FINAL REPORT

Diseases of abalone

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The University of Queensland

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Summary

In 1985 and 1986 many greenlip abalone *Haliotis laevigata* near Edithburg in the St Vincent Gulf died. The protozoan parasite, *Perkinsus olseni*, was suspected to be the cause. We showed that *Perkinsus olseni* was seasonally abundant in greenlip from the edge of the die-back area. It was also common in three other species of abalone, *H. rubra*, *H. cyclobates* and *H. scalaris*, and was recovered from 4 species of bivalves from the same area (*Barbatia pistachia*, *Chlamys bifrons*, *Katylesia rhytiphora* and *Pinna bicolor*).

In July, 1987, 140 healthy adult abalone were transplanted from Hardwicke Bay to Stansbury at the centre of the die-off in an attempt to recolonise the area. This was done with the help of commercial abalone divers. In the following October, a subsample indicated the transplants were uninfected and were growing well. However, by March 1988, many of the abalone had recently died and the remainder were heavily infected.

In July, 1989, 195 greenlip from Tipara Reef were tagged and transplanted to the same site. A subsample the following March indicated that there was good growth, very little infection, and no sign of recent deaths. The epizootic had evidently passed and these abalone were successfully recolonising the area. However a follow-up sample a year later, in April, 1992, indicated that many of the animals had become infected though there was no sign of recent deaths.

The die-back area is near the northern limit for greenlip abalone in the Gulf of St Vincent. Laboratory experiments showed that abalone infected with Perkinsus died more frequently than uninfected abalone and that stress such as high temperature may predispose the abalone to disease. The time of the orginal die-back corresponded to warm winters on the Yorke Peninsula.

Laboratory and field observations suggest that the parasite is common in greenlip in late summer. During winter abalone are able to contain and eliminate the infection. We conclude that the parasite is widespread around the Yorke Peninsula in hosts other than greenlip, and that greenlip near the upper limit of their temperature range are likely to contract fatal infections.

Background

Soft yellowish abscesses were noticed in the flesh of blacklip abalone in the 1970s. The protozoan parasite *Perkinsus olseni* Lester & Davis, 1981 was found to be the cause. It was named after Mr A.M. Olsen, Chief Fisheries Officer, South Australia, who first brought the infection to our attention.

In the mid 1980s, greenlip abalone *Haliotis laevigata* disappeared from much of the western shore of the Gulf of St Vincent, South Australia. No unusual traces of pollutants or heavy metals could be found (Shepherd, 1985). Disease was suspected when Mr Andrew Geering, a commercial abalone diver, sent moribund abalone to The University of Queensland and I found them to be heavily infected with *Perkinsus* sp. (Lester, 1986).

Prior to the description of *Perkinsus olseni*, only one *Perkinsus* species was known, *P. marinus*, which occurs in oysters *Crassostrea virginica* along the eastern coast of the United States and apparently did not occur in any other host. Since then two other species have been described, *P. atlanticus* Azevedo 1989 from clams in Portugal, and *P. karlssoni* McGladdery et al., 1991 from scallops in eastern Canada.

Members of the genus *Perkinsus* have been variously classified as fungi, slime moulds and protozoans. They are currently placed in the protozoan phylum Apicomplexa along with gregarines, coccidia and malaria parasites though morphological, developmental and recent

DNA studies (Goggin & Barker, 1993) suggest the Perkinsidae are probably more closely related to dinoflagellates.

Objectives

Four objectives were stated in the original application. They were to: monitor the spread of *Perkinsus*, determine the range of hosts for the parasite, determine what predisposed abalone to fatal infection, and attempt to rehabilitate abalone in the devastated area.

Technical information

Diagnosis of *Perkinsus* infection is sometimes based on histological sections. However, a much more sensitive and accurate diagnosis is the Ray test (Ray, 1966). Pieces of abalone are cultured in fluid thioglycollate medium for 2 to 8 days then transferred to sea water and stained with Lugol's iodine. If a *Perkinsus* species is present, blue-black hypnospores can be seen dotting the surface of the tissue.

Methods

Molluscs were examined for *Perkinsus* using the Ray Test. A piece of muscle, digestive gland and gill was dropped into 15 ml fluid thioglycollate. Antibiotic was added and the vials left for 4 to 7 days prior to examination. Greenlip from the edge of the die back area were also investigated histologically to detect other infections.

Adult greenlip (140) for the first translocation experiment were collected by Mr Andrew Stevens from Hardwicke Bay on 6 July, 1987, driven across the Yorke Peninsula and put back in the water the same day 1 km off Stansbury. Twenty of them were tagged using numbered yellow plastic tags and superglue.

For the second translocation experiment, 195 adult greenlip were collected from Tipara Reef, again by Andrew Stevens, and driven to Edithburg. Here they were put aboard the RV Ngerin, tagged, and dropped off in 4m water 1 km off Stansbury as before.

For the laboratory experiments, uninfected abalone were collected from West Island, S.A., and 120 hatchery-reared greenlip were purchased from Adrian Cuthbertson, Tasmania.

Results

Objective 1. <u>Survey to monitor the spread of the infection</u>

Greenlip abalone occur as far north as Black Point in the Gulf of St Vincent (Fig. 1). However, at the start of the project most had disappeared north of Troubridge Shoals. On the Shoals, particularly Ryans Ground, Bobs Ground, Halftide Rock and Marion Reef, which corresponded to the edge of the die-back area, heavily infected greenlip were common. The few greenlip that could be found further north were generally infected. However, along the southern coast of the Peninsula west of Troubridge Point where greenlip were still common, only 1 of 127 greenlip sampled were infected (see Appendix 1 and Lester et al., 1990). *Perkinsus* was the only pathogen consistently found in histological sections of greenlip abalone from the Shoals. On one occasion a dead abalone which still contained some meat was recovered and *Perkinsus* was isolated from this. Thus there was a strong correlation between the die-back area, dead abalone and *Perkinsus* infection.

Perkinsus was recovered from multiple sites off the Yorke Peninsula in 1986, 1988, 1989, 1990 and 1992. Significant annual variation was not detected apart from low infection off Stansbury in 1990. Samples from Marion Reef suggest seasonal variation occurs as

prevalence in greenlip was higher in late summer than in late winter (9/20 (45%) in March, 7/25 (28%) in July, 13/157 (8%) in September and 6/35 (17%) in January.

Objective 2. Range of hosts

Nine mollusc species were found infected. These included four abalone, *Haliotis laevigata* (greenlip), *H. rubra* (blacklip), *H. cyclobates*, and *H. scalaris*, and five bivalves, *Chlamys bifrons* (scallop), *Barbatia pistachia* (ark shell), *Katelysia rhytiphora* (cockle), *Cleidothaerus* sp. (false oyster or jewel box), and *Pinna bicolor* (razorfish or penshell). Laboratory work described below suggests that these molluscs all contain the same species of *Perkinsus*. The data from different hosts, for January to August, have been combined in Fig. 1 to give an overall picture of the distribution of *Perkinsus* around the Yorke Peninsula. In all hosts the parasite is prevalent from Troubridge Point to Stansbury and much less common along the southern coast and in Hardwicke Bay. Infected animals were not found close inshore; samples from in and around the Edithburg jetty and the Stenhouse Bay jetty were free of infection.

Cross infection experiments in the laboratory showed that *Perkinsus* isolated from *Haliotis laevigata* would infect *H. cyclobates*, *H. scalaris* and even bivalves such as *Pinctada sugillata* and *Anadara trapezia*. Furthermore, isolates from the bivalve *Anadara trapezia* would develop in *Haliotis scalaris* and then reinfect other bivalves (Goggin et al., 1989). From this we concluded that one or several species of *Perkinsus* in Australia had very low host specificity. We were unable to determine how many species of *Perkinsus* were present.

Morphological differences noted in histological sections, such as size of trophozoite or type of host response, were found to be related to the host rather than the parasite and so these features could not be used to separate species. Dr Goggin, a postdoctoral fellow in the Department, sequenced the two internal transcribed spacer regions of the ribosomal RNA gene of *Perkinsus* spp. from three sources: *H. laevigata* from Marion Reef, *Anadara trapezia* from Moreton Bay (Qld.), and *Chama pacificus* from the Great Barrier Reef (Heron Island). Out of 600+ bases only 1 base differed between the *Perkinsus* from *C. pacificus* and the others, and one base between the *Perkinsus* from *A. trapezia* and the others (Goggin, in press). This strongly suggests that the isolates all belonged to one species, *Perkinsus olseni*. DNA of *Perkinsus marinus* sent from the USA differed at 73 locations indicating that it is clearly a separate species.

Dead queen scallops were observed in January 1992 off Black Point, in the north of the Gulf of St Vincent. No *Perkinsus* was found in 10 scallops examined, though it was present in 2 greenlip collected in the same area. The cause of death of the scallops remains unknown.

Objective 3. Factors associated with fatal infection in abalone

Preliminary observations on *Haliotis rubra* which had been infected in the field and then held in the laboratory indicated that dead parasites predominated in animals held at 15oC whereas at 20oC healthy parasites were circulating freely in the haemolymph (Lester & Davis, 1981). We therefore kept infected *H. laevigata* at these two temperatures.

Infection in *H. laevigata* caused high mortality at both 15 and 20oC. Of 30 hatchery raised animals exposed to zoospores of *P. olseni* and kept at 20 ℃, 26 had died within 2 months, whereas only 6/30 controls had died. At 15 ℃, 23/23 died in the exposed group compared to 3/23 in the controls (Goggin, 1990). Infection in the exposed animals, and absence of infection in the controls, was confirmed by the Ray test. Greenlip kept at the higher temperature had more parasites in their tissues than those at the lower temperature.

In other experiments, the cockle *Anadara trapezia* was used in place of the abalone. We found that at 20oC there was no significant difference in mortality between infected and control cockles whereas at 27-30 ℃, 86% of 140 exposed cockles died compared to 33% of

140 controls (Goggin, 1990). Thus, cockles were more likely to die when they were subjected to both *Perkinsus* infection and high temperature.

A third stress was applied to infected cockles by decreasing water exchange in their tanks. Again, more animals with *Perkinsus* infections died than uninfected cockles (32/50 compared to 3/50; Goggin 1990).

These results indicate that infections by *Perkinsus olseni* can kill abalone and cockles, and that death is more likely if the animal is stressed.

Objective 4. Experimental recolonisation of die-back area

In July, 1987, 140 healthy adult greenlip were transplanted from Hardwicke Bay to Stansbury at the centre of the die-off in an attempt to recolonise the area. In the following October, a subsample of 20 indicated that the transplants were uninfected and growing well. However, by March 1988, many of the abalone had recently died and most of those left were heavily infected (15/20). We therefore left any further transplant attempts for at least a year.

In July, 1989, 195 greenlip from Tipara Reef were tagged and transplanted to the same site off Stansbury. A subsample the following March indicated that there was slight infection (1/33), no recent deaths, and signs of good growth (Fig. 2). The growth rates appear to be underestimates because most of the dead shells (Fig. 2, circled) were smaller than the original length recorded. Evidently the method of measuring varied slightly between the two trips. The epizootic had evidently passed and these abalone were successfully recolonising the area. A follow-up sample a year later, in April, 1992, indicated that 14 of 19 animals had become infected though there was no sign of recent deaths.

Discussion

All the objectives were achieved. *Perkinsus olseni* is evidently widespread in South Australia and needs to be taken into account in future abalone husbandry. Two surveys in Tasmania for *Perkinsus* sp., examining a range of hosts, failed to locate any infections (Goggin & Lester, 1988; Goggin et al., 1989). *Perkinsus* sp. has not been reported from Victoria, New South Wales, Western Australia or the Northern Territory, though surveys have not been carried out. From its distribution in Queensland and in South Australia it is likely to be all around Australia in warm temperate and tropical waters.

The low host specificity of *Perkinsus olseni* contrasts with that of *P. marinus* which is generally believed to be restricted to *Crassostrea virginica* though there is a report of *P. marinus* being recovered from the tissues of the gastropod *Boonea impressa* (White et al., 1987). The DNA sequence data of Goggin (in press) suggests that *Perkinsus atlanticus*, which is found in the cockle *Ruditapes decussatus* in Portugal, is closely related to, if not the same as, *Perkinsus olseni* and quite different from *P. marinus*.

Perkinsus olseni infects several abalone species and apparently becomes a problem only when wild abalone are stressed by some other factor such as higher than normal temperatures. However, in the laboratory we found that heavy infections would kill *Haliotis laevigata* even at the more moderate temperature of 15oC. In the USA, *Perkinsus marinus* kills oysters during the summer (Andrews, 1965).

The reintroduction of greenlip into the die back area may have helped to re-establish a greenlip population there as the second group thrived through at least one spawning season. Adult greenlip move little. Shepherd (1973) found they remained on the same rock for months at a time. McShane et al. (1988) concluded that recruitment of *Haliotis rubra* to a reef was related to the abundance of adults on that reef. Thus colonisation of areas denuded of abalone is slow. The Stansbury site was 25 km from the nearest population of greenlip which was on the Troubridge Shoals.

Implications and recommendations

Our results suggest that disease caused by *Perkinsus olseni* will continue to be important in the management and future development of the abalone industry, particularly in regards to aquaculture and abalone ranching. Reduction of losses from this parasite are likely to arise through improved husbandry methods, vaccines, genetic engineering, biological control, or some combination of these.

Several husbandry approaches could be considered. Moving the abalone into cool water during January and February may avoid mortality. A survey for *Perkinsus* at the ranching site may reveal that the main reservoir of infection is not the abalone but another mollusc such as *Pinna* (razorfish). The numbers of *Pinna* at the site could be reduced by damage or removal. Other mechanisms may be available to reduce the numbers of other shellfish if they were the main source of infection.

When many abalone are present, they themselves are likely to consititute an important reservoir of infection. The flagellated stage of *Perkinsus* that transmits the infection requires several days in dead tissue to develop. Thus to inhibit transmission, infected abalone should be removed from the site before they die, or soon after. Removal may only need to be done in January and February because as the water cools, the abalone are likely to recover from the infection.

As many abalone are able to recover, stimulation of their non-specific immune response may increase this ability. There are products on the market today that stimulate the response of prawns so that, for example, prawns dipped in the solution have an improved chance of surviving *Vibrio* infection than non-treated prawns ('Vibrogen S', Aqua Health Ltd., Bangkok). The protection lasts about 1 month. If a stimulant was found for abalone, the animals could be dip-treated before being laid on the bottom.

Possibly strains of abalone that are resistant to *Perkinsus olseni* could be developed by selective breeding, though this may be difficult because the parasite has low host specificity and is able to attack many mollusc species. In the future, abalone strains developed through genetic engineering may have a greater chance of success.

Some form of biological control may be possible. Because *Perkinsus olseni* is widespread, it is likely to have its own pathogens. Dinoflagellates are major pathogens of marine invertebrates, indeed *Perkinsus* itself is probably a dinoflagellate as discussed above. Many parasitic dinoflagellates develop in free-living dinoflagellates so it is likely *Perkinsus* itself is host to one or more dinoflagellates. When one is found, the main challenge would be to produce the large quantities needed to protect an abalone farm.

Probable viral particles have been reported from *Perkinsus atlanticus* by Azevedo (1990). A virus of *Perkinsus* could form the basis of a vaccine to be applied to abalone before they are released into the field. If the virus was relatively benign to *Perkinsus*, it is likely its pathogenicity could be increase by genetic transformation. How long a virus would remain in the tissues of abalone and confer protection is unknown.

Drugs have not yet been tested for their efficacy to control infection in abalone. Parasites away from the host are resistant to chlorine though hypnospores on the bottom of tanks are killed within seconds by freshwater. The parasites survive freezing (Goggin et al., 1990).

Perkinsus olseni is likely to be a part of the abalone industry for some time. It is clear that with more intensive management of populations, there are many approaches that could be used in the development of techniques to control the disease.

The project was initiated by Mr Andrew Geering, commercial abalone diver, and the Abalone Divers Association of South Australia, Central Zone, in 1986. It was supported by FIRTA from 1987 to 1990. From 1986 to 1988, most animals were collected with the help of Andrew Geering, Andrew Stephens and Chris Johnson. The Department of Fisheries, South Australia, strongly supported later field work. From 1988 to 1992 their officers, particularly Kevin Brandon, Andrew Dalgetty and Brian Davies, together with Kim Sewell and Louise Goggin from the University of Queensland, and Andrew Geering, collected and examined animals aboard the RV Ngerin. Dr Scoresby Shepherd coordinated activities in South Australia and supplied uninfected abalone from West Island. A related project on *Perkinsus* on the Great Barrier Reef was supported by the Australian Research Council (ARC Postdoctoral Fellowship and ARC Grant to Dr L. Goggin, University of Queensland). All these people made an important contribution to the project; without their help much less would have been achieved.

Intellectual property

Most of the information given above has already been published in the scientific literature. Papers arising from the work:

Goggin, C.L. and R.J.G. Lester 1987. The occurrence of *Perkinsus* sp. (Protozoa: Apicomplexa) in bivalves on the Great Barrier Reef. Austasia Aquaculture Magazine **2**: 7.

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Technical summary

Prevalence of *Perkinsus olseni* in molluscs from Yorke Peninsula, South Australia.

Haliotis laevigata

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22.3.86   Troubridge Pt 6/6 infected with Perkinsus
22.3.86   Bobs Ground   8/11
22.3.86   Halftide R.   3/7
22.3.86   Marion Reef   4/9
22.3.86   Hardwicke B   0/15
 6.7.87   Stansbury     1/2, other had dead Perkinsus
 4.7.87   Troubridge P  0/14
 6.7.87   Hardwicke B
                20 tagged plus 120 not-tagged moved to Stansbury.
5.10.87   Stansbury transplants  0/20
5.10.87   Coobowie B.   0/16
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14.3.88 Stansbury transplants 15/20
14.3.88 Ryans Ground 1/10 (near Troubridge shoal)
2.8.88 Marion Reef 1/20
3.8.88 Troubridge Pt 0/22
3.8.88 Suicide Pt 0/1
3.8.88 Pt. Yorke 0/45
2.8.88 Stansbury transplants 2/20, non-trans. 3/7
3.8.88 Hillock Pt. 0/8
3.8.88 Althorpe Is 0/30
3.8.88 Stenhouse B 0/23
15.3.89 Port Julia 0/2
15.3.89 Stansbury 3/9
15.3.89 Marion Reef 9/20
15.3.89 Tipara Reef 1/20
15.3.89 Wardang Is. 1/6
15.3.89 Hardwicke B. 0/20
15.3.89 Althorpe Is 1/20
15.3.89 S.Neptune Is 0/20
13.7.89 Tipara Reef 1/26 (origin of shipment)
14.7.89 195 transplanted from Tipara Reef to Stansbury
14.7.89 Stansbury 3/6
14.7.89 Marion Reef 7/25
27.7.89 Marion Bay 0/8 (frozen)
26.9.89 Marion Reef 13/157
10.1.90 Marion Reef 6/35
22.3.90 Stansbury, transplant from Tipara Reef 11.7.89
1/33 (plus 7 empty tagged shells)
22.3.90 Stansbury, juvenile 0/1
12.1.92 Black Point 2/2
27.4.92 Stansbury 14/19
30.4.92 Hardwicke Bay (frozen) 2/29
TOTAL 114/764 15%

Haliotis rubra
14.3.88 Ryans Gd 1/1
2.8.88 Troubridge P 0/1
2.8.88 Suicide Pt 0/1
2.8.88 Pt. Yorke 0/6
2.8.88 Hillock Pt. 1/3
2.8.88 Althorpe I 1/5
3.8.88 Stenhouse B 0/17
15.3.89 Marion Reef 0/1
15.3.89 Tipara Reef 2/11
15.3.89 Wardang Is 3/20
15.3.89 S.Neptune I 0/17
15.3.89 Althorpe I 2/20
TOTAL 10/103 10%

Haliotis cyclobates
22.3.86 Troubridge Pt 2/3
22.3.86 Bobs Gd. 1/4
22.3.86 Halftide R. 0/5
22.3.86 Marion Reef 1/3
6.7.87 Stansbury 0/3
6.7.87 Troubridge P 0/8
5.10.87 Stansbury 0/1
5.10.87 Coobowie B. 0/29
14.3.88 Stansbury 1/3
14.3.88 Ryans Gd 1/20

2.8.88	Stansbury	2/21
2.8.88	Troubridge P	0/9
2.8.88	Marion Reef	0/20
2.8.88	Edithburg jty	0/7
3.8.88	Davenport	0/3
3.8.88	Hillock Pt	1/3
15.3.89	Ardrossan	0/20
15.3.89	Port Julia	0/20
15.3.89	Stansbury	8/20
15.3.89	Marion Reef	1/20
15.3.89	Tipara Reef	1/20
15.3.89	Wardang I	1/4
15.3.89	Hardwicke B.	0/4
27.7.89	Marion Bay	0/6 (frozen)
26.9.89	Marion Reef	0/5
10.1.90	Marion Reef	0/4
22.3.90	Stansbury	0/6
12.1.92	Black Point	0/1
30.4.92	Hardwicke Bay (frozen)	0/1

TOTAL 20/273 7%

Haliotis scalaris

4.7.87	Troubridge P	0/5
4.10.87	Stansbury	0/1
4.10.87	Coobowie B	0/2
14.3.88	Ryans Gd	0/2
2.8.88	Stansbury	0/5
3.8.88	Troubridge P	1/10
3.8.88	Suicide Pt	0/1
3.8.88	Pt.Yorke	0/8
3.8.88	Hillock Pt	0/1
3.8.88	Althorpe I	0/2
3.8.88	Stenhouse B	0/16
15.3.89	Stansbury	1/6
15.3.89	Marion Reef	17/20
15.3.89	Tipara Reef	2/20
15.3.89	Wardang I	2/20
14.7.89	Stansbury	9/25
22.3.90	Stansbury	4/8

TOTAL 36/152 24%

Other molluscs

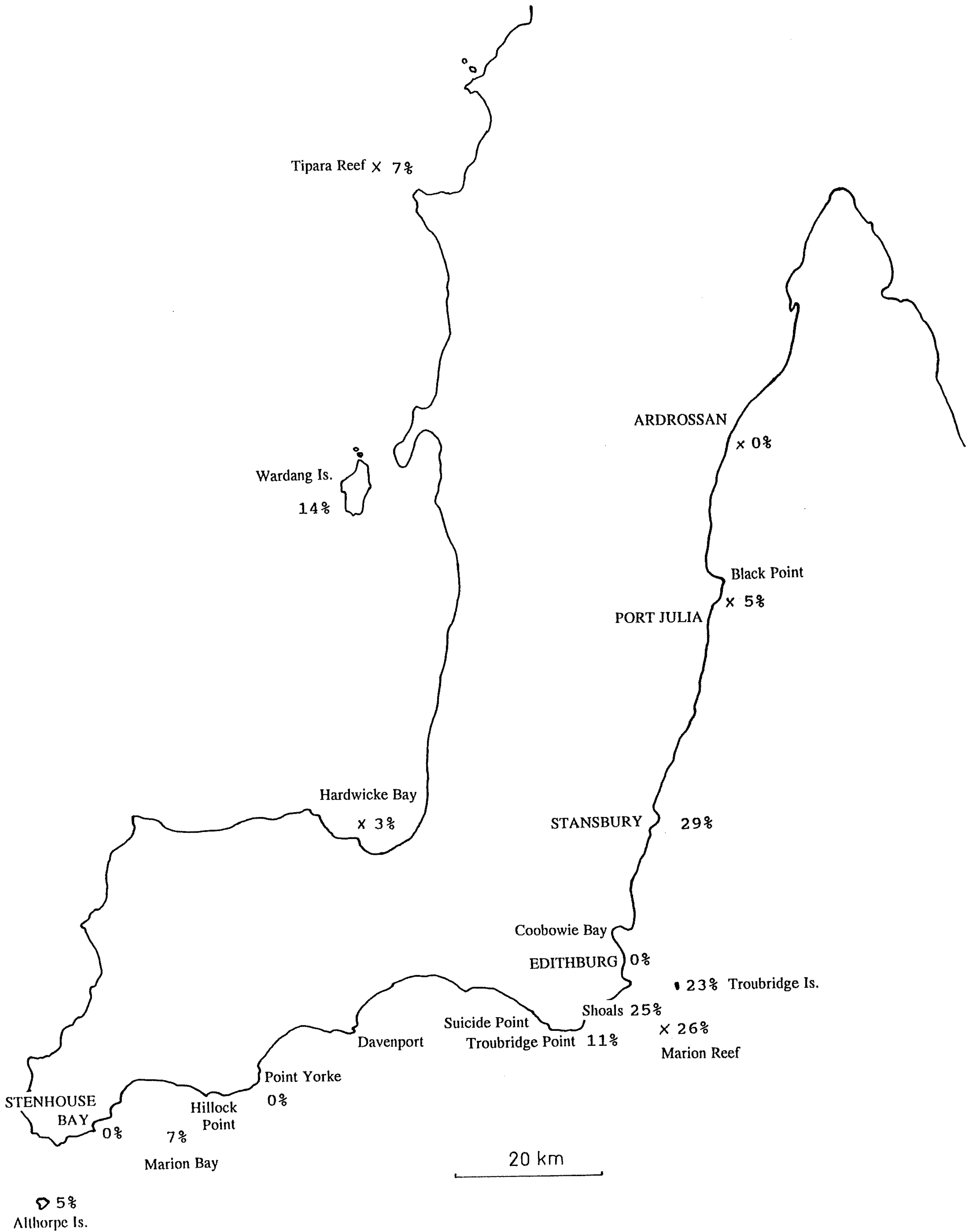
22.3.86 Troubridge Pt Elephant foot 0/1
22.3.86 Bobs Ground whelk 0/1, limpet 0/1
22.3.86 Halftide Rock limpet 0/3
14.3.88 Ryans Gd *Chlamys bifrons* 0/1
14.3.88 Edithburg Bay *Chlamys* 0/12, *Pinna bicolor* 0/2
2.8.88 Stansbury *Barbatia pistachia* 2/5, *Katelysia rhytiphora* 3/21, *P.bicolor* 0/13, *Malleus meridianus* 0/2, *Chlamys* 0/10, *Scutus* 0/1, *Chama* 0/1, *Brachidontes rostratus* 0/11.
2.8.88 Edithburg jetty *P.bicolor* 0/10, *Barbatia* 0/10 *Chama* (=*Cleidothaerus*?) 0/6, *M.meridianus* 0/8.
2.8.88 Marion Reef *Scutus* 0/1
2.8.88 Troubridge P *B. pistachia* 0/4, trochid *Granata* 0/3, *Malleus* 0/2, *Chama ruderalis* 0/2, clam *Mactra pura* 0/1
2.8.88 Davenport *Pinna* 0/3, cockle *Callucina lacteola* 0/1.
2.8.88 Althorpe I. *Pinna* 0/7, *Malleus* 0/6, *Cleidothaerus* 0/3.

2.8.88	Stenhouse Bay waraner *Turbo undulatus* 0/2, *Cleidothaerus* 0/1
15.3.89	Ardrossan *P. bicolor* 0/20
15.3.89	Port Julia *P. bicolor* 1/20, *Chlamys* sp. 0/9, *B. pistachia* 0/1
15.3.89	Stansbury *P. bicolor* 7/19
15.3.89	Wardang I *Cleidothaerus* sp. 1/1
14.7.89	Stansbury *Chlamys bifrons* 7/13, *Pinna bicolor* 3/10
14.7.89	Troubridge Shoal *K. rhytiphora* 7/30
27.7.89	Marion Bay (frozen) *Chlamys* 0/1
22.3.90	Stansbury *Chlamys* 2/9, *Pinna* 2/6
12.1.92	Black Point, Queen scallops 0/10
	TOTAL 35/304 12%

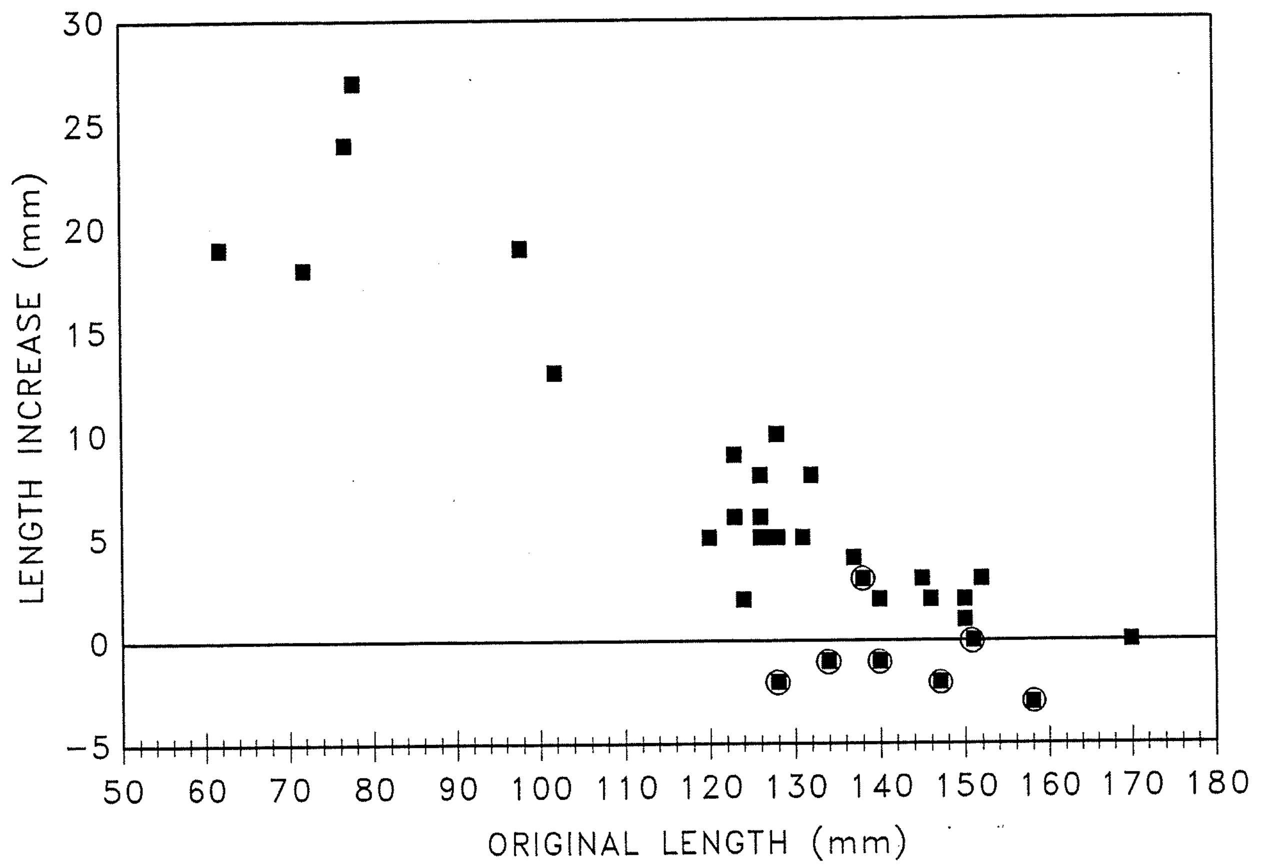
FIGURE CAPTIONS

Figure 1. Yorke Peninsula, South Australia, showing the localities mentioned in the text and the overall prevalence of *Perkinsus olseni* in 8 species of molluscs (4 *Haliotis* spp., *Chlamys* sp., *Barbatia* sp., *Katylesia* sp. and *Pinna* sp.). Sample size 30 to 320 total mollusc, taken January to August.

Figure 2. Original shell length of tagged *Haliotis laevigata* plotted against their increase in length after being off Stansbury for 8 months. Circles indicate dead shells.



Tipara Reef
7%
ARDROSSAN
0%
Wardang Is.
14%
Black Point
5%
PORT JULIA
Hardwicke Bay
3%
STANSBURY
29%
Coobowie Bay
EDITHBURG
0%
23%
Troubridge Is.
Shoals 25%
Suicide Point
26%
Davenport
Troubridge Point
11%
Marion Reef
Point Yorke
0%
STENHOUSE BAY
Hillock Point
0%
7%
Marion Bay
20 km
5%
Althorpe Is.



LENGTH INCREASE (mm)
30
25
20
15
10
5
0
-5
50
60
70
80
90
100
110
120
130
140
150
160
170
180
ORIGINAL LENGTH (mm)

FINAL REPORT

Diseases of abalone

FIRC: 87/009

The University of Queensland

Principle Investigator: Dr R.J.G. Lester, Department of Parasitology, The University of Queensland, Brisbane, Qld. 4072.
Tel: (07) 365-3305. Fax: (07) 365-1588.

FISHING INDUSTRY RESEARCH TRUST ACCOUNT

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FISHING INDUSTRY RESEARCH TRUST ACCOUNT

Summary

In 1985 and 1986 many greenlip abalone *Haliotis laevigata* near Edithburg in the St Vincent Gulf died. The protozoan parasite, *Perkinsus olseni*, was suspected to be the cause. We showed that *Perkinsus olseni* was seasonally abundant in greenlip from the edge of the die-back area. It was also common in three other species of abalone, *H. rubra*, *H. cyclobates* and *H. scalaris*, and was recovered from 4 species of bivalves from the same area (*Barbatia pistachia*, *Chlamys bifrons*, *Katylesia rhytiphora* and *Pinna bicolor*).

In July, 1987, 140 healthy adult abalone were transplanted from Hardwicke Bay to Stansbury at the centre of the die-off in an attempt to recolonise the area. This was done with the help of commercial abalone divers. In the following October, a subsample indicated the transplants were uninfected and were growing well. However, by March 1988, many of the abalone had recently died and the remainder were heavily infected.

In July, 1989, 195 greenlip from Tipara Reef were tagged and transplanted to the same site. A subsample the following March indicated that there was good growth, very little infection, and no sign of recent deaths. The epizootic had evidently passed and these abalone were successfully recolonising the area. However a follow-up sample a year later, in April, 1992, indicated that many of the animals had become infected though there was no sign of recent deaths.

The die-back area is near the northern limit for greenlip abalone in the Gulf of St Vincent. Laboratory experiments showed that abalone infected with Perkinsus died more frequently than uninfected abalone and that stress such as high temperature may predispose the abalone to disease. The time of the orginal die-back corresponded to warm winters on the Yorke Peninsula.

Laboratory and field observations suggest that the parasite is common in greenlip in late summer. During winter abalone are able to contain and eliminate the infection. We conclude that the parasite is widespread around the Yorke Peninsula in hosts other than greenlip, and that greenlip near the upper limit of their temperature range are likely to contract fatal infections.

Background

Soft yellowish abscesses were noticed in the flesh of blacklip abalone in the 1970s. The protozoan parasite *Perkinsus olseni* Lester & Davis, 1981 was found to be the cause. It was named after Mr A.M. Olsen, Chief Fisheries Officer, South Australia, who first brought the infection to our attention.

In the mid 1980s, greenlip abalone *Haliotis laevigata* disappeared from much of the western shore of the Gulf of St Vincent, South Australia. No unusual traces of pollutants or heavy metals could be found (Shepherd, 1985). Disease was suspected when Mr Andrew Geering, a commercial abalone diver, sent moribund abalone to The University of Queensland and I found them to be heavily infected with *Perkinsus* sp. (Lester, 1986).

Prior to the description of *Perkinsus olseni*, only one *Perkinsus* species was known, *P. marinus*, which occurs in oysters *Crassostrea virginica* along the eastern coast of the United States and apparently did not occur in any other host. Since then two other species have been described, *P. atlanticus* Azevedo 1989 from clams in Portugal, and *P. karlssoni* McGladdery et al., 1991 from scallops in eastern Canada.

Members of the genus *Perkinsus* have been variously classified as fungi, slime moulds and protozoans. They are currently placed in the protozoan phylum Apicomplexa along with gregarines, coccidia and malaria parasites though morphological, developmental and recent

DNA studies (Goggin & Barker, 1993) suggest the Perkinsidae are probably more closely related to dinoflagellates.

Objectives

Four objectives were stated in the original application. They were to: monitor the spread of *Perkinsus*, determine the range of hosts for the parasite, determine what predisposed abalone to fatal infection, and attempt to rehabilitate abalone in the devastated area.

Technical information

Diagnosis of *Perkinsus* infection is sometimes based on histological sections. However, a much more sensitive and accurate diagnosis is the Ray test (Ray, 1966). Pieces of abalone are cultured in fluid thioglycollate medium for 2 to 8 days then transferred to sea water and stained with Lugol's iodine. If a *Perkinsus* species is present, blue-black hypnospores can be seen dotting the surface of the tissue.

Methods

Molluscs were examined for *Perkinsus* using the Ray Test. A piece of muscle, digestive gland and gill was dropped into 15 ml fluid thioglycollate. Antibiotic was added and the vials left for 4 to 7 days prior to examination. Greenlip from the edge of the die back area were also investigated histologically to detect other infections.

Adult greenlip (140) for the first translocation experiment were collected by Mr Andrew Stevens from Hardwicke Bay on 6 July, 1987, driven across the Yorke Peninsula and put back in the water the same day 1 km off Stansbury. Twenty of them were tagged using numbered yellow plastic tags and superglue.

For the second translocation experiment, 195 adult greenlip were collected from Tipara Reef, again by Andrew Stevens, and driven to Edithburg. Here they were put aboard the RV Ngerin, tagged, and dropped off in 4m water 1 km off Stansbury as before.

For the laboratory experiments, uninfected abalone were collected from West Island, S.A., and 120 hatchery-reared greenlip were purchased from Adrian Cuthbertson, Tasmania.

Results

Objective 1. Survey to monitor the spread of the infection

Greenlip abalone occur as far north as Black Point in the Gulf of St Vincent (Fig. 1). However, at the start of the project most had disappeared north of Troubridge Shoals. On the Shoals, particularly Ryans Ground, Bobs Ground, Halftide Rock and Marion Reef, which corresponded to the edge of the die-back area, heavily infected greenlip were common. The few greenlip that could be found further north were generally infected. However, along the southern coast of the Peninsula west of Troubridge Point where greenlip were still common, only 1 of 127 greenlip sampled were infected (see Appendix 1 and Lester et al., 1990). *Perkinsus* was the only pathogen consistently found in histological sections of greenlip abalone from the Shoals. On one occasion a dead abalone which still contained some meat was recovered and *Perkinsus* was isolated from this. Thus there was a strong correlation between the die-back area, dead abalone and *Perkinsus* infection.

Perkinsus was recovered from multiple sites off the Yorke Peninsula in 1986, 1988, 1989, 1990 and 1992. Significant annual variation was not detected apart from low infection off Stansbury in 1990. Samples from Marion Reef suggest seasonal variation occurs as

prevalence in greenlip was higher in late summer than in late winter (9/20 (45%) in March, 7/25 (28%) in July, 13/157 (8%) in September and 6/35 (17%) in January.

Objective 2. Range of hosts

Nine mollusc species were found infected. These included four abalone, *Haliotis laevigata* (greenlip), *H. rubra* (blacklip), *H. cyclobates*, and *H. scalaris*, and five bivalves, *Chlamys bifrons* (scallop), *Barbatia pistachia* (ark shell), *Katelysia rhytiphora* (cockle), *Cleidothaerus* sp. (false oyster or jewel box), and *Pinna bicolor* (razorfish or penshell). Laboratory work described below suggests that these molluscs all contain the same species of *Perkinsus*. The data from different hosts, for January to August, have been combined in Fig. 1 to give an overall picture of the distribution of *Perkinsus* around the Yorke Peninsula. In all hosts the parasite is prevalent from Troubridge Point to Stansbury and much less common along the southern coast and in Hardwicke Bay. Infected animals were not found close inshore; samples from in and around the Edithburg jetty and the Stenhouse Bay jetty were free of infection.

Cross infection experiments in the laboratory showed that *Perkinsus* isolated from *Haliotis laevigata* would infect *H. cyclobates*, *H. scalaris* and even bivalves such as *Pinctada sugillata* and *Anadara trapezia*. Furthermore, isolates from the bivalve *Anadara trapezia* would develop in *Haliotis scalaris* and then reinfect other bivalves (Goggin et al., 1989). From this we concluded that one or several species of *Perkinsus* in Australia had very low host specificity. We were unable to determine how many species of *Perkinsus* were present.

Morphological differences noted in histological sections, such as size of trophozoite or type of host response, were found to be related to the host rather than the parasite and so these features could not be used to separate species. Dr Goggin, a postdoctoral fellow in the Department, sequenced the two internal transcribed spacer regions of the ribosomal RNA gene of *Perkinsus* spp. from three sources: *H. laevigata* from Marion Reef, *Anadara trapezia* from Moreton Bay (Qld.), and *Chama pacificus* from the Great Barrier Reef (Heron Island). Out of 600+ bases only 1 base differed between the *Perkinsus* from *C. pacificus* and the others, and one base between the *Perkinsus* from *A. trapezia* and the others (Goggin, in press). This strongly suggests that the isolates all belonged to one species, *Perkinsus olseni*. DNA of *Perkinsus marinus* sent from the USA differed at 73 locations indicating that it is clearly a separate species.

Dead queen scallops were observed in January 1992 off Black Point, in the north of the Gulf of St Vincent. No *Perkinsus* was found in 10 scallops examined, though it was present in 2 greenlip collected in the same area. The cause of death of the scallops remains unknown.

Objective 3. Factors associated with fatal infection in abalone

Preliminary observations on *Haliotis rubra* which had been infected in the field and then held in the laboratory indicated that dead parasites predominated in animals held at 15oC whereas at 20oC healthy parasites were circulating freely in the haemolymph (Lester & Davis, 1981). We therefore kept infected *H. laevigata* at these two temperatures.

Infection in *H. laevigata* caused high mortality at both 15 and 20oC. Of 30 hatchery raised animals exposed to zoospores of *P. olseni* and kept at 20 °C, 26 had died within 2 months, whereas only 6/30 controls had died. At 15 °C, 23/23 died in the exposed group compared to 3/23 in the controls (Goggin, 1990). Infection in the exposed animals, and absence of infection in the controls, was confirmed by the Ray test. Greenlip kept at the higher temperature had more parasites in their tissues than those at the lower temperature.

In other experiments, the cockle *Anadara trapezia* was used in place of the abalone. We found that at 20oC there was no significant difference in mortality between infected and control cockles whereas at 27-30 °C, 86% of 140 exposed cockles died compared to 33% of

140 controls (Goggin, 1990). Thus, cockles were more likely to die when they were subjected to both *Perkinsus* infection and high temperature.

A third stress was applied to infected cockles by decreasing water exchange in their tanks. Again, more animals with *Perkinsus* infections died than uninfected cockles (32/50 compared to 3/50; Goggin 1990).

These results indicate that infections by *Perkinsus olseni* can kill abalone and cockles, and that death is more likely if the animal is stressed.

Objective 4. Experimental recolonisation of die-back area

In July, 1987, 140 healthy adult greenlip were transplanted from Hardwicke Bay to Stansbury at the centre of the die-off in an attempt to recolonise the area. In the following October, a subsample of 20 indicated that the transplants were uninfected and growing well. However, by March 1988, many of the abalone had recently died and most of those left were heavily infected (15/20). We therefore left any further transplant attempts for at least a year.

In July, 1989, 195 greenlip from Tipara Reef were tagged and transplanted to the same site off Stansbury. A subsample the following March indicated that there was slight infection (1/33), no recent deaths, and signs of good growth (Fig. 2). The growth rates appear to be underestimates because most of the dead shells (Fig. 2, circled) were smaller than the original length recorded. Evidently the method of measuring varied slightly between the two trips. The epizootic had evidently passed and these abalone were successfully recolonising the area. A follow-up sample a year later, in April, 1992, indicated that 14 of 19 animals had become infected though there was no sign of recent deaths.

Discussion

All the objectives were achieved. *Perkinsus olseni* is evidently widespread in South Australia and needs to be taken into account in future abalone husbandry. Two surveys in Tasmania for *Perkinsus* sp., examining a range of hosts, failed to locate any infections (Goggin & Lester, 1988; Goggin et al., 1989). *Perkinsus* sp. has not been reported from Victoria, New South Wales, Western Australia or the Northern Territory, though surveys have not been carried out. From its distribution in Queensland and in South Australia it is likely to be all around Australia in warm temperate and tropical waters.

The low host specificity of *Perkinsus olseni* contrasts with that of *P. marinus* which is generally believed to be restricted to *Crassostrea virginica* though there is a report of *P. marinus* being recovered from the tissues of the gastropod *Boonea impressa* (White et al., 1987). The DNA sequence data of Goggin (in press) suggests that *Perkinsus atlanticus*, which is found in the cockle *Ruditapes decussatus* in Portugal, is closely related to, if not the same as, *Perkinsus olseni* and quite different from *P. marinus*.

Perkinsus olseni infects several abalone species and apparently becomes a problem only when wild abalone are stressed by some other factor such as higher than normal temperatures. However, in the laboratory we found that heavy infections would kill *Haliotis laevigata* even at the more moderate temperature of 15oC. In the USA, *Perkinsus marinus* kills oysters during the summer (Andrews, 1965).

The reintroduction of greenlip into the die back area may have helped to re-establish a greenlip population there as the second group thrived through at least one spawning season. Adult greenlip move little. Shepherd (1973) found they remained on the same rock for months at a time. McShane et al. (1988) concluded that recruitment of *Haliotis rubra* to a reef was related to the abundance of adults on that reef. Thus colonisation of areas denuded of abalone is slow. The Stansbury site was 25 km from the nearest population of greenlip which was on the Troubridge Shoals.

Implications and recommendations

Our results suggest that disease caused by *Perkinsus olseni* will continue to be important in the management and future development of the abalone industry, particularly in regards to aquaculture and abalone ranching. Reduction of losses from this parasite are likely to arise through improved husbandry methods, vaccines, genetic engineering, biological control, or some combination of these.

Several husbandry approaches could be considered. Moving the abalone into cool water during January and February may avoid mortality. A survey for *Perkinsus* at the ranching site may reveal that the main reservoir of infection is not the abalone but another mollusc such as *Pinna* (razorfish). The numbers of *Pinna* at the site could be reduced by damage or removal. Other mechanisms may be available to reduce the numbers of other shellfish if they were the main source of infection.

When many abalone are present, they themselves are likely to consititute an important reservoir of infection. The flagellated stage of *Perkinsus* that transmits the infection requires several days in dead tissue to develop. Thus to inhibit transmission, infected abalone should be removed from the site before they die, or soon after. Removal may only need to be done in January and February because as the water cools, the abalone are likely to recover from the infection.

As many abalone are able to recover, stimulation of their non-specific immune response may increase this ability. There are products on the market today that stimulate the response of prawns so that, for example, prawns dipped in the solution have an improved chance of surviving *Vibrio* infection than non-treated prawns ('Vibrogen S', Aqua Health Ltd., Bangkok). The protection lasts about 1 month. If a stimulant was found for abalone, the animals could be dip-treated before being laid on the bottom.

Possibly strains of abalone that are resistant to *Perkinsus olseni* could be developed by selective breeding, though this may be difficult because the parasite has low host specificity and is able to attack many mollusc species. In the future, abalone strains developed through genetic engineering may have a greater chance of success.

Some form of biological control may be possible. Because *Perkinsus olseni* is widespread, it is likely to have its own pathogens. Dinoflagellates are major pathogens of marine invertebrates, indeed *Perkinsus* itself is probably a dinoflagellate as discussed above. Many parasitic dinoflagellates develop in free-living dinoflagellates so it is likely *Perkinsus* itself is host to one or more dinoflagellates. When one is found, the main challenge would be to produce the large quantities needed to protect an abalone farm.

Probable viral particles have been reported from *Perkinsus atlanticus* by Azevedo (1990). A virus of *Perkinsus* could form the basis of a vaccine to be applied to abalone before they are released into the field. If the virus was relatively benign to *Perkinsus*, it is likely its pathogenicity could be increase by genetic transformation. How long a virus would remain in the tissues of abalone and confer protection is unknown.

Drugs have not yet been tested for their efficacy to control infection in abalone. Parasites away from the host are resistant to chlorine though hypnospores on the bottom of tanks are killed within seconds by freshwater. The parasites survive freezing (Goggin et al., 1990).

Perkinsus olseni is likely to be a part of the abalone industry for some time. It is clear that with more intensive management of populations, there are many approaches that could be used in the development of techniques to control the disease.

The project was initiated by Mr Andrew Geering, commercial abalone diver, and the Abalone Divers Association of South Australia, Central Zone, in 1986. It was supported by FIRTA from 1987 to 1990. From 1986 to 1988, most animals were collected with the help of Andrew Geering, Andrew Stephens and Chris Johnson. The Department of Fisheries, South Australia, strongly supported later field work. From 1988 to 1992 their officers, particularly Kevin Brandon, Andrew Dalgetty and Brian Davies, together with Kim Sewell and Louise Goggin from the University of Queensland, and Andrew Geering, collected and examined animals aboard the RV Ngerin. Dr Scoresby Shepherd coordinated activities in South Australia and supplied uninfected abalone from West Island. A related project on *Perkinsus* on the Great Barrier Reef was supported by the Australian Research Council (ARC Postdoctoral Fellowship and ARC Grant to Dr L. Goggin, University of Queensland). All these people made an important contribution to the project; without their help much less would have been achieved.

Intellectual property

Most of the information given above has already been published in the scientific literature. Papers arising from the work:

Goggin, C.L. and R.J.G. Lester 1987. The occurrence of *Perkinsus* sp. (Protozoa: Apicomplexa) in bivalves on the Great Barrier Reef. Austasia Aquaculture Magazine **2**: 7.

Goggin, C.L. and R.J.G. Lester 1987. The occurrence of *Perkinsus* species (Protozoa, Apicomplexa) in bivalves from the Great Barrier Reef. Diseases of Aquatic Organisms **3**: 113-117.

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Technical summary

Prevalence of *Perkinsus olseni* in molluscs from Yorke Peninsula, South Australia.

Haliotis laevigata

22.3.86 Troubridge Pt 6/6 infected with Perkinsus
22.3.86 Bobs Ground 8/11
22.3.86 Halftide R. 3/7
22.3.86 Marion Reef 4/9
22.3.86 Hardwicke B 0/15
6.7.87 Stansbury 1/2, other had dead Perkinsus
4.7.87 Troubridge P 0/14
6.7.87 Hardwicke B
20 tagged plus 120 not-tagged moved to Stansbury.
5.10.87 Stansbury transplants 0/20
5.10.87 Coobowie B. 0/16

14.3.88 Stansbury transplants 15/20
14.3.88 Ryans Ground 1/10 (near Troubridge shoal)
2.8.88 Marion Reef 1/20
3.8.88 Troubridge Pt 0/22
3.8.88 Suicide Pt 0/1
3.8.88 Pt. Yorke 0/45
2.8.88 Stansbury transplants 2/20, non-trans. 3/7
3.8.88 Hillock Pt. 0/8
3.8.88 Althorpe Is 0/30
3.8.88 Stenhouse B 0/23
15.3.89 Port Julia 0/2
15.3.89 Stansbury 3/9
15.3.89 Marion Reef 9/20
15.3.89 Tipara Reef 1/20
15.3.89 Wardang Is. 1/6
15.3.89 Hardwicke B. 0/20
15.3.89 Althorpe Is 1/20
15.3.89 S.Neptune Is 0/20
13.7.89 Tipara Reef 1/26 (origin of shipment)
14.7.89 195 transplanted from Tipara Reef to Stansbury
14.7.89 Stansbury 3/6
14.7.89 Marion Reef 7/25
27.7.89 Marion Bay 0/8 (frozen)
26.9.89 Marion Reef 13/157
10.1.90 Marion Reef 6/35
22.3.90 Stansbury, transplant from Tipara Reef 11.7.89
1/33 (plus 7 empty tagged shells)
22.3.90 Stansbury, juvenile 0/1
12.1.92 Black Point 2/2
27.4.92 Stansbury 14/19
30.4.92 Hardwicke Bay (frozen) 2/29

TOTAL 114/764 15%

Haliotis rubra

14.3.88 Ryans Gd 1/1
2.8.88 Troubridge P 0/1
2.8.88 Suicide Pt 0/1
2.8.88 Pt. Yorke 0/6
2.8.88 Hillock Pt. 1/3
2.8.88 Althorpe I 1/5
3.8.88 Stenhouse B 0/17
15.3.89 Marion Reef 0/1
15.3.89 Tipara Reef 2/11
15.3.89 Wardang Is 3/20
15.3.89 S.Neptune I 0/17
15.3.89 Althorpe I 2/20

TOTAL 10/103 10%

Haliotis cyclobates

22.3.86 Troubridge Pt 2/3
22.3.86 Bobs Gd. 1/4
22.3.86 Halftide R. 0/5
22.3.86 Marion Reef 1/3
6.7.87 Stansbury 0/3
6.7.87 Troubridge P 0/8
5.10.87 Stansbury 0/1
5.10.87 Coobowie B. 0/29
14.3.88 Stansbury 1/3
14.3.88 Ryans Gd 1/20

2.8.88	Stansbury	2/21	
2.8.88	Troubridge P	0/9	
2.8.88	Marion Reef	0/20	
2.8.88	Edithburg jty	0/7	
3.8.88	Davenport	0/3	
3.8.88	Hillock Pt	1/3	
15.3.89	Ardrossan	0/20	
15.3.89	Port Julia	0/20	
15.3.89	Stansbury	8/20	
15.3.89	Marion Reef	1/20	
15.3.89	Tipara Reef	1/20	
15.3.89	Wardang I	1/4	
15.3.89	Hardwicke B.	0/4	
27.7.89	Marion Bay	0/6	(frozen)
26.9.89	Marion Reef	0/5	
10.1.90	Marion Reef	0/4	
22.3.90	Stansbury	0/6	
12.1.92	Black Point	0/1	
30.4.92	Hardwicke Bay (frozen)	0/1	

TOTAL 20/273 7%

Haliotis scalaris

4.7.87	Troubridge P	0/5
4.10.87	Stansbury	0/1
4.10.87	Coobowie B	0/2
14.3.88	Ryans Gd	0/2
2.8.88	Stansbury	0/5
3.8.88	Troubridge P	1/10
3.8.88	Suicide Pt	0/1
3.8.88	Pt.Yorke	0/8
3.8.88	Hillock Pt	0/1
3.8.88	Althorpe I	0/2
3.8.88	Stenhouse B	0/16
15.3.89	Stansbury	1/6
15.3.89	Marion Reef	17/20
15.3.89	Tipara Reef	2/20
15.3.89	Wardang I	2/20
14.7.89	Stansbury	9/25
22.3.90	Stansbury	4/8

TOTAL 36/152 24%

Other molluscs

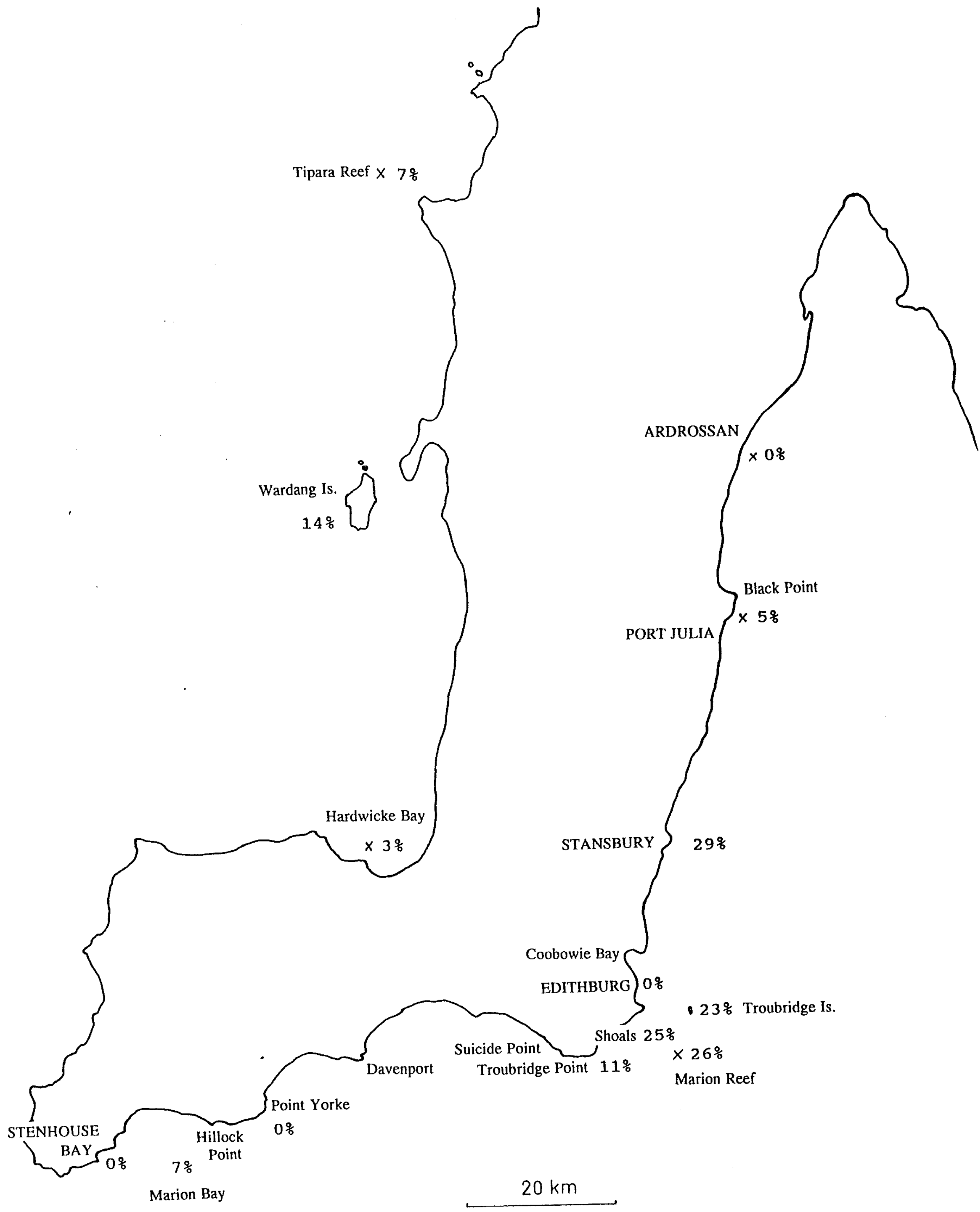
22.3.86 Troubridge Pt Elephant foot 0/1
22.3.86 Bobs Ground whelk 0/1, limpet 0/1
22.3.86 Halftide Rock limpet 0/3
14.3.88 Ryans Gd *Chlamys bifrons* 0/1
14.3.88 Edithburg Bay *Chlamys* 0/12, *Pinna bicolor* 0/2
2.8.88 Stansbury *Barbatia pistachia* 2/5, *Katelysia rhytiphora* 3/21, *P.bicolor* 0/13, *Malleus meridianus* 0/2, *Chlamys* 0/10, *Scutus* 0/1, *Chama* 0/1, *Brachidontes rostratus* 0/11.
2.8.88 Edithburg jetty *P.bicolor* 0/10, *Barbatia* 0/10 *Chama (=Cleidothaerus?)* 0/6, *M.meridianus* 0/8.
2.8.88 Marion Reef *Scutus* 0/1
2.8.88 Troubridge P *B. pistachia* 0/4, trochid *Granata* 0/3, *Malleus* 0/2, *Chama ruderalis* 0/2, clam *Mactra pura* 0/1
2.8.88 Davenport *Pinna* 0/3, cockle *Callucina lacteola* 0/1.
2.8.88 Althorpe I. *Pinna* 0/7, *Malleus* 0/6, *Cleidothaerus* 0/3.

2.8.88	Stenhouse Bay waraner *Turbo undulatus* 0/2, *Cleidothaerus* 0/1
15.3.89	Ardrossan *P. bicolor* 0/20
15.3.89	Port Julia *P. bicolor* 1/20, *Chlamys* sp. 0/9, *B. pistachia* 0/1
15.3.89	Stansbury *P. bicolor* 7/19
15.3.89	Wardang I *Cleidothaerus* sp. 1/1
14.7.89	Stansbury *Chlamys bifrons* 7/13, *Pinna bicolor* 3/10
14.7.89	Troubridge Shoal *K. rhytiphora* 7/30
27.7.89	Marion Bay (frozen) *Chlamys* 0/1
22.3.90	Stansbury *Chlamys* 2/9, *Pinna* 2/6
12.1.92	Black Point, Queen scallops 0/10
	TOTAL 35/304 12%

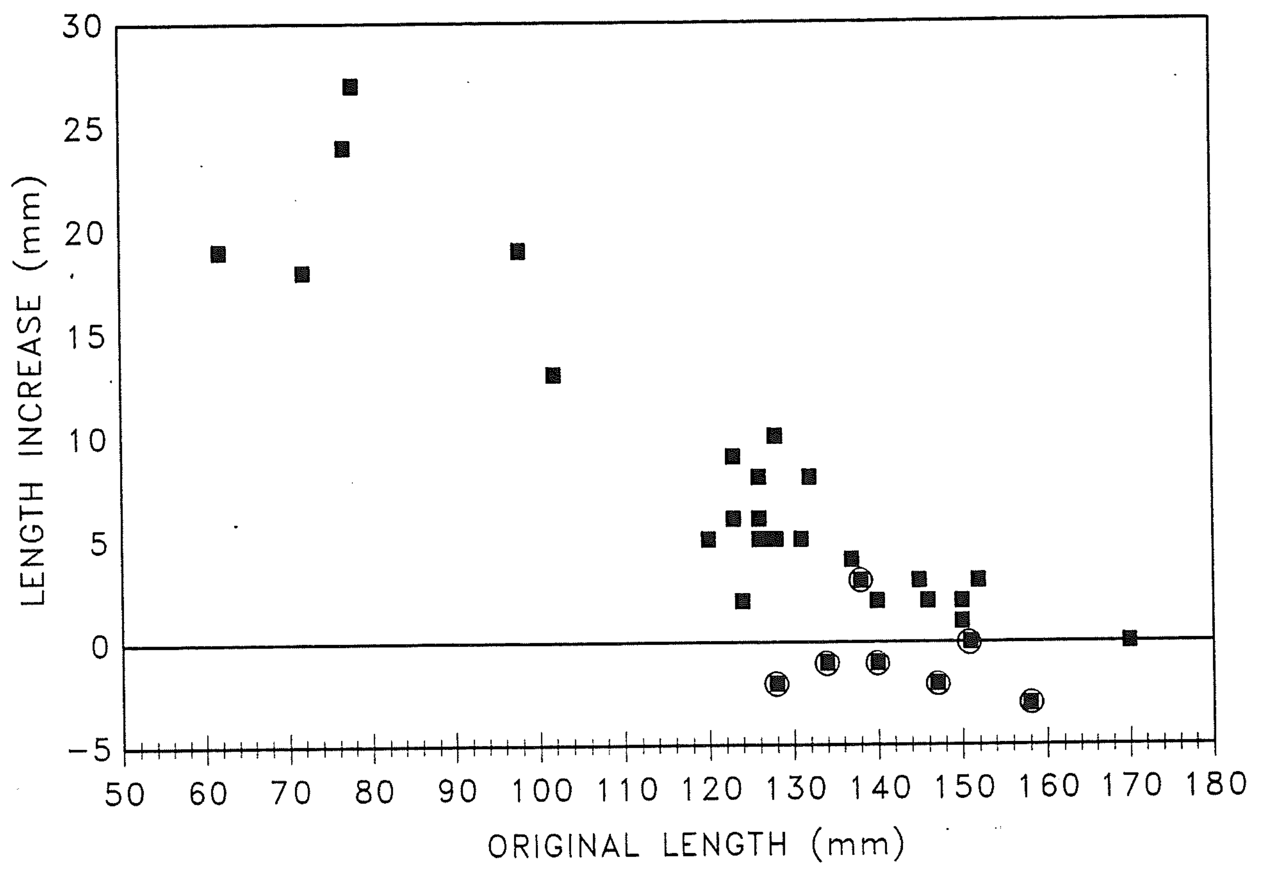
FIGURE CAPTIONS

Figure 1. Yorke Peninsula, South Australia, showing the localities mentioned in the text and the overall prevalence of *Perkinsus olseni* in 8 species of molluscs (4 *Haliotis* spp., *Chlamys* sp., *Barbatia* sp., *Katylesia* sp. and *Pinna* sp.). Sample size 30 to 320 total mollusc, taken January to August.

Figure 2. Original shell length of tagged *Haliotis laevigata* plotted against their increase in length after being off Stansbury for 8 months. Circles indicate dead shells.



Tipara Reef x 7%
Wardang Is.
14%
ARDROSSAN
x 0%
Black Point
x 5%
PORT JULIA
Hardwicke Bay
x 3%
STANSBURY
29%
Coobowie Bay
EDITHBURG
0%
23% Troubridge Is.
Shoals 25%
x 26%
Marion Reef
Suicide Point
Troubridge Point 11%
Davenport
Point Yorke
0%
STENHOUSE
BAY
0%
Hillock
Point
7%
Marion Bay
20 km
5%
Althorpe Is.



LENGTH INCREASE (mm)
30
25
20
15
10
5
0
-5
50
60
70
80
90
100
110
120
130
140
150
160
170
180
ORIGINAL LENGTH (mm)