PARASITISM AND INVASIVE SPECIES:
AN ECOLOGICAL STUDY OF MUSSEL POPULATIONS

THESIS

Submitted in fulfilment of the

requirements for the Degree of

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by

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS

Despite the fact that the acknowledgements are usually the most read part of any thesis (pers. obs.), most people leave them for the last minute rush, and I am not an exception. There are many people who, at one time or another, gave me a hand and I just hope I will not leave anybody out, if so.... pleeze forgive me!

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Humm... I think I did all the work for the reproduction chapter myself, but if I'm forgetting somebody, please forgive me... can't remember when last I got some decent sleep.

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DECLARATION

The work described in this thesis was carried out in the Department of Zoology and Entomology, Rhodes University under the supervision of Professor Christopher D. McQuaid. This study represents original work by the author and has not been submitted in any form to another University.

ABSTRACT

The Mediterranean mussel *Mytilus galloprovincialis*, was introduced accidentally to South Africa and has since become invasive. One possible explanation for the success of this species is that it has been released from the effects of parasites which affect the indigenous species of mussels. The aim of this work was to examine the parasitic load of different mussel populations along the southern African coast and to assess the effects of parasites on their hosts.

A survey was done to quantify the levels of parasitism in indigenous populations of *Perna perna* from Southern Africa and of *Mytilus galloprovincialis* from South Africa (where it is exotic) and Spain (where it is indigenous). This survey was carried out at three different geographic scales: small scale (metres), studying the incidence of parasites within a mussel bed; medium scale (kilometres to tens of kilometres), comparing prevalences of infection among different localities; and large scale (hundreds to thousands of kilometres), examining infection rates along the Southern African coast, as well as the coast of the Basque Country (North Spain). During this survey metazoan parasites other than trematodes were rare. Four species of trematodes were commonly found infecting the indigenous mussel *Perna perna*. These were metacercariae of the genus *Proctoeces*, bucephalid sporocysts, encysted metacercariae on the labial palps and gelatinous cysts with metacercariae inside. A detailed description of *Proctoeces* is given; the other parasites are described elsewhere. No parasites were found in *M. galloprovincialis* either in South Africa or in Spain.

This thesis concentrates on the study of prevalences of *Proctoeces* and bucephalid sporocysts

and their effects on *Perna perna*. Infection rates with *Proctoeces* are highly dependent on the sex of the host, with more females than males being infected, and are also size dependent, though only for females. Identification of the sex of mussels infected with bucephalid sporocysts is often not possible since the sporocysts spread over the gonad and replace it. Prevalence of infection with this parasite also increases with the size of the host.

To study the influence of *Proctoeces* and bucephalid sporocysts on the ecological fitness of *Perna perna*, their effects on survival and competitive ability were tested. The results showed significant negative effects. Both parasites significantly depressed condition but only after spawning, when the mussels were already stressed. In order to check for effects on host survival, the effects of both parasites on mortality rates, gaping behaviour and water loss of mussels exposed to air were also examined. Neither parasite affected mortality rate or gaping behaviour of *Perna perna*. *Proctoeces* did not affect the amount of water lost by mussels, but the bucephalid sporocysts did. Mussels infected with sporocysts lost significantly more water than non-infected individuals. This increase in water loss was not related to the gaping behaviour, but a test of the strength of the adductor muscles showed that less force was needed to open mussels with bucephalid sporocysts than non-infected mussels. This was not the case for mussels infected by *Proctoeces*. Weaker mussels will fail to seal the valves properly, resulting in an increase of water loss on exposure to air by evaporation.

Another factor that will have an obvious effect on a population is the reproductive output of the animals. Histological sections of the gonad of infected and non-infected females were cut to study the effects of both parasites on reproduction. Statistical tests comparing the numbers and sizes of oocytes in females infected with *Proctoeces* and non-infected females showed

no significant differences. However, bucephalid sporocysts have a dramatic effect on reproduction by castrating the host, leaving no trace of sex products.

One of the major factors shaping the composition of a mussel bed is competition for space and food, with smaller mussels being at a competitive disadvantage. Thus, in order to examine effects of both parasites on the competitive ability of *Perna perna*, summer and winter growth rates were compared for infected and non-infected mussels. *Proctoeces* reduced growth both in summer and in winter whilst bucephalid sporocysts had no significant effect.

Both growth and reproduction are important components of the energy budget of an animal, and each is affected by either *Proctoeces* or the bucephalid sporocysts. In an attempt to test if *Perna perna* compensates energetically for these negative effects, filtration rates and oxygen consumption of mussels with and without parasites were measured. Neither parasite had a significant effect on filtration rates or oxygen consumption of the host.

All these results indicate that both *Proctoeces* and the bucephalid sporocysts have a detrimental effect on their host, and that the mussels do not compensate for these negative effects. There is neither an increase in filtration, nor a decrease in respiration to balance the energy lost to the parasite.

The two parasites studied affect the host in different but complementary ways. The effects of both parasites are concentrated on those size classes of mussel which channel most energy into the portion of the energy budget affected by the parasite. *Proctoeces* affects growth only in the smaller individuals, which under normal conditions would put most energy into growth;

and the bucephalid sporocysts castrate the bigger mussels, which would expend most energy on reproduction. By reducing growth rates of small mussels or castrating large mussels, these parasites effectively remove them from the breeding population and reduce their competitive abilities. These negative effects, together with the high prevalence of both parasites in *Perna perna* along the South African coast and their absence in *Mytilus galloprovincialis*, suggest that parasites may be an important reason for the success of *Mytilus*.

THESIS STRUCTURE

Chapter one is a general introduction to the thesis, presenting the original hypothesis and putting the subject into context.

Chapter two studies the prevalence of infection by trematode parasites in indigenous and exotic mussel populations in South Africa, and compares the infection rates of the mussel *Mytilus galloprovincialis* in exotic populations in South Africa and indigenous populations from the North of Spain.

Chapter three clarifies the life-cycle of one of the parasites found during this study using molecular techniques.

The rest of the thesis is devoted to assessing the effects of the two most important parasites on the ecological fitness of their host.

Chapter four examines the effects of the parasites on the general fitness and survival of the brown mussel *Perna perna*, whilst Chapters five to seven deal with several factors that may affect the dynamics of the host population by affecting the competitive ability of the mussels (i.e. reproductive output, growth, filtration rates and oxygen consumption).

Finally, Chapter eight is a general discussion which summarizes the findings and draws some conclusions.

CHAPTER 1.

GENERAL INTRODUCTION.

The word ecology (from the Greek "oikos" for home and "logos" for wisdom) was coined by the German biologist Ernst Haeckel in 1866 to express his idea of the varied animals of a place evolving to live together, sharing a home and its resources (Colinvaux, 1993). Krebs (1978) defined ecology as the scientific study of the interactions that determine the distribution and abundance of organisms. There are five main categories of interactions among organisms: competition, predation, parasitism, mutualism and detritivory (Begon *et al.*, 1990). Even though it is generally accepted that there are more parasitic than free living species, and that those organisms that are not parasites are usually hosts (Schmidt and Roberts, 1989), ecology textbooks rarely dedicate more than a few pages to the subject of parasites (Ricklefs, 1973; Krebs, 1978; Begon *et al.*, 1990; Colinvaux, 1993). Most ecological research has emphasized predation and competition (Connell, 1975, 1983; Schoener, 1983; Herbold and Moyle, 1986), while host-parasite interactions have been relatively unstudied.

Exceptions to this were the works of Elton, who advocated ecological surveys of parasites on the same principles as for free living animals (see Williams and Jones, 1994), Hegner, who suggested that the object of parasitology should be the study of the inter-relationships between the host and the parasite (see Dogiel, 1964), and Dogiel. Dogiel was the founder of Soviet ecological parasitology (Dogiel *et al.*, 1961). He said that ecological parasitology is concerned with the study of the relationship between the parasite fauna treated as a unit and the changes in the environmental and physiological conditions of the host (Dogiel, 1964). For him, parasitism was an ecological concept; consequently parasitology should concern itself not only

with the parasite and the host, but also with the relationships and adaptations between them. Unfortunately most ecologists did not follow these examples, and until the 1970s few parasitologists realized the importance of adopting an ecological approach to the study of parasites. In the last 20 years more and more authors have realized the importance of bringing the disciplines of ecology and parasitology together (Rohde, 1982; Kinne, 1983; Toft, 1991; Jensen and Mouritsen, 1992; Huxham *et al.*, 1993), and there has been increasing interest in the impact of parasites at a population and community level (Dobson and Hudson, 1986; McCallum and Dobson, 1995).

An important term used in the discusion of parasitism from an ecological perspective is "*regulation* ". Regulation refers to how much the parasite depresses the host population, at equilibrium, compared to a host population that is parasite free (Toft, 1991). If regulation occurs, it may affect the interaction of the host with other species, such as competitors or predators, and the structure of host communities. Traditionally, the major determinants of community structure were assumed to be competition and predation (Connell, 1975, 1983; Schoener, 1983), nevertheless recent theoretical, experimental and field studies indicate that parasitism is also important (Brown *et al.*, 1988; Minchella and Scott, 1991). In 1978, Anderson and May published two theoretical papers which provided a clear demonstration of the potential of parasites to regulate host populations (Anderson and May, 1978; May and Anderson, 1978). Over recent years several studies have suggested that macroparasites can regulate host population abundance (Kuris, 1974; Anderson, 1978; Anderson and Crombie, 1984; Scott and Anderson, 1984; Blower and Roughgarden, 1987). Parasites can influence host survival directly or indirectly by increasing the susceptibility of the infected host to

predation or by reducing its competitive fitness (Holmes and Bethel, 1972; Rohde, 1982; Giles, 1983; Rau, 1983a; 1983b; Milinski, 1984; Scott and Anderson, 1984; Brown and Brown, 1986; Price *et al.*, 1986; Wilson and Edwards, 1986; Dobson, 1988; Figueras and Fisher, 1988; Scott, 1988; Keymer and Read, 1991; Minchella and Scott, 1991; Stewart, 1991; Hudson *et al.*, 1992; Jensen and Mouritsen, 1992; Kuris and Lafferty, 1992; Lafferty, 1992). Price *et al.* (1986) reviewed different ways in which parasitism may affect the outcome of competition, and concluded that mediation by parasites is very common in nature and must be regarded as one of the major types of interaction in ecological systems.

The importance of parasites as potential regulatory agents has always been controversial. May (1983) and Scott (1988) expressed the opinion that there is no single factor which acts as *the regulator* , nevertheless the role of parasites must not be ignored or underestimated. By now, there is little doubt that parasites have the potential to reduce the fitness of their hosts. However, for many years ecologists have carried out their studies at the community level without taking into consideration the importance of parasitism (Price, 1980; May, 1983; Minchella and Scott, 1991), and Lauckner (1986) goes as far as to doubt the validity of marine ecological data and concepts which do not take into account the effects of parasites.

One question ecologists have busied themselves with for many years is that of biological invasions, and many books have been written on the subject (e.g. Macdonald *et al.*, 1986; Mooney and Drake, 1986; Drake *et al.* 1989; Hengeveld, 1989 and Ramakrishnan, 1991). The introduction of a new animal into an ecosystem usually creates general concern about its effects on the native fauna. Aliens may influence the community by changing species

diversity, community structure and function and/or the ecological processes that are dependent on the interaction between organisms, e.g. competition (Breytenbach, 1986; Bruton and van As, 1986; Ramakrishnan and Vitousek, 1989).

The concern about species introduction has increased in recent years due to the greater extent and speed of transport, which makes the introduction of more species possible (Carlton, 1974; Moyle, 1991; Stewart, 1991; Kennedy, 1993). And more and more authors have become aware of the risk of introducing pests and pathogens with other species (Bruton and Merron, 1985; Balon and Bruton, 1986; Ebenhard, 1988; Stewart, 1991; Kennedy, 1993; Sindermann, 1993; Occhipinti Ambrogi, 1994). Bain (1993) developed a checklist for assessing potential environmental impacts of introduced species, emphasizing fish. He included biological effects, habitat alterations, hybridization, population control and diseases and parasites as indicators of impact.

An introduced species may become invasive due to the absence of natural controls (e.g. predators, parasites and diseases) or to its ability to adopt alternative life styles in response to a new environment (Bruton, 1986).

When an animal is introduced into a new ecosystem, there are three possibilities regarding parasites:

1- the animal leaves its usual parasites behind (Dobson and May, 1986; Dobson, 1988; Lafferty and Kuris, 1994);

2- the animal brings parasites with it (Barton, 1994);

3- the animal acquires the indigenous parasites after its introduction (Barton, 1994).

The first two possibilities would more than likely result in a competitive advantage for the invader. In relation to hypothesis one, Dobson (1988) said that some parasites become lost during the colonization process, presumably due to the absence of specific vectors. Clearly parasites with intermediate hosts are less likely to colonize a new area (Eberhard, 1988; Kennedy, 1993). An example of this situation could be the introduction of the European zebra mussel *Dreissena polymorpha* into North America, although this has not been proved. Zebra mussels were introduced only in their planktonic veliger stage, which is free of parasites, thus parasites were not introduced with them (Conn and Conn, 1995). Obviously any species would benefit from a lack of parasites. But Dobson and May (1986) concluded that, although this is an attractive hypothesis, there is little evidence to support it. Even in cases when a reduction in associated parasite species has been reported for invaders (e.g. the House sparrow and the European starling in North America), related benefits have not been demonstrated (see Dobson and May, 1986 for references).

In the case of invasive animals bringing parasites with them, this implies an extra problem for indigenous species if they are attacked by those parasites (Leberg and Vrijenhoek, 1994). It is often argued that indigenous species are more susceptible to the diseases of introduced species since they have never been exposed to them and therefore they have no immunity (Eberhard, 1988). A classic example of the potential effects of an introduced parasite on a susceptible host population is the introduction of the monogenean *Nitzschia sturionis* in the Aral Sea, where it caused the decline of the spiny sturgeon *Acipenser nudiventris* (reviewed by Rohde, 1984). Sindermann (1993) reviewed several examples of diseases introduced with exotic marine animals and gave a series of recommendations to deal with the problem of introduced diseases.

Freeland (1983) concluded that for an invasion to be successful, parasites of the invading and indigenous species must be different. Invaders are usually few and, if both species are susceptible to the same parasites, the probability of indigenous parasites wiping out the invader species is higher than the probability of invaders bringing parasites that will affect the natives.

Regardless of the mechanisms, it seems clear that exotics can affect the viability of native species, and that they pose an additional threat if they harbour parasites that also attack indigenous species.

Up to twelve marine molluscs are thought to have been introduced to South Africa; five of them were deliberately introduced and are commercially cultured and the other seven are known or suspected to have been accidentally introduced (Griffiths *et al.*, 1992). The Mediterranean mussel *Mytilus galloprovincialis* is the only one that has become invasive (Griffiths *et al.*, 1992). We have just seen how parasites may affect the result of competition between invasive and indigenous species. The fact that *M. galloprovincialis* has become invasive implies that it is competitively superior to the indigenous species, but few studies have been done on the parasitic load of mussels in South Africa (Lasiak, 1989, 1993).

Mussels are common hosts for many parasites. Cheng (1967) listed nine protozoans, ten trematodes, two gastropods, five copepods and three crustaceans that have been found in *M. edulis*, four parasite species in *M. galloprovincialis* and three in *M. californianus*.

The aims of this work were to examine the parasitic load of populations of an invasive and an indigenous mussel species in South Africa and to assess the effects of the parasites on their hosts. Since digenetic trematodes are the most frequent and most important metazoan parasites

of bivalves (Cheng, 1967; Kinne, 1983) this study concentrated on this group of parasites. Digenetic trematodes are the most common and abundant flatworms. Their development occurs in at least two hosts; the first is usually a mollusc, and the final host is usually a vertebrate. Many species include a second or even a third intermediate host in their life cycle. The host for the adult is the definitive or final host and the others are called intermediate or paratenic hosts.

Trematode life-cycles are notoriously complex and variable, but a "typical" life cycle would be as follows: a ciliated, free swimming larva, the miracidium, emerges from the egg and penetrates the first intermediate host. This can be done by active penetration or passive ingestion of the miracidium by the host (Shoop, 1988). The miracidium metamorphoses into a sacklike form, the sporocyst. Within the sporocyst a number of germ balls develop asexually by mitotic division to become rediae. The redia is more differentiated than the sporocysts, possessing for example a pharynx and a gut. Additional germ balls develop within the redia, and these become cercariae, which can already be considered young adults. The cercaria may remain in that host, although this only occurs in one family (Shoop, 1988). In most cases the cercaria emerges from the host and infects the new host, where it develops into a metacercaria which usually encysts. If the host with the metacercaria is eaten by the final host, the metacercaria escapes from the cyst and develops into the adult form (Cheng, 1986; Barnes, 1987; Schmidt and Roberts, 1989). Many variations of this generalized life cycle are known.

In the last few years, with the increasing concentration on ecological parasitology there has also been increasing concern about the use and misuse of some terminology. In 1981 a

committee was created to establish working definitions of some of the terms most commonly used (Margolis *et al.*, 1982). The terms prevalence, incidence, intensity, site and locality are used throughout this thesis in the sense explained by this committee. Prevalence refers to the number of individuals of a host species infected with a particular parasite species divided by the number of hosts examined, whilst intensity refers to the number of individuals of a particular parasite species in each infected host. Incidence refers to the number of new cases of infection appearing in a population within a given period of time divided by the number of uninfected individuals in the population at the beginning of the time period. The term site is used to indicate the part of the host where the parasite was found whilst locality indicates the geographical place of collection of the host (Margolis *et al.*, 1982).

CHAPTER 2.

PREVALENCE OF PARASITES

IN MUSSEL POPULATIONS.

2.1 INTRODUCTION

Of the four most common mussel species in South Africa, the brown mussel *Perna perna* is the most abundant in the warmer waters of the east and south coasts of Southern Africa, from Barra Falsa (22^0 55'S) in central Mozambique to False Bay (34^0S, 18^0E) in South Africa (Berry, 1978; van Erkom Schurink & Griffiths, 1990). The water on the west coast is much cooler due to the Benguela Upwelling System (Andrews and Hutchings, 1980) and *P. perna* is very rare on this coast, becoming abundant again in northern Namibia (van Erkom Schurink & Griffiths, 1990). Archaeological records and recent studies have shown that *P. perna* has been used as food by man in South Africa for several thousand years, and it is still heavily exploited in some areas of the country, such as the Transkei region of the east coast (Lasiak & Dye, 1989; Lasiak, 1991, 1992, 1993; Lasiak & Field, 1995). The survival of this mussel seems to be threatened by an alien mussel, *Mytilus galloprovincialis*, which has become invasive in South Africa (van Erkom Schurink and Griffiths, 1990).

Mytilus galloprovincialis is believed to have originated in the Mediterranean sea (Gosling, 1992) and its distribution in Europe ranges from the Black Sea to the Atlantic coasts of France and the British Isles. It is a highly invasive species which has been successfully introduced to California (McDonald and Koehn, 1988), Japan (Wilkins *et al.*, 1983), Hong Kong (Lee and Morton, 1985), Korea (McDonald *et al.*, 1990), Australia (McDonald *et al.*,

1991) and South Africa (Grant and Cherry, 1985; Beaumont *et al.*, 1989). *M. galloprovincialis* is believed to have been accidentally introduced on the west coast of South Africa in the late 1970s (Grant and Cherry, 1985) and it has since became invasive. In 1988 de Moor and Bruton described it as the most abundant alien organism that has invaded the marine environment in South Africa. In 1990 it was reported to be the most abundant intertidal mussel species on the west coast of South Africa (van Erkom Schurink and Griffiths, 1990), and in 1992 it was estimated that *M. galloprovincialis* constituted over 70% of the intertidal mussel biomass on that coast, having displaced the slower growing *Aulacomya ater* (Griffiths *et al.*, 1992). It is now spreading rapidly onto the south and east coasts (Phillips, 1994) where it has the potential to compete with the indigenous mussel *Perna perna* (van Erkom Schurink and Griffiths, 1990).

The fact that *Mytilus galloprovincialis* has become invasive in South Africa implies that it is competitively superior to the indigenous species. One possible explanation for this phenomenon is that it is not subject to the parasites from its area of origin and/or is less susceptible to the native parasites of the area where it has been introduced.

As a first step to testing this hypothesis, a survey was carried out comparing the parasite loads of the indigenous *Perna perna* and the exotic *Mytilus galloprovincialis*, both in one of its countries of origin (Spain) and in South Africa, where it has been introduced.

2.2 MATERIALS AND METHODS

2.2.1 Epidemiology

Specimens collected during the survey were dissected and examined for parasites in the field whenever this was possible. Otherwise they were opened, by either heating or freezing, and placed in 70% alcohol or 10% sea water-formalin until examination was possible. The total length of the animal was measured, and once the tissues were hardened they were removed and examined for parasites under a dissecting microscope. The sex of the animal was recorded when possible on the basis of the external anatomy of the gonad, as was the presence of any trematodes.

A) *Perna perna*

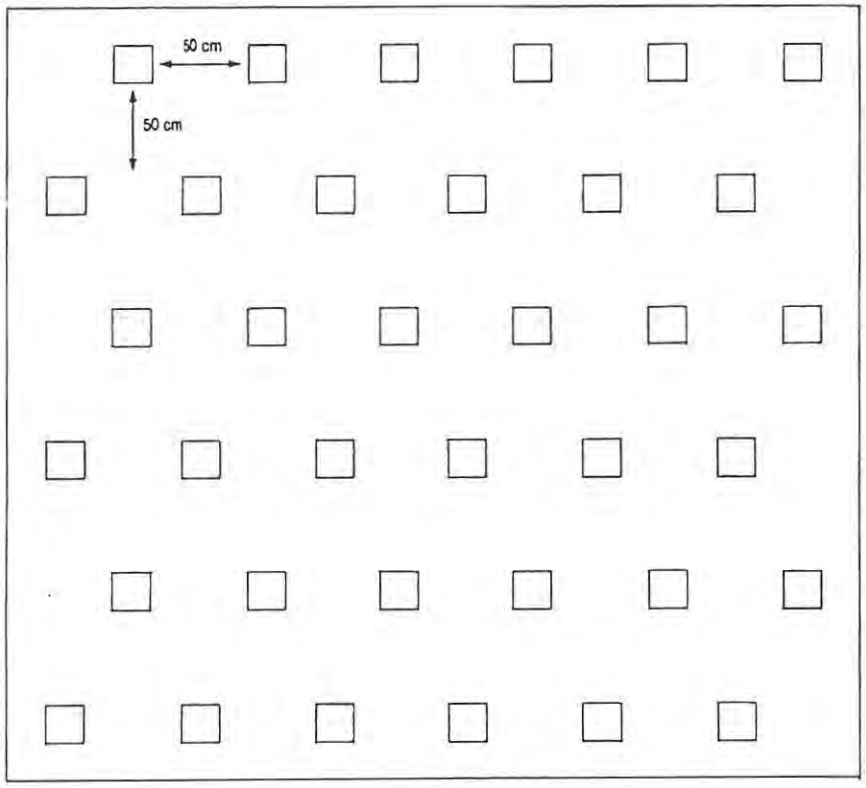
In the case of the indigenous mussel *Perna perna*, the survey was carried out at different spatial scales:

A.1- Small scale (metres).

To derive a valid sampling strategy for comparisons on a geographical scale, it was necessary first to understand the distribution of parasites among mussels on a small scale, that is, within a mussel bed population. In order to assess the distribution patterns of the most common trematode species found in *Perna perna*, a survey was carried out using a small scale grid. In an area of approximately 14 m^2, 36 quadrats of 20x20 cm separated from each other by

50 cm were collected in a chess-board pattern, as shown in Fig. 2.1. Presence of parasites is often dependent on host size (see A.2), therefore a narrow size range was used. Twenty mussels between 60 and 90 mm length were collected from each quadrat and examined for parasites. A 6x6 contingency table was used to compare parasite prevalences through the area sampled.

Fig. 2.1. Diagram of the area sampled during the small scale survey.



A.2- Medium scale (kilometres).

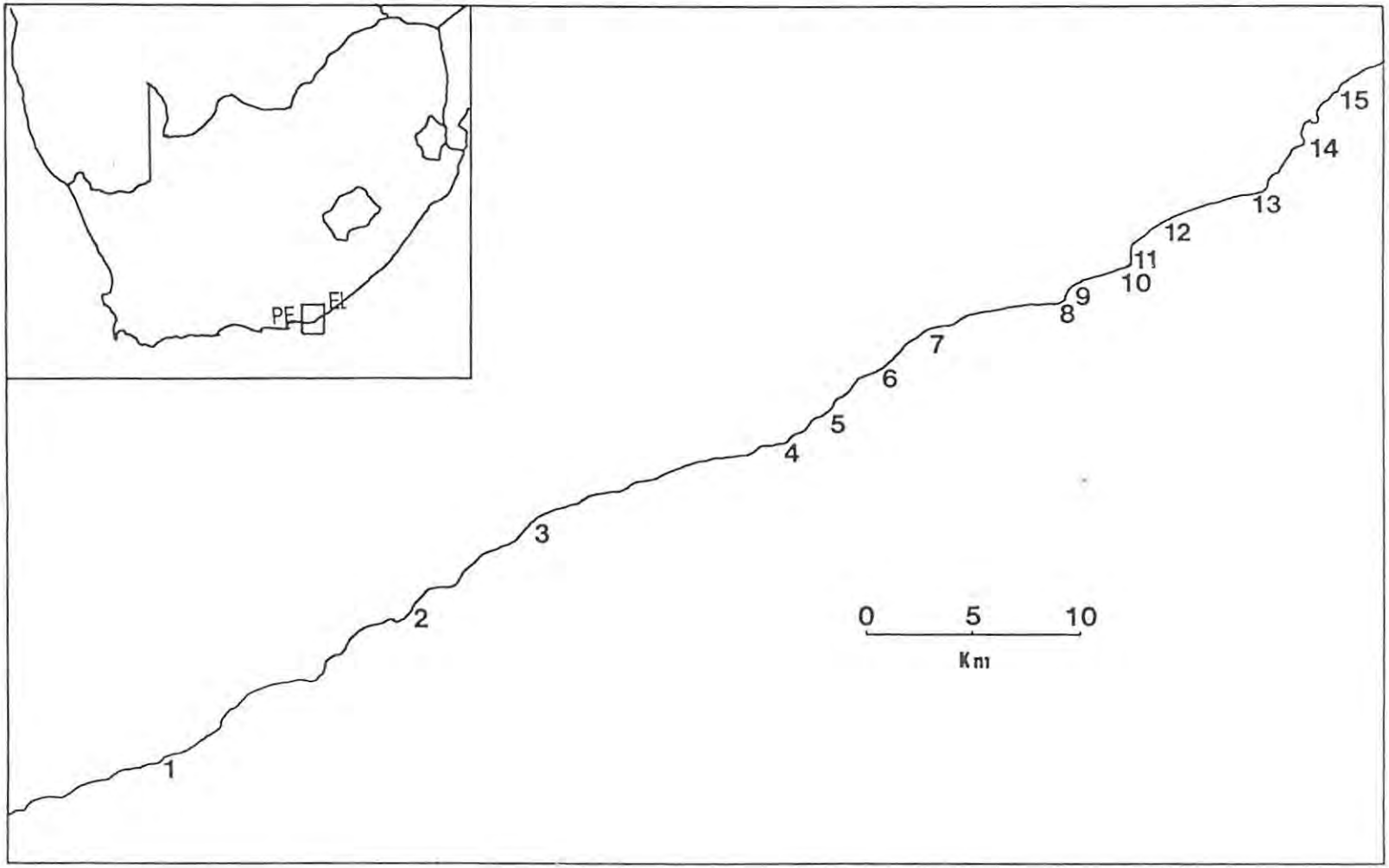
Locality

Samples were taken from localities along a stretch of coast of about 60 km on the south coast of South Africa. The thirteen localities were spaced at intervals of a few kilometres (3-8 km) apart (Fig. 2.2). Between fifty and a hundred animals of 60 to 90 mm were taken randomly from an area of a few square metres in the middle of the mussel bed at each locality and examined for parasites.

Sex dependence

In those cases where identification of the host sex was possible, a 2x2 contingency table based on the sex of the mussel and the presence or absence of parasites was used for each locality to test the relationship between infection rates and sex. The data were analyzed using a Chi-square statistic with Yates correction for continuity (Zar, 1984). Some parasites proved to be sex dependent whilst other parasites were not. In addition to performing the thirteen separate Chi-square tests, for parasites for which prevalence of infection was sex-dependent a heterogeneity analysis of 2x2 contingency tables was done to decide if differences in infection rates between sexes varied at different localities (Zar, 1984). Chi-square tests for the prevalence of infection were done among localities. For parasites for which the 2x2 contingency tables proved that infection rates were sex-dependant, the sexes were analyzed separately.

Fig. 2.2. Localities sampled during the medium scale survey (1=Cannon Rocks, 2=Kenton-on-Sea, 3=Kasouga, 4=Kowie Point, 5=Beach Crescent, 6=Sandstone Ridge, 7=Beacon Rocks, 8=Riet Point, 9=Riet River Mouth, 10=Three Sisters, 11=between Three Sisters and Seafield, 12=Seafield, 13=Great Fish Point, 14=Fish River Mouth, 15=Old Women's River, EL=East London, PE=Port Elizabeth).



Size dependence

The influence of the size of mussels on the prevalence and intensity of infection was also tested. Two hundred mussels of between 20 and 100mm length were randomly collected from one locality (Kowie Point). The mussels were divided into eight size classes (20-29.9mm, 30-39.9mm, 40-49.9mm, 50-59.9mm, 60-69.9mm, 70-79.9mm, 80-89.9mm and >90mm) with 25 mussels in each size class, and examined for parasites. A Chi-square test was used to test for size dependent prevalences. Spearman's rank correlation coefficient was used to test intensity of infection versus size of mussel.

Subsequent results indicated that, due to the low infection rates in small mussels, a sample size of 25 was not large enough to detect infections in animals smaller than 40 mm, thus, 100 mussels between 20-30 mm and 100 between 30-40 were examined for parasites.

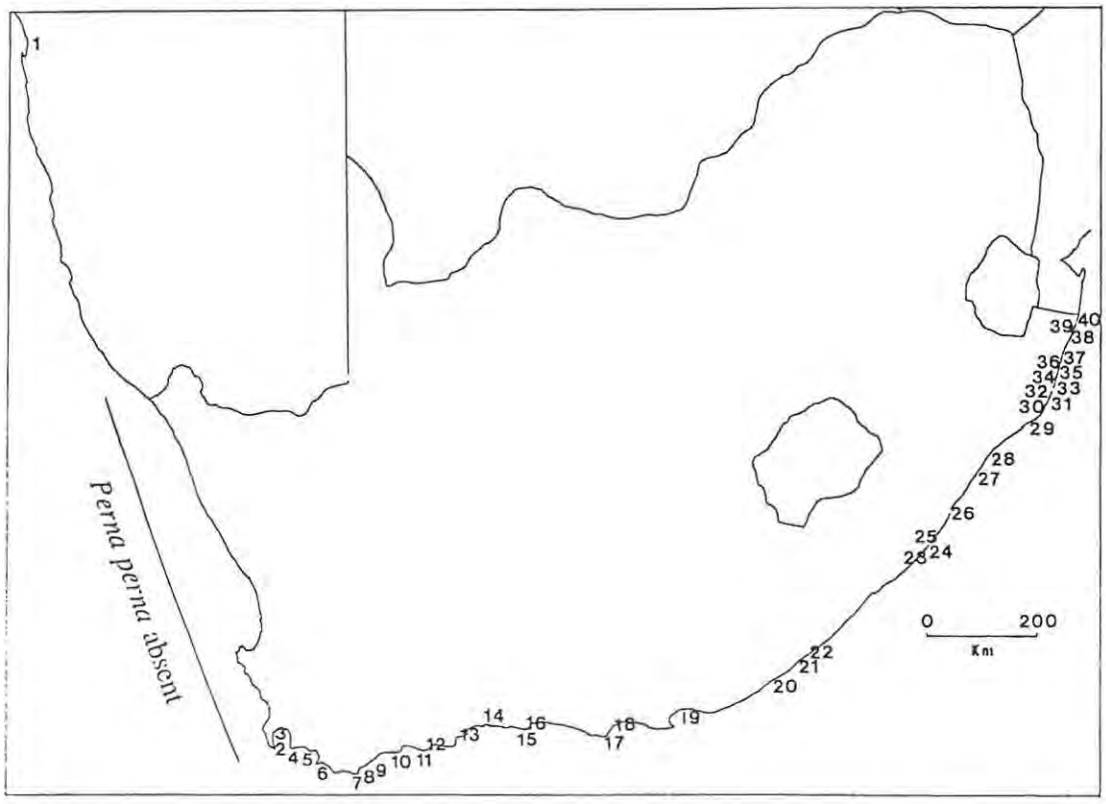
Seasonality

Samples were also collected at Kowie Point every two months from June 1991 till October 1992 to assess seasonal changes in infection rates. A 2x2 contingency table was calculated for each month to test the effects of sex on prevalence of parasites, and then a heterogeneity analysis was undertaken to determine if the differences between sexes varied from month to month. Chi-square tests were used to test if there were seasonal variations in prevalence rates. If the results of initial analyses showed that infection was not sex dependent, data were pooled, otherwise the sexes were treated separately.

A.3- Large scale (hundreds of kilometres)

Fifty mussels between 60 and 90 mm were collected as for the survey at medium scale from a further 40 localities along the west, south and east coasts of Southern African (Fig. 2.3) and processed as above.

Fig. 2.3. Sample sites for *Perna perna* during the large scale survey (1=Walvis Bay, 2=Simonstown, 3=St. James, 4=Betty's Bay, 5=Hermanus, 6=Pearly Beach, 7=Cape Agulhas, 8=Struisbaai, 9=Arniston, 10=Cape Infanta, 11=Groot Jongersfontein, 12=Still Bay, 13=Mossel Bay, 14=Wilderness, 15=Robberg, 16=Plettenberg Bay, 17=Cape St. Francis, 18=Jeffrey's Bay, 19=Hougham Park, 20=Kayser's Beach, 21=Queensberry Bay, 22=Haga Haga, 23=Port Edward, 24=Ramsgate, 25=Oslo Beach, 26=Umkomaas, 27=Umdloti Beach, 28=Zinkwazi Beach, 29=Dawson's Rocks, 30=Mapelane, 31=First Rocks, 32=Mission Rocks, 33=between Mission Rocks and Cape Vidal, 34=Cape Vidal, 35=between Cape Vidal and Leven Point, 36=Leven Point, 37=Sodwana, 38=Island Rock, 39=Bhanga Nek and 40=Kosi Mouth).



B) *Mytilus galloprovincialis*

Data of prevalence of parasites on the west coast already exist (S. Webb, pers. comm.). Between 45 and 80 mussels of 50 to 90 mm were randomly collected from nine localities on the west and south coasts of South Africa (Fig. 2.4) and processed as above.

In order to check if infection rates of *Mytilus galloprovincialis* in South Africa were different from infection rates in an area where it is indigenous, thirty mussels were collected from natural mussel beds from nine localities on the coast of the Basque Country in North Spain (Fig. 2.5) and examined for parasites.

Fig. 2.4. Sample sites for *Mytilus galloprovincialis* in South Africa (1=Bloubergstrand, 2=Betty's Bay, 3=Hermanus, 4=Pearly Beach, 5=Arniston, 6=Mossel Bay, 7=Plettenberg Bay, 8=Cape St. Francis and 9=Jeffrey's Bay).

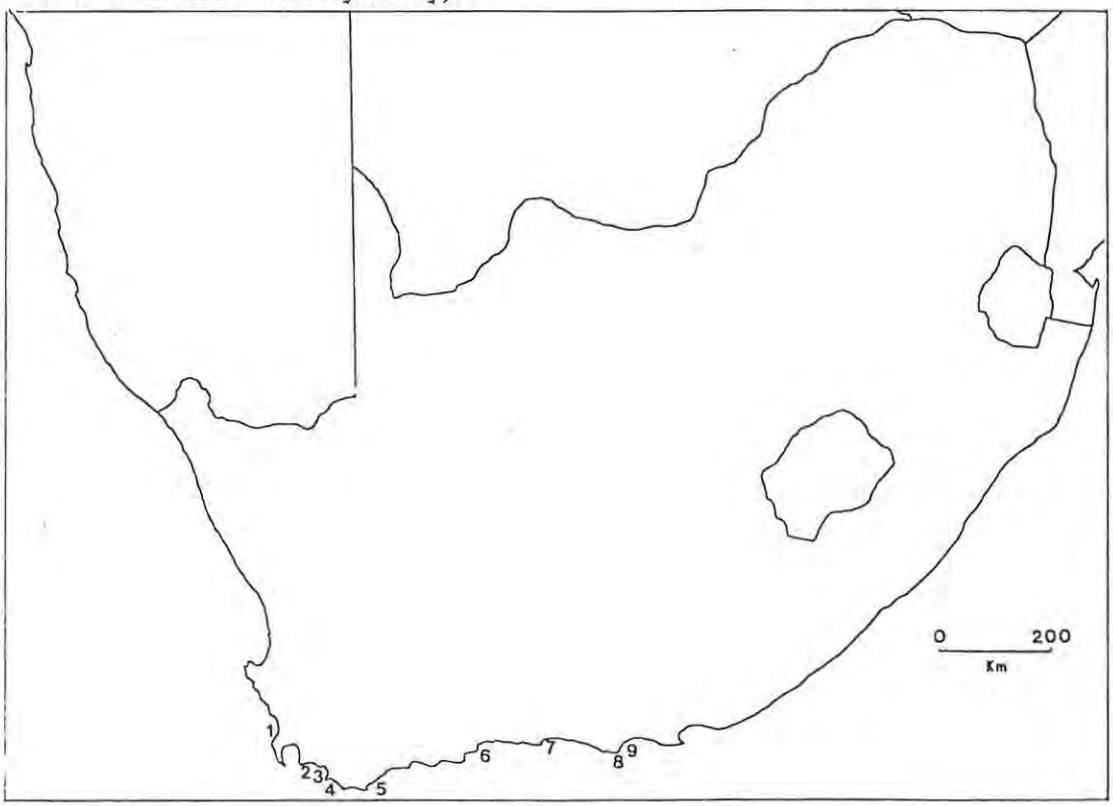
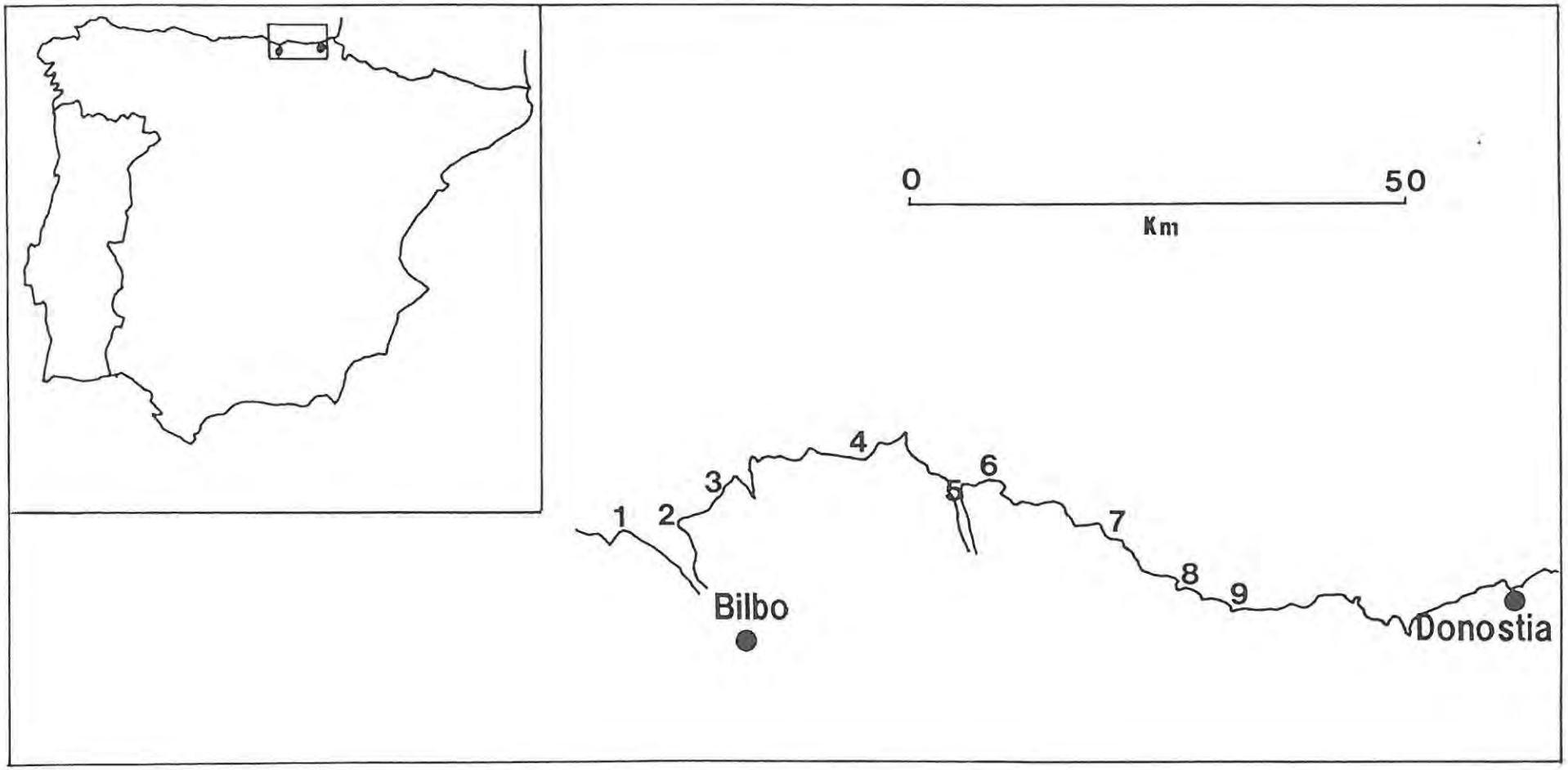


Fig. 2.5. Sample sites for *Mytilus galloprovincialis* in the Basque Country (North Spain) (1=Zierbana, 2=La Galea, 3=Meñakoz, 4=Bakio, 5=Txatxarramendi, 6=Laga, 7=Lekeitio, 8=Ondarroa and 9=Deba).



2.2.2 Descriptions

Descriptions of the parasites were made based on slides of living specimens, since the size and shape of the body and internal organs, as well as their positions, are affected by the way the specimens are treated (Berland, 1982; Bakke, 1988). Parasites were placed on a microscope slide in a drop of salt water and covered with a coverslip. The slides were examined 30-60 minutes after preparation because some features become more clearly defined just before death (Webb, 1991). Acetic orcein and neutral red were used as intravital stains to define some of the features (Bergan, 1955; Webb, 1991).

Measurements of the parasites were taken only in non-fixed specimens, since considerable stretching and shrinkage of characters occurred in specimens subjected to other treatments, such as heat killed animals or those fixed in Berland's fluid. Ten measurements were taken of each feature. Body width was measured at three places, 25%, 50% and 75% along the length of body starting from its anterior end.

For more detailed observations some specimens were prepared following a method suggested by B. Berland (pers. comm.). Parasites were fixed in Berland's fluid where the specimens swell/stretch and become fairly transparent. They were overstained in Carmine, differentiated in HCl-ethanol and washed in several changes of distilled water. After dehydratation with glacial acetic acid, Creosote was used as an intermedium, and the specimens were then mounted in Canada balsam.

Some specimens were fixed with a cold solution of 2.5% glutaraldehyde and prepared for scanning and transmission electron microscopy (SEM and TEM) by conventional methods.

2.3 RESULTS

No parasites were found in *Mytilus galloprovincialis* (Tables 2.1 & 2.2).

Table 2.1. Prevalence of infection with digenetic trematodes in the Mediterranean mussel *Mytilus galloprovincialis* along the coast of the Basque Country (Spain) (Localities listed from west to east, see Fig. 2.5).

Locality	Number examined	% infected
(1) Zierbana (Nov '91)	30	0
(2) La Galea (Nov '91)	30	0
(3) Meñakoz (Nov '91)	30	0
(4) Bakio (Nov '91)	29	0
(5) Txatxa. (Nov '91)	30	0
(6) Laga (Nov '91)	30	0
(7) Lekeitio (Nov '91)	30	0
(8) Ondarroa (Dec '91)	29	0
(9) Deba (Dec '91)	27	0

Table 2.2. Prevalence of infection with digenetic trematodes in the Mediterranean mussel *Mytilus galloprovincialis* in South Africa (Localities listed from west to east, see Fig. 2.4).

Locality	Number examined	% infected
(1) Blouberg. (Aug '91)	42	0
(2) Betty's B. (Dec '92)	50	0
(2) Betty's B. (Nov '94)	44	0
(3) Hermanus (Apr '94)	46	0
(4) Pearly Beach (Apr '94)	46	0
(4) Pearly Beach (Nov '94)	45	0
(5) Arniston-1 (Dec '92)	25	0
(5) Arniston-1 (Nov '94)	50	0
(5) Arniston-2 (Dec '92)	65	0
(5) Arniston-2 (Nov '94)	49	0
(6) Mossel Bay-1 (Nov '94)	50	0
(6) Mossel Bay-2 (Nov '94)	34	0
(7) Plettenberg (Nov '94)	50	0
(8) St. Francis (Nov '94)	7	0
(9) Jeffrey's Bay (Nov '94)	6	0

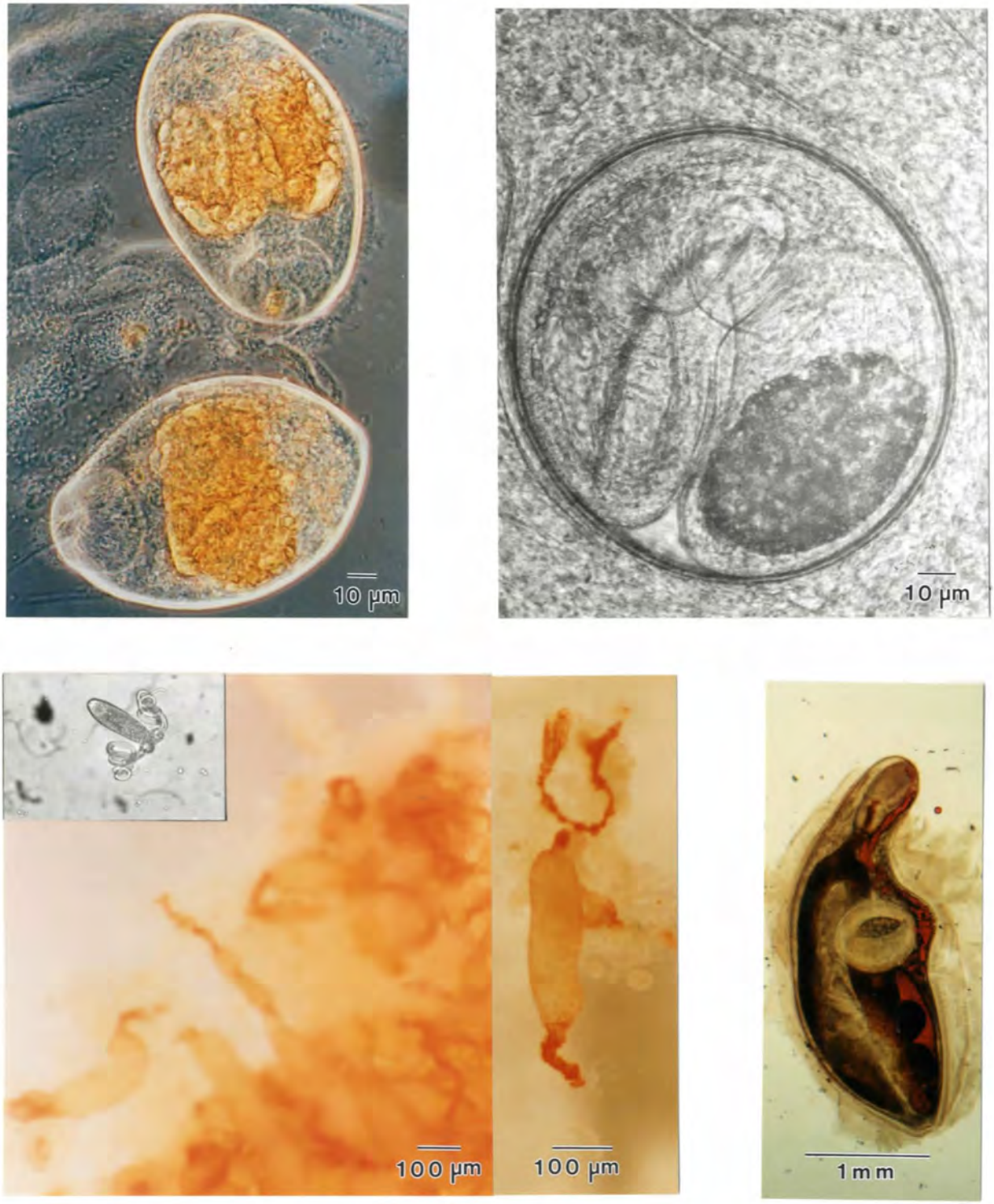
As a result of this survey some copepods, pea-crabs and four species of trematodes were found in the indigenous mussel *Perna perna*. The pea-crabs were extremely rare (only two in a few thousand mussels) and the copepods were also very rare. The trematodes were more abundant, and they included two kinds of cysts: gelatinous cysts on the gonad (Fig. 2.6 A) and hard cysts on the labial palps (Fig. 2.6 B); gasterostome sporocysts (Fig. 2.6 C) and metacercariae of the genus *Proctoeces* (Fig. 2.6 D). The gasterostome sporocysts and the two types of cysts were also found by S. Webb on the west coast (pers. comm.), and their descriptions are dealt with in a separate paper (Webb and Calvo-Ugarteburu, in prep.). The fourth trematode, metacercariae of *Proctoeces*, was only found in localities east of Cape Agulhas (34^0 50'S, 20^0 01'E). This species was very common and was selected for more detailed consideration.

2.3.1 **Gelatinous cysts and Labial cysts**

A) Description

These two encysted metacercariae, one on the gonad and the other on the labial palps of the mussels (Fig. 2.6 A and B), have also been found on the west coast and are described by Webb and Calvo-Ugarteburu (in prep.).

Fig. 2.6. Digenetic trematodes commonly found parasitizing *Perna perna* in South Africa. A. Gelatinous cyst. B. Labial palp cyst. C. Bucephalid sporocysts. D. Metacercaria of *Proctoeces*.



B) Epidemiology

Prevalence of infection by these two parasites was examined only at medium scale. The results of the Chi-square test between sexes showed that prevalences of both parasites were independent of the sex of the host at all the localities examined ($p>0.05$), therefore infection rates of males and females were combined together for the geographical and seasonal tests.

Examination of the geographical data showed that there were no significant differences in the prevalence of infection with gelatinous cysts among localities ($\chi^2=5.08$, $p>0.05$), with infection rates being very high (80-100%) at all localities examined (Fig. 2.7). Similarly, infection rates of this parasite were constant throughout the year ($\chi^2=0.56$, $p>0.05$) (Fig. 2.8). In the case of encysted metacercariae on the labial palps of the mussels, infection rates differed significantly from locality to locality ($\chi^2=84.76$, $p<0.01$), with prevalences varying from 18 to 100% (Fig. 2.9). The Chi-square test showed that there were also seasonal differences in prevalence rates of this parasite ($\chi^2=11.56$, $p<0.05$), with prevalences being higher during winter (Fig. 2.10).

Fig. 2.7. Prevalence of infection with gelatinous cysts on *Perna perna*. Sample sizes in brackets. (See Fig. 2.2 for names of localities).

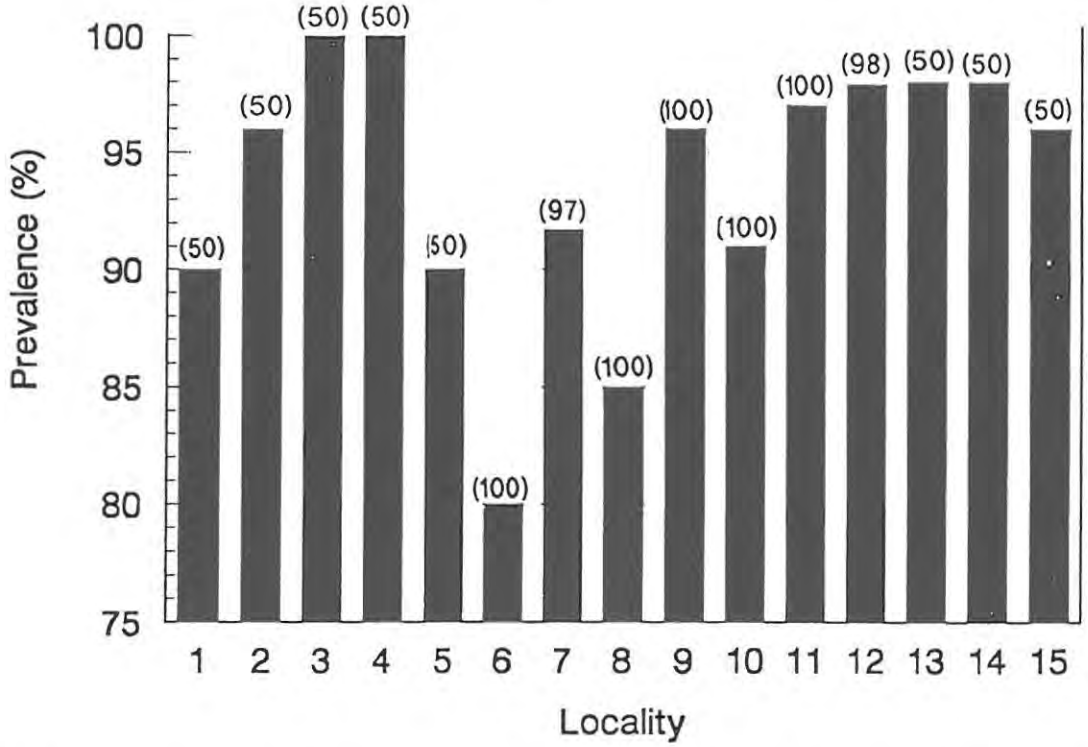


Fig. 2.8. Seasonal variations in prevalence of gelatinous cysts on *Perna perna*. Sample sizes in brackets.

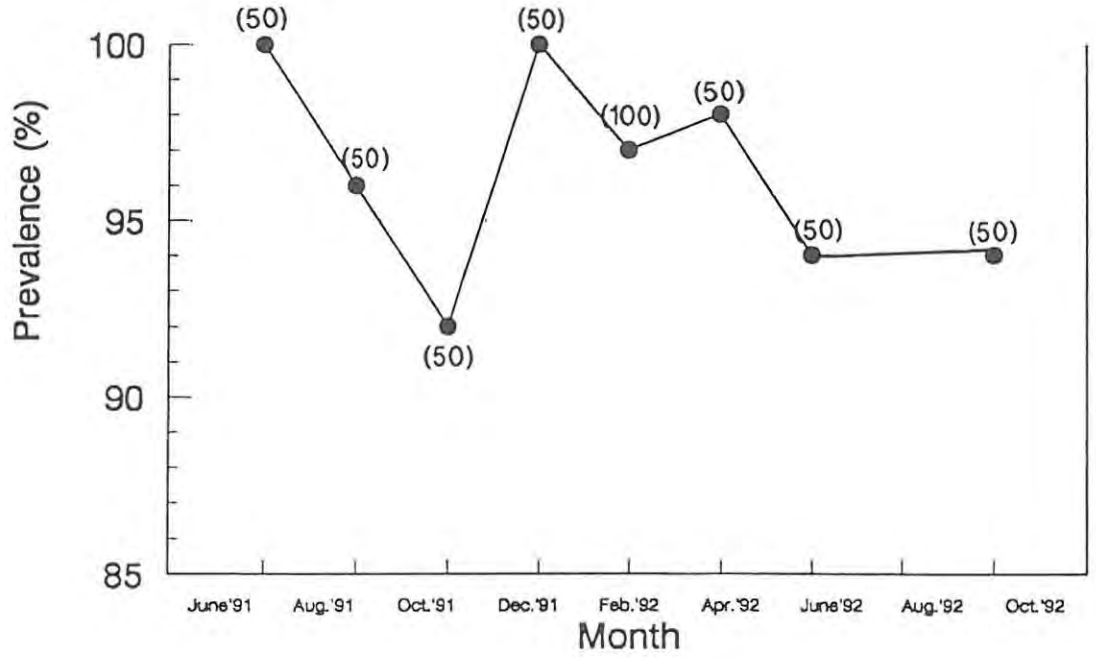


Fig. 2.9. Prevalence of infection with cysts on the labial palps of *Perna perna*. Sample sizes in brackets. (See Fig.2.2 for names of localities).

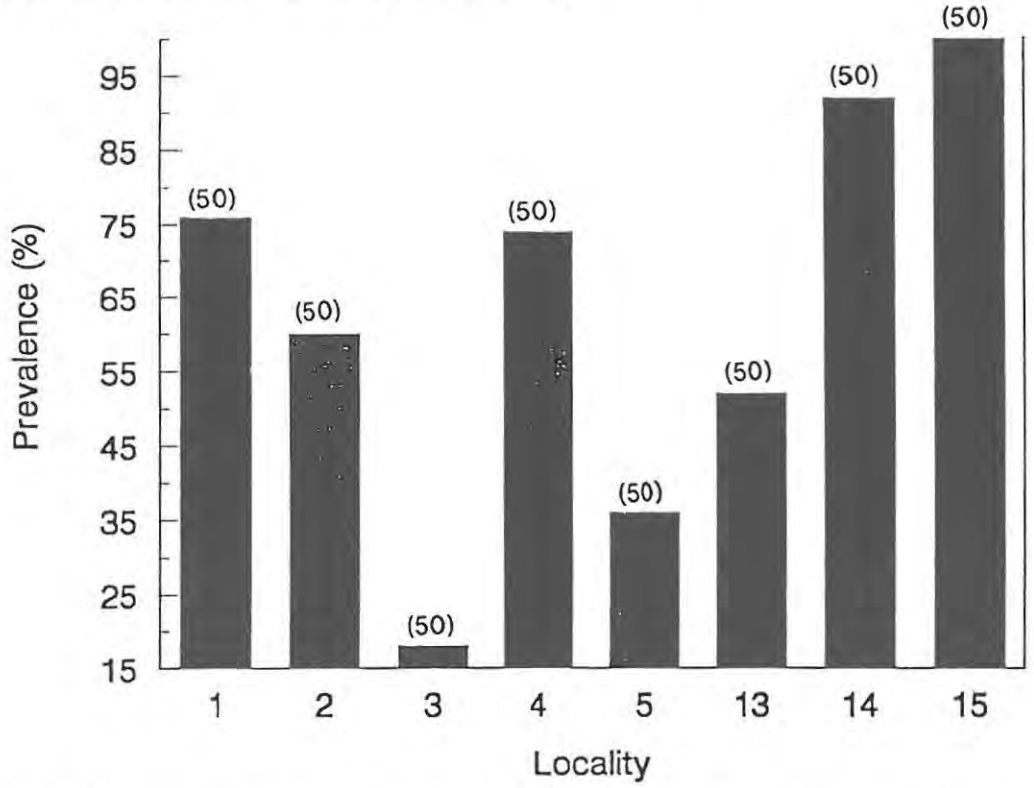
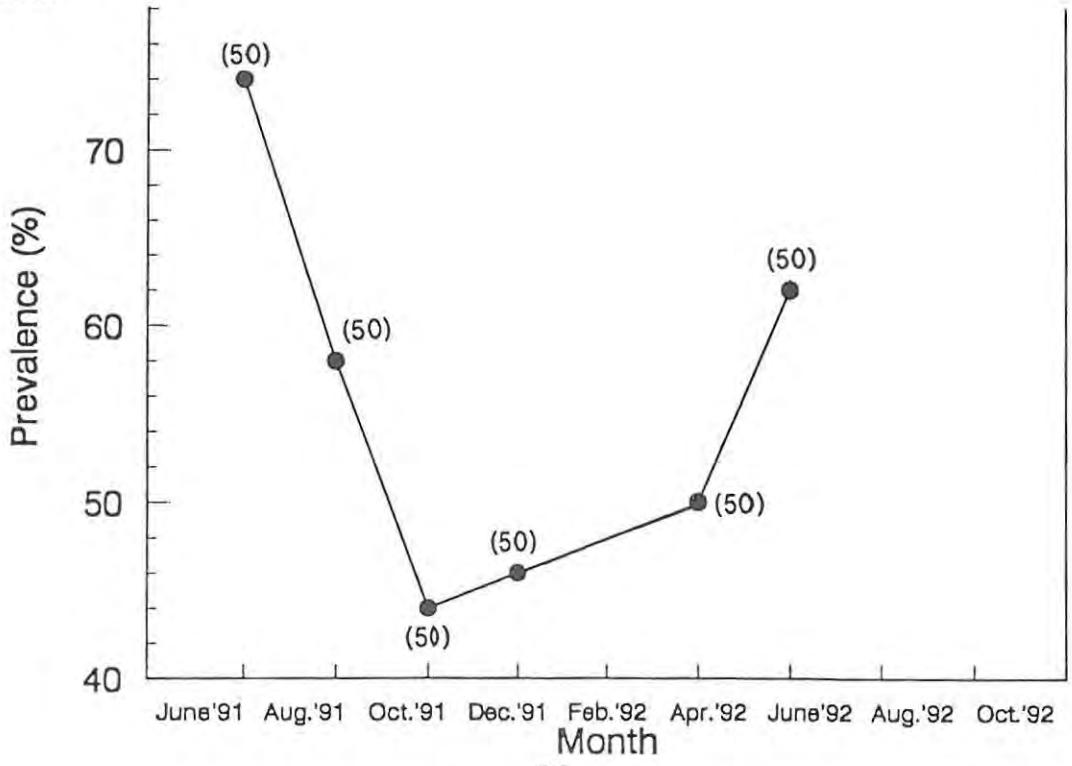


Fig. 2.10. Seasonal variations in prevalence of labial cysts on *Perna perna*. Sample sizes in brackets.



2.3.2 Gasterostome sporocysts

A) Description

Gasterostome sporocysts, probably belonging to the genus *Bucephalus* von Baer, 1827, were also found on *Perna perna* along the west coast of South Africa; see Webb and Calvo-Ugarteburu (in prep.) for their description. Since identification of bucephalids to generic level from either sporocysts or cercarial stages is not possible (Lasiak, 1991) this parasite will be referred to simply as a bucephalid.

B) Epidemiology

B.1 - Small scale

Table 2.3 shows the number of mussels infected by bucephalid sporocysts in the small scale area sampled. Since prevalence rates were very low or zero in most quadrats, the Chi-square test was calculated using the number of mussels without parasites in each quadrat. The results showed that there were no significant differences among the different quadrats (χ^2=0.61, p>0.05), therefore the probability of collecting the same number of infected and non-infected mussels through random sampling was uniform throughout the area sampled.

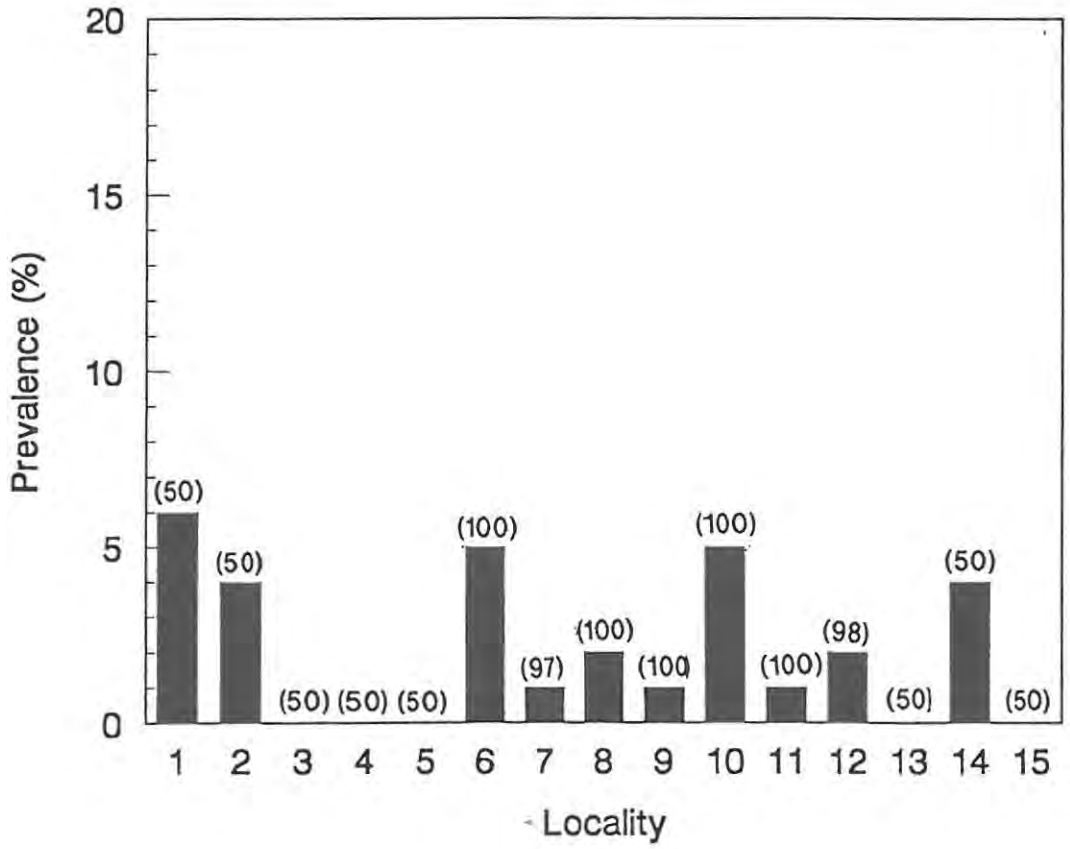
Table 2.3. Number of *Perna perna* infected with bucephalid sporocysts in the quadrats shown in Fig. 2.1 (n=20 for each quadrat).

	1	2	3	4	5	6
1	0	0	1	0	1	0
2	1	1	0	1	1	1
3	1	0	2	1	2	1
4	1	3	0	0	0	1
5	0	0	0	1	0	0
6	0	0	0	0	0	0

B.2 - Medium scale

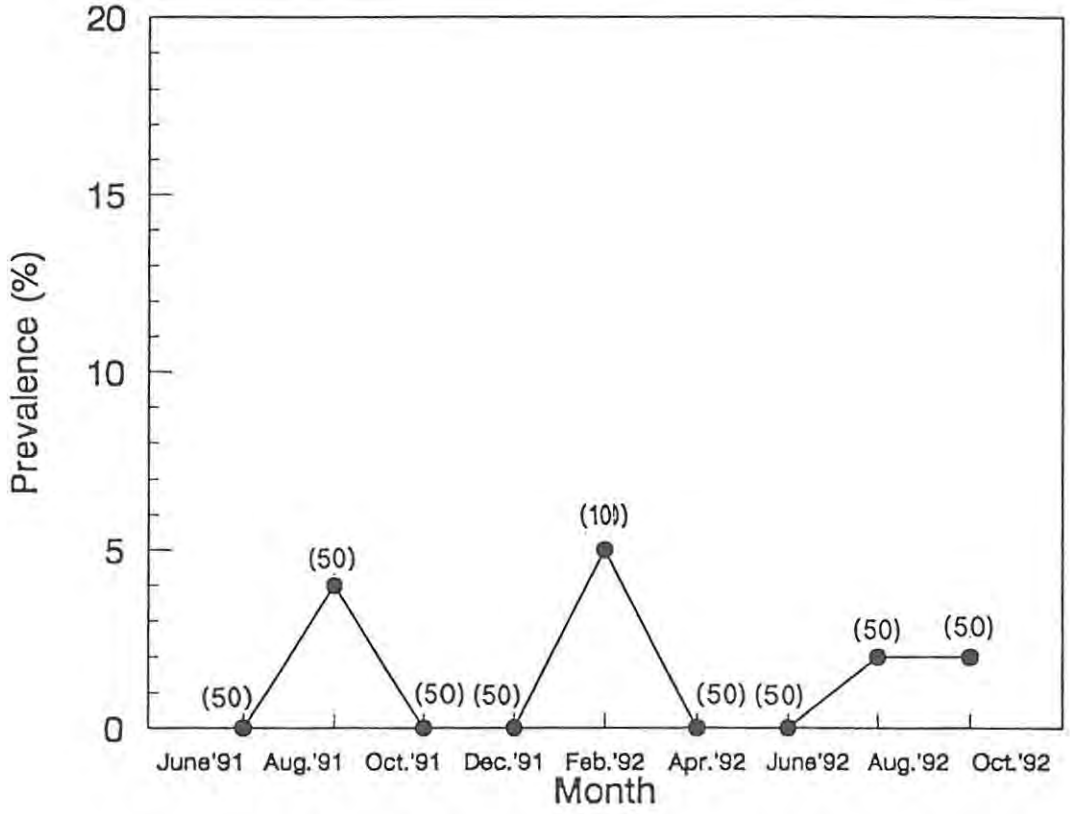
The prevalence of infection with bucephalid sporocysts along the south coast of South Africa was very low, varying from 0 to 6% (Fig 2.11). In light infections sporocysts were found only on the gonad, but as intensity of infection increased, they spread onto other tissues. In most cases gonadal tissue was completely replaced by branching sporocysts, and identification of the sex of the host was not possible. A Chi-square test was done using the data for mussels of both sexes together, and the results showed that there were no significant differences in prevalence rates among the different localities sampled in this survey (χ^2=0.665, p>0.05).

Fig. 2.11. Prevalence of infection by bucephalid sporocysts on *Perna perna*. Sample sizes in brackets. (See Fig. 2.2 for names of localities).



Seasonal infection rates with this sporocyst at Kowie Point are shown in Fig. 2.12. There were no seasonal differences throughout the year (χ^2=0.319, p>0.05).

Fig. 2.12. Seasonal variations in prevalence of infection with bucephalid sporocysts on *Perna perna*. Sample sizes in brackets.



B.3 -Large scale

Infection rates with this parasite were low at most of the localities sampled, although infection rate rose to 49% in one locality (Table 2.4).

Ninety eight mussels were collected at one locality (Hougham Park, 33^0 47'S, 25^0 44'E) with high prevalences (22.45%), to study size dependent prevalences. Prevalence of infection was highly dependent on the size of the mussel (χ^2=104.29, $p<0.01$), with over 50% of the mussels larger than 90mm being infected with this parasite (Fig. 2.13).

Table 2.4. Prevalence of infection with bucephalid sporocysts on *Perna perna* along the Southern African coast. Localities are listed from west coast to east coast (See Fig. 2.3).

Locality	Mussels examined	% infected
(1) Walvis Bay (Aug '92)	25	0.0
(2) Simonstown (Aug '91)	30	0.0
(3) St. James (Aug '91)	30	0.0
(4) Betty's Bay (Dec '92)	50	0.0
(4) Betty's Bay (Nov '94)	44	0.0
(5) Hermanus (Apr '94)	18	0.0
(6) Pearly Beach (Apr '94)	46	4.3
(6) Pearly Beach (Nov '94)	45	0.0
(7) Agulhas (Dec '92)	50	2.0
(7) Agulhas (Apr '94)	50	2.0
(7) Agulhas (Nov '94)	50	2.0
(8) Struisbaai (Apr '94)	50	0.0
(9) Arniston-1 (Dec '92)	75	1.3
(9) Arniston-1 (Apr '94)	52	1.9
(9) Arniston-1 (Nov '94)	50	0.0
(9) Arniston-2 (Dec '92)	50	1.0
(9) Arniston-2 (Nov '94)	47	12.0
(10) Cape Infanta (Dec '92)	50	30.0
(10) Cape Infanta (Apr '94)	50	36.0
(11) Jongersfontein (Nov '94)	50	2.0
(12) Still Bay (Apr '94)	51	5.9
(13) Mossel Bay-1 (Dec '92)	50	0.0

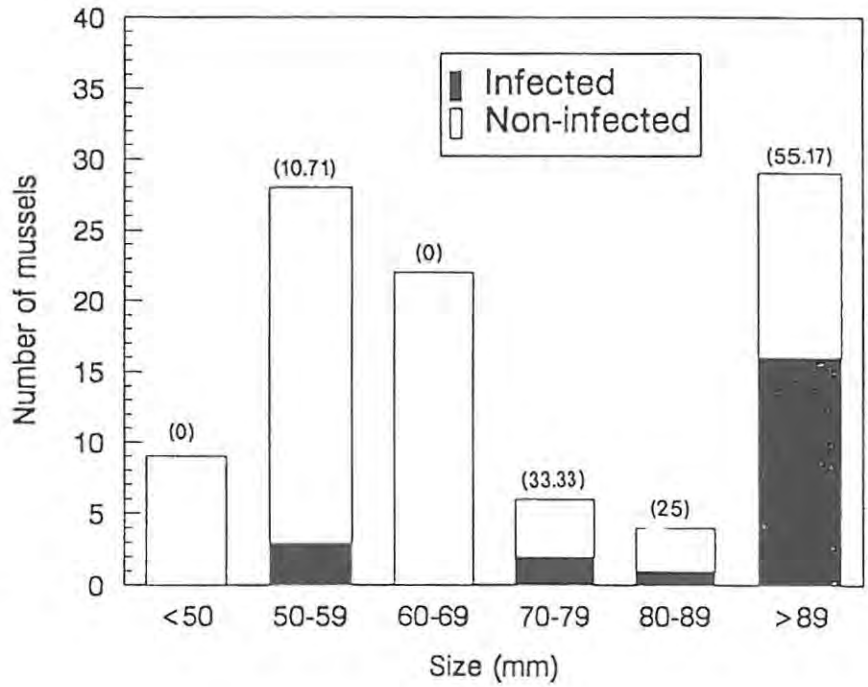
Table 2.4. (Cont.)

(13) Mossel Bay-1 (Nov '94)	49	0.0
(13) Mossel Bay-2 (Nov '94)	34	2.9
(14) Wilderness (Dec '92)	50	12.0
(15) Robberg (Dec '92)	35	2.9
(16) Plettenberg (Nov '94)	56	3.6
(17) St. Francis (Dec '92)	50	14.0
(17) St. Francis (Nov '94)	57	49.1
(18) Jeffrey's Bay (Nov '94)	59	10.2
(19) Hougham Park (Oct '92)	98	22.4
(20) Kayser's Beach (Oct '92)	50	4.0
(21) Queensberry B. (Oct '92)	48	2.0
(22) Haga Haga (Oct '92)	50	2.0
(23) Port Edward (Nov '93)	50	4.0
(24) Ramsgate (July '94)	50	12.0
(25) Oslo Beach (July '94)	50	28.0
(26) Umkomaas (July '94)	50	18.0
(27) Umdloti (July '94)	50	0.0
(28) Zinkwazi (July '94)	50	0.0
(29) Dawson's Rocks (July '94)	50	2.0
(30) Mapelane (Dec '95)	50	6.0
(31) First Rocks (Dec '95)	50	6.0
(32) Mission Rocks (Feb '95)	50	8.0
(32) Mission Rocks (Dec '95)	50	4.0
(33) MR-CV (Dec '95)	50	4.0
(34) Cape Vidal (Dec '95)	50	6.0
(35) CV-LP (Dec '95)	49	2.0

Table 2.4. (Cont.)

(36) Leven Point (Dec '95)	50	4.0
(37) Sodwana (Dec. '95)	50	2.0
(38) Island Rock (Dec. '95)	48	0.0
(39) Banga Nek (Dec. '95)	57	0.0
(40) Kosi Mouth (Dec. '95)	52	0.0

Fig. 2.13. Histograms showing the prevalence of bucephalid sporocysts related to the size of the mussels examined. Percentage of infection in brackets.



2.3.3 *Proctoeces*

A) Description

The following description is based on both living animals and slides of stained animals. The results of the measurements are tabulated, giving descriptive statistics of feature variability (Table 2.5). The drawing of an entire metacercaria (Fig. 2.14) is based on numerous slides of both living and stained specimens. The body is globular to elongate; body surface appears striated, although this is probably the result of body movements. Both suckers are globular, the oral sucker is subterminal and consistently smaller than the ventral sucker, which lies between one third and half way along the body, depending on the degree of stretching. Very short prepharynx, which is not visible in most specimens. Well developed globular pharynx. Short oesophagus. Two wide digestive caecae reaching to the posterior end of the body. Two globular testes, one slightly anterior to the other, located in the posterior part of the body, halfway between the ventral sucker and the excretory pore. A *vas efferens* leaves each testis and they join to form the *vas deferens* just before entering the cirrus sac. Cirrus sac is elongated and usually lies next to the ventral sucker. Cirrus sac contains a coiled seminal vesicle, the ejaculatory duct, the *pars prostatica* and a muscular papilla next to the opening. The genital pore opens close to the ventral sucker, approximately level with the intestinal bifurcation. The ovary is globular and lies anterior to testes in the anterior hindbody. The excretory pore is terminal and leads into a Y shaped vesicle which divides at the level of the testes into two excretory ducts which reach to the level of the pharynx.

Fig. 2.14. Metacercaria of *Proctoeces* from *Perna perna*. CS=cirrus sac, DC=digestive caeca, ED=excretory ducts, EV=excretory vesicle, MP=muscular papilla, O=ovary, OE=oesophagus, OS=oral sucker, P=pharynx, SV=seminal vesicle, T=testes, U=uterus, VE=vas efferens and VS=ventral sucker.

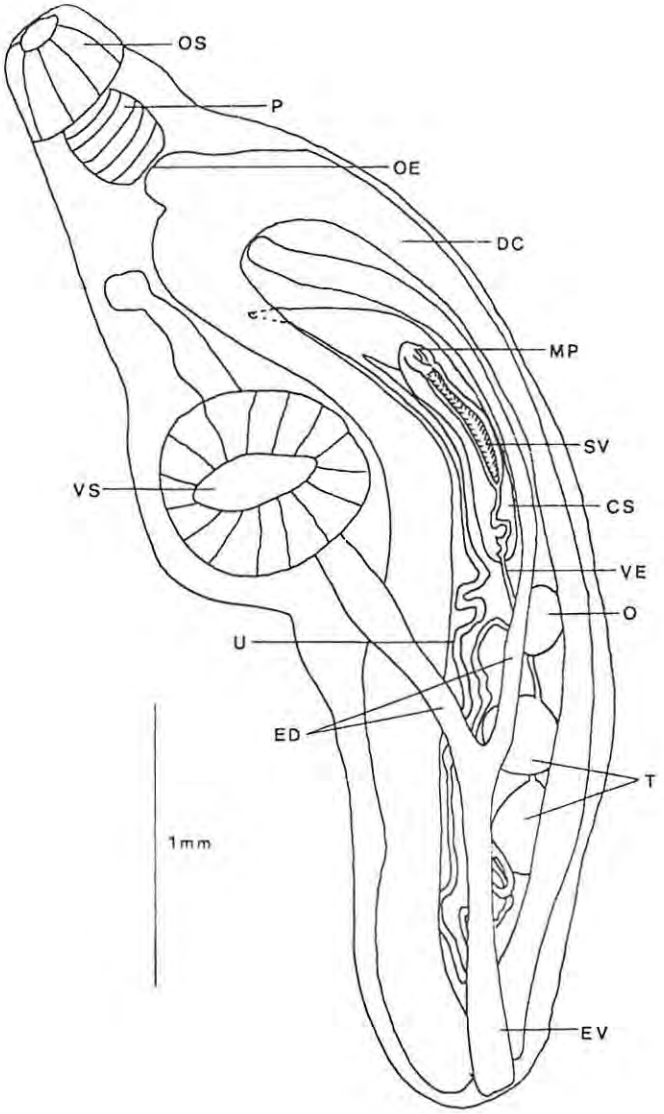
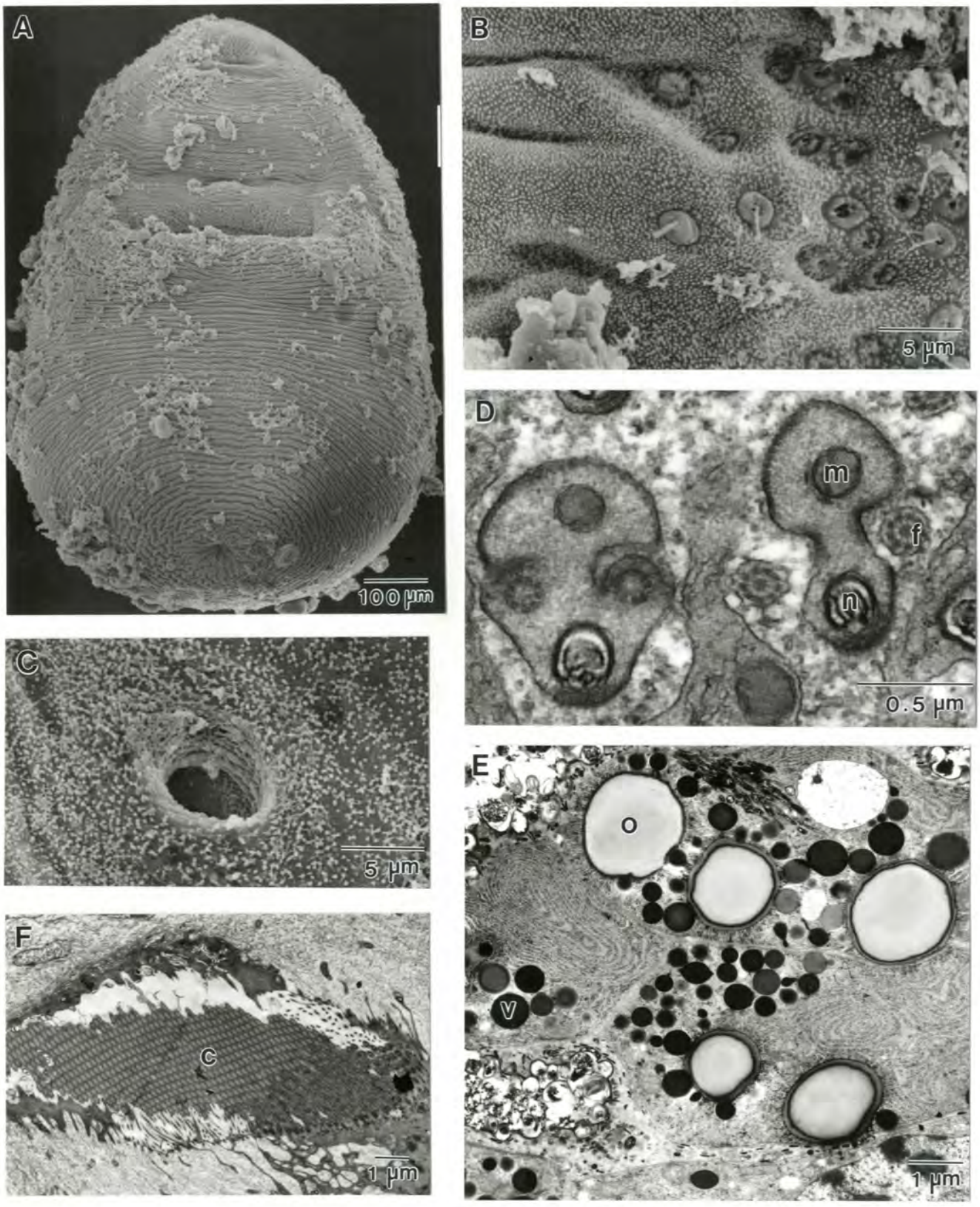


Table 2.5. Dimensions of *Proctoeces* from *Perna perna* in South Africa (all measurements in mm).

Organ	n	X	SD	Range
Body Length	10	3.65	0.53	2.50-4.32
Width (25%)	7	0.80	0.17	0.52-1.00
Width (50%)	10	1.36	0.25	1.12-1.88
Width (75%)	7	0.88	0.14	0.65-1.08
Oral sucker Length	10	0.38	0.07	0.32-0.47
Width	10	0.42	0.07	0.29-0.54
Prepharynx	2	0.03	0.01	0.03-0.04
Pharynx Length	10	0.26	0.06	0.17-0.39
Width	10	0.28	0.05	0.17-0.36
Oesophagus	6	0.09	0.01	0.07-0.11
Ventral sucker Length	10	0.63	0.13	0.42-0.86
Width	10	0.66	0.10	0.51-0.82
Ovary Length	10	0.20	0.03	0.17-0.25
Width	10	0.21	0.04	0.15-0.27
Testes Length	10	0.25	0.04	0.18-0.30
Width	10	0.29	0.06	0.21-0.40
Length	10	0.28	0.07	0.15-0.40
Width	10	0.29	0.05	0.22-0.38

Scanning electron microscopy showed the surface of the metacercariae to be striated in appearance (Fig. 2.15 A), probably the result of contraction during fixation. Gland cells are present over the whole body surface, but are concentrated around the suckers (Fig. 2.15 B).

Fig. 2.15. Scanning and transmission electron micrographs of *Proctoeces*. (A) SEM of metacercaria; (B) Gland cells around the ventral sucker; (C) Laurer's canal opening on the dorsal surface; (D) TEM of cross section through sperm: f=flagellum, m=mitochondria, n=nucleus; (E) TEM of cross section through the ovary: o=oocyte, v=secretory vesicles; (F) TEM of cross section through a flame cell: c=cilia.



Laurer's canal opens mid-dorsally at approximately mid hindbody, just below the level of the ventral sucker (Fig. 2.15 C). Transmission electron microscopy revealed the presence of biflagellate sperm in the testis and in the sperm ducts (Fig. 2.15 D). Sections through the ovary showed a number of large oocytes (Fig. 2.15 E). Flame cells were found at various positions throughout the animal (Fig. 2.15 F).

B) Epidemiology

B.1 - Small scale

The number of mussels infected by *Proctoeces* in the area sampled in the small scale survey is shown in Table 2.6. The results of the Chi-square test showed that there were no significant differences among the different quadrats (χ^2=20.30, p>0.05), indicating that infected mussels were randomly distributed.

Table 2.6. Number of *Perna perna* infected with *Proctoeces* in the quadrats shown in Fig. 2.1 (n=20 for each quadrat).

	1	2	3	4	5	6
1	10	11	9	9	9	8
2	12	4	13	14	10	11
3	11	7	9	8	10	12
4	11	14	8	13	14	9
5	6	7	8	15	13	12
6	7	6	9	11	4	7

B.2 - Medium scale

The results of the medium scale survey are summarized in Fig. 2.16. Chi-square tests showed that there were significant differences in infection rates between sexes at all the localities examined ($p<0.01$ at all localities), with a mean of 75.6% (SD=12.2) of females being infected whilst only 13.1% (SD=8.4) of males were infected. The heterogeneity analysis showed that the differences between sexes did not vary from locality to locality ($\chi^2$12.36, $p>0.05$). Since the presence of *Proctoeces* was sex dependent, Chi-square tests comparing localities were done separately for males and females. Prevalences of parasites in females varied from locality to locality ($\chi^2=26.18$, $p<0.05$), but in the case of males there were no significant differences in the prevalence of parasites among localities ($\chi^2=11.04$, $p>0.05$).

Fig. 2.16. Percentage of male and female *Perna perna* infected with *Proctoeces* in each one of the localities sampled. Sample sizes in brackets. (See Fig. 2.2 for names of localities).

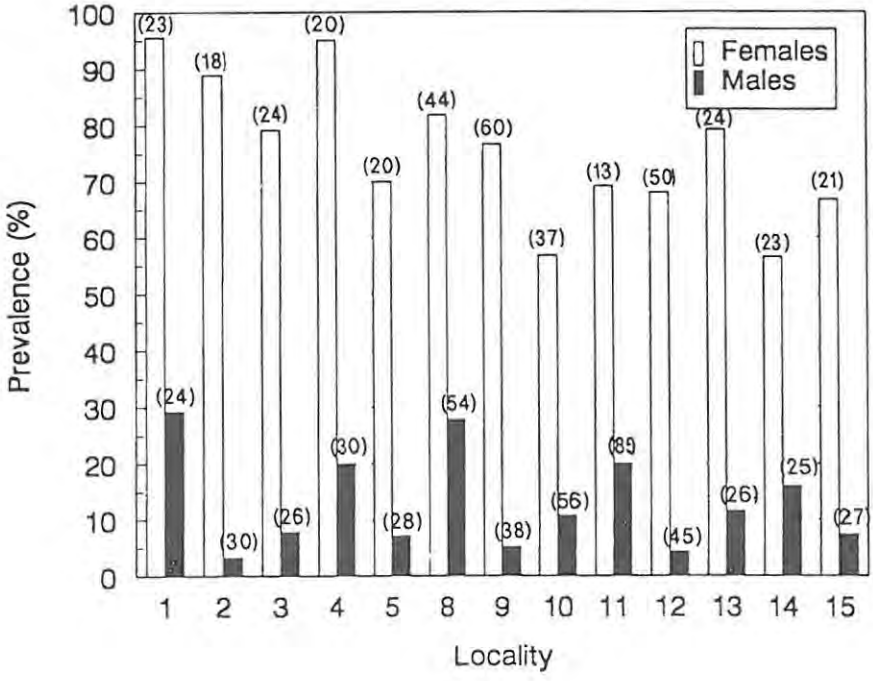


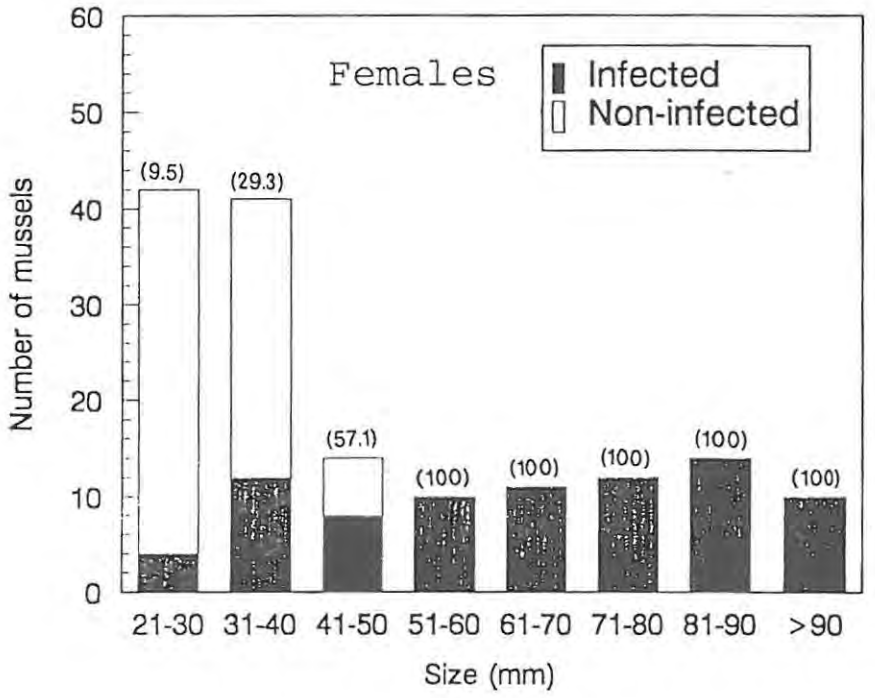
Table 2.7 summarizes the data on size dependent prevalences and intensities of infection. A Chi-square was calculated for each sex to determine size dependence of prevalence. With a sample size of 25 mussels in each size class, infection rates of 0% for males and females between 20 and 30mm and 0% for males and 44% for females between 30 and 40 mm were found. Nevertheless, examination of 200 mussels between 20 and 40 mm showed higher infection rates in these groups than the first analysis indicated. When the sample size was increased to 100 animals in each size class, infection rates were found of 0% for males and 9.5% for females between 20 and 30 mm and 3.4% for males and 29% for females between 30 and 40 mm. The results showed that prevalence of infection was highly dependent on the size of the mussel in the case of females (χ^2=127.82, $p<0.001$), but that it was not dependent on size in the case of males (χ^2=8.46, $p>0.05$) (Fig. 2.17). Spearman's rank correlation tests were used separately for males and females and showed a positive correlation between size of mussel and intensity of infection in both sexes (n=92, r=0.24, $p<0.05$ for males and n=87, r=0.72, $p<0.001$ for females).

Patterns of seasonality of infection with *Proctoeces* are shown in Fig. 2.18. The results of the Chi-square tests for each month showed that there were significant differences between males and females in all the months sampled ($p<0.01$ for all months). The heterogeneity analysis indicated that differences between sexes stayed constant throughout the year ($\chi^2$7.9, $p>0.05$). Chi-square tests showed that there were no seasonal variations in prevalence of infection with *Proctoeces* in either females (χ^2=9.8, $p>0.05$) or males (χ^2=4.1, $p>0.05$).

Table 2.7. Prevalence and intensity of infection of male and female *Perna perna* with metacercariae of *Proctoeces* related to the length of the mussels.

Shell length (mm)	Number examined	Prevalence (%)	Intensity	
			Mean	Range
Females				
20-29	42	9.50	1.25	1-2
30-39	41	29.00	1.25	1-2
40-49	14	57.14	1.87	1-3
50-59	10	100.00	6.30	2-15
60-69	11	100.00	9.00	2-18
70-79	12	100.00	6.75	2-18
80-89	14	100.00	12.64	3-10
>90	10	100.00	10.60	2-28
Males				
20-29	58	0.00	0.00	0
30-39	59	3.40	1.00	1
40-49	11	9.09	1.00	1
50-59	12	0.00	0.00	0
60-69	13	0.00	0.00	0
70-79	12	25.00	2.00	1-4
80-89	11	0.00	0.00	0
>90	14	21.43	2.30	2-3

Fig. 2.17. Histograms showing the prevalence of *Proctoeces* metacercarial infections relative to the size classes of *Perna perna* examined. Percentage of infection in brackets.



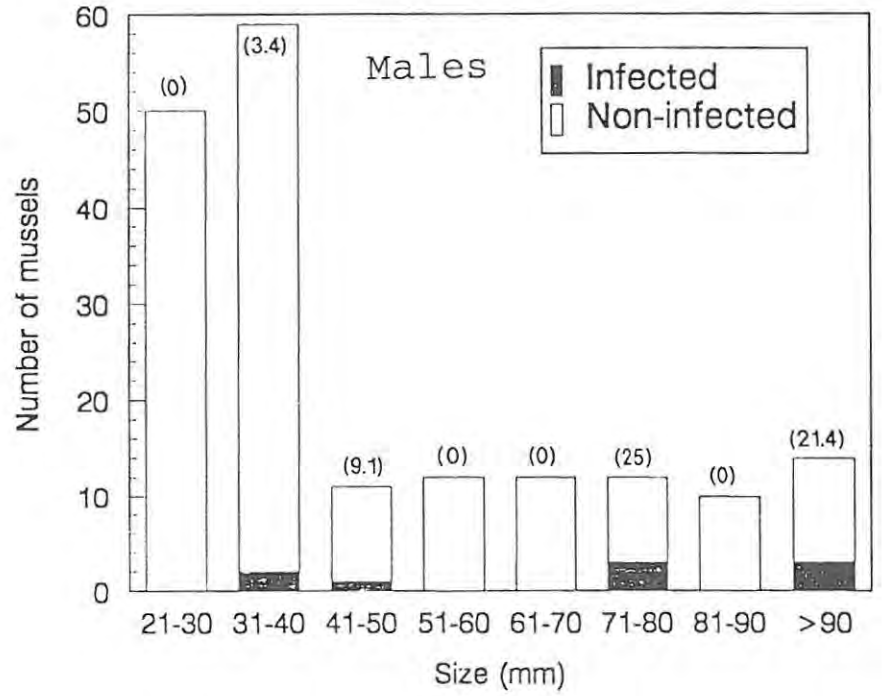
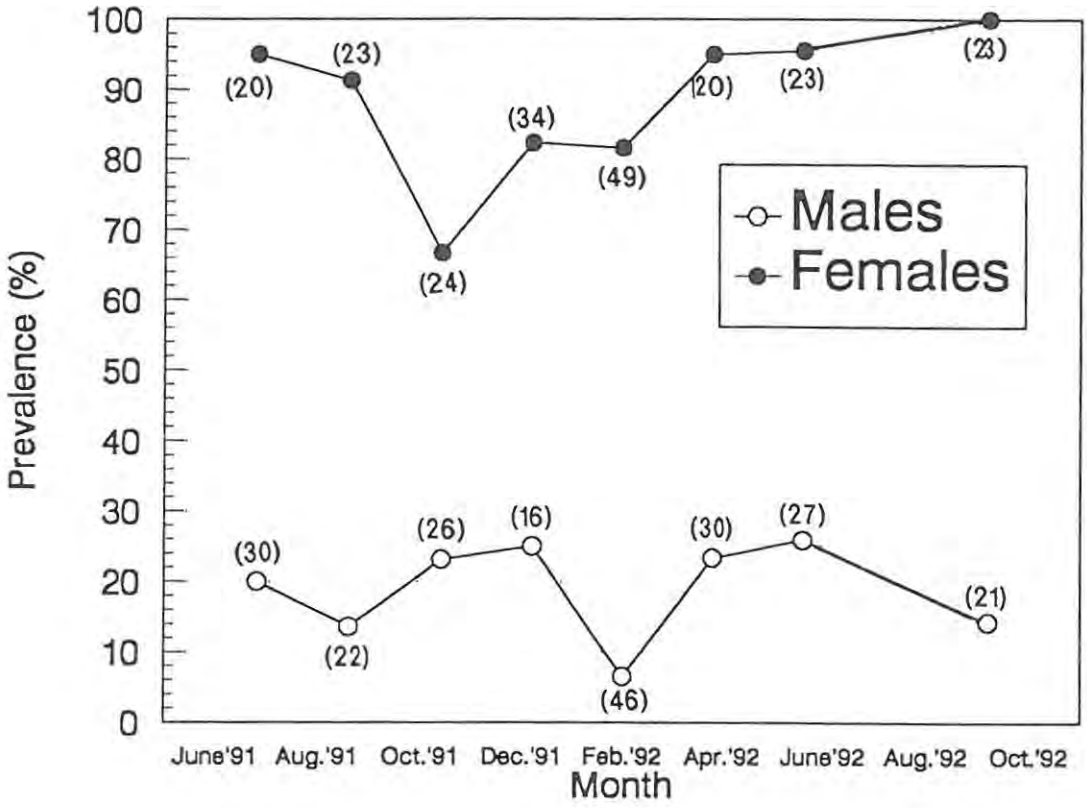


Fig. 2.18. Infection rates of male and female *Perna perna* infected with *Proctoeces* between June '91 and October '92. Sample sizes in brackets.



B.3 - Large scale

At a larger scale, prevalence data for this parasite along the Southern African coast show that it is present nowhere west of Cape Agulhas (locality 7 on Fig. 2.3) (Table 2.8, Fig. 2.19). Infection rates were highest on the south coast of South Africa, between Cape Agulhas and Durban, and decreased sharply north of Cape Vidal, with no parasites present north of Sodwana (Table 2.8).

Table 2.8. Prevalence of infection with *Proctoeces* on *Perna perna* along the Southern African coast. Locations listed from west to east (see Fig. 2.3).

Locality	Number examined	Number males	Number females	% infected	% males infected	% fem. infected
(1) Walvis Bay (Aug '92)	25	13	8	0.00	0.00	0.00
(2) Simonstown (Aug '91)	30	19	10	0.00	0.00	0.00
(3) St. James (Aug '91)	30	15	15	0.00	0.00	0.00
(4) Betty's Bay (Dec '92)	50	18	32	0.00	0.00	0.00
(4) Betty's Bay (Nov '94)	44	22	28	0.00	0.00	0.00
(5) Hermanus (Apr '94)	18	8	10	0.00	0.00	0.00
(6) Pearly Beach (Apr '94)	46	22	22	0.00	0.00	0.00
(6) Pearly Beach (Nov '94)	45	24	21	0.00	0.00	0.00
(7) Agulhas (Dec '92)	50	30	19	0.00	0.00	0.00
(7) Agulhas (Apr '94)	50	32	17	4.00	0.00	11.76
(7) Agulhas (Nov '94)	50	24	25	0.00	0.00	0.00
(8) Struisbaai (Apr '94)	50	30	20	6.00	3.33	10.00
(9) Arniston-1 (Dec '92)	75	37	37	56.00	29.73	83.78
(9) Arniston-1 (Apr '94)	52	23	28	54.00	13.04	89.29

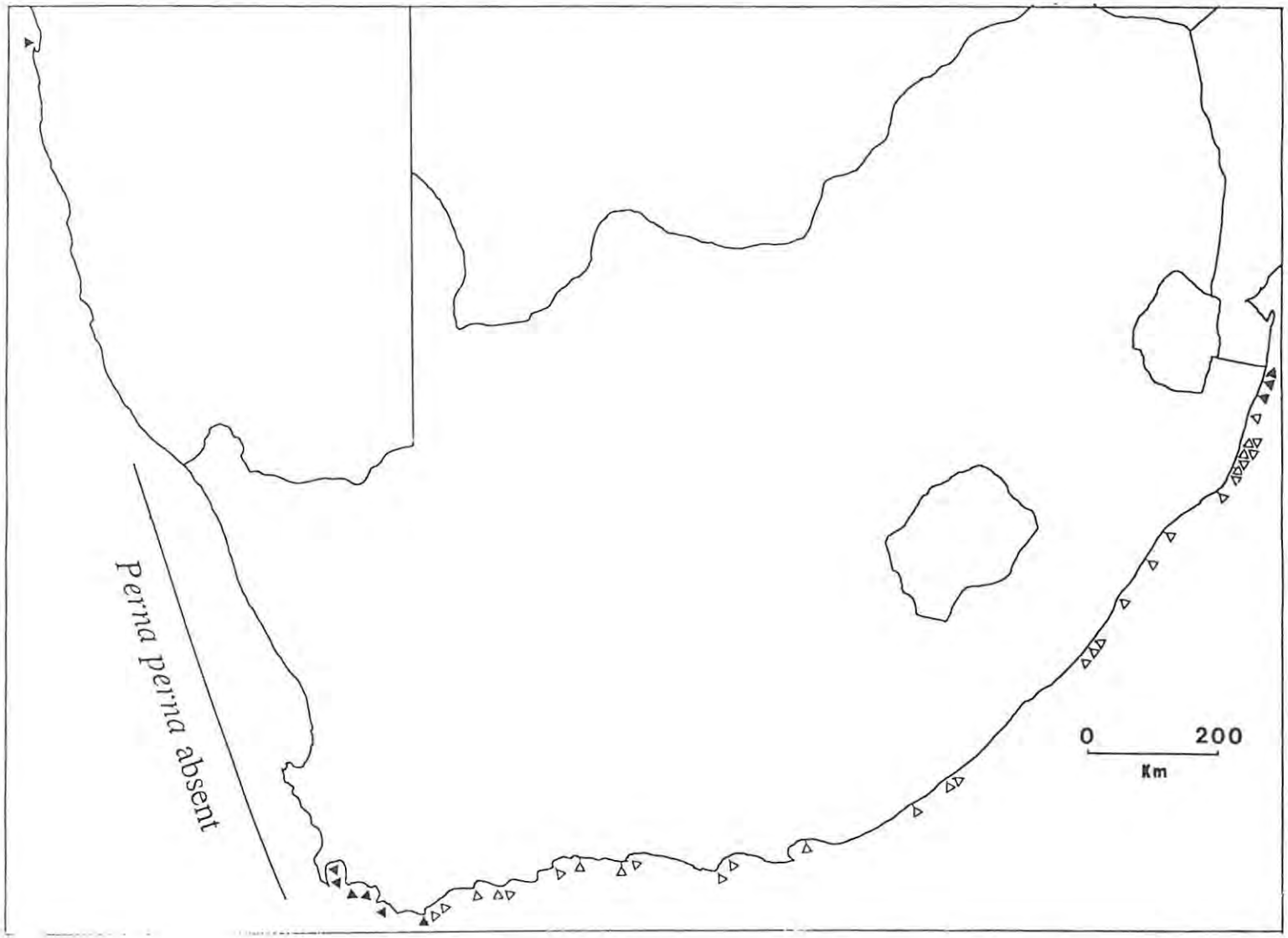
Table 2.8. (Cont.)

(9) Arniston-1 (Nov '94)	50	17	33	72.00	47.06	84.85
(9) Arniston-2 (Dec '92)	50	20	25	40.00	5.00	76.00
(9) Arniston-2 (Nov '94)	47	28	13	21.00	7.14	61.54
(10) Cape Infanta (Dec '92)	50	15	21	54.00	13.30	100.00
(10) Cape Infanta (Apr '94)	50	20	15	40.00	25.00	100.00
(11) Jongersfontein (Nov'94)	50	31	18	6.00	6.45	5.55
(12) Still Bay (Apr '94)	51	31	15	10.00	0.00	33.30
(13) Mossel Bay-1 (Dec '92)	50	28	22	22.00	3.57	45.45
(13) Mossel Bay-1 (Nov '94)	49	24	24	10.00	0.00	20.83
(13) Mossel Bay-2 (Nov '94)	34	17	15	53.00	17.65	100.00
(14) Wilderness (Dec '92)	50	22	22	58.00	18.18	100.00
(15) Robberg (Dec '92)	35	17	18	17.00	5.88	27.78
(16) Plettenberg (Nov '94)	56	32	22	0.00	0.00	0.00
(17) St. Francis (Dec '92)	50	27	17	38.00	18.52	70.59
(17) St. Francis (Nov '94)	57	17	13	49.00	52.94	92.31
(18) Jeffrey's Bay (Nov '94)	59	31	23	46.00	16.13	95.65
(21) Queensberry B (Oct '92)	48	23	24	50.00	13.04	87.50
(22) Haga Haga (Oct '92)	50	25	23	36.00	8.00	69.56

Table 2.8. (Cont.)

(23) Port Edward (Nov '93)	50	20	24	54.00	20.00	95.83
(24) Ramsgate (July '94)	50	26	19	30.00	0.00	78.95
(25) Oslo Beach (July '94)	50	19	18	28.00	0.00	72.22
(26) Umkomaas (July '94)	50	18	22	34.00	0.00	77.27
(27) Umdloti (July '94)	50	31	19	24.00	0.00	63.16
(28) Zinkwazi (July '94)	50	24	26	48.00	0.00	92.31
(29) Dawson's Rocks (July '94)	50	28	20	14.00	3.57	30.00
(30) Mapelane (Dec. '95)	50	30	20	36.00	6.67	80.00
(31) First Rocks (Dec. '95)	50	32	15	32.00	9.37	86.67
(32) Mission Rocks (Dec. '95)	50	33	16	20.00	3.03	56.25
(33) MR-CV (Dec. '95)	50	27	22	46.00	7.41	95.45
(34) Cape Vidal (Dec. '95)	50	30	17	44.00	23.33	88.23
(35) CV-LP (Dec. '95)	49	16	32	4.08	0.00	6.25
(36) Leven Point (Dec. '95)	50	36	12	6.00	2.78	16.67
(37) Sodwana (Dec '95)	50	23	27	8.00	0.00	14.81
(38) Island Rock (Dec '95)	48	32	16	0.00	0.00	0.00
(39) Bhanga Nek (Dec '95)	57	36	21	0.00	0.00	0.00
(40) Kosi Mouth (Dec '95)	52	41	11	0.00	0.00	0.00

Fig. 2.19. Prevalence of infection with *Proctoeces* in *Perna perna* along the Southern African coast (▵=infected, ▲=not infected) (see legend Fig. 2.3 for names of localities).



2.4 DISCUSSION

2.4.1 *Perna perna*

Of the four trematode species found in *Perna perna*, two are encysted metacercariae which have not been reported in the area. The other two, metacercariae of *Proctoeces* and bucephalid sporocysts, have been found infecting *P. perna* in South Africa before (Lasiak, 1989, 1993). Both encysted metacercariae and the bucephalid sporocysts have also been found on the west coast (S. Webb, pers. comm.), and their taxonomic status and epidemiology are dealt with in detail elsewhere (Webb and Calvo-Ugarteburu, in prep). Although prevalence of cysts in some localities is rather very high (Figs. 2.7 and 2.9), they are not likely to have any serious harmful effect on the mussels, therefore, the rest of this discussion will deal with *Proctoeces* and the bucephalid sporocysts.

Bucephalid sporocysts

During the survey *Perna perna* was frequently found to be infected by bucephalid sporocysts (Fig. 2.11 and Table 2.4). Bucephalid sporocysts have commonly been found parasitizing the gonad and digestive gland of bivalve molluscs (Tennent, 1906; Woodhead, 1929, 1930; Cole, 1935; Cheng and Burton, 1965; Breton, 1970; Stunkard, 1974; Umiji *et al.*, 1976; Joseph, 1978; Lasiak, 1991, 1993; Taskinen *et al.*, 1991, 1994; Gibson *et al.*, 1992). The taxonomic status of the sporocysts found in this study is unclear. They belong to the family Bucephalidae Poche. Von Baer in 1827 erected the genus *Bucephalus* to include some cercarial forms

(Kniskern, 1952). Von Siebold in 1848 used the generic name *Gasterostomum* to refer to some adult parasites from fish. It was not till 1858 that Wagener noticed the relationship between *Bucephalus* cercariae and *Gasterostomum* adults based on anatomical resemblances (Tennent, 1906; Kniskern, 1952). Kniskern (1952) reviewed the literature on the family Bucephalidae and enumerated a series of criteria for the erection of genera and the differentiation of species within the family. He cautioned against conclusions drawn from morphological characters in cases where life histories were not known. Tennent (1906) was the first to complete a life-cycle experimentally, and although by 1950 Yamaguti already recognized 145 species of adult bucephalids parasitizing freshwater and marine fishes, experimental life-cycle studies have still been carried out on only a few species (Taskinen *et al.*, 1994). The few Bucephalidae life-cycles known to date are all fairly uniform. The sporocysts and cercariae invariably occur in bivalves, the metacercariae in teleost fishes and the adults in predatory fishes (Lauckner, 1983).

So far nothing is known about the other stages of the life-cycle of the sporocysts found in this study, which would be necessary to identify them to specific level. The identification and study of the life cycle of this parasite is a major job on its own. For the purpose of this thesis this parasite will be refered to as bucephalid sporocysts, no attempt was made to resolve their life-cycle or clarify their taxonomic status.

Lasiak (1993) found bucephalid sporocysts in *Perna perna* similar to those found in this study. She found no apparent geographic trends in infection, with parasites being present on both the south and east coasts of South Africa, as was found in this survey. Lasiak (1993)

found low prevalences of infection (from 1,7% to 12%). Most of the localities sampled during this study also showed low prevalences (<12%) (Table 2.4), but prevalences went up to nearly 50% in some localities, e.g. Cape St. Francis. The results from the small scale survey showed that this parasite is randomly distributed throughout the mussel bed (Table 2.3), which is in agreement with Lasiak's (1993) conclusion that parasitized individuals were distributed at random. Also in agreement with Lasiak's results are the results for size related prevalences. In both studies a positive correlation was found between the size of the mussels and the infection rates (Lasiak, 1993; Fig. 2.13). The evidence on seasonality is ambivalent. Whilst Lasiak (1993) concluded that the prevalence of infection was not independent of the season, no seasonal differences were found in this study (Fig. 2.12). Like Lasiak, Tennent (1906) and Joseph (1978) found a peak of infection by bucephalid sporocysts in summer months, from June/July to October, though Tennent (1906) found infected oysters every month. On the other hand, Taskinen *et al.* (1994) found no significant differences between monthly prevalences of either of the two bucephalid parasites they studied. The lack of seasonal differences in this study could be the result of a methodological error resulting in insufficient statistical power. The seasonal study was done in a locality with very low infection rates (Kowie Point), and infection rates remained low or non-existent all year round. In order to clarify if there are seasonal variations on the prevalence of the bucephalid sporocysts found in *P. perna* in South Africa, a monthly survey in one of the localities with higher prevalences, e.g. Cape St. Francis, Hougham Park or Oslo Beach, is necessary.

Proctoeces

Lasiak (1989) found metacercariae of a fellodistomid trematode in all the mussel samples examined along the South African coast with the exception of those samples from the western Cape. Both the parasite found by Lasiak, and the one found during this study were identified by R. Bray as metacercariae of the genus *Proctoeces*, possibly *P. maculatus*.

A) Taxonomy

Proctoeces maculatus was first described as *Distomum maculatum* by Loss in 1901 from the hindgut of a labrid fish, and by Linton in 1907 from a sparid fish (Bray and Gibson, 1980). In 1911 it was placed in the genus *Proctoeces* by Odhner (Prevot, 1965; Bray and Gibson, 1980). The first record of the molluscan host of the genus *Proctoeces* was reported by Fujita in 1925 (Shimura and Egusa, 1979). He found a metacercaria in an oyster in Japan and described it as a new species, *P. ostreae*. This species was considered a synonym of *P. maculatus* by Bray (1983). The taxonomic status of the genus *Proctoeces* is confused even now, some 80 years after its erection. Bray and Gibson (1980) concluded that of the 14 species assigned to this genus, seven were synonyms of *P. maculatus* and four were incorrectly placed in this genus. They only accepted two species, *P. maculatus* and *P. lintoni*, and considered a third species, *P. magnorus*, as a *species inquirendum*. In his review in 1983, Bray listed 21 synonyms for *P. maculatus*, including *P. lintoni* as one of them. Shimazu (1984) concluded that *P. magnorus* is different from *P. maculatus* in that the oral sucker is bigger than the ventral sucker, and therefore accepted it as a separate species.

Bray (1983) indicated that *Proctoeces maculatus* had a worldwide distribution. Different larval stages have been found in the Black Sea, the Mediterranean and Atlantic coasts of Europe, Japan, Chile and the U.S.A (reviewed by Bray, 1983). *Proctoeces* employs a variety of hosts and life cycle patterns. Adults have been recorded in 59 species of teleosts, one polychaete, three bivalves and fourteen gastropods; metacercariae in one amphineuran, six gastropods, nine bivalves, one cephalopod, three polychaetes, and one echinoderm; and sporocysts in three species of bivalves (Bray, 1983). Wolf *et al.* (1987) and Lasiak (1989) found *Proctoeces* sp. in Australia and the south and east coasts of South Africa respectively, thus confirming that the distribution of this genus in molluscs is world-wide.

From the description given above it appears that the metacercariae found in this study and that described by Bray and Gibson (1980) and Bray (1983) as *Proctoeces maculatus* are identical, and specimens from this work were identified by R. Bray (pers. comm.) as possibly *P. maculatus*. The size of these metacercariae (2.5-4.32 mm) (Table 2.5) falls within the size range of most *Proctoeces* described (e.g. Stunkard and Uzmann, 1959; Shimazu, 1979; Bray and Gibson, 1980; Shimazu, 1984), although there is a positive relationship between the size of the mussels and the size of the metacercariae (personal observation). This may very well explain why some authors have found *Proctoeces* as big as 8 mm (Shimura and Egusa, 1979; Oliva, 1984). Oliva and Zegers (1988) showed that the morphometrics of a parasite are strongly affected by its host, therefore they concluded that morphological characters that have previously been considered of taxonomical importance, such as body length, size of gonads and shape of the ovary, are not very reliable. They concluded that the only characters that do not vary significantly are the diameter of the suckers and the eggs. The oral to ventral sucker

ratio of metacercariae from this study is 1:1.3-1.8, which agrees with most of the literature for *P. maculatus* (Shimazu, 1979; Shimura and Egusa, 1979; Bray and Gibson, 1980; Shimazu, 1984).

Also, there is no reason to believe that the metacercariae found in this study are a different species from that found by Lasiak (1989) since, although she did not describe her parasites, the results from this work conform to her findings. She found similar infection rates to those in this study, and also found higher prevalence and intensity of infection in females than in males, a significant relationship between prevalence and intensity of infection and the size of the host and no seasonal changes in prevalence. Therefore, until further morphological and life cycle studies are done in order to clarify the specific status of this parasite, it will be referred to as *Proctoeces* sp.

B) Prevalence

Prevalence data around the world are extremely variable. There is no apparent pattern either by host species, larval stage or geography, and various authors have found infection rates ranging from less than 1% up to 99%. Extreme values of less than 10% (Uzmann, 1953; Canzonier, 1972; Shimura, 1980; Winstead and Couch, 1981; Wolf *et al.*, 1987 and Robledo *et al.*, 1994) or more than 80% (Shimura, 1980; Oliva and Diaz, 1988, 1992 and Bretos and Chihuailaf, 1993) are quite common, but mostly they range between 5 and 80% (Uzmann, 1953; Ichihara, 1965; Lang and Dennis, 1976; Wardle, 1980; Pondick, 1983; Osorio *et al.*, 1986; Turner, 1986 and Figueras *et al.*, 1991).

These differences in prevalence have sometimes been explained as a consequence of a latitudinal effect. For example, Oliva and Diaz (1992) found that prevalence of infection of *Fisurella limbata* with *Proctoeces lintoni* in Chile increased with latitude (from 14 to 99%), as did the degree of progenesis of the parasites. Aitken-Ander and Levin (1985) applied the term "progenetic" to sexually mature, ovigerous larval stages of digenetic trematodes occurring in invertebrates. Progenesis has commonly been reported in the genus *Proctoeces* (Dollfus, 1964; Prevot, 1965; Lang and Dennis, 1976; Tripp and Turner, 1978; Winstead and Couch, 1981; Bray, 1983; Aitken-Ander and Levin, 1985; Wolf *et al.*, 1987; Oliva and Diaz, 1992), and appears to occur mostly in cold waters. Bray (1983) pointed out that, apart from eliminating the necessity of the normal fish host, progenesis allows opportunities for adaptation to new hosts, since gravid specimens can temporarily live in several fishes. This would help to explain the large number of fish species that have been recorded as hosts of *Proctoeces.*

C) Seasonality

From the literature it appears that there are no clear patterns in seasonality of infection with *Proctoeces* sp, or in sex and size linked prevalence. Lasiak (1989) concluded that prevalence of infection in South Africa did not change from season to season, although she only used a quarterly sampling interval. This agrees with several other authors who found little variation in the prevalence of infection throughout the year, with no significant differences among different months (Shimura, 1980; Pondick, 1983; Oliva and Diaz, 1992 and Bretos and Chihuailaf, 1993). In contrast, Lang and Dennis (1976), Tripp and Turner (1978), Winstead

and Couch (1981) and Turner (1986) found seasonal changes in prevalence. Both Lang and Dennis (1976) and Tripp and Turner (1978) agreed that infection with adult *Proctoeces* was highest in autumn, dropped in winter and was very low in summer. But whilst Lang and Dennis (1976) explained the peak in autumn to be due to cercariae being released from mature sporocysts which have maximum incidence during summer, Tripp and Turner (1978) found no seasonal changes in incidence of sporocysts. Turner (1986) on the other hand found that prevalence of infection with sporocysts increased in July and disappeared by September. Uzmann (1953) reported that maturation of cercarial generations of *Cercaria milfordensis* occurred during late winter and spring, when the water temperature was at its lowest (2-8^0 C).

The result of the present study showed that the prevalence of infection was dependent on the sex of the host throughout the year, but there were no seasonal changes in prevalence (Fig. 2.18). This agrees with most of the literature referring to seasonal infections of *Proctoeces*.

D) Sex and size linked prevalence

Prevalence of infection with metacercariae of *Proctoeces* was considerably higher in females than in males at all the localities examined (Fig. 2.16) and throughout the year (Fig. 2.18). This agrees with Lasiak (1989) and with Aitken-Ander and Levin (1985) who found higher prevalences and intensities of infection in females than males. On the other hand, Shimura (1980), Pondick (1983), Osorio *et al.* (1986), Oliva and Diaz (1988, 1992) and Bretos and Chihuailaf (1993) found no relationship between prevalence of infection and host sex. These apparently contradictory results may be explained in terms of the larval stage that was studied.

This study, as well as both Lasiak's and that of Aitken-Ander and Levin, were on metacercariae whilst all the others were on adults.

Lasiak (1989) only used mussels larger than 30 mm in her studies since preliminary data showed no infections in mussels smaller than 30 mm. When size dependent prevalences were first examined with a sample size of only 25 mussels in each size class the results obtained conformed to Lasiak's. But when the sample size was increased to 100 in each of the two smaller size classes (i.e. 20-30 mm and 30-40 mm), higher prevalences were found (Fig. 2.17). Lasiak (1989) also found 100% infection in females larger than 65 mm, which corresponds completely with the results from this work (100% infection in females >50mm, Fig. 2.17), and that intensity of infection increased with host size. The present study showed that prevalence of infection was dependent on size for females but not for males, and that intensity was dependent on size in both sexes (Tables 1.6 & 1.7). Infection rates seem also to be dependent on the size of the host in other life history stages such as sporocysts (Lang and Dennis, 1976) and adults (White, 1972; Shimura, 1980; Osorio *et al.*, 1986; Oliva and Diaz, 1992 and Bretos and Chihuailaf, 1993). Exceptions to this are given by Windstead and Couch (1981), who found no direct correlation between the size of the host and the prevalence of metacercariae of *Proctoeces*, and Lang and Dennis (1976), who concluded that the incidence of adult *P. maculatus* did not increase with the size of the mussels.

1.4.2 *Mytilus galloprovincialis*

Despite the fact that mussels are commonly infected by trematodes, *Mytilus galloprovincialis*

seems to be surprisingly free of them. Cheng (1967) listed only two trematode species found in this mussel compared to ten species reported in *M. edulis*. Coustau *et al.* (1990) compared rates of parasitism by *Prosorhynchus squamatus* in *M. edulis* and *M. galloprovincialis* from the same locality in France, and found higher prevalences in *M. edulis* than in *M. galloprovincialis* throughout the year.

The absence of trematodes in *M. galloprovincialis* is unlikely to be due to a lack of studies on this species. Spain is the world's largest producer of mussels (Robledo *et al.*, 1995), with 200,000 metric tons of *M. galloprovincialis* being produced annually in Galicia (NW Spain) (Figueras, 1989). Due to the importance of this industry, several workers have been involved in the study of mussel culture in this area (e.g. Perez and Roman, 1979; Roman and Perez, 1979 and Figueras, 1989) but it appears that parasites are not a major problem for the culture of mussels in Spain (Figueras, 1989). Figueras *et al.* (1991) reported only one species, *Proctoeces maculatus*, parasitizing *M. galloprovoncialis* in Galicia and said that it was only rarely found. On the other hand Canzonier (1972) found 4% infection rates of *M. edulis* with *Cercaria tenuans* in the same area.

The present survey of parasites on this mussel has shown that *Mytilus galloprovincialis* is free of trematodes both in the area where it has been recently introduced in South Africa (Table 2.1) and in the Basque Country where it is indigenous (Table 2.2). One must realise that this apparent absence of parasites on *M. galloprovincialis* could be the result of small sample sizes. Although a sample size of 50 mussels per locality was enough to detect parasitism in *Perna perna*, this may not be enough for *M. galloprovincialis* since very low prevalences have been reported for this mussel. Cajaraville (1991) found 3 out of more than 400 mussels

examined to be infected with *Proctoeces maculatus* (0.73%). Her samples were also taken on the coast of the Basque Country. Nevertheless, it is clear that prevalences of trematodes in *M. galloprovincialis* are much lower than in *P. perna.*

SUMMARY

A survey of trematodes was done at different scales for populations of the indigenous mussel *Perna perna* and the invasive *Mytilus galloprovincialis* in South Africa, as well as for indigenous populations of *M. galloprovincialis* in Spain. Both populations of *M. galloprovincialis* were found to be free of parasites, whilst *P. perna* was commonly infected by trematodes. Four species of trematodes were found parasitizing *P. perna*, namely two types of encysted metacercariae, bucephalid sporocysts and metacercariae of *Proctoeces*. Prevalences of the two encysted metacercariae were independent of the sex of the host at all localities examined. There were no significant differences in the prevalence of infection with gelatinous cysts among the localities examined, nor seasonal variations. Infection rates of the cysts from the labial palps differed from locality to locality, with prevalences varying from 18 to 100%. Prevalence rates of this parasite were significantly higher during the winter months.

Both *Proctoeces* and the bucephalid sporocysts are randomly distributed throughout the mussel bed. Bucephalid sporocysts are found in very low prevalences at most sites along the South African coast. Prevalences are highly dependent on the size of the mussel, and there are no seasonal variations in infection rates with this parasite. *Proctoeces* is only found

between Cape Agulhas and Sodwana Bay, and it infects more females than males. Prevalence of infection was highly dependent on the size of the mussel in the case of females but not in the case of males. There were no seasonal variations in prevalence of infection with *Proctoeces* in either females or males. Metacercariae of *Proctoeces* were described.

CHAPTER 3.

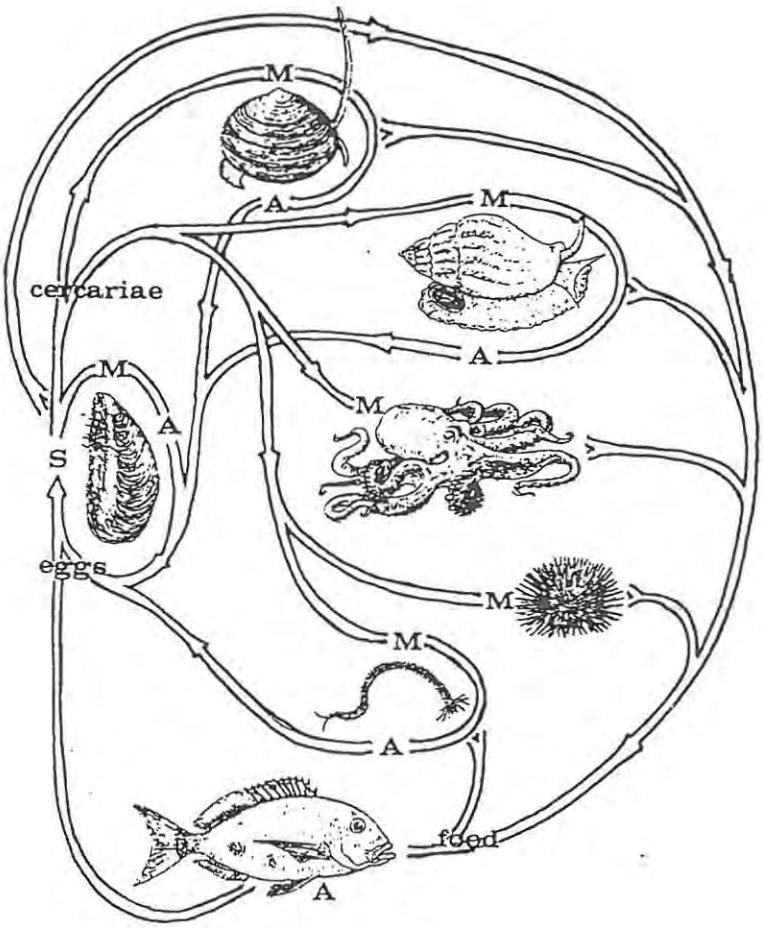
LIFE-CYCLE OF *Proctoeces*

3.1 INTRODUCTION

The digenetic metacercariae found during this work have been placed in the genus *Proctoeces*, and they are probably *Proctoeces maculatus* (R. Bray, personal communication). Unfortunately the taxonomic status of this genus is still confused (Bray and Gibson, 1980; Bray, 1983), and a more complete knowledge of the parasite's life-history is needed before a definitive classification can be made.

The life-history of *Proctoeces maculatus*, type-species of the genus *Proctoeces*, is fairly complex and has been reviewed by Bray and Gibson (1980) and Bray (1983). *P. maculatus* usually utilizes marine pelecypods as primary hosts and fishes as definitive hosts. Several other invertebrates may act as secondary hosts. Figure 3.1 (after Bray, 1983) shows different alternatives for its life-cycle. In a "typical" life-cycle the sporocysts are found in a bivalve mollusc, and there can be one or two generations of sporocysts. The cercariae are microcercous, but with a tendency to lose their tails. The mollusc can be ingested by a shallow water fish which becomes the final host. In this case the metacercarial stage may be omitted. The most common final host for this parasite is a teleost fish, normally of the family Labridae or Sparidae (Bray and Gibson, 1980; Bray, 1983). There may also be transfer of cercariae from one mollusc to another, or the entire life-history may occur within one host. Progenesis applies to sexually mature ovigerous digenetic trematodes occurring in invertebrates, and it is a common phenomenon observed among specimens of this genus.

Fig. 3.1. Diagram showing the complexities possible in the life-cycle of *Proctoeces maculatus*. A=adult, M=metacercaria, S=sporocyst. (After Bray, 1983).



Progenesis can be complete (Dollfus, 1964; Lang and Dennis, 1976; Windstead and Couch, 1981; Bray, 1983; Aitken-Ander and Levin, 1985; Wolf *et al.*, 1987 and Oliva and Diaz, 1992) or incomplete (Prevot, 1965; Turner, 1986). In the case of the metacercariae found in this study, although there were sperm and ova present in the metacercariae, these animals do not seem to be progenetic to the point of completing their whole life cycle in the mussels. They exhibit an incomplete progenesis, since there were no shelled eggs in the uterus.

Geographical patterns of distribution of this parasite along the South African coast seem to indicate that its distribution is linked to the presence of other hosts necessary for the completion of the life cycle. Prevalence data showed that there were no infected mussels west of Cape Agulhas, and that infections were more abundant on the south coast, and started to decline in Natal. There was a dramatic reduction in infection rates north of Cape Vidal, and no *Proctoeces* were found north of Sodwana (Fig 2.3, Chapter 2). Further evidence in favour of this hypothesis is the fact that Lasiak (1989) found the highest intensities of infection in the two marine reserves that she sampled. Whilst the majority of mussels elsewhere had low intensities (from 1 to 20 metacercariae per parasitized individual), intensities went up to 134 metacercariae in some mussels from Hluleka and 337 metacercariae per individual in Dwesa. Although there are no data available to support this hypothesis, the fact that these two places are marine reserves suggests that both the number of species present and the number of individuals per species are greater, making it easier for a parasite to complete its life cycle. Since the metacercaria is not a free living stage, but it must be eaten by the final host, a search for a sparid or labrid fish endemic to this area which feeds on mussels was carried out. After a short selection process the list of possible hosts was reduced to a single candidate, the

musselcracker *Sparadon durbanensis* (Smith and Heemstra, 1988; Buxton and Clarke, 1991; C. Buxton, pers. comm.). Microscopical examination of a few stomachs of this fish showed the presence of adult trematodes of the family Fellodistomidae, which were subsequently identified by R. Bray (pers. comm.) as belonging to the genus *Proctoeces*, again possibly *P. maculatus*.

The opportunity was taken here to attempt to clarify the life cycle of *Proctoeces* in *Perna perna* using a molecular biological technique. The application of molecular approaches to clarify the relationships within and between different species, including parasitic species, has increased in the last few years (Nadler, 1992; Lumb *et al.*, 1993; Fernandez *et al.*, 1994; Humbert *et al.*, 1994). Randomly Amplified Polymorphic DNA fingerprinting (RAPD) has proved to be a useful tool for the identification of species (Humbert *et al.*, 1994). The RAPD method employs short random oligo-deoxynucleotide primers ir a polymerase chain reaction (PCR) to generate polymorphic markers. The products of amplification are rur under an electric field on a gel, resulting in a profile referred to as a DNA fingerprint (Chambers, 1994). These profiles may be compared and similarity indices may be calculated among the samples.

In this Chapter the technique of RAPD-PCR DNA-fingerprinting was used to investigate the hypothesis that the parasites infecting *Perna perna* utilize musselcrackers as the final host. The null hypothesis was that the metacercariae that infect the mussels are related to the adult stages from the musselcracker. If the analysis of the fingerprints resolves the parasites from the mussels and those from the musselcrackers separately one can reject the null hypothesis.

On the other hand, if the dendrograms show mixed clustering of fingerprints from mussel and fish parasites one can tentatively accept the null hypothesis, although one cannot reject the hypothesis that they may still be different species. This Chapter was done in collaboration with other researches as it required specialist techniques. The DNA extraction and amplification were undertaken by R. Chambers and J. Seaman, and J. Seaman carried out the electrophoresis and analysis of the gels.

3.2 MATERIALS AND METHODS

3.2.1 Sample collection

Ten mussels (*Perna perna*) and ten musselcrackers (*Sparadon durbanensis*) infected respectively with metacercariae and adults of the genus *Proctoeces* were collected from Kenton-on-Sea (33^0 41'S, 26^0 41'E). Two parasites per animal were removed and placed in 70% alcohol for DNA analysis. A small piece of tissue was dissected from each host to check for possible tissue contamination. In addition, ten metacercariae were removed from one of the above mussels, and ten adults from one fish to study relationships among individual parasites within the same host. All the parasites were preserved in 70% alcohol for DNA extraction.

3.2.2 DNA extraction

The specimens were removed from the alcohol and ground into a powder, after freezing with

liquid nitrogen. The tissue was then suspended in STE buffer (0.1M sodium hydroxide, 50mM Tris, 1mM EDTA, pH 7.5) and 10 µl of 10% sodium dodecylsulphate (SDS) were added to lyse the cells. The DNA was then extracted twice using an equal volume of phenol:chloroform:isoamylalcohol (25:24:1). The supernatent was extracted once with an equal volume of chloroform:isoamylalcohol (24:1). The DNA was precipitated overnight using two volumes of ice-cold absolute ethanol and 1/10 volume of 2M sodium chloride. The precipitated DNA was recovered through centrifugation at 4^0C and resuspended in TE (10mM Tris-base; 1mM EDTA, pH 8) (Nxomani *et al.*, 1994). The presence and concentration of DNA within the extracted sample were determined by reading the absorbance of each sample at 260nm using a SHIMADZU uv-visible recording spectrophotometer. This was carried out on a 1/100 dilution of the actual sample and the concentration of DNA in each sample was estimated using the assumption that when $A_{260} = 1$, approximately 50 µg/µl of DNA is present (Bezuidenhout, pers. comm.).

3.2.3 Amplification and electrophoresis

Amplification of the DNA samples was carried out using 50 pmol/µl of primer 2 (code 3'-5'TGG ATG AAC G) and primer 6 (code 3'-5' AAC CGA TGC T); each with a GC ratio of 50%. The amplification procedure was done using a reaction mixture containing: 40 µmol deoxynucleotides (DNTP'S), 1x Taq polymerase reaction buffer IV (Biotech), 16mM magnesium chloride (Biotech), ~50ng/µl of DNA template and 0.5 units/µl of Taq polymerase (Biotech). The amplification mixture was covered with liquid paraffin and forty cycles of a PCR reaction were carried out. Table 3.1 shows the parameters used during the PCR cycles.

Table 3.1. Parameters used during the PCR cycles.

Initial denaturation	94°C	180 sec
Denaturation	94°C	30 sec
Primer annealing	37°C	30 sec
Extension	72°C	60 sec
Final extension	72°C	240 sec

Each amplified sample was then separated under an electric field. The gels used in the preparation of the RAPD fingerprints were cast in vertical Hoefer slab gel equipment (Model SE 400) as well as Tall Mighty Smalls and comprised ofacrylamide: bis acrylamide (29%:1% in Milli Q water). 0.08% ammonium persulphate and 0.05% N,N,N',N'-tetramethyl-ethylendiamin (TEMED) were added to the acrylamide mixture for the initiation of polymerisation. The resolving gel was prepared in 1.5M Tris-HCL, 0.4% SDS pH 8 while the stacking gel was prepared in 0.5M Tris-HCl, 0.4% SDS pH 6.8. Each gel was run for approximately 7 hours (approximately 4 hours for Tall Mighty Smalls) in 1x TBE (90mM Tris-borate; 90mM boric acid; 2mM EDTA, pH 8) (C. Bezuidenhout, pers. comm.).

After electrophoresis the gels were incubated in Buffer A (10% (v/v) ethanol, 0.5% (v/v) acetic acid) for at least 10 minutes. The gels were stained with 0.1% silver nitrate for exactly 10 minutes. The gels were developed in an alkaline solution of Buffer B (1.5% (w/v) sodium hydroxide, 0.1% (w/v) sodium borohydride and 0.15% (v/v) formaldehyde (C. Bezuidenhout,

pers. comm.). Gel to gel variation was corrected for by running a reference marker of pBR322 along with the RAPD fingerprints.

3.2.4 Analysis

Final polyacrylamide gels were scanned using the uvp gel system (GDS 2000) and stored as TIFF files. The individual fingerprints were then analyzed using a fully automatic computer programme known as Gel-Manager (Version 1.5). Similarity matrices were generated and dendrograms produced by Pearson's product moment correlation coefficient and unweighted pair group method with averages (UPGMA) (Nxomani *et al.*, 1994).

3.3 RESULTS

Each primer produced fingerprints with a large number of bands (Fig. 3.2) which were used to produce the dendrograms. Dendrograms for primers 2 and 6 were drawn initially including the tissue samples from the host and then without them to test for possible contaminations (Figures 3.3, 3.4, 3.5 and 3.6).

Fig. 3.2. Examples of RAPD patterns from primer 2 (A) and primer 6 (B).

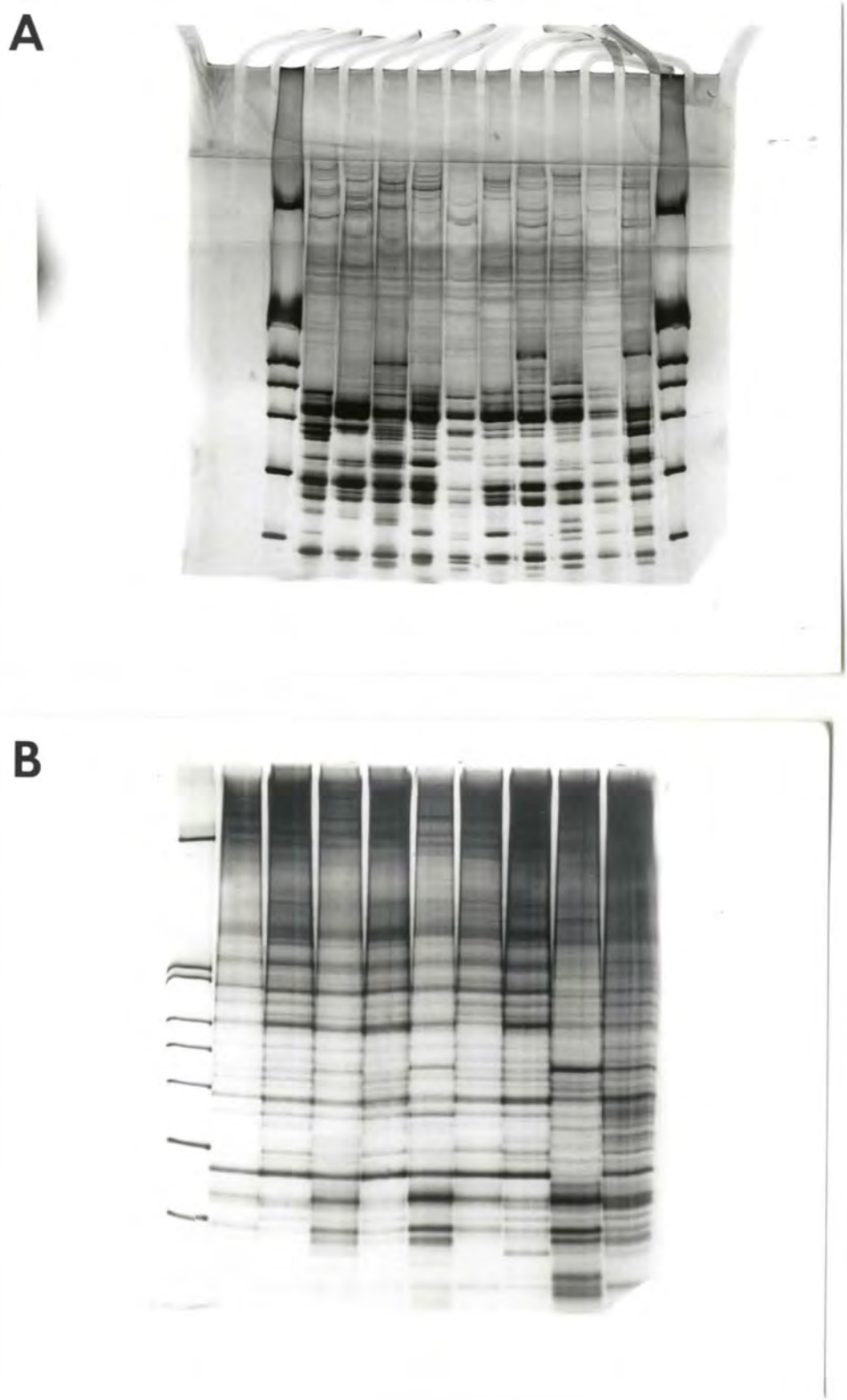


Fig. 3.3. Gel manager dendrogram based on primer 2, using the host tissues as controls. Abbreviations: m1 to m10 = tissue from mussels one to ten; m0-1 to m0-10 = ten metacercariae from one mussel; m1-1 and m1-2 = the two metacercariae from mussel one; etc. The adults from the fish are labelled the same way, using f instead of m. See text for significance of marked individuals.

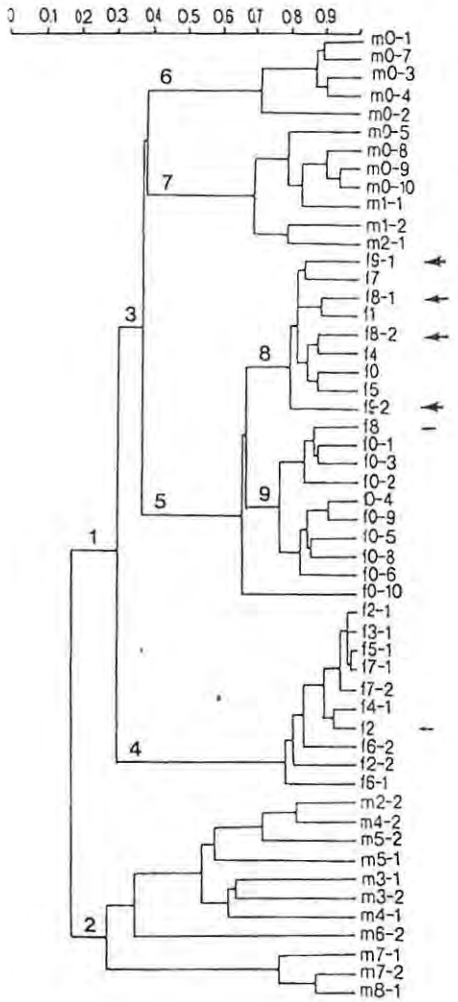


Fig. 3.4. Gel manager dendrogram based on primer 6, using the host tissues as controls. See Fig. 3.3 for abbreviations, and text for significance of marked individuals.

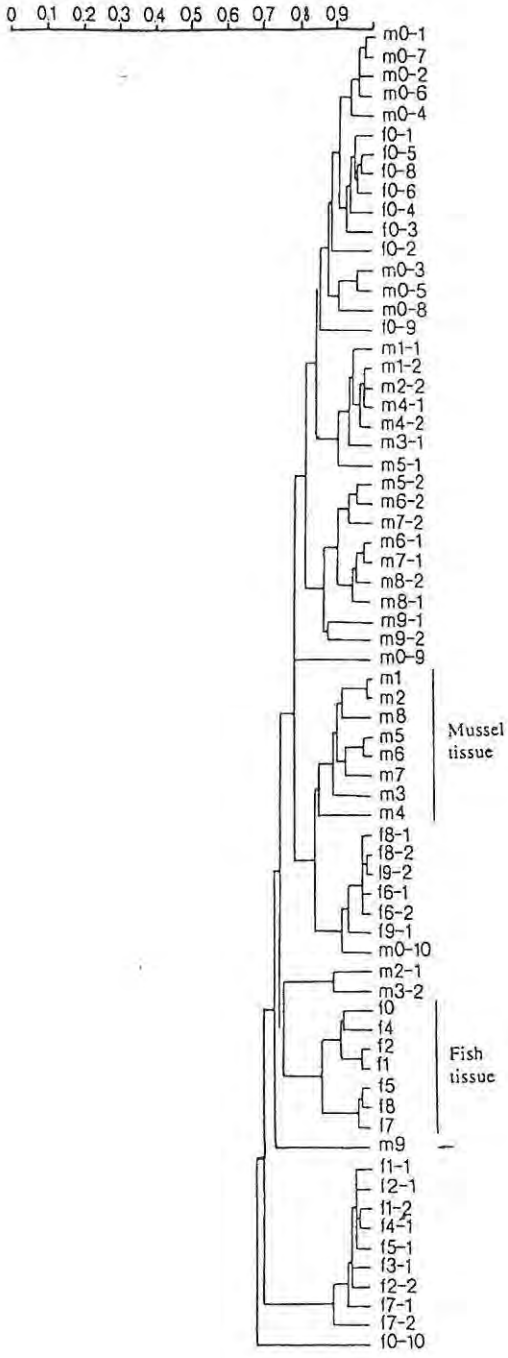


Fig 3.5. Gel manager dendrogram based on primer 2, without host tissue. See Fig. 3.3 for abbreviations and text for significance of marked individuals.

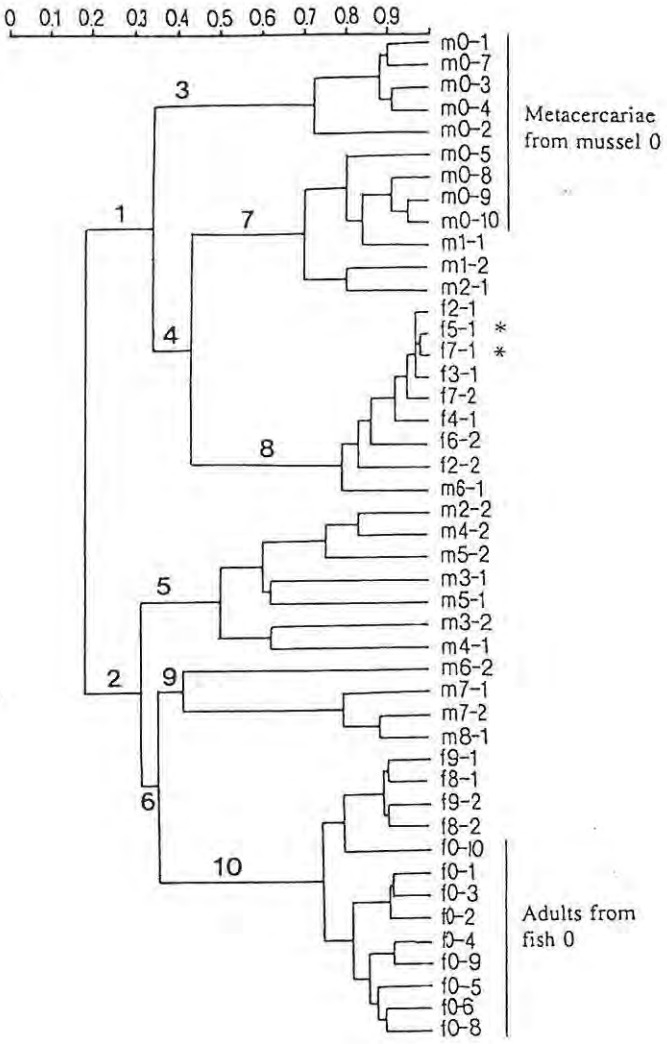


Fig 3.6. Gel manager dendrogram based on primer 6, without host tissue. See Fig. 3.3 for abbreviations and text for significance of marked individuals.

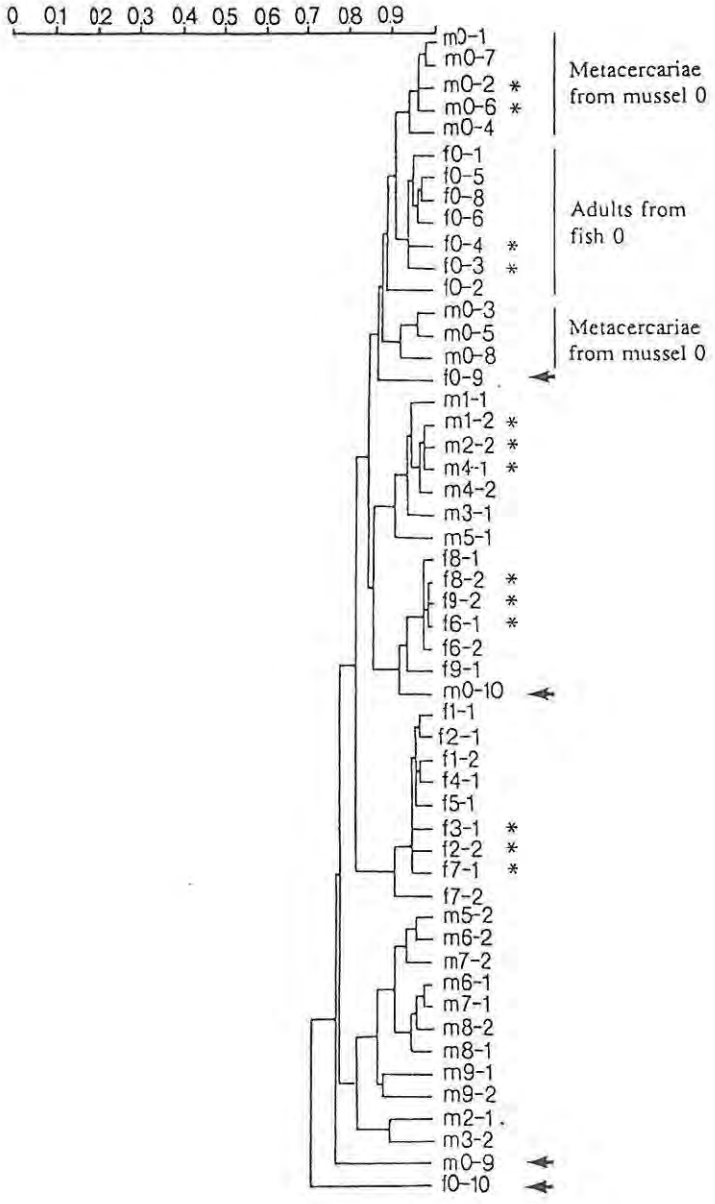


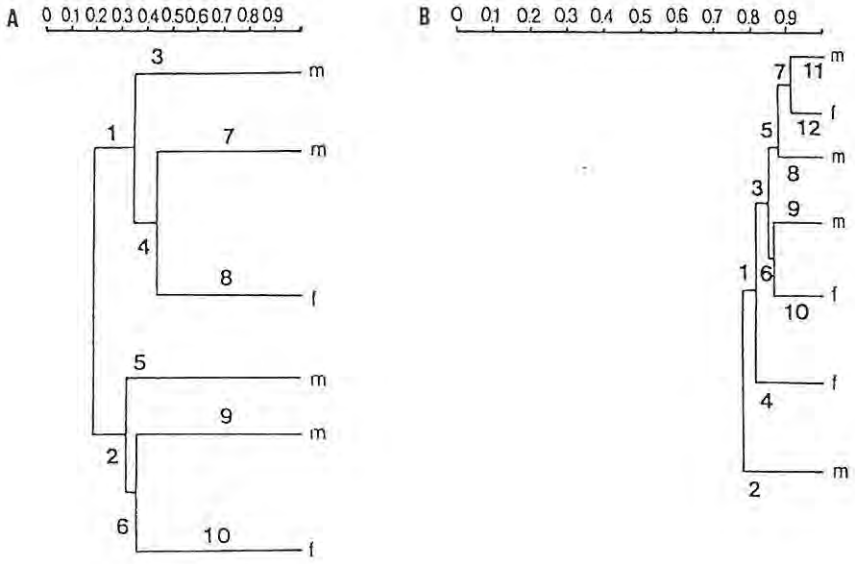
Figure 3.3 shows that, although the pattern is not perfect, e.g. f2 and f8 are in different clusters, most tissue samples from the musselcracker clustered together (cluster 8). Parasites from two of the fish (f9-1, f8-1, f8-2 and f9-2) grouped together with the tissue samples in cluster 8, which could be an indication of possible contamination of fish tissue within the samples. However, in the case of f8-1 and f8-2, the tissue from the fish they had parasitized (f8) is in a separate group (cluster 9), indicating that this is not the case. Unfortunately there is no tissue from fish 9 (f9) to confirm this.

In the case of primer 6, tissue from both the mussels and the musselcrackers group in two separate clusters (with the sole exception of m9), with no parasite samples amongst them, showing that there was no contamination of host tissue within the parasite DNA extraction (Fig 3.4).

Figure 3.7 shows schematic representation of the dendrograms from Figures 3.5 and 3.6. The dendrogram for primer 2 shows two nearly symmetric branches (1 and 2 of Fig. 3.7 A), each one comprising approximately half metacercariae (from mussels) and half adults (from fish). Each of these branches is in turn divided into a branch comprising a cluster of metacercariae from the mussels (3 and 5) and another branch (4 and 6) which separates into two groups, one including only metacercariae (7 and 9) and the other including only adults (8 and 10).

Both dendrograms without the tissue controls (Figs. 3.5 and 3.6) show that some of the metacercariae are more closely related to some of the adults than to other metacercariae from

Fig. 3.7. Schematic representation of the dendrograms of (A) Fig. 3.5 and (B) Fig. 3.6. Grouping in (A) corresponds almost exactly to Fig. 3.5; i.e. it is almost a "perfect' scheme. In (B) the grouping is not perfect, e.g. f0-9 and f0-10 group together with mussel parasites and m0-10 groups with fish parasites (see Fig. 3.6).



the same host. For example, branch 7 of Fig. 3.7 A is closer to branch 8 than to branch 3, and branch 10 of Fig. 3.7 B is closer to branch 9 than to branch 12. With rare exceptions, adults from the same host cluster together in both dendrograms (Figs. 3.5 and 3.6), although they are more closely related to some metacercariae than to the other adults. Similarity indices of 0.43 and 0.35 were found among some metacercarie and some adults within primer 2, and of 0.91 and 0.86 using primer 6 (Fig. 3.7 A and 3.7 B), whilst some metacercariae only linked to other metacercariae at values of 0.19 for primer 2 (Fig. 3.7 A) and of 0.79 for primer 6 (Fig. 3.7 B). In the same way, similarity indices among some adults were much lower than among some metacercariae and some adults (0.19 for primer 2 and 0.83 for primer 6). The fact that there is a higher similarity index among some metacercariae and some adults than

metacercariae and some adults than among all metacercariae or all adults using both primers (branches 7-8 and 9-10 of Fig. 3.7 A and 9-10 and 11-12 of Fig. 3.7 B) suggests that the two life-cycle stages are very closely related. A comparison of Fig. 3.5 and Fig. 3.6 shows that the grouping differs from primer to primer. Although parasites from mussels and parasites from fish were in mixed groups in both cases, the similarity patterns varied. For example, primer 6 grouped metacercariae from mussel 0 (m0) with the adults from fish 0 (f0) (Fig. 3.6), whilst these two groups are widely separated using primer 2 (Fig. 3.5).

Finally, some samples were found to be identical to others. For example, Fig. 3.5 shows that one of the adult parasites from a fish was identical to an adult from another fish (f7-1 and f5-1). The number of identical pairs was much higher for primer 6 (Fig. 3.6). This included two of the metacercariae from the same mussel (m0-2 and m0-6) and two of the adults from one of the fish (f0-4 and f0-3). Identical individuals were also found from different hosts. For example, m1-2 is identical to m2-2 and m4-1. These individuals were all from different mussels. Likewise, f8-2 is identical to f9-2 and f6-1; and f3-1 is identical to f2-2 and f7-1. Again all these individuals were dissected from different fish.

3.4 DISCUSSION

Similarity indices among some metacercariae and some adults are higher than among all the metacercariae or all the adults, suggesting that parasites from the mussels and those from the fish are very closely related. This result is supported by the geographical ranges of both metacercariae and fish. The musselcracker, *Sparadon durbanensis*, is endemic to South Africa,

occurring in shallow littoral waters from False Bay to Natal (Smith and Heemstra, 1988; Buxton and Clarke, 1991). Although the geographic range of *Perna perna* is larger, i.e. from False Bay to central Mozambique (Berry, 1978), this mussel is infected by metacercariae of *Proctoeces* only within the range of the musselcracker (Fig. 2.3, Chapter 2). The eastern limits of both metacercariae and fish coincide within 100 km of Sodwana (Fig 2.3, Chapter 2). No musselcracker catches were reported north of Sodwana during 1995 (W. Bartholomew and S. Hoseck, unpublished data and pers. comm.). The absence of parasites west of Cape Agulhas, even though musselcrackers are present in this region, suggests that another host is necessary for the completion of the life-cycle.

The presence of genetically identical parasites in the same host as well as identical parasites in different hosts can be explained by the life cycle of the parasite (Fig. 3.1). In a "typical" life-cycle of a digenetic trematode, a free-swimming miracidium emerges from the egg and penetrates the first intermediate host. It metamorphoses into a saclike form, the sporocyst, which may give place to a second generation of sporocysts or to rediae. Within the sporocysts or the rediae a number of embryos develop asexually to become cercariae. Once the cercariae are mature they leave the primary host and enter a secondary host, usually a mollusc, where they develop into metacercariae. All metacercariae originating from cercariae from the same sporocyst are effectively clones and therefore genetically identical. The metacercariae do not leave the host and they enter the next host only when the infected molluscs are ingested. In the case of *Proctoeces* in South Africa, *Perna perna* is the secondary host, harbouring the metacercarial stage. Neither the primary nor the final host are currently known. When the cercariae leave the primary host they swim till they find a secondary host, in this case

mussels. More than one cercaria from the same primary host may enter the same secondary host, which would explain the presence of two identical parasites such as m0-2 and m0-6 in the same mussel (Fig. 3.6). On the other hand cercariae from the same primary host may infect several mussels, explaining the presence of identical parasite individuals in different mussels (e.g. m1-2, m2-2 and m4-1; Fig 3.6). This is especially possible in view of the small area from which specimens were collected. Since all the mussels and musselcrackers were collected from the same locality, it is possible that more than one fish had identical parasites or that the same fish was infected by more than one identical parasite. If two musselcrackers ate two mussels infected with metacercariae originating from the same sporocyst those metacercariae would develop into identical adults (e.g. f8-2, f9-2 and f6-1, and f3-1, f2-2 and f7-1; Fig. 3.6). If a musselcracker eats a mussel which is infected by identical metacercariae (i.e. a mussel which has been infected by more than one cercariae from the same primary host) these will develop into identical adults in the same fish (e.g. f0-4 and f0-3; Fig. 3.6).

Clearly the situation is complicated and it is not possible to draw definitive conclusions without rRNA analysis. Nevertheless, the results from this DNA analysis support the hypothesis that the metacercariae from *Perna perna* and the adults from *Sparadon durbanensis* belong to the same species. This case demonstrates the advantages of molecular techniques as an aid to elucidation of parasite life-cycles, but it also brings out the difficulties of applying genetic approaches to organisms which have complex life-cycles including cloned larval stages.

CHAPTER 4.

EFFECTS OF TREMATODES ON GENERAL CONDITION AND SURVIVAL OF *Perna perna*

4.1 INTRODUCTION

Chapter 2 showed that the indigenous mussel *Perna perna* is commonly infected by trematodes, whilst the exotic mussel *Mytilus galloprovincialis* is free of trematodes. The rest of the thesis deals with the effects of the two most important parasites (metacercariae of *Proctoeces* and bucephalid sporocysts) on *Perna perna.*

If a parasite is going to have an effect on the dynamics of the host population, it will do so by affecting the survival and/or reproduction of host individuals (Sousa and Gleason, 1989; Huxham *et al.*, 1993). The effects on survival can be direct (increasing or decreasing the mortality of the hosts) or indirect (affecting the interactions of the host with other individuals such as predators and competitors). This chapter studies the effects of these two parasites on several aspects of the general condition and survival of their host. The effects of each parasite species were studied separately. However, heavy infections by bucephalid sporocysts may mask the presence of *Proctoeces*. Obvious cases of double infections were omitted, since, when different species of trematodes occupy the same host, interactions amongst them may be expected (Curtis and Hubbard, 1993). Nevertheless, there is no doubt that some of the animals infected with sporocysts may have been hosts for *Proctoeces* as well. This possibility also applies to animals used in later chapters.

The most direct way to estimate the deleterious effect of a parasite on the ecological fitness of a bivalve host is to measure the reduction in the rate of somatic and germinal growth (Newell and Barber, 1988). Various indices of condition have been proposed through the years (see Lawrence and Scott, 1982 and Davenport and Chen, 1987 for examples), but the most widely used one is Hopkin's condition index (Lawrence and Scott, 1982; Davenport and Chen, 1987; Newell and Barber, 1988). This is the ratio of dry flesh weight to the internal cavity volume of the shell, and is a prime indicator of how well an animal has utilized the volume available for tissue growth. In this chapter, the effects of *Proctoeces* and bucephalid sporocysts on the condition index of mussels were studied. Since several authors have reported a high correlation between the condition index and the reproductive state of an animal (Walne, 1970; Gee *et al.*, 1977; Pekkarinen, 1991) the condition index of infected and non-infected mussels was measured before and after spawning.

The condition index may give an idea of the state of health of an individual, but it does not contribute much information at a population level. On the other hand, energetic values are the most meaningful units of biomass for ecological research, as they give an indication of the energy that can be potentially passed through the ecosystem (Odum, 1971). Therefore, the effects of both parasites on the energetic content of the host were also considered.

It is generally accepted that parasites decrease fitness (Poulin and Vickery, 1993), and often a higher mortality rate and an increased susceptibility to poor environmental conditions have been found in molluscs infected with parasites (Rothschild, 1941b; Moose, 1963; Etges and Gresso, 1965; Sturrock, 1966; Howell, 1967; Sturrock and Sturrock, 1970; Lo, 1972;

Meuleman, 1972; Berger and Kondratenkov, 1974; Tallmark and Norrgren, 1976; Makanga, 1981; Minchella, 1985; Skorping, 1985; Lauckner, 1986; Ward and Langdon, 1986; Ruiz, 1991; Jonsson and Andre, 1992; Huxham *et al.*, 1993; Lafferty, 1993; Schwartz and Cameron, 1993). However, there is no general agreement on the matter. For example, Hodasi (1972), Wilson and Denison (1980), Sluiters *et al.* (1980), Sluiters (1981) and Copeland *et al.* (1987) all concluded that infection appears to have no significant effect on survival of the host, and other authors have shown that infected hosts live longer than uninfected ones (McClelland and Bourns, 1969; Baudoin, 1975). Loker (1979) and Sousa and Gleason (1989) said that the effects of parasites on host survival depend on the parasite-host system of study, and Sousa and Gleason (1989) concluded that they also depend on the level of stress experienced by the host.

A variety of environmental stressors may reduce host resistance to disease-causing agents and mortality. For example, Zuk (1987) found significant differences in longevity and weight of infected and non-infected crickets only when they were kept on a low-food regime, and Scott (1988) concluded that the impact of infection on the host depends not only on the pathogenicity of the infection but also on the interactions between infection and other factors such as nutritional status and stress (see Newell and Barber, 1988 for further references).

Intertidal bivalves are exposed to air during receding tides. During these periods they have two options: either they close their valves tightly, isolating themselves from the environment and switching to anaerobic respiration, or they may interact with the aerial environment allowing aerobic metabolism (Burnett, 1988; McMahon, 1988). The first option has the

advantage of minimizing the risk of desiccation and predation, but it results in inefficient utilization of energy stores (McMahon, 1988), and it may also lead to a great reduction in carbohydrates, since they become the main source of energy under anaerobic conditions (de Zwaan and Wijsman, 1976). Although high littoral molluscs are primarily aerobic during normal tidal cycles, they have been reported to shift to anaerobic metabolism in cases of extended periods of emersion (McMahon, 1988). Marshall and McQuaid (1993) concluded that during long periods of exposure to air, the intertidal mussel *Perna perna* in South Africa alternates periods of gaping with periods of shell closure, relying on aerobic metabolism for its survival. The last section of this chapter is concerned with the effect of parasites on the gaping behaviour, water loss and mortality rates of *P. perna* under extended periods of emersion.

The gaping of bivalves exposed to air may be related to the oxygen consumption and metabolic demands of the organism, but it may also be related to the state of health of the animal. Howell (1967) reported that the adductor mussels of oysters heavily infected with *Bucephalus longicornutus* showed signs of weakening and the valves were easily opened. Similarly, Lauckner (1983), Ward and Langdon (1986) and Jonsson and Andre (1992) also reported abnormal valve movement patterns in infected animals.

To conclude this chapter, the effects of both parasites on the strength of the adductor muscle of mussels were evaluated by measuring the force necessary to open the valves of infected and non-infected mussels.

4.2 MATERIALS AND METHODS

Infection rates of trematodes in mussels can only be determined *post hoc* by dissection of the animals. To reduce the number of animals needed in the experiments, mussels were collected from natural populations with high infection rates. Mussels from Kowie Point (33^0 38'S, 26^0 52'E) showed about 50% infection rates with *Proctoeces*, and approximately half of the mussels bigger than 70 mm from Hougham Park (33^0 47'S, 25^0 44'E) were infected with bucephalid sporocysts (Fig. 2.2 and 2.3, Chapter 2).

All the data were tested for normality and homogeneity of variances prior to statistical analysis. If they did not fulfill the conditions for parametric tests, the appropriate transformations were done (Zar, 1984). If, after the transformation the conditions were still not fulfilled, non-parametric tests were used as specified.

4.2.1. Condition index

Since several authors have reported a high correlation between the condition index and the reproductive state of bivalves (Walne, 1970; Gee *et al.*, 1977; Pekkarinen, 1991), the condition index for populations of infected and non-infected mussels was measured before and after the major spawning period, i.e. April-May. Mussels were collected from Kowie Point (length 60-85 mm) and Hougham Park (length 90-110 mm), and their condition was calculated as the ratio of dry weight to shell cavity volume. The total volume of each individual was measured using the water displacement method. Each mussel was placed in

turn in a 100 ml measurement cylinder containing water. A reference level was chosen prior to the introduction of the mussels, and the water was returned to the reference level by pipetting. The total length and weight of the animals were also measured prior to dissection. Shell volume was measured by the same method, and the shell cavity volume was calculated as the total volume of the animal minus the volume of the shell.

The soft tissues were carefully removed, weighed and examined for parasites. Afterwards they were dried in an oven at 60°C for at least 48 hours to measure the dry weight. The condition index was calculated as dry weight in grams divided by the shell cavity volume in millilitres multiplied by a hundred. The condition indices of animals with and without *Proctoeces* and bucephalids were compared using t-tests. Separate tests were done for populations collected before and after spawning.

During the collection of data for the calculation of the condition indices the maximum length, total weight and volume of the whole animal and the fresh flesh weight were measured. These data were used for the calculation of regression lines for total weight *vs* length, volume *vs* length and flesh weight *vs* length for parasitized and non-parasitized mussels both before and after spawning. Slopes and intercepts of the regression lines for parasitized and non-parasitized individuals were compared using a test for equality of regressions (Kleinbaum *et al.*, 1988).

4.2.2. Energetic values

After weighing, the dry tissues were finely ground with a mortar and pestle in order to homogenize the different components, and subsamples were bombed in a Gallenkamp ballistic bomb calorimeter. The energetic contents per gram of dry weight of mussels with and without parasites were compared using t-tests.

4.2.3. Gaping, water loss and survival

Mussels between 60 and 90 mm long were collected from Kowie Point and Hougham Park. One hundred mussels from each locality were weighed and placed standing upright in the laboratory, simulating their position in the field, with the anterior end down. Relative humidity was kept between 10 and 30% and temperature between 20 and 30^{0}C. Observations were made at two-hour intervals to record gaping. All animals with the valves parted were recorded as gaping, without taking into account the width of the gape. Mussels were weighed every six hours to measure loss of water. Under natural conditions *Perna perna* is never exposed to air for more than twelve hours. Therefore, the number of infected and non-infected mussels that died in the first twelve hours and the amount of water that mussels with and without parasites lost in that period were compared using a Student's t-test. A Spearman's correlation test was carried out to check for possible relationships between the number of times the mussels gaped in the first twelve hours and the number of parasites they contained.

In order to check if the effects of the parasites were more marked under stressful situations,

the experiment was continued until the mussels died. The experiment lasted 84 hours, by which time all the animals had gaped and failed to react to probing, therefore they were considered to be dead. The final weight of each mussel was recorded as it was declared dead. Mussels were then dissected and examined for parasites, and the tissues were placed in an oven at 60^0 C for a period of 48 hours. For each mussel the time that it took to die, the number of times that it gaped and the loss of weight during that time, the presence or absence of *Proctoeces* and bucephalid sporocysts, the number of *Proctoeces* metacercariae present and the dry weight were recorded.

Total mortality rates, and the mean amount of water lost by infected and non-infected animals were compared using t-tests. The relationships between the presence/absence of parasites and/or the number of parasites and the number of times the mussels gaped were studied using Spearman's correlation coefficient.

4.2.4. Adductor muscle strength

Mussels from Kowie Point (65-85 mm) and Hougham Park (85-105 mm) were collected in order to compare the strength of the adductor mussel for individuals infected and those not infected with *Proctoeces* or bucephalid sporocysts. The force needed to open infected and non-infected mussels was measured with an Instron 4301 apparatus. Two small hooks (5-10 mm long) were inserted on the byssus opening on the anterior ventral surface of the mussels and attached to the jaws of the machine. The machine was run with a uniform speed of separation of the jaws of 100±20 mm per minute until the mussel stopped presenting a

resistance. The highest force reached during this procedure was recorded. The mussels were then dissected and examined for parasites. Flesh tissues were placed in an oven at 60°C for at least 48 hours and dry weights were recorded. The strength of the mussels was recorded as Newtons needed to force them open per gram of dry weight. The force needed to open mussels with and without parasites was compared using a t-test.

4.3 RESULTS

4.3.1. Condition Index

The condition indices of mussels with and without *Proctoeces* and sporocysts before and after spawning are shown in Table 4.1. The results of the t-tests showed that mussels infected with either parasite had a significantly lower condition index than non-infected mussels only in the samples taken after spawning ($p<0.05$ for both parasites). Neither parasite had a significant effect on the condition of the host before spawning ($p>0.05$ for both parasites). Nevertheless, since, contrary to expected, mussels without sporocysts appeared to be in better condition after spawning than before spawning, one should therefore treat these results with caution.

Table 4.2 shows the regression equations for all the variables measured in animals with and without parasites before and after spawning. These curves are given in Figs. 4.1 and 4.2. The F-values for the comparisons between regression equations for parasitized and non-parasitized mussels before and after spawning are shown in Table 4.3. There were no significant differences between the slopes or intercepts of any of the lines for non-infected mussels and

mussels infected by either parasite, indicating that they are likely to belong to the same statistical population.

Table 4.1. Condition indices of *Perna perna* with and without *Proctoeces* and bucephalid sporocysts before and after spawning.

	Yes *Proctoeces*	No *Proctoeces*	Yes sporocysts	No sporocysts
Before spawning				
Sample size	37	43	15	22
Mean	6.19	6.09	5.11	5.67
Std. deviation	1.12	1.13	1.00	1.22
	p>0.05 n.s.		p>0.05 n.s.	
After spawning				
Sample size	52	22	7	35
Mean	4.77	5.57	5.11	6.39
Std. deviation	1.18	1.35	1.43	1.53
	p=0.01 *		p<0.05 *	

Table 4.2. Regression equations of length *vs* volume (L *vs* V), length *vs* total weight (L *vs* TW) and length *vs* fresh flesh weight (L *vs* FW) of *Perna perna* infected and not infected with *Proctoeces* (*Proc.*) and bucephalid sporocysts (spor.) measured before and after spawning.

		Before Spawning	After Spawning
L *vs* V	Yes *Proc.*	Y = -14.0+0.5 X (r^2 = 75.3%)	Y = -27.8+0.7 X (r^2 = 85.5%)
	No *Proc.*	Y = -17.0+0.5 X (r^2 = 79.4%)	Y = -30.7+0.7 X (r^2 = 83.7%)
	Yes spor.	Y = -38.3+0.9 X (r^2 = 56.3%)	Y = -66.1+1.2 X (r^2 = 86.3%)
	No spor.	Y = -62.9+1.1 X (r^2 = 77.7%)	Y = -58.0+1.1 X (r^2 = 71.9%)
L *vs* TW	Yes *Proc.*	Y = -29.7+0.8 X (r^2 = 72.5%)	Y = -43.4+1.0 X (r^2 = 81.31%)
	No *Proc.*	Y = -29.8+0.8 X (r^2 = 78.8%)	Y = -35.8+0.9 X (r^2 = 64.1%)
	Yes spor.	Y = -54.0+1.2 X (r^2 = 66.9%)	Y =-116.0+1.9 X (r^2 = 91.6%)
	No spor.	Y = -81.3+1.5 X (r^2 = 67.2%)	Y = -67.0+1.3 X (r^2 = 60.4%)
L *vs* FW	Yes *Proc.*	Y = -4.4+0.2 X (r^2 = 53.2%)	Y = -5.9+0.2 X (r^2 = 62.9%)
	No *Proc.*	Y = -6.4+0.2 X (r^2 = 72.0%)	Y = -12.1+0.3 X (r^2 = 73.1%)
	Yes spor.	Y = -11.8+0.3 X (r^2 = 34.2%)	Y = -19.4+0.4 X (r^2 = 81.4%)
	No spor.	Y = -20.0+0.4 X (r^2 = 58.8%)	Y = -16.1+0.3 X (r^2 = 42.4%)

Fig. 4.1. Regression lines of length *vs* volume (A and B), length *vs* total weight (C and D) and length *vs* fresh weight (E and F) of *Perna perna* with and without *Proctoeces* metacercariae. A, C and E were measured before spawning; B, D and F after spawning.

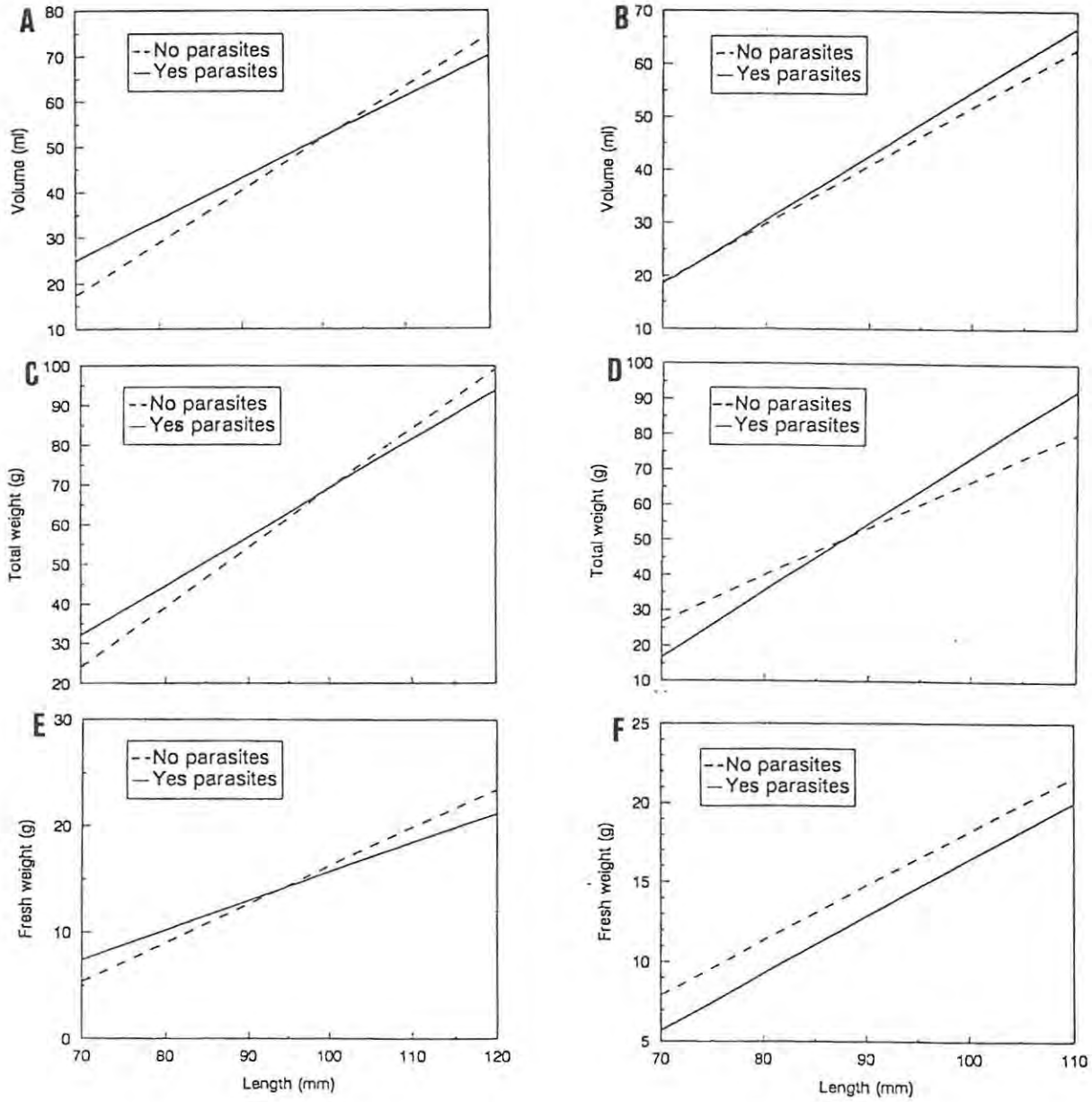


Fig. 4.2. Regression lines of length *vs* volume (A and B), length *vs* total weight (C and D) and length *vs* fresh weight (E and F) of *Perna perna* with and without bucephalid sporocysts. A, C and E were measured before spawning; B, D and F after spawning.

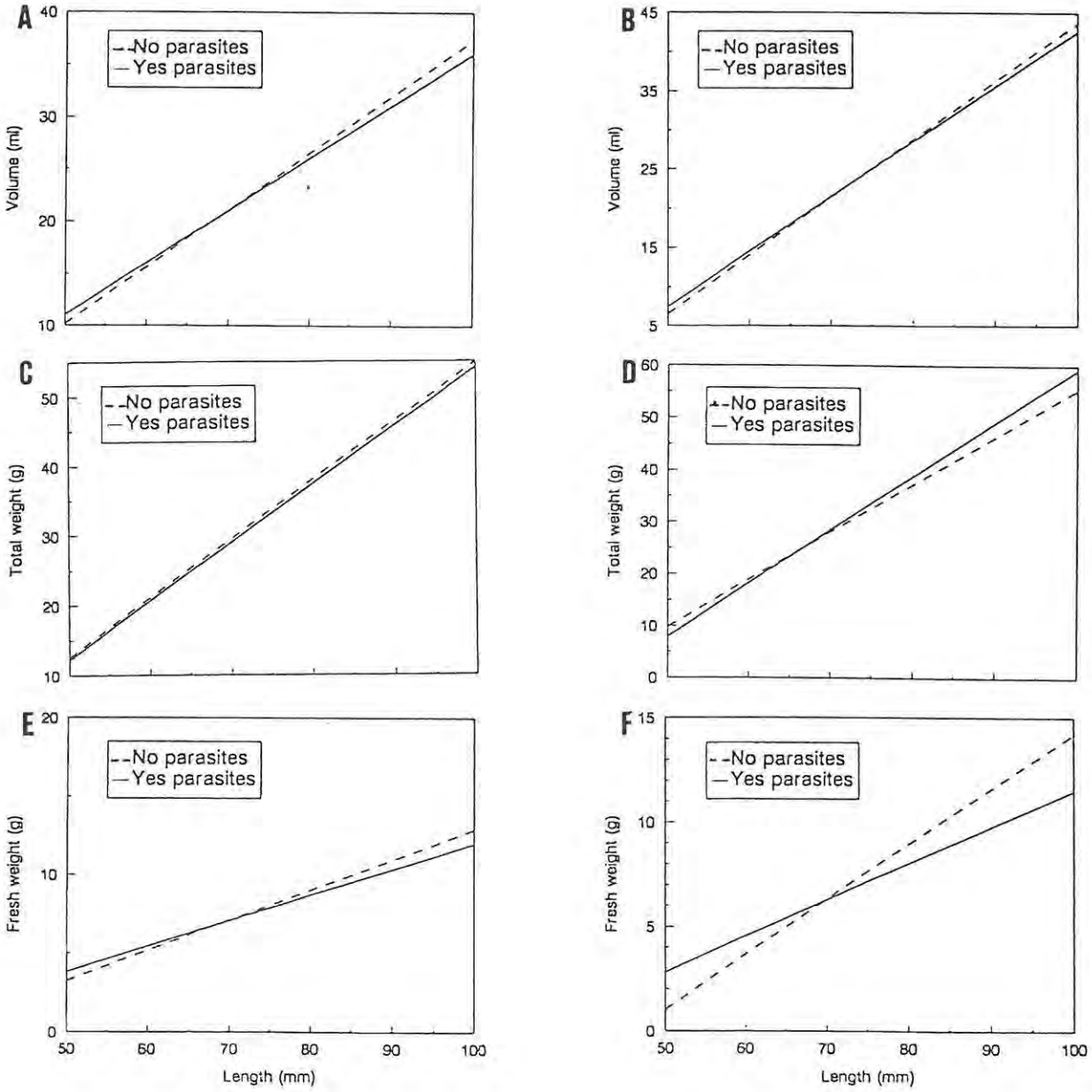


Table 4.3. Comparison of the slopes and intercepts for the equations from Table 4.2.

		Slopes	Intercepts
Before Spawning	*Proc.* L *vs* V	F=0.47, p>0.05	F=0.01, p>0.05
	L *vs* TW	F=0.01, p>0.05	F=0.48, p>0.05
	L *vs* FW	F=0.86, p>0.05	F=0.02, p>0.05
	Spor. L *vs* V	F=0.96, p>0.05	F=0.30, p>0.05
	L *vs* TW	F=0.57, p>0.05	F=0.06, p>0.05
	L *vs* FW	F=0.52, p>0.05	F=0.32, p>0.05
After Spawning	*Proc.* L *vs* V	F=0.23, p>0.05	F=0.00, p>0.05
	L *vs* TW	F=0.56, p>0.05	F=0.45, p>0.05
	L *vs* FW	F=5.21, p>0.05	F=1.01, p>0.05
	Spor. L *vs* V	F=0.21, p>0.05	F=1.16, p>0.05
	L *vs* TW	F=2.30, p>0.05	F=0.52, p>0.05
	L *vs* FW	F=0.01, p>0.05	F=3.38, p>0.05

4.3.2. Energetic values

Table 4.4 gives the energetic content of mussels with and without parasites before and after spawning. Neither *Proctoeces* nor the sporocysts had a significant effect on the kilojules per gram dry weight of mussels before or after spawning (p>0.05 in both cases).

Table 4.4. Energetic content of *Perna perna* with and without *Proctoeces* or bucephalid sporocysts measured before and after spawning.

	Yes *Proctoeces*	No *Proctoeces*	Yes sporocysts	No sporocysts
Before spawning				
Sample size	37	43	15	22
Mean	23.47	23.79	22.71	23.52
Std. deviation	1.93	2.17	1.70	2.70
	p>0.05 n.s.		p>0.05 n.s.	
After spawning				
Sample size	34	15	7	35
Mean	24.84	24.55	21.10	22.10
Std. deviation	2.26	2.08	2.27	1.76
	p>0.05 n.s.		p>0.05 n.s.	

4.3.3. Gaping, water loss and survival

Table 4.5 summarizes the amount of water lost per gram of dry weight. The variances of the amount of water lost by animals infected with *Proctoeces* and non-infected animals after 12

hours were not homogeneous, even after logarithmic transformation, so a Mann Whitney U-test was used to compared water lost by infected and non-infected animals. The result showed no significant differences ($p>0.05$). Neither were there significant differences in the amount of water lost by animals with/without bucephalid sporocysts during the first 12 hours of the experiment (t-test, $p>0.05$). Similarly, the amount of water lost by mussels infected with *Proctoeces* and non-infected mussels at the end of the experiment was not significantly different (t-test, $p>0.05$). On the other hand, animals infected with bucephalid sporocysts lost significantly more water than non-infected animals by the time the experiment finished (t-test, $p<0.01$ after logarithmic transformation of the data, Table 4.6).

Table 4.5. Amount of water lost (g water/g dry weight) by animals with and without parasites in the first twelve hours of the experiment on gaping.

	Yes *Proctoeces*	No *Proctoeces*	Yes sporocysts	No sporocysts
Sample size	51	41	18	79
Mean	2.72	2.09	2.43	2.52
Std. deviation	2.92	1.27	1.57	2.09
	p>0.05 n.s.		p>0.05 n.s.	

Table 4.6. Amount of water lost (g water/g dry weight) by mussels with and without parasites by the end of the experiment on gaping.

	Yes *Proctoeces*	No *Proctoeces*	Yes sporocysts	No sporocysts
Sample size	51	41	18	80
Mean	13.79	12.98	13.44	7.63
Std. deviation	7.23	5.61	5.55	2.64
	$p>0.05$ n.s.		$p<0.01$ **	

Spearman's correlation test for data on all animals showed a significant positive correlation between the amount of water lost during the first twelve hours and the number of times that the animals gaped (Table 4.7). However, there was no significant relationship between the presence or number of parasites and either the amount of water lost or the number of times the mussels gaped during the first twelve hours (Table 4.7). This situation changed by the end of the experiment, by which stage there was no significant relationship between the water lost and the gaping behaviour of the mussels (Table 4.7). At the end of the experiment, again, there was no significant correlation between the presence or number of *Proctoeces* and either the amount of water lost by the mussels or the number of times they gaped (Table 4.7). However, the presence of bucephalid sporocysts was associated with a significant increase in the amount of water lost by the mussels (Table 4.7).

Table 4.7. Values of r generated by Spearman's correlation tests on the relationships among parasitism, gaping behaviour and water loss of mussels after 12 hours of exposure to air and at the end of the experiment on gaping.

	12 hours		End	
	Gaping	Water lost	Gaping	Water lost
Presence *Proctoeces*	0.19 (p>0.05)	0.08 (p>0.05)	0.15 (p>0.05)	0.01 (p>0.05)
Number *Proctoeces*	0.14 (p=0.05)	0.07 (p>0.05)	0.13 (p>0.05)	0.01 (p>0.05)
Water lost (all animals)	0.34 (p<0.01) **		0.04 (p>0.05)	
Sporocysts	0.04 (p>0.05)	0.001 (p>0.05)	0.15 (p>0.05)	0.48 (p<0.01) **
Water lost (all animals)	0.34 (p<0.01) **		0.15 (p>0.05)	

Figure 4.3 represents the survival of mussels with and without parasites exposed to air. No mussels died during the first 12 hours. The t-tests comparing the mean time that infected and non-infected mussels took to die showed no significant differences for either *Proctoeces* or bucephalid sporocysts (p=0.68 and p=0.23 respectively, Table 4.8).

Fig. 4.3. Survival of mussels with and without *Proctoeces* (A) and bucephalid sporocysts (B) exposed to air.

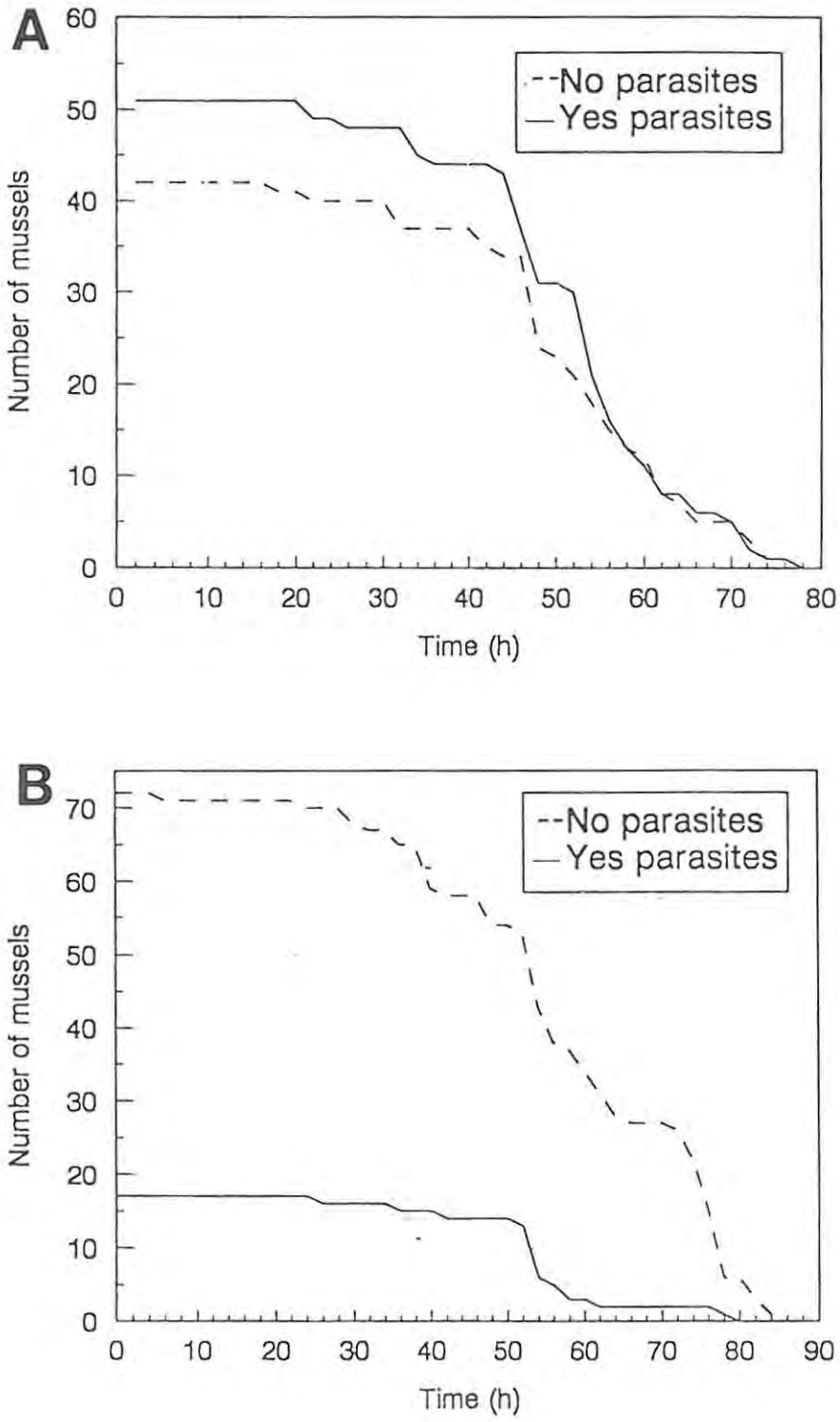


Table 4.8. Average time (hours) that infected and non-infected animals survived in exposure to air.

	Yes *Proctoeces*	No *Proctoeces*	Yes sporocysts	No sporocysts
Sample size	51	41	18	80
Mean	52.59	53.66	53.33	58.87
Std. deviation	12.45	12.24	13.28	18.33
	p>0.05 n.s.		p>0.05 n.s.	

4.3.4. Adductor muscle strength

Table 4.9 shows the force (Newtons/gram of dry weight) needed to open mussels with and without *Proctoeces* or bucephalid sporocysts. The test for homogeneity of variances showed that in the case of animals infected with *Proctoeces* the variances are not homogeneous, therefore the data were logarithmically transformed and compared with a t-test. The results of the t-tests showed no significant difference in the force required to open *Proctoeces* infected and parasite free mussels ($p>0.05$). In the case of the sporocysts, a significantly greater force was needed to open non-infected mussels than infected ones ($p=0.03$). More force was needed to open mussels from Kowie Point than mussels from Hougham Park, even though they were smaller.

Table 4.9. Newtons per gram of dry weight needed to force open infected and non-infected mussels.

	Yes *Proctoeces*	No *Proctoeces*	Yes sporocysts	No sporocysts
Sample size	41	21	18	47
Mean	85.84	76.29	51.43	59.70
Std. deviation	22.38	14.16	14.81	13.40
	$p>0.05$ n.s.		$p<0.05$ *	

4.4. DISCUSSION

4.4.1. Condition Index

Condition indices are considered to be good indicators of the health status of bivalves, although they may be affected by many factors. Fluctuations of condition have been reported to result from environmental stressors, such as thermal and starvation stress (Gee *et al.*, 1977) and exposure to parasites (Kent, 1979; Pregenzer, 1981; Bierbaum, 1985; Theisen, 1987; Gauthier *et al.*, 1990). However, this may not always be the case. For example Hickman

(1978) failed to find changes in the condition of *Perna canaliculus* infected with *Pinnotheres novazelandiae*.

Pekkarinen (1991) noticed that there were seasonal changes in the condition of *Mytilus edulis*, and both Walne (1970) and Gee *et al.* (1977) agreed that the cycle of gametogenesis is the main cause of such seasonal variations. Walne (1970) concluded that the decline in condition in summer is probably the result of weight losses due to spawning combined with an inability of flesh growth to keep up with shell growth during this period. Kent (1979) also found seasonal changes in condition of mussels. He concluded that condition was highest in late summer, declining during winter when food avalability was lowest and consumption could not compensate for metabolic losses of energy reserves. Condition then reached a minimum in spring, possibly coinciding with the spawning period. This same seasonal pattern was found by Baird (1966).

Previous studies on the effects of bucephalids on the condition of the host have shown contradictory results. Whilst Gauthier *et al.* (1990) found that oysters infected with *Bucephalus* tend to have low values of condition index, Pekkarinen (1993) concluded that unionid mussels parasitized with bucephalid sporocysts did not always have a lower condition than non-parasitized individuals. This could be a result of seasonal differences in the timing of the two studies. The results of this chapter show that there was no apparent effect of either *Proctoeces* or bucephalid sporocysts on the condition index of *Perna perna* before spawning, but that both parasites had a negative effect on the condition of the host after spawning (Table 4.1). This would agree with the results of those authors who have found a deleterious effect

of parasites only under stressful situations (Hepper, 1955; Bayne *et al.*, 1978). Spawning can be considered a natural stressor since it can cause a reduction in filtration rate and postspawning animals are frequently depleted of energy reserves (see Newell and Barber, 1988 for references).

It must be realised that the opposite situation is also possible. Instead of stress making animals more susceptible to parasites, parasites may increase the animals' susceptibility to stress. Esch *et al.* (1975) studied the relationship between stress and parasitism. They concluded that parasitism can act as a stressor input which may affect the density of the host species by affecting natality or mortality rates. This may in turn create a new stressor input which may affect the parasite. In other words, the whole system may be regulated by a series of negative feedbacks.

Even if parasites affect the host only when it is already under some sort of environmental stress, the importance of these effects must not be underestimated. As Zuk (1987) pointed out, parasites which exert detrimental effects only when environmental conditions are stressful may have a profound effect on host biology.

4.4.2. Energetic value

Table 4.4 shows that there were no significant differences in mean calorific value of animals with and without *Proctoeces* or bucephalid sporocysts, nor between measurements taken before and after spawning. Although it may seem surprising not to find any seasonal

variations in calorific values, this has been noticed by other authors. Berry (1978) found a low annual variation in calorific values of *P. perna* in Natal (South Africa). Griffiths and King (1979) found no seasonal fluctuations in the energy value of the flesh of *Aulacomya ater*, and Cheung (1991, 1993) concluded that the calorific values of somatic tissues of *P. viridis* were not significantly different between seasons.

Although calorific values for *Perna perna* determined in this work (Table 4.4) are within the range of values previously reported for this mussel in South Africa (Berry, 1978; van Erkom Schurink and Griffiths, 1991), the mean calorific value found in this study (23.3 kJ g^{-1} dry weight) is higher than that previously reported (20.3 kJ g^{-1} dry weight in both studies). Calorific values found for other species of mussels are very similar to those found in this work, e.g. 22.1 kJ g^{-1} dry weight for *Aulacomya ater* (Griffiths and King, 1979); 20.8 kJ g^{-1} dry weight for *A. ater*, 20.4 kJ g^{-1} dry weight for *Mytilus galloprovincialis*, and 19.3 kJ g^{-1} dry weight for *Choromytilus meridionalis* (van Erkom Schurink and Griffiths, 1991); 20.59-24.85 kJ g^{-1} dry weight for *P. viridis* (Cheung, 1991, 1993).

4.4.3. Gaping, water loss, survival and adductor muscle strength

With respect to mortality, there were no significant differences in the time that infected and non-infected mussels took to die when exposed to air (Table 4.8). Furthermore, not one mussel died in the first twelve hours of the experiment, and 50% of the mussels died only after 50 hours of exposure to air (Fig 4.3). Therefore, this observation is not likely to have any serious ecological meaning, since mussels are never exposed to air for so long under

natural conditions.

Mussels are facultative anaerobes, and when exposed to air they have two alternatives: they may close the valves and switch to anaerobic metabolism, minimizing the risk to desiccation, but being less productive; or they may carry on with aerobic metabolism. Marshall and McQuaid (1993) suggested that *Perna perna* relies on aerobic metabolism during air exposure, which implies a certain degree of shell gaping. Under natural conditions, mussels do not gape all the time, but open and close the valves according to their oxygen demands, losing some water every time they gape. The results of this experiment showed that there was a correlation between the number of times the mussels gaped and the amount of water they lost during the first twelve hours (Table 4.7).

This situation changed as the time of exposure to air increased. By the end of the experiment there was no significant relationship between the amount of water lost per gram of dry weight and the number of times that the mussels gaped. In the case of animals from Kowie Point, the amount of water lost at the end of the experiment was not related to the time that the mussels spent out of water. Nor were there significant differences between the amount of water that mussels with and without *Proctoeces* lost. On the other hand, animals from Hougham Park showed different behaviour. There was still no significant relationship between the amount of water that these mussels lost and the number of times they gaped, but the longer the mussels were exposed to air the more water they lost. Also, the results from the t-test showed that animals infected with bucephalid sporocysts lost significantly more water than non-infected animals. This increase in water loss could have been the result of an abnormal gaping behaviour, but this is not the case since the relationship between presence

of parasites and the number of times the mussels gaped was not significant.

Another possible explanation is that the mussels did not seal the valves properly and continuous evaporation of water took place. This conforms with the results from the experiment on adductor muscle strength. Less force was needed to open animals infected with bucephalid sporocysts than non-infected animals. This means that infected mussels were weaker, which could result in a failure to close the valves properly, with a subsequent increase in water loss by evaporation. Weakening of infected animals has been noticed before (Howell, 1967; Canzonier, 1972) and apart from the effect on the amount of water lost it has obvious ecological implications by rendering the host more susceptible to certain predators. This debilitation of the mussels would not affect predation by some predators. For example, if an octopus fails to pull open a mussel it drills a hole into it, injects saliva, which paralyses the adductor muscles, and then pulls it open (McQuaid, 1994). Weakening of the adductor muscles may, however, facilitate predation by other animals such as starfish. Norberg and Tedegren (1995) measured the force applied by *Asteria rubens* trying to open mussels and found that they applied a maximum force between 7.6 and 70 N. Jonsson and Andre (1992) noticed that *Cerastoderma edule* heavily infected with *Cercaria cerastodermae* were slow to close the valves and on several occasions they found the whelk *Nassarius reticulatus* attacking gaping cockles that were not yet dead. Similarly Howell (1967) observed shrimps probing between the valves of gaping oysters which were heavily infected with *Bucephalus longicornutus*.

Although bucephalid sporocysts do not directly affect mortality of the mussels, it is possible

that, through weakening the adductor muscle, this parasite is responsible for higher death rates due to causes that have yet to be assessed.

SUMMARY

Proctoeces and bucephalid sporocysts had limited effects on the aspects of survival examined. Regression analyses showed that neither parasite affected the relationships between shell length and total weight, total volume the fresh flesh weight. Nor did they significantly affect calorific content of *Perna perna* before or after spawning. Both parasites did have a significant negative effect on the condition of the host, but only after spawning, when the mussels were already stressed.

A survival experiment showed that there were no significant differences in the amount of time that mussels with and without parasites survived out of the water, nor in the number of times that they gaped. *Proctoeces* had no significant effect on the amount of water that mussels lost, but animals infected with bucephalid sporocysts lost significantly more water than non-infected animals. This could be explained by the results of the adductor muscle strength. Whilst there were no significant differences in the force needed to open mussels infected with *Proctoeces* from that needed to open non-infected mussels, less force was needed to open mussels infected with bucephalid sporocysts than non-infected mussels. This means that mussels with sporocysts are weaker, which could result in a failure to close the valves properly, with the consequent increase in water loss due to evaporation, or in an increased susceptibility to predators.

CHAPTER 5.

EFFECTS OF TREMATODES

ON REPRODUCTIVE OUTPUT OF *Perna perna*

5.1 INTRODUCTION

A parasite may influence the population dynamics of its host mainly by inducing mortality or by affecting the reproductive output of host individuals. Chapter 3 showed that, although neither *Proctoeces* nor the bucephalid sporocysts directly affect the survival of their host, both parasites have a negative effect on the condition of the host at least when the host is already stressed. Sporocysts may also have an indirect effect by weakening the adductor muscle, making the valves easier to open.

This chapter deals with the effects of parasites on the reproductive output of the host. Parasites may have direct or indirect effects on host reproduction (see Scott, 1988 for references). Parasites that castrate the host will obviously have a major impact on the reproduction of the host individual, but some parasites also have an indirect effect on host reproduction by delaying growth, which leads to delayed age at sexual maturity and decreased reproductive life-span (Scott, 1988). Trematodes affect the reproductive output of their host mainly in three ways: they may cause a reduction in reproductive output (parasitic castration), an increase in reproductive output (fecundity compensation), or they may induce a change of sex.

Parasitic castration

True castration is an irreversible destruction of the reproductive system (Hurd, 1990). However, most of the literature uses the term parasitic castration to refer to the more-or-less complete blockage of reproductive output. A useful definition of parasitic castration was given by Baudoin (1975): parasitic castration is "a destruction or alteration of gonad tissue, reproductive behaviour, hormonal balance, or other modification that results in a reduction of host reproductive effort above and beyond that which results from a non-selective use of energy reserves by the parasite".

Cheng *et al.* (1973) and Sullivan *et al.* (1985) divided parasitic castration into several different types:

a) Mechanical: due to ingestion, abrasion or pressure exerted on the gonad. Examples are: pressure exerted by sporocysts and cercariae of *Glypthelmins pennsilvaniensis* in the genital tract of *Helisoma trivolvis* (Cheng and Cooperman, 1964); ingestion of the gonad of *Littorina littorea* by rediae (Robson and Williams, 1971); physical destruction of the gonad of *Lymnaea truncatula* by larval stages of *Fasciola hepatica* (Wilson and Denison, 1980); direct ingestion of the gonad of *Helisoma anceps* by rediae of *Halipegus occidualis* (Crews and Esch, 1986); and destruction of the gonad of *Fissurella crassa* caused by the attachment of *Proctoeces lintoni* by its acetabulum (Oliva, 1992, 1993).

b) Chemical: due to secretion of lytic, toxic or endocrinologically antagonistic molecules by the parasite. Chemical castration can in turn be divided into:

b.1. Direct: the parasites live in the immediate vicinity of the gonad (e.g. Cheng *et al.*, 1973, sporocysts of *Zoogonius rubellus* secrete a substance that destroys the sex cells of *Nassarius obsoletus*).

b.2. Indirect: with the parasites located at a distance from the gonad (e.g. McClelland and Bourns, 1969, hormonal castration of *Lymnaea stagnalis* infected by *Trichobilharzia ocellata*).

Sullivan *et al.* (1985) defined both mechanical and chemical (direct and indirect) effects as primary effects, and they said that on top of these, there may also be secondary effects, i.e. disturbances of the host's physiology that interfere with gonadal development and/or survival. An example of this could be the action of sporocysts on the hepatopancreas of *Littorina littorea*. The sporocysts constrict the flow of haemolymph, reducing the flow of nutrients to the digestive gland and gonad and allowing the accumulation of excretory products (Robson and William, 1971). Another example would be the drain of nutrients observed by Makanga (1981) in *Biomphalaria glabrata* infected with *Schistosoma mansoni*.

Parasitic castration is the most commonly reported effect of parasites on host reproduction. Many trematodes have been found to reduce the reproductive output of their host (Rothschild, 1936; Uzman, 1953; Menzel and Hopkins, 1955; Sturrock, 1966, 1967; Cheng and Cooperman,1964; Etges and Gresso, 1965; McClelland and Bourns, 1969; Robson and Williams, 1971; Hodasi, 1972; Meuleman, 1972; Cheng *et al.*, 1973; Dupoy and Martinez, 1973; Tallmark and Norrgren, 1976; de Jong-Bring *et al.*, 1979; Wilson and Denison, 1980; Makanga, 1981; Meier and Meier-Brook, 1981; Sluiters, 1981; Lauckner, 1983; Pearson and

Cheng, 1985; Crews and Esch, 1986, 1987; Joose and van Elk, 1986; Kabat, 1986; Copeland *et al.*, 1987; Fernandez and Esch, 1991; Jonsson and Andre, 1992; Oliva, 1992, 1993; Jokela *et al.*, 1993), and it has been suggested that they can act as regulators of host populations (e.g. Kuris, 1974; Kuris and Lafferty, 1992). In addition to an obvious direct effect on the population by reducing the number of offspring produced, parasitic castration also has an indirect effect. Castrated animals remain alive and use the same resources as unparasitized individuals (Lafferty, 1993), which results in competition between unparasitized and castrated animals.

Fertility compensation

Infected hosts may suffer from increased mortality or reduced reproduction. Therefore, if a host is exposed to a parasite, whether or not it becomes infected, it may increase its reproductive output in order to compensate for expected future loss in reproductive success in the case of successful infection. Evidence for this comes from experimental exposure of mussels to larval parasites by which they are not subsequently infected (Minchella and Loverde, 1981; Minchella, 1985 and Thornhill *et al.*, 1986). Fertility compensation can be considered a strategy of the host acting as a second line of defence against the parasite (Minchella, 1985).

5.2 MATERIALS AND METHODS

Survey data showed that the prevalence of *Proctoeces* was much higher in females that in males (Chapter 2); indeed in most places it was difficult to find females without parasites or to find infected males. Nevertheless there is no evidence to suggest that *Proctoeces* induces sex change from male to female. Thus, only females were used to study the effects of parasites on reproduction. Four females infected with *Proctoeces* and four non-infected females were dissected and the soft tissues were carefully removed and weighed. A small portion of the gonad was weighed and placed in Davidson's fixative for histological examination. These samples were then dehydrated in a series of alcohols of increasing concentrations (70%, 96%, 96%, 100% and 100%), left overnight in methyl benzoate and then placed in benzene before embedding in paraplast for use in the microtome. Five 7µm sections 70µm apart were cut from each gonad and stained with haematoxylin-eosin to study the reproductive state of the animal. The number of oocytes present in three random microscope fields per section was counted, and 20 oocytes that had been cut through the nuclei were measured in each section using a graduated ocular. The number of oocytes and mean oocyte size in infected and non-infected females were tested using a nested ANOVA (Zar, 1984).

In animals infected with bucephalid sporocysts the gonad is almost obliterated and it is often not possible to determine the sex of the host. Ten animals with parasites, and ten non-infected females were dissected and processed as above.

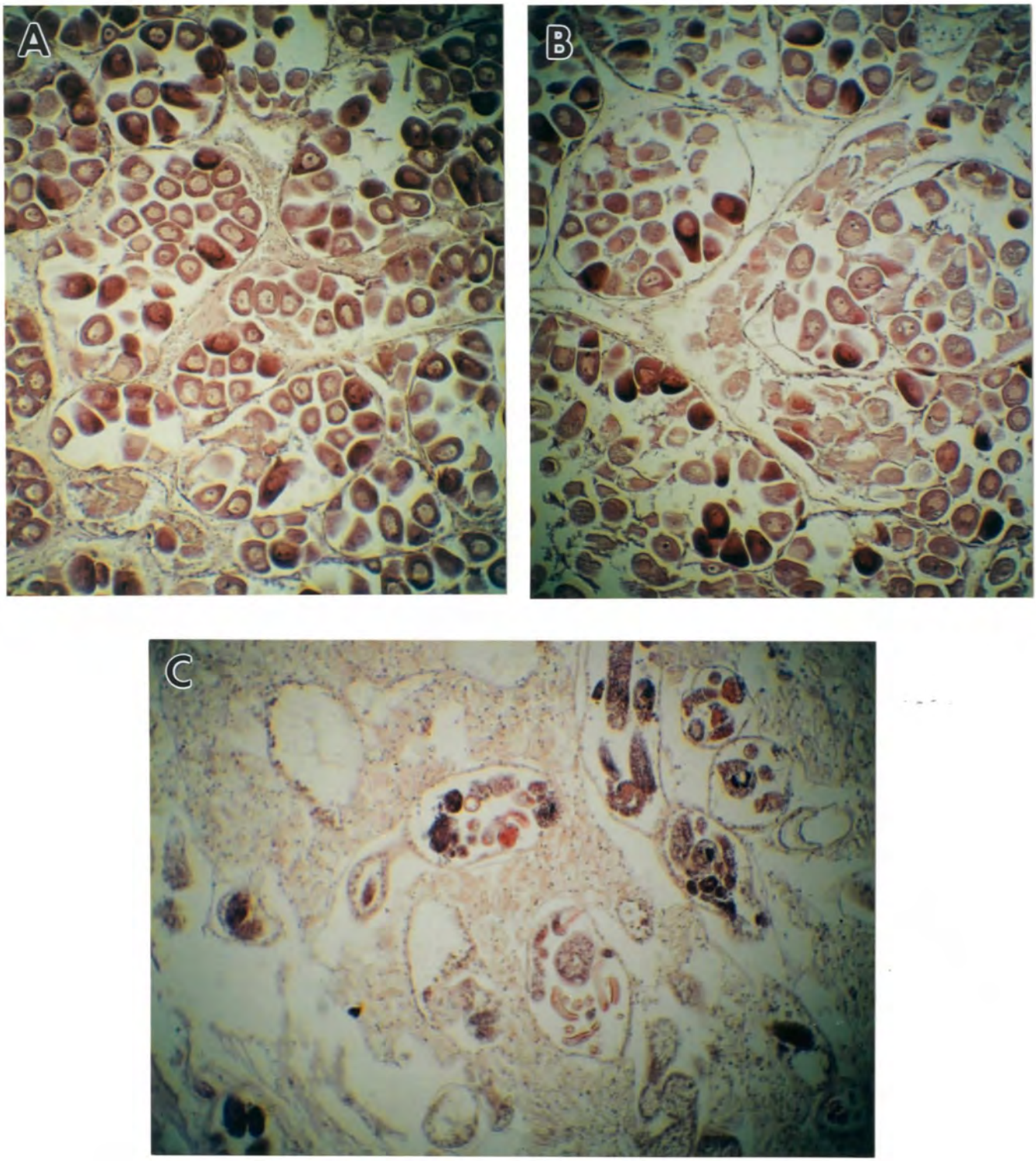
5.3 RESULTS

Histologically and morphologically, the ovaries of animals infected with *Proctoeces* look identical to those of non-infected individuals (Fig. 5.1 A & B). The nested ANOVA comparing the number of oocytes in infected and non-infected females showed no significant differences in the oocyte number owing to *Proctoeces*; most of the variation observed was due to the microscope field examined (Table 5.1).

Table 5.1. Nested ANOVA showing the variation in the number of oocytes due to the microscope field, section or mussel examined and the presence or absence of *Proctoeces*.

Source of variation	Sum of Squares	d.f.	Variance component	Percent
Proctoeces	760.03	1	7.39	5.42
Mussel	1901.27	6	14.60	10.70
Section	3133.73	32	0.00	0.00
Field	9152.67	80	114.41	83.88

Fig. 5.1. Cross sections through the gonad of (A) a female infected with *Proctoeces*, (B) a non-infected female and (C) a mussel infected with bucephalid sporocysts.



Likewise, nested ANOVA indicated that mean oocyte diameter did not vary significantly between individual mussels (Table 5.2), nor was it affected by the presence of *Proctoeces* (Table 5.2).

Histological examination of animals infected with bucephalid sporocysts showed that all of them were completely castrated, with no trace of sex products left (Fig 5.1 C), so it was not possible to count or measure oocytes. Furthermore, one of the animals which had been classified as a non-infected female had a light infection of sporocysts, which means that infection rates of this parasite have been underestimated.

Table 5.2. Nested ANOVA showing the variation in oocyte size owing to the section or mussel examined and to the presence or absence of *Proctoeces*.

Source of variation	Sum of squares	d.f.	Variance component	Percent
Proctoeces	55.12	1	0.116	1.29
Mussel	52.09	6	0.031	0.34
Section	180.00	32	0.000	0.00
Error	6730.00	760	8.855	98.37

5.4 DISCUSSION

Trematode metacercariae do not normally cause parasitic castration, and for many years they have been erroneously believed to cause little or no harm to their host (Lauckner, 1983). Lasiak (1989) found that metacercariae of *Proctoeces* commonly infected female *Perna perna* in South Africa. She concluded that, since these parasites ingest material from the host tissue, they will affect the host's growth and reproduction. Fig. 5.1 A & B show that the gonads of infected and non-infected females are morphologically identical. There were no significant differences in either oocyte densities (Table 5.1) or oocyte size (Table 5.2) between infected and non-infected females. All the infected females used in this study had less than 10 metacercariae, which may account for the lack of effects on reproduction. Although the metacercariae ingest the gonad of their hosts, the fact that infected females have the same number of oocytes as non-infected females probably means that mussels regenerate the oocytes as quick as parasites destroy them. This situation will no doubt change in the case of high intensities of infection. However, although some females harbour over 50 metacercariae (pers. obs.) and this number can go up to over 300 in some localities (Lasiak, 1989), the majority of the females showed low intensities of infection with metacercariae of *Proctoeces* (less than 15, pers. obs.). Therefore, it appears that in most cases of infection with metacercariae of *Proctoeces* the effect of the parasite on the reproductive output of the host is negligible. Although the possibility that infected females have the same number of oocytes per square centimeter of section but less gonad tissue may not be discounted, there was no evidence of this.

Most infections by bucephalid sporocysts and cercariae in bivalve molluscs have been reported to start in the gonad, with the sporocysts eventually replacing all the gonadal tissue and spreading into other organs (see Howell, 1967 and Lauckner, 1983 for references). One exception to this rule was found by Cheng and Burton (1965) who noted that *Bucephalus* sp. infected mainly the digestive gland of the oyster *Crassostrea virginica.*

In the case of the bucephalid sporocysts found in this study, infection started in the gonad and spread into other organs. During the first stages of infection, the sex of the mussel was still recognizable, but in most cases the gonad was completely replaced by sporocysts, making identification of the sex impossible. In cases of heavy infestation, sporocysts could be found even between the adductor muscle fibres, which is probably the cause of the weakening of this muscle by this parasite (Chapter 4).

In all cases of bucephalid infections found in the literature, there was a reduction of reproductive capacity of the host that could lead to total castration (e.g. Cousteau *et al.*; Menzel and Hopkins, 1955; Gauthier *et al.*, 1990; Jokela *et al.*, 1993). This is in agreement with the results of this study. Even though with light infections gametes are still produced, infection with bucephalid sporocysts invariably leads to total cessation of gamete production.

Even if parasitic castrators reduce the reproductive effort of the host individual, their effect on host populations is still controversial. Although there is no doubt that, as a result of parasitic castration, infected individuals may be excluded from the breeding population, several authors have argued that this does not necessarily affect the population. In most cases castration occurs only after the first breeding season, giving the host a chance to reproduce

at least once (James, 1965; Robson and Williams, 1971; Rohde, 1982; Lauckner, 1986; Jokela *et al.*, 1993). This is true in the case of bucephalid sporocysts from this study. Results from Chapter 2 showed that the prevalence of this parasite increased with the size of the animal, with mussels bigger than 70mm having up to 70% infection rates in one location (i.e. Hougham Park, Chapter 2). Thus, most mussels have gone through one or more breeding seasons before becoming infected. In fact, since *Perna perna* only live two years (Berry, 1978), most mussels would only lose one breeding season. On the other hand, it must also be recognised that these large mussels are the ones which are putting most energy into reproduction, sometimes over 90% of their total energy budget (see review by Seed and Suchanek, 1992). Therefore, in places with high prevalences of bucephalid sporocysts, castration of large mussels may seriously reduce the breeding potential of the mussels and have a definitive impact on the mussel population.

SUMMARY

Histological sections through the gonad of infected and non-infected mussels were used to study the effects of *Proctoeces* and bucephalid sporocysts on the reproductive output of *Perna perna*.

Statistical tests comparing the number and size of oocytes in females infected with *Proctoeces* and in non-infected females showed no significant differences in either the number nor the size of the oocytes. On the other hand, identification of the sex of mussels infected with bucephalid sporocysts was not possible. Histological examination of infected animals showed that they were completely castrated, with no trace of sex products left.

CHAPTER 6.

EFFECTS OF TREMATODES

ON GROWTH RATES OF *Perna perna*

6.1 INTRODUCTION

Many studies have been done on the effects of parasites on mollusc growth. Initially they concentrated on studying the effects of trematode-gastropod systems of medical and veterinary importance. For example, there are many studies of the effects of different schistomatids on the growth of several snail hosts in the genera *Biomphalaria* and *Bulinus*, with extremely variable results. Sturrock (1966, 1967) found that both *Schistosoma mansoni* and *S. haematobium* significantly increased the growth of *Biomphalaria pfeifferi* and *Bulinus nasutus productus* respectively, whilst other authors found a negative effect on growth (Sturrock and Sturrock, 1970; Meier and Meier-Brook, 1981; Raymond and Probert, 1993) or no significant effect at all (Crews and Yoshino, 1989). Meier and Meier-Brook (1981) found a significant reduction of growth only in young snails. This seems to contradict the results of Raymond and Probert (1993) who said that *B. natalensis* infected with *S. margrebowiei* always grew less than uninfected individuals, regardless of the age at which infection took place. Raymond and Probert (1993) summarized this situation by saying that the effects of parasitism by Schistosomids vary depending on the species or even geographic strain of parasite studied. Still this does not explain the fact that Moose (1963) only found differences in growth in female snails, whilst there were no significant differences in the growth of males. Perhaps the most interesting finding of Moose (1963) and Raymond and Probert (1993) is that in both studies, exposed but uninfected small snails grew as poorly as

did parasitized snails.

There are also many studies on snails of the genus *Lymnaea* with equally variable results. Loker (1979) found that *L. catascopium* infected with *Schistosomatium douthitti* grew faster in the first two months, then slower, and after eight months they were smaller than non-infected snails. However, the majority of studies have found an increase in growth of infected hosts which has been explained in different ways. McClelland and Bourns (1969) found that *L. stagnalis* infected with *Trichobilharzia ocellata* grew more than non-infected snails, whilst Sluiters *et al.* (1980) studying the same host-parasite system (*L. stagnalis* - *T. ocellata*) found that gigantism occurred only when there was high cercaria production. Sluiters (1981) concluded that the growth acceleration in *L. stagnalis* with *T. ocellata* was a consequence of a reduction in reproductive activity. Hodasi (1972) found an increase in growth rate only in young *L. truncatula* infected with *Fasciola hepatica* and suggested that it might be due to the release of some growth factor by the rediae. Wilson and Denison (1980) also found that *L. truncatula* snails infected with *F. hepatica* had longer shells than the controls, but they said that this gigantism had a nutritional origin. Joose and van Elk (1986) found an increase in body wet weight in *L. stagnalis* infected with *T. ocellata* due to an increase of the haemolymph volume in infected snails, with no significant differences in dry weights.

Subsequent to those initial studies, parasitologists showed some interest in brackish water and marine molluscs, mainly gastropods and bivalves of economic importance infected with trematodes or crustacea. Again, the results ranged from significant growth increases (gigantism) (e.g. Rothschild, 1936, 1941a, 1941b; Cheng *et al.*, 1983; Crews and Esch, 1986

and Mouritsen and Jensen, 1994, studying effects of trematodes on molluscs) to significant growth reductions (Cannon, 1979; Sousa, 1983; Huxham *et al.*, 1993 and Lafferty, 1993, studying trematodes; Menzel and Hopkins, 1955, studying fungus and Kruczynsky, 1972, studying pea crabs). On the other hand, several other studies showed no significant effect on host growth (Hughes and Answer, 1982; Copeland *et al.*, 1987 and Fernandez and Esch, 1991 studying trematodes in molluscs; Bierbaum and Ferson, 1986 studying pea crabs on mussels).

In general, the literature indicates that trematodes do not have uniform effects on the growth of their hosts. The results may depend not only on the parasite and host species, but also on the conditions under which the experiment took place (laboratory *vs.* field, age at which infection occurs, *etc.*). Another factor that may account for the different results is the way the measurements were taken (Mouritsen and Jensen, 1994). The majority of the studies assess growth as an increase in shell size, however, it is not clear that this is a valuable measure (Hurd, 1990). Cheng (1971) stated that enhanced growth was due to increased calcium deposition in the shells of parasitized hosts and Joose and Van Elk (1986) concluded that the greater gain in body wet weight of infected snails was not the result of a more rapid increase in dry matter; the total dry weight of parasitized and non-parasitized animals was similar. Kruzcynsky (1972) on the other hand found a lower mean dry weight for scallops infected with pea crabs, whilst Wilson and Denison (1980) found that the dry mass of *Lymnaea truncatula* infected with *Fasciola hepatica* was twice as much as that of the controls.

While a variety of effects have been shown, most studies indicate that trematodes cause an increase in molluscan host growth. There have been several explanations for this. Wesenberg-

Lund (1934) was the first to point out that infected snails are sometimes abnormally large and explained this as a result of an increase of food ingested to satisfy the demands of the parasites. Since then several hypotheses have been proposed to explain an abnormal increase in host size due to parasitism. Wright (1971), Wilson and Denison (1980) and Sluiters (1981) among others explained gigantism as a side effect of parasitic castration, i.e. the energy that is not used for reproduction is used in growth. But this hypothesis leaves several unanswered questions. For example, some parasites may cause partial or total castration but there is no evidence of extra growth. Therefore, enhanced growth is a potential, but not a certain, consequence of parasitic castration (Cheng *et al.*, 1983). Furthermore, if this is the case, why does the destruction of the gonad of *Littorina* by X-rays not lead to increased growth (Wright, 1971)?

Baudoin (1975) considered increased host growth as a result of parasitic castration to be favourable to the parasite. Parasitic castration decreases host reproductive effort increasing therefore the energy available for non-reproductive purposes (i.e. fat storage and growth), which often results in an increased host (and therefore parasite) survivorship. Against this, Fernandez and Esch (1991) argued that if the host uses the energy available after parasitic castration for somatic growth it is limiting the energy available to the parasite.

Sousa (1983) predicted that gigantism will occur in short-lived, semelparous species. He carried out a field study on the influence of trematode parasites on the growth of a long-lived marine gastropod, *Cerithidea californica*, and concluded that there is no evidence to suggest that parasitism causes *C. californica* to grow faster than if it were uninfected. On the contrary,

some of the parasite species stunted growth in juvenile hosts, and although they probably have the same effect in adults, it was less obvious because adults grow much slower than juveniles. He explained the apparently contradictory results found in his study and in early studies in terms of differences in the life histories of the hosts. When the animals are infected before reproductive age, even complete destruction of the gonad by the parasite does not free extra energy for growth since at this stage the host dedicates no energy to reproduction. What is more, the parasite may have a negative effect on the growth of the host due to the extra energy needed to repair the damage caused by the parasite. Once the host reaches reproductive age, the effect of parasites will depend on the amount of energy that the animal would have spent on reproduction. Most short-lived animals have only one breeding season and, therefore, they put a high reproductive effort into it. In this case, parasitic castration will leave a large proportion of energy available to be used in growth. On the other hand, long-lived species spread their reproductive effort over several breeding seasons. Thus, only a small amount of energy is available for growth, and in many cases the cost of repairing the damage caused by parasites is even higher. Sousa (1983) therefore concluded that long-lived species will seldom exhibit gigantism and will more often have their growth stunted by infections of parasitic trematodes.

The findings of Huxham *et al.* (1993) agree with Sousa's (1983) predictions as the long-lived, iteroparous snail *Littorina littorea* shows parasite induced growth stunting. On the other hand, Fernandez and Esch (1991) did not find gigantism in the semelparous snail *Helisoma anceps* after parasitic castration.

Minchella (1985) proposed another hypothesis of gigantism as a counteradaptation of long-lived hosts in response to parasitic castration. He said that gigantism may improve survivorship and therefore allow the hosts to outlast the effects of trematode infection. If this is true, gigantism should be observed only in long-lived hosts and only when reproduction is completely inhibited. He also made the assumption that the damage caused by the parasite is reversible. *Biomphalaria glabrata* parasitized with *Schistosoma mansoni* often lose their infections after several months (A. Kuris, pers. comm.), but this may not always be the case, e.g. Sturrock and Sturrock (1970), Howell (1967) and Sousa (1983).

The preceding chapter showed that whilst *Proctoeces* has no apparent effect on the reproductive output of *Perna perna*, buchephalid sporocysts invariably castrate *P. perna*. As we have seen, the effects of parasites on growth have many times been related to effects on reproduction. This chapter is concerned with the effects of both parasites on the growth rates of the brown mussel *P. perna* and attempts to link these effects to those on reproduction.

Several authors have reported that growth in bivalves is markedly seasonal, although this varies depending on the species. For example, Arnold *et al.* (1991) found slow growth in the clam *Mercenaria mercenaria* during summer and autumn and rapid growth during winter and spring. They related growth inversely to temperature, fast growth with cold temperature and vice versa. This pattern has also been found by other authors in the same animal (e.g. Peterson *et al.*, 1983; Jones *et al.*, 1990). However, most studies on mussels agree that they show maximum growth during spring and summer, with slower or even negative growth during autumn and winter (Andreu, 1965; Dare and Edwards, 1976; du Plessis, 1977; Berry,

1978; Crawford and Bower, 1983; Rodhouse *et al.*, 1984; Page and Hubbard, 1987; Anwar *et al.*, 1990; Richardson *et al.*, 1990; Cheung, 1991, 1993; Sukhotin and Kulakowski, 1992 and van Erkom Schurink and Griffiths, 1993).

This seasonal variation in growth has been correlated with temperature (Cheung, 1991, 1993; Sukhotin and Kulakowski, 1992). But since variations in water temperature may be strongly correlated with phytoplankton abundance (Andreu, 1965; Page and Hubbard, 1987) it is difficult to separate the effects of temperature and food availability. Wallace (1980) and Page and Hubbard (1987) suggested that food availability is the main factor whilst van Erkom Schurink and Griffiths (1993) concluded that temperature rather than food availability is the variable controlling growth rates in *Mytilus galloprovincialis* and *Perna perna.*

Although there is no general agreement on an explanation for these seasonal differences, there is no doubt that growth rates of mussels differ in summer and in winter. Therefore the effects of parasites on the growth of *Perna perna* were studied in both seasons.

6.2 MATERIALS AND METHODS

In order to compare growth rates of parasitized and non-parasitized animals, small mussels (less than 15 mm) that are parasite-free (Chapter 2) were collected. The intention was to infect half of them experimentally, keeping the other half as controls, and grow them in a sterilized recirculating system in the laboratory. There were two major obstacles to this. The first was with respect to the growth of the mussels: mussels are filter feeders, and in their

natural environment phytoplankton and organically-rich detrital particles are their main source of nutrients. Since the water from the system had to be kept totally sterile to prevent new parasite larvae from infecting the mussels, food supply become a great problem. Mussels were fed with cultures of the unicellular algae *Dunaliella salina*, but although they stayed alive for over a year they did not grow. The second problem was that, even if the mussels had grown in the laboratory, the hosts for the previous larval stages of both parasites had to be found before experimental infestation would be possible. In view of these problems natural mussel populations were used and the growth experiment was carried out in the field.

Populations of mussels from Kowie Point (33° 38'S, 26° 52'E) and Hougham Park (33° 47'S, 25° 44'E) (Figs. 2.2 & 2.3, Chapter 2) showed approximately 50% infection rates by *Proctoeces* and bucephalid sporocysts respectively (Chapter 2). A size range of mussels (20-90mm) was collected in October from these two populations (Kowie Point, n=1300 and Hougham Park, n=260). The shell of each mussel was cleaned and measured and individuals were marked by glueing a small plastic tag to the shell. The mussels were then placed in mesh cages and hung in the sea at approximately 2m depth in a mussel farm in Port Elizabeth (33° 58'S, 25° 38'E). Mesh size was 1cm, which is large enough to ensure a continuous flow of water through them and small enough to prevent the smaller mussels from falling off.

In order to check whether the mussels acquired new infections during the experiment, a random subsample (n=10-20) was taken every month and rates of infection were checked. The size of each individual was recorded monthly during summer (October 1992- March 1993). At the end of this period the mussels were dissected and examined for parasites. Mean growth

rates for infected and non-infected mussels were compared using analysis of covariance, with the initial size of the mussels as a covariate, since it has been demonstrated that growth rates decrease with increasing size (du Plessis, 1977; Kautsky, 1982; Crawford and Bower, 1983; Cheung, 1991). The presence or absence of parasites and, when possible, the host sex were the main effects. Mussels were divided into six 10 cm size classes (Kowie Point: smallest size class <30, largest class >70mm; Hougham Park: <60 to >100mm) to study size dependent effects of the parasites. As the data were unbalanced, the results were analyzed separately for each size class using the Mann-Whitney U-test. In order to assess whether the effects of the parasites differed between seasons the experiment was repeated during winter (May 1993 - November 1993). During the winter experiment initial sample sizes were 700 for each population and the statistical design was different. Size dependent effects were examined by dividing samples from each locality into seven size classes (21-30mm, 31-40mm, 41-50mm, 51-60mm, 61-70mm, 70-80mm and >80mm), with 100 animals in each size class for each locality. The results were analyzed using ANCOVA and Mann-Whitney U-tests as above.

6.3 RESULTS

Data on infection rates in the subsamples of 10-20 mussels taken every month showed that infection rates stayed constant during the whole experiment (Chi-square test, $p>0.05$). Growth rates of mussels with and without *Proctoeces* or bucephalid sporocysts are shown separately for summer and winter months in Fig. 6.1. An Analysis of Covariance using the sex of the mussels and the presence/absence of *Proctoeces* as factors showed that sex had no significant effect on growth (Table 6.1). However, mussels infected with *Proctoeces* grew significantly

less than non-infected animals both in summer and in winter (Table 6.1)

Fig. 6.1. Growth rates of animals with and without *Proctoeces* and bucephalid sporocysts both in summer (A and C) and in winter (B and D). Means ± SD.

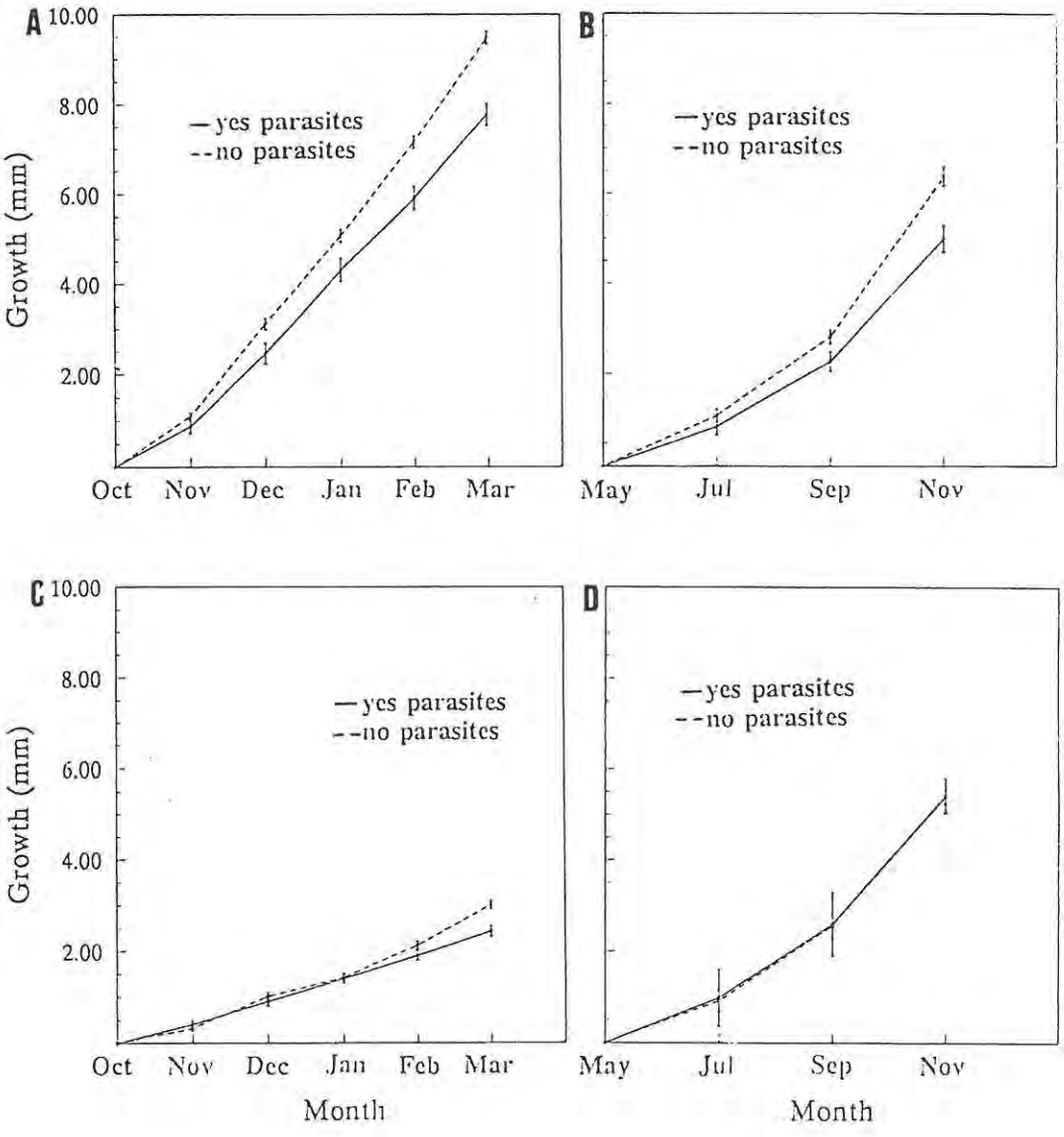


Table 6.1. Results of the Analysis of Covariance (using the initial size as a covariate) comparing growth rates of *Perna perna* with and without *Proctoeces* during summer and winter months.

Source of variation	Sum of Squares	d.f.	F-ratio	Signif. level
SUMMER				
Covariate				
Size	4320.66	1	180.01	<0.01 **
Effects				
Sex	45.36	1	1.89	>0.05 ns
Parasite	124.66	1	5.19	<0.05 *
Interactions	30.23	1	1.26	>0.05 ns
Residual	10681.28	445		
WINTER				
Covariate				
Size	4071.87	1	219.36	<0.01 **
Effects				
Sex	4.37	1	0.23	>0.05 ns
Parasite	76.93	1	4.14	<0.05 *
Interactions	128.15	1	6.90	<0.01 **
Residual	6719.52	362		

The Mann-Whitney U-tests comparing growth rates of animals with and without parasites in each of the six size classes during summer showed that *Proctoeces* had a detrimental effect on the growth of only the smallest animals ($p<0.05$ for animals <30 mm, $p>0.05$ for all the other size classes). Although parasitized mussels grew less than non-parasitized individuals in most of the other size classes, the differences were not statistically significant (Fig. 6.2 A). The results of the winter experiment showed exactly the same pattern (Fig. 6.2 B). Infected mussels smaller than 30mm grew significantly less than non-infected mussels of the same size ($p<0.05$). In the size classes between 30 and 70mm infected animals grew slightly but not significantly less than non-infected animals ($p>0.05$).

In the case of animals infected with bucephalid sporocysts, it was not possible to determine the sex of infected animals in most cases, therefore the analysis used only parasitism as a main effect. The presence of parasites had no significant effect on the growth of the host either in summer or in winter (Table 6.2). When separate size classes were considered, the Mann-Whitney U-tests showed that size class had an effect on growth. Animals of 71 to 80mm with sporocysts grew significantly less than non-infected individuals of the same size ($p<0.05$ both in summer and in winter; Figs. 6.2 C and D).

Fig. 6.2. Growth rates of *Perna perna* of different initial sizes with and without *Proctoeces* (A and B) and bucephalid sporocysts (C and D) during summer (A and C) and winter (B and D). ■ Infected, □ Non-infected.

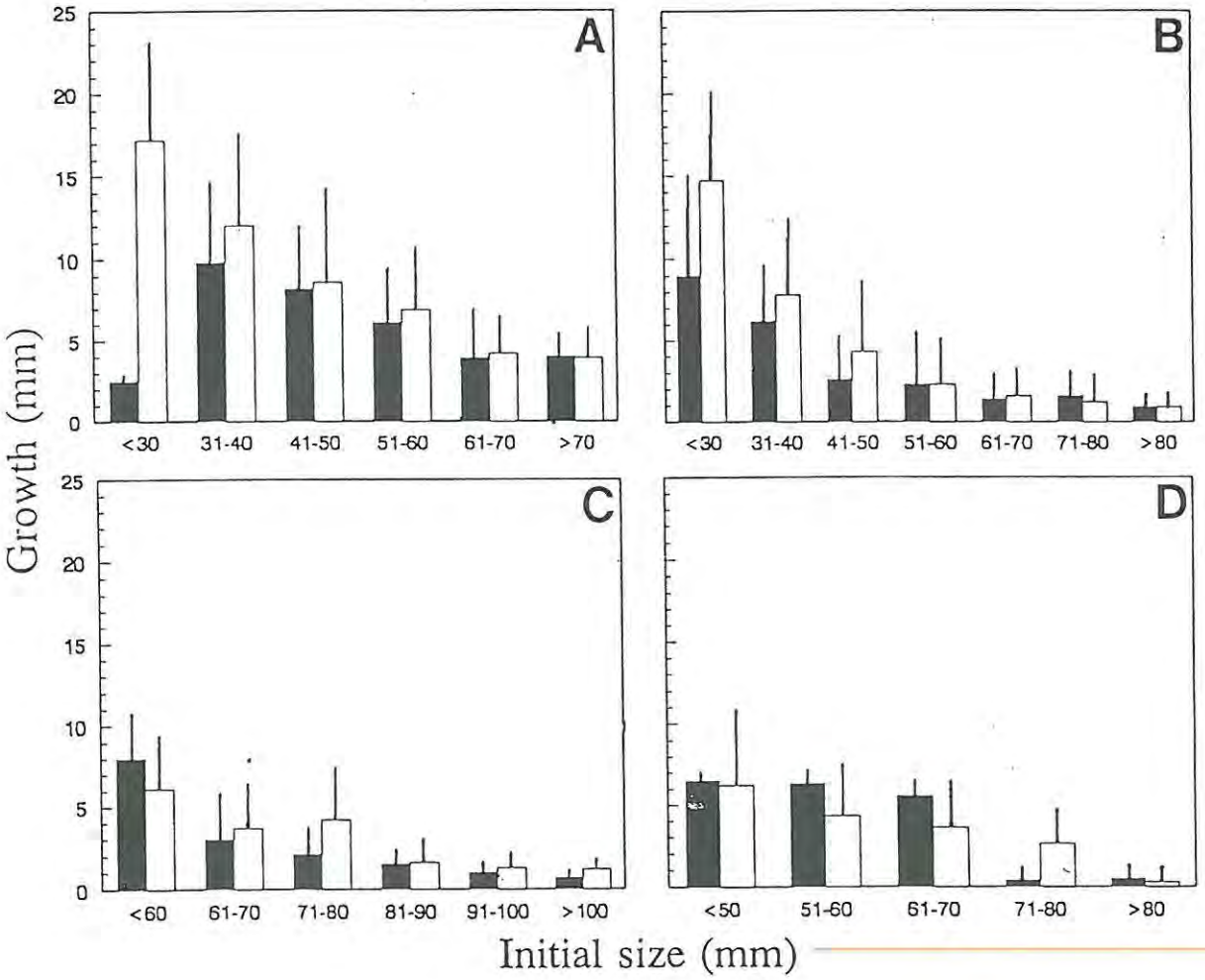


Table 6.2. Results of the Analysis of Covariance (using the initial size as a covariate) comparing growth rates of *Perna perna* with and without bucephalid sporocysts during summer and winter months.

Source of variation	Sum of Squares	d.f.	F-ratio	Signif. level
SUMMER				
Covariate				
Size	326.67	1	75.17	<0.01 **
Effects				
Parasite	12.02	1	2.77	>0.05 ns
Residual	604.01	139		
WINTER				
Covariate				
Size	5659.82	1	301.08	<0.01 **
Effects				
Parasite	0.02	1	0.001	>0.05 ns
Residual	6109.56	325		

6.4 DISCUSSION

Lasiak (1989) found prevalences of *Proctoeces* metacercariae in *Perna perna* at several places along the South African coast similar to those found during this work. She concluded that, being an active growth phase in the life-cycle, the metacercariae probably ingest material from the host's tissues, disturbing its growth and reproduction.

Parasites may have different effects depending on the size of the host either because of reproduction (see Introduction) or because the energy used for growth shifts with age (Griffiths and King, 1979; Seed and Suchanek, 1992).

Results from this chapter showed that *Proctoeces* had a harmful effect on the growth of mussels, especially of those smaller mussels which, under normal conditions, would be expending most of their energy on growth (see Seed and Suchanek, 1992 for references). Although Chapter 2 showed that *Proctoeces* did not have a direct effect on the mortality of the mussels, parasites may indirectly affect the survival of the host by affecting their relationships with predators and competitors. In a mussel bed mussels are densely packed, competing for space and food, with slower growing mussels having a competitive disadvantage over those mussels that grow faster (Kautsky, 1982). Therefore, any depression of growth rate is likely to affect the competitive ability of the mussels. Furthermore, although *Proctoeces* has no direct effect on mussel reproduction (Chapter 3), it may have an indirect effect by reducing growth rates, since fecundity is size related and smaller mussels spend less energy on reproduction than bigger ones (Seed and Suchanek, 1992).

In the case of bucephalid sporocysts, the effect on growth is presumably less ecologically important, as it decreases growth only of large mussels (71-80mm). These animals channel most of their energy into reproduction and exhibit low growth rates anyway.

Mytilids are widely reported to have different seasonal growth rates (Andreu, 1965; Dare and Edwards, 1976; du Plessis, 1977; Berry, 1978; Crawford and Bower, 1983; Rodhouse *et al.*, 1984; Page and Hubbard, 1987; Anwar *et al.*, 1990; Richardson *et al.*, 1990; Cheung, 1991, 1993; Sukhotin and Kulakowski, 1992 and van Erkom Schurink and Griffiths, 1993). Therefore the growth experiment in the present study was done both in summer and in winter. The present study showed that there were variable results between seasons. Animals from Kowie Point showed slower growth rates in winter than in summer months (Fig. 6.1 A and B). In the case of animals from Hougham Park, although growth was higher in winter than in summer (Fig. 6.1. C and D), this was probably the result of the smaller initial size of the mussels used in the winter experiment (Fig. 6.2 C and D). The possibility of the effects of parasites on mussel growth being confounded by infections picked up and/or lost during the course of the experiment was minimized by taking a monthly subsample. If mussels were infected during the study, a change on infection rates with the time would be expected (unless the number of new infections acquired equaled the number of infections lost, which is very unlikely). Since infection rates stayed constant through the duration of the experiment it is assumed that new infections were not acquired. The effects of the parasites were consistent between seasons; bucephalid sporocysts had no significant effect on growth in either summer or winter (Fig 6.1. C and D) whilst *Proctoeces* significantly depressed growth of small mussels both in summer and in winter (Fig 6.1 A and B).

Some of the controversy on the effects of trematodes on growth could be due to the different methodologies used. This may very well explain, for example, why McClelland and Bourns (1969) found that, under laboratory conditions, infected snails grew faster than uninfected controls, whilst Wright (1971), in a field experiment, concluded that these same snails, *Lymnaea stagnalis*, grew slower when infected than when uninfected (in Fernandez and Esch, 1991). Fernandez and Esch (1991) pointed out that the relationship between growth rates and parasitism is mainly an energetic problem and therefore laboratory studies are unrealistic because the animals are well fed and without limited resources.

This discrepancy goes even further in the case of Sousa (1983) and Minchella (1985). The first hypothesized that gigantism occurs only in the case of short-lived species, whilst Minchella concluded that it occurs only in long-lived species. Interestingly, both of them used the same host species (*Hydrobia ulvae*) to support their hypothesis (Mouritsen and Jensen 1994).

The controversy refers not only to the effects of the parasites on host growth, but also to the mechanisms to explain these effects. However, the bulk of the literature agrees on an association between the effects of trematodes on the host growth and effects on reproduction, specifically of parasitic castration. Castration may lead to an increase in growth rate and/or gigantism as energy formerly allocated by the host to reproduction is put into somatic growth (e.g. Wilson and Denison, 1980; Wright, 1971; and Sluiters, 1981).

The results from this study, which applied identical techniques to two parasites, indicate very

clearly that there is no fixed relationship between the effects of parasites on growth and reproduction; the effect depends on the parasite-host system studied. In this case, two parasites affected different components of the host energy budget. *Proctoeces* directly affected growth but not reproduction, and the bucephalid sporocysts affected reproduction but (except for the largest mussels) not growth.

Partitioning of energy in mussels varies with size, with small mussels spending more energy on growth and big mussels putting most energy (sometimes over 90%) into reproduction (see review by Seed and Suchanek, 1992). In the present study *Proctoeces* reduced growth, and affected principally the smallest mussels, whilst the bucephalids affected principally the biggest mussels by castrating them. Each parasite affects only one major component of the energy budget, and in both cases the effects of the parasites are strongest in those size classes which channel most energy into the portion of the energy budget affected by the parasite.

Both growth and reproduction are important components of the energy budget of mussels. Assimilated energy is allocated into respiration, production (with two components: growth and reproduction) and excretion. This and the preceding chapter have shown that parasites have a deleterious effect on *Perna perna*, decreasing either growth or reproduction. The next step would be to check if *P. perna* reallocates or compensates for the energy lost to growth and/or reproduction.

SUMMARY

In order to check the effects of parasites on host growth, growth rates of *Perna perna* infected and not infected with either *Proctoeces* or bucephalid sporocysts were compared during both summer and winter seasons.

Mussels infected with *Proctoeces* grew significantly less than non-infected mussels both in summer and in winter. However, when the effect of *Proctoeces* on mussel growth was examined within mussels of different size classes, it was seen that *Proctoeces* only affects growth in the smaller size class. In contrast, with the exception of one size class, bucephalid sporocysts did not have a significant effect on mussel growth in either summer nor winter.

CHAPTER 7.

EFFECTS OF TREMATODES ON FILTRATION AND OXYGEN CONSUMPTION OF *Perna perna*

7.1. INTRODUCTION

One way to study the effects of a parasite on the individual and on the population ecology of its host is to examine the effects on the host's energy budget (Munger and Karasov, 1989). Since survival and reproduction of an individual depend on its use of energy, the study of the effects of parasites on the energy budget of individual hosts should also provide information on the likely impact of parasites on the host population.

Acquired energy is allocated for respiration and production (Munger and Karasov, 1989). By the first law of thermodynamics, energy entering the system must equal energy stored plus energy leaving the system, therefore, any factor affecting one side of this equation is going to have a measurable effect on the other side. For example, a decrease in efficiency of energy acquisition must be compensated for either by a decrease in rate of energy expenditure or by the use of stored energy (Munger and Karasov, 1989).

Chapters 5 and 6 have shown how both *Proctoeces* and bucephalid sporocysts have a negative effect on the portion of energy allocated to production by affecting either growth or reproduction. The aim of this chapter was to examine whether, in infected individuals, the lost energy normally allocated to growth or reproduction is reallocated within the mussels energy budget, or whether it is lost to the parasite. If reallocation within the mussel occurs, one

would expect either a decrease in energy acquisition or an increase in the other major form of energy expenditure, respiration. If reallocation does not occur, the energy is lost to the parasite, in which case the next question would be: "Do the mussels compensate for that loss of energy, i.e. is there an increase in energy acquisition or a reduction in respiration?".

7.2 MATERIALS AND METHODS

Perna perna were collected from Kowie Point (60-85mm length) and Hougham Park (70-95mm length), two locations known to have high infection rates of *Proctoeces* and bucephalid sporocysts respectively (Chapter 2). The animals were then transferred to the laboratory, cleaned of fouling organisms and maintained in a recirculating seawater system at a temperature of 17-20 ^{0}C and a salinity of 35% . The mussels were fed twice a day with a suspension of the unicellular alga *Dunaliella salina* until they were used in experiments.

A flow-through system was used to determine oxygen consumption and filtration rates simultaneously. The system consisted of a header tank, from which water was distributed to sixteen 500ml flasks. Fourteen of the flasks were occupied by one mussel each; the remaining two were acted as controls and had no mussels. Each flask had an outgoing pipe which siphoned out the water from the immediate vicinity of the mussel. The outgoing water was collected in a bottom tank, from where it was pumped back to the header tank. The animals were introduced in the system, with great care not to leave any air bubbles which would interfere with the oxygen readings, and then left for an hour to acclimatize before readings were taken.

Oxygen consumption was measured using an automatic multi-channel respirometer. Each of the outgoing pipes was connected in turn to a Clark oxygen electrode type E5046 (Radiometer) which was calibrated using the two control flasks. After allowing a couple of seconds to stabilize, the oxygen concentration from the water in the vicinity of the mussels was measured for four minutes. The difference in oxygen concentration between the control and the experimental flasks was considered to be the oxygen consumed by the mussels. For each mussel the oxygen consumption was noted after one, three and five hours.

Filtration rates were measured simultaneously from the difference in algal concentration between the control and experimental flasks. Sinking of algae was insignificant and the decrease in algal concentration was assumed to be due solely to the feeding activity of the mussels. After measuring the respiration, 10ml of water were collected from the outgoing pipe of each flask, diluted with 10ml of electrolyte, and the number of algae cells was counted using a Coulter counter model ZM with an aperture of 5 µm.

The flow rate through each of the flasks was kept between 2 and 20ml/min.

Filtration and respiration rates were calculated according to the following equations (A. Dye, pers. com.):

$$F = [\text{flow rate} \times (1 - \text{final cell concentration/initial cell concentration})]$$

$$R = \Delta PO_2 \times \text{flow rate} \times 12.487$$

After all the measurements were taken the mussels were dissected and examined for parasites. Mussel tissues were then placed in an oven at 60°C for at least 48 hours to determine the individual dry weights. Filtration rates and oxygen consumption were normalised to dry

weight for each individual, and the data then were tested for normality and homogeneity of variances. Raw filtration rates deviated significantly from the assumption of homoscedasticity and were therefore logarithmically transformed. Filtration rates and oxygen consumption for animals with and without *Proctoeces* and bucephalid sporocysts after one, three and five hours of experiment were compared using a two-way ANOVA, with the presence or absence of parasites and the time as main effects.

7.3 RESULTS

Figure 7.1 represents filtration rates ($l.h^{-1}.g^{-1}$) of *Perna perna* with and without metacercariae of *Proctoeces* and bucephalid sporocysts after one, three and five hours of experiment. The results of the two-way ANOVA for filtration rates of parasitized and non-parasitized individuals after logarithmic transformation of data are summarized in Tables 7.1 and 7.2. Neither *Proctoeces* nor the sporocysts had a significant effect on the filtration rates of the mussels. In both cases filtration was highly dependent on time, with lower filtration rates as time increased. At the beginning of the experiment, non-infected animals filtered more water than animals infected with either parasite (Fig. 7.1), although this difference was not statistically significant. After three hours in the experimental flasks, this situation reversed, with mussels infected either by *Proctoeces* or bucephalid sporocysts having higher filtration rates than non-infected individuals. By the end of the five hours, filtration rates of parasitized and non-parasitized mussels were very similar (Fig. 7.1). Although Fig. 7.1 suggests that there were interactions between the effects of parasites and of the time, the results of the ANOVA showed that this was significant only in the case of mussels parasitized by *Proctoeces*.

Fig. 7.1. Filtration rates of *Perna perna* with and without *Proctoeces* (A) and bucephalid sporocysts (B). Means ± SD.

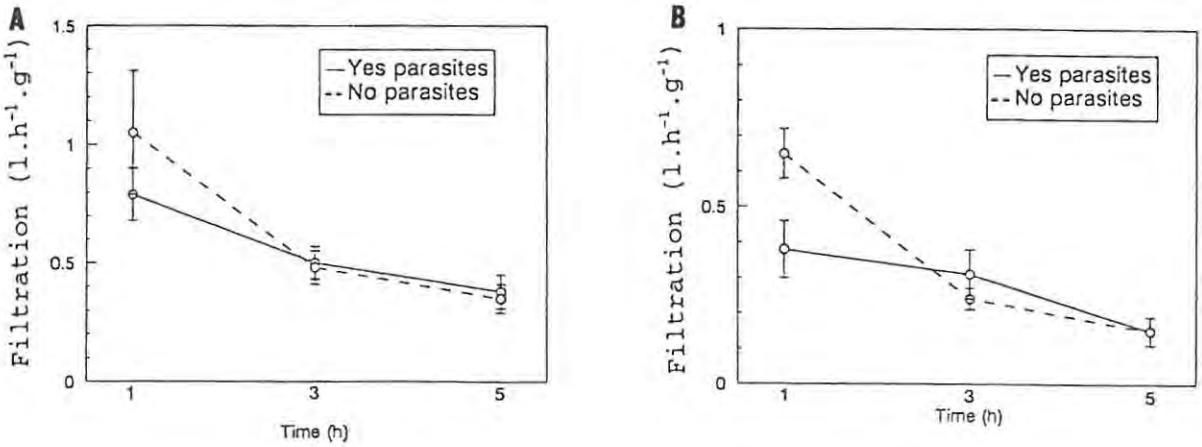


Table 7.1. Results of the Analysis of Variance comparing filtration rates of mussels infected and not infected with metacercariae of *Proctoeces* after 1, 3 and 5 hours.

Source of variation	Sum of Squares	d.f.	F-ratio	Sig. level
Parasites	0.013	1	0.50	0.49
Time	0.436	2	34.22	<0.01
Interactions	0.060	2	4.69	0.01
Residual	0.656	103		

Table 7.2. Results of the Analysis of Variance comparing filtration rates of mussels infected and not infected with bucephalid sporocysts after 1, 3 and 5 hours.

Source of variation	Sum of Squares	d.f.	F-ratio	Sig. level
Parasites	0.003	1	0.26	0.61
Time	0.208	2	18.11	<0.01
Interactions	0.016	2	1.43	0.24
Residual	0.471	82		

Oxygen consumption of non-infected mussels and mussels infected with *Proctoeces* or bucephalid sporocysts is shown in Figure 7.2. A two-way ANOVA showed that respiration was not significantly influenced by either time or the presence of parasites (Tables 7.3 and 7.4). Respiration rates of non-infected mussels were slightly higher than those of infected mussels at all times, but the difference was not statistically significant. There were no significant interactions between the effects of the parasite and time for either *Proctoeces* or the sporocysts.

Fig. 7.2. Oxygen consumption of *Perna perna* with and without *Proctoeces* (A) and ɔucephalid sporocysts (B). Means ± SD.

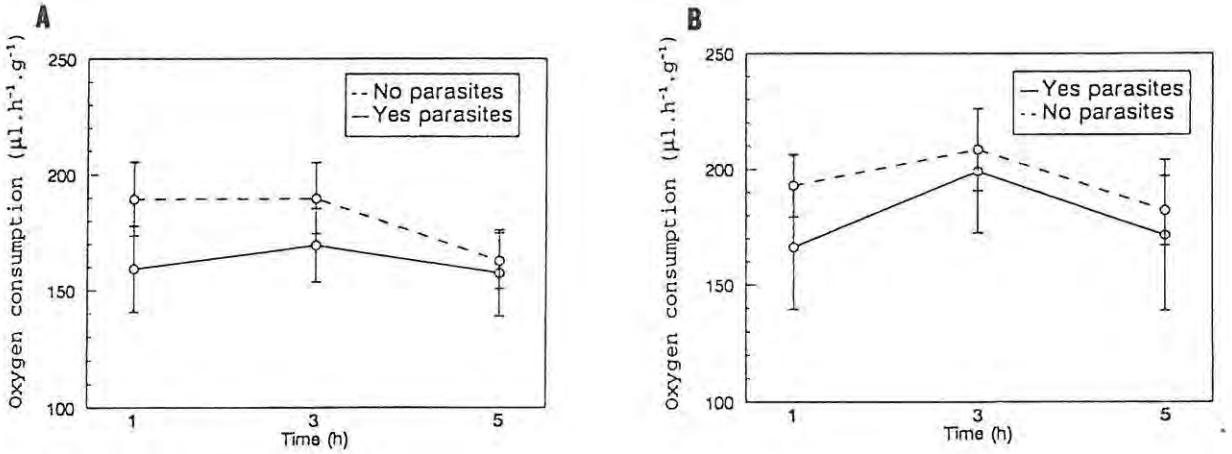


Table 7.3. Results of the ANOVA comparing oxygen consumption (after 1, 3 and 5 hours) of mussels infected and not infected with metacercariae of *Proctoeces*.

Source of variation	Sum of Squares	d.f.	F-ratio	Sig. level
Parasites	16222.10	1	2.91	0.09
Time	1715.87	2	0.27	0.76
Interactions	12866.08	2	2.04	0.14
Residual	125885.40	40		

Table 7.4. Results of the ANOVA comparing oxygen consumption (after 1, 3 and 5 hours) of mussels infected and not infected with bucephalid sporocysts.

Source of variation	Sum of Squares	d.f.	F-ratio	Sig. level
Parasites	6187.76	1	0.72	0.41
Time	7034.07	2	1.28	0.29
Interactions	2716.22	2	0.49	0.61
Residual	115161.76	42		

7.4 DISCUSSION

Many studies have been done on the physiological responses of mussels to different environmental conditions (e.g. Winter, 1973; Andreu, 1976; Cabanas *et al.*, 1979; Kiorboe *et al.*, 1980; Navarro and Winter, 1982; Hawkins *et al.*, 1985; Meyhofer, 1985; Clarke and Griffiths, 1990; Grant and Thorpe, 1991; Navarro *et al.*, 1991; van Erkom Schurink and Griffiths, 1992; Widdows and Page, 1993). Most of these works have found filtration rates between 0.1 and 5.3 $l.h^{-1}$ depending on the size of the mussels and the environmental conditions such as temperature, particle concentration and flow rate at which the measurements were made (Winter, 1973; Cabanas *et al.*, 1979; Andreu, 1976; Kiorboe *et al.*, 1980; Meyhofer, 1985; Clarke and Griffiths, 1990; Navarro *et al.*, 1991; van Erkom Schurink

and Griffiths, 1992 and Widdows and Page, 1993). Although filtration rates found in this study were very low (Fig. 7.1), they were within the range of values previously reported for mussels. These low values could be the result of very low flow rates through the experimental flasks (Walne, 1972).

In comparison, very little is known about the effects of parasites on molluscan host physiological processes, and the results are often contradictory. Ishak *et al.* (1970) found that schistosome-infected *Biomphalaria alexandrina* showed a consistently lower rate of oxygen consumption than uninfected individuals, whilst Meakins (1980) concluded that larval *Schistosoma mansoni* elevated the oxygen consumption of *Biomphalaria glabrata.* Several authors have used changes in filtration or respiration to explain changes in other parameters of the energy budget. For example, Wesenberg-Lund (1934) explained gigantism as a result of an increase in the amount of food eaten, and Williams and Gilbertson (1983) found that *Biomphalaria glabrata* infected with *Schistosoma mansoni* for 33 days fed more often that uninfected snails. On the other hand, this has been disproved in several studies. For example, Meuleman (1972) found that infected snails consumed less food than controls from the third week after infection onwards.

With respect to bivalves, the effects of parasites seem to be more homogeneous. Bayne *et al.* (1978) found lowered filtration rates in *Mytilus edulis* infected with *Mytilicola intestinalis*, although only under extreme conditions of temperature and or ration. Ward and Langdon (1986) demonstrated that parasitism by naturally occurring densities of *Boonea impressa* deleteriously affects *Crassostrea virginica* by significantly reducing filtration rates, and

Bierbaum and Shumway (1988) found lower respiration and filtration rates in *Mytilus edulis* infected with *Pinnotheres maculatus* than in non-infected individuals, though they failed to find any differences in the assimilation efficiency.

Results from this chapter show that infection with neither *Proctoeces* nor bucephalid sporocysts had a significant effect on filtration rates or oxygen consumption of *Perna perna* (Tables 7.1, 7.2, 7.3 and 7.4), even though each parasite affected either reproduction or growth (Chapters 5 and 6). This means that there was no reallocation or compensation for the energy lost to production. The energy was not reallocated to other parameters of the energy budget such as filtration or respiration, but was lost to the parasite. Nor did *P. perna* either increase its food consumption or decrease its oxygen consumption in an attempt to compensate for the energy lost to the parasite. The fact that the effects of the parasites are directed to that fraction of the energy budget allocated to production instead of to maintenance could be considered as a parasitic adaptation. Host survival is necessary for parasite survival, therefore the parasite should influence the metabolism of the host as little as possible (Davis and Farley, 1973; Jokela *et al.*, 1993; J. Taskinen, pers. comm.).

SUMMARY

The two previous chapters have shown that both *Proctoeces* and bucephalid sporocysts have a negative effect on either growth or reproduction of *Perna perna*. In an attempt to explain whether the mussels reallocate or compensate for that energy lost, filtration rates and oxygen consumption of *P. perna* with and without parasites were measured. Neither *Proctoeces* nor

the bucephalid sporocysts had a significant effect on either filtration or respiration. Therefore it can be concluded that *P. perna* does not reallocate the energy, rather it is lost to the parasite. Nor do the mussels compensate for the energy lost.

CHAPTER 8.

SYNTHESIS

The importance of parasites as potential regulatory agents of populations has always been controversial. Since the work of Anderson and May in the late 1970s (Anderson and May, 1978; May and Anderson, 1978) many authors have suggested that macroparasites can regulate host population abundance (Kuris, 1974; Anderson, 1978; Anderson and Crombie, 1984; Scott and Anderson, 1984; Blower and Roughgarden, 1987). Despite this there is a lack of ecological bias in parasitological studies.

Many studies have been done on the effects of trematodes on molluscan hosts, but most of them are purely descriptive. They describe the effects of a certain parasite on a certain host and conclude by saying whether parasites affect different parameters such as growth, reproduction or filtration, without entering into the ecological implications of these effects. A recent review by Poulin (1994) on effects of helminth parasites in host behaviour concluded that parasites significantly affect the behaviour of the host. These changes in host behaviour induced by parasites are associated with a reduction in host fitness in most cases. A classical example from the terrestrial environment is that of lizard malaria. A long term study on fence lizards carried out by Schall and coauthors and reviewed by Keymer and Read (1991) showed that although direct effects on survivorship, body size and growth rates were difficult to detect, infected animals were anaemic. This translated into a decrease in oxygen transport capacity, aerobic activity and stamina. Since social interaction requires high metabolic activity, parasitized animals spent less time interacting with females and maintaining territories. Thus, parasitism of males made them less attractive to females and resulted in a

decrease in reproductive success.

Although there is no doubt nowadays that parasites affect the behaviour of the host, the interactions among parasites, hosts and other species such as predators and competitors have also been largely ignored by general ecologists and parasitologists alike for many years (Price, 1990). Nevertheless, in the last few years there has been a considerable increase in this type of study. Many examples of how parasites may alter the behaviour of their host in such a way as to leave it more vulnerable to predators are provided in a book edited by Barnard and Behnke (1990) using examples from both terrestrial and aquatic ecosystems. Two typical marine cases are those described by Granovitch (1992) and Jonsonn and Andre (1992). Granovitch showed that trematodes affect the migration patterns of the intertidal snail *Littorina saxatilis*, preventing them from making their normal tidal migrations. Those snails that do not migrate during low tides are much more susceptible to predation by birds. Jonsonn and Andre found an interesting correlation between trematode infestation and loss of burrowing ability in the cockle *Cerastoderma edule*. Again, this impaired burrowing ability increases the susceptibility of cockles to predators.

Price *et al.* (1986) reviewed different ways in which parasitism may affect the outcome of competition, and concluded that mediation by parasites is very common in nature and must be regarded as one of the major types of interaction in ecological systems. One of the few marine studies which considers the interactions among parasites, predators and competitors was done by Elner and Rafaelli (1980) who examined the interactions between two intertidal snails, a predator and a parasite. *Littorina rudis* and *L. nigrolineata* compete for food, but

other species influence this interaction. *L. rudis* is preferred prey of the crab *Carcinus maenas*, and both of them are linked by the parasite *Microphallus similis*. In classic fashion *M. similis* uses *C. maenas* as final host and *L. rudis* as intermediate host. *M. similis* also reduces fecundity of *L. rudis*, putting it at a competitive disadvantage against *L. nigrolineata*.

Competition between indigenous and introduced species is central to the study of biological invasions and it has been suggested that release from parasites is important in allowing an introduced species to become invasive (Bruton, 1986). The Mediterranean mussel *Mytilus galloprovincialis* was accidentally introduced to South Africa in the late seventies and it has since become invasive (van Erkom Schurink and Griffiths, 1990; Hockey and van Erkom Schurink, 1992). *M. galloprovincialis* is a highly invasive species which has been successfully introduced to many places, from the Atlantic to the Pacific (see Chapter 2 for references). Comparative studies performed by van Erkom Schurink and Griffiths (1991, 1993) and Hockey and van Erkom Schurink (1992) on the four major species of mussels present in South Africa nowadays indicate that *M. galloprovincialis* exhibits all the characteristics of an aggressive invasive species: it has a rapid growth rate under a wide range of environmental conditions, a high level of tolerance to physiologically limiting factors which allows it to colonize marginal areas, and a higher reproductive potential than those of the three indigenous species. All these characteristics have been considered by Erlich (1989) to be attributes of a successful invasive.

Parasitic infestations have been recognized as one of a number of stressful factors which may lower the resistance of the host and its ability to adapt to changing environmental conditions

(Williams and Jones, 1994). Whether the reason for the success of *Mytilus galloprovincialis* is its competitive superiority or its lack of parasites, there is no doubt that this species has become successfully established on the west coast of South Africa, and has the potential to displace the indigenous *Perna perna* on the south coast, where it has recently appeared (Phillips, 1994).

In this study, for the first time in South Africa, the topics of parasitology and marine ecology have been brought together, examining specific mechanisms by which parasites may affect the invasiveness of *Mytilus galloprovincialis* as a possible example of parasite mediated competition. A pre-requisite for parasite mediated competition is that the competing species must have different susceptibilities to parasites (Price *et al.*, 1986). Low infection rates of *M. galloprovincialis* by parasites have been reported by other authors. Hepper (1957) concluded that *M. galloprovincialis* was more resistant to the copepod *Mytilicola intestinalis* than was *Mytilus edulis*, and Seed (1969) found that infection rates with pea crabs were lower in *M. galloprovincialis* than in *M. edulis*. Chapter 2 showed that the indigenous mussel *Perna perna* in South Africa is commonly infected by trematodes, one of which (*Proctoeces*) appears to have the musselcracker (*Sparadon durbanensis*) as a final host (Chapter 3). The invasive *M. galloprovincialis* on the other hand is free of trematodes. A question that immediately comes to mind is "Why does *Perna* have parasites while *Mytilus* does not, even in areas where the two species coexist?". Combes (1991) mentioned that the survival of parasites in a host depends on two processes: compatibility of the parasite with the host or a "compatibility filter", and the likelihood of encountering that host or an "encounter filter". Since *Mytilus* and

Perna share the same resources in many areas in this country, the possibility of an encounter filter is very unlikely in this case. *M. galloprovincialis* may be protected against infection through its immune system, or parasites may simply not recognize it as a potential host.

Having examined epidemiology, the next step was to assess the effects of those parasites on *Perna perna*. Chapters 4 to 7 provided evidence that the two trematodes studied in this case (*Proctoeces* and bucephalid sporocysts) have a number of deleterious effects on their host. Apart from a parasite induced reduction in condition after spawning and weakening of the adductor muscle by bucephalid sporocysts, the most important effects are on growth and reproduction. Kautsky (1982) concluded that intraspecific competition for food and space leads to competitive suppression of small mussels. Therefore, the fact that mussels infected with *Proctoeces* show reduced growth rates will affect their competitive abilities. Mussels infected with bucephalid sporocysts are completely castrated, with consequent reproductive loss for the population. This is especially important, as infection rates of this parasite increase with the size of the mussel, and the largest mussels are those which direct most energy into reproduction (Seed and Suchanek, 1992). Several authors have suggested that the relationship between infection and size is due to parasite-induced gigantism (see Chapter 6 for references). However, results from Chapter 6 showed that infection with bucephalid sporocysts has no significant effect on mussel growth, therefore infection rates are likely to be age related. Older animals have been exposed longer and also they consume more food, thus increasing the chances of filtering parasites with their food (Curtis and Hurd, 1983; Liebman, 1983; Rohde, 1984; Minchella *et al.*, 1985; Taskinen and Valtonen, 1995). Therefore, even though there is no apparent direct effect of either of the trematodes on survival of their host (Chapter 4),

infection seems to be associated with a substantial reduction in fitness. By castrating large individuals (Chapter 5) or reducing growth rates of small individuals (Chapter 6), both parasites have a negative effect the mussel population. Chapter 7 has shown a lack of compensation for these negative effects. The energy lost for growth or reproduction is not replaced by an increase in filtration or compensated for by a reduction in respiration. Rather it is simply lost to the parasite.

In the near future populations of *Perna perna* in South Africa are likely to be threatened by *Mytilus galloprovincialis*. Not only is *Mytilus* competitively superior to *Perna*, but it is also free of parasites. Mussel beds composed only of *P. perna* already have intraspecific competition in which parasitized individuals are at a disadvantage. In the event of *M. galloprovincialis* and *P. perna* entering into competition, the competitive stress to which parasitized *P. perna* will be subjected will be even greater. Consequently the parasites are likely to have an even greater effect. There is ample evidence in the literature to support the idea that the effects of parasites are more pronounced under conditions of stress (Hepper, 1955; Anderson, 1975; Cromptom and Nesheim, 1982; Bierbaum and Ferson, 1986; Zuk, 1987; Sousa and Gleason, 1989). Likewise, *Proctoeces* and bucephalid sporocysts affect the condition of *Perna perna* only when *P. perna* is under stress (Chapter 4).

In this study we have seen how parasites affect the indigenous mussel *Perna perna* and are likely to affect its interactions with the invasive *Mytilus galloprovincialis*. Since for a parasite community the resource base is the host population (Price, 1990), this situation raises the question: "If parasites affect the competitive ability of *P. perna* to the point where this species

is competitively displaced by *M. galloprovincialis*, what will then happen to the parasite community?. Will they in turn also become extinct or will they develop in *Mytilus*?".

Survival of the parasite implies new adaptations for infecting new hosts. Combes (1991) outlined three possible outcomes for a case like this:

1- The parasite may develop in the new host incidentally, without dynamic or genetic consequences for the parasite;

2- The parasite may develop in both hosts, this means an increase in host spectrum with possible dynamic consequences for the parasite;

3- The parasite may develop in the new host, but its lineage may diverge genetically and undergo speciation.

In conclusion, the lack of trematodes in *Mytilus galloprovincialis*, together with their high prevalences and negative effects on *Perna perna* may help to explain the success of *Mytilus* in South Africa. It remains to be seen whether in the future either parasite will develop in *M. galloprovincialis*, in which case this mussel may lose some of its competitive advantage; or whether *M. galloprovincialis* will drive both *Perna perna* and its parasites to extinction.

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APPENDIX

If there is something I have learnt after so many years of doing a doctorate, it is the different ways of cooking mussels, therefore it is only fair for me to share this piece of knowledge. But before starting, and to answer a question that I have been asked many times, I'll just quote Menzel and Hopkins (1955, p.341):

> "*Bucephalus cuculus* is actually beneficial from the gastronomic standpoint. In southern waters, normal oysters spawn so heavily and so long that they become emaciated and nearly tasteless by late summer, but *Bucephalus*-infected oysters, being castrated or "caponized", remain fat and retain an excellent flavour all summer. Personal tests have proved that the *Bucephalus* sporocysts taste even better than the oyster"

And if this is true for the oysters I don't see why it shouldn't be for the mussels.

To clean mussels scrape the shells with a small knife to remove any organisms that may be growing on them. Then drop them into a bucket with cold water and let them soak for a couple of hours to get rid of the sand.

ROGER PETER'S MUSSEL SOUP

Gather 60 mussels. Clean and boil in 2 -3 cups water until shells open. Remove mussels from the pot and reserve one cup of strained mussel stock. Slice 2 or 3 large/medium onions. Put

onions into a large pot with one cup water and approximately 200 grams butter or margarine. Bring to boil and allow to simmer until water has almost boiled away. Take off stove and mix in one cup cake flour. Add one cup mussel stock, one and a half cups milk, one cup white wine and approximately one and a half litres of water.

Bring to boil slowly and allow to simmer, stirring regularly. Add a few shakes of Aromat and one crumbled chicken stock cube. Add parsley and garlic to taste. Place chopped and whole mussels in the pot and allow to simmer until ready to serve. If too thick, add more water. If too thin, add some Maizena mixed with milk. This can be done according to choice.

BOILED MUSSELS WITH VINEGAR

Gather mussels. Clean and place in pot. Add enough water to cover the bottom of the pot. Cover and steam mussels until shells open. Allow to cool. Remove mussels from shells and remove any "beards" remaining in the mussels. Mix one measure vinegar to half measure water, depending on the amount of mussels. Mussels must be covered in vinegar/water mixture. Add salt and pepper to taste and serve. Mussels will keep in vinegar in refrigerator for up to five days.

MUSSEL SALAD

Boil the mussels till open, adding some vinager and thyme to the water. Remove them from the shells and place them on a bowl. Toss with onion, parsley, oil, vinager and salt and

pepper to taste. Serve on lettuce leaves.

OVEN CRUMBED MUSSELS

Gather, clean and cook 30 mussels. Remove mussels from shells and keep 30 half shells. Melt 250ml butter with lemon juice to taste (approximately 50ml). Add one large clove crushed garlic. Place one mussel per shell into half shells and pour 2 teaspoons of liquid over each mussel. Sprinkle fresh bread crumbs over each mussel and place under grill until browned. Serve hot.

FRIED MUSSELS

Put the mussels in a large pot with a bit of white wine, some finely chopped onion, a bit of parsley and coarse pepper. Cover and cook till the mussels open. Remove the mussels from the shells and strain the broth. Roll each mussel in flour, beaten egg and bread crumbs. Deep fry them until golden. They may be served with tomatoe sauce mixed with the strained broth.

And now, here are two of my favourites:

1- Put the mussels in a pot with a bit of water and steam them till they open. Take of one of the shells leaving the meat in the other, and place on a tray. Fry some garlic, chilli, onions and tomatoes in a pan and mush them. Add a glass of white wine and bring to boil for about five minutes. Pour the sauce over the mussels, and decorate with parsley.

2- Insert a knife next to the byssus threads of the mussels and cut the mussel ligament to open the valves. Remove one of the shells leaving the meat in the other and place on an oven tray. Put some garlic butter on top and bake in a medium oven for about 10 minutes, when they are nearly ready add some grated cheese on top and put back into the oven till melted.

All this as far as fancy recipes, but remember it is not too difficult to enjoy a good plate of mussels. For example, nothing easier than putting them on the braai after a day of heavy sampling on the rocks, or if you have a smoker even better. And finally, if you are lazy, in a hurry, or simply can't stand cooking, you can just throw them in a pot with a bit of water till they open and the flesh comes off the shell easily.

So, you know, bonne appetite and.... don't forget to check the parasites!