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A survey of some parasites and diseases of several species of bivalve mollusc in northern Western Australia

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ABSTRACT: Pteriid oysters (*Pinctada maxima, Pinctada margaritifera, Pinctada albina, Pteria penguin*), rock oysters (*Saccostrea glomerata, Saccostrea cucullata, Saccostrea echinata*) and representatives of other taxa (Malleidae, Isognomonidae, Pinnidae, Mytilidae, Spondylidae, Arcidae) from the wild, and 4670 hatchery-reared *P. maxima*, from northern and Western Australia, were examined for parasites and diseases. Rickettsiales-like inclusions and metacestodes of *Tylocephalum* occurred in most species. Intranuclear virus-like inclusions occurred in 1/415 wild *P. maxima*, 1/1254 *S. cucullata*, 3/58 *Isognomon isognomum*, 1/80 *Pinna bicolor* and 1/45 *Pinna deltodes*. *Perkinsus* was histologically observed in 1/4670 *P. maxima* spat, 2/469 *P. albina*, 1/933 *S. glomerata*, 16/20 *Malleus meridianus*, 12/58 *I. isognomum*, 1/45 *P. deltodes*, 5/12 *Spondylus* sp., 1/16 *Septifer bilocularis* and 3/6 *Barbatia helblingii*. One of 1254 *S. cucullata* was heavily systemically infected with *Perkinsus* merozoites, meronts and schizonts, and was patently diseased. Other potentially serious pathogens included *Haplosporidium* sp. in 6/4670 *P. maxima* spat, *Marteilia sydneyi* from 1/933 *S. glomerata*, and *Marteilia* sp. (probably *M. lengehi*) (1/1254) and *Haplosporidium* sp. (125/1254) from *S. cucullata*. The latter were associated with epizootics on offshore islands, with heaviest prevalence (45%) in oysters with empty gonad follicles. *Marteilioides* sp. infected the oocytes of 9/10 female *S. echinata* from Darwin Harbour. Details of geographical distribution and pathology are given, and the health of the bivalves examined is discussed.

KEY WORDS: Bivalves · Australia · *Marteilia* · *Marteilioides* · *Haplosporidium* · *Perkinsus* · Distribution

INTRODUCTION

Studies on the parasites and diseases of bivalves on the east coast of Australia have shown the presence of serious and potential pathogens. Two pathogens of Sydney rock oysters *Saccostrea glomerata*, *Marteilia sydneyi* causing QX disease (Perkins & Wolf 1976, Wolf 1977a, 1979, Roubal et al. 1989), and *Mikrocytos roughleyi* causing winter mortality (Roughley 1926, Farley et al. 1988) have affected production in aquaculture (Roughley 1926, Potter 1983), and both are OIE-listed notifiable diseases. *Marteiloides branchialis* infections (Anderson & Lester 1992), and a *Steinhausia*-like infection of oocytes (Anderson et al. 1995) have also been reported from *S. glomerata*. Following the identification of *Perkinsus olseni* as the cause of mass mortalities among abalone *Haliotis* spp. in South Australia (Lester & Davis 1981, O'Donoghue et al. 1991), *Perkinsus* was found in many bivalves on the Great Barrier Reef (Goggin & Lester 1987, Lester & Sewell 1989), and to be transmissable between diverse groups of bivalves (Goggin et al. 1989). More recent studies have shown pearl oysters *Pinctada maxima* in eastern Australia to be infected by *Perkinsus* (Norton et al. 1993c) and a papova-like virus (Norton et al. 1993d), and giant clams *Tridacna crocea* and *Hippopus hippopus* to be infected with Rickettsiales-like organisms (Goggin & Lester 1990, Norton et al. 1993b) and a *Marteilia*-like organism (Norton et al. 1993a). Bodies previously considered to be parasites in Australian

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pearl oysters (Wolf & Sprague 1978) were subsequently identified as the residual bodies of lysed digestive epithelial cells (Pass & Perkins 1985).

In Western Australia, pearl oysters *Pinctada maxima* have been moved from collection sites to farms between Exmouth Gulf and King Sound for several decades (see below for locations). Studies on bivalve diseases in Western Australia have been limited to a study on vibriosis in pearl oysters (Dybdahl & Pass 1985, Pass et al. 1987), herpesvirus infections in *Ostrea angasi* (Hine & Thorne 1997), and *Haplosporidium* sp. in *P. maxima* (Hine & Thorne 1998). During 1992 to 1994, when this study was conducted, the companies co-operating in this research (see 'Acknowledgements') obtained their *P. maxima* stocks either from quota-limited wild adult oysters taken by diving, or from spat reared in hatcheries at Oyster Creek, Carnarvon, and Darwin Harbour, Northern Territory, and moved to quarantine sites at Sam's Creek at the eastern base of the Dampier Archipelago, in Pender Bay and King Sound, and then on to farms in King Sound. For this study, 415 adult *P. maxima* were collected during 1993 to 1994 from sites in the vicinity of hatcheries (Oyster Creek, Darwin Harbour), quarantine (Sam's Creek, King Sound) and farm sites (King Sound), or opportunistically as part of other research programmes (80 Mile Beach), or pearl company activities (Bynoe Harbour). Some were 'vomit' shell that had rejected nuclei after seeding, and therefore came from farm sites. Their infections are compared with those of 4670 spat examined as part of a translocation protocol during 1992 to 1994, prior to movements from hatcheries to quarantine sites, and from quarantine sites on to farms. This study was also carried out to identify the infections present in similar bivalves (Pteriidae, Malleidae, Isognomonidae) in quarantine and grow-out areas, to determine whether they may act as reservoirs of infection. Finally, the study was carried out to identify the infections of edible oyster species that may form the basis of new aquaculture ventures, and to compare the parasites and diseases of Sydney rock oysters *Saccostrea cucullata*, which are experimentally cultured in the hatchery at Oyster Creek (n = 758), with wild oysters taken from rocks in other areas.

MATERIALS AND METHODS

All bivalves sampled were opportunistically collected during other research programmes, or by co operating pearl oyster companies during their operations. Live bivalves were opened and tissues fixed in 10% formol saline or Davidson's fixative, in the field or in the laboratory. Sections (5 to 7 µm thick) were cut and stained with haematoxylin and eosin. Ovarian tissue of 4 *Saccostrea echinata* from Darwin Harbour that were histologically found to have parasites in their eggs was prepared for transmission electron microscopy (TEM) by fixation in 2.5% glutaraldehyde made up in 0.22 µm filtered seawater, post-fixation in 1% OsO_4 buffered with 0.1 M phosphate buffer, pH 7.2 for 1 to 1.5 h, stained with 2% uranyl acetate for 10 min and 5% lead citrate for 5 to 6 min, and examined on a Philips CM10 transmission electron microscope. Paraffin embedded tissue was prepared for TEM by dewaxing in xylene overnight, hydrating through 2 changes of 100, 90 and 70% ethanol, and post-fixing and staining 1 mm^3 blocks as above. The bivalves sampled, and their infections, are given by area in Tables 1 to 3, and the locations of those areas are given

Table 1 Prevalence of parasites in pearl oysters. IVI = intranuclear virus-like inclusions. RLOs = Rickettsiales-like organisms. The numbers in bold under each area are the total number of specimens examined from that area

Species/infection	Oyster Creek	Exmouth Islands	Dampier Archipelago	80 Mile Beach	King Sound	Darwin-Bynoe	All area %
Pinctada maxima	**n = 21**	**1**	**0**	**148**	**228**	**17**	**415**
IVI	0	1	–	0	0	0	0.2
Pinctada albina	**n = 20**	**2**	**55**	**197**	**195**	**0**	**469**
RLOs	0	0	0	0	2	–	0.4
Ancistrocomid ciliates	0	0	0	7	0	–	1.5
Perkinsus sp.	0	0	2	0	0	–	0.4
Tylocephalum sp.	0	1	11	32	13	–	12.2
Sporocysts	0	0	1	0	0	–	0.2
Pinctada margaritifera	**n = 11**	**0**	**18**	**0**	**0**	**0**	**29**
All negative	0	–	0	–	–	–	
Pteria penguin	**n = 0**	**0**	**69**	**19**	**0**	**0**	**88**
RLOs	–	–	1	0	–	–	1.1
Tylocephalum sp.	–	–	2	11	–	–	14.8
Tetrabothriate cestodes	–	–	0	6	–	–	6.8

Table 2. Prevalence of parasites in *Saccostrea* spp. IVI = intranuclear virus-like inclusions. RLOs = Rickettsiales-like organisms. The numbers in bold under each area are the total number of specimens examined from that area

Bivalve	Oyster Creek	Exmouth Islands	Dampier Archipelago	King Sound	Darwin-Bynoe	All areas %
Saccostrea glomerata	**n = 758**	**50**	**117**	**8**	**0**	**933**
RLOs	3	0	0	0	–	0.3
Marteilia sydneyi	0	0	1	0	–	0.1
Perkinsus sp.	0	0	0	1	–	0.1
Ancistrocomid ciliates	13	0	0	0	–	1.4
Sphenophyra-like ciliates	12	0	1	0	–	1.4
Tylocephalum sp.	3	13	0	1	–	1.8
Sporocysts	0	1	0	0	–	0.1
Saccostrea cucullata	**n = 22**	**769**	**430**	**33**	**0**	**1254**
IVI	0	1	0	0	–	0.1
RLOs	0	1	0	0	–	0.1
Haplosporidium sp.	0	121	4	0	–	10.0
Marteilia sp.	0	1	0	0	–	0.1
Perkinsus sp.	0	0	1	0	–	0.1
Ancistrocomid ciliates	0	3	4	0	–	0.6
Nematopsis sp.	0	1	0	0	–	0.1
Tylocephalum sp.	0	0	9	0	–	0.7
Nematode larvae	0	4	0	0	–	0.3
Saccostrea echinata	**n = 0**	**0**	**0**	**12**	**94**	**106**
Marteilioides sp.	–	–	–	0	9	8.5
Ancistrocomid ciliates	–	–	–	0	5	4.7
Tylocephalum sp.	–	–	–	0	13	12.3
Sporocysts	–	–	–	0	2	1.9

Table 3. Prevalence of parasites and diseases in hammer shells (Malleidae), razor shells (Isognomonidae) and pen shells (Pinnidae). IVI = intranuclear virus-like inclusions. RLOs = Rickettsiales-like inclusions. *Includes 65 spat. The numbers in bold under each area are the total number of specimens examined from that area

Bivalve/infection	Oyster Creek	Exmouth Islands	Dampier Archipelago	80 Mile Beach	King Sound	All areas %
Malleidae						
Malleus malleus	**n = 0**	**5**	**4**	**2**	**0**	**11**
Tylocephalum sp.	–	5	1	0	–	54.5
Sporocysts	–	1	0	0	–	9.1
Malleus meridianus	**n = 0**	**2**	**2**	**0**	**16**	**20**
Perkinsus sp.	–	0	1	–	15	80.0
Tylocephalum sp.	–	2	1	–	8	50.0
Proctoeces sp.	–	0	1	–	0	5.0
Isognomonidae						
Isognomon isognomum	**n = 0**	**0**	**36**	**0**	**22**	**58**
IVI	–	–	1	–	2	5.2
RLOs	–	–	2	–	0	3.4
Perkinsus sp.	–	–	7	–	5	20.7
Tylocephalum sp.	–	–	0	–	2	3.4
Sporocysts	–	–	1	–	0	1.7
Pinnidae						
Pinna bicolor	**n = 0**	**9**	**71***	**0**	**0**	**80**
IVI	–	0	1	–	–	1.3
RLOs	–	9	10	–	–	23.8
Ancistrocomid ciliates	–	3	0	–	–	3.8
Tylocephalum sp.	–	4	16	–	–	25.0
Pinna deltodes	**n = 0**	**0**	**0**	**29**	**16**	**45**
RLOs	–	–	–	0	1	2.2
Perkinsus sp.	–	–	–	0	1	2.2
Tylocephalum sp.	–	–	–	13	2	33.3

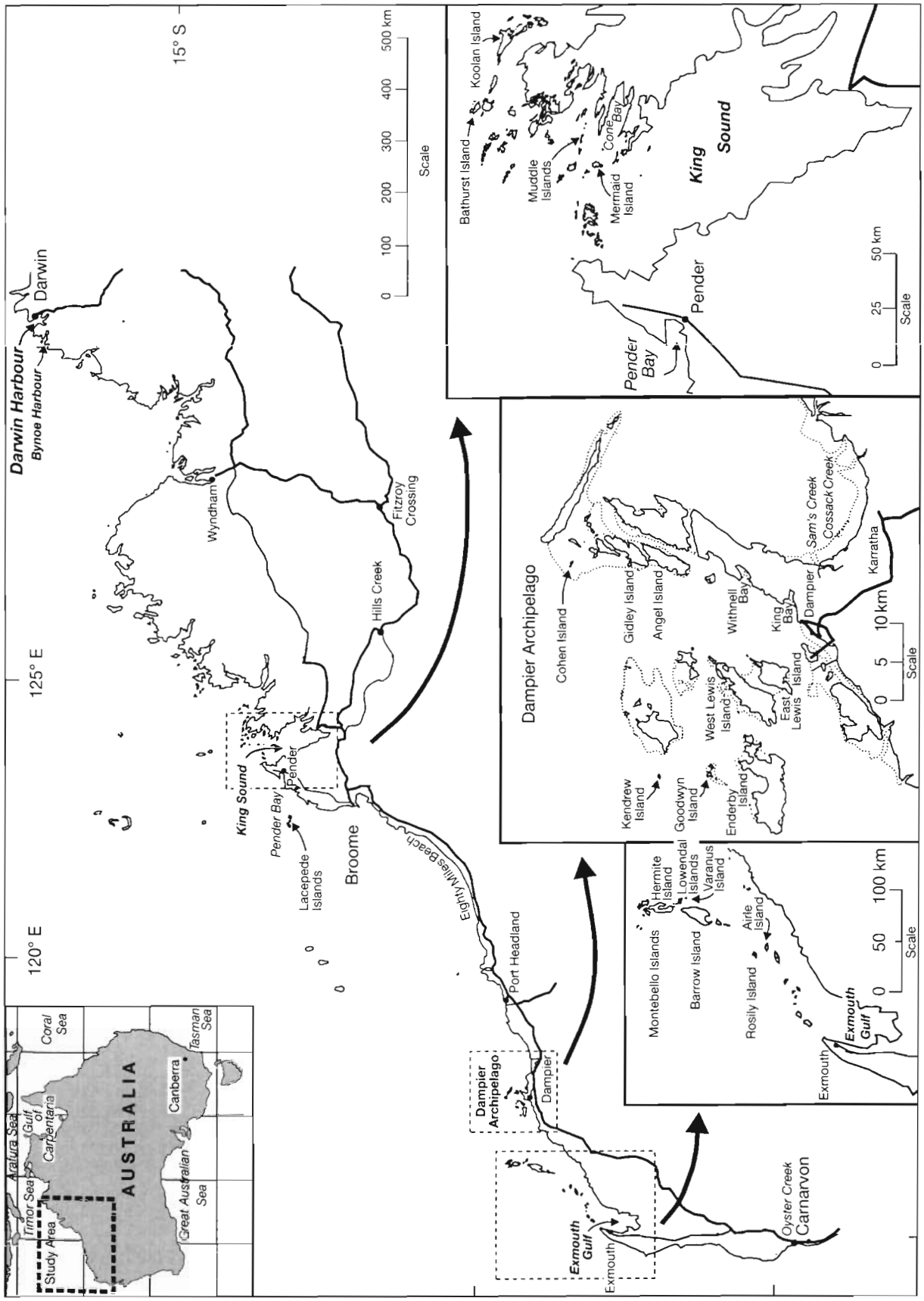

Fig. 1. Location of sampling sites mentioned in the text

in Fig. 1: (1) Oyster Creek, Carnarvon; (2) Exmouth Gulf and outlying islands; (3) The Dampier Archipelago; (4) 80 Mile Beach; (5) King Sound; (6) Darwin Harbour and Bynoe Harbour, Northern Territory. More detailed information on the occurrence of specific infections within each area is given in the text, and the locations within the areas are shown in Fig. 1.

RESULTS

Pearl oysters (Pteriidae)

Adult *Pinctada maxima* (n = 415) were uninfected, except for 1 oyster collected from Exmouth Gulf, which had round to ovoid eosinophilic intranuclear virus-like inclusions (IVI) 6 to 7 µm across in diverticular epithelial cells (Fig. 2a, Table 1). Hatchery-reared *P. maxima* (n = 4670) were infected with the basophilic inclusions usually caused by Rickettsiales-like organisms (RLOs) (0.9%), a coccidian oocyst containing >13 sporozoites in the heart (0.3%) (Fig. 2b), ancistrocomid ciliates in the gut (2.2%) (Fig. 2c), *Haplosporidium* sp. in the connective tissue but not epithelium (0.1%) (Fig. 2d), and *Tylocephalum* metacestodes (0.9%) in connective tissue of the digestive gland and mantle. One of several *P. maxima* originating from a hatchery in Darwin, and being held in quarantine in Sam's Creek, Dampier Archipelago, was infected with a schizont of *Perkinsus*, 19 µm across, in connective tissue at the base of the gills. No host reaction was apparent.

Pinctada albina (n = 469) were infected with RLOs (0.4%) and ancistrocomid ciliates in the gut (1.5%) (Table 1). *Perkinsus* schizonts, 20 to 30 µm across, occurred in small groups, 40 to 48 µm across enclosed by a thin layer of brown cells, in the connective tissue of 2/13 (15.4%) *P. albina* from between West and East Lewis Islands, Dampier Archipelago. *Tylocephalum* metacestodes were more prevalent in *P. albina* (12.2%) (Table 1) than in *P. maxima* (0%), and 30/58 (51.7%) of *P. albina* examined from Shark Bay near Carnarvon were infected with *Tylocephalum* (data not shown). Granulomas comprising cells with indistinct pale eosinophilic cytoplasm enclosed *Tylocephalum* metacestodes between the digestive diverticulae or gonad follicles, but cells were well-defined with weakly

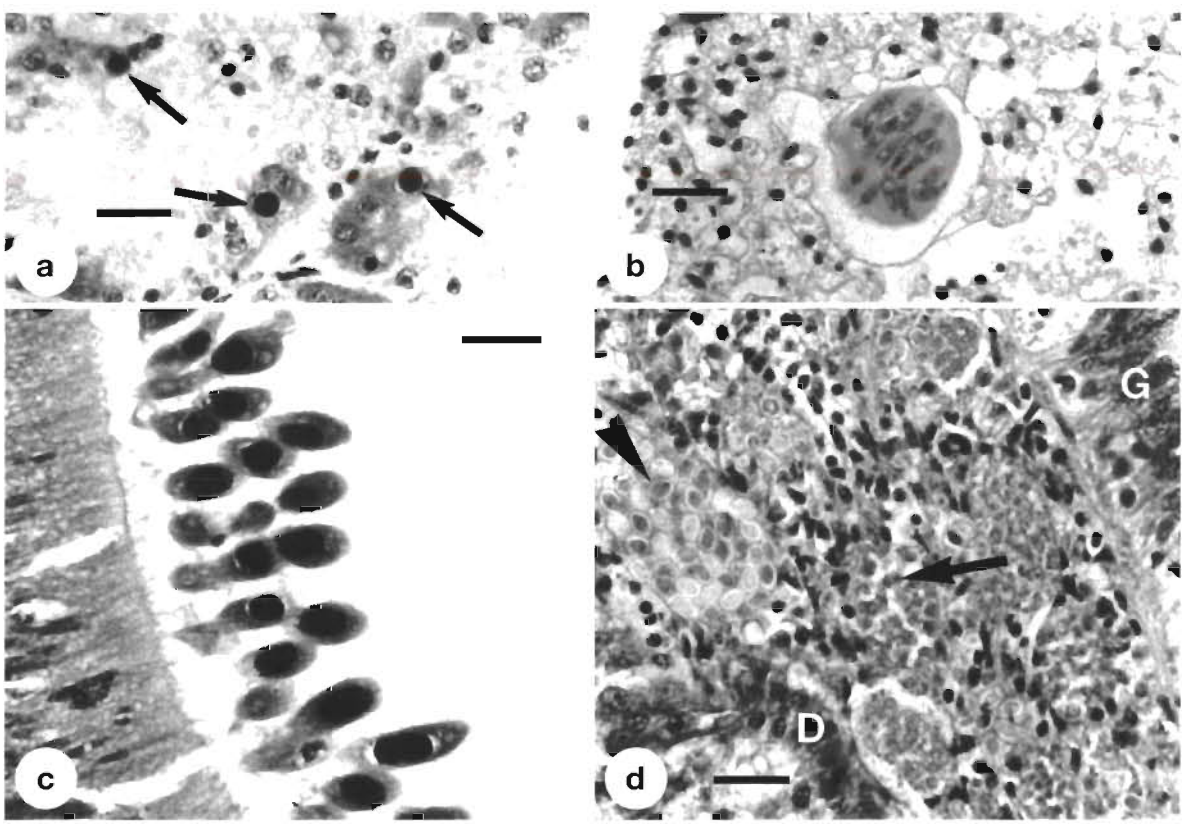

Fig. 2. Infections in *Pinctada maxima*. (a) IVI in diverticular epithelial cells (arrows); scale bar = 14 µm. (b) Coccidian oocyst with sporozoites in the heart; scale bar = 23 µm. (c) Ancistrocomids in the gut. The gaps in the epithelium are sectioning artifacts; scale bar = 19 µm. (d) Developmental stages of *Haplosporidium* sp. between the gut (G) and diverticular (D) epithelia. Stages present include multinucleate syncytia (arrow) and maturing spores (arrowhead); scale bar = 11 µm

basophilic cytoplasm when encystment occurred in connective tissue near the gut. Granuloma cells formed a narrow band around the parasite (39 ± 10 µm in diameter; n = 6), and lesions were small (253 ± 46 µm). *Tylocephalum* appeared to be normal in all sections examined, and were presumably viable at the time of fixation.

Pinctada margaritifera (n = 29) examined from Oyster Creek, Goodwyn Island and Flying Foam Passage, Dampier Archipelago, were uninfected. One of 45 (2.2%) *Pteria penguin* from buoys between West and East Lewis Islands, Dampier Archipelago, was infected with RLOs. Other parasites found in *P. penguin* (n = 88) included *Tylocephalum* metacestodes encysted in the connective tissue of the digestive gland (14.8%), and immature tetrabothriate cestodes in the gut (6.8%) (Table 1).

Edible oysters (Ostreidae)

The pattern of infection in Sydney rock oysters *Saccostrea glomerata* differed between those hatchery-reared at Oyster Creek (n = 758) and those collected from the wild (n = 175) (Table 2). Hatchery-reared *S. glomerata* had higher levels of RLOs (0.4%), ancistrocomid-like ciliates in the gut (1.7%) and *Sphenophyra*-like ciliates in small groups on the gills (1.6%) than oysters collected from the wild (0.0, 0.0 and 0.6%, respectively). Neither taxa of ciliates had any apparent effect on the host. *Tylocephalum* was more common in wild oysters (8.0%) than in Oyster Creek stocks (0.4%).

The diverticular epithelium of 1/22 (4.5%) *S. glomerata* from a bay south of King Bay, Dampier Archipelago, was infected with *Marteilia sydneyi* (Fig. 3a). *Perkinsus* schizonts 8.5 to 9.0 µm in diameter occurred in connective tissue beneath the epithelium of the lateral gill filaments, and in connective tissue of the mantle among a few agranular haemocytes, in 1/4 *S. glomerata* from Cone Bay, King Sound. There was no apparent host reaction. In one *S. glomerata* from Exmouth Gulf, trematode sporocysts occupied about one-third of the connective tissue in the digestive gland, with hyalinocytes and a few eosinophilic granulocytes around sporocysts that were shrunken and necrotic, suggesting that they were degenerating.

Saccostrea cucullata (n = 1254) were infected with IVI (1/26 [3.8%] from the Montebello Islands), RLOs (1/129 [0.8%] from Barrow Island), ancistrocomid-like ciliates in the gut (1/129 [0.8%] from Barrow Island, 2/332 [0.6%] from the Lowendal Islands, 1/205 [0.5%], Exmouth Islands), and 1/205 (0.5%) from King Bay and 3/30 (10.0%) from Goodwyn Island, Dampier Archipelago, *Nematopsis* sp. (1/129 [0.8%] from Barrow Island, Exmouth Islands), and *Tylocephalum* (9/205 [4.4%] in King Bay, Dampier Archipelago) (Table 2). *Marteilia* sp. occurred on and superficially in the epithelium of the distal and part of the proximal digestive diverticulae, but not in the main digestive tract (Fig. 3b), of 1/26 (3.8%) *S. cucullata* from the Montebello Islands, Exmouth Islands.

In June 1993, epizootics among *Saccostrea cucullata* around Airlie Island, Exmouth Islands, in which 50 to 70% of oysters appeared freshly dead, were associ-

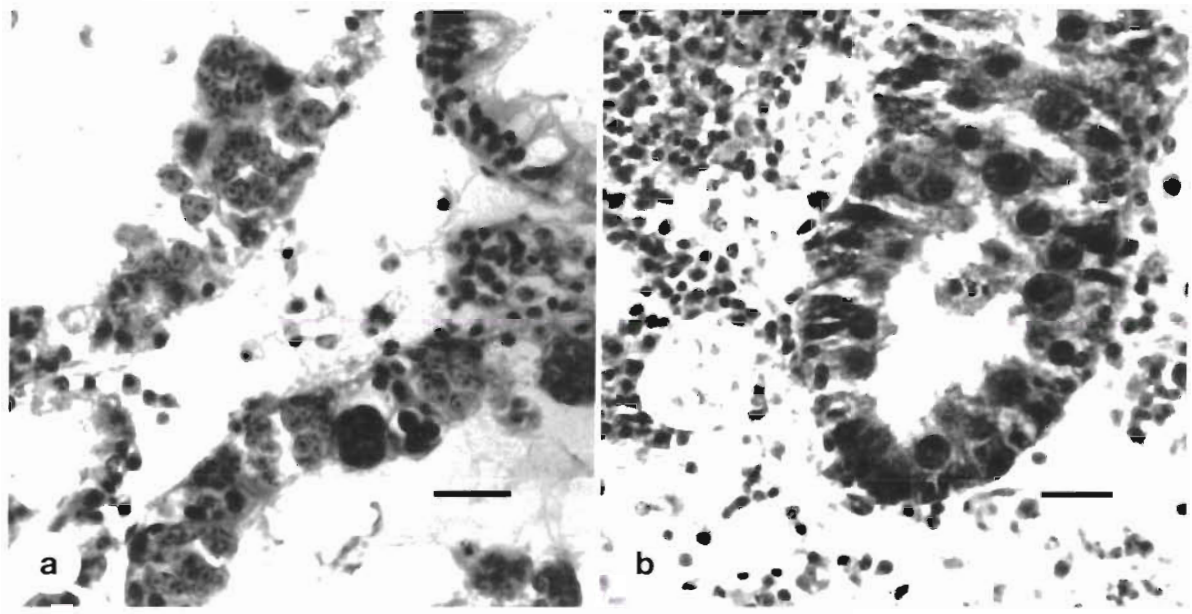

Fig. 3. *Marteilia* spp. (a) *M. sydneyi* in the diverticular epithelium of *Saccostrea glomerata*; scale bar = 28 µm. (b) *Marteilia* sp. in the diverticular epithelium of *S. cucullata*; scale bar = 19 µm

ated with a parasite tentatively identified as *Haplosporidium* sp. Subsequent investigation showed *Haplosporidium* sp. to be most prevalent in the Lowendal Islands (90/332 [27.1%] of oysters), with lower prevalence in the Montebello Islands (4/26 [15.4%]), on Airlie Island (26/211 [12.3%]), East Lewis Island (3/44 [6.8%]), and Rosily Island (1/31 [3.2%]).

One of 44 (2.3%) *Saccostrea cucullata* from East Lewis Island was heavily infected with *Perkinsus* and appeared shrunken with a pale translucent digestive gland. Meronts, merozoites and schizonts of *Perkinsus* were intracellular within haemocytes or extracellular (Fig. 4a) in haemolymph sinuses or replaced connective tissue throughout the oyster. Merozoites, 4.0 to 6.8 µm in diameter, developed to meronts, 4.6 to 6.8 µm in diameter, containing a vacuoplast. Intranuclear virus-like particles (VLPs), 45 to 54 nm in diameter, which occasionally appeared hexagonal in section (Fig. 4b), were seen in ~90% of nuclei at both stages. About a third of the nuclei were irregular in shape and, in about half the cells, the nuclear membrane appeared to be broken. Schizonts, 8.0 to 9.0 µm in diameter, frequently contained >10 merozoites per section (Fig. 4c), many of which contained vacuoplast material.

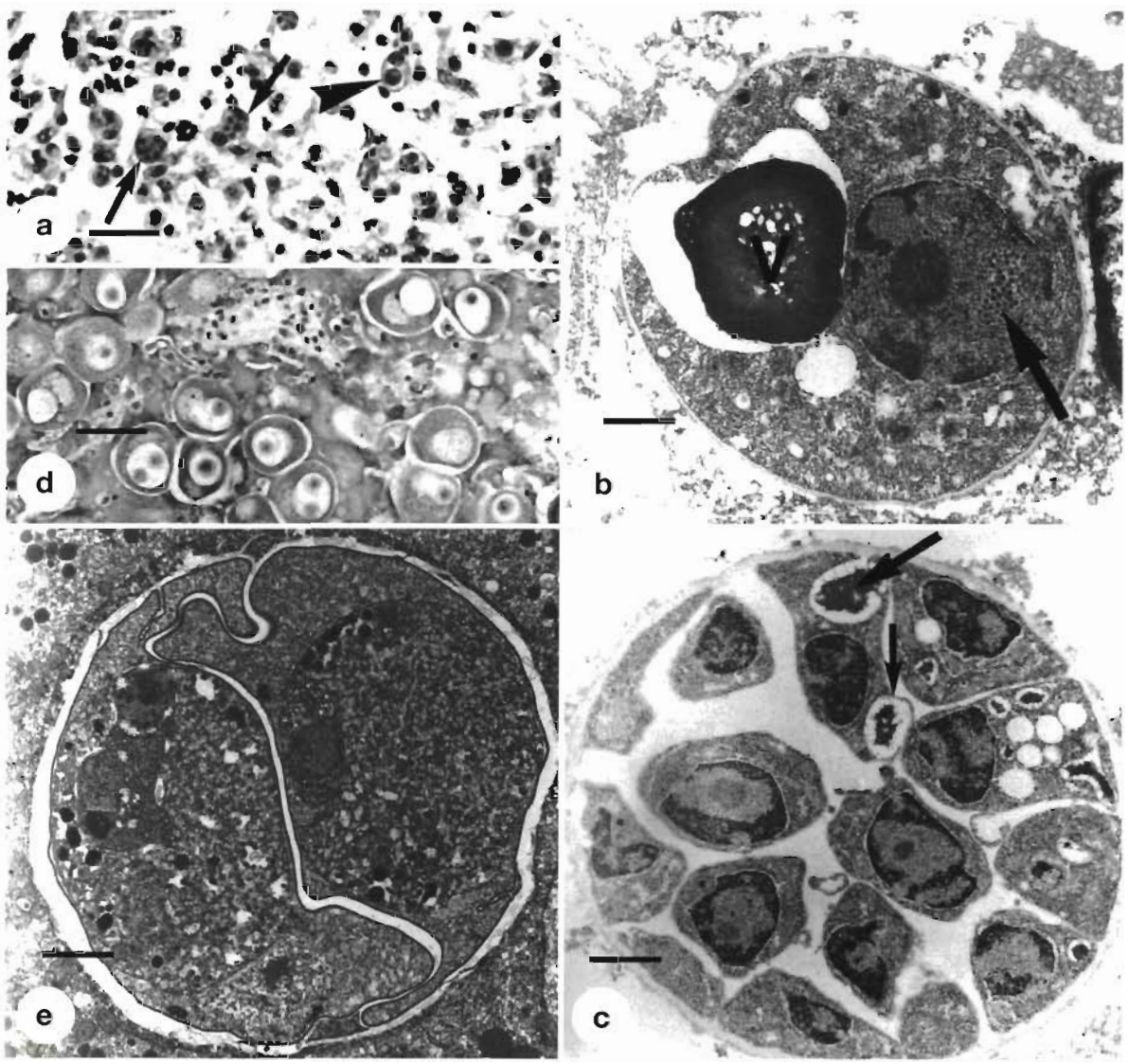

Fig. 4. Protozoan infections of *Saccostrea* spp. (a) Section through the digestive organ of *S. cucullata* infected with *Perkinsus* sp., showing a phagocytosed meront (arrowhead) and extracellular schizonts (arrows); scale bar = 19 µm. (b) *Perkinsus* sp. meront with vacuoplast (V) and intranuclear VLPs (arrow); scale bar = 0.6 µm. (c) *Perkinsus* sp. schizont enclosing merozoites with remnants of vacuoplast material (arrows); scale bar 0.9 µm. (d) Ovacytes of *S. echinata* infected with *Marteilioides* sp. showing apparent presence of 1 or 2 spores; scale bar = 42 µm. (e) Two sporangial cells lying in a vacuole in an ovacyte of *S. echinata*; scale bar = 18 µm

None of the 12 *Saccostrea echinata* from King Sound (Cone Bay 0/2, Koolan Island 0/10) were infected, but ancistrocomid-like ciliates in the gut (5.3%), *Tylocephalum* (13.8%), and unidentified sporocysts (2.1%) occurred in the connective tissue of *S. echinata* (n = 94) from Darwin Harbour and Bynoe Harbour (Table 2). An ovarian parasite, tentatively identified as *Marteilioides* sp., occurred in 9/43 (20.9%) (9/10 females with ova) *S. echinata* from Darwin Harbour, but was not seen in 51 (0/12 females with ova) *S. echinata* from Bynoe Harbour. Under the light microscope the parasite appeared to lie within a vacuole (Fig. 4d) and consisted of cells within cells. Sometimes 2 stages were present in a 69 configuration. Ultrastructurally, endogenous budding within a primary cell produced a secondary cell, giving rise to 1 or 2 sporangial (tertiary) cells from each of which 1 spore develops (Fig. 4e) (Dr Tim Anderson, Department of Parasitology, University of Queensland, pers. comm.). Parasite development did not appear to affect the ova, even at the ultrastructural level.

Hammer shells (Malleidae), razor shells (Isognomonidae) and pen shells (Pinnidae)

All 5 *Malleus malleus* examined from Exmouth Gulf were heavily infected with *Tylocephalum* metacestodes, but these were less prevalent at other sites (Table 3). *Malleus meridianus* (n = 20) also showed high prevalence of *Tylocephalum* in Exmouth Gulf (2/2), Port Robertson, Dampier Archipelago, (1/2) and Cone Bay, King Sound (8/16). The most prevalent infection of *M. meridianus* in Cone Bay was *Perkinsus* (14/15) (93.3%), which was also detected at Port Robertson (1/2), Dampier Archipelago. Schizonts 15 to 28 µm in diameter occurred in connective tissue of the digestive gland without apparent host reaction.

Among 58 *Isognomon isognomum* examined, eosinophilic IVI 5 µm in diameter were observed in nuclei of diverticular epithelial cells of *I. isognomum* from Goodwyn Island (1/2), Dampier Archipelago, Datum Bay in Cone Bay (1/2) and from the Muddle Islands (1/6), King Sound. *I. isognomum* from Sam's Creek, Dampier Archipelago, were infected with RLOs (2/23) (Table 3) and *Perkinsus* (7/23). The latter also infected *I. isognomum* in Cone Bay (3/13) and Mermaid Island (2/3), King Sound. *Perkinsus* schizonts 13 to 22 µm in diameter infected the connective tissue throughout the digestive gland (Fig. 5a) and near the base of the gills. They were frequently surrounded by halo and without apparent host reaction. Brown cells were numerous throughout the connective tissue but were never congregated around schizonts. *Tylocephalum* was less prevalent in *I. isognomum* than *Malleus* spp. (Table 3).

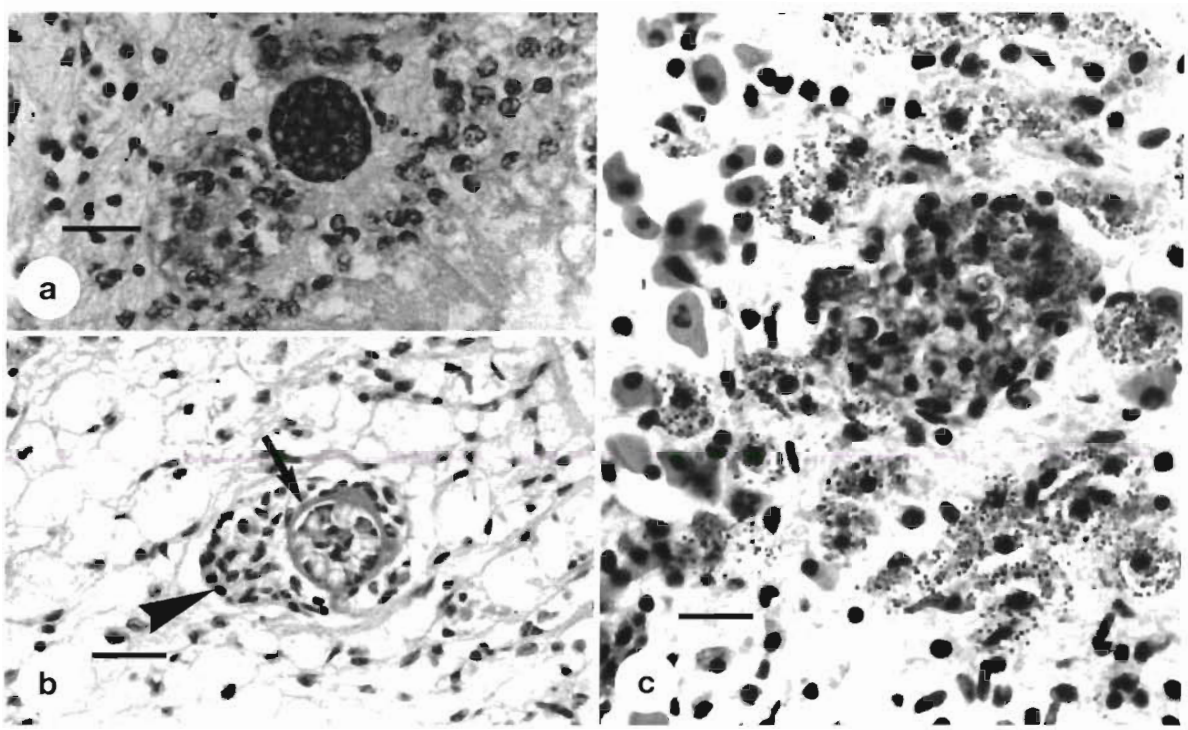

Fig. 5. *Perkinsus* sp. (a). Schizont in *Malleus meridianus*, with no apparent host reaction; scale bar = 12 µm. (b) Schizont in *Spondylus* sp. enclosed in a capsule (arrow), next to an accumulation of haemocytes (arrowhead); scale bar = 12 µm. (c) Loose merozoites in *Barbatia helblingii*, surrounded by brown cells; scale bar = 28 µm

Among 80 *Pinna bicolor* examined, of which 9 were from Exmouth Gulf, 9 (100%) were infected with RLOs, 3 (33%) with ancistrocomid ciliates in the gut, and 4 (44%) with *Tylocephalum* (Table 3). Ancistrocomids occurred at high intensities, with their apices among the brush border of columnar epithelial cells. No haemocytic response was apparent in underlying Leydig tissue. Eosinophilic IVI 5 µm in diameter occurred in the nuclei of diverticular epithelial cells of 1/13 *P. bicolor* from Sam's Creek, Dampier Archipelago. *Pinna deltodes* (n = 45) were also infected with RLOs (2.2%) and *Tylocephalum* (33.3%), and 1/14 (7.1%) *P. deltodes* from Cone Bay was infected with *Perkinsus* (Table 3).

In *Malleus* spp. and *Pinna* spp., *Tylocephalum* metacestodes occurred in granulomas in the connective tissue, most frequently among digestive diverticulae or in the mantle. *M. malleus* and *P. bicolor* were heavily infected with ~18 and ~16 encysted *Tylocephalum* metacestodes per section, respectively. In *Malleus* spp. the concentric layers of granulomatous cells surrounding the parasites were of similar width, 77 ± 18 µm across (n = 13), and granulomatous lesions were 301 ± 60 µm across, but there was no evidence of compression of surrounding diverticulae.

Other species

Perkinsus infections also occurred in *Spondylus* sp. (probably *S. violascens*) (Spondylidae) from 80 Mile Beach (2/5), Cone Bay (2/6), and Koolan Island (1/1), as encapsulated schizonts, 25 to 46 µm in diameter in Leydig tissue, often associated with an accumulation of haemocytes (Fig. 5b). One of 16 (6.3%) *Septifer bilocularis* (Mytilidae) from Cone Bay was infected with *Perkinsus* schizonts, 18 µm in diameter, in the kidney. Four *S. bilocularis* from Flying Foam Passage were uninfected. *Stavilia horrida*, another mytilid from 80 Mile Beach was infected with RLOs (3/13) (23.1%) (Table 2). *S. horrida*. was also infected with *Tylocephalum* sp. (1/13) (7.7%), and 2/13 (15.4%) had immature unidentified cestodes with 4 suckers in the gut. *Barbatia helblingii* (Arcidae) from Cone Bay and the Muddle Islands were infected with RLOs (1/6) (16.7%) and *Perkinsus* (3/6) (50.0%). *B. helblingii* was more heavily infected with *Perkinsus* than other hosts, with foci of brown cells surrounding merozoites in connective tissue between the digestive tract, gonads and digestive diverticulae (Fig. 5c); however schizonts were not observed. Five of 21 (23.8%) *Dendostrea folium* (Ostreidae) from Cone Bay were infected with *Tylocephalum*. No parasites were detected from giant clams; 4 *Hippopus hippopus* from Mermaid Island and Cone Bay, 1 *Tridacna squamosa* from the Muddle Islands, and 3 *Tridacna maxima* from Port Robertson.

DISCUSSION

The only infection observed in 415 wild *Pinctada maxima* was IVI in diverticular epithelium, similar to the inclusions reported from Western Australian *P. maxima* by Pass et al. (1988) that contained virus-like particles (Perkins 1993). Hatchery-reared spat also showed low prevalence of minor infections, except for heavy infection by *Haplosporidium* sp. in 6/105 spat from the hatchery at Oyster Creek (Hine & Thorne 1998). The life-cycle of *Haplosporidium* sp. is unknown but *H. nelsoni* cannot be directly transmitted, possibly due to an alternative host in the life-cycle (Haskin & Andrews 1988, Ford 1992). The infected *P. maxima* spat were hatchery-bred from adults collected from Exmouth Gulf, and were continuously held in the hatchery in unfiltered Oyster Creek water. Therefore, if a second host is involved in the life-cycle, it may be planktonic. The single *P. maxima* infected by *Perkinsus*, which was derived from the hatchery in Darwin Harbour and was being held at a quarantine site at Sam's Creek, probably acquired the infection while in quarantine, as *Perkinsus* was not detected at the hatchery, and 7/23 *Isognomon isognomum* in Sam's Creek were infected by *Perkinsus*. *P. maxima* appears to have little susceptibility to *Perkinsus* sp. infection (Norton et al. 1993c).

Perkinsus was not observed in 29 *Pinctada margaritifera* and in only 2 of 469 *P. albina* examined here, in contrast to 3/26 infected *P. margaritifera* and 1/4 infected *P. albina* (= *P. sugillata*) on the Great Barrier Reef (Goggin & Lester 1987). Great Barrier Reef *P. albina* were also readily experimentally infected with *Perkinsus* (Goggin et al. 1989). The low prevalence of *Perkinsus* from *P. albina* cannot be attributed to the absence of *Perkinsus* from the collection sites, as 247 *P. albina* were sampled from sites at which *Perkinsus* occurred in other species. Apparent absence may be due to differences in strains or species of *Perkinsus* (Lester et al. 1990, Goggin & Lester 1995), susceptibility of the host, environmental factors affecting host or parasite, or non-detection of low intensity infections using histology, rather than incubation in fluid thioglycollate as used by Goggin & Lester (1987) and Goggin et al. (1989).

Non-detection of *Perkinsus* by histology is doubtful, however, because of the large number of *Pinctada albina* examined, and histological detection in other species were at similar levels to those detected by Goggin & Lester (1987). In this study the highest prevalences of *Perkinsus* infection were in *Malleus meridianus* (16/20; 80%; Malleidae), *Barbatia helblingii* (3/6; 50%; Arcidae), *Spondylus* sp. (5/12; 42%; Spondylidae), *Isognomon isognomum* (12/58; 21%; Isognomonidae), *Septifer bilocularis* (1/16; 6%; Mytilidae), and *Pinna deltodes* (1/45; 2%; Pinnidae). Comparable prevalences on the Great Barrier Reef, calculated

from Table 1 in Goggin & Lester (1987), were *Malleus regula* 2/14 (14%), arcids 24/41 (59%), spondylids 12/37 (32%) and isognomonids 3/41 (7%). Although the Pteriidae, Malleidae and Isognomonidae are grouped in the Superfamily Pteriacea, *Malleus* and *Isognomon* appear more susceptible to *Perkinsus* infection than *Pteria* or *Pinctada*.

Schizonts varied greatly in size from 8 to 9 µm in *Saccostrea* spp. to 48 µm in *Spondylus* sp. It is unclear whether there is 1 *Perkinsus* sp., as suggested by cross-infection experiments (Goggin et al. 1989), or more than one, as suggested by morphology (Lester et al. 1990, Goggin & Lester 1995). *Perkinsus marinus* may exist as different strains varying in virulence that infect genotypically different stocks of *Crassostrea virginica* varying in resistance to infection (Bushek & Allen 1996). In light of this, and ready cross-infection by Australian *Perkinsus* (Goggin et al. 1989), it seems likely that 1 or 2 species of Australian *Perkinsus* exist, exhibiting either substantial intraspecific variation or lack of host specificity. In this study, *Perkinsus* apparently only caused disease in *Saccostrea cucullata*, in which merozoites, meronts and schizonts occurred, but development appeared to be static, as schizonts only, in other hosts.

In *Saccostrea glomerata*, infection levels differed between hatchery-reared and wild stocks. Ancistrocomid-like ciliates are common in oysters (Bower et al. 1994), and the semi-enclosed system in the hatchery at Oyster Creek may have allowed build up in prevalence and intensity of these directly transmitted parasites. Alternatively, the stress of culture may affect the oysters, predisposing them to infection, as demonstrated in experimentally infected oysters (Pauley et al. 1967). As elasmobranchs are the definitive hosts of *Tylocephalum* (Butler 1987), the greater number of metacestodes in wild, rather than hatchery-reared, oysters probably reflects proximity of wild oysters to definitive hosts. The most potentially serious infection encountered in *S. glomerata* was *Marteilia sydneyi* (QX disease), seen in only 1 of 933 oysters. The life cycle of this parasite is unknown (Roubal et al. 1989), but the inclusion of infected individuals in intensive culture may lead to the build up of infection, and result in the high mortalities experienced in culture in eastern Australia (Potter 1983, Lester 1986). This study extends the known distribution of *M. sydneyi* more than 3000 km to the west.

Saccostrea cucullata was infected by 3 genera, (*Perkinsus*, *Haplosporidium* sp., *Marteilia* sp.) of protozoans, including species known to cause serious disease. *Perkinsus olseni*, the cause of epizootics in abalone in South Australia, differs from *Perkinsus* in *S. cucullata*, in having larger merozoites, meronts and schizonts (13 to 16 µm diameter) (Lester & Davis 1981), and an infrequently observed vacuoplast (Goggin & Lester 1995). In size, the *Perkinsus* of *S. cucullata* is more similar to trophozoites of *P. marinus* (5 to 7 µm diameter), *Perkinsus* in *Tridacna maxima* (2 to 6 µm) and *Chama pacifica* (2 to 4 µm) (Lester et al. 1990). The 45 to 54 nm diameter VLPs observed in *Perkinsus* from *S. cucullata* resemble, in size and appearance, the 46 to 53 nm diameter VLPs reported from *P. marinus* (Perkins 1996).

Haplosporidium sp. of *Saccostrea cucullata* most closely resembles *Haplosporidium* sp. from *Crassostrea gigas* in Korea (Kern 1976) and from Japan (Friedman et al. 1991) in spore size, but it differs from both as it only infects connective tissue, and not diverticular epithelium. The *Marteilia* sp. in *S. cucullata* is similar to *M. lengehi* from *S. cucullata* in the Persian Gulf (Comps 1976), but differs in the lack of infection of the main digestive tract in this study. Despite this, the Persian Gulf and Australian *Marteilia* appear similar and infect the same host. If the *Marteilia* here is *M. lengehi*, this species may occur in *S. cucullata* across the Indian Ocean.

The parasite in ova of *Saccostrea echinata* in Darwin Harbour has previously been reported by Wolf (1977b). The development of 1 spore from each sporont places the parasite in the genus *Marteilioides* Comps et al., 1986, redefined by Anderson & Lester (1992). It resembles *Marteilioides chungmuensis* in *Crassostrea gigas* from Korea (Chun 1979, Comps et al. 1986), but differs in that the sporont in *M. chungmuensis* derives from the secondary cell (Comps et al. 1986), whereas here it appeared to derive from a tertiary cell. A similar parasite occurs in the ova of *C. gigas* in Japan (Imai et al. 1968), where it causes the development of grossly enlarged egg masses (Matsuzato et al. 1977). An ovarian parasite in *C. gigas* from Humboldt Bay, California, has similar stages to *M. chungmuensis* and the parasite reported here, particularly the 69 configuration of developing spores (Fig. 12 of Becker & Pauley 1968), and may also be a species of *Marteilioides*.

In declining prevalence, *Tylocephalum* metacestodes occurred in *Malleus meridianus* (55%), *M. malleus* (55%), *Pinna deltodes* (33%), *P. bicolor* (25%), *Pinctada albina* (17%), *Pteria penguin* (15%), *Saccostrea echinata* (12%), *S. horrida* (8%), *S. glomerata* (4%), *Isognomon isognomum* (3%), and *S. cucullata* (1%). Prevalence was high in the few *Spondylus* (5/12), and *Barbatia helblingii* (3/6) examined, but was less frequently seen in *Septifer* (1/16). None of 29 *Pinctada margaritifera* and 425 wild *P. maxima* were infected, and only 4 of 4670 *P. maxima* spat (<0.1%) from hatcheries were infected. The lack of host specificity of *Tylocephalum* metacestodes has often been reported (Lauckner 1983). Although *Tylocephalum* has previously been reported in *Saccostrea* in Australia (Wolf

1976), the prevalence and intensity of *Tylocephalum* infection observed here does not support the assertion that *Pinctada* spp. and *Saccostrea* (= *Crassostrea*) spp. are particularly susceptible hosts (Lauckner 1983).

The identity of immature helminths with 4 suckers in the digestive tracts of *Pteria* and *Stavilia* from 80 Mile Beach could not be determined with certainty. However, although inverted tentacles were not observed, the presence of the 4 suckers and position in the stomach is similar to that of tentaculo-plerocercoids of the lecanicephalid cestode *Polypocephalus*, which parasitize skates and rays (Cake 1979).

The bivalves studied here often had low prevalences and intensities of infection, and some had a small range of infections when compared with temperate bivalves. This may be related to the harshness of the environment. In northern Western Australia the tidal fluctuations can cover ~8 m, and with gently sloping coastline, many hundreds of metres of coast are exposed to the tropical sun when tides fall. In many areas the desert extends to the sea and there is negligible organic run-off. Sea temperatures are high (Pass et al. 1987) and at very high tides the sea over-runs coastal salt pans, resulting in a hypersaline surface layer of warm (<40°C) water when the tide drops. This may not only limit the diversity of the coastal fauna, but also that of their parasites and diseases.

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LITERATURE CITED

Anderson TJ, Lester RGJ (1992) Sporulation of *Marteilioides branchialis* n. sp. (Paramyxea) in the Sydney rock oyster, *Saccostrea commercialis*: an electron microscope study. J Protozool 39:502–508

Anderson TJ, Hine PM, Lester RJG (1995) A *Steinhausia*-like infection in the ovocytes of Sydney rock oysters *Saccostrea commercialis*. Dis Aquat Org 22:143–146

Becker CD, Pauley GB (1968) An ovarian parasite (*Protista incertae sedis*) from the Pacific oyster, *Crassostrea gigas*. J Invertebr Pathol 12:425–437

Bower SM, McGladdery SE, Price IM (1994) Synopsis of infectious diseases and parasites of commercially exploited shellfish. Annu Rev Fish Dis 4:1–199

Bushek D, Allen SK (1996) Host-parasite interactions among broadly distributed populations of the eastern oyster *Crassostrea virginica* and the protozoan *Perkinsus marinus*. Mar Ecol Prog Ser 139:127–141

Butler SA (1987) Taxonomy of some tetraphyllidean cestodes from elasmobranch fishes. Aust J Zool 35:343–371

Cake EW (1979) *Polypocephalus* sp. (Cestoda; Lecanicephalidae): a description of tentaculo-plerocercoids from bay scallops of the northeastern Gulf of Mexico. Proc Helminthol Soc Wash 46:165–170

Chun SK (1979) Amoeba infection in oyster (*Crassostrea gigas*). Bull Korean Fish Soc 12:281–285

Comps M (1976) *Marteilia lengehi* n. sp. parasite de l'huître *Crassostrea cucullata* Born. Rev Trav Inst Pêches Marit 40: 347–349

Comps M, Park MS, Desportes I (1986) Étude ultrastructurale de *Marteilioides chungmuensis* n.g. n.sp. parasite des ovocytes de l'huître *Crassostrea gigas* Th. Protistologica 22: 279–285

Dybdahl R, Pass DA (1985) An investigation of mortality of the pearl oyster, *Pinctada maxima*, in Western Australia. Report no. 71, Fisheries Department of Western Australia

Farley CA, Wolf PH, Elston RA (1988) A long-term study of 'microcell' disease in oysters with a description of a new genus, *Mikrocytos* (g. n.), and two new species, *Mikrocytos mackini* (sp. n.) and *Mikrocytos roughleyi* (sp. n.). Fish Bull 86:581–593

Ford SE (1992) Avoiding the transmission of disease in commercial culture of molluscs, with special reference to *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX). J Shellfish Res 11:539–546

Friedman CS, Cloney DF, Manzer D, Hedrick RP (1991) Haplosporidiosis of the Pacific oyster, *Crassostrea gigas*. J Invertebr Pathol 58:367–372

Goggin CL, Lester RJG (1987) Occurrence of *Perkinsus* species (Protozoa, Apicomplexa) in bivalves from the Great Barrier Reef. Dis Aquat Org 3:113–117

Goggin CL, Lester RJG (1990) Rickettsiales-like infection in the gills of *Tridacna crocea* from the Great Barrier Reef. J Invertebr Pathol 56:135–138

Goggin CL, Lester RJG (1995) *Perkinsus*, a protistan parasite of abalone in Australia: a review. Mar Freshw Res 46: 639–646

Goggin CL, Sewell KB, Lester RJG (1989) Cross-infection experiments with Australian *Perkinsus* species. Dis Aquat Org 7:55–59

Haskin HH, Andrews JD (1988) Uncertainties and speculations about the life cycle of the eastern oyster pathogen *Haplosporidium nelsoni* (MSX). In: Fisher WS (ed) Disease processes in marine bivalve molluscs. Am Fish Soc Spec Publ 18:5–22

Hine PM, Thorne T (1997) Replication of herpes-like viruses in haemocytes of adult flat oysters *Ostrea angasi* (Sowerby, 1871): an ultrastructural study. Dis Aquat Org 29:189–196

Hine PM, Thorne T (1998) *Haplosporidium* sp. (Haplosporidia) in hatchery-reared pearl oysters, *Pinctada maxima* (Jameson, 1901), in north Western Australia. J Invertebr Pathol 71:48–52

Imai T, Mori K, Sugawara Y, Tamate H, Oizumi J, Itikawa O (1968) Studies on the mass mortality of oyster in Matsushima Bay. VII. Pathogenetic investigation. Tohuku J Agric Res 19:250–264

Kern GC (1976) Sporulation of *Minchinia* sp. (Haplosporida, Haplosporidiidae) in the Pacific oyster *Crassostrea gigas* (Thunberg) from the Republic of Korea. J Protozool 23: 498–500

Lauckner G (1983) Diseases of Mollusca: Bivalvia. In: Kinne O (ed) Diseases of marine animals, Vol II. Biologische Anstalt Helgoland, Hamburg, p 477–961

Lester RJG (1986) Field and laboratory observations on the oyster parasite *Marteilia sydneyi*. In: Cremin M, Dobson C, Moorhouse D (eds) Parasite lives. University of Queensland Press, Brisbane, p 33–40

Lester RJG, Davis GHG (1981) A new *Perkinsus* species (Apicomplexa, Perkinsea) from the abalone *Haliotis ruber*. J Invertebr Pathol 37:181–187

Lester RJG, Sewell KB (1989) Checklist of parasites from Heron Island, Great Barrier Reef. Aust J Zool 37:101–128

Lester RJG, Goggin CL, Sewell KB (1990) *Perkinsus* in Australia. In: Perkins FO, Cheng TC (eds) Pathology in marine science. Academic Press, New York, p 189–199

Matsuzato T, Hoshina T, Arakawa KY, Masumura K (1977) Studies on the so-called abnormal egg-mass of Japanese oyster, *Crassostrea gigas* (Thunberg)—I. Distribution of the oyster collected in the coast of Hiroshima Pref., and parasite in the egg-cell. Bull Hiroshima Fish Exp St 8:9–25

Norton JH, Perkins FP, Ledua E (1993a) *Marteilia*-like infection in a giant clam, *Tridacna maxima*, in Fiji. J Invertebr Pathol 61:328–330

Norton JH, Shepherd MA, Abdon-Nagutt MR, Lindsay S (1993b) Mortalities in the giant clam *Hippopus hippopus* associated with rickettsiales-like organisms. J Invertebr Pathol 62:207–209

Norton JH, Shepherd MA, Perkins FP, Prior HC (1993c) *Perkinsus*-like infection in farmed golden-lipped pearl oyster *Pinctada maxima* from the Torres Strait, Australia. J Invertebr Pathol 62:105–106

Norton JH, Shepherd MA, Prior HC (1993d) Papovavirus-like infection of the golden-lipped pearl oyster, *Pinctada maxima*, from the Torres Strait, Australia. J Invertebr Pathol 62:198–200

O'Donoghue PJ, Phillips PH, Shepherd SA (1991) *Perkinsus* (Protozoa: Apicomplexa) infections in abalone from South Australian waters. Trans R Soc South Aust 115:77–82

Pass DA, Perkins FO (1985) 'Protistan parasites' or residual bodies in *Pinctada maxima*. J Invertebr Pathol 46:200–201

Pass DA, Dybdahl R, Mannion MM (1987) Investigations into the causes of mortality of the pearl oyster, *Pinctada maxima* (Jamson), in Western Australia. Aquaculture 65:149–169

Pass DA, Perkins FO, Dybdahl R (1988) Viruslike particles in the digestive gland of the pearl oyster (*Pinctada maxima*). J Invertebr Pathol 51:166–167

Pauley GB, Chew KK, Sparks AK (1967) Experimental infection of oysters (*Crassostrea gigas*) with thigmotrichid ciliates. J Invertebr Pathol 9:230–234

Perkins FO (1993) Infectious diseases of molluscs. In: Couch JA, Fournie JW (eds) Pathobiology of marine and estuarine organisms. Advances in fisheries science. CRC Press, Boca Raton, FL, p 255–287

Perkins FO (1996) The structure of *Perkinsus marinus* (Mackin, Owen and Collier, 1950) Levine, 1978 with comments on taxonomy and phylogeny of *Perkinsus* spp. J Shellfish Res 15:67–87

Perkins FO, Wolf PH (1976) Fine structure of *Marteilia sydneyi* sp. n.—haplosporidan pathogen of Australian oysters. J Parasitol 62:528–538

Potter MA (1983) Growth rates of cultivated Sydney rock oysters, *Saccostrea* (*Crassostrea*) *commercialis*, in two estuaries in subtropical southern Queensland. J Agric Anim Sci 40:137–140

Roubal FR, Masel J, Lester RJG (1989) Studies on *Marteilia sydneyi*, agent of QX disease in the Sydney rock oyster, *Saccostrea commercialis*, with implications for its life cycle. Aust J Mar Freshw Res 40:155–167

Roughley TC (1926) An investigation of the cause of an oyster mortality on the Georges River, New South Wales, 1924-5. Proc Linn Soc N S W 51:446–491

Wolf PH (1976) Occurrence of larval stages of *Tylocephalum* (Cestoda: Lecanicephaloidea) in two oyster species from northern Australia. J Invertebr Pathol 27:129–131

Wolf PH (1977a) Diseases and parasites in Australian commercial shellfish. Haliotis 8:75–83

Wolf PH (1977b) An unidentified protistan parasite in the ova of the black-lipped oyster, *Crassostrea echinata*, from northern Australia. J Invertebr Pathol 29:244–246

Wolf PH (1979) Life cycle and ecology of *Marteilia sydneyi* in the Australian oyster, *Crassostrea commercialis*. Mar Fish Rev 41:70–72

Wolf PH, Sprague V (1978) An unidentified protistan parasite of the pearl oyster, *Pinctada maxima*, in tropical Australia. J Invertebr Pathol 31:262–263

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