The very small size, 4-5mm in adults adds to the likelihood of inadvertent translocation, believed to have occurred between South Africa and California.

Fortunately, larvae of this species are all crawling larvae that do not settle on dead shell but may infect shells of other species of gastropods. Bivalves rarely become infected. The restriction of substrate to live gastropod shells reflects the growth habit of this parasite that settles on the margins of actively growing shells, instead of burrowing in the shell like spionids. The sabellid larvae depend on the host reaction to cover the parasite creating a tunnel from which the brachial crown protrudes on the external surface during feeding. In the non-feeding or dried state, these burrows are seen as pinpoint lesions on the exterior surface. The deposition of host shell as well as stunting, which may result from reduced growth rate, increases the height of the shell at the margins. In severely affected animals of the Californian species, this produces a characteristic capped-shaped shell. This deformity is not characteristic of infection in the natural species, *H. midae* (A Mouton pers.com.). Information on this parasite has been summarised by Ruck and Cook (1998) and Bower (2004d). Previous surveys have found no evidence of this parasite in Australia.

ABALONE AQUACULTURE SUBPROGRAM: A NATIONAL SURVEY OF DISEASES OF COMMERCIALLY EXPLOITED ABALONE SPECIES TO SUPPORT TRADE AND TRANSLOCATION ISSUES AND THE DEVELOPMENT OF HEALTH SURVEILLANCE PROGRAMS.

Western Australian Report

John Creeper, Fran Stephens, and Kylie Freeman,

Western Australian Department of Fisheries

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ABALONE AQUACULTURE SUBPROGRAM

Department of Fisheries
Government of Western Australia

Fish for the future

5.2 A SURVEY OF DISEASES OF ABALONE IN WESTERN AUSTALIA

5.2.1 Introduction: Abalone distribution in Western Australia

Abalone are distributed along most of the Western Australian coastline ranging from intertidal zones to depths of 80 metres. The temperate species extend from Shark Bay in the north to the border with South Australia on the south coast. (Figure 5.2.1)

Three commercially exploited species - the greenlip abalone (*Haliotis laevigata*), the brownlip abalone (*H. conicopora*), and Roe's abalone (*H. roei*) occupy ecologically distinct habitats. Greenlip abalone are widely distributed along the south coast to Cape Naturaliste in water depths from five to 50 metres. Brownlip abalone are distributed along the south coast of WA, in similar depths to greenlip abalone, from around the head of the Great Australian Bight through to the lower parts of the west coast, although they are uncommon north of Cape Leeuwin. East of the Great Australian Bight, brownlip abalone is replaced by the closely related blacklip abalone (*H. rubra*). Roe's abalone can be found from Victoria to the Zuytdorp Cliffs (north of Kalbarri) on inter-tidal platforms to depths of five metres.

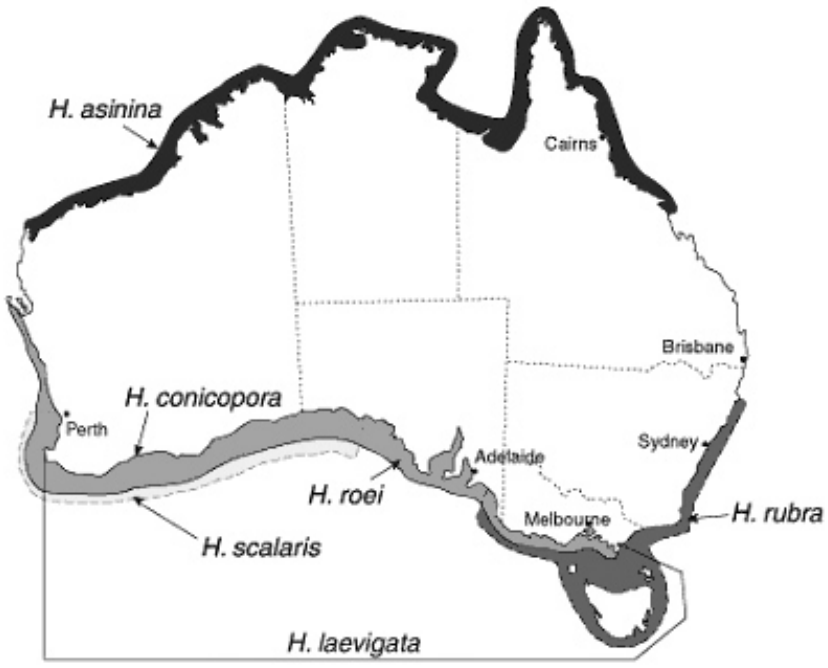

Figure 5.2.1. Distribution of commercially exploited *Haliotis* sp.

Greenlip abalone occur in granite or limestone rock areas in depths of five to 50 m with high wave action and water movement, where their major food supply of drift red algae is abundant. Brownlip abalone are found in similar depths to the greenlip, but generally occur in areas of lower water movement, often living deep in caves and fissures in granite rocks. Roe's abalone inhabit areas of high wave action and are most abundant on inter-tidal and shallow sub-tidal limestone platforms on the west coast. They also occur at relatively low densities

under granite boulders, or in crevices on open rock faces on the south coast (Freeman, 2001, Shepherd et al., 1995)

5.2.2 Methods

5.2.2.1 Sample collection

During the period between March 2003 and April 2004, 687 abalone were collected from six coastal zones representing 22 distinct sampling sites in Western Australia (Figure 5.2.1). With the exception of thirty Roe's abalone each collected from Perth and Geraldton, and twelve donkey ear abalone (*H. asinina*) collected from Broome, all abalone were collected from the southern coast extending from Augusta to Esperance. The commercial greenlip abalone farms based at Albany and Bremer Bay contributed 102 and 60 specimens respectively – collections being made at different times in an attempt to include seasonal influences.

Wild caught samples were collected by professional divers and staff from the Western Australian Department of Fisheries and comprised the three temperate species found in Western Australia. A single sample of the northern tropical species, the Donkey-ear abalone was included in the survey and comprised 10 specimens collected from Broome. All wild-caught samples were considered to be clinically normal. One submission of 30 moribund greenlip abalone from a commercial farm was submitted because of ill-health.

Full details of the sampling regime are provided in Figure 5.2.2. and Table 5.2.1.

5.2.2.2 Examination methods

Shell was examined for the presence of commensal fouling organisms and boring and parasitic shell diseases. Any incidental or gross pathological abnormalities of the abalone were assessed at the time of sampling.

Harvested abalone were formalin fixed as soon as practicable after capture. Block sections for histological processing and paraffin embedding were made according to the survey standard methodology (section 4.3), and detailed guidelines given at the initial training workshop. Five micron haematoxylin and eosin stained sections were prepared by conventional methods. At the commencement of the survey, abalone that had been collected along limestone formations and from concrete raceways in the commercial farms had accumulated gritty calcarific material within the digestive gland that damaged microtome blades and resulted in poorer quality paraffin sections. As collections moved to granite rock areas this became less of a problem.

Where electron microscopy was performed, sections of tissue were deparaffinised, embedded in epoxy resin and sectioned and stained by routine methods.

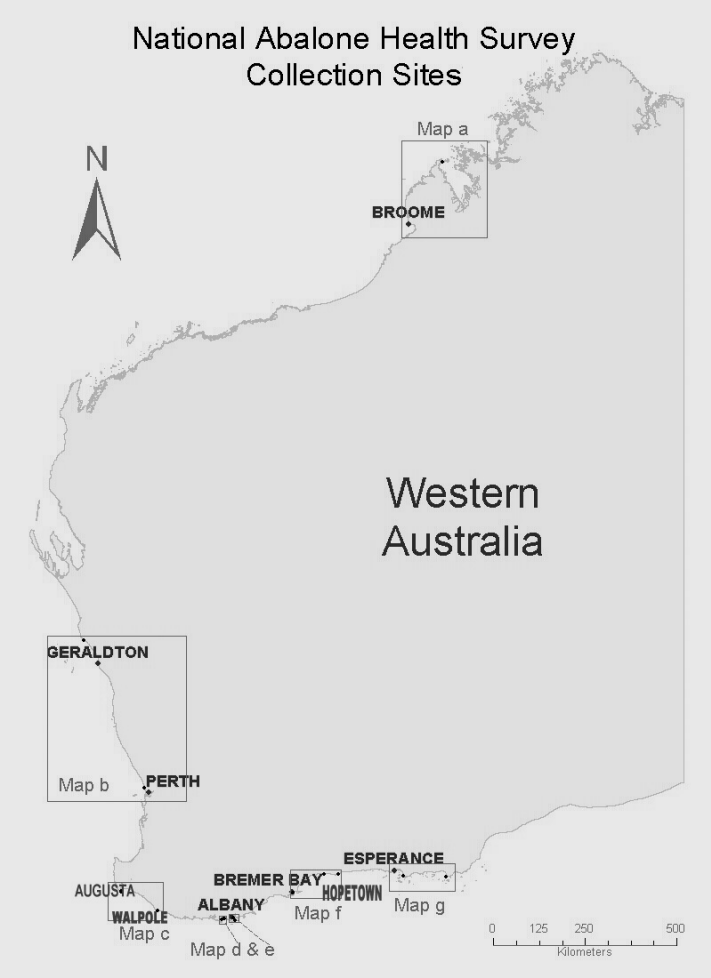

Figure 5.2.2: Abalone collection sites in Western Australia (maps a – g provided in Appendix 3).

5.2.3 Results

5.2.3.1 Shell diseases

Mudworms are defined as segmented marine worms belonging to the Polychaete class and the Spionid family, which invade the shells of molluscs. So far the most serious effects on abalone are reported with *Boccardia knoxi* and *Polydora hoplura* (Lleonart, 2001). Where facilities were available at the commercial farms, a distinction between Spionid and other polychaete parasites was made. In wild-caught abalone, shell damage was assessed but not directly attributed to either species. Scoring was made on a 1 to 4 scale. Severe mudworm infestation was classified as score 4 and was defined by blistering to 30% or more of the inside shell surface area.

Full details of mudworm damage is detailed in WA Appendix 2 (within 8.4). All submissions showed at least minor damage to the shell resulting from mudworm parasitism. Severe mudworm damage was seen in all brownlip examined, albeit in a small sample size. Roe's abalone appeared to be more prone to severe mudworm damage than greenlip abalone. (Table 5.2.2.)

Table 5.2.1: Collection sites for Western Australian abalone health survey

Site	Location	Species	Farm / Wild	No of animals	Collected
1	Great Southern Marine Hatcheries	Greenlip	F	30	1-Apr-03
2	Bayside Abalone Farms	Greenlip	F	30	2-Apr-03
3	Hope Town - Masons	Greenlip	W	30	26-Apr-03
4	Hope Town - Two Mile	Greenlip	W	30	26-Apr-03
5	Hope Town - Two Mile	Roe's	W	30	27-Apr-03
6	Windy harbour (in close)	Greenlip	W	30	7-May-03
7	Augusta - Cosy Corner	Greenlip	W	30	14-May-03
8	Augusta - Bay area	Greenlip	W	30	8-May-03
9	Mutton Bird Island near Albany	Greenlip	W	30	24-Feb-04
10	Cosy Corner near Albany	Greenlip	W	30	24-Feb-04
11	NW side Mutton Bird Island	Roe's	W	30	24-Feb-04
12	Near Beach near Mutton Bird Island	Roe's	W	30	24-Feb-04
13	Cosy Corner near Albany	Roe's	W	30	24-Feb-04
14	Boat Ramp Frenchman's Bay, Albany	Roe's	W	30	26-Feb-04
15	Great Southern Marine Hatcheries - period 2	Greenlip	F	30	9-Mar-04
16	Great Southern Marine Hatcheries - period 2	Greenlip	F	30	10-Mar-04
17	Marmion Beach - Perth	Roe's	W	30	16-Mar-04
18	Wylie Point - Esperance	Roe's	W	30	17-Mar-04
19a	Middleton Beach Albany/Seasick Bay	Greenlip	W	29	22-Mar-04
19b	Middleton Beach Albany	Brownlip	W	4	22-Mar-04
220	One Arm Point - Broome (Tropical)	Donkey Ear	W	12	16-May-04
21a	Thomas Fishery Esperance	Greenlip	W	25	27-Mar-04
21b	Thomas Fishery Esperance	Brownlip	W	5	27-Mar-04
22	Port Gregory Roe's (Geraldton)	Roe's	W	30	3-Apr-04
23	Bayside Abalone Farms - period 2	Greenlip	F	40	2-Apr-04

Table 5.2.2. Prevalence of mudworm damage

Location	**Greenlip**	Roe's	Brownlip
Albany	Prevalence	Prevalence	Prevalence
- Hatchery	30 %		
- Muttonbird Island	25 %	70 %	
- Middleton Beach	0 %	46 %	100%
- Windy Harbour	13 %	66 %	
Bremer Bay	0 %		
Hopetown	7 %		
Augusta			
-Bay Area	10 %		
- Cosy Corner	60 %		
Esperance	4 %	10 %	100 %
Geraldton		6 %	
Perth		10 %	

5.2.3.2 Perkinsus Testing

Perkinsus testing was performed using the Ray's fluid thioglycollate test on eighteen collections. Positive results were detected in one sampling site from Cosy Corner, Augusta in 30 Greenlip abalone. No histological evidence of Perkinsosis disease was seen. Perkinsus, but not Perkinsus disease, has been described previously in Western Australia (Hine and Thorne, 2000).

5.2.3.3 Summary of infectious abalone diseases detected by histological examination.

Helminth
- Cestodes
- Trematodes
- Nematodes

Protistan
- Ciliates
- Apicomplexan like parasites
  - Suspected coccidia
  - Cryptosporidian-like parasites of intestinal epithelium
- Intracellular parasites of the digestive gland.

Eubacteria
- Rickettsiales - Rickettsia-Like Organisms

Bacterial
- *Vibrio* sp.

Viral-like inclusions
- Digestive Gland and Intestine
- Intestinal

5.2.3.4 Helminth parasitic diseases

The intermediate stages of metazoan (helminth) parasites were the most commonly encountered parasitic disease in all submissions. These were most commonly seen within the stroma of loose connective tissues beneath the epithelium of the mantle and foot. In the majority of cases they were attended by a granulomatous response composed of haemocytes and lesser numbers of brown cells (Figures 5.2.4 & 5.2.5) that produced a mantle of cells around the encysted parasite. In a small number of cases however there was minimal inflammatory response and metacestodes were lined by pallisading haemocytes. Some were identified as metacestodes. Others clearly showed a digestive system (not present in cestodes) and were identified as trematode stages (metacercaria), see figure 5.2.6

All seven of the wild sites around the Albany area contained infected abalone, highest prevalences were detected in Roe's abalone with a 30% prevalence seen at one site. Roe's abalone were over represented and six of the eight Roe's abalone collections showed cestode infection. Greenlip abalone showed a lower severity and lower prevalence in all areas except Hopetown where 33% of the abalone sampled showed metacestode parasite infection.

Cestode diseases:

The metacestodes, or more accurately plerocerci, measured 20-30 μ × 10 μ and did not contain a bladder. Taxonomically the plerocerci are probably located with one of two genera (genus *Polypocephalus* or *Tylocephalum*) in the Family Lecanicephalidea (Hine and Thorne, 2000). The plerocercid represents the second intermediate host of the cestode, the first intermediate host is not known in Australia, however a bird is believed to be an intermediate host in South Africa. No metazoan infection was seen in farmed abalone.

Trematode parasitic diseases

The metacercarial intermediate host of the trematode fluke was detected in abalone from 12 sites, representing a site prevalence of 15%. The prevalence within each site ranged from 6% to 45% and infections were usually systemically widespread within individual abalone and invaded and replaced much of the parenchymal tissues (Figure 5.2.7). Where infections were confined to individual organ systems, the right kidney and adjacent gonadal tissue were always involved and the gonad appeared to be the initial site of infection (Figure 5.2.8).

Roe's abalone were over represented with seven of the nine infected samples from temperate sites being *H. roei* with prevalence rates up to 30%. Two Donkey Eared abalone sampled from Broome were infected. Histologically, infection with metacercariae appeared to be progressive and likely to lead to organ failure and death. Very little inflammatory response was associated with infection and active development of stages was evident in all infected abalone examined.

Nematode parasites

No adult nematodes were seen. Fragments of eggs like bodies somewhat resembling nematode eggs were identified embedded within the loose connective tissues of the mantle in two abalone. The identity was uncertain due to similarity of these to gregarine cysts, which were also present (Figure 5.2.9).

5.2.3.5 Protistan diseases

Ciliates

Two distinct ciliated protozoans were identified associated with the gills and digestive gland.

Digestive gland ciliate:

A ciliate 5-8 µ in length with multiple macronuclei were seen from abalone from two sites. In one site the prevalence was 22% with moderate numbers of the ciliate free and adherent to the mucosa of the digestive gland (Figure 5.2.10). In some instances the underlying digestive tubule epithelial cells appeared hyperplastic, presumably in response to the presence of the parasite.

Gill holotrichous cilate :

A large 20-25µ long, elongated holotrichous ciliate with a large bean shaped nuclei and a fringe of cilia surrounding the entire surface was seen adherent to the gill lamellae in large numbers without inciting an inflammatory response (Figure 5.2.11). The ciliate was identified in brownlip, greenlip and Roe's abalone with a prevalence exceeding 80% in one sample of Roe's abalone from Albany. In this latter heavy infestation, ciliates were also observed within the eosophagus and stomach.

Apicomplexan-like parasites

Cryptosporidian-like parasites

A unicellular parasite was observed adherent to the intestinal mucosa in four abalone. On the basis of morphology and intraepithelial location was considered to resemble a cryptosporidial apicomplexan, though this has not been confirmed. Clusters of the 10-15 µ diameter, roughly circular parasite with a rim of eosinophilic cytoplasm, were observed in segmental areas of the intestine (Figure 5.2.12)

Coccidian–like parasite

Cells considered to resemble a coccidian-like parasite was observed in the intestinal mucosa from several Roe's abalone from different collection sites. The morphology is suggestive of the macrogametocyte stage of an apicomplexan parasite (Figure 5.2.13). Attempts to further identify the parasite using electron microscopy of affected tissue was unsuccessful.

Uncharacterised gut tubule intracellular parasite

Dumb bell shaped intra-cytoplasmic encapsulated structures 15 µ in length with a refractile cell wall was seen from two sampling sites of greenlip abalone. In one submission, 25% of animals were affected. In heavily infected abalone most epithelial cells in a single digestive gland tubule appeared to be infected, with multiple affected tubules in the worst affected animals (Figure 5.2.14). Overall impact appeared to be minimal, although this infection was seen in animals in poor condition from one site where gross stunting was evident.

5.2.3.6 Eubacterial infections

Rickettsia-like –organisms

Structures consistent with a rickettsiales was identified within the cytoplasm of epithelial cells in the digestive gland and intestine in 10 animals from 4 samples (Figures 5.2.15 and 5.2.16), and in the gills of 9 animals from 4 samples (Figure 5.2.17). They were characterised by variably sized, to 30µ diameter, stippled basophilic structures. The histological appearance of

the ricksettsiales-like organisms and the characteristic histological changes in digestive tubules is quite dissimilar to those described with Withering Syndrome. This is further supported by the lack of clinical evidence of Withering Syndrome was described in submitted animals.

5.2.3.7 Bacterial infections

Suspected Vibrio spp. infection

Two collections from each of the two commercial farms showed a high prevalence (50 % and 60%) of focal and focally extensive areas of granulomatous inflammation within the digestive gland. Severely affected glands were collapsed and replaced by the inflammatory cell infiltrate (Figure 5.2.18). No intra-lesional infectious agents were identified. Because testing of the farmed abalone had not detected the presence of metazoan infection, it was concluded the lesions could be most likely attributed to a prior bacterial infection. As *Vibrio* spp. had been recovered from the farms in the past it was believed this would be the likely bacterial species involved.

Haemocytic granulomas affecting the foot and mantle were common findings in wild-caught stock. Whilst no infectious agent was identified within the granulomas, the lesions were consistent with either a response to a metacestode or bacterial infection. Acute, haemocytic accumulations in the foot were considered to be due to a bacterial rather than a parasitic infection.

5.2.3.8 Virus like findings

Intestinal viral inclusions

Putative viral infections of the intestine were identified by the presence of densely basophilic intra-cytoplasmic and intranuclear inclusion bodies within the oesophagus and / or intestinal mucosa (Figure 5.2.19 and 5.2.20). The presence of the inclusions was not accompanied by evidence of inflammation or cellular damage. There appears to be two distinct types of cytoplasmic inclusion bodies seen in the main gut and suggests the presence of 2 distinct processes. Because the inclusions were present in low numbers, and although attempts were made, identification of the nature of the inclusion bodies by ultrastructure was not successful.

Digestive gland viral-like inclusions

Densely basophilic-magenta staining inclusions were seen in the digestive gland of two abalone (Figures 5.2.21 and 5.2.22). In one of the abalone similar inclusion bodies were identified within the intestinal mucosa suggesting that the same process was affecting both organs.

(Following pages)

Figure 5.2.3. Perkinsus type dinoflagellate spores growing on gill in Ray's fluid thioglycollate medium (dissecting microscope x 40).

Figure 5.2.4*:* encysted metacestode within mantle

Figure 5.2.5: metacestode with mantle of Haliotis leavigata, likely to be of the genus Polypocephalus or Tylocephalum (Lecanicephalidae).

Figure 5.2.6. Somewhat similar parasite showing digestive system section.

Figure 5.2.7. Trematode rediae, cercariae and sporocysts replacing gonad and right kidney tissue in *Haliotis roei* (H&E ×120).

Figure 5.2.8. Metacercarial stages of trematode parasite within the right kidney of *Haliotis roei* (H&E × 360).

(next page)

Figure 5.2.9. Nematopsis like gregarine within loose connective tissues of mantle.

Figure 5.2.10. Ciliate adherent to the luminal surface of the digestive gland tubular epithelial cell.

Figure 5.2.11. A large holotrichous ciliate adherent to gill epithelial cells.

Figure 5.2.12. Numerous Cryptosporidia-like protistans intimately associated with the intestinal enterocytes and appearing to hold an intraepithelial location.

Figure 5.2.13. Coccidia-like cell with stippled appearance and several other smaller structures, possibly meronts, with an amorphous basophilic appearance within the intestine from *Haliotis roei.*

Figure 5.2.14. Numerous intracytoplasmic dumbbell shaped parasites within digestive gland epithelial cells.

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Figure 5.2.15. Irregularly shaped and densely basophilic staining intracytoplasmic Rickettsia-like structure in enterocyte.

Figure 5.2.16. Basophilic-stippled Rickettsiales-like structure in the cytoplasm of a digestive gland tubule epithelial cell. (Inset – ruptured cell, showing bacterial nature).

Figure 5.2.17. Rickettsiales-like organisms in gills.

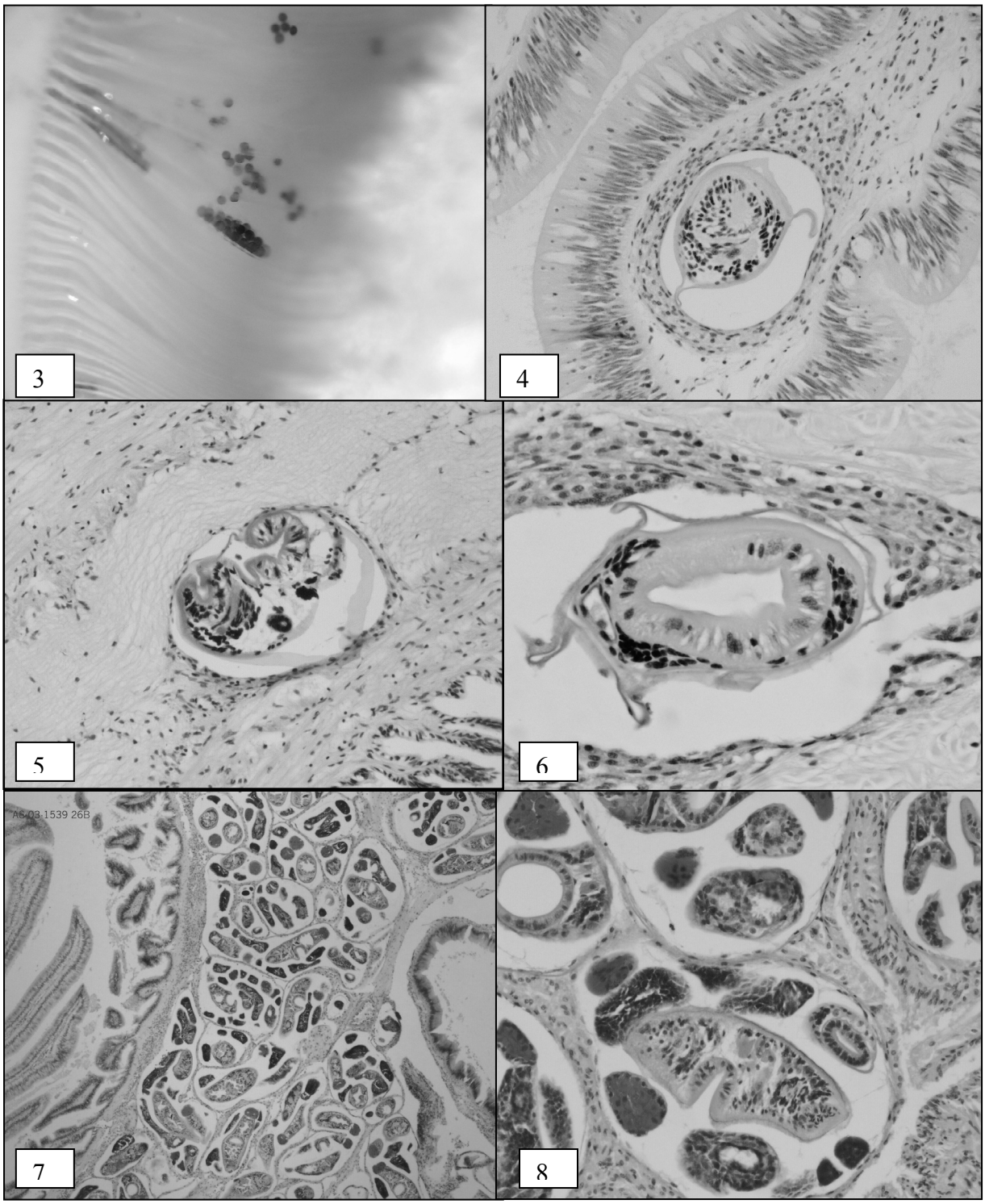
Figure 5.2.18. Intense haemocytic inflammatory infiltrate replacing and separating digestive gland tubules, suspected to reflect recent bacterial infection.

Figure 5.2.19. Two basophilic intracytoplasmic inclusion bodies within enterocytes.

Figure 5.2.20. Single large basophilic intracytoplasmic inclusion bodies within enterocytes.

Figure 5.2.21*:* Intensely basophilic, large, circular intranuclear inclusion bodies within enterocytes.

Figure 5.2.22. Densely basophilic-magenta inclusion body within an epithelial cell of the digestive gland.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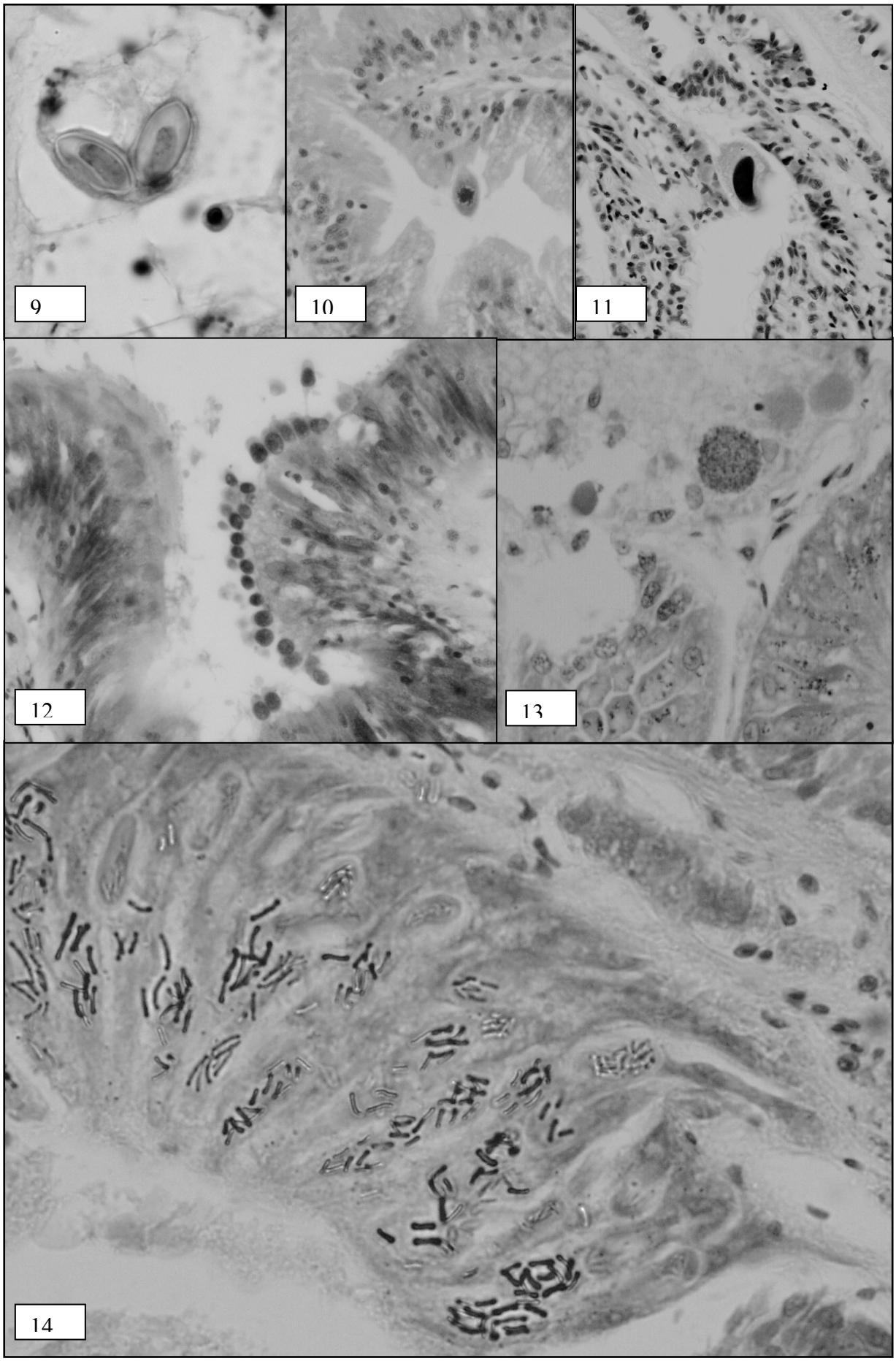
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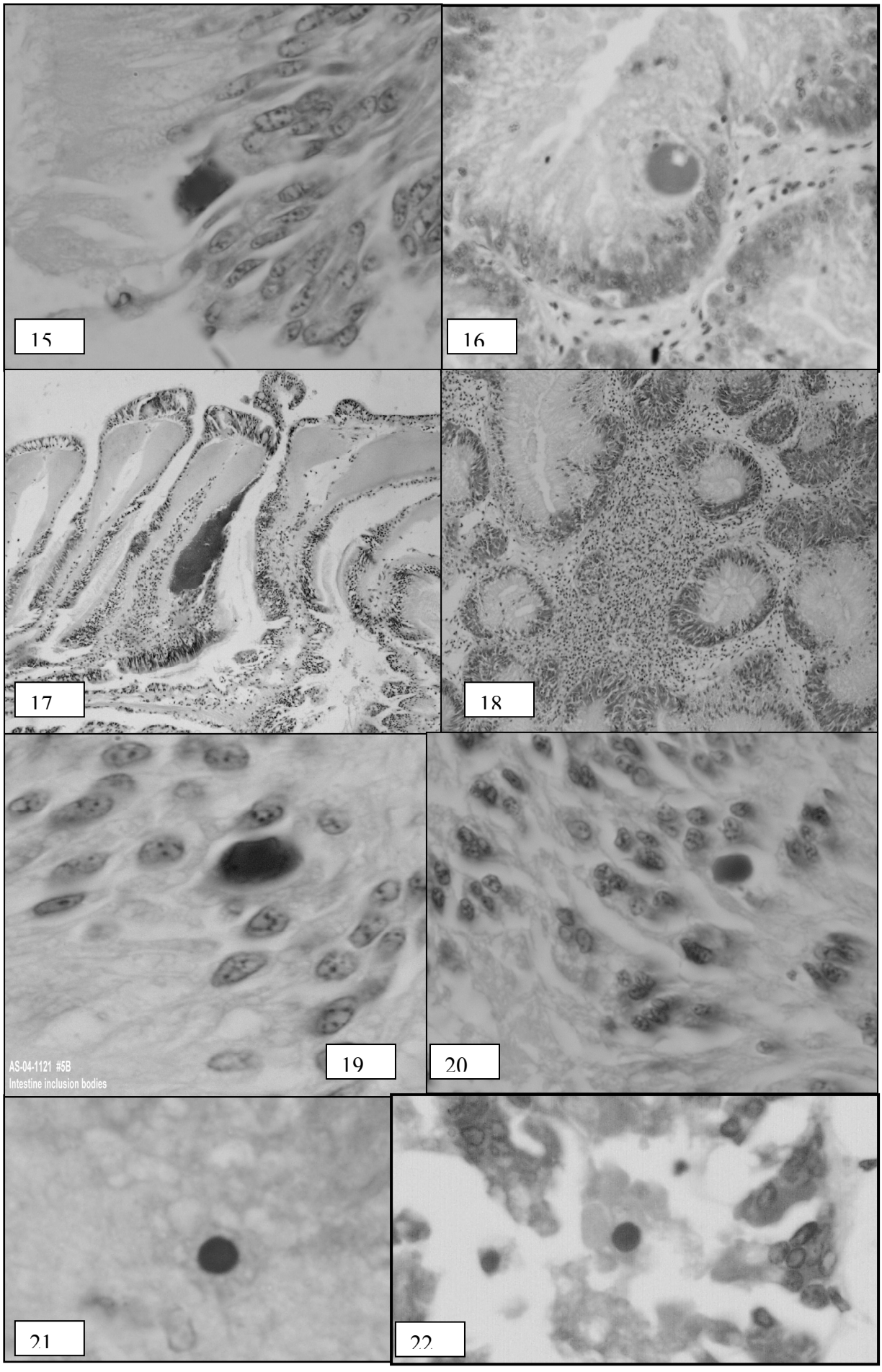
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Intestine inclusion bodies
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5.2.3.9 Summary of Lesions due to physical or physiological causes (non-infectious)

Physiological Diseases
Bloat
Oedema Digestive Gland
Proteinaceous Haemolymph
Non-Infectious Inflammatory Diseases
Left Kidney Nephropathy
Brown Cell Hyperplasia
Pedal Ganglioneuritis
Brown cell Gonad replacement
Haemocytic Granulomas
Reactions to Bacillariophyta - Diatoms
Diseases of Unknown Origin
Brick shaped haemocyte nuclei
Pseudo-inclusions digestive gland

5.2.3.10 Physiological diseases

Bloat

The dilation of the digestive gland tubules and/or the intestine, presumably by air, was a common finding in several samples, with one sample having 50% prevalence. The lesion was present in both farmed and wild stocks. This particular sample set was reported to be moribund and it not known if the intestinal bloat was a sequel or initiating cause of the animal's condition. A common underlying factor in bloated animals appears to be poor water quality either during collection at farms or in transport tanks following collection from wild sites. Figure 5.2.23 shows marked dilation, but no associated tissue changes.

Oedema of the digestive gland interstitium

Oedema of dilated interstitium was seen in only one abalone that is likely to have been a moribund specimen and was collected with abalone affected by a high prevalence of bloat (Figure 5.2.24).

Proteinaceous haemolymph fluid in multiple organs

The accumulation of highly eosinophilic staining haemolymph in vascular channels of multiple organs systems was a consistent abnormal finding throughout all samples examined. The accumulation was most pronounced and most consistently seen in the sinuses of the gills and left kidney, although other organs including the intestine were also seen (Figures 5.2.25 to 5.2.26)

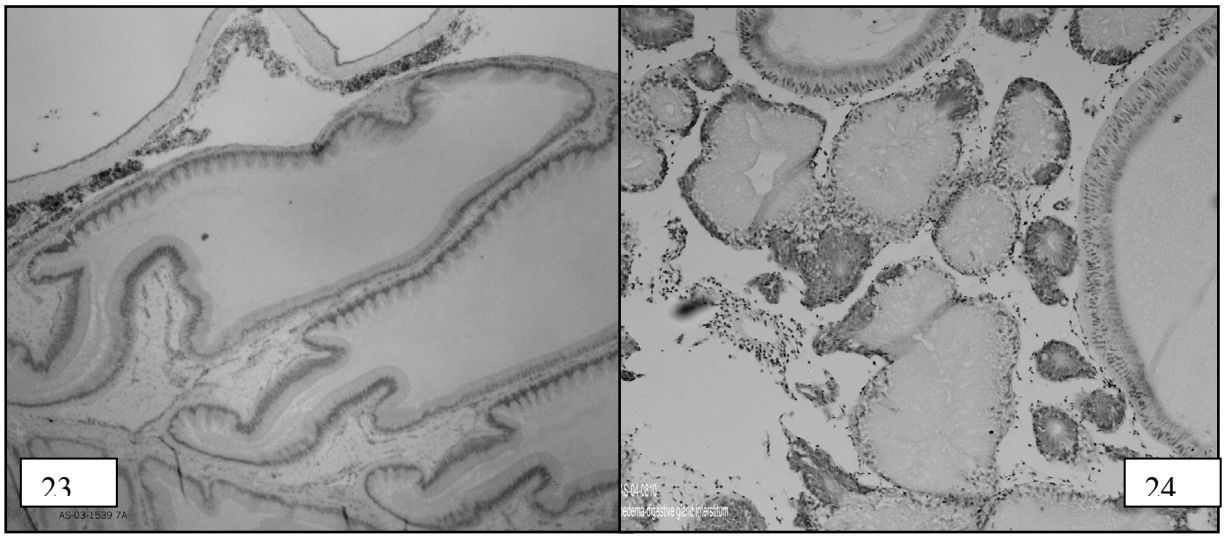

Figure 5.2.23 Massively dilated intestinal lops by air (bloat).

Figure 5.2.24. Oedematous fluid expanding the interstitium and separating the glands of digestive tubules.

5.2.3.11 Non specific inflammatory diseases.

Phylum bacillariophyta – Reactions to diatom and sponge spicules

Refractile silicaceous spicules derived either from diatoms or sponges were detected in animals from five wild sites and involved Roe's (two sites), brownlip (one site) and greenlip (one site) from sites extending along the southern coast from Augusta to Esperance.

Spicules were found within the mantle and attended by an intense haemocytic and brown cell inflammatory response typical of a foreign body reaction (Figure 5.2.27). In general, most spicules were up to 5 μ in length, much greater than the usual diatom size. This suggests a sponge spicule is the most likely source of the spicules.

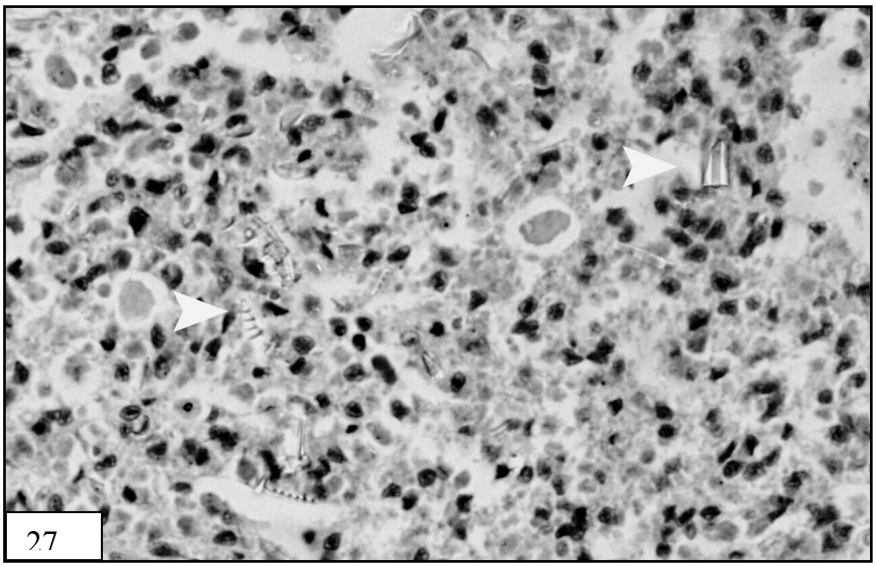

Figure 5.2.27. Granulomatous foreign body inflammatory response surrounding refractile spicules of a diatom.

Degeneration of the left kidney / nephrosis

Abalone with degenerative lesions of the kidney was seen in one farmed submission and two wild-caught submissions. The lesion appeared to be an extension or a more severe form of proteinaceous fluid accumulation within the fimbriae of the left kidney and were characterised by a reduction in the number and architectural distortion of the fimbriae and accompanied by an intense inflammatory cell infiltrate within the interstitium of the fimbriae (Figure 5.2.28). Large lakes of eosinophilic amorphous staining material within sinuses was interpreted to be haemocytic condensations analogous to mammalian fibrin thrombi.

Brown cell hyperplasia

Accumulation of haemocytes containing densely brown granular cytoplasmic pigment was seen in at least one abalone from 65% of sites surveyed and appeared to infect all age and sized animals. Figure 5.2.29 shows aggregations of pigmented cells in the right kidney.

Pedal ganglioneuritis

Inflammatory cell infiltrates around the perineural loose connective tissues around the pedal nerve and pedal ganglia was seen in at least one abalone from 50% of the sites sampled (Figures 5.2.30-31). In most instances, the inciting cause of the inflammatory cells was not evident however in several cases metazoan parasites were in close proximity (Figure 5.2.5).

Haemocytic granulomas

These were located most commonly within the mantle or foot and were composed of roughly circular aggregates of haemocytes and lesser numbers of brown cells. Except as mentioned above, there were no foreign bodies or parasites visible in the histological section to give an indication as to what was the initial inciting cause.

Brown cell replacement of male gonad

The formation of roughly circular structures to 300 μ diameter and comprised of brown cells within the male gonad parenchyma (Figure 5.2.32) was a common finding and was seen in five of the 22 submissions. The lesion was interpreted to be an incidental finding.

5.2.3.12 Lesions of unknown aetiology

Pseudoinclusions in digestive gland

Mineralised concretions were seen in the cytoplasm of the digestive gland epithelial cells in five submissions and interestingly, the highest incidence was seen in farmed abalone suggesting a dietary influence. Ultrastructural studies showed the structures to be composed of lamellated mineral and degenerate membrane material (Figure 5.2.33).

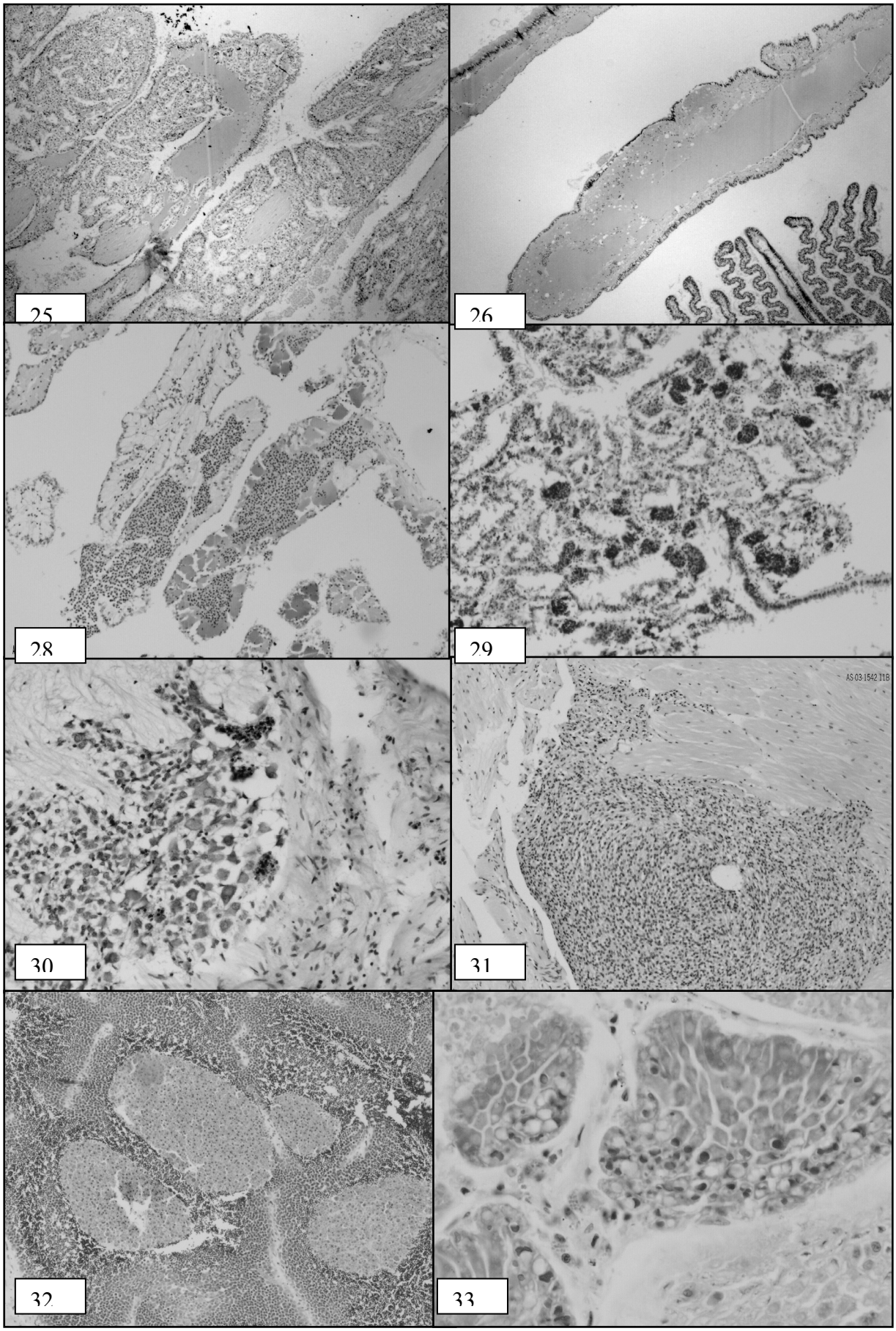
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Figure 5.2.25. Dilated right kidney haemolymph sinuses containing eosinophilic, proteinaceous fluid.

Figure 5.2.26. Proteinaceous haemolymph in mantle.

Figure 5.2.27. Haemocytic condensations and densely eosinophilic haemolymph within a distorted fimbriae of the left kidney.

Figure 5.2.29. Massive accumulations of brown pigment and "brown cells" in kidney.

Figure 5.2.30. Haemocytes and brown cells infiltrating between ganglion cells of the pedal nerve.

Figure 5.2.31. Well organised haemocytic aggregations within the foot having a chronic time frame.

Figure 5.2.32. Discrete accumulations of brown cells within the male gonad.

Figure 5.2.33. Mineralized concretions accumulating within the cytoplasm of digestive gland epithelial cells.

5.2.4 Summary and discussion

The survey of Western Australian abalone concentrated on the three commercially exploited species and identified no potentially serious pathogens. Shell damage associated with Polychaete and Spionid marine worms affected all brownlip abalone examined with greenlip being least affected of the three species.

Infection by trematode metacercariae appeared to be progressive and associated with very little inflammatory response. Invasion and replacement of kidney and gonadal tissue could lead to organ failure and eventual death in infected animals.

Over 80% of wild caught abalone showed evidence of metazoan parasite infection – usually either the presence of an encysted trematode or focal haemocytic granulomas within the foot, mantle or digestive gland. Farmed abalone appeared to be free of these parasites.

Inflammatory lesions surrounding the pedal nerve of the foot were likely to be secondary to metazoan infestation in adjacent tissues. No evidence was seen of neural lesions consistent with amytrophia as described in juvenile Japanese black abalone (Nakatsugawa et al., 1999).

The ciliates associated with the gills and digestive gland induced minimal host response and are likely to be commensals. Ciliates with similar morphological features have been described in South Africa as scyphidiid peritrichs of the genus Mantoscyphidia and occurring in large numbers of the gills of *Haliotis* species (Botes, 1999). It was concluded the ciliates are most likely not parasitic.

5.2.4.1 APPENDICES

APPENDIX WA 1: Summary of gross and histological lesions (appended, following page)

APPENDIX WA 2: Detailed gross findings of shell lesions including shell measurements (Electronic)

APPENDIX WA 3: State map of sampling sites (Electronic)

APPENDIX WA 4 Maps of individual collection sites (Electronic)

Appendix WA 1.

Appendix 1. WESTERN AUSTRALIAN ABALONE HEALTH SURVEY - SUMMARY OF RESULTS

								Virus	Eubacteria				Protistans						Helminth			Bacteria		Non-Infectious Causes									
CASE NUMBER	Map ID	Farmed or wild	Species	No samples	Length range	Weight Range	Perkinsus	Intestinal viral inclusion bodies	Viral Inclusions Digestive Gl.	GIT Rickettsia	Gill rickettsia	Microsporidia DG ciliate in digestive gland			spicules mantle tissues	cryptosporidian-like intestine	Coccidian-like intestine	microsporidia Digestive Gl.	nematodes	cestodes	trematodes	Granulomas foot/mantle No agent seen	Granulomas digestive Gland	bloat	Proteinaceous haemolymph	Oedema DG	kidney degeneration	Brown cell hyperplasia kidney and systemic	Pedal neuritis	degenerating ovary	Gonad replacement by brown cells	mineralisation of Digestive Gland
WA04/1539	13	wild	Roe's	30	58-88		Negative								1				2	3	1	2		4	7			1				
WA04/1542	8	wild	greenlip	30	138-159		Negative	1	1													3			6			1				
WA04/0807	3	wild	roe's	30	49-80		Negative				1			7						7	2	1			2		1	1				
WA04/1540	12	wild	greenlip	30	79-148		Negative													11	1	2			8			1	3			
WA04/1120	20	wild	brownlip	4	168-187		Negative								1																	1
WA04/1120	20	wild	greenlip	29	97-129		Negative													4		1			5		1	1	2			
WA04/0911	15	wild	roe's	30	45-66		Negative			2							1					2			5							
WA04/1541	11	wild	greenlip	30	95-154		Negative													7	2	6			5			2	2			
WA04/1158	16	wild	roe's	30	55-73		Negative						1				1							3	1			2	2		4	2
WA04/1121	19	wild	brownlip	5	120-151		Negative							3						3			1 (gill)	2								
WA04/1121	19	wild	greenlip	25	120-138		Negative	1				6						6		6	1	4		1	4	1						
WA04/0810	6	wild	roe's	30	30-78		Negative													1	10											
WA04/806	1	wild	roe's	30	49-80		Negative				1			13		1				6	1	3			8			1	1		1	
WA04/809	5	wild	roe's	30	30-78		Negative							24						10	2	2			2							
WA04/1686	17	wild	tropical	12	64-88		Negative													3											2	
WA04/945	18	wild	roe's	30	57-92		Negative			1				7	1					2	1									4		2
WA04/808	4	wild	greenlip	30	65-140		Negative													3				16	1							
WA04/805	2	wild	greenlip	30	85-133		Negative													5		4	2	15				3				
WA03 2584	10	wild	greenlip	30	112-160	184.5-693.5	Negative			6	1				2			1				4	12					4			6	9
WA03/1660	9	wild	greenlip	30	108-130		Perkinsus Positive			1	6				1							4	3					1				1
All Farmed		Farmed	greenlip	162			Negative		1				7			1						3	36	15	4		3	8	2		1	
			TOTAL	687																												

ABALONE AQUACULTURE SUBPROGRAM: A NATIONAL SURVEY OF DISEASES OF COMMERCIALLY EXPLOITED ABALONE SPECIES TO SUPPORT TRADE AND TRANSLOCATION ISSUES AND THE DEVELOPMENT OF HEALTH SURVEILLANCE PROGRAMS.

Victorian Report

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Fisheries Research and Development Corporation

ABALONE AQUACULTURE SUBPROGRAM

5.3 SURVEY OF DISEASES OF ABALONE IN VICTORIA

5.3.1 Introduction

Two species of abalone are harvested in Victoria for human consumption, *Haliotis laevigata* (greenlip) and *Haliotis rubra* (blacklip).

Greenlip abalone are distributed from Corner Inlet near Wilson's Promontory in Victoria along the south coast of Australia to Cape Naturaliste in Western Australia and islands in Bass Strait. They inhabit rocky reefs and live at depths of 10m to 40m. They are often found in or near sea grass beds and have a patchy distribution along the coast and the distance between populations may be as much as tens of km. Blacklip abalone are distributed from Coff's Harbour in NSW around the southern coast to Rottnest Island in WA including Bass Strait and Tasmania. They are most common in waters under 5 m in depth but around Tasmania they can live in waters 40m deep. They inhabit rock faces and crevices. Their distribution is more or less continuous along rocky coastlines. (Kailola et al., 1993)

5.3.1.1 The Victorian industry

Divers work off many locations along the coastline and harvest both greenlip and blacklip species but blacklip abalone predominate in the catch. Greenlip fetch premium prices in the export markets.

There are abalone farms distributed along the coast in Victoria. At the time of the survey there were five operations included in the survey.

The value to the Victorian economy in 2003/04 of the wild harvest of blacklip abalone was $46.293M and wild harvest of greenlip abalone was $0.08M. Farmed abalone is reported to have a value of $3.554M in 2003/04 (Anon, 2004). It is the second biggest abalone industry in Australia behind Tasmania.

5.3.2 Materials and methods

5.3.2.1 Sampling

A statistically significant number of 30 individual adult and juvenile animals were randomly selected from all farmed and multiple wild sites from coastal Victoria. The wild abalone were collected by fisheries officers during a census.

Wild blacklip abalone were sampled from five sites during January and February 2003: Discovery Bay, Lady Julia Percy Is, Port Phillip Bay, Cape Schank and Western Port Bay, and wild greenlip abalone from Walkerville and Portland during the summer of 2004. They were placed whole in formalin almost immediately.

Farmed abalone were collected from Narrawong, Leopold, Port Fairy, Lara and Indented Head in the summer of 2004 and placed in formalin.

Details of numbers and sites are provided in Table 5.3.1 and Figure 5.3.1.

5.3.2.2 Testing

The formalin fixed abalone were separated from their shells by twisting them by the foot muscle in an anticlockwise direction. A total of 450 abalone were examined grossly. The entire foot muscle of each abalone was examined after slicing at 1cm intervals.

All 450 abalone were examined histologically, after standard processing and sectioning at about 5 microns and stained with haemotoxylin and eosin. The screening included examination of the foot muscle, epipodium, gills (ctenidia), oesophagus, digestive gland, left and right kidneys, heart and gonad.

Small abalone were fitted directly into the cassette horizontally and sectioned whole.

For larger abalone, sections were taken according to the survey guidelines. To achieve this, and include all the organs above, section preparation was standardised for Victorian samples according to the following method. One gill was removed and placed in a cassette. The first cut was made at two thirds of the way from the anterior end and before the commissure of the gill chamber. It was directed at right angles to the depth of the foot muscle. This cut included the posterior digestive gland and sometimes its diverticula, right kidney and sometimes gonad. The second cut was made just posterior to the gill chamber commissure and directed at a slight angle (20^{o}) posteriorly to the depth of the foot muscle. This section included the left kidney on more than 90% of occasions, rectum, heart, right kidney, digestive gland and occasionally gonad. The third cut was at the most posterior aspect of the abalone opposite the mouth and directed at right angles to the surface to the depth of the foot muscle. This section included the gonad, digestive gland and intestine. The fourth cut was made at right angles to the mouth on the right side at right angles to the surface into the foot muscle to a depth of 2-3cm. The cut included the epipodium and the plantar aspect of the foot muscle.

Muscle from 30 abalone was cultured in thioglycollate broth using formalin fixed muscle from 6 blacklip abalone from each of the following sites: Discovery Bay, Lady Julia Percy Is, Port Phillip Bay (Pt Cook), Cape Schank and Western Port Bay (Pyramid Rock).

5.3.3 Results

5.3.3.1 Problems encountered in the survey

Good fixation of the wild specimens was a problem in that the large abalone were fixed with insufficient formalin (the recommended amount is 10 times the volume of 10% formalin to that of abalone). It is difficult to ascertain whether the collectors did not follow the instructions or whether the instructions were insufficient. Histological sections were over stained with haematoxylin as tissues had a predominantly bluish hue.

5.3.3.2 Gross examination

No abscesses or granulomas were observed in foot muscles of any abalone sampled for histopathology.

The shells from the wild abalone were variable in quality with evidence of fractures, mudworm damage and erosions. Farmed abalone shells were generally of better quality.

The shells have been offered to a university zoology department for study of polychaete infestation and quality.

5.3.3.3 Thioglycollate culture for Perkinsus.

Examination of 30 wild blacklip abalone by culture in thioglycollate broth for *Perkinsus sp.* proved negative.

5.3.3.4 Histopathological examination for Perkinsus

No *Perkinsus olseni* were detected in 450 abalone.

Table 5.3.1. Sampling summary for Victoria

Sample ID	Farmed or wild	Species	No Examined	Thioglycollate
Vic03205A	Wild Pt Cook	Blacklip	30	6
Vic03205B	Wild C Schank	Blacklip	30	6
Vic03205C	Wild LadyJPI	Blacklip	30	6
Vic03205D	Wild WP Bay	Blacklip	30	6
Vic03205E	Wild Disc Bay	Blacklip	30	6
Vic041063B	Wild Wlkvlle	Greenlip	30	
Vic042188B	Wild Ptlnd	Greenlip	30	
Vic041045A	Farm 1	Blacklip	30	
Vic041045B	Farm 1	Greenlip	30	
Vic041235B	Farm 2	Greenlip	30	
Vic041236A	Farm 3	Blacklip	30	
Vic041236B	Farm 3	Greenlip	30	
Vic041581A	Farm 4	Blacklip	30	
Vic041581B	Farm 4	Greenlip	30	
Vic041582 B	Farm 5	Greenlip	30	

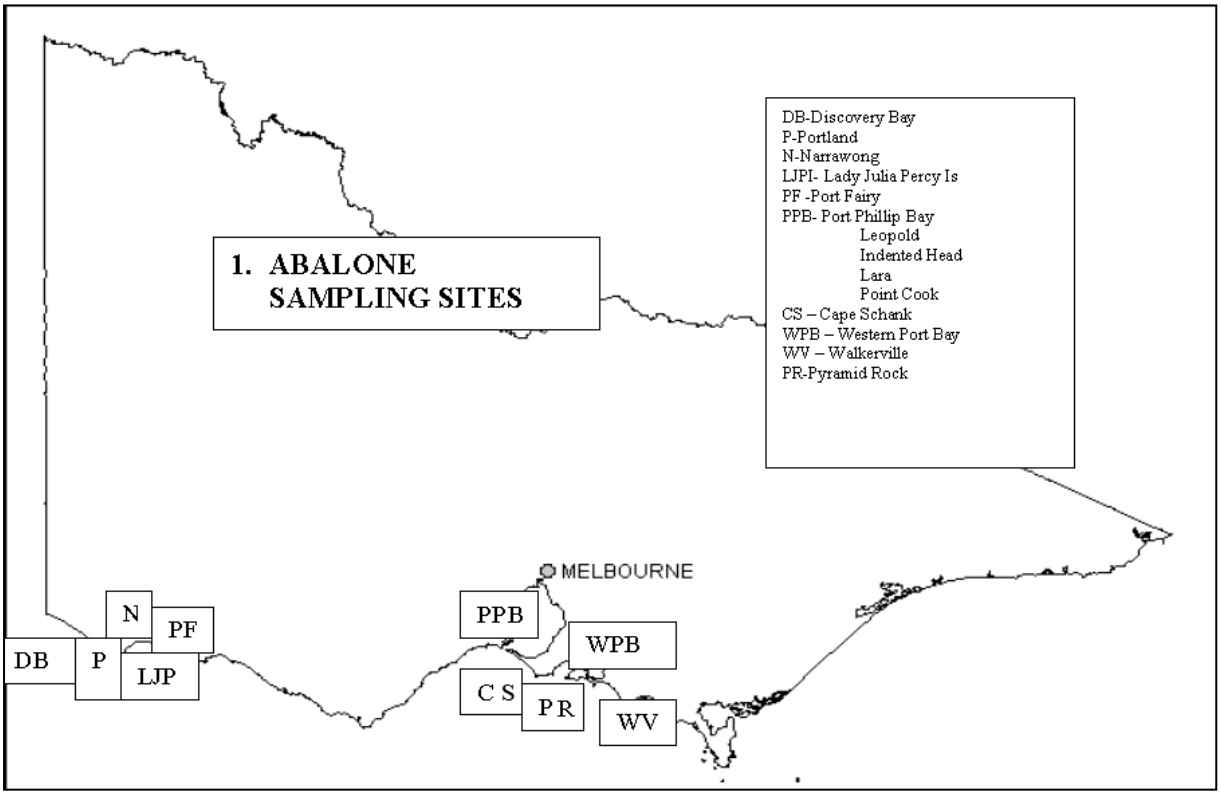

Figure 5.3.1. Victorian sampling sites.

5.3.3.5 Histopathology summary for each population

Wild populations

Pt Cook In Port Phillip Bay (Vic03205A) – wild blacklip abalone:

Ciliates were observed in gills in 3/30, metacercaria in 1/30 foot muscle. High protein concentrations were observed in many specimens in gills and left and right kidneys. A dark pigment in the right kidney was common.

Cape Schank (Vic03205B – wild blacklip abalone:

Ciliates were observed in gills in 26/30, metazoan parasites were observed in 1/30 gill cavities and the foot muscle of one abalone. Haemocyte aggregates were observed in 3/30.

Lady Julia Percy Island (Vic03205C) – wild blacklip abalone:

Ciliates were observed in gills in 13/30. Crystalline material (sand) 1/30, metacercaria in foot muscle 1/30 and aggregates of haemocytes 1/30 were observed in connective tissue of organs.

Western Port Bay (Vic03205D) – wild blacklip abalone:

Ciliates were observed in gills 3/30.

Discovery Bay (Vic03205E) – wild blacklip abalone:

Residual bodies in digestive gland were observed in 16/30. Metazoan parasites widely distributed in the organs in1/30.

Walkerville (Vic041063B) – wild greenlip abalone:

High protein concentrations in gill and kidney vessels, pigment in organs and also residual bodies were commonly seen. There was a single cell protozoan observed in the oesophageal mucosa.

Portland (Vic042188B) – wild greenlip abalone:

Residual bodies in digestive glands, high protein concentrations in organs and pigments were observed.

Farmed populations

Farm 5 greenlip abalone (*Vic041582B),*

Farm 3 blacklip abalone (*Vic041236A)* and greenlip abalone (*Vic041236B)*

Protein fluid was common in gills, left and right kidneys. Pigment was also common in organs including left and right kidneys and oesophagus. Residual bodies in digestive glands were apparent in many animals.

Farm 2 greenlip abalone (*Vic 041235 B)*

Residual bodies in digestive glands and high protein concentrations in gills and kidneys were common. Thickening of ctenidia (gills) and a putative protozoan oesophageal parasite with granulomas and congestion in the left kidney were seen.

Farm 1 greenlip abalone (*Vic041045B)* blacklip abalone (*Vic041045A)*

Residual bodies in digestive gland were present but not common. High protein concentration in vessels was moderately common.

Farm 4 greenlip abalone (*Vic041581B)*

High protein concentrations in vessels were moderately common.

Farm 4 blacklip abalone (*Vic041581A)*

Residual bodies in digestive glands were very common.

Protein in vessels was present in a range of organs.

Ciliates were observed to be large oval cells with nuclear material and sometimes cilia could be seen. They were usually found in the spaces between the ctenidia, see Figure 5.5.2. Occasional small protozoa were observed in and near oesophageal epithelium (Figure Fig 5.3.3).

Metazoan parasites were seen as sections of multicellular organisms with organ structures, sometimes including an obvious gut cross section, and a cuticle. Metacercaria within cercarial stages clearly visible in parasites within the right kidney (see Figure 5.3.4), though the structure of metazoan parasites in other tissues such as foot (Figure 5.3.5) was not always obvious in the sections observed. Lodges parasites elicited a variable level of reaction, sometimes resulting in blockage of gill vessels and gill (Figure 5.3.6).

Black and yellow black granules were seen in epithelial cells.

Residual bodies were observed in intact cells in the digestive gland epithelium. At the microscopic level the appeared as amorphous inclusion in the cytoplasm with a slight refractile appearance and a halo, see Figure 5.3.7.

Table 5.3.2 is a summary of histological findings.

5.3.4 Summary and discussion of findings from the survey

Few infectious agents were detected. No *Pekinsus olseni* were detected. Gill ciliates and trematode metacercaria were observed in wild populations but not in the farmed populations, and an oesophageal protozoan was seen in one farmed abalone. Its identity and significance is unknown. Farm mortality associated with *Vibrio harveyi* has recently been diagnosed on a Victorian farm. Fungal muscle infection of wild abalone has also recently been seen in a Victorian holding system, though whether this was of Tasmanian origin (as suspected) or arise after these animals had been translocated to Victoria was not established.

Residual bodies were a common but not a universal finding and probably represent the result of metabolism of the cells of the digestive glands at fixation.

High protein concentrations in vessels in the organs particularly the left and right kidneys and the gills may represent a change due to stress prior to fixation. Removing the abalone from its habitat and taking it out of water prior to fixation may be sufficient for this stress reaction to occur.

5.3.5 Post Script to survey

Three other examinations were included in the study, following the completion of formal sampling.

5.3.5.1 *Vibrio harveyi* associated mortality.

A mortality problem has been found in farmed greenlip abalone where *Vibrio harveyi* has been cultured and inflammatory lesions were seen histologically. This came to light through routine investigation of mortalities on the farm on the *ad hoc* submission of the farm management.

5.3.5.2 Fungal disease in wild caught abalone in a holding system

Wild caught abalone of Tasmanian origin showed mortality and rapidly progressing focal foot lesions in a holding system (Figure 5.3.8). Histologically these animals showed extensive inflammation that under close observation was found to contain poorly stained fungal hyphae of variable thickness. The pathology was regarded as typical of the condition described as fungal tubercle disease.

5.3.5.3 Feed associated mortality

Examination of farmed greenlip abalone stocks with high mortality and reduced feeding shortly after provision of an imported feed were examined by two laboratories. A number of other farms in two States were similarly affected at the same time. Mortality was reported 10-21 days after feeding the imported food. These findings were from examinations in Victorian and Tasmanian laboratories.

All farms involved received the same batch of feed and shared information. The farm supplying samples for investigation had been informed of mortalities on other farms and noted a reduction of feeding and had therefore ceased feeding this food several days prior to mortalities commencing. Animals had resumed feeding by the time of examination, though

mortalities continued. On this farm mortalities were seen only in the largest (spawning) fish, smaller immature animals were not apparently affected.

Animals examined were greenlip abalone from tanks experiencing mortality after receiving this feed, packed either live or fixed and classified as either moribund or apparently unaffected. The animals packed live showed diffuse, slightly red soft "blisters" at the margins of an otherwise contracted bled-out foot. Similar gross findings were reported from another farm (James Harris, pers. comm.). Histological examination showed changes ranging from marked dilation and depletion of the peri-gut haemocyte beds to extensive granulomatous lesions within the gut and surrounding tissue beds.

The major lesion seen in animals that were apparently healthy at collection was a haemocyte bed and intra-epithelial reaction, characterised by marked aggregation of the defence cells in affected areas (Figure 5.3.9). Intra-epithelial reaction (in oesophagus, sometimes oesophageal pouch, intestine and stomach / crop, digestive gland ducts but rarely the glands), resulted in an early granulomatous appearance. The intra-epithelial reaction was florid in some animals, though frank epithelial cell damage was rare. Similar granuloma-like reactions were seen to a lesser extent in other visceral organs such as kidneys. Granulomatous reactions appear more marked in later samples.

Moribund animals were more likely to show marked dilation and depletion of the peri-gut haemocyte beds, especially of haemocytes. Foci of pycnotic cells and what appeared to be an early granulomatous reaction were seen in or associated with various gut epithelia and kidneys, and in some areas the interstitium. As well as frank pycnosis, other apparently degenerative nuclear changes were seen in some haemocytes, prompting electron microscopic examination for viral exclusion. There was no direct histological evidence of infectious agents. Bacteriology of transport stressed animals showed mixed *Vibrio* species. Although this included *V. splendidus* this was not of the strain previously associated with disease outbreaks. (Carson, 2004). Electron microscopy of granulomatous gut epithelium and associated sub-epithelial reactions of two abalone did not result in visualisation of any viral type particles.

Monitoring of affected farms confirmed that while deaths continued for some time at lower levels, no secondary wave of mortality occurred in affected tanks, and deaths did not spread to adjacent tanks not fed this food.

Discussion:

These findings suggest animals that mount a granuloma like response may be less likely to die. Unfortunately there was no further monitoring to assess the effect of digestive system damage on production.

Table 5.3.2 Summary of histopathological findings for Victoria

Origin	WB	WB	WB	WB	WB	WG	WG	FG	FB	FG	FG	FG	FB	FG	FB
Source code	**Pt Cook**	**Cape Schank**	Lady Julia Percy Is	Western Port Bay	Discovery Bay	Walkerville	Portland	Farm 5 Greenlip	Farm 3 Blacklip	Farm 3 Greenlip	Farm 2 Greenlip	Farm 1 Greenlip	Farm 1 Blacklip	Farm 4 Greenlip	Farm 4 Blacklip
Ciliates	+/-	+	+	+/-											
Metazoans	+/-	+/-	+/-		+/-										
Protein excess in organs	+					+	+/-	+	+	+	+	+	+	+	+/-
Pigments in organs	+/-					+	+/-	+	+	+					
Oesophageal protozoa						+/-					+/-				
Residual bodies Digest gland					+	+	+/-	+	+	+	+	+/-	+/-		+
Haemocyte Aggregates		+/-													

\+ >20%

+/- < 20%

WB = wild blacklip abalone

FB = farmed blacklip abalone

WG = wild greenlip abalone

FG = farmed greenlip abalone

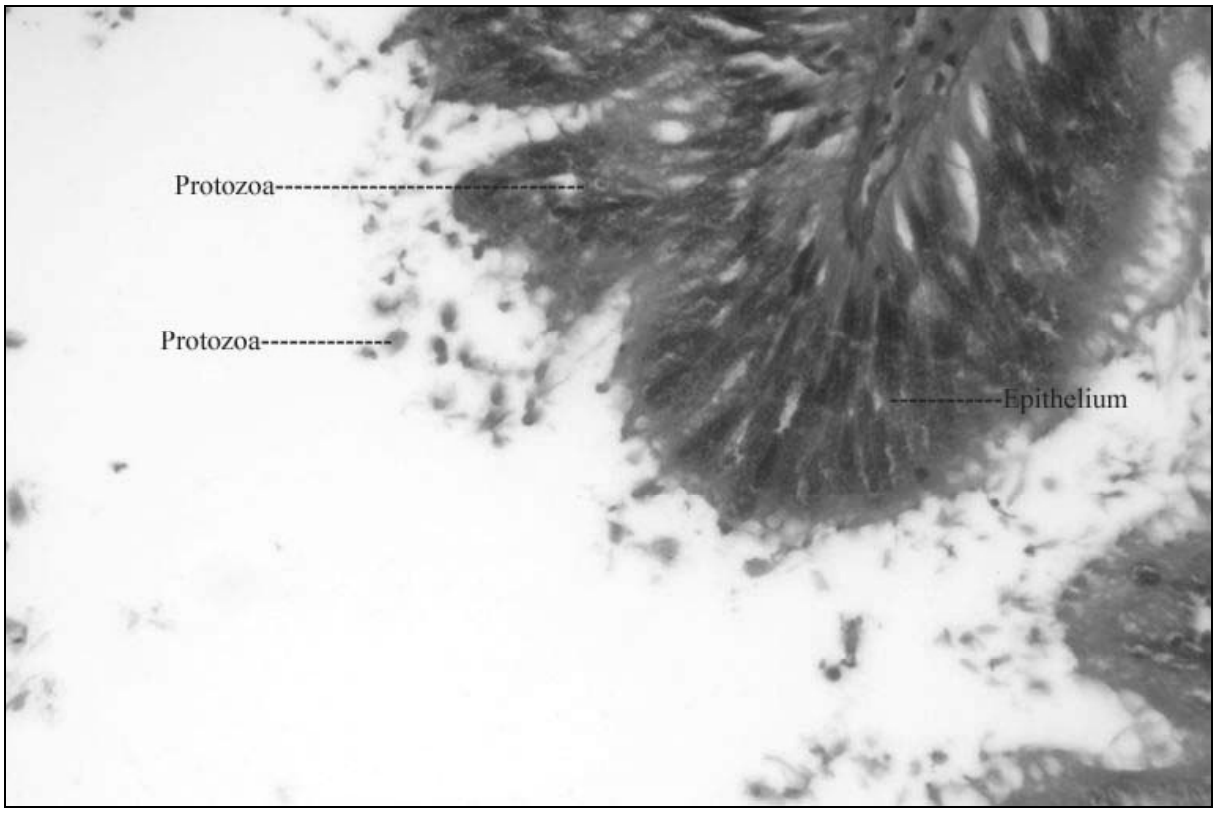

Fig 5.3.2 Oesophageal protozoa.

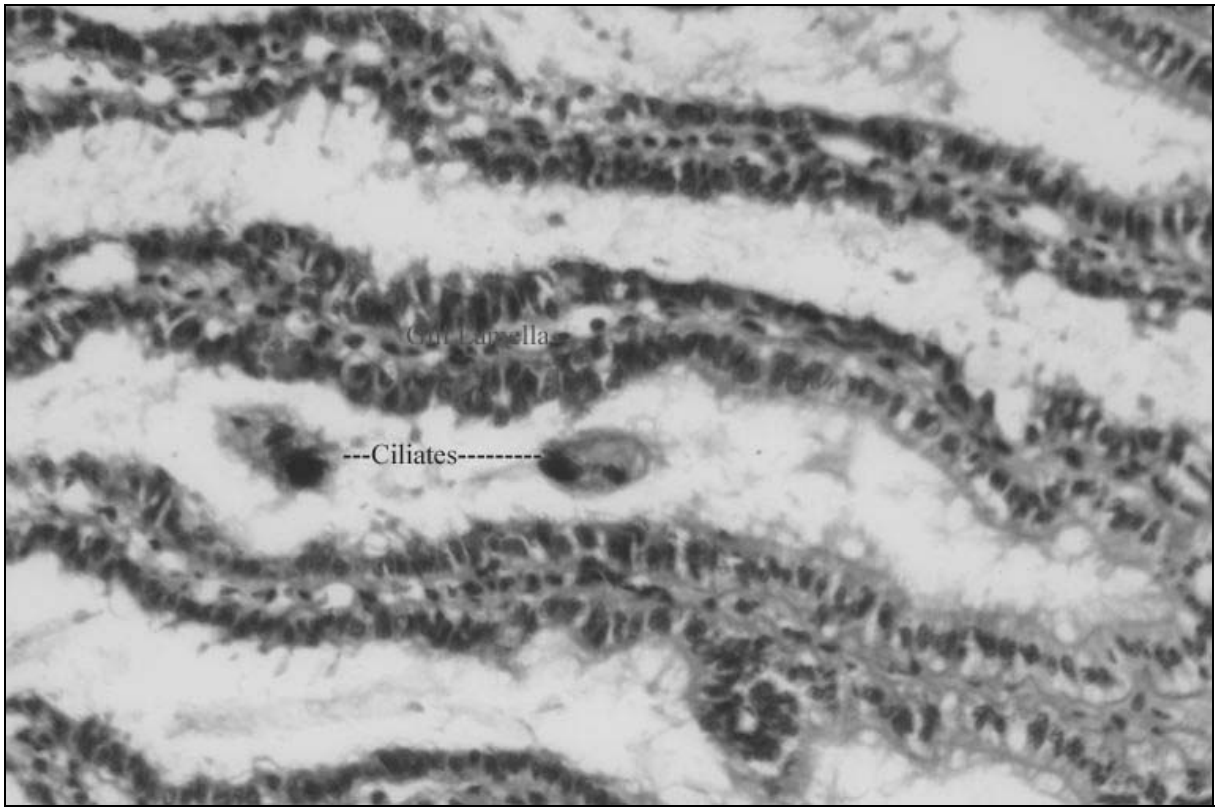

Fig 5.3.3. Ciliates in gill.

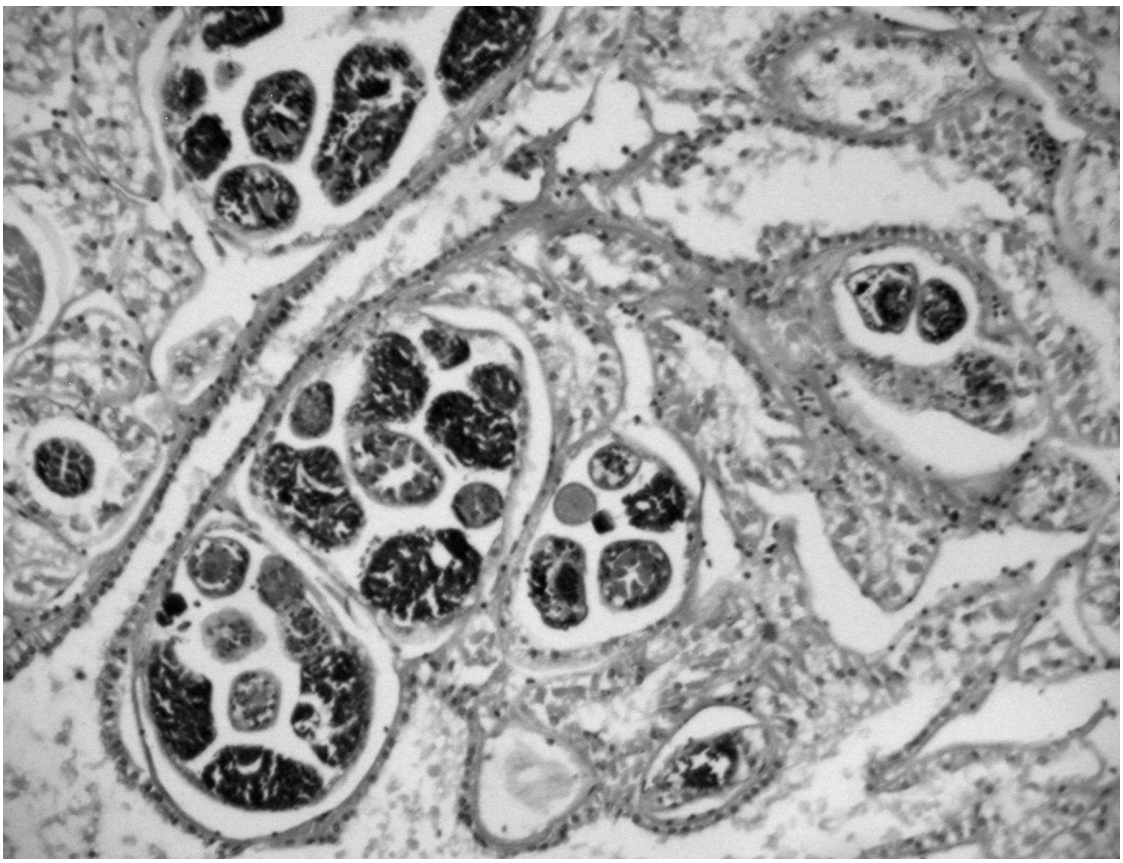

Fig 5.3.4 Metazoan in right kidney.

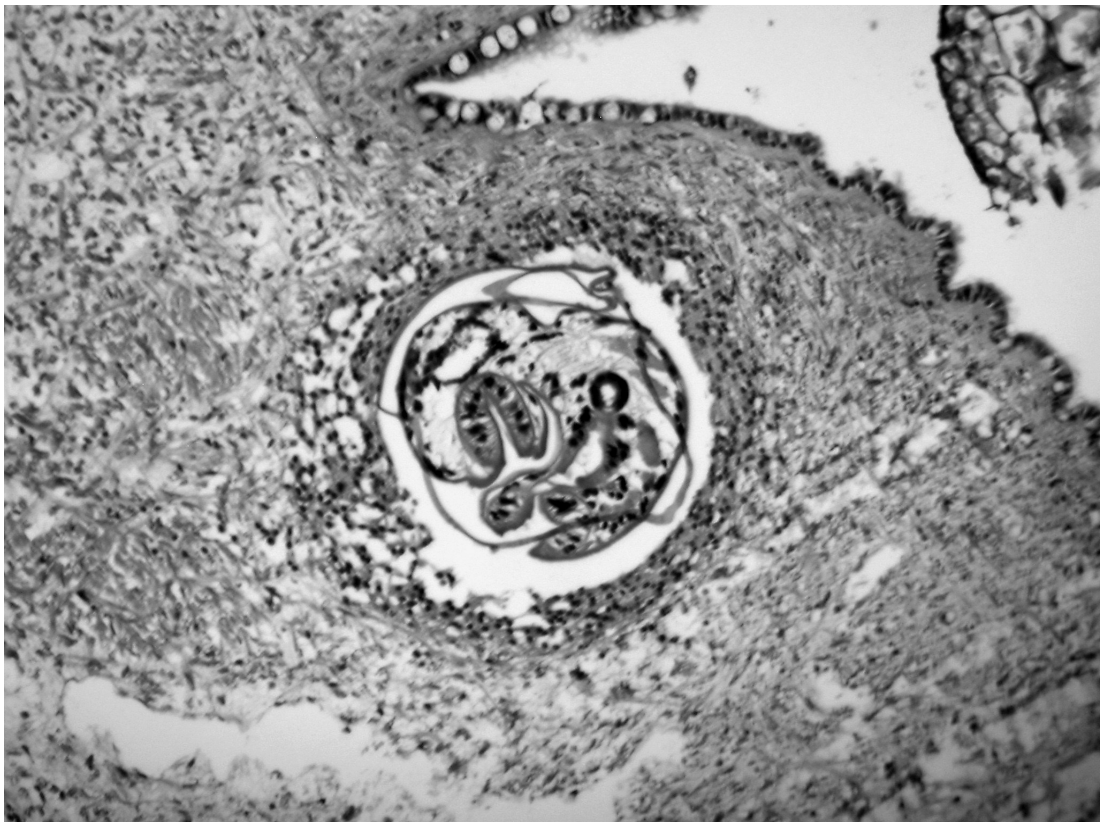

Figure 5.3.5. Live metazoan parasite in foot muscle, surrounded by slight inflammatory reaction.

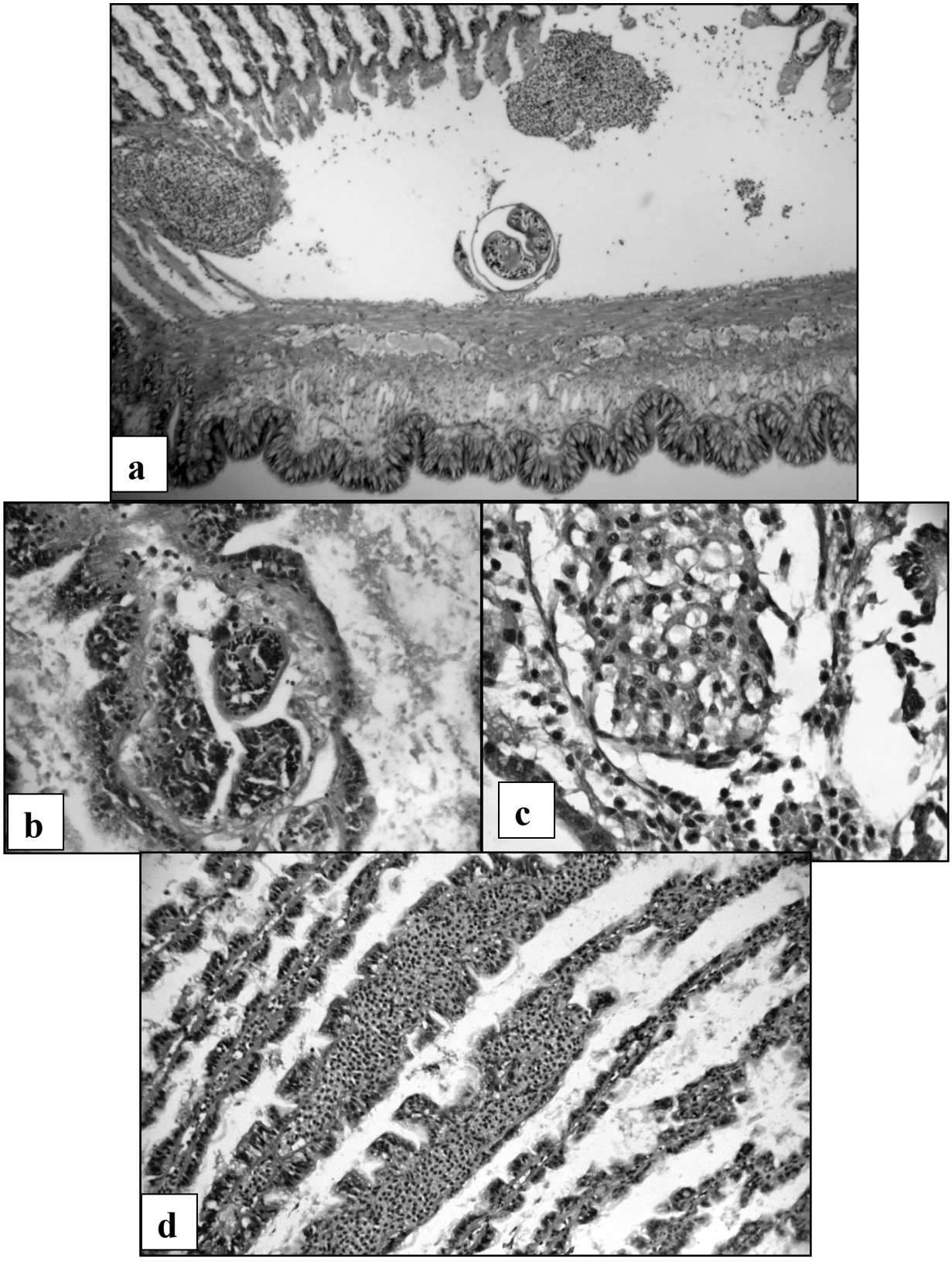

Fig 5.3.6. Gill pathology and lodged metazoans

5.1.6a. Metazoan parasite free within major gill vessel.
5.1.6b Metazoan parasite lodged within minor gill vessel.
5.1.6c Focal gill reaction suggestive of localised blockage (possibly metazoan).
5.1.6d More generalised gill reaction where the cause is not obvious (metazoan or external insult such as shell damage suspected).

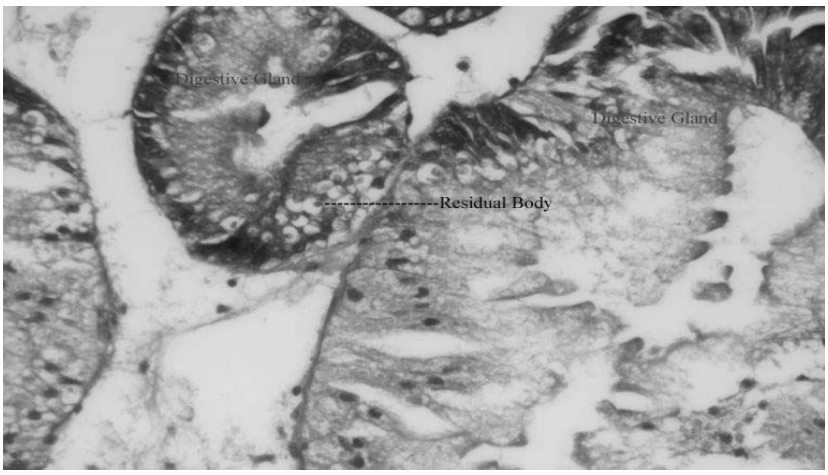

Fig 5.3.7. Residual bodies in digestive gland.

Fig 5.3.8. Gross appearance of fungal lesions on wild caught blacklip abalone of Tasmanian origin from a Victorian holding system.

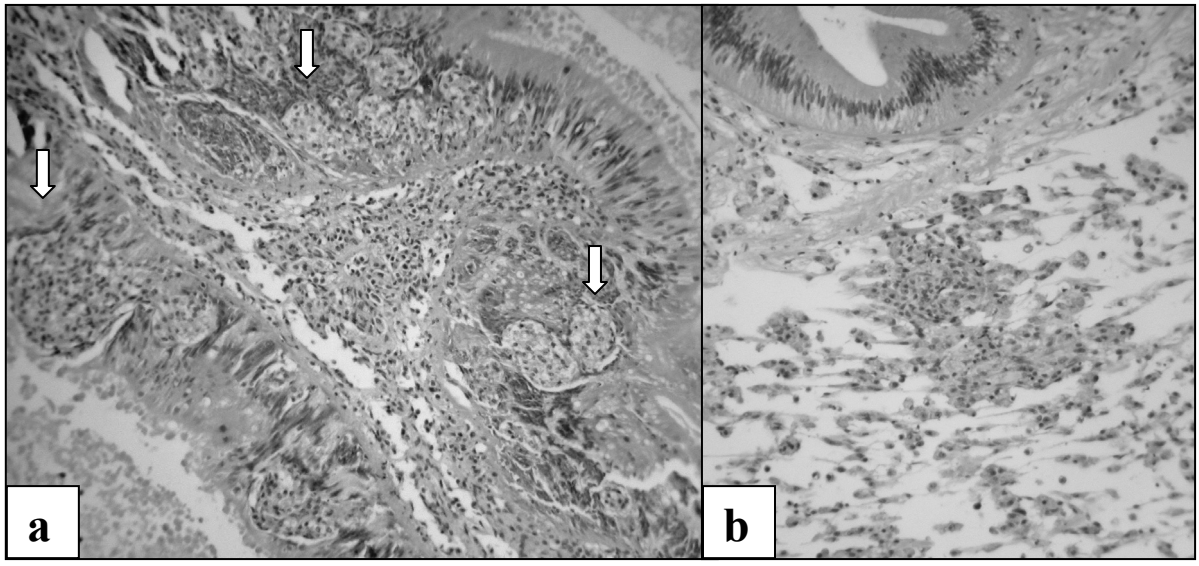

Figure 5.3.9. Epithelial granulomas (a, arrows) and haemolymph aggregates in interstitium (a and b, higher magnification) in animals from imported feed related mortality episode.

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South Australian Report

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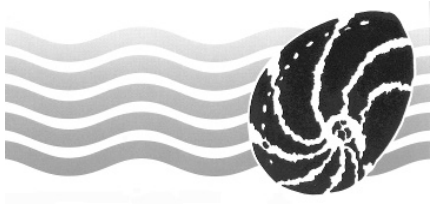


ABALONE AQUACULTURE SUBPROGRAM

A SURVEY OF DISEASES OF ABALONE IN SOUTH AUSTRALIA

PRIMARY INDUSTRIES AND RESOURCES SA

5.3.6 Introduction

The abalone fauna of South Australia includes five common species: *Haliotis laevigata* (greenlip abalone), *H. rubra* (blacklip abalone), *H. scalaris* (staircase abalone), *H. roei* (Roe's abalone) and *H. cyclobates* (cyclo abalone). Small populations of brownlip abalone (*H. conicopora*) and tiger abalone (*H. laevigata* x *H. rubra*) exist in isolated environments within the state.

Of these species, only *H. laevigata* and *H. rubra* are subject to commercial harvest (the remaining species do not usually grow sufficiently large to reach the size limits set in the different zones); the fishery is regulated under the *Fisheries (Scheme of Management—Abalone Fisheries) Regulations 1991* and is divided into the Southern, Central and Western Zones. Catch in these zones is summarised in the table below. The fishery has existed since 1967.

Table 5.4.1. Total catch and effort for the three zones of the South Australian abalone fishing industry

Zone	Year	Species	Total catch (t)	Effort (hrs)
Southern	2002/03	Blacklip	144.95	1430
		Greenlip	3.0	
Central	2003	Blacklip	36.02	1982
		Greenlip	142.62	
Western	2003	Blacklip	304.58	6269
		Greenlip	247.44	

Abalone aquaculture in South Australia has expanded over the last 15 years. In 2002/03 the State's 17 land based abalone farms produced approximately 40 t of abalone and generated $4.6m. At the time the survey was conducted in 2004/05,15 marine sites were licensed but had not produced sufficient product or been operating for long enough to be assessed separately, but these farms will provide increased production over the following years.

Abalone farming sites are situated from Streaky Bay on the State's west coast to the south-east of the State. There is a concentration of both land-based and in-sea farms on the East Coast of Eyre Peninsula.

5.3.7 Health survey information

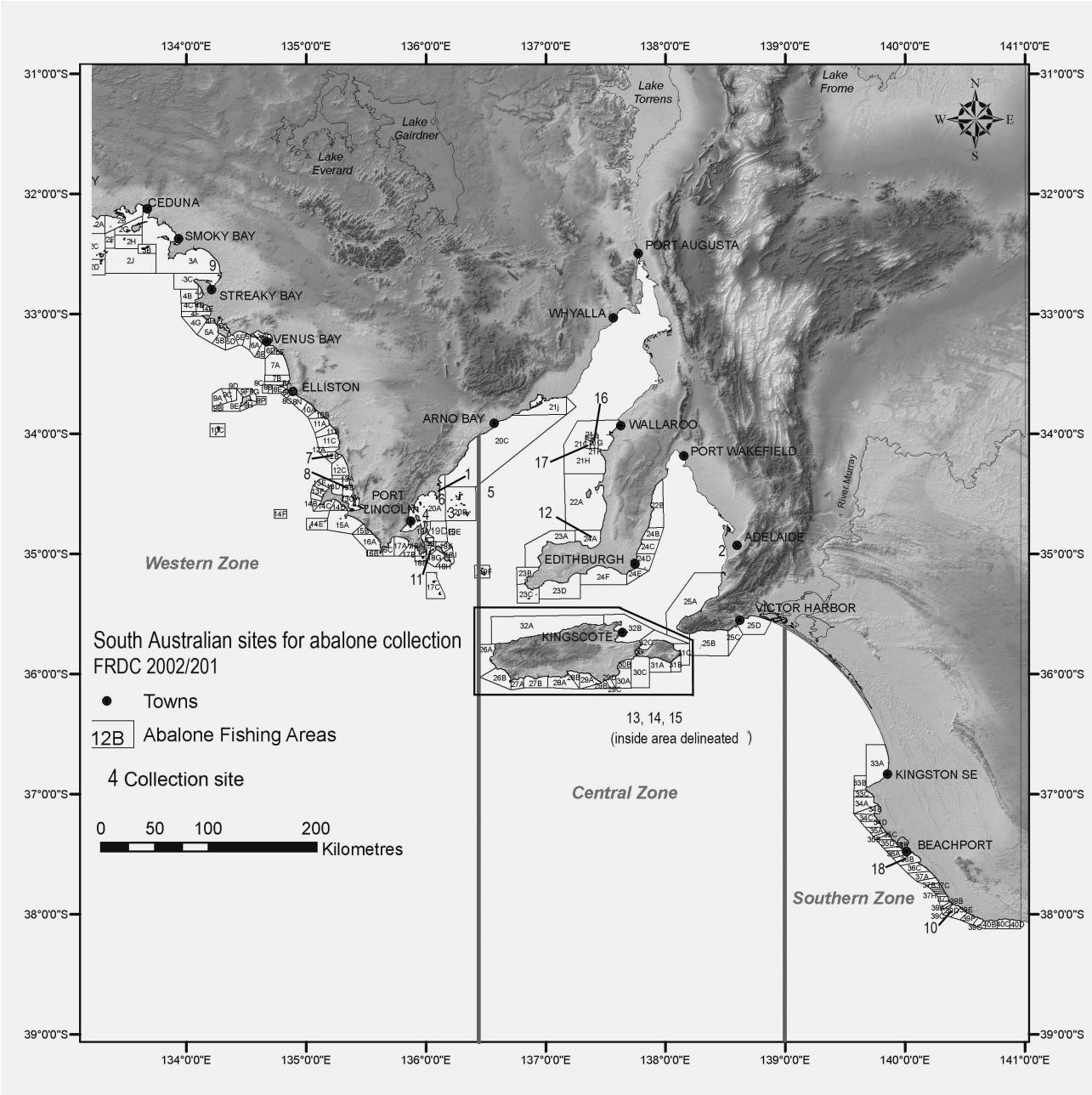
A total of 236 farmed abalone from 9 farm sites and 274 abalone from 9 sites in the wild-capture fishery were collected between January 2003 and March 2005. All land-based farms were invited to participate; all but two land-based farms that held stock at the time of the survey provided material.

Farmed abalone were all *H. laevigata*. Different *Haliotis* spp. have distinct environmental preferences (Shepherd, 1973), although at some sites both species are captured by fishers. Wild-

capture abalone were 57% (n= 156, 3 batches) *H. rubra*, with the remaining 43% (n=118, 4 batches) were *H. laevigata*. Two batches of abalone from the wild capture component included a mixture of *H. laevigata* (n= 4, 1) and *H. rubra* (n= 26, 30). Collection sites (numbered as per Table 5.4.2) are identified on Map 5.4.1. (PIRSA retains more detailed information.)

Table 5.4.2. **Details of abalone sampled in the South Australian survey**

Date	Site #	Species	Farmed (F) / wild (W)	n	Zone (wild only)
29/01/03	1	*H. laevigata*	F	39	
17/02/03	2	*H. laevigata*	F	2	
25/02/03	3	*H. laevigata*	F	16	
02/12/03	4	*H. laevigata*	F	29	
05/12/03	5	*H. laevigata*	F	30	
15/12/03	6	*H. laevigata*	F	30	
23/06/04	7	*H. laevigata*	F	30	
30/06/04	8	*H. laevigata*	F	30	
09/07/04	9	*H. laevigata*	F	30	
10/07/04	10	*H. rubra*	W	30	Southern
17/08/04	11	*H. laevigata*	W	32	Western
04/09/04	12	*H. laevigata*	W	31	Central
21/09/04	13	*H. rubra*	W	31	Central
21/09/04	14	*H. laevigata* *H. rubra*	W	4 26	Central
21/09/04	15	*H. laevigata* *H. rubra*	W	30 1	Central
10/12/04	16	*H. laevigata*	W	29	Central
10/12/04	17	*H. laevigata*	W	30	Central
24/03/04	18	*H. rubra*	W	30	Southern



Map 5.4.1. Abalone health survey collection sites in South Australia.

5.3.8 Methods

The shell of each animal was examined for the presence of fouling organisms (commensal and phoretic) and for parasitic shell diseases (recorded as present or absent). Pathological findings were noted on arrival at the laboratory.

Harvested abalone were formalin fixed as soon as possible after arrival at the laboratory. Block sections for histological processing and paraffin embedding were made according to the guidelines set out in the survey standard methodology (section 4.3), and guidelines provided at the training workshop. Sections were cut at 5 μm and stained with haematoxylin and eosin using conventional methods. Special stains were applied when further elucidation was deemed necessary. Sections were viewed using a compound microscope.

An additional histopathology sample of farmed abalone that had been samples in November 2003 and examined by two pathologists at that time (Mouton and Handlinger) was made available by the farm. This sample compared several age groups with differing health history.

5.3.9 Results

5.3.9.1 Shell diseases

Mudworm

The common mudworm species in South Australia are *Polydora hoplura* and *P. armata* and *Boccardia chilensis*. *Boccardia knoxi* is regarded as exotic to South Australia. Mudworms were not identified to species in this survey but had been examined in detail previously (M Deveney, unpublished). Of the nine farmed samples examined, only four had any evidence of mudworm infection, while all nine samples of wild caught abalone were infected.

Shell fouling organisms

All but one sample of farmed animals had shells fouled with spirorbid and sabellid polychaetes, including some similar to *Pomatoceros* sp. Wild caught animals carried an abundant fauna of varieties of algal growth, sponges and some molluscan organisms. In one instance, shell penetration by sponge-like organisms with a significant host response was noted. This was possibly due to *Cliona* sp. (Shepherd, 1973).

5.3.9.2 Helminth parasitic diseases

Trematode metacercarial infection

A metacercaria, possibly of an opecoelid (these are uncommon in molluscs as metacercariae) or apocreadiid digenean was present in 3 wild-caught abalone, each from a different site: sites 10 (from the Southern region), 11 (Western region) and 13 (Central region). One was within or in juxtaposition with a nerve fibre in the foot of one animal while the other 2 were within the sub-epithelial stoma. Only one parasite had a mild mantle of haemocytes (Figure 5.4.1). The first intermediate host is a mollusc and the definitive host is likely to be a predatory fish.

Nematodes

A cystic space containing sections of a large nematode was present in the connective tissue near the mouth/oesophagus in one wild abalone from site 17 (Figure 5.4.2). None of the sections included reproductive tissue so it could not be ascertained if the nematode was sexually mature or a larval stage. The cyst space was occupied by degenerate haemocytes and granulocytes, which also formed an inflammatory corona around the apparently fibrotic well. A small nematode (larval?) was also detected within a granuloma in the gut of a wild abalone from site 12.

Table 5.4.3. Distribution of mudworm between *H. laevigata* and *H. rubra* at the 9 wild-caught sites sampled

Site	Mudworm prevalence (%)	Mudworm prevalence (%)
	H. laevigata	***H. rubra***
10	-	30
11	19	-
12	16	-
13	-	65
14	25	35
15	63	100
16	62	-
17	10	-
18	-	60

5.3.9.3 Protistan diseases

Phylum Bacillocophyta diatoms and sponges

Numbers were not recorded but occasional blacklip and greenlip abalone from wild-capture sites had mantle granulomas with refractile spicules at their core. These spicules appeared similar to those of sponges.

Ciliates

Of 30 blacklip abalone submitted from site 18, 10 had moderate numbers of elongate holotrichous ciliates within the gill chamber (Figure 5.4.3). No inflammatory response was

noted in 9 of these animals with 1 showing branchiitis that may have been associated with the presence of the ciliates.

Suctorial ciliates were present on the foot surface of two wild-capture animals from site 10 (Figure 5.4.4). These were not associated with lesions and appeared to be commensals, or incidental findings.

Some poorly preserved large protozoa (possibly ciliates) were present within the sub-epithelial connective tissue of the feet of two animals from site 10 (Figure 5.4.5).

Perkinsus testing

Pooled tissues of five of the nine farm submissions and seven of the nine wild-capture submissions were examined for *Perkinsus* using Ray's fluid thyoglycollate medium (RFTM). All RFTM tests for *Perkinsus* were negative. No *Perkinsus* was detected histologically. *Perkinsus olseni* is known to be present in SA waters (Lester and Davis, 1981; O'Donoghue, Phillips & Shepherd, 1991).

Apicomplexa

Suspected coccidia

A suspected coccidean-like parasite was detected in parsitophorous vacuoles in digestive gland epithelium in two animals from two farm sites. Though the parasitic nature of these cells has not been confirmed, they are recorded for future reference (Figure 5.4.6).

Intra-epithelial digestive tubule parasite.

Oval basophilic organisms were found in clusters in the digestive gland of wild-capture animals. They were present in animals from five wild-capture sites (12, 13, 14, 15, 16) and one farm site (9). Two forms were seen, with many of the basophilic bodies contained refractile bodies with a dumbbell bacillary appearance that was somewhat reminiscent of Microsporidia, but without this structure (Figure 5.4.7), while others showed only the small clearly nucleated forms (Figure 5.4.8). The presence of these organisms elicited no apparent response by the host.

Nematopsis-like gregarines

Gregarine-like organisms suggestive of *Nematopsis* sp. were seen in five animals from wild-capture site 16 and four from site 12. No tissue reaction was seen in association with these organisms (Figure 5.4.9).

5.3.9.4 Eubacterial infections

Intracytoplasmic, round, basophilic stippled structures of up to 30 μm diameter within epithelial cells of the digestive gland and gut were detected in 13 wild abalone from sites 12, 15, 16 and 17. Four of these infected abalone were from site 12. Though these were rickettsia-like in appearance (Figure 5.4.10), none of the abalone had the cellular evidence of Withering Syndrome and the characteristic clinical signs of Withering Syndrome have never been observed in South Australia.

5.3.9.5 Bacterial infections

Although animals from all sites had some apparent haemocytic inflammatory responses, both diffuse and granulomatous, no bacteria were identified in association with this in wild populations. In animals from one farm site (4) small bacilli (probably *Vibrio* sp.) were noted in the mantle of a single animal and phagocytosed bacilli in the para-intestinal haematopoietic tissue of another. Nine animals from this site had marked post-mortem invasion by mixed bacilli.

5.3.9.6 Physiological conditions / lesions due to non-infectious causes

Eosinophilic haemolymph

Highly eosinophilic haemolymph was present in vascular channels of numerous tissues and organs in many animals. This was observed most often in gills (Figure 5.4.11) and kidneys.

Brown cell hyperplasia

Many animals of both species and all ages from farmed and wild animals had accumulations of haemocytes with condensed brown, granular pigment which stained positively with Schmorls stain for melanin (Figure 5.4.12). While blacklip abalone had more pigmented tissues, the formation of these melano-haemocytic nodules were spread across both species and could appear in most tissues.

Pedal ganglioneuritis

Inflammatory cells specifically targeting neural tissue, particularly in the foot, were seen rarely and appeared to have no connection with pathogens or inflammation of other tissues. Note the one metacercaria in close apposition to a nerve sheath mentioned above.

Concretions in foot tissue

Mineralised concretions of unknown aetiology were seen in the feet of one large wild capture abalone and a single, small farmed animal (Figure 5.4.13).

Oedema and angiectasis

Lesions such as those of Figure 5.4.14 were observed in many abalone, but is not known whether this is a pre- or post-capture/collection condition, or even if it occurs pre- or post-mortem.

Granulomas of the digestive system

While granulomas of the digestive glands and other digestive system organs was not a feature of this survey, high levels were found in the November 2003 samples made available by one of the farms (J. Handlinger & A. Mouton, pers com), and this lesion has been seen previously in South Australia. Most granulomas were in the digestive glands. No infectious agents were seen in association with these lesions. Some of the affected groups had a history of poor performance, with spionid mudworm infection recognised as one contributing factor, but the cause of this lesion was unresolved.

5.3.10 Summary and discussion

The wild-capture areas sampled in this survey included bias for the willingness of commercial divers to assist in the collection of material for scientific purposes. Despite being the most productive of South Australia's abalone fishing areas, only a single sample was obtained from the Western Zone (site 11). Further confounds in the wild-capture animals include avoidance of areas known for heavy *Perkinsus* infection (divers avoid these areas to avoid a high discard rate of captured abalone) and a bias towards larger, commercial-sized or near commercial-sized animals. Small abalone in the wild are highly cryptic and are difficult to locate and capture.

The distances between Adelaide and the areas where abalone are farmed and caught introduced some artefacts to this study. Some animals, particularly the wild-capture abalone, arrived dead and had considerable post-mortem artefacts.

This survey failed to find *Perkinsus olseni* in wild abalone, despite this pathogen being known to be enzootic in South Australia (Lester et al., 1990). Infection with *P. olseni* has been recorded in South Australia in association with abalone mortalities (Lester, 1986) and the infection has been described as having a patchy distribution (Lester et al., 2001). Material collected outside this survey from an area known for high *P. olseni* prevalence showed up to a 93.3% (n= 30; animals with clinical signs collected preferentially) prevalence of infection when tested by RFTM (Lester and Haywood, 2005) Losses to the wild-capture fishery continue due to poor marketability of infected animals (which are typically discarded). In addition to the bias described above in terms of avoidance of high-prevalence areas by commercial divers, *P. olseni* is known to have a patchy distribution within the state (O'Donoghue et al., 1991; Hayward et al., 2002) with infected sites presumably characterised by specific environmental factors (Hayward et al., 2002).

Shell damage due to mudworms was minimal in all samples examined although all nine wild sites and four of nine farmed sites were infected, though mudworm related health problems have been seen on farms and were evident to some extent in the samples collected in November 2003. Some areas of the State (particularly Spencer Gulf and the Gulf of St Vincent) have elevated salinity (averaging 40 ppt) and this may have contributed to the relatively low prevalence and intensity of mudworm infections observed. The low overall prevalence of mudworm in the abalone from the Southern and Western Zones, outside the reverse estuaries of South Australia's gulfs may be due to other specific environmental factors such as hard substrates, high current and wave action and low organic load in the water (Lleonart, 2001).

Of the wild-capture abalone, three animals were infected by trematodes. No farmed abalone were found to be infected by trematodes. The overall prevalence of trematodes and indeed, all parasitic infections may have been underestimated due to the sampling method used in the study. Various lesions such as haemocytic granulomas may speculatively been due to parasite infections past or present.

Other infectious agents such as the presumed coccidia, *Nematopsis*-like organisms and rickettsia-like organisms did not elicit any inflammation or other host response and appeared to have no ill effects on their hosts.

Overall, the generally good health of the populations of abalone examined was reflected histologically with low numbers of largely innocuous organisms found and occasional incidental findings which are currently mostly curiosities, but may be further investigated in

the future. Bacterial related mortalities have been reported previously on SA farms, including *V. harveyi* (Reuter & McCorist, 1999), but in general have not been well defined.

5.3.11 Acknowledgments (SA)

The authors thank the FRDC for funding this work, divers and commercial farm operators for submitting animals, the planning committee which set up the study (especially locally Dr Ruth Reuter), and the many technical staff who assisted in the processing of the material.

(Following pages)

Fig 5.4.1. Metacercaria within mantle of haemocytes in foot muscle.

Fig 5.4.2. Section of nematode near mouth of abalone.

Fig 5.4.3. Holotrichous ciliates between gill filaments.

Fig 5.4.4. Suctorial ciliates on foot surface.

Fig 5.4.5. Unidentified possible ciliates within and beneath foot epithelium.

Fig 5.4.6. Suspected coccidia-like organism in parasitophorous vacuole in digestive gland.

Fig 5.4.7. Oval basophilic bodies within digestive gland, containing brown refractile longated, dumbbell shaped bodies

Fig 5.4.8 Basophilic stage of this parasite in a digestive gland tubule from another animal

(next page)

Fig 5.4.9. *Nematopsis*-like organism within foot.

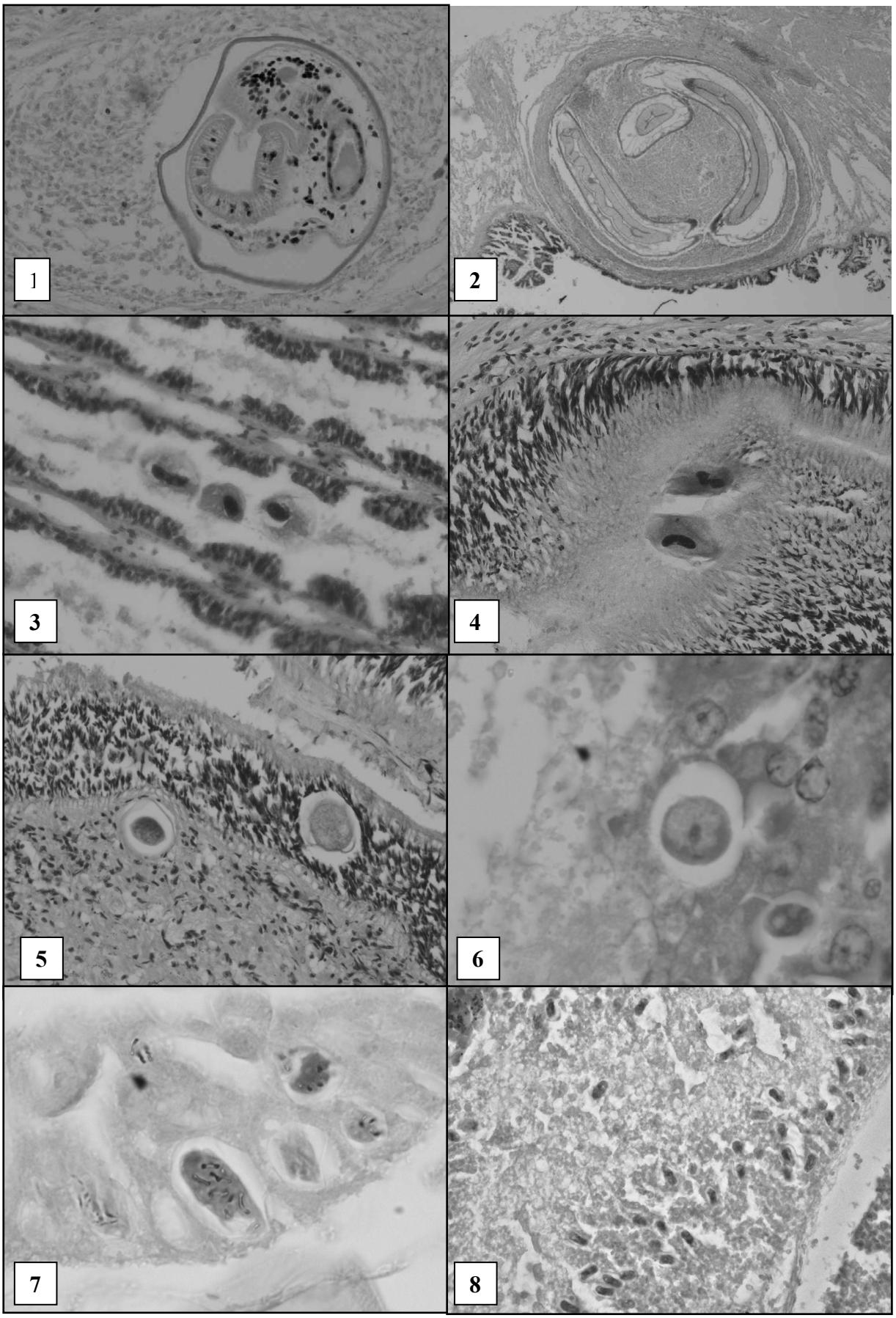
Fig 5.4.10. Stippled basophilic round body (*Rickettsia*-like) in digestive gland.

Fig 5.4.11. Eosinophilic haemolymph in gill filaments.

Fig 5.4.12. Melano-haemacytic nodule in kidney.

Fig 5.4.13. Concretions (intravascular?) within foot.

Fig 5.4.14. Oedema of foot.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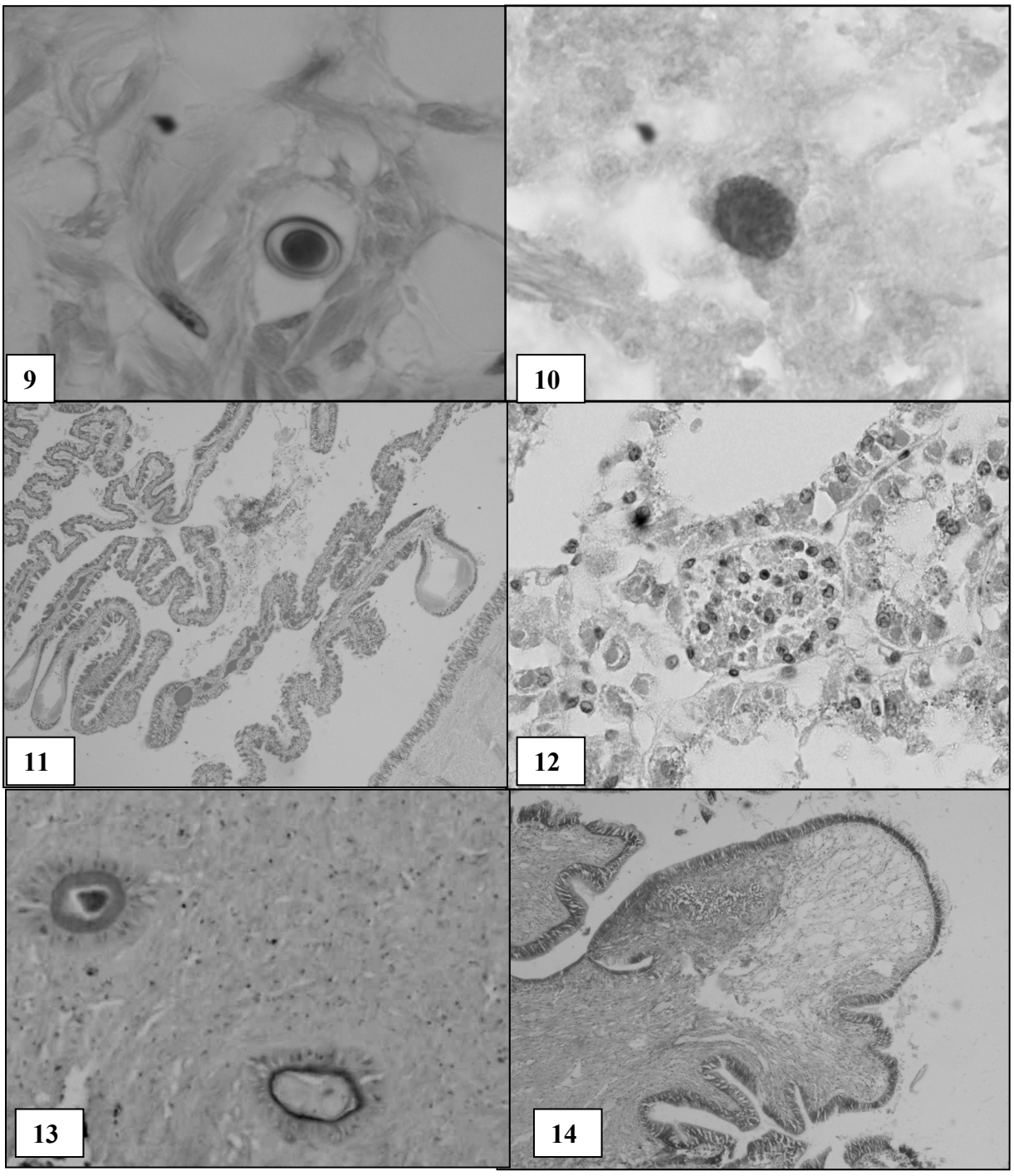
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ABALONE AQUACULTURE SUBPROGRAM: A NATIONAL SURVEY OF DISEASES OF COMMERCIALLY EXPLOITED ABALONE SPECIES TO SUPPORT TRADE AND TRANSLOCATION ISSUES AND THE DEVELOPMENT OF HEALTH SURVEILLANCE PROGRAMS.

New South Wales Report

Richard Callinan and Matthew Landos

NSW Department of Primary Industries

Final Report May 2006

In FRDC Project No. 2002/201

Australian Government

Fisheries Research and Development Corporation

ABALONE AQUACULTURE SUBPROGRAM

NSW DEPARTMENT OF **PRIMARY INDUSTRIES**

5.4 A SURVEY OF DISEASES OF ABALONE IN NEW SOUTH WALES

5.4.1 Introduction: the New South Wales Industry

NSW has a semi-continuous (habitat limited) population of blacklip abalone *(Haliotis rubra*) from Yamba in the north to the southern border. This species forms the basis of the commercial harvest industry in NSW that is valued at around $8 million a year. *H. coccoradiata* is a smaller abalone that is found from Coffs Harbour in the north to the southern border. It is not commercially harvested.

The zone between Port Stephens and Jervis Bay is currently part of a fishery closure. *Perkinsus* infection is under investigation in this area, and was a major focus of this study. The remainder of the coast is open for commercial fishing managed by NSW Fisheries with Total Allowable Catch (TAC) limits in place.

5.4.2 Materials and Methods

A total of 407 wild *H. rubra* were collected from 15 sites (20-30 animals per site) listed in Table 5.5.1 below in north to south order. NSW Fisheries dive teams undertook collections from Jervis Bay north in April-May 2002. Locations south of Jervis Bay to Disaster Bay were collected in April / May 2003. All sampling took place in conjunction with the NSW Fisheries Stock Assessment Program / Perkinsus Survey project.

Each site typically comprised about 100m of reef and in some cases there were two sites in a particular locality (such as 'Batemans Bay-Rich' and 'Batemans Bay-Mossy Point'); in these cases the sites were from 500m to several km apart. In general, however, distances between sites ranged from approx 10 km apart in the south (where abalone more concentrated) to 100 km apart in north (eg Crowdy Head to Seal Rocks/Foster). Site locations are identified in Figure 5.5.1.

Abalone were maintained live on board dive boats and preserved in 10% formalin fixative upon return to land before transportation to the laboratory.

Additionally hatchery reared *H. rubra* (n=99) and *H. coccoradiata* (n=98) were sampled from clinically normal stock at the NSW Fisheries Port Stephens, Tomaree Abalone Experimental Hatchery, fixed in 10% formalin seawater on site, and shipped to the laboratory. This hatchery was involved with experimental work on intensive breeding and reseeding research. Unfortunately, moribund animals were unavailable for sampling.

No farm samples were included as presently (May 2005), there are no active commercial abalone aquaculture ventures in NSW. One grow out facility is currently moving through the approval process.

All animals were processed and examined by histological examination of H & E sections of all abalone tissues.

Shells were examined for the presence and prevalence and of a range of changes and organisms including: length, weight (total animal), mudworms (brown shell blisters dissected and any worms found identified); sponges (% covering shell soft and hard combined); cunjevoi, barnacles, oysters, spirobids, F. Serpulidae (*Pomatoceros* sp.), F. Sabellaridae, growth checks, weed % cover. Each shell was photographed and linked to the spreadsheet

entry. Assistance in identifying shell organisms was provided by Lexi Walker (Southern Cross University) Biology Department and Ben Walsh (SCU student).

Selected animals north of Jervis Bay had gill biopsies individually cultured in Ray's thioglycollate media and iodine stained for presence of Perkinsus hypnospores.

Table 5.5.1: Abalone examined from NSW

Sample ID	**Farmed or wild**	**Species**	**No abalone examined (HP)**	**RFTM test results**	**Perkinsus detection in histo**	Other tests
Crowdy Head North and South	Wild	*H rubra*	30	0/30	0/30	none
Fishermans Bay Port Stephens	Wild	*H. rubra*	9	1/9	2/9 (22%)	none
Fingal Island South	Wild	*H. rubra*	21	5/21	4/21 (19 %)	none
Sydney	Wild	*H. rubra*	30	0/30	0/30	none
Kiama	Wild	*H. rubra*	28	Na	2/28 (7.1 %)	none
South Jervis Bay	Wild	*H. rubra*	27	6/27	8/27 (30 %)	none
Ulladulla South	Wild	*H. rubra*	31	Na	0/31	none
Batemans Bay-Rich	Wild	*H. rubra*	29	Na	0/29	none
Batemans Bay-Mossy Point	Wild	*H. rubra*	20	Na	0/20	none
Mystery Bay/ Bermagui	Wild	*H. rubra*	23	Na	0/23	none
Tathra	Wild	*H. rubra*	43	Na	0/43	none
Merimbula	Wild	*H. rubra*	32	Na	0/32	none
Eden	Wild	*H. rubra*	32	Na	0/32	none
Disaster Bay	Wild	*H. rubra*	25	Na	0/25	none
Port Stephens Fisheries Centre-Tomaree	Hatchery	*H. rubra*	99	Na	0/99	none
Port Stephens Fisheries Centre Tomaree	Hatchery	*H. coccoradiata*	98	Na	0/98	none

Figure 5.5.1 Sampling locations for NSW.

5.4.3 NSW results

5.4.3.1 Thioglycollate culture for *Perkinsus* hypnospores.

Results are summarised in Table 5.5.2. All positives were recorded in the closed zone between Port Stephens and Jervis Bay. There were no gross *Perkinsus* abscesses.

5.4.3.2 Histopathology observations for Perkinsosis

Organisms consistent in size and morphological appearance with *Perkinsus olseni* were detected in 16 abalone from 5 of 15 sites with all positive sites falling between Port Stephens and Jervis Bay. Perkinsosis was not detected from outside this zone to the north or south. Various stages of perkinsus (merozoites of 2-4μ, meronts of 13-16μ) were identified in a range of tissues of affected abalone. In most cases the presence of perkinsus was associated with significant tissue necrosis and infiltrations of hemocytes (Figure 5.5.2). Organisms were identified in hemolymph vessels (Figure 5.5.3) throughout the abalone, in the gill (Figure 5.5.4), the sub-enteric hemocyte beds (Figure 5.5.5), right kidney (see Figure 5.5.3), gonad, hypobranchial gland, and within foot muscle (Figure 5.5.6) Most commonly affected area of the animal appeared to be the hemocyte beds beneath the mucosa of the gut. In some cases, infections at this site had led to disruption of the gut epithelium and sloughing of mucosa and

Perkinsus organisms into the lumen (see Figure 5.5.7 and 5.5.8). Similarly, proliferation of *Perkinsus* organisms within gill vessels sometimes resulted in vessel blockage (infarction) and gill necrosis (Figure 5.5.9).

Animals affected by perkinsosis often had significant intra-haemocytic pigmentation throughout tissues including, sub-epithelial intestinal hemocyte beds (Figure 5.5.10 and 5.5.11), right kidney, digestive gland and foot. Perkinsosis prevalence within sites ranged from 0-25%.

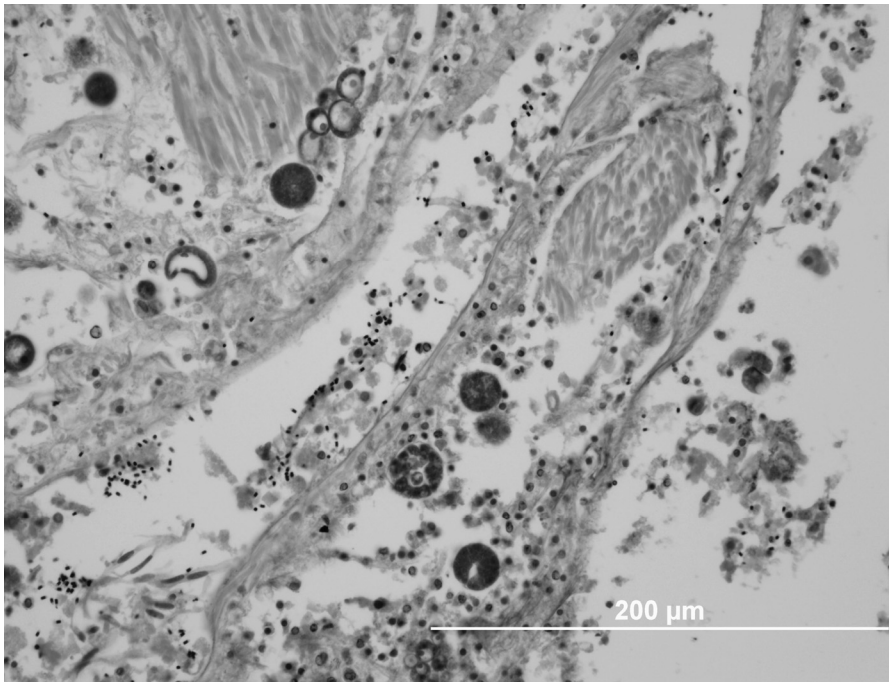


Figure 5.5.2. *Perkinsus* organisms surrounded by necrosis and tissue reaction.

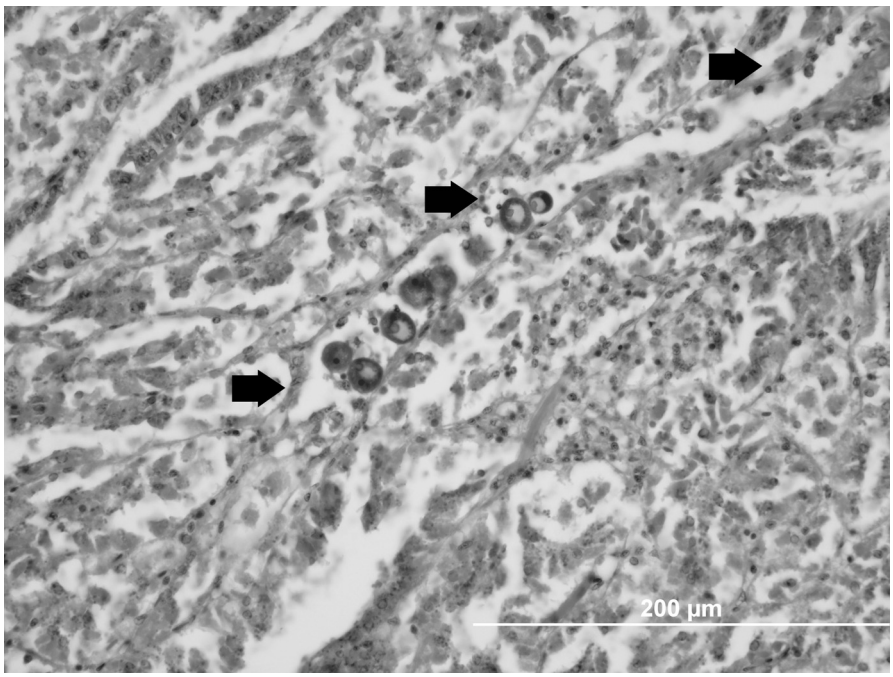


Figure 5.5.3. *Perkinsus* organisms in haemolymph vessel (arrows).

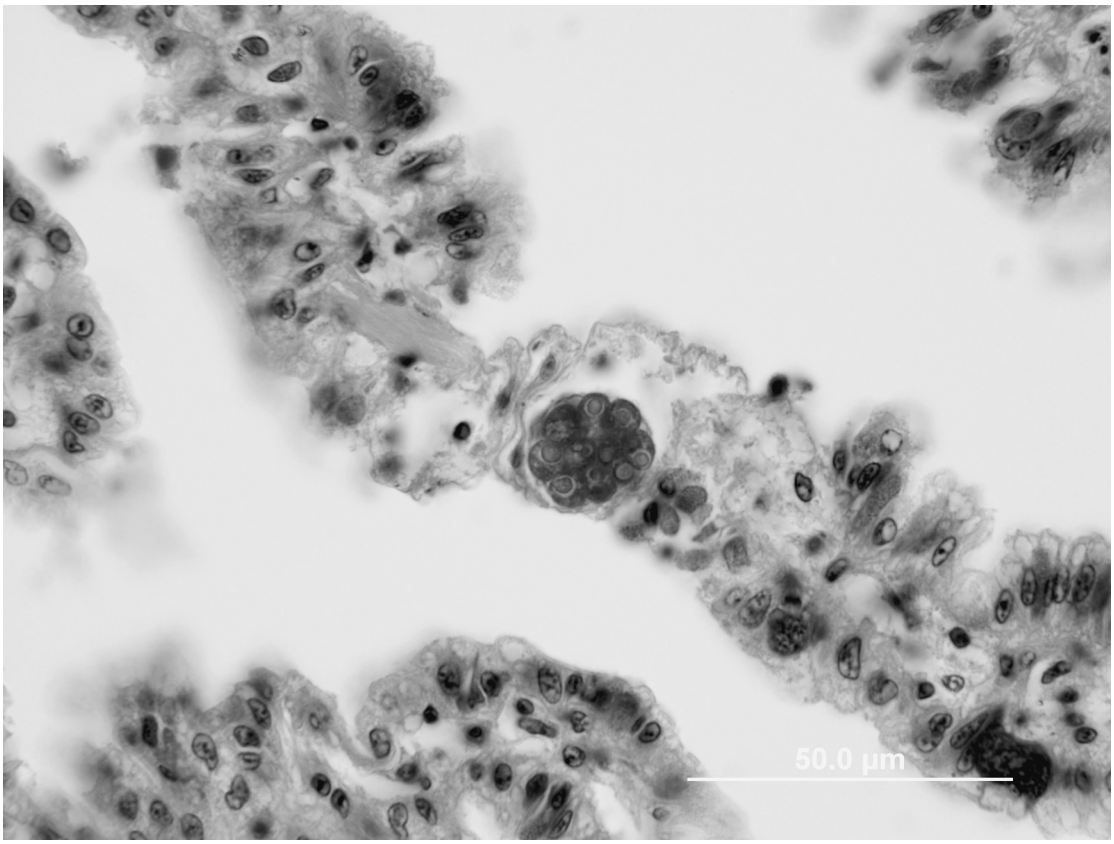


Figure 5.5.4. Dividing *Perkinsus* parasite in gill vessel.

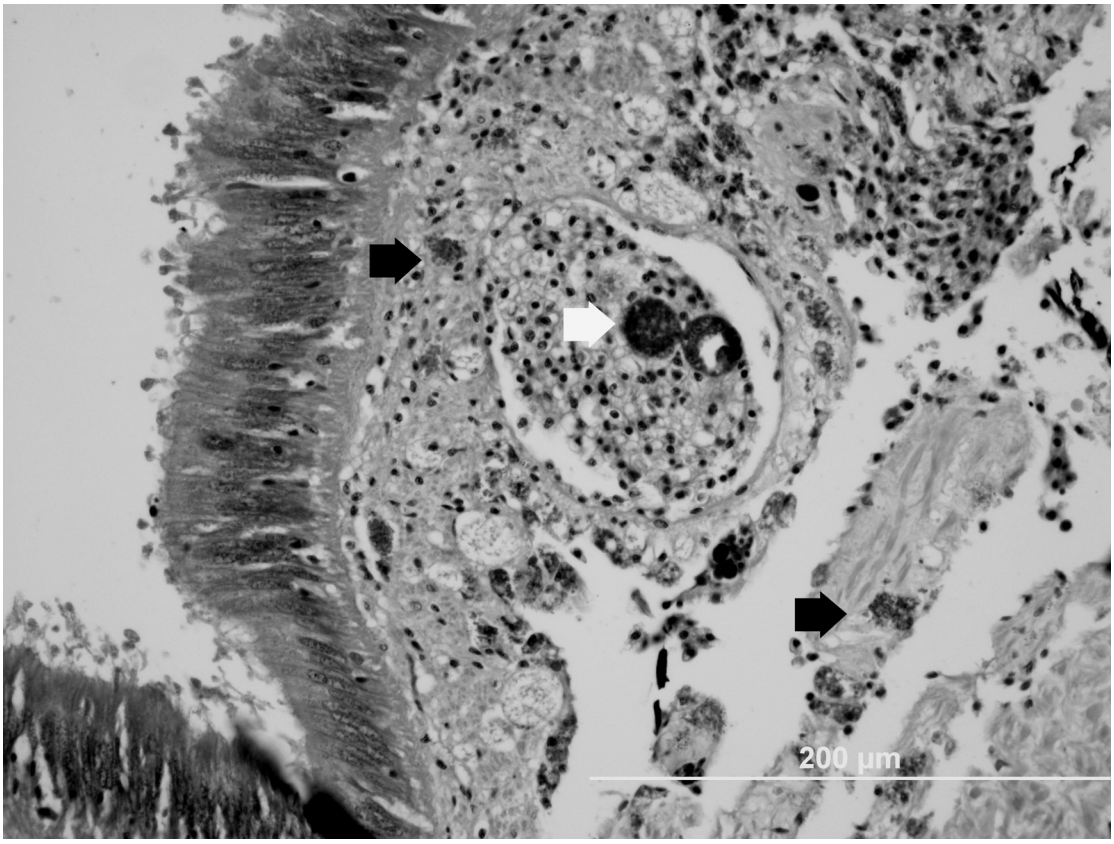


Figure 5.5.5. *Perkinsus* parasites (white arrow) and reaction within vessel beneath gut. Increased pigment in granulocytes (black arrows).

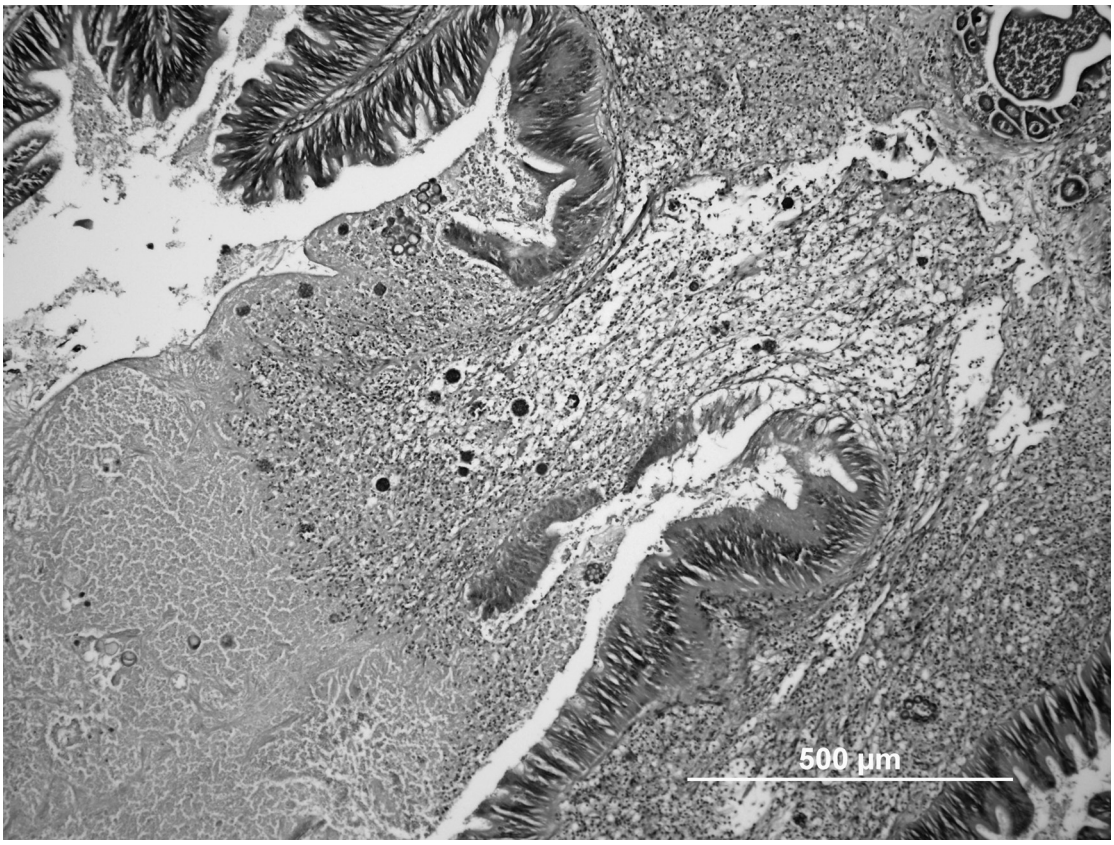


Figure 5.5.6. *Perkinsus* organisms and extensive inflammation under gut epithelium, leading to erosion of the epithelium and ulceration of the gut.

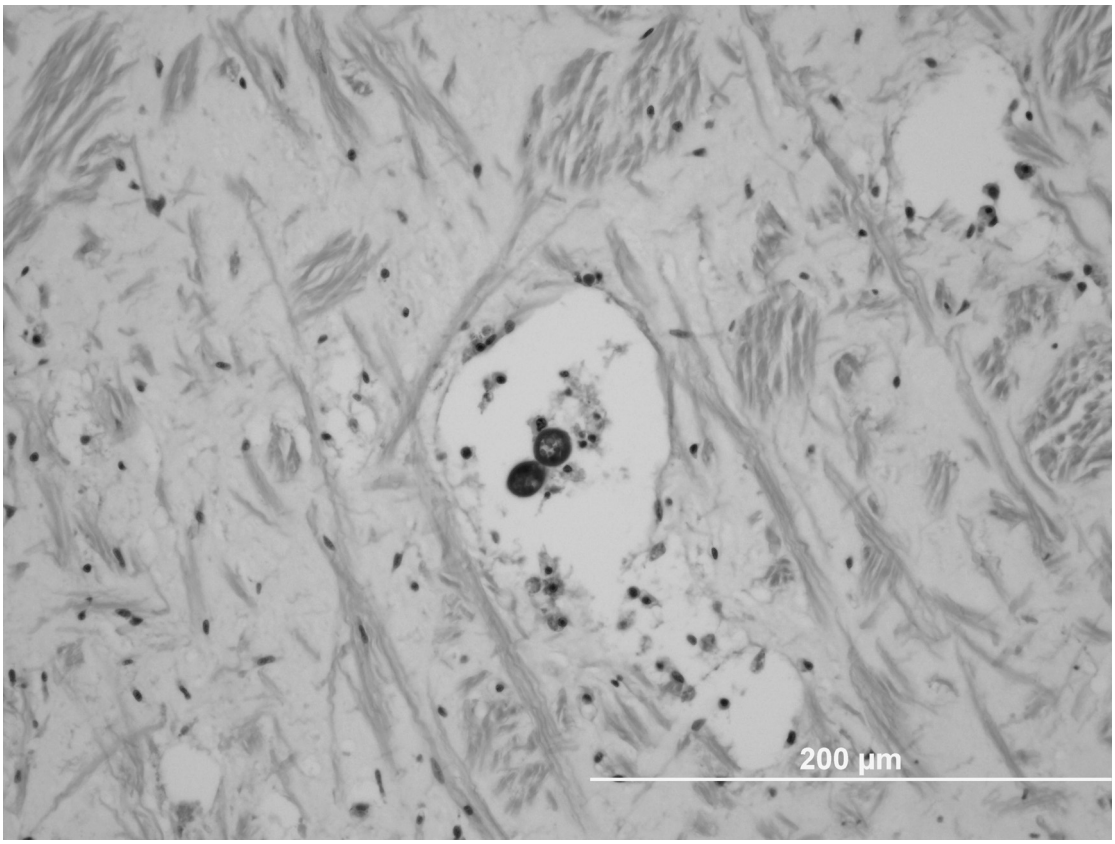


Figure 5.5.7. *Perkinsus* organisms within foot muscle vessel.

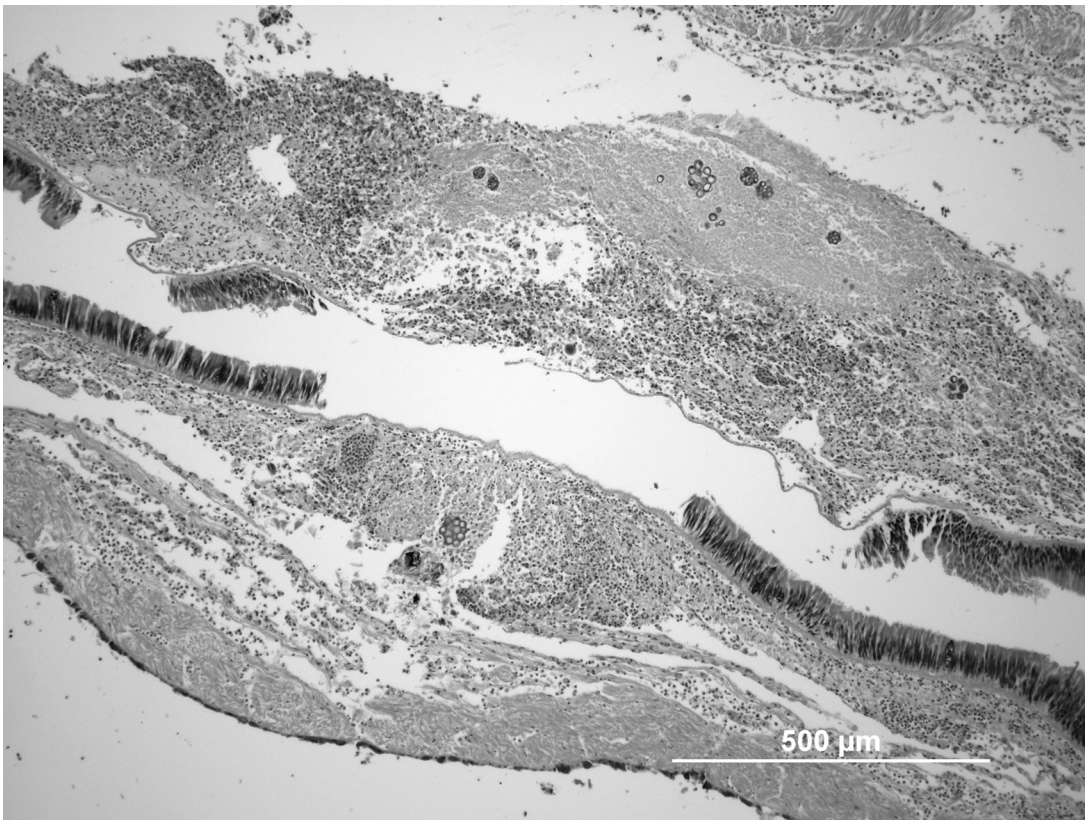


Figure 5.5.8. Similar gut pathology with erosion of both surfaces of the gut.

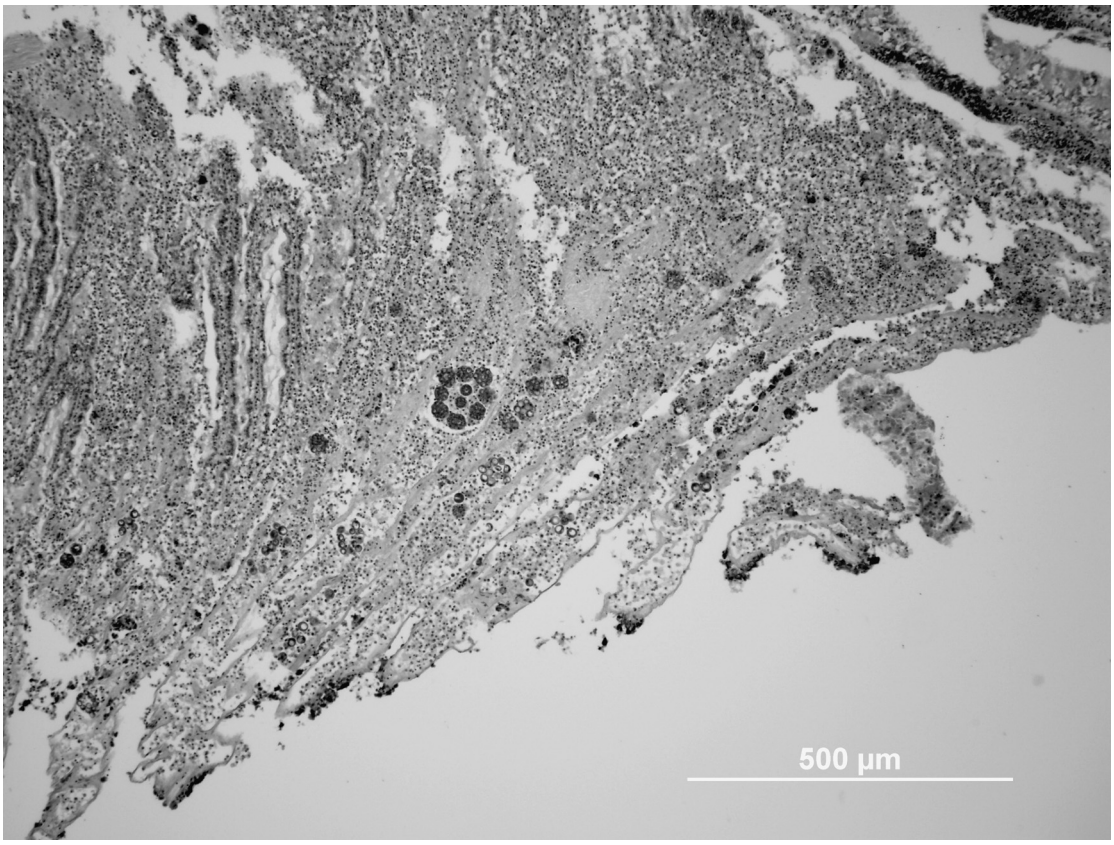


Figure 5.5.9. Multiple *Perkinsus* organisms blocking gill vessels, resulting in necrosis (paler areas with loss of detail).

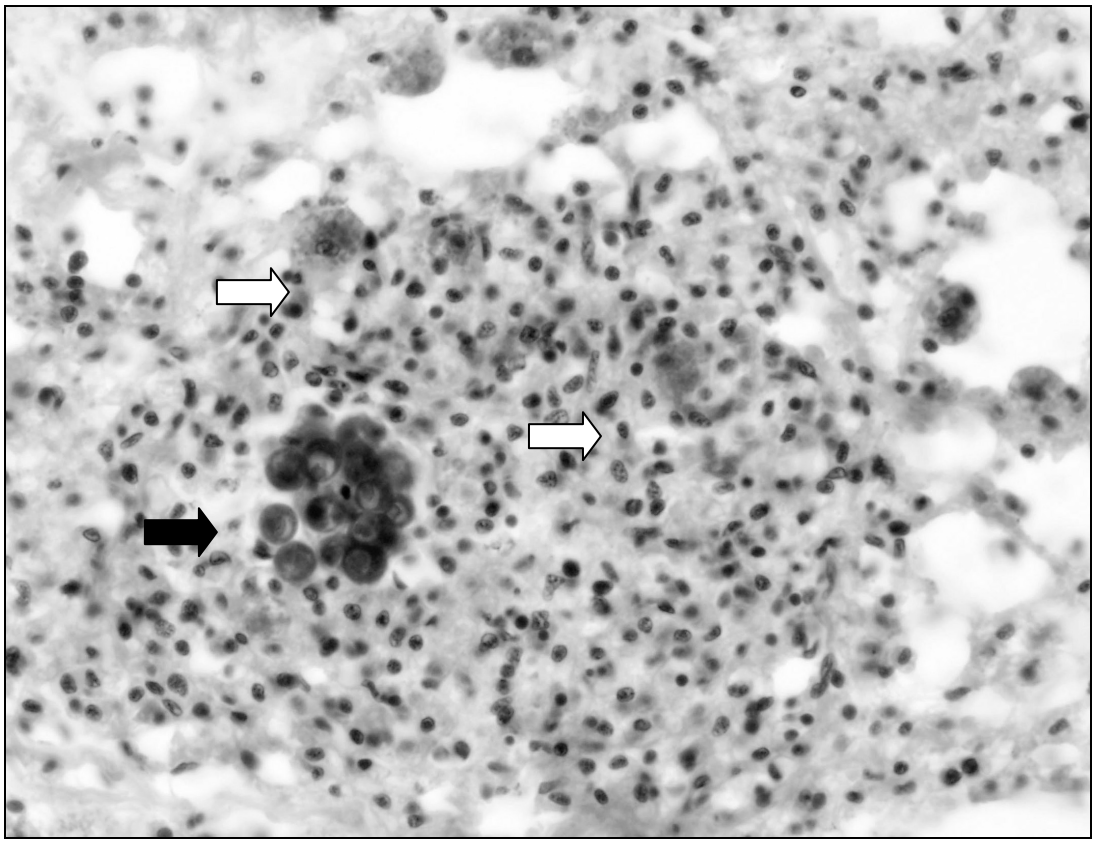


Figure 5.5.10. Brown cells (white arrows) in reaction surrounding *Perkinsus* organisms (black arrow).

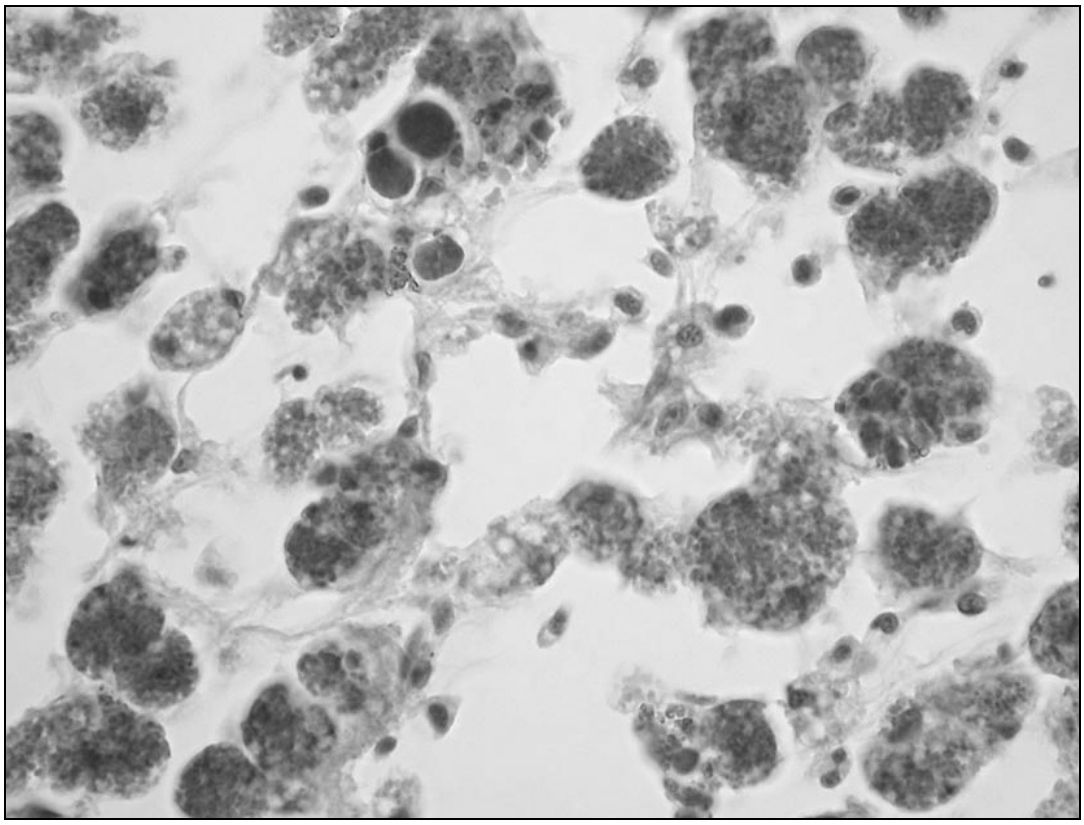


Figure 5.5.11. Very heavy generalized pigmentation of granulocytes in an abalone from a *Perkinsus* infected population.

5.4.3.3 Other Histopathology findings

Gill ciliates

Mild to moderate numbers of a 25-30µ ciliated protistan parasite were identified in association with gill tissue in a large proportion of wild and hatchery reared animals sampled (11 animals). The organism had a basophilic bean-shaped nucleus. There was little, if any host reaction to the presence of these ciliates. It is suggested that this may be a commensal organism.

Gregarines

Gregarines were observed in a range of tissues in small numbers (21 animals in total). There was typically no host reaction associated with their presence (Figure 5.5.12).

Metazoans

Encysted metazoa (trematodes) were seen in two abalone (Figure 5.5.13).

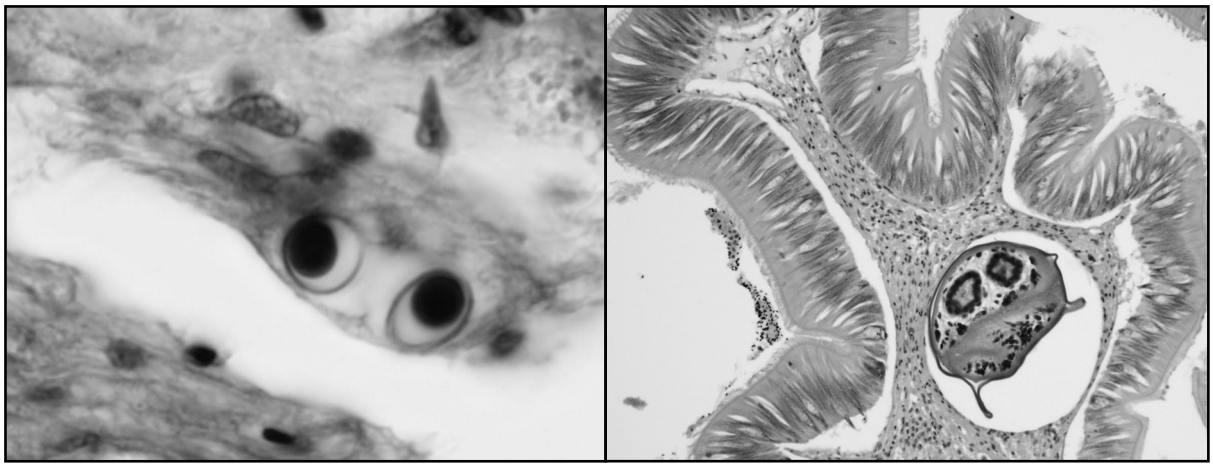


Figure 5.5.12. Two *Nematopsis* like gregarines in interstitial tissue.

Figures 5.5.13. Trematode in lamina propria of the oesophagus.

5.4.3.4 Observations from shell examinations

Shell boring polychaetes

Approximately 30% of wild abalone had brown shell blisters on the internal surface of their shells. Prevalence of blisters from each site varied between 0% and 69%. Infrequently a worm was identified during dissection of the tunnels that allowed definitive identification of the causative polychaete. Species identified from the *Spionidae* included *Boccardia knoxi,* and *Polydora haswelli.*

Mudworms

Within each site of collection there was similarity in the types of organisms present on the shell of the abalone. Between sites the assemblage was variable and not correlated to geographic location.

Both *Boccardia knoxi* and *Polydora haswelli* were found in association with shell lesions, and tunnels through the abalone shell. Brown pigmented blisters on the inside of shells were identified in abalone across the range of collection. Blisters varied in size from 2mm, through to 40mm. The blisters were raised up to 5mm above the normal internal contour of the shell, and had a variable thickness. Upon dissection of blisters only a small minority contained the apparent inciting cause- mudworms. The causation of other blisters was not able to be definitively identified. The blisters represent a chronic host response to an irritant, and it is possible many of these blisters are slowly resolving lesions from attack by mudworms that may have been eliminated or unable to be excavated from their tunnels. Given the equal distribution of this type of lesion in animals across the range, it is unlikely that TBT has any role in blister formation. The shell blisters observed in NSW stocks were consistent in appearance with those in Tasmania and the FRDC mudworm publication, which have been definitively linked with the invasion of mudworms.

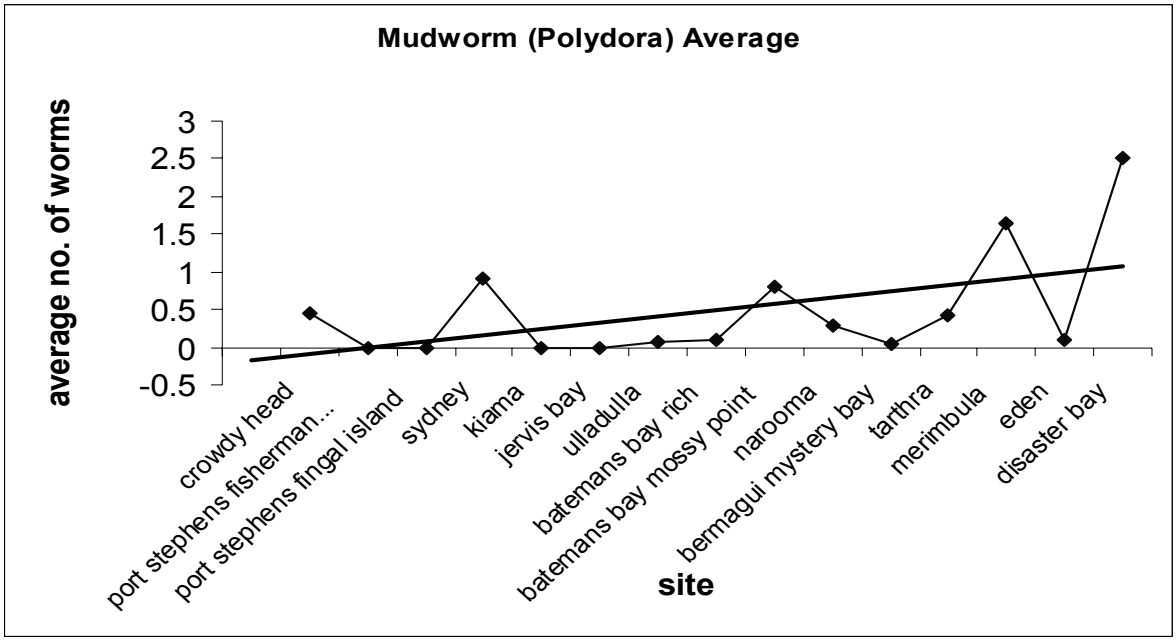


Figure 5.5.14. Mean mudworm levels over the region sampled.

Boring sponges

Hard and soft sponges (species not determined) causing pinpoint punctate shell lesions, in severe cases resulting in weakened shells.

Other commensal organisms

Fouling organisms not regarded as causing significant shell damage included the following:

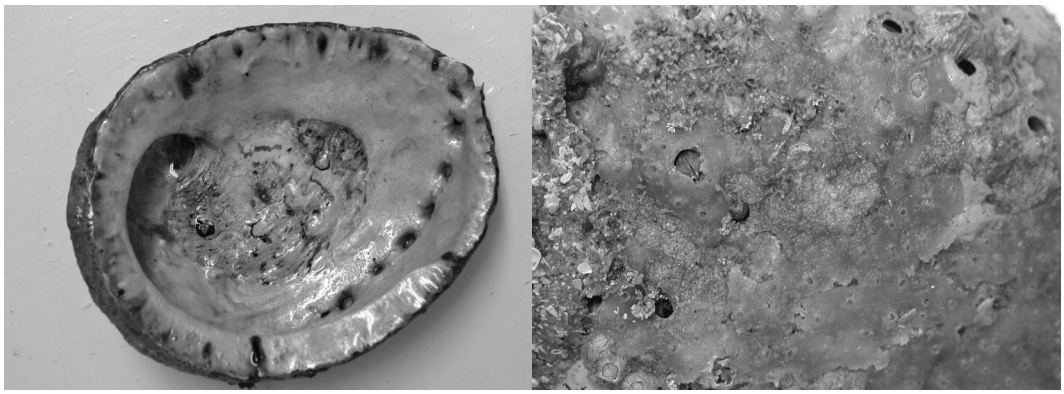
Spirorbids
F. Serpulidae: *Pomatoceros sp.*
F Sabellaridae
Oyster
Barnacle
Cunjevoi
Unidentified mollusc

5.4.3.5 Shell growth checks

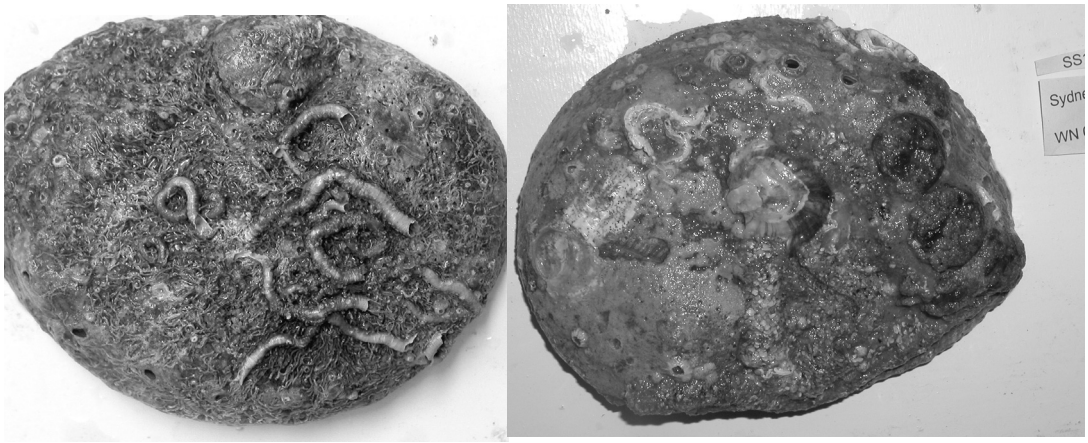
Significant checks in growth were recorded based on the appearance of radial notches in the shell (see Figure 5.5.15, lower right), and mean growth checks per sampling region in Figure 5.5.16. The worst affected groups of abalone (both increased severity of infection and increased prevalence of infection) were in the northern end of NSW. Some of the growth checks do approximately correlate with the timing of the first reported perkinsosis outbreaks at Port Stephens, using conservative estimates of shell deposition over time. Abundance of abalone in the Port Stephens area was documented to have commenced declining around 1997-1998 through the NSW Fisheries Abalone Stock Assessment program. By 2001 the local stock was in a massive decline, as measured by the abundance of abalone on key stock assessment reefs. It was not until around this time, that investigations led to identification of parasites consistent with *Perkinsus olseni,* in association with moderate to severe tissue damage in infected animals. The 2002 survey collections detected through RFTM and histopathology an elevated prevalence of perkinsosis in the Port Stephens stocks compared to other areas of NSW affected by Perkinsus, It is speculated that this may represent a more recent infection in this population compared to areas around the Central Coast where the organism was linked to die backs of abalone as early as 1992. However there are numerous other explanations that cannot be confirmed due to an absence of data. Across the local Port Stephens population, the higher proportion of growth checks suggest there had been some impediment to growth, which may have been environmental, nutritional or disease related. There is insufficient data to speculate whether the inciting cause of the growth checks came prior to the perkinosis outbreak or was manifest in animals that have recovered from the infection.

Growth checks in some Tasmanian stocks were linked to the presence of a haemocytic parasite. NSW stocks were assessed to be free of the haemocytic parasite, first identified in the Tasmanian Kent Group samples, after thorough examination. The Veterinary Pathologists undertaking examinations of NSW samples were trained in the identification of the parasite, through photographic material generated during the project by the Principal Investigator.

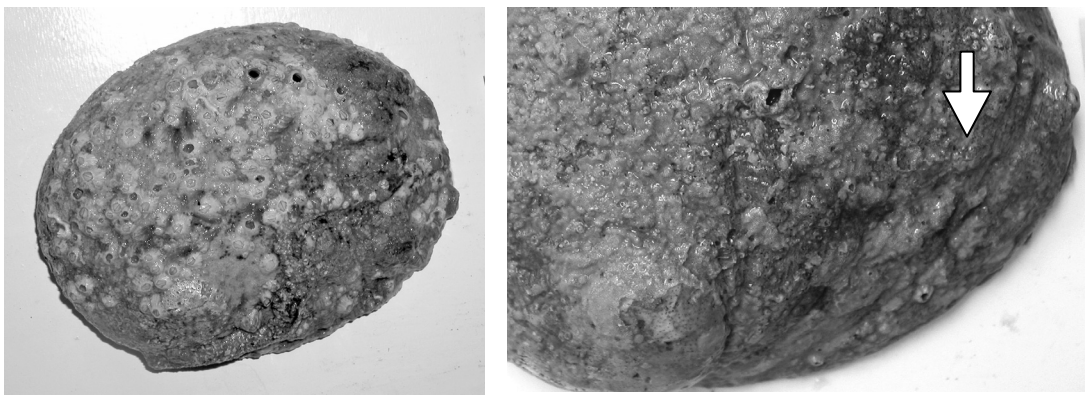
Boccardia knoxi



Polydora haswelli Sponges and barnacles & F Sabellaridae



F. Serpulidae: Pomatoceros *sp* Cunjevoi



Barnacles Growth check (arrow)

Figure 5.5.15. Shell changes.

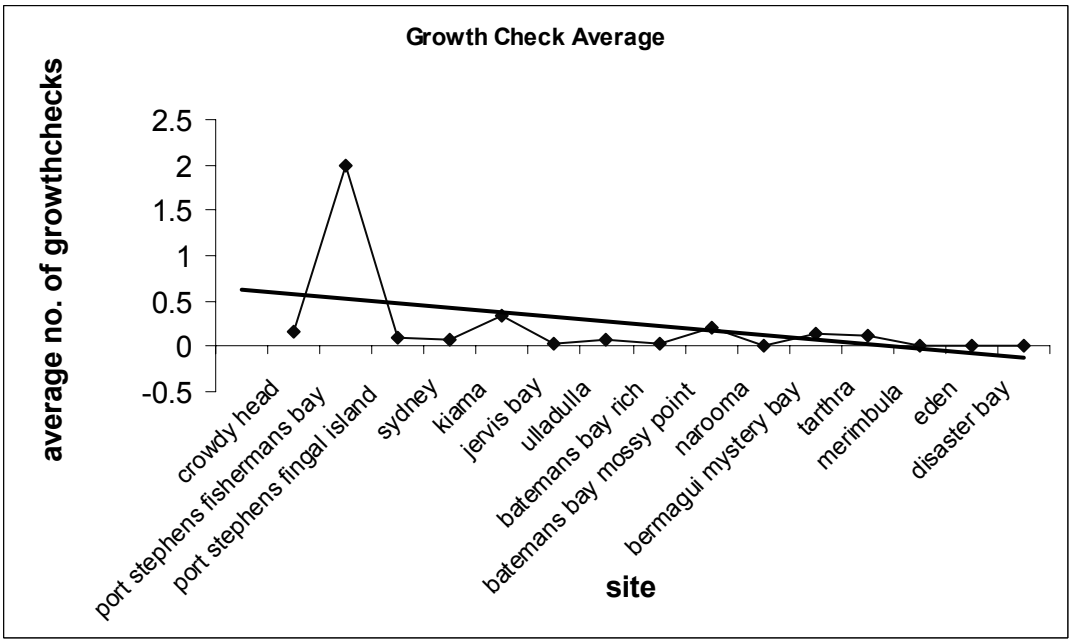


Figure 5.5.16. Growth checks in NSW abalone over the north to south range.

5.4.3.6 Weight to length ratio

Fixed animals were weighed inclusive of shell weight, and measured along their longest axis. Over the range from north to south a reduction in weight to length was observed, as shown in Figure 5.5.17.

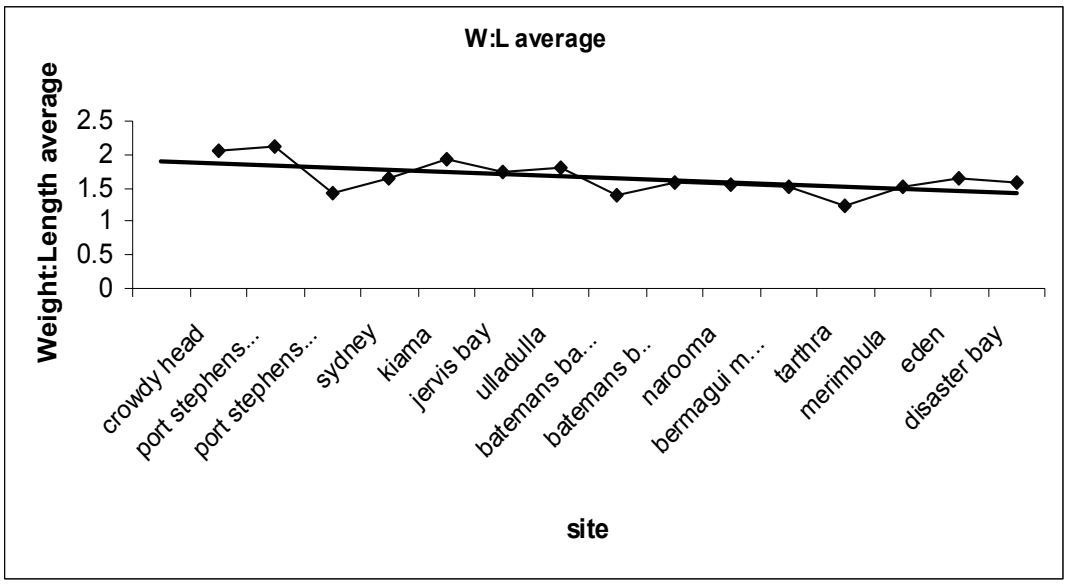


Figure 5.5.17. Weight to length ratio over the north-south range.

5.4.4 Discussion

Perkinsosis was a major focus for the NSW survey, and only pathogen identified that appeared to be responsible for significant damage to the host. The first outbreak of Perkinsosis in NSW was reported in 1992 at Terrigal, just north of Sydney. Data from Abalone Stock Assessment surveys of NSW has recorded a massive decline, up to 95%, in abalone populations between Port Stephens and Jervis Bay from this time until the present. This decline can be tightly linked to outbreaks of perkinsosis in some sites (Port Stephens, Jervis Bay). There is insufficient data to provide such a link at other sites. No other significant infectious or non-infectious diseases were identified to explain the stock declines.

The triggers for the outbreaks of *Perkinsus* related mortality are not known. It appears likely that *Perkinsus* infection has been a major factor in the decline of abalone stocks over the infected range. The NSW Fisheries Abalone Stock Assessment program identified a tight correlation between the presence of Perkinsus, assessed by positive RFTM tests, and areas of stock decline. Areas south of Jervis Bay have been resurveyed in 2004-5 and at this time no histological evidence of *Perkinsus* infection has been recorded. The 2002/3 survey suggested the pathogen may be confined to this area of depletion and that the progressive depletion (reported anecdotally by divers, over recent years) may have been due to expansion of the range of the pathogen during times of favourable conditions for disease epizootics.

The pattern of mortality is not one that has been matched in other abalone populations, where *Perkinsus* is known to be present, either in the extent of the stock decline or the nature of the lesions. This suggests that either the NSW abalone are more susceptible (perhaps a more stressed population), or the pathogen is a more virulent strain, or that other factors have increased the natural susceptibility of NSW abalone to this diseases. This survey found no evidence of increased susceptibility due to intercurrent disease. A more widely held hypothesis is that environmental conditions prevailing in recent years are more conducive to the propagation of a perkinsosis epizootic event, with environmental temperature the most commonly suggested environmental factor involved. It was outside the scope of this project to investigate the potential links between seasonal fluctuations in water temperature and *Perkinsus* outbreaks. However, an overview of recent sea surface temperature profiles does not indicate a marked difference in the absolute temperature or the duration of elevated temperature as this has not increased over the last 10 years. It is difficult to explain the apparent progression of the disease both north and south, with only water temperature as the primary risk factor.

With regard to variations in pathogen virulence, it should be noted that DNA sequencing of the *Perkinsus* strains has not been performed to check whether the WA, Qld, SA and NSW strains are identical. Hence a strain difference cannot currently be excluded as an explanation for the NSW mortalities. An important area of work is to determine the similarity of NSW strains of *Perkinsus* from epizootic areas with *Perkinsus* strains from SA and SE Queensland where propagating epizootics have not been reported.

The prevalence of perkinsosis across the NSW affected range provided a low-grade correlation that the highest infection rates were present at the margins of the infected zone. This would be epidemiologically consistent with a propagating epizootic, entering a naïve population of stock. There is some data from the more recent NSW *Perkinsus* survey (2004-5) where increased sampling at the northern margin of the infected zone, Port Stephens, detected a change in prevalences at the five sites, previously sampled in the 2002 survey. In 2002 the northern most reef sampled was free of *Perkinsus*, with the southern four reefs having some of the highest prevalences of all NSW abalone stocks. In the 2004-5 survey, the prevalence of perkinsosis on the four southern reefs had markedly declined, however the northern most reef,

had one of the highest disease prevalences. Although the strength of the sample numbers is low with n=20 on each site, the pattern of change is worthy of note.

An alternative hypothesis is that the pathogen has been present along the entire NSW coast in abalone, or other mollusc populations for a long time, and that since 1992, conditions have favoured disease outbreaks. Given the presence of the pathogen in Victoria, Western Australia, South Australia and Southern Queensland a broad distribution of *Perkinsus olseni* would seem likely. It is also likely that where disease prevalence was low (eg less than 10%), the disease was unlikely to be reliably detected in the 2002/3 work due to the relatively low level of sampling (~20-30 animals per site). Further survey work may therefore be necessary to clarify the true distribution of the pathogen.

Further survey work may also provide information on environmental factors likely to explain a progressing margin of apparent spread in both a northerly and southern direction. The recent experimental results of work by Haywood and Lester, 2005 would support the view that water temperature is not a primary risk factor affecting susceptibility to perkinsosis, although fluctuations in temperature may be a relevant stress. They were unable to establish *Perkinus olseni* infection with prolonged temperature stress, but were able to do so more reliably with fluctuating stress levels. It is postulated that other factors may be more significant in disease progression and expression in NSW than water temperature alone.

Further work on the epidemiology and pathogenesis of perkinsosis in blacklip abalone is warranted to adequately explain the losses in wild NSW blacklip abalone fisheries. Confirmation of an epidemiological pattern typical of a propagating epizootic entering a naïve population would strongly suggest that either assumptions of the widespread background distribution of *Perkinsus olseni* are incorrect, and that this has a discontinuous distribution, or that strains widespread and endemic to this area in previous decades were not pathogenic to abalone. The implications of this (that either the pathogenic strain was translocated to this area, possible from SA, or that a new pathogenic strain has arisen *de novo*), both suggest that the risk of translocation of molluscs from areas of abalone *Perkinsus* infection are higher than might be anticipated from the apparent widespread distribution of the parasite. Some work to further define the epidemiological pattern is currently underway, repeating and enhancing the 2002/3 survey.

With regard to general pathology indicators, it was noted that a significant proportion of NSW abalone across the range had brown pigmentation throughout the tissues of the animal, although most notably in the haemocyte bed beneath the gut epithelium and in the digestive gland parenchyma. The pigment was both intracellular and extracellular and was sometimes dark brown in colour. These cells sometimes contained unusually large numbers of granules (as seen in Figure 5.5.11). Unfortunately there was not scope with the time frames of this project, within the other commitments of NSW DPI, to further investigate these findings in relation to factors such as geographic locality, animal size/age, shell pathogens or *Perkinsus* infection. Some of this data has been recorded in the NSW data-base (submitted as part of Appendix V) but has not been analysed. However, there was no clear correlation between the level of pigmentation and general body condition. The complete glass slide, shell photo and histological findings data remain as a resource, should funds become available to undertake further investigations on the NSW material.

The usefulness of the weight to length ratios data is reduced as the animals were weighed including their shells, including all the shell fouling organisms growing on them. It is recognised now that it would have been more appropriate to measure a meat weight to shell length ratio. Despite this, there appeared to be a weak correlation between the latitude of collection and the ratio. The further south in the range animals were collected the lower the weight to length ratio became. The cause of this trend is speculative and may be related to water temperature influencing feeding behaviour and hence shell growth. It has been observed

in farmed abalone that shell shape is influenced by growth rate. Shells tend to be rounder (hence reduced shell length) in slower growing animals. Speculatively, the ratio may suggest slower growth in the northern latitudes of NSW if this same rounded shell effect occurs in wild stocks also. The shape of shells was not recorded for NSW abalone, although all shells were photographed uniformly, so this work could be done from recorded materials.

The other organisms identified on the exterior of abalone shells were not thoroughly speciated due to the difficulty of classifying sponges and other marine fouling organisms. An empirical assessment of damage was undertaken through visual appraisal of the distortion of the normal shell profile that was associated with the fouling organism. Only the soft and hard sponges appeared to cause significant shell damage. However, there was not a severe impact on the abalone in most cases, with most animals having a similar weight to length ratio, and no gross atrophy of the foot musculature. It is speculated that the major effect may be a slowing of growth, through increased metabolic demand on the host to repair the punctuate shell damage caused by the invasive sponges. It was noted that the prevalence and severity of sponge damage on shells was not correlated to the presence or absence of perkinsosis. The severity of sponge damage varied locally between reefs, but was similar within an individual reef population of stock. A similar pattern of variation was observed with spionid infestations, between stocks of abalone. Hence it would seem that stresses associated with shell lesions are not sufficient necessary cause alone to precipitate a *Perkinsus* outbreak.

The observation of growth checks appeared to be widely distributed in NSW stocks, with some evidence for a local reef effect, rather than necessarily a regional or latitude effect, with the exception of the Port Stephens stocks. The causation of a shell growth check in wild abalone is not well understood. Some potential causes postulated include: sub-optimal seasonal nutrient deficiency manifest through either a shortage of appropriate kelp availability, or a decline in the quality of feed available; increased challenge from disease/parasitic agents (eg *Perkinsus*, boring sponges), and inappropriate thermal conditions for optimal growth. The elevated prevalences on some localised reefs suggest that it is more likely to be a micro-environmental factor (eg nutrition or disease) than a macro factor, such as the water temperature.

5.4.5 Contributors

Dr Richard Callinan (histopathology and photographs)
Matt Landos (histopathology, report and photos)
Steve Pepper (tissue processing technician)
Abalone Stock Assessment team, NSW DPI (sample collection and RFTM tests)
Ben Walsh (shell survey)
Lexi Walker (shell survey)

ABALONE AQUACULTURE SUBPROGRAM: A NATIONAL SURVEY OF DISEASES OF COMMERCIALLY EXPLOITED ABALONE SPECIES TO SUPPORT TRADE AND TRANSLOCATION ISSUES AND THE DEVELOPMENT OF HEALTH SURVEILLANCE PROGRAMS.

Tasmanian Report

Judith Handlinger, Jeremy Carson, Richmond Loh, Stephen Pyecroft,

Department of Primary Industries, Water & Environment

Final Report May 2006

In FRDC Project No. 2002/201

Australian Government

Fisheries Research and Development Corporation

ABALONE
AQUACULTURE
SUBPROGRAM

Tasmania

DEPARTMENT of PRIMARY INDUSTRIES, WATER and ENVIRONMENT

Tasmanian Aquaculture & Fisheries Institute

University of Tasmania

5.5 SURVEY OF DISEASES OF ABALONE IN TASMANIA

5.5.1 Abalone distribution and the Tasmanian abalone industry

The Tasmanian abalone fishery is the largest wild abalone fishery in the world, providing approximately 25% of the annual world harvest (Anon, 2005). This fishery is a major contributor to the Tasmanian economy, with 450 people having direct interests in abalone quota, and more than 3,000 Tasmanians, directly or indirectly, benefit financially from the industry. During the last few years, the market for live abalone has expanded rapidly. A significant portion of the catch (estimated as approximately half of the fishery) is now exported live to interstate and Asian markets. Processors are located throughout the State, and most send trucks to pick up fish from a range of locations to freight the catch to their processing premises. Tasmania also possesses a small but growing aquaculture sector, located in the southern, eastern, northern and Bass Straight areas. The following information is taken from the DPIWE web site (Anon, 2005).

Two species of abalone are harvested in Tasmania: *Haliotis rubra* (blacklip abalone) and *Haliotis laevigata* (greenlip abalone). Both species are mobile bottom dwellers that graze on drift seaweeds and algae on rock surfaces. They occur on rocky bottoms, mainly within the littoral zone in depths of 5–30 meters, although they are found from the shallows down to 40 meters.

Blacklip abalone are distributed around Tasmania and across southern Australia on suitable rocky bottoms. They occur on rocky reefs in both exposed and sheltered waters. Their distribution is variable but less patchy than greenlip abalone.

Greenlip abalone are very patchy in their distribution and occur across southern Australia. Greenlip abalone in Tasmania tend to frequent less complex and patchy reefs and are most abundant along the north coast and around the Bass Strait islands.

Tasmanian abalone stocks were not significantly fished before 1963, when modern diving equipment and the identification of Asian markets provided the basis of the current industry. The industry is recognised as being managed in a sustainable manner, with harvest levels controlled through quota management and size limits. In 2000, following a further review of management, fishing zones were introduced into the fishery. 'Zoning' was introduced to counteract a continuing trend of catches becoming more concentrated towards the (more assessable) east coast, with catches decreasing in other areas. The zones introduced in 2000 were a 'eastern zone' and a 'western zone' for blacklip abalone, and a separate total allowable catch for greenlip abalone which could be taken anywhere around the State (in practice in the north of the State). In 2001 a further blacklip zone in the north of the State was also introduced. For the 2003 season a new blacklip zone encompassing the small Bass Strait islands and the central north coast was introduced. A reduced size limit of 114mm was set for the Bass Straight zone, in recognition of the smaller size achieved at maturity in this location. The current zones and size limits are shown in Figure 5.6.1. and 5.6.2.

5.5.2 Methods for the Tasmanian survey

5.5.2.1 Sample collection

As far as possible wild samples were collected in conjunction with abalone stock assessment surveys, though a dedicated sampling was required for samples from the Bass Straight blacklip zone. Wild sampling sites are shown in Figure 5.2.3.

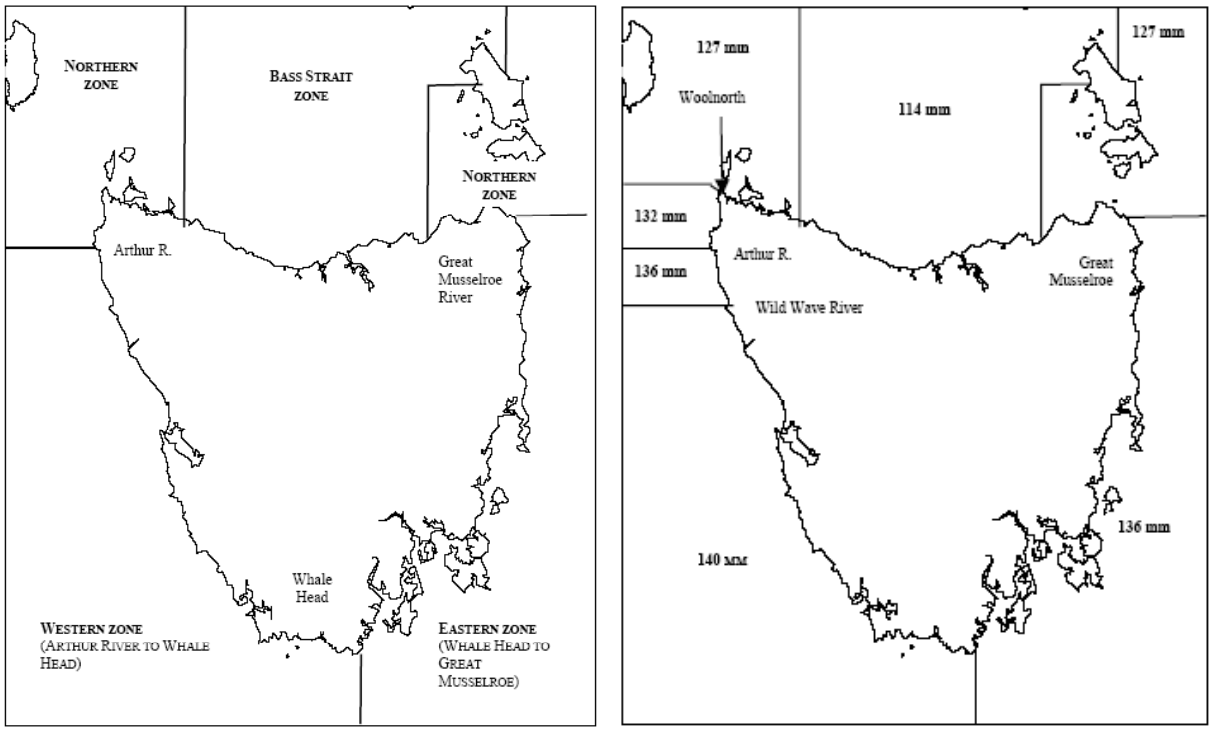


Figure 5.6.1 and 5.6.2: Fishing zones and size limits for the Tasmanian commercial blacklip abalone fishery.

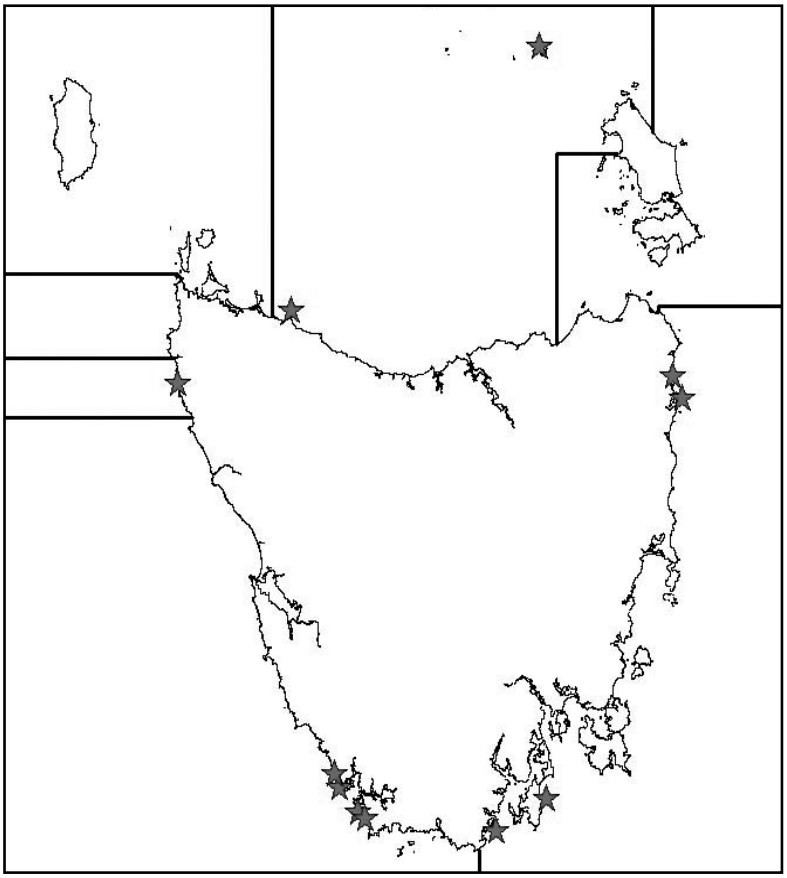


Figure 5.2.3. Wild blacklip sampling sites * , relative to zone boundaries.

Wild samples were held live on board in a flow through system, shipped to the laboratory and examined as soon as practical after receipt, although when several batches were received together they were held in recirculation tanks overnight or occasionally longer, with stress and some deaths evident in the initial batch of multiple samples.

No systematic sampling of the less abundant wild greenlip abalone was undertaken, though one small sample of recently caught greenlip abalone undergoing mortality in a holding system was made available. Another population sample of greenlip abalone of wild origin held for some months in an experimental system was also sampled.

Samples were also collected from all active farms with commercial quantities of abalone (2 small licence holders with small experimental populations on marine shellfish leases were not included). There was a heavy bias towards sick fish, or groups with a history of losses. Because samples submitted for a primary diagnosis were included, farms samples sometimes comprised several sub-samples.

5.5.2.2 Examination procedure

After gross assessment for activity (for animals held submerged), active animals were sedated with AQUI-S anaesthesia (according to product instructions) measured, examined for the presence and location of any shell or foot lesions, including overall level of fouling, shell discolouration, spionid tunnels, foot erosions or blisters, and (where visible) the sex and maturity of the animal. A decision to conduct bacteriology and haemocyte counts was made primarily on the basis of clinical findings. In populations where such tests were not indicated by clinical examination, a minimum of 10% of animals were generally cultured routinely, to obtain background data on normal flora and bacterial carriage under stress conditions. From up to 10 % of the animals (including any considered in ill-health), approximately 1 ml of haemolymph was collected aseptically from the cephalic sinus (Jorgensen et al., 1984) for bacterial culture and for haematology. For the latter, an Improved Neubauer haemocytometer chamber immediately filled with undiluted haemolymph and haemocyte counts carried out immediately on five 1mm^2 squares. Any clotted samples were rejected. Where haemolymph abnormalities were detected or suspected, a haemolymph smear was prepared by placing a slide with one drop of haemolymph into a wet chamber for at least 10 minutes to allow haemocytes to adhere to the glass, then fixed in 10% formalin seawater or other fixative prior to staining. The remainder of the haemolymph sample was used for bacterial culture. At this stage appropriate additional samples were taken for bacteriological culture from significant gross external lesions.

Thioglycollate cultures for Ray's test was used to test for *Perkinsus* organisms for approximately 20% (or more) of wild caught animals, using pools of tissue from several animals. Screening for *Perkinsus olseni* was also undertaken during histological assessment.

Where high levels of spionid mudworm lesions were present, spionids were induced to leave the shells and identified according to the methods of Lleonart (2001).

Staining of haemocyte smears

Fixed slides with adhered haemocytes were transferred to 70% ethanol until stained with Haematoxylin and Eosin according the schedule given in Table 5.6.1 (as provided by C. Freedman, pers. com.).

Table 5.6.1. Staining schedule for haemocyte smears

Step	Contents	Time
1	95% ETOH	1 min
2	70% ETOH	1 min
3	50% ETOH	1 min
4	30% ETOH	1 min
5	Distilled Water	2 min
6	Haematoxylin	4 min Agitate
7	Distilled Water	4 min
8	Clarifier	1-3 min Agitate
9	Distilled Water	3 min
10	Bluing Reagent	5 min
11	Distilled Water	3 min
12	70% ETOH	2 min
13	95% ETOH	2 min
14	Eosin	30 sec Agitate
15	95% ETOH	5 secs
16	95% ETOH	5 secs
17	100% ETOH	10 sec Agitate
18	100% ETOH	10 sec
19	Hemo-De	2 mins
20	Hemo-De	2 mins
21	Hemo-De	2 mins
	Total time	37.3 Minutes

5.5.2.3 Bacteriological methods

Bacterial sampling was primarily conducted on detectable lesions and / or abalone showing ill-health. Although at a sub-sample of 10% of the abalone (generally) were sampled to provide background data on the level and nature of non-detectable bacterial carriage as well as bacterial assessment of grossly detectable lesions.

For culture of external lesions or blisters, swabs from the site were cultured on plates of selective Shieh's culture medium (CRC for Aquaculture proprietary formulation) for marine flavobacteria. Haemolymph or samples from internal lesions were cultured on blood agar with 2% NaCl and TCBS and Johnson's marine agar (JMA) for heterotrophic bacteria (Johnson, 1968) or ZoBell's marine agar 2216E (ZMA) (ZoBell, 1946) culture plates. Cultures were incubated at 25°C for 48 hours. Smears were prepared from all primary material cultured and Gram stained. Primary cultures were assessed on the basis of purity and abundance and predominant bacterial forms were subcultured and purified on JMA or ZMA. Nearly all isolated bacteria were determined to be Vibrionaceae. These were identified initially using MicroSys® V48 and latterly V36 identification system for *Vibrio* and related species (Dept. Primary Industries, Water & Environment, Launceston). MicroSys is a miniaturised identification system using biochemical tests. Identification was undertaken by matching the profile of an unknown against a database of reactions for known species. Matching is undertaken by probabilistic methods using the software package PIBWin (Bryant, 2004) and the VibEx6 database for MicroSys V36. An identification was accepted if the Willcox probability score ≥0.99.

5.5.2.4 Histological examination

In general, most abalone were fixed in 10% formalin in seawater as this had proved to be satisfactory and practical fixative for large volume fixation. Small animals were fixed whole. Larger animals were initially fixed whole, or as one large block with trimming of excess tissue round tissues to be sectioned, with further fixation (usually within the histology cassette) after trimming to section block size. After fixation, three standard sections were taken from most animals, although small animals were sectioned whole. The most anterior section included the oesophagus, mouth cartilages and foot area. Oesophageal parts were included rather the more interior salivary glands where possible, especially once a parasite of the oesophageal pouch was detected, although this had not initially been a target organ. The most distal section through the mantle cavity was slanted to include, if possible, the heart and left kidney, as well as gill and the digestive gland. A second foot section was also included.

Each tissue was examined and scored according to the following criteria.

The foot was scored for epithelial loss, sub-epithelial dilations, sub-epithelial cellular reactions, focal lesions such as abscesses, general condition including muscle density and vascular dilation and the presence of parasites.

Gills were examined for parasites, protein precipitation, dilation and congestion of haemolymph vessels. Protein precipitation and haemocytes pooling were also assessed in the heart and left kidney. The right kidney was scored for the level of pigmentation and dilation. The main gut, oesophagus and oesophageal pouch were assessed for the level of parasites, haemocyte infiltrates and changes to the lamina propria. In the lamina propria and other interstitial locations, granulocytes and the smaller circulating haemocytes were scored separately. The granulocytes were scored for abundance and granular staining. Haemocytes were scored for abundance, the presence of aggregations of adhered and clotted cells, the presence of parasites and the general distribution pattern. This tissue was also assessed for the level of tissue distension and protein pooling. The gut tubules (digestive gland) were scored for tubular morphology, level of pigment, the presence of granulomas or concretions, the overall level of granularity or vacuolation of tubule cells, the presence of parasites, and the degree of separation of tubules from each other by interstitial tissue representing a decreasing condition.

The gonads were assessed for maturity and nervous tissue, hyper-branchial gland and other tissues assessed for general pathology.

As both normal tissue variations and the pathology of abalone are poorly defined, tissue changes over and above the presence of pathogens and parasites were examined in detail and tabulated, using a scoring system of 0 to 3 (absent, minimal, moderate, severe or abundant). This provided a basis for assessment of the impact of the disease agents detected, and provides a background database for health assessments of abalone examined in the future.

5.5.2.5 Electron microscopy

Electron microscopy was carried out as required for clarification of the nature and identity of pathogens detected, on residual formalin fixed tissue from the animals in which these were detected or by re-fixation and re-embedding from the routine paraffin blocks, according to the following procedure:

The following steps for reprocessing from were performed on an Automatic Mixer.

1. Tissue for EM impregnated with wax is placed overnight into glass vial containing xylene. Xylene removed and replaced with fresh xylene (24 hours total).
2. Xylene removed and absolute ethanol added to vial overnight. Following morning, fresh absolute ethanol x2 changes (18 hours total).

3. Remove absolute ethanol. Rehydrate with ethanol to distilled water via 1 hour of each 95%, 70%, 50%, 30% distilled water. Finish with 1 hour of cacodylute buffer.
4. Dissect into 0.5mm^2 blocks.
5. Fix in 2.5% glutenaldehyde in 0.1M NaCaCO Buffer.
6. Rinse with buffer.
7. Post- fix and process as per routine electron microscopy samples as follows. Post fixation in 1% osmium tetroxide for 1 hour at room temperature, rinse with distilled water then add uranyl acetate for 30 minutes and rinse again. Dehydrate tissue to 100% ethanol, clear in 100% propylene oxide, infuse with 50:50 mix resin/propylene oxide on rotator for 2-3 hours, embed in resin. Thin sections were routinely stained and examine in Hitachi H300 electron microscope.

5.5.3 Results for the Tasmanian abalone survey

5.5.3.1 Samples collected

A total of 912 animals were examined, comprising 425 of wild origin and 487 farmed abalone.

The wild abalone consisted of 396 blacklips (*H. rubra*) and 29 greenlips (*H. laevigata*) of wild caught origin. Wild samples collected specifically for the survey consisted of random samples from ten blacklip sites from all regions of the State. One small sample of greenlips (n=8) was submitted from animals dying in holding systems. A further 30 animals, comprising 21 greenlips and 9 blacklips, which had been collected from the Bass Strait area and held in a research facility, and were examined retrospectively.

Farmed samples were from the nine active farms holding commercial quantities of abalone. They consisted primarily of 30 animals from each of the distinct populations present, plus animals that were submitted to the laboratory for diagnostic purposes. As the primary purpose was to detect the diseases present rather than estimate prevalence, this gave the opportunity to study more diseased animals in greater detail. The farmed samples consisted of 159 blacklip, 215 greenlip, 81 hybrids and 32 animals of mixed species (the latter being from diagnostic submissions).

Participating farms, wild sites and samples are summarised in Table 5.6.2

Of the 912 animals, length and weight were measured on 94% of wild stock and 43 (length) and 40% (weight) of farmed stock. Bacteriology was carried out on 24% of wild stock (102 animals) and 20 % of farmed stock (94 animals). Thioglycollate cultures was undertaken on 43 % of wild stock, 17 % of farmed stock, giving an overall count of 29.6 % of animals tested for *Perkinsus* by this method, in addition to the histological assessment for this parasite. Haemocyte counts were carried out on 28.6% of wild stock (120 animals) and 15% of farmed stock (73 animals).

This Chapter summarises the infectious agent and changes suggestive of infections agents detected in Tasmanian abalone. General tissue changes not directly associated with these agents were recorded and are analysed further in Chapter 5.7.

Table 5.6.2. Tasmanian samples examined

Participating Farms
Abalone Aquafarms, Stanley
Abalone Farms Australia
ABTAS Seafoods
Coastal Seafoods
Cold Gold
Furneaux Aquaculture, Flinders Is.
Puncheon Head, Cape Barron Is
Springbay Seafoods, Triabunna
Tas Abalone Farms, Swansea

Wild Sample Site	Zone	Species	Number
Acteon Is	East (south part)	Blacklip	40
Bay of Islands	East (south part)	Blacklip	30
Bottom Sound, C Barron/Clarke Islands	Greenlip (Flinder's Is)	Greenlip	8
Couta Rock, South of Arthur Rocks	West (northern part)	Blacklip	33
Flinders Island, Kent Group	Bass Strait	Blacklip	33
Fluted Cape, Bruny Island	East (south part)	Blacklip	45
Garden Island	East (northern part)	Blacklip	38
Hobbs Island nth of Port Davey	Western (south part)	Blacklip	30
Mutton Bird Island, south of Port Davey	Western (south part)	Blacklip	31
Rocky Cape, Smithton	Northern (west part)	Blacklip	37
St Helens Pt	East (northern part)	Blacklip	37
Ex Bass St Count Research stock	*ex* Bass Straight	Greenlip	21
Ex Bass St Research stock	*ex* Bass Straight	Blacklip	9
West Pyramid by Port Davey	Western (south part)	Blacklip	33
Total			**425**

5.5.3.2 Examinations for Perkinsus

No *Perkinsus* organisms were detected in any of the abalone examined histologically, or the 266 abalone (80 farmed, 186 wild) cultured in Ray's thioglycollate medium.

5.5.3.3 Virus and virus-like diseases

No diseases were associated with viruses, but two virus-like changes were seen and investigated further.

Intranuclear changes suggestive of viral inclusions

Dark intranuclear material resembling intranuclear viral inclusion bodies (Figure 5.6.4a) or smaller dark bodies suspected to be of similar type were seen in 29 animals. All were in wild blacklip animals. Of these, ten were in the Fluted Cape, Bruny Island area in the second 2004 collection. This change was also present in two animals in the ten examined from the initial November 2003 collection. Nine were from the Flinders Island stunted blacklip collection of July 2004 (not all good examples), two from the Rocky Cape/Smithton collection of November 2003 and three from the Couta Rocks collection of the same date. This change was also seen in three animals from the Bay of Islands in February 2003. These inclusion-like bodies were usually detected in the oesophagus epithelium, although they are occasionally in other parts of the digestive tract, and were usually present as isolated cells with inclusion-like bodies in the nucleus. In only one animal were these bodies common enough to warrant electron microscopy follow-up studies. Electron microscopy examination (conducted in Tasmania and the Australian Animal Health Laboratory) of this animal showed the material within the nuclei to consist of fine fibular particles of 10 to 12nm. This is regarded as too small for virus particles. The material also lacked virus-related structure (Figure 5.6.4b and 5.6.4c).

Health of animals with virus-like intranuclear inclusions:

No significant pathology was associated with these bodies, and no consistent bacteriological findings. Many of these showed no growth on culture, though *V. spendidus* was isolated from two animals. There were no consistent shell findings, many being without any noted lesions though occasionally sponges, fouling, bryozoan, or brown spot discolouration on the inside of the shell were seen. In general, shells were mostly good. There was no consistency of foot lesions. Those animals from Flinders Island with these changes showed poorer condition. There were no consistent findings in other organs.

Haemocyte counts were generally within the normal range, though several were low. It is unclear if the low counts were due to a real pre-mortem change or artefact. Low counts were common from the Flinders Island group, apparently related to other parasites. Haemocyte counts for those with the inclusions averaged 6192 (s.d. = 2775, n=14, minimum count = 1000). The remaining animals averaged 7437 (s.d. = 4314, n=193, minimum count = 650).

Virus-like intracytoplasmic inclusions of fixed tissue granulocytes

A second cell inclusion with some resemblance to virus inclusions was seen occasionally in large fixed tissue granular sites as basophilic amorphous irregularly shaped masses in the cytoplasm of these cells. This change was seen only in wild blacklips from nine animals. It was seen in both the submissions from Fluted Cape, Bruny Island (southern part of the Eastern Zone), from one animal from the ten examined initially in the 2003 and four from the larger sample of 2004. It was also seen from St Helens Point in the north of the Eastern Zone in two animals, in one animal from the Bay of Islands and in one animal from the Couta Rocks area in the northern part of the Western Zone. One animal from Fluted Cape showed large numbers of these inclusions and was examined by electron microscopy, whereas many of the others showed only occasional cells with these inclusions. Electron microscopy showed the inclusions to consist of a crystalline-like array of slightly larger particles (Figure 5.6.5). Rather than viral particles, these may be similar to the crystalline arrays seen occasionally in mammalian granular cells such as mast cells undergoing regeneration (Handlinger and Rothwell, 1984). There was no obvious pathology in these animals.

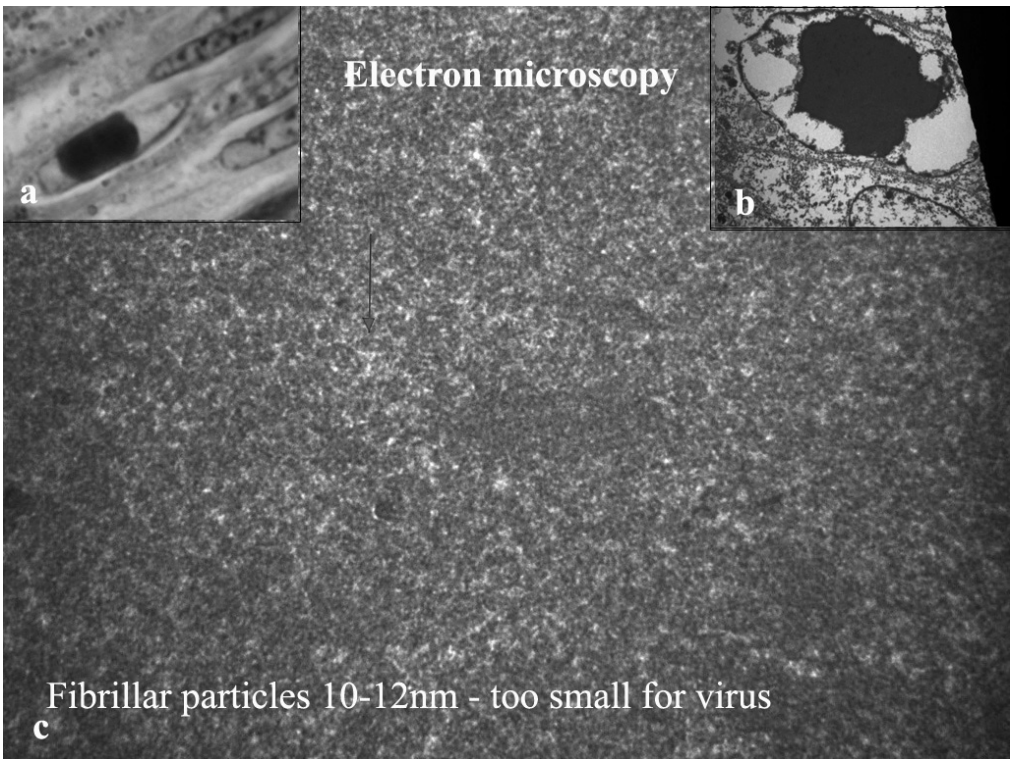


Figure 5.6.4. Intranuclear inclusions of the oesophagus, a) light microscopy appearance, b) low power electron microscope appearance, c) details at high power.

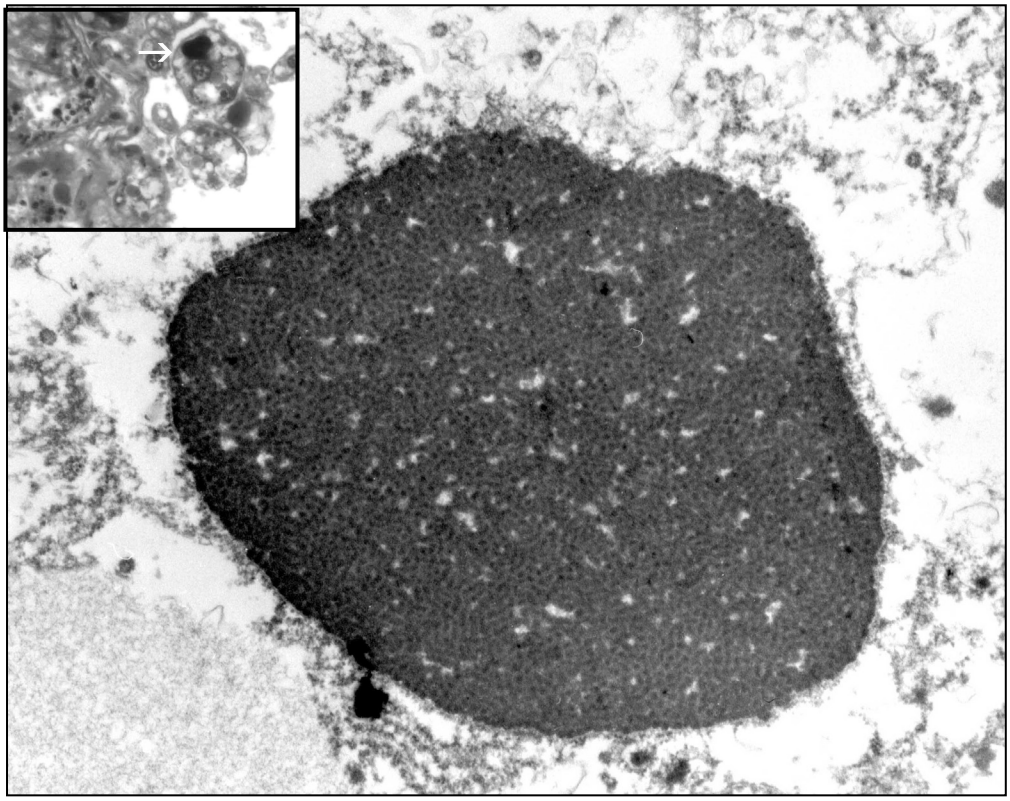


Figure 5.6.5: Electron micrograph showing details of an intracytoplasmic granulocyte inclusion. Inset: light microscopy appearance showing cytoplasmic location of inclusion (resin section, toluodine blue stain).

5.5.3.4 Bacterial infections

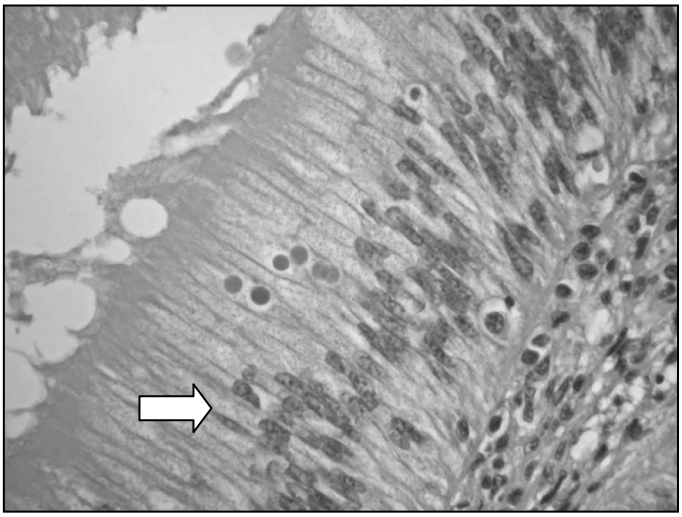
In addition to histological examinations, bacteriology cultures were carried out on 94 farmed animals and 102 wild animals. Findings are summarised in Table 5.6.3.

Table 5.6.3. Bacteria isolated from Tasmanian abalone

Bacteria isolated from haemolymph of farmed abalone	**Total haemolymph isolations***	**Isolation pattern**
Vibrio harveyi (Phenon 32)	28	
Vibrio splendidus I (Phenon 46)	16	
Vibrio. splendidus I as virtually pure isolate		10
Mixed Vibrio spp. Including *V. splendidus* I		4
Mixed Vibrio spp. Including *V. splendidus* I & *V. nereis*		1
With *Vibri. navarrensis* (major isolate haemolymph) & *V. splendidus* I (haemolymph +, abscess +)		1
Vibrio lentus	2	
Single haemolymph isolate, not identifiable	2	
Mixed *Vibrio* spp. incl. *Vibrio chagasii*	1	
Mixed *Vibrio* spp. many unidentifiable	18	
Total positive haemolymph cultures	**67**	
No growth	**54**	
Total haemolymph cultured (farmed)	*121*	
Bacterial isolates from gut of farmed abalone (haemolymph negative)		
Vibrio aestuarianus and *V. splendidus* I		3
V. aestuarianus and *V. navarrensis*		1
Mixed *Vibrio* sp. Including *V. splendidus* I		2
Bacterial isolated from haemolymph of wild abalone		
V. splendidus I (pure growth)		3
Mixed *Vibrio* spp. including *V. splendidus*		10
Mixed *Vibrio* spp. (unidentified) / No significant growth		7
Total positive haemolymph cultures	**20**	
No growth	**80**	
Total haemolymph cultured (Wild)	*100*	
Bacteria isolated from other organs of wild abalone		
Marine *Flavobacterium*-like sp. from foot surface lesion		2
Cultures from shell lesions – no significant growth		2

Rickettsia-like organisms

Rickettsia / chlamydia-like organisms (RLO) were seen in low numbers in the oesophagus (Figure 5.6.6) or digestive tubules of seven wild animals and five farmed animals from four locations. Of the wild stocks, three greenlips from Cape Barren showed these cells (37.5 %) but in other populations only sporadic individual animals showed them (from Couta Rocks, Garden Island and in the experimental animals derived earlier from Bass Strait). No animal had large enough cell numbers for follow-up tests to confirm their identity, though their bacterial nature was usually visible (Figure 5.6.7). No pathology was associated with these cells and none were at levels suggesting they were likely to be detected in any follow-up tests.



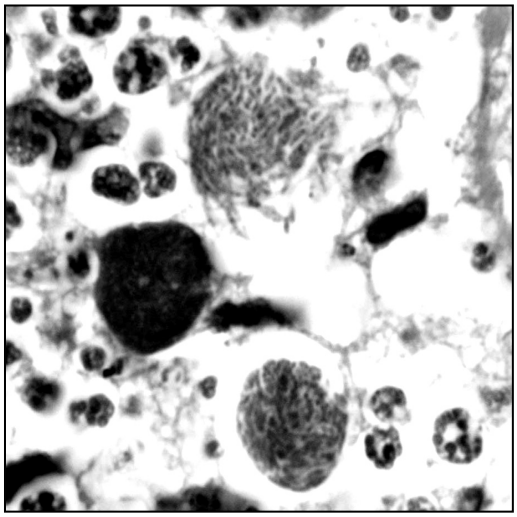


Figure 5.6.6. Occasional cells in oesophagus containing rickettsia-like organisms (arrow).

Figure 5.6.7. Two similar cells in the digestive gland. One cell is rupturing showing the bacterial nature of the inclusions.

Vibriosis

The two most significant culture results, and the only *Vibrio* species associated with disease were *Vibrio harveyi* and *V. splendidus* I. *V. harveyi* was only cultured from farmed animals, and only from farms with a previous history of *V. harveyi* related mortality. *V. spendidus* I was cultured from both farmed and wild species, and was the most common isolate, except from farms with a history of *V. harveyi* mortality. Other bacteria in general appeared to be associated with a terminal increase in bacterial load in stressed animals, rather than pre-collection bacterial infection, the pattern being difficult to distinguish from post-mortem invasion except where associated with definite pre-mortem lesions such as oesophageal villus tip erosion and reaction. Bacteria were occasionally detected in the lumen of digestive gland tubules in animals that were alive at the time of sampling. It was seen in two wild animals from different submissions and two farmed animals. The significance is uncertain but appears minimal.

The pathology of these two infections was as previously described (Handlinger et al., 2002, 2005), with *V. harveyi* infection showing both septicaemic lesions and abscessation, while *V. splendidus* and mixed bacterial infections showed a more septicaemic pattern. *V. splendidus* occasionally showed more definite focal localisation in a variety of tissue, but with most bacteria being detected in the interstitial haemocyte / granulocyte beds. Inclusion of findings from diagnostic samples in the survey results enabled a comparison of *V. harveyi* infection in

blacklip (*H. rubra),* which are regarded as very susceptible to this disease, when present, and greenlip-blacklip hybrids. In this instance, hybrids showed much better survival, but showed large numbers of partially walled-off abscesses (Figure 5.6.8 and 5.6.9) which were likely to have provided a continued source of farm contamination, and may thus act as a significant reservoir on mixed farms. In contrast *H. rubra* typically showed little effective walling-off, with bacteria being confined by haemocytes alone (Figure 5.6.10). To better understand the disease pattern, the pathology of these infections was investigated further (see Chapter 5.7).

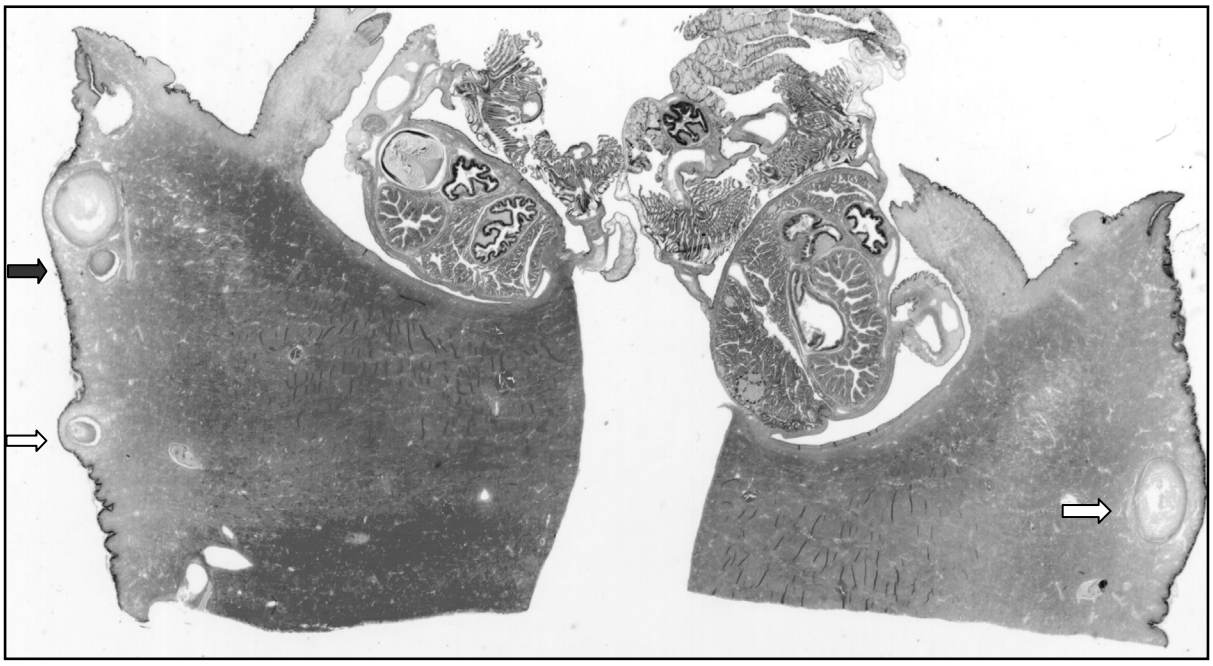


Figure 5.6.8. Multiple large *Vibrio harveyi* abscesses in the foot of a hybrid abalone. Abscesses are present in both sections (arrows).

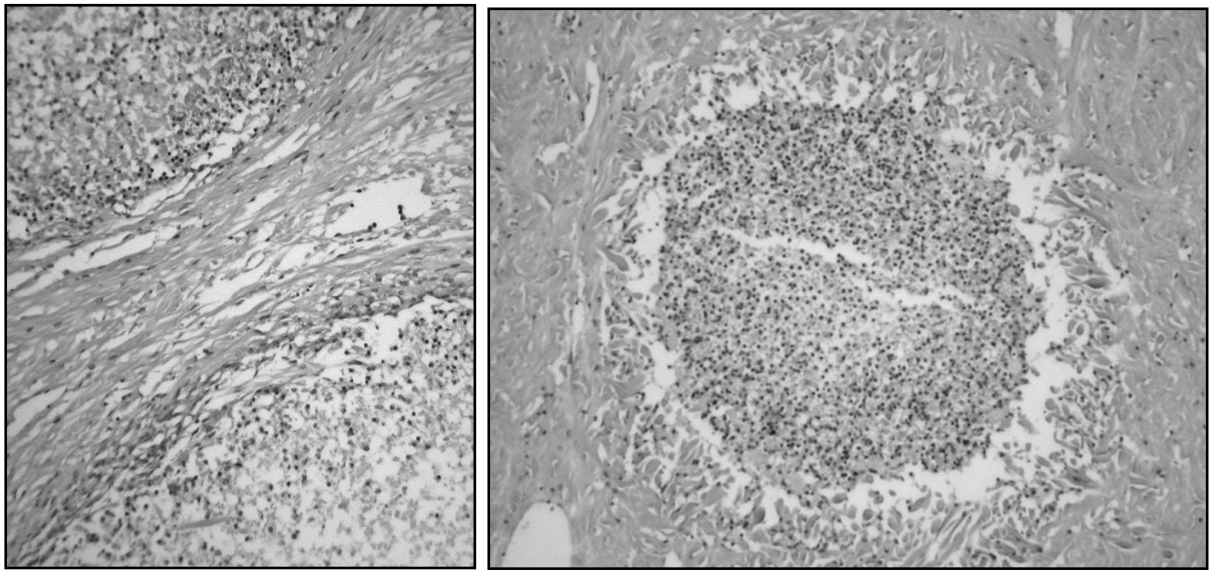


Figure 5.6.9. Details of the margin of the adjacent sections (black arrow, previous figure), showing a well-formed wall.

Figure 5.6.10. Typical abscess from the foot of a blacklip abalone *H. rubra,* showing retention of bacteria by haemocyte mass only, without development of a fibrous wall.

Flavobacterial disease

Marine *Flavobacterium*-like infections were rare in both wild and farm groups. They were cultured on two occasions from wild animals. There was evidence of this type of infection occasionally in farmed animals. Both syndromes described previously were seen (Handlinger et al., 2002, 2005). The large invasive form was seen twice. Once the haemocyte parasite was seen in conjunction with variable *Vibrio* infections in a holding facility of wild caught greenlips. This was considered a significant fact in the observed mortalities. The invasive form was also seen in two blacklip animal on one farm in conjunction with *V. harveyi* infection (Figure 5.6.11) and on another farm with fungal invasion (Figure 5.6.12). Superficial epithelial erosion associated with smaller *Flavobacteria* forms were seen in Tasmania in one farmed animal and one wild animal with mixed shell and tissue parasites.

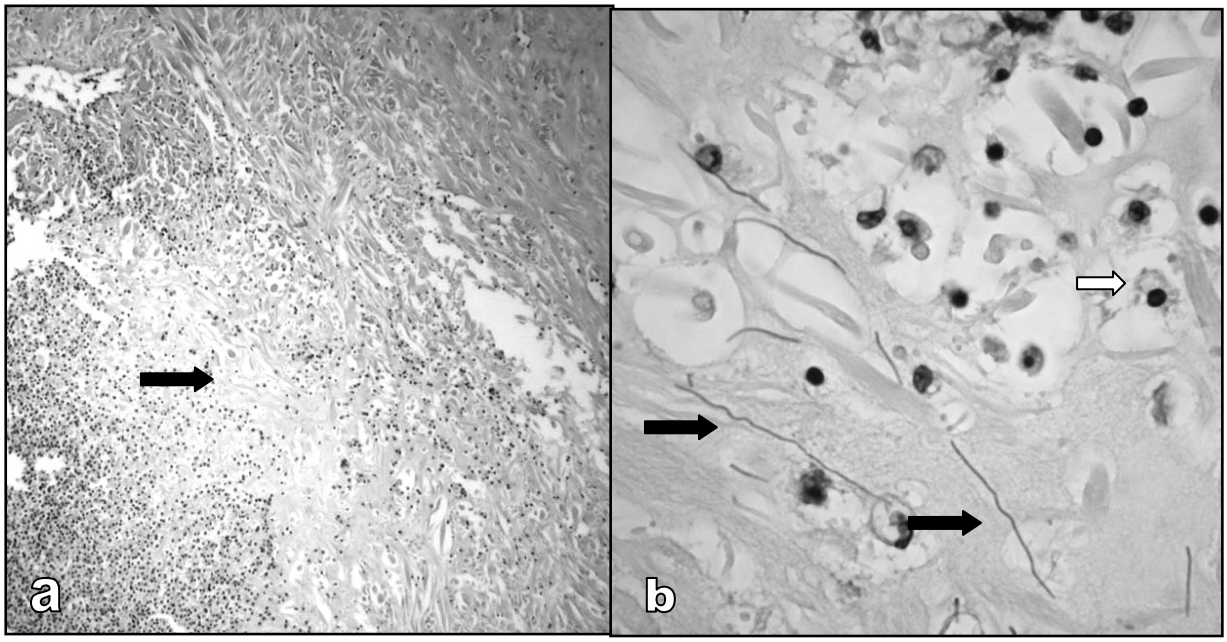


Figure 5.6.11. Necrotic area at the margin of a large *V. harveyi* abscess (11a, at arrow), containing large *Flavobacteria* forms (11b black arrow), as well as the smaller *Vibrio* bacteria free and in haemocytes (white arrow).

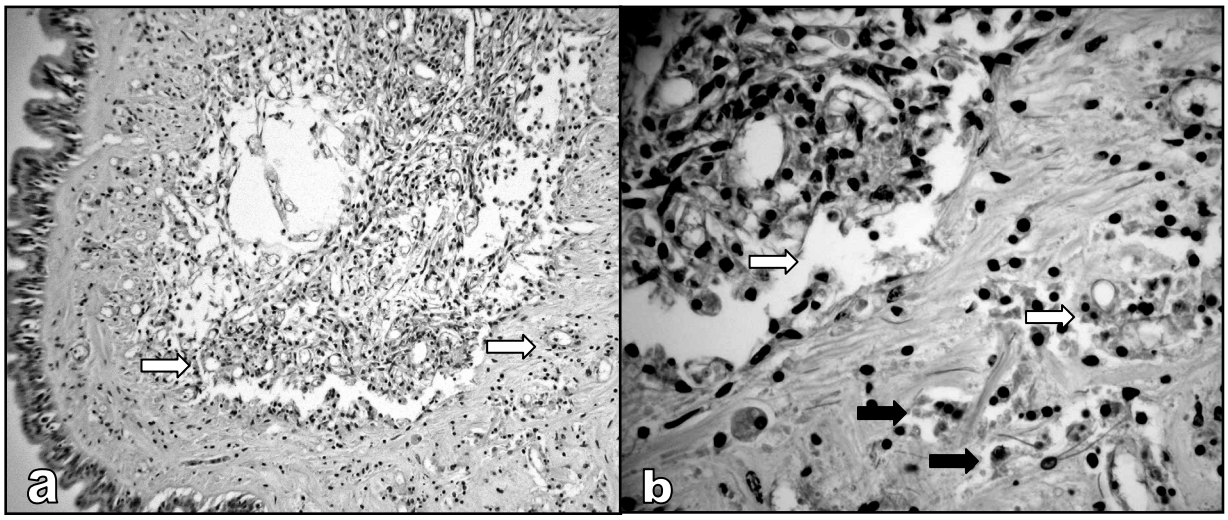


Figure 5.6.12. Fungal lesion (12a) extending from skin (not shown) containing liquefied necrotic core with both fungi (white arrows), and large *Flavobacterial* forms (black arrows).

5.5.3.5 Fungal lesions

Fungal infection was seen in this survey only in the mixed infection shown in Figure 5.6.12, although such infections are known to occur in wild stock from Tasmania and New South Wales, and probably Victoria though the origin of previously affected animals from Victoria was also unknown (Handlinger, unpublished). Typically this occurs as small tubercular reactions of foot surfaces, in which the thin walled hyphae of variable thickness may be poorly stained and difficult to detect, as described for Victoria. Animals reported in the Victorian survey (post-script) were of Tasmanian origin, and may have been infected prior to movement.

5.5.3.6 Protozoa and protozoa like parasites seen previously in Tasmanian abalone

Ciliates

Gill ciliates

Ciliates, similar to those seen in other States, were detected on the gills of 10% of the animals (79 animals), representing 23.1% Flinders Island wild stock and 1.3% of farmed stock. Ciliates were detected on the gills of all wild zones except for Mutton Bird Island in the south of Port Davey, with prevalences ranging from 5.56 to 51.6 % on the Kent Group. Gill ciliates were detected in three of the nine farmed sites (2.2, 3.6 and 7.7 % of animals).

Ciliates appeared to be free-living. Ingested food was readily discerned, including sloughed host cells, bacteria and sperm, providing an indication of gill conditions. In general, no pathology was associated with gill ciliates, although they were occasionally more common in animals with shell lesions and some associated gill damage.

Ciliates in oesophageal pouch

Holotriche ciliates were detected in the oesophageal pouch and appeared similar to those on the gills. It was seen in 20 % of wild abalone but present in only one farmed animal. In the wild abalone submissions, ciliates were detected in all but two submissions, with prevalence varying from one affected animal (3.7 %) to 48.6 % at Acteon Island. Five submissions had this parasite in 25 % of the animals or greater. No pathology was obvious for these parasites. Very occasional suctorian (peritrich) ciliates were seen in the mouth or upper oesophagus region (Figure 5.6.13).

Ciliates in other locations

Free-living ciliates were detected on the surface of the foot of two animals, from Acteon Island and Rocky Cape.

Intra-epithelial ciliates (suspected)

In the oesophagus and less commonly the digestive gland, cells that superficially resembled the small intra-epithelial ciliates common in some other molluscs were occasionally seen. They were classified mainly on the prominent ciliate-like nuclear appearance within the epithelium (luminal forms shown in the Western Australian survey were not seen). These cells were present in six of the wild sites (including those with intranuclear inclusions plus two other groups), and in four farm samples (10 farmed animals in total). Some wild animals showed both the suspicious intra-nuclear inclusions in the oesophagus epithelium and these smaller bodies. It is suspected they may represent the same change rather than ciliates. The putative epithelial ciliates were seen in the gut tubules only from seven wild animals and two farmed animals, with the majority being from the Fluted Cape, Bruny Island areas.

Cryptosporidium-like parasites adhered to hind gut epithelium

In the main gut (predominantly the rectum, occasionally the intestine) a coccidian-like parasite adhered to the epithelium in patches was seen in six wild animals (1.44 %) and 16 farmed animals (3.52 %). This was intimately associated with the surface of the epithelium in a manner resembling that of cryptosporidia and suggesting a similar intraepithelial location (Figure 5.6.14). They were most commonly seen in the rectum, often at the margins of the typhlocole, but were also seen in a similar location in the lower intestine and in the upper intestinal loop.

These parasites were seen in compromised wild stock (blacklip and greenlip) from the Flinders Island area (two animals from the Flinders Island/Kent Group sample and three of the eight greenlip animals from Bottom Sound), and in healthy sea-caged stock from the same area. In other wild stock they were suspected in one animal in Aceteon Island as several small cells in the anterior loop of the intestine, though it is unclear if these cells represent the same parasite. The cryptosporidia-like parasites have previously been seen in Tasmania in sea-caged stock from Flinders Island. In previous decades it had also been seen in farmed stock in other locations, but only in isolated individuals, in stock fed wild collected macroalgae on farms with some wild-caught brood stock. Thus the affected farms are those that in many ways resemble a sea-based environment.

In individual animals, there was no overall indications of an effect of this parasite on animal health. However the epithelium was thinned and, in some places, eroded to the basement membrane in areas affected by the parasite. A small intra-epithelial reaction, including haemocytes and pigmented cells, was evident. In both farmed and wild affected groups from Flinders Islands, other animals showed similar inflammatory infiltrates, focal inflammatory infiltrates in the intestine and hind gut. This suggests that the prevalence of this parasite in the populations may have been higher than that recorded. This type of lesion was not seen in other sites.

This cryptosporidium-like parasite has not been fully characterised in Australia. It is seen more commonly in South African farmed stock, and is currently under study (A. Mouton, pers. com.), and so no further examinations were undertaken. Analysis of infection patterns in South Africa confirms the increases susceptibility of animal with access to natural feed or the marine environment (Mouton, 2006).

Incidental suspected parasites of the gut epithelium

Other less well-defined cells suggestive of inter-epithelial parasites and protozoa were seen in the intestine of 6% of wild stock (25 animals) and 1.8 % of farmed stock (8 animals), and in the oesophagus of one animal (Couta Rocks) and two sea-farmed animals. The nature of these parasites has not been defined and may indeed vary in type, though some resemble findings in SA as shown in Figures 5.4.5. These may even include some ingested non-parasitic cells. The maximum prevalence in wild stock was 18 % in the Kent Group. It was suspected that these samples could include the haemocyte parasite, as cells were generally smaller than those from other locations. In the farmed stock, these cells were seen in six of the animals from Flinders Island and one animal from each of two other farms in the Southern Region and East Coast. In no animal were these cells sufficiently common to warrant follow-up investigations.

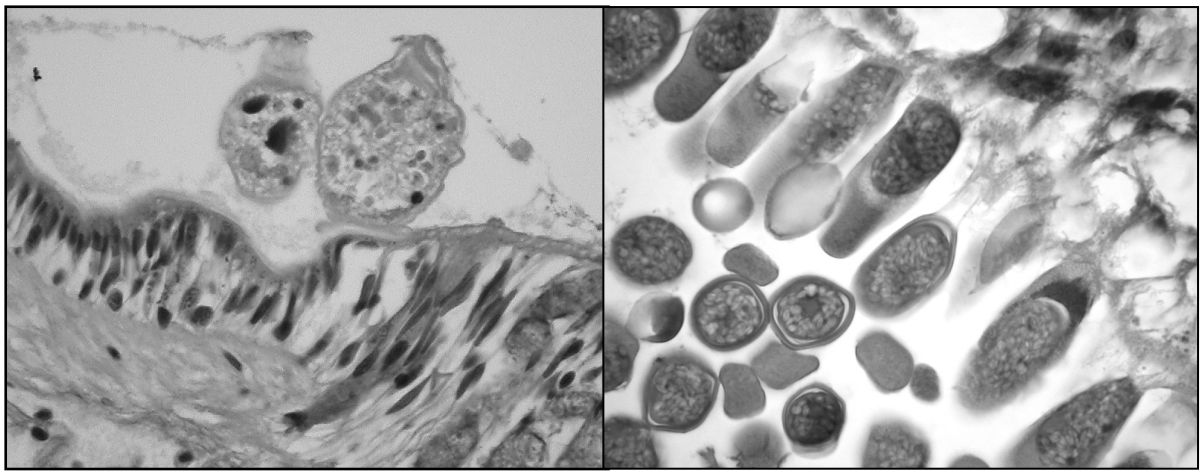


Figure 5.6.13. Rare suctorian ciliate in anterior buccal area.

Figure 5.6.14. Cryptosporidia like parasite of the rectum.

Intra-epithelial parasites of the digestive tubules

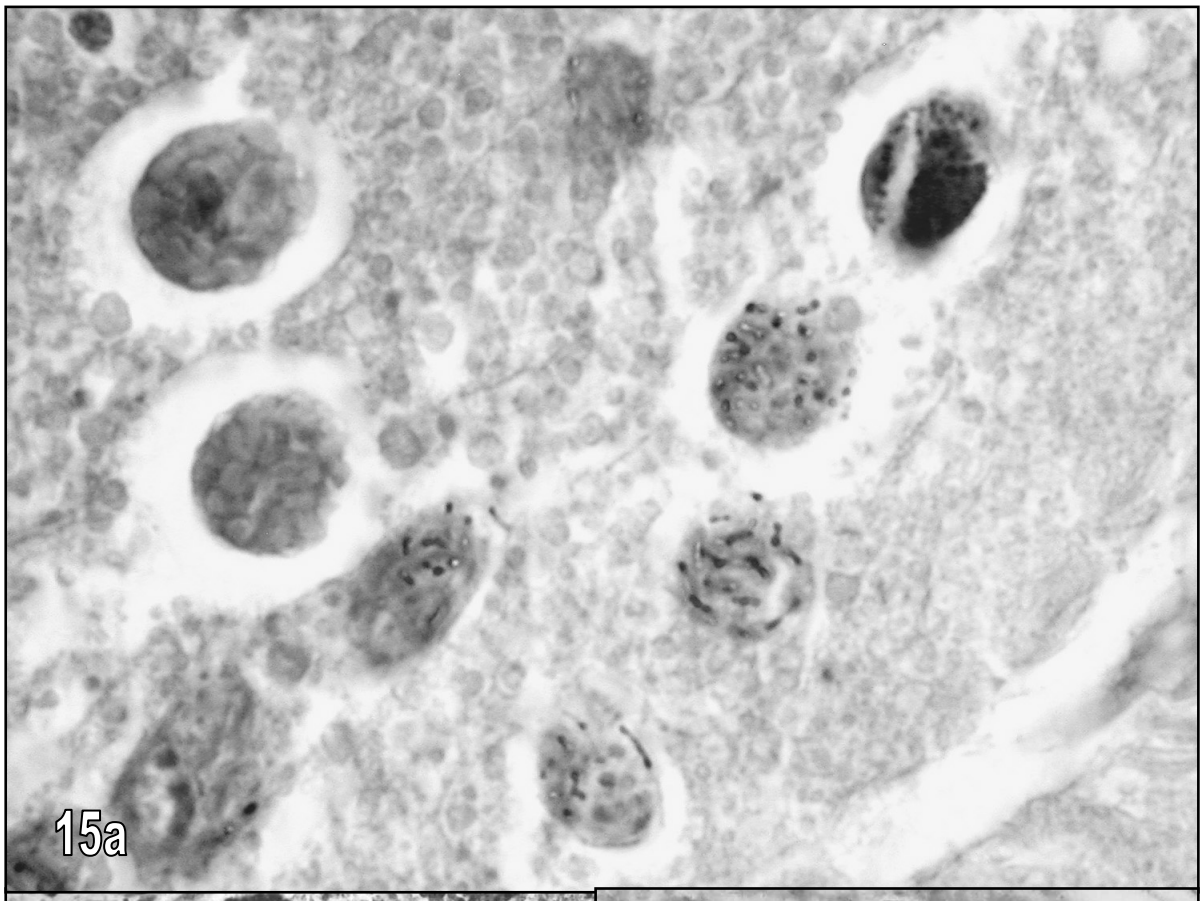
Enigmatic parasites of the gut tubules have been seen on rare occasions previously in Tasmania and confirmed in 13 wild abalone and one sea caged farmed abalone in this survey. They were found in 10 wild blacklips from five locations (Acteon Island, Couta Rocks, Fluted Cape on Bruny Island (Eastern zone, southern part), Garden Island on the northern part of the East Coast zone and Rocky Cape at Smithton). The highest number in blacklips was seen at Fluted Cape, with four affected animals (9.1 %). They were also seen in three of the eight greenlips from Bottom Sound on Cape Barren, Cape Barren Island (35 %).

Although all were identified by the elongated, slightly dumbbell shaped spore-like form as shown from other States, they appear to have three main forms – the highly refractile elongated to dumbbell shaped putative spore, large round granules filling the host cell, and small elongated uni-nucleate gamont like form (Figure 5.6.15). The pale blue round granule form appeared to precede the formation of the spore like stage.

Although the number of parasites in an individual tubule was often high, very few tubules were affected in any single animal. There was no obvious pathology associated with these parasites (further assessed Chapter 5.7).

Renal coccidia

One wild animal from Hobbs Island (southern part of Western zone) showed a single left kidney folia with coccidian parasite resembling *Pseudoklossia* of other mollusc species (Figure 5.6.16). One previous example of this type of parasite, again in a single left kidney fold of a wild abalone, had been seen in previous years in Tasmania (see section 5.1.1). Though this type of parasite is common in many molluscs, including some abalone species, the rarity of the finding suggests this may be an aberrant infection with a more common usual host. There was no associated pathology.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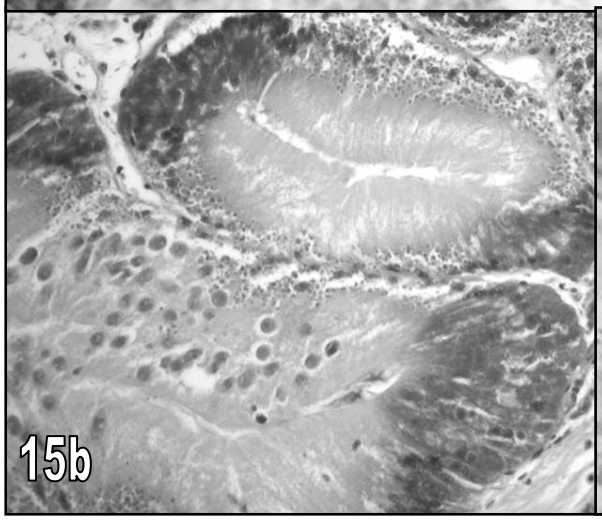
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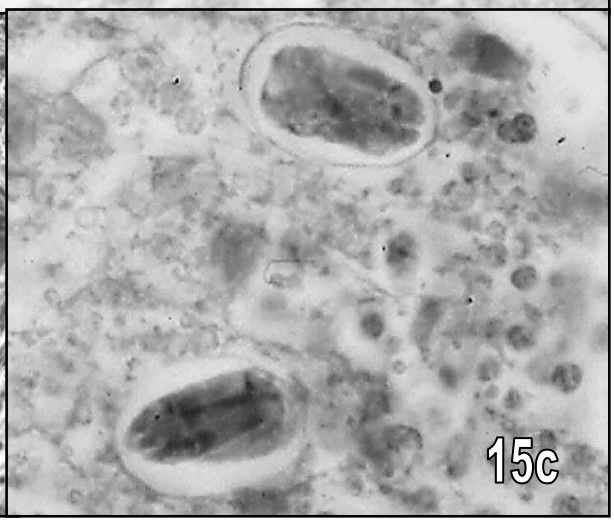
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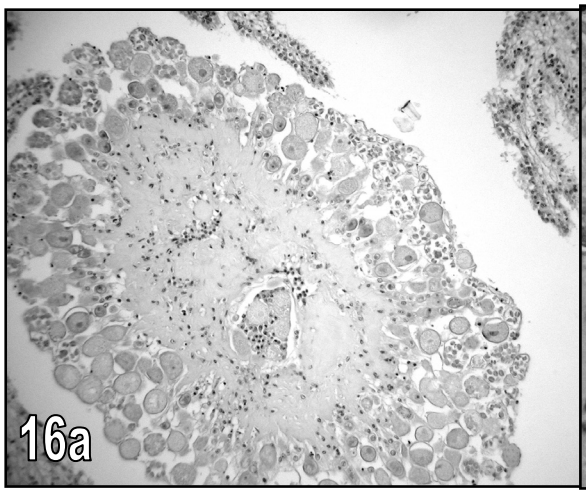
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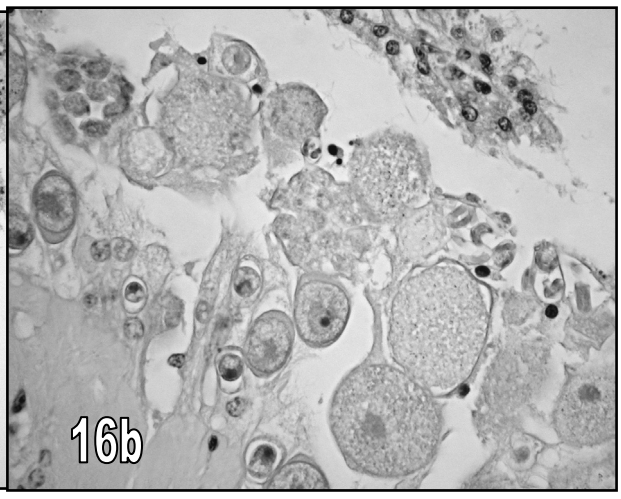
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Figure 5.6.15 Intracellular parasite of the digestive tubules, showing short dumbbell shaped spore like bodies and rounder pale blue staining forms (5.6.15a); typical heavy infection in a single tubule, with adjacent tubules unaffected (5.6.15b), and the meront-like elongated forms (5.6.15c).

Figure 5.6.16. *Pseudoklossia*-like left kidney coccidian showing the single affected folium (5.6.16a), with details of the maturation.

Gregarines

Gregarines were detected in the interstitial tissues on 11 occasions from farmed animals and 12 occasions from wild stock. Only occasional reactions and, in general, no pathology was associated with these organisms. The 11 detections from farmed animals were from four locations (two locations showing four affected animals per farm) but only in greenlip, hybrid and mixed stock from both onshore and sea-based farms. Of the 12 wild animals with gregarines present, three were in greenlip stock that had been moved from the Bass Strait to the Eastern Zone, three were blacklips from the Eastern Zone (two from St Helens in the northern part of the Eastern Zone, one from the south-east), one blacklip from the Western Zone, two from the Rocky Cape (eastern margin of the Northern Zone), three from the Bass zone. Thus this parasite was more common overall in geenlips, but both species carried it.

5.5.3.7 Protozoan Parasites not previously detected

Protozoan-like parasites of the oesophageal pouch

Specific parasites were detected in the oesophageal pouch of abalone from most wild sites and from one sea-caged farm. They were detected in 47 wild animals (16.3%) and 8 of 60 animal from a sea-farm (13 % from this farm). Parasites were not confirmed from other farms, though cells suggestive of dead or dying parasites were occasionally present on 6 farms, including one group with granulomas in this organ. Once these parasites were detected, the oesophageal pouch region was selectively included in sections in preference to other parts of the upper digestive tract such as salivary glands. Consequently, approximately 70 % of pouches were examined.

The typically single-celled parasites varied in size but the majority were slightly larger than host epithelial cells, individual cells being embedded in the epithelium. As well as the simple single-cell form, multi-nucleate stages (rare) and cells containing a number of much smaller form (presumably from internal division of the multinucleate stages) were also present, suggesting nuclear division followed by cytoplasmic division. Large forms with thick walls and refractile extensions suggested differentiation into a different stage of a complex life cycle. Very small forms were also seen singly within cells (suggesting further local spread). These parasites tended to be present as multi-focal infections of the pouch with replacement of epithelial cells in affected patches, but little other pathological consequences evident (Figure 5.6.7 and 5.6.18).

Haemocyte parasite

Parasites of the haemocytes were detected initially in blacklip abalone from the recognisable stunted stock from the Kent Group near Flinders Island. They were subsequently detected in greenlip abalone that had been collected from the near Cape Barren area, due to mortalities in the holding system. Stock derived from these Bass Strait stocks and held for some months in an experimental holding system were subsequently examined. All other abalone were also examined closely for these parasites.

No haemocyte parasites were detected in farmed stock. The prevalence in the Kent Group samples was 51.5 % and in the dying greenlips from Cape Barren, 75 % (6 of the 8 animals). They were also subsequently detected in 33 % of the Bass Strait derived experimental holding system samples and in 55 % of the samples from Rocky Cape at the eastern margin of the Northern Zone.

The parasites were present as round to spindle shaped uni-nucleate forms in the cytoplasm of haemocytes, one to several parasites per cell (Figure 5.6.19). They were first detected in haemocyte smears made following the detection of abnormalities of haemocytes in wet examination during a haemocyte count. They were readily detected when in large numbers, but were easily overlooked when present in low numbers. Indeed, initial examination had not detected the parasites in the Rocky Cape sample.

These haemocyte parasites were associated with pathology. On initial examination, a variety of gross lesions and sometimes poor foot muscle tone were noted in these animals. Initial haemocyte counts showed an atypical haemocyte pattern as well as variable and sometimes very low haemocyte counts. At the gross level, the recognised stunting was detectable, particularly in the initial Kent Group samples, which were a more rounded shape with a greater width to length of shell, and occasionally by growth check lines in the shell, which were sometimes multiple. Many of the animals showed an increase brown colouration of the foot muscle tissue grossly as well as histologically in the form of an increase in brown pigmentation of tissue granulocytes (Figure 5.6.20).

The above changes were also common in animals from the Kent Group populations in which no haemocyte parasites were detected. Gross findings of this nature in the foot muscle were not seen in other populations. A marked increase in brown pigmentation was relatively rare in other wild populations, and was not seen in farmed animals (Chapter 5.7). Other changes noted in animals with the haemocyte parasite and their cohorts were marked variations in abundance of haemocytes in tissue beds (Figure 5.6.21), with distinct haemocyte aggregation in some animals. Haemocytosis and reaction were also seen in other vascular beds, including protein and cellular increase in left kidneys, sometimes with visible parasites, and small foci of vascular reaction through the foot (Figure 5.6.22).

Though in general ciliates were the only parasites identified on gills, other suspected parasites were detected on the gills of animals in groups with this haemocyte parasite, and in other epithelia. These may resemble unidentified small cells suspected to be incidental parasites of other epithelia. Consequently, gill findings were compared with unaffected groups to determine the likelihood that parasites may occasionally pass into the epithelia. Unrelated parasites were also present in animals from the Kent Group, including severely damaged shells with variable spionid mudworm lesions, which have been demonstrated to adversely affect health and growth (Lleonart, 2002, Handlinger et al., 2004), and often marked shell invasion by sponges and other organisms. As the internal tissue changes were present in apparently uninfected animals, and the effects on health could not necessarily be ascribed to the parasite alone, tissue changes in affected groups and their cohorts were analysed further, following a general assessment of tissue changes (see Chapter 5.7).

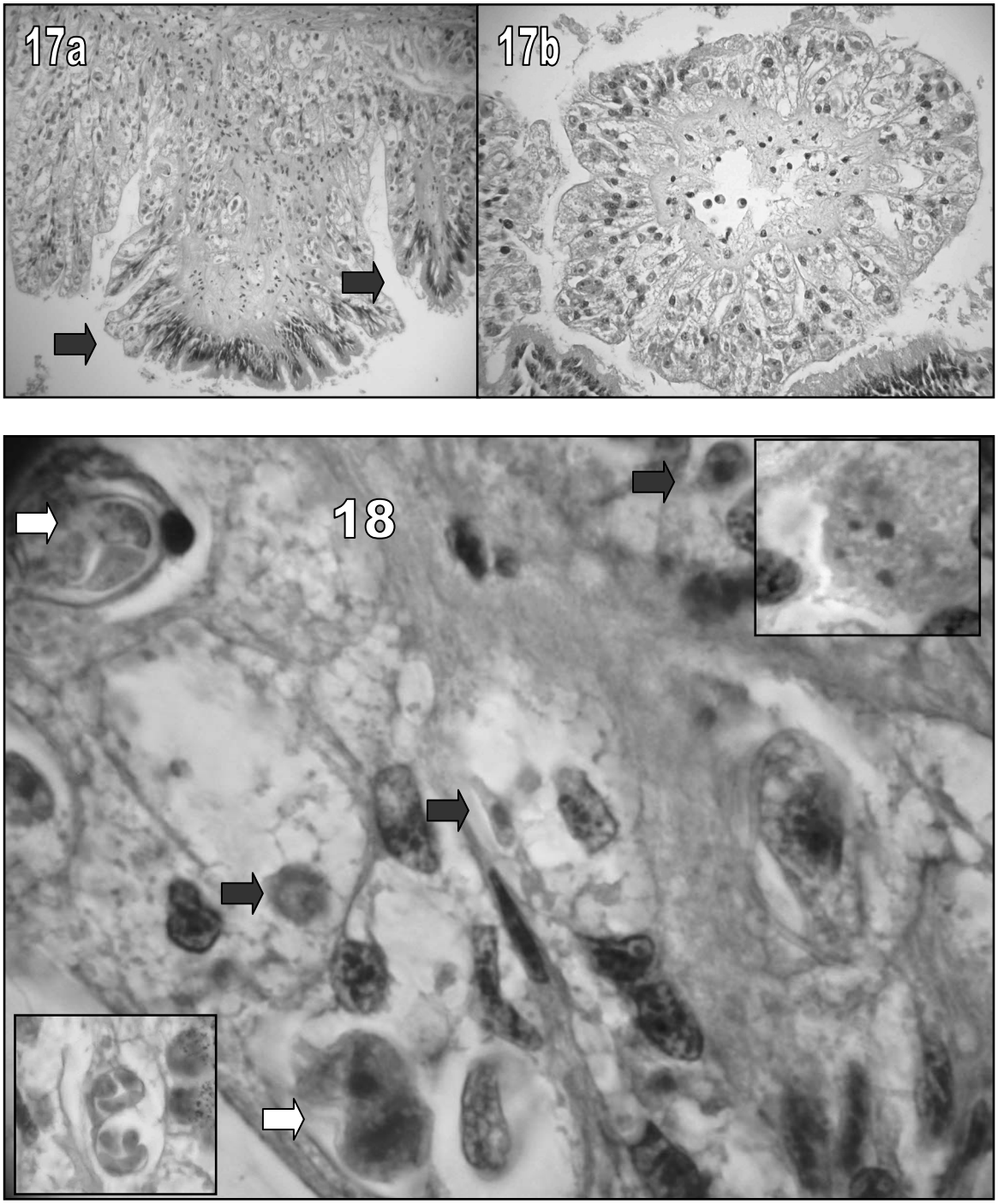


Figure 5.6.17. Two patches of oesophageal pouch heavily infected with the intra-epithelial parasite, showing sharp margins of the affected and unaffected areas.

Figure 5.6.18. High power view of various stage of this parasite. Common small forms (black arrows), large refractile forms (white arrows), rarer multinucleate forms (top inset), and multiple small forms that appear to result from cytoplasmic division of these.

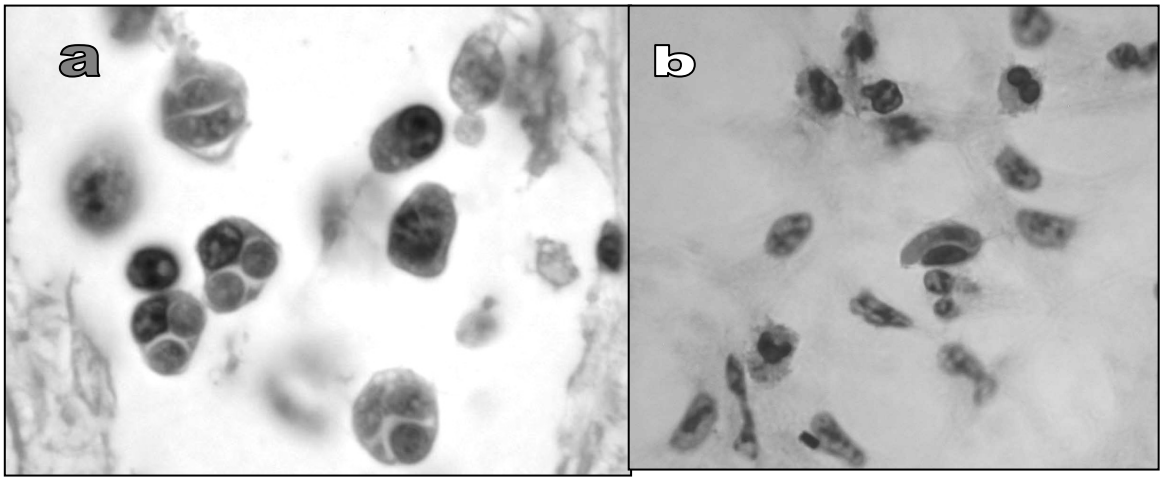


Figure 5.6.19. The histological appearance of the haemocyte parasite (a) in sections, and (b) in haemolymph smear.

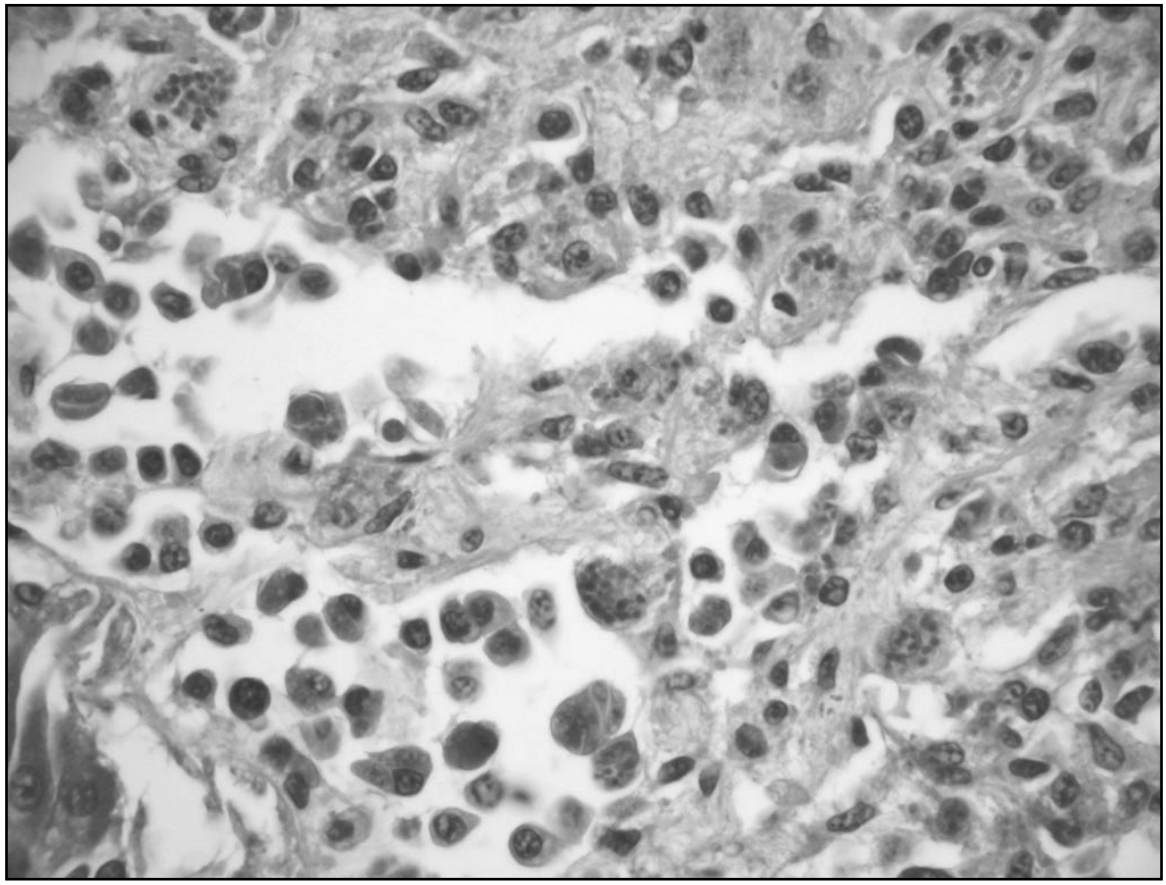
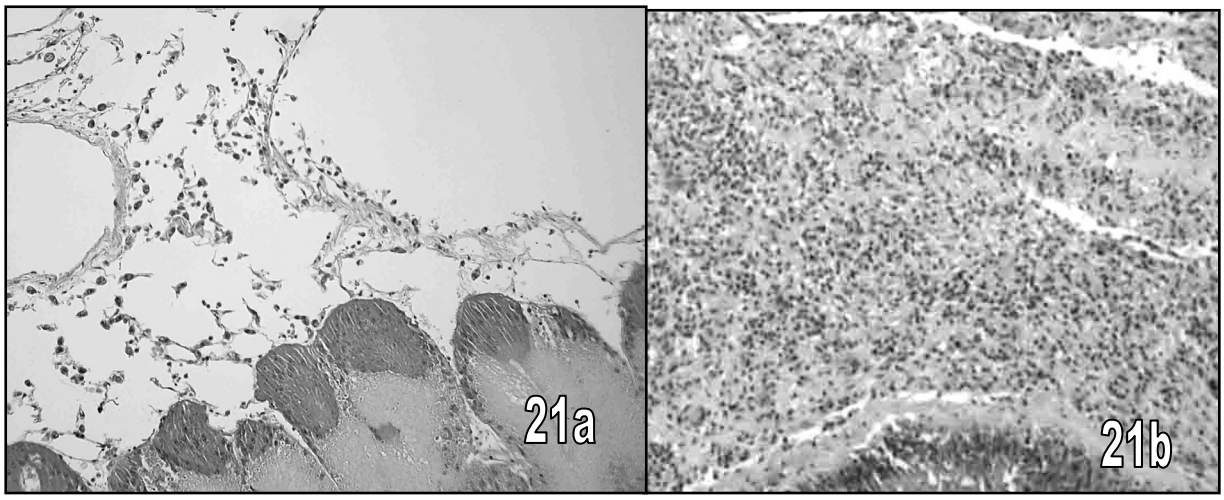


Figure 5.6.20. Section of peri-gut tissue showing haemocyte parasites in circulating haemocytes and in the adjacent bed, plus brown granulocyte pigmentation.



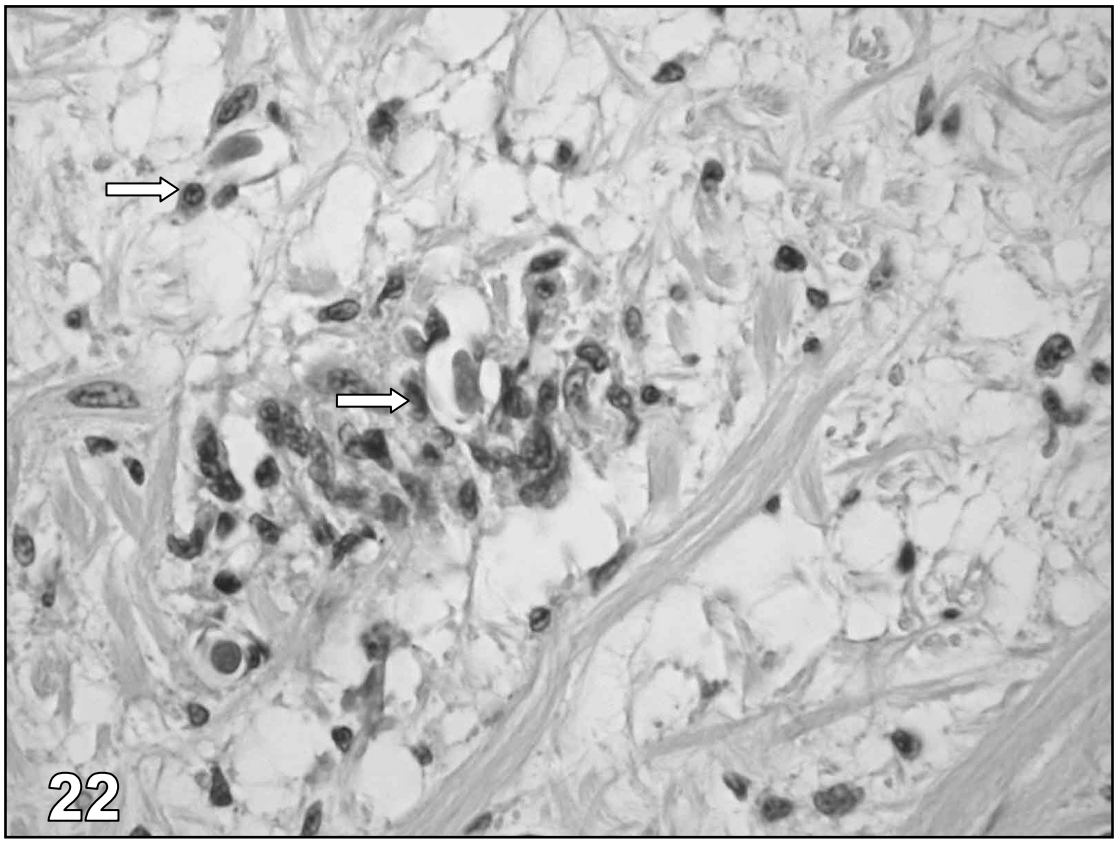


Figure 5.5.21. Pathology associated with the haemocyte parasite. Figure 21a shows a reduction, and 21b an increase, in the density of the interstitial haemocyte and granulocyte bed.

Figure 5.5.22 shows a focal reaction in the foot muscle, with several parasites visible (arrows).

5.5.3.8 Electron microscopic examination of parasites

Intraepithelial parasite of digestive tubules

Electron microscopy was carried out on a single animal from Tasmania with an intense infection. Toluidine blue stained, 1µ resin sections suggested the rounder forms that stain pale blue in H& E sections contained a fine dumbbell core within a toluidine blue exclusive capsule. The organisms were electron dense and little detail of the granules was obtained, although the cells showed a complex wall (Figure 5.6. 23). The morphology did not conform to common pathogenic parasites and the nature of the organism has not been identified.

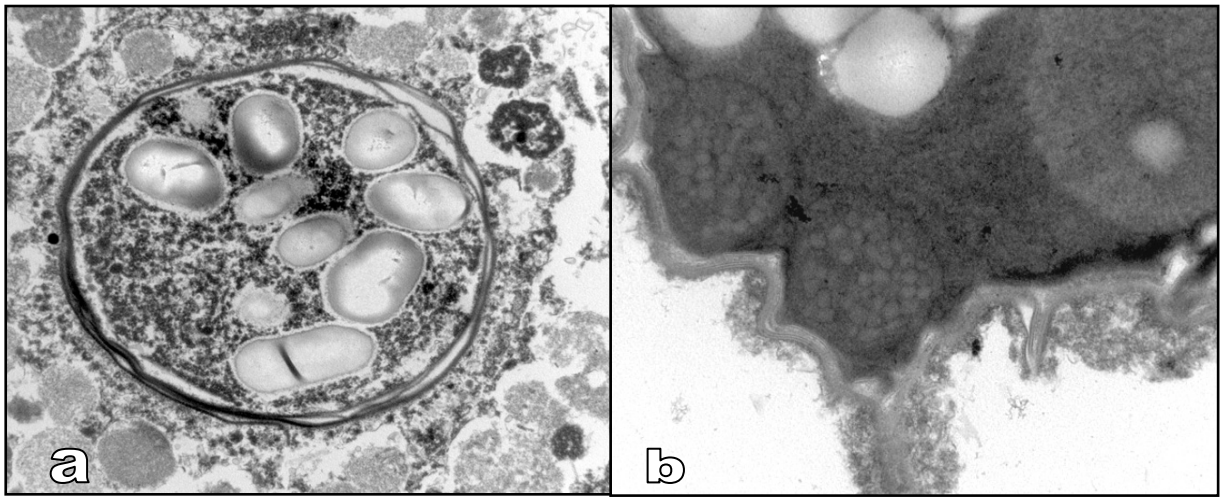


Figure 5.6. 23 a & b. Electron microscope appearance of the intra-epithelial digestive tubule parasite, showing electron lucent nature of granules and detail of complex wall.

Oesophageal pouch parasite

Electron microscopy of the oesophageal parasite showed a number of characteristics that will aid in characterisation of the parasite, although no definitive identification has yet been possible (Figure 5.6.24).

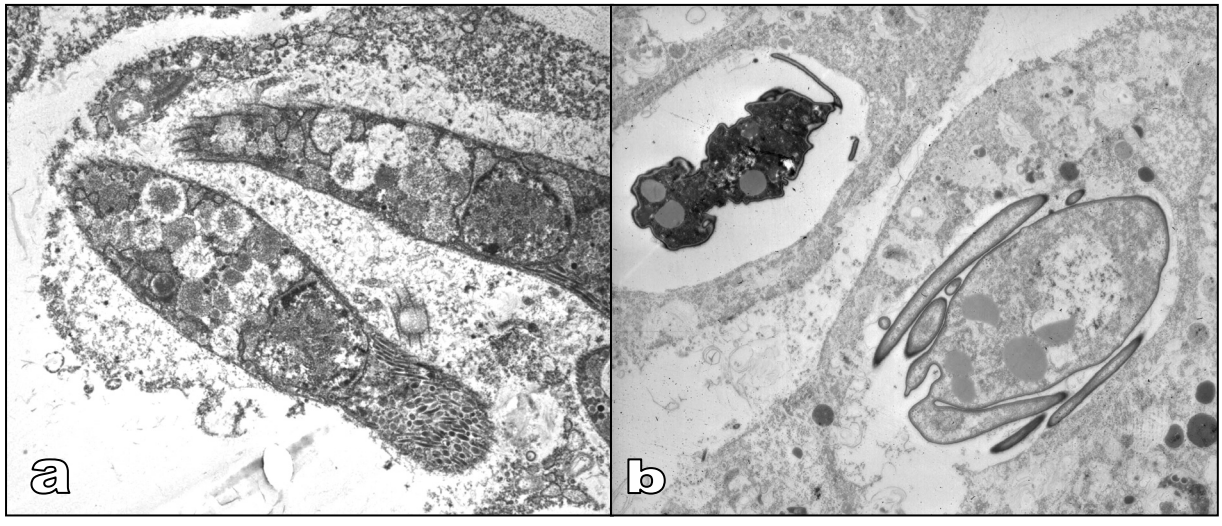


Figure 5.6. 24 a & b. Electron microscope detail of the oesophageal pouch parasite, showing complex cytoplasmic structures extending through the surface of small forms (a) and folded smooth surface of larger forms (b).

Haemocyte parasite

Electron-microscopy was carried out on one animal from the Kent Group sample that showed a large number of parasites within haemocytes (Figure 5.6.25). Initial findings suggested an apicomplexan nature, and several rudimentary apical complexes, seen as an apical concavity were subsequently found in this sample (Figure 5.6.25b).

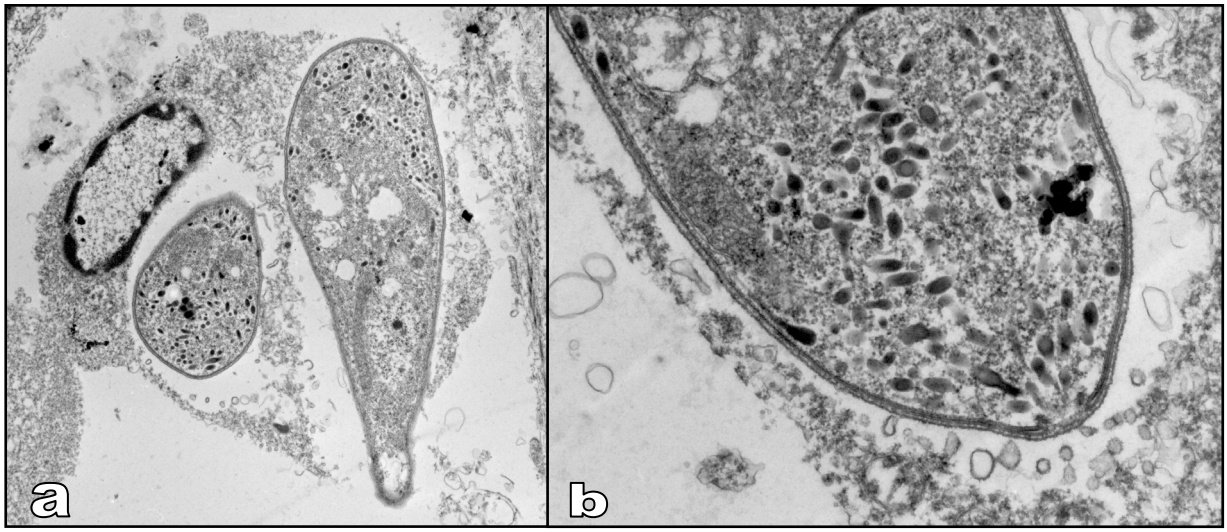


Figure 5.6.25 a & b. Electron microscopic appearance of the two haemocyte parasites within a host haemocyte (a), with detail of the apical concavity (b).

5.5.3.9 Metazoan parasites of internal tissue

Metazoan parasites of the foot

Metazoan parasites (trematodes) were occasionally detected in the foot (six wild animals and three farmed animals), with some times multiple parasites present. These parasites were distributed in five wild sites (the maximum number of affected animals being two), with virtually all areas of the State being represented. Of the farm samples, there was one affected animal in young stock recently introduced to a marine location and two in older animals. They appeared to be trematodes, with an appearance similar to those described from other States. They were usually surrounded by a mild granulomatous response. Similar metazoa were seen in the interstitial tissue surrounding the intestine (five animals).

One larger embedded metazoan (suspected nematode) was seen, in an animal from Acteon Island. One animal from the experimental-system held wild-caught group, showed an unidentified metazoan parasite in the lumen of the right kidney surrounded by a mild reaction and increased mucus. The identity was not clear.

In this survey, no trematode larvae were detected in organs such as the kidney and gonad, although this parasite pattern had been seen occasionally on previous occasions (Handlinger, 2001).

5.5.3.10 Shell diseases

Spionid mudworm lesions

Shell lesions detected in this survey were of several types. Spionid mudworm-related lesions, including burrows, blisters and occasionally chimneys, were detected in 11.9% of farmed

stock (54 animals) and 8.8 % of wild stock (10.25 % overall). Within the individual source populations, the level of infection varied significantly. Chimneys were rarely detected.

Six of the wild populations examined showing no obvious evidence of mudworm infestation. Two showed only one animal with mudworm lesions (3 %). The Bay of Islands and Couta Rocks populations showed ten and 12 % infected respectively and three populations (the two North and East Coast samples – at Garden Island and St Helens and the Bass Strait derived experimental stock) showed higher prevalences of 26.7, 74 and 31.5 % respectively. Note that poor survival after harvest had been reported as an industry concern from one but not both of the two East Coast locations.

In farmed stock, three patterns were found. Three farms showed no mudworms in the stock examined. Three farms showed only occasional animals affected with mudworm, and three populations showed mudworm prevalence of 26.7, 31.5 and 74%. Note that the latter group were all highly skewed to groups that had a history of poor survival associated with mudworm. Some of these populations have been grown for at least part of their lives in sea cages. Sea cages appear to be a susceptibility factor, but not all sea caged populations showed high levels of mudworm.

Of the mudworm species, one group with moderate levels of mudworms showed only *Polydora hoplura.* Another showed a mixture, with *Polydora hoplura* dominant but occasional *Polydora websterii*, and unidentified worms, possibly of other species. Only one farm with sea based culture showed *Boccadia knoxii* infection. In this population *B. knoxii* chimneys were present in the majority of the mudworm infected animals and represented the highest level of mudworm infection. This group had been recognised as a residual mudworm infected group and therefore highly skewed.

Other shell lesions

Other shell lesions were more variable and were seen in 19.5 % of farmed stock and 28 % of wild stock (overall mean 23.6 % for this survey). The level in individual wild populations varied from zero to 67 %. This represents a variety of observations, not all of them pathogenic. Bryozoa were detected on 11 occasions from the East and St Helens areas. Of these, two also showed sponge invasion, and one showed low levels of mudworms. Bryozoa was seen in five animals from the Western Zone, one from Hobbs Island, four from Muttonbird Island with no other shell lesions and three animals from the northern Kent Group. These animals showed little other shell change. In wild animals, chipped shells were seen only from the Eastern Garden Island (one animal) and St Helens areas –(six animals). These chips occurred prior to collection.

Occasional cracked shells were seen, one each from the Bay of Islands and Hobbs Island. Discoloured shells were occasionally seen from the Western area, one each from Muttonbird Island and West Pyramid. Heavy fouling, usually with algae, was seen in eight animals (three from the Western, two from the Northern and three from the Eastern zones). Limpets were detected only in seven animals from the Garden Island area, north of St Helens area. Limpets were occasionally embedded, causing local distortions of the shell and eroded surface scrapes. However, there was little other shell change. Only one of these animals showed mild mudworm blisters. Occasional heavy spirorbid fouling was found in individual animals at St Helens (two), Garden Island (one), Muttonbird Island (one).

Sponges were particularly heavy in a number of animals from the Kent Group, very heavy lesions leading to crumbling shells in four animals from this site. Sponge and mudworms were seen occasionally from Couta Rocks, as well as the Kent Group. Sponge was also seen in occasional animals from St Helens Point and Acteon Island.

Superficial coloured spotting on the internal surface of the shell was seen in a total of 46 wild abalone, the colour ranging from brown in the majority and occasionally green, golden, grey and in one case, red. These were seen at most locations. Shallow shell spotting was seen in two animals from Garden Island, nine animals from St Helens Point (only one of these was associated with mudworms in each group) and in 12 animals from the Bay of Islands (only one of which had apparent mudworm). Spotting was recorded in four animals from the Kent Group, although the prevalence could have been higher as many shells were grossly abnormal. It was seen in six animals from Rocky Cape, none of them associated with mudworm. Minor spotting was seen in one animal only from the Hobbs Island samples. Eight animals with significant spotting, occasionally quite heavy, was recorded from West Pyramid, near Pt Davey. These were not associated with overt mudworms. There was similar spotting in two animals from Muttonbird Island.

Shell lesion other than spionid mudworms were found in 95 farmed animals (19.5 %) making the total overall mean 23.6 % for this survey. This is an over-estimate of the overall mean, and not an accurate estimate of growing stock, as it includes minor fouling recorded for small hatchery stock from one farm, which had the highest level of shell lesions of all the farmed stock. In hatchery stock, the levels of fouling were slightly higher in poorer growing animals. The primary cause of the slower growth was related to diet, not fouling. Brown spots were relatively common at this farm. On another farm there was also mudworm infestation, and occasionally split and fouled shells. One farm had high levels of chipping in the shell in one subgroup, another showed several broken shells within one subgroup and smaller chips within another. Heavy to moderate spirorbid and barnacle fouling was an occasional finding in farmed stock. These were rare in sea caged stock. Spirorbids were more common in the heavily mudworm-infected sea cage stock, which is consistent with previous findings. (Lleonart, 2002, Handlinger et al., 2004).

In terms of abalone species susceptibility to mudworms, mudworms were found in 23.3 % of the 159 farmed blacklips examined and 7.9 % of the 215 greenlips examined and was not seen in the hybrid groups, which totalled 113 animals. Interpretation of these figures needs to take into consideration the skewed sampling towards older, mudworm affected blacklip groups. In the wild stock, mudworm was in 7.1 % of blacklip, representing a true prevalence and in 38 % of the greenlip examined, although virtually all greenlips were selected from populations of concern, and so should be regarded as skewed.

For similar reasons, the other shell lesions were virtually all observed in blacklips in the wild. In the farmed stock, shell lesions other than mudworm were found in 25.8 % of blacklips, 19.5 % of greenlips, 14.8 % of the hybrids and from none of the 31 mixed groups. These included surface fouling (especially noted in hatchery stock), chipped and occasionally cracked shells, occasional spirobids and bryozoa and occasional dark spots. The latter were seen most often in groups with some mudworm, or from sea farms.

5.5.3.11 Lesions not clearly associated with specific infectious agents

Several enigmatic sporadic findings were suspected to be associated with infectious agents, though no specific agents were identified. Apparently non-infectious changes were more common, particularly protein precipitation in the haemolymph and mild gut degeneration changes that were suspected to be related to stress during the transport of samples to the laboratory.

Possible infectious lesions included abscesses in two wild abalone, granulomas predominantly in the oesophagus from one farm, and granulomas in the digestive gland.

Foot abscesses in wild stock

Abscesses seen on farms were generally *V. harveyi* related, with bacteria generally being readily visible in section. No *V. harveyi* was isolated from wild stock, and only two wild animals showed abscesses, in which no bacteria were visible. Both showed small cells with pale eosinophilic nuclei in the reactive margin of abscesses but it was unclear if these were parasites or degenerating host cell fragments.

Of the two wild animals with abscesses (one from Garden Island caught and one from St Helens), one had a relatively minor lesion and the other two large abscesses. The latter (from Garden Island) had a high haemocyte count (19,350/ml), periwinkle damage near the anterior of the shell and mudworm burrows in the shell. There was a surface inflammatory reaction present, an erosion and some surface healing as well as the deeper abscesses. This animal also showed some tip necrosis and rupture pre-mortem in the oesophagus although that was relatively recent and probably post-collection origin. The large fixed haemocytes were moderately brown and vacuolated, as were cohorts. The surface reactions in this animal were noted to have many haemocytes but these were not clotting except at the margin of the lesion. The deeper formed abscesses had a well-developed reactive wall plus a necrotic core. The small cells of uncertain character were present in this reactive zone. The location of these lesions suggested they might have been an extension from a wound.

The other animal showed a chip from the anterior right margin of the shell and a few small blisters on the inner surface of the shell and on the foot attachment. Otherwise there were no external lesions, but the animal was in relatively poor condition. The one abscess showed with no visible bacteria, *Perkinsus* or other parasites, and only occasional bodies were present, which appeared to be degeneration products.

Apparently non-infectious lesions

The following apparently non-infectious changes were noted, some apparently representing specific conditions (such as bloat and digestive gland granulomas), others of a more general nature. The pattern of these changes were analysed to determine their potential value as general indicators of the nature, severity and duration (acute or chronic) of insults, and are discussed in detail in Chapter 5.7.

Other occasional findings were interstitial bed reactions around spicules of apparent algal or sponge origin (one animal from Couta Rocks).

Major general tissue changes and lesions not associated with specific infectious agents.

Physiological Diseases

Bloat
Oedema and dilation of the interstitial tissue, including the foot
Proteinaceous haemolymph
Pooling of proteinaceous haemolymph and or haemocytes in left kidney

Non-Infectious Changes

Generalised pigment increase in granulocytes
Focal pigment in granulocytes and through organs
Generalised pigment increase in right kidney

Diseases of Unknown Origin

Granulomas of the digestive gland tubules
Granulomas of other digestive system organs
Pseudo-inclusions digestive gland

5.5.4 Discussion

Infectious agents found in Tasmania could (in common with other States), best be classified as either agents of little concern common in other molluscs (surface and epithelial ciliates, gregarines, trematodes, and the non-pathogenic rickettsia-like agent); ubiquitous agents with at least secondary pathogen potential (spionid mudworms, sponges, most *Vibrio* species and *Flavobacterial* bacteria); infections agents recognised previously from Australian abalone with little pathogenic potential (the cryptosporidia-like and intracellular digestive gland parasites); new agents whose pathogenicity needs to be determined; and pathogens of undoubted primary pathogenic potential and probably limited distribution (*V. harveyi,* possibly pathogenic strains of *V. splendidus* I, and possibly the spionid *Boccardia knoxi).*

This classification is somewhat subjective, given the snap-shot nature of the survey. The lack of reaction associated with ciliates and gregarines, for example, is typical of these infections in bivalves. The lack of associated pathology suggests the very few rickettsia-like organisms were of the ubiquitous variety rather than withering syndrome, but if heavier infections with these organisms are detected in the future, this may need to be confirmed.

The distribution of *Boccardia knoxi* is still not fully established, though it appears not to be present in all abalone growing areas. Although complicated by sampling bias and the small number of wild-caught greenlip abalone, the species comparison tends to confirm previous findings of reduced susceptibility of greenlip to mudworm infestation, compared to blacklips. Other shell lesions were more difficult to quantify (or speciate), and hence to assess for significance, include sponges (sometimes associated with severe shell changes), bryoazoa (less apparent effects on shells), limpets and small internal shell discolourations which were not fully defined but showed some similarities to fungal-like lesions reported elsewhere. Further investigation of the nature and significance of these lesions would also appear warranted. In general further investigation of shell conditions requires taxonomy skills beyond that currently available to this survey. The tissue effect of these changes are further assessed in Chapter 5.7.

Vibrio harveyi was the major bacterial disease encountered during the Tasmanian survey, but all isolates were from farms with clinical signs or history indicative of *V. harveyi*-related mortality. Where less specific indications of disease were present, or random bacterial cultures carried out during the survey, *Vibrio splendidus* I strains were the most common isolates (Table 5.6.3). Other bacteria including mixed infections were isolated less frequently, but without evidence of pathogenicity. In general these were present either at low levels, indicating bacterial carriage rather than pathogenic infections, or were judged to be peri-mortem invaders.

The clinical significance of the *V. harveyi* on farms is well established, but the *V. harveyi* organism is generally regarded as ubiquitous. However recent findings from a related FRDC Project (2001/628) suggest that the strains isolated from Tasmanian abalone are specific strains which so far have been isolated only from diseased abalone (Carson, 2004). As they have been seen only on farms, and in this survey only on farms with a previous history of outbreaks of *V. harveyi* related mortalities, these strains may not be ubiquitous. If this is the case they may represent a potential translocation risk. Whether this strain is present in other States is uncertain as there is little information on bacteria isolated from States other than Tasmania. Although *V. harveyi* infection has been seen in South Australia (Reuter and McOrist, 1999), Western Australia (Nicki Buller, pers. com.) and recently in Victoria during this project, no strains from these States have yet been available for comparison with the Tasmanian strain using the criteria established for this strain. Similarly, Carson (2004), reported that the *V. splendidus* I strain isolated from disease outbreaks in abalone was also a new specific strain. Although this organism was more widely isolated, and less closely associated with disease outbreaks, more information on strains of this organism associated

with diseased abalone is needed, and a coordinated approach to bacterial identification across States is highly desirable.

One of the new parasites detected, the undefined protozoan-like parasite of the oesophageal pouch, appears to have minimal clinical significance. It is wide-spread in clinically healthy and highly productive Tasmanian stocks. A very occasional, apparently similar parasite was recorded in Victoria, and an apparently similar organism, described as coccidian-like on light microscopic appearance, has been reported from New Zealand (Diggles et al., 2002). The proportion of pouches examined in other States is not known. This will be influenced by the exact site of section and the oesophageal pouch was not a specific target organ. Consequently the number of pouches included in sections appears to have varied between States and between batches. In addition, the areas of infection are small and easily overlooked.

Other findings were suspected to represent new pathogens, such as the suspected viral inclusions and at least some of the suspected intra-epithelial ciliates, but where shown on further investigation to represent tissue changes that are probably unrelated to infectious agents. The knowledge that such reactions occur is valuable to avoid misdiagnosis if these changes are seen in conjunction with disease, and prompt further investigation before a definitive diagnosis is given.

The other new parasite, that of the haemocytes, was more restricted in location within Tasmania, was not detected by surveys in other States, was present at higher prevalence in infected populations, was associated with areas of long-term poor growth and indications of poor condition, and in some cases with current clinical disease. These factors suggest that this parasite would meet the survey criteria of a significant pathogen (capable of producing a clinically significant effect on 10% or more of a population). However as there were a number of parasites present in these populations, including some severe shell lesions in the worst affected groups, and generalised lesions apparently associated with the haemocyte parasite were also present in apparently uninfected animals, the pathology of this infection is analysed further in the following Chapter.

The multiple findings from these populations highlight another common finding during this survey, of multiple infections in chronically sick animals, including individual animals. Examples include the association of the larger *Flavobacteria* forms with bacterial or fungal infection. Another example was an individual wild blacklip with shell mudworm lesions, poor overall condition, and superficial *Flavobacterial* erosions of some duration, from which multiple bacteria were isolated. At the time of sampling, this animal had a very low haemocyte count, which may readily explain the multiple infections, highlighting the need for comprehensive examination of sick animals and an understanding of the pathological interactions.

These conditions are investigated further in Chapter 5.7, and will be illustrated further in the project Atlas.

5.5.5 Contributors

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The Fish Technical Officer team, DPIWE
Craig Mundy and the abalone collection team, TAFI.
Dane Hayes, DPIWE, (tissue processing, slide preparation and electron microscopy)
Alex Hyatt, CSIRO Animal Health Laboratory (additional electron microscopy)

ABALONE AQUACULTURE SUBPROGRAM: A NATIONAL SURVEY OF DISEASES OF COMMERCIALLY EXPLOITED ABALONE SPECIES TO SUPPORT TRADE AND TRANSLOCATION ISSUES AND THE DEVELOPMENT OF HEALTH SURVEILLANCE PROGRAMS.

Analysis of pathology findings from Tasmania to assess disease significance

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Tasmanian Aquaculture & Fisheries Institute, University of Tasmania.

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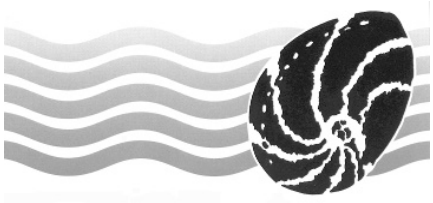
Fisheries Research and Development Corporation



ABALONE AQUACULTURE SUBPROGRAM

Tasmania

DEPARTMENT of PRIMARY INDUSTRIES, WATER and ENVIRONMENT

Tasmanian Aquaculture & Fisheries Institute

University of Tasmania

5.6 ANALYSIS OF PATHOLOGY FINDINGS FROM TASMANIA TO ASSESS DISEASE SIGNIFICANCE

5.6.1 Introduction

Tissue changes such as those described here have been noted and interpreted for a number of years by extension from other species, although the validity of the interpretations has been uncertain. The large number of animals systematically examined during this survey provided an opportunity to verify the suspected associations between stressors and the pathology seen, and determine, in general terms, the likelihood that a change will reflect pre-existing pathology (rather than sampling or post-mortem induced changes or normal variation). An understanding of this relationship is essential for assessing the severity of the pathogens detected in the survey in the individual animals infected, and inferring from this their likely impact in the future. Although general pathology findings were reported from other State surveys (and these observations were taken into account), detailed information was recorded only for the Tasmanian samples, representing therefore the primary data for this assessment. As apparent relationships could be spurious when considering one snapshot survey, the assessment was restricted to qualitative assessment, but the form of the data means this is available to enable more detailed retrospective analyses in the future when more data is available.

The pattern of each of the commonly recorded tissue changes was assessed individually against the pattern in the whole survey and against subgroups where history and other indicators of general health suggested compromised health, to evaluate the usefulness of each change as an indicator of disease.

These changes, together with haemocyte counts, history and gross observations, were then compared between animals with specific infectious agents and the equivalent general population without that agent, to assess the overall impact of the parasites and infections detected. Changes regarded as useful under this assessment were then examined, together with the prevalence of the putative pathogen and the severity of local lesions directly associated with the agent, to evaluate the overall impact of the agent in infected animals.

5.6.2 Methods for general assessment of tissue changes

Tissue changes that were sufficiently common and distinct to be recognised as a specific change were scored for severity during the systematic histopathology examinations of all abalone of the Tasmanian survey. The scoring system, given in Table 5.6.1, was generally from 0 to 3, where 0 = no change, 1= mild or scattered foci only, 2= medium or widespread change, 3= heavy or uniform change. (Although the score system made some provision for higher scores in some categories, scores of this level were not recorded and are probably inconsistent with life.) Each change was then assessed against the overall survey results for usefulness as an indicator of pre-mortem pathology. Individual disease agents were then assessed against the suite of useful pathology indicators.

In the foot, changes that were sufficiently common and distinct to be scored were epithelial loss, sub-epithelial cellular reactions, changes in muscle density and vascular dilation, as well as major lesions of the foot such as abscesses and granulomas. The presence of obvious brown stained granular cells (termed “brown cells” due to their histological and probable

functional similarity to bivalve haemocytes with residual pigment), were also recorded, together with the degree of granule pigmentation. In some cases these were present in sufficient number to impart a grossly evident brown colour to the foot muscle.

Dilation and protein precipitation were scored in the gill, protein precipitates in the heart and left kidney. The presence of apparent calcium precipitates was also scored in these organs.

The degree of dilation as well as the level of pigment was recorded in right kidney tubules. Right kidney pigment was divided into uniform pigmentation of tubule epithelial cells and focal aggregates of pigmented cells (probably haemocytes and / or granulocytes).

Table 5.7.1. Scoring system for tissue changes

ORGAN	SCORE 0	SCORE 1	SCORE 2	SCORE 3	SCORE 4
Foot epithelium	no epithelial loss	Minimal loss, possibly with vacuolation.	At least one slough to the basement membrane, <10 of epithelium lost	10-40% loss of epithelium	40-70% loss (Score 5, > 70 % loss was never seen)
Sub-epithelial dilation	none	minimal	2 focally obvious	3 widespread	
subepithelial cellular reaction	no increase in haemocyte aggregation,	some increased cellularity,	generalised increase in haemocytes, no clustering. Little to no tissue distension	generalised increase in haemocytes, occasional clustering, may be distension	marked increase in haemocytes, prominent clustering, moderate haemolymph distension
protein deposition	none	mild, scattered foci only	medium	heavy	
Haemolymph channel dilations	none	mild, scattered foci only	moderate, several foci	marked	cavernous
Pigmentation (kidney, gut)	no pigment	sparse pigment	moderate pigment	marked pigment	
Pigmentation of granulocytes	Pink / white granules	Pink & brown	Uniform light brown	Dark brown	

In the digestive system, changes recorded included the extent and nature of degenerative changes in the oesophagus. These included an increase in the lamina propria between the villus folds, protein precipitation and calcium precipitates in the lamina propria, and degeneration and increased pigmentation of the epithelium. Similar changes were scored (but were less common) in the intestine.

In the oesophageal pouch, the major change other than parasites recorded were the presence of granulomas, though slightly pigmented concretion-like cytoplasmic contents were also seen (and were at times suggestive of debris from killed intra-epithelial parasites). Tissue changes recorded in the digestive tubules included concretions, granulomas and the presence of bacteria within the lumen. Tubular spacing was also recorded as an indicator of condition. Gonad maturation and degeneration were generally the only criteria recorded for this organ. Interstitial tissue was scored for parasites, the level of overall dilation of interstitial spaces, the staining and granule characteristics of the fixed tissue granular sites, plus the overall abundance of haemocytes and whether there was evidence of clumping or other haemocyte pathology.

5.6.3 Methods for assessing the significance of pathology associated with specific disease agents

Tissue changes considered as useful general indicators of pathology were then used to assess the impact in individual infected animals of each of the pathogens associated with overt pathology, and each of the new parasites. This was done by comparing the proportion of infected animals showing that change, compared with the proportion of animals not infected with that pathogen. Note that the latter population did not exclude animals with other diseases or parasites. However neither was duel infection with other pathogens excluded from the infected group. However co-infection levels were also considered as a potentially useful indicator, as animals with compromised health may have increased susceptibility to other diseases. This comparison was designed to provide indicative indications only.

5.6.4 Results and discussion

Despite efforts to obtain standard sections (in animals large enough for this approach), there was some variation in the actual organs recovered, especially with the smaller organs such as the heart, and what were originally non-target organs such as the oesophageal pouches, an in very small animals sectioned whole at differing orientations. Of the 924 animals examined (425 of wild origin and 487 farmed), the following organs were recovered and examined.

The digestive system examination was divided into the oesophageal pouch and upper oesophagus, the oesophagus and crop, the main gut comprising intestine and rectum, and the digestive tubules. With the standard sections used, the stomach was rarely included. Tissues that were examined from virtually all animals included the foot muscle and epithelium, the gills (86%), right kidney, main gut, oesophagus, fixed tissue granular sites and circulating haemocytes. Nerves were assessed in the foot muscle when major ganglia were not included. Results for these organs are expressed as a percentage of the total number of animals examined.

Of the other organs, heart was recovered in 80% of wild animals (366 animals) and 76% of the smaller farmed animals (370), an overall average of 78%. The left kidney was recovered less often, as anticipated, being examined in 81% of wild animals (344) but only 35% (170) of the smaller farmed animals, (overall, 57% recovery). The oesophageal pouch was recovered from 70 % (297) of wild stock and 64 % (311) of farmed stock (67 % overall). Gut tubules were recovered in 92 % (391 animals) and 89 % (433) respectively (91 % overall). The gonad was observed in 66 % of wild stock and 48 % of farmed stock, this representing both recovery

and level of maturity affecting this value. Results for these organs are expressed as a percentage of the actual number of organs examined.

5.6.4.1 Evaluating of tissue changes as general indicators of pathology

Tissue changes of the foot

Foot lesions were examined for an indication of the proportion of changes that were due to sample collection, compared to underlying pathology, as a background to interpretation of routine samples. The foot was also a useful site to assess general vascular changes as the proportion of other interstitial cells such as granulocytes is generally low. As the foot muscle was examined in 903 of the 907 total animals (over 99.5 % of both farmed and wild groups), findings are expressed as a percentage of the total animals examined.

Foot epithelial loss

Foot epithelial loss was detected in 35.1 % of the farmed animals and 30.2 % of wild stock, with levels varying between submissions. Of the farmed stock, the proportion of animals showing any degree of foot epithelial loss varied between submissions from 18.5 % to 72.7 % and for the wild stock, epithelial loss varied from 5 % at Acteon Island to 57 % at Couta Rocks.

Overall the epithelial loss score appeared to be a better indicator of severity than most of the other changes, although positive scores were noted to reflect a variety of insults. These insults included apparent sample handling abrasions (some of which were associated with slight healing reactions), occasional *Flavobacterial* infections, systemic bacterial infection in farmed animals, and occasionally early post-mortem changes or changes close to death. The latter is consistent with signs recognised by the industry of epithelial loss approach death. In farm animals, some epithelial loss was associated with severe blister formation and ruptures of blisters. Mild blisters, on the other hand, were generally sub-epithelial, varying from a slight increase in tissue spaces (apparently related to slight trauma) to sites of obvious infection.

As well as providing an indicator of severity of foot lesions in individuals, high levels of epithelial loss appeared to be a more robust indicator than level 1 scores (for demonstrating differences between groups of animals. That is, that many animals showed small areas of loss and this should probably be regarded as background handling levels. The mean epithelial score for those animals showing this lesion was 1.23 for wild animals and 1.44 for farmed animals. In the wild submissions, eight sites showed either no animals with loss or very occasional animals with a foot epithelial loss score of greater than 1. A mean score of 1.8 was found from the Bay of Islands, 1.25 for Couta Rocks, 2 for Hobbs Island and 1.36 for West Pyramid. That these sites are furthest from the laboratory suggests the epithelial loss may reflect post-collection effects as well as direct trauma at the time of sample collection. This is consistent with the problems encountered in receiving a large number of samples at the same time from a single collection trip, with consequent delays in processing some of these animals. Farmed animal mean scores for epithelial loss ranged from 1.1 to 2 with the highest scores being in farmed animals showing bacterial infections.

In summary, foot epithelial loss appears to be a robust measure of real pre-mortem stress, provided background levels of minor loss (score 1) are discarded. Significant epithelial loss may reflect either collection trauma, when early healing may be evident, post collection stress, or bacterial infection. In the absence of bacterial infection, epithelial loss may prove

useful as a means of assessing the severity of post-collection stress as well as direct collection trauma between groups (or operators).

Foot sub-epithelial dilations

Sub-surface dilations were found in 49.1 % of farmed animals and 37.9 % of wild stock. The ranges for submissions of farmed sources was from 31.7 % to 72.3 %, and wild sources from 5 % at Acteon Island to 68 % at Garden Island in the Eastern Region.

Sub-epithelial dilations were thus very common but in isolation appeared to be a poor indicator of overall changes. The average score of animals showing this change was 1.23 for wild stock and 1.29 for farmed stock. Levels were low in the two wild submissions in which epithelial loss was suspected to be due to post-collection handling. A mean score of 2 was found from the *Flavobacteria* affected fish from Clarke Island, Bottom Sound. Relatively high scores were found at the Bay of Islands (1.5) and Garden Island (1.52). In farmed stock, scores of five submissions were close to 1, that is, very few animals had a score above 1, with one site showing a mean score of 1.6 in association with bacterial infections. Two farms showed a mean score of 1.4, also in association with bacterial infections and one farm with a mean score of 1.4 that did not have this association.

In general these dilations were due to fluid separation of muscle fibres near the surface. Although rupture of fibres is likely to be a factor, some dilations may simply reflect fluid flux due to muscle action. The presence of fine basophilic particles or protein precipitates in some dilations is more likely to represent pathology, with the fine precipitates suggestive of at least local loss of fluid cation levels and the protein deposits apparently similar to those in other organs. It is possible both changes may reflect reduced local circulation.

Sub-epithelial foot reactions

Subepithelial foot reactions or an increased density of the subepithelial cellularity was seen overall in 20.9 % of farmed stock and 10 % of wild stock (range for farmed sites, 10 to 45.5 % and for wild sites, from 5 % to 32.3 %).

Sub-epithelial reactions were generally mild with the exception of the *Flavobacteria* affected group from Bottom Sound, which showed a mean score of 2. The Garden Island (East Coast) sample with a mean score of 1.5 reflects the small number of animals with responses to fluke and other parasites near the epithelial surface and the reaction associated with the single abscess. Sub-epithelial scores in farmed stock were all 1, except for one farm with a score of 1.1. Thus, virtually all animals showed only slight increases in this criterion, and more severe lesions can be interpreted as significant with confidence.

Foot abscesses

Abscesses were detected in 36 farmed stock (7.4 % of the total) and two wild abalone. Virtually all these samples were from two sites biased towards current bacterial problems (31 % from one site and 25.9 % of the other site showed well-developed abscesses). The proportion of animals affected also varied between groups within these sites, some severely affected groups showing a very high proportion of animals with abscesses. Both these problems were associated with *V. harveyi* type infection. One abscess was reported on gross examination. Minor reactions were detected histologically from one farm with a few animals showing mixed infections, including *V. splendidus* I.

Severity of abscess formation was also scored on the basis of number and size of abscesses present. Of the farmed stock, the mean abscess score for *V. harveyi* affected animals were 2, 2.6 and 3 (that is, multiple abscesses were common). The two abscesses in wild stock have already been discussed.

Peri-neural reactions

Oedema and slight cellular reactions in the nerve sheaths (which was not scored specifically in all animals but became recognised as a significant indicator of early circulatory changes in the foot), was seen as the presence of oedema and very slight blood vessel reactions. Mild reactions of this nature would previously be regarded as insignificant. These were recorded in farmed stock (up to 15.6 % of the stock) but less commonly in wild stock (for example, 12 % of the sick wild animals from Bottom Sound, Flinders Island).

Generalised foot oedema and slight blood vessel reactions

Oedema dilation of blood vessels associated with slight blood vessel reactions was detected in both wild and farmed groups. This condition was present in 11.4% of blacklips and 41.4% of greenlips, the latter being biased towards groups with a history of disease. Within individual populations, the numbers were quite variable. In farm stock this change was present in 28.1% of stock, and was recorded in most groups. Only one population showed zero count, but three other populations showed change in occasional animals (2 to 4 animals or < 7 %). Levels were higher in the remaining populations (22, 45, 55 and 64 %). This included the groups with investigation of bacterial disease. This possible relationship will be explored further below.

In the wild animals, two populations (Bay of Islands and West Pyramid) showed no general oedema scores and only occasional animals (1 to 3) were seen in most of the other blacklip populations. The highest count was found in Muttonbird Island Port Davey group (68 %), in the Flinders Island Kent Group (24 %), the experimental group (33 %) and with lower numbers from Couta Rocks (15 %) and Rocky Cape (9.4 %). This change was also detected in the two animals from Clarke Island where complete foot samples were available.

The mean score for affected animals for foot oedema and slight blood vessel reactions was 1.43 for wild stock and 1.5 for farmed stock. Of the wild stock, the change was most marked in the Bottom Sound greenlips and the Bass Strait derived animals held in an experimental establishment. These two haemocyte parasite affected groups had a mean scores of 3 and 2 respectively. This change was also present in the similarly affected Flinders Island/Kent Group and Rocky Cape, but at lower levels (mean group scores of either 1 or close to 1). Similar levels were seen at the Garden Island and St Helens areas.

Brown cells in the foot muscle

Brown cells in the foot were detected in significant numbers in some wild populations, but particularly in those groups with haemocyte parasites. It was present in 85 % of the Kent Group population, 33 % of the experimental-held group, in both animals submitted with an intact foot from the Clarke Island submission and 21.8 % from Rocky Cape. Other populations showed this change in no more than three animals. This change was not seen in any farmed animals. In some animals from the Kent Group, the severity was such to impart a brown colour visible grossly to the affected foot. Brown discolouration has also been recognised by processors in animals from these areas. Overt brown cell increase in the foot is thus an uncommon change overall, and can be regarded as a severe indicator of tissue turnover.

Pallor of foot muscle

Pallor of the foot muscle was another indicator of poor tissue reserves and has been reported previously (Handlinger et al., 2004, Lleonart, 2002). There appears to be a high correlation between the pallor of the foot and haemocyte parasites but was not restricted to these populations. It is recognised that this is a somewhat more objective score, dependent on uniformity of staining and subject to both within and between laboratory variations. Increased

foot pallor (compared to the staining of other tissues) was often semi-focal. However assessment should be restricted to the dense muscle areas as the foot margins are generally paler and less dense. Stain variations may also reflect oedematous changes (related to circulatory changes or loss of muscle tone).

Muscle pallor was seen overall in 11 % of farmed stock and 23.4 % of wild stock. In farmed stock, the prevalence varied from zero in one population to 22 %. There was not a clear association with major pathology (bacterial infections), which is as expected as this would usually be of short duration. In the wild populations, foot pallor was seen in 100 % of samples from the Kent Group, 36.7 % from the experimentally held group, and those animals in which the foot was available from the Clarke Islands. Relatively high levels were also seen from Couta Rocks (27 %), Bay of Islands (16.6 %) and West Pyramid (21 %). Overall, it was considered that this change was real and, when severe, a very good indicator of poor condition. But due to the subjective nature, this was a less useful indicator than the brown cell and oedema scores. The objectivity of this indicator can be increased by increasing the range of stains such as periodic acid shift (PAS) stain for glycogen (as illustrated by Lleonart, 2002).

Changes of the oesophageal pouch

Apart from the presence of parasites and other putative pathogens, the major changes in the oesophageal pouch were the presence of granular concretions and occasionally, granuloma formation within the epithelium. As well, the holotrich and sessile ciliates were seen in and attached to the epithelium as discussed above.

As indicated previously, pouch parasites were confirmed in 16 % of wild stock overall, and on one sea-farm. Oesophageal pouch granulomas were recorded mainly from one farm group, the cause not determined. All but two of the granulomas were found within two subgroups from this farm.

Granular changes the pouch epithelial cells varied from scattered individual cells with large refractile to pigmented granules, the presence of a highly granular epithelium, and occasional cells with bright eosinophilic granules suggestive of mucus cells but of an intensely eosinophilic nature.

Granulomas were detected in the oesophageal pouch of 21 of these animals, plus 2 animals from other farms. As granulomas were present in other organs also, these will be considered below.

Oesophageal pouch concretions

Concretions (large refractile to pigmented granules) were detected in scattered oesophageal pouch cells in 31 wild animals (7.38 % of pouches examined), and 19.9% of farmed pouches. Possible correlations with parasites and other pathology were explored.

In wild animals, these were common in 3 of the 4 haemocyte parasite affected groups (34.4%, 23.8 %, 10% of pouches examined). None were seen from the Bottom Sound group (only 4 pouches examined as most animals submitted as fixed incomplete tissues.) In these groups, most animals with concretions did show the haemocyte parasite. However the concretions were also common at Couta Rocks (25%) and St Helens (16.7 %), which were negative for the haemocyte parasite, and were present in most other populations.

Only 6 of the 31 pouches from wild animals also contained the pouch parasite (plus one classed as suspicious for the parasite). Eight of these pouches contained ciliates.

With regard to other general body changes, most of the animals with pouch concretions showed some increase in granulocyte pigment, and 19 (61%) showed some increase in digestive gland tubule separation, compared to the population mean of 39.3 % with gut tubule separation (34 % of animals without concretions). Thus it appears more likely that this change reflects a more general insult or range of insults, than a reaction to any of the parasites considered. Such an insult would be of a chronic nature, and possibly linked to nutritional status or general energy balance.

The 61 farmed animals with concretions fell into several categories, with no obvious correlation with tubule spacing or other chronic indications of low condition. Of these, 9 were from the group with pouch granulomas, which were detected in 7 of these animals. However most of the 22 animals with pouch granulomas showed no pouch concretions. As granulomas were present in other organs also, these will be considered below. Another group (15 animals with concretions) was small hatchery stock with relatively high levels of external fouling. Fourteen animals with pouch concretions were from sea-farms where some pouch parasites were present. Only two animals showed both the parasite and concretions. Concretions were present in the pouches of 5 of the 12 animals from other locations suspicious for dead for dying parasites. These parasites were not confirmed in any animal of this group, though the status remained suspicious. The pigmented to refractile globules were more widespread in this group. One of these farms, a marine farm with a single animal suspicious for the oesophageal pouch parasites had granules in 40 % of the animals.

Oesophageal pouch epithelial cell concretions without suspicion of oesophageal parasites within the population were seen in only one farm and one wild site at low levels (two animals). The presence of the parasite without the granules was recorded in three wild sites, although this may be an underestimation as the granules may not have been recorded in early examinations.

The presence of concretions appeared to correlate with the presence, but not necessarily the level, of the oesophageal parasites, or with the presence of granulomas of unknown origin. This suggests that at least some of these concretions or fused pigmented granules are the result of host activity, possibly against the pouch parasite, though additional inciting causes have not been excluded. The correlation with infected or suspicious groups, rather than infected animals, suggests this may represent a successful response to eliminate the parasite.

Eosinophilic goblet cells

The presence of bright eosinophilic cells within the oesophageal pouch epithelium was recorded in 2 % of the wild stock and 4 % of the farmed stock, with a high correlation between this change and the presence of the refractile to pigmented granules. The relationship between these two changes is unknown. As the exact function of the pouches is also unknown, assessment of the significance of damage to this organ is difficult.

Changes of the oesophagus.

The oesophagus was examined in over 95 % of animals and results are expressed as findings per total animals examined. Oesophageal changes seen included oesophageal degeneration (usually acute), granulomas and other evidence of inflammation within the oesophageal epithelium, and a sequence of changes in the lamina propria that generally appeared to precede these changes.

Bloat like oesophageal degeneration

Oesophageal degeneration was a common finding in the early wild animals samples that suffered post-transport stress following collection, due to distance and inability to process the samples promptly in the laboratory. The resemblance of these lesions to those of bloat and the presence of bloat in some samples submitted from farms, enabled the sequence of degenerative changes to be better defined. In general, it was found that degenerative changes of the epithelium commenced at the villus tips. Early changes associated with this, or preceding this change, often included protein deposition in the underlying lamina propria or oedematous separation of this area. This observation led to an increase in the volume of lamina propria being recognised as an indication of early oesophageal pathology. It was found that, in animals regarded as essentially healthy without acute stress lesions, apposing epithelia of the oesophageal loops were separated by a very thin layer of lamina propria. Stressed animals showed an increase of fluid in this area, followed by an increase in haemocytes. These changes were present in epithelia affected by either bloat-like degeneration or degeneration in association with bacterial infection. In the latter, bacteria were usually detected in the lamina propria of both the oesophagus and the main gut.

In wild stock, degenerative bloat-like changes was generally seen in animals held under adverse conditions with a full gut. In contrast, farm stock affected by bloat frequently showed empty oesophagus and main gut, the dilated lumen being filled with protein rich or gassy fluid. However a similar sequence of changes was seen. Protein deposition (sometimes seen only as a thin eosinophilic line at the basement membrane) appeared to be followed by degeneration of these cells, slight deposition of calcium along the basement membrane, increased lamina propria (fluid then increased cellularity), followed by increased transmigration of haemocytes into the degenerating epithelium. Haemocyte plugs (clots) were present in many degenerate tips. These changes will be illustrated in detail and colour in the electronic pathology atlas. Only some of these changes have previously been recognised as bloat related.

Lamina propria increase and occasional oesophageal granulomas were present also in the group showing oesophageal pouch granulomas, although not to the same extent.

Changes of the intestine and associated lamina propria

There were no specific indicators of pathology in the intestinal regions of the main gut in the Tasmanian survey, other than presence of parasites and directly associated degenerative and reactive changes as previously discussed. Granulomas were rarely detected in this organ (4 animals total). Two were minor aggregates approaching granuloma formation in the rectum of farmed animals with granulomas in the oesophageal pouch and oesophagus. Another was also from this group. No granulomas were seen in the main gut of wild animals. However the presence of marked granuloma-like formation in all parts of the digestive tract of Victorian post-survey samples under investigation for feed-related mortality was noted.

Changes of the digestive tubules

As well as the presence of parasites and occasional rickettsia-like organisms, tissue variations observed in the digestive tubules included variations in pigment levels and in the type of granularity of digestive gland cells. This has not been analysed in detail as the criteria was too uncertain, though the impression was of larger coarser granules in animals of longer term poor nutritional status, and sometimes pale poorly defined granules in animals suspected to have recently ceased feeding. Examination of granulation under more controlled conditions is

required to fully interpret granule changes. Basophilic concretions in basal epithelial cells, granulomas, and an increase in interstitial space between the digestive gland tubules were recorded. Bacterial were detected in the digestive gland lumen of two farmed animals from different sites and two wild animals from two sites showed low numbers.

Tubule spacing

The tubule spacing scoring system was based on levels suggested by Dr Anna Mouton, based on South African farmed health surveillance programs (Mouton, 2003). Under this system, any separation of spaces between the tubules is regarded as abnormal, and regarded as indicating less than optimum conditions. Using these criteria, tubules separation scores of 1 or more were recorded from 46.8 % of wild animals (sites varying from 6.9 to 100 %, but only 1 sites less than 10 %), and in 52.5 % of farmed stock (sites varying from 38 to 72.4 %).

In the farmed stock, 3 animals (from different farms) had a tubule separation score of 3 (0.6 %), 49 (10 %) with a score of 2, 170 (35 %) with a score of 1. Although bacterial disease was relatively common in those with higher scores, there was no clear correlation with disease or other indicators of poor condition. This may reflect difficulties in differentiation of shrunken inactive tubules and reduced interstitial stores from acute oedematous separation.

In wild stock, the acute changes were less common and high scores relatively more common. There were 11 animals (2.3 %), with a tubule separation score 3, 57 animals (13 %) with score 2, and 119 (28 %) with score 1. The majority of groups showing between 30 and 55 % of animals with at least some areas of spacing detected in wild stock. The animals from the Kent Group showed increased scores in 90 % of animals and those from Clarke Island scored 100 %. Hobbs Island and Acteon Island showed very few animals with any detectable separation of the digestive tubules.

There was a higher (but not absolute) correlation with parasites and other indicators of disease. For example, haemocyte parasites were detected in 27% of those with score 3, 17.5 % of animals with score 2, 16 % of animals with score 1, and only 8 % of animals with score 0. More animals with high gut separation scores showed high levels of granulocyte pigment, although this was occasionally seen in animals with little or no gut tubule separation.

Overall, the distribution of scores of two or greater was much more restricted and probably represents a more accurate estimate of poor condition. The criteria used in South Africa appeared to be outside the usual observations of farmed stock in Australia. Therefore it was concluded that a score of 1 with slight separation of tubules in at least some areas may not indicate an abnormality.

Concretions of digestive gland cells

Concretions were present (usually at the margins of the basal gland cell mass) of nine wild animals, representing 2.3 % overall. However, the majority of these were in the sick animals from Clarke Island greenlip submissions, where all but one of these animals showed this change. The change was seen in one animal from St Helens Point and one in the experimentally held animals. The animal from St Helens was unremarkable except for a large round lesion covering many of the breathing holes of the shell, some congestion of the left kidney and a very granular oesophageal pouch with red mucus-like cells within that epithelium. However, the concretions were regarded as somewhat atypical being more fibrillar than in the other animals. The animal from the experimental group showed typical lesions (as recorded by other States and by Diggles et al., 2002). This was a greenlip animal affected with the haemocyte parasite, as were the animals from Clarke Island. Although penetration of fixative and the status of the animal at the time of sampling have been suggested as a factor in these concretions, these findings suggest either the moribund state or

chronic compromises to health could be a factor. The animals from Clarke Island were sick at the time of sampling. Some were also subject to some compromise of fixation (fixed on-site but with less than optimal quantity of fixative) as the available resources for this opportune sample collection were limited. The change was seen in one the two animals submitted live (moribund). Moribund animals due to acute changes in other groups did not show this change.

In the farmed stock, such concretions were present in 16 animals (3.8 %). The majority (9 animals) were from one submission. This was in general unremarkable, except that the animals had not been eating. Many of the animals showed thrombi and occasional bacteria in the gill although bacterial disease was not indicated in this group overall. Two other groups, one being a sea cage, showed three animals with these concretions. One animal from the oesophageal pouch granuloma group showed small concretions of this type.

Overall the findings suggest perhaps both physiological state of the cells and the rate of fixative penetration may influence this change. As mollusc tissues are osmoconforming, thermoconforming, capable of maintenance on anaerobic respiratory systems for some time, and with relatively slow metabolic rate, the rate of post-mortem degeneration may vary between tissues, with some tissues maintaining cellular activity for some time. Thus slow fixative penetration may result in sufficient time for alterations within still-active cells. The digestive gland of animals which are actively eating are generally very enzymatically active, and degenerate rapidly, which may prevent the formation of these concretions. This combination may explain the development of these apparently post-mortem concretions.

Granulomas of the digestive gland

These were detected in the digestive tubules in low numbers in three wild animals from different areas and in 45 farmed stock. Of these, 28 were present in the group that showed oesophageal and pouch granulomas and in which granulomatous disease were tracked, 11 were present in another site with mild losses associated with mixed *Vibrio* infection in some animals and a suggestion of possible oil contamination of the intake water. Occasional granulomas were found in five farmed animals from four other sites. No organisms were identified in any of these granulomas.

Overview of granulomas of the digestive system

The pattern of this change differed between wild and farmed groups. Digestive system granulomas were seen as sporadic findings in wild animals. In farmed groups they were usually rare, but when present were generally seen affecting multiple animals within a group, with multiple lesions, although differences were seen between affected groups in the range of organs affected.

In the wild stock, three granulomas were seen in digestive gland tubules, none in the oesophageal pouch, two in the oesophagus. These were all at low levels, from different submissions.

Four farmed animals showed granulomas in the main gut. All of these also showed granulomas in the oesophagus. Three of these were from the group with oesophageal pouch granulomas. Of the 19 showed granulomas in the oesophagus, 14 were from the granuloma group source, the other five from three different sources. A total of 24 animals showed granulomas in the oesophageal pouch, 22 of these from the same source. One also showed granulomas in the digestive gland from the group with suspected oil toxicity. The other isolated case showed very poorly defined granulomas and long-standing *V. harveii* infection. Of these, nine animals showed granulomas also in the digestive tubules. In all, 45 animals showed granulomas in the digestive tubules, 11 from the oil group, 30 from the granuloma

study source, some from the feed trial group, some associated with bloat, and at least one sea-caged. Greenlips, hybrids and blacklips were all represented. Only one to two animals showed granulomas within the other sources. A total of 13 animals had granulomas elsewhere in the gut but not in the tubules. Of these, seven were from tracked oesophageal granuloma group and six from other sources.

The history of affected groups suggests that granulomas within the epithelium of the digestive system may be associated with food or water contaminants such as petrochemicals. Although the range of potential causes has not been defined, the pattern suggests that multiple granulomas should be regarded as a useful indicator of significant pathology.

Further information from the Victorian post-survey feed related incident, the high number of granulomas in the November 2003 opportunistic samples from South Australia, and the information from other States confirmed that granulomas were rare as a finding in individual animals but usually affected a number of animals in a group.

Overall the findings suggest this is a general response of the gut epithelial tissues, with several possible inciting causes, possibly including pollutants. Although the range of inciting causes has not been defined, the findings suggest that multiple granulomas be regarded as significant,

Changes of the circulatory system (gills, heart and left kidney)

Although occasional inflammatory lesions were seen, especially in gills, the most common findings in these organs were gill vessel dilation and protein pooling. The latter was also observed in other locations, such as the lamina propria between gut loops. In acutely affected animals this was seen as distended spaces filled by smooth eosinophilic material. In the days following an acute insult (such as transport to the laboratory or bloat), contraction of this material into smaller more intensely stained globules was seen, allowing some assessment of lesion age. Less frequently, fine stippled material suspected to be calcium precipitates were seen within the precipitated protein.

In farmed stock, gill dilation and gill protein was detected in 55 and 64 animals respectively, generally in animals under investigation for acute bacterial diseases. Bacteria were detected in the gills in 17 animals, from three of these groups.

Protein was detected in the heart in 34 animals and in the left kidney in 92 animals, confirming that while this is usually part of a generalised response, the left kidney may be the most sensitive organ for detecting this change.

Finely stippled basophilic deposits were seen in the left kidney in ten animals, the heart in six animals and once in the gill (farmed stock). Granuloma-like changes were seen in two hearts and 12 left kidneys overall, usually as part of a more generalised response.

In wild animals, gill dilation was detected in 76 animals, gill protein in 57. These were generally from those animals furthest from the laboratory rather than sick animals. In fact, the levels in the worst affected groups overall were quite low. This is regarded as an indication of recent acute damage. Protein pooling was detected in 22 hearts and 156 left kidneys in wild animals. Calcium deposits in gills, hearts and kidneys were 1, 4 and 7 respectively, confirming the pattern in farmed animals with regard to organ sensitivity.

Bacteria were detected in 20 left kidneys from farmed animals, but none from the wild animals.

Changes of the right kidney:

In the right kidney, the major changes were the level of dilation of the tubules and pigment. Pigment showed either a uniform dispersed pattern in renal tubal cells or focal distribution, possibly within cells of haemocyte origin.

In farmed animals, dispersed pigment was seen in 148 animals, with focal pigment seen in only 16. Dilation of the right kidney was seen in 301 animals (all groups, 18-100 %).

The distribution of right kidney diffuse pigment varied considerably between submissions with two farmed submissions showing no dispersed pigment and one only very low levels in two animals. Three sites showed moderate levels of 16 to 19 affected animals and three showing much higher levels. This will be assessed further in conjunction with specific pathogens.

In wild stocks, dispersed right kidney pigment was seen in 110 animals, focal pigment in 45 and dilated right kidneys in 135. Focal pigment was relatively uniformly distributed throughout the groups, although three groups showed no focal right kidney pigment, with the highest levels being in the held experimental stock (11 animals). Dispersed right kidney pigment was again much more variable between submissions, with the highest levels being present in the Kent Group –(22 %), Muttonbird Island (24 %), Rocky Cape (18 %) and Couta Rocks (16 %).

Although dilation of the kidney are regarded in the South African health surveillance program and by Harris et al. (1998b), as an indicator of poor water quality, it is suspected that in many cases this is an acute change induced by the conditions of transport. In both wild and farmed p populations, the highest proportion of dilated kidneys were seen in submissions with a long transit time. Thus, this change should be evaluated based on whether animals are fixed on site or transported first.

There appeared to be some correlation of dilated right kidneys with dispersed pigment score, although at this stage, separation of transport-induced and background changes has not been attempted.

Changes in interstitial tissues

In the interstitial tissues, the major indicators of pathology were separation and apparent oedema, changes in the abundance, uniformity of distribution and degree of aggregation of small circulating haemocytes and the abundance and granular appearance of fixed granulocytes. While these have been tabulated, their importance is obvious. They have only so far been analysed in conjunction with specific pathogens discussed further below. Granulocytes were scored for the level of pigment in granules, which ranged from 0 (pink granules), through mixed pink and brown granules, to increasing levels of brown pigment. Vacuolation of the granulocytes was another common findings, apparently independent of the level of pigmentation though more common in paler granulocytes (especially farmed groups). In some animals (especially farmed) fixed tissue granulocytes with indistinct granules (smudged) were present.

5.6.4.2 Assessment of the pathology associated with disease agents detected

Pathology associated with the haemocyte parasite

Prevalence of tissue changes

The histological evidence for a significant impact of the haemocyte parasite on individual infected animals was assessed by comparing the total scores for each useful pathology indicators in the wild animals with haemocyte parasites (48 animals confirmed, suspected in 3), animals from the same source populations in which no parasites were detected (57), and animals from other wild source populations with no evidence of the parasite (317). Animals in which the parasite was detected comprised 11.3 % of the total. Apparently unaffected animals from the same population comprised 13.4 %.

Overall, the animals from affected source populations were slightly smaller than the total population sample. Mean length from unaffected populations was 123.5mm, while animals with the parasite were 101.9mm. Mean length of unaffected animals from these populations was 105.8mm. It is possible that the slight differences between infected and uninfected cohorts, together with slightly smaller mean weight of animals without the parasite, could represent an age-related effect.

The mean haemocyte count was lower in infected animals, averaging 5680 compared to 7317 in the unaffected population. The unaffected animals from the uninfected group were slightly lower at 6068 (See figure 5.7.1). Though severely affected animals were initially recognised by very low haemocyte counts (as well as haemocyte abnormalities visible on wet examination), haemocyte counts showed a wide range in all groups and the overall group differences were less than anticipated.

Infected animals were slightly more likely (35 %) to show foot epithelial loss than unaffected populations (30 %). Unaffected animals from the infected group were slightly less likely to show this change (25%).

Infected animals were more likely than apparently uninfected animals from infected populations to show sub-epithelial dilations compared to cohorts and unaffected populations (48 % compared to 35 and 36 %).

Similarly, infected animals were more likely to show sub-epithelial reactions, and apparently uninfected animals less likely to show this change, than control populations (29, 10.5 and 15.5 % respectively). Infected animals were much more likely to show oedema and slight reactions along the blood vessel tracts. These reactions were found in 31.3 % of the infected animals, 14 % of the apparently uninfected animals from infected populations and 10.7 % from other non-infected populations.

As well as the prevalence of these changes, the overall level of severity was compared. Comparison of mean scores for the animals positive for each tissue change showed that mean scores in the infected animals were slightly higher for sub-epithelial reactions and oedema and blood vessel reactions. Mean sub-epithelial reaction score was 1.21 compared to 1.08 in the general population, with the unaffected cohort little different to the latter. Similarly, the mean oedema score for infected animals was 1.33, while the other two groups had a mean score of 1 (all mild lesions). Mean scores per affected animal were no higher than the uninfected group for foot epithelial loss or foot epithelial dilation.

Almost all of the brown cell scores in the foot were seen in the affected populations, with a similar proportion between infected and apparently uninfected populations. This change was

seen in 44 % of infected animals, 46 % of other animals from this population and 3.8 % from uninfected populations.

Foot pallor, although a more subjective score, also varied between groups of different status with regard to this parasite. Feet were scored as pale in 52 % of animals with the haemocyte parasite, in 47 % of other animals from infected populations, plus two of the three animals with suspicious lesions, but in only 15 % of unaffected populations.

Infected animals were likely to show left kidney protein and their uninfected cohorts slightly more likely than non-infected populations (54, 39 and 34 % respectively). A similar trend was seen in heart protein precipitants, although numbers were low. Four infected animals and three uninfected animals showed granulomas and/or old reactions in the left kidney compared to a total of two from the unaffected populations (8.3 and 5 % compared to 0.6 %). There was no tendency for these animals to show calcium deposits.

Both infected and non-infected animals from the same population were considerably more likely to show dispersed right kidney pigment and focal right kidney pigment, but only slightly more likely to show dilated right kidneys. The dispersed pigment was seen in 48 and 42 % of infected and non-infected animals from the same source, compared to 9.7 % in the general population. Dilated right kidney tubules were seen in 42 and 33 % in infected and non-infected animals from the same-source respectively, compared to 30 % overall.

All of the gut tubule concretions were seen in infected populations. This comprises all the Bottom Sound greenlips, except for one greenlip in the experimental holding group.

A high score for gut tubule spacing was more common in both infected animals and uninfected animals from this population (54 and 53 %, compared to unaffected wild populations, 38.5 %). However, looking at the actual score in animals showing increased spaces, both infected animals and others from that population showed an increased score of 1.6 compared to 1.4 for the population. The mean score across all animals was 0.85 in infected animals, 0.84 in uninfected animals from these populations and 0.53 in the population as a whole.

Animals from the infected population showed a slightly higher level of the intra-nuclear inclusion-like changes in the oesophagus epithelium. There changes were more prevalent in the unaffected animals (8.3 and 14 % compared to 5 % in the general population). The nature of this change is uncertain. None of the infected population showed cytoplasmic inclusion-like change of the granulocytes.

Other changes related to the gut epithelium and lamina propria increases were relatively rare in infected populations and similar to uninfected populations.

Co-infection with other parasites

There was some evidence of an overall increase in general parasite load in the population with haemocyte parasites. Seven uninfected animals from the infected population (12.3 %) showed inter-epithelial parasites in the main gut, though there was no increase in the infected animals (6.3 % compared to 5% in the general population). Infected populations also showed a high proportion of the adhered gut parasites in the hind-gut (five in total compared to one animal, possibly atypical from the non-infected wild populations). This could simply reflect the geographic distribution of the gut surface parasites as occasional animals from farmed stock in this region also showing the adhered gut parasite (but not that of the haemocytes).

Infected animals were also slightly more likely to show other muscle parasites (6 % compared to 3 and 2 % in the other two groups). They were slightly more likely to show gill ciliates than

the general population (21 % compared to 16 %), but much less likely than the apparently unaffected animals in infected populations, which showed ciliates in 40 % of animals.

One of the parasite infected animals and five of the apparently unaffected animals in the infected group showed putative protozoan parasites in the gill epithelium. These were regarded as possibly similar to the haemocyte parasites, leaving a question whether these were related. Possible gill epithelial parasites were seen in three animals from other populations, but whether these were of similar type was uncertain. Frank haemocyte parasites were seen in gill vessels in four animals, making this a less likely location for their detection than other vessels. Both the infected animals and their apparently uninfected cohorts were less likely to show gill dilation and gill protein than the uninfected populations (15 and 5 % compared to 21 % for dilation; and 10.5 % compared to 14.5 % for protein).

Infected animals were no more likely and uninfected animals from these populations less likely than uninfected populations to show oesophageal pouch parasites (infected 14.6 %, general population 12 % and uninfected from infected source, 3.5 %). However, animals in the infected groups were slightly more likely to show concretions in the oesophageal pouch than uninfected populations (6.7 % and 10.5 % compared to 5 %). These animals did not show the red mucus cells occasionally encountered in this organ.

Though numbers were low, they did appear more likely to show gregarines in the interstitial tissue, especially the uninfected animals (8.3% and 12.3 % compared to 1.3 % in other populations). Though numbers were low, infected but not uninfected animals also appeared slightly more likely to show the digestive gland parasites (6.3 % compared to 1.8 % uninfected animals and 2.8% in the general population).

Animals with the parasite were slightly more likely to have mudworm infestations. Mudworm was seen in 21 % of those with the parasite, one of the three with suspected parasite infection. In contrast, animals from these populations that did not show the parasite had a similar level of mudworm to unaffected sources, (12 % compared to 14 % in unaffected sources). Animals in these populations with the parasite had a similar level of other (non-mudworm) shell changes to the overall population (35 % for both groups). This did not account for severity of the change, however, with many shell lesions in the Bass Straight group in particular being severe. Animals without the parasite from these source populations showed less shell changes, being found in only 19 % of the animals.

Granulocyte and haemocyte relationships to haemocyte parasite

The relationship between haemocyte parasites, abalone species, circulating haemocytes, tissue haemocyte abundance, and the level of fixed tissue granulocyte pigmentation is shown in Figures 5.7.2, 5.7.3 and 5.7.4.

Though some very low circulating haemocyte counts were seen in infected animals, the overall variability was high (Figure 5.7.2). Tissue haemocyte levels (Figure 5.7.3) indicates both the level of variability in the overall wild population, and an apparent bi-phasic response to the parasite, with many animals showing decreased numbers of haemocytes, while a smaller number show a marked increase.

The biphasic response in tissue haemocyte reserves was seen in both species, and in infected animals and their cohorts. Indeed the sometimes florid increase in haemocytes was more marked in uninfected cohorts than visibly infected animals.

A comparison of granulocyte characteristics of these same groups (Figure 5.7.4) reinforced the similarity of visibly infected and apparently uninfected animals from the same populations, compared to the blacklip population at large.

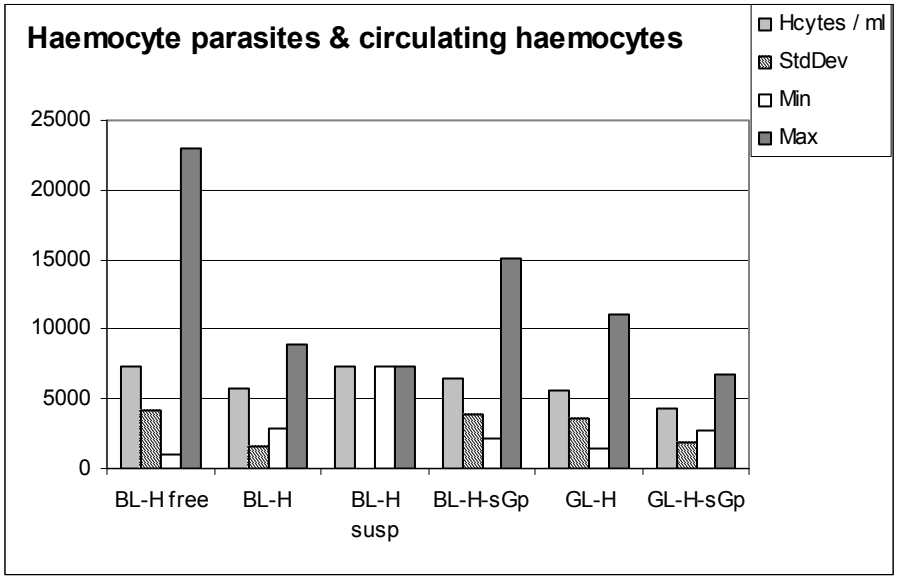


Figure 5.7.2. Comparison of haemocyte counts in blacklip (BL) and greenlip (GL) abalone from populations that are free of the haemocyte parasite (free), infected with this parasite (H), from the same populations with suspicious infections (H susp), or cohort of the infected animals (sGp). Suspicious infections were those where occasional parasite like cells were seen but morphology was poor and the nature uncertain. (Haemocyte counts were not carried out on all animals).

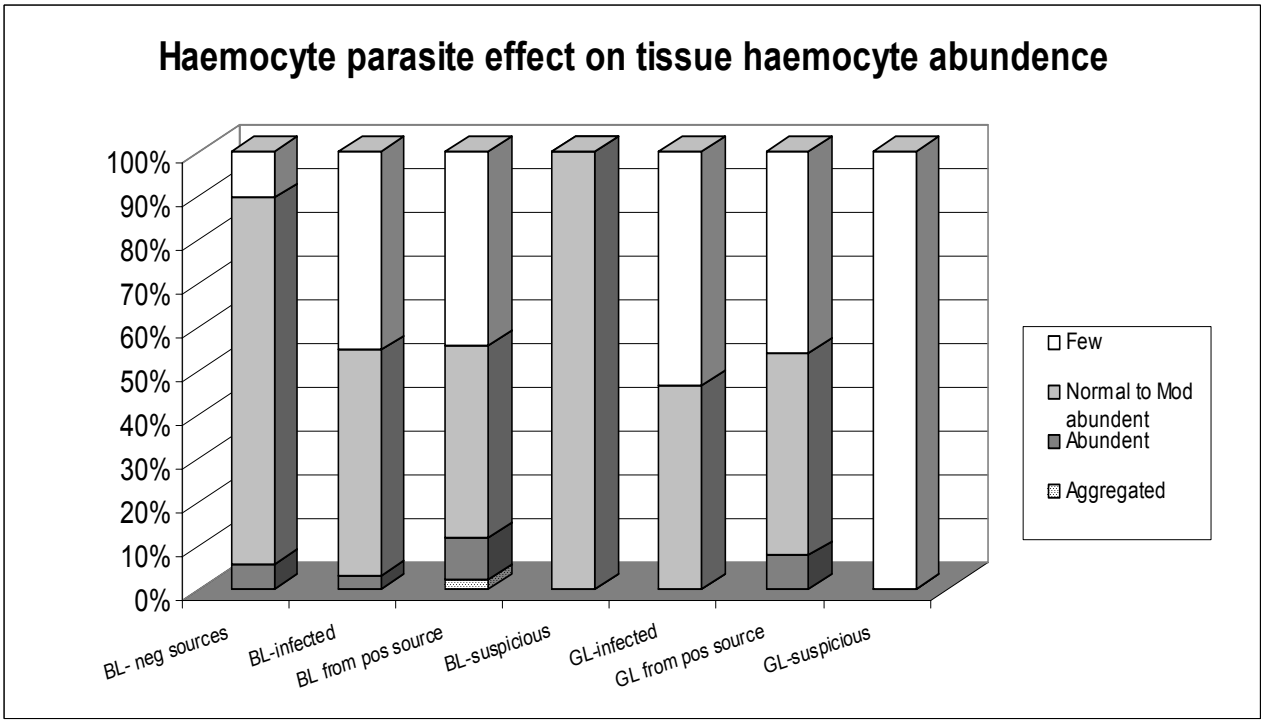


Figure 5.7.3. Comparison of tissue haemocyte abundance in wild blacklip (BL) and greenlip (GL) from populations free of the haemocyte parasite, infected animals, those suspicious for infection, and apparently uninfected cohorts from positive sources. Suspicious infections were those where occasional parasite like cells were seen but morphology was poor and the nature uncertain. (n as for Figure 5.7.2)

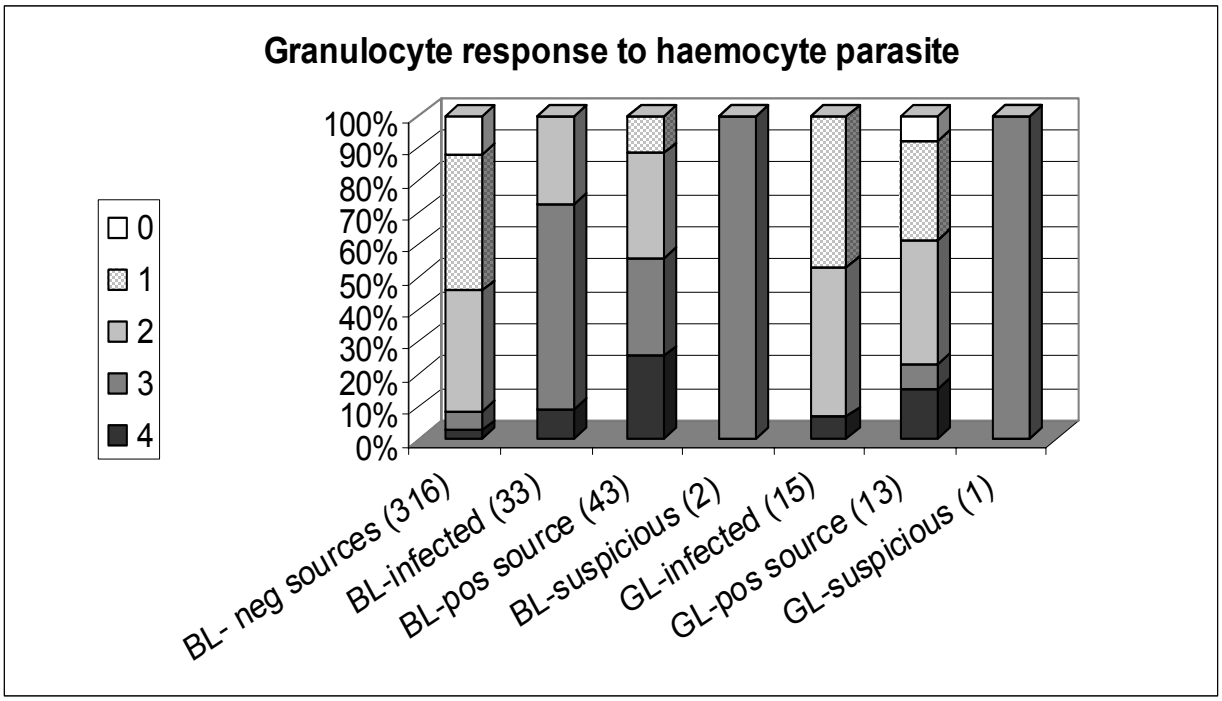


Figure 5.7.4. Comparison of fixed tissue granulocyte pigmentation levels of infected and uninfected wild blacklip (BL) and greenlip (GL) populations, compared to wild blacklip sources negative for this parasite. Level 0 = no pigmentation (pink to red granules, or little colour); Level 1= mixed pink & brown granules; Level 2= predominantly light brown granules; Level 3, moderately brown; Level 4 dark brown.

These comparisons indicate an increase in animals with few haemocyte reserves, and of increased granulocyte pigment, in both the visibly infected animals and their cohorts, to a similar degree, although both the haemocyte and granulocyte patterns were more variable in the animals in which no parasites were visualised. The apparently uninfected group did show occasional animals with low granulocyte pigment, but more animals with heavy tissue reactions of a similar nature to the visibly infected group. Marked tissue reactions were in general more common in animals with few parasites, rather than heavily infected animals, suggesting this is a late or post-crisis stage of infection. The associated foot pigmentation and pallor and gross lesions extending into both the visibly infected and cohort populations. The results are consistent with perceptions during examination that many of the apparently uninfected animals are likely to be either lightly infected or recently infected with this parasite.

Pathology associated with bacterial infection in farmed animals

Few cultures were carried out in States other than Tasmania. Histological evidence of bacterial infection was rare in any State except in groups with a known bias towards current ill-health. This is consistent with recognised disease patterns, as only transient infections of sufficient magnitude to be detectable are expected in clinically normal animals, except where bacteria are retained in walled off abscesses. There was concern, however of a possible association of suspected recent bacterial infection and granulomas of the digestive gland found in Western Australia and Tasmania. A similar granulomatous pattern was seen in additional farm samples from SA from a problem group sampled in November 2003, which were made available to the survey. The suspected relationship has not been confirmed.

To date only *V. harveyi* and *V. splendidus* I have been implicated in primary septicaemic disease in Australia, and the pathology of these two infection has been described (Handlinger et al., 2002, 2005). *V. harveyi* infections show both septicaemia and localisation, usually in the foot musculature, with abscess formation. *V. splendidus* showed a more septicaemic pattern, with some focal localisation in a variety of tissue, but with less focal host response and most bacteria in the interstitial haemocyte / granulocyte beds or vasculature. Inclusion of findings from diagnostic samples in the survey results enabled a more detailed comparison of *V. harveyi* infection in blacklip (*H. rubra),* which are regarded as very susceptible to this disease, and greenlip-blacklip hybrids. Hybrids showed much better survival, but displayed a large number of partially walled-off abscesses. It is postulated that these were likely to have provided a continued source of farm contamination, and may thus act as a significant reservoir on mixed species farms. This also enabled further assessment of other infections hitherto regarded as terminal invaders.

Granulocyte and haemocyte relationships to bacterial infection on farms

While the clinical significance of specific strains of *V. harveyi*, and to a lesser extent some *V. splendidus* infections is well established, this is not true of other *Vibrio* species, which often appear as terminal invaders of little overall significance. In order to better understand the disease pattern, haemocyte counts and tissue levels of farmed abalone with *V. harveyi, V. splendidus* I or with infections of mixed or other *Vibrio* species were compared (Figures 5.7.5, 5.7.6 and 5.7.7).

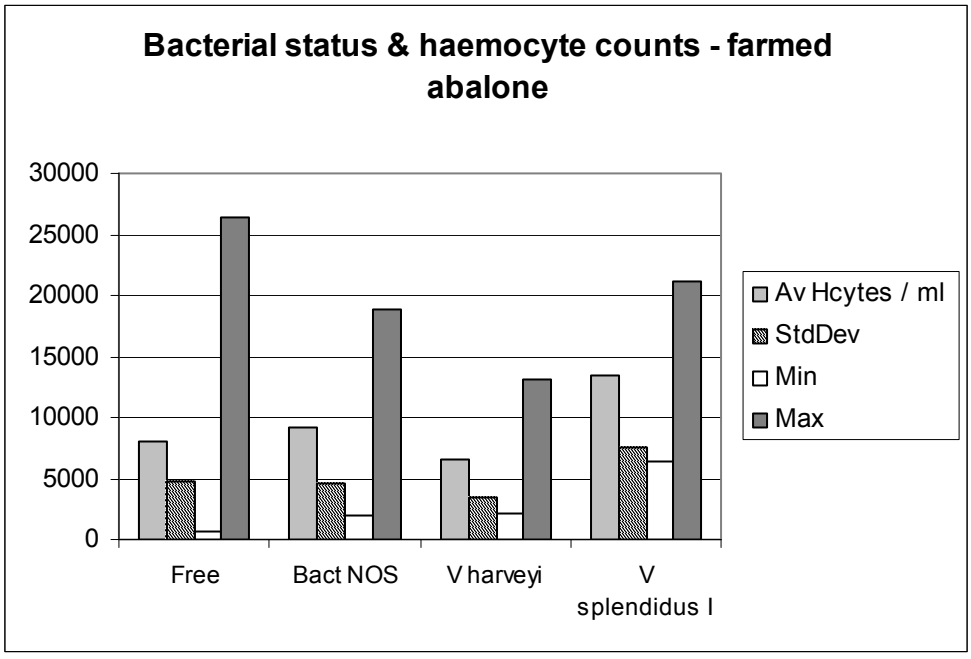


Figure 5.7.5. The effect of bacterial infections on circulating haemocyte counts of abalone free of any signs of infection, or infected with *V. harveyi, V. splendidus* I, or other or mixed bacterial infection not otherwise specified (NOS).

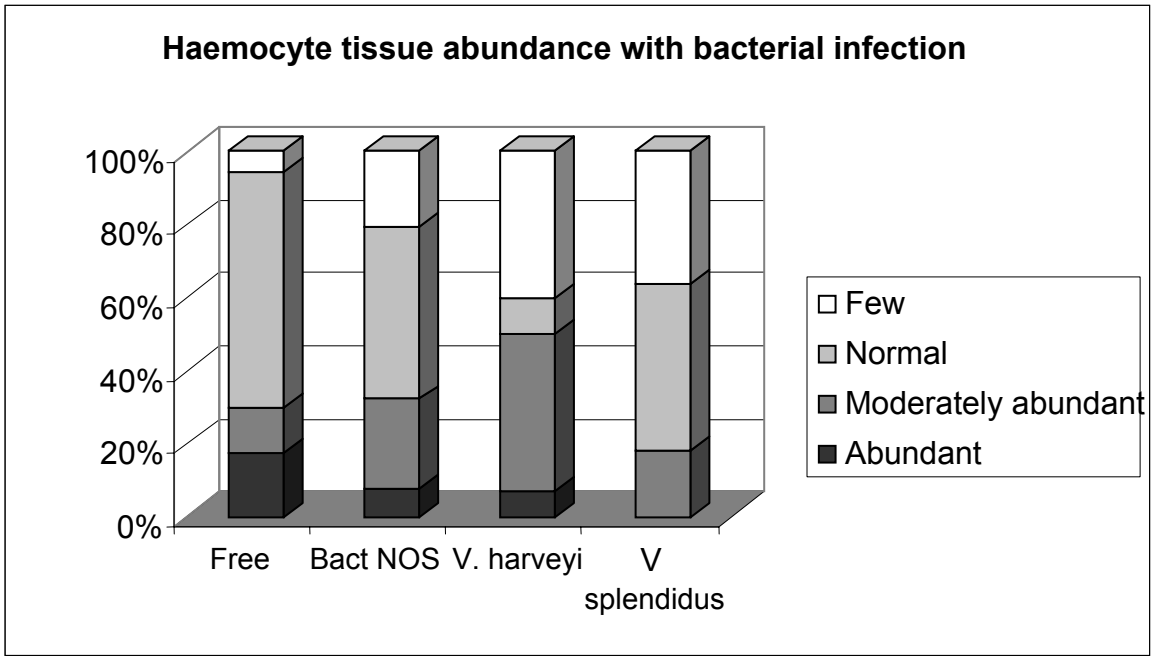


Figure 5.7.6. The effect of bacterial infection on the abundance of haemocyte within the major interstitial beds of farmed abalone.

The overall haemocyte count pattern in farmed animals (Figure 5.7.5) is similar to the haemocyte parasite comparison for wild animals, with the level of within-population variability, even in normal animals, reducing the usefulness of haemocyte counts under these sampling conditions. This comparison has not identified other conditions (including transport stress), likely to affect haemocyte counts or otherwise excluded abnormal counts from the population used as controls for assessing bacterial infection ("unaffected by bacteria", rather than "healthy"). Nevertheless a slight overall reduction in circulating haemocytes was seen with *V. harveyi* infections (where many haemocytes are often sequestrated into abscesses), and an increase in circulating haemocyte in *V. splendidus* infection, which is largely as septicaemic condition. However the histological assessment (Figure 5.7.6) showed an overall decline in tissue abundance in all three types of infection. It appears likely that circulating haemocyte levels are maintained at the expense of haemocyte reserves.

This comparison indicates that all the bacterial infections resulted in a decline in overall haemocyte reserves, although this was not obvious with haemolymph counts due to continued stimulus to mobilise reserves into the circulating pool. Thus direct tissue observation is a more sensitive measure, in terms of timing, for assessing the overall haemocyte competency in abalone. It is uncertain to what extent simple oedematous separation of the interstitial beds would complicate this assessment, but there is evidence that this also occurs (for example, the initial cell-poor increase in lamina propria seen with early bloat-like lesions).

Comparison of granulocytes was undertaken on farmed samples, comparing the level of pigment with and without bacterial infection (Figure 5.7.7), plus other granule and cytoplasmic criteria that were not evident in parasitised wild stock, and rarely evident in wild populations (Figure 5.7.8). This included red staining granules and granules of poor definition giving a diffuse "smudged" appearance to the cytoplasm of these cells.

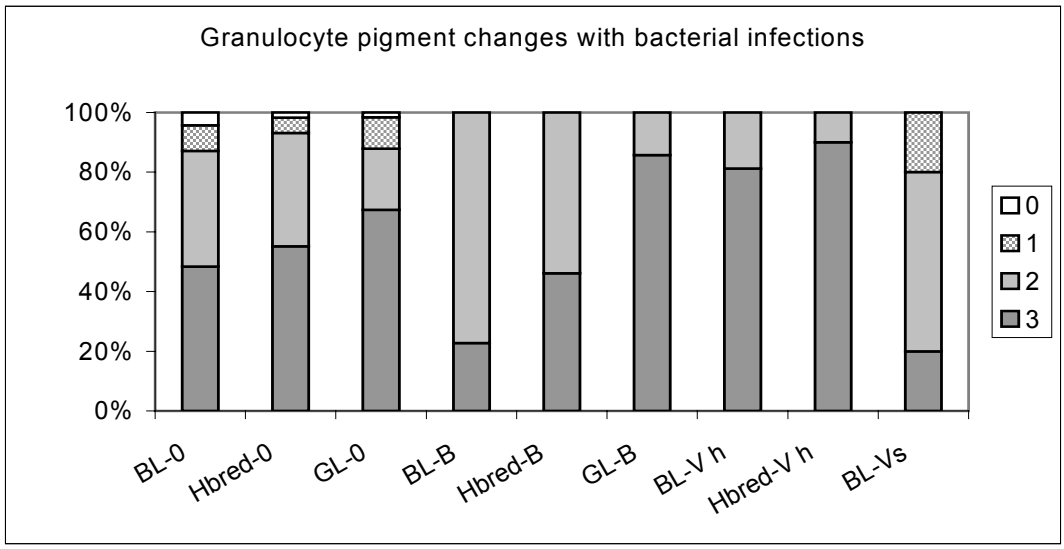


Figure 5.7.7. Comparison of fixed tissue granulocyte pigmentation levels of blacklip (BL), hybrid (Hbred), and greenlip (GL) animals free of bacterial infection (-0), or infected with *Vibrio harveyi (*V h), *V. splendidus* (Vs) on other bacteria (B). Pigmentation Level 0 = no pigmentation (pink to red granules, or little colour); Level 1= mixed pink & brown granules; Level 2= predominantly light brown granules; Level 3, moderately brown; Level 4 dark brown. (n as for Figure 5.7.7)

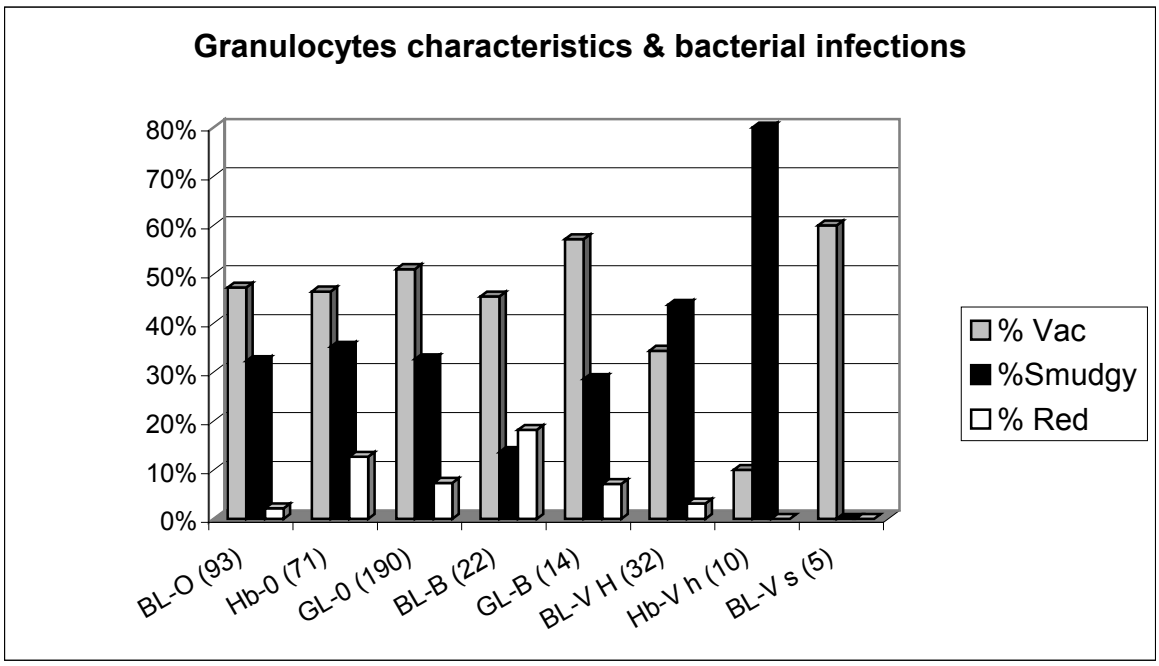


Figure 5.7.8. Comparison of fixed tissue granulocyte granule characteristics of blacklip (BL), hybrid (Hbred), and greenlip (GL) animals free of bacterial infection (-0), or infected with *Vibrio harveyi (*V h), *V. splendidus* (Vs) of other bacteria (B).

The comparison shown in Figure 5.7.7 demonstrates that the overall level of pigment in granulocytes of farmed animals was considerably lower than for wild stock, but how much of this is attributable to age, and how much to the high nutrition status or other farm conditions is uncertain. Pigment levels were considerably lower in the uninfected animals. That the susceptible blacklip population with *V. harveyi* infection shows pigment levels closest to the uninfected animals may appear initially unexpected, but this may reflect the short duration of infection in this species, with a higher proportion of the similarly infected hybrids showing chronic infections. It is notable that animals of all species with other infections (which were generally regarded as opportunistic), also showed a decline in cells without pigmentation, confirming that these are in general likely nevertheless to be pre-mortem infections with some host response. To what extent the granulocyte changes are due to the bacterial infection directly, or reflect other stresses that may have lead to opportunistic infection is not clear. However the different levels of heavier pigment between the species does suggest differences in the dynamics of such infections between the species.

Tabulation of the granulocyte characteristics (Figure 5.7.8) gave unexpected results, compared to impressions from clinical examinations, but interpretation of the variations is unclear.

Both vacuolation and "smudgy" or smudged granules would appear to be normal under the sampling conditions of this survey, though the apparent partial degranulation classed as "smudged" was suspected to be in part related to delays in fixation as well as bacterial infection and possibly pre-sampling stress including transport. All of these could have altered the physiological state of these cells prior to sampling. The current data do not allow a determination of whether either of these impressions is true, and if so whether they are independent or interacting. Further observation during on-going disease investigations is required to clarify this, though the current data-base may assist in this process. Despite the higher than expected background level of the "smudged" change (and the score system did not allow quantification of this), there is a clear increase with *V. harveyi* infection in the hybrids, but not the blacklips. This needs to be considered, in conjunction with the more chronic infections in the hybrids, as to whether this represents a difference in species or in the duration of infection. This appears to be reduced considerably with blacklips undergoing other bacterial infections. Vacuolation appears to be specifically reduced in hybrids with *V. harveyi* infections. I tis not known if this difference in innate, is due to their overall more successful responses compared to blacklips (at least in the two major groups compared from one outbreak), or due to a longer time-frame in hybrids surviving with well circumscribed abscesses.

The deeper red granule staining is still an enigma. It was suspected that this could possibly represent granule regeneration (or new granulocytes), but there is little evidence to support or refute this from the figures shown.

Assessment of parasites of low prevalence

Whether parasites regarded as minor were correlated with any of the general pathology indicators was assessed to determine whether these might have a potential impact on the general health of abalone, beyond the local pathology directly associated with the parasites. The proportion of animals with specific tissue changes and minor parasites were examined for apparent correlations. No unexpected correlations of tissue changes were detected.

Oesophageal pouch parasite

The proportion of specific tissue changes were the same for oesophageal pouch as for animals without these parasites, confirming that these have little impact in infected animals and therefore no expected impact on the population as a whole.

Intra-epithelial digestive gland parasite

A similar analysis was undertaken on animals with the intra-epithelial digestive gland parasites. No obvious effect on health was demonstrated. Half of the 10 affected blacklips showed detectable separation of digestive gland tubules (recognised as a sign of less than optimum feeding – Mouton, 2003). However some dilation of interstitial spaces were more common in wild stock (as is usual for wild animals of any species, compared to optimally fed farmed animals) and only three of the affected animals showed a spacing score > 1. Haemocytes in these animals were normal to moderately abundant. There was some variation in brown pigmentation of granulocytes but only one animal showed marked pigmentation. Occasionally granulocytes were vacuolated, one with red granules. None of them showed the oesophageal pouch parasites so these did not appear to be related. There was very little right kidney pigment to focal to dispersed, three kidney slightly dilated. Overall the impact appears minimal at the levels seen,

Adhered intestinal parasite

With the adhered gut parasite, often associated with foci epithelial degeneration, sometimes to the basement membrane, an impact could be expected if the parasite is widespread throughout the intestine. This possibility is of concern to South African farmers, with an adaptation and build-up of the parasite under intensive farm conditions not unexpected (A. Mouton, pers. comm.).

A comparison of general tissue changes was confounded by the low numbers of positive animals and the apparent geographic restriction within the Tasmanian populations to the Bass Straight area. This presents challenges for transport to the laboratory, and samples from the affected area have therefore undergone transport stress. The affected farm populations are sea farms, which may differ from land based farms in background levels of other parasites. Wild populations with this parasite were also affected with the more pathogenic haemocyte parasite. Nevertheless no unexpected general pathology was observed. The farmed animals with the adhered parasite (12 or 2.5 % of the total farmed population) had slightly higher levels right kidney dilation and diffuse pigment (4 % of each total), and 5 % of the foot sub-epithelial dilations and reactions, but these could reflect the transport stress. The low levels currently present even on sea-farms are not of current concern, but perhaps caution should be applied to any re-entry of sea-caged stock to land-based farms that are currently free of the parasite (in Tasmania, currently all of the land based farms are free).

Assessment of significance of shell lesions

Spionid mudworm shell diseases

Animals with shell lesions were compared with uninfected animals for correlations that warrant further analysis or research, taking severity of lesion into account where possible. This was predominantly undertaken on wild blacklip animals. The sample of wild greenlips was heavily skewed, and farm mudworm problems have already been investigated (Handlinger et al., 2004). Wild animals also showed a greater variety of shell lesions.

The majority (88 %) of wild animals were clear of mudworm. In all, 30 animals were scored as mudworm level one, 16 as two, four as level three (7.1, 3.8, 0.9 % respectively). Thus, mudworm affected animals represented in total 11.8 % of wild stock. As indicative values only, the total counts were compared for a variety of criteria. Mudworm affected animals represented 17 % of those also with foot epithelial loss and 18.2 % with sub-epithelial dilation. Sub-epithelial reactions were not increased (8 % in of mudworm affected animals compared to 10 % for the total wild population). These results suggest that there was no on-going direct surface damage due to mudworms. Both of the wild animals with foot abscess lesions also had mudworm infestations.

Mean foot brown cells were slightly higher overall in mudworm affected animals, but this was due to inclusion of animals with the haemocyte parasite. If the Bass Strait populations were removed, only one animal mudworm affected animal showed this change.

Mudworm affected animals were more likely to have muscle parasites (28 % had muscle parasites in the mudworm affected group). This could be an age-related relationship. Mudworm affected animals were slightly less likely to have gill ciliates (only in 7.1 %), which is unexpected as it was thought mudworm damage increased surface food supplies for commensals such as ciliates.

There appeared to be no relationship with protein deposits. Mudworm affected animals included only 10.3 % of the animals with protein deposition in the gills, 13.6 % of animals with protein deposition in the heart, and 5.1 % of those with this change in the left kidneys. They were slightly more likely to show gill dilation (15.8 % of the affected animals). These correlations did not include any animals with a high score 3 mudworm.

Perhaps surprisingly, mudworm affected animals with dispersed right kidney pigment represented only 7.1% of animals. Lleonart (Lleonart, 2002, Handlinger et al, 2004) found this to be a consistent finding in animals with active shell blisters (that is, those showing active lesions on the interior surface of the shell). This is consistent, though, with other indications from this comparison suggesting the many of the mudworm infections may not be impacting on the health of the abalone at the time of sampling. There was no correlation with animals with a dilation of the right kidney, which is expected as this is regarded as an acute effect, and only 4.6 % of those with focal pigment.

Mudworm affected animals were less likely to show lamina propria changes in the oesophagus (5.6 % of such changes). As this change in wild caught animals is seen as an indication of degeneration of gut contents in recently fed animal held in air, this suggests they could be less likely to have been feeding heavily before collection. However the score for oesophageal degeneration in lightly affected animals was higher (2 compared to 1.1 for other animals with this change). Although two of the four wild animals showing bacteria in this location had mudworms, they were no more likely to show oesophageal degenerative changes.

Mudworm affected animals showed a slightly higher proportion of oesophageal pouch parasites (16.7 %) and gregarines (18.8 %). They were slightly less likely to show perigut reactions (5.4 % of such cases), or gut tubular parasites (in only one mudworm affected animal). Mudworm affected animals appeared to be less likely to show the putative inter-epithelial parasites of the hind gut (7.7 % of these changes). None showed the adhered gut parasites or the flukes in the gut area.

The proportion of animals with an increase in gut tubular spacing appeared unaffected by mudworm presence. However mudworm affected animals included a number of animals with scores of 2 or 3 (that is, marked gut tubular dilation). Both the proportion of animals with increased tubule spacing, and the mean severity of this change were greater at moderate

mudworm levels (mudworm score 2), than with mild infection or mudworm score-3. Indeed if the Bass Strait populations are excluded, none of the four mudworm score-3 animals showed tubule separation. (With the Bass Strait populations included, animals without mudworm had a mean score of 1.4, with light mudworm infestation, 1.5, with heavier mudworm infestations, 1.7.) These differences are likely to be more marked if a tubule separation score of 1 is regarded as normal for wild animals. Overall these findings are consistent with the other indications suggesting that many heavily infected shells (in animals that survived long enough for such levels of infection to build up), were likely to have healed internal shell lesions, not directly affecting the health of these animals.

Other shell lesions such as discoloured shell spots (green to brown) were relatively common in lightly mudworm infested animals. Excluding the skewed Bass Strait populations, other shell lesions were seen in half of the 28 animals with Score-1 mudworm, 12 of 16 animals with Score-2, mudworm infection. These were mainly discoloured spots in animals with light mudworm infestations (plus one limpet, one with barnacles, one with sponge). Animals with moderate infestation (Score-2) showed spots, occasional deformities, limpets, bryozoa, and occasional barnacles. Heavily infected animals showed very few other discernible shell changes other than one animal with sponge and algae.

Bacteriology was carried out on 12 mudworm affected animals. *V. Splendidus* was isolated in significant numbers from one animal from Garden Island. The remainder were negative.

Haemocyte counts were carried out on 11 animals with mild mudworms, three with a mudworm score of 2, plus 120 without mudworm infestation. No haemocyte counts were carried out on heavily infected (score 3) animals, largely because levels of mudworm infestation could not be assessed in live animals. Mean haemocyte counts in the unaffected animals was 6450 (SD=3588), the mildly mudworm infected animals, 10,009 (SD=5405), and in the moderately affected animals, 8,533 (SD=351). This result suggests a bi-phasic response. The severity of infestation was generally less than that of the stunted animals reported to have very low haemocyte counts studied previously (Lleonart, 2002, Handlinger et al., 2004). The high level of variability was due to occasional animals in these groups with very low counts and other apparent pathology. One of these was affected by acute congestive lesions in the gut, slight calcium deposition, and heavy focal lesions at the edge of the epipodium (possibly mudworm related). Another low count was associated with haemocyte parasites from the Kent Group.

Granulocyte appearance was quite variable in mudworm affected animals. An occasional animal from Couta Rock and Rocky Cape showed darkly pigmented granulocytes, as did two animals from the Kent Group. The remainder varied from light to moderately pigmented. Quite a number were pink and brown, brown or vacuolated. The range of appearances recorded varied more by site than by mudworm status. This suggests that mudworm is not the usual determining factor for granulocyte changes. (Note neither was granulocyte pigment indicative of mudworm infection in the Lleonart studies referred to above, despite other indications of chronic ill-health.) The majority showed unremarkable haemocyte distribution, other than occasional focal lesions especially near the mantle that could be directly mudworm related. An occasional animal showed a sparsity of haemocytes. This included one animal that was considered to have bled out after collection, and two unremarkable animals from St Helens Point and an occasional animal from Garden Island and Couta Rocks.

Animals with mild mudworm infestation were of similar length to the population average, averaging 121mm with a weight/length ratio of 2.6 in both unaffected and mildly affected groups. Moderately affected animals were slightly bigger on average (126mm), heavily affected animals were slightly smaller, (99mm, n=4). Given that mudworm infestations generally progress if the animals survive, but that active lesions of the internal shell surface

may be covered by nacre which reduces or eliminates a direct effect on the host, it appears likely that moderately affected animals may be slightly older animals generally coping well with the infection, but that at least some of the heavily infected animals are either younger and unlikely to survive or stunted.

5.6.5 Discussion

The correlations between infectious processes and tissue changes provide the basis for interpretation of pathology and diagnosis of disease. It also provides an understanding of disease interactions. There is still little known overall of abalone response to different disease agents. As well as observable tissue changes, it is likely that a number of specific haemocyte factors are stimulated by specific pathogens (such as innate antibacterial factors previously studied). However, it is clear that most pathogens produce a drain on haemocyte resources, resulting in increased susceptibility to other pathogens, and that the larger fixed tissue granulocytes are also involved with host responses. In these, granulocyte pigment is seen as an indication of the level of on-going rather than very recent damage. The comparisons reported and discussed in section 5.7.4.1 advance knowledge of pathological processes that will aid future diagnoses, as well as providing the basis for the assessment of pathogens detected in the survey (section 5.7.4.2).

The assessment of individual diseases helped to better define the pathology directly and indirectly associated with the invader, which will aid future diagnosis of these infections. Such general indicators may be particularly important for the haemocyte parasite, which may be very hard to detect otherwise, if present in low numbers. Although apparent correlations between an invading organism and general pathology from a single snap-shot survey such as this could be spurious, this assessment goes some way towards an overall prediction of the likely effect of these pathogens on populations. This is valuable for managing endemic infections, as well as being essential knowledge for assessing the risks associated with disease translocation. Defining the pathology associations will provide a useful framework for consistency in pathology reporting of on-going health surveillance, which will aid in fully defining the nature of these changes and a better understanding of the immune responses of abalone.

While general tissue changes such as protein pooling, right kidney tubule dilation, and variations in haemocyte levels have been discussed previously (Edwards et al, 2003, Handlinger et al, 2002, 2005b, Mouton 2003), the examination of pathology indicators clarified the interpretation of several changes. Observations of gill dilation and protein pooling within and around the major vessels have been seen previously, for example with bacterial infection and anaesthetic damage. It appears likely that both are changes of congestion reflecting slower circulation, as occurs with vertebrates. Contraction of the apparently precipitated protein in the days following an acute insult has been observed, sometimes with apparent precipitation of fine calcium deposits, and allows for some aging of these lesions They changes were very common in this survey in most States, almost always as diffuse rather than contracted protein as shown for Western Australian samples (Figure 5.2.23) which is consistent with the suggestion that many are changes induced by transport stress, which may include compromises in water quality, exposure to air or handling damage. Sample handling needs to be assessed in interpretation of such changes. This change was also associated with overt pathology, however, with contracted globules and calcium deposition following protein precipitation in the gut lamina propria.

Bacteria were detected in 20 left kidneys from farmed animals during disease outbreaks, but none from the wild animals. This has also been reported previously during overt bacterial infection (Handlinger et al., 2005b). It is not known whether this is a functional filtration site to remove bacteria via the urine, or an incidental anatomical accident. However this pattern of bacteria within the left kidney would appear to be a reliable indicator of pre-mortem infection, which may be difficult to establish with abundant post-mortem invasion.

The criteria used in South Africa for separation of digestive gland tubules as a measure of starvation appeared to be outside the usual observations of farmed stock in Australia. Part of this may be due to the variability noted between the several parts of the gland usually examined in the survey. This may not be appreciated on routine scoring using one standard section, and suggests finite comparisons between surveillance programs must ensure they utilise the same standard section, or take this into account. Another possible variable may reflect the aim or the South African farm program, which was to obtaining maximum growth. It is uncertain whether the high number of animals scored as showing some increase in tubule spacing reflects species differences, or farmed animals and wild animals at a lower level of nutrition than seen in the South African farms. If the latter, and as most animals were healthy and productive, further work may be needed to determine whether a higher level of nutrition is correlated with more robust health or the reverse.

Both haemocyte comparisons (wild animals with and without the haemocyte parasite, and farmed animals with and without bacterial infections, showed haemocyte counts to be a surprisingly poor measure of overall haemocyte abundance, and therefore immune competency, compared to direct assessment in peri-gut haemocyte bed. Circulating haemocyte levels are likely to be dynamic and influenced by acute stresses such as transport, as well as by chronic infections. The counts are likely to be more uniform if the test was undertaken at the point of collection, and have been used previously as useful clinical indicators. However unless other causes of variability can be identified and taken into consideration, or variability reduced by sampling at source or with minimal post-collection stress, counts on small numbers of animals may be too variable to detect the relatively slight changes seen with both the infections studies in the population as a whole. It is known that this may vary with the nutritional status (Lleonart, 2002, Handlinger et al., 2004), bacterial infection, antibiotic treatment (Handlinger et al., 2006), and other infections (Shields et al., 1996). For haemocyte counts to be useful for clinical assessment (which usually is carried out on small numbers of animals), there is a need to establish the normal range for abalone under different conditions, and to better identify the range of acute handling factors that may affect this.

The overall findings from haemocyte parasite infected populations suggest that the majority of animals in these populations have been impacted by the parasite, with long-term effects on growth and some increase in susceptibility to other parasites and diseases. It is possible that some of these associations, such as the apparent increased susceptibility to gregarine infection, may be spurious, possibly reflecting an increased average age in the source population, which grown slowly and has been little harvested. The high proportion of animals affected, plus the long-term impacts with a consistent increase in tissue pigment and sometimes multiple growth checks visible in shells suggest on-going infection in most animals, with a reduction in numbers (whether total or not has not been established) after a haemocyte crisis. The likelihood of this is reinforced by the somewhat similar levels of infection persisting in the animals held for some months in the experimental system. Increased mortalities were seen with some intakes of these animals, and in the Clarke Island group, but overall the effect would appear to be one of poor productivity than of mortality. This would seem to be reflected in the reduced legal size for blacklip animals from the Bass

Straight zone (Figure 5.6.3). Note that the adjacent Northern zone (shown to be infected at least near the Western boundary of the Bass Straight zone, though with less severity), also has a slightly reduced legal size. Further sampling in this zone is needed to establish the geographic margins of the infection.

These changes will be illustrated in detail in the accompanying Atlas of Diseases and Pathology of Abalone (from an Australian Perspective).

5.7 SUMMARY OF SURVEY RESULTS ACROSS AUSTRALIA

5.7.1 Overview of infectious diseases and parasites

Samples examined are summarised in Table 5.8.1, The results of all disease agents detected are summarised in Table 5.8.2 (wild stock), and Table 3 (farmed stock). Each agent has been discussed in details in State survey reports, with an overview below.

Table 5.81. Samples examined by State, species and origin

	Wild sites	Farmed sites	No. abalone	Total
Tas	11 *H. rubra* 2 *H. laevigata*	9 (Blacklip, Greenlip & hybrids)	387 wild *H. rubra* 9 *H. rubra* of wild origin (held several months) 8 wild *H. laevigata* 21 *H. laevigata* of wild origin (held several months) 159 farmed *H. rubra* 215 farmed *H. laevigata* 113 farmed hybrids & mixed	912
Vic	5 *H. rubra* 2 *H. laevigata*	5	150 wild *H. rubra* 60 wild *H. laevigata* 90 farmed *H. rubra* 150 farmed *H. laevigata*	450
SA	9 sites: 3 *H rubra* *4* H. laevigata *2* mixed	9 farms *H. laevigata*	118 wild *H. rubra* 156 wild *H. laevigata* 236 farmed *H. laevigata*	510
WA	22 sites	2	240 *H. roei*, 264 wild *H. laevigata* 9 wild *H. conicopora (*2 sites) 12 wild tropical *H. asinina* 162 farmed *H. laevigata*	687
NSW	15 Blacklip (Eden to Crowdy Head)	2 (hatchery)	407 wild *H. rubra* 99 *H. rubra* hatchery 1 y.o 98 *H. Coccoradiate* spat	604
Total			Plus opportune extras*	3163*

*Opportune extra samples (not included in the above) include the SA farm samples examined at the start of the survey by Dr Anna Mouton and Dr J. Handlinger, and post-survey samples from Victoria examined after feed related mortality and fungal infection.

Table 5.8.2. Pathogens and parasites recorded from wild stock

Findings - wild stock	TAS	NSW	WA	SA	Vic
	425	**407**	**525**	**274**	**210**
Perkinsus					
Perkinsus culture	Negative	pos (12)	pos (1 population)	Negative (known to occur)	Negative
Perkinsus by histology	Negative	pos (16, 0-25%)	Negative	Negative	Negative
Inclusion-like bodies					
Virus-like inclusion I - GIT	Uncommon (30, 5 BL sites, max 27%)		Rare		
Virus like inclusion II - granulocytes	Rare (9, 4 sites, max 11%)				
Bacterial infection					
GIT Rickettsia like organisms	Rare (8, max 3 of 7 sick GL)		Rare (10)	Rare 13, 4 sites)	
Gill Rickettsia like organisms	0		Rare (9)		
Vibio harveyi type bacterial abscesses	0				
Vibrio type bacteria NOS	Rare 16, incl. moribund, sampling)				
Flavobacterial disease	Rare (3, max 2/2 live sick GL)				
Fungal infection					
Foot tubercle lesions	0 (known to occur)	0 (known previously)			Post-survey of uncertain origin.
Protozoa & protozoan like parasites					
Holotrich ciliates on gill and / or foot	Common (84, max 48%)	Common most groups	Common (max 80%)	Usually rare (33%, 1 site)	Variable (0 to 87%)
Holotrich ciliates in upper GIT	Moderate (61, 12 of 13 sites, max 43%, healthy)				
Peritrich attached ciliates	v Rare			Rare (2)	
Intrapithelial / digestive gland small ciliates	? artefact or inclusion (34,		Uncommon (2 sites, up		

gland small ciliates	max 44%)		to 22%)		
Intracellular parasites of digestive gland	Rare (13, max BL 9%, GL 3 of 8 sick - 38%)		Uncommon (2 sites, Greenlip up to 25%)	Rare (5 sites)	
Suspected digestive gland coccidia			(Rare, uncertain)		
Adhered cryptosporidia-like parasites of GIT	Rare (6, 3 sites)		Rare (4)	Rare (9 from 2 sites)	
Oesophageal pouch epithelial parasite	Moderate (48, 12 of 13 sites, max 24%)				Rare (1)
GIT Epithelial parasites NOS	Rare (26, 9 sites, max 7 of 40)				
Haemocyte apicomplexan	Restricted (2 BL 33 & 53% & GL, 33% & 6/8 sick)				
Nematopsis-like Gregarines in interstitium	Rare (16)	Rare	Rare (2)		
Renal coccidia	Rare (1)				
Surface associated Parasite NOS	Rare (9)				
Metazoan parasites					
Fluke metacercaria of gonads & kidney	o (known to occur)		Common (12 of 22 sites, up to 45% / site)		Rare (3)
Fluke metacercaria / metacestodes of foot & interstitium	Rare (19)		Common (Most wild sites, up to 33% / site)	Rare (3)	Rare (2)
Nematodes	0			Rare (2)	

Table 5.8.3. Pathogens and parasites from farmed stock

Findings - farmed stock	TAS	NSW	WA	SA	Vic
	487	**197 hatchery**	**162**	**236**	**240**
Perkinsus		No pathogens reported			
Perkinsus culture	Negative		Negative	Negative	Negative

Perkinsus by histology	Negative	Negative	Negative	Negative	Negative
Inclusion-like bodies					
Virus-like inclusion I - GIT	0		Rare (1)		
Virus like inclusion II - granulocytes	0				
Bacteria					
GIT Rickettsia like organisms	Rare (5)				
Gill Rickettsia like organisms	0				
Vibrio harveyi type bacterial abscesses	42 (biased investigation samples, 2 farms)				
Vibrio type bacteria NOS	64 (biased investigations)		Past infection Suspected	Rare	
Flavobacterial disease	Rare (3)				
Fungal infection					
Foot tubercle lesions	Rare (1)				
Protozoa & like parasites					
Holotrich ciliates on gill and / or foot	Rare (5)				
Holotrich ciliates in upper GIT	Rare (1, pouch)				
Peritrich attached ciliates	0				
Intraepithelial / digestive gland small ciliates	suspected (10)		Rare (1 farm, 23%)		
Intracellular parasites of digestive gland	Rare (1, sea farm)			Rare (1 farm)	
Suspected digestive gland coccidia				Rare (2)	
Adhered cryptosporidia-like parasites of GIT	Sea farms (12 & 4 suspicious)		Rare (1)	Rare (9 from 2 sites)	
Oesophageal pouch epithelial parasite	1 sea-farm (8), sporadic				Rare (1)

	suspicion (12)				
GIT epithelial parasites NOS	Rare (7)				
Haemocyte apicomplexan	0				
Nematopsis-like gregarines in interstitium	Rare (13, max<1%)	Rare	Rare (2)		
Renal coccidia	0				
Surface associated parasite NOS	Rare (3, gill)				
Metazoan parasites					
Fluke metacercaria of gonads & kidney	0				Rare (3)
Fluke metacercaria / metacestodes of foot & interstitium	Rare 3 (1 sea farm)			0	Rare (2)
Nematodes	0				

5.7.2 Overview of non-infectious insults and tissue changes

The summary from the Western Australian survey, that general tissue changes and findings of a non-infectious nature included frequent accumulation of highly eosinophilic staining haemolymph in vascular channels of multiple organs systems, and digestive system distension and related degeneration related to bloat or retained gut contents, was found to be common to samples in most States, both wild and farmed. These changes are interpreted as an indication of acute tissue damage, and is suspected in some cases to reflect farming system stresses, such as poor water quality, and in others the stress of handling post collection where immediate on-site fixation was not possible. Protein pooling was also seen with bacterial infections.

More chronic changes of a non-specific nature included granulomatous reaction through the foot or gut. Some of these cases were related to ingestion of spicules of algae or sponge, while recent bacterial infection was suspected in other cases. Indicators of general poor condition included separation of relatively inactive tubules of the digestive glands, and pigment accumulation in fixed granulocytes, in focal cellular accumulations, in epithelial cells of the right kidney, and occasionally around nerve ganglia.

Mineralised concretions were seen in the digestive glands. While other influences including diet are suspected, this change may in part be an artefact of delayed fixative penetration.

These changes were analysed in more detail for the Tasmanian samples in Chapter 5.7, but the general results of that analysis would be applicable to all States.

6 GENERAL DISCUSSION

6.1 Rationale for sampling strategy

6.1.1 Rationale for sample size

Except for small populations, the number of samples required for detection of a disease with any desired level of confidence is largely independent of population size, but is dependent on the expected prevalence of the pathogen, if present (Simon and Schill, 1984). The primary aim of the sampling strategy was to maximise the likelihood of detection of serious pathogens, defined as those capable of achieving a biologically significant prevalence (defined for this survey as 10%) of a population. If associated with significant pathology, diseases at this prevalence could be expected to have a serious outcome at the population level. In 95% of such cases, infection of at least one infected animal within the population would be detectable in a single random sample of 30 animals, provided there is a high test efficiency (Cannon and Roe, 1982). Diseases at higher prevalence are likely to be detected with greater confidence in a sample of 30 animals.

6.1.2 Population sampling

Larger samples (for example, the pooled results of several clusters within a population) will also increase the overall level of confidence. Natural abalone stocks are semi-continuous and overlapping. Animals are sedentary, with few known natural borders between populations. With these characteristics, individual populations within the stock are difficult to define (and may not exist). Genetic studies in progress suggest that for both major species there is a broad population continuum, though with varying degrees of gene flow among sites and some genetic drift over distance. These population characteristics increase the confidence of the survey as in many respects each species could be considered as one large population, possibly ranging from NSW to WA.

Given the need to collect samples by diving, neither true individual random sampling across the whole population nor individual systematic sampling were realistic options for this survey. To offset this limitation, a higher level of sampling of sub-populations (defined by site for sedentary animals) was generally carried out. Cluster sampling (in this case, random sampling of 30 animals from within groups of abalone, at various locations, from farmed or wild stocks) is a recognised and recommended alternative to simple random sampling for selection of individuals or sample sites (Steel and Torrie, 1980, Tietjen, 1986, Thompson, 1992).

Results have been collated into data bases that can be further analysed on the assumption each cluster represent discrete populations, or that they represent a cluster within a larger population. Using the former assumption, the data is primarily referable to the individual site, and the sample size of 30 provides limited data on each site. Analysis as a cluster, rather than a discrete population, is based on the assumption that animals from each site are more similar to each other than to the population generally, but are still part of the same population. If cluster samples are small (eg 10 animals), the results can only be analysed with confidence regarding the larger population, with little information derived from individual sites.

The analysis of cluster samples is more complex than with simple random sampling, but standard statistical analysis techniques, like generalised linear mixed modelling, are available for such data. Selection of a sample size of 30 for each site provides prevalence information for individual sites (for diseases with a high expected prevalence, around 10%). This would also need to take into account an estimate of test sensitivity, which will vary between diseases. Definitive knowledge of sensitivity is not always possible, but the best estimate is likely to be made during or at the completion of the survey using techniques such as multiple sections if estimation is critical. This sample size also allows analysis of site effects within the context of the larger population, provided there are no major spatial differences in prevalence. If there were spatial differences, this would indicate the species has become differentiated into true sub-populations, at least at this point in time, with regard to a particular pathogen. Typically cluster sampling may utilise either random or systematic sampling for site selection. In most States, selection of sites was systematic, as samples were collected in conjunction with existing population surveys. Such surveys generally have established reference sites selected on a systematic basis over habitats suitable for the species.

6.1.3 Rationale for bias

Biasing towards diseased individuals and populations is an internationally accepted technique to counter errors resulting from failure to achieve true random sampling (which tend to decrease confidence in such results), to ensure maximum confidence in negative results, and is in line with OIE general recommendations for mollusc sampling (Anon, 2000), that sites be selected "in the most practical way" to maximise the chances of detecting pathogens.

Advice from epidemiologists and biometricians, both externally (Dr Rob Cannon of Agriculture, Fisheries and Forestry, Australian and Dr Chris Baldock of AusVet Animal Health Services) and from within DPIWE, was that this strategy is appropriate for maximising the detection of serious diseases in populations with these characteristics. The results are suitable for epidemiological and statistical analysis on an as-needs basis, to quality the likelihood of a particular infection in a particular area for translocation risk assessment, for example. Estimates of the level of confidence over larger areas can be gained through pooling of results from adjacent sample sites of continuous populations without natural population boundaries.

This survey was a preliminary study, with the aim of maximising knowledge of diseases present, rather than meeting international standards of proof of absence. However, it is noteworthy that the survey strategy provides data similar to that used by which France to claim recognition of disease freedom for Pacific oysters (Thebault et al., 2001). In this survey, the French used cluster sampling of 30 animals per site for 100 sites.

6.2 Constraints

6.2.1 Constraints in obtaining samples from *Perkinsus* affected areas

Limited funding for sample collection presented difficulties in ensuring uniformity of sampling strategy and sample quality for wild abalone. Available opportunities for sample collection ranged from specific sampling trips funded by State Government and other bodies, sample collection during stock assessment, and collection by commercial divers during normal

commercial activities. Collection in conjunction with commercial dive activity (as used in South Australia) resulted in a bias away from areas of poor production, and an omission of areas of known *Perkinsus olseni* infection. This prevented further comparison of pathology and secondary factors in South Australian and New South Wales *Perkinsus* infected areas. Given the apparent differences in pathological expression, and the limited histological examination of abalone from the SA affected areas since the initial studies, it is recommended that this comparison be continued whenever samples from this area are available, and that future studies of *Perkinsus* from South Australia include histological samples.

6.2.2 Optimising fixation

Collection during routine stock assessment, as used in Victoria, resulted in a delay before submission to the laboratory, overcome by fixation of whole animals by survey collection teams. Restrictions on the use and storage of fixative on board and the need for gross examination and comprehensive histological sampling limited the opportunity for further sample preparation on site. The delay in penetration due to retention of the shells compounded general problems of adequate fixation for the soft tissues of large abalone, particularly of the digestive gland. With the high digestive enzyme level of the digestive gland, these cells generally show some degree of degeneration with normal fixation regimes, particularly with large animals. This is compounded if there is a reduced volume or concentration of fixative, as apparently occurred with some samples from Victoria. Attempts to reduce the penetration time by sectioning the digestive gland prior to fixation (a standard practice with tissues of most animals), is problematic in this species as the soft gland tends to fragment readily. For this reason, the digestive gland has sometimes been omitted in abalone disease or histology studies. An example is the initial studies of withering syndrome; this resulted in a delay in recognition that the digestive gland was the primary organ affected by this disease. Excessive trimming of solid muscle tissue resulted in distortion of the soft tissue during fixation that prevented standardisation of sections. Optimal fixation of the digestive gland for this survey (where maximum section size of multiple organs was required to maximise pathogen detection) involved shell removal, trimming of excess muscle, and sectioning from areas near the surface of the digestive gland not covered by active gonad. For specific examination of the digestive gland under non-survey conditions, where uniformity of the gland is assumed and smaller sections are sufficient for examination, a suggested method is short fixation with such a preparation to harden the tissue and prevent distortion or fragmentation, followed by fixation in the cassette to maximise penetration rate.

Fixation also appeared to be a factor in the development of the refractile residual bodies or concretions of digestive gland cells, although the increased prevalence of these in some groups of sick animals suggested the possibility that the internal cell changes induced by slow fixation could also occur pre-mortem. Slow penetration time is exacerbated where the animals are fixed whole without shell removal, as occurred with wild Victorian samples collected from remote sites where fixation on site in bulk was the only practical method of obtaining samples in a timely manner, as fixative can penetrate from only one surface. This may have been compounded by a dilution error in some samples. Coincident with this was the finding of high numbers of so-called residual bodies or concretions. These bodies have been described previously been by Diggles et al., 2002, whose electron microscopic examination confirmed the inanimate nature of these particles as concretions within the digestive gland cells. Residual bodies were also common in farmed stock from Victoria, which may also indicate fixation differences as these animals were regarded as otherwise healthy. In Tasmania this change was virtually restricted to farmed hybrid and greenlip animals with bacterial infection and wild-

origin greenlips with the haemocyte parasite and other infections. This suggests that the physiological state of the digestive gland cells at the time of sampling may also be a factor. It is considered likely that these bodies represent the result of metabolism of the cells of the digestive glands at or near the time of fixation. At this stage it is not known whether this may occur pre-mortem, or may be induced by continued enzyme activity during slow fixation. They are regarded as a potentially useful indicator of physiological status of these cells, but are not infectious.

Slow fixation may also in part account for the increased basophilic staining of Victorian survey sample sections compared with those from other sources and tissues from other types of animals at this laboratory. Such differences in staining are a common finding with aquatic animal tissue, though rarely to the level seen in these samples. It is recognised that generalised stain shifts of this nature may reflect slight differences in stain composition or aging between laboratories, which cannot be standardised despite using overall comparable methodology. Though the exact reasons are rarely determined, laboratories are usually able to overcome these with slight changes to staining procedures, as indeed this laboratory has now done for subsequent abalone samples. It is likely therefore that there was a reduction in the sensitivity of at least the initial Victorian samples due to a combination of fixation factors and laboratory stain variability, which could have compromised the detection of low numbers of small parasites. However this would not apply to larger parasites or to associated pathology indicative of significant disease.

6.2.3 Availability of taxonomic expertise

This survey tabulated the extent of shell lesions, and of internal metazoan parasites. In most States, an attempt was made to speciate the major spionid species present, though in Victoria the shells were made available to other programs and no results are currently available. The other organisms identified on the exterior of abalone shells were not thoroughly speciated due to the difficulty of classifying sponges and other marine fouling organisms. Similarly, only perfunctory identification of metazoan parasites was carried out on metazoan parasites of soft tissues. This was also partly due to a lack of availability of specific expertise in this area, and partly due to inherent difficulties in identification of such parasites where only the intermediate stages of infection are available. Therefore the data to allow identification of such parasites is limited, and any differences in species distribution between areas would not be apparent.

6.3 Overview of pathogens and parasites detected

This focus of this survey was to define the disease agents present, but particularly diseases of economic significance. A prevalence of > 10% and clinical significance in these animals may be regarded as economically significant. Few of the agents detected reached this level of infection, or were demonstrated to have a significant adverse effect in infected individuals, other than pathogens known previously, such as the known bacterial diseases of farmed stock (described by Handlinger et al., 2002, 2005), *Perkinsus* infection in NSW (also recognised in South Australia), shell diseases, and one new parasite of the haemocytes from an areas of Bass Strait with historical evidence of stunted stock.

Infectious agents found could best be classified as either agents of little concern that are common in other molluscs, ubiquitous agents with at least secondary pathogen potential, incidental agents of minimal importance recognised previously from Australian abalone, abalone specific parasites with little pathogenic potential, new agents whose pathogenicity needs to be determined, and pathogens of undoubted potential and probably limited distribution.

6.3.1 Infectious agents of minimal concern

Agents of little concern common to other molluscs were generally found throughout the survey, and included surface and epithelial ciliates, gregarines, trematodes, and the non-pathogenic rickettsia-like agent. The only finding of note is the relatively high level of metazoan infection (mainly trematodes) from the Western Australian population. The reason for this is not apparent. Nor is it practical to speciate the parasites based on their histological appearance, and the number of species involved (in any area) is uncertain. Despite the markedly higher levels found in WA, no significance was attributed to these parasites, other than mild inflammation in migratory tracts, which sometimes included the per-neuronal sheaths. Such parasites are common in most mollusc groups, and are of little consequence unless infection levels are sufficiently high to either affect product quality through visible gross effects, or to result in sterilisation if present at high levels in gonads, or in some cases to prevent the ability of bivalves to close the shell.

6.3.2 Parasites that appear to be specific to abalone

Agents that appear to be specific to abalone include the cryptosporidia-like and intracellular digestive gland parasites, and probably the oesophageal pouch parasite, none of which could be fully classified by light or electron microscopy. These had been reported only from abalone in the Southern Hemisphere, and were considered likely to represent a suite of parasites restricted to this group and therefore suggestive of a prolonged common heritage. However the adhered cryptosporidia-like rectal parasite has been seen in red abalone in the United States (Friedman, pers. com.), and in red abalone in Chile derived from imports from the United States (Avilés, 2006).

The cryptosporidia-like rectal parasite and the intracellular parasite of the digestive tubules are both enigmatic parasites of unknown affinity, that have nevertheless been seen commonly in abalone farms in South Africa (Mouton, 2000). The digestive gland parasite appears also to have been seen previously in New Zealand (B. Jones, pers. comm.). Both are rare, and currently very rare on farms, though sea caged stock is more vulnerable. There is little pathology associated with either, though the epithelial erosion associated with the cryptosporidia-like parasite is likely to be significant if high levels of the parasite are present. There is some concern by South African farms that these parasites may become more adapted to farm conditions, and may represent the pathogens of the future (A. Mouton, pers. comm.). For this reason it is recommended that where possible these parasites be excluded from farms, or their spread on farms limited. This is likely to be a realistic aim for land based farms.

The other new parasite detected, the undefined protozoan like parasite of the oesophageal pouch, appears to have minimal clinical significance. It is widespread in clinically healthy and highly productive Tasmanian stocks, and is apparently present in Victoria, though at low

levels. Whether this defines the range of this parasite is uncertain as the oesophageal pouch was not a specific target organ, and the areas of infection are small and easily overlooked. It would appear likely that this parasite is the same parasite as that described as coccidia in the oesophageal pouch in New Zealand abalone by Diggles et al., (2002).

6.3.3 Ubiquitous agents with secondary pathogen potential

Ubiquitous agents with at least secondary pathogen potential probably included the majority of the spionid mudworm found, sponges, most *Vibrio* species and *Flavobacterial* bacteria. The distribution of most of these bacterial species is not established as few were speciated and criteria to do so are not readily available for many species, though this is under study for *Vibrio* species (Carson et al., 2004). Only sporadic cases of the *Flavobacterium*-like infections were seen in this survey, conforming to the two patterns of pathology that have been described previously from Tasmania. No pathology other than apparently terminal effects was seen in abalone from with *Vibrio* species were isolated, other than as described below.

6.3.4 New parasites of apparent pathogenicity.

The various parasites recorded in this survey in general appear to have little significance though some caution has been voiced on the long-term advisability of excluding them from intensive farms if possible. The apparent exception is the haemocyte parasite from Bass Strait, which was shown to be capable of infecting at least 50% of animals in a population (and possibly a higher proportion in populations of very low numbers) with an apparently persistent infection. Historical and clinical evidence suggests this is associated with poor growth, poor quality meat and poor survival following movement stress. However mortality appears low in unstressed stock as large stocks of stunted animals are still present in infected areas and several animals showed multiple growth checks in the shell, suggesting prolonged or repeated infection. The overall effect on productivity appears to be marked, with minimal harvest from the most affected area over a prolonged period, although it is uncertain if this is due only to this parasite. The geographic margin of the infected area is unknown, but it may be of significance that the legal harvest size for *H. rubra* is not only decreased in the recognised stunted Bass Strait zone, but also in the adjacent Northern Zone, where the parasite has been confirmed in both Eastern and Western portions near the margin with the Bass Strait Zone.

Given the prevalence in infected areas, this parasite should be considered as a translocation risk from these areas, with likely effect on productivity in both wild and farmed stocks, unless proved otherwise. The life cycle of this parasite is unknown. Establishing this may be necessary to accurately assess the translocation risk and some mollusc parasites depend on apparently geographically restricted intermediate hosts. If direct mortality is low, and the life cycle permissive of translocation, infection in recipient populations is likely to be difficult to detect until high levels of infection have been established. Further studies of this parasite are indicated, particularly with regard to direct mortality, life cycle and seasonality of infection level, to define over what periods test for this haemocyte parasite are appropriate.

Given the difficulty of detecting low levels of infection, further studies of the distribution are also recommended, with emphasis on stunted stock and those showing similar tissue reactions. Stunting is recognised in Victorian stocks, though they were not apparently included in the survey or samples submitted during a recent kill-out operation. Stunting of ill-defined cause was also seen in the WA population returning a positive Ray's thioglycollate test on gill sampling. This test is used to detect sporulating stages of *Perkinsus* infection, but as the

specificity is low and there was no histological evidence of established internal infection, in the initial sample or on re-sampling, it is not known if the Ray's test detected *Perkinsus* or a similar dinoflagellate organism. Retrospective examination did not detect the haemocyte parasite. However whether the time of sample collection was appropriate for this parasite is as yet unknown. Thus the cause of stunting in this population is still unknown, and a general recommendation would be to further investigate the cause of stunting in all such population.

6.3.5 Pathogens with established disease potential

Pathogens of undoubted disease potential and probably limited distribution include *Perkinsus olseni,* possibly pathogenic strains of *V. harveyi* and of *V. splendidus* I, and possibly the spionid *Boccardia knoxi.*

6.3.5.1 Perkinsosis

Perkinsosus in NSW was the major wild disease studied (presumably due to *Perkinsus olseni)*. *Perkinsus* was the only pathogen identified by the NSW study that appeared to be responsible for significant damage to the host> it was concluded that this disease is a significant factor in the 95 % decline in abalone populations between Port Stephens and Jervis Bay since the first recorded NSW outbreak of Perkinsosis in 1992 at Terrigal, just north of Sydney. The pathology differed from that reported in South Australia, with the NSW blacklips apparently unable to wall off and contain the parasites, dying without the abscesses seen in SA. This pattern of mortality is not one that has been matched in other infected abalone populations where *Perkinsus* is known to be present. This suggests that either the NSW host is more susceptible or the pathogen is more virulent, or both.

Apart from the presence of the parasites, histological indicators for NSW *Perkinsus* affected stocks were somewhat similar to those of the Tasmanian animals chronically infected with the haemocyte parasite, though there were some differences, such as the larger number of granule in some pigmented fixed granulocytes. While this confirms the general nature of these pathology indicators, it is still uncertain if these are a direct consequence of infection, or reflect other stresses that may favour *Perkinsus* infection.

The triggers for the outbreaks of *Perkinsus* related mortality events are not known. Anecdotal reports over recent years suggest that the progressive depletion may have been due to expansion of the range of the pathogen during times of favourable conditions for disease epizootics. The findings from this survey suggest the prevalence of perkinsus across the NSW affected range is highest at the margins of the infected zone. This would be epidemiologically consistent with a propagating epizootic, entering a naive population of stock.

The hypothesis of exposure of a naive population exposed to a new pathogen could reflect differences in species or strain, as well as new incursions of *Perkinsus*. *Perkinsus* organisms have been found to be widely but not uniformly distributed around Australian mainland coast. Organisms con-specific with *Perkinsus olseni* have affected abalone in South Australia and at least one other Australian mollusc. It has also infected a range of mollusc in New Zealand, Korea, Japan, Portugal, France, Italy and Spain, based on analysis of two relevant DNA sequences (Anon, 2003). However uncertainties must still remain as to whether all the Australian *Perkinsus* organisms are of this species, or whether more minor strain differences play a part in determining pathogenicity in different host species. DNA sequencing of the *Perkinsus* strains has not been performed to determine to whether the WA, Qld, SA and NSW

strains are identical. Hence a strain difference cannot currently be excluded as an explanation for the NSW mortalities. If strain differences do play a part in pathogenicity, translocation or *de novo* strain transformation of an abalone-adapted strain into a naive population could also explain this pattern.

An alternative hypothesis is that the pathogen has been present along the entire NSW coast in abalone, or other mollusc populations for a long time, but that conditions in recent years have favoured the parasite (or disadvantaging the abalone). Given the historical evidence of organisms of this type in Victoria, Western Australia, South Australia and Southern Queensland, and the multiple species of potential hosts, a broad distribution of the parasite is quite possible. Due to the relatively low level of sampling per site (~20-30 animals) and limited host range tested (only abalone), the disease would be unlikely to be detected in this survey if present at low levels. Further survey work may clarify the true distribution of the parasite. This survey, however, has defined the current distribution of the disease perkinosis in abalone, and confirmed that when internal invasion is present, this is likely to be present at relatively high prevalence (mean prevalence per infected population of 19 %). The true nature of the positive Ray's test is also uncertain, as is the implications for translocation if *Perkinsis* organisms are present as surface inhabitants.

Thus further work on the epidemiology and pathogenesis of perkinsosus in blacklip abalone is warranted to adequately explain the losses in wild NSW blacklip abalone fisheries, and to better define the overall risk pattern for infection in this species. Some work is currently under-way, repeating and enhancing the 2002/3 survey.

Another potentially useful general finding from this survey was that histopathology, using this range of sections, appeared to be at least as sensitive as the Ray's thioglycollate test for detecting *Perkinsus* infection. The major advantage of the Ray's test is generally regarded as one of reduced cost, rather than sensitivity, although previous studies with *Perkinsus marinus* in oysters suggested histopathology to be a less sensitive technique than sub-sample thioglycollate tests for that organism (Ford and Tripp, 1996). The relative sensitivities will be influenced by the size of *Perkinsus* stage present and the ease of visualisation, the relative proportion of tissue examined by both methods, the expertise of the pathologist and the likelihood of indicative lesions if infection is present. As *P. marinus* is a smaller organism, and usually systemic rather than associated with distinct focal lesions, these are all factors that suggest histological diagnosis for *P. olseni* infection in abalone may compare favourably with this technique for *P. marinus* diagnosis. As the numbers tested from infected populations was low, further information to confirm the sensitivity of histopathology for this disease would be valuable, especially where diagnostic histopathology is routinely undertaken for health monitoring. This would enable to level of assurance of *Perkinsus* status to be more easily determined without the additional cost of thioglycollate tests.

Development of specific tests for this pathogen, with specificity criteria, would assist in work to explain both the NSW and equivocal Western Australian findings, and confirm the species involved in NSW. In general it is accepted (Anon, 2003), that in areas with no previous history of a disease, or in other situations where specificity is critical, a positive finding on a test with low specificity (such or a test detecting DNA fragments or morphological features that could be shared by other organisms), should be confirmed with a second type of test. This was attempted using histopathology to confirm the positive thioglycollate test on superficial gill tissue in WA (which could have been due to other similar dinoflagellates), without success. This may have be due to the relatively insensitivity of both tests, to very low levels of *Perkinsus* infection, to surface colonisation only with *Perkinsus*, or to non-*Perkinsus* organisms. Thus more specific tests of high sensitivity would be valuable for confirmation of suspicious cases.

It was hoped that a direct comparison could be made during this survey of detailed pathology and tissue responses of *Perkinsus* infected abalone in NSW and South Australia, but resource for collection of samples from the SA affected area were not available. Should this be possible in the future, it is recommended that samples be collected for a direct histological comparison, plus strain comparison if possible. Understanding the apparent conundrum between an apparently widely distributed parasite and sporadic but dramatic mortality is essential for determining the level of risk through stock translocations, and the extent of cross-species risk.

6.3.5.2 Bacterial diseases of significance

Survey results indicate that the other OIE listed abalone disease, withering syndrome, is not present in Australia, though low levels of rickettsia / Chlamydia like organisms were present, unassociated with pathology. Should higher levels of these organisms be found in the future, specific tests to exclude the withering syndrome organism may be advisable.

Results confirmed that the major current problems on farms are those relating to bacterial infections, often with a non-infectious stress component. Although the most significant pathogen is *V. harveyi*, there is little information on bacteria from States other than Tasmania. *V. harveyi* infection was seen in Tasmania and Victoria during this project, is reported to have occurred in Western Australia (N. Buller, pers. com.) and has previously been reported from South Australia (Reuter and McOrist, 1999). Parallel research indicated that the *V. harveyi* and *V. splendidus* strains isolated from Tasmanian abalone are specific strains which so far have been isolated only from diseased abalone (Carson, 2004) However there is no information on strain type of isolates from other States, and therefore no indication of whether these are widespread strains or represent a disease translocation risk. However as *V. harveyi* infection has been shown to persist, probably within abscesses, with a risk of secondary outbreaks and further spread following stress, farms so far free of this disease should in any case exercise caution with stock from infected farms. *V. splendidus* infection has not been shown to be persistent. There is some evidence from gut cultures from this survey that *V. spendidus* may be a common gut inhabitant, but there is insufficient data to establish whether this is normal, or whether this includes the strain isolated from sick animals.

It is highly recommended that diagnostic and health surveillance programs include comparison of strains of these *Vibrio* species from other States with the Tasmanian strains, and with overseas strains of *V. harveyi* showing similar pathology, such as those in France.

6.3.5.3 Shell diseases of significance

While this survey attempted to define the extent of shell lesions, and their distribution, further studies would be needed to accurately define their nature as this is beyond the resources or expertise of the current study. Spionid mudworm infection is the best studied of the shell diseases with regard to effects on abalone health (Lleonart, 2002, Handlinger et al., 2004). Species identification of spionids was variably undertaken, reflecting individual State concerns and available expertise, although the expertise for identification of the common spionids was generally available. Identification of other shell invaders such as sponges was hampered by a lack of reference taxonomists for these organisms.

Although most of the spionids affecting Australian abalone appear ubiquitous within Australian waters, and are not considered of translocation concern, *Boccardia knoxi* was thought to have limited Australian distribution and has therefore become a translocation issue. This spionid was detected in Tasmania and NSW, but not SA. Data on spionid identification is not available from WA or Victoria. This mudworm species has otherwise only been reported

from New Zealand. The hypothesis has been suggested (D. Ogburn, pers. com.), that some of the problem mudworm species, possibly including this species, may have been translocated from New Zealand during the late 19th and early 20th centuries with oyster stock relayed temporarily in Australian waters for rehydration prior to market. Such temporary relaying is known to have occurred in Tasmanian, NSW and Victorian waters. This hypothesis could explain the presence of *B. knoxi* in at least two eastern States, and apparent absence from SA. Further work to complete the identification of spionid species in WA and Victoria may be necessary to better define the distribution of this species if it remains of translocation concern. However, although this species was initially implicated in mudworm related mortality during early sea-based abalone culture in Tasmania, the work of Lleonart, 2002 and Handlinger et al., 2004, indicated that other spionid species regarded as endemic across Australia may show equal or greater pathogenicity.

Overall findings suggest that other shell lesions may similarly affect abalone health, including sponges. The small shell lesions detected as discolouration have similar gross characteristics (though smaller) to those attributed to fungi in New Zealand (Grindly et al., 1998). However, their significance has not been established.

6.4 General aspects

As well as defining the diseases present in Australia, this survey provided the opportunity to expand knowledge of general abalone responses. The opportunity for systematic examination of large numbers of abalone, both wild and farmed, healthy and in poor health, using standard sampling criteria, allowed apparent correlations between tissue changes and disease conditions to be recorded by each State, and assessed further by comparison between groups with specific diseases in Chapter 5.7., where specific correlations were discussed. This enabled definition of the acute changes that may results from the sampling process, and defined the levels of change that may be regarded as significant. Exclusion of viruses as a cause of two types of cell inclusions will also aid timely and accurate diagnosis where these changes are seen.

This data adds to the depth of expertise for abalone disease diagnosis within Australia that has been fostered by the collaborative nature of the survey. Indeed the project aim to involve at least one or two pathologists in each of the five participating States was exceeded, with the direct involvement of 14 veterinary pathologists and others with different types of diagnostic expertise. Diagnostic laboratories with a considerable background in abalone diseases now exist in each of the southern States. These laboratories also work within an effective network for inter-laboratory referrals. The data-bases and slide collections generated are also available for further analysis. The two workshops held in conjunction with this project fostered this relationship, as well as establishing network and an exchange of knowledge with other abalone health professionals such as researchers and non-laboratory veterinarians. This has provided a much expanded expertise base for the abalone health monitoring and surveillance programs under development by the industry, and abalone disease control. Retention and expansion of the current levels of available diagnostic expertise will be further enhanced by the electronic atlas of abalone diseases and pathology being developed from this project. However if the value of this project is to be utilised, industry must recognise the need for non-laboratory disease investigation expertise, a framework for such activities and for capture of health related information across this industry, and the need to ensure familiarity of those working in the aquaculture and wild harvest industries with disease issues.

7 BENEFITS

Major benefits are the increased knowledge of diseases present, their pathology and distribution, data to support claims regarding Australia's health status and the *Perkinsus* status of most areas, and a greatly expanded pool of diagnostic expertise available to these industries. The value of electronic teaching material resulting from this survey to maintain this pool is demonstrated by the loss of at least 3 participating pathologists prior to the completion of the survey.

This work has been of significant benefit to the NSW wild harvest industry *Perkinsus* investigations, defining the pathology as different to that seen in SA, and suggesting that different factors are likely to be affecting disease expression. This will also benefit on-going studies.

The increased knowledge of diseases present in Australia, and their associated pathology, plus the expanded pool of diagnostic expertise available will enable the aquaculture industry to meet their objectives of preventing or limiting disease in their stock and meeting future market expectations in regard to health accreditation. This knowledge is also being used to evaluate translocation risks and develop a national abalone translocation policy that will enable farms to purchase or move stock to improve their gene base or utilise hatcheries in a more efficient manner.

This information is also seen as essential to underpin health monitoring and surveillance programs under development that are expected to improve collation of health findings across the industry, and further expand knowledge of abalone diseases. Such programs will also provide assurance to the wild industry on the health of farmed animals. Inclusion of a wild industry health monitoring program would potentially similarly provide farms with assurances on wild stocks interacting with farms. The advice provided through this program has facilitated the development of these programs, and of assessment of translocation risks.

8 FURTHER DEVELOPMENTS

The major area for future development is the establishment of on-going health surveillance programs for both the farmed industry (progressing) and the wild fishery, and the development of national translocation guidelines for abalone. The latter is already under way (FRDC 2005/640), utilising the results of this survey to define and quantify translocation risks. This report outlines a number of areas where further information is required, both on the distribution of the agents detected and on assessment of their pathogenic potential. This includes the need for on-going study of the *Perkinsus olseni* mortality problem in NSW, which should best include a comparison with the South Australian infection. Comparison of bacterial strains isolated from disease outbreaks in each State, and further studies of the extent of the haemocyte parasite distribution in Bass Strait and in other stunted stocks, are also highly recommended.

Much of this information can be gained through on-going health monitoring programs that are planned by the aquaculture sector. The need for studies of *Perkinsus* infection in wild stock, and studies of the haemocyte parasite and stunted wild stocks generally, highlights the need

for an overall industry health surveillance program to include the wild harvest industry. Advice provided to both industry sectors has highlighted the need for inclusion of wild industry health surveillance to establish a verified early warning system for disease incursions and new diseases and limit the impact of disease, and the enhanced value of routine health monitoring for establishing an official status as free from a specified disease. Health surveillance and monitoring programs for the aquaculture sector are under development at both national and State levels.

9 PLANNED OUTCOMES

The planned outcomes were as follows:

1. Knowledge of abalone diseases present in Australia and moderate knowledge of their current distribution and prevalence.

This outcome was achieved, although there is only limited knowledge of the distribution of some parasites and specific bacterial strains. There most remain some uncertainty regarding parasites difficult to detect at low levels (particularly the haemocyte parasite); parasites of non-target or small organs which were not routinely included in the survey sections (the oesophageal pouch parasite); and parasites present at low prevalence and consequently the sample size from individual sites was too small to establish a true distribution. Of these, only the haemocyte parasite is likely to be of concern, and only stunted populations, which tended to be avoided by sampling strategies in some States, would be of sufficient concern to warrant targeted further sampling. Knowledge of the distribution of abalone-specific pathogenic strains of *V. harveyi,* and possibly *V. spendidus* is limited by the level of bacteriology examinations during disease outbreaks in some States, and, until recently, by the lack of availability of methods for differentiating such strains.

2. **Significantly improved diagnostic capability available to both abalone aquaculture and wild fisheries managers, as a result of the direct survey findings, the related training activities, and the collective approach to collation and interpretation of these and previous findings.**

This outcome has been achieved, with participation in the project of considerably more than the expected number of pathologists and other abalone health service providers, though there is concern regarding the retention rate of this expertise. The project framework fostered development of an inter-laboratory network that is ongoing.

This network was further expanded to include other health service providers by the Abalone Specialist Health Workshop held in Sydney on September 30-October 31, 2004, attended by 34 people. Participants included 8 of the pathologists involved in the survey, 2 pathologists involved with abalone disease surveys in other countries (Dr Anna Mouton from South Africa, and Dr Ben Diggles who undertook the recent New Zealand abalone disease survey). In addition there were three field-based veterinarians, three regulatory health specialists, two diagnostic research specialists (in microbiology and molecular test development), five scientists who have or are working in abalone health related research, four abalone fisheries staff, five abalone farmers, one abalone ecologist and two mollusc immunologists. Fifteen participated as speakers, ensuring that the workshop aim of reviewing all factors affecting abalone health was achieved.

Maintaining an on-going pool of expertise will be facilitated by circulation of an electronic

Proceedings from this workshop to participants, and the electronic *Atlas of Diseases and Pathology of Abalone: A guide to diagnosis from an Australian Perspective*, developed within a framework provided by collaboration with TAFE Tasmania MultiMedia students.

3. **A framework and sound basis for on-going health surveillance programs for the abalone farming sector to provide cost-effective access to diagnostic services, improved feedback for addressing on-farm factors affecting abalone health, and better assurances with regard to abalone health for farm planning, supply of other farm customers, and market access.**

Advice on a framework for health surveillance programs for this industry, and steps to develop this, have been provided in the Abalone Aquaculture Subprogram "Ab-brief" Newsletter, and in the Workshop Proceedings for 2005, together with the survey results of diseases present and their distribution on which these were based (Handlinger 2005, Handlinger et al., 2005a). State Abalone Growers Associations are working towards such programs. This process will be aided by development of a framework for EMS systems for the industry (Marshall, 2005), and by the recent formation of the Australian Abalone Growers Association.

4. **Reduced production costs from reduction in losses and improved health of farmed abalone.**

While there is no direct measure of this available, the information from this survey, and the improved services as a result of the collaborative learning experience should enable this.

5. **A sound basis for decision making with regard to movement and testing protocols for interstate and intra-state movements, quarantine issues, and reseeding operations (which will benefit both the farmed and wild abalone industries) and improve public confidence in farming and reseeding operations.**

Similarly, this project has provided the information required to achieve this outcome. A consideration of a coordinated national approach has been undertaken in the form of a linked project FRDC 2004/080.

6. **A significantly improved basis with regard to trade access issues, claims of disease freedom, and likely disease related quality risks to trade.**

Achieved. Both the knowledge gained directly from the survey, and the on-going improved basis for health surveillance, will contribute to this.

7. **Improved knowledge of the likelihood of paralytic shellfish poisoning issues occurring in abalone.**

This outcome has not yet been achieved, though a strategy to achieve this has been developed. The planned methodology to achieve this was through identification of episodes of paralytic shellfish poisoning (PSP) toxin accumulation in filter feeding molluscs, when a small number of abalone samples from resident abalone would be analysed for PSP toxins. The only periods of PSP related blooms that were identified during the survey were within the Huon area of Tasmania. The 2004 bloom period was missed, and plans for collection of samples during 2005 were aborted when the bloom lasted for a shorter period than expected on past history, and did not extend to areas where resident abalone were readily available. To the date of printing there has been no PSP related bloom in 2006. The only known previous detection of Paralytic Shellfish Poison (PSP) toxins in Australian abalone was their identified in low to moderate concentrations in the foot and gut tissues of a limited number of abalone sampled from Lorne in western Victoria during mid-July 1992 (Gorfine, 2002), soon after cysts of the PSP-producing dinoflagellate *Gymnodinium catenatum* were detected in the water column and sediments (Todd, 2001)

8. **A sound basis for prioritising future research needs such as further investigation of specific diseases, or extending parts of the survey to meet international standards for official freedom from certain diseases.**

The knowledge base for this activity has been considerably increased.

10 CONCLUSION

This survey achieved all the major objectives including increased knowledge of abalone diseases and parasites in Australia and the extent and basis of their pathological effects; increased general understanding of abalone pathology; markedly increased the availability of aquatic animal pathologists with experience in abalone, and considerably increased the familiarisation of other aquatic animal health service providers with this species; providing this information for both aquaculture and wild fisheries industries and regulators; and capturing this information in an electronic format for future training. Additional work has been recommended on several diseases, some of which can occur within a routine health surveillance framework.

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12 APPENDICES

12.1 APPENDIX 1: INTELLECTUAL PROPERTY

There are no intellectual property issues regarding the data in this report, Intellectual property issues regarding the Pathology Atlas output from this project will be addressed elsewhere.

12.2 APPENDIX 2: STAFF

Judith Handlinger, (Principal Investigator, veterinary pathologist)
Stella Bastianello (veterinary pathologist)
Richard Callinan (veterinary pathologist)
John Creeper (veterinary pathologist)
BS Oyay (veterinary pathologist)
Celia Hooper (veterinary pathologist)
Fran Stephens (veterinary pathologist)
Matt Landos (veterinary pathologist)
MJ Lancaster (veterinary pathologist)
Peter Phillips (veterinary pathologist)
Richmond Loh, DPIWE, (veterinary pathologist)
Stephen Pyecroft, DPIWE, (veterinary pathologist)
WM Forsyth (veterinary pathologist)
Jeremy Carson, DPIWE (reference microbiologist)
Marty Deveney (SA survey coordinator & parasitologist)
Kylie Freeman (WA survey coordinator)
Brian Jones (mollusc pathologist)
Dane Hayes, DPIWE, (slide preparation and electron microscopy)
Alex Hyatt, CSIRO Animal Health Laboratory (additional electron microscopy)
Craig Mundy and the abalone collection team, TAFI.
Abalone Stock Assessment team, NSW DPI (sample collection and RFTM tests)
Ben Walsh (shell survey, NSW)
Lexi Walker (shell survey, NSW)

Technical teams in each laboratory including:
The Fish Technical Officer team, DPIWE
Steve Pepper (tissue processing technician, NSW)

12.3 APPENDIX 3: COLOR REFERENCE MATERIAL (electronic format)

Reference material in colour format will be submitted in electronic format, to augment the report. This will include the report in PDF format, including colour pages, reference maps in colour, and additional reference photographs of conditions seen in each State.

12.4 APPENDIX 4 : RAW DATA (electronic format)

Raw data to be submitted in electronic format will include detailed databases of findings from State disease surveys, which will be available for further analysis and future research. Further detailed information on sample sources will be held by the State laboratories, as agreed with relevant industry sectors. Individual farms will not otherwise be identified, though this data can be sourced from the participating laboratories should the need arise on an as-needs basis, for such issues as translocation. It may be made available for further research on agreement with the owners.

12.5 APPENDIX 5: ABALONE DISEASES AND PATHOLOGY TEACHING CD (Electronic format).

The "Atlas of Abalone Diseases and Pathology from an Australian Perspective" will be submitted in electronic format, for sale or otherwise distribution by the Abalone Aquaculture Subprogram, as specified in the Project Agreement. This will include all conditions found in this survey, plus reference material of exotic infectious diseases and other non-infectious conditions of abalone. The inclusion of material from other countries is of value for exotic disease diagnosis, and broadens the information base from a national to an international perspective. There is considerable interest the availability of this resource outside Australia, both within the Asia-Pacific region, and internationally through the recently formed international Abalone Disease Expert Group.

Industry has also requested a poster version, restricted to the gross findings in abalone.

12.6 APPENDIX 6: PROCEEDINGS OF THE ABALONE HEALTH WORKSHOP, SEPTEMBER 2004. (electronic format)

An Electronic Proceedings for the abalone health workshop held in September 2004, incorporating the Power Point presentations with accompanying text, is to be circulated to participants (although this not a project requirement). This will also be lodged with the Abalone Aquaculture Subprogram of FRDC. As this contains the intellectual property of other participants, it is not for general distribution but is intended as a reference of abalone health expertise within the Australian region.