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Occurrence of *Perkinsus* species (Protozoa, Apicomplexa) in bivalves from the Great Barrier Reef

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ABSTRACT: Bivalves from the Great Barrier Reef were examined for *Perkinsus* infection using the thioglycollate method (Ray 1966). They were taken from Heron, Lizard, Orpheus, North Direction and Thetford Reefs. Thirty of the 84 species examined were infected (160 out of 644 individuals). They belonged to 23 families. The 4 most frequently infected families, in descending order, were Chamidae, Arcidae, Tridacnidae and Spondylidae. All of 4 moribund *Tridacna gigas* examined were infected.

INTRODUCTION

The best known member of the class Perkinsea, *Perkinsus marinus* (Mackin et al. 1950), was previously classified with the fungi under the names *Dermocystidium marinum* and *Labrinthomyxa marina*. It was placed in the phylum Apicomplexa by Levine (1978) on the recognition of an apical complex in the biflagellated zoospores (Perkins 1976).

Perkinsus marinus is homoxenous. The schizont divides in the oyster to produce trophozoites which have a large eccentric vacuole. Under the anaerobic conditions which prevail as the host tissue dies, or in thioglycollate culture media (Ray 1966a), the trophozoite swells to produce prezoosporangia. On return to aerobic seawater the prezoosporangium develops a discharge tube and through this motile zoospores are released. These motile zoospores then initiate new infections (Perkins & Menzel 1967).

Perkinsus species have historically been considered the cause of extensive mortalities in commercially important molluscs (Ray & Chandler 1955). *P. marinus* has caused mass mortalities of the oyster *Crassostrea virginica* along the east coast of America (Andrews & Hewatt 1957) and in the Gulf of Mexico (Ray 1966b). A similar species of *Perkinsus* has been thought to be the cause of mortalities of *C. virginica* in Hawaii (Kern et al. 1973) and more recently has been associated with mortalities of the clam *Venerupis decussata* and other bivalves in the Mediterranean (Da Ros & Canzonier 1985). In Australia, *P. olseni* parasitises the gastropod *Haliotis ruber* causing muscle abscesses and probably host mortality (Lester & Davis 1981). A similar *Perkinsus* species may be the cause of recent widespread mortalities in *H. laevigata*, in South Australia (Lester 1986).

Unidentified *Perkinsus* species are common in bivalves. Perkins (in press) reported that 34 species of bivalve molluscs from the Pacific and Atlantic Ocean and the Mediterranean Sea harbour *P. marinus* or related organisms.

On the Great Barrier Reef, Perkins (1985) found *Perkinsus* spp. in *Tridacna maxima* and *Saccostrea cuccullata* (= *Crassostrea amasa*). The present survey was initiated after our discovery of a *Perkinsus* infection in a moribund *T. gigas* from Lizard Island. Over a third of the giant clams at Lizard Island have died in the 3 yr up to 1987, apparently the result of an epizootic of unknown cause (Alder et al. 1986). The clams were a major tourist attraction and were estimated to be at least 20 yr old.

MATERIALS AND METHODS

Apparently healthy bivalves were collected from Lizard, Orpheus and Heron Reefs (Fig. 1). Four moribund *Tridacna gigas* were also collected, two from Thetford Reef and one each from Lizard and North Direction Reefs.

The whole bivalve, or part of the digestive gland, mantle and gill, was immersed in fluid thioglycollate medium (FTM) as described by Ray (1966a) to permit

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the development of prezoosporangia. After incubation at room temperature (23 to 30 °C) for 4 d, the tissue was removed from the vials, stained with Lugol's iodine and examined under a dissecting microscope. Prezoosporangia became visible as blue-black spheres. The intensity of the infection was graded on a scale from 0 to 4, depending on the highest number of prezoosporangia found: 0 = none; 1 = 1 to 10 per field of 20 mm^2; 2 = 11 to 50 per field; 3 = 51 to 100; 4 = 100+.

RESULTS

A total of 644 bivalves representing 84 species from 23 families were tested for *Perkinsus* infection. Of these, 25 % were infected (Table 1).

Heavy infections (levels 3 and 4) were found only in the Tridacnidae, Spondylidae, Arcidae and Chamidae.

Of the 104 tridacnids sampled, 38 (37 %) were infected. Infections in *Tridacna gigas, T. maxima* and *T. crocea* from Lizard Island all registered level 3 or 4. Level 2 infections were found in *T. maxima* from Heron Island.

Tissues from 4 moribund clams were examined. Two *Tridacna gigas* from Thetford Reef were heavily infected with *Perkinsus* sp. (level 4). A single dying *T. gigas* sampled from North Direction Reef registered an infection level of 3, and a moribund *T. gigas* from Lizard Island was infected at an unknown level.

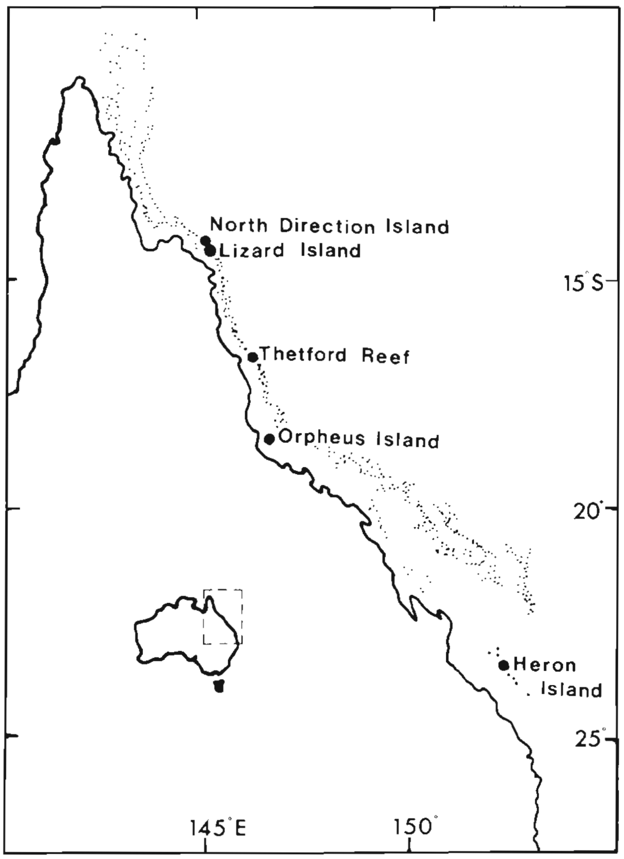

Fig. 1. Location of collection sites

A total of 34 % of spondylids sampled were infected. *Spondylus nicobaricus, S. squamosus* and *S. nicobaricus* form *gracilis* from Lizard Island had levels of 3 or 4. Heron Island spondylids had a maximum infection level of 1.

Of all chamids sampled, 86 % were infected. Many had infection levels of 3 or 4, particularly on Heron Island. Of the 59 % of arcids infected, *Barbatia corallicola* was the most heavily infected, and had infection levels of 3 or 4 on both Lizard and Heron Islands.

Infections were found in 30 % of *Codakia paytenorum* (F. Lucinidae) sampled from Heron Island. Levels 3 and 4 were recorded in December 1986, when 6 of 12 *C. paytenorum* were found infected.

Only 4 of 88 Ostreidae tested were infected. The positive *Alectryonella plicatula* and *Malleus regula* from Lizard Island both had an infection level of 1. None of 23 *Saccostrea cucculata* from Lizard Island were infected.

In general, infection levels were heaviest on Lizard Island except for the Chamidae. *Chama pacificus* and *C. sulphurea* (F. Chamidae) were heavily infected on Heron Island.

DISCUSSION

Perkinsus spp. were widespread in bivalves from the Great Barrier Reef and were particularly abundant in bivalves on Lizard Island where the current giant clam mortalities are occurring. Moribund *Tridacna gigas* were quickly consumed by scavenger fish. Hoese (1964) showed that *P. marinus* was still viable after passage through the gut of the fishes *Gobiosoma bosci, Chasmodes bosquianus* and *Opsanus tau*. It is possible the parasites in the clams could be dispersed in a similar way. Whether the parasite can cause the death of tridacnids is not clear at the moment and is the subject of our current studies.

Many Chamidae were infected at levels 3 and 4. These high levels and the apparent absence of recently dead shells suggest a low level of pathogenicity for the parasite in these hosts. However, the Chamidae are inconspicuous members of the reef community and recent mortalities may have gone unnoticed.

Many Spondylidae and Arcidae were found infected though mostly at a low level. These families could be reservoir hosts for one of the *Perkinsus* species.

Only 4 of 88 Ostreidae tested were infected. These

Table 1. Prevalence of *Perkinsus* spp. in bivalves from 5 reefs: Heron, Lizard, Thetford, North Direction and Orpheus. (No. of infected/No. of sampled bivalves)

Species	Heron	Lizard	Thetford	N. Direction	Orpheus
TRIDACNIDAE					
Tridacna gigas		3/16	2/2	1/1	1/13
Tridacna maxima	3/17	10/20			
Tridacna crocea		11/13			
Tridacna derasa		0/4			
Tridacna squamosa		0/1			
Hippopus hippopus		7/17			
OSTREIDAE					
Saccostrea cucullata	0/13	0/23			
Alectryonella plicatula		2/8			
Hyotissa hyotus	0/14	0/1			
Dendostrea folium	0/10				
Crassostrea sp. 2*	0/1				
Crassostrea sp. 3*	0/1				
Lopha cristagalli		0/3			
Malleus regula		2/14			
SPONDYLIDAE					
Spondylus lamarcki	0/1	1/1			
Spondylus nicobaricus	2/6	1/1			
Spondylus squamosus	1/11	7/13			
Spondylus violascens	0/1	0/1			
Spondylus candidus	0/1				
Spondylus barbatus	0/1				
S. nicobaricus from *gracilis*		1/1			
ARCIDAE					
Barbatia corallicola	4/8	16/27			
Barbatia fusca		3/4			
Anadara antiquata		1/2			
CHAMIDAE					
Chama iostoma	4/4	12/19			
Chama pacificus	40/42				
Chama sulphurea	1/1				
MYTILIDAE					
Septifer bilocularis	0/2	2/27			
Trichomya hirsuta		0/1			
Lithophaga obesa	0/1				
Trapezium rostratum		1/1			
ISOGNOMONIDAE					
Isognomon isognomon	0/20	0/11			
Electroma alacorvi	2/6	1/4			
CARDIDAE					
Fragum fragum	0/16	0/8			
Fragum unedo		0/5			
Acrosterigma unicolor		1/5			
Acrosterigma rosemariensis		0/1			
Fulvia australe		0/1			
Fulvia radiatum		0/1			
PECTINIDAE					
Chlamys irregularis	0/1				
Chlamys lentiginosa		1/5			
Pedum spondyloideum		0/14			
Gloripallium pallium		0/1			

Table 1 (continued)

Species	Heron	Lizard	Thetford	N. Direction	Orpheus
PTERIDAE					
Pinctada margaritifera	1/8	2/18			
Pinctada sugillata	0/1	1/3			
Pinctada peruviridis		0/3			
Pinctada sp. 17*		0/1			
Pteria cypsellus		0/2			
Pteria penguin		0/1			
LUCINIDAE					
Ctena reevei	1/1				
Codakia paytenorum	6/20				
Codakia tigerina	0/3				
Codakia simplex		0/2			
Divaricella ornata		0/20			
GLYCYMERIDAE					
Glycydonta marica	0/1	0/2			
GASTROCHAENA					
Gastrochaena lamellosa	0/1				
LIMIDAE					
Limaria fragilis	0/1				
MESODESMATIDAE					
Atactodea striata	0/6	0/20			
CARDITIDAE					
Benguina semiorbiculata		0/6			
MACTRIDAE					
Mactra maculata		0/3			
PSAMMOBIDAE					
Asaphis violascens		0/1			
FIMBRIDAE					
Fimbria fimbriata	0/1	0/8			
PINNIDAE					
Atrina pectinata	1/2	0/1			
Atrina vexillum	0/1	0/1			
Pinna deltodes		0/1			
Pinna muricata		1/1			
TELLINIDAE					
Tellina triradiatum	0/1				
Tellina staurella	0/1				
Tellina quoyi	0/1				
Tellina crucigera	0/1				
Tellina tenuilirata		0/1			
Tellina vulsella		0/1			
Tellina exculta		0/1			
Obtellina bougeri	0/1				
Quadrans gargadia	0/1	0/1			
SEMELIDAE					
Semele cuspidariaeformis	0/2				
VENERIDAE					
Irus irus	1/1				
Lioconcha castraensis		0/13			
Lioconcha hieroglyphica		0/1			
Lioconcha tigerina		0/1			
Lioconcha ornata		0/5			
Callista semisulcata		1/1			
Periglypta puerpera		1/1			
Pitar inflata		0/1			

* Unidentified bivalves have been lodged in the Queensland Museum as follows: *Crassostrea* sp. 2, MO.17018; *Crassostrea* sp. 3, MO.17019; *Pinctada* sp. 17, MO.17020

were the subtidal oysters *Alectryonella plicatula* and *Malleus regula* from Lizard Island. Perkins (1985) found *Perkinsus* sp. on Lizard Island in 5 out of 25 *Saccostrea cuccullata* (= *Crassostrea amasa*), an intertidal oyster. The 23 *S. cuccullata* from Lizard Island examined in this study were not infected. The infection found by Perkins may be localised or seasonal.

The variability in prevalence and intensity of *Perkinsus* sp. suggests a difference in the pathogenicity of the parasite in different hosts, possibly because more than one species of *Perkinsus* occurs on the reef. Preliminary morphological observations during parasite development following thioglycollate culture suggest this is likely.

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