Pearl Oyster (*Pinctada maxima*) Aquaculture: Health Survey of Northern Territory, Western Australia and Queensland Pearl Oyster Beds and Farms

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OBJECTIVES:

1. To detect and document the serious diseases and significant pathogens of farmed pearl oysters in Western Australia, Northern Territory and Queensland.

2. Develop a data base of pearl oyster disease, location and prevalence that can assist both government and farmers in making informed decisions about translocation of stock.

SUMMARY

A comprehensive health survey of pearl oysters *Pinctada maxima* was undertaken across northern Australian marine waters in a collaborative project between fisheries organisations and pearl producers in Northern Territory (NT), Queensland (Qld) and Western Australia (WA). The majority of animals examined in the study represented mature animals from the wild or from pearl culture farms from NT, Qld and WA (4502 animals). The study also reports on 22 batches of 150 spat, examined after spending a minimum of 6 weeks in open water sites in WA as part of the regulatory controls in place controlling oyster movements in the State. A low number of mature and immature animals examined for disease investigations and following placement in sea cages/panels in NT were also included in the study.

The study established the occurrence, prevalence and distribution of a taxonomically diverse range of microbial, protozoan and metazoan agents associated with pearl oysters in Australian waters and, within the limits of the study, ascribed pathogenic significance to these agents. In some cases, the prevalence and distribution of agents identified in earlier studies were established. The majority of animals examined were free from infectious agents which may adversely impact upon oyster growth and pearl production. A proportion of oysters carried agents which were not considered significant pathogens. A number of microbial, protozoan and metazoan agents were identified in the shell matrix or in the tissues of the oyster which were considered to have potential to adversely impact upon the breeding, rearing and

production of pearl oysters in Australian tropical waters.

Pathogenic or potentially pathogenic agents identified in mature *P. maxima* from clinically normal populations in the study included a papova-like virus of the palp associated with epithelial hypertrophy and cilia loss, viral-like inclusion bodies in the digestive gland associated with tubular degeneration, enigmatic protozoan-like bodies associated with severe degenerative and inflammatory lesions in the digestive gland of mature oysters and a copepod associated with oesophageal occlusion and epithelial erosion. The shell matrix was also a target for potentially pathogenic boring bivalves, invasive sponges and mudworms, resulting in shell denaturation and blistering.

In the first 6 weeks of exposure of juvenile oysters to the marine environment in WA, a *Haplosporidian* sp. with high morbidity was detected, together with a heart apicomplexan, palp virus, rickettsiales-like agent in the digestive gland, viral-like inclusion bodies in the digestive gland, a copepod in the digestive gland, *Ancistrocomid*-like ciliates in the alimentary tract and gills.

Sequential examination of batches of juvenile oysters up to 23 weeks after placement in the sea in panels in the NT demonstrated progressive colonisation by a range of unidentified protozoan and metazoan organisms.

Examination of diseased mature and juvenile oysters in NT associated mortalities with *Vibrio* spp., an enigmatic protozoan-like agent and abnormal environmental parameters.

A number of agents showed marked differences in distribution between states and between regions within states. The establishment of a restricted geographic distribution of potentially pathogenic agents in Australian *P. maxima* provides a basis on which rational quarantine may be implemented to avoid introduction of deleterious agents or pests when considering translocations or introductions of oyster stocks from different regions within Australia.

The study established normal histological criteria for *P. maxima* and defined a range of host responses to injury. These studies provide a basis on which the normal structure of the pearl oyster may be differentiated from the structure altered by disease, thus establishing criteria for disease diagnosis in pearl oysters. The normal histological criteria and histopathological changes associated with infectious and non-infectious conditions found in the study are to form the basis of an FRDC atlas of pearl oyster morphology and pathology.

The study included a comprehensive review of infectious and non-infectious agents, conditions and disease states of pearl oysters *Pinctada* spp. This review provides an international comparative basis on which to diagnose infections and disease states in Australian oysters and also provides an international perspective if introductions from elsewhere are contemplated.

All findings of the study have been collated on a relational database which can be utilised to determine the prevalence, occurrence and distribution of all agents and conditions identified and by which correlations between variable factors and specific agents or conditions can be made. It is intended that the database be made available to interested parties.

The study provides essential baseline data on disease occurrence and prevalence and a basis for the diagnosis of infectious and non-infectious diseases of *P. maxima.* Avenues for further investigation of infectious agents are suggested.

CHAPTER 1: GENERAL INTRODUCTION

BACKGROUND

The commercial harvesting and farming of pearl oysters of the genus *Pinctada* comprises an expanding industry in tropical marine environments worldwide. Earlier methods of harvesting oysters for their shell and for natural pearls has generally been superseded by farming of wild harvested oysters, by the hatchery production of seed stocks and by the cultivation of pearls. Pearl farming and the cultivation of pearls has been practiced for many years in a number of countries, including Japan, Middle Eastern countries and French Polynesia. More recently, pearl oyster farming has been investigated and pursued in other Pacific nation countries (Dalzell and Adams 1995).

Contemporary pearl farming necessitates a supply of new oyster stocks to replenish oysters harvested for their pearls, shell and meat. Such activities demand introductions of seed stock from hatcheries, or introductions of wild-harvested oysters. The introduction of living aquatic animals, especially molluscs is well recognised as a major means of introducing unwanted diseases, parasites and pest species (Andrews 1980; Stewart 1991; Humphrey 1993, 1994, 1995). A long history of severe adverse impacts on aquaculture industries or on the aquatic environment following such introductions exists. Thus, the demand for replenishment stock to support the pearl industry presents potential risks with respect to introductions of unwanted organisms and the establishment of disease.

The harvesting of tropical pearl oysters for their shell and for their natural pearls has long been a traditional aspect of Australian fisheries and Australian maritime lore. Since the 1960's, commercial farming of wild-harvested pearl oysters and seeding of these stocks for cultured pearl production have substantially replaced earlier methods, with demands for stock replenishment leading to hatchery production of seed pearl (McCue 1992; Knuckey 1995).

NEED

Despite the current and future economic importance of the pearl farming industry, little is known of the diseases and parasites which may adversely impact upon pearl oyster production and the culture of quality pearls in Australian waters. No systematic study of pathogens, parasites or diseases of pearl oysters has been undertaken in Australia and such information is fundamental to protecting the industry from the introduction of diseased stock, to formulate policies relating to translocation of stock and to develop diagnostic procedures for the rapid recognition of diseases when they occur.

OBJECTIVES

In order to redress this situation, a comprehensive study of wild-harvested and farmed pearl oysters *Pinctada maxima* commenced in 1994 to determine the occurrence, prevalence and distribution of pathogens, parasites and diseases of pearl oysters in northern Australian waters. In addition to determining disease data, the study sought to establish and collate histological and histopathological reference data relating to pearl oysters. The study, funded

by the Fisheries Research and Development Corporation, is a joint project between the Northern Territory Department of Primary Industry and Fisheries, the Queensland Department of Primary Industries, and the Fisheries Department of Western Australian. The study was based on gross and microscopic or histological examinations of pearl oysters to further define the spectrum of pathogens and potential pathogens which exist in *P. maxima* across tropical Australia and to determine the major disease problems likely to be encountered. In addition, data was collected on the occurrence and prevalence of major fouling and boring organisms, on commensals and epiphytic organisms.

The principle objective of the study was to determine the occurrence, prevalence and distribution of pathogens, parasites and diseases of pearl oysters in northern Australia, with a view to:

- Improving the fragmented knowledge on diseases and potential diseases which may adversely impact on the pearl oyster culture industry of Australia.

- Providing a scientific basis on which to implement regional and national quarantine to prevent spread or introductions of disease, based on the established occurrence and distribution of potentially infectious agents.

- Developing diagnostic tests and procedures to assist in the recognition of exotic and endemic diseases of pearl oysters.

- Developing guidelines and strategies which may be implemented to avoid or mitigate against infectious and non-infectious diseases, should these occur in farmed Australian pearl oysters.

- Identifying infectious agents which may limit pearl oyster production as subjects for further research.

This report details the nature, occurrence, prevalence and distribution of microbial, protozoan and metazoan agents infecting *P. maxima* and identifies potential pathogens. In addition, specific and non-specific pathological and histopathological changes in the tissues of the oyster are described. The report also identifies regional differences in distribution of infectious agents and provides a basis on which pathogens, parasites and diseases exotic to Australian pearl oysters may be determined. A basis for the development and implementation of quarantine guidelines to protect existing stocks from introduced agents is discussed.

CHAPTER 2: INFECTIOUS AND NON-INFECTIOUS DISEASES OF PEARL OYSTERS - A REVIEW

INTRODUCTION

In order to optimise production in any intensive animal industry, a knowledge of those factors which limit, reduce or preclude production is essential. Aquaculture of pearl oysters is no exception; an understanding of potentially infectious, pathogenic agents, as well as non-infectious and environmental factors which may impact adversely on the production and management of pearl oysters is an essential basis on which to develop a sound industry practice. Such knowledge is of special importance in developing and implementing reliable methods for hatchery production of pearl oysters to supplement collection of wild oysters for commercial pearl production (Mills 1997). Further, a knowledge of occurrence, distribution and prevalence of infectious agents and non-infectious conditions is central to the development and implementation of quarantine policies and strategies to protect against introductions of disease without imposing unnecessary barriers to trade. This knowledge also provides a basis for the definition of those diseases exotic or non-endemic to a region. A knowledge of the morphology of pearl oysters, as well as the pathogenicity, pathology, epidemiology, taxonomy and growth characteristics of infectious agents is an essential pre-requisite in the development of tests and procedures for the identification of pathogenic agents and their rational control, treatment or prevention.

Unlike edible oysters and other commercially important bivalve molluscs in which numerous pathogens, parasites and diseases have been described and reported (Sindermann 1990), few such agents or conditions have been described in pearl oysters of the genus *Pinctada*. Wada (1991) noted that damage associated with a mudworms, sponges and a trematode parasite of *P. fucata martensii* have been the cause of major damage to the Japanese pearl oyster industry since 1952. As well, a number of other reports of predators, commensals, parasites and diseases and epiphytic or fouling organisms of pearl oysters are variously documented. Parasites were noted to be major causes of decreased production by Crossland (1957) who reported a 25% reduction in the value of the marketed crop of pearl shell caused by such agents.

Those infectious micro-organisms, parasites, diseases, predators, commensal organisms and epiphytic or fouling organisms which have been described from pearl oysters *Pinctada* spp. are reviewed in this chapter. Their listing forms a basis on which exotic diseases may be determined for quarantine purposes and to assist in their identification. A checklist of these agents is presented in Tables 2-1 to 2-4.

Non-infectious and environmental factors are also well established as causing or contributing to mortalities and disease in pearl oysters. Mechanisms by which such factors may be recognised and strategies to mitigate against the impacts of such factors are clearly lacking in the aquaculture of pearl oysters. New approaches to routine practices may also be necessary. The use of anaesthesia, for example, in invasive procedures (Mills *et al.* 1997) may alleviate stress and tissue damage in mature oysters. In particular, seasonal fluctuations in temperature and salinity, and altered environmental conditions during transport and handling appear to be important causes of losses in pearl farming. Non-infectious and environmental factors are reviewed in relation to adverse impacts on pearl oyster growth and production. Toxic algal blooms, including "red tides", are also well described and are reviewed under non-infectious

and environmental factors.

Subsequent to the increasing world wide interest and expansion of commercial pearl oyster aquaculture and pearl production, it is likely that as yet unreported infectious agents and non-infectious conditions will be described in association with decreased pearl oyster production and reduced pearl quality. The occurrence of such events should be anticipated by farm managers, fisheries biologists and molluscan pathologists in addressing diseases in pearl oysters.

INFECTIOUS DISEASES AND AGENTS

Viruses and viral diseases

Viruses representing members of the families Birnaviridae, Herpesviridae, Iridoviridae, Papovaviridae, Reoviridae and Retroviridae as well as several viral-like agents have been reported in molluscs other than the genus *Pinctada* (Sindermann 1990). Some, notably the iridoviruses, are major molluscan pathogens (Sparks 1985). Few reports of viruses or viral-like agents are recorded in *Pinctada* spp. Two such agents have been recorded from *P. maxima* in Australia.

Intranuclear inclusions containing virus-like particles were commonly observed in digestive gland epithelium in adult *P. maxima* by Pass *et al.* (1988). No disease or pathology was attributed to the presence of the agent. Norton *et al.* (1993a) described a Papovavirus-like infection of the palp epithelium from a population of adult *P. maxima* from the Torres Strait. Infected oysters showed massive hypertrophy of infected cells in the ciliated columnar epithelium, with nuclear enlargement and loss of cilia. The virus was detected in apparently normal oysters.

High mortalities attributed to a 'virus' disease were reported in *P. margaritifera* in French Polynesia. There is no evidence that a viral agent was involved in these mortalities and the term virus appears to have been used in a generic sense to imply a spreading disease with high mortality. Subsequent examinations associated the mortalities with the presence of a gregarine parasite although it is premature to invoke a causal link between the gregarine and the deaths (Chagot *et al.* 1993). Adverse environmental conditions have also been associated with the deaths (Cabral 1989a; Bernadac *et al.* 1980; Vacelet *et al.* 1996), including high levels of zinc associated with galvanised platforms (Remoissenet 1995). The condition is discussed below under Protozoa and Protozoal Diseases and Diseases of Uncertain Aetiology.

Bacteria and bacterial diseases

Infections with *Vibrio* spp., especially *Vibrio harveyi* have been the cause of post-transport mortalities in pearl oyster *P. maxima* in Australia. Such infections were associated with poor water circulation and decreased water temperature during post-collection transport (Lester 1990; Dybdahl and Pass 1985; Pass *et al.* 1987).

A spectrum of bacteria have been isolated from *P. maxima* during cultural examinations in Western Australia. These include *Vibrio* spp., *V. pelagicus V. mediterranei, V. alginolyticus, V. anguillarum V. splendidus* II *V. parahaemolyticus Photobacterium* sp., *Cornebacterium* sp. and *Erwinia hebicola.*

Marine *Vibrio* tend to localise in the heart, inciting haemocytic inflammatory lesions (Pass *et*

al. 1987), an observation explained by Suzuki (1995) who demonstrated that fixed phagocytes of the pearl oyster in the auricle are a primary site of antigen localisation, with entrapment of antigen within six hours. Experimentally, Mannion (1983) investigated the relationship of water temperature to infection and mortalities of *P. maxima* by marine *Vibrio* spp. This author concluded that lower temperature enhances bacterial disease and demonstrated invasion of oyster tissues *by Vibrio harveyi* at 21°C. Invasion of mature and immature pearl oysters by marine *Vibrio* spp. is also commonly associated with mortalities. Stress, including low salinity, is considered to play a major role in such infections.

Protozoa and protozoal diseases

Perkinsus-like protozoa were described by Norton *et al.* (1993b) in focal granulomatous lesions in the tissues of adult *P. maxima* from a population undergoing a high mortality in Torres Strait, Australia. While affected oysters showed multifocal granulomatous systemic lesions which contained the protozoan, a causative role in the mortalities could not be established. A *Perkinsus* sp. is commonly isolated from a wide range of apparently healthy bivalves on the Great Barrier Reef, including at least two species of pearl oyster, *P. margaritifera*, and *P. sugillata*, (Goggin and Lester 1987). Goggin *et al.*(1989) induced heavy experimental infection of *Perkinsus* sp. in *P. sugillata* and demonstrated that the protozoan from *P. sugillata* was infective for other Australian molluscan species.

Chagot *et al.* (1993) associated high death rates with the presence of a sporozoan gregarine parasite in the alimentary tract of *P. margaritifera* in French Polynesia. The parasite was intracellular and the digestive epithelium was described as the target tissue, although its presence in sub-epithelial connective tissue in heavily parasitised individuals was noted. The parasite was associated with local destruction or flaking of parasitised cells. In some individuals, the high number of parasitised cells resulted in complete destruction of the rectal epithelium. A causative role was not, however, clearly established and the finding may be incidental.

The occurrence of a *Haplosporidium* sp. in pearl oysters was described in *P. maxima* from Western Australia by Hine (1996) and Hine and Thorne (1998). Jones (1996 - Unpublished) reported the same Haplosporidan in the gut of 4.6 % of 150 pearl oyster spat examined prior to translocation from a quarantine facility to a farm site in Western Australia. There were no apparent mortalities observed at the site at the time when the infected oysters were sampled, however, the almost total replacement of digestive gland epithelium with spores in all stages from immature to mature spores, strongly suggested that the spat would not have survived the loss of digestive gland tissue. The agent is distinct from *Haplosporidium nelsoni* (Hine 1996).

The batch infected with *Haplosporidium* sp. was considered to have acquired the infection locally as testing prior to release from the hatchery failed to demonstrate the organism. Testing of other spat from the same batch reared elsewhere showed no evidence for infection. Electron microscope examination of the tissue has confirmed that the parasite is an undescribed haplosporidan belonging to the genus *Haplosporidium*. It is the same parasite as that observed in a batch of spat from the Carnarvon hatchery in 1993 (Hine 1996). Organisms from this hatchery reported as *Nematopsis* sp. in SCFH (1993) were subsequently identified as *Haplosporidium* sp. (Jones - Unpublished).

An Apicomplexan parasite has been reported on two occasions in the heart of *P. maxima* in Australia (Jones 1998- unpublished).

Mikrocytos sphas been described from Australian pearl oysters *P. maxima,* apparently

unassociated with disease (SCFH 1993) but details are sparse.

Algae and algal diseases

Diseases associated with algae are discussed under Non-infectious and Environmental Diseases and Conditions.

Platyhelminths and platyhelminth diseases

A turbellarian (class Turbellaria) predator consistently associated with mortalities of *P. maculata* a fouling pearl oyster is discussed under Predators and Predation.

Tapeworms (class Cestoda) have long been recognised as parasites of the pearl oyster *Margartifera vulgaris* and were initially believed to be the cause of natural pearl formation (Sparks 1985). It was subsequently shown that invasion by larval trematodes was more important in natural pearl formation than larval cestodes (Sindermann 1990). Crossland (1957) reported a larval tapeworm in *P. vulgaris* in the Red Sea. Similarly, Nasr (1982) described larval infections by the cestode *Tylocephalum* sp. in *P. margaritifera* from the Red Sea resulting in an intense focal inflammatory response in the oyster. Larval *Tylocephalum*, which are not considered to be host specific, may occur at high prevalence and intensities in pearl oysters and edible oysters and may reduce the condition of their molluscan host (Sindermann 1990).

Wada (1991) reported that infection by larval fluke (class Trematoda) *Bucephalus varicus* affected up to 40% of pearl oysters and that infected oysters cannot be used for pearl production.

DISEASES AND CONDITIONS OF UNCERTAIN AETIOLOGY

Mass mortalities in French Polynesia

Mass mortalities of *P. margaritifera* occurred in French Polynesia in 1985 and 1986 with 50-80% losses in adult, juvenile and spat (Chagot *et al.* 1993; Remoissenet 1995). The occurrence of the mortalities is reviewed by Remoissenet (1995), and considerable uncertainty exists regarding the cause or causes. The mortalities were initially reported in the Gambier group of islands and subsequently on Takapoto Atoll, Tuamoto Group. A "virus" was alleged to be the cause, but no such agent was isolated or visualised. The disease spread in cultured black lip pearl oysters in the Tuamoto Group following transfer of stocks among atolls (SPC 1985, 1988; Cabral 1989a, 1989b; Brayley 1991; Eldredge 1994). Brayley (1991) also reported the disease in other bivalve molluscs in the Tuamotu Group. Earlier, Bernadec *et al.* (1980) considered the decreased numbers of oysters in this group to be due to over-exploitation and not to algal blooms as was initially suggested by some workers. Severe socio-economic losses were associated with the apparent disease. More recently Cabral (1989b) considered the aetiology to be a complex of uncharacterised factors while Chagot *et al.* (1993) associated a sporozoan gregarine parasite with the losses, although a causal relationship appears unlikely (see Protozoa and Protozoan Diseases). Marin and Dauphin (1991) reported microstructural and biochemical alterations of the shell associated with changes in the amino acid content of soluble and insoluble organic matrices of the nacreous layer in affected oysters. Remoissenet (1995) in reviewing the mass mortalities presented evidence which suggested that zinc eluted from galvanised platforms may have had a causative role.

Vacelet *et al.* (1996) studied the nutrient concentrations of the aquatic environment of Takapoto Lagoon and found both nitrogen and phosphorus to be limiting. These authors reported the density of the pearl oysters appeared to exceed the nutritional potential of the lagoon and due to the oligotrophic conditions, phytoplankton and even bacteria were unable to sustain their feeding, suggesting that nutritional inadequacy was associated with the disease in *P. margaritifera.*

Mass mortalities in Australian *P. maxima*

Mass mortalities of larval, juvenile and adult Australian pearl oysters are well recognised, but the cause or causes of such losses have, in many cases, not been fully defined. Wolf and Sprague (1978) reported that mass mortalities had caused great concern in some commercial pearl farms in the years 1968-1978, yet the cause remained unknown. Mass mortalities attributed to overcrowding occurred in Torres Strait in 1966 and 1968 whilst in 1969, high mortalities attributed to domestic sewage and chemical pollution occurred adjacent to Thursday Island (Pyne 1972). Widespread mass mortalities were reported in *P. maxima* following the grounding of the oil tanker Ocean Grandeur on 3rd March 1970 in Torres Strait. Mortalities of up to 80% were recorded in new shell in the 1970 season following transport to farms and surviving oysters were in weak condition and had depressed growth rates. Surviving oysters developed a double-backed shell abnormality and showed abnormal nacre deposition on half pearls. The oil spill and the use of a non-biodegradable detergent were seen as significant causes of the mortalities by some observers (Yamashita 1986) but other investigations suggested an uncharacterised infectious agent as the cause (Pyne 1972).

Dybdahl and Pass (1985) and Pass *et al.* (1987) investigated mortalities involving up to 80% of harvested shell following removal from collecting grounds in Western Australia and concluded that such losses were associated with marine *Vibrio* infection related to inadequate water circulation and lowered water temperature during transportation. Norton *et al.* (1993b) investigated a mortality involving 85% of adult oysters in a farm at Torres Strait and identified a *Perkinsus*like organism in affected oysters. Subsequent data (J. Norton 1996 - unpublished) indicates that mortalities may continue on some farms. While such mortalities are not generally investigated, the losses may be caused by similar factors described in Western Australia (Pass *et al.* 1987), including marine *Vibrio* spp., and intercurrent deficiencies in hygiene, handling and transport procedures. Recently, high mortalities at a farm in Northern Territory were reported to have a seasonal basis, possibly associated with decreased salinity and decreased water temperature.

Unpublished accounts of massive mortalities in juvenile stock and occasional mass mortalities in larval stock are also reported. Again, the cause or causes are largely undefined, but likely involve both infectious and environmental/husbandry factors.

Heavy losses of juvenile grow-out spat from pearl oyster hatcheries have been experienced on pearl farms in northern Australia. Causes of losses include the escape of spat from mesh baskets, the clumping of juvenile spat within the baskets causing secondary starvation, and reduced water flow into the baskets from the use of very small mesh which became fouled. Survivors from one batch of spat had a papova virus-like infection of the gills and a non-specific enteritis (Norton - unpublished data).

Periodic high mortalities of *P. maxima* in Australia appear to be relatively common. Such mortalities appear to largely have gone un-investigated and the only evidence of disease is anecdotal.

Protistan parasites/residual bodies

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

BIOFOULING AND BORING ORGANISMS

The external shell surfaces of the pearl oyster are readily colonised by a taxonomically diverse range of organisms which grow on the shell or invade the shell matrix. Fouling of shells and cages by these organisms and shell damage caused by organisms which bore into or otherwise invade the shell are a major problem of cultured oysters worldwide (Mohammed 1972; Wada 1991; Doroudi 1996; Dharmaraj and Chellam 1983; Dharmaraj *et al.* 1987). Pearl oysters growing in natural oyster beds are also subject to similar fouling and invasion by boring organisms (Dourodi 1996). Biofouling and shell invasion may impact adversely in a number of ways which may severely retard oyster growth and pearl production. Death and destruction of oysters, decreased oyster growth, decreased pearl quality, poor or downgraded shell quality, reduced water flows, decreased feeding, weakened shell structure, competition for food, and spat mortalities have been associated with fouling and boring organisms (Wada 1973, Mohammed 1976, Dharmaraj *et al.* 1987). Major costs are incurred by the industry in cleaning of fouled shells and the implementation of husbandry procedures to mitigate against fouling.

Unchecked, fouling occurs progressively throughout the year by accumulation of biota on shell and panels. Seasonal variation in the species and abundance of fouling and boring organisms may occur (Dharmaraj and Chellam 1983; Dharmaraj *et al.* 1987). Settlement of fouling organisms is also to some extent dependent on the water depth of panels. Dharmaraj and Chellam (1983) found settlement of barnacles to be greater with depth to two metres during early months of settlement, whereas later, settlement towards the surface was greater. Blisters associated with boring organisms are generally fewer in younger oysters (Dharmaraj and Chellam 1983).

Taxa associated with fouling and boring organisms include the algae, barnacles, sponges, bivalves, gastropods, nematodes, polychaete worms, ascidians, bryozoans, crabs, starfish, coelenterates, nemerteans, turbellarians and temnocephalans. Table 2-2 lists the fouling and boring organisms reported or observed in association with pearl oysters *Pinctada* spp.

Boring organisms comprising polychaetes, sponges, molluscs and isopods cause considerable damage to the shells of pearl oysters (Dharmaraj *et al.* 1987). *Polydora* spp. are notable invaders, resulting in simple and compound blisters on the inner shell, and causing nodular protrusions into the shell cavity. Tracts and cavities associated with polychaetes and other boring organisms progressively fill with mud and detritus, giving rise to the name "mud blisters". Other species may inhabit the shell between layers of periostracum, weakening the shell. (Dharmaraj *et al.* 1987). Doroudi (1993) described specific destruction of the shell of the pearl oyster *P. margaritifera* following infestation by the sponge *Cliona* sp. which was found

to be the most common parasite in the Persian Gulf. The condition "red bum" in Australian pearl oysters is generally thought to result from invasion of the shell matrix by sponges.

Barnacles

Barnacles are highly modified, sessile marine crustaceans with a free-swimming larval stage (Buchsbaum 1948). Barnacles may be significant biofouling agents. In Japan, Wada (1991) reported the dominant species of barnacles fouling pearl oysters as *Balanus variegatus tesselatus* and *B. amphritrite*, and Cohn (1949) reported four species of *Balanus* as fouling organisms of *P. martensii*. Dharmaraj and Chellam (1983) reported *B. amphridite* as a predominant fouling organism in the Gulf of Mannar, India.

Dharmaraj *et al.* (1987) identified barnacles as the most dominant group of fouling organisms with *B. amphitrite variagatus* the most common, and *B. amphitrite venustus* and *B. amphitrite communis* of less importance. Doroudi (1996) also noted that barnacles were major fouling organisms of farmed oysters.

Crabs

The crustaceans *Alpheus* spp., *Phymodius ungulatus*, *Liomera rugatus*, *Trapezia digitalis*, *Petrolisthes lamarckii*, *Actea hispidus* and a representative of the family Pinnotheridae were described by Nasr (1982) as fouling organisms on oyster beds of *P. margaritifera* in the Red Sea. Dharmaraj *et al.* (1987) reported crabs as fouling organisms of pearl oysters in the Gulf of Mannar, India.

Bryozoans

Bryozoans are colonial, plant-like organisms, encased in a calcareous or horny case, many of which form encrusting growths. Bryozoa of several genera were identified as the sub-dominant group of fouling organisms by Dharmaraj *et al.* (1987). The bryozoan *Zoobotryon verticillatum*, together with other unspecified bryozoa, were commonly found as fouling organisms on farmed *P. fucata martensii* in Korea by Je *et al.* (1988).

Molluscs

Numerous molluscs have been identified as fouling organisms (Table 2-2). Several species bore into the shell matrix, resulting in holes, tracts and cavities with concomitant damage to the shell and nacreous surfaces. *Martesia* sp. and *Lithophaga* sp. are recognised as destructive boring molluscs of pearl oysters (Doroudi 1996, Dharmaraj *et al.* 1987). Dharmaraj *et al.* (1987) described heavy settlement and carpet-like formations over the surface of cages with *Avicular vexillum* on farmed oysters.

Je *et al.* (1988) described the bivalve molluscs *Mytilus edulis galloprovincialis*, *Crassostrea gigas*, *Anomia chinensis* and other unspecified bivalve and gastropod molluscs as relatively common fouling organisms of farmed *P. fucata martensii* in Korea. Crossland (1957) also described *Lithophaga* sp., a bivalve mollusc to be a common boring organism in the shell of cultured *P. margaritifera* in the Red Sea. Dharmaraj *et al.* (1987) reported that *Avicula vexillum* and spat of *Crassostrea* sp. as numerous fouling organisms in farmed pearl oysters in the Gulf of Mannar, India. They noted that carpet-like formations of *Avicular vexillum* formed on cages limiting water exchange. Doroudi (1996) also noted that spat of edible oysters were major fouling organisms in the Persian Gulf.

Polychaetes or mudworms

The Polychaetes or marine bristle worms comprise a large class of Annelids and are amongst the most common marine animals. Polychaetes are major fouling organisms on pearl oysters and a number of species are also major boring organisms of the shell matrix.

Manchenko and Radashevsky (1994) reported the polychaete annelid *Polydora vulgaris* as a commensal borer of the oysters *P. margaritifera* from the South China Sea. Similarly, Crossland (1957) and Nasr (1982) reported *Polydora* spp. as a parasite of *P. margaritifera* in the Red Sea, resulting in shell damage characterised by production of purplish blisters in the nacreous lining of the shell. Wada (1991) described the formation of a mud tube and blisters caused by *Polydora* spp. in several species of bivalves including *P. fucata martensii*. Wada (1991) also reported the major parasite to be *Polydora ciliata*, and noted that infection results in fatigue and mortalities of infected oysters. Doroudi (1996) noted that tubiculous polychaetes formed a major component of fouling organisms in farmed oysters in the Persian Gulf.

Mohammad (1972) noted that 33-35% of cultivated *P. margaritifera* were invaded by *Polydora pacifica* in the South Seas. Nasr (1982) recorded nereid, sabellid and other tube worms as fouling organisms in beds of oysters *P. margaritifera* in the Red Sea. Mohammed (1972) reported infestations of mudworm *Polydora vulgaris* in 4.68% of 20,733 *P. margaritifera* from the Arabian Gulf, with a higher infestation rate (14.77 %) in older animals compared with young (0.28%), and a negative correlation between infestation rate and pearl weight.

Echinoderms

The echinoderms *Holothuria atra*, *H. impatens*, *H. paradalis*, *Linckia multiflora*, *Opniothrix* spp., *Synaptula reciprocans* and *Ophiolepis superba* were described by Nasr (1982) as fouling organisms of beds of oysters *P. margaritifera* in the Red Sea.

Porifera

Sponges are well recognised fouling and boring organisms and are considered to be amongst the major organisms invading pearl shell. Dharmaraj *et al.* (1987) described profuse growths of the sponges *Callyspongia fibrosa* and *Haliclona exigua* as completely covering oysters in the Gulf of Mannar, although apparent damage was minimal. Dharmaraj *et al.* (1987) also described three species of *Cliona* as boring sponges, resulting in shell perforation and ultimately a weakened fragile shell. Crossland (1957) reported five species of boring sponge to cause serious shell disease in *P. margaritifera* in the Red Sea. The most common of these species, a red sponge, produced holes 1-2 mm diameter in the shell. The boring sponge *Cliona* sp. was reported by Nasr (1982) as a parasite of the shell of *P. margaritifera* in the Red Sea. Je *et al.* (1988) described unspecified sponges as fouling organisms of *P. fucata martensii* in Korea. Wada (1991) reported that sponges, together with mudworms and a trematode, were the major parasites adversely affecting pearl production in Japan.

Algae

Algae and seaweed are commonly reported as fouling organisms of pearl oysters. Brown seaweed *Padina* spp., green algae *Caulerpa* spp., calcareous algae *Halimeda* spp. and various red algae were recorded as fouling organisms of beds of *P. margaritifera* in the Red Sea by Nasr (1982). Similarly, Cohn (1949) recorded five species of *Codium* as fouling organisms of

P. martensii; in Japan. Dharmaraj *et al.* (1987) noted that a number of species of algae were common fouling organisms in the Gulf of Mannar (Table 2-2).

Coelenterates

Coelenterates are characterised by a hollow sac-like body with a single opening or mouth typically surrounded by tentacles. The phylum includes hydras, jellyfish and sea anemones (Beckett 1984). Coelenterates are significant as fouling organisms of farmed pearl oysters. Various hydroids and members of the family Actinidae were among fouling organisms of beds of oysters *P. margaritifera* in the Red Sea (Nasr 1982). Unspecified hydrozoan and anthozoan coelenterates, including *Actinaria*, were reported by Je *et al.* (1988) as fouling organisms of farmed pearl oysters *P. fucata martensii* in Korea. Similarly, Dharmaraj *et al.* (1987) reported a range of hydroids and anthozoans as fouling organisms of pearl oysters in the Gulf of Mannar.

Nemertea

Je *et al.* (1988) reported unspecified Nemertinea as common fouling organisms of farmed pearl oysters *P. fucata martensii* in Korea.

Nematodes

Je *et al.* (1988) reported unspecified nematodes to be occasional fouling organisms of farmed pearl oysters *P. fucata martensii* in Korea.

Ascidians

The Subphylum Urochordata includes the tunicates and ascidians, invertebrate chordates which have a tough outer tunic and are generally sessile, growing permanently attached to the substrate (Buchsbaum 1948). Ascidians are reported as fouling organisms of pearl oysters in the Gulf of Mannar, sometimes occurring in large numbers (Dharmaraj *et al.* 1987).

Turbellarians and temnocephalans

Je *et al.* (1988) reported unspecified turbellaria as common fouling organisms of farmed pearl oysters *P. fucata martensii* in Korea.

COMMENSAL AND SYMBIOTIC ORGANISMS

Nasr (1982) described the commensal Pontoniine shrimp *Conchodytes meleagrinae* typically found as one small male and one large female in the mantle of *P. margaritifera* in the Red Sea. Approximately 50% of oysters had such shrimps. *Conchodytes meleagrinae* was earlier described as a commensal of *P. margaritifera* in Australia by Bruce (1977). Bruce (1989) subsequently described *Conchodytes maculatus* as a bivalve associate of *P. maxima* from the north west shelf.

Dix (1973) reported the pea crab *Pinnotheres villosulus* in the mantle of *P. maxima* in Australia, where it induced crateriform cavities without any apparent harm to the host.

Pearl fish identified as *Onuxodon margaritiferae* by Dr Barry Hutchings, Western Australian Museum are uncommonly observed in *P. maxima* collected at the Compass Rose pearling

grounds, Western Australia (Serena Sanders, Broome Pearls - Personal observation). There is no apparent harm caused by this fish to the host.

PREDATORS AND PREDATION

Predation of pearl oysters is a widely reported problem in farmed oysters and in natural oyster beds by a range of fishes, rays, octopus, starfish, molluscs and crustaceans (Dharmaraj 1987). Juvenile pearl oysters are particularly vulnerable to predation; up to 50% mortalities are recorded for unprotected spat (Gervis and Sims 1992).

A number of species of fish genera including *Lethrinus*, *Chrysophrys*, *Pagrus*, *Tetradon*, *Serranus* and *Balistes* are known predators of *Pinctada* spp. (Table 2-3). Young oysters below one year are especially susceptible (Crossland 1957, Gervis and Sims 1992, Dharmaraj *et al.* 1987). Rays are also predators of older pearl oysters (Dharnaraj *et al.* 1987). In general, fish predators are not a problem if the oysters are protected by a cover (Gervis and Sims 1992).

Crustaceans, molluscs and echinoderms may be serious predators of pearl oysters (Table 2-3). Crossland (1957) reported several species of crabs and a hermit crab as attacking oysters of up to 100 mm diameter, but noted that attacks were usually on very young stages up to approximately 12 mm diameter. Dharmaraj *et al.* (1987) also described crabs as serious predators, with the larval stages entering cages. Gervis and Sims (1992) and Dharmaraj *et al.* (1987) described octopus, starfish, crabs and a variety of gastropods as predatory species. The mollusc *Murex virgineus* is described as a voracious predator of *P. fucata* (Gervis and Sims 1992) and *M. anguliferus* was reportedly the worst predator in unprotected beds of *P. margaritifera* in the Red Sea (Crossland 1957, Gervis and Sims 1992). Chellam *et al.* (1983) described severe mortality in *P. fucata* following predation by the gastropods *Cymatium cingulatum* and *M. virgineus*. *M. ramosus* has also been implicated in predation of pearl oysters (Gervis and Sims 1992). The crab *Charybdis* sp. was reported by Gervis and Sims (1992) as destroying entire cages of *P. fucata* in India.

Ranillid and muricid gastropods generally do not pose a problem once spat cannot migrate from cages and if off-bottom culture techniques are used (Gervis and Sims 1992). Gastropods of the family Ranellidae (Cymatidae) are described as serious pests in the culture of *P. margaritifera* and *P. maxima* in Okinawa and other tropical areas (Gervis and Sims 1992). *Cymatium cingulatum* preys on *Pinctada* spp. in India. In laboratory studies in the Solomon Islands, *C. muricinum*, *C. aquatile*, *C. nicobaricum* and *C. pileare* have been observed to prey on smaller *Pinctada* spp (Gervis and Sims 1992).

Newman *et al.* (1993) described a turbellarian predator, *Stylochus* (*Imogene*) *matatasi* as being consistently associated with mortalities of the cultured giant clam, *Tridacna gigas* and the fouling pearl oyster *P. maculata* in the Solomon Islands.

NON-INFECTIOUS AND ENVIRONMENTAL DISEASES AND CONDITIONS

Non-infectious and environmental factors are well established as important causes of mortalities and production losses in pearl oysters. Such factors may act in their own right, or

may contribute to disease occurrence by stressing the oysters or rendering them more susceptible to infectious agents which might otherwise not cause disease.

Feeds and feeding

Bivalves acquire energy for growth and metabolism by feeding on suspended particulate matter. The filtration rate of pearl oysters is an indication of feeding activity, and is temperature dependent. Filtration rate in *P. fucata martensii* increases up to 28°C, above which a dramatic reduction in filtration rate is reported (Numaguchi 1995a). The metabolic rate of *P. fucata martensii* also increases with increasing water temperature. As a consequence, food intake via filtration may be insufficient to maintain metabolic requirements at temperatures above 28°C, and oysters may lose condition. (Numaguchi 1995a).

Following a period of extended food deprivation, Numaguchi (1995a) reported increased mortalities in mature *P. fucata martensii* commencing at 70 days, with a cumulative mortality of 95% at day 115. Unfed oysters showed early decrease in adductor muscle glycogen content, decreased crystalline style weight, decreased dry meat weight and decreased condition index, ie, the ratio of dry to wet oyster meat weight.

Temperature

Water temperature plays a major role in maintaining normal physiological function and in eliciting pathophysiological effects on the oyster. As noted, filtration rate increased in *P. fucata martensii* up to 28°C after which a marked reduction occurred (Numaguchi 1995a). Lowered water temperatures below the optimum for a particular species may also cause serious losses. Such losses have been reported in *P. martensii* in Japan (Cohn 1949).

Effects of water temperature on unfed pearl oysters *P. fucata martensii* was investigated by Numaguchi (1995b) who reported a decreasing condition index and dry meat weight with increasing temperature, especially above 28°C. The results suggested that the degree of catabolic losses of pearl oysters is depended on the ambient seawater temperature in unfed oysters, and that unfed pearl oysters can survive by utilising energy reserves from their meat for up to 60 days at water temperatures of 15-28°C.

Increasing water temperature and increasing metabolic rate also increases the requirement for oxygen, especially above 27°C (Numaguchi 1995b). Factors which limit oxygen availability to the oyster, including fouling, decreased water exchange and decreased dissolved oxygen, may all adversely impact on the metabolism of the animal.

Decreased activity of host inflammatory processes at lower environmental temperature and favourable temperatures for growth of marine *Vibrio* bacteria were considered by Dybdahl and Pass (1985) and Pass *et al.* (1987) to be a major factor in deaths of transported *P. maxima.* Mannion (1983) examined the influence of temperature to experimental infection with the bacteria *Vibrio harveyi* and *Pseudomonas putrificiens* in *P. maxima* at 19°C and 29°C. Oysters held at the lower temperature showed a markedly greater incidence of disease and mortality.

Salinity

Reductions in salinity associated with heavy rainfall and/or run-off from rivers and estuaries may cause serious losses to cultures of oyster stocks. Cohn (1949) also noted that stratification of waters of different salinity may occur under such conditions, with 1-2 metres of fresh or less saline water overlying sea water of normal specific gravity. High losses were

described by Cohn (1949) in Japan in *P. martensii* under these conditions.

Ota and Fukushima (1961) associated decreased salinity with poor shell growth and poor quality of pearls, likely resulting from decreased food consumption.

Decreased irradiance

Cohn (1949) noted indirect damage to oysters caused by algal blooms associated with reduction of light penetration and subsequent effects on metabolism.

Phytoplanktonic blooms (algal or dinoflagellate blooms, red tides)

Phytoplanktonic blooms are reported causes of direct and indirect losses of pearl oysters, the Japanese pearl oyster industry particularly suffering severe losses as a result of such blooms on numerous occasions (Sparks 1985). Such losses may be due to decreased oxygen, suffocation associated with clogging of gills or direct toxic effects (Cohn 1949; Sparks 1985).

Matsuyama *et al.* (1995) described large-scale red tides of the toxic dinoflagellate *Heterocapsa* sp. (Dinophyceae) which occurred in Japan during the summer and again in the autumn of 1992. Both red tides were preceded by heavy rainfall and/or vertical mixing of the seawater associated with a typhoon. The red tides caused mass mortality of pearl oysters *P. fucata*. Oysters closed their shells tightly and died despite a high concentration of dissolved oxygen.

Sparks (1985) reported an earlier mass mortality in 1910 probably caused by *Gymnodinium mikimoto* Cohn (1949) described algal blooms of *Gymnodinium* in Japan in 1917 and in 1934 which caused extensive damage to fish and shellfish, including pearl oysters. Cohn (1949) reported that the predominant organisms occurring in the red tides in Japan were the dinoflagellates genera *Gymnodinium*, *Gonyaulax*, *Peridinium*, *Ceratium* and. *Prorocentrum* and the distomacean genus *Chaetocerae*.

Nagai *et al.* (1996) investigated the role of the toxic red tide dinoflagellate *Heterocapsa circularisquama* in a mass mortality in two month old pearl oysters *P. fucata*. Toxicity appeared to be a direct effect of *H. circularisquama* cell numbers. Oysters rapidly contracted their mantles, closed their shell valves, contracted their gills and experienced irregular heartbeat patterns until the heart stopped permanently.

NEOPLASIA

Few neoplasms have been described in *Pinctada* species. Dix (1972) described two polypous mesenchymal tumours in *P. margaritifera* from Queensland, Australia.

Table 2-1. A checklist of pathogens, parasites and diseases reported or observed in pearl oysters *Pinctada* spp.

Viruses and Viral Diseases

Aetiological Agent	Disease/Condition	Host Species	Location	Reference/s
Papovavirus-like	Epithelial hypertrophy	*P. maxima*	Australia	Norton *et al.* 1993a
Virus-like agent	Apparently healthy	*P. maxima*	Australia	Pass *et al.* 1988

Bacteria and Bacterial Diseases

Aetiological Agent	Disease/Condition	Host Species	Location	Reference/s
Vibrio harveyi	Mortalities	*P. maxima*	Australia	Lester 1990; Pass *et al.* 1987
Vibrio harveyi	Mortalities	*P. maxima*	Australia	Dybdahl & Pass 1985; Pass *et al.* 1987
Vibrio sp.	Mortalities	*P. maxima*	Australia	Dybdahl & Pass 1985;
Vibrio sp.	Normal	*P. maxima*	Australia	Jones - Unpublished
Mixed bacteria	Septicaemia	*P. maxima*	Australia	SCFH 1991
Vibrio pelagicus	Normal	*P. maxima*	Australia	Jones - Unpublished
Vibrio mediterranei	Normal	*P. maxima*	Australia	Jones - Unpublished
Vibrio alginolyticus	Normal	*P. maxima*	Australia	Jones - Unpublished
Vibrio anguillarum	Normal	*P. maxima*	Australia	Jones - Unpublished
Vibrio splendidus II	Normal	*P. maxima*	Australia	Jones - Unpublished
Vibrio parahaemolyticus	Normal	*P. maxima*	Australia	Jones - Unpublished
Photobacterium sp.	Normal	*P. maxima*	Australia	Jones - Unpublished
Cornebacterium sp.	Normal	*P. maxima*	Australia	Jones - Unpublished
Erwinia hebicola	Normal	*P. maxima*	Australia	Jones - Unpublished

Protozoa and Protozoal Diseases

Aetiological Agent	Disease/Condition	Host Species	Location	Reference/s
Gregarines	Apparently healthy	*P. maxima*	Australia	Cui 1997
Gregarines	Mortalities (incidental finding ?)	*P. margaritifera*	French Polynesia	Chagot *et al.* 1993
Haplosporidium sp.	No disease reported	*P.maxima*	Australia (WA)	Hine 1996; Hine & Thorne 1998
Mikrocytos sp.	No disease reported	*P. maxima*	Australia	SCFH 1993
Perkinsus sp.	No disease reported	*P. margaritifera*	Australia	Goggin & Lester 1987; Lester & Sewell 1989
Perkinsus sp.	No disease reported	*P. sugillata*	Australia	Goggin & Lester 1987
Perkinsus-like sp.	Mortalities	*P. maxima*	Australia (Qld)	Norton *et al.* 1993b
Protistan-like bodies	"Residual" bodies	*P. maxima*	Australia (WA)	Pass & Perkins 1985; Wolf & Sprague 1978

Algae and Algal-Associated Diseases

Aetiological Agent	Disease/Condition	Host Species	Location	Reference/s
Heterocapsa sp.	"Red tide" Mortalities	*P. fucata*	Japan	Matsuyama *et al.* (1995)

Table 2-1(continued). A checklist of pathogens, parasites and diseases reported or observed in pearl oysters *Pinctada* spp.

Annelids and Annelidan Diseases

Aetiological Agent	Disease/Condition	Host Species	Location	Reference/s
Polychaete	High mortality	*P. maxima*(?)	PNG	Eldredge 1993

Platyhelminths and Platyhelminth Diseases

Aetiological Agent	Disease/Condition	Host Species	Location	Reference/s
Cestoda				
Tylocephalum sp.	Parasitism	*P. margaritifera*	Red Sea	Nasr 1982
Unspecified larvae	Parasitism	*P. vulgaris*	Red Sea	Crossland 1957
Trematoda				
Bucephalus varicus	Parasitism	*P. fucata*	Japan	Wada 1991

Diseases of Unknown Aetiology

Aetiological Agent	Disease/Condition	Host Species	Location	Reference/s
Uncharacterised	Mass mortalities	*P. margaritifera*	French Polynesia	SPC 1985, 1988; Brayley 1991; Cabral 1989a, 1989b; Eldredge 1993; Vacelet *et al.* 1996
Uncharacterised	Mass mortalities	*P. margaritifera*	Red Sea	Nasr 1982

Table 2-2. Epiphytic, fouling and boring organisms reported or observed in association with pearl oysters *Pinctada* spp.

Taxon	Fouling (F) or Boring (B)	Host Species *	Geographic Location	Reference/s
Algae				
Padina sp.	F	Pm	Red Sea	Nasr 1982
		Pm; Pr	Persian Gulf	Doroudi 1996
Caulerpa sp	F	Pm	Red Sea	Nasr 1982
Halimeda sp.	F	Pm	Red Sea	Nasr 1982
Dictyota divaricata	F	Pr	Persian Gulf	Doroudi 1996
Ulva sp.	F	Pm; Pr	Persian Gulf	Doroudi 1996
Sargassum spp.	F	Pm; Pr	Persian Gulf	Doroudi 1996
Spyridia filamentosa	F	Pmx	Australia	Steinberg (pers. comm.)
Unspecified red algae	F	Pm	Red Sea	Nasr 1982
Bryozoa				
Zoobotryon verticillatum	F	Pfm	Korea	Je et al 1988
Membranipora sp.	F	Pf	Gulf of Mannar	Dharmaraj *et al.* 1987
Thallamoporella sp.	F	Pf	Gulf of Mannar	Dharmaraj *et al. 1987*
Lagenipora sp.	F	Pf	Gulf of Mannar	Dharmaraj *et al.* 1987
Watersipora sp.	F	Pf	Gulf of Mannar	Dharmaraj *et al.* 1987
Bugula sp.	F	Pf	Gulf of Mannar	Dharmaraj *et al.* 1987
Unspecified	F	Pfm	Korea	Je *et al.* 1988
Unspecified		Pm; Pr	Persian Gulf	Doroudi 1996
Annelida				
Polychaeta				
Polydora ciliata	B	Pf	Gulf of Mannar	Dharmaraj & Chellam 1983
Polydora ciliata	B	Pfm	Korea	Je *et al.* 1988
Polydora vulgaris	B	Pm	South China Sea	Manchenko & Radashevsky 1994
Polydora sp.	B	Pm	Red Sea	Nasr 1982; Crossland 1957
		Pm; Pr	Persian Gulf	Doroudi 1996
Nereid Annelids	F	Pm	Red Sea	Nasr 1982
Nereis spp.		Pm; Pr	Persian Gulf	Doroudi 1996
Perinereis spp.		Pm; Pr	Persian Gulf	Doroudi 1996
Sabellid Annelids	F	Pm	Red Sea	Nasr 1982
Leonates sp.		Pm; Pr	Persian Gulf	Doroudi 1996
Hydroides sp.		Pm; Pr	Persian Gulf	Doroudi 1996
Serpula spp.		Pm; Pr	Persian Gulf	Doroudi 1996
Euthalenessa sp.		Pm; Pr	Persian Gulf	Doroudi 1996
Eunice sp.		Pm; Pr	Persian Gulf	Doroudi 1996
Lumbrineris sp.		Pm; Pr	Persian Gulf	Doroudi 1996
Unspecified polychaetes	F	Pm	Red Sea	Nasr 1982
	F	Pmx	Indonesia	Taylor 1997
		Pm; Pr	Persian Gulf	Doroudi 1996

* **Pm** = ***Pinctada margaritifera*** **Pfm** = ***Pinctada furcata martensii***
Pmx = ***Pinctada maxima*** **Pf** = ***Pinctada fucata***
Pr = ***Pinctada radiata***

Table 2-2 (continued). Epiphytic, fouling and boring organisms reported or observed in association with pearl oysters *Pinctada* spp.

	Fouling (F) or Boring (B)	Host Species *	Geographic Location	Reference/s
Platyhelminths				
Stylochus spp.		Pm; Pr	Persian Gulf	Doroudi 1996
Unspecified	F	Pfm	Korea	Je *et al.* 1988
Nemertea				
Unspecified	F	Pfm	Korea	Je *et al.* 1988
Nematodes Aschelminthes				
Unspecified	F	Pfm	Korea	Je *et al.* 1988
Porifera				
Callyspongia fibrosa	F	Pf	Gulf of Mannar	Dharmaraj *et al.* 1987
Chalina sp		Pm; Pr	Persian Gulf	Doroudi 1996
Cliona sp.	B	Pm;Pmx	Red Sea, Australia	Nasr 1982; Authors obs
Cliona vastifica	B	Pm; Pr	Persian Gulf	Doroudi 1996
Cliona vastifica	B	Pf	Gulf of Mannar	Dharmaraj & Chellam 1983
Cliona carpenteri		Pm; Pr	Persian Gulf	Doroudi 1996
Cliona margaritifera		Pm; Pr	Persian Gulf	Doroudi 1996
Halicliona exigua	F	Pf	Gulf of Mannar	Dharmaraj *et al.* 1987
Halicliona spp.		Pm; Pr	Persian Gulf	Doroudi 1996
Unspecified	F	Pfm	Korea	Je *et al.* 1988
Unspecified	F	Pm	Red sea	Crossland 1957 citing Herdman 1903)
Coelenterates				
Hydrozoa				
Unspecified	F	Pfm	Korea	Je *et al.* 1988
Various hydroids	F	Pm	Red Sea	Nasr 1982
Anthozoa				
Actinidae	F	Pm	Red Sea	Nasr 1982
Unspecified Actinaria	F	Pfm	Korea	Je *et al.* 1988
Unidentified coral		Pm; Pr	Persian Gulf	Doroudi 1996
Unidentified anemones		Pm; Pr	Persian Gulf	Doroudi 1996
Echinoderms				
Linckia multiflora	F	Pm	Red Sea	Nasr 1982
Opniothrix sp.	F	Pm	Red Sea	Nasr 1982
Holothuria atra	F	Pm	Red Sea	Nasr 1982
Holothuria impatens	F	Pm	Red Sea	Nasr 1982
Holothura paradalis	F	Pm	Red Sea	Nasr 1982
Synaptula reciprocans	F	Pm	Red Sea	Nasr 1982
Ophiolepis superba	F	Pm	Red Sea	Nasr 1982
Unspecified	F	Pm; Pr	Persian Gulf	Doroudi 1996

* **Pm** = ***Pinctada margaritifera*** **Pfm** = ***Pinctada furcata martensii***
Pmx = ***Pinctada maxima*** **Pf** = ***Pinctada fucata***
Pr = ***Pinctada radiata***

Table 2-2 (continued). Epiphytic, fouling and boring organisms reported or observed in association with pearl oysters *Pinctada* spp.

Taxon	Fouling (F) or Boring (B)	Host Species *	Geographic Location	Reference/s
Mollusca				
Avicular vexillum	F	Pf	Gulf of Mannar	Dharmaraj *et al.* 1987
Arca sp.		Pm; Pr	Persian Gulf	Doroudi 1996
Mytilus sp.		Pm; Pr	Persian Gulf	Doroudi 1996
	F	Pmx	Indonesia	Taylor 1997
Mytilus edulis galloprovincialis	F	Pfm	Korea	Je *et al.* 1988
Crassostrea gigas	F	Pfm	Korea	Je *et al.* 1988
Crassostrea sp.	F	Pf	Gulf of Mannar	Dharmaraj *et al.* 1987
	F	Pmx	Indonesia	Taylor 1997
Saccostrea cucullata	F	Pm; Pr	Persian Gulf	Doroudi 1996
Anomia chinensis	F	Pfm	Korea	Je *et al.* 1988
Gastrochaena cuneiformis	B	Pmx	Australia	NT Museum Record
Lithophaga divaricalyx	B	Pmx	Australia	NT Museum Record
Lithophaga malaccana	B	Pm; Pr	Persian Gulf	Doroudi 1996
	B	Pmx	Australia	NT Museum Record
Lithophaga hanleyana	B	Pm; Pr	Persian Gulf	Doroudi 1996
Lithophaga teres	B	Pmx	Australia	NT Museum Record
Lithophaga sp.	B	Pm	Red Sea	Crossland 1957
Modiolus metcalfei	F	Pf	Gulf of Mannar	Dharmaraj *et al* 1987
Pinctada fucata	F	Pm; Pr	Persian Gulf	Doroudi 1996
Pinctada fucata	F	Pf	Gulf of Mannar	Dharmaraj *et al.* 1987
Pinctada sugillata	F	Pf	Gulf of Mannar	Dharmaraj *et al.* 1987
Pinctada chemnitzii	F	Pf	Gulf of Mannar	Dharmaraj *et al.* 1987
Pinctada spp.	F	Pmx	Indonesia	Taylor 1997
Pinna spp.	F	Pmx	Indonesia	Taylor 1997
Pteria penguin	F	Pm; Pr	Persian Gulf	Doroudi 1996
Pteria spp.	F	Pmx	Indonesia	Taylor 1997
Murex sp.		Pm; Pr	Persian Gulf	Doroudi 1996
Cypraea sp.		Pm; Pr	Persian Gulf	Doroudi 1996
Vermetus sp.		Pm; Pr	Persian Gulf	Doroudi 1996
Terebra sp.		Pm; Pr	Persian Gulf	Doroudi 1996
Trochus sp.		Pm; Pr	Persian Gulf	Doroudi 1996
Loffiidae sp.		Pm; Pr	Persian Gulf	Doroudi 1996
Vermicularia sp.		Pm; Pr	Persian Gulf	Doroudi 1996
Unidentified chiton		Pm; Pr	Persian Gulf	Doroudi 1996
Unspecified bivalves & gastropods	F	Pfm	Korea	Je *et al.* 1988
Martesia sp.	B	Pf	Gulf of Mannar	Dharmaraj & Chellam 1983
Crustacea				
Decapoda				
Alpheus sp.	F	Pm	Red Sea	Nasr 1982
Phymodius ungulatus	F	Pm	Red Sea	Nasr 1982
Liomera rugatas	F	Pm	Red Sea	Nasr 1982

* **Pm** = ***Pinctada margaritifera***
Pmx = ***Pinctada maxima***
Pr = ***Pinctada radiata***
Pfm = ***Pinctada furcata martensii***
Pf = ***Pinctada fucata***

Table 2-2 (continued). Epiphytic, fouling and boring organisms reported or observed in association with pearl oysters *Pinctada* spp.

Taxon	Fouling (F) or Boring (B)	Host Species *	Geographic Location	Reference/s
Crustacea (cont)				
Trapezia digitalis	F	Pm	Red Sea	Nasr 1982
Petrolisthes lamarckii	F	Pm	Red Sea	Nasr 1982
Actaea hispidus	F	Pm	Red Sea	Nasr 1982
Conchodytes meleagrinae	F	Pm	Red sea	Nasr 1982; Chase and Bruce 1993
	F	Pm; Pr	Persian Gulf	Doroudi 1996
Conchodytes maculatus	F	Pmx	Australia, Phillippines	Bruce 1989; Chace and Bruce 1993;
Pinnotheres villosus	F	Pmx	Australia	Dix 1973;
Cirripedia				
Balanus amphitrite	F	Pm; Pr	Persian Gulf	Doroudi 1996
	F	Pmx	Australia	Steinberg (pers. comm.)
Balanus amphitrite variegatus	F	Pf	Gulf of Mannar	Dharmaraj & Chellam 1983; Dharmaraj *et al.* 1987
Balanus amphitrite communis	F	Pf	Gulf of Mannar	Dharmaraj & Chellam 1983; Dharmaraj *et al.* 1987
Balanus amphitrite venustus	F	Pf	Gulf of Mannar	Dharmaraj & Chellam 1983; Dharmaraj *et al.* 1987
Balanus tintinnabulum	F	Pm; Pr	Persian Gulf	Doroudi 1996
Isopoda				
Sphaeroma sp.		Pm; Pr	Persian Gulf	Doroudi 1996
Decapoda				
Scyllarus sp.		Pm; Pr	Persian Gulf	Doroudi 1996
Chordata (Ascidians)				
Ascidia depressiscula	F	Pf	Gulf of Mannar	Dharmaraj *et al.* 1987
Botrilloides spp.	F.	Pf	Gulf of Mannar	Dharmaraj *et al.* 1987
Botryllus sp.	F	Pm; Pr	Persian Gulf	Doroudi 1996
Dicarpa sp.	F	Pf	Gulf of Mannar	Dharmaraj *et al.* 1987
Didemnum sp.	F	Pm; Pr	Persian Gulf	Doroudi 1996
Diplosoma sp.	F	Pf	Gulf of Mannar	Dharmaraj *et al.* 1987
Leptoclinides sp.	F	Pm; Pr	Persian Gulf	Doroudi 1996
Unidentified spp.	F	Pm; Pr	Persian Gulf	Doroudi 1996
Pisces				
Onuxodon margaritiferae	F	Pmx	Australia	WA Museum Records

* **Pm** = ***Pinctada margaritifera***
Pmx = ***Pinctada maxima***
Pr = ***Pinctada radiata***
Pfm = ***Pinctada furcata martensii***
Pf = ***Pinctada fucata***

Table 2-3. Reported predators of pearl oysters *Pinctada* spp.

Taxon	Host Species	Location	Reference/s
Platyhelminths			
Turbellaria			
Stylochus matatasi	*P. maculata*	Solomon Islands	Newman *et al.* 1993
Mollusca			
Gastropoda			
Cymatium cingulatum	*P. fucata*	Gulf of Mannar	Dharmaraj *et al.* 1987; Chellam *et al.* 1983
Murex virgineus	*P. fucata*	Gulf of Mannar	Dharmaraj *et al.* 1987; Chellam *et al.* 1983
Murex ramosus	*Pinctada* sp.		Dharmaraj *et al.* 1987
Murex anguliferus	*P. margaritifera*	Red Sea	Crossland 1957
Bivalvia			
Sistrum spectrum	*Pinctada* sp.	Gulf of Mannar	Dharmaraj *et al.* 1987
Pinaxia coronata	*Pinctada* sp.	Gulf of Mannar	Dharmaraj *et al.* 1987
Nassa sp.	*Pinctada* sp.	Gulf of Mannar	Dharmaraj *et al.* 1987
Purpurea sp.	*Pinctada* sp.	Gulf of Mannar	Dharmaraj *et al.* 1987
Turbinella sp	*Pinctada* sp.	Gulf of Mannar	Dharmaraj *et al.* 1987
Crustacea			
Decapoda			
Charybdis lucifera	*P. fucata*	Gulf of Mannar	Dharmaraj *et al.* 1987
Atergatis integerrisimus	*P. fucata*	Gulf of Mannar	Dharmaraj *et al.* 1987
Leptodius exaratus	*P. fucata*	Gulf of Mannar	Dharmaraj *et al.* 1987
Neptunus spp.	*P. fucata*	Gulf of Mannar	Dharmaraj *et al.* 1987
Thalamita spp	*P. fucata*	Gulf of Mannar	Dharmaraj *et al.* 1987
Unspecified crabs	*P. margaritifera*	Red Sea	Crossland 1957
Chordata			
Pisces			
Lethrinus karwa	*P. margaritifera*	Red Sea	Crossland 1957
Lethrinus spp.	*P. fucata*	Gulf of Mannar	Dharmaraj *et al* 1987
Chrysophrys bifurcatus	*P. margaritifera*	Red Sea	Crossland 1957
Pagrus sp.	*P. margaritifera*	Red Sea	Crossland 1957
Balistes viridescens	*P. margaritifera*	Red Sea	Crossland 1957
Balistes mitis	*P. fucata*	Gulf of Mannar	Dharmaraj *et al.* 1987
Balistes stellaris	*P. fucata*	Gulf of Mannar	Dharmaraj *et al.* 1987
Balistes maculatus	*P. fucata*	Gulf of Mannar	Dharmaraj *et al.* 1987
Balistes sp.	*P.margaritifera; P.radiata*	Persian Gulf	Doroudi 1996
Serranus spp.	*P. fucata*	Gulf of Mannar	Dharmaraj *et al.* 1987
Tetradon stellatum	*P.margaritifera; P.radiata*	Persian Gulf	Doroudi 1996
Rhinoptera javanica	*P. fucata*	Gulf of Mannar	Dharmaraj *et al.* 1987
Ginglymostoma spp.	*P. fucata*	Gulf of Mannar	Dharmaraj *et al.* 1987
Unspecified octopus & starfish	*P. fucata*	Gulf of Mannar	Dharmaraj *et al.* 1987

Table 2-4. A checklist of pathogens, parasites, pests, diseases and commensals recorded or observed in Australian pearl oysters *Pinctada* spp.

	Aetiological Agent	Disease	Reference/s
Viruses and Viral Diseases	Papovavirus-like	Epithelial hypertrophy of palp	Norton *et al.* 1993a
	Intranuclear virus	No disease reported	Pass *et al.* 1988
Bacteria and Bacterial Diseases	*Vibrio harveyi*	Mortalities	Mannion 1983; Lester 1990; Pass *et al.* 1987; Dybdahl & Pass 1985;
	Vibrio sp	Mortalities	Dybdahl & Pass 1985;
	Vibrio sp.	Normal	Jones - Unpublished
	Vibrio pelagicus	Normal	Jones - Unpublished
	Vibrio mediterranei	Normal	Jones - Unpublished
	Vibrio alginolyticus	Normal	Jones - Unpublished
	Vibrio anguillarum	Normal	Jones - Unpublished
	Vibrio splendidus II	Normal	Jones - Unpublished
	Vibrio parahaemolyticus	Normal	Jones - Unpublished
	Photobacterium sp.	Normal	Jones - Unpublished
	Cornebacterium sp.	Normal	Jones - Unpublished
	Erwinia hebicola	Normal	Jones - Unpublished
	Pseudomonas putrefaciens	Mortalities	Mannion 1983
	Mixed bacteria	Septicaemia	SCFH 1991
Protozoa and Protozoal Diseases	Gregarines	No disease reported	Cui 1997
	Haplosporidium sp.	No disease reported	Hine 1996; Hine & Thorne 1998
	Mikrocytos sp.	No disease reported	SCFH 1993
	Perkinsus sp.	No disease reported	Goggin & Lester 1987; Lester & Sewell 1989
	Perkinsus-like	Mortalities (adults)	Norton *et al.* 1993b
	Protistan-like sp.	"Residual" bodies	Pass & Perkins 1985; Wolf 1993b; Wolf & Sprague 1978
Crustacea and Crustacean Diseases	*Conchodytes maculatus*	No disease reported	Bruce 1989; Chace and Bruce 1993;
	Pinnotheres villosus	No disease reported	Dix 1973
Fishes	*Onuxodon margaritiferae*	No disease reported	WA Museum Record (Hutchings 1998).
Algae	*Spyridia filamentosa*	Fouling	Steinberg (pers. comm.)
Porifera	*Cliona* sp.	Fouling	Authors Obs.
Mollusca	*Gastrochaena cuneiformis*	Boring	NT Museum Record
	Lithophaga divaricalyx	Boring	NT Museum Record
	Lithophaga malaccana	Boring	NT Museum Record
	Lithophaga teres	Boring	NT Museum Record
Cirripedia	*Balanus amphitrite*	Fouling	Steinberg (pers. comm.)

CHAPTER 3: DISEASES AND PARASITES OF AUSTRALIAN PEARL OYSTERS: A PATHOLOGICAL EVALUATION

INTRODUCTION

A sound understanding of the occurrence, distribution and prevalence of disease causing agents infecting pearl oysters is fundamental to disease diagnosis, management of disease and the implementation of local, regional and national quarantine programs to prevent spread of diseases. The development of diagnostic tests and procedures to assist in the recognition of exotic and endemic diseases also necessitates a sound understanding of the histological structure of pearl oysters and their pathological responses to infectious or non-infectious diseases.

Despite the size, social and economic importance of the Australian cultured pearl oyster industry and accounts of diseases and production problems, little information exists detailing the occurrence, prevalence and distribution of infectious or non-infectious agents which may cause disease or reduce productivity in Australian pearl oysters. No prospective study has been undertaken to establish the pathogenic significance of such agents and no study has been undertaken which describes in detail the histological structure of Australian pearl oysters.

This study reports on a comprehensive pathological survey of wild-harvested and farmed pearl oysters undertaken specifically to identify the spectrum of parasites, pathogens and disease which may be commonly encountered in pearl oysters from northern Australian waters and which may adversely impact pearl oyster production.

MATERIALS AND METHODS

Source and collection of pearl oysters

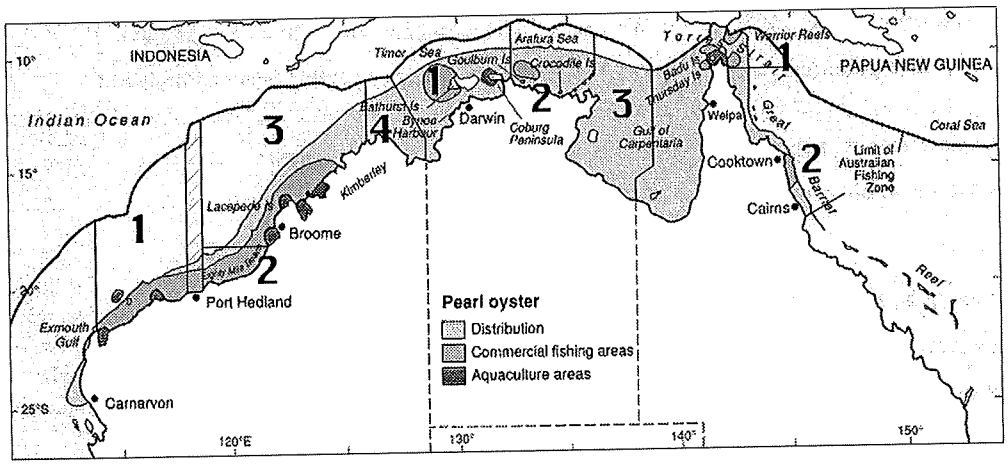
Representative samples of mature pearl oysters *P. maxima* were collected from populations in defined geographical zones in NT, Qld and WA over a three year period between 1994 and 1996 (Figure 1). Oysters examined in the study were derived from wild-harvest and farmed sources and represented clinically normal animals, animals used for half or round pearl production, animals discarded for rejecting seed pearls or animals at the end of their effective pearl production. Where possible, 150 oysters were collected from each site on two occasions representing different seasons. The origin, history and date(s) of collection are given in Table 3.1.

Processing and fixation

Oysters were dissected free of their shells by severing of the adductor muscles followed by immersion in fixative, or were fixed *in-situ* by wedging open the shells with subsequent immersion in 10% buffered formalin. Oysters were fixed either immediately following harvesting, or following transportation live in the whole shell to the laboratory. Where possible, several incisions were made in the animal to facilitate tissue penetration of fixative. Shells sent to the laboratory were stored dry for subsequent measurements and assessment of

shell damage associated with epiphytic and boring organisms. Alternatively, shells were assessed at the collection site. This was not always possible because of on farm practices and or time constraints.

Figure 1: Map depicting locations of pearling zones in northern Australia. (Numbers denote zones within each state)



Pathological examination

Shell morphometry and assessment of damage

Northern Territory. Where available, length, width and height of each shell, together with the dry weight of both shells of each oyster were recorded. Shells were assessed in a semi-quantitative manner for damage associated with boring or other epiphytic fauna. In particular full thickness or near full thickness penetration of the nacreous layer, and agents associated with such penetrations, were noted and characterised.

Internal shell surfaces were evaluated, and damage categorised as nil, mild, moderate, or severe, based on the nature, extent and number of blemishes or defects as follows.

Evaluation of Damage	Criteria
Nil	No defects, abnormalities or blemishes
Mild	< approx. 5 defects and/or < approx. 5% of the surface affected
Moderate	Approx. 5-20 defects and/or <approx 20% of the surface affected
Severe	> approx. 20 defects and/or > approx 20% surface affected

The extent and severity of damage on external shell surfaces was assessed on the number of holes and/or cavitations evident on gross examination and classified as occasional (+), moderate (++), and numerous (+++).

Queensland. The extent of boring sponges, boring organisms, holes and shell damage in the external shell was assessed on a scale as follows: 5%, 5-20%, >20% of shell area. Oysters

with nacre marks and/or retracted mantles were also recorded. Shell dimensions and weights were not recorded.

Western Australia. The extent and severity of damage associated with epiphytic and boring organisms was evaluated in a semi-quantitative manner with the occurrence of fouling organisms, mudworm, boring bivalves and sponges noted. Shell dimensions and weights were recorded in most instances.

Collection and identification of epiphytic organisms

Organisms associated with holes or cavities in the shell were dissected free from formalised shell, or were collected following emigration after placing shells in fresh water. Fresh organisms were fixed in 10% formalin awaiting identification. Similarly, epiphytic or commensal crustaceans associated with gills and mantle, fixed in 10% buffered formalin, were also held awaiting identification.

Representative epiphytic and boring organisms were forwarded to Dr. Richard Willan, Curator of Molluscs and Echinoderms, Museum and Art Gallery of the Northern Territory for identification.

Representative specimens of shrimps and pea crabs found in the mantle cavity were submitted for identification to Ms. Karen Coombes, Collections Manager, Natural Sciences, Museum and Art Gallery of the Northern Territory.

Assessment of gross pathological changes

Gross morphological or pathological changes of the entire animal were noted and recorded prior to dissection and collection of tissue samples for histopathological examination.

Histopathology and histopathological examination

Representative sections of tissue of each oyster, including gills, digestive gland, stomach, midgut, hindgut, mantle, adductor muscle, gonad, palps, heart, pericardial gland, foot, kidney and oesophagus as well as associated interstitial tissue, were collected. Typically, five sections were taken from each animal at different levels such that in each case all tissues were represented. In a low number of cases, not all tissues were represented. Tissues were embedded in paraffin wax and sections cut and stained with haematoxylin and eosin for routine histological examination. Where appropriate, lesions or abnormalities observed grossly were also sectioned in a similar manner.

Histological sections were examined for normality, and changes of histopathological significance recorded for each organ or tissue.

Data storage and analysis

Data collected in the study was collated and entered on a database (Filemaker Pro). This database was utilised to determine the prevalence of pathogens, parasites and histopathological changes and to make comparisons between populations of oysters from different regions.

RESULTS

Numbers and source of animals in study

A total of 4502 animals were examination from 22 separate locations or farm sites. Sampling was conducted on 35 occasions between September 1994 and September 1996. The source, identification, history, collection dates and numbers of oysters examined are shown in Table 3-1.

Northern Territory. 1280 oysters were examined from eight separate locations, representing four wild harvest locations and four farmed sites. Altogether, ten samplings were conducted. Animals were derived from three defined geographical zones.

Queensland. 1068 oysters were examined from six separate locations, representing two wild harvest locations and four farmed sites. Altogether, ten samplings were conducted. Animals were derived from two defined geographical zones.

Western Australia. 2154 oysters were examined from nine separate locations, representing three wild harvest locations and six farmed sites. Altogether, 15 samplings were conducted. Animals were derived from three defined geographical zones, and were collected during colder months "cold" or warmer months "warm" from these zones.

Shell morphometry

Abnormalities of shell structure and growth

Northern Territory. In the four series of oysters examined (WP, SM, A and D2 Series), 63 - 95% of larger, older oysters showed mild to severe shell damage, while less damage was evident in smaller, younger oysters (Table 3-2, 3-3).

On the internal or nacreous surface of the shells, abnormalities of shell structure and growth were characterised by focal to irregular brown-black discoloured nodules, plaques and tracts or "mud blisters" beneath the nacre. These areas were commonly overlaid by varying thicknesses of nacre, considered to represent walling off or isolation of damaged areas by the oyster and suggesting damage at different times. Lesions varied in size from one mm to 10 cm or more in extent, and varied in number from single or occasional, to numerous, often coalescing and involving the majority of the shell surface. On cut sections of shell, separation of overlying nacre from dark pigmented melanotic material was usually evident, representing an earlier invasion by burrowing or invading organisms. One or both shells were involved, with varying degrees of damage on different sides of the oyster.

Gross and sub-gross examination of the shells showed the cause of the lesions to be multi factorial, but resulting in similar lesions and varying levels of nacre deposition. Boring molluscs and burrowing and boring marine worms were commonly and readily observed, the latter often producing convoluted tracts beneath the nacre arising from the shell margins. Boring molluscs frequently produced dark sub-nacreous plaques in the central regions of the shell. Multiple fine holes and tracts (<1mm) of uncertain cause were often associated with the pigmented subnacreous lesions. On occasions, branching mycelial-like brown pigmented subnacreous plaques with no apparent connection to external surfaces were present.

Organisms identified as major biofouling and boring organisms in Northern Territory pearl oysters include the boring molluscs *Lithophaga malaccana, L. teres, L. divaricalyx* and *Gastrochaena cuneiformis* (Dr R.C. Willan).

Queensland. The prevalence of holes in the external shell valves caused by boring sponges and other agents was low to moderate and was mainly seen in aged, wild oysters and is shown in Table 3-2.

In one series of 148 wild pearl oysters which were collected from Torres Strait and which had a dorso-ventral size range of 13 to 23 cm, 36 had marked nacre (mostly mudworm induced) while 27 had external holes presumably made by boring sponges; of these 27 oysters, 11 had mild lesions (<5% shell area), 8 had moderate lesions (5-20%) shell area) and 8 had severe lesions (>20% shell area). Of 148 wild oysters collected from Torres Strait, 6 had double backs.

In a second series of 126 wild pearl oysters which were collected from the coast off Cooktown and which had a dorso-ventral size range of 22 to 23 cm, 38 had marked nacre (mostly mudworm induced), while 28 had external holes presumably made by boring sponges. Of these 28 oysters, 18 had mild lesions (<5% shell area), 8 had moderate lesions (5 to 20% shell area) and 2 had severe lesions (>20% shell area).

The prevalence of shell lesions was very low in farmed oysters. Farm A (Torres Strait) had only 4 oysters with nacre marks and 1 double backed oyster out of 36 oysters. Out of 150 oysters, Farm B (Torres Strait) had 20 oysters with marked nacre. On both farms, holes in the external shell were insignificant. The nacre marks were mainly mudworm induced.

Western Australia. A total of approximately 19 percent of oysters collected from WA had damage associated with boring and fouling organisms (Table 3.2). A range of boring sponges, boring bivalves and mudworm blisters was found in Western Australian oysters similar to NT and Qld. Approximately 17 % of oysters showed mudworm damage, varying from mild to severe. Approximately 8 % of shells showed damage associated with sponge invasion of the matrix, commonly referred to as "red bum".

Shell cavity organisms: commensals and symbionts

Northern Territory. Approximately 85% of one population of oysters contained pea crabs and approximately 72 % of another population contained shrimps present as commensal or symbiotic organisms inhabiting the region below the gills and mantle (Table 3-4). Shrimp were identified as *Conchodytes maculatus* and pea crabs as *Pinnotheres* sp. (Ms K. Coombes). Often, crabs were located in a deep depression within the mantle tissue adjacent to the palps, surrounded by a hypertrophic tissue response. It was unusual to find both crabs and shrimps together in the one animal, and crabs were usually solitary, whereas shrimps were commonly found in pairs, or in threes. Unidentified metazoa were present at a low occurrence. Pea crabs were common in wild pearl oysters collected in water depths <20 meters whereas shrimp were common in oysters collected at depths >20 meters.

Queensland. In one series of 148 wild pearl oysters from Torres Strait, 26 oysters (18%) carried pea crabs (*Pinnotheres* sp.) and 17 oysters (12%) carried shrimp (*Conchodytes* sp.). Both pea crabs and shrimp were present in farmed pearl oysters from Torres Strait, but their prevalence's were not recorded. In one series of 126 wild pearl oysters collected off Cooktown, only shrimps were found.

Western Australia. Pea crabs were the common finding in oysters in Western Australia.

Gross pathology

Northern Territory. Gross pathological examinations of oysters samples from clinically normal populations were generally unremarkable. Oedema of mantle and foot tissues was a common finding in several populations. Crateriform depressions in mantle and gill tissues containing pea crabs have been noted above.

Queensland. The oysters surveyed were normal and no significant gross pathology was seen.

Western Australia. No significant gross pathological changes were observed.

Histopathology

The occurrence and nature of histopathological changes and microbial, protozoan and metazoan agents observed in the study, together with their prevalence and distribution within tissues of oysters, and between populations and regions, are presented in Tables 3-5, 3-6, 3-7 and 3-8.

Table 3-5 shows the overall proportion of oysters in populations examined from NT, Qld and WA which show one or more significant histological change or infectious agent.

Table 3-6 shows the overall occurrence of inflammatory, degenerative and proliferative histopathological changes, together with those microbial, protozoan and metazoan agents observed in each population of oyster examined from each geographical zone or region in each state or territory.

Table 3-7 details the occurrence, prevalence and distribution of those histopathological changes and those microbial, protozoan and metazoan agents identified in the study in each population of oysters arranged according to organ or tissue.

Table 3-8 shows the overall prevalence and distribution of histopathological changes and infectious agents in populations of oysters grouped according to defined geographical zone or region.

Overall, 38.6% of the oysters examined showed one or more changes of morphological or pathological significance. Oysters collected from Qld showed the highest proportion of significant changes (74.3%), with 43.8% and 24.7% showing changes in oysters from NT and WA, respectively. Histological changes varied from 16.6% to 92% in populations of clinically normal mature oysters examined in the study (Table 3-5). In the majority of cases, the change or changes observed were mild in extent or severity.

The spectrum of inflammatory, degenerative, and proliferative histopathological responses, together with protozoan and metazoan agents observed in populations of oysters from NT, Qld and WA are shown in Table 3-6. Specific changes were observed in response to the presence of microbial, protozoan or metazoan agents while similar changes also appeared in the apparent absence of such agents. In many cases, the presence of microbial, protozoan or metazoan agents failed to elicit any demonstrable histopathological response, especially where the agent was external to the stromal or interstitial tissues.

Inflammatory changes were characterised by infiltrations or accumulations of haemocytic cells in stromal tissues and/or were associated with regions of tissue damage. At least two morphologically distinct haemocytic cell types were evident in such responses, together with giant cells in some oysters. Degenerative changes were characterised by degeneration of epithelial surfaces, often accompanied by necrosis, by mineralisation and epithelial atrophy. Proliferative changes were characterised by hyperplasia of epithelial surfaces and occasionally by neoplasia. Oedema and hyperpigmentation were interpreted as likely representing a degenerative process, or a result of a previous inflammatory response.

Non-specific changes (tables 3-6, 3-7)

Non-specific inflammatory changes. Focal or diffuse haemocytic inflammatory cell infiltrations occurred in oysters from all populations in each State/Territory in the absence of apparent inciting causative agents. On occasions, haemocytic infiltrations were associated with regressing gonads. A mild, diffuse infiltration of stromal tissues with low numbers of haemocytic cells was considered a normal feature of *P. maxima*.

Focal and regional haemocytic accumulations. Focal or multifocal, discrete, usually circular accumulations of haemocytic cells were frequently observed in tissues, especially in the stroma of mantle and palp, unassociated with an aetiological agent. Single or multiple foci occurred at an overall prevalence of between 0.7 to 26.3 % in all but two populations (Table 3-6). Many foci resembled those observed associated with metazoan parasites (focal granulomas), and often occurred in the same animals in which focal parasitic granulomata were present. In many cases, fibrous encapsulation was apparent peripheral to the cellular accumulation. Foci were single or multiple. Encapsulated, focal discrete haemocytic accumulations with a peripheral capsule were occasionally observed as "abscesses" or granulomas with central necrosis in the heart.

Regional or focally extensive infiltrations of haemocytes were observed. Such infiltrations tended to be irregular in form and less intense in nature than focal accumulations noted above. Infiltrations occurred in all tissues, including gonad, kidney and adductor muscle. More commonly, regional infiltrations of cells were associated with the stromal tissues of the hind and mid gut. Intense focally extensive haemocytic infiltrations were occasionally seen associated with degeneration of digestive gland epithelium, in the absence of an obvious inciting cause. Small irregularly focal haemocytic accumulations were occasionally seen in the heart. Regional or focally extensive haemocytic infiltrations occurred in populations of pearl oysters from all regions

Diffuse or generalised inflammation. A generalised infiltration of the organ or tissue was reported in 19 of the 35 populations examined at a prevalence between 0.7 to 8.8 % (Table 3-6). In most cases, no obvious causative agent was apparent, however, diffuse inflammation appeared to be associated with regressing gonads.

Multifocal "nests" of haemocytes in the interstitium. Small, loosely arranged, focal or multifocal aggregates of haemocytes were frequently observed in the interstitial tissues. These are considered to be a normal histological feature and possibly represent germinal centres of haemocyte production.

Eosinophilic granular bodies in digestive gland epithelium. Single or multiple, ovoid eosinophilic bodies approximately 4-5 microns in diameter were present in, or closely associated with the epithelium of the digestive gland diverticular in four populations from the NT at a prevalence of up to 3.3% (Table 3-6). On occasions, such bodies showed fine basophilic granular internal structure. Occasionally, a moderate haemocytic inflammatory

response was associated with these bodies and with digestive gland epithelial degeneration. The relationship between these bodies, the protozoan-like bodies associated with microgranulomata in the digestive gland and the viral-like eosinophilic inclusions described below in pearl oysters from WA is unclear.

Amphiphilic bodies; digestive gland. Ovoid bodies, approximately 5-7 microns diameter, were occasionally seen associated with digestive gland epithelium. Their nature is uncertain.

Multinucleate cells in interstitium. Infrequently, multinucleate cells were present in the interstitial tissues of oysters, generally in association with a diffuse haemocytic infiltration. Multinucleate cells were observed in three populations at a prevalence between 1.3 to 6.5%.

Dilation of digestive gland diverticular. Marked dilation of digestive gland diverticulae, with flattening or attenuation of epithelium, was observed. The change was generally regional as opposed to generalised, involving irregular segments of the digestive gland. The dilation was noted in three populations of oyster from NT at a prevalence between 6.2 to 14 % (Table 3-6).

Oedema. Oedematous changes characterised by marked dilation of haemocytic sinusoids and irregular spongiform change in stromal tissues was observed primarily in palp and mantle tissues. Oedema occurred in six populations at a prevalence between 0.7 to 4.2 % (Table 3-6).

Eosinophilic granule cell infiltrations/accumulations. In two populations, stromal tissues of oysters were infiltrated by large numbers of eosinophilic granule cells. Prevalence of affected oysters was 1.8 to 3.9 % (Table 3-6).

Hyperpigmentation. Generally, macrophages containing pigment were relatively common at low numbers in tissues other than heart. Hyperpigmentation of the auricle of the heart is a normal feature. Occasionally, pigmented cells similar to those in the heart extended into the kidney. Occasionally, large numbers of pigmented macrophages or "brown cells" were observed in interstitial tissues, especially associated with stroma adjacent to the alimentary tract. These cells are generally considered to contain metabolites of cell degeneration. Nine populations showed increased pigmentation at a prevalence of 0.5 to 2.4%.

Mineralisation/Lamelliform mineralisation. Focal areas of mineralisation were occasionally observed in the gill. Concentric, lamelliform, mineralised foci were occasionally observed in a variety of tissues, including the kidney, gonad, adductor muscle and foot.

Unidentified cysts. Cystic structures were reported on the gills of oysters from two populations of wild harvested oysters from Torres Strait at a prevalence of 2.0 to 2.5 % (Table 3-6).

Fibroma/Neurofibroma. Two proliferative lesions morphologically consistent with fibroma or neurofibroma were recorded, one each from NT and WA. Both cases originated from wild-harvested oysters (Table 3-6)

Specific changes (tables 3-6, 3-7)

Metazoa in stromal tissues. A number of metazoan agents were observed within the stromal tissues of the host from each State/Territory.

(i). Focal metazoan granulomata (Tylocephalum-like sp.*).* Focal concentric haemocytic accumulations peripheral to a central metazoan agent, approximately 50-100 microns diameter, often with peripheral fibrous encapsulation, were observed. These focal metazoan

granulomata were especially prominent in the stroma of palp, oesophagus, stomach and mantle tissues. The metazoa are typical of the cestode larvae *Tylocephalum* sp. On occasions, multiple metazoan granuloma were present in the one animal. Granuloma occurred in oysters from 14 populations at a prevalence between 0.5 to 18.0 % (Table 3-6).

(ii). Diffuse metazoan inflammation. Metazoan parasites in stromal tissues from WA were associated with diffuse inflammatory cellular responses in four populations at prevalence between 0.7 to 2.0 % (Table 3-6).

(iii). Stromal metazoa; no inflammation. Occasionally, metazoan agents were encountered in the tissues of oysters without an appreciable inflammatory response.

Metazoa; external. A variety of metazoan agents were found associated with the external surfaces or epithelium of the gills and occasionally the palp epithelium. Generally, there was no associated inflammatory or degenerative responses associated with these agents. Such metazoa occurred at a prevalence of up to 4.1 % (Table 3-6).

Metazoa; Intra-lumenal or intra-sinusoidal. Morphologically diverse metazoan agents were observed in the lumen of the stomach, hind gut, midgut, digestive gland, kidney, heart, oesophagus and haemolymph sinusoids. In general, these appeared to incite no tissue damage and no inflammatory response. Occasionally, metazoa in the digestive gland were associated with atrophy of the epithelium, a haemocytic response and mineralisation. Intra-lumenal or intra-sinusoidal metazoa occurred in nine populations at a prevalence of up to 18.6 % (Table 3-6).

In two populations of oysters from Northern Territory, copepods were commonly observed in the oesophageal lumen, associated with epithelium degeneration and necrosis. These organisms were observed on occasions apparently ingesting the oesophageal epithelium. The copepods were identified as *Anthessius pinctadae* (Jones - unpublished).

Intranuclear viral-like inclusions; digestive gland epithelium. These inclusions, consistent with the viral inclusions described by Pass *et al.* (1988) were a common finding. On occasions, individual animals showed particularly high occurrence rates of these bodies, with in excess of 20 inclusion bodies per high power field in some areas. Hyperplasia and degeneration of digestive gland epithelium were occasionally associated with the presence of these bodies, especially where individual glands were heavily infected. In general, however, inclusions appeared sporadically and were unassociated with any inflammatory, degenerative or proliferative changes. These inclusions occurred in 31 of the 35 populations examined, at a prevalence between 1.3 and 52.9 % (Table 3-6).

Regional haemocytic accumulations. Focally extensive or regional accumulations or infiltrations of haemocytes were observed occasionally associated with rod-shaped bacterial-like organisms. Tissues affected included digestive gland and heart, with attendant degeneration and necrosis. In the digestive gland, the inflammatory response extending into the lumen of the diverticulae.

Cryptosporidia-like bodies; digestive gland. On one occasion, multiple small (2-3 microns) basophilic bodies with no obvious internal structure were present in and associated with the epithelium of the digestive gland diverticula of a single oyster from the NT. No specific pathology was attributed to the agents.

Enigmatic protozoan-like microgranlomata Organisms resembling the protozoan *Haplosporidium* were infrequently visualised in the digestive gland and gill tissues of adult

oysters. These organisms, were characterised by their ovoid, eosinophilic appearance and size, approximately 7-10 microns diameter. The organisms were associated with epithelial degeneration and an intense focal inflammatory cell response. They were reported from two populations in WA and two populations in NT at a prevalence between 0.7 to 2.9 % (Table 3-6).

Rickettsiales-like bodies. Rickettsiales-like bodies, characterised by fine granular, basophilic bodies approximately 20-30 microns diameter, occupying epithelial cells or intimately associated with epithelial cells, were seen primarily in the digestive gland diverticular epithelium and also in gill and gonad. There was no apparent tissue damage or inflammatory response associated with these bodies. Rickettsia were reported in 19 populations at a prevalence between 0.5 to 17.1 % (Table 3-6).

Ancistrocomid-like ciliates. These elongate, ciliate protozoa approximately 25-30 microns in length were present in the alimentary tract. No tissue damage or inflammatory response was attributable to these agents. *Ancistrocomid*-like ciliates were reported in six populations of oysters, at a prevalence between 0.6 to 56.3 % (Table 3-6).

Turbellarian-like ciliates. These large ciliated metazoan agents approximately 300-400 microns in length were present in the palps of wild oysters from Queensland from both Torres Strait and the North-East Coast at a prevalence of 1.6 to 12.7 percent (Table 3-6). No tissue damage was apparent with these agents.

Gregarine Protozoa. Gregarine protozoa were commonly seen in oysters as non-ciliated, indented, ovoid bodies approximately 10-15 microns in length. The main sites of colonisation were the epithelium of the digestive gland and stomach. Occasionally they were seen in the epithelium of the midgut. No tissue damage was associated with the agents. Gregarine protozoa were observed only from Queensland from both Torres Strait and from the North-East Coast at a prevalence between 8.7 and 100 % (Table 3-6).

Papovavirus-like inclusions. Inclusion bodies and associated epithelial hyperplasia and hypertrophy were commonly reported in the epithelium of the palps of oysters. This agent caused marked epithelial hypertrophy and loss of cilia in affected cells, and in some cases involved expansive regions of the palp. The agent was recorded only from Queensland at a prevalence of 6.0 to 50.0 % (Table 3-6).

Regional differences in prevalence of changes and agents

Differences in occurrence and prevalence between geographic zones and between each state or territory for a number of non-specific and specific pathological responses and infectious agents were noted. Conversely, some changes appeared to be common to states and geographic zones. These similarities or differences are tabulated in Table 3-8.

Focal or regional non-specific inflammation was a finding common to each zone and state/territory. Similarly, focal or regional inflammation associated with the presence of *Tylocephalum*-like metazoa was common to all zones and states/territory.

Diffuse or generalised inflammation was uncommon in NT, was not recorded in Qld and was reported in oysters in all zones in WA. This may reflect differences in interpreting the extent of the inflammatory response between different states.

Oedema was noted in a low number of animals in four zones in the NT and one zone in WA. This change was not recorded in Qld.

Gill rickettsiales-like bodies were observed in two zones in NT, two zones in WA and in both zones in Qld. Rickettsial-like bodies were commonly associated with the epithelium of the palp in both regions in Qld, but were not observed in this location in WA or NT. Rickettsiales-like bodies associated with the epithelium of the digestive gland were recorded in all zones but one in NT.

The copepod *Anthessius pinctadae* associated with the oesophageal lumen, was found in a high proportion oysters from two zones in NT, up to 16.5%, but not in oysters from elsewhere.

Gregarine protozoa were reported in association with the epithelium of the stomach, digestive gland and midgut in populations of oysters from both zones in Queensland, but were not recorded in oysters elsewhere.

Microgranulomas in the digestive gland associated with enigmatic protozoan-like (*Haplosporidian*-like) bodies were recorded in one zone in NT and in all zones in WA, but were not recorded in Qld.

Ancistrocomid-like ciliates were recorded in a single NT oyster. None were recorded in WA oysters, whereas they were recorded at a high prevalence in oysters from both zones in Qld.

Seasonal variation in prevalence

No obvious differences between the prevalence of specific and non-specific changes were evident in populations that were sampled at differing times.

DISCUSSION

This comprehensive histological and morphological study of Australian pearl oysters *P. maxima* provides valuable information relating to the normal histological structure of this species and provides fundamental baseline data relating to the occurrence, prevalence and distribution of non-specific and specific pathological changes, morphological features and infectious agents associated with *P. maxima* in Australia.

The study sites selected for collection of oysters in the survey represent a diverse range of marine environments across northern Australia. Included are both wild harvest sites and pearl oyster farms. As such, observations arising from the study are considered to be representative of pathological changes and parasites which might be encountered over the whole of the northern Australian pearl oyster fisheries and farming regions.

In terms of identifying potentially infectious, pathogenic organisms, the study was limited in that clinically normal animals were principally studied. Cultural examinations for virus, fungi or protozoa are generally not available in molluscs, and fungi and protozoa are usually identified by histopathological examination. Further, bacteriological cultural examination frequently results in the isolation of large numbers of commensal organisms which cannot readily be distinguished from potential pathogens. In planning the study, the difficulties in undertaking and interpreting microbiological cultural studies were acknowledged, as well as the

potential of fish and shellfish to carry potential pathogens in an asymptomatic or latent state. The study, in identifying a range of specific and non-specific pathological and morphological changes, as well as a range of infectious agents, provides a morphological basis for further studies addressing specific aspects of pearl oyster microbiology, pathology or ecology.

Infestation with bio-fouling organisms and with boring agents was clearly a problem in those populations in which such agents were assessed in the present study. Wild-harvested oysters generally showed more extensive biofouling and shell invasion than farmed animals (Table 3-2), with up to 29% of animals showing moderate to severe damage. Biofouling and epiphytic organisms are known to cause severe deleterious impacts on shell quality, oyster growth and pearl quality, formation and production (Nasr 1982; Wada 1991; Doroudi 1996; Je *et al.* 1988; Dharmaraj *et al.* 1987).

A taxonomic study of biofouling and boring organisms was not a major objective of the study, however, a number of significant organisms were identified and described. The boring molluscs *Lithophaga* spp. were common in pearl oysters and produced large holes to 1-2 cm, sometimes extending down and disrupting or breaching the nacreous layer. Polychaete worms invading the shell nacre were also common, resulting in "mud blisters". Collectively, such organisms can be expected to result in losses to the industry, as is reported elsewhere.

Pea crabs and shrimps were found to be common commensals of pearl oysters with up to 85 % and 72% of populations containing these organisms respectively. Apart from local oedema in the mantle caused principally by the pea crabs, no pathology was associated with these organisms, supporting the findings of Dix (1973) that these agents are essentially commensals.

Few gross pathological lesions were observed, as might be expected in a study of clinically normal oysters. The oedema of mantle and foot tissues were possibly associated with prolonged time between collection of the oysters and fixation. Crateriform depressions in mantle and gill tissues containing pea crabs did not appear to have any detrimental impact on the animals.

Histological assessment of the oysters demonstrated the relatively common occurrence, up to 43.8%, of inflammatory degenerative or proliferative changes and/or the presence of infectious agents in nominally healthy oysters. The study demonstrated a spectrum of non-specific inflammatory changes and inflammatory changes associated with specific agents. The study also identified a range of microbial, protozoan and metazoan agents, some of which were associated with histopathological lesions. Such agents were generally asymptomatic in the present study, but under certain conditions of stress, environmental change, altered ecological relationships or in younger animals, might manifest as pathogens.

Non-specific inflammatory changes, characterised by infiltrations or accumulation of haematocytic cells in tissues, was a common finding in all populations, unassociated with obvious causative agents. Humoral and cellular components of the inflammatory process are well described in bivalve molluscs (Feng 1988). The identification of a fibronectin-like molecule in haemolymph and its secretion by amebocytes of *P. fucata*(Suzuki and Funakoshi 1992), the demonstration of approximately 30 haemolymph proteins in *P. fucata* including two glycoproteins and one glycolipoprotein (Suzuki 1990), the demonstration by Suzuki and Mori (1991) that the granular cells under the mantle epithelium of *P. fucata martensii* secrete haemolymph lectin, the demonstration of haemagglutinin activity in haemolymph *of P. fucata martensii* (Yamaguchi and Mori 1988), and the presence of a galactose-specific lectin from the haemolymph of *P. fucata martensii* by Suzuki and Mori (1989) suggest that the humoral inflammatory process in *Pinctada* spp. is analogous to other molluscan species. In other

species, cellular elements which participate in the inflammatory response and wound repair include agranular amoebocytes, basophilic granulocytes and eosinophilic granulocytes, with a typical response of aggregation, phagocytosis, hyperplasia and encapsulation (Feng 1988). It is likely that *Pinctada* species share common features of the inflammatory response with other bivalves and that the haemocytic accumulations, infiltrations and the granuloma formations observed in the absence of obvious aetiological agents represent a spectrum of past or existent inflammatory responses to a variety of antigens, including soluble antigens, not visible histologically. The significance of these cellular responses is difficult to determine in the present study. In those animals in which a high prevalence of metazoan granulomas occurred, the presence of focal microgranulomas probably represents a response to such agents in adjacent tissues, or to degenerate parasites. Other lesions likely represent a response to bacterial infection, especially *Vibrio* spp., including focal abscesses in the heart. The presence of multinucleate cells in interstitial tissues in some oysters possibly represents a degenerative response associated with prolonged stress and tissue damage. Giant cells were observed predominantly in one population which had been transported from the oyster grounds to the laboratory over a period of days, with considerable potential for overheating.

Low to moderate numbers of diffusely arranged haemocytes in the interstitial tissues of the oysters was common and appeared to be part of the normal histological structure.

Degenerative changes identified histologically were also relatively common, apparently unassociated with causative agents. Such changes included oedema, increased pigmentation in macrophages in interstitial tissues and kidney and mineralisation. Oedema in mantle tissues was conspicuous in a population which had been removed from water and held at ambient temperature for some time prior to examination. It is believed the oedema is a degenerative response in a physiologically compromised oyster. The significance of increased pigmentation in macrophages is unclear, but is generally recognised as reflecting prior cellular breakdown. Pigmentation of the heart, and the epithelium of the mantle tissue is part of the normal spectrum of histological features of the pearl oyster. Lamellar mineralisation in a number of cases appeared analogous to pearl formation, with the concentric deposition of nacre in tissues. Mineralisation may occur, however, without nacre formation.

Specific inflammatory cell infiltrations were associated with the presence of metazoa in tissues, with degenerative lesions of the digestive gland epithelium, and with putative microbial, protozoan and metazoan agents. In many cases, however, the presence of a putative infectious agent failed to elicit an inflammatory response, or cause notable tissue damage.

Intranuclear viral-like inclusion bodies were present in digestive gland epithelium of oysters from all zones at a prevalence up to 52.9%. These intranuclear inclusions have been described earlier by Pass *et al.* (1988) as containing virus-like particles, but were not associated with disease or pathology. In the present study, mild to moderate digestive gland epithelial hyperplasia and degeneration appeared to be associated with heavy infections in some cases, suggesting the agent may be pathogenic under some circumstances.

Papova-like viral inclusions were widespread and occurred commonly in the epithelium of the palp in wild harvested and farmed oysters from Qld (Table 3-7). Inclusions were not observed in oysters from the NT or WA. Prevalence in populations varied between 7-31%. Hyperplastic and degenerative epithelial changes in the palp arising from infection might well result in decreased feeding activity, especially in younger oysters, with consequent decreased productivity.

Rickettsiales-like bodies were commonly associated with digestive gland epithelium and less commonly with gill and palp epithelia. Prevalence varied from 0.6 to 17% in the digestive gland, 0 to 2.4% in the gill and 0 to 13.8% in the palps. No obvious pathology was associated with the presence of these agents in tissues. Rickettsiales-like organisms are recognised as asymptomatic infections in a range of molluscan species including *Crassostrea gigas*, *C. virginica* and *Mya arenaria* and have been associated with mass mortalities in the scallop *Placopecten magellanicus* (Sparks 1985). The pathogenic significance of the Rickettsiales-like organisms in the current study warrants further investigation.

Metazoan agents were relatively common in some populations and were found in one or more of three locations; in the interstitial or stromal tissues, in the lumen of the alimentary tract, haemolymph sinuses or kidney, or on the external surfaces of the oyster. The taxonomy of the agents remains uncertain and should be the subject of further investigations.

The metazoa associated with discrete focal or multifocal granulomata in interstitial tissues are morphologically consistent with the larval cestode, *Tylocephalum* sp. Lesions were particularly prevalent in tissues peripheral to the oesophagus, palps and stomach. Members of this tapeworm group are recognised as parasites of the pearl oyster *Margartitifera vulgaris* and were initially believed to be the cause of natural pearl formation (Sparks 1985). It was subsequently shown that invasion by larval trematodes was more important in natural pearl formation than larval cestodes (Sindermann 1990). Larval *Tylocephalum* sp. have been recorded in molluscs including the oysters *C. virginica*, *C. gigas* and the clam *Tapes semidecussata* (Sparks 1985, Sindermann 1990). In Australian waters, *Tylocephalum* has been reported in *Saccostrea commercialis* from New South Wales and Qld, and in *Crassostrea echinata* from NT (Wolf 1976, 1977). Larval *Tylocephalum* are not considered to be host specific, may occur at high prevalence and intensities in pearl oysters and edible oysters, and may reduce the condition of their molluscan hosts (Sindermann 1990). Adults occur in the digestive tract of elasmobranchs (Sindermann 1990) and at least one adult form, *Tylocephalum campanulatum* has been recorded in Qld in *Rhina ancylostonus* (Butler 1987). The significance of infections in the current study are uncertain, but high numbers of larvae may cause dysfunction of affected tissues. The taxonomy and the life cycle of these agents, and their pathogenic potential, especially for young or stressed animals should be investigated further.

A range of metazoa were observed to be present in the lumen of the stomach, digestive gland, mid and hind gut. Occasionally, metazoa in the lumen of the digestive gland appeared to result in occlusion, with degeneration and an associated inflammatory response. Generally, these metazoa did not appear to elicit tissue damage or an inflammatory response in the animals examined. Clarification of the taxonomy, life cycle and the pathogenic potential of these metazoa appears warranted.

Metazoa on gill epithelium also did not appear to invoke a host response. In some cases, a chitinous cuticle around these agents suggested they were sections of pea crabs or shrimps observed grossly.

The copepod *Anthessius pinctadae* was recorded at a high prevalence and intensity in the oesophageal lumen in two populations from NT. Epithelial damage associated with feeding was evident in some animals. This agent would appear to be potentially pathogenic and further studies appear warranted to determine its life cycle and pathogenic potential.

Turbellarian-like ciliates were reported from the epithelial surfaces of the palps of oysters in Qld, but not elsewhere. These agents elicited no host response and were not considered pathogens.

Gregarine protozoa were commonly associated with the epithelium of the stomach, digestive gland and midgut in Qld oysters, but were not observed in oysters from NT or WA. These agents appeared innocuous, causing no apparent damage or host response and have been characterised by Cui (1997).

Microgranulomata in digestive gland tissue, characterised by epithelial degeneration and necrosis and an intense haemocytic infiltration, were infrequently associated with enigmatic ovoid, protozoan-like bodies in oysters from NT and WA. The ovoid bodies appeared to have internal structure and did not resemble host tissue. Possibly, these agents represent members of the Phylum Ascetospora and should be investigated further to determine their nature and pathogenic significance. Ascetosporan or Haplosporan parasites of molluscs are the cause of serious and economically important molluscan diseases including bonamiaisis caused by *Bonamia ostreae*, the cause of heavy mortalities in *Ostrea edulis* in all European oyster growing areas (Bucke *et al.* 1984; Sparks 1985). Haplosporidiosis of the gaper clam *Tresus capax*, a lethal is caused by an uncharacterised haplosporidian (Sparks 1985). Disease in olympic oysters *Ostrea lurida* has been associated with a haplosporidian parasite of uncertain pathogenic significance (Mix and Sprague 1974; Sparks 1985). Oyster seaside haplosporidiosis caused by *Haplosporidium costalis* (*Minchinia costalis*) results in recurrent annual epizootic mass mortalities in *Crassostrea virginica* (Andrews and Castagna 1978; Sparks 1985; Perkins 1979). American oyster haplosporidiosis or MSX disease caused by *Haplosporidium nelsoni* (*Minchinia nelsoni*) results in seasonally recurrent, catastrophic epizootics in American oysters (Perkins 1979; Sparks 1985; Ford and Haskin 1982). European oyster marteiliasis or Aber disease, caused by *Marteilia refringens* results in epizootic mortalities in European oysters (Cahour 1979; Perkins 1979; Sparks 1985). European oyster minchiniasis is caused by *Minchinia armoricana* (VanBanning 1979; Sparks 1985). In Australia, epizootic mortalities in Sydney rock oysters *Saccostrea (Crassostrea) commercialis*, with poor body condition and massive parasitic invasion of digestive tubules are caused by the haplosporidian *Marteilia sydneyi* (Perkins 1979; Sparks 1985).

Ancistrocomid-like ciliates. These were frequently observed in the midgut of Qld oysters and only rarely in the midgut of NT oysters. There presence was unassociated with obvious pathological changes. *Ancistrocomid*-like ciliates were present in spat examined in WA.

Differences in the distribution of certain agents, especially those agents identified as pathogens or potential pathogens clearly indicates that zones or regions currently free of such agents should maintain this status.

Dilation of digestive gland diverticula is considered a normal process associated with lack of digestive activity, that is, no available food. In intertidal molluscs, dilation of digestive tubules is not an uncommon observation. The digestive tubule goes through four morphological phases associated with tidal rhythm and feeding activity (Winstead 1995). These are a holding phase and an absorptive stage, both with a tall columnar epithelial cells and a narrow lumen; and a disintegrating phase and reconstituting phase characterised by a wide lumen and relatively squamous epithelium. While animals are feeding the digestive tubules are in the first two stages, and as the tide recedes and feeding stops the tubules revert to the last two phases (Morton 1970a, b, and Owen 1970). In subtidal oysters the first two phases predominate (Mathers 1976; Wilson & LaTouche 1978; Robinson & Langton 1980) and may involve up to 90% of tubules in *Mytilus* (Langton 1975). Dilation of digestive glands can also be the result of stress (Thompson *et al.* 1974).

Table 3-1. Source, identification, history and total number of mature pearl oysters *Pinctada maxima* examined in study

Northern Territory

Identification	Geographic Origin	Zone	History	Farm Code	No.	Date/s of Collection	Lab. Access. No/s.
SM series	Western Grounds	NT 1	Wild harvest rejects		76	28-30/10/ 94	95/2131
WP series	Western Grounds	NT 1	Wild harvest / klunkers		168	24/10/94	94/1977, 1946, 1960, 1971, 1998
A series A1-6	Bynoe Harbour	NT 1	Half pearl harvest	C1	148	23/9/94	95/214
A7-8						12/94	
A9-148						12/1/95	
C series	Bynoe Harbour	NT 1	Half pearl harvest	C1	103	24/06/95	95/1363
D2 series	Coburg Peninsula	NT 1	Round pearl harvest	C3	150	26/9/95	96/153
E Series	Coburg Peninsula	NT 1	Triple vomit shells	C4	150	6/5/96	96/1080
L Series	Bynoe Harbour	NT 1	Half pearl harvest	C1	85	26/6/96	
M Series	Coburg Peninsula	NT 1	Farmed oyster rejects	C2	150	13/9/96	96/2475
N Series	Far Eastern Grounds	NT 3	Wild harvest		153	7-9/9/96	97/0188
O series	Eastern Grounds	NT 2	Wild harvest		97	11-12/9/96	
Total Examined					**1280**		

Queensland

Identification	Geographic Origin	Zone	History	Farm Code	No.	Date/s of Collection	Lab. Access. No/s.
Wild	Barrier Reef, Cooktown	NE Coast	Wild Harvested		126	7/2/95	95-40794, 95-40682
Farm C	Cairns	NE Coast	Half pearl culture	Farm C	150	30/5/96	96-44231
Farm C	Cairns	NE Coast	Round pearl culture	Farm C	6	24/12/94	95-41991
Farm D	Cairns	NE Coast	Half pearl culture	Farm D	29	2/95 to 7/95	95-45605
Farm A	Torres Strait	Torres Strait	Half pearl culture	Farm A	36	16/11/94	94-52033
Farm A	Torres Strait	Torres Strait	Half pearl culture	Farm A	150	21/11/95	95-50096
Farm B	Torres Strait	Torres Strait	Half pearl culture	Farm B	150	20/2/95	95-41213
Farm B	Torres Strait	Torres Strait	Half pearl culture	Farm B	151	27/2/96	96-41140
Wild	Torres Strait	Torres Strait	Wild harvest		148	5/11/94	94-52041
Wild	Torres Strait	Torres Strait	Wild harvest		122	20/11/95	95-50100
Total Examined					**1068**		

Table 3-1 (Continued). Source, identification, history and total number of mature pearl oysters *Pinctada maxima* examined in study

Western Australia

Identification	Geographic Origin	Zone	History	Farm Code	No.	Date/s of Collection	Lab. Access. No/s.
Wild	Exmouth Gulf	Zone 1: Warm	Wild harvest		151	27/1/95	P95/328
Wild	Exmouth Gulf	Zone 1: Cold	Wild harvest		150	23/6/95	P95/1874
Farm E	Exmouth Gulf	Zone 1: Cold	Farmed	Farm E	148	26/8/95	P95/2642
Farm F	Dampier	Zone 1: Cold	Farmed	Farm F	150	25/8/95	P95/2645
Wild	80 Mile Beach	Zone 2: Warm	Wild harvest		153	13/3/95	P95/836
Wild	80 Mile Beach	Zone 2: Cold	Wild harvest		153	21/5/95	P95/1671
Farm G	Roebuck Bay	Zone 2: Warm	Farmed	Farm G	56	28/3/95	P95/1070
Farm G	Roebuck Bay	Zone 2: Cold	Farmed	Farm G	150	18/8/95	P95/2644
Farm H	Roebuck Bay	Zone 3: Warm	Farmed	Farm H	124	28/3/95	P95/1072
Wild	Lacepede Is.	Zone 3: Warm	Wild harvest		192	7/2/95	P95/683
Farm I	Kuri Bay	Zone 3: Warm	Farmed	Farm I	162	29/3/95	P95/1071
Farm I	Kuri Bay	Zone 3: Cold	Farmed	Farm I	162	16/8/95	P95/2643
Farm J	Cygnet Bay	Zone 3: Warm	Farmed	Farm J	116	27/3/95	P95/1069
Farm J	Cygnet Bay	Zone 3: Cold	Farmed	Farm J	150	21/8/95	P95/2646
Farm K	Cone Bay	Zone 3: Cold	Farmed	Farm K	137	17/8/95	P95/2626
Total Examined					**2154**		

Table 3-2. Shell damage associated with boring and fouling organisms

Northern Territory

		Degree of Shell Damage					
		Nil	Mild	Moderate	Severe	Unscored	Total Damaged Shells
Population	Origin	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Wild (SM Series) (n=75)	Western Ground	28 (37)	41 (55)	6 (8)			47 (63)
Wild (WP Series) (n=167)	Western Ground	26 (15)	72 (44)	48 (29)	21 (12)		141 (84)
Farm C1 (A Series) (n=5)	Bynoe Harbour	4 (80)			1 (20)		1 (20)
Farm C3 (D2 Series) (n=150)	Coburg Pen.	20 (13)	30 (20)	51 (34)	49 (33)		130 (87)
TOTAL DAMAGED							319 (80)

Queensland

		Degree of Shell Damage					
		Nil	Mild	Moderate	Severe	Unscored	Total Damaged Shells
Population	Origin	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Wild (n=148)	Torres Strait	121 (82)	11 (8)	8 (5)	8 (5)		27 (19)
Farm A (n=36)	Torres Strait	32 (89)	4 (11)				4 (11)
Farm B (n=150)	Torres Strait	130 (87)	20 (13)				20 (13)
Wild (n=126)	NE Coast	60 (48)	56 (44)	8 (6)	2 (2)		66 (52)
Wild (n=122)	Torres Strait	41 (34)	71 (58)	4 (3)	6 (5)		81 (66)
Farm A (n=150)	Torres Strait	148 (99)	2 (1)				2 (1)
Farm B (n=151)	Torres Strait	137 (91)	14 (9)				14 (9)
TOTAL DAMAGED							214 (24)

Note: Damage in Queensland *P. maxima* mainly caused by boring sponges and mudworms

Table 3-2 (continued). Shell damage associated with boring and fouling organisms

Western Australia

		Degree of Shell Damage					
		Nil	Mild	Moderate	Severe	Unscored	Total Damaged Shells
Population	Origin	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Dampier P95-1065 (n=151)	WA Zone 1					3 (2.0)	3 (2.0)
Exmouth P95-328 (n=151)	WA Zone 1					31 (20.5)	31 (20.5)
Exmouth Gulf 1874 (n=150)	WA Zone 1					42 (28.0)	42 (28.0)
Exmouth Gulf P95-2642 (n=148)	WA Zone 1					23 (15.5)	23 (15.5)
Dampier P95-2645 (n=150)	WA Zone 1					51 (34.0)	51 (34.0)
Roebuck Bay P95-1070 (n=150)	WA Zone 2					43 (28.7)	43 (28.7)
Roebuck Bay P95-2644 (n=150)	WA Zone 2					24 (16.0)	24 (16.0)
80 Mile Beach 1671 (n=150)	WA Zone 2					13 (8.7)	13 (8.7)
80 Mile Beach P95-836 (n=160)	WA Zone 2					2 (1.3)	2 (1.3)
Cygnet Bay P95-1069 (n=150)	WA Zone 3					10 (6.7)	10 (6.7)
Cygnet Bay P95-2646 (n=150)	WA Zone 3					89 (59.3)	89 (59.3)
Kuri Bay P95-1071 (n=150)	WA Zone 3					3 (2.0)	3 (2.0)
Kuri Bay P95-2643 (n=150)	WA Zone 3					30 (20.0)	30 (20.0)
Cone Bay P95-2626 (n=136)	WA Zone 3					70 (51.5)	70 (51.5)
Lacepede Island P95-683 (n=192)	WA Zone 3	182 (94.8)	4 (2.1)	6 (3.1)			10 (5.2)
TOTAL DAMAGED							444 (19.4)

Table 3-3. Size/age associated, epibiont induced abnormalities of shell growth and development

Northern Territory

Population (No)	SM Series (N=75)		WP Series (n=167)	
Shell length (mm)	<150	>150	<150	>150
Degree of Shell Damage	No. (%)	No. (%)	No. (%)	No. (%)
Mild	0 (0)	41 (55)	2 (10)	70 (48)
Moderate	0 (0)	6 (8)	0 (0)	48 (33)
Severe	0 (0)	0 (0)	0 (0)	21 (14)
Total Damaged	0 (0)	47 (63)	2 (10)	139 (95)

Table 3-4. Occurrence of commensal or symbiotic epifauna within the shell cavity

Northern Territory

	Geographic Origin	Peacrabs	Shrimps	Other Invertebrates
Population		No. (%)	No. (%)	No. (%)
Wild (WP Series) (n=168)	NT 1: Western Grounds	19 (11.3)	121 (72)	0 (0)
Wild (SM Series) (n=76)	NT 1: Western Grounds	65 (85.5)	0 (0)	5 (6.6)
Farm C1 (A Series) (n=148)	NT 1: Bynoe Harbour	9 (6.1)	2 (1.4)	0 (0)
Farm C1 (C Series) (n=103)	NT: Bynoe Harbour	At least one crab in each oyster		
Wild (N series) (n=150)	Far Eastern Grounds		Most with Shrimps	
Wild (O series) (n=97)	Eastern Grounds		Most with Shrimps	

Table 3-5. Proportion of oysters showing significant histopathological changes

Northern Territory

Identification	Zone	Total	No. with changes (%)
Wild (SM Series)	NT 1: Western Grounds	76	46 (60.5)
Wild (WP Series)	NT 1: Western Grounds	168	98 (58.3)
Farm C1 (A Series)	NT 1: Bynoe Harbour	148	65 (45.9)
Farm C1 (C Series)	NT 1: Bynoe Harbour	103	54 (52.4)
Farm C3 (D2 Series)	NT 1: Coburg Peninsula	150	47 (31.3)
Farm C4 (E Series)	NT 1: Coburg Peninsula	150	53 (35.3)
Farm C1 (L Series)	NT 1: Bynoe Harbour	85	39 (45.9)
Farm C2 (M Series)	NT 1: Coburg Peninsula	150	58 (38.7)
Wild (N Series)	NT 3: Far Eastern Grounds	153	61 (39.9)
Wild (O Series)	NT 2: Eastern Grounds	97	39 (40.2)
TOTAL		**1280**	**560 (43.8)**

Queensland

Identification	Zone	No.	No. with changes (%)
Wild: 7/2/95	NE Coast	126	89 (70.6)
Farm C: 30/5/96	NE Coast	150	138 (92.0)
Farm C: 24/12/94	NE Coast	6	5 (83.3)
Farm D: 2/95-7/95	NE Coast	29	20 (69.0)
Farm A: 16/11/94	Torres Strait	36	12 (33.3)
Farm A: 21/11/95	Torres Strait	150	127 (84.7)
Farm B: 20/2/95	Torres Strait	150	122 (81.3)
Farm B: 27/2/96	Torres Strait	151	127 (84.7)
Wild: 5/11/94	Torres Strait	148	83 (56.1)
Wild: 20/11/95	Torres Strait	122	70 (57.4)
Total Examined		**1068**	**793 (74.3)**

Western Australia

Identification	Zone	Total	No. with changes (%)
Wild (P95-328)	1: Exmouth Gulf	151	25 (16.6)
Farm J (P95-1069)	3: Cygnet Bay	116	38 (39.7)
Farm F (P95-2645)	1: Dampier	150	30 (20.0)
Wild (P95-836)	2: 80 Mile Beach	153	57 (37.3)
Farm I (P95-1071)	3: Kuri Bay	162	28 (17.3)
Wild (P95-683)	3: Lacepede Island	192	70 (36.5)
Farm E (P95-2642)	1: Exmouth Gulf	148	28 (18.9)
Farm G (P95-2644)	2: Broome	150	27 (18.0)
Farm J (P95-2646)	3: Cygnet Bay	150	40 (26.7)
Farm I (P95-2643)	3: Kuri Bay	162	29 (17.9)
Farm K (P95-2626)	3: Cone Bay	137	36 (26.3)
Wild (P95-1874)	1: Exmouth Gulf	150	43 (29.7)
Farm H (P95-1072)	2: Broome	124	27 (21.8)
Wild (P95-1671)	2: 80 Mile Beach	153	30 (19.6)
Farm G (P95-1070)	2: Broome	56	24 (42.9)
TOTAL		**2154**	**532 (24.7)**

Table 3-6. Overall prevalence of specific and non-specific histopathological changes in oysters examined in the study:
Northern Territory

Population/Farm Series/ Identification	Farm C1 A	Farm C1 C	Farm C1 L	Wild SM	Wild WP	Farm C2 M	Farm C3 D2	Farm C4 E	Wild N	Wild O
Date/s of Collection (Month/Year)	9/94-1/95	6/95	6/96	10/94	10/94	9/96	9/95	3/96	9/96	9/96
Geographic Zone or Region	NT 1	NT 1	NT 1	NT 1	NT 1	NT 1	NT 1	NT 1	NT 3	NT 2
Number of Oysters Examined	(n=148)	(n=103)	(n=85)	(n=76)	(n=168)	(n=150)	(n=150)	(n=150)	(n=153)	(n=97)
Number & Percentage with Changes	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Histopathological Change										
Focal inflammation: non-specific	20 (13.5)	18 (17.5)	7 (8.2)	20 (26.3)	28 (16.7)	4 (2.7)	26 (17.3)	20 (13.3)	10 (6.5)	4 (4.1)
Diffuse inflammation; non-specific	1 (0.7)	3 (2.9)			7(4.2)				1 (0.7)	
Multinucleate cellular accumulations				1 (1.3)	11 (6.5)					
Regional dilation; digestive gland						21 (14.0)			16 (10.8)	6 (6.2)
Oedema; stromal		1 (1.0)		1 (1.3)	7 (4.2)	5 (3.3)		1 (0.7)		
Eosinophilic granule cells, numerous										
Hyperpigmentation	2 (1.4)	1	1	1 (1.3)	4			1 (0.7)		
Unidentified cysts										
Fibroma/neurofibroma					1 (0.6)					
Focal inflammation; metazoan	1 (0.7)			1 (1.3)		3 (1.8)	12 (8.0)	14 (9.3)	2 (1.3)	
Diffuse inflammation; metazoan										
Rickettsiales-like bodies		2 (1.9)		13 (17.1)	13 (7.7)				2 (1.3)	4 (4.1)
Ancistrocomid-like ciliates; alimentary			1 (1.2)							
Cryptosporidia-like bodies; digestive gland								1 (0.7)		
Microgranuloma, Protozoan-like		3 (2.9)							1 (0.7)	
Papovavirus-like inclusions, epithelial; palp										
Turbellarian-like ciliates; epithelial; palp										
Gregarine protozoa; alimentary										
Metazoa: stromal										
Metazoa; intralumenal or sinusoidal	2 (1.4)	1 (1.0)	2 (2.4)		5 (3.0)	11 (7.3)		1 (0.7)	25 (16.3)	18 (18.6)
Metazoa, external	1 (0.7)			1 (1.3)	2 (1.2)	1 (0.7)		2 (1.3)	2 (1.3)	4 (4.1)
Viral-like, intranuclear inclusions, epithelial; digestive gland	24 (16.2)	37 (35.9)	24 (28.2)	16 (21.1)	33 (19.6)	11 (7.3)	9 (6.0)	7 (4.7)		4 (4.1)
Intracytoplasmic inclusions, epithelial, eosinophilic; digestive gland										
Eosinophilic ovoid bodies; epithelial; digestive gland	4 (2.7)		1 (1.2)				5 (3.3)	1 (0.7)		

Table 3-6 (Continued). Overall prevalence of specific and non-specific histopathological changes in oysters examined in the study:
Queensland

Population/Farm Series/ Identification	**Wild NE Coast**	**Farm C NE Coast**	**Farm C NE Coast**	**Farm D NE Coast**	**Farm A Torres St**	**Farm A Torres St**	**Farm B Torres St**	**Farm B Torres St**	**Wild Torres St**	**Wild Torres St**
Date/s of Collection (Month/Year)	2/95	5/96	12/94	2/95-7/95	11/94	11/95	2/95	2/96	11/94	11/95
Geographic Zone or Region	NE Coast	NE Coast	NE Coast	NE Coast	Torres St	Torres St	Torres St	Torres St	Torres St	Torres St
Number of Oysters Examined	(n = 126)	(n = 150)	(n = 6)	(n = 29)	(n = 36)	(n = 150)	(n = 150)	(n = 151)	(n = 148)	(n = 122)
Number & Percentage with Changes	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Histopathological Change										
Focal inflammation: non-specific	16 (12.7)	30 (20.0)	1 (16.7)		5 (13.9)	42 (28.0)	47 (31.3)	52 (34.4)	23 (15.5)	24 (19.7)
Diffuse Inflammation; non-specific						1 (0.6)				
Multinucleate cellular accumulations										
Regional dilation; digestive gland										
Oedema; stromal										
Eosinophilic granule cells, numerous										
Hyperpigmentation									3 (2.0)	3 (2.5)
Unidentified cysts										
Fibroma/neurofibroma										
Focal inflammation; metazoan										
Diffuse inflammation; metazoan										
Rickettsiales-like bodies	2 (1.6)	4 (2.7)		5 (17.2)		12 (8.0)	1 (0.7)	7 (4.6)	12 (7.4)	11 (9.0)
Ancistrocomid-like ciliates; alimentary		84 (56.3)		2 (0.6)		79 (52.7)		24 (15.8)		10 (8.2)
Cryptosporidia-like bodies; digestive gland										
Microgranuloma, Protozoan-like										
Papovavirus-like inclusions, epithelial; palp	24 (19)	20 (13.4)	3 (50.0)	9 (2.6)		19 (12.7)	9 (6.0)	12 (7.9)	18 (12.2)	9 (7.4)
Turbellarian-like ciliates; epithelial; palp	16 (12.7)									2 (1.6)
Gregarine protozoa; alimentary	15 (11.9)	90 (60.0)	5 (83.3)	10 (34.5)	4 (11.1)	13 (8.7)	101(67.3)	108(71.5)	52 (35.1)	31 (25.4)
Metazoa: stromal		2 (1.3)				1 (0.6)				
Metazoa; intralumenal or sinusoidal										
Metazoa, external										
Viral-like, intranuclear inclusions, epithelial; digestive gland		79 (52.9)	1(16.7)		2 (5.6)	41 (27.5)	3 (2.0)	11 (7.3)	2 (1.4)	4 (3.3)
Intracytoplasmic inclusions, epithelial, eosinophilic; digestive gland										
Eosinophilic ovoid bodies; epithelial; digestive gland										

Table 3-6 (Continued). Overall prevalence of specific and non-specific histopathological changes in oysters examined in the study:
Western Australia

Population/Farm Series/ Identification	Wild Exmth.	Wild Exmth.	Farm E Exmth.	Farm F Dampier	Wild 80 Mile	Wild 80 Mile	Farm G Broome	Farm G Broome	Farm H Broome
Date/s of Collection (Month/Year)	1/95	6/95	8/95	8/95	3/95	5/95	3/95	8/95	3/95
Geographic Zone or Region	Zone 1	Zone 1	Zone 1	Zone 1	Zone 2	Zone 2	Zone 2	Zone 2	Zone 2
Number of Oysters Examined	(n = 151)	(n = 150)	(n = 148)	(n = 150)	(n = 153)	(n = 153)	(n = 56)	(n = 150)	(n =124)
Number & Percentage with Changes	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Histopathological Change									
Focal inflammation: non-specific	3 (1.9)	6 (4.0)	7 (4.7)	1 (0.7)	16 (10.4)	10 (6.5)	4 (7.1)	3 (2.0)	3 (2.4)
Diffuse inflammation; non-specific	6 (4.0)	1 (0.7)	3 (2.0)	2 (1.3)	3 (2.0)	4 (2.6)	2 (3.6)	3 (2.0)	9 (7.3)
Multinucleate cellular accumulations							1 (1.8)		
Regional dilation; digestive gland									
Oedema; stromal							1 (1.8)		
Eosinophilic granule cells, numerous					6 (3.9)		1 (1.8)		
Hyperpigmentation									
Unidentified cysts									
Fibroma/neurofibroma									
Focal inflammation; metazoan		2 (1.3)			28 (18.3)	8 (5.2)	3 (5.4)		1 (0.8)
Diffuse inflammation; metazoan					3 (2.0)	1 (0.7)	1 (1.8)		1 (0.8)
Rickettsial-like bodies	2 (1.3)				1 (0.7)	2 (1.3)	1 (1.8)		
Ancistrocomid-like ciliates; alimentary									
Cryptosporidia-like bodies; digestive gland									
Microgranuloma, Protozoan-like					2 (1.3)			1 (0.7)	
Papovavirus-like inclusions, epithelial; palp									
Turbellarian-like ciliates; epithelial; palp									
Gregarine protozoa; alimentary									
Metazoa: stromal	1 (0.7)	5 (3.3)			2	4 (2.6)	9		
Metazoa; intralumenal or sinusoidal	2 (1.3)								
Metazoa, external	1 (0.7)								
Viral-like, intranuclear inclusions, epithelial; digestive gland		26 (17.3)	17 (11.5)	27 (16.0)	9 (5.9)	2 (1.3)	4 (7.1)	21 (14.0)	9 (7.3)
Intracytoplasmic inclusions, epithelial, eosinophilic; digestive gland	10 (6.6)	3 (2.0)			1 (0.7)				
Eosinophilic ovoid bodies; epithelial; digestive gland	0	0	0	0	0	0	0	0	0

Table 3-6 (Continued). Overall prevalence of specific and non-specific histopathological changes in oysters examined in the study: *Western Australia*

Population/Farm Series/ Identification	**Wild L'pede I**	**Farm I Kuri B**	**Farm I Kuri B**	**Farm J Cygt. B**	**Farm J Cygt. B**	**Farm K Cone B**
Date/s of Collection (Month/Year)	2/95	3/95	8/95	3/95	8/95	8/95
Geographic Zone or Region	Zone 3	Zone 3	Zone 3	Zone 3	Zone 3	Zone 3
Number of Oysters Examined	(n = 192)	(n = 162)	(n = 162)	(n = 116)	(n = 150)	(n = 137)
Number & Percentage with Changes	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Histopathological Change						
Focal inflammation: non-specific		2 (1.2)	1 (0.6)	6 (5.2)	1 (0.7)	3 (2.2)
Diffuse inflammation; non-specific	14 (7.3)	1 (0.6)	8 (4.9)	8 (6.7)	3 (2.0)	12 (8.8)
Multinucleate cellular accumulations						
Regional dilation; digestive gland						
Oedema; stromal						
Eosinophilic granule cells, numerous	30 (15.6)	12 (7.4)		1 (0.9)		3 (2,2)
Hyperpigmentation	1 (0.5)		3 (1.9)		1 (0.7)	
Unidentified cysts						
Fibroma/neurofibroma						
Focal inflammation; metazoan		3 (1.9)		1 (0.9)	4 (2.7)	
Diffuse inflammation; metazoan	3 (1.6)					1 (0.7)
Rickettsiales-like bodies	1 (0.5)					2 (1.5)
Ancistrocomid-like ciliates; alimentary						
Cryptosporidia-like bodies; digestive gland						
Microgranuloma, Protozoan-like	1 (0.5)			1 (0.9)		
Papovavirus-like inclusions, epithelial; palp						
Turbellarian-like ciliates; epithelial; palp						
Gregarine protozoa; alimentary						
Metazoa: stromal						
Metazoa; intralumenal or sinusoidal						
Metazoa, external						
Viral-like, intranuclear inclusions, epithelial; digestive gland	13 (6.8)	7 (4.3)	17 (10.5)	17 (14.7)	30 (20.0)	13 (9.5)
Intracytoplasmic inclusions, epithelial, eosinophilic; digestive gland						
Eosinophilic ovoid bodies; epithelial; digestive gland						

Table 3-7. Histopathological changes in oysters examined in the study: *Northern Territory*

	Population	A Series: Bynoe Farm C1	C Series: Bynoe Farm C1	L Series: Bynoe Farm C1	SM Series: Western Grounds: Wild	WP Series: Western Grounds: Wild	M Series: Coburg Peninsula Farm C2	D2 Series: Coburg Peninsula Farm C3	E Series: Point Coburg Peninsula Farm C4	N Series: Far Eastern Grounds: Wild	O Series: Eastern Grounds: Wild
	Geographic Zone or Region	NT 1	NT 1	NT 1	NT 1	NT 1	NT 1	NT 1	NT 1	NT 3	NT 2
	Date of Collection	Sep-94 to Jan 95	Jun-95	Jun-96	Oct-94	Oct-94	Sep-96	Sep-95	May-95	Sep-96	Sep-96
	Laboratory Accession No.	95-214	95-1363		95-2131		96-2475	96-153	96-1080	97-0188	
	Number of Oysters Examined	(n=148)	(n=103)	(n=85)	(n=76)	(n=168)	(n=150)	(n=150)	(n=150)	(n=153)	(n=97)
	Number & Percentage with Changes	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Organ/Tissue	Histopathological Change										
Mantle	Inflammation, focal or regional	1 (0.7)	1 (1.0)	2 (2.4)	1 (1.3)	2 (1.2)		12 (8.0)	5 (3.3)	1 (0.7)	2 (2.1)
	Inflammation, diffuse or generalised						2 (1.3)	3 (2.0)	3 (2.0)		
	Inflammation, focal, metazoan										
	Inflammation, diffuse, metazoan		1 (1.0)		1 (1.3)	3 (1.8)					
	Oedema						2 (1.3)	3 (2.0)	3 (2.0)		
	Metazoa in stroma										
	Macrophages, pigmented, numerous										
	Eosinophilic granule cells, numerous										
Gill	Unidentified cysts		1 (1.0)			4 (2.4)					
	Rickettsiales-like bodies; epithelial	1 (0.7)			1 (1.3)	2 (1.2)			2 (1.3)	2 (1.3)	1 (1.0)
	Metazoa, external	1 (0.7)									
	Inflammation, focal or regional										
	Inflammation, focal, metazoan										
	Inflammation, diffuse, metazoan										
	Metazoa in stroma										
	Eosinophilic granule cells, numerous										
	Fibroma/Neurofibroma										
Palp	Papovavirus-like inclusions, epithelial										
	Rickettsiales-like bodies; epithelial						1 (0.7)	5 (3.3)	7 (4.7)		1 (1.0)
	Metazoa in stroma						1 (0.7)				1 (1.0)
	Metazoa on surface										

Table 3-7 (continued). Histopathological changes in oysters examined in the study: *Northern Territory*

	Population	A Series: Bynoe Farm C1	C Series: Bynoe Farm C1	L Series: Bynoe Farm C1	SM Series: Western Grounds: Wild	WP Series: Western Grounds: Wild	M Series: Coburg Peninsula Farm C2	D2 Series: Coburg Peninsula Farm C3	E Series: Coburg Peninsula Farm C4	N Series: Far Eastern Grounds: Wild	O Series: Eastern Grounds: Wild
Palp (cont)	Turbellarian-like ciliates; epithelial										
	Inflammation, focal or regional	3 (2.0)	1 (1.0)		1 (1.3)		3 (2.1)	9 (6.0)	8 (5.3)	1 (0.7)	
	Inflammation, diffuse or generalised										
	Inflammation, focal, metazoan						1 (0.7)	5 (3.3)	7 (4.7)	1 (0.7)	
	Inflammation, diffuse, metazoan										
	Oedema								1 (0.7)		
	Eosinophilic granule cells, numerous										
	Microgranulomata; protozoan-like										
Oesophagus	Metazoa in stroma								2 (1.3)	1 (0.7)	
	Inflammation, focal or regional				1 (1.3)				1 (0.7)	2 (1.3)	1 (1.0)
	Inflammation, focal, metazoan								2 (1.3)	1 (0.7)	
	Inflammation, diffuse, metazoan										
	Metazoa in lumen/mouth									22 (13.5)	16 (16.5)
Stomach	Gregarine protozoa, epithelial/lumenal								4 (2.7)		
	Metazoa in stroma										1 (1.0)
	Metazoa in lumen			1 (1.2)		1 (0.6)	1 (0.7)				
	Inflammation, focal or regional				1 (1.3)	5 (3.0)	1 (0.7)		6 (4.0)	1 (0.7)	
	Inflammation, diffuse or generalised										
	Inflammation, focal, metazoan								2 (1.3)		
	Inflammation, diffuse, metazoan										
Digestive Gland	Viral-like, intranuclear inclusions, epithelial	24 (16.2)	37 (35.9)	24 (28.2)	16 21.1	33 (19.6)	11 (7.3)	9 (6.0)	7 (4.7)		4 (4.1)
	Intracytoplasmic inclusions, epithelial, eosinophilic										
	Eosinophilic ovoid bodies; epithelial	4 (2.7)		1 (1.2)				5 (3.3)	1 (0.7)		
	Rickettsiales-like bodies; epithelial		1 (1.0)		13 17.1	9 (5.4)				2 (1.3)	4 (4.1)
	Cryptosporidia-like bodies								1 (0.7)		
	Gregarine protozoa, epithelial/lumenal										
	Metazoa in stroma						1 (0.7)	1 (0.7)			
	Metazoa in lumen	2 (1.4)	2 (1.9)	1 (1.2)			4 (2.7)			3 (2.0)	1 (1.0)

Table 3-7 (continued). Histopathological changes in oysters examined in the study: *Northern Territory*

	Population	A Series: Bynoe Farm C1	C Series: Bynoe Farm C1	L Series: Bynoe Farm C1	SM Series: Western Grounds: Wild	WP Series: Western Grounds: Wild	M Series: Coburg Peninsula Farm C2	D2 Series: Coburg Peninsula Farm C3	E Series: Coburg Peninsula Farm C4	N Series: Far Eastern Grounds: Wild	O Series: Eastern Grounds: Wild
Digestive Gland (cont)	Inflammation, focal or regional	6 (4.1)	14 (13.6)	5 (5.9)	3 (3.9)	3 (1.8)	1 (0.7)	1 (0.7)	1 (0.7)	5 (3.3)	2 (2.1)
	Inflammation, focal, metazoan							1 (0.7)			
	Inflammation, diffuse, metazoan									1 (0.7)	
	Microgranulomata; protozoan-like		3 (3.1)				21 (14.0)			16 (10.8)	6 (6.2)
	Regional dilation of glands										
	Inflammation, diffuse or generalised										
	Multinucleate cellular accumulation										
	Oedema										
	Macrophages, pigmented, numerous										
Midgut	Gregarine protozoa in lumen			1 (1.2)							
	Ancistrocomid-like ciliates in lumen	4 (2.7)	2 (1.9)		7 (9.2)	12 (7.2)		2 (1.3)		1 (0.7)	1 (1.0)
	Inflammation, focal or regional							1 (0.7)			
	Inflammation, focal, metazoan										
	Inflammation, diffuse, metazoan	1 (0.7)	1 (1.0)			2 (1.2)	1 (0.7)				
	Metazoa in lumen							2 (1.3)			
	Metazoa in stroma										
	Inflammation, diffuse or generalised					3 (1.8)					
Hindgut	Inflammation, focal or regional					2 (1.2)	5 (3.3)			3 (1.3)	
	Metazoa in lumen										
	Metazoa in stroma										
	Inflammation, diffuse or generalised										
	Inflammation, focal, metazoan										
	Inflammation, diffuse, metazoan										
Gonad	Inflammation, focal or regional	5 (3.4)	6 (6.2)		2 (2.6)	17 (10.2)					
	Metazoa in stroma							1 (0.7)			
	Inflammation, diffuse or generalised										
	Inflammation, focal, metazoan										
	Inflammation, diffuse, metazoan							1 (0.7)			
	Macrophages, pigmented, numerous										
Heart	Metazoan parasites										
	Inflammation, focal or regional		1 (1.0)		1 (1.3)	2 (1.2)			1 (0.7)		

Table 3-7 (continued). Histopathological changes in oysters examined in the study: *Northern Territory*

	Population	A Series: Bynoe Farm C1	C Series: Bynoe Farm C1	L Series: Bynoe Farm C1	SM Series: Western Grounds: Wild	WP Series: Western Grounds: Wild	M Series: Coburg Peninsula Farm C2	D2 Series: Coburg Peninsula Farm C3	E Series: Coburg Peninsula Farm C4	N Series: Far Eastern Grounds: Wild	O Series: Eastern Grounds: Wild
Kidney	Inflammation, focal or regional		1 (1.0)	1 (1.2)					2 (1.3)		
	Pigmentation			1 (1.2)					1 (0.7)		
	Metazoa in tubules								1 (0.7)		
Interstitial Tissues	Metazoa in stroma	1 (0.7)			1 (1.3)			1 (0.7)			1 (1.0)
	Inflammation, focal or regional	2 (1.4)	2 (1.9)		4 (5.3)	18 (10.7)	1 (0.7)	1 (0.7)		2 (1.3)	
	Inflammation, diffuse or generalised	2 (1.4)	1 (1.0)		1 (1.3)	11 (6.5)			1 (0.7)		
	Inflammation, focal, metazoan	1 (0.7)			1 (1.3)			1 (0.7)			
	Inflammation, diffuse, metazoan										
	Multinucleate cellular accumulations				1 (1.3)	11 (6.5)					
	Oedema		1 (1.0)		1 (1.3)	5 (3.0)	5 (3.3)				
	Fibroma/neurofibroma					1 (0.6)					
	Macrophages, pigmented, numerous	2 (1.4)	1 (1.0)		1 (1.3)	4 (2.4)				1 (0.7)	
	Metazoa in haemolymph sinus										
	Eosinophilic granule cells, numerous										
	Microgranuloma, Protozoan-like										
Foot	Inflammation, focal or regional					1 (0.6)					
	Metazoa in stroma										
	Eosinophilic granule cells, numerous									1 (0.7)	2 (2.1)
	External metazoa in groove								2 (1.3)		
Adductor Muscle	Inflammation, focal or regional			1 (1.2)							
	Oedema					2 (1.2)					

Table 3-7 (Continued). Histopathological changes in oysters examined in the study: *Queensland*

	Population	N.E, Coast Wild	N.E. Coast Farm C	N.E. Coast Farm C	N.E. Coast Farm D	Torres Strait Farm A	Torres Strait Farm A	Torres Strait Farm B	Torres Strait Farm B	Torres Strait Wild	Torres Strait Wild
	Geographic Zone or Region	NE Coast	NE Coast	NE Coast	NE Coast	Torres Strait	Torres Strait	Torres Strait	Torres Strait	Torres Srrait	Torres Strait
	Date of Collection		30-5-96	23-12-96	2/95-7/95	16-11-94	21-11-95	20-2-95	22-2-96	5-11-94	20-11-95
	Laboratory Accession No.										
	Number of Oysters Examined	(n = 126)	(n = 150)	(n = 6)	(n = 29)	(n = 36)	(n = 150)	(n = 150)	(n = 151)	(n = 148)	(n = 122)
	Number & Percentage with Changes	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Organ/Tissue	Histopathological Change										
Mantle	Inflammation, focal or regional	1 (0.8)	6 (4.0)			2 (5.6)	1 (0.7)	2 (1.3)	10 (6.6)	5 (3.4)	4 (3.3)
	Inflammation, diffuse or generalised										
	Inflammation, focal, metazoan										
	Inflammation, diffuse, metazoan										
	Oedema										
	Metazoa in stroma										
	Macrophages, pigmented, numerous										
	Eosinophilic granule cells, numerous									3 (2.0)	3 (2.5)
Gill	Unidentified Cysts								1 (0.7)	1 (0.7)	3 (2.5)
	Rickettsiales-like bodies; epithelial	1 (0.8)	1 (0.7)					1 (0.7)	1 (0.7)	1 (0.7)	13 (10.7)
	Metazoa, external				1 (0.3)						
	Inflammation, focal or regional	4 (3.2)					2 (1.3)	5 (3.4)	1 (0.7)	5 (3.4)	2 (1.6)
	Inflammation, focal, metazoan										
	Inflammation, diffuse, metazoan										
	Metazoa in stroma										
	Eosinophilic granule cells, numerous										
	Fibroma/Neurofibroma										
Palp	Papovavirus-like inclusions, epithelial	24 (19)	20 (13.4)	3 (50.0)	9 (2.6)		19 (12.7)	9 (6.0)	12 (7.9)	18 (12.2)	9 (7.4)
	Rickettsial-like bodies; epithelial		2 (1.3)		4 (1.2)		5 (3.4)		5 (3.3)		1 (0.8)
	Metazoa in stroma	1 (0.8)	10 (6.7)				1 (0.7)		2 (1.3)		
	Metazoa on surface										2 (1.6)
	Turbellarian-like ciliates; epithelial	16 (12.7)									
	Inflammation, focal or regional	2 (1.6)	3 (2.0)			1 (2.8)	4 (2.7)	9 (6.0)	11 (7.3)	4 (2.7)	2 (1.6)

Table 3-7 (Continued). Histopathological changes in oysters examined in the study: *Queensland*

	Population	N.E, Coast Wild	N.E. Coast Farm C	N.E. Coast Farm C	N.E. Coast Farm D	Torres Strait Farm A	Torres Strait Farm A	Torres Strait Farm B	Torres Strait Farm B	Torres Strait Wild	Torres Strait Wild
Palp (cont)	Inflammation, diffuse or generalised										
	Inflammation, focal, metazoan										
	Inflammation, diffuse, metazoan										
	Oedema										
	Eosinophilic granule cells, numerous										
	Microgranulomata; protozoan-like										
Oesophagus	Metazoa in stroma		1 (0.7)						6 (4.0)		
	Inflammation, focal or regional								12 (7.9)		
	Inflammation, focal, metazoan										
	Inflammation, diffuse, metazoan										
	Metazoa in lumen/mouth										
Stomach	Gregarine protozoa, epithelial/lumenal		6 (4.0)	2 (33.3)		1 (2.8)	1 (0.7)	17 (11.4)	20 (13.2)	15 (10.2)	4 (3.3)
	Metazoa in stroma										
	Metazoa in lumen										1 (0.8)
	Inflammation, focal or regional		1 (0.7)			1 (2.8)	1 (0.7)	3 (2.0)	1 (0.7)	2 (1.4)	
	Inflammation, diffuse or generalised										
	Inflammation, focal, metazoan										
	Inflammation, diffuse, metazoan										
Digestive Gland	Viral-like, intranuclear inclusions, epithelial		79 (52.9)	1 (16.7)		2 (5.6)	41 (27.5)	3 (2.0)	11 (7.3)	2 (1.4)	4 (3.3)
	Intracytoplasmic inclusions, epithelial, eosinophilic										
	Eosinophilic ovoid bodies; epithelial										
	Rickettsiales-like bodies; epithelial	2 (1.6)	1 (0.7)		1 (0.3)		8 (5.4)	1 (0.7)	2 (1.3)	10 (6.8)	6 (4.9)
	Cryptosporidia-like bodies										
	Gregarine protozoa, epithelial/lumenal	26 (20.6)	89 (59.6)	4 (66.7)	10 (2.9)	4 (11.2)	12 (8.0)	93 (62.3)	106 (70.0)	50 (34.0)	32 (26.2)
	Metazoa in stroma	8 (6.3)	1 (0.7)						1 (0.7)		
	Metazoa in lumen			1 (16.7)							
	Inflammation, focal or regional	4 (3.2)	13 (8.7)			1 (2.8)	6 (4.0)	14 (9.4)	14 (9.2)	5 (3.4)	10 (8.2)
	Inflammation, focal, metazoan										
	Inflammation, diffuse, metazoan										
	Microgranulomata; protozoan-like										
	Regional dilation of glands										
	Inflammation, diffuse or generalised										

Table 3-7 (Continued). Histopathological changes in oysters examined in the study: *Queensland*

	Population	N.E, Coast Wild	N.E. Coast Farm C	N.E. Coast Farm C	N.E. Coast Farm D	Torres Strait Farm A	Torres Strait Farm A	Torres Strait Farm B	Torres Strait Farm B	Torres Strait Wild	Torres Strait Wild
Digestive Gland (cont)	Multinucleate cellular accumulation										
	Oedema										
	Macrophages, pigmented, numerous										
Midgut	Gregarine protozoa in lumen							2 (1.3)			
	Ancistrocomid-like ciliates in lumen		84 (56.3)		2 (0.6)		79 (52.7)		24 (15.8)		10 (8.2)
	Inflammation, focal or regional						1 (0.7)	1 (0.7)			2 (1.6)
	Inflammation, focal, metazoan										
	Inflammation, diffuse, metazoan										
	Metazoa in lumen										
	Metazoa in stroma										
	Inflammation, diffuse or generalised										
Hindgut	Inflammation, focal or regional	1 (0.8)									
	Metazoa in lumen										
	Metazoa in stroma										
	Inflammation, diffuse or generalised										
	Inflammation, focal, metazoan										
	Inflammation, diffuse, metazoan										
Gonad	Inflammation, focal or regional		1 (0.7)					1 (0.7)			1 (0.8)
	Metazoa in stroma										
	Inflammation, diffuse or generalised										
	Inflammation, focal, metazoan										
	Inflammation, diffuse, metazoan										
	Macrophages, pigmented, numerous										
Heart	Metazoan parasites		1 (0.7)								
	Inflammation, focal or regional	2 (1.6)	2 (1.3)	1 (16.7)		1 (2.8)	28 (18.8)	11 (7.4)	10 (6.6)		6 (4.9)
Kidney	Inflammation, focal or regional						1 (0.7)	8 (5.4)			
	Pigmentation										
	Metazoa in tubules										
Interstitial Tissues	Metazoa in stroma		1 (0.7)							3 (2.0)	3 (2.5)
	Inflammation, focal or regional		3 (2.0)				5 (3.4)		5 (3.3)		1 (0.8)
	Inflammation, diffuse or generalised						1 (0.7)				

Table 3-7 (Continued). Histopathological changes in oysters examined in the study: *Queensland*

	Population	N.E, Coast Wild	N.E. Coast Farm C	N.E. Coast Farm C	N.E. Coast Farm D	Torres Strait Farm A	Torres Strait Farm A	Torres Strait Farm B	Torres Strait Farm B	Torres Strait Wild	Torres Strait Wild
Interstitial Tissues (cont)	Inflammation, focal, metazoan										
	Inflammation, diffuse, metazoan										
	Multinucleate cellular accumulations										
	Oedema										
	Fibroma/neurofibroma										
	Macrophages, pigmented, numerous										
	Metazoa in haemolymph sinus										
	Eosinophilic granule cells, numerous										
	Microgranuloma, Protozoan-like										
Foot	Inflammation, focal or regional	2 (1.6)				1 (2.8)	6 (4.0)	1 (0.7)			
	Metazoa in stroma										
	Eosinophilic granule cells, numerous										
	External metazoa in groove										
Adductor Muscle	Inflammation, focal or regional		1 (0.7)					2 (1.3)			1 (0.8)
	Oedema										

Table 3-7 (Continued). Histopathological changes in oysters examined in the study: *Western Australia*

	Population	Exmouth Gulf Wild Harvest	Exmouth Gulf Wild Harvest	Exmouth Farmed	Dampier Farmed	80 Mile Beach Wild Harvest	80 Mile Beach Wild Harvest	Broome Farmed	Broome Farmed	Broome Farmed
	Geographic Zone or Region	Zone 1	Zone 1	Exmouth Zone 1	Dampier Zone 1	Zone 2	Zone 2	Zone 2	Zone 2	Zone 2
	Date of Collection	27-01-95	23-06-95	26-08-95	25-08-95	13-03-95	21-05-95	27-03-95	18-08-95	29-03-95
	Laboratory Accession No.	P95-328	P95-1874		P95-2645	P95-836	P95-1671	P95-1070	P95-2644	P95-1072
	Number of Oysters Examined	(n = 151)	(n = 150)	(n = 148)	(n = 150)	(n = 153)	(n = 153)	(n = 56)	(n = 150)	(n =124)
	Number & Percentage with Changes	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Organ/Tissue	Histopathological Change									
Mantle	Inflammation, focal or regional		4 (2.6)	2 (1.4)		2 (1.3)	3 (2.0)	2 (3.6)	1 (0.7)	1 (0.8)
	Inflammation, diffuse or generalised			1 (0.7)	1 (0.7)	4 (2.6)	2 (1.3)			2 1.6)
	Inflammation, focal, metazoan					17 (11.1)	3 (2.0)			
	Inflammation, diffuse, metazoan						1 (0.7)			
	Oedema							1 (1.8)		
	Metazoa in stroma		1 (0.7)			22 (14.3)	7 (4.6)	4 (7.1)		1 (0.8)
	Macrophages, pigmented, numerous								1 (0.7)	
	Eosinophilic granule cells, numerous					6 (3.9)				
Gill	Unidentified Cysts									
	Rickettsiales-like bodies; epithelial					2 (1.3)	1 (0.7)			
	Metazoa, external									
	Inflammation, focal or regional		1 (0.7)				1 (0.7)			
	Inflammation, focal, metazoan					2 (1.3)				
	Inflammation, diffuse, metazoan									
	Metazoa in stroma		1 (0.7)			2 (1.3)				
	Eosinophilic granule cells, numerous									
	Fibroma/Neurofibroma						1 (0.7)			
Palp	Papovavirus-like inclusions, epithelial									
	Rickettsial-like bodies; epithelial									
	Metazoa in stroma		3 (2.0)			17 (11.1)	4 (2.6)	6 (10.7)		5 (4.0)
	Metazoa on surface									
	Turbellarian-like ciliates; epithelial									
	Inflammation, focal or regional					8 (5.2)		1 (1.8)	1 (0.7)	1 (0.8)

Table 3-7 (Continued). Histopathological changes in oysters examined in the study: *Western Australia*

	Population	Exmouth Gulf Wild Harvest	Exmouth Gulf Wild Harvest	Exmouth Farmed	Dampier Farmed	80 Mile Beach Wild Harvest	80 Mile Beach Wild Harvest	Broome Farmed	Broome Farmed	Broome Farmed
Palp (cont)	Inflammation, diffuse or generalised					1 (0.7)		2 (3.6)		2 (1.6)
	Inflammation, focal, metazoan		2 (1.3)			16 (10.4)	2 (1.3)			1 (0.8)
	Inflammation, diffuse, metazoan					1 (0.7)	1 (0.7)			1 (0.8)
	Oedema									
	Eosinophilic granule cells, numerous					6 (3.9)		1 (1.8)		
	Microgranulomata; protozoan-like									
Oesophagus	Metazoa in stroma									
	Inflammation, focal or regional									
	Inflammation, focal, metazoan									
	Inflammation, diffuse, metazoan									
	Metazoa in lumen/mouth									
Stomach	Gregarine protozoa, epithelial/lumenal									
	Metazoa in stroma					1 (0.7)				
	Metazoa in lumen									
	Inflammation, focal or regional						1 (0.7)			
	Inflammation, diffuse or generalised									
	Inflammation, focal, metazoan									
	Inflammation, diffuse, metazoan									
Digestive Gland	Viral-like, intranuclear inclusions, epithelial	9 (6.0)	26 (17.3)	17 (11.4)	27 (18.0)	9 (5.9)	2 (1.3)	4 (7.1)	21 (14.0)	9 (7.3)
	Intracytoplasmic inclusions, epithelial, eosinophilic		3 (2.0)			1 (0.7)				
	Eosinophilic ovoid bodies; epithelial									
	Rickettsiales-like bodies; epithelial	2 (1.3)				1 (0.7)	1 (0.7)	1 (1.8)		
	Cryptosporidia-like bodies									
	Gregarine protozoa, epithelial/lumenal									
	Metazoa in stroma		3 (2.0)				3 (2.0)			
	Metazoa in lumen	1 (0.7)				1 (0.7)				
	Inflammation, focal or regional	10 (6.6)	2 (1.3)	4 (2.7)	1 (0.7)	4 (2.6)	4 (2.6)	5 (8.9)	2 (1.3)	1 (0.8)
	Inflammation, focal, metazoan						3 (2.0)			
	Inflammation, diffuse, metazoan									
	Microgranulomata; protozoan-like					2 (1.3)			1 (0.7)	
	Regional dilation of glands									

Table 3-7 (Continued). Histopathological changes in oysters examined in the study: *Western Australia*

	Population	Exmouth Gulf Wild Harvest	Exmouth Gulf Wild Harvest	Exmouth Farmed	Dampier Farmed	80 Mile Beach Wild Harvest	80 Mile Beach Wild Harvest	Broome Farmed	Broome Farmed	Broome Farmed
Digestive Gland (cont)	Inflammation, diffuse or generalised		1 (0.7)	3 (2.0)			1 (0.7)	1 (1.8)	2 (1.3)	1 (0.8)
	Multinucleate cellular accumulation							1 (1.8)		
	Oedema								1 (0.7)	
	Macrophages, pigmented, numerous									
Midgut	Gregarine protozoa in lumen									
	Ancistrocomid-like ciliates in lumen						2 (1.3)			
	Inflammation, focal or regional	1 (0.7)					2 (1.3)			
	Inflammation, focal, metazoan									
	Inflammation, diffuse, metazoan									
	Metazoa in lumen	1 (0.7)					2 (1.3)			
	Metazoa in stroma						1 (0.7)			
	Inflammation, diffuse or generalised						2 (1.3)			
Hindgut	Inflammation, focal or regional									
	Metazoa in lumen						2 (1.3)			
	Metazoa in stroma						1 (0.7)			
	Inflammation, diffuse or generalised						2 (1.3)			
	Inflammation, focal, metazoan									
	Inflammation, diffuse, metazoan									
Gonad	Inflammation, focal or regional	1 (0.7)					1 (0.7)			
	Metazoa in stroma			1 (0.7)	1 (0.7)				1 (0.7)	4 (3.2)
	Inflammation, diffuse or generalised									
	Inflammation, focal, metazoan									
	Inflammation, diffuse, metazoan									
	Macrophages, pigmented, numerous									
Heart	Metazoan parasites									
	Inflammation, focal or regional									
Kidney	Inflammation, focal or regional									
	Pigmentation									
	Metazoa in tubules									
Interstitial Tissues	Metazoa in stroma					6 (3.9)				
	Inflammation, focal or regional					11 (7.2)				

Table 3-7 (Continued). Histopathological changes in oysters examined in the study: *Western Australia*

	Population	Exmouth Gulf Wild Harvest	Exmouth Gulf Wild Harvest	Exmouth Farmed	Dampier Farmed	80 Mile Beach Wild Harvest	80 Mile Beach Wild Harvest	Broome Farmed	Broome Farmed	Broome Farmed
Interstitial Tissues (cont)	Inflammation, diffuse or generalised									
	Inflammation, focal, metazoan					6 (3.9)				
	Inflammation, diffuse, metazoan									
	Multinucleate cellular accumulations									
	Oedema									
	Fibroma/neurofibroma									
	Macrophages, pigmented, numerous									
	Metazoa in haemolymph sinus									
	Eosinophilic granule cells, numerous					6 (3.9)				
	Microgranuloma, Protozoan-like									
Foot	Inflammation, focal or regional					2 (1.3)				
	Eosinophilic granule cells, numerous					3 (2.0)				
	Metazoa in stroma					1 (0.7				
	External metazoa in groove									
Adductor Muscle	Inflammation, focal or regional									
	Oedema									

Table 3-7 (Continued). Histopathological changes in oysters examined in the study: *Western Australia*

	Population	Lacepede Island Wild Harvest	Kuri Bay Farmed	Kuri Bay Farmed	Cygnet Bay Farmed	Cygnet Bay Farmed	Cone Bay Farmed
	Geographic Zone or Region	Zone 3	Zone 3	Zone 3	Zone 3	Zone 3	Zone 3
	Date of Collection	7-02-95	29-03-95	16-08-95	27-03-95	21-08-95	17-08-95
	Laboratory Accession No.	96-683	95-1071	95-2643	95-1069	95-2646	95-2626
	Number of Oysters Examined	(n = 192)	(n = 162)	(n = 162)	(n = 116)	(n = 150)	(n = 137)
	Number & Percentage with Changes	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Organ/Tissue	Histopathological Change						
Mantle	Inflammation, focal or regional	2 (1.0)			1 (0.9)		1 (0.7)
	Inflammation, diffuse or generalised	4 (2.1)	2 (1.2)	4 (2.5)	2 (1.7)	1 (0.7)	4 (2.9)
	Inflammation, focal, metazoan					2 (1.3)	1 (0.7)
	Inflammation, diffuse, metazoan	2 (1.0)					
	Oedema						
	Metazoa in stroma	2 (1.0)				2 (1.3)	1 (0.7)
	Macrophages, pigmented, numerous	1 (0.5)		2 (1.2)	1 (0.9)	1 (0.7)	
	Eosinophilic granule cells, numerous	30 (15.6)	6 (3.7)		1 (0.9)		1 (0.7)
Gill	Unidentified Cysts						
	Rickettsiales-like bodies; epithelial	1 (0.5)					2 (1.5)
	Metazoa, external						
	Inflammation, focal or regional						
	Inflammation, focal, metazoan						
	Inflammation, diffuse, metazoan						
	Metazoa in stroma						
	Eosinophilic granule cells, numerous		6 (3.7)				2 (1.5)
	Fibroma/Neurofibroma						
Palp	Papovavirus-like inclusions, epithelial						
	Rickettsiales-like bodies; epithelial						
	Metazoa in stroma	2 (1.0)	1 (0.6)		1 (0.9)		
	Metazoa on surface						
	Turbellarian-like ciliates; epithelial						
	Inflammation, focal or regional	1 (0.5)	1 (0.6)		3 (2.0)		

Table 3-7 (Continued). Histopathological changes in oysters examined in the study: *Western Australia*

	Population	Lacepede Island Wild Harvest	Kuri Bay Farmed	Kuri Bay Farmed	Cygnet Bay Farmed	Cygnet Bay Farmed	Cone Bay Farmed
Palp (cont)	Inflammation, diffuse or generalised	2 (1.0)					4 (2.9)
	Inflammation, focal, metazoan				1 (0.9)		
	Inflammation, diffuse, metazoan	2 (1.0)					
	Oedema						
	Eosinophilic granule cells, numerous	30 (15.6)	10 (6.2)				1 (0.7)
	Microgranulomata; protozoan-like	1 (0.5)					
Oesophagus	Metazoa in stroma						
	Inflammation, focal or regional						
	Inflammation, focal, metazoan						
	Inflammation, diffuse, metazoan						
	Metazoa in lumen/mouth						
Stomach	Gregarine protozoa, epithelial/lumenal						
	Metazoa in stroma						
	Metazoa in lumen						
	Inflammation, focal or regional						
	Inflammation, diffuse or generalised						
	Inflammation, focal, metazoan						
	Inflammation, diffuse, metazoan						
Digestive Gland	Viral-like, intranuclear inclusions, epithelial	13 (6.8)	7 (4.3)	17 (10.5)	19 (16.4)	30 (20.0)	13 (9.5)
	Intracytoplasmic inclusions, epithelial, eosinophilic						1 (0.7)
	Eosinophilic ovoid bodies; epithelial						
	Rickettsiales-like bodies; epithelial	1 (0.5)					
	Cryptosporidia-like bodies						
	Gregarine protozoa, epithelial/lumenal	1 (0.5)					
	Metazoa in stroma					2 (1.3)	
	Metazoa in lumen					1 (0.7)	
	Inflammation, focal or regional			1 (0.6)	5 (4.3)	1 (0.7)	1 (0.7)
	Inflammation, focal, metazoan					2 (1.3)	
	Inflammation, diffuse, metazoan						
	Microgranulomata; protozoan-like				1 (0.9)		
	Regional dilation of glands						

Table 3-7 (Continued). Histopathological changes in oysters examined in the study: *Western Australia*

	Population	Lacepede Island Wild Harvest	Kuri Bay Farmed	Kuri Bay Farmed	Cygnet Bay Farmed	Cygnet Bay Farmed	Cone Bay Farmed
Digestive gland (cont)	Inflammation, diffuse or generalised						3 (2.2)
	Multinucleate cellular accumulation						
	Oedema			3 (1.9)			
	Macrophages, pigmented, numerous						1 (0.7)
Midgut	Gregarine protozoa in lumen						
	Ancistrocomid-like ciliates in lumen						
	Inflammation, focal or regional						
	Inflammation, focal, metazoan						
	Inflammation, diffuse, metazoan						
	Metazoa in lumen						
	Metazoa in stroma						
	Inflammation, diffuse or generalised						1 (0.7)
Hindgut	Inflammation, focal or regional						
	Metazoa in lumen						
	Metazoa in stroma						
	Inflammation, diffuse or generalised						2 (1.5)
	Inflammation, focal, metazoan						
	Inflammation, diffuse, metazoan						
Gonad	Inflammation, focal or regional				1 (0.9)		
	Metazoa in stroma						
	Inflammation, diffuse or generalised	9 (4.7)		4 (2.5)	1 (0.9)	2 (1.3)	3 (2.2)
	Inflammation, focal, metazoan						
	Inflammation, diffuse, metazoan						
	Macrophages, pigmented, numerous	1 (0.5)					
Heart	Metazoan parasites						
	Inflammation, focal or regional						
Kidney	Inflammation, focal or regional						
	Pigmentation						
	Metazoa in tubules						
Interstitial Tissues	Metazoa in stroma						
	Inflammation, focal or regional				3 (2.6)		

Table 3-7 (Continued). Histopathological changes in oysters examined in the study: *Western Australia*

	Population	Lacepede Island Wild Harvest	Kuri Bay Farmed	Kuri Bay Farmed	Cygnet Bay Farmed	Cygnet Bay Farmed	Cone Bay Farmed
Interstitial Tissues (cont)	Inflammation, diffuse or generalised				4 (3.4)		
	Inflammation, focal, metazoan						
	Inflammation, diffuse, metazoan						
	Multinucleate cellular accumulations						
	Oedema						
	Fibroma/neurofibroma						
	Macrophages, pigmented, numerous	1 (0.5)		1 (0.6)			
	Metazoa in haemolymph sinus						3 (2.2)
	Eosinophilic granule cells, numerous		11 (6.8)				
	Microgranuloma, Protozoan-like	1 (0.5)			1 (0.9)		
Foot	Inflammation, focal or regional						
	Eosinophilic granule cells, numerous	30 (15.6)					
	Metazoa in stroma						
	External metazoa in groove						
Adductor Muscle	Inflammation, focal or regional						
	Oedema						

Table 3-8. Overall prevalence of histopathological changes in oysters examined in the study from Northern Territory, Queensland and Western Australia, according to zone or region

	State/ Territory of Origin	Northern Territory				Queensland			Western Australia				TOTAL
	No. examined / State / Territory	1280				1068			2154				4510
	Zone or Region	NT 1	NT 2	NT 3	NT Total	NE Coast	Torres Strait	QLD Total	WA Zone 1	WA Zone 2	WA Zone 3	WA Total	TOTAL
	No. examined per zone	**1030**	**97**	**153**	**1280**	**311**	**757**	**1068**	**599**	**636**	**919**	**2154**	
	No. & Percentage with Changes	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Organ or Tissue	**Histopathological Change**												
Mantle	Inflammation, focal or regional	32 (3.1)	2 (2.1)	1 (0.7)	35 (2.7)	7 (2.3)	24 (3.2)	31 (2.9)	6 (1.0)	29 (4.6)	7 (0.8)	42 (2.0)	108 (2.4)
	Inflammation, diffuse or generalised	1 (0.1)			1				2 (0.3)	9 (1.4)	19 (2.0)	31 (1.4)	31 (0.7)
	Oedema	4 (0.4)			4 (0.4)					1 (0.2)		1 (0.1)	6 (0.1)
	Metazoa in stroma	8 (0.8)			8 (0.6)				1 (0.2)	34 (5.3)	5 (0.5)	40 (1.9)	48 (1.1)
	Macrophages, pigmented, numerous									1 (0.2)	5 (0.5)	6 (0.3)	6 (0.1)
	Eosinophilic granule cells, numerous									6 (0.9)	38 (4.1)	44 (2.0)	44 (1.0)
Gill	Unidentified cysts						6 (0.8)	6 (0.6)					6 (0.1)
	Rickettsiales-like bodies; epithelial	5 (0.5)			5 (0.4)	2 (0.6)	5 (0.7)	7 (0.7)		3 (0.5)	3 (0.3)	6 (0.3)	18 (0.4)
	Metazoa, external	6 (0.6)	1 (1.0)	2 (1.3)	9 (0.7)	1 (0.3)	16 (2.1)	17 (1.6)					26 (0.6)
	Inflammation, focal or regional	1 (0.1)			1 (0.1)	4 (1.3)	15 (2.0)	19 (1.8)	1 (0.2)	3 (0.5)		4 (0.2)	24 (0.5)
	Metazoa in stroma								1 (0.2)	2 (0.3)		3 (0.1)	3 (0.1)
	Eosinophilic granule cells, numerous										8 (0.9)	8 (0.4)	8 (0.2)
	Fibroma/Neurofibroma									1 (0.2)		1 (0.1)	1 (<0.1)
Palp	Papovavirus-like inclusions, epithelial					56 (18.0)	67 (8.9)	123 (11.5)					123 (2.7)
	Rickettsiales-like bodies; epithelial					6 (1.9)	11 (1.5)	17 (1.6)					17 (0.4)
	Metazoa in stroma	13 (1.3)	1 (1.0)		14 (1.2)	11 (3.5)	3 (0.4)	14 (1.3)	3 (0.5)	32 (5.0)	4 (0.4)	39 (1.8)	68 (1.5)
	Metazoa on surface	1 (0.1)	1 (1.0)		2 (0.2)								2 (<0.1)
	Turbellarian-like ciliates; epithelial					16 (5.1)	2 (0.3)	18 (1.7)					18 (0.4)

Table 3-8 (continued). Overall prevalence of histopathological changes in oysters examined in the study from Northern Territory, Queensland and Western Australia, according to zone or region

Organ or Tissue	Histopathological Change	NT 1	NT 2	NT 3	NT Total	NE Coast	Torres Strait	QLD Total	WA Zone 1	WA Zone 2	WA Zone 3	WA Total	TOTAL
Palp (cont)	Inflammation, focal or regional	38 (3.7)		2 (1.3)	40 (3.1)	5 (1.6)	31 (4.1)	36 (3.4)	2 (0.3)	31 (4.9)	6 (0.7)	39 (1.8)	115 (2.6)
	Inflammation, diffuse or generalised									7 (1.1)	8 (0.9)	15 (0.7)	15 (0.3)
	Oedema	1 (0.1)			1 (0.1)								1 (<0.1)
	Eosinophilic granule cells, numerous									7 (1.1)	41 (4.5)	48 (2.2)	48 (1.1)
	Microgranulomata; protozoan-like										1 (0.1)	1 (0.1)	1 (<0.1)
Oesophagus	Metazoa in stroma	2 (0.2)		1 (0.7)	3 (0.2)	1 (0.3)	6 (0.8)	7 (0.7)					10 (0.2)
	Inflammation, focal or regional	4 (0.4)	1 (1.0)	3 (2.0)	8 (0.6)		12 (1.6)	12 (1.1)					20 (0.4)
	Metazoa in lumen/mouth		16 (16.5)	22 (13.5)	38 (3.0)								38 (0.8)
Stomach	Gregarine protozoa, epithelial/lumenal					8 (2.6)	58 (7.7)	66 (6.2)					66 (1.5)
	Metazoa in stroma	4 (0.4)			4 (0.3)								4 (0.1)
	Metazoa in lumen	3 (0.3)	1 (1.0)		4 (0.3)		1 (0.1)	1 (0.1)		1 (0.2)		1 (0.1)	6 (0.1)
	Inflammation, focal or regional	15 (1.5)		1 (0.7)	16 (1.3)	1 (0.3)	8 (1.1)	9 (0.8)					25 (0.6)
	Inflammation, diffuse or generalised									1 (0.2)		1 (0.1)	1 (<0.1)
Digestive Gland	Viral-like, intranuclear inclusions, epithelial	161 (15.6)	4 (4.1)		165 (12.9)	80 (25.7)	63 (8.3)	143 (13.4)	79 (13.2)	45 (7.1)	99 (10.8)	223 (10.4)	531 (11.8)
	Intracytoplasmic inclusions, epithelial, eosinophilic								3 (0.5)	1 (0.2)	1 (0.1)	5 (0.2)	5 (0.1)
	Eosinophilic ovoid bodies; epithelial	11 (1.1)											
	Rickettsiales-like bodies; epithelial	23 (2.2)	4 (4.1)	2 (1.3)	29 (2.3)	4 (1.3)	27 (3.6)	31 (2.9)	2 (0.3)	3 (0.5)	1 (0.1)	6 (0.3)	66 (1.5)
	Cryptosporidia-like bodies	1 (0.1)											
	Gregarine protozoa, epithelial/lumenal					129 (41.5)	297 (39.2)	426 (39.9)			1 (0.1)	1 (0.1)	427 (9.5)
	Metazoa in stroma	2 (0.2)			2 (0.2)	9 (2.9)	1 (0.1)	10 (0.9)	3 (0.5)	3 (0.5)	2 (0.2)	8 (0.4)	20 (0.4)
	Metazoa in lumen	9 (0.9)	1 (1.0)	3 (2.0)	13 (1.0)	1 (0.3)		1 (0.1)	1 (0.2)	1 (0.2)	1 (0.1)	3 (0.1)	17 (0.4)
	Inflammation, focal or regional	35 (3.4)	2 (2.1)	5 (3.3)	42 (3.3)	17 (5.5)	50 (6.6)	67 (6.3)	17 (2.8)	19 (3.0)	10 (1.1)	46 (2.1)	155 (3.4)
	Microgranulomata; protozoan-like	3 (0.3)		1 (0.7)	1 (1.0)					3 (0.5)	1 (0.1)	4 (0.2)	5 (0.1)
	Regional dilation of glands	21 (2.0)	6 (6.2)	16 (10.8)	43 (3.4)								43 (1.0)
	Inflammation, diffuse or generalised								3 (0.5)	5 (0.8)	3 (0.3)	11 (0.5)	11 (0.2)
	Multinucleate cellular accumulation									1 (0.2)		1 (0.1)	1 (<0.1)
	Oedema										1 (0.1)	1 (0.1)	1 (<0.1)
	Macrophages, pigmented, numerous									1 (0.2)	1 (0.1)	2 (0.1)	2 (<0.1)

Table 3-8 (continued). Overall prevalence of histopathological changes in oysters examined in the study from Northern Territory, Queensland and Western Australia, according to zone or region

Organ or Tissue	Histopathological Change	NT 1	NT 2	NT 3	NT Total	NE Coast	Torres Strait	QLD Total	WA Zone 1	WA Zone 2	WA Zone 3	WA Total	TOTAL
Midgut	Gregarine protozoa in lumen						2 (0.3)	2 (0.2)					2 (<0.1)
	Ancistrocomid-like ciliates in lumen	1 (0.1)			1 (0.1)	86 (27.7)	113 (14.9)	199 (18.6)					200 (4.4)
	Inflammation, focal or regional	28 (2.7)	1 (1.0)	1 (0.7)	30 (2.4)		4 (0.5)	4 (0.4)	1 (0.2)	4 (0.6)		5 (0.2)	39 (0.9)
	Metazoa in lumen	5 (0.5)			5 (0.4)				1 (0.2)			1 (0.1)	6 (0.1)
	Metazoa in stroma	2 (0.2)			2 (0.2)					2 (0.3)		2 (0.1)	4 (0.1)
Midgut (cont)	Inflammation, diffuse or generalised									1 (0.2)	1 (0.1)	2 (0.1)	2 (<0.1)
Hindgut	Inflammation, focal or regional	3 (0.3)			3 (0.2)	1 (0.3)		1 (0.1)		4 (0.6)		4 (0.2)	8 (0.2)
	Metazoa in lumen	7 (0.7)		3 (1.3)	10 (0.8)								10 (0.2)
	Metazoa in stroma									2 (0.3)		2 (0.1)	2 (<0.1)
	Inflammation, diffuse or generalised									1 (0.2)	2 (0.2)	3 (0.1)	3 (0.1)
Gonad	Inflammation, focal or regional	30 (2.9)			30 (2.4)	1 (0.3)	2 (0.3)	3 (0.3)	1 (0.2)		1 (0.1)	2 (0.1)	35 (0.8)
	Metazoa in stroma	1 (0.1)			1 (0.1)					1 (0.2)		1 (0.1)	2 (<0.1)
	Inflammation, diffuse or generalised								2 (0.3)	5 (0.7)	19 (2.1)	26 (1.2)	26 (0.6)
	Macrophages, pigmented, numerous										1 (0.1)	1 (0.1)	1 (<0.1)
Heart	Metazoan parasites					1 (0.3)		1 (0.1)					1 (<0.1)
	Inflammation, focal or regional	5 (0.5)			5 (0.4)	5 (1.6)	56 (7.4)	61 (5.7)					64 (1.4)
Kidney	Inflammation, focal or regional	4 (0.4)			4 (0.3)		9 (1.2	9 (0.8)					13 (0.3)
	Pigmentation	2 (0.2)			2 (0.2)								2 (<0.1)
	Metazoa in tubules	1 (0.1)			1 (0.1)								1 (<0.1)
Interstitial Tissues	Metazoa in stroma	2 (0.2)	1 (1.0)		3 (0.2)	1 (0.3)	6 (0.8)	7 (0.7)		6 (0.9)		6 (0.3)	16 (0.4)
	Inflammation, focal or regional	31 (3.0)		2 (1.3)	33 (2.6)	3 (1.0)	11 (1.5)	14 (1.3)		17 (2.7)	3 (0.3)	20 (0.9)	67 (1.5)
	Inflammation, diffuse or generalised	16 (1.6)			16 (1.3)		1 (0.1)	1 (0.1)			4 (0.4)	4 (0.2)	21 (0.5)
	Multinucleate cellular accumulations	12(1.2)			12 (0.9)								12 (0.3)
	Oedema	12 (1.2)			12 (0.9)								12 (0.3)
	Fibroma/neurofibroma	1 (0.1)			1 (0.1)								1 (<0.1)
	Macrophages, pigmented, numerous	8 (0.8)			8 (0.6)						2 (0.2)	2 (0.1)	10 (0.2)
	Metazoa in haemolymph sinus			1 (0.7)	1 (0.1)								1 (<0.1)
	Eosinophilic granule cells, numerous									6 (0.9)	14 (1.5)	20 (0.9)	20 (0.4)
	Microgranuloma, Protozoan-like										1 (0.1)	1 (0.1)	1 (<0.1)

Table 3-8 (continued). Overall prevalence of histopathological changes in oysters examined in the study from Northern Territory, Queensland and Western Australia, according to zone or region

Organ or Tissue	Histopathological Change	NT 1	NT 2	NT 3	NT Total	NE Coast	Torres Strait	QLD Total	WA Zone 1	WA Zone 2	WA Zone 3	WA Total	TOTAL
Foot	Inflammation, focal or regional	1 (0.1)			1 (0.1)	2 (0.6)	8 (1.1)	10 (0.9)		2 (0.3)	1 (0.1)	3 (0.1)	13 (0.3)
	Metazoa in stroma									1 (0.2)		1 (0.1)	1 (<0.1)
	Eosinophilic granule cells, numerous									3 (0.5)	30 (3.3)	33 (1.5)	33 (0.7)
	External metazoa in groove		2 (2.1)	1 (0.7)	3 (0.2)								3 (0.1)
Adductor	Inflammation, focal or regional	3 (0.3)			3 (0.2)	1 (0.3)	3 (0.4)	4 (0.4)					7 (0.2)
Muscle	Oedema	2 (0.2)			2 (0.2)								2 (<0.1)

CHAPTER 4: INFECTIOUS AGENTS AND DISEASES OF AUSTRALIAN PEARL OYSTERS; LABORATORY INVESTIGATIONS 1995-1997

INTRODUCTION

A spectrum of pathogenic or potentially pathogenic infectious agents have been reported worldwide in or on pearl oysters *Pinctada* species (Chapter 2). These include viruses, bacteria, protozoa and metazoa which invade the tissues of the oyster, a range of agents which colonise and invade the shell and a number of predators that feed in or on the oyster itself. The role, significance and mechanisms of such agents in eliciting disease is, however, poorly understood, as is the role of adverse environmental conditions in initiating or contributing to disease states.

Based on a knowledge of the comparative pathology and microbiology of other aquatic animals, including bivalve molluscs, it is likely that a range of microbial and metazoan agents will cause disease in their own right in pearl oysters, or in association with stressing factors, including adverse environmental parameters.

It is usual to investigate occurrences of disease or production losses in terrestrial, avian and aquatic animal species, especially those of commercial importance. Such investigations include pathological and microbiological examinations to determine the cause of the disease or production loss, and may include monitoring of environmental parameters. Once the cause or causes are determined, a basis for the treatment, control or prevention of such disease may then be established. These principles of disease diagnosis in aquatic animals are well described, but appear rarely to have been applied to *Pinctada* species in Australia or elsewhere, despite the occurrence of mortalities and production losses.

Major practical difficulties exist in examining diseased pearl oysters and determining the cause or causes of disease. Anecdotal evidence has indicated that batches of hatchery reared juvenile oysters may incur high mortalities, 40-100%, following transplant into sea cages for rearing, yet such batches of oysters have seldom been subjected to pathological or microbiological examination. The rapid loss of tissue by predation on oysters which gape and cannot maintain shell closure, the rapid decomposition of tissues which occurs in tropical waters and logistical difficulties in submitting oysters for laboratory examination combine to hindering critical pathological and microbiological examination. Often, the aquaculturalist or examining pathologist is confronted by a cage of decomposing or empty shells.

It is highly likely that pathogens, parasites or pests will accompany movements of *Pinctada* species as has been the case with uncontrolled movements of other bivalve molluscs, with potential to introduce pathogens, parasites or pest species (Andrews 1980; Stewart 1991; Humphrey 1995). In order to mitigate against the introduction of such agents, some measure of quarantine and health certification on the population to be moved is essential. Movements of larval or juvenile pearl oysters in Australia have generally been accompanied by laboratory examinations of spat prior to shipment to new geographic regions in northern Australia to verify freedom from adventitial agents.

This chapter reviews and records results of laboratory studies or examinations conducted on *P. maxima* in Australia in recent years. Three major oyster groups which were subjected to laboratory studies are reviewed:

1. Diseased adult and juvenile oysters submitted for investigations during the years 1995-1997.

2. Sequential laboratory examinations of juvenile oysters placed in sea cages for growout.

3. Oyster spat examined prior to and following translocation between biogeographical regions

The results of these studies are presented to augment data obtained in the survey of clinically normal oysters (Chapter 3) and to further extend knowledge on the pathology, microbiology and diseases in *Pinctada* species.

MATERIALS AND METHODS

Source of oysters

Diseased adult and juvenile oysters.

Farmed oysters determined to have undergone production losses and oysters which were clinically diseased were submitted live or fixed in preservative to the laboratory. Where possible, gross pathological examinations were conducted, and representative samples of tissues fixed in 10% seawater formalin and processed for histopathological examination. Diseased spat were derived from hatchery oysters observed to undergo high mortalities. Table 4-1 shows details of oysters examined.

Sequential examination of caged juvenile oysters.

Juvenile hatchery reared oysters from two separate spawnings were transferred to a sea cage for rearing at 110 days post spawning (Table 4-4). Rearing was conducted in Bynoe Harbour, NT. Samples of the population were collected at periodic intervals following introduction and examined histologically for lesions, morphological changes or the presence of infectious agents (Table 4-5).

Translocated spat.

Subsamples of approximately 150 larval, hatchery reared oysters were randomly collected from populations destined for translocation from NT. In all cases, histopathological examinations were undertaken on these animals and in some cases, examination by culture for *Perkinsus* sp. was undertaken.

A further 22 batches of 150 spat were examined after spending a minimum of 6 weeks in open water sites in WA as part of the regulatory controls in place controlling oyster movements in that State.

Clinical examination, history and gross pathology

Where possible, details relating to the losses, including environmental data, were collected, and a description of the nature of the disease obtained. The occurrence of lesions or abnormalities in shell structure or in the animals were recorded.

Histopathological examination

Tissues were collected in 10% phosphate buffered formalin (NT) or Davidson's fixative or seawater formalin (WA), embedded in paraffin and sections cut and stained with haematoxylin and eosin for preliminary histopathological examination. A full range of tissues and organs, including mantle, gonad palps, digestive gland, kidney, heart, stomach, midgut, hindgut, gills and adductor muscle were examined from each oyster. Spat were fixed whole and processed as above.

Microbiological examination

Examination for bacteria was conducted by culture of samples of tissues or haemolymph on marine blood and TCBS agar. Examination for *Perkinsus* sp. was conducted by culture according to the technique of Ray (1966).

RESULTS

Diseased adult oysters

Results of examinations on adult oysters are summarised in Table 4-2.

Clinical examination, history and gross pathology: Oysters examined from populations reported to be "sick", or with a history of mortalities showed a range of gross changes. In some cases, no obvious changes were evident. In other cases, dead oysters were present, characterised by the presence of empty shells, or shells containing remnants of tissue. These were unsuitable for further examination. In other cases, living, diseased oysters showed mantle retraction, weak adductor muscle tone, and/or poor mantle colouration. Brownish discolouration of exposed nacre associated with the retracted mantle was a common feature of these animals. Mantle tissues were typically pale and retracted and gonadal development appeared poor in some affected animals.

Histopathology: Significant histopathological findings from populations of oysters exhibiting signs of disease are summarised in Table 4-2. No single major pathogen was incriminated in outbreaks of disease investigated in the study. Microabscessation were associated with bacteria, and granulomas were associated with a protozoan-like agent. The range of generalised or focal degenerative tissue changes and the occurrence of bacteria in such tissues indicate that the cause of disease may be multi-factorial, involving sub-optimal environmental parameters, nutritional inadequacy or other stressing factors, with secondary bacterial invasion.

A spectrum of non-specific and specific inflammatory and degenerative changes were also present, together with the occurrence in some cases of microbial, protozoan or metazoan agents.

Many oysters showed focal haemocytic accumulations in the stromal or interstitial tissues. In most cases, these accumulations were not associated with an observable causative agent. Occasionally, multinucleate cells were present in the stromal tissues.

Occasional intranuclear basophilic inclusion bodies were present in the digestive gland epithelium of one oyster (AB series).

Microgranulomas characterised by an intense haemocytic infiltration, digestive gland degeneration and the presence of multiple ovoid bodies were present in one animal. These bodies appeared identical to the enigmatic protozoan-like agents seen as described in Chapter 3.

One animal (NT 96/2143) showed bacterial invasion of tissues with rod-shaped bacteria and erosion of gastric epithelium consistent with *Vibrio* sp.

Diseased Spat

Results of examinations on diseased spat are summarised in Table 4-3.

Clinical examination, history and pathology: Two cases were investigated. in the first case, 25,000 plus 30 day old spat from a hatchery were reported to be undergoing 95% mortality. No gross lesions were reported. In the second case, "sickness" and death over a period of a few weeks were reported in 135 day old spat.

Spat and juvenile oysters (<40mm) are susceptible to *Vibrio* infections both in hatcheries and in sea cages. Starvation seems to be common in sea cages. Environmental effects such as temperature shock may also be implicated though data is still anecdotal. High silt loads in the water following cyclones have caused deaths due to starvation and animals expend their energy filtering sand which has very little available nutrient.

Histopathology: Severe, generalised tissue necrosis was present in some individuals, together with multinucleate cells and haemocytic infiltrations. In a number of individuals, multinucleate, ovoid bodies of uncertain character were present.

Sequential examination of caged juvenile oysters

The dates of collection and age at collection of spat collected are shown in Table 4-4 and results of examinations on spat are summarised in Table 4-5.

The sequential appearance of infectious agents is shown in Table 4-5. At the time of placement (Day 0), no lesions or infectious agents were present in the spat (F and G1 series).

After 31 days in the sea, one series (H) showed no lesions or agents. After this time, however, the G2 series were observed to have acquired a number of protozoan and metazoan species. The alimentary tract of 7 of the oysters contained morphologically similar metazoan agents; Three morphologically disparate protozoan-like agents were also present in the alimentary tract of approximately half of the oysters. These agents conformed to;

- Large ovoid to elongate ciliated organisms approximately 100-150 microns in size.
- Ovoid single celled bodies surrounded by a cast or cuticle, approx 50-60 microns.
- Smaller single celled bodies approx 25 microns.

Translocated spat

Northern Territory: Cultural examinations for *Perkinsus* sp., and histological examination for other pathogens or parasites were conducted on some 1200 hatchery reared larvae prior to translocation in 1993. No *Perkinsus* spp. or histological abnormalities were recorded.

Western Australia: A total of 7350 spat were examined between Jan. 1995 and Sept. 1996. These showed a low proportion of infectious agents. Ten animals had viral-like inclusion bodies in the digestive gland epithelium, 25 had an uncharacterised copepod in the digestive gland, one animal had a viral-like inclusion in the palp epithelium and two animals had rickettsiales-like organisms in the digestive gland. One population showed a high proportion of *Haplosporidium* sp. whilst not associated with mortality, the high level of organisms in infected animals suggested that pathology and disease were likely sequelae. In other spat, a heart apicomplexan, rickettsiales-like agent and inclusions in the digestive gland were seen from the Broome area, inclusions and copepods in the digestive gland from Pender Bay, and *Ancistrocomid*-like ciliates in the alimentary tract from a quarantine site at the 80 mile beach and also in the gills from a pearling site in zone 1. Note that in all cases these hatchery produced spat were grown in aseptic conditions and checked negative for pathogens prior to leaving the hatchery. These infections were picked up in the first six weeks of exposure to the marine environment.

These studies demonstrate that Australian *P. maxima* are subject to a spectrum of disease states in which the cause or causes is poorly understood. Juvenile oysters are sequentially colonised by protozoan and metazoan agents shortly after leaving the hatchery and may, even as spat, carry potentially infectious agents with potential to establish these in new locations consequent to movement of the animals.

DISCUSSION

Diseased mature oysters

In a number of cases the cause of the "sickness" was not apparent. In such cases there was no direct evidence of infectious disease or parasitism. Oedema was considered to relate to prolonged handling stress prior to fixation associated with, for example, overheating and/or hypoxia. The inflammatory cell infiltrates and the vacuolar change may indicate earlier low-grade infection with *Vibrio* sp. or may reflect other stressing factors including rough handling.

In cases such as this where no infectious agent is apparent, non-infectious causes of illness, including poor nutrition, toxicoses, or previous stressing factors including rough handling and environmental factors should be considered.

Disseminated bacterial microgranulomatous abscessation was present in the oysters following shell engraving, likely due to invasion with marine *Vibrio* spp. following heat stress and/or direct tissue damage.

The presence of focal inflammation in the heart and bacterial colonies in and associated with the digestive tract is consistent with bacterial septicaemia, especially *Vibrio* spp.

The presence of smaller, uniform bacterial organisms in microabscesses in older animals supports a case for systemic vibriosis. Rapid onset of autolysis and invasion by a large

number of different bacterial species is to be expected following death or debilitation from any cause. Death in molluscs may be difficult to determine as different parts of the animal may be viable while other parts show advanced necrotic as a result of pathological or noxious processes. Thus, bacterial invasion may occur regionally in debilitated animals. In general, the syndrome of bacterial invasion appears to be consistent with stress and secondary bacterial invasion, probably *Vibrio* spp. in the first instance, followed by a mixed range of saprophytic marine bacteria. Other causes should be considered, including viruses, exposure to toxicants or toxic marine algae.

The microgranulomata associated with the enigmatic protozoan-like bodies and the tissue destruction in a diseased oyster represents a significant finding with respect to the nature and pathogenicity of the agent. These agents also seen in healthy populations (Chapter 3) should be the subject of further investigations.

Diseased spat

The pathological response in diseased spat examined was clearly centred on the digestive gland epithelium and digestive gland interstitial tissues. There is little evidence to suggest an infectious agent. Viral inclusions, protozoan and metazoan agents were not observed. The significance of the cocco-bacillary bodies is enigmatic. These bodies may represent bacteria or rickettsial agents, but they were not a common feature in all spat observed, and may represent a secondary invasion. Infectious agents, especially viruses, which cannot be visualised by light microscopy, may play a primary role and the possibility of such agents should not be ignored. Examination of any remaining formalin fixed spat by electron microscopy should be undertaken.

The possibility of exposure to water-borne toxic factors in diseased spat was considered. Direct exposure to a toxin appears unlikely, as all epithelial surfaces appeared normal with the exception of the digestive gland, although the relative susceptibility of different epithelial structures to toxins is not known. Indirect exposure to a toxic substance might explain the histopathological changes observed, if a putative toxin was delivered to the digestive gland tubules in some way, either a small particulate matter or incorporated in algal cells or bacterial cells through bio-accumulation.

While the number of samples examined was relatively few, no unequivocal evidence for a single cause of the mortalities was present. The ovoid multinucleate bodies in the sample S1 are likely degenerate and necrotic tissue cells. The invasion of the tissues in spat S2 appears to be a peri-mortem event if not occurring earlier.

Sequential examinations of oysters

This short study demonstrated the colonisation of juvenile oysters by a range of protozoan and metazoan agents following exposure to the natural marine environment. Further long-term studies are warranted to monitor such colonisation, especially by potential pathogens, and to associate any mortalities with the appearance of these agents.

Translocated oysters

The low prevalence of potentially infectious agents carried by apparently healthy spat examined as part of quarantine and health certification procedures demonstrates the potential to introduce pathogens into new areas, concurrent to the introduction of spat. An understanding of the nature and distribution of such agents is essential in determining whether to proceed with an introduction or to prevent the release of potentially infected animals.

Table 4-1. Source, identification, history and total number of pearl oysters *Pinctada maxima* submitted for disease investigations in Northern Territory 1994-1997

Geographic Origin and History	No.	Date/s of Collection	Identification *(Lab. Access. No.)*	Age and Type
Bynoe Harbour *Diseased*	7		AB series *(94/1998)*	Mature
Coburg Peninsula *High mortalities*	4	02-02-95 (Acc date)	95/175	Mature
Bynoe harbour *High mortalities*	8	30-04-95/ 01-05-95	95/687	1-12 months
Bynoe harbour *High mortalities*	6	04-05-95	95/733	12 months
Coburg Peninsula *"Diseased"*	1		95/439	Mature
Coburg Peninsula *"Diseased"*	1		95/439	Mature
Bynoe Harbour *"Diseased"*	3		D series*(95/1362)*	2-3 years
Bynoe Harbour *Engraving deaths*	7		95/527	Mature

Table 4-2. Source, history and pathological findings of mature diseased pearl oysters examined in study from Northern Territory.

Identification	Date of Collection	Location	History	Gross Pathology	Histopathology
95/1362 D1	4-7-95	NT1; Farm C1	"Sick"	nsf*	Mild mantle oedema; focal eosinophilic granular cell infiltration of kidney.
95/1362 D2	9-7-95	NT1; Farm C1	"Sick"	nsf	Diffuse interstitial inflammation of midgut and stomach; mild to moderate mantle oedema.
95/1362 D3	9-7-95	NT1; Farm C1	"Sick"	nsf	Moderate mantle oedema; focally extensive inflammation in mantle.
95/439-1		NT1; Farm C3	"Sick"	nsf	Mild mantle oedema.
95/439-2		NT1; Farm C4	"Sick"	nsf	Mild mantle oedema.
95/527/1-5		Darwin Aquaculture Centre	Abscess development following engraving of shells	Abscesses in mantle reported.	Multifocal microgranulomas throughout the tissues with numerous bacterial colonies.
96/2143/P7	7/8/96	NT1; Farm C4	Animals in poor condition.		nsf
96/2143/P8		NT1; Farm C4	Animals in poor condition.		Focal inflammation in heart associated with bacteria; erosion and inflammatioin of stomach epithelium.
95/687 / Y1-Y5	30/4/95 to 1/5/95	NT1; Farm C1	25% mortality in 12 mth old oysters.		Severe autolysis, digestive gland inflammation and a heavy mixed bacterial invasion are present.
95/733-Y1	4/5/95	NT1; Farm C1	12 mth old oysters showing 60-90% mortality, with large number dying suddenly.		nsf
95/733-Y2					nsf
95/733-Y3					Focal inflammation in mantle and peripheral to the midgut.
95/733-3A					Inflammation in gills and mantle, with haemocyte necrosis and bacterial invasion.
95/733-3B					Marked vacuolation of digestive gland epithelium.
95/733-3C					Marked vacuolation of digestive gland epithelium.

Table 4-2 (continued). Source, history and pathological findings of mature diseased pearl oysters examined in study from Northern Territory.

Identification	Date of Collection	Location	History	Gross Pathology	Histopathology
95/175-1	2/2/1995	NT1; Farm C2	Mortalities in seeded pearl oysters.	Mantle retraction, with discolouration of exposed nacre; pale mantle tissue.	Moderate to intense inflamation in interstitium; epithelium erosion of mantle, stomach anf palps, with bacterial invasion.
95/175--2		NT1; Farm C2		Regional mantle pallor	Regional erosion of mantle epithelium.
95/175-3		NT1; Farm C2		Gaping, weak, poor adductor muscle tone, minimal mantle retraction.	Mantle erosion and intense interstitial inflammation.
95/175-4		NT1; Farm C4		Slight gaping and mantle retraction.	Focal inflammation in heart and mantle.

* nsf = no significant findings

Table 4-3. Source, history and pathological findings of juvenile diseased pearl oysters examined in study from Northern Territory.

Identification	Date of Collection	Farm	Location	History	Histopathology
96/1398/1-8		Darwin Aquaculture Centre		135 day old spat became sick and died over a period of a few weeks.	Multifocal inflammation in digestive gland stroma with haemocyte necrosis and tubular degeneration associated with small cocco-bacillary like bodies.
95/687 S1	30/4/95 to 1/5/95			95% mortality in hatchery reared spat	Severe, generalised tissue necrosis with numerous ovoid basophilic and eosinophilic bodies are present, some of which are multinucleate and resemble protozoa.
95/687 S2					Intense haemocytic infiltration with microabscessation. Invasion of tissues by large spirilliform bacteria is conspicuous and small, basophilic multinucleate bodies are present.
95/687 S3					Severe generalised necrosis and deeply staining ovoid bodies are present.

Table 4-4. Sequential examination of juvenile pearl oysters *Pinctada maxima* following introduction to sea cages/panels in Bynoe Harbour, Northern Territory

Identification	Lab. Accession No.	History	No. Sampled	Date of Collection	Age at Collection (days)	Days post-placement
F series	96/537	Ex hatchery	60	16/1/96	128	18
H series	96/538	Ex sea cage	60	16/2/96	141	31
G1 series	96/539	Ex hatchery	60	16/1/96	110	0
G2 series	96/539	Ex sea panels	42	16/2/96	141	31
G3 series	96/539	Ex sea panels	44	28/3/96	181	71
G4 series	96/1081	Ex sea panels	40	16/5/96	230	120
G5 series		Ex sea panels	58	26/6/96	271	161

Table 4-5. Results of sequential examinations of juvenile pearl oysters *Pinctada maxima* following introduction to sea cages/panels in Bynoe Harbour, Northern Territory

Identification (Series)	F	H	G1	G2	G3	G4	G5
Days post placement	18	31	0	31	71	120	161
Age (days)	128	141	110	141	181	230	271
No. examined	60	60	60	42	44	40	58
Shell length (mean)			10.6	24	28.6	37.8	38.3
Hinge length (mean)			12.9	25.5	30.7	36.8	39.3
Morphological features	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Alimentary tract metazoan Type 1 (large)				7 (16.7)	1 (2.3)	5 (12.5)	
Alimentary tract metazoan Type 2 (small)					4 (9.1)	1 (2.5)	
Alimentary tract unicellulate Type 1				+	+		
Alimentary tract unicellulate Type 2				+	+	+	
Alimentary tract unicellulate Type 3				+	+		
Digestive gland focal inflammation					2 (4.5)	1 (2.5)	2 (3.4
Digestive gland lumenal metazoa					1 (2.3)		
Gill metazoa Type 1 (small)					5 (11.4)		
Gill metazoa Type 2 (large)					1 (2.3)		
Mantle metazoan (small)					1 (2.3)		
Mantle metazoan (large segmented)						3 (7.5)	
Mantle focal inflammation							1 (1.7)

+ = present in population

CHAPTER 5: MORTALITY EVENTS IN AUSTRALIAN PEARL OYSTERS

INTRODUCTION

Mass mortalities of larval, juvenile or adult pearl oysters appear well recognised in Australia and with few exceptions, do not appear to have been described or details published. Such mortalities appear to have largely gone un-investigated and their cause or causes have in many cases not been fully defined. Wolf and Sprague (1978) reported that mass mortalities had caused great concern in some commercial Australia pearl farms in the years 1968-1978, yet the cause remained unknown. Mass mortalities attributed to overcrowding occurred in Torres Strait in 1966 and 1968, whilst in 1969 high mortalities attributed to domestic sewage and chemical pollution occurred adjacent to Thursday Island (Pyne 1972). Widespread mass mortalities were reported in *P. maxima* following the grounding of the oil tanker Ocean Grandeur on 3rd March 1970 in Torres Strait. Mortalities of up to 80% were recorded in new shell in the 1970 season following transport to farms and surviving oysters were in weak condition and had depressed growth rates. Surviving oysters developed a double-backed shell abnormality and abnormal nacre production on half pearls. The oil spill and the use of a non-biodegradable detergent were seen as significant causes of the mortalities by some observers (Yamashita 1986) but other investigations suggested an uncharacterised infectious agent as the cause (Pyne 1972).

Dybdahl and Pass (1985) and Pass *et al.* (1987) undertook detailed investigations of mortalities involving up to 80% of harvested shell following removal from collecting grounds in WA and concluded that such losses were associated with marine *Vibrio* infection related to inadequate water circulation and lowered water temperature during transportation. Norton *et al.* (1993b) investigated a mortality involving 85% of adult oysters in a farm at Torres Strait and identified a *Perkinsus*-like organism in affected oysters. Subsequent data (Norton 1996 - unpublished) indicates that mass mortalities continue in farms. While such mortalities are not generally investigated, the losses may be caused by similar factors described in WA (Pass *et al.* 1987), including marine *Vibrio* spp., and intercurrent deficiencies in hygiene, handling and transport procedures. Recently, high mortalities at a farm in NT were reported to have a seasonal basis, possibly associated with decreased salinity and decreased water temperature.

Unpublished accounts of massive mortalities in juvenile stock and occasional mass mortalities in larval stock are also reported. The cause or causes are largely undefined, but likely involve both infectious and environmental/husbandry factors.

Heavy losses of juvenile grow-out spat from pearl oyster hatcheries have been experienced on pearl farms in northern Australia. Causes of losses include the escape of spat from mesh baskets, the clumping of juvenile spat within the baskets causing secondary starvation, and reduced water flow into the baskets from the use of very small mesh which readily becomes fouled (Tlili 1996; Norton, Jones - unpublished). Survivors from one batch of spat had a papova virus-like infection of the gills and a non-specific enteritis (Norton - unpublished).

Mass mortalities in Queensland pearl oysters

Norton (1996 - unpublished) documented investigations into five major mortality events in pearl oysters in Queensland as follows:

Event A: Adult pearl oysters had been kept on the sea bed in Torres Strait at a collection point or dump for many months without being cleaned or turned. These oysters were then placed in panels on a long line close to an island in Torres Strait. During August 1996 the oysters were seeded for round pearls on the island where on-shore tanks were used during the operation. At the same time, several large sea turtles were slaughtered on the beach and the waste products were flushed into the sea where the oysters were held on the long lines. Within 1 to 2 days of the above events, oysters started to die. Some 1600 oysters died. About 100 oysters which did not open in the land-based tanks and which were not operated on, did not die. Pearl oysters on long lines about 100 metres away also did not die. Histopathology was done on 5 oysters. Non-specific inflammatory lesions were found in 4 of the oysters. Bacteriology was inconclusive as to the bacterium involved. It was concluded that a bacterial infection was the most likely cause of the histopathology and of the deaths. The losses were probably the result of several stresses.

Event B: In April 1996, 60 adult pearl oysters were collected from the panels of a long line on a farm on the Great Barrier Reef off the Qld coast. They were transported in coolite containers on a large commercial pleasure cruiser to the coast over a few hours. There they were held overnight in tanks belonging to a commercial aquarium fish and lobster dealer. The next day, the oysters were placed back into the coolite containers and transported by aircraft over a 1 hour trip. They were placed in clean seawater tanks prior to being placed into panels on a long line in the sea. Deaths occurred within 2 days of transport and a majority of the oysters were dead within a week of arrival. Histopathology was done on 6 of the surviving oysters. Non-specific inflammatory lesions were present in a range of organs. No bacteriology was done. It was concluded that the oysters had suffered a bacterial infection that was most probably contracted in the tanks of the commercial aquarium dealer

Event C: During September 1994, pearl oysters were collected off the eastern Qld coast by using an aluminium speed boat. Of 50 oysters collected, almost half died over the following month. The seas had been very rough during the trips done to collect these oysters and the oysters in their coolite containers had been subjected to the constant "thump, thump, thump" of the boat as it rode over the waves during the two hour trip from collection site to the coast. Previous collection trips had been made in much larger boats and in relatively calm weather and no losses had been experienced.

To test the effect of this stress, a group of 14 healthy adult oysters was divided into two groups. Seven oysters were placed in each of two coolite containers. One container was raised 60 cm above the bench and then dropped onto the bench. This was repeated every 20 seconds for 2 hours to simulate the speed boat stress. The other container of oysters was left on the bench untouched. The "dropped" oysters were all dead within 7 days of the experiment. This result was not unexpected since oyster tissue is made up of delicate cells which are "bags of fluid" which, if dropped without the support of the surrounding seawater, will tend to rupture. The result is tissue damage, loss of haemolymph and death.

Event D: In March 1995, 600 hatchery-reared spat (10mm) were placed in 0.25 inch net panels and cleaned every 2 to 4 days with a gentle spray. After 3 months, losses started and at the time of sampling in July 1995, 500 spat had died. Minimum water temperature for the period was 24.5°C. The water was reported to have been very muddy and the panels were usually covered with a layer of silt. Histopathology was done on nine spat. The spat had a generalised, non-specific gastroenteritis. In addition, some spat also had non-specific inflammatory lesions of the mantle and gills. Papovavirus-like inclusions were present on the palps. It was concluded that the muddy water had carried bacteria into the spat and that this had caused the inflammatory lesions in the spat. It is uncertain whether the viral infection of

the palps was a primary or a secondary effect in the mortality, or possibly just a coincidental infection.

Event E: A batch of 2000 adult pearl oysters were purchased by a farm in Torres Strait from a pearl oyster collector. The oysters had originated off Cooktown on the north-east coast of Qld and had been transported in tanks on a large carrier vessel over 3 days.

Over the following 12 months, heavy mortalities occurred. By late 1991, 1700 of the 2000 oysters had died. Histopathology was done on 14 of the surviving oysters. A *Perkinsus*-like protozoan associated with an inflammatory reaction was found in the connective tissue of 3 of the oysters. Areas of non-specific inflammation were also seen in various tissues/organs of these oysters.

It was concluded that a bacterial infection associated with inadequate hygiene and handling procedures was the primary cause of the losses. The *Perkinsus*-like infection would appear to be a secondary infection. The remaining oysters were destroyed because of the potential danger of the *Perkinsus*-like protozoan spreading on the farm.

CHAPTER 6: GENERAL DISCUSSION

INTRODUCTION

This study set out to identify and characterise the spectrum of infectious agents and conditions which may adversely impact upon or restrict the breeding, rearing and production of the pearl oyster *P. maxima* in Australian tropical waters. This was achieved primarily through the gross and histological examination of healthy and diseased oysters from farmed and wild sources across northern Australian waters. The study established the prevalence and distribution of a range of microbial, protozoan and metazoan agents found in association with pearl oysters. Temporal variations in prevalence of these agents in certain populations were examined by repeat studies on separate occasions. In addition to identifying infectious agents, the study established baseline morphological parameters for normal oysters and histopathological host responses following invasion by infectious agents or conditions.

The study established criteria for the diagnosis or identification of pathogenic or potentially pathogenic agents and diseases and their occurrence and distribution in nominally healthy or diseased animals. By establishing the geographical distribution of infectious agents within Australian waters and through a comprehensive review of literature on infectious agents of pearl oysters, the study sought to provide data for quarantine and health certification strategies which may be used to mitigate against introduction of deleterious agents or pests when translocating or introducing oyster stocks. Finally, the study sought to identify specific infectious agents, conditions or factors which have the potential to restrict oyster growth and pearl production which might be further investigated.

Overall, 4,502 pearl oysters were examined in the study, originating from defined coastal zones in the NT, Qld and WA waters. Oysters were harvested from wild sources and from commercial farming operations consequent to cultured pearl operations and procedures. To meet the needs of the study, oyster tissues were preserved as soon as possible after collection for transportation to the laboratory. Where possible, data on shell morphometry and shell damage associated with fouling and boring organisms was assessed.

A relatively abundant literature exists describing the taxonomically diverse species which inhabit the shell surfaces or shell matrix of *Pinctada* spp. or which are predators of pearl oysters. Many of these organisms are known to impose severe adverse impacts on oyster farming and pearl production. The study included a comprehensive review of literature describing recognised pathogens, parasites, fouling and boring organisms and predators of pearl oysters and checklists of such agents arranged according to taxonomic classification. This information, described in detail in Chapter 2, is presented as an adjunct to the recognition of many of the taxonomically diverse and complex agents which infect, invade or colonise *P. maxima.* The current study was not intended, however, as a detailed taxonomic survey of fouling and boring organisms, although where these caused obvious or severe damage, their presence was noted and identifications made.

The current study focussed primarily on those invasive microbial, protozoan or metazoan agents which were considered to be pathogens or potential pathogens of *P. maxima* which may compromise oyster growth or pearl production. In contrast to other commercially important molluscan species, there are few published reports describing the pathogens, parasites or diseases of pearl oysters *Pinctada* spp. or the histopathological responses of pearl oysters to infection or disease. In addition, the normal histological structure of *Pinctada* spp. is neither well described or readily available. Collectively, information on the nature of

agents infecting or colonising pearl oysters and information on the normal histological appearance and the host response to infection is fundamental to the diagnosis of disease or understanding disease processes. The study undertook to provide this information to assist pathologists, microbiologists and biologists working on pearl oyster health and production.

As a basis for the study, detailed pathological and histopathological examination of pearl oysters from pearl farming operations and from wild-harvested pearls across tropical Australia were undertaken. This approach was taken in the first instance to identify pathogens, potential pathogens, parasites or commensals present in Australian *P. maxima*, to establish the normal histological appearance of pearl oysters, to evaluate histopathological changes associated with the presence of infectious or potentially infectious microbial, protozoan and metazoan agents and to evaluate and describe non-specific histopathological changes in oyster tissues. While such an approach is limiting with regard to fully characterising the range of agents detected, the study clearly defined a range of potentially harmful agents at different localities which may be subject to further studies or research to more fully characterise their nature and pathogenic significance.

The study recognised special considerations in the histological interpretation of oyster tissues. The point of death in oysters is problematic. Because of the relatively simple organisation of the tissues which are bathed in an oxygenated marine saline, tissues can remain viable for relatively long periods after the organism as a whole has suffered a lethal insult. Examples are the advanced autolysis which can occur when live oysters are plunged in formalin. The shell remains clamped shut until autolysis has begun. Mantle tissues can remain apparently viable, while digestive gland tissue has become autolytic.

In the majority of cases in the study, mature oysters which were exhibiting no evidence of disease were examined. As such, infectious agents detected in these animals were considered latent infections. Of importance in these cases is the pathogenic potential of such agents to cause disease under different physiological or environmental conditions, in different population densities or in different age classes. Data derived from examinations of "diseased" oysters made during the course of the study supplemented information on the role and significance of such latent infections and provided some insight into the pathogenic potential of a number of the agents observed.

Ancillary information collected during the study suggested that periodic high mortality events have occurred, are common and continue to be experienced in farmed pearl oysters in northern Australian waters (Chapter 4 and 5). Several major mortality events were investigated during the course of the study. While certain contributory factors were identified, the full spectrum of contributory causes remains unknown. Such mortalities are in part associated with poor transport conditions, changes in water temperature, salinity and infection by pathogenic marine bacteria (Pass *et al.* 1987).

Detailed data on prevailing or pre-existing environmental conditions were not collected in the study and associations between oyster health, production or disease were not made. The role of adverse environmental conditions in compromising pearl oyster health and production is, however, well documented (Chapter 2) and the adoption and implementation of a rigorous program of environmental monitoring, laboratory surveillance of dead or moribund oysters and a system of record keeping would appear of value in defining the extent and cause of production losses and mortalities and offer strategies which may prevent or minimise future losses.

The study is the first comprehensive review and investigation of pathogens, parasites and diseases of pearl oysters undertaken. The findings of the study are discussed in relation to the clinical and pathological significance of agents identified, in relation to industry protection through disease diagnosis, quarantine and health certification and in relation to further avenues for research to improve growth, production and pearl quality.

POTENTIAL PATHOGENS

The study established that a range of taxonomically diverse microbial, protozoan and metazoan agents invade the tissues, colonise the internal or external epithelial surfaces or colonise or invade the shell surfaces and shell matrix of Australian pearl oysters *P. maxima.* The study re-confirmed the occurrence of several agents described in earlier studies and provided prevalence data for these agents. The finding of a spectrum of infectious agents in *P. maxima* is consistent with similar studies in other molluscs, especially those of commercial importance. As such it is apparent that principles and strategies used for disease diagnosis, disease control and disease prevention are applicable to pearl oysters.

The full spectrum of agents visualised in the study are discussed in Chapter 3. Many of the agents appeared to be innocuous, with no evidence of disease associated with their presence and an absence of an ability to incite a host inflammatory response. As noted, however, primarily normal healthy oysters were examined and the presence of such agents under different circumstances may result in disease.

A number of agents which, based on observations of diseased oysters or on host or tissue responses in clinically normal oysters, are considered pathogens or potential pathogens are discussed below, with comments on their likely pathogenic significance and further studies which may be undertaken to elucidate their true pathogenic potential.

Viruses and virus-like agents and inclusions

Queensland papova-like virus. Norton *et al.* (1993a) described Papova viral-like lesions on the palps of *P. maxima* from Torres Strait, Qld. Similar lesions were found to be common in both wild and farmed oysters in the study at a prevalence rate of between 7 to 31 % in Qld only. Histopathological examination of infected oysters showed marked hypertrophy of the epithelium of the palp tissue, with loss of cilia. The full pathogenic significance of this agent yet to be determined, but it appears likely that in heavily infected oysters, dysfunction of the palps may occur, with potential adverse effects on feeding and growth. Young animals may be especially susceptible. The pathogenic significance of this virus should be investigated as a potential serious pathogen of pearl oysters. As the agent does not appear to be present in NT or WA oysters, efforts should be implemented to restrict its movement and to prevent spread of the agent to uninfected areas.

Western Australian palp inclusions. Inclusion bodies in the palp epithelium were seen in a small number of oysters from WA. The morphology of these inclusions appeared different to that observed in Qld oysters, with an absence of hypertrophy and hyperplasia. Electron microscopic studies are warranted to determine the nature of this agent and to compare it with the Qld papova-virus like agent.

Intranuclear amphiphilic viral-like inclusions. Intranuclear inclusion bodies similar to those described by Pass *et al.* (1988) were recorded from the majority of populations

examined from all locations. The pathogenic significance of these bodies is uncertain. In some cases large numbers of inclusions appeared to be associated with degeneration of the digestive gland epithelium and an associated inflammatory response. The pathogenic significance of this putative virus should be clarified. While there was little evidence of a major pathogenic role in this study or the study by Pass *et al.* (1988), the agent may prove of increased pathogenic significance for adult or juvenile oysters or oysters undergoing physiological stress.

Eosinophilic intracytoplasmic inclusions. A high proportion of oysters in WA waters showed intracytoplasmic inclusions in the epithelial cells of the digestive gland diverticulae. These were only recorded in WA waters. The nature of these inclusions requires clarification by electron microscopy, as they may be viral in origin and of pathogenic potential.

Bacteria and bacterial diseases

Colonisation of healthy molluscs by potentially pathogenic marine bacteria is well recognised (Lauckner 1983; Humphrey 1988), as is invasion of stressed molluscs by pathogenic marine bacteria (Sparks 1985). As such, culture for bacteria was not undertaken as part of the survey of nominally healthy oysters and limited cultural examinations were undertaken on diseased oysters. Observations on bacterial agents was thus restricted to histological observations and to limited cultural examinations on diseased oysters.

Inflammatory lesions, abscesses and degenerative lesions in which bacterial organisms could be seen were identified in the study. Focal granulomas or abscesses were seen in mature healthy oysters, while massive bacterial colonisation of tissues and tissue necrosis was observed in larvae or spat undergoing mortalities. While the nature of these bacteria could not be positively identified in most cases, the lesions were typical of those described for bacterial invasion of *P. maxima* and other shellfish (Lauckner 1983; Sparks 1985). In mature oysters, focal accumulations of haemocytes were present in interstitial tissues and myocardium in which rod-shaped bacteria could be seen. In larvae, overwhelming invasion of tissues by bacteria and associated tissue necrosis were evident. These changes are typical of marine *Vibrio* infection in molluscs generally (Sparks 1985) and were similar to lesions described in *P. maxima* following *Vibrio* spp. infection by Pass *et al.* (1987). The occurrence of *Vibrio* sp. was confirmed in oysters undergoing high mortalities, but the role of this agent as a primary pathogen in such cases remains uncertain.

Rickettsiales-like agents. Cysts containing fine basophilic stippling and morphologically typical of Rickettsiales-like agents or related organisms were relatively common in oysters from all locations. Cysts were observed commonly associated with the digestive gland epithelium, extending into the lumen. On occasions they were also observed associated with the gill epithelium. Cysts were generally solitary or several in number and no obvious pathogenic associations could be made with respect to the health of the oyster or associated inflammatory response. Rickettsiales-like and chlamydial organisms are reported in a range of mollusc species, generally in the digestive tubular epithelium and unassociated with significant pathology or disease (Sparks 1985). Mortalities associated with Rickettsiales-like organisms have been described in molluscs including the giant clam *Hippopus hippopus* (Norton *et al.* 1993) and the scallop *Placopecten magellanicus* (Sparks 1985). As with other agents, the pathogenic significance of Rickettsiales-like organisms should be determined for Australian *P. maxima* under differing conditions and different age groups.

Protozoa and protozoan diseases

Pathogenic protozoa are among the most serious of all molluscan pathogens, with major losses incurred in edible oyster populations, often following the introduction of imported or translocated oysters (VanBanning 1979; Andrews 1980; Sparks 1985; Stewart 1991). The occurrence of protozoa in *Pinctada* spp. should be viewed with particular concern, especially if accompanied by mortalities. The study identified several protozoa or protozoal-like agents of high pathogenic potential. In WA, a *Haplosporidian* sp. was associated with a high morbidity in spat following translocation. While deaths were not apparent, the intensity of infection suggested that clinical disease may result from the infection. In other cases in NT and WA, *Haplosporidian*-like bodies were associated with focally extensive inflammatory lesions in apparently healthy oysters and in oysters from populations undergoing mortalities as discussed below. The ultrastructural characterisation of these bodies by electron microscopy is essential to determine their true nature and pathogenic potential. A range of protozoan agents which appeared to be non-pathogenic were identified in the study and are described in Chapter 3. There was no evidence of the pathogen *Perkinsus* sp. earlier associated with diseased or healthy *P. maxima* from Qld waters (Goggin and Lester 1989; Norton *et al.* 1993b) in any of the healthy or diseased animals examined.

Haplosporidian-like bodies. Ovoid, enigmatic, protozoan-like organisms were identified in healthy mature oysters in NT and WA and in mature oysters derived from populations undergoing mortalities on one occasion from NT. In the latter case, the agent was associated with severe necrosis and granuloma formation in the digestive gland. The agent may represent a major potential pathogen and should be the subject of further investigation to define its exact nature and pathogenic significance. As noted, ultrastructural characterisation of these bodies is needed to determine their true nature and pathogenic significance.

***Haplosporidium* sp. in Western Australian spat.** The presence of a Haplosporidan parasite in spat in WA following importation from NT and release into sea cages is reported. Infection is believed to have been acquired in WA. This agent is considered to represent a serious pathogen of pearl oysters in Australia. The agent has been characterised by Hine (1996) and Hine and Thorne (1998). Further studies are required to establish the pathogenic potential of this agent and to determine its full distribution.

Metazoa and metazoan agents

Metazoa were observed in the tissues, in the lumen of internal organs and on the epithelial surfaces of the oysters and numerous metazoa were present on the shell surfaces or invading the shell matrix.

Within the tissues of the oysters, metazoan agents were present in the lumen of the alimentary tract. In the majority of cases, these agents were unassociated with significant histopathological changes. On occasions, metazoa in the digestive tubules appeared to be occluding the lumen, undergoing degeneration and eliciting an associated inflammatory response. These were generally seen as isolated incidental lesions. Of potential pathogenic significance is the high prevalence of a copepod *Anthessius pinctadae* in the oesophagus and mouth parts of mature oysters from NT (Chapter 3). This agent appeared to be actively feeding on the oesophageal epithelium and occluding the lumen. This agent warrants further investigation as a significant potential pathogen.

As discussed in Chapter 3, the metazoa associated with discrete focal or multifocal granulomata in interstitial tissues are morphologically consistent with the larval cestode, *Tylocephalum* sp. Lesions were particularly prevalent in tissues peripheral to the oesophagus, palps and stomach. Members of this tapeworm group have long been recognised as parasites of pearl oysters *Margartifera vulgaris* (Sparks 1985). The significance of infections in the current study are uncertain, but high numbers of larvae may cause dysfunction of affected tissues. The taxonomy and the life cycle of these agents, and their pathogenic potential, especially for young or stressed animals, should be considered for further research.

Crustacean commensals

The high prevalence of either symbiotic or commensalistic pea crabs or small shrimps in the pearl shells appears of no concern. The was no evidence of deleterious effects of such animals, even where crateriform changes in the mantle were conspicuous grossly. Dix (1973) found approximately 67% of 126 *P. maxima* to have a single pea crab, and/or a concave mantle thickening and described a causative relationship between the presence of pea crabs and the occurrence of these changes. Histologically, displacement of muscle fibres, and thickening due to connective tissue matrix containing individual muscles fibres were present.

Fouling and boring organisms.

The external shell surfaces or shell matrix of the pearl oyster are readily colonised by numerous, taxonomically diverse marine organisms. Fouling of shells and cages, and shell damage caused by these organisms are significant problems of cultured oysters worldwide. They are noted to cause retarded oyster growth, poor pearl quality, oyster mortalities, poor or downgraded shell quality, death, reduced water flows, decreased feeding, weakened shell structure, competition for food, and spat mortalities (Chapter 2). In addition, the cleaning of fouled shells and the implementation of husbandry procedures to mitigate against fouling require ongoing intensive labour on pearl farms with major costs to the cultured pearl industry.

The study did not investigate or describe the full spectrum of fouling and boring organisms which occur on, or invade the shell matrix of *P. maxima*. Shell damage associated with such organisms was conspicuous in many of the oysters collected in the study, especially wild harvested animals. Boring molluscs, sponges and marine worms appear to be the major causes of such damage.

In all regions, holes in the shell matrix associated with boring sponges and molluscs were present in wild shell, especially in the larger and older specimens. Stained nacre caused by mudworms was a problem in both wild and farmed shell. In NT, many wild oysters harvested for subsequent seed pearl inoculation or half pearl production are rejected because of shell damage associated with a range of shell organisms. Older oysters from otherwise healthy farmed populations in NT locations showed many animals with severe shell damage due to epiphytic organisms. Such damage elsewhere in cultured edible oysters is a well recognised cause of decreased productivity and mortalities. In the current survey, many of the shells would appear to be unsuited for half pearl production at least, and the extent of damage would seriously downgrade the shell if intended for other purposes.

In shells examined from the NT, the study found that smaller oysters had less shell damage than larger oysters, supporting the findings of Dharmaraj and Chellam (1983) who reported blisters associated with boring organisms to be generally fewer in younger oysters.

ENVIRONMENTAL FACTORS

As with other molluscan species, optimal environmental conditions are necessary for pearl oysters for optimal growth, production and resistance to disease. Monitoring of environmental conditions at the time of sampling of oysters for the study, or environmental monitoring associated with disease events investigated during the course of the study was not undertaken. Sub-optimal and adverse environmental factors are, however, well established as causes of mortalities, production losses, poor shell growth and poor pearl quality in pearl oysters and should be considered in any investigation of disease events, sub-optimal production or poor pearl quality. Adverse environmental factors in addition, may predispose oysters to disease by compromising specific and non-specific immune factors.

Environmental factors which impact upon pearl oyster health and production are reviewed in Chapter 2, and include salinity, water temperature, available nutrients and phytoplanktonic blooms.

Reduced salinity and stratification associated with heavy rainfall and/or run-off from rivers and estuaries are associated with mortalities, production losses and poor pearl quality (Cohn 1949, Ota and Fukushima 1961). Reduced salinity may also induce decreased food consumption, further exacerbating poor production (Ota and Fukushima 1961). Water temperature also plays a major role in maintaining normal physiological function. Sub-optimal water temperatures may cause serious losses (Cohn 1949). Decreased environmental temperatures are thought to reduce host inflammatory and immune functions, thereby compromising the ability of the host to resist infection while at the same time presenting favourable environmental temperatures for growth and invasion of pathogenic marine bacteria, especially *Vibrio* spp. (Mannion 1983). Such a mechanism was considered by Dybdahl and Pass (1985) and Pass *et al.* (1987) to be a major factor in deaths of transported mature *P. maxima.* Increased water temperature may be an important adverse environmental factor. Increasing water temperature increases the metabolic rate of pearl oysters and consequently increases the requirement for oxygen, especially above 27°C (Numaguchi 1995b). Factors which limit oxygen availability to the oyster, including fouling, decreased water exchange and decreased dissolved oxygen, may all adversely compromise the metabolism of the animal, leading to reduced production and possibly increased susceptibility to infection or disease.

Adequate available food and food intake is a major environmental factor influencing growth and production. Bivalve molluscs acquire energy for growth and metabolism by feeding on suspended particulate matter. The filtration rate of pearl oysters is an indication of feeding activity, and is temperature dependent. Filtration rate in *Pinctada fucata martensii* increases up to 28°C, above which temperature a dramatic reduction in filtration rate is reported. (Numaguchi 1995b). The metabolic rate of *P. fucata martensii* also increases with increasing water temperature. As a consequence, food intake via filtration may be insufficient to maintain metabolic requirements at temperatures above 28°C, and oysters may loose condition. (Numaguchi 1995).

Food depravation is known to have severe adverse consequences for pearl oysters. Numaguchi (1995a) reported increased mortalities in mature *P. fucata martensii* deprived of food, with associated decreases in adductor muscle weight, body condition and muscle glycogen content.

Blooms of toxic dinoflagellate phytoplankton cause losses of pearl oysters, either directly due to decreased oxygen or suffocation associated with clogging of gills or direct toxic effects (Cohn 1949; Sparks 1985; Matsuyama *et al.* 1995), or indirectly as a result of shell closure (Ota and Fukushima 1961), or associated with reduction of light penetration and subsequent effects on metabolism (Cohn 1949). Such blooms may follow heavy rainfall events and vertical mixing of the seawater and may have a seasonal occurrence, especially during summer and autumn (Ota and Fukushima 1961).

It is clear that adverse environmental factors play a major role in determining the health and viability of pearl oysters. Considerable benefits would accrue in interpreting and managing production data from ongoing environmental monitoring of salinity, temperature, irradiance and chlorophyll as a measure of available feed.

MANAGEMENT AND HUSBANDRY

Handling and trauma

Despite their outward appearance of a robust animal, pearl oysters, on close histopathological examination have an extremely fragile structure and anatomy. A range of delicate, soft tissues and organs are contained within the tough and heavy shells. Many of the tissues are separated from the environment by a thin, delicate epithelium and breaching of such epithelia may readily result in loss of haemolymph and infection by bacteria or other pathogens. Rough handling, overheating and oxygen deprivation may readily result in denaturation or damage to tissues.

The oedema commonly seen in the mantle tissues of normal individuals in the study likely resulted from tissue damage associated with prolonged transport, overheating and oxygen deprivation prior to fixation. Apart from physiological stress, it would appear that rough handling, especially chipping, dropping or rough scraping, such as occurs when cleaning oysters, could damage epithelium and render the animals susceptible to pathogenic bacteria present in the marine environment. It is noteworthy that significant reductions in mortalities of pearl oysters have been achieved in Qld by improved handling and transport techniques (Norton - unpublished). Gentle handling and optimal transport conditions should be a fundamental basis of sound management practices on farms.

Grow-out spat losses

Major losses have been reported during farm grow-out of hatchery reared spat in both NT and Qld. Further investigation in to the causative factors behind these losses is required.

Sequential colonisation of larval oysters by a range of protozoa and metazoa was demonstrated in the study. The importance of colonisation by such agents with respect to potential pathogens and spat losses is an area for further investigation, especially in waters with those potential pathogens described above.

HISTOLOGY AND HISTOPATHOLOGY

As a basis for the interpretation of histopathological changes, a sound knowledge of the normal anatomy and histology of the pearl oyster is essential. A number of studies have been published describing the general anatomy of pearl oysters *Pinctada* sp., including those of Velayudhan and Gandhi (1987) in the English language, Fougerouse-Tsing and Herbaut (Undated) in the French language, and Takemura and Kafuku (1957) and Shiino (1952) in the Japanese language. Other studies have focussed on the anatomy or histology of certain organs, including the studies of Dix (1973), Suzuki (1985) and Garcia-Gasca *et al.* (1994). While not specifically seeking to describe anatomical or histological features of *P. maxima*, the present study identified several histological features of importance to the diagnostician.

On a comparative morphological basis, the tissues of the mature pearl oyster *P. maxima* resemble the tissues of other bivalve molluscs. Moderate numbers of haemocytes located diffusely or in focally extensive areas in the interstitium, especially adjacent to the alimentary tract, appears to be a normal histological feature. Similarly, low numbers of macrophages containing brown pigment appear a normal component of the interstitium.

Inflammatory (haemocytic) responses

The study demonstrated that *P. maxima*, like other bivalve molluscs, was readily capable of mounting haemocytic inflammatory responses which likely play a major role in internal defence mechanisms as well as other biological and physiological functions (Auffret 1988). This response typically consisted of intense, focal or focally extensive accumulations or infiltrations of haemocytic cells. In many cases, no causal agent or lesion was evident. At least two morphologically distinct haemocytes were evident in such responses, a larger cell resembling a macrophage and a smaller cell with distinct nucleus and scant cytoplasm. Further studies to clarify the nature, role and morphogenesis of these cells and their role in physiology and defence in *P. maxima* appears warranted.

Non-specific histopathological changes

Inflammatory and degenerative changes, apparently unrelated to the presence of microbial, protozoan or metazoan agents were common in oysters from all locations and included oedema, haemocytic accumulations, abscessation and granulomata, pigmentation, multinucleate cells in interstitial tissues, mineralisation, digestive gland epithelial degeneration and associated haemocytic infiltration and dilation of digestive glands. Such findings are basic to an understanding of the histopathological responses of the pearl oyster. On two occasions neoplastic proliferative changes resembling fibroma or neurofibroma were seen.

The histological and histopathological data obtained in the study is seen as providing a comprehensive reference basis on the normal histology of the pearl oyster *P. maxima* and is essential knowledge for any prospective histopathological examinations on diseased oysters. It is essential that such information is made readily available for producers, research workers an diagnosticians as fundamental information on pearl oyster morphology and pathology. The information gained by individual investigators in the current study is to be made available in the form of an atlas of histology in order that the findings of the study be made as widely available as possible. The production of the atlas is to be funded by the Fisheries Research and Development Corporation (Project No 96/226)

DISEASE AND DISEASE DIAGNOSIS

Disease in aquaculture is ultimately expressed as the net result of complex interactions between the host species, infectious agents and environmental factors and recognises such factors as water quality, stocking density, feed availability and physiological stress (Plumb 1992). In order to adequately diagnose disease, all contributing factors should be determined, including a detailed laboratory examination to determine the presence and role of infectious agents, together with a sound knowledge of the physiological requirements and gross and histological morphology of the cultured species and the recognition of deviations from these requirements.

The principles of disease diagnosis which apply to domestic animals and birds also apply to aquatic animals. The study provides the diagnostician with a range of specific and non-specific changes in oysters which may be referred to in the investigation of outbreaks of disease. In addition a sound knowledge of comparative pathology assists in the detection of disease in a novel species. These principles were applied to investigations of mortalities in a number of cases during the period of the study.

Bivalve molluscs are sessile animals which may show few apparent visual changes indicative of disease and are relatively difficult to assess in a clinical sense. Fouling organisms may mask visible signs of disease. The study highlighted such problems for producers in monitoring the health of oyster stocks and in determining the magnitude and chronology of disease events. Following disease or death, the soft tissues of molluscs in tropical waters are readily and rapidly degraded by environmental micro-organisms and predators. Typically, oysters are examined periodically at cleaning and in many cases, by the time a disease or mortality event is detected, affected oysters have degenerated to a point whereby the level of putrefaction or predation precludes meaningful examination. Commonly, empty shells with remnants of adherent tissue are the remaining evidence of a mortality or disease event.

Oysters may be located in remote locations and cannot readily be transported to a laboratory for examination. Attendant delays and potential damage and trauma in transit further compromise the oyster and mitigate against a rapid and conclusive disease diagnosis.

Critical inspection and evaluation of oysters during cleaning or other operations, accurate monitoring of losses, detection and sampling of oysters in the early stages of disease and rapid transport of samples to an appropriate diagnostic laboratory would greatly facilitate the detection and diagnosis of disease events.

REGIONAL DIFFERENCES IN DISTRIBUTION AND QUARANTINE

A number of agents were identified in the study which appear to have a restricted distribution. The regional occurrence of such agents has major implications for the introduction of diseases consequent to the introduction or translocation of oysters, especially where potential pathogens occur in one region but not in another. Such situations demands the implementation of quarantine and health certification procedures to prevent the introduction of deleterious agents into new areas.

Infectious agents with apparently restricted distribution identified in the study include the following.

Palp: Papovavirus-like inclusions. This potentially pathogen appears restricted to Qld waters.

Palp: Rickettsiales-like bodies. These agents were observed in the epithelium of the palp of Qld oysters only. While they may be similar to Rickettsia identified in NT and WA, the unusual distribution on the oyster in Qld is noteworthy, and may reflect a separate strain of organisms with a predilection for palp epithelium.

Palp: Turbellarian-like ciliates. These agents appeared in the palp epithelium of Qld oysters only.

Oesophagus: Lumenal metazoa. Single or multiple copepods, *Anthessius pinctadae* were found in the oesophagus of oysters from two populations in NT only. These agents appeared histologically to graze on the epithelium, inducing erosion, and in large numbers appeared to occlude the lumen. These agents appear to be potential pathogens.

Digestive gland: Eosinophilic intracytoplasmic inclusions. These bodies, possibly representing viral inclusion bodies, were reported in WA oysters only. The nature and pathogenic significance of these agents should be determined.

Digestive gland: Gregarine-like protozoa. A high prevalence of these agents was found in the digestive tubules and elsewhere in Qld oysters, with occasional similar agents described in WA oysters.

Digestive gland: Microgranuloma, haplosporidian-like. An enigmatic ovoid body resembling protozoan haplosporidia was associated with a microgranulomatous reaction in the digestive gland and elsewhere of NT and WA oysters. These agents may represent significant pathogens. In at least one case, lesions were seen in mature oysters undergoing a disease event.

Ancistrocomid-like ciliates. A high prevalence was found in the midgut of Qld oysters, and in oysters from WA. One oyster with similar agents was observed in the NT. This oyster is believed to have originated from Qld (unpublished data).

Haplosporidiosis. Haplosporidiosis of larval oysters was reported in WA. This represents a serious potential for disease and restriction of spread of the agent is warranted.

The regional distribution of these agents warrants caution when planning translocations or introductions of pearl oysters. In general, there appears to be a need to protect Qld oysters from agents reported in WA and NT, but not in Qld, and a need to protect oyster populations in NT and WA from agents present in Qld oysters.

CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

The study established for the first time in Australia the occurrence, prevalence and distribution of a range of microbial, protozoan and metazoan agents which may invade pearl oysters *P. maxima* and which may result in disease. A histological basis on which disease diagnosis may be based is established. The study provides occurrence data which may be used to plan and implement health certification and quarantine procedures to mitigate against the spread or introduction of disease.

The study shows that pearl oysters, like other molluscs, are subject to a range of potential pathogens, commensals and epiphytic colonising organisms. The taxonomic complexity of these agents is recognised. The pathogenic potential of most of the agents identified in the study remains uncertain.

Recommendation 1. Specific studies are warranted to establish the taxonomy and pathogenic significance of infectious agents identified in the study, especially in larval and juvenile oysters.

Difficulties in the prompt recognition and diagnosis of disease may be encountered due to poor sample availability and quality of tissues when disease is recognised. Failure to submit appropriate samples for pathological and microbiological examination severely compromises early and/or accurate disease diagnosis.

Recommendation 2. On-farm disease investigation kits should be available on farms and on boats to facilitate appropriate sampling of oysters determined to be diseased. As a minimum, kits should contain 10% seawater formalin, sterile tubes and dissection equipment for collecting tissue samples in an appropriate manner.

The study recognised the importance of adverse environmental parameters in the initiation of disease events.

Recommendation 3. Ongoing environmental monitoring at farm sites should be implemented to provide background information for production and for correlation with disease events. As a minimum monitoring should include water temperature, salinity, irradiance, feed availability and oxygen. Stratification should also be monitored.

The study noted disease events associated with adverse environmental conditions and noted the presence of potential pathogens associated with adult and juvenile oysters. Stress and a sub-optimal environmental parameters are clearly important factors in pearl oyster aquaculture yet their role in predisposing oysters to disease is poorly defined.

Recommendation 4. Studies to evaluate the pathophysiological effects of sub-optimal salinity, temperature and oxygen on Australian pearl oysters, and the role of sub-optimal environmental parameters in predisposing pearl oysters to disease should be undertaken. Further, physiological markers which indicate the nature and severity of stress in Australian pearl oysters should be investigated.

The study identified a diverse range of infectious agents and non-infectious conditions which may be associated with disease. Occurrence of disease in molluscs is often complex and multifactorial, with the full spectrum of interacting factors poorly understood.

Recommendation 5. Workshops for producers addressing the nature of disease, sampling and collection techniques and dispatch of samples to laboratories to facilitate disease diagnosis should be organised through industry/government organisations.

The normal histology of the pearl oyster is poorly described, as is the response to infectious or non-infectious agents. An atlas of normal histology and histopathology of the pearl oyster would be of considerable benefit to assist in interpretation of histological changes.

Recommendation 6. An atlas of normal histology and histopathology of the pearl oyster should be developed to assist in interpretation of histopathological changes, as an essential reference for disease diagnosticians and for research personnel investigating the biology and production of pearl oyster and pearl production.

The study demonstrated that, despite the heavy external shell, the tissues of the pearl oyster are particularly fragile and that inappropriate handling or environmental conditions may seriously damage these fine tissues. Inappropriate handling includes rough treatment whilst out of water and over-heating.

Recommendation 7. Producers should examine handling techniques with a view to minimising stress and trauma.

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