Assessment of predation by the West Coast rock lobster (*Jasus lalandii*): relationships among growth rate, diet and benthic community composition, with implications for the survival of juvenile abalone (*Haliotis midae*).

University of Cape Town

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DECLARATION

This thesis documents original research carried out in the Marine Biology Research Institute within the Zoology Department at the University of Cape Town. It has not been submitted in whole or in part for any degree at any other university. Much of the information presented here is original, and all other sources are fully acknowledged and referenced. Any assistance I have received has been fully acknowledged. All uncited interpretations are my own, and I bear their full responsibility.

Signed by candidate

S. Mayfield
30-04-98

To my parents, Sheila and Gordon Mayfield, for their continued support.

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"I know there's fish out there but where God only knows... They say these waters aren't what they used to be."

Lyrics by Billy Joel
The Downeaster 'Alexa'
Storm Front 1989

University of Cape Town

TABLE OF CONTENTS

Acknowledgements 1

Abstract 2

General Introduction 4

Chapter 1: A literature review of the main techniques available for dietary analyses, and their suitability for assessing the diet of the West Coast rock lobster, *Jasus lalandii*. 10

Chapter 2: The application of an immunological technique to determining the diet of the West Coast rock lobster, *Jasus lalandii*. 22

Chapter 3: Influence of rock-lobster size, depth of capture and season (stage of moult cycle) on the diet of the West Coast rock lobster, *Jasus lalandii*. 55

Chapter 4: West Coast rock-lobster diet: comparisons between geographic locations with different rates of growth and benthic community composition. 84

Chapter 5: Prey species preference, sea urchin consumption and predation on abalone by captive West Coast rock lobsters, *Jasus lalandii*. 121

Chapter 6: Inter-relations among rock lobsters, sea urchins, winkles and juvenile abalone: implications for community management. 146

General conclusions 192

References 195

University of Cape Town

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ABSTRACT

This thesis focused on two aspects of predation by the West Coast rock lobster (*Jasus lalandii*). These were (1) the problem of slow growth currently observed in adult male rock lobsters and the putative link between growth rate and food availability, and (2) the influence of rock-lobster predation on the benthos, but more specifically on sea urchins and juvenile abalone.

Rock lobsters feed on a wide range of prey types, usually across several phyla. However, direct visual examination of gut contents is often hampered both by the reduction of prey items to small unidentifiable fragments, and the rapid digestion of soft prey. Because an accurate analysis of the diet of *J. lalandii* was vital for both components of this thesis, techniques available for dietary analysis other than direct observation of gut contents were reviewed. An immunological technique, the ELISA, was selected as the most appropriate one to employ for accurate determination of the diet of this species. Species-specific antisera were developed against 26 potential prey species using New Zealand rabbits and cross-reactions between the different prey species were reduced to less than 4%. However, interference from rock-lobster digestive enzymes prevented application of the technique to determine the diet of *J. lalandii.* Several methods used to remove these digestive enzymes from the gut samples proved unsuccessful. Thus, this immunological technique was ultimately unsuitable as a tool for determining the diet of *J. lalandii*, although the reasons for its failure were of scientific interest in their own right.

Based on the alternative visual technique of assessing gut contents, rock lobsters showed large differences in diet between large and small individuals. The diet of small rock lobsters (carapace length (CL) < 75mm) comprised a wide range of species including, in order of importance, coralline algae, barnacles, sponges and ribbed mussels. By contrast, large rock lobsters (CL > 80mm) ate fewer species, with fish and ribbed mussels being the most abundant prey items. Diets of mature male rock lobsters (70 - 80mm CL) varied minimally with depth and season at two sites of contrasting growth rate, although cannibalism was highest at both sites during the male rock lobster moulting period. For the same large animals, the gut fullness index showed seasonal variability at the fast growth site only. The proportion of rock lobsters feeding showed similar trends at both fast-growth and slow-growth sites and was highest during the early intermoult and lowest during the pre-moult periods.

Prey species composition of male *J. lalandii* (70 - 80mm CL) was also determined from eight sites of contrasting growth rate. There was no difference in either stomach fullness or the proportion feeding between the eight sites. Bray-Curtis similarity and multidimensional scaling plots suggested that rock-lobster diets were also similar at all eight sites. An analysis comparing rock-lobster diet and benthic community composition at two sites of contrasting growth rate showed that despite vast difference in prey availability, the diet of adult male rock lobsters from these two sites was similar. An identical analysis conducted on immature rock lobsters at two different sites (also of contrasting growth rate) yielded the same result. It was concluded that

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growth rate was not related to diet *per se*, but rather to the availability of preferred prey species.

Laboratory experiments showed that rock lobsters are highly selective foragers with a preference for (1) mussels over sea urchins and winkles, and (2) juvenile abalone over sea urchins. When offered only sea urchins, small rock lobsters (CL < 68mm) were incapable of feeding on them, but larger rock lobsters could kill any size of sea urchin offered. All sizes of rock lobsters (CL > 68mm) preferentially ate small sea urchins rather than large ones when offered a choice.

In the field, the distribution of juvenile abalone was strongly linked to that of sea urchins and densities of juvenile abalone increased with sea urchin density below a threshold of 25 sea urchins.m^{-2}. Beyond this level, no further increases in juvenile abalone densities were recorded. It was estimated that a sea urchin density of 5 sea urchins.m^{-2} would support 0.27 ± 1.92 juvenile abalone.m^{-2}, but a density of at least 25 sea urchins.m^{-2} was estimated as being necessary to enhance the commercial abalone fishery. Sea urchin densities were strongly negatively associated with the density of large rock lobsters (CL > 68mm). A density of 5 sea urchins.m^{-2} corresponded with a density of 0.08 large rock lobsters.m^{-2}. The current rock lobster density in the commercial abalone fishing area is three times higher than this. To reduce the rock lobster density to a level at which sea urchin density will be high enough to support sufficient juvenile abalone to supply the commercial abalone fishery in future requires a massive initial rock-lobster harvest.

In conclusion, it is suggested that (1) other variables (*e.g.* water temperature and dissolved oxygen content) be investigated as possible contemporary causes of the coastwide depression in rock-lobster growth rate; (2) long-term experiments on the relationship between preferred food availability and growth rate be conducted in captivity and (3) rock lobsters in the abalone fishing grounds be harvested (at an unyet determined level) sustainably, preferably by local, well organised and well controlled fishing groups, to offset any future losses due to the immanent collapse of abalone recruitment caused by rock-lobster predation. Re-seeding of abalone and sea urchins should only be considered when rock lobster densities are sufficiently reduced, either naturally or by exploitation.

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GENERAL INTRODUCTION

Spiny lobsters form the basis of important crustacean fisheries in nearly all of the world's oceans (Booth & Phillips 1994). Members of the cosmopolitan genus *Jasus*, which occur almost ubiquitously in cool, shallow-water habitats between 27° and 45° S (Heydorn 1969, Pollock 1995) are certainly no exception to this rule.

The West Coast (spiny) rock lobster, *Jasus lalandii* (H. Milne Edwards), has been harvested for at least 10 000 years. Collections began with the Khoi-San as they scoured the coasts in search of food (Buchanan 1988). Since the turn of the 20th century, commercial exploitation of *J. lalandii* has increased rapidly, and this species has formed the mainstay of South Africas' rock-lobster industry for many decades. Three other species (*Panulirus homarus*, *Palinurus gilchristi* and *Palinurus delagoae*) contribute about a third of the total annual rock-lobster catch (Cockcroft & Payne 1997). Commercially exploitable densities of *J. lalandii* occur principally between Lüderitz (Namibia) and Cape Point, although the distribution of this species ranges from Walvis Bay (Namibia) in the North to just East of East London (Figure 1; Barnard 1950, Cockcroft & MacKenzie 1997). In South Africa, the fishing industry for *J. lalandii* is regulated by a total allowable catch, a minimum legal size (75 mm carapace length) and a closed season.

Commercial landings of about 10 000 tons were recorded in the early 1950's after which catches declined steadily to seemingly stable levels in the mid 1980's (Cockcroft & Payne 1997). This represented a total catch of about 8.5 million individuals per annum (*ca.* 3700t) of which 90% were males (Pollock 1986). This was equivalent to about 15% of the total value of all South Africa's commercial fisheries (Payne and Crawford 1989). Today, the West Coast rock-lobster fishery is valued in excess of R145 million per annum and provides jobs for upwards of 4000 people (Mr P Foley, West Coast rock lobster Sea Management Association, pers. comm.). However, this resource is continually under increasing threat. The current pressures of poaching, an enlarging recreational fishery (Cockcroft & MacKenzie 1997) and massive local mortalities from red tide events (Newman & Pollock 1974a, Matthews

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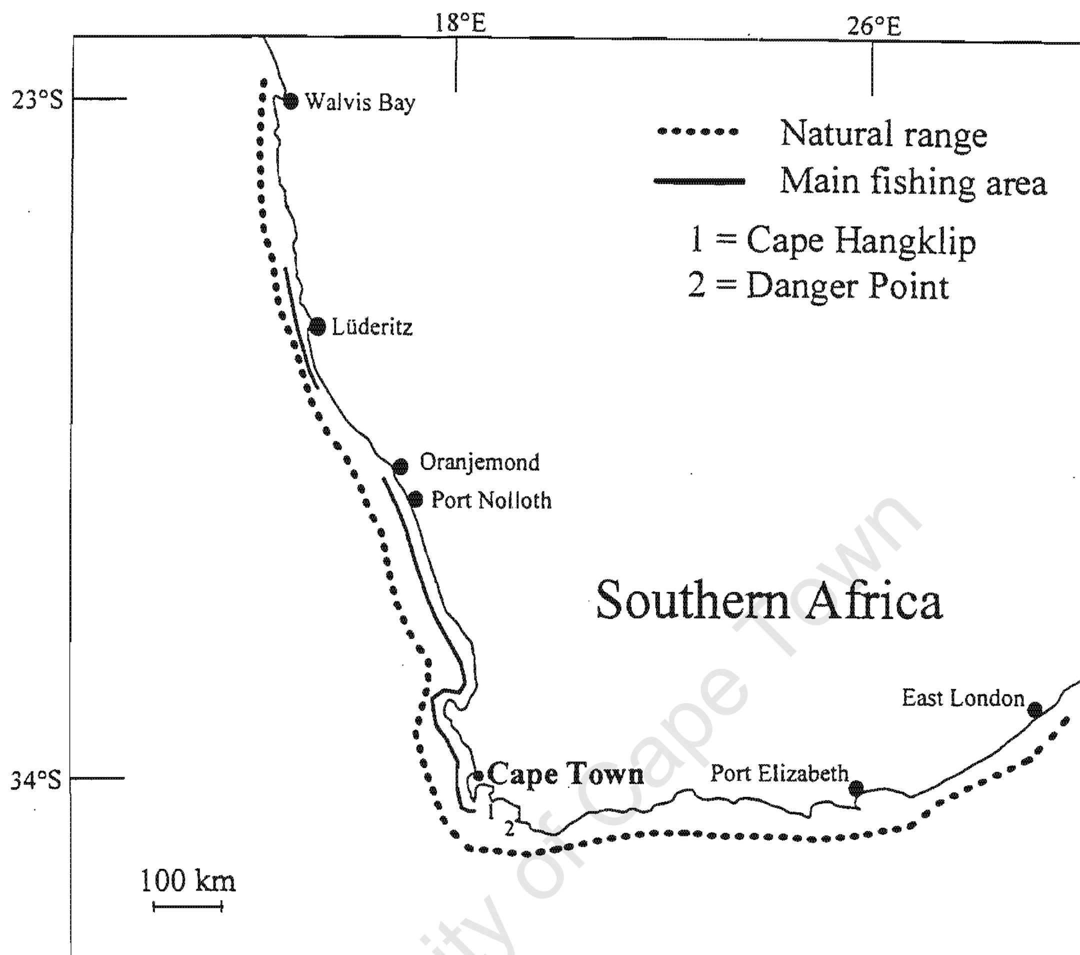

Figure 1: Map of southern Africa to show the natural range of and extent of the commercial fishery for the West Coast rock lobster, *Jasus lalandii*.

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& Pitcher 1996) are likely to be compounded in the near future. This is because increased demand from disadvantaged communities for access to the resource will become an ever increasing issue (Cockcroft & Payne 1997).

As in all arthropods, growth in *J. lalandii* occurs at moulting, with growth rate influenced by both moult increment and moult frequency. As the intermoult period for adult *J. lalandii* is relatively constant with moulting occurring once annually (Pollock 1986), growth of adults is largely dependent on annual moult increments. Annual variation in male *J. lalandii* growth rate (Melville-Smith *et al.* 1995) and differences in growth rates between areas are not unusual (Pollock & Beyers 1981, Goosen & Cockcroft 1995). However, between 1987/1988 and 1994/1995 a large decrease in the growth rate of all size classes of male rock lobsters beyond the previously observed natural variation was recorded along the entire coastline between Port Nolloth and Cape Hangklip (Goosen & Cockcroft 1995; Figure 1). Subsequent analysis showed this drop in moult increment to be far more serious than originally thought (Cruywagen 1997). Simultaneously, there was an increase in the frequency of negative growth in male rock lobsters and a reduction in the size at 50% sexual maturity for female rock lobsters (Cockcroft & Goosen 1995). The drop in growth rate had profound implications for both the minimum legal size and annual total allowable catch, as the exploitable biomass of this resource is very sensitive to changes in male growth increment (Bergh & Johnston 1992, Cockcroft & Payne 1997). To date, there has been limited recovery and the condition of slow growth seems to be affecting both juvenile and adult individuals.

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Meanwhile, the cause of this slow growth remains poorly understood. Two theories have been put forward to explain it. The first suggests that an increase in the prevalence and a change in the distribution of low oxygen water has caused the reductions in growth rate (Pollock 1987, Pollock & Shannon 1987, Tomalin 1993). Chronic exposure to water low in dissolved oxygen can have severe physiological effects on feeding and growth rate, and mortality increases (Pollock & Shannon 1987). This was shown experimentally for *J. lalandii* (Beyers *et al.* 1994) and *Nephrops norvegicus* (Baden *et al.* 1990). Additionally, crowding of rock lobsters into

the shallow oxygen-rich waters adjacent to the shore (which occurs as they try to avoid the low oxygen waters) may increase competition for food (Pollock & Beyers 1981).

An alternate hypothesis is the postulation that prevailing environmental conditions, predominantly as a result of the 1990/1991 *El Niño*, resulted in a failure of mussel recruitment (Pollock *et al.* 1997), and a consequent reduction in both the quantity and quality of mussels available as prey. Mussels are usually considered the prey of choice for *J. lalandii*. Numerous studies have shown (1) food availability to be important in determining growth rate (*e.g.* Chittleborough 1975, McKoy & Esterman 1981, Beyers & Goosen 1987), and (2) rock lobsters from areas with different growth rates to consume different prey (*e.g.* Newman & Pollock 1974b, Pollock *et al.* 1982, Joll & Phillips 1984). These are usually interpreted as a result of differences in benthic community structure. However, the relationship between diet, growth rate and food availability is not yet clearly established (Joll & Phillips 1984, Elner & Campbell 1987). This triangular link, which has to date remained virtually unconsidered for *J. lalandii*, forms the first of two *foci* in this thesis.

The South African commercial abalone (*Haliotis midae*) fishery is currently valued at around US$15 million (Oakes & Ponte 1996). The main fishing area lies between Cape Hangklip and Danger Point (Tarr 1992), and provides employment to several small towns along the Cape South Coast. In addition, this species supports an ever enlarging recreational fishery which also provides economic input into the same area. Abalone stocks have recently shown dramatic declines (Moloney 1997, Plagányi & Butterworth 1997), primarily due to heavy poaching. Observations have shown encrusting coralline algae to be essential for abalone settlement (Tarr 1989), while more recent field surveys (Tarr *et al.* 1996) and extensive field and laboratory experiments (Day 1998) have shown that sea urchins are essential for the survival of juvenile abalone.

Anecdotal reports suggest that rock lobster numbers have increased substantially since about 1987 in the area 150km eastwards from Cape Hangklip. Lobsters around the

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world tend to be voracious predators which have long been seen as an ecologically important species (Paine 1969) with the ability to strongly influence the abundance and population structure of their prey (Mann & Breen 1972, Breen & Mann 1976a, Tegner & Levin 1983, Robles *et al.* 1990, Andrew & MacDiarmid 1991). The South African species is no exception (Pollock 1979, Griffiths & Seiderer 1980, Barkai & Branch 1988a, Barkai *et al.* 1996), even to the extent of creating alternate stable states on two nearby islands (Barkai & McQuaid 1988). Suggestions of a quantitative increase in rock lobster numbers and a concurrent decline in sea urchin densities (and hence juvenile abalone numbers) East of Cape Hangklip since 1986, led Tarr *et al.* (1996) to propose that immigrating rock lobsters had consumed the sea urchins, thereby causing a precipitous decline in juvenile abalone numbers. Recent work (Day 1998) has unravelled the crucial relationships among sea urchins, abalone and coralline algae. As poaching is currently intensifying rather than abating, any further reduction of abalone stocks brought about by reductions in juvenile survival become critical if this species is to withstand commercial extinction. The role of rock lobsters as a predator in this system, and their influence in controlling sea urchin population structure and abundance, forms the second focus of this thesis.

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Thus, this thesis is about two aspects of rock lobster predation. In the first instance, it focuses on the relationship between rock-lobster diet, food availability and growth rate. In the second, it deals with the impact of rock lobsters on sea urchins, and the consequent ripple effect on juvenile abalone.

Both of these threads require an accurate assessment of rock lobster diet. Determining the diet of crustaceans is never easy as the combined actions of the mandibles and the gastric mill ossicles reduce once macroscopic food to small fragments (Williams 1981). Although some prey may have diagnostic fragments (Elner & Campbell 1987), accuracy of analyses are still dependent on the level of expertise of the analyst (Sunderland 1988). For this reason, the first chapter reviews currently available methods of dietary analysis while the application of the most appropriate of these techniques for the determination of the diet of *J. lalandii* forms the subject of Chapter 2

Diet in crustaceans has been shown to vary with many factors. Analysis of the relationship between diet, food availability and growth rate for adult and juvenile *J. lalandii* (the focus of Chapter 4) required sampling to be standardised. For this reason, some of the factors which may influence the results of such a dietary analysis (*e.g.* depth, stage of moult cycle, size of rock lobster) were first tested in Chapter 3.

Any measure of the impact of rock lobster predation requires additional knowledge of prey choice. For this reason, Chapter 5 focused on experiments involving feeding preferences shown by captive rock lobsters. As such it was not intended to be a large multifactorial feeding experiment. Rather, it has two roles. The first is to be a service chapter for Chapter 6, which deals with the influence of predation by *J. lalandii* on sea urchins (and the ripple effect on juvenile abalone) and other components of the benthos, as well as exploring current management issues. The second role is to allow a comparison of the natural diet (Chapter 4) with prey choices observed in the laboratory.

Like all science, this thesis has yielded conclusive answers to some of the questions addressed, left others open and raised new questions. For this reason, the thesis is concluded by a brief synthesis that draws together the threads, summarises what has been accomplished and proposes other avenues of research.

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Chapter 1

A literature review of the main techniques available for dietary analyses, and their suitability for assessing the diet of the West Coast rock lobster, *Jasus lalandii*.

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1. INTRODUCTION

Dietary analysis is an important tool used by ecologists in order to determine food web interactions (Berg 1979, Feller *et al.* 1979) and to understand the structure and functioning of communities. Both detection and quantification of predator-prey relationships (Pickavance 1971, Healy & Cross 1975, Emsweiler-Rose *et al.* 1984, Sunderland 1988, Ohman *et al.* 1991, Theilacker *et al.* 1993), and information pertaining to nutritional requirements (Williams 1981) are facilitated by a knowledge of animal diet.

Determining the diet of decapods is frequently difficult. This is primarily because although most prey consumed are macroscopic, few identifiable fragments remain post-mastication. This has been highlighted over the last 20 years in the case of the West Coast rock lobster, *Jasus lalandii*. Previous studies on the diet of this species have all been based on visual assessments of gut contents, and despite numerous investigations (*e.g.* Heydorn 1969, Newman & Pollock 1974b, Barkai & Branch 1988a), the diet of the West Coast rock lobster remains controversial. Hence, a more accurate technique for diet analysis is desirable.

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Several reviews dealing with methods of determining the diet of a predator have been compiled. However, these reviews have tended to focus on one or other of the two main approaches used, namely visual (Berg 1979, Hyslop 1980, Williams 1981) or non-visual (Boreham & Ohiagu 1978, Calver 1984). Few authors appraise the relative merits and drawbacks of both techniques simultaneously (see Sunderland 1988), and reviews of non-visual methods tend to be restricted to serology (Calver 1984).

By far the vast majority of diet analyses have been performed visually, with the direct examination of stomach contents the most widely used technique (Berg 1979). However, several disadvantages associated with this approach (Theilacker *et al.* 1986) have necessitated the development of alternative techniques. These new methods focused on non-visual approaches, with serology, and in particular ELISAs (enzyme

linked immunosorbent assay) and Western-Blot systems becoming increasingly advanced.

This chapter presents the main techniques available for dietary analysis, their advantages and limitations, and the situations to which they are applicable. Furthermore, this chapter makes a recommendation on which techniques may be most suited to accurately determining the diet of *J. lalandii*.

2. VISUAL TECHNIQUES

Traditionally, dietary analyses have been undertaken using visual techniques. Such techniques include direct observation of the predator in the field (*e.g.* Myrberg 1973, Garshelis *et al.* 1986, Karnofsky *et al.* 1989; Robles *et al.* 1990, Kuitek *et al.* 1992), the examination of either stomach contents (*e.g.* Butler 1989, Diehl 1992, Hill & O'Keefe 1992, Cerda & Wolff 1993) or faecal pellets (*e.g.* Aurioles *et al.* 1984, Corbett 1989, Weaver 1993), and *in situ* experiments (Holmes 1984, Barkai & Branch 1988a). Post-mortem methods tend to be preferred because consumption by the predator is not influenced by the presence of the investigator, thus ensuring that any "results apply directly to an undisturbed, natural system" (Sunderland 1988, p206).

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Examination of stomach contents is the most widely used visual technique (Berg 1979, Dickman & Huang 1988), despite some noteworthy drawbacks. Most important of these drawbacks is the fact that post-maceration identification of prey is difficult (Williams 1981, Theilacker *et al.* 1986), because prey species lose their morphological identity (Feller *et al.* 1979), rendering most of the stomach contents unidentifiable (Weaver 1993). This problem is further compounded in the Decapoda as the combined actions of the gastric mill and mouthparts tend to reduce once macroscopic prey to tiny fragments (Williams 1981).

One way to alleviate this problem is to develop an index of diagnostic fragments (Elner & Campbell 1987). However, this fails to consider the presence of soft-bodied

organisms in the diet which causes a bias towards organisms with hard body components (Young 1973, Calver 1984, Dickman & Huang 1988). This still fails to account for differences in digestibility of prey items (Calver 1984). Furthermore, identification of prey remains are dependant on the abilities of the examiner (Sunderland 1988). Stomach content analysis is also unsuitable for very small animals such as planarians (Pickavance 1971), as well as for liquid feeders (*e.g.* mosquitoes), although cytological characteristics of bloodmeals have been used (Washino & Tempelis 1983). Thus with visual techniques, the accuracy to which natural diet may be assessed is frequently low (Williams 1981). On the other hand, various indices of gut fullness and volumetric or numeric contribution of different prey species do allow quantitative comparisons to be made.

3. NON-VISUAL TECHNIQUES

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Non-visual techniques have numerous advantages over visual methods of diet analysis. The most obvious is that all these approaches are objective, thus removing the problems of operator bias and expertise (Sunderland 1988). Many non-visual methods allow analysis of microscopic organisms (Shapiro *et al.* 1989), dietary analysis of liquid feeders (Washino & Tempelis 1983), and the identification of soft-bodied prey (Davies 1969, Calver 1984) which are all rarely possible in visual analyses. Most non-visual techniques have the potential for untrained personnel to process large numbers of samples with relatively simple apparatus (Crook & Sunderland 1984). Two spheres of non-visual techniques may be identified: serological and non-serological methods.

3.1 Non-serological techniques

Non-serological methods include the use of stable isotopes, radioactive isotopes and autoradiography, chromatography and electrophoresis to facilitate gut content identification.

3.1.1 Stable isotopes

Variations in the ratio of stable isotopes (Carbon, Nitrogen, Sulphur) do have potential in the study of animal diets in natural systems (Fry *et al.* 1978, Peterson & Fry 1987). Although not directly suitable for the analysis of specific gut contents, stable isotope analysis has often been used to distinguish between general categories of food with different isotope signals. Perhaps this is because several conditions need to be met prior to use, including a knowledge of potential prey, isotopic contrasts in those prey, and that samples are well preserved (Schwarcz 1991). Furthermore, this approach to diet analysis can become complex if numerous food sources exist (Rau *et al.* 1991), predominantly because widely differing isotope ratios are required between prey species before discernment of prey in the diet is possible.

3.1.2 Radio-isotope labelling

The basic principal behind this technique is that prey labelled with radioactive isotopes are released into the field, and, after a period of time, autoradiography is used to detect the presence of the isotopes in the gut contents or soma of the predators (Sunderland 1988). This approach has been used with some success. For example, Baldwin & Newell (1991) using a dual radioisotope labelling technique (^{3}H & ^{14}C), were able to identify the particle diet and obtain data on clearance rate and food selectivity of the planktotrophic larvae of the eastern oyster *Crassostrea virginica*. Employing similar techniques, but using ^{32}P labelled eggs and 1st, 2nd and 3rd stage larvae of lepidopteran pests, and exposing these to potential arthropod predators in field cage experiments, McCarty *et al.* (1980) were able to identify their predators using autoradiography. This technique can be used quantitatively (Sunderland 1988), but with several difficulties needing to be overcome to achieve this (Southwood 1978). However, Giller (1982), amongst others, raised concerns about the use of radioactive tracers, given that they are difficult to control, at times unpredictable and can be very costly. Perhaps though, the largest drawback of this technique is that although it is useful for controlled experiments, the natural diets of predators are almost impossible to elucidate by this means.

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3.1.3 Chromatographic techniques

Chromatographic methods involve the separation of mixtures of proteins on the basis of protein size, usually observed as the rate of migration through some medium (*e.g.* filter paper). Putman (1965, 1967) attempted to use paper chromatography to identify mite prey species consumed by spiders, but was hampered by an inability to separate prey species and to quantify rates of predation. This simple technique is useful only for very basic predator-prey systems, and is even further limited in that quantifying predation is rarely possible (Putman 1965). More recently, however, Knutsen & Vogt (1985a,b) have successfully studied the diet of clawed lobsters, *Homarus gammarus*, using a more sophisticated approach - a gas chromatographic technique. They compared the pattern obtained from the stomach contents of starved and fed juvenile lobsters with patterns obtained from a cultured prey organism (Knutsen & Vogt 1985a). Also analysed were numerous infaunal species and detritus which facilitated the identification of stomach contents (Knutsen & Vogt 1985b). Despite this approach providing accurate dietary analysis, it is very time intensive in both the initial set up and sample processing.

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3.1.4 Electrophoresis

In a similar manner to chromatographic methods, electrophoresis again relies on the differential rates of protein migration, usually through a polyacrylamide gel (Sunderland 1988). In this case, however, protein movements are induced by the presence of an electric field. The aim is to locate protein bands as 'fingerprints' that characterise each potential prey species (Giller 1982), with esterases being preferred due to their high allele diversity (Giller 1984). This method is a cheap and rapid approach to diet analysis, but with limited potential for quantification of prey. Although Giller (1986) used this method successfully to identify 18 prey species for two predatory waterbugs (*Notonecta glauca* and *N. viridis*), other authors have experienced difficulties in separating groups (Griffiths 1983 as cited by Sunderland 1988). This method, like chromatography, appears to be suitable only for simple predator-prey interactions, where the potential for finding a unique 'fingerprint' band for each prey species is substantial. It is also the most frequently

used technique for locating potential species specific protein bands for serological diet analysis (Sunderland 1988).

3.2 Serological techniques

Serological techniques may be defined as "analytical techniques using antibodies... for the selective determination of sample components" (Hage 1993, p420). In principal, each prey species comprises several chemical substances (antigens), some of which will be unique to that species. Antisera raised in mammals (usually mice or rabbits) against species-specific antigens may then be applied in serological methods to detect the presence of those unique antigens in the gut contents of predators. The specificity of antibody and antigen is based on the unique molecular configurations of the antigenic determinants which comprise a short section of each protein (Monroe 1984). Good reviews encompassing preparation of antigen, production of antiserum and methods of storing the gut contents of predators have been compiled by Boreham & Ohiagu (1978) and Washino & Tempelis (1983).

Serology is a useful tool for dietary analyses for several reasons. The relationship between antibody and antigen is highly specific (Calver 1984, Monroe 1984), extremely sensitive (An *et al.* 1990), and there is no interference with the natural environment prior to sampling (Davies 1969). Furthermore, these tests often require little apparatus, reagents are easily prepared and procedures and interpretation of results are simple (Washino & Tempelis 1983). Problems associated with serological approaches include antibody specificity (*i.e.* quality, Feller 1992), cross-reactivities (where an antibody recognises an antigen different from the one against which it was raised), and the assumptions necessary for determining quantities of prey consumed (Boreham & Ohiagu 1978, Sunderland 1988).

Serological approaches have been used to facilitate diet analysis, and can conveniently be divided into precipitation tests (where a positive result is indicated by the formation of a precipitate) and non-precipitation tests (where a positive result is usually observed as a colour change in a microwell plate or on nitrocellulose paper). Non-precipitation tests include radioimmunoassays, enzyme immunoassays and

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fluorescence immunoassays. These are more sensitive analyses than precipitation tests, often by up to three orders of magnitude (Washino & Tempelis 1983). An additional advantage of non-precipitation tests is that quantification of different prey items in the diet can be measured (Van Emon & Lopez-Avilla 1992).

3.2.1 Precipitation tests

Essentially two types of precipitation tests have been used in diet analyses (Davies 1969, Boreham & Ohiagu 1978, Calver 1984, Sunderland 1988) and both are attractive to ecologists because of their simplicity (Davies 1969). In principal, when a soluble antigen and its specific antibody combine, a visible precipitate forms (Boreham & Ohiagu 1978). These tests may be carried out in liquids or in gels and because they are based on serology, they are specific, highly sensitive and cross-reactions can be reduced.

The first test, the capillary ring test (= Ascoli test) consists of carefully pipetting the antigen onto a layer of antiserum in a test-tube, ensuring that no mixing takes place (Ascoli 1902, as cited by Healy & Cross 1975). If the antigen and antiserum match, a line of precipitate forms at the interface. This technique has been used successfully in the analysis of the natural prey of three species of *Anthocoris* (Heteroptera), which feed on broom (Dempster 1963, 1964).

The second, and by far the most widely used precipitation test, is carried out either in a test-tube (Oakley-Fulthorpe test, Boreham & Ohiagu 1978, Sunderland 1988) or on a microscope slide (Ouchterlony test, Sunderland 1988), and involves the migration of antigen and antibody towards each other in a gel matrix, usually agar. Titova (1970) employed both this method and the Ascoli test to examine the predator-prey interactions between the pest species *Eurygaster integriceps* and several predatory arthropods. Pickavance (1970, 1971) used it to determine the diet of planarians. More complex predator-prey systems have also been elucidated. Also working on planarians, Boddington & Mettrick (1974) examined the feeding habits of an immigrant species, *Dugesia polychroa*, in Toronto Harbour. Young (1973) examined the prey and predators of *Phaenocora typhlops* (another planarian) while

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Feller *et al.* (1979) employed it successfully to analyse the food web of a soft-bottom intertidal community and Lund & Turpin (1977) investigated larval consumption by two ground beetles.

Some drawbacks of this method are the length of time it takes for diffusion to yield a visible precipitation band (Davies 1969; Sunderland 1988), and the volume of antiserum required for each test. These problems may be reduced by using a simplified 'micro'-Ouchterlony, as described by Crowle (1958). Thus, despite historic success, lack of sensitivity, obscurity of some results and time difficulties with this approach (Healy & Cross 1975) have limited the application of precipitation tests. However, the biggest drawback will remain the lack of quantitativeness of this method.

3.2.2 Non-precipitation tests

3.2.2.1 Radioimmunoassays and immunofluorescence

Both of these techniques are based on the ELISA principal (4.2.2.2), but instead of using an enzyme bound to an antibody to change the colour of a substrate, detection of antigen is carried out by either radio-labelled or fluorescent antisera. Radioimmunoassays are based on the analysis of radio-labelled antiserum and comprise a high proportion of current immunoassays (Hage 1993), despite the drawbacks highlighted by Voller & Bidwell (1986), which include the short shelf life of the isotopes used and administrative inconveniences. Non-isotopic immunoassays are more common in ecological literature. In the fluorescence immunoassay, antibodies are labelled with organic compounds such as fluorescein (Hage 1993) and bound to the antibodies by glutaraldehyde (Monroe 1984). However, the need for trained staff and the subjective nature of analysis have limited the use of fluorescence immunoassays (Voller & Bidwell 1986). Campbell *et al.* (1983) used an immunofluorescent technique to identify marine chroococcoid cyanobacteria in water samples and to provide information on the seasonal variations of different bacterial strains in a temperate zone estuary. The same method was used by Shapiro *et al.* (1989) to assay small eukaryotes in sea water samples without the need to resort to electron microscopy which often distorts cells during preparation, thus

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hampering identification. A recent commercial application involved the examination of brewing yeast for microbial contaminants which could result in product spoilage (Whiting *et al.* 1992).

3.2.2.2 ELISA and related techniques

The idea of coupling enzymes to antibodies or antigens is a relatively new, but popular technique (Washino & Tempelis 1983, Hage 1993). This approach is typically termed an Enzyme Linked Immunosorbent Assay (ELISA), and although several varieties exist (see Voller *et al.* 1979), the basic principal is that enzymes are linked to antibodies, such that the resulting conjugate retains both enzymatic and immunological activity (Sunderland 1988). This became practical when gluteraldehyde was used in 1969 to bind enzymes to antibodies (Monroe 1984). The most common enzymes currently utilised are horseradish peroxidase and alkaline phosphatase (Hage 1993). These enzymes catalyse specific reactions, which when monitored (usually through a substrate colour change), provide an indication of the abundance of that particular antibody, and thus of the antigen. The reactions can be conducted either in microwell ELISA plates or on Nitrocellulose paper, where the catalysed reaction usually results in the formation of a staining or fluorescent precipitate (essentially a Western-Blot). ELISA's are extremely sensitive (Sunderland 1988), quantitative (Engvall & Perlmann 1972, Hentschel & Feller 1990, Ohman *et al.* 1991), and are easily automated (Washino & Tempelis 1983). However, the drawbacks are similar to those already described for serological techniques, namely antibody specificity and cross-reactivities.

Animal diets and predator-prey relationships have been investigated with ELISA. Crook & Sunderland (1984) made use of a sandwich ELISA to detect and quantify the remains of several species of aphids in the gut contents of predatory insects and spiders. The food-chain links between euphausiids, larval anchovy (*Engraulis mordax*) and ciliate protists have also been resolved using this approach (Theilacker *et al.* 1986, 1993, Ohman *et al.* 1991). Antisera raised against a single prey species have successfully been utilised to identify that prey in the stomach contents of several predators using both the ELISA (Arnold *et al.* 1996) and

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immunoelectroosmophoresis approaches (Healy & Cross 1975, Schultz & Clarke 1995). Furthermore, the detection of multiple, phylogenetically closely related prey species in the diet of a single predator, *Octopus vulgaris*, has been achieved (Grisley & Boyle 1988).

There are several other applications for ELISAs. For example, ELISAs have been commercially developed to analyse food for the presence of Salmonella (Emsweiller-Rose *et al.* 1984, D'Aoust & Sewell 1986). The advantage of this technique relative to traditional methods is a shorter analysis time. Detection of species composition of food products is receiving ongoing attention (Sotelo *et al.* 1993, Berger *et al.* 1988). On the medical front, Cousland & Poxton (1984) described an ELISA to examine the surface antigens of *Bacteroides fragilis*, a frequent pathogen in wound infections. In water quality analysis, the presence of harmful bacteria (Fuhrman & Wollum 1985, Tamplin *et al.* 1991) and environmental contaminants (Thurman *et al.* 1990, Van Emon & Lopez-Avilla 1992) can both be detected using an ELISA. Taxonomic analysis has also been undertaken - Kirby & Rybicki (1986) considered taxonomic relationships between 14 *Streptomyces* strains, finding this method quicker, less subjective and more quantitative than Ouchterlony assays.

Thus, although ELISA may be limited by the quality of the available antiserum, it is a sensitive, rapid, quantitative and easily automated approach to diet analysis. Overall it is potentially the most efficient of the non-visual techniques.

4. CONCLUSIONS AND RECOMMENDATIONS

To achieve accuracy in assessing the diet of *J. lalandii* requires a novel approach. A summary of the techniques discussed here and their major advantages and associated problems are given in Table 1.1. Visual methods were ruled out largely due to difficulties in identifying prey and because they tend to be non-quantitative, and while radioimmunoassays are highly quantitative, they are expensive and insensitive when compared to ELISA. Even with the limitations described for visual techniques, they do have a few advantages: provided the prey is not reduced to an unrecognisable

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Table 1.1: A list of the diet analyses methods reviewed, their disadvantages and advantages.

Method	Advantages	Disadvantages
Visual analysis	Low time Low costs Can be quantitative Needs no pre-assumption about which species may be prey	Inaccurate Unable to detect 'soft' prey Biased by operator experience
Isotopes	Accurate Can determine paleodiet	Expensive Difficult to control Not quantitative
Chromatograhy	Can be acurate Simple technique	Suitable only for simple systems Time consuming Not quantitative
Electrophoresis	Inexpensive Rapid	Suitable only for simple systems Not quantitative
Precipitation tests	Simple	Need a large volume of antisera Only semi-quantitative Time consuming
Radioimmunoassays	Accurate Quantitative	Requires well trained personnel Reagents have a short shelf life Isotopes expensive
ELISA	Sensitive Accurate Quantitative Rapid once established	Reasonably expensive Initial setup labour intensive

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mass, all prey items can be detected and identified. Serological techniques overcome the problem of unrecognisable prey, but only if antisera have been developed to allow their detection. It is often impossible to do this for **all** prey available in the environment. The two techniques are thus complementary and both were pursued in assessing the diet of *J. lalandii*.

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Chapter 2

The application of an immunological technique to determining the diet of the West Coast rock lobster, *Jasus lalandii*.

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1. INTRODUCTION

Detection and quantification of predator-prey relationships (Pickavance 1971, Healy & Cross 1975, Emswiller-Rose *et al.* 1984, Sunderland 1988, Ohman *et al.* 1991, Theilacker *et al.* 1993), as well as information pertaining to an organisms' nutritional requirements (Williams 1981), are facilitated by a knowledge of their diet. Accurate analyses of diet are never simple. As highlighted in the previous chapter, despite several visual assessments of the diet of the West Coast rock lobster, there is still considerable disagreement about the diet of this species (Heydorn 1969, Newman & Pollock 1974b, Pollock 1979, Barkai & Branch 1988a, Barkai *et al.* 1996). While several studies have suggested that mussels are the prime food source (*e.g.* Heydorn 1969, Newman & Pollock 1974b, Pollock 1979), other studies have shown lobsters to be opportunistic when mussels are scarce (Barkai & Branch 1988a).

Visual techniques have become the traditional method of diet analysis for rock-lobsters (Berg 1979, Dickman & Huang 1988, Medellin 1988), despite their having numerous drawbacks, which are further compounded in the Decapoda because of the crushing activity of the mouthparts and gastric mill ossicles (Williams 1981). The identification of prey items with no hard body components, such as Cnidaria, is almost totally excluded (Calver 1984, Young 1973). Even for those prey species which have persistent and identifiable (or diagnostic) hard components (Elner & Campbell 1987), analysis is biased both by the varying rates of digestion and egestion of these components (Corbett 1989) and the level of expertise of the analyst (Sunderland 1988), to such an extent that sometimes even qualitative prey identification can be precluded (Feller & Ferguson 1988). Given the limitations of these visual methods, alternative methods of diet analysis urgently require consideration.

Non-visual methods of diet analysis have the potential to circumvent most, if not all of the problems highlighted above. They allow for the identification of prey which have no hard or diagnostic body components (Davies 1969), but perhaps their most important advantage is the alleviation of operator bias as the techniques are all objective (Sunderland 1988). Additionally, non-visual techniques have been suggested to be both time and cost efficient (D'Aoust & Sewell 1986), as well as having the potential for untrained personnel to

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process large numbers of samples with little or simple apparatus (Crook & Sunderland 1984).

Serological techniques, and in particular enzyme-linked immunosorbent assays (ELISA), are amongst the most useful of the non-visual techniques. They have several advantages in that they tend to be both highly specific and extremely sensitive (Calver 1984, Monroe 1984). Furthermore, they are quantitative (Engvall & Perlman 1972) and easily automated (Washino & Tempelis 1983).

Most dietary studies that have employed ELISAs have been restricted to simple predator-prey relationships. To my knowledge, only Feller *et al.* (1979) and Grisley & Boyle (1988) have considered predators with more than one potential prey species, and both papers reported successful prey species identification and differentiation. Given their success, and the specificity of antibodies to their antigenic determinants (Monroe 1984), the original aims of this chapter were to (1) develop and apply a serological technique, the Enzyme Linked Immunosorbant Assay (ELISA), to determine the diet of the West Coast rock lobster *Jasus lalandii* and (2) to compare the results of the this technique with those obtained using the more traditional visual analysis approach. In the final event, unexpected difficulties precluded success, but the reasons for this are of interest in their own right. A central flow of this chapter is thus the achievements and the limitations of this serological technique.

2. MATERIALS AND METHODS

All immunological work was carried out in the Department of Immunology at the University of Cape Town's Medical School. The procedure is a complex one involving many steps. For clarity, these are summarised in Figure 2.1 which outlines the sequential stages.

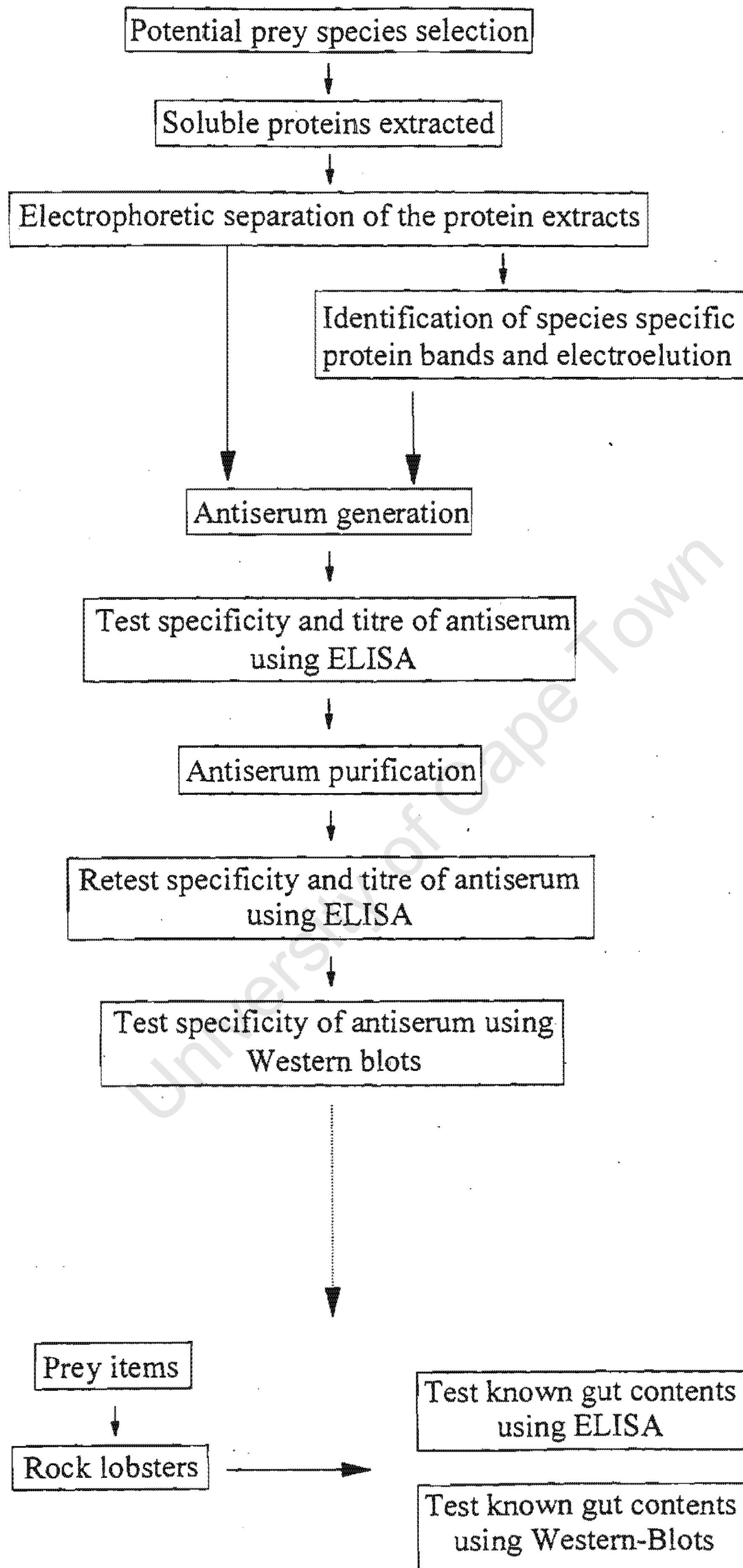

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Figure 2.1: A flow chart to illustrate the process carried out from acquisition of prey items to the testing of known gut contents.

2.1 Prey item collection

The choice of the potential rock-lobster prey items used in this study for the production of antisera was based on previous published dietary information (Heydorn 1969, Newman & Pollock 1974b, Pollock 1979, Barkai & Branch 1988a, Barkai *et al.* 1996), and a consideration of the dominant species present in inshore West Coast benthic communities which could be available to *J. lalandii*. Twenty-six potential prey items, ranging across six phyla from sponges to arthropods were selected (Table 2.1). Of these, less than half had been previously recorded as components of the stomach contents of rock lobsters, but the remaining species were abundant and potential prey that were suspected as having been overlooked in previous (visual) assessments of diet.

Potential rock-lobster prey items were collected by SCUBA diving on the West Coast of South Africa. Specimens were held in filtered sea water in constant temperature aquaria (16°C, 12h:12h light:dark cycle) for 3 days to facilitate gut evacuation for the removal of extraneous proteins (Leone 1947). They were then quickly frozen, finely chopped, hand-homogenised and mixed with phosphate buffered saline (PBS, 1.4M NaCl, 10mM $Na_2HPO_4 \cdot 2H_2O$, 3mM KCl, 2mM KH_2PO_4, pH = 7.4) at a ratio of 1:5 (v/v). These solutions were tumbled overnight at 4°C, and then centrifuged at 12,000rpm for 15 minutes at 4°C. The supernatant was retained, and the protein concentration determined using a Protein Assay (Pierce kit BCA 23225) against a Bovine Serum Albumin Fraction 5 (BSA) standard. Samples were aliquoted and stored at -20°C.

2.2 Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis (SDS-Page)

SDS-Page was carried out after An *et al.* (1988) and Wang *et al.* (1992). SDS is an anionic detergent which binds to proteins and gives them a negative charge. Approximately 1.4g of SDS binds to 1g of protein (Reynolds & Tanford 1970), thus charging the proteins according to their weight, ensuring they separate solely according to size when run on polyacrylamide gels (Weber & Osborne 1969). An 11% SDS resolving gel (Heussen & Dowdle 1980) was poured between two glass plates (16cm x 14cm x 1mm), and allowed to polymerise at room temperature. Once set

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Table 2.1: List of the 26 species selected, their common names and concentrations of the immunogens used for injection and antiserum titre. Scientific names are given in full here but have been abbreviated in all subsequent tables, figures and plates.

	Species	Common name	Extract concentration mg/ml	Antiserum dilution (thousands)
Crustacea	*Jasus lalandii*	Cape rock lobster	9.00	1/256
	Plagusia chabrus	Cape rock crab	9.70	1/256
	Notomegabalanus algicola	Barnacle	2.00	1/200
Molluscs	*Aulacomya ater*	Ribbed mussel	4.00	1/512
	Mytilus galloprovincialis	Mediterranean mussel	6.00	1/512
	Choromytilus meridionalis	Black mussel	4.30	1/64
	Patella compressa	Kelp limpet	4.60	1/200
	Patella barbara	Bearded limpet	3.60	1/64
	Oxystele variegata	Periwinkle	0.80	1/128
	Oxystele tigrina	Periwinkle	2.50	1/120
	Turbo sarmaticus	Giant periwinkle	7.00	1/128
	Argobuccinum pustulosum	Whelk	2.00	1/256
	Burnupena papyracea	Whelk	2.60	1/128
	Burnupena cincta	Whelk	4.90	1/512
	Haliotis midae	Abalone	5.00	1/64
Echinoderms	*Parechinus angulosus*	Sea urchin	5.30	1/256
	Marthasterias glacialis	Starfish	10.70	1/128
	Henricia ornata	Starfish	5.00	1/10
	Ophioderma wahlbergi	Brittlestar	1.00	1/50
	Patiria granifera	Starfish	6.60	*
Sponges	*Leucosolenia sp.*	Sponge	1.00	1/50
	Polymastia mammilliaris	Sponge	5.00	1/120
	Hymeniacedon perlevis	Sponge	4.70	1/128
Cnidaria	*Pseudactinia varia*	Sea anemone	3.50	1/1024
	Bunodactis reynaudi	Sea anemone	5.40	1/256
Kelp	*Laminaria pallida*	Kelp	1.00	1/64
	Ecklonia maxima	Kelp	3.00	1/32
Purified extracts	*Choromytilus meridionalis*	Black mussel	<1	1/5
	Aulacomya ater	Ribbed mussel	<1	1/8
	Mytilus galloprovincialis	Mediterranean mussel	<1	1/32
	Rock lobster gut lining		12.40	1/64

* denotes no usable antiserum obtained

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(approximately 90 minutes), a 4% SDS stacking gel with castellated wells was carefully cast on top of the separation gel (Heussen & Dowdle 1980). Each lane was loaded with 50μl of sample at a concentration of $1.0mg.ml^{-1}$ in reducing sample buffer. One lane was always reserved for a molecular weight marker (BDH Electran MW marker (#44264 2L) consisting of Cytochrome C (12.3kD), Myoglobin (17.2kDa), Carbonic Anhydrase (30.0kDa), Ovalbumin (42.7kDa), Albumin (66.2kDa) and Ovotransferrin (77.0kDa)). Electrophoresis was done at 4°C in a vertical slab unit. A current of 4mA was applied across the gel until the tracking dye front had entered the resolving gel, after which it was increased to 8mA. Once the tracking dye had reached the bottom of the resolving gel, the gel was removed and stained with Coomassie Blue (0.05% w/v) in destain solution (30%Ethanol, 10% Acetic acid, 60% Water) for 20 minutes. Thereafter the gel was removed from the stain solution and washed overnight in 10 volumes of destain solution.

2.3 Electroelution

For six closely related potential prey species whose incubation could have led to the raising of cross-reacting antibodies, species-specific protein bands were identified from the Coomassie-stained SDS-Page gels. Large quantities of these protein bands were obtained by running thick (3mm) gels as described above. After staining, the protein band was excised from the gel using a sterile scalpel, rinsed overnight in five volumes of distilled water, and then fragmented by using a 5ml syringe to push the gel through a 0.5mm gauge needle. Electroelution of these proteins was carried out in a horizontal electrophoresis chamber after Harlow & Lane (1988). The chamber (1l) was filled with running buffer (0.1M Tris base, 1M Glycine, 1.5mM SDS) with 1ml 0.5M dithiothreitol, and bordered with Spectrapore membrane tubing (5kDa cutoff size). A current of 100mA was applied across the chamber for 2hr, after which the eluted proteins were removed and frozen (-20°C). The remaining gel fragments were re-stained with 0.05% coomassie blue to ensure all proteins had been eluted.

2.4 Immunisation procedure and antiserum generation

All antisera were raised against the prey extracts in 10-week-old female New Zealand albino rabbits following ethics approval from the University of Cape Town. Injection

University of Cape Town

procedures followed that of Harlow and Lane (1988). One millilitre of blood was collected from the marginal ear vein of each rabbit prior to immunisation. The initial injection consisted of 1mg of protein, emulsified with Freund's complete adjuvant (DIFCO Laboratories) to form a total volume of 1ml, divided equally between five subcutaneous injection sites on the dorsal surface (Berger *et al.* 1988). To ensure high titre and affinity from the small immunogen doses available following electroelution, the method described by Vaitukaitis (1981) was followed - 20 dorsal subcutaneous injection sites rather than just 5 were used. After 4 and 7 weeks incubation, 1ml of blood was obtained from each rabbit via the marginal ear vein (Bush & Tai 1994) and tested for antibody titre using the ELISA system described below. Simultaneously, booster injections were administered ($0.5 mg.ml^{-1}$), emulsified in Freund's incomplete adjuvant (DIFCO Laboratories). Terminal bleeds by cardiac puncture (Calver 1984) following administration of an anaesthetic were done by the University of Cape Town's Animal Unit staff 10 weeks after the initial injection. The blood was allowed to clot overnight at 4°C, centrifuged (12,000rpm, 30 minutes, 4°C), and the serum retained, aliquoted and frozen at -20°C (Calver 1984).

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2.5 Enzyme-Linked Immunosorbent Assays (ELISA)

The ELISAs were carried out using Greiner (# 655101) PS-microwell plates following methods described by Voller & Bidwell (1986) and An *et al.* (1990) and were initially used to determine the detection limit or sensitivity (titre) of the antisera. Wells were coated overnight at room temperature with 100µl of prey extracts ($10 \mu g.ml^{-1}$) in PBS. The extracts were then discarded and the wells washed three times with PBS-T (PBS with 0.05% Tween 20). 200µl of blocking solution (PBS-T with 4% BSA ($8.4 mg.ml^{-1}$)) was then added to each well and left for 45 minutes at room temperature. Wells were then washed as before. The first antibody, (*i.e.* the antiserum from the rabbit) was then added to duplicate wells in serial dilution from 1/2,000 to 1/1,024,000 at $100 \mu l.well^{-1}$ and incubated at room temperature for 90 minutes, after which the wells were washed as above. 100µl of the second antibody (Biotinylated, affinity isolated goat anti-rabbit antiserum, DAKO, Lot: 014) was then added to each well at a dilution of 1/5,000 in PBS for a 30 minute incubation time at room temperature. Following this incubation, wells were washed as described above. The

penultimate incubation was with Horse-radish Peroxidase (HRP)-conjugated streptavidin (DAKO, Lot: P397) for 30 minutes at room temperature (dilution 1/10,000 in PBS; 100µl.well^{-1}). Wells were then washed at least 10 times with PBS-T. Finally a colourimetric substrate, 2,2-azino-di-(3 ethylbenzthiazoline sulfonic acid) (ABTS, 100µl, 20mg.ml^{-1}) in 10ml Citrate buffer (0.1g.ml^{-1} citric acid, pH = 4.0) with 10µl hydrogen peroxide (H_2O_2) was added at 100µl.well^{-1} for 30 minutes at room temperature. Absorbance (optical density) of the green substrate was read at 405nm using an ELISA plate reader (Organon Teknika Microwell System). From this optical density reading, the dilution of antiserum which gave an optical density reading of 1 after 30 minutes incubation with ABTS was determined. On all plates four negative and two positive control wells were also run. Two wells were incubated with normal rabbit serum (pre-antigen-incubation serum) as the first antibody, and two wells with no first antibody. The positive control consisted of the second antibody (goat anti-rabbit) being bound to the ELISA plate well overnight (step 1).

2.6 Specificity testing and antiserum purification

The specificity of each antiserum was tested by incubating each potential prey item extract with each antiserum produced (at the antiserum dilution already determined to give an absorbance reading of 1 after 30 minutes incubation with ABTS) using the ELISA system described above. Antisera were considered cross-reacting to antigens other than the one against which they were raised when the optical density reading was 3 times higher than the negative controls.

Following identification of the cross-reactions, 20 cross-reacting pairs of antibodies for which it was considered important to eliminate the cross-reaction were selected. A pre-absorbance procedure, described below, was used to reduce cross-reactivities (after Ausubel *et al.* 1994). 25ml Sepharose CL-4B slurry (Pharmacia) was washed in a sintered glass funnel with 10 volumes of distilled water. The beads were transferred to a polypropylene tube and resuspended in 25ml 0.2M Na_2CO_3. CnBr (0.5g) was dissolved in 1ml acetonitrile and while stirring slowly, added dropwise to the beads over one minute. The slurry was stirred for another seven minutes, transferred to a sintered glass funnel and washed first with 10 volumes of ice-cold 1mM HCl and then

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two volumes of ice-cold 0.1mM HCl. The final wash was done with two volumes of coupling buffer (0.1M $NaHCO_3$, 0.5M NaCl, pH = 8.3). The beads were then transferred immediately to an equal volume of coupling buffer containing the antigen ($10mg.ml^{-1}$) to be coupled. Tubes were tumbled for 2hr at room temperature, then centrifuged (8,000rpm) for five minutes and the supernatant discarded. The sepharose beads were then quenched by blocking with Glycine buffer (50mM Glycine.HCl, 0.5M NaCl, pH = 2.5) for 2hr at room temperature. The tubes were again centrifuged for five minutes (8,000rpm), the supernatant discarded and the beads equilibrated in PBS.

Antisera containing cross-reacting antibodies were pre-incubated with an equal volume of sepharose beads bound with those antigens to which they cross-reacted. Antisera and antigens were allowed to react at room temperature for 30 minutes. The tubes were then centrifuged for five minutes (8,000rpm) and the supernatant retained. The resultant 'purified' antiserum was retested for titre and specificity as described above, and the operating dilution adjusted as necessary.

University of Cape Town

2.7 Western-Blotting

Western-Blots were carried out after Towbin *et al.* (1979) and Wang *et al.* (1992) to check banding patterns of antisera binding to prey species extracts. SDS-Page gels were performed as described above and the proteins transferred electrophoretically (1hr, 100mv) from the gel to nitrocellulose paper (Hybond-C, 0.45µm, AMERSHAM) in ice-cold transfer buffer (25mM Tris, 200mM glycine and 20% (v/v) methanol, pH = 8.3). After protein transfer, the blot was dried to ensure protein adhesion, then re-wet with methanol and washed three times with PBS-T. Thereafter it was blocked using 1% PVP (Polyvinylpyrrolidone, 360 kDa) in PBS-T for 40 minutes at room temperature. The blot was washed again as above, and antiserum applied for two hours (1/2,000 dilution) at room temperature. The blot was then washed as above, and incubated for 30 minutes at room temperature with biotinylated goat anti-rabbit antiserum at a dilution of 1/4,000. Thereafter the blot was washed again and then incubated with a 1/5,000 dilution of HRP-conjugated streptavidin for 30 minutes at room temperature. Subsequently, the blot was washed 10 times as above, allowed to

wash overnight and developed with Chemiluminescence substrate which generates a luminescent signal with HRP activity. Exposure of the blots to X-ray film produces a hard copy.

2.8 Protein gels

An SDS gel containing 1% w/v gelatin was run as described above to test for rock-lobster digestive enzyme activity in the digested prey extracts. After the gel had been run to completion, it was allowed to develop overnight in PBS at room temperature, and then stained with 4M sodium acetate stain and viewed against a dark background (Higgins & Dahmus 1979). The positive control used was the enzyme collagenase (*Clostridium histolyticum*).

University of Cape Town

2.9 Testing animals fed known diets

Male West Coast rock lobsters (*Jasus lalandii*, 60-70mm carapace length) were obtained from baited long-line lobster traps off Cape Town and maintained in constant temperature aquaria (16°C, 12h:12h light:dark cycle). The rock lobsters were offered pilchards (*Sardinops sagax*) on a daily basis. Prior to being offered food test samples, lobsters were starved for three days to allow complete gut evacuation (Zoutendyk 1988a) and then offered single prey items, for a digestion period of less than two hours (Schultz & Clarke 1995). Rock lobsters were exsanguinated following submersion in an ice-water slush to anaesthetise them. Stomachs were removed, the contents flushed into a test tube, and soluble proteins extracted overnight in PBS as described for the prey items above (2.1). Gut samples of rock lobsters fed known prey were then tested using the ELISA system.

3. RESULTS

3.1 Prey items

Twenty-six potential prey species were selected and collected. Of these, four were echinoderms, there were two each of crustaceans, cnidarians and kelp, three species of sponges and the remainder were molluscs (Table 2.1). The soluble protein extract concentrations prepared in PBS ranged between ~0.5mg.ml^{-1} and 12.4mg.ml^{-1}, with an

average of 4.7mg.ml^{-1} (Table 2.1). For each potential prey species, the soluble proteins separated out on the SDS-Page gels with unique banding patterns, as exemplified for seven of the species in Plate 2.1. Similar SDS-page gels were run for all 26 potential prey species, and species-specific bands identified in this way. The species-specific bands for production of specific antibodies were selected for just six potential prey species as being 54kDa & 27kDa (for the whelk, *B. cincta*), 60kDa (ribbed mussel, *A. ater*), 40kDa (Mediterranean mussel, *M. galloprovincialis*), 29kDa (kelp limpet, *P. compressa*), 47kDa (black mussel *C. meridionalis*) and 20kDa (bearded limpet, *P. barbara*).

3.2 ELISA

The antiserum titres for all potential prey species in the rabbits increased over time in a similar pattern to that shown by way of illustration for *C. meridionalis* antiserum development (Figure 2.2). All titre curves were negatively sigmoidal in shape, and linear when intersecting the Y = 1 line, except for four weeks incubation which did not reach the Y = 1 line. Final 10-week titres ranged between 1/10,000 and 1/1,024,000 for the crude extract incubations (see Figure 2.3 for examples and Table 2.1 for a complete list). Usable antisera when rabbits were injected with the purified protein extracts were only obtained for the three mussel species. The titre of these 'specific' antisera ranged between 1/5,000 and 1/ 32,000 (Table 2.1). Proteins from the three whelk and two limpet species induced no suitable antiserum production.

3.3 Cross-reactivity

Cross-reactions were observed in 43 (6.1%) of the 702 possible interactions between antisera and undigested antigens (27 antisera times 27 extract antigens minus 27 self reactions) when testing for antiserum specificity. Thirty-eight (88%) of the 43 cross-reactions were between the molluscs (Table 2.2). Twenty of these cross-reactions were selected as being important for resolving. The cross-reactions between the two mussel species most likely to be preyed upon were reduced using the 'specific' antiserum (Table 2.3), while other cross-reacting antibodies were resorbed by incubation using the absorption process described above. Of these 20 cross-reactions, 17 were completely eliminated, and three not reduced at all. Total cross-reactivity was reduced to less than 4%.

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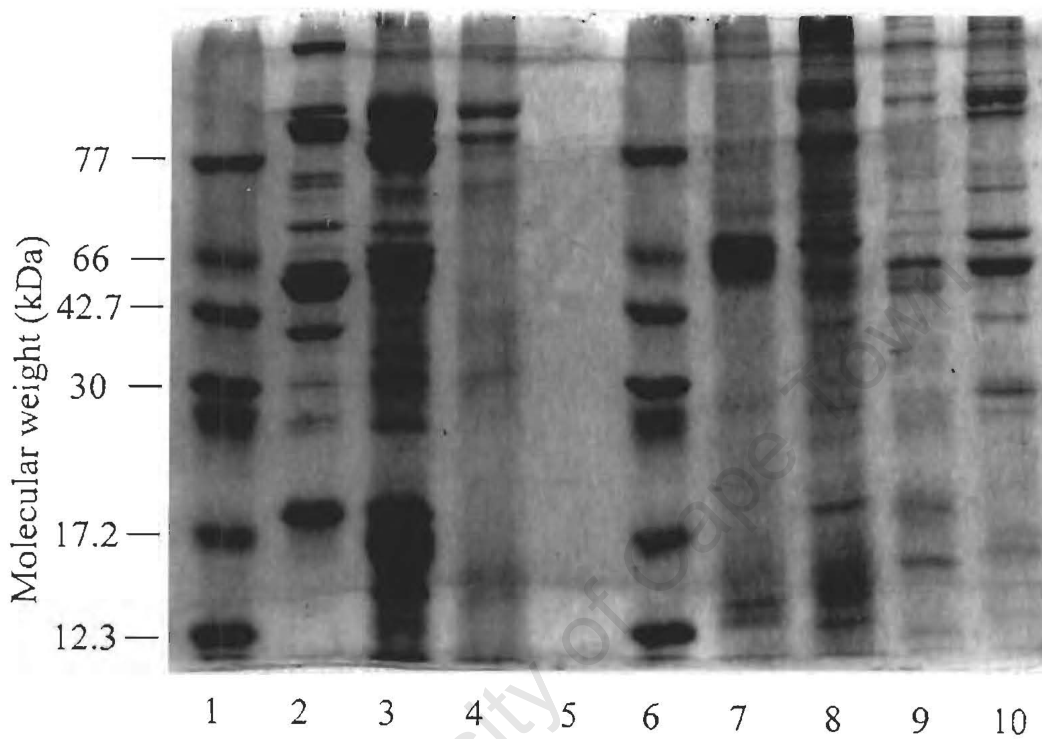

Plate 2.1: SDS gel stained with 1% coomassie blue to show the banding pattern of eight of the potential prey items. Lanes 1 and 6 are molecular weight markers. The rest of the lanes are 2: *J. lalandii*, 3: *P. chabrus*, 4: *N. algicola*, 7: *A. ater*, 8: *C. meridionalis*, 9: *H. Midae* and 10: *P. compressa*.

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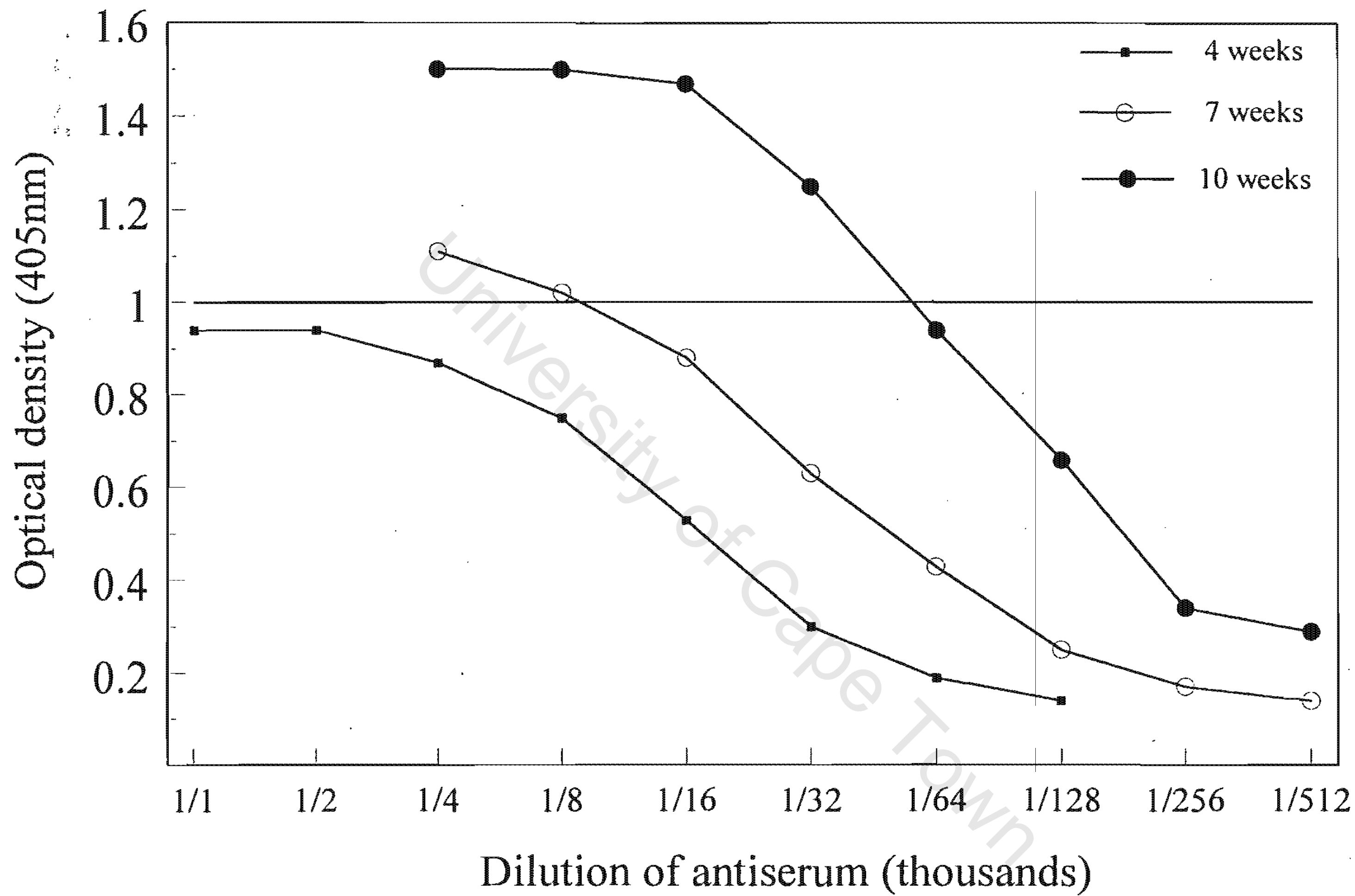

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Figure 2.2: Absorbance values (at 405nm) observed in the ELISA wells following a 30 minute incubation with ABTS for varying dilutions of *C. meridionalis* antiserum at 4, 7 and 10 weeks following the initial injection of immunogen.

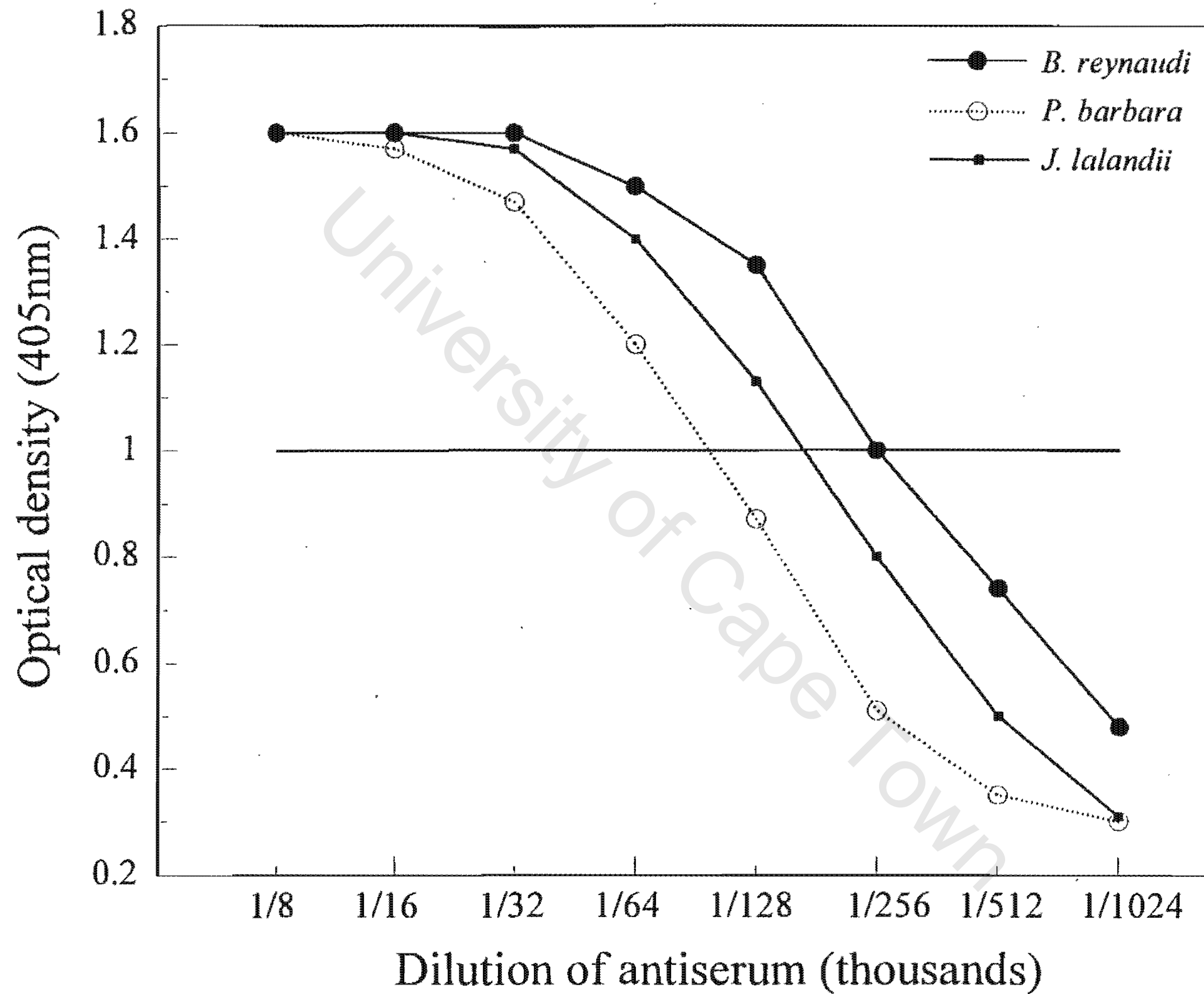

Figure 2.3: Absorbance values (at 405nm) observed in the ELISA wells following a 30 minute incubation with ABTS at varying dilutions of three antisera after 10 weeks incubation in the rabbit.

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Table 2.2: List of the cross-reactivities observed when the different antisera (top row) were incubated against the different (undigested) potential prey items (left hand column) to test for antiserum specificity using the ELISA. Clear cells indicated no cross-reactivity. All shaded cells indicate a strong binding between antiserum and antigen. Those blocks in the diagonal are self-reactions. All other shaded blocks are cross-reactivities. Dotted blocks refer to cross reactivities that were eliminated while blocks with diagonal lines indicate cross-reactivities we tried to reduce, but failed. No attempt was made to eliminate the cross-reactivities shaded black.

	Crustacea				Molluscs												Echinoderms				Sponges			Cnidaria		Kelp	
	J. lalandii	Gut lining	*P. chabrus*	*N. algicola*	*A. ater*	*C. meridionalis*	*M. galloprovincialis*	*P. compressa*	*P. barbara*	*O. variegata*	*O. tigrina*	*T. sarmaticus*	*A. pustulosum*	*B. papyracea*	*B. cincta*	*H. midae*	*P. angulosus*	*M. glacialis*	*H. ornata*	*O. wahlbergi*	*Leucosolenia spp.*	*P. mammiliaris*	*H. perlevis*	*P. varia*	*B. reynaudi*	*L. pallida*	*E. maxima*
J. lalandii	■	▨																									
Gut lining		■	░															░									
P. chabrus		▨	■																								
N. algicola				■																							
A. ater					■	░					░		░														
C. meridionalis					░	■			░		░			░													
M. galloprovincialis					░	░	■				░		░	▨		░		░									
P. compressa								■	■					■		■											
P. barbara								■	■		■			■		■											
O. variegata										■	■		■	■													
O. tigrina										■	■					■											
T. sarmaticus									■		■	■															
A. pustulosum									■				■														
B. papyracea													■	■	■												
B. cincta													■	■	■												
H. midae									■	■	■	■				■											
P. angulosus																	■										
M. glacialis																		■									
H. ornata								░											■								
O. wahlbergi																				■							
Leucosolenia spp.																					■						
P. mammiliaris																						■					
H. perlevis																							■				
P. varia																								■			
B. reynaudi																									■		
L. pallida																										■	
E. maxima																										░	■

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Table 2.3: Absorbance values obtained when different mussel antisera (top row) were incubated with different mussel antigens (first column) using the ELISA. Figures in bold highlight the reduction in cross-reactivity achieved using specific antisera.

Species	*A. ater*	*A. ater* (specific)	*C. meridionalis*	*C. meridionalis* (specific)
A. ater	0.42	0.69	0.31	**0.11**
C. meridionalis	0.64	**0.16**	1.3	0.75

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Antisera subjected to the absorption process had a titre loss between 2.3% and 33% (Table 2.4).

3.4 Digested extracts

Having established the sensitivity and specificity limits of the antisera, the appropriate level for the concentration of antigen for the initial overnight coating of the ELISA plate was determined. All antiserum-antigen interactions tested produced log-phase curves, and the optimal level of antigen concentration for maximal sensitivity and minimum interference was determined to be $5\mu g.ml^{-1}$ (see Figure 2.4 for examples of two species). The absorbance reading for all interactions was, however, much smaller for the digested extracts when compared with non-digested (crude) extracts (Figure 2.4).

More importantly, when 12 different antisera were tested against rock-lobster gut contents from rock-lobsters fed eight different known diets in the laboratory, there was a much higher level of cross-reactivity than that incurred when doing the same test with undigested prey species. Of 88 possible interactions, 43 (49%) cross-reacted (Table 2.5). Some antisera even failed to recognise the digested version of the immunogen against which they were produced, *e.g. P. chabrus* and *N. algicola*. All antisera tested also recognised digested material from at least some species other than the one against which they were generated. Thus, whereas undigested prey could be identified with a high degree of accuracy and with very little cross-reactivity, once digestion had occurred, gut contents with known prey could no longer be distinguished (or even recognised, in some cases).

3.5 Western-Blots

When undigested extracts or digested gut remains of the same prey species were incubated simultaneously with 'their' antiserum using Western-Blots, a much greater binding response was evident on the undigested extract lane when compared with the lane containing digested remains (Plate 2.2). Further, there was a high level of binding by the antiserum generated against the rock-lobster gut lining, with all digested food extracts, while there was a complete lack of binding of this antiserum to undigested extracts (Table 2.2 & Plate 2.3). SDS-Page electrophoresis patterns revealed after coomassie staining, showed that irrespective of the food offered to the lobster, the same protein

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Table 2.4: Pre- and post-adsorption absorbance values from antisera incubated with their immunogen, and the loss of sensitivity recorded.

Antiserum	Pre-adsorption	Post-adsorption	% difference
A. pustulosum	1.30	1.27	2.30
M. glacialis	1.46	1.50	2.60
E. maxima	1.40	1.35	3.50
H. ornata	1.36	1.50	9.30
H. midae	1.22	1.36	10.30
N. algicola	1.21	1.43	15.30
P. chabrus	0.60	0.73	17.80
P. barbara	1.03	1.29	20.10
B. papyracea	1.08	1.50	28.00
O. tigrina	1.27	0.85	33.00

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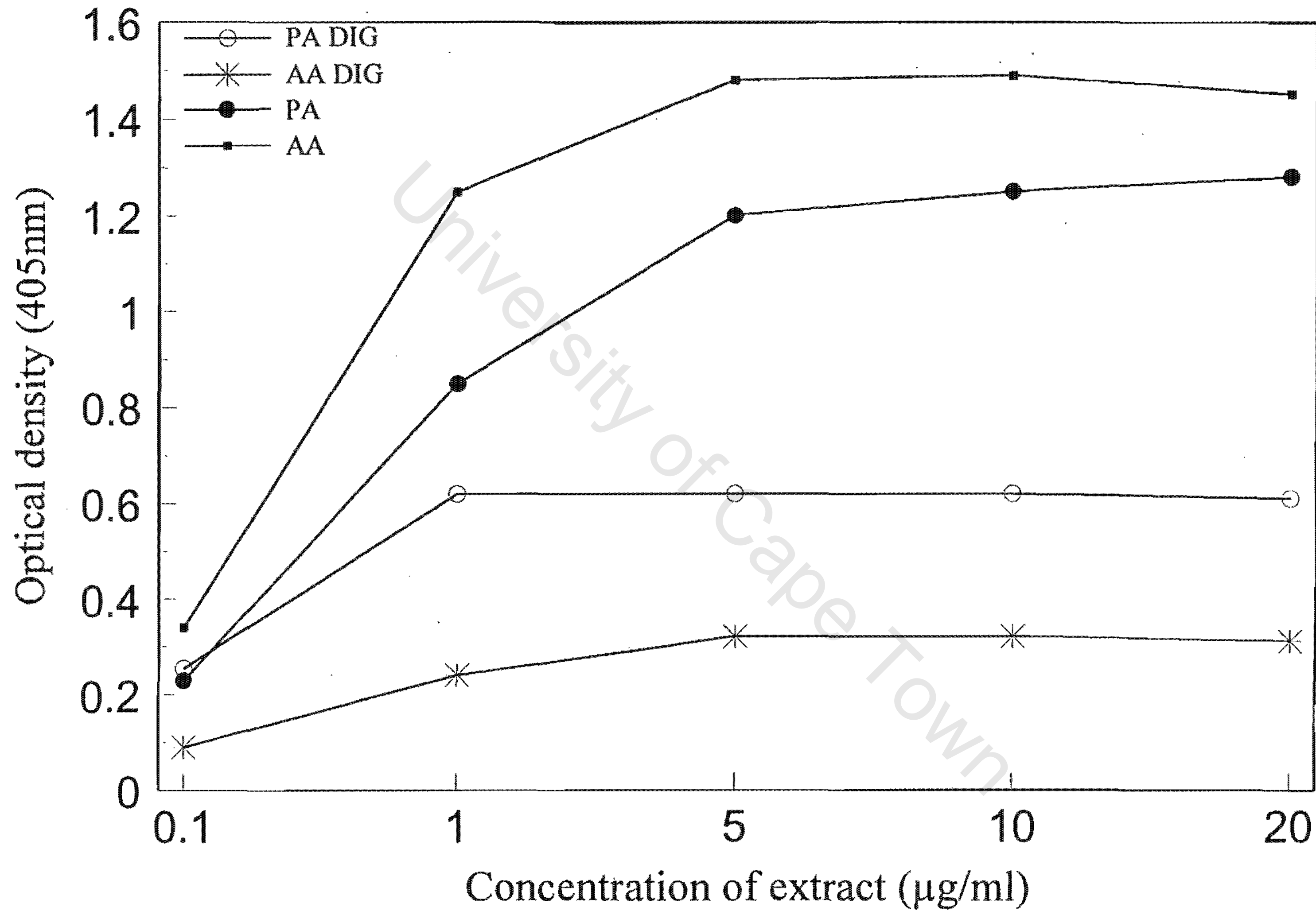

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Figure 2.4: Absorbance values (at 405nm) observed in the ELISA wells following a 30 minute incubation with ABTS for various initial concentrations of the immunogen ($\mu g.ml^{-1}$). PA and AA refer to undigested *Parechinus angulosus* and *Aulacomya ater* and PADIG and AADIG to digested extracts of these respective species.

Table 2.5: Absorbance values obtained from incubating different antisera (top row) with eight different prey items (left hand column) following their digestion by *J. lalandii*. Values in bold are high enough to be considered cross-reactivities. ND = no data. Self-reactions are underlined.

	Antisera											
Extracts	*A. ater*	*C. meridionalis*	*P. angulosus*	*P. chabrus*	*J. lalandii*	*P. compressa*	*N. algicola*	*H. midae*	*T. sarmaticus*	*M. glacialis*	Sponge	Gut lining
A. ater	**0.58**	**0.55**	**0.18**	0.10	**0.18**	0.08	**0.38**	0.14	0.09	0.13	**0.33**	**0.34**
C. meridionalis	**1.32**	1.39	**0.80**	**0.45**	**0.57**	**0.98**	**0.87**	**1.37**	**0.88**	**1.18**	**0.97**	**0.67**
P. angulosus	0.26	0.28	0.42	0.07	0.10	0.09	0.11	0.09	0.09	0.11	0.14	**0.22**
P. chabrus	**0.45**	**0.56**	0.09	0.09	**0.14**	0.07	**0.31**	0.17	0.09	0.08	**0.18**	ND
J. lalandii	0.22	**0.30**	0.10	**0.38**	**0.43**	0.07	**0.60**	0.09	0.08	0.08	0.14	**0.18**
P. compressa	**0.28**	0.25	0.07	0.07	0.10	**0.32**	0.12	**0.50**	0.13	0.07	**0.20**	ND
N. algicola	**0.28**	0.22	0.08	0.06	0.09	0.07	0.12	0.09	0.08	0.07	**0.18**	ND
Fish	**0.31**	**0.33**	**0.19**	0.08	**0.14**	0.08	**0.27**	0.11	0.08	0.08	**0.22**	ND

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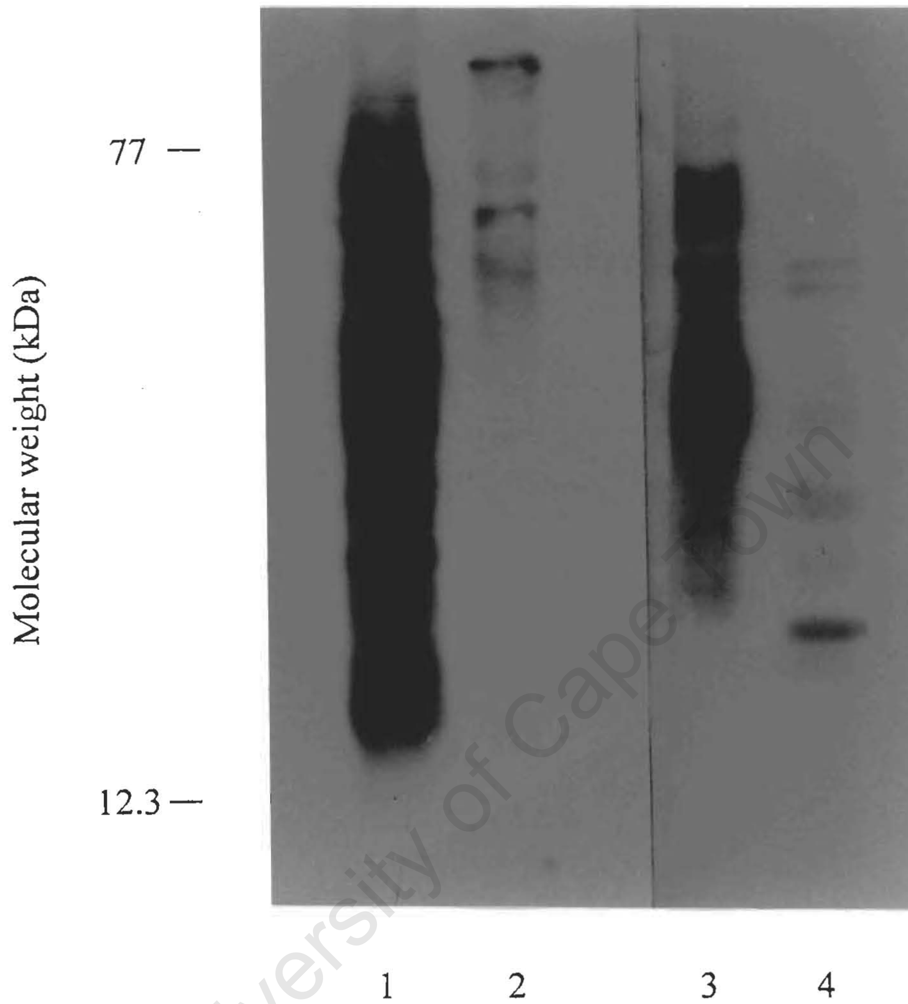

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Plate 2.2: Western-Blot to demonstrate the binding of antisera to digested and undigested prey items. Lanes 1-2 and lanes 3-4 were incubated with antiserum developed against *A. ater* and *N. algicola* respectively. Lanes 1 & 3 were undigested extracts of *A. ater* and *N. algicola* respectively, and lanes 2 & 4 were digested extracts of *A. ater* and *N. algicola* respectively.

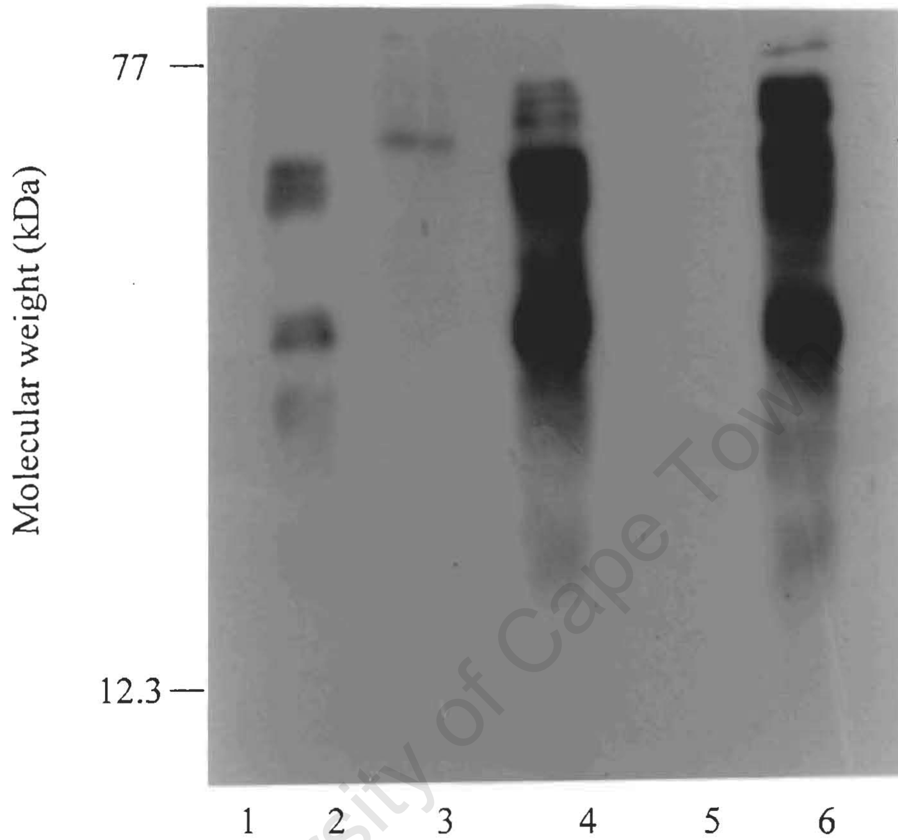

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Plate 2.3: Western-Blot to demonstrate the binding of the antiserum developed against rock lobster gut lining with undigested (lanes 1, 3 & 5) and digested (lanes 2,4 & 6) prey items. Lanes 1 & 2, 3 & 4 and 5 & 6 were *P. angulosus*, *C. meridionalis* and *A. ater* respectively.

banding pattern resulted when the gut content remains were separated on the gel (Plate 2.4). A separate analysis using a protein gel confirmed the presence of proteolytic enzymes (probably rock-lobster in origin) in all the prey extracts obtained from rock-lobster stomachs, because proteins incorporated into the gel were digested at the same molecular weight positions as the suspected rock-lobster enzymes (Plate 2.5).

4. DISCUSSION

Usable antisera were produced against all 27 of the crude prey extracts and three of the six purified immunogens in the rabbits, allowing their specific and unique identification. Negative sigmoidal titre curves were observed for all antibody-antigen interactions, and antiserum titre increased with time after immunisation (Figure 2.2). Only 43 cross-reactions out of a possible 702 interactions between antisera and undigested prey species were recorded - a figure of only 6.1%. Of these, the majority were between the molluscan species (Table 2.2). Cross-reactions between phylogenetically close species were expected due to the similarities in protein structure between animals in taxonomically related groups (Grisley & Boyle 1988). The level of cross-reaction was much less than that reported for various snake venoms (Berger & Bhatti 1989), for antisera raised against *Vibrio* spp. (Mutharia *et al.* 1993), and for cross-reactivities between sera raised against 20 different invertebrate species (59%, Feller *et al.* 1979). It was, however, a lot greater than that observed during studies on the recognition of food types in octopus (Grisley & Boyle 1988). Through absorptive processes and the use of the specific antisera, cross-reactivity was reduced to 4%. An extremely low titre-loss was observed during this purification process, probably due to the high specificity of the developed antisera. Thus, at this stage of the procedure, the ELISA could sensitively and specifically distinguish the (undigested) tissues of virtually all the potential prey species examined.

4.1 Difficulties with the identification of gut contents

Problems arose when the antisera developed were tested against extracts derived from the gut contents of rock lobsters fed a known diet in the laboratory. During these tests, a much higher rate of cross-reactivity was observed between the various antisera used and the digested extracts tested (Table 2.5). Of 88 interactions in the trial, 43 (49%) showed cross-

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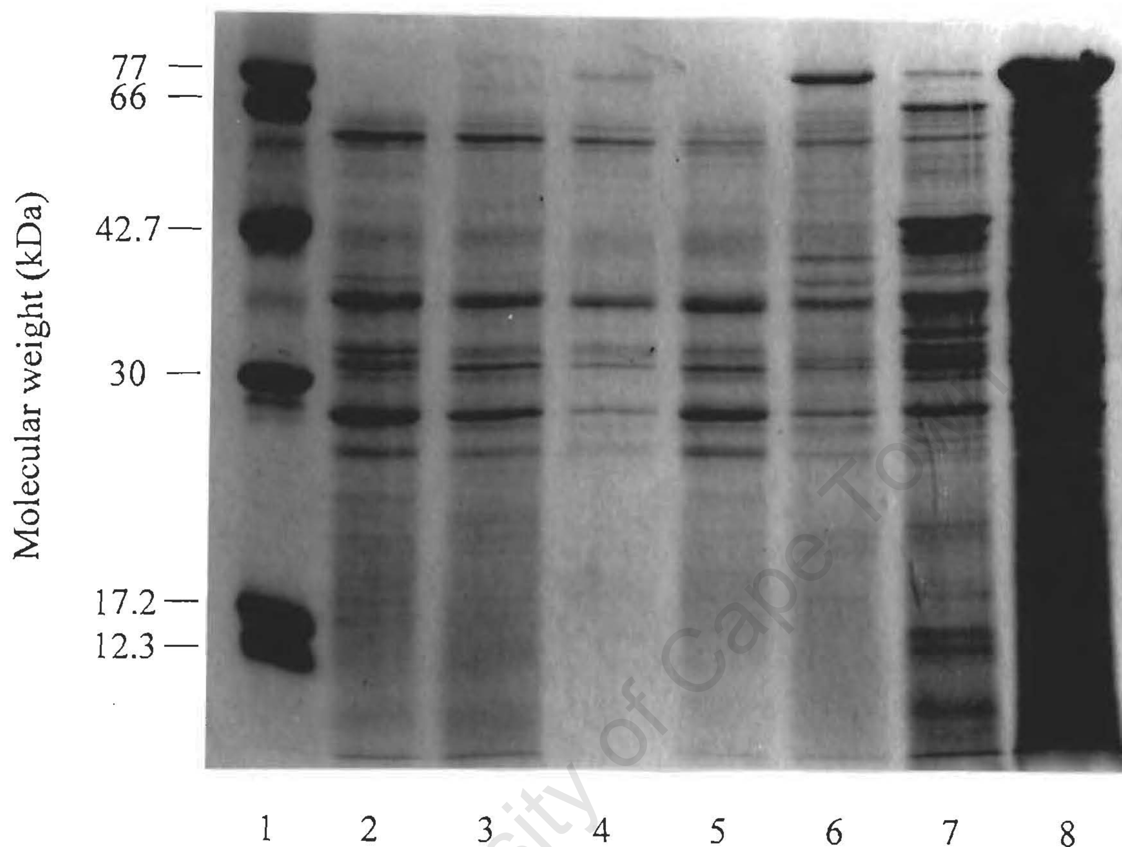

Plate 2.4: SDS gel stained with 1% coomassie blue to show the banding pattern of six prey items following their digestion for 30 minutes by rock lobsters. Lane 1 is a molecular weight marker. Lanes 2-8 are *A. ater*, *C. meridionalis*, *J. lalandii*, *P. angulosus*, *H. midae*, *P. chabrus* and rock lobster digestive gland respectively.

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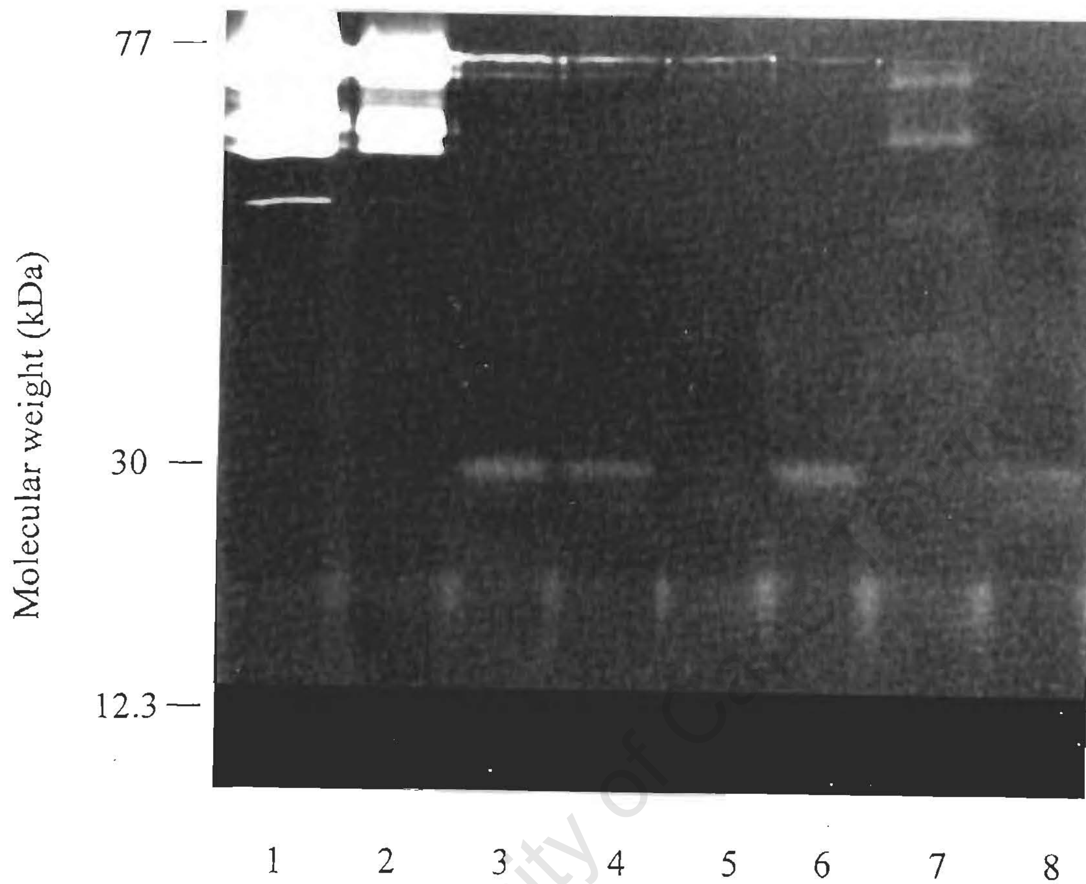

Plate 2.5: 1% gelatin gel stained with sodium acetate to show activity of proteases in the digested extracts. Lanes 1 & 2 are controls (collagenase). Lanes 3-8 are *A. ater, C. meridionalis, J. lalandii, P. angulosus, H. midae* and rock lobster digestive gland respectively.

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reactions, a figure nearly 10 times that obtained from trials with undigested prey items (Table 2.2). Antisera recognised and bound to proteins against which they were not developed, even across different phyla. For example, the antiserum generated against rock-lobster (*J. lalandii*) tissue which recognised no other immunogen in the initial cross-reactivity experiments recognised not only digested *J. lalandii*, but also digested mussel (*A. ater* and *C. meridionalis*), digested rock crab (*P. chabrus*), and digested fish (*S. sagax*) remains, displaying a complete loss of antiserum specificity. Also, antisera developed to detect sea urchin, (*P. angulosus*) remains, which showed no cross-reactivity with undigested material, recognised and bound to proteins from the digested remains of mussels and fish. This was completely unexpected, because of the generally high specificity of antisera (Monroe 1984), upon which the entire principal of prey identification by ELISA is based.

Furthermore, several of the antisera failed to recognise the digested version of the prey item against which they were developed. Two of these were the antisera developed to detect for the presence of rock crab (*P. chabrus*) and barnacles (*N. algicola*, Table 2.5). Thus, it was not even possible to use all the antisera that had been developed to recognise their own immunogens, and certainly not to reliably separate digested material between different species - even those from phylogenetically distant groups. Thus, the antisera displayed a complete lack of specificity when detecting digested material. Schultz & Clarke (1995), using an immunological procedure, found that detection of juvenile red drum remains in predators' stomach contents was not possible after four hours of digestion. That problem is unlikely to be applicable in the present case because the rock lobsters were killed within two hours of consuming the prey offered them and, furthermore, the same complications were experienced even when the rock lobsters were killed within 15 minutes of consuming prey. Thus, digestion of prey proteins is unlikely to have led to the loss of antiserum specificity.

4.2 Identification of the causes of the problem

Given that ELISA has been successfully employed to identify the gut contents of many other predators (*e.g.* Crook & Sunderland 1984, Ohman *et al.* 1991, Arnold *et al.* 1996), various methods were used to try to establish why there were problems with both detection

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and cross-reaction of digested prey items from the guts of *J. lalandii*. Separating proteins extracted from digested material on SDS-gels showed that irrespective of the prey item offered to the lobster and irrespective of the duration of digestion (15 min to 24hr), the same banding pattern was observed on the gels (Plate 2.4). Not only were the banding patterns from all digested species identical, but they were similar to that for rock-lobster digestive gland and rock-lobster gut lining extracts. Although some breakdown products from digestion must have been present in the lanes on the gel, the banding pattern observed suggested that rock-lobster digestive enzymes were, volumetrically, the dominant proteins in the extracts that were obtained from the rock-lobster stomachs and were masking proteins from the prey.

This supposition was confirmed with the results obtained from Western-Blots and the protein gel. The Western-Blots showed strong binding of the antisera to the undigested proteins against which they were developed, but almost no binding to the digested version (Plate 2.2). In contrast, and by way of support, the antiserum developed against rock-lobster gut lining (representative of lobster digestive enzymes) showed high binding with all digested prey extracts, and very little binding to the undigested prey items (Plate 2.3). The antiserum raised against rock-lobster gut lining cross-reacted with all digested prey against which it was tested (Table 2.5), but it cross-reacted only to extracts obtained from one other crustacean when incubated with undigested material (Table 2.2).

The protein gel (Plate 2.5), which consisted of an SDS-Polyacrylamide gel with 1% w/v gelatin, showed that the bands observed on the SDS gel (Plate 2.4) were rock-lobster gut enzymes because they digested the gelatin when incubated overnight in PBS, leaving clear bands when the gel was stained with sodium acetate. The observed activity of these proteins suggested that they could only be enzymes, and their most likely source was the rock-lobster gut contents. Barkai *et al.* (1996) state that they were able to obtain several millilitres of digestive fluid from each rock lobster when they were determining the digestive capabilities of rock lobsters. Relative to the size of rock lobsters, this is an extremely large amount of digestive fluid. Such high amounts of digestive enzymes may allow rock lobsters to feed opportunistically, and digest food rapidly.

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To sum up thus far, it has been shown that the development of antisera to a range of potential prey items is possible, and that in the case of undigested prey proteins they showed high specificity and thus low cross-reactivity (< 4%). However, the same success was not evident when the antisera were tested against the gut contents of rock lobsters fed a known diet. The antisera showed less sensitivity, less specificity and (thus) more cross-reactivity (~50%) than was either expected or predicted. Western-Blot and protein gel techniques suggest that the reason this technique did not work was not because the antibodies fail to recognise the digested prey, but rather that the digestive enzymes of the rock lobster are so abundant that they simply prevent any digested prey proteins which may have been in the extracts from being detected.

4.3 Attempts to resolve the problem

To try to overcome this problem, several other techniques were tried. The first approach was to run the ELISA system using different ELISA plates, and using a different binding buffer ($HC0_3^-$, pH = 9.0). The original experiments had all been conducted in Greiner microwell plates using PBS (pH = 7.4) as the binding buffer. Polysorp (NUNC), Maxisorp (NUNC) and Falcon plates were all tested. None of these plates, nor changing the buffer, made any improvement on the results already discussed above.

The second approach was to develop and test a sandwich ELISA. The principal involved is to bind the antisera produced in the rabbits to the ELISA plate microwells in the first step of the ELISA. In theory, these antibodies then attract and bind to the specific proteins of the prey when they are added to the system in the form of undigested prey (the positive control) or digested prey. In the third step, the same antiserum used in the first step is bound to biotin (akin to the biotinylated goat anti-rabbit antiserum) and added to the wells. The ELISA was then carried out as described in 2.5.

Biotin was added to each of the rabbit-generated antibodies as follows. The antisera were separated from other proteins in the serum using a Caprilic acid purification method (Reik *et al.* 1987) and then concentrated following Wolfson's precipitation method using Saline Ammonium sulphate solution (after Weir 1973, Garvey *et al.* 1977). The isolated antibodies were then biotinylated using long-arm biotin (Biotinyl-N-hydroxy-succinimide,

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SIGMA B2643) after Stähli *et al.* (1983). However, the results did not show any improvement in the detection of rock-lobster stomach contents.

The possibility was then considered that the rock-lobster digestive enzymes were not only digesting the prey proteins substantially during soluble protein extract preparation, but also breaking down the antisera in the ELISA wells. To each gut content sample, enzyme inhibitors were added to try to alleviate this problem. PMSF (1mM) as well as both Pepstatin ($1\mu g.ml^{-1}$) and Aprotein ($1\mu g.ml^{-1}$) were added to each stomach sample to serve as general protease inhibitors. This however, changed neither the banding pattern on the SDS gels, nor the Western-Blot or ELISA results. The experiment was repeated with doubled concentrations of Pepstatin and Aprotein ($2\mu g.ml^{-1}$), but the results were unchanged.

One idea for dealing with the excessive amounts of digestive enzymes was to wash the stomach contents in several volumes of PBS before incubating it to make the soluble-protein extract of the gut contents, in an attempt to remove, or at least reduce the amounts of digestive enzymes in the extract. Again, subsequent Western-Blots and the ELISA showed similar patterns to those in Table 2.5 and Plates 2.2 & 2.3, indicating that washing gut remains had no effect on eliminating these digestive enzymes. Thus, the enzymes must have been bound strongly to the bolus.

Performing an inhibition ELISA showed that there were proteins in the digested extracts that the antisera recognised and bound to (Figure 2.5). In principal, this process is conducted as described in 2.5 above, except that the first antibody (*i.e.* from the rabbit) is incubated with a range of concentrations of a particular extract (either digested or undigested) prior to that antiserum being added to the ELISA well. If the concentration of antiserum is kept constant as the concentration of proteins in the extract is increased, then one expects a reduced signal on the ELISA for antisera incubated with higher concentrations of extract (as fewer antisera are available for binding to the proteins already bound to the plate). If there is no binding between the antiserum and extract during the pre-incubation there will be no signal attenuation on the ELISA. When the antiserum generated against *C. meridionalis* was pre-incubated with both digested and undigested

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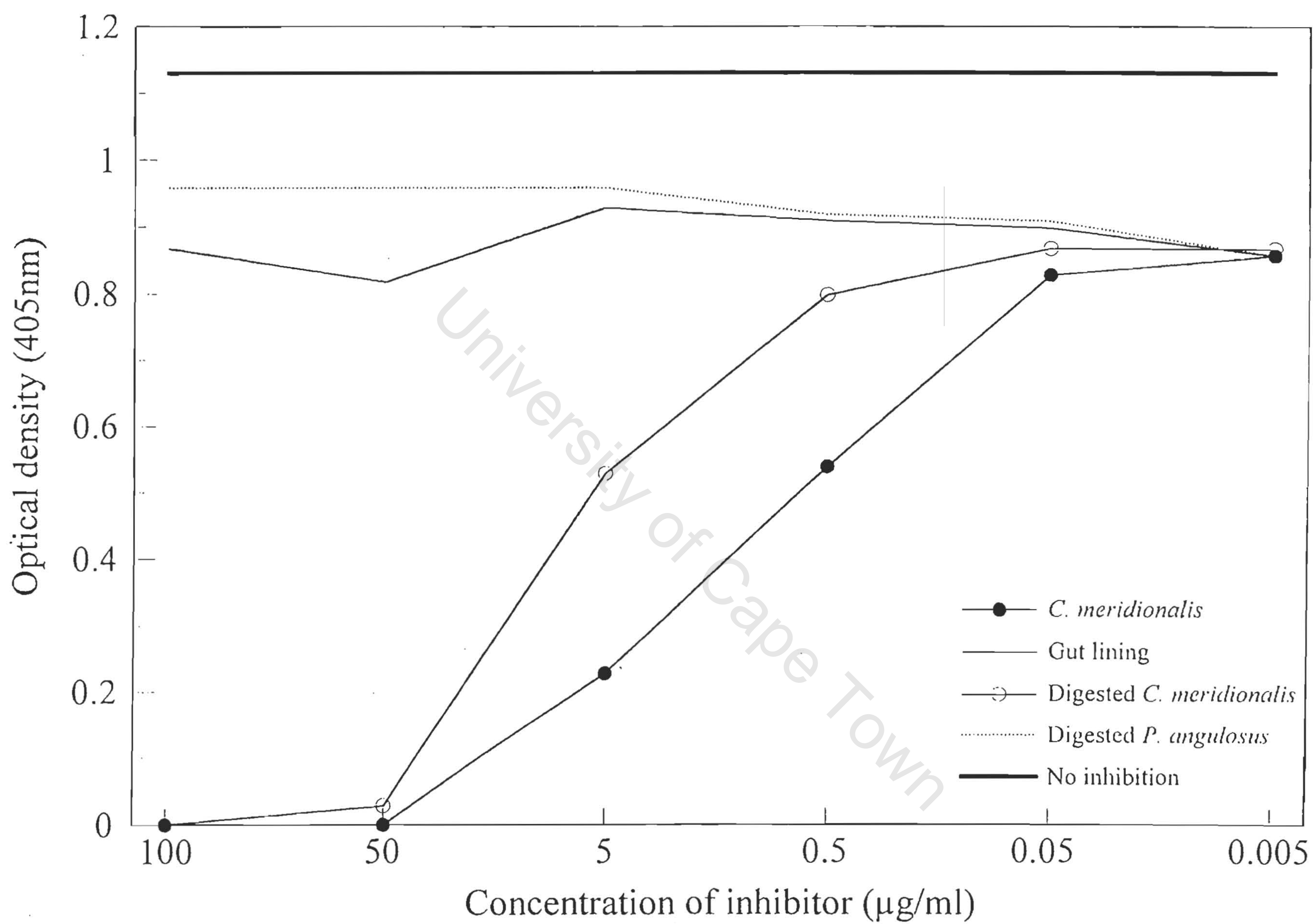

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Figure 2.5: Absorbance values (at 405nm) observed in the ELISA wells following a 30 minute incubation with ABTS for the inhibition ELISA at varying dilutions of the inhibitor.

C. meridionalis, inhibition occurred in both cases on the ELISA. However, when pre-incubated with gut lining extract and digested *P. angulosus*, no inhibition was evident. This proved that antisera were recognising prey protein remains from the digested extracts, even if this recognition was being masked by digestive enzymes.

The last procedure tried was to use ultrafiltration of the digested extracts in an attempt to remove the digestive enzymes, and thus increase the detection of prey remains as suggested by performing the inhibition ELISA. SDS-Page gels had shown that the bulk of the digestive enzymes causing the contamination were between 50kDa and 30kDa, and the rationale behind using this technique was to separate proteins between these two molecular weights out of the digested extracts using differential ultrafiltration.

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The ultrafiltration was done under pressure, using Nitrogen gas to force the digested extracts through Diaflo micropore filters (AMICON). The first attempt at filtration was to force the digested extract through a 5000Da filter to try to remove small molecular weight proteins from the extract in an attempt to enhance binding efficiency of prey item protein remains over digestive enzymes on the ELISA plate. This was unsuccessful, however, and digested extracts were still unidentifiable. The next step was to use a 50kDa and a 30kDa filter in series, as the majority of the enzymes were between 45kDa and 35kDa. Detection of digested material was greatest for the >50kDa fraction, with a low signal being recorded for the 30-50kDa fraction, and almost no response from the <30kDa fraction. However, although this appeared to alleviate the problems, the background and cross-reactivites for other antisera were also highest for the >50kDa fraction. Thus, ultrafiltration also did not solve the problems which were being faced.

Finally, banding patterns were obtained for different predators, including the European shore crab (*Carcinus maenas*), the Super klipvis (*Clinus superciliosus*) and the whelk (*Bullia laevissima*), when offered a single prey species, the black mussel *Choromytilus meridionalis*. This was done to see if different predators showed similar breakdown products for the same prey species in order to determine if this immunological technique could be applied to diet studies of other species. These predatory species were maintained under similar conditions to those of the rock-lobsters in an aquarium and fed black mussels

after a starvation period of 48 hours. The banding patterns for *J. lalandii* and for *Carcinus maenas*, were extremely similar (Plate 2.6). Banding patterns for other predators showed large differences to those observed for these decapods, but further work will be required to test if the system which was developed will be useful in assessing the diet of these predators non-visually, because the banding pattern observed may simply be their own pattern of digestive enzymes.

5. CONCLUSION

All these approaches failed to overcome the problems encountered and, despite the development of many antisera with high specificity to undigested prey items, the system failed to work for the assessment of the diet of the West Coast rock lobster, *J. lalandii*, when employed on digested gut contents. It was concluded that the high amounts of rock-lobster digestive enzymes present in the extracts taken from rock-lobster stomachs prevented analysis of the diet using immunological methods, because of physical interference. Unless a method can be found to remove these proteins from the protein pool obtained from the digested prey extracts, researchers will have to continue relying on a visual, and thus less precise method of diet analysis for this species. Nevertheless, although the ELISA method failed as a means of identifying gut contents, the fact that the guts of *J. lalandii* contain such large amounts of digestive enzymes is in itself of interest. There is a strong tendency in the literature to publish only positive results, and the failure of the ELISA system may well not be unique, if only successful cases of its application have been reported. In any event, its failure here led to the exclusive use of visual assessments of diet in the remaining chapters of this thesis.

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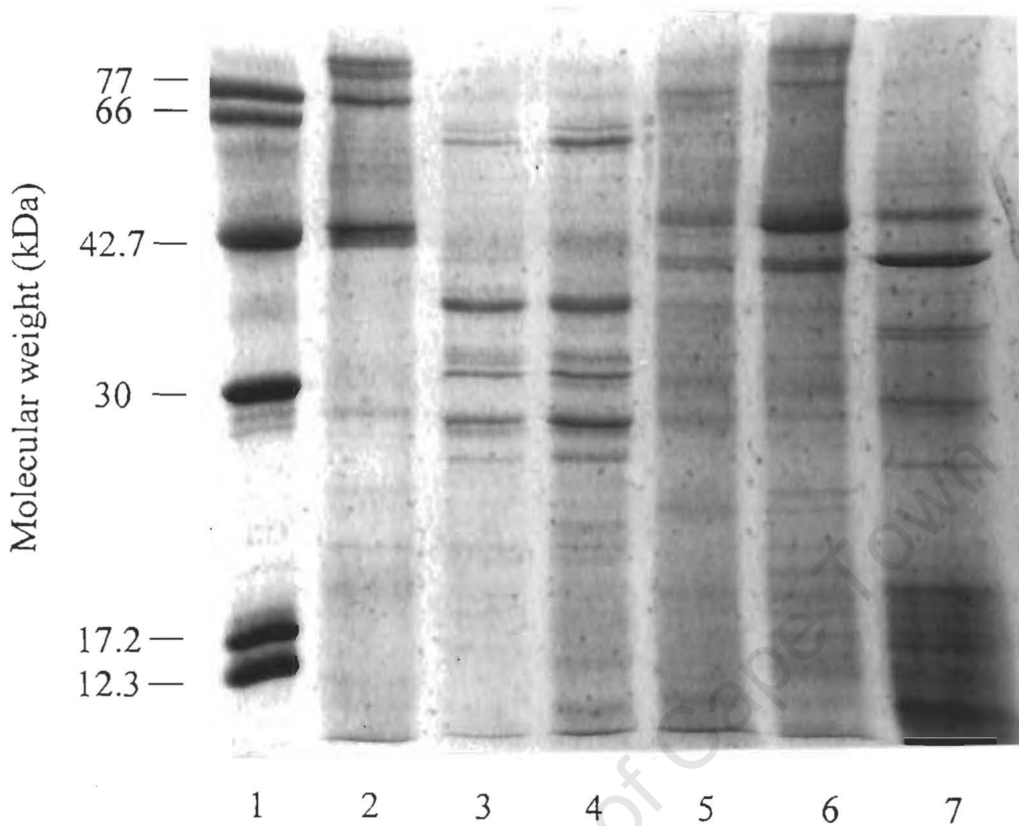

Plate 2.6: SDS gel stained with .1% coomassie blue to show the banding pattern of four different predators fed *C. meridionalis*. Lane 1 is a molecular weight marker. Lane 2 is *C. meridionalis* extract (undigested), lanes 3 & 4 are digested extract replicates from *J. Lalandii*, and lanes 5-7 are digested extracts from *Carcinus maenas* (crab), *Bullia laevissima* (whelk) and *Clinus superciliosus* (fish) respectively.

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Chapter 3

Influence of rock-lobster size, depth of capture and season (stage of moult cycle) on the diet of the West coast rock lobster, *Jasus lalandii*.

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1. INTRODUCTION

Studies on the feeding patterns of various lobster species have shown variation in diet with lobster size, capture depth and moult cycle. Ontogenic changes in diet have been observed in *Homarus americanus* stomach contents (Lawton & Lavalli 1995), as well as *Panulirus homarus* (Berry 1971), *Polycheles typhlops* and *Stereomastis sculpta* (Cartes & Abelló 1992). Temporal variation in diet on a seasonal basis was demonstrated for *H. americanus* (Ennis 1973), while depth-related dietary changes have been observed in *Polycheles typhlops* and *Stereomastis sculpta*, where diet differed as prey species abundance changed (Cartes & Abelló 1992). The diets of immature lobsters have been well studied, particularly for *Panulirus cygnus* (Joll & Phillips 1984, Jernakoff *et al.* 1993) and *H. americanus* (Carter & Steele 1982, Lawton & Lavalli 1995), and shown to differ from the diets of adults. Similar dietary variation in the West Coast rock lobster, *Jasus lalandii*, may be expected.

Dietary assessments on *J. lalandii* have been made over a wide geographical range (Barkai *et al.* 1996), as well as smaller, more localised areas (Newman & Pollock 1974b, Pollock 1979, Barkai & Branch 1988a) and in the laboratory (Griffiths & Seiderer 1980, Zoutendyk 1988a,b, van Zyl *et al.* 1998). However, all of these diet studies on *J. lalandii* (except for Barkai & Branch 1988a) have failed to consider aspects such as the diet of juvenile lobsters (< 65mm carapace length (CL)), and variation in diet with rock-lobster size. The influence of moult cycle stage and capture depth on the diet of *J. lalandii* has been completely ignored.

Recent declines in the growth rate of *J. lalandii* (Goosen & Cockcroft 1995, Cruywagen 1997) have been tentatively blamed on varying food resources (Pollock *et al.* 1997). Although the link between rock-lobster condition and growth is now well established (Cockcroft 1997), it is the links between rock-lobster diet, food availability and condition (hence growth) that remain poorly understood. Before examining these links (Chapter 4), it is necessary to standardise the effects of size, season (moult stage) and depth. This is required to ensure that any differences in diet observed between rock lobsters collected from areas of contrasting growth rates may

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not simply be attributed to other variables. Thus, the relationship between rock-lobster diet, growth rate and food availability can be determined reliably only if these variants are explored first.

This chapter investigates the influence of all of these aspects on rock-lobster diet. Based on the literature examined, it is predicted that: (1) The diet of juvenile rock lobsters will be different from that of mature conspecifics; more specifically, smaller rock lobsters will have higher levels of gut fullness, a higher prey diversity, less cannibalism and a higher intake of calcium (and other inorganic materials) in their diet relative to their body mass. This is because small rock lobsters moult frequently and can feed on a diverse array of small prey. (2) Diet will vary through the moult cycle, showing reduced consumption during the 'hard old' (early pre-moult) and 'hard new' (early intermoult) shell states and being dominated by prey species high in inorganic components during the early intermoult period. (3) Diet will vary bathymetrically, showing low diversity in the deeper stations.

2. METHODS

Logistics precluded the possibility of sampling all combinations of dates, sizes and depths simultaneously, so each of these factors was investigated in isolation, holding the other variables constant. Assigned stages in the moult cycle were based on shell state criteria (Heydorn 1969, Cockcroft 1997).

2.1 Seasonal changes in diet

Monthly samples were collected (when possible) from two localities, namely Olifantsbos (an area where rock lobsters grow slowly (SG)) and Dassen Island (where rock lobsters grow fast (FG), Goosen & Cockcroft 1995), between 15 and 25m water depth between February 1994 and July 1995 (see Figure 3.1 for localities). These sites were selected because (1) they were accessible, (2) good data exist on growth rates there, (3) they represent extremes of fast and slow growth and (4) they are important commercial fishing grounds.

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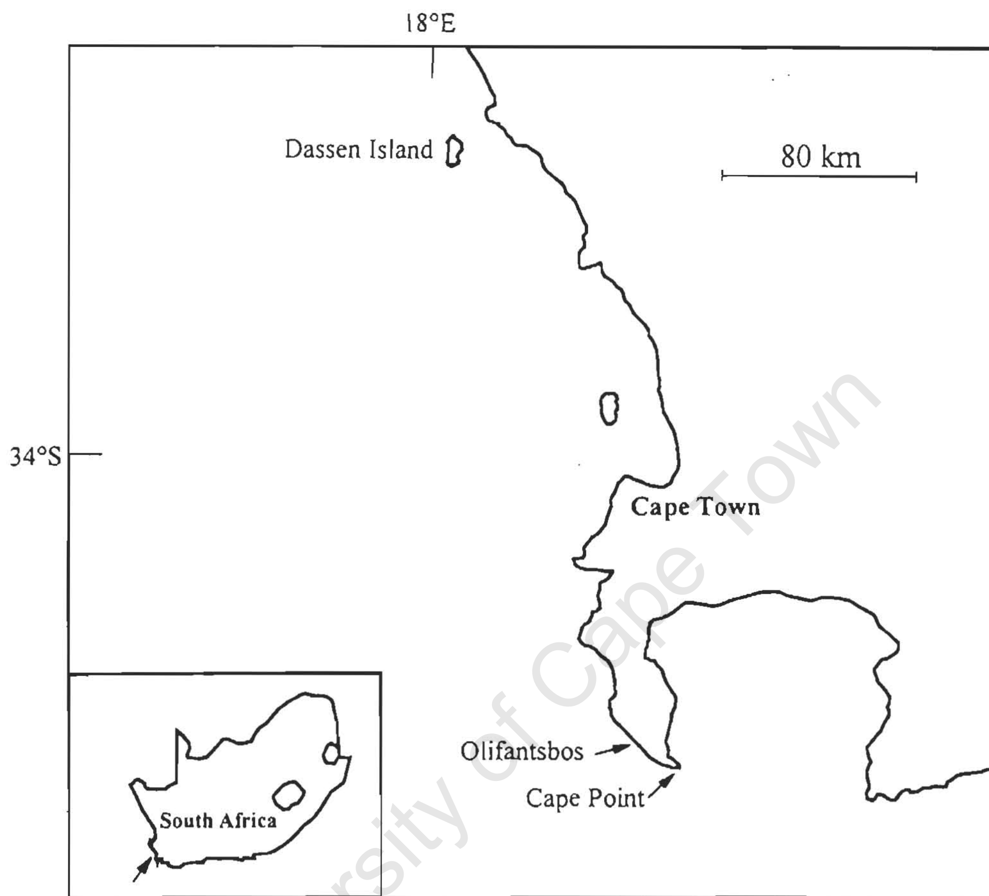

Figure 3.1: Map of the study area to show the location of the study sites.

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In certain months, violent storms prevented sampling. At both localities, during each sampling trip, 60 rock lobsters standardised to male only and 70-80mm carapace length (CL) were collected using bait-protected long-line lobster traps deployed at night at Olifantsbos, and by SCUBA divers operating shortly after dawn at Dassen Island. To deny the rock lobsters access to the baits, the bait was protected inside a 500μm mesh bag. The difference in dietary composition, relative proportions of prey items, bolus weight and percentage gut fullness between diver- and trap-caught specimens is negligible (Griffiths 1996), provided that account is taken of species that are not normally consumed by rock lobsters but which fall prey to them when lured into bait-protected traps. This applies particularly to the isopod *Cirolana* which is attracted to, and consumed in, traps (Griffiths 1996). Using these two methods for rock lobster collection may, however, have biased the percentage of rock lobsters feeding because trap sampling necessitates the attraction of rock lobsters to the fish bait, which is not a random sample.

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All rock lobsters were immediately anaesthetised in an ice-water slush, exsanguinated, and the first 30 full stomach samples (minimum volume of $1cm^3$) frozen at -20°C for further analysis. The proportion of the sample feeding was calculated as:

$$\frac{\text{number of rock lobsters with full stomachs}}{\text{number of rock lobsters collected}} \times 100$$

When analysed, all stomachs were defrosted, blotted dry and then weighed to 0.001g (Mettler AE 100 electronic balance). Gut contents were then flushed into a Petri dish and the gut membrane re-weighed as above. The gut fullness index was calculated as:

$$\frac{\text{total stomach weight (g) - stomach membrane weight (g)}}{\text{total stomach weight (g)}} \times 100$$

Prey species composition was determined by identification of diagnostic fragments when viewing the stomach content remains under a binocular microscope (8 X magnification). Gut contents were assessed subjectively, and the percentage contribution of each prey species to the total gut volume was estimated (after

Hyslop 1980, Williams 1981). Frequency of occurrence of each prey species (*i*) was calculated after Berg (1979) as

$$\frac{\text{number of stomachs containing species } i}{\text{number of stomachs examined}} \times 100$$

2.2 Effects of rock lobster size

To investigate the effect of rock-lobster size on diet, 60 male rock lobsters from each of five size classes (*viz.* 60-64mm, 65-70mm, 75-79mm, 80-85mm and >85mm CL) were collected from one site, Olifantsbos (SG), at a standardised depth of 20m using bait-protected long-line lobster traps in April 1994, as described above. The timing of the sampling (April) was chosen to ensure that all rock lobsters would be in the intermoult stage of their annual moult cycle and none would be reproductively active. This eliminated moult stage and reproductive effects. For logistic reasons the decision was made to sample only one site but to sample it intensively. I recognise that this does limit generalisation of the results to other sites. The intention of the study was, however, primarily to explore the effects of size on diet to determine the validity of restricting subsequent aspects of the work to males of one specific size range. Rock lobster stomachs were collected as above, and gut fullness and prey species composition were calculated as previously described. The *a priori* hypothesis stating that the composition of the diet would vary with rock-lobster size was tested using analysis of similarity (ANOSIM). Subsequently, Shannon-Wiener diversity (H) and Margalefs' species richness (D) indices were calculated for prey species diversity (data for all individuals of each size class pooled) after Clarke & Warwick (1994) as

$$H = \Sigma P_i (\log_e P_i)$$

and

$$D = S - 1 / (\log_e N)$$

where P_i is the proportion of the biomass attributed to the ith species, S is the total number of species and N is the number of individuals, using Primer v4.0 (Plymouth Marine Laboratory). The contribution of inorganic material by every prey item to each bolus was estimated by multiplying the bolus weight by the proportion of the bolus comprising that species. These weights were then converted first to a dry mass and

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then an inorganic material mass (based on ash free dry mass), using the conversions of Field *et al.* (1980). The mass of inorganic material per gram of bolus was then calculated. These values, the gut fullness levels and the absolute bolus masses between the five size classes were compared using a Kruskal-Wallis ANOVA by ranks (Zar 1984), followed, where appropriate, with a Newman-Keuls multiple range test ($\alpha = 0.05$), using Statistica v5.

2.3 Effects of depth

To examine the potential influence of capture depth on rock lobster diet, rock lobsters (males only; 70-80mm CL) were collected on a once-off basis during December 1995 at night from both Cape Point (FG) and Olifantsbos (SG) at a shallow (15m) and a deep (50m) site, using bait-protected long-line lobster traps. The date of sampling again ensured that all animals were in the intermoult stage at both sites and would not have been influenced by reproductive activity. Rock lobsters were dissected and stomachs collected as previously described. The *a priori* hypothesis, stating that diet will vary bathymetrically, was tested for dependence between diet composition and capture depth using analysis of similarity (ANOSIM), and then analysed using Bray-Curtis similarity and an MDS plot (Primer v4.0, Plymouth Marine Laboratory). Prey species richness and diversity indices were calculated as described above.

3. RESULTS

Much less seasonal variation in rock lobster diet was observed at Dassen Island when compared with Olifantsbos (Figures 3.2 & 3.3 respectively). At Dassen Island, cannibalism was more prevalent during winter (June), spring (August) and to a lesser extent early summer (October - November). The most commonly encountered prey items in the gut contents from this site were ribbed mussels (*Aulacomya ater*) and barnacles (*Notomegabalanus algicola*). At Olifantsbos, ribbed mussels were more abundant in the diet of rock lobsters during summer (November - March), and barnacles comprised a large component of the diet during the pre-moult and early intermoult periods. Rock lobsters captured during winter (June - July) and late spring (October) at Olifantsbos had a high occurrence of fish in the diet, and cannibalism

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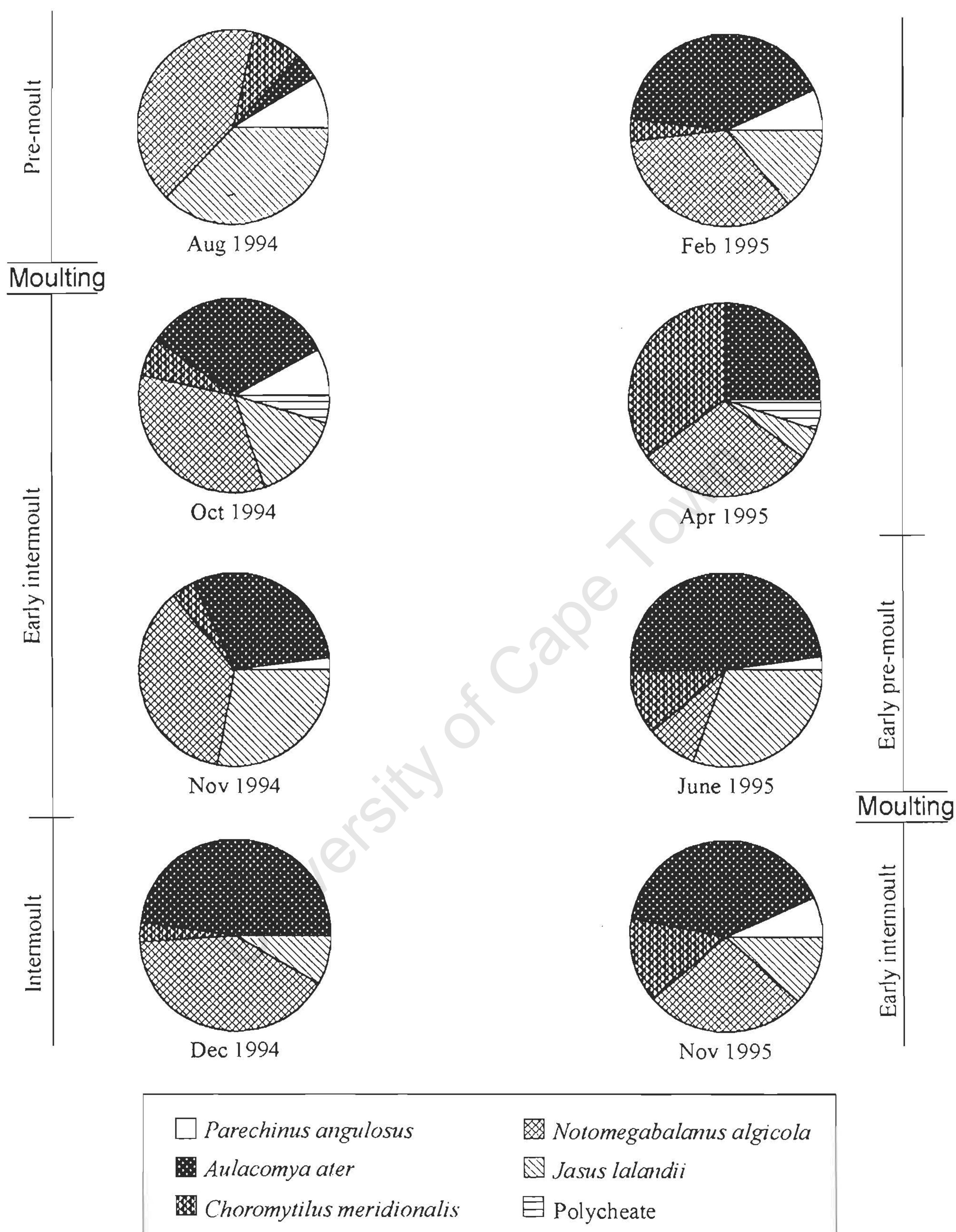

Figure 3.2: Diet, by frequency of occurrence, of male rock lobsters (70-80mm CL) sampled from Dassen Island between August 1994 and November 1995.

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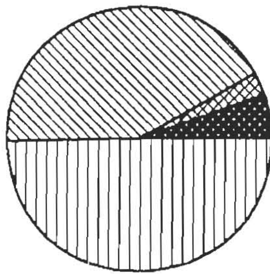
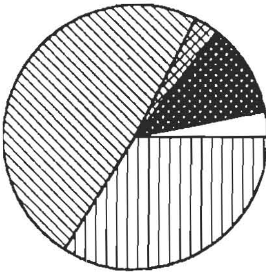
Moulting

Early intermoult

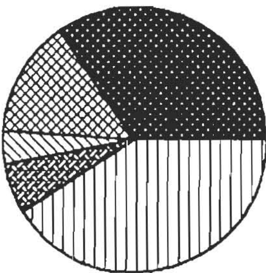


Oct 1994

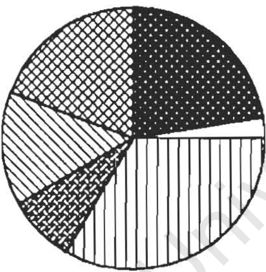


Nov 1994

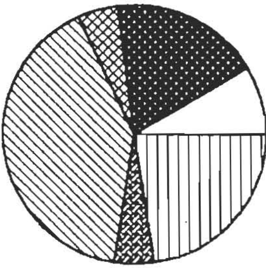
Intermoult



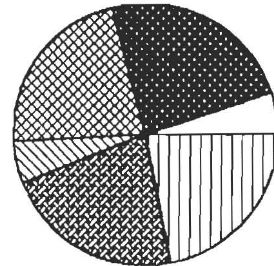
Dec 1994



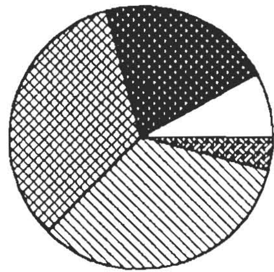
Jan 1995



Feb 1995

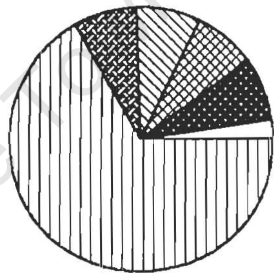


Mar 1995

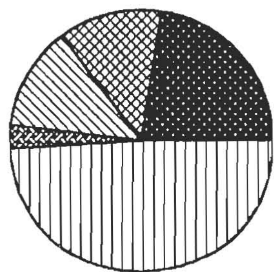


May 1995

Early pre-moult



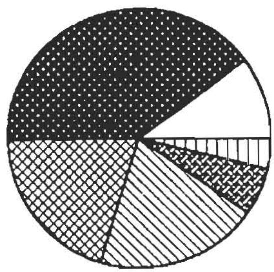
June 1995



July 1995

Moulting

Early intermoult



Nov 1995

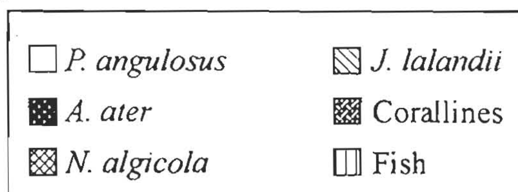


Figure 3.3: Diet, by frequency of occurrence, of male rock lobsters (70-80mm CL) sampled from Olifantsbos between October 1994 and November 1995.

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(not consumption of exuviae), although encountered in samples taken throughout the year, was most prevalent during late spring to early summer (October and November).

The proportion of rock lobsters feeding at both sites was low during the late inter-moult period and peaked in the early intermoult stage (Figure 3.4). Although the gut fullness index showed no seasonal variation at Olifantsbos, it was much lower during summer at Dassen Island when compared to other times of the year (Figure 3.5) – and throughout the sampling period was consistently higher at Olifantsbos when compared to Dassen Island. At Dassen Island, gut fullness tracked the proportion of rock lobsters feeding.

Rock lobsters of different sizes had significantly (Global R = 0.09, $P < 0.05$) different proportions of prey items in their stomach contents when the diets of lobsters from a size range of individuals were compared (Figure 3.6). Ribbed mussels (*Aulacomya ater*) and fish dominated the diet of rock lobsters larger than 80mm CL. The diet of small rock lobsters (< 70mm CL) comprised numerous species, but coralline algae, barnacles and rock-lobster remains were the dominant prey items observed in their gut contents. Rock lobsters between 75mm and 79mm CL showed a diet intermediate between these two groups and the highest rate of cannibalism. Moreover, the Shannon-Wiener index and Margalef's species richness indices were much higher for smaller rock lobsters (1.58 and 1.12 respectively) when compared to their larger conspecifics (1 and 0.46 respectively, Table 3.1). There were no significant differences in gut fullness between the five size groups (Kruskal-Wallis ANOVA by ranks, H = 1.56, $P > 0.05$, Table 3.1), nor in the amounts of inorganic material (g inorganic material.g bolus weight^{-1}) in the gut contents (H = 3.75, $P > 0.05$, Table 3.1). Large rock lobsters (> 85mm CL) did however have significantly larger bolus masses when compared to the other size classes (H = 12.545, $P < 0.05$) which were statistically indistinguishable from each other (Table 3.1).

Rock lobster diet also varied with capture depth. Ribbed mussels and coralline algae were the most common prey items in the stomachs of rock lobsters from the shallows off Cape Point, while at depth, fish were the most commonly encountered prey item

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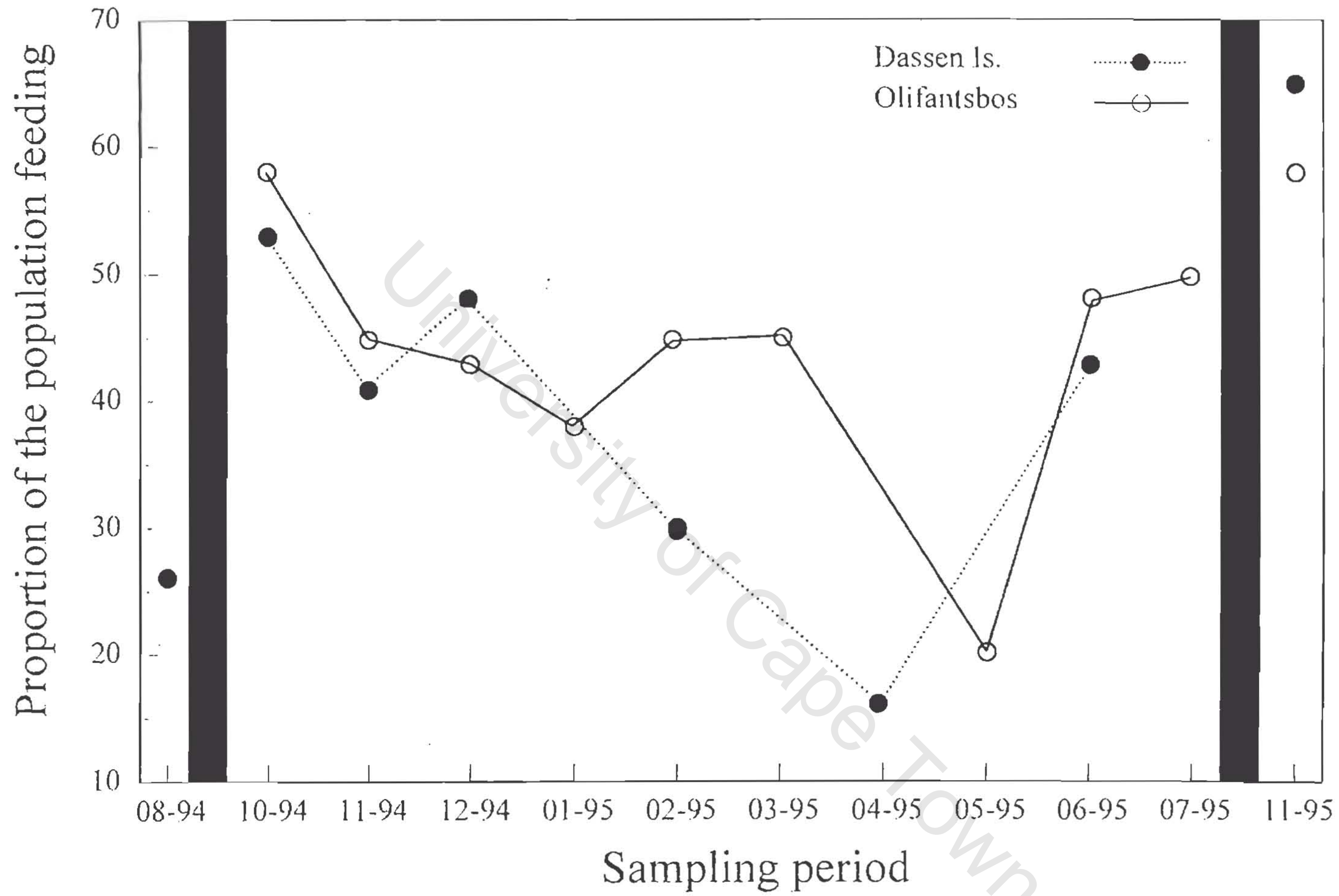

Figure 3.4: Proportion of the rock lobster population feeding over the sampling period for rock lobsters caught at Dassen Island and Olifantsbos. The shaded area shows the male rock lobster moult period.

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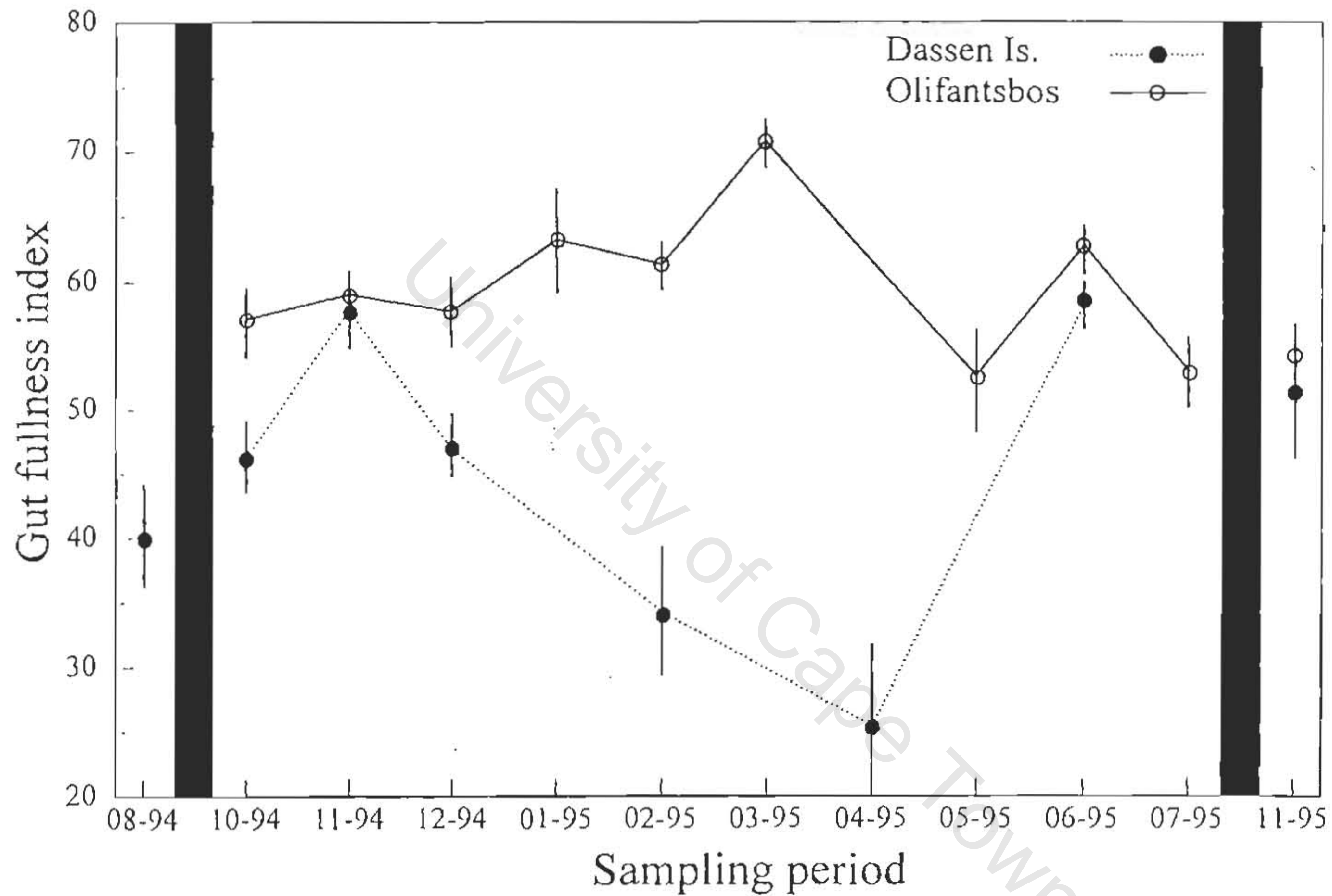

Figure 3.5: Gut fullness index calculated over the sample period for rock lobsters caught at Dassen Island and Olifantsbos. Values shown are the means ± standard error. The shaded area shows the male rock lobster moult period.

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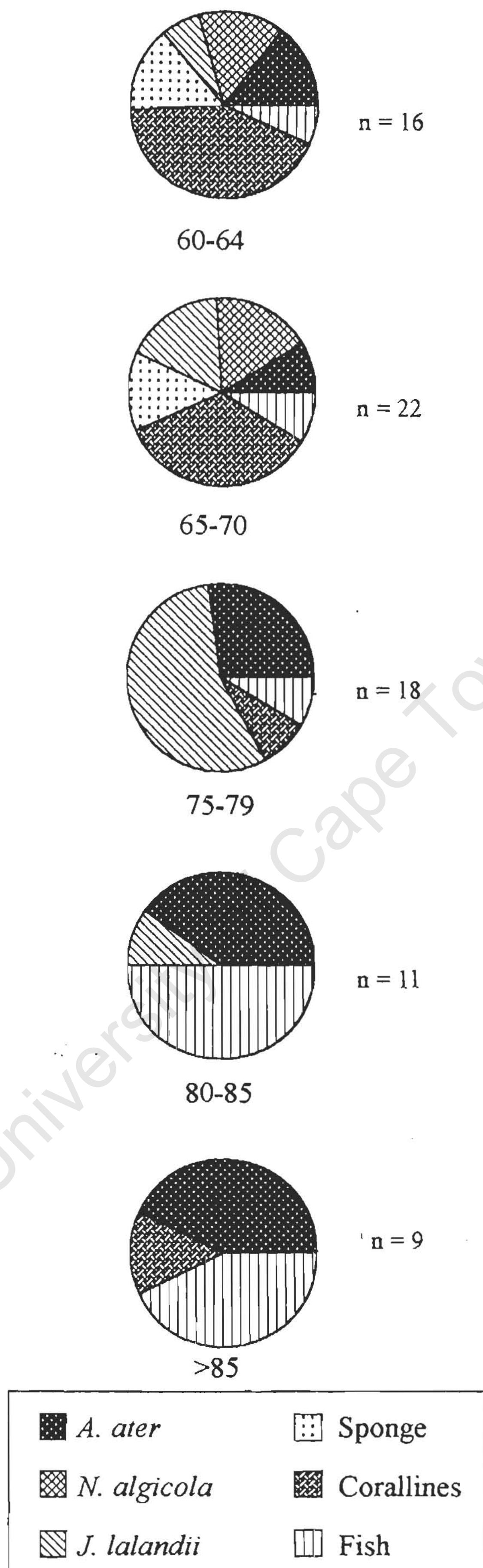

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Figure 3.6: Variation in the diet of rock lobster (*Jasus lalandii*), by frequency of occurrence, with increasing size. Numbers under each pie-chart refer to the size class represented. N = sample size.

Table 3.1: Margalef's and Shannon-Wiener diversity indices, gut fullness index (%), bolus weight (g), inorganic component of the bolus only (g), ratio of inorganic component to bolus weight (g inorganic material.g bolus weight-1), mass of inorganic material in the bolus per unit body weight (mg inorganic material. g wet weight^{-1}) and bolus weight as a proportion of body weight (mg bolus weight. g body weight^{-1}) for rock lobsters of different sizes. The rock lobster length-wet weight regression equation was obtained from Chapter 6 (equation 4).

	Rock lobster size class (mm CL)				
	60-64	65-70	75-79	80-85	>85
Margalef's Index	1.12	1.08	0.736	0.444	0.461
Shannon-Wiener Index	1.58	1.67	1.11	0.93	1.00
Gut fullness	54.7	51.8	55.5	46.8	64.5
Bolus weight	0.84	0.91	1.74	1.61	3.41
Bolus inorganic content	0.24	0.14	0.17	0.59	0.63
g inorganic.g bolus^{-1}	0.31	0.22	0.13	0.27	0.23
mg bolus.g body weight^{-1}	6.6	5.2	6.6	5.3	8
mg inorganic.g body weight^{-1}	1.9	0.8	0.7	1.9	1.7

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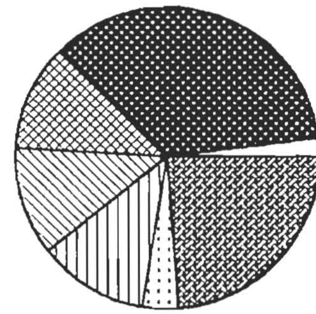
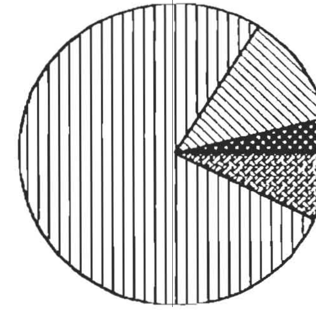
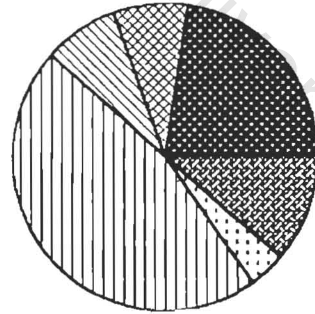


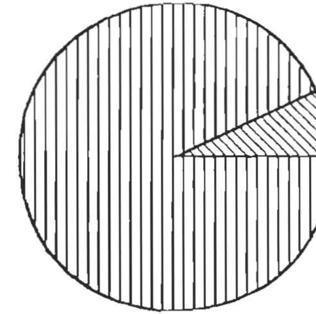
Cape Point (shallow)



Olifantsbos (shallow)



Cape Point (deep)



Olifantsbos (deep)

n = 30 for all samples

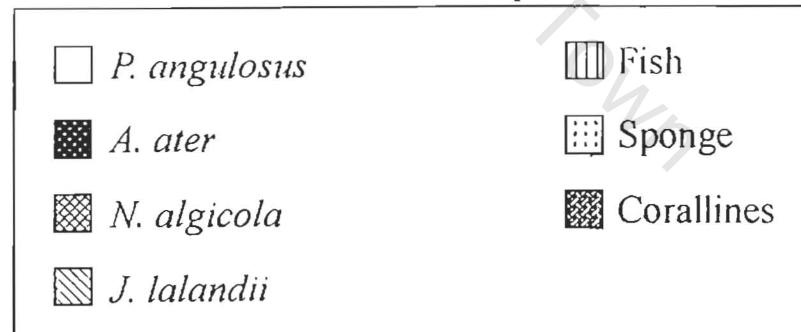


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Figure 3.7: Diet, by frequency of occurrence, of male rock lobsters (70-80mm CL) sampled from a shallow (20m) and deep (50m) station at two localities.

Table 3.2: Margalef's and Shannon-Wiener indices for the diet of rock lobsters collected from shallow and deep stations at Olifantsbos and Cape Point.

	Cape Point shallow	Cape Point deep	Olifantsbos shallow	Olifantsbos deep
Margalef's Index	1.152	1.124	0.681	0.266
Shannon-Wiener Index	0.854	0.818	0.557	0.365

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Figure 3.8: Bray-Curtis similarity dendrogram showing two groups separating out with less than 30% similarity when stomach samples of rock lobsters collected at two sites and from two depths were analysed. a & b = Cape Point shallow and deep respectively. c & d = Olifantsbos shallow and deep respectively.

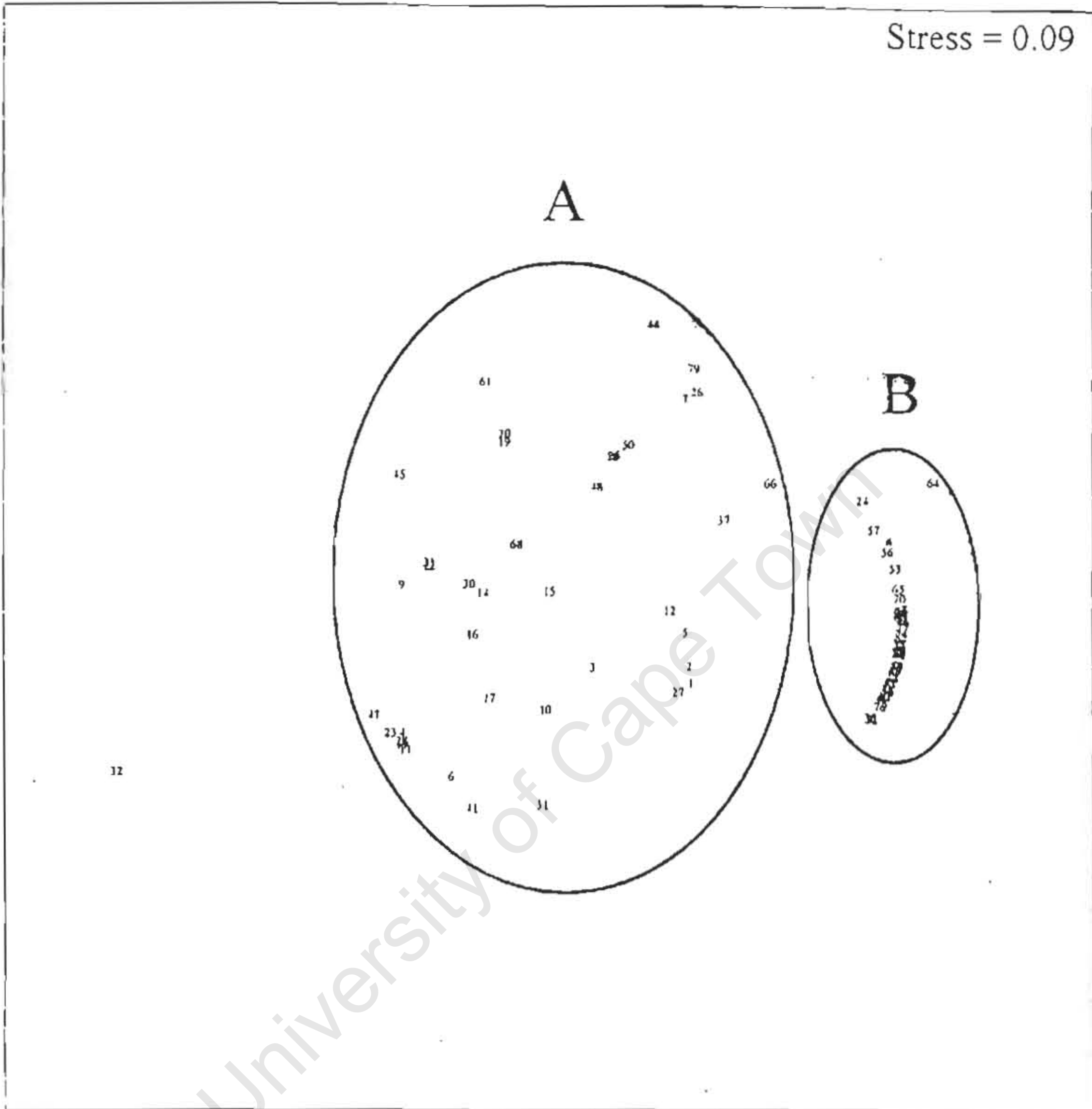

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Figure 3.9: Multi-dimensional scaling plot to show groupings of the rock lobster stomach samples from rock lobsters collected at two sites and from two depths. A and B are the two groups from Figure 3.8.

(Figure 3.7). At Olifantsbos, the number of prey items in the diet at the shallow station was double that of the deep station, although fish dominated the diet from both depths (Figure 3.7). For both sites, the species richness and prey diversity index were lower for the deeper station when compared to the shallow station (Table 3.2), although the difference was only marginal in some cases. Nevertheless, an analysis of similarity (ANOSIM) for both Cape Point and Olifantsbos suggested there were no significant differences between the diet of rock lobsters caught at different depths (Global R = 0.019, $P > 0.05$). Subsequently however, Bray-Curtis similarity (Figure 3.8) and the associated MDS plot (Figure 3.9) suggested that there was more difference in rock- lobster diet between Olifantsbos and Cape Point than between the two depth zones sampled. The dendrogram separated into two groups with less than 20% similarity. These groups reflected samples from both depths at Cape Point (group A) and Olifantsbos (group B) respectively. The same distinction was evident on the MDS plot. Analysing these two figures more closely confirms that only small differences in diet between rock lobsters caught at the two depths were observed.

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4. DISCUSSION

The diet of the West Coast rock lobster, *Jasus lalandii*, varied between rock lobsters of different sizes, but only to a limited extent between rock lobsters captured at two different depths. Furthermore, rock lobster diet varied through the course of the sampling period, showing seasonal (*i.e.* moult cycle) variation. However, before considering these patterns in more detail one question needs to be addresses: whether trap-effects influenced the gut contents of the animals examined.

4.1 Possible Trap effects on the diet

Large numbers of rock lobsters collected during the seasonal sampling at Olifantsbos and the depth-related sampling at both Cape Point and Olifantsbos contained high volumes of fish in their gut contents (Figures 3.3 & 3.7). Concerns were raised during initial sampling, that rock lobsters exposed to fish baits in the baited-traps might have a high abundance of fish-bait remains in the stomach contents, thus masking other

prey items. To prevent the rock lobsters gaining access to the baits, all traps thus had the bait protected with bags (mesh size = 500μm). Furthermore, only one bait species was used (Maasbanker, *Trachurus trachurus*), and all bait fish were between 25 and 35cm total length. Fish remains in the stomach contents usually consisted of fin rays and vertebrae, and although they could not all be identified to species level, none of the remains were determined to be either *T. trachurus* or *Merluccius capensis* (Hake – the only other typically used bait fish, Dave Japp, Sea Fisheries Research Institute, Cape Town). A concurrent study considering other aspects of trap-effects on the diet of *J. lalandii* also noted high levels of fish remains in the diet of trap-caught rock lobsters (Griffiths 1996). Again, these fish remains did not come from the bait species used in the experiment nor from Hake, and were probably derived from small clinids (Steve Lamberth, University of Cape Town, pers. com.). Small fish are consumed by captive rock lobsters (pers. obs.), and several divers have reported catching rock lobsters which are holding small fish in their claws. The capture of live fish by *Homarus americanus* (Lawton & Lavalli 1995) and some deep-water clawed lobsters (*Metanephrops* spp., Wassenberg & Hill 1989) has also been reported.

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The stomach samples of trap-caught *J. lalandii* also contained large numbers of isopods, but their consumption by the rock lobsters has been shown to be due to a trap effect (Griffiths 1996). Consequently, isopods were excluded from any dietary analyses and corrections made to the gut fullness indices and proportion of animals feeding. Thus, dietary patterns relating to rock-lobster size, seasonality and depth were unlikely to have been biased by trap effects.

4.2 Ontogenic changes in diet

It was anticipated that the diet of the rock lobsters would vary as the size of the rock lobsters increased. More specifically, it was predicted that juvenile rock lobsters would have higher gut fullness levels, a higher prey diversity, less cannibalism and a greater dietary intake of inorganic components per unit body weight. The diet of individuals smaller than 70mm CL was dominated by coralline algae, with barnacles and sponges being other important prey items (Figure 3.6). In contrast, the gut contents of large rock lobsters (> 80mm CL) were dominated by the ribbed mussel

and fish. Rock lobsters between 70 and 80mm CL had a mixed diet between the two extremes highlighted above, their gut contents containing large amounts of rock lobster remains and coralline algae (similar to the small rock lobsters) as well as fish and ribbed-mussel fragments (akin to the large rock lobsters). Coralline algae and barnacles are rich in inorganic components, whereas mussels and fish tend to be more protein rich (Field *et al.* 1980). So, it seemed that the diet of small rock lobsters contained prey with a higher inorganic content than those in the diet of larger specimens. However, further analysis did not confirm this. Use of a non-parametric ANOVA, showed that rock lobsters of different sizes consumed the same mass of inorganic material per unit mass of bolus and the ratio between mg inorganic content of the bolus and g body weight was similar across all sizes of rock lobsters (Table 3.1). Thus the initial prediction that small individuals would have a diet with a higher inorganic content was not upheld by the data. It is possible that coralline algae is ingested incidentally – they are certainly epiphytic on mussels. However, few if any mussel remains were covered in coralline algae.

Relative to large individuals, small rock lobsters have a high growth rate (Pollock 1979). Thus, it was predicted that juvenile rock lobsters would have a relatively higher gut fullness. It was also predicted that they would have a higher diversity of prey. There are two possible reasons for this. First, there is a greater range of species in the smaller size-spectrum of prey than among large prey. Second, if preferred prey are eliminated or reduced by competition with large rock lobsters, then small individuals may have to diversify to meet their energy needs - which are likely to be higher per unit mass because of their fast growth and rapid moulting (Beyers *et al.* 1994). Griffiths & Seiderer (1980) have produced good evidence that the size range of mussels (the preferred prey) available to small rock lobsters is often depleted due to predation. For large rock lobsters, which can consume most prey, and a much greater size range of that prey, competition should be less and they can concentrate on preferred prey so that their diversity indices will be smaller. The former prediction was not borne out in the data as no statistical difference was found between the gut fullness levels of the five different size classes, and the ratio between bolus mass and body weight showed no trend, ranging between 5.2 and 8 (Table 3.1).

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However, the diversity of prey was greater in smaller animals. Small rock lobsters contained a much higher number of prey species in the stomach content - up to six in one lobster. By contrast, the gut contents of larger lobsters tended to be dominated by one prey item. This conclusion was supported by the Shannon-Wiener and Margalef's indices calculated for the five size-class groups (Table 3.1). Results showed that both species richness and diversity of prey items were greater in small than in large rock lobsters.

Cannibalism has been shown to be prevalent amongst adult *H. americanus* (Elner & Campbell 1987, Lawton & Lavalli 1995) but (exuviae excluded), almost absent among small specimens (Carter & Steele 1981). Thus, one of the predictions of this chapter was that cannibalism would be highest in the largest size classes of rock lobsters considered. Indeed, cannibalism was low among small rock lobsters, but was most prevalent in the intermediate sized rock lobsters rather than in large rock lobsters. Rock-lobster remains were encountered in over 50% of the stomachs of intermediate-sized individuals, but only 5% of large animals. It is surprising that so few of the large rock lobsters were eating their own species. Previous studies have recorded high levels of cannibalism in *J. lalandii* (*e.g.* Pollock 1979, Barkai & Branch 1988a, Barkai *et al.* 1996, Mayfield & Branch 1996). However, if it is true that larger individuals suffer less competition for food, their lower rates of cannibalism may be a reflection of this.

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Studies on variation in diet with size are not new to crustaceans, nor even to Decapods. Ontogenic changes in diet have previously been shown for individuals of *J. lalandii* caught at Marcus and Malgas Islands (Barkai & Branch 1988a). In their study, the diet of large lobsters was dominated by mussels at both islands. Smaller rock lobsters at Marcus Island consumed mainly sea urchins (*Parechinus angulosus*), whereas barnacles, mysids, algae and sponge comprised their diet at Malgas Island. A similar ontogenic diet pattern has been described for the South African East Coast rock lobster, *Panulirus homarus*. Berry (1971) found that the diet of immature rock lobsters was dominated by barnacles with a small amount of mussel remains, when compared with adult *P. homarus* which fed mainly on mussels. Other lobster species

such as *Polycheles typhlops*, *Stereomastis sculpta* and *Homarus americanus* have also shown clear ontogenic changes in diet (Cartes & Abelló 1992, Lawton & Lavalli 1995). In these species, the dietary switch was similar to that observed in this study with small lobsters consuming less fish and mussels when compared with their larger conspecifics. Studies on other crustacean species have highlighted shifts in diet with size for, amongst others, prawns (O'Brien 1994) and crabs (Cerda & Wolff 1993).

Some discussion on the possible reasons for the observed ontogenic differences observed in the diet of West Coast rock lobsters is merited here. Juveniles moult more frequently than their larger conspecifics - up to three times per year (Beyers *et al.* 1994) - while adults tend to moult annually (Heydorn 1969, Cockcroft & Goosen 1995). It has been suggested that prey items of large rock lobsters are unavailable to smaller specimens because of their physical inability to open mollusc shells (Griffiths & Seiderer 1980, Elner & Campbell 1981, Moody & Steneck 1993, Lawton & Lavalli 1995), thus leading to observed differences in diet. However, in this study, both small and large individuals had molluscs (including mussels) in their gut contents, just in very dissimilar proportions (Figure 3.6). An alternative hypothesis is that small lobsters, because of their shorter inter-moult period, require a fundamentally different diet to the large rock lobsters - one rich in inorganic constituents to facilitate frequent exoskeleton replacement. Dietary intake of prey items high in mineral constituents has been shown to be highest in pre- and post-moult specimens of *H. americanus* (Leavitt *et al.* 1979, Ennis 1973), and post-moult *J. lalandii* fed predominantly on barnacles and small non-bivalve molluscs (Barkai & Branch 1988a). This suggests that foods high in calcareous components are important in moulting. The inorganic components of barnacles, ribbed mussels, rock lobsters and fish are reported to be 0.82, 0.88, 0.38, and 0.18g.g dry mass^{-1} (Field *et al.* 1980), and that for coralline algae 0.7g.g dry mass^{-1} (Manneveldt 1995). Despite juvenile rock lobsters feeding predominantly on mineral rich prey, the intake of inorganic material per unit body mass showed no trend with rock-lobster size. Thus, the data support neither of these hypotheses and leaves the question as to why ontogenic changes in diet were observed in this species still unanswered.

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4.3 Bathymetric variation in diet

The diets of male *J. lalandii* of standardised size (70-80mm CL) showed, as predicted, some differences with capture depth (Figure 3.7). At the shallow station at Cape Point, the diet was diverse, but consisted principally of ribbed mussels and coralline algae. At 50m depth in the same area, the diet was quite different: fish remains were found in more than 45% of stomach samples examined, although ribbed mussels and coralline algae were still encountered. For both depths, the species richness and diversity indices were similar, but slightly depressed for the deeper station (Table 3.2). By contrast, the diet of rock lobsters collected from the shallow station at Olifantsbos showed twice the prey richness when compared to the deep station, although fish were the dominant prey item at both depths. This was confirmed by the Margalef's and Shannon-Wiener indices, both of which were about 50% lower at the shallow when compared with the deep station. Ribbed mussels and coralline algae were also consumed at the shallow station, but the only other prey item at 50m was rock-lobster remains. Concerns were raised that the high proportion of fish remains observed in the stomach contents from Olifantsbos were the result of the rock lobsters feeding on the bait in the baited-traps. However, any possible trap effects have already been eliminated (see 4.1), and the regular consumption of fish is a finding of great interest in its own right.

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Despite the differences between dietary diversity at different depths, when the data were subjected to an analysis of similarity (ANOSIM), the result was non-significant (Global R = 0.019, $P > 0.05$), indicating little dependence of diet on capture depth. Analysis using Bray-Curtis similarity and MDS plots suggested that the reason for this non-significant result was because much larger differences were observed between the diets of rock lobsters caught at the two sites (Cape Point and Olifantsbos), than between the two depths at each site (groups A & B, Figures 3.8 & 3.9). Closer analysis of the data using only those stomach samples collected from Olifantsbos (one of the sites selected for seasonal diet analysis) showed that there was very little within-depth grouping of the samples (group B, Figure 3.8). It is possible that the rock lobsters were not foraging in the vicinity of the dens – similarity in diet

may have resulted from the deep (>50m) rock lobsters moving inshore to feed. Such nightly movements have already been shown in *Panulirus cygnus* (Jernakoff & Phillips 1986), but similar research has not yet been undertaken on *J. lalandii*.

Limited information is available on bathymetric differences in rock lobster diet, primarily because of the difficulties involved in sampling lobsters below 100m depth. Cartes & Abelló (1992) observed that the diet of the polychelid lobster *Polycheles typhlops*, differed when comparing a depth range of 550-750m to a range of 1000-1200m. At the shallow depth, macrura and euphausids were dominant prey items, while at the 1000-1200m station, polychaetes, mysids and isopods became increasingly important. These authors showed that depth-related changes in diet reflected changes in prey availability. The deep sea Mediterranean crab, *Geryon longipes*, also showed bathymetric variation in diet, with shallow-water individuals consuming more pteropods, polychaetes and echinoids (Cartes 1993a). Teleosts and then polychaetes become increasingly important components of the diet at greater depths. Similar results were obtained for pandalid (Cartes 1993b) and aristeid shrimps (Cartes 1994). For other decapod crustaceans, bathymetric variation in diet has also been shown (Wassenberg & Hill 1989). Thus, changes in diet with depth are the norm in crustaceans. In the case of *J. lalandii* some depth-related differences do exist (notably a shift to increased consumption of fish at greater depth), but there were much greater inter-site differences recorded for the two localities examined. However, dietary diversity did decline with depth. For other lobsters, it appears that diet variation with depth tracks prey species availability. That is likely to be the case here, although (as discussed later in this thesis) *J. lalandii* exhibits strong preference for particular prey over others. Future studies need to look at the diet of this species at deeper depths.

4.4 Temporal variation in diet

Rock-lobster diet varied seasonally at both Dassen Island and Olifantsbos over the 18-month sampling period. At Dassen Island, barnacles and ribbed mussels were the dominant prey items in the gut contents virtually throughout the sampling period (Figure 3.2), but cannibalism was more prevalent during winter (June) and spring

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(August) and to a lesser degree early summer (October - November), which corresponds to the female and male moulting periods respectively (Heydorn 1969, Newman & Pollock 1974c). Less than 1% of the cannibalism involved consumption of exuviae – the rest involved the consumption of (usually small) rock lobsters. In 1994, most male rock lobsters moulted in September (Cockcroft 1997) - thus the high cannibalism observed around this period may have been the consumption of early and late moulters. Male rock lobsters show high synchronicity during moulting, ostensibly to prevent high mortalities due to cannibalism during this period.

Considerably more seasonal variation in diet was observed at Olifantsbos (Figure 2.3). The most commonly-encountered prey remains in the stomachs were fish (except in May and November 1995). Ribbed mussels and sea urchins were also common. Although barnacle and rock lobster remains were observed in nearly all samples, barnacle remains were much more abundant during the intermoult period of reserve accumulation (December to May 1995, Cockcroft 1997). Cannibalism peaked during late spring (October & November 1994) and again in winter (May 1995) - a trend similar to that previously described for Dassen Island. Again, those individuals not moulting with the bulk of the rock lobsters probably account for a large proportion of this observed cannibalism. At both Dassen Island and Olifantsbos, rock lobsters were consuming prey items rich in inorganic components during the pre- and post-moult months, thus upholding the prediction that diet will vary according to physiological requirements. This was presumably in preparation for enlarging the exoskeleton during the forthcoming moult. Most interestingly, aside from fish, rock lobsters at both these sites were consuming similar prey items, despite their growth rates being very different. The idea that diet and growth are linked will be developed in the next chapter (Chapter 4).

Seasonal variation in diet has previously been reported for *J. lalandii* (Barkai & Branch 1988a), with cannibalism being shown to be highest during summer (presumed to be during the early intermoult phase). At Malgas Island, peak consumption of ribbed mussels was observed in winter, and barnacle ingestion was highest during late spring/summer (Barkai & Branch 1988a). This accords to the data

presented here, and corresponds to the need to increase ingestion of inorganic material to allow for moulting (Scarratt 1980, as cited by Lawton & Lavalli 1995). Temporal shifts in diet also occur in *Homarus americanus*, with a shift towards a more calcium-rich diet during the moulting season in late summer (Ennis 1973) when sea urchins, chitons and crabs became more abundant in the diet (Carter & Steele 1982, Elner & Campbell 1987, Lawton & Lavalli 1995). It has been suggested that this dietary pattern follows the physiological requirements of moulting, rather than simply reflecting prey availability (Leavitt *et al.* 1979), and that it involves active selectivity of prey species by *H. americanus* (Lawton & Lavalli 1995). In contrast, for *Panulirus cygnus*, no seasonal variation in diet was noted (Joll & Phillips 1984). Thus seasonal variation in diet appears to be species-specific and may be related to the geographical range of the species. Lobster species inhabiting areas of high latitiude which tend to have clearly defined seasons may shown more seasonal variation in diet. However, despite the similarity between this and previous studies it needs noting that environmental conditions (e.g. swell) can alter feeding behaviour and may have impacted on my results due to the infrequency of sampling.

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Although the proportion of rock lobsters feeding and the gut fullness index are crude indices, they are valuable indicators of both the level of feeding activity in the rock lobster population and the quality of the food environment. It was predicted that both these indices would show seasonal variation, being depressed just before, during and after moulting. It must be noted though that the sampling resolution here was probably not fine enough to completely examine these hypotheses.

The proportion of rock lobsters feeding showed marked seasonality at both sites. Low numbers were foraging during winter (late intermoult), but this figure increased immediately prior to moulting and remained high in the post-moult period (Figure 3.4). Additionally, at Dassen Island, stomach fullness showed an almost identical seasonal pattern to that of the proportion of rock lobsters feeding, although the same trend was not observed at Olifantsbos (Figure 3.5). Gut fullness ranged between 24 and 71% for both sites combined. Previous studies on *J. lalandii* have reported cessation of feeding for up to six weeks prior to and for five weeks following

moulting (Heydorn 1969, Zoutendyk 1988b, Beyers *et al.* 1994), although the current consensus is that feeding stops for a maximum of 21 days (Dr A Cockcroft, SFRI, pers. comm.). As male rock lobsters moulted during September 1994 (Cockcroft 1997), cessation of feeding should have taken place between August and October. The data show that this was not the case. The gut fullness index for Dassen Island was lower then than during the early intermoult period (Figure 3.5), but the remaining data show no suppression of feeding around the moult (Figures 3.4 & 3.5). However, as the sampling resolution is so coarse it is likely that the moulting window (and thus rock lobsters immediately pre- and post-moult) was missed. Thus, while not entirely supporting the original hypothesis, some depression in feeding was observed during the time surrounding moulting. The depression of male rock lobster feeding (Figure 3.4) during Winter 1995 is probably due to the female rock-lobster moult cycle. As this is the period when reproduction is thought to take place (*i.e.* while the females are soft), it is necessary for the male rock lobsters to be focussed on reproduction rather than on feeding during this time. The proportion of rock lobsters feeding tracked the commercial CPUE index for both Olifantsbos and Dassen Island (Table 3.3) giving some credence to their reliability. Rock lobsters not feeding will not be attracted to baited traps. Conversely it helps explain seasonal variations in CPUE.

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Studies on *H. americanus* suggest a similar seasonal pattern in gut fullness to that observed here for *J. lalandii*, with a high gut fullness index just prior to moulting and during the early pre-moult phase (Ennis 1973, Carter & Steele 1982, Elner & Campbell 1987). Thus, cessation of feeding prior to moulting does not take place in this species of lobster. Seasonal variation in gut tract fullness has also been observed in *Panulirus cygnus* (Joll & Phillips 1984). By contrast, in this species the degree of foregut filling was extremely low in both pre- and post-moult individuals, from two sites of contrasting growth rate. Thus, seasonal variation in gut fullness has been shown in other lobster species, rendering *J. lalandii* no exception. Such variations are most likely related to the difficulty of feeding when the lobsters are soft shelled (pre- and post-moult). Further field work on the gut fullness of *J. lalandii* will be necessary to determine if cessation of feeding occurs in the wild, and not only in captive

Table 3.3: Commercial catch per unit effort (CPUE, kg.trap^{-1}) for Dassen Island and Olifantsbos from November 1994 to November 1995. ND = no data.

Month	Dassen Is.	Olifantsbos
Nov-94	4.39	10.25
Dec-94	5.02	8.71
Jan-95	3.04	10.66
Feb-95	3.52	8.96
Mar-95	3.48	5.89
Apr-95	2.82	5.99
May-95	3.24	6.28
Jun-95	1.83	9.96
Jul-95	ND	9.51
Aug-95	ND	6.7
Sep-95	ND	9.94
Oct-95	ND	ND
Nov-95	6.36	15.2

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individuals (Zoutendyk 1988b), but my data suggest feeding is actually highest immediately before and after moulting (Figures 3.4 & 3.5) even if it is halted during moulting.

5. CONCLUSION

In conclusion, this study has shown that the diet of the West Coast rock lobster, *Jasus lalandii* varied significantly with lobster size and season and that some depth-related differences in diet are also evident for this species.

Small rock lobsters consumed a larger diversity of prey, but did not have higher gut fullness indices nor a larger intake of inorganic material per unit body mass when compared with larger individuals. No significant differences in mean gut fullness were observed between lobsters of different sizes although very large lobsters (> 85mm CL) did have significantly larger food boluses. Cannibalism was highest for the rock lobsters between 70 and 80mm CL.

The gut fullness index, the proportion of rock lobsters feeding at any one time, and the diet varied temporally. The proportion of rock lobsters feeding at any one time tracked the commercial CPUE for both Dassen Island and Olifantsbos. Noting problems with sampling resolution, at the times when cessation of feeding was predicted, peak rock-lobster feeding was taking place.

Depth-related differences in diet were masked by the large differences in diet between Olifantsbos and Cape Point. However, both the richness and diversity of the diet was reduced for the deeper station. This change was more pronounced at Olifantsbos. At both sites, fish became increasingly more important in the diet with depth.

Future studies into rock-lobster diets need to take into consideration factors such as capture depth, moult stage and rock-lobster size when making comparisons. Thus any geographical comparisons of rock-lobster diet, or studies on interactions between diet and growth must be conducted in a narrow depth range, over a short period of time and be restricted to a narrow rock lobster size class if valid comparisons are to be made. These standardised comparisons form the body of the following chapter, where they are related to growth rate.

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Chapter 4

West Coast rock lobster diet: comparisons between geographic locations with different rates of growth and benthic community composition

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1. INTRODUCTION

It has long been known that along the West Coast of South Africa, there are areas where the growth of the rock lobster, *Jasus lalandii*, is fast (high growth, FG) and other areas where it is slow (low growth, SG) (Goosen & Cockcroft 1995). However, in 1988, a dramatic decline was observed in the overall growth rate of *J. lalandii* (Goosen & Cockcroft 1995) which was subsequently shown to be more serious than originally thought (Crywagen 1997). This appeared to be a coast-wide phenomenon (Melville-Smith *et al.* 1995, Pollock *et al.* 1997), and was associated with a concurrent increase in the frequency of 'negative growth' in male rock lobsters (Cockcroft & Goosen 1995). Only during the 1995/1996 fishing season did the growth rate show signs of a recovery (Sea Fisheries Research Institute, Cape Town, unpublished data). Slow rock-lobster growth severely affects the commercial rock lobster fishing industry because growth of male rock lobsters regulates the productivity of the resource (Bergh & Johnston 1992) and hence the exploitable biomass (which translates to the total allowable catch, TAC).

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Two theories have been proposed to account for the observed depression of growth rate. The first suggests that an increased prevalence, and a change in the distribution, of low-oxygen water has led to the slower growth rate (Pollock 1987, Pollock & Shannon 1987, Tomalin 1993). Chronic exposure to water low in dissolved oxygen appears to have serious consequences for the growth rates of rock lobsters (Beyers 1979, Beyers *et al.* 1994). Reduced growth rates and higher natural mortalities of *Panulirus longipes cygnus* have been demonstrated during conditions of low-oxygen (Chittleborough 1975), and similar responses occur in juvenile *J. lalandii* (Beyers *et al.* 1994). Furthermore, *J. lalandii* has frequently been observed aggregating at very shallow depths (1 - 4m) on the Cape West Coast in times of low oxygen concentration (Pollock & Beyers 1981). Consequently, large numbers of rock lobsters are forced to forage in a small area, resulting in a lower *per capita* food intake and leading to a density-dependent reduction in the growth rate. A similar idea has been postulated for *P. gilchristi*, for which slow growth occurs in an area with the highest CPUE (Groeneveld 1997). It is also possible that *J. lalandii* may simply stop

feeding under sub-optimal oxygen conditions, as shown for the Norway lobster (*Nephrops norvegicus*), which seems to die of starvation rather than from a lack of oxygen (Baden *et al.* 1990). Of nearly 45 rock lobster stomachs examined during a red tide event in 1997, only 2 rock lobsters had any food remains in their stomachs (Mayfield & Branch, unpublished data).

An alternate hypothesis is that the observed drop in annual growth increment is linked to food availability. Several studies have indicated that food availability is an important factor - possibly even the major one - regulating growth rate (*e.g.* Newman & Pollock 1974b, Chittleborough 1975, 1976, McKoy & Esterman 1981, Pollock & Beyers 1981, Beyers & Goosen 1987). An apparent reduction in mussel recruitment on the West Coast as a direct result of changes in the normal wind patterns because of the 1990/1991 *El Niño* was postulated to have initiated the period of slow *J. lalandii* growth observed recently. It follows that the abundance and quality of food resources available to *J. lalandii* may have been lower during the period of slow growth (1988 - 1996) than in previous years (Pollock *et al.* 1997). Although lobsters from areas with contrasting growth rates have been shown to feed on different prey items, probably as a result of differences in benthic community structure (Newman & Pollock 1974b, Pollock *et al* 1982, Joll & Phillips 1984), the link between growth rate, rock-lobster diet and food availability has not yet been clearly established (Joll & Phillips 1984, Elner & Campbell 1987), especially for *J. lalandii.*

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This chapter considers the diet of rock lobsters from eight areas of contrasting rock-lobster growth rate (5 FG, 3 SG) using data collected during the austral summer of 1994-1995. Subsequently, a detailed comparison is made between the diets of both immature and adult male rock lobsters at two sites each (1 FG, 1 SG), for which information on the composition of the benthos was obtained. On the assumption that food availability influences rock-lobster growth rate, it was predicted that: (1) the proportion of rock lobsters feeding and the gut fullness indices would be consistently higher in fast-growth areas when compared with slow-growth areas; (2) rock-lobster diet would be different between areas of contrasting growth rate, with rock lobsters in slow-growth areas consuming more foods of 'poor' quality; (3) the rock-lobster diet

should reflect the composition of the benthic community - *i.e.* assuming rock lobsters are non-selective feeders; and (4) there should be a higher biomass of edible (defined here as nutritionally beneficial) benthos in areas of fast growth than those of slow growth.

2. METHODS

Sixty male rock lobsters (70 - 80mm carapace length (CL)) were collected from each of eight sites (FG: Cape Point; Robben Island; Dassen Island; Vondeling Island and Elands Bay; SG: Olifantsbos; the Knoll and North Blinder, Figure 4.1) during a short time period during December 1994, the summer period, when energy reserves are being accumulated (intermoult period, Cockcroft 1997). Slow-growth areas were defined as those in which the average annual moult increment of 70-79mm CL *J. lalandii* was less than 2mm over the previous five years (data from Goosen & Cockcroft 1995). Additional adult rock lobster stomach samples were collected from Dassen Island (FG) and Olifantsbos (SG), also during summer, in November 1994 and February 1995, and juvenile rock lobster stomach samples obtained from the Cape Town harbour wall (SG) and Mouille Point (FG) during September 1997. All rock lobsters were caught at night (because of their crepuscular feeding pattern Paterson (1960)), using bait-protected long-line commercial rock-lobster traps in 15-20m water depths, with the exception of the November 1994 and February 1995 collections at Dassen Island which were conducted at dawn using SCUBA in the same depth range. All stomach samples of immature rock lobsters were collected using SCUBA in 5-7 meters of water. Dietary differences between diver- and trap-caught rock lobsters have been shown to be minimal (Griffiths 1996). Rock lobsters were anaesthetised by immersion in a water-ice slush, exsanguinated, and their stomachs removed. The first 30 full gastric-mill (stomach) samples were frozen at -20°C for further analysis. Full guts were defined as those with a bolus of at least $1cm^3$ (adults) and $0.5cm^3$ (juveniles).

All rock-lobster stomachs were weighed (wet weight) before and after the removal of the bolus to determine the fullness of the digestive tract (Berg 1979), following

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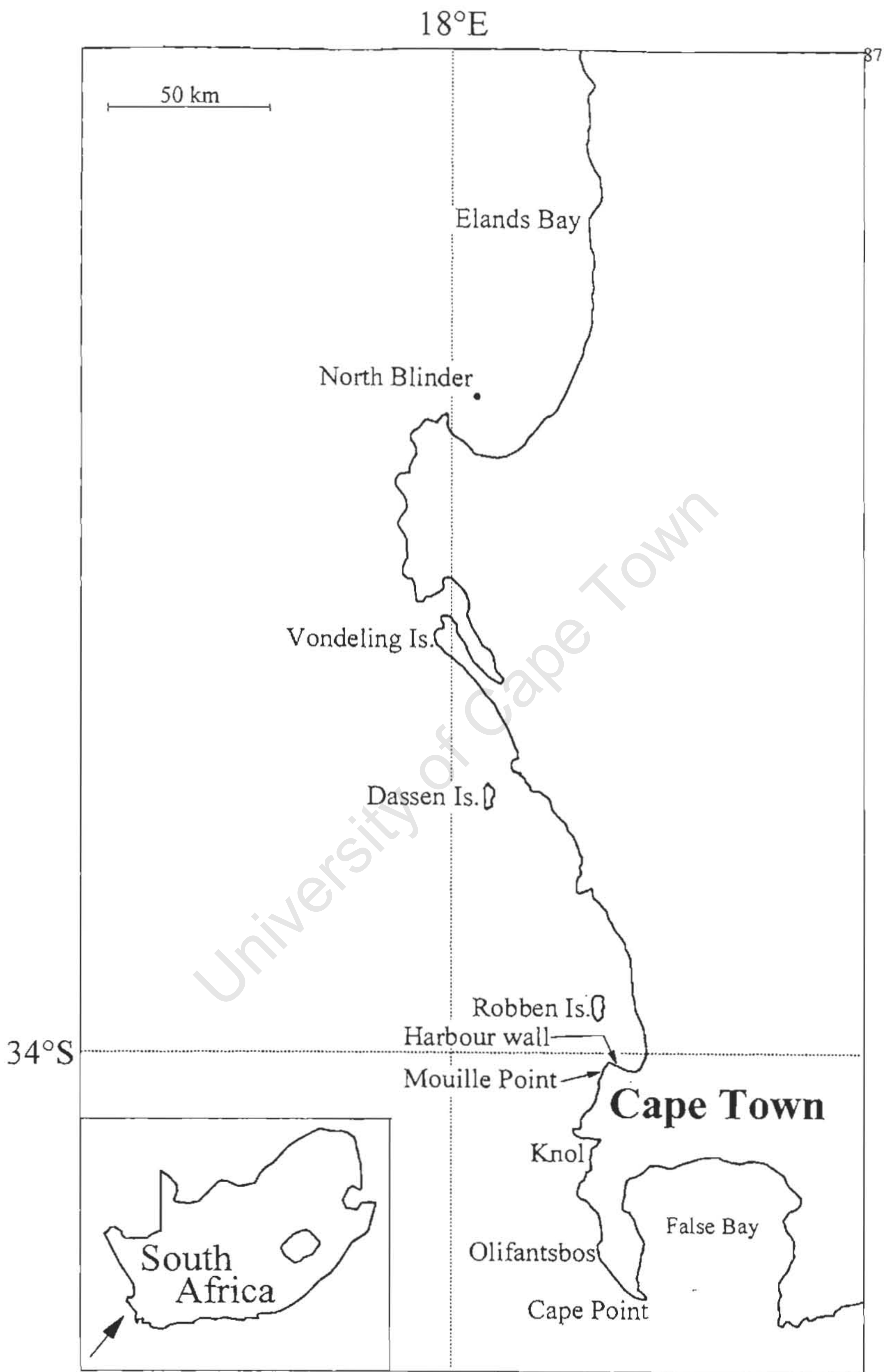

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Figure 4.1: Map of the West Coast of South Africa to show the locations of the 10 sites sampled.

removal of excess water by blotting the stomachs on absorbent paper (Hyslop 1980). The bolus was then flushed into a Petri dish, spread out, and examined using a Nikon binocular dissecting microscope (8 X magnification) to identify diagnostic fragments from prey remains. Gut contents were assessed visually and the percentage contribution of each prey species to the total gut volume was estimated (after Hyslop 1980, Williams 1981). The gut fullness index was taken as:

$$\frac{\text{total stomach weight (g) - stomach membrane weight (g)}}{\text{total stomach weight (g)}} \times 100$$

The proportion of rock lobsters in the sample that were feeding was obtained by:

$$\frac{\text{number of full stomachs obtained}}{\text{number of rock lobsters examined}} \times 100$$

The frequency of occurrence of each prey item (i) was calculated after Berg (1979):

$$\frac{\text{number of stomachs with item } i}{\text{number of stomachs examined}} \times 100$$

Differences in mean gut fullness were tested between the eight sites sampled using a one-way analysis of variance (ANOVA), after testing for homoskedasticity using Bartlett's test (Zar 1984). Post-hoc comparisons were done using a 95% Newman-Keuls Multiple Range Test. Gut fullness indices were combined from all slow and fast growth sites respectively and compared using a Students t-test (homoskedasticity shown: F test, $F_{104,59} = 1.44$, $P > 0.05$, Zar 1984).

For each bolus examined, the estimated proportional contribution of each prey item to the total volume of the stomach contents was multiplied by the bolus weight (g) to give an estimate of individual prey species wet mass. Bray-Curtis similarities (based on prey wet mass estimates and following root-root data transformation) were calculated to compare the groupings of the diets from each of the eight sites (Primer v4.0, Plymouth Marine Laboratory) and an MDS plot drawn using only the information of every second rock lobster to avoid the large number of samples

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obscuring interpretation. This was a systematic sample from a haphazardly distributed population and thus approximates a random sampling procedure.

Dietary data for Olifantsbos and Dassen Island from November 1994 and February 1995 were initially assessed for within site consistency and between site differences using a log-likelihood (G) ratio for contingency table analysis (which approximates the χ^2 distribution) after Zar (1984). Thereafter the data were treated as described above and analysed together using Analysis of Similarity (ANOSIM, α set at 1% due to the small sample sizes), Bray-Curtis similarity dendrograms and a MDS plot (Primer v4.0, Plymouth Marine Laboratory). Prey preference and randomness of feeding for both adult and juvenile rock lobsters were assessed using a χ^2 contingency table (after Schneider 1981, Zar 1984) based on the null hypothesis (H_o) that the proportion of foods in the stomach contents would equal their proportional abundance in the available benthos. Dietary data for juveniles collected at Mouille Point and the harbour wall were also analysed using a Bray-Curtis similarity dendrogram and MDS plot as described above, and prey species composition compared using a log-likelihood (G) ratio for contingency table analysis.

The benthos at Olifantsbos and Dassen Island was sampled during December 1994, using an underwater monochrome video camera (after Carleton & Done 1995, Hughes & Atkinson 1997). Briefly, the camera (Panasonic CCD B/W, WV-BL90, LENS = WV-LA4R5A (4.5mm, f1.4)) was mounted in a stainless steel frame, tethered to the ship and linked to a ship-board black and white monitor through a video-tape recording system (Mayfield *et al.* in prep.). The image from the camera encompassed a quadrat of 0.125m^2. As the camera was lowered to the sea floor, an overall view of the reef was recorded to identify species that would not be observed at the quadrat scale, such as kelp and rock lobsters. Fifteen quadrat images were recorded from Dassen Island and 29 from Olifantsbos. These images were viewed on a large-screen television and percentage cover estimates assigned to each species in each quadrat. The percentage cover estimates were subsequently converted to biomass values (after Velimirov *et al.* 1977, Field *et al.* 1980). Benthic data for the harbour wall and Mouille Point were obtained by SCUBA divers. Using a quadrat (1000cm^2),

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the percentage cover of each species comprising each quadrat was estimated. Some values exceeded 100% due to secondary cover (primarily of barnacles) also being included. The percentage cover estimates were converted to biomass as described above.

A comparison between the benthos of Dassen Island and Olifantsbos was made both on the basis of species abundance, and by using a Bray-Curtis similarity cluster analysis (based on species biomass) following root-root data transformation to compare the groupings of the video image samples from the two localities. An MDS plot was constructed and SIMPER analysis used to identify species responsible for the observed groupings. The data for Mouille Point and the harbour wall were analysed using a Bray-Curtis similarity dendrogram, MDS plot and SIMPER (Primer V4.0, Plymouth Marine Laboratory). An analysis of variance (ANOVA) was employed to compare differences in the available edible biomass across all four sites (Homoskedasticity shown: Brown-Forsythe test, $F_{70,3} = 1.15$, $P > 0.05$).

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3. RESULTS

3.1 Rock lobster diet

The percentage of adult rock lobsters with full stomachs did not vary between sites of contrasting growth rate, with both fast- growth and slow-growth sites exhibiting high and low percentages of rock lobsters feeding (Table 4.1). Furthermore, the average percentage of rock lobsters feeding at fast- and slow-growth sites was virtually identical. The gut fullness indices (Table 4.1) had equal variance (Bartlett's Chi-Squared test, $B_c = 12.381$, df = 7, $P > 0.05$) and the means varied significantly amongst the eight sites (ANOVA, $F_{7,157} = 7.26$, $P < 0.01$). However, rock lobsters from fast-growth sites did not all exhibit high gut fullness indices, nor did those from slow-growth sites consistently have low indices. Pooling data for all individual rock lobsters for fast- and slow-growth sites respectively, showed that the average gut fullness observed in rock lobsters caught from slow-growth sites (52.1%) was significantly different (Students *t*-test (2-tailed), $t = 2.56$, df = 163, $P < 0.05$) to that for fast-growth sites (44.7%).

Table 4.1: Proportion of lobsters with full stomachs, gut fullness indices and results of the Newman-Keuls *post hoc* comparison for the stomach contents from lobsters collected at eight sites.

		% Rock lobsters	Gut fullness index			Newman-Keuls multiple	
Locality	Growth Rate	with full guts	Mean	SE	n	range test	
North Blinder	SG	39	35.22	4.77	9	*	
Knol	SG	64	54.76	2.42	29		*
Olifantsbos	SG	58	55.51	3.37	22		*
Elands Bay	FG	62	35.07	3.72	28	*	
Vondeling Is.	FG	68	37.77	3.33	22	*	
Dassen Is.	FG	65	51.37	4.91	19		*
Robben Is.	FG	47	58.72	2.75	18		*
Cape Point	FG	27	47.06	3.09	18	*	*
Overall mean	SG	53.6	52.10	2.01	61		
Overall mean	FG	53.8	44.70	1.85	105		

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The Bray-Curtis similarity analysis of diet showed no apparent within site or within growth-rate groupings when the diets of rock lobsters collected from the eight sites were compared (Figure 4.2). Many groups were apparent in the dendrogram, with 16 outliers. All the groups showed a mix of stomach samples obtained from rock lobsters collected from both fast- and slow-growth areas. Because of the high number of samples (n = 177), the MDS plot was based on the data obtained from every second rock lobster (Figure 4.3), and supports the results of the dendrogram in that no clear groupings either within site or within growth rate were observed.

There was a geographic trend in the gut fullness index (Table 4.1), with the three lowest values being recorded for the northern-most sites. With one exception, this division was supported by the Newman-Keuls test. Analysis of the dominant components of the gut contents (Figure 4.4) failed to reveal any obvious differences between fast- and slow-growth areas, but, once again, there were geographic trends. The most striking change was the disappearance of black mussels (*Choromytilus meridionalis*) from the diet south of Dassen Island, where they were replaced by sponges. The ribbed mussel, *Aulacomya ater*, was a ubiquitous and dominant element in the diet. Sea urchins (*Parechinus angulosus*) were recorded at all but one of the sites (*i.e.* North Blinder - which also had, by far, the lowest diversity of prey). Cannibalism occurred at all sites except North Blinder, and there was no suggestion that it occurred more often at slow- *versus* fast-growth sites: indeed its mean contribution to the proportional frequency in the diet was considerably less at slow-growth (7.3%) than at fast-growth sites (20.5%).

The diets of adult *J. lalandii* at the two sites of contrasting growth rate (Dassen Island and Olifantsbos) for which benthic data were also obtained are shown in Figure 4.5. For both sites, at both times, the diet mainly comprised ribbed mussels, rock lobsters and barnacles (*Notomegabalanus algicola*), with large numbers of Olifantsbos individuals consuming fish, and barnacles much more prevalent in the diet at Dassen Island. The log-likelihood ratio showed that the diet of rock lobsters at each site was consistent through the sampling period (Dassen Is.: $G = 4.72$, $df = 7$, $P > 0.05$;

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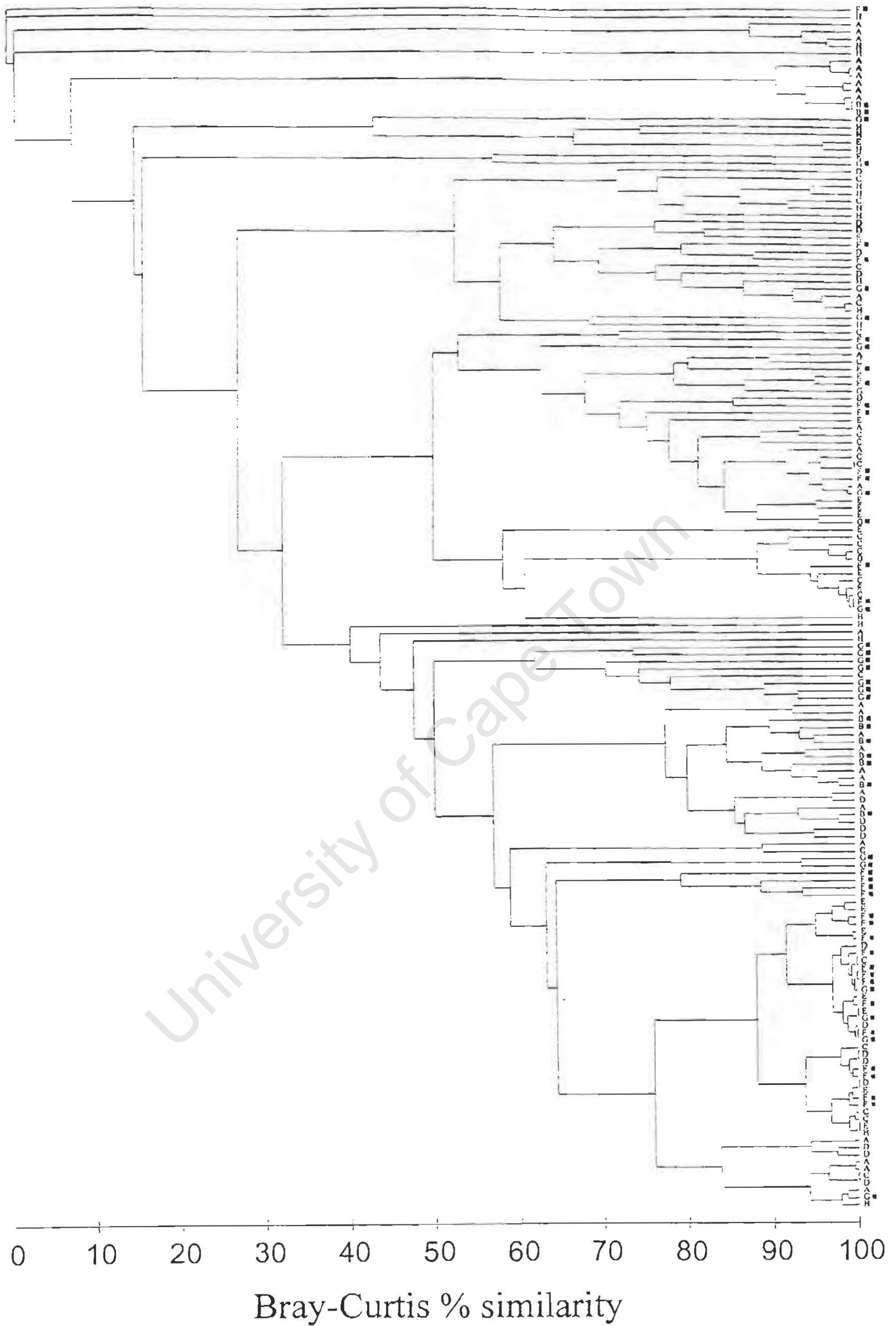

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Figure 4.2: Bray-Curtis similarity dendrogram based on the wet weight of prey items observed in the gut contents of rock lobsters caught from eight sites. The letters B, F, & G refer to slow-growth sites, and A, C, D, E, & H refer to fast-growth sites. Each letter refers to one rock lobster stomach examined and all slow-growth sites are marked with a shaded square next to the letter.

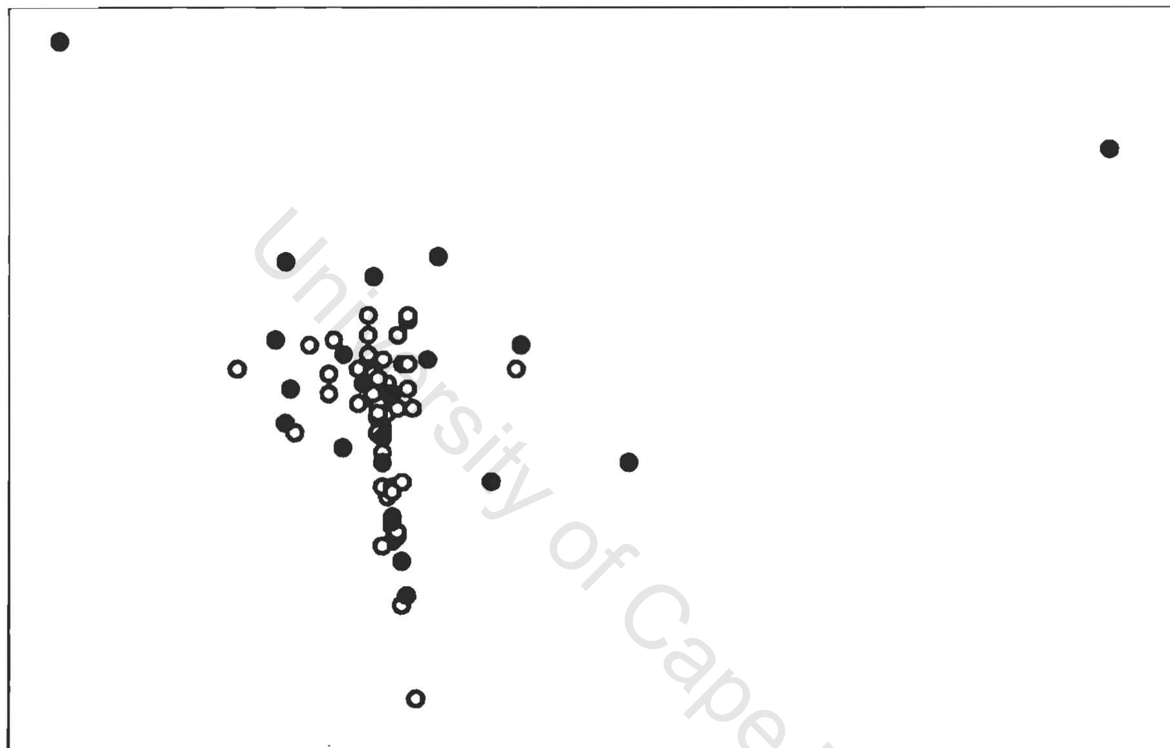

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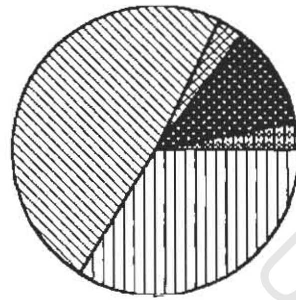
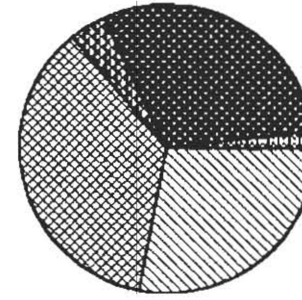
Figure 4.3: MDS plot to compare the groupings of the diet of lobsters caught from eight sites. Each dot represents one rock lobster stomach examined, but only every second rock lobster stomach is shown. Shaded dots refer to individuals from fast-growth sites.

Olifantsbos (SG)

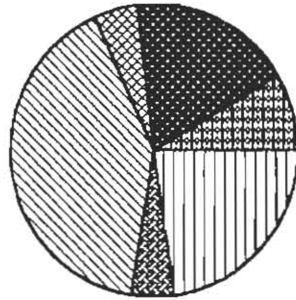
Dassen Island (FG)



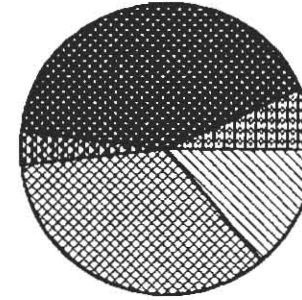
November 1994



November 1994



February 1995



February 1995

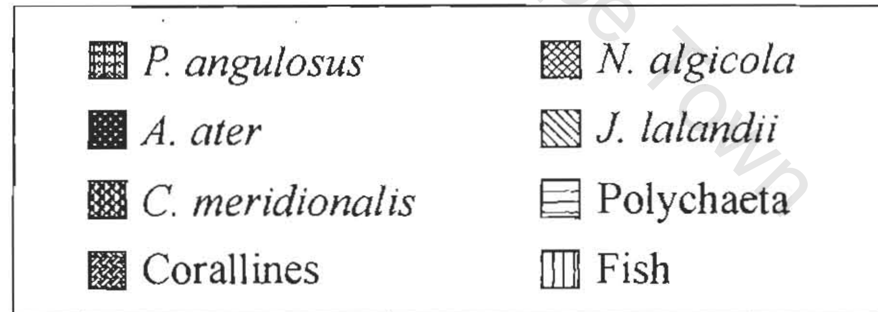


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Figure 4.5: Diet of rock lobsters from Olifantsbos and Dassen Island during November 1994 and February 1995, based on the frequency of occurrence index.

Olifantsbos G = 5.69, df = 7, $P > 0.05$), but when the two sets of data for each site were pooled and the similarity of the rock-lobster diet tested the diet was found to be statistically different at the two sites (G = 27.5, df = 7, $P < 0.05$). Alternate (and arguably stronger) multivariate analyses based on the wet weight of each prey species in the gut contents of individual rock lobsters gave a different result. Although the diets of rock lobsters collected from Dassen Island and Olifantsbos were statistically different (thus preventing pooling of the data) at the two sampling times (ANOSIM, Dassen Island: Global R = 0.09, $P < 0.01$; Olifantsbos: Global R = 0.049, $P < 0.01$), the diets of rock lobsters were statistically similar across the two sites for the February 1995 sample (Global R = 0.16, $P > 0.01$). Analysis of Bray-Curtis similarity suggested that rock lobsters at Dassen Island (FG) and Olifantsbos (SG), were eating similar foods in the same proportion (Figure 4.6). The dendrogram showed two groups separating with less than 20% similarity, and although group 'A' was dominated by stomach samples from Dassen Island, and group 'B' by samples from Olifantsbos, both groups were 'contaminated' almost 25% by samples from the other locality. This was further supported by the MDS plot which showed almost no separation between the two sites (Figure 4.7).

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The diet of juvenile rock lobsters collected from the harbour wall and Mouille Point was dominated by the ribbed mussel, with barnacles and sea urchins also being important dietary components (Figure 4.8). Bray-Curtis similarity analysis suggested the existence of three groups with less than 50% similarity, with groups 'A' and 'B' being homogeneous and group 'C' comprising 50% each of samples from the harbour wall (c) and Mouille Point (m, Figure 4.9). The corresponding MDS plot (Stress = 0.10) showed a similar discrimination but also a large overlap in the diet from the two sites (Figure 4.10). The chi-square analysis showed the composition of the diet from the two sites to be statistically similar (G = 5.1, df = 4, $P > 0.05$).

Although the mean gut fullness index for juvenile rock lobsters was greater for the slow growth site (49.7%) than for the fast growth site (46.3%), these values were not significantly different (Students t-test (2-tailed), $t = 0.421$, df = 22, $P > 0.2$).

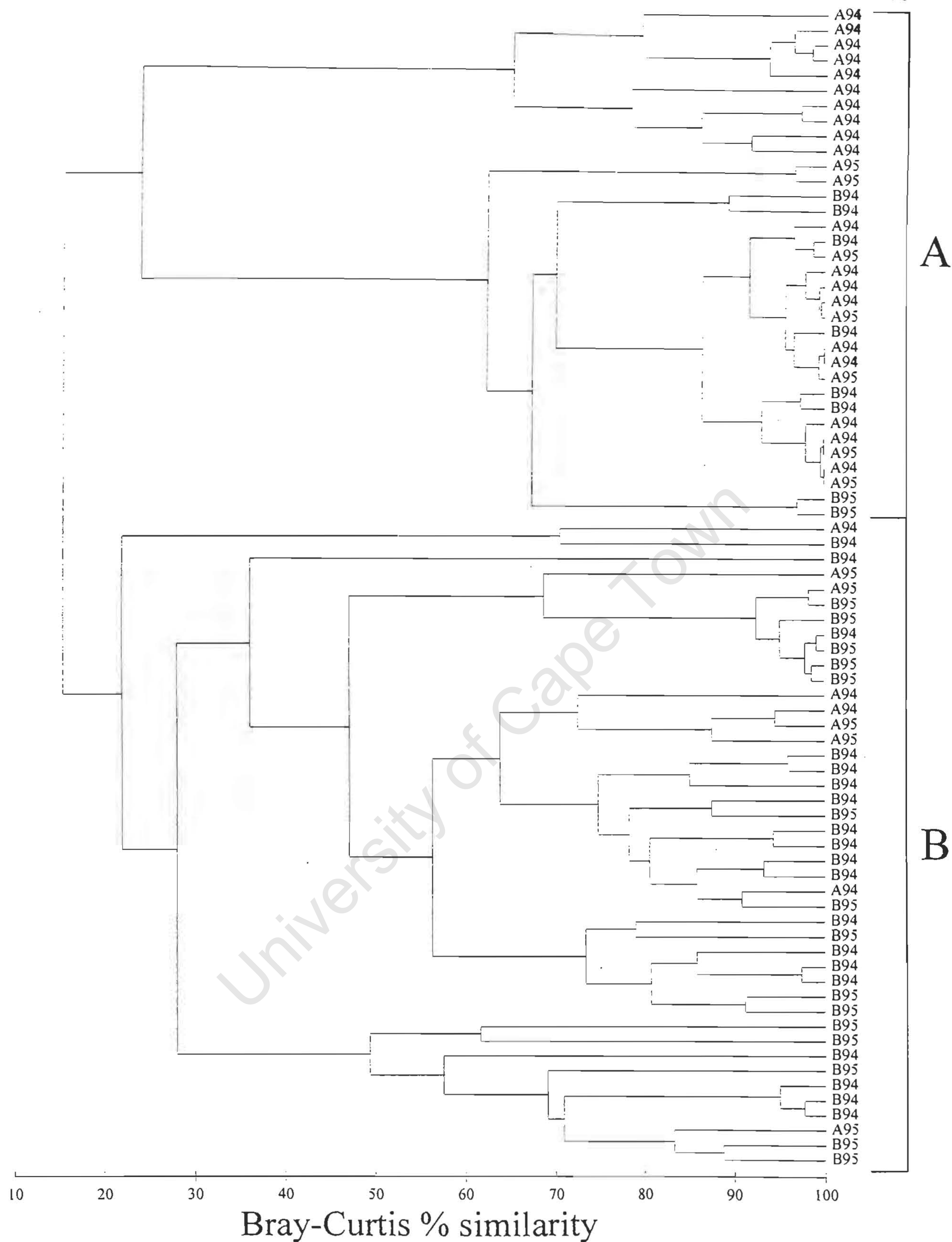

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Figure 4.6: Bray-Curtis similarity dendrogram based on the wet weight of prey items observed in the gut contents of rock lobsters caught from Dassen Island (A) and Olifantsbos (B). 94 and 95 refer to November 1994 and February 1995 respectively.

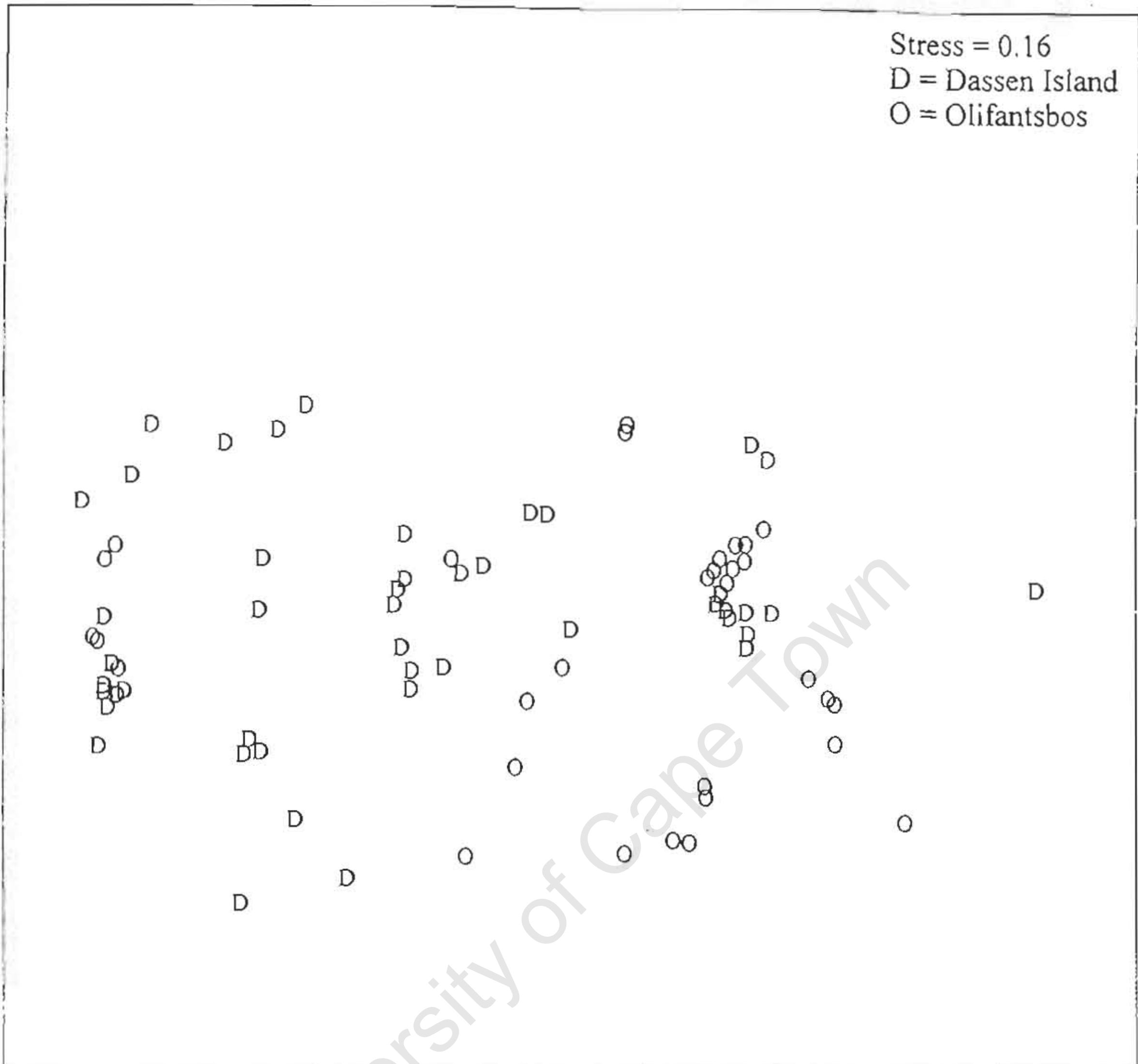

Figure 4.7: MDS plot to compare the groupings of the diet of rock lobsters caught from Dassen Island and Olifantsbos during November 1994 and February 1995 based on the wet weight of prey items observed in the gut contents.

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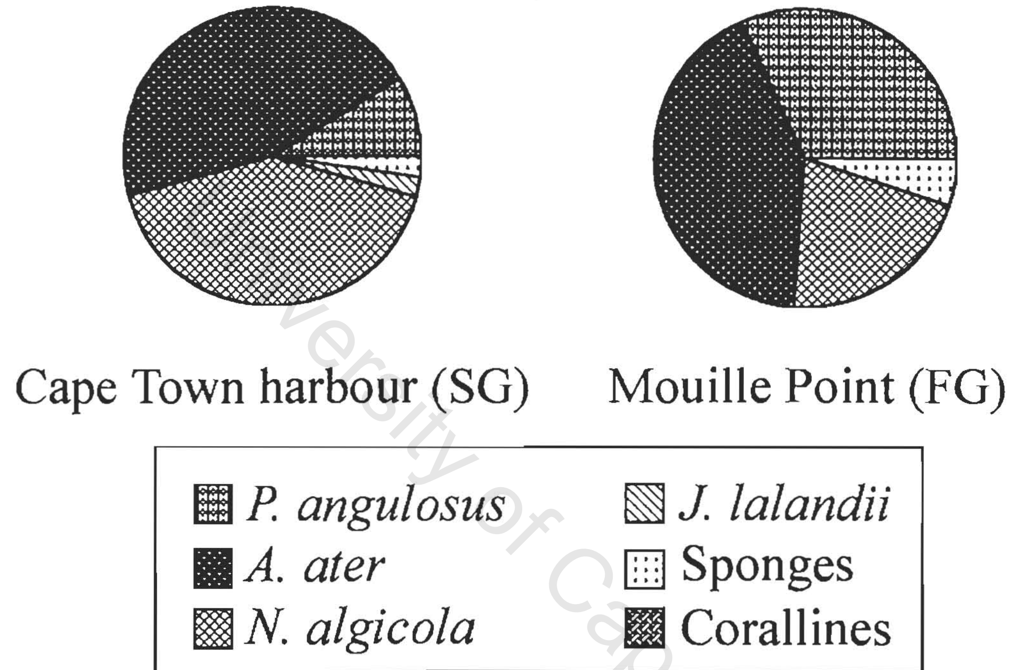

Figure 4.8: Diet of juvenile rock lobsters from the harbour wall and Mouille Point for September 1997, based on the frequency of occurrence index.

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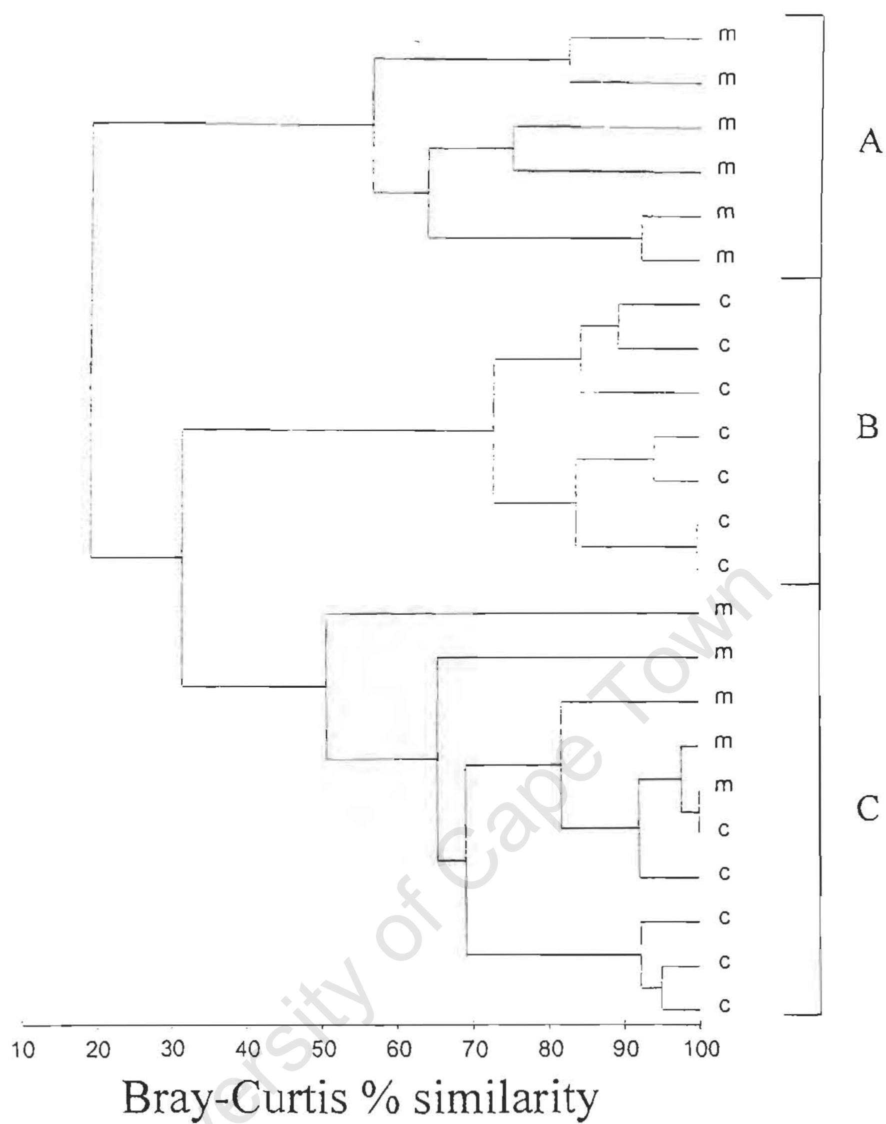

Figure 4.9: Bray-Curtis similarity dendrogram based on the wet weight of prey items observed in the gut contents of rock lobsters caught from Mouille Point (m) and the harbour wall (c) during September 1997.

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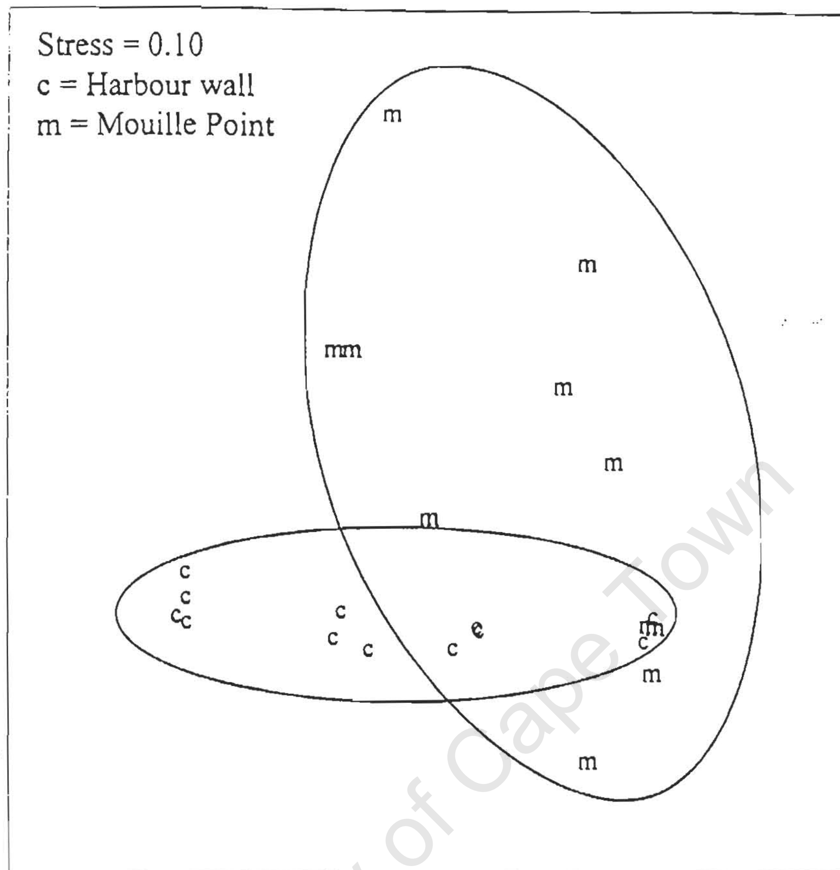

Figure 4.10: MDS plot to compare the groupings of the diet of rock lobsters caught from Mouille Point (m) and the harbour wall (c) caught during September 1997. The analysis is based on the wet weight of prey items observed in the gut contents.

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3.2 Benthos

Twenty-three species were identified from the analyses of the benthic video-image samples obtained from Dassen Island and Olifantsbos. Differences in the benthic community structure were apparent when comparisons were made on the basis of species abundance (Figure 4.11). The benthic community at Olifantsbos was dominated by *Tethya* sp. and other sponges, barnacles and encrusting coralline algae. In contrast, barnacles, mussels (*Aulacomya ater* and *C. meridionalis*) and foliar algae were the dominant macrobenthos at Dassen Island. The Bray-Curtis similarity dendrogram and the MDS plot support these findings (Figures 4.12 & 4.13 respectively). Four groups separating out with less than 50% similarity were evident on the dendrogram. Of these, groups 'A', 'B' & 'D' consisted primarily of samples collected at one of the two sites, while group 'C' contained samples obtained from both sites. The MDS plot suggests the groupings shown by the dendrogram are robust, as the Dassen Island and Olifantsbos samples group out separately, with very little overlap (Figure 4.13). The two sites were shown to be 67% dissimilar, with sponge, barnacles and encrusting coralline algae the species determining the majority (65%) of the observed dissimilarity (SIMPER analysis).

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Fewer species were identified in the benthic analysis of the Cape Town harbour wall and Mouille Point. These included coralline algae, barnacles, sea urchins, ribbed mussels, sponges and several species of foliar algae (Figure 4.14). The benthic community at Mouille Point was dominated by the ribbed mussel, with coralline algae and sea urchins being less common. By contrast, coralline algae, and to a much lesser extent, mussels and sea urchins comprised the major components of the benthic community on the harbour wall. SIMPER analysis suggested that the benthic communities of these two sites are 44% dissimilar, and this was reinforced by the Bray-Curtis dendrogram and MDS plot which show substantial separation between the two sites (Figures 4.15 & 4.16 respectively).

Biomass values (g) for the dominant benthic species (or groups) and the edible component of the benthic community are shown in Table 4.2 (wet mass.m^{-2}). What is most obvious from the data is that the biomass of mussels (a preferred prey species -

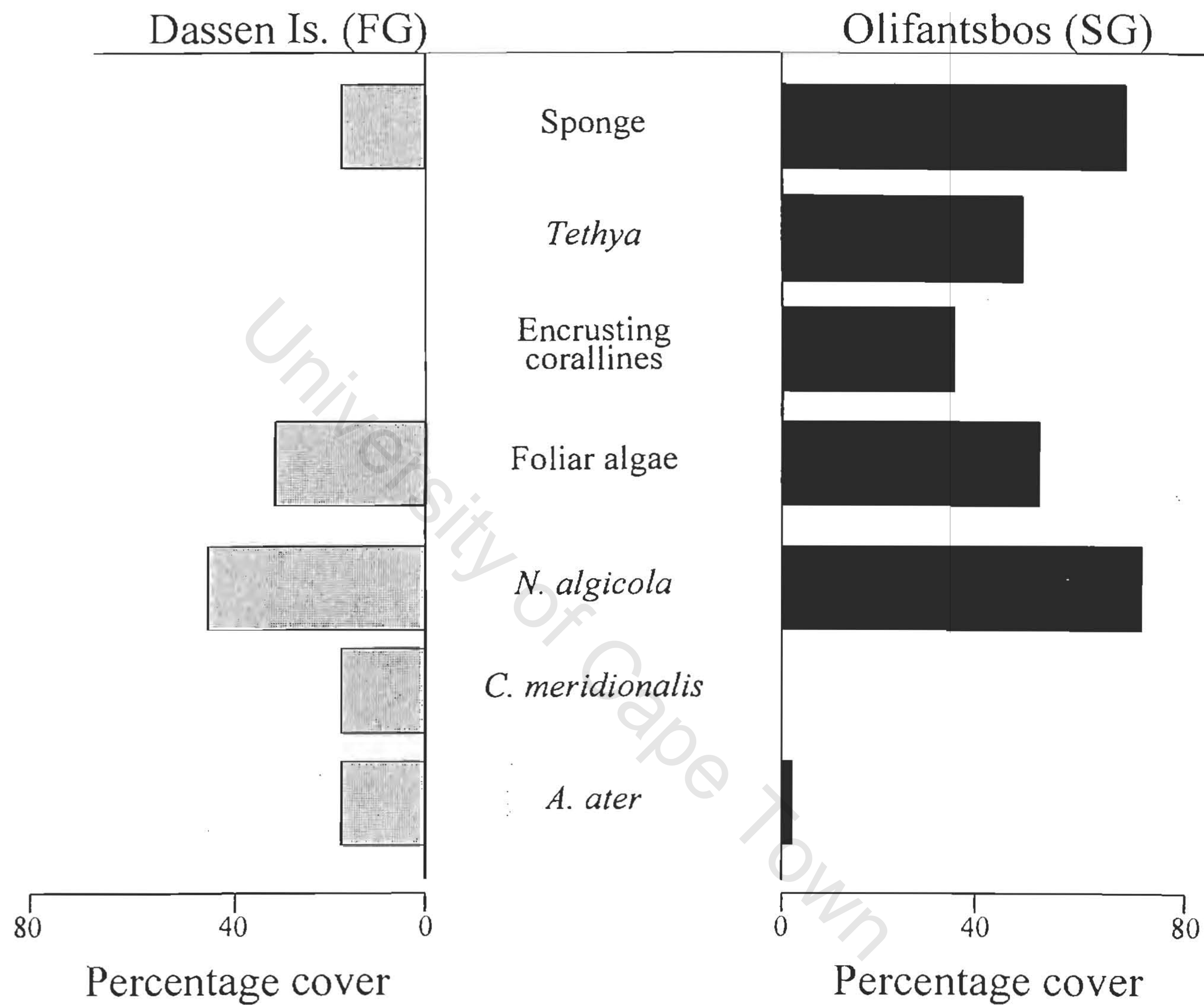

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Figure 4.11: Percentage cover of benthic organisms at Olifantsbos and Dassen Island determined from underwater video images.

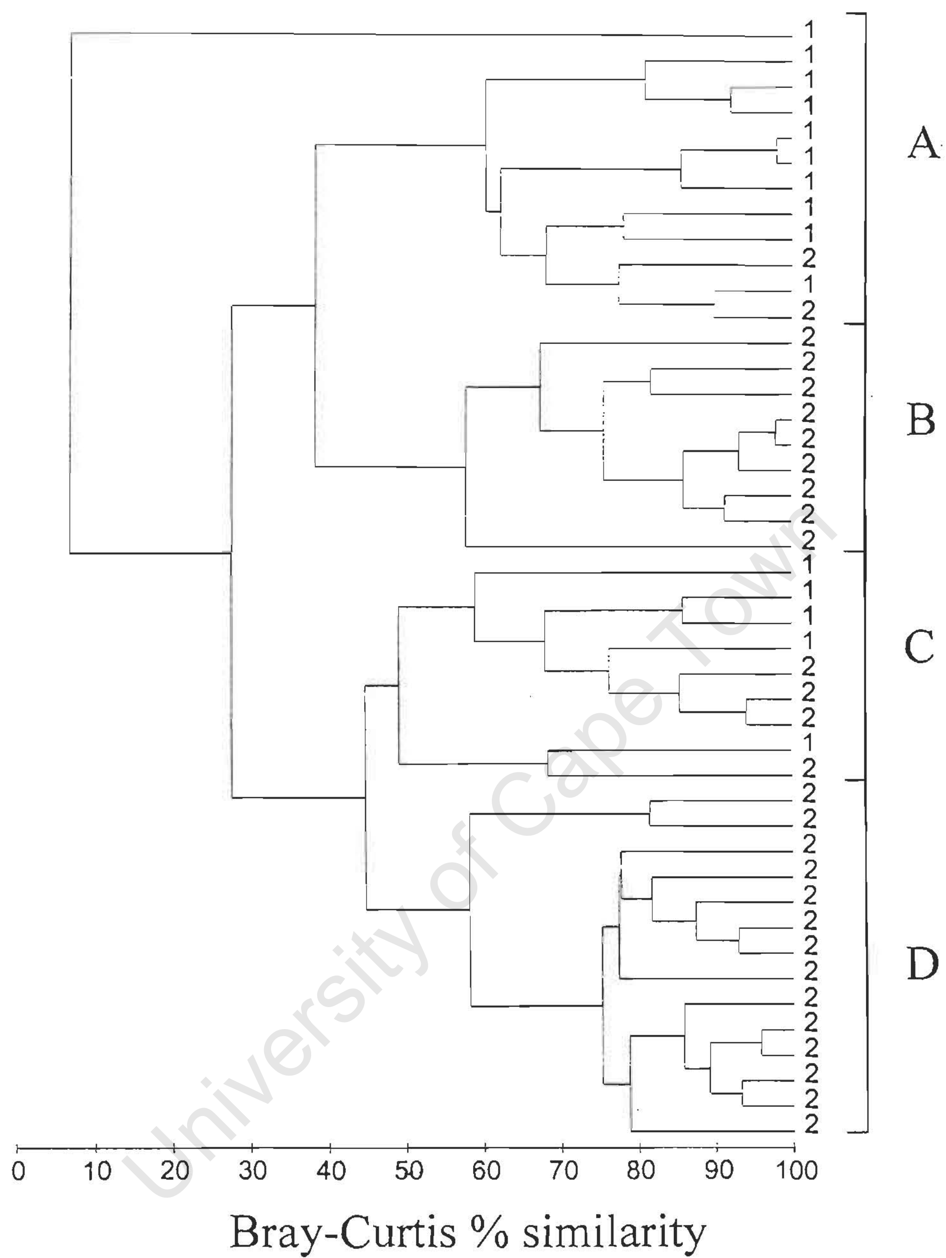

Figure 4.12: Bray-Curtis similarity dendrogram based on species biomass to compare the groupings of the benthic samples obtained from Dassen Island (1) and Olifantsbos (2).

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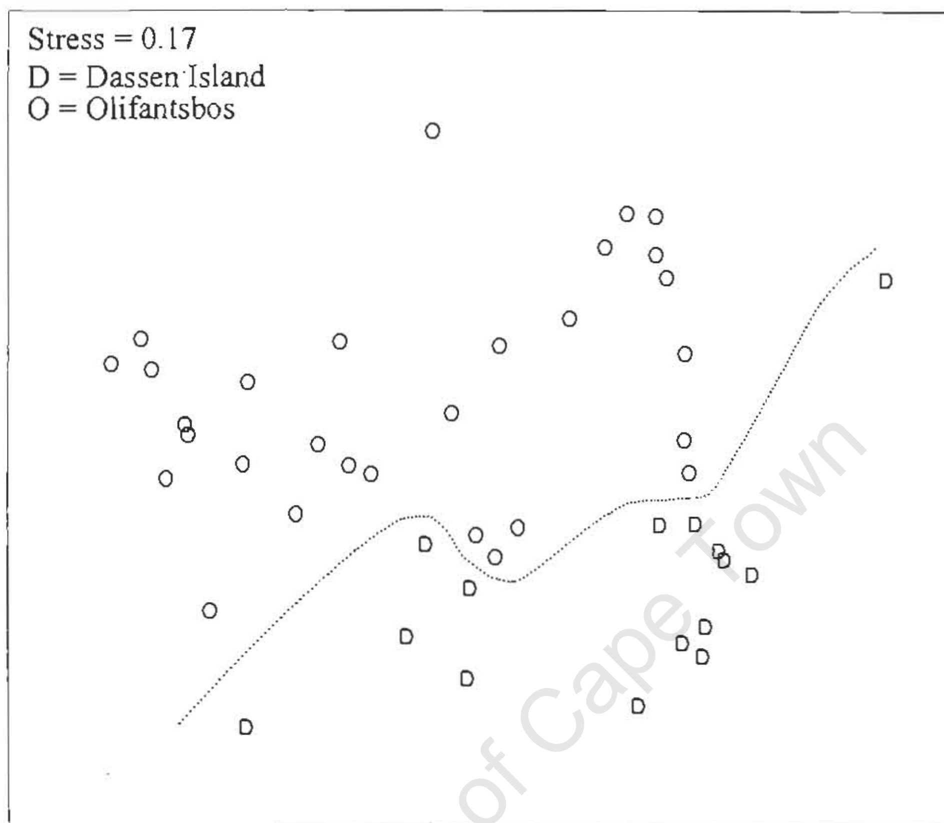

Figure 4.13: MDS plot to compare the groupings of the benthic samples obtained from Dassen Island and Olifantsbos. The analysis is based on species biomass.

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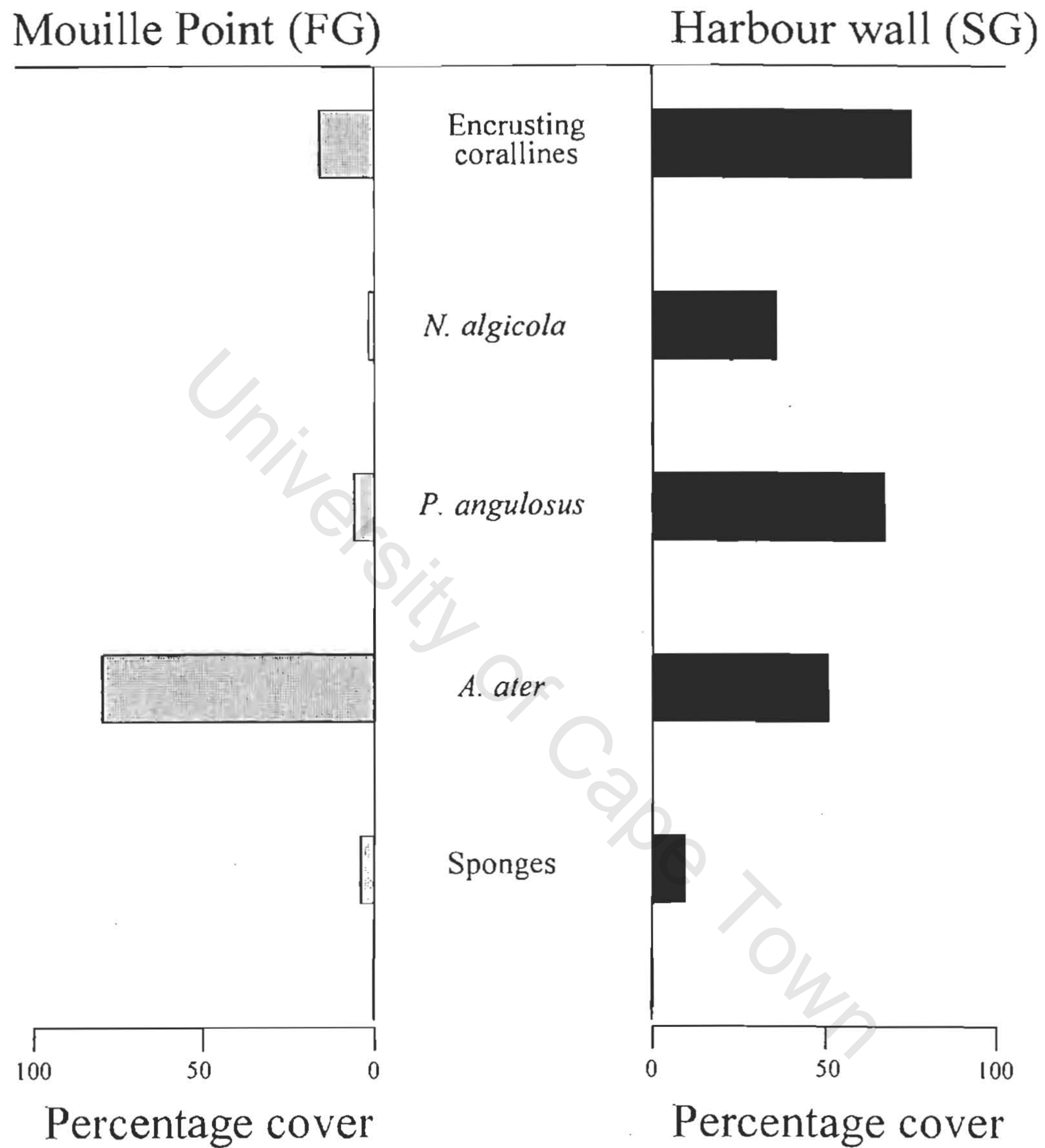

Figure 4.14: Average percentage cover of benthic organisms at the harbour wall and Mouille Point.

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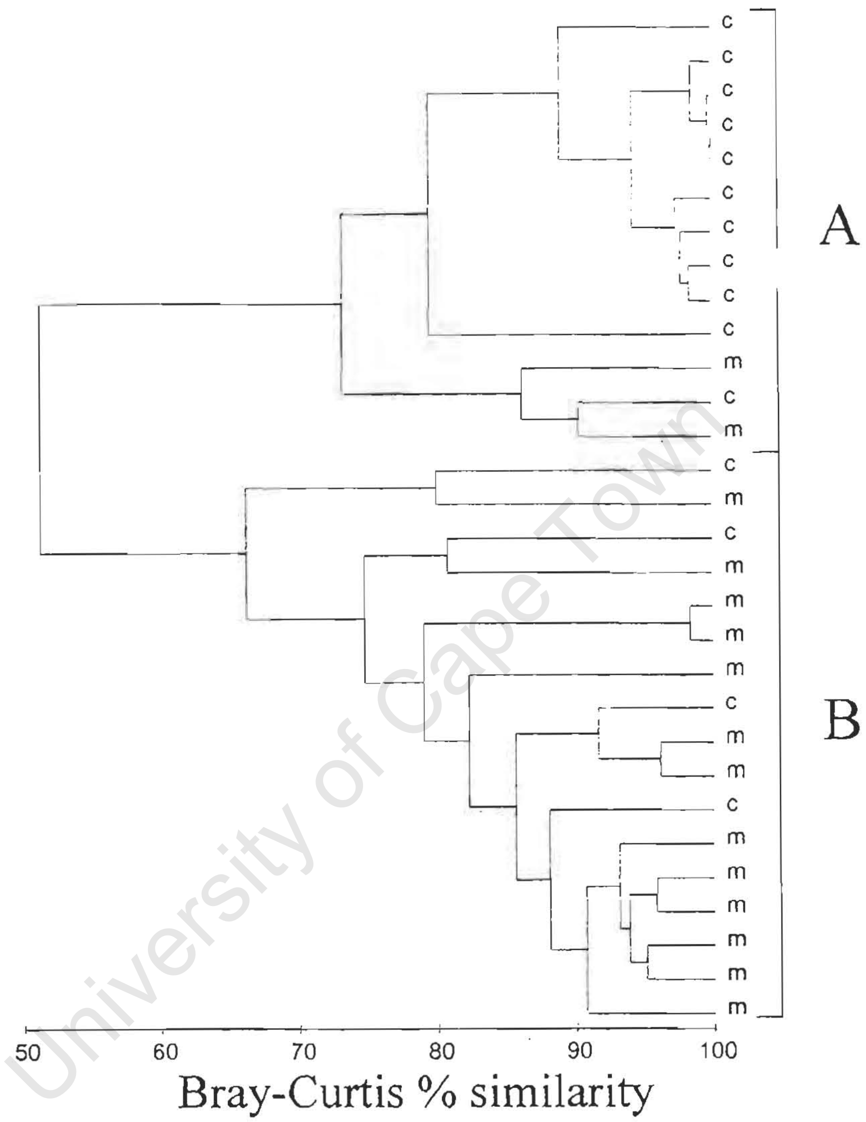

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Figure 4.15: Bray-Curtis similarity dendrogram based on species biomass to compare the groupings of the benthic samples obtained from Mouille Point (m) and the harbour wall (c).

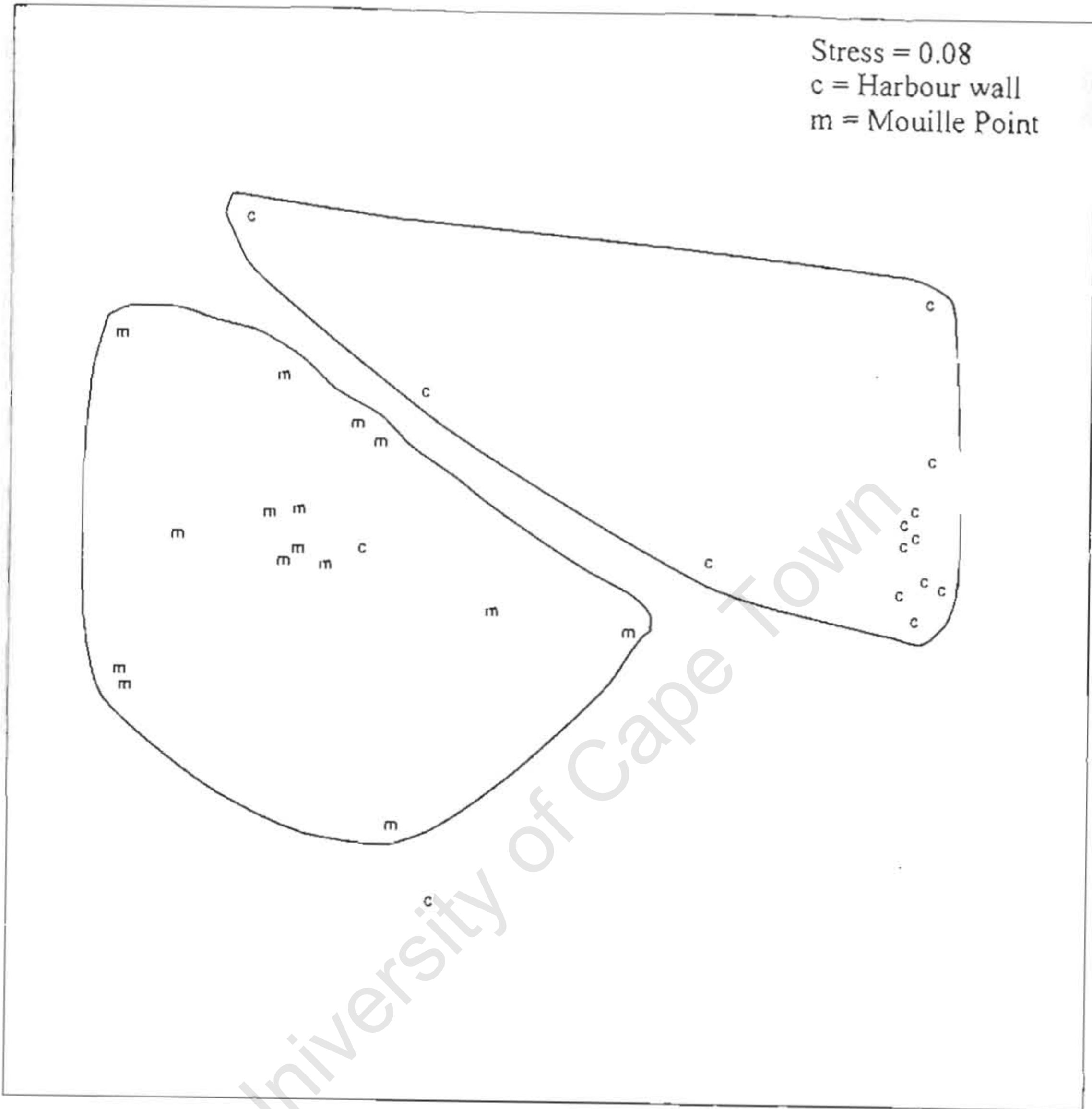

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Figure 4.16: MDS plot to compare the groupings of the benthic samples obtained from Mouille Point and the harbour wall. The analysis is based on species biomass.

Table 4.2; Biomass and standard error (in bold) of various potential prey species at the four sites studied (g wet mass.m^{-2}), including an estimate of the amount of edible prey. Benthic species marked with a * were considered edible. The Newman-Keuls multiple range test showed the average edible biomass at Mouille Point to be statistically greater than at the other three sites, which were statistically indistinguishable from one another.

		Harbour wall		Mouille Pt.		Olifantsbos		Dassen Is.	
		SG		FG		SG		FG	
	Coralline algae	2235	**289**	506	**192**	623	**188**	-	-
	Foliar algae	<1	**0.29**	1	**0.21**	<1	**0.2**	<1	**0.3**
*	*P. angulosus*	334	**75**	259	**51**	-	-	-	-
*	*A. ater*	826	**391**	3303	**279**	<1	**0.1**	97.8	**40**
*	*C. meridionalis*	-	-	-	-	-	-	64.3	**37**
	Sponges	36	**7**	16	**8**	1745	**430**	640	**273**
*	*N. algicola*	81	**34**	10	**7**	576	**132**	830	**168**
	Edible component	1247	**425**	3572	**277**	576	**132**	991	**173**
	Sample Number	15		15		29		15	

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than

see below and Chapter 5) was much higher at Dassen (FG) the Olifantsbos (SG) where adult rock lobsters were sampled, and higher at Mouille Point (FG) then the Harbour wall (SG) where juveniles were sampled. However, mussel biomass at the Harbour wall was nevertheless greater than at Dassen Island. A similar pattern was evident when the wet mass of the total edible component was compared between sites. ANOVA showed there were statistically significant differences between the edible benthic component of the four sites ($F_{70,3} = 29.04$, $P < 0.01$). However, the subsequent Newman-Keuls multiple range test showed that only Mouille Point differed from the other three sites.

3.3 Prey species selectivity

Relative to the available benthos, rock lobsters at Dassen Island, Olifantsbos, the harbour wall and Mouille Point were highly selective with respect to food choice (Olifantsbos: $\chi^2 = 55.61$, df = 2, $P < 0.01$; Dassen Island: $\chi^2 = 86.8$, df = 2, $P < 0.01$; harbour wall: $\chi^2 = 189.63$, df = 4, $P < 0.001$; Mouille Point: $\chi^2 = 282.03$, df = 4, $P < 0.001$). Juvenile rock lobsters consumed more barnacles and much less coralline algae than was predicted (null hypothesis: rock lobsters are non-selective foragers). Adult rock lobsters at Dassen Island and Olifantsbos were actively selecting ribbed mussels with barnacles (abundant in the benthos) being under-represented in their diets.

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4. DISCUSSION

Two sets of questions can be addressed by the data. First is a series of questions relating to the diet of rock lobsters - does food intake and dietary composition differ between areas of fast *versus* slow growth; failing this, are there any geographical patterns in diet? Second, in relation to the benthos - are there differences in the edible benthos available to rock lobsters in fast- *versus* slow-growth areas and do the diets of these rock lobsters reflect the general availability of prey?

A crude indication of the frequency of feeding can be obtained by calculating the proportion of animals collected with full stomachs. This index has largely been

ignored in most studies on rock-lobster diet, but it remains a valuable indicator of the level of feeding activity. It does have some limitations however, being influenced by environmental conditions (including whether the rock lobsters can physically forage as well as water clarity, temperature and dissolved oxygen concentration), as well as feeding periodicity and patterns (Paterson 1960, Zoutendyk 1988b) and stage in the moult cycle. Because rock lobsters are considered opportunistic feeders (Weiss 1980, as cited by Lawton & Lavalli 1995), it was predicted that the index would be consistently higher in fast-growth areas, which were also predicted to have abundant food. In other words, if rock lobsters are food limited, then fast-growth areas should be characterised by a high abundance of preferred prey, and this should be reflected by a high value for the feeding-proportion index. However, at the FG and SG sites, there were no obvious differences in the percentage of animals with full guts (SG: mean = 53.6, n = 3; FG: mean =53.8, n = 5), although more regular sampling or the sampling of more sites may have elucidated differences. In their study on *Panulirus cygnus*, Joll & Phillips (1984) demonstrated that consistently higher numbers of individuals were feeding at a slow-growth site than at an adjacent fast-growth site. They attributed this result to the need for rock lobsters to consume more and feed more frequently in a poor food environment (SG site). Rock lobsters at both fast- and slow-growth sites appear to be equally successful in finding food (as evidenced by the equal proportions of rock lobsters with full guts), but it may just take longer to obtain a full meal at the SG sites.

Digestive tract fullness, or the gut fullness index, gives a more refined indication of food uptake, as it is influenced by several factors, including competition for food, food shortage, health of the individual and environmental conditions (Berg 1979). However, it fails to consider both changes in digestion rate with temperature and differential rates of digestion of different prey items (Ennis 1973). In the case of *J. lalandii*, average gut fullness ranged from 35% to 59% and was significantly different between fast- and slow-growth sites when data were pooled ($P < 0.05$), with gut fullness being higher at the slow-growth sites. These data are comparable to those obtained for *Homarus americanus*, which exhibited gut fullness indices between 29.7% and 57.9% (Elner & Campbell 1987). If food availability in the environment is

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important in determining the growth rate of rock lobsters, and preferred food is generally 'rare' at the slow-growth sites, the rock lobsters in these areas may need to feed to a high level of gutfullness (*i.e.* to gluttony) whenever they encounter any suitable prey - whereas individuals in fast-growth areas may only need to feed to satiation as food will always be available when desired.

Bray-Curtis similarity and MDS analyses both suggested that there were no consistent differences in rock-lobster gut contents between sites of contrasting growth rate (Figures 4.2 & 4.3), a conclusion supported by the frequency of occurrence of prey items encountered at these sites (Figure 4.4). Although it was predicted that diet would differ between areas of contrasting growth rate, rock lobsters from FG *versus* SG sites showed similar dietary patterns. The one site that stood out as being 'different' was North Blinder which is currently recovering from complete benthic devastation following a black tide event in St. Helena Bay during April 1994 (Matthews & Pitcher 1996). Rock lobsters from North Blinder showed the lowest diversity in terms of dietary items, which is most probably due to the low species richness of the benthic community that presently consists primarily of ribbed and black mussels. At all other sites, dietary diversity was high, and although some prey items were rare in the stomach contents, and thus not reflected in Figure 4.4, up to 10 prey species were encountered per site.

There were, however, some trends in *J. lalandii* diet over the geographical range of the samples (Figure 4.4). There was an almost complete loss of black mussels from the stomach contents of animals south of Dassen Island with replacement by sponges, a result similar to that of Barkai *et al.* (1996). The abundance of subtidal sponges has been shown to be higher in the South (Field *et al.* 1980). Furthermore, cannibalism was greater South of North Blinder, and the amount of food consumed (gut fullness), was also higher in the four southernmost sites (Table 4.1). Barnacles were present in the rock-lobster gut contents at five of the eight sites sampled. Sea urchins were a common prey item in the stomach samples from all sites excluding North Blinder at which they were not part of the available prey spectrum (pers. obs.).

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The results of dietary analysis of *J. lalandii* in the present study do not differ markedly from those of previous investigations (*e.g.* Heydorn 1969, Newman & Pollock 1974b, Pollock 1979, Barkai & Branch 1988a, Barkai *et al.* 1996). Dominated by ribbed mussels, the diet also included sea urchins, black mussels, sponges and crustacean remains, especially *J. lalandii.* Despite their importance in the diet of the East Coast rock lobster, *Palinurus delagoae* (Berry 1971), barnacles had not been reported as a dietary constituent for *J. lalandii* prior to 1988 (see *e.g.* Heydorn 1969, Newman & Pollock 1974b, Pollock 1979, Pollock *et al.* 1982). Nevertheless, they constituted a major component of the dietary items in the present study. This supports more recent work (Barkai & Branch 1988a, Barkai *et al.* 1996) which found barnacles to be an important prey item for *J. lalandii*, as well as a component of the gut contents of the introduced European shore crab, *Carcinus maenas* (Le Roux *et al.* 1990). The abundance of sea urchins encountered in the stomach contents of this survey conflicts with previous findings, which recorded their scarcity in stomach samples (*e.g.* Heydorn 1969, Pollock 1979, Barkai & Branch 1988a) or even absence from them (Pollock *et al.* 1982). The apparent increase in the amount of barnacles and sea urchins consumed may reflect a reduction in the availability of mussels, generally considered to be the preferred food source (Pollock 1979, Griffiths & Seiderer 1980, Pollock *et al.* 1982). This high occurrence of sea urchins in the diet may have serious implications for abalone. Juvenile abalone live almost exclusively beneath sea urchins (Day 1998, Tarr *et al.* 1996) from which they derive not only protection, but also food (Day 1998). Feeding preferences of rock lobsters and their predation on sea urchins, and the possible implications of this for abalone recruitment, form the basis of Chapters 5 & 6 respectively.

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When reviewing the diet of lobsters from other geographical regions, it appears that crustaceans, molluscs and echinoderms are generally the dominant prey items of this group (Ennis 1973, Elner & Campbell 1987, and see Lawton & Lavalli 1995 for a review). Only the Norway lobster, *Nephrops norvegicus*, differs in that polychaetes are dominant in their diet (Baden *et al.* 1990). Thus, the diet of *J. lalandii* seems consistent with that of most other lobsters from vastly different geographical regions. Most likely this is because these ubiquitous prey are common groups in most shallow

subtidal areas. The similarities are surprising because they include comparisons of species that are functionally rather different, embracing both spiny and clawed lobsters.

Thus far, it has been established that, irrespective of the growth rate exhibited by rock lobsters in different areas, they consume similar prey in approximately equal proportions. If prey availability differs from area to area, the relative consistency of the diet suggests that rock lobsters are selective foragers, and satisfy their dietary needs by consuming specifically targeted prey species. Perhaps then, the link between diet and growth rate is less important than the link between food availability and growth rate (Newman & Pollock 1974b). In other words, slow growth rate at particular locations may be due to a shortage of suitable, or targeted prey. Thus, living in a 'poor' food environment leads to energetically more expensive foraging, even if the rock lobsters succeed in filling their guts to an equal (or greater) amount when compared to conspecifics living in areas with a 'good' food source.

The relationship between growth rate and the availability of preferred prey items in the benthos has received little attention in the past, and can best be described as tenuous (Elner & Campbell 1987). However, simultaneous sampling of both rock-lobster gut contents and benthic community structure permits not only a closer examination of this relationship, but also information on prey item selectivity (*i.e.* prey choice). It was with this in mind that simultaneous surveys were made of both the benthos and the diets of rock lobsters at four sites - Olifantsbos (SG), Dassen Island (HG), Mouille Point (HG) and the Cape Town harbour wall (SG). Diet analysis was restricted to adults (males only, 70-80mm CL) at Dassen Island and Olifantsbos, and to juveniles (CL < 50mm) at Mouille Point and the harbour wall.

When comparing the benthic flora and fauna of Olifantsbos and Dassen Island, large differences in community structure were observed, in terms of both species abundance (Figure 4.11) and biomass (Figures 4.12 & 4.13). The communities were only 33% similar (SIMPER analysis). The dominant macrobenthic organisms at Olifantsbos were sponges and barnacles, with smaller amounts of foliar algae and encrusting

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corallines occupying primary space between the sponge colonies - an observation consistent with that of Field *et al.* (1980). Mussels, both black and ribbed were rare or absent. By contrast, large areas of primary space on the subtidal reefs off Dassen Island were occupied by barnacles. The next most prevalent species were mussels, while the abundance of sponges was low. This benthic community was consistent with previous reports from Dassen Island (Newman & Pollock 1974b), and similar to that of nearby rocky areas such as Kreeftebaai and Melkbosstrand (Field *et al.* 1980). Thus, while Olifantsbos reefs are depauperate in mussels, mussels are common at Dassen Island. Furthermore, the biomass of the edible component of the benthos was on average nearly twice as high at Dassen Island as at Olifantsbos (Table 4.2).

Having demonstrated differences in the benthic communities, the focus shifts to the diet of rock lobsters at these two localities. Based on the frequency of occurrence of prey items, the diet of rock lobsters collected from Olifantsbos (SG) and Dassen Island (FG) during November 1994 and February 1995 (Figure 4.5) was not dissimilar to that presented for November 1995 (Figures 3.2 & 3.3), although the frequency of cannibalism was lower in November 1995 at Olifantsbos. Thus, rock lobsters at both sites showed both temporal and spatial similarities in diet composition and proportion during the summer periods of two consecutive years. The diet of rock lobsters at Dassen Island (there are no previous dietary data available for Olifantsbos) also accords well with that of previous findings (Newman & Pollock 1974b).

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Based on the Bray-Curtis similarity and the MDS analyses (Figures 4.6 & 4.7) of the wet weight of prey items, similarity of diet was also observed between individual rock lobsters collected at Olifantsbos and Dassen Island. Although stress in the MDS plot was high (0.16), rendering the plot potentially misleading (Clarke 1993), the combined information in Figures 4.6 & 4.7, coupled with the ANOSIM result ($P > 0.01$) shows similar diets at the two sites.

For the juvenile (CL < 50mm) rock lobsters both dietary and benthic information was also obtained from two sites of contrasting growth rate - Mouille Point (FG) and the harbour wall (SG, Hazell *et al.* in prep). The benthic analyses for the harbour wall and

Mouille Point all suggested large differences between these sites - indeed, SIMPER analysis suggested these two sites to be only 44% similar. Based on percentage cover analysis (Figure 4.14), the harbour wall was dominated by coralline algae, with ribbed mussels and sea urchins much rarer components of the benthic community. By contrast, mussels were by far the dominant benthic component at Mouille Point, with sea urchins and coralline algae less common. This conclusion was confirmed by the Bray-Curtis and MDS analyses (Figures 4.15 & 4.16), both of which showed clear separation between the two sites. Furthermore, as was the case with the previous analysis, the biomass of the edible component of the benthos was more than twice as high at the FG site (Mouille Point, Table 4.2). Biomass of edible prey at the Harbour wall (a slow growth site) was, however, on a par with that at Dassen Island and Olifantsbos.

The gut contents of juvenile *J. lalandii* were statistically similar at both the FG and SG sites examined ($P > 0.05$). At both localities, ribbed mussels were the dominant dietary component, and whereas barnacles were the second-most common prey item in stomachs obtained from rock lobsters captured on the harbour wall, both barnacles and sea urchins were of equal importance in the diet of rock lobsters caught at Mouille Point (Figure 4.8). Cannibalism and the consumption of both sponges and coralline algae was low at both sites. Subsequent to this analysis, the MDS plot suggested a large overlap in the diets of rock lobsters captured from these two sites (Figure 4.10).

There are no previous studies examining the diet of juvenile *J. lalandii*, although the diet of small individuals of other rock lobster species is well documented. The diet of post-puerulus western rock lobsters (*P. cygnus*) was dominated by coralline algae, crustaceans and molluscs (Jernakoff *et al.* 1993). For juvenile individuals of the same species, molluscs, coralline algae and sea grass were the most frequent food items (Joll & Phillips 1984). Stomach samples for immature *H. americanus* (12-73mm CL) contained a high diversity of prey items. These included sea urchins, mussels, rock crabs, polychaetes and brittlestars (Carter & Steele 1982, Lawton & Lavalli 1995).

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There is evidence of spatial dissimilarity in dietary composition of *P. cygnus* (Joll & Phillips 1984), whereas that of *J. lalandii* is often very similar (Newman & Pollock 1974b, Pollock *et al.* 1982) even between areas with contrasting growth rates. However, this contrast needs to be viewed in the light of the availability of preferred food items in the environment before any conclusions can be drawn about the influence of rock-lobster diet on growth rate. Two previous studies on *J. lalandii* reported that rock lobsters from two areas of both contrasting growth rate and benthic community structure, showed similar diet composition (Newman & Pollock 1974b, Pollock *et al.* 1982), and the findings here concur with the results of those studies. I believe that the difference in growth rate observed between Dassen Island and Olifantsbos (adults) and between Mouille Point and the harbour wall (juveniles), is more likely to be related to the availability of preferred food, rather than to diet *per se*. Both juvenile and adult rock lobsters seem to acquire both the same amount (quantity) and type of food (quality) at the two pairs of sites studied. However, because edible (profitable) food was scarcer and would have been more difficult to find at Olifantsbos and the harbour wall, the rock lobsters at these two sites may have to forage for longer, and therefore for less overall energetic reward relative to effort expended when compared with individuals at Dassen Island and Mouille Point. If the rewards are smaller, the energy reserves during moulting will be less and, consequently, either a smaller moult increment or longer intermoult period will result.

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Predation by *J. lalandii* has been suggested to be one of the crucial factors influencing species diversity in the South African West Coast subtidal communities, and preferred prey species are likely to be in low abundance in areas where rock-lobster populations are large (Barkai *et al.* 1996). It was predicted that rock lobsters would be non-selective foragers. But, despite differences in the benthos, (1) adult rock lobsters at Dassen Island (FG) and Olifantsbos (SG) consumed the same prey in similar proportion during the austral summers of 1994-1995 and (2) juvenile rock lobsters at Mouille Point (FG) and the harbour wall (SG) were eating the same amounts of the same prey during September 1997. Thus the prediction failed to be upheld by the data. This suggests that *J. lalandii* exhibits strong prey selectivity, because if they were

non-selective foragers, then gut contents would simply reflect the benthic communities at each site.

A prey selectivity test was conducted on both the adult and juvenile rock lobsters. In these tests, the relative abundances of prey occurring in both the benthic community and the stomach contents were compared (for the four sites at which data are available) based on the null hypothesis (H_0) that the abundances would be the same in both cases. The results clearly showed that *J. lalandii* adults were highly selective foragers (for Dassen Island χ^2 = 55.61, $P < 0.01$ and for Olifantsbos χ^2 = 86, $P < 0.01$) - a definite preference for the ribbed mussel was shown. For example, at Olifantsbos, ribbed mussels were extremely rare, but comprised 15% of gut volume, while barnacles, which were very common in the benthos, made up less than 5% of stomach volume. Similar trends in prey preference were also observed for Dassen Island. Furthermore, juvenile rock lobsters were shown to be even more selective (for the harbour wall χ^2 = 189, $P < 0.001$ and for Mouille Point χ^2 = 282, $P < 0.001$). At both sites, for example, barnacles were extremely rare in the benthos, but comprised 34% and 10% of the gut fullness for the harbour wall and Mouille Point respectively. Thus, some prey species low in abundance in the available benthos showed high abundance in stomach contents, and this pattern was consistent for both small and large rock lobsters. This is clearly evidence for some form of selective foraging. These results both support and conflict with the earlier findings of Barkai *et al.* (1996), which showed mussels to be the most, and barnacles amongst the least preferred prey species. In my case, positive selection for barnacles by juvenile rock lobsters was shown. However, the issue remains unclear - other studies have suggested that *J. lalandii* feeds opportunistically (Barkai & Branch 1988a). Active prey selection has also been proposed for *H. americanus* (Lawton & Lavalli 1995), although surveys are conflicting. This species has been shown to have both a diet reflecting prey species abundance (Miller *et al.* 1971), and to show the same diet from two areas of completely contrasting benthic community structure (Elner & Campbell 1987). Laboratory preference tests have shown *J. lalandii* to be highly selective as regards both size and species when offered different mussels (Griffiths & Seiderer 1980) and

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winkles (van Zyl *et al.* 1998), and the question of prey selectivity is further pursued by way of laboratory experiments in Chapter 5.

5. CONCLUSION

In conclusion, data collected in this study suggest that rock lobsters from a wide range of geographical locations and contrasting growth rates consume similar prey species in roughly equal proportion. More specifically, adult rock lobsters at Dassen Island (FG), Olifantsbos (SG), and juvenile rock lobsters at the harbour wall (SG) and Mouille Point (FG) were eating the same foods in similar proportions despite vast differences in prey species availability. Consequently, rock lobsters seem to be highly selective foragers. Hence, rock lobsters appear to 'target' certain food groups, and the abundance of these 'preferred' prey may influence growth. Whereas gut fullness indices were similar in areas of FG and SG, the amount of time and energy devoted to obtaining a full meal must be greater in areas where preferred food is scarce. This implies that energetic considerations are probably more important in determining growth rate, rather than diet *per se*. Thus the data support the second of the two theories outlined earlier - *i.e.* that limitation in food availability is most probably the reason for the currently observed slow growth rate in the West Coast rock lobster.

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Chapter 5

Prey species preference, sea urchin consumption and predation on juvenile abalone by captive West Coast rock lobsters, *Jasus lalandii*.

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1. INTRODUCTION

Laboratory studies on lobsters other than *Jasus lalandii* have focused primarily on physiology (*e.g.* Dall 1970, Taylor & Whiteley 1989, Baden *et al.* 1990, Lellis & Russell 1990, Jury *et al.* 1994a,b), tagging methods (Guan 1997), den selection (Spanier & Zimmer-Faust 1988), activity rhythms (Lawton 1987), and studies on various aspects of diet and prey selection. These include experiments on feeding behaviour (Moody & Steneck 1993), bait choice (Fielder 1965), growth rates and survival under different feeding regimes (Kurmaly *et al.* 1990), prey species preference (Evans & Mann 1977, Tegner & Levin 1983), prey size selection (Tegner & Levin 1983, Lawton 1987, Andrew & MacDiarmid 1991, Takahashi *et al.* 1995) and levels of cannibalism in captivity (Sardà & Valladares 1990).

One of the earliest laboratory experiments on *J. lalandii* was carried out by Heydorn (1969), who examined moulting, growth and reproduction, demonstrating amongst other things that captive rock lobsters took about 25 days to moult and that they grew slower than tagged rock lobsters in the field. Through a series of feeding trials, Griffiths & Seiderer (1980) showed that *J. lalandii* has a preference for the black mussel (*Choromytilus meridionalis*) over the ribbed mussel (*Aulacomya ater*). In addition, they determined the maximum size of each mussel species that could be cracked by different-sized rock lobsters, and showed that prey-size preference lay well below the maximum size that could be consumed. The long-term consumption rates and feeding efficiency of *J. lalandii* have also been examined (Zoutendyk 1988a,b). In both these studies, prey choice was restricted to a single species - the black mussel. Oxygen consumption at various temperatures (Zoutendyk 1989), and the effects of low oxygen levels on juvenile *J. lalandii* with respect to growth, feeding and mortality have been determined (Beyers *et al.* 1994). More recently, preference by *J. lalandii* for abalone (*Haliotis midae*) over keyhole limpets (*Fissurella mutabilis*) was shown, with the consumption of both these species being dramatically reduced if sea urchins (*Parechinus angulosus*) were introduced into the experimental tanks (Scott *et al.* in press.). A similar study demonstrated preference for the pink-lipped topshell (*Oxystele sinensis*) over the smooth turban shell (*Turbo cidaris*), and a distinct selection for small individuals of both these species of winkles (van Zyl *et al.* 1998). Several

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unpublished feeding studies on, for example, condition factors and their impact on growth have also been conducted (Dr A Cockcroft, Sea Fisheries Research Institute, pers. comm.).

Although the results of the analysis of the diet of *J. lalandii* in the current study (Chapter 4) did not differ vastly from previous published investigations, one point of departure was the high incidence and abundance of sea urchins observed in many of the gut samples. The almost ubiquitous finding of sea urchins in the dietary samples is of major current relevance to the inshore abalone (*Haliotis midae*) fishery in the south-western Cape. This is because field surveys (Tarr *et al.* 1996) and both laboratory and field experiments (Day 1998) have highlighted the strong dependence of juvenile abalone on sea urchins. This relationship is strongest when the sea urchins have access to drift kelp. They then feed on it, and some becomes available to the juvenile abalone beneath the spiny canopy. Rock-lobster numbers are thought to have recently increased in the area of South Africa's main commercial abalone fishing grounds (the area extending about 200km East of Cape Hangklip, see Figure 1), and to have consumed most of the sea urchins and resulted in the observed precipitous decline of abalone juveniles (Tarr *et al.* 1996). However, before concluding whether the current decline in sea urchin numbers (and hence juvenile abalone numbers) could be attributable to the change in the rock-lobster population (Chapter 6), specific laboratory feeding preference and size selectivity experiments were considered necessary not only for evaluating the potential impact of rock lobsters of different sizes on sea urchins, but also for direct comparison with the diet of rock lobsters from the field (Chapter 4).

This chapter thus explores two distinct avenues. The first and main avenue is the consumption of sea urchins by captive rock lobsters (including maximum size eaten, preferred size range and feeding rate), and the potential role of sea urchins in mediating predation on juvenile abalone. The second avenue examines prey preferences by rock lobsters when offered several prey species simultaneously, with the choice of prey species offered being based on both the observed diet (Chapter 4) and species likely to be found in the diet of rock lobsters East of Cape Hangklip

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(Chapter 6). By testing of the specific hypotheses defined below, this chapter not only provides a service for Chapter 6, but also allows comparison with the natural situation already described in Chapter 4.

Based on previous literature, the following specific hypotheses were tested: (1) small rock lobsters will be incapable of consuming large sea urchins; (2) *J. lalandii* would show size selectivity for sea urchins, as previously demonstrated for mussels and winkles, with small sea urchins being favoured over large individuals; (3) the consumption rate on sea urchins and both the maximum size and average size consumed should increase with rock-lobster size; (4) sea urchins will offer protection to juvenile abalone from predation by rock lobsters, and the magnitude of this protection will be greatest in the presence of drift kelp; and (5) rock lobsters will prefer prey species either rich in energy, easy to consume, or both.

2. METHODS

All of the laboratory experiments were conducted in flow-through aquaria systems between December 1996 and April 1997. Water temperature and salinity were held constant at 13 ± 1°C and 35 ± 0.5ppt, respectively. The aquaria were maintained on a 12h:12h light:dark cycle, with the light phase starting at 6am.

All the rock lobsters used in these experiments were obtained from baited long-line rock lobster traps set overnight at Olifantsbos (Figure 4.1). Rock lobsters (males only, size range 38 – 93mm CL) were collected using traps to prevent unnecessary damage to limbs, and to ensure they were eating at the time of capture. All specimens were transported 'dry' to the aquaria, and placed in the filtration ponds to acclimate for at least 7 days before beginning the experiments. In the filtration ponds, the rock lobsters were fed pilchards (*Sardinops sagax*) on an *ad lib* basis. This was to prevent any learning process affecting the results obtained (Wright *et al.* 1990, Gosselin & Chia 1996). Because all rock lobsters were captured from the same area during the same time period, their physiological states were assumed to be similar. Furthermore, as they were caught during Summer, they would all have had the same feeding

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regime, and been in the same phase of the annual cycle, *i.e.* the reserve accumulation phase (Cockcroft 1997).

The prey items offered to the rock lobsters were maintained in glass tanks in the same flow-through system under the same light:dark cycle, and were all alive and undamaged when offered to the rock lobsters. Black mussels (*Choromytilus meridionalis*), sea urchins (*Parechinus angulosus*) and the periwinkles (*Oxystele sinensis* and *Turbo cidaris*) were collected from Blouberg, Millers Point and Onrus respectively. Juvenile abalone (*Haliotis midae*) were obtained from the Hermanus Seafarms hatchery.

2.1 Determination of the maximum size of sea urchins eaten by rock lobsters

To determine the maximum sizes of sea urchins that rock lobsters are capable of consuming, 22 rock lobsters ranging between 43 and 91mm carapace length (CL) were transferred into separate experimental tanks measuring 32 X 32 X 60cm, and supplied with a flow-through system of water. The water level was maintained at ¾ capacity to prevent sea urchins climbing over the sides of the tanks and escaping. Rock lobsters were acclimated in these tanks for 72hr prior to the commencement of the experiment, during which period they were starved to ensure equal hunger levels at the initiation of the experiment. Beginning with urchins 10 - 15mm in size (test diameter, TD), successively larger sea urchins were offered to the rock lobsters singly and in sequence. Sea urchins were replaced daily if consumed. This continued until either (1) the rock lobsters had consumed the largest sized sea urchins offered to them (approximately 61mm), or, (2) the rock lobster did not consume any sea urchins for 48hr. Following this they were offered a mussel to check that they were capable of feeding during the experiment. A distinction has been made here between the partial and total consumption of sea urchins. Partial consumption refers to the sea urchin being flipped over, its Aristotle's lantern removed, and portions of the digestive and gonadal material scooped out. Total consumption implies the breaking up and consumption of the whole sea urchin, including the test.

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2.2 Sea urchin size selection

To investigate the size preferences for sea urchins by a range of sizes of rock lobsters, two separate experiments were performed. All rock lobsters were transferred to the same holding tanks and acclimated to the experimental facility as described in 3.1. In the first experiment, 14 rock lobsters covering a size range of 69-92mm CL were offered different numbers of sea urchins of known size from each of five size classes (*viz.*: 10-19mm; 20-29mm; 30-39mm; 40-49mm and 50-59mm TD). The number of sea urchins offered in each size class varied so that all size classes occupied an equal surface area of the aquarium, so that there was an equal chance of the rock lobster encountering sea urchins of the different size classes. The area chosen was that equivalent to one sea urchin with a test diameter of 55mm (*i.e.* 2375mm^2), the midpoint of the largest size class (Table 5.1). Thus sea urchins covered a total area of 5 X 2375mm^2 which was equal to about 2% of the surface area of the experimental tank. The experiment was run for four days, with daily replacement of dead sea urchins by others of comparable size.

In the second experiment, eight rock lobsters of 69 to 115mm CL were offered six sea urchins in each of the five size classes defined above. Consumed sea urchins were replaced daily, and the experiment was run for three days. Both of these experiments were used to estimate the total number of sea urchins consumed per day, the average size consumed, the gross energy gain from consuming those sea urchins, as well as gaining information on the sizes of urchins selected.

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2.3 Prey species preference

To determine whether preference is shown for any particular prey species, rock lobsters were transferred to the experimental tanks and acclimated for 96hr as described in 2.1. In the first of two experiments, eight rock lobsters (size range 68-102mm CL) were offered five each of black mussels, sea urchins and the two periwinkles with daily replacement, until at least 20 prey items had been consumed. All items offered were known to be consumed by rock lobsters (mussels: Griffiths & Seiderer 1980, winkles: van Zyl *et al.* 1998, sea urchins: this chapter). The experiment was run for a maximum of 5 days. The data were analysed using a χ^2 contingency

Table 5.1: Size classes and number of sea urchins used in experiment 1 on rock lobster predation on sea urchins. These data were used to calculate the number of sea urchins in each size class (test diameter) that would be equivalent in surface area to one sea urchin with a test diameter of 55mm.

Sea urchin size class (mm)	Midpoint of size class (mm)	Test radius (mm)	Area of one individual (mm^2)	# of individuals equivalent to 1 urchin of size 55mm
10-19	15	7.5	176.7	~13
20-29	25	12.5	490.9	~5
30-39	35	17.5	962.1	~3
40-49	45	22.5	1590.4	~2
50-59	55	27.5	2375.8	1

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table (2 X 4), based on the null hypothesis (H_o) that rock lobsters would show equal preference for all four of the prey items offered with α set at 1% to eliminate type 1 statistical errors (after Zar 1984).

In the second experiment, the preference between juvenile abalone and sea urchins was determined. Each of nine rock lobsters of 69-115mm CL was offered a minimum of four sea urchins (20-35mm TD) and three juvenile abalone (5-28mm shell length). All juvenile abalone were placed under sea urchins at the start of the experiment. Consumed items were replaced daily for 3 days. As juvenile abalone tend to leave the shelter of sea urchins less often if kelp strands are present in the tanks (Day 1998), the experiment was repeated with kelp present. In this case, the urchins (n = 18) and abalone (n = 12) were allowed to acclimate in each experimental tank for 72hr, before rock lobsters in the size range 68-102mm CL were introduced individually into these tanks. There was no daily replacement and remaining sea urchins and abalone were counted after 48 hours.

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2.4 Calorimetry

Forty-four sea urchins obtained from Millers Point (see above) were weighed (wet weight) to an accuracy of 0.001g and dried to constant weight at 60°C (approximately 4 days). They were subsequently ground using an electric mill and forced through a mesh of 250µm. The resulting powder was combined with a known amount of flour (16.04 $kJ.g^{-1}$) and completely combusted under excess oxygen in a DDS 500 bomb calorimeter. Calorific estimates for the two winkle species, black mussels and for abalone were obtained from Griffiths 1981 and Field *et al.* (1980). The resulting mean calorific value for sea urchins and values derived from the literature for other species considered in this chapter are summarised in Table 5.2. The relationship between sea urchin TD (mm) and calorific value per individual (kJ) was described by the equation:

$$\text{calorific value} = -6.06 + \exp(1.18 + 0.04\text{TD}) \quad (r = 0.916; P < 0.01) \qquad \text{......(1)}$$

This equation was used when calculating the total daily calorific intake of rock lobsters. Because the sea urchin test contains very little energy when compared with the gonadal and digestive tissue, no distinction between partially and totally consumed sea urchins was made when calculating calorific intake.

Table 5.2: Calorific content of the different prey items offered to and consumed by rock lobsters during these feeding experiments.

Species	Calorific content (kJ.g^{-1})	Source
Sea urchin, *P. angulosus*	1.1	This Chapter
Black mussel, *C. meridionalis*	2.55	Griffiths (1981)
Abalone, *H. midae*	6.18	Field *et al*. (1980)
Winkle, *T. cidaris*	0.81	Field *et al*. (1980)
Winkle, *O. sinensis*	2.17	Field *et al*. (1980)

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3. RESULTS

3.1 Maximum size of sea urchins consumed

Rock lobsters smaller than 68mm CL were not able to partially or totally consume sea urchins (Figure 5.1). However, almost all rock lobsters above this size were capable of at least partially consuming all sizes of sea urchins offered (usually by removing the Aristotle's lantern and scooping out the gonad and digestive material). The maximum size of sea urchin totally consumed increased significantly with rock lobster size above 68mm CL (r = 0.629, df = 9, $P < 0.05$). By extrapolation, a rock lobster larger than 105mm CL should be able to totally consume any size of sea urchin, the maximum size being *ca.* 61mm TD.

3.2 Sea urchin size selection

Results presented below (3.2.1 to 3.3) make no distinction between total and partial consumption of sea urchins.

3.2.1 Experiment 1

Rock lobsters of all sizes (69 - 93mm CL) selected sea urchins on the basis of prey size when offered a selection (Figure 5.2). All rock lobsters tested were able to consume sea urchins across the full size spectrum offered (from 10 - 60mm TD), with one exception (the 69mm individual). All rock lobsters preferentially consumed small sea urchins.

3.2.2 Experiment 2

A similar result was obtained when equal numbers of each size class of sea urchin were offered to the rock lobsters - again there was a definite preference shown for small sea urchins (TD < 30), but sea urchins from all size classes were consumed (Figure 5.3). If sea urchins had been consumed at random, then proportional consumption should have been 20% for each of the five size classes. For all cases combined, sea urchins greater than 40mm TD were significantly under-represented

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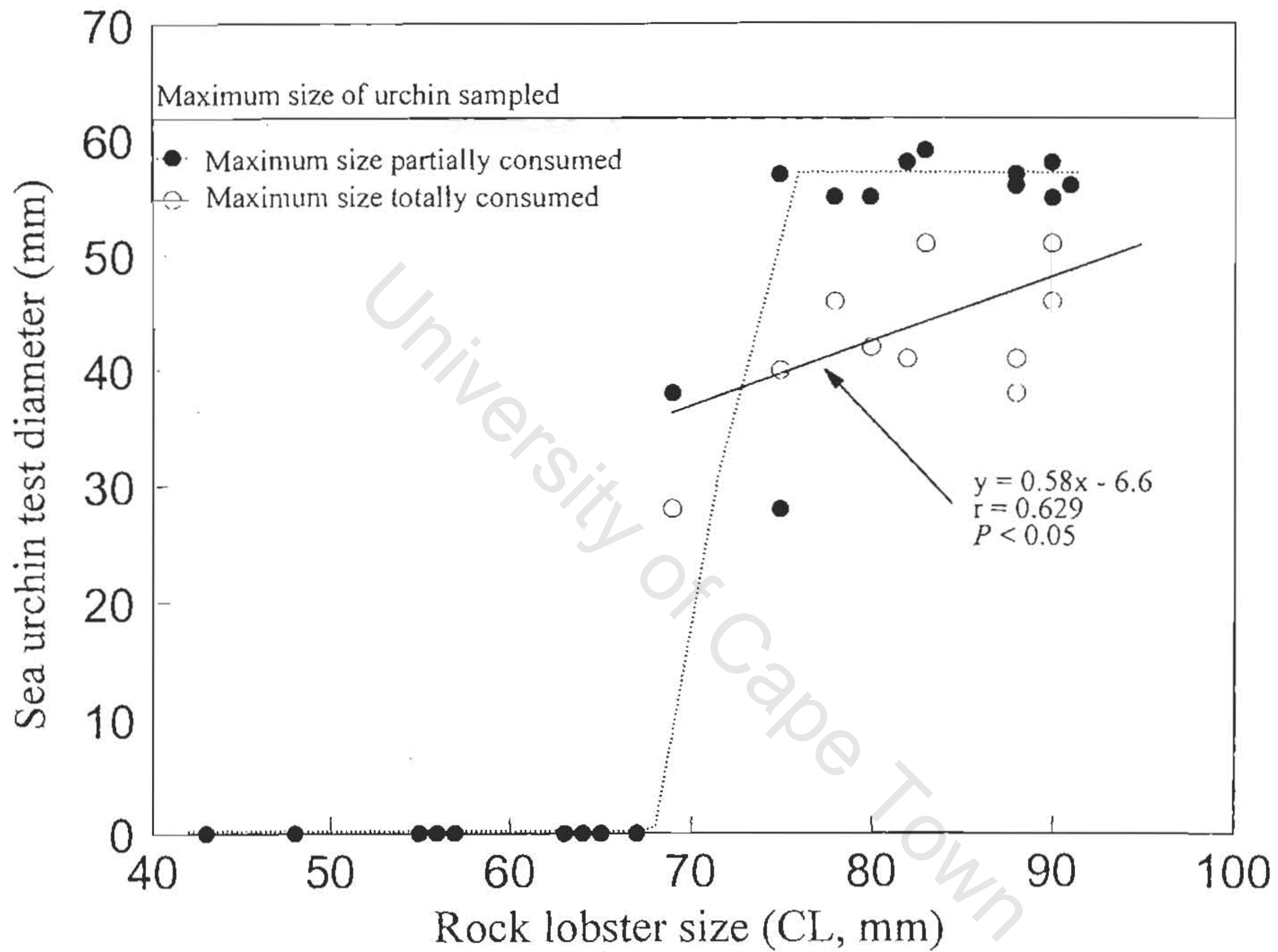

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Figure 5.1: Maximum sizes of sea urchins partially or totally consumed by rock lobsters of different sizes.

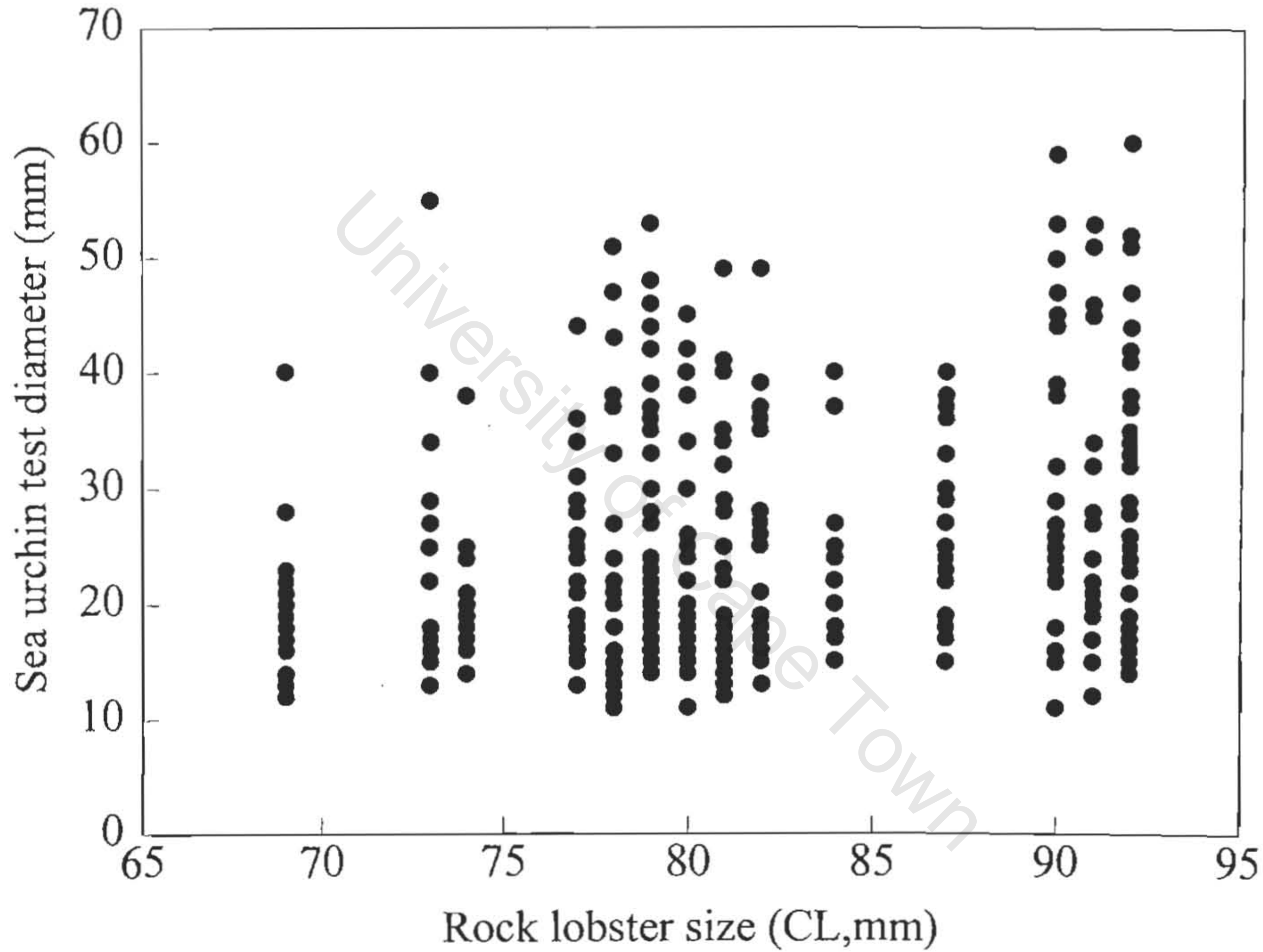

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Figure 5.2: Sizes of sea urchins consumed by 14 individual rock lobsters when they were offered different numbers of sea urchins in each size class (in the ratio shown in Table 1). Each dot represents one sea urchin consumed.

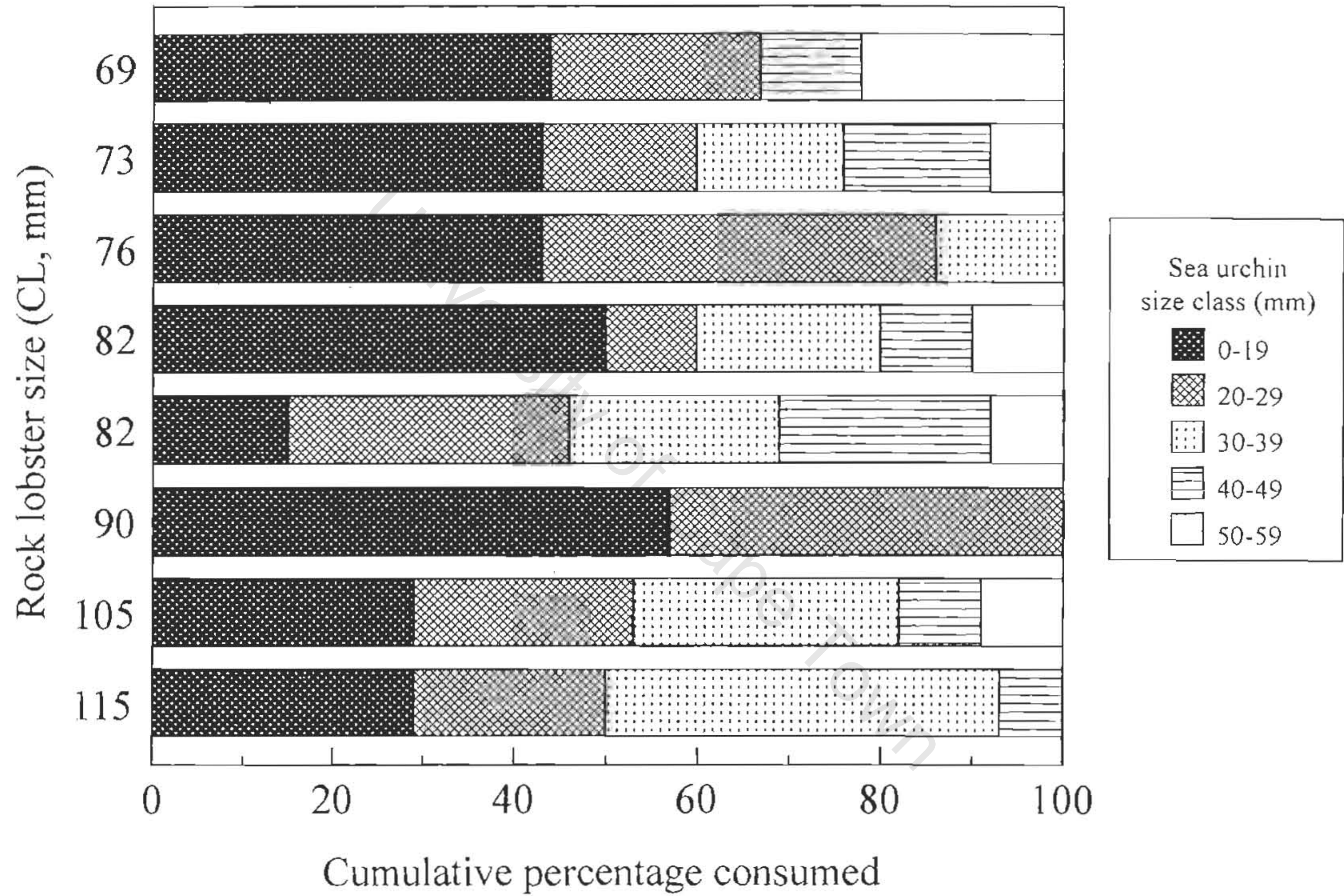

Figure 5.3: Sizes of sea urchins consumed by 8 rock lobsters when offered six sea urchins in each of five size classes, with daily replacement.

among those consumed, and those less than 30mm TD significantly over-represented ($\chi^2 = 24.49$, df = 4, $P < 0.001$).

3.3 Consumption rates

There were fluctuations in the rate of consumption, with cessation or reduction of feeding following periods of high consumption. On average there was no significant correlation between the number of sea urchins consumed daily and the size of rock lobsters (average consumption = 6.11, $r = 0.413$, $P > 0.05$, Figure 5.4). Large rock lobsters did consume sea urchins of significantly larger mean size than smaller individuals ($r = 0.606$, df = 16, $P < 0.01$, Figure 5.4). Using equation 1, ingestion rates of rock lobsters feeding on sea urchins (data from both experiments 3.2.1 & 3.2.2) were used to calculate the total energy intake of each rock lobster (kJ.day^{-1}). This daily energy intake was linearly correlated with rock lobster size ($r = 0.605$, df = 16, $P < 0.01$, Figure 5.5).

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3.4 Prey preferences

When rock lobsters were offered four different prey species simultaneously, all individuals preferentially selected black mussels. Few sea urchins and even fewer of the winkle *Oxystele sinensis* - and no *Turbo cidaris* - were eaten (Figure 5.6). For all rock lobsters, the χ^2 analysis showed highly significant prey selection (P always $<< 0.01$). The number of sea urchins consumed increased with size of rock lobster.

Rock lobsters preferentially ate juvenile abalone when offered a choice between these and sea urchins, both in the presence and absence of kelp (Figure 5.7). Although larger rock lobsters again tended to consume more sea urchins than smaller individuals, in only two cases (14%) did rock lobsters consume equal or higher numbers of sea urchins than abalone.

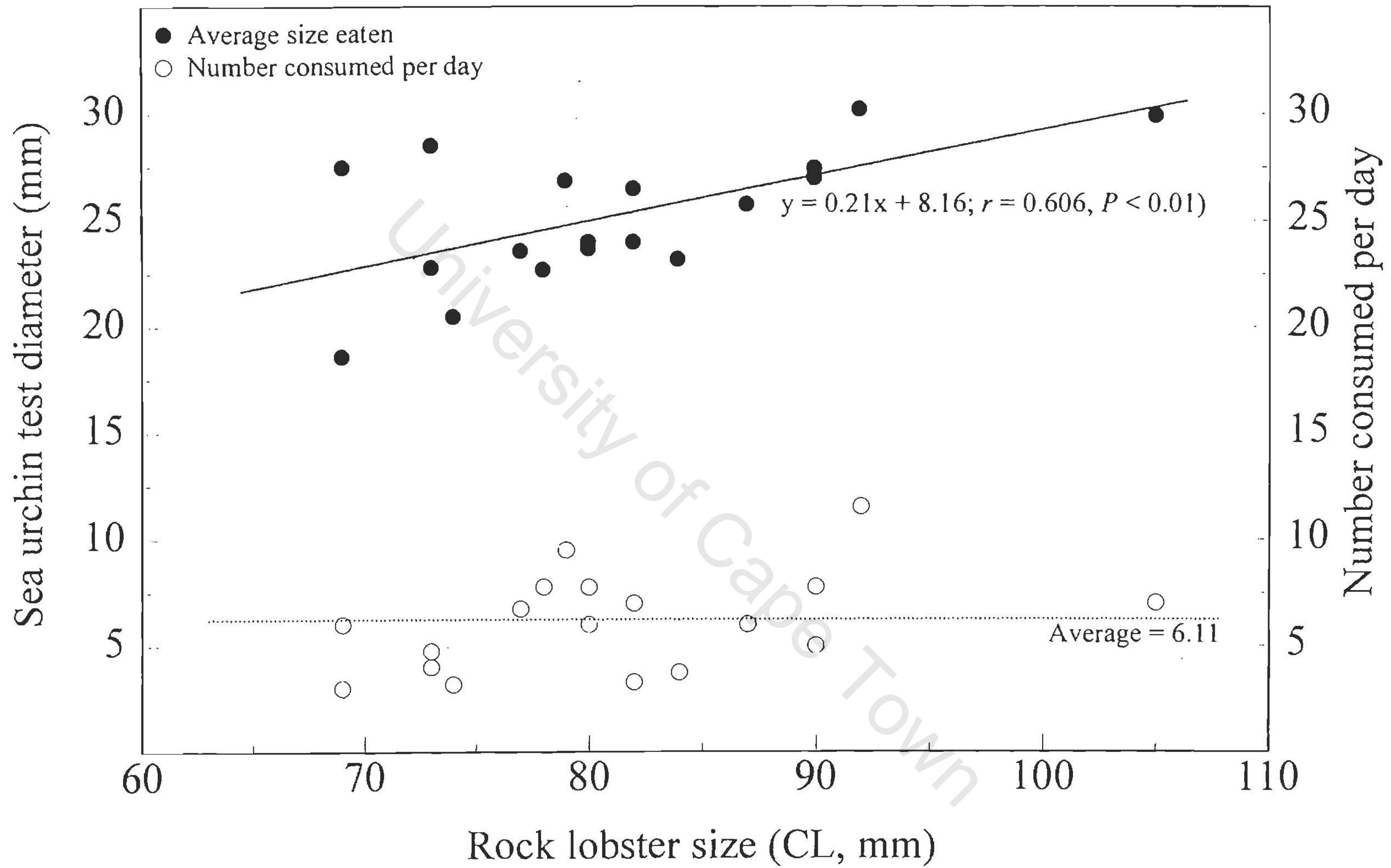

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Figure 5.4: Average size and number of sea urchins consumed daily by rock lobsters ranging in size from 69 to 105mm CL.

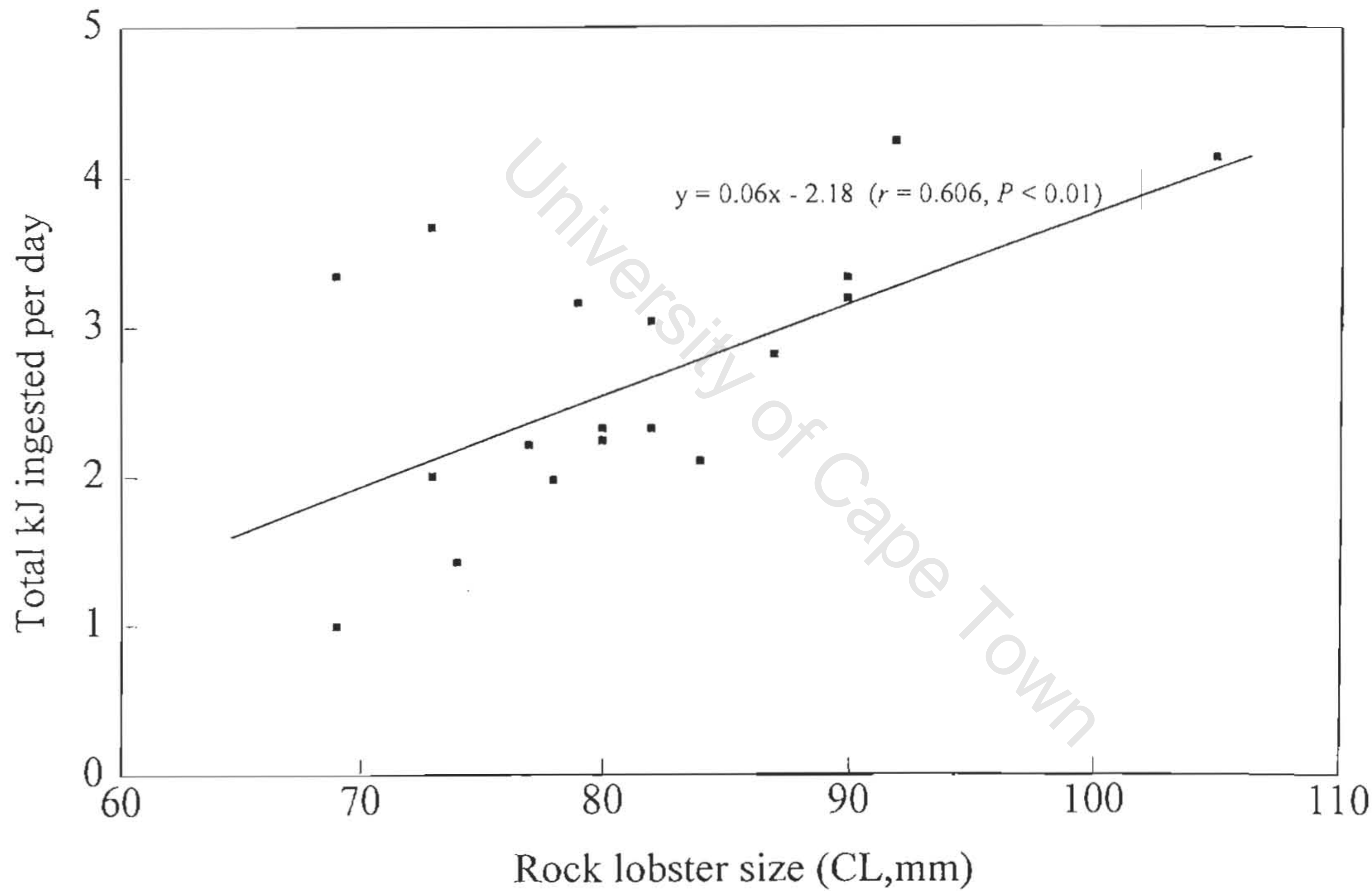

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Figure 5.5: Total calorific intake per rock lobster per day in relation to rock lobster size.

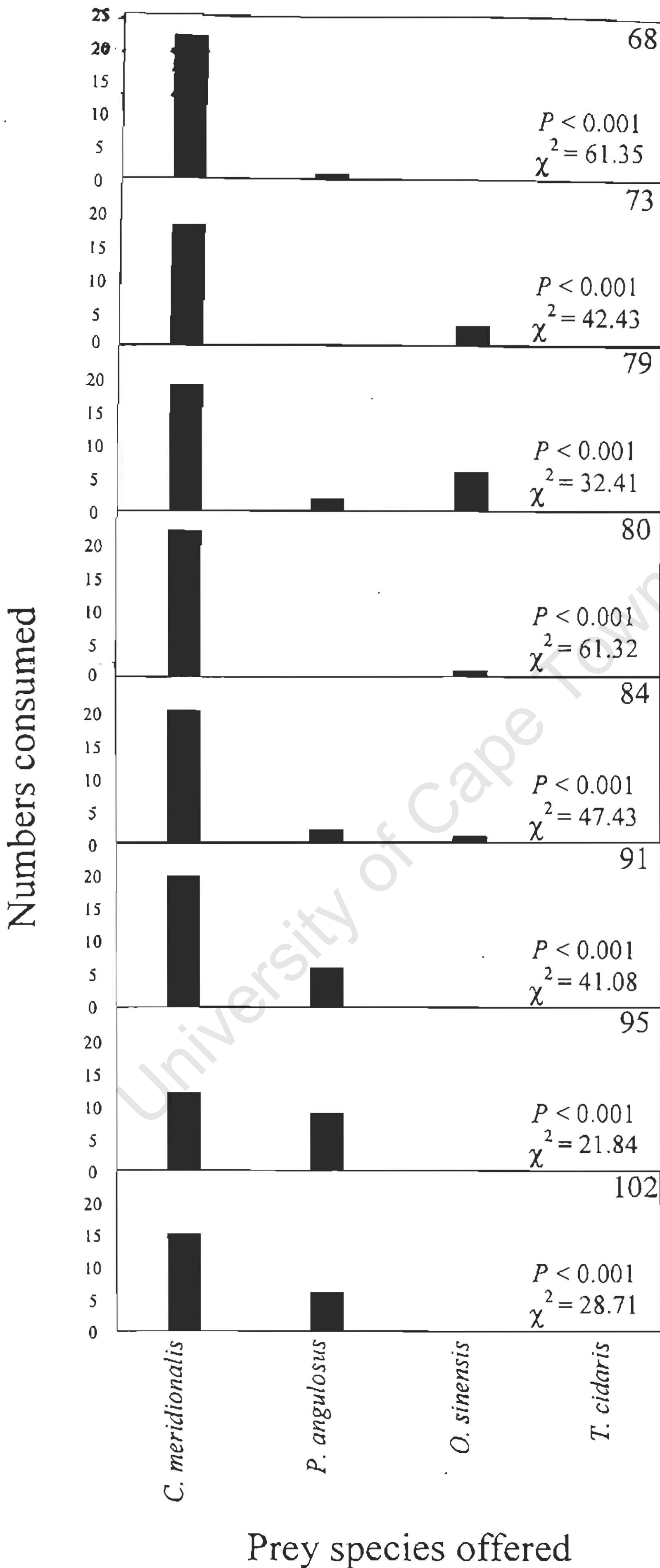

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Figure 5.6: Numbers of each prey type consumed by eight rock lobsters ranging from 68 - 102mm CL. Rock lobster sizes (mm) are indicated in the top right hand corner of each block. Significant departures from random selection are indicated by probability values for χ^2 tests (α set at 1%).

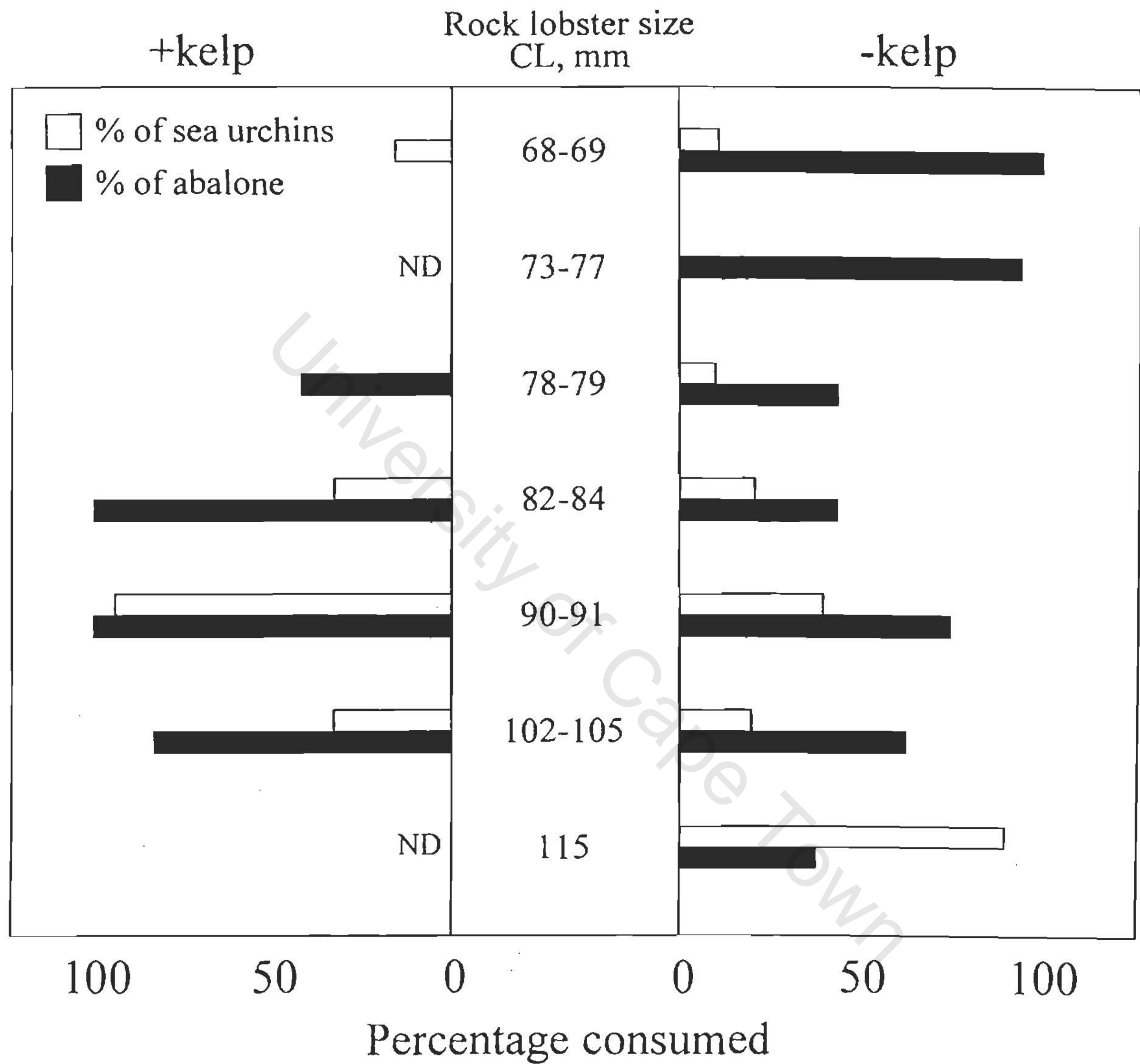

Figure 5.7: Percentages of sea urchins and juvenile abalone consumed by rock lobsters in the presence or absence of kelp. The rock lobsters have been grouped into seven size categories. N.D. = no data for that size class.

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4. DISCUSSION

The central thread of this chapter is the consumption of sea urchins and prey selection by captive rock lobsters. Although several papers have already dealt with prey selection in *Jasus lalandii*, none to date have considered the impact of this species on sea urchins, or indeed its prey preferences when offered a widely different range of prey. The data obtained can be used to answer these and other questions, as well as to address the hypotheses described in the introduction.

Initially, for the experiments concerning the maximum sizes of sea urchins consumed, a distinction was made between the partial and total consumption of sea urchins by rock lobsters. This is significant if considering the abilities of rock lobsters. In terms of the remainder of the experiments and of the impact of rock lobsters on sea urchins, the distinction is irrelevant since either partial or total consumption is fatal.

Several factors may influence a predator's choice of prey. These include cost-benefits (Brunner *et al.* 1992), risk (Hughes *et al.* 1995), considerations of long term energy and mineral intake (Lankford & Targett 1997) and food quality (Irons *et al.* 1988, Chambers *et al.* 1991). Because food quality of each prey species was kept constant (all individuals of each prey species were collected simultaneously), and predation risk was zero during these experiments, only cost-benefit and long term considerations should have influenced the rock lobster's choices. The phase of the moult cycle also influences choice of prey, but all rock lobsters were in the same phase of their moult cycle, namely the reserve accumulation phase prior to moulting in October (Cockcroft 1997).

4.1 Maximum sizes of sea urchins consumed

It was hypothesised that small rock lobsters would be incapable of handling and consuming large sea urchins. Small rock lobsters (< 68mm CL) failed to consume any sea urchins in the experimental tanks (Figure 5.1). It has previously been shown that small *J. lalandii* are incapable of feeding on either large mussels (Griffiths & Seiderer 1980) or large specimens of the winkle *Oxystele sinensis* (van Zyl

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et al. 1998). Given that gut contents of small rock lobsters collected from the field do contain sea-urchin remains, albeit a very small proportion (< 10%, Chapters 3 & 4), the rejection of all sizes of sea urchins by these rock lobsters in captivity is probably due to a physical inability to consume them. Previous studies have alluded to the physical inability of small decapods to crush prey (*e.g.* Griffiths & Seiderer 1980) and this has been used as a means to explain ontogenic changes in diet (see Lawton & Lavalli 1995).

Large (CL > 68mm) rock lobsters consumed all the sea urchins offered to them, and with the exception of two individuals (69mm and 74mm CL) they were able to at least partially consume sea urchins offered to them up to the maximum size (61.5mm TD) (Figure 5.1). Thus, large *Parechinus angulosus* are not immune to predation by *J. lalandii*. However, the maximum size of sea urchin completely consumed increased significantly with rock lobster size ($P < 0.05$). Similar results were obtained when *J. lalandii* was offered mussels (Griffiths & Seiderer 1980) and winkles (van Zyl *et al.* 1998), and when other lobster species have been offered sea urchins (Tegner & Levin 1983, Andrew & MacDiarmid 1991), large gastropods (Takahashi *et al.* 1993) or mussels (Robles *et al.* 1990). Prey consumption by other predators such as fish (*e.g.* Lechanteur 1992) also follows this pattern. The results were not unexpected as the size of potentially edible prey tends to increase with increasing lobster size (Tegner & Levin 1983, van Zyl *et al.* 1998).

4.2 Sea urchin size selection and consumption rates

In the first size-selection experiment, rock lobsters were offered a choice of different sized sea urchins, divided into size classes. To ensure that sea urchins from each size class had an equal chance of being encountered by a randomly feeding predator, the number of sea urchins in each size class was different - but the surface area of the tank occupied by the sum of the individuals in each size class was the same (Table 5.1). Rock lobsters consumed the different sized sea urchins in approximately the same ratio in which they were offered (Figure 5.2). Initially this makes it appear that rock lobsters were consuming the sea urchins at random. In actual fact, equal areas of

University of Cape Town

different sized groups were supplied, yet disproportionately large numbers of small sea urchins were eaten. The result is strongly re-inforced by the second experiment.

In the second size-selection experiment, rock lobsters were offered equal numbers of sea urchins (six) in each of five size classes (thus small sea urchins occupied a very small area of the experimental tank). Although rock lobsters ate sea urchins from all size classes, on average over 65% of the sea urchins consumed were less than 29mm TD (Figure 5.3), despite these small sea urchins comprising only 40% of those available (and taking up less than 15% of the total area occupied by sea urchins). Conversely, consumption of large sea urchins (> 40mm TD) was significantly less than would have been predicted on the basis of their availability. Thus, a definite preference for smaller sea urchins was shown, supporting the conclusions of experiment 1. Despite the energetic value of large sea urchins being relatively much greater than small sea urchins, crushing and consuming large urchins is probably more expensive (in terms of handling time and energy). Targeting small sea urchins may be a way of maximising the gain for a given energy or time input. Thus the first two predictions have been upheld - small rock lobsters were not only incapable of consuming large sea urchins, but any sea urchins at all; and rock lobsters showed active selection for small sea urchins.

Selection for small prey has been previously reported for *J. lalandii*. In laboratory experiments, both small and large rock lobsters preferentially consume small mussels (Griffiths & Seiderer 1980) and small winkles (van Zyl *et al.* 1998) rather than large ones, and these authors suggest that energetic considerations are responsible for the choices observed. Selection of small mussels over large was not observed in the case of juvenile *Homarus americanus*, perhaps because these animals are central-place foragers (Lawton 1987). However, other species of lobsters have also shown strong selection for small prey and a reluctance to consume larger prey (Tegner & Levin 1983, Robles *et al.* 1990, Andrew & MacDiarmid 1991), and a preference for prey well below the critical size (Griffiths & Seiderer 1980, Robles *et al.* 1990). This behaviour is not limited to lobsters: Dungeness crabs, *Cancer magister*, have been reported to eat more clams from the smallest size class offered (Juanes &

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Hartwick 1990), and the giant clingfish (*Chorisochismus dentex*) appears to select prey well below the maximum size it can consume (Lechanteur 1992).

Although selection of sea urchins was primarily for small individuals, on average, larger rock lobsters ate significantly larger sea urchins than smaller rock lobsters (Figure 5.4). This result supports the earlier prediction that smaller rock lobsters should consume smaller sea urchins. Larger prey take longer to consume (Griffiths & Seiderer 1980, Takahashi *et al.* 1995), and may require either a greater expenditure of energy (Griffiths & Seiderer 1980) or a greater risk (Hughes & Ward 1993, McNamara & Houston 1994, Hughes *et al.* 1995) prior to any gain. Thus small rock lobsters can be expected to consume smaller prey - a trend already demonstrated for *J. lalandii* feeding on both winkles (van Zyl *et al.* 1998) and mussels (Griffiths & Seiderer 1980), and now demonstrated for sea urchins.

Despite fluctuations in the rate of feeding, the average number of sea urchins consumed per rock lobster per day did not vary significantly with predator size ($P > 0.05$, average = 6.11 urchins.rock lobster^{-1}.day^{-1}, Figure 5.4). The fluctuations in feeding rate were comparable to those recorded by Zoutendyk (1988a) during long-term feeding experiments on *J. lalandii*. He considered fluctuations to be residual from the nutritional condition of rock lobsters when collected from the field - where weather can play an important role in determining whether or not foraging can take place. Certainly baited traps tend to catch few rock lobsters when the sea is rough, but catch extremely high numbers immediately following the abatement of strong surface conditions (Zoutendyk 1988b). Zoutendyk (1988a) reasoned that when weather conditions allow foraging, rock lobsters may feed to satiation, following which they may cease feeding for a period of time. The fluctuations of feeding observed in the laboratory may be a reflection of this.

Different sized rock lobsters consumed essentially the same number of sea urchins, but because larger rock lobsters ate generally larger sea urchins and consumed them completely, the total calorific intake increased linearly with rock lobster size (Figure 5.5). A similar linear result was obtained for previous experiments on

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J. lalandii fed gastropods (van Zyl *et al.* 1998). However, the daily energetic intake of rock lobsters eating gastropods was nearly five times that of rock lobsters eating sea urchins. This is most likely related to gastropods having a relatively high calorific value of 10-20kJ.g flesh weight^{-1} (Field *et al.* 1980) when compared to sea urchins (1.1kJ.g^{-1}). If rock lobsters are limited in the total volume of food that can be ingested, then those feeding on gastropods will enjoy a greater calorific intake than individuals consuming the same volume of sea urchins. Rock lobsters fed on mussels (22kJ.g flesh weight^{-1}) also showed an increase in calorific intake with rock lobster size (Griffiths & Seiderer 1980), although the relationship was exponential rather than linear in this case. Thus, although sea urchin consumption rate did not increase with rock-lobster size, both the average and the maximum size consumed, and the daily energy intake did increase with rock-lobster size.

4.3 Prey species preferences

When offered a choice between four different prey species, rock lobsters overwhelmingly selected black mussels (*Choromytilus meridionalis*), which constituted 80% of the prey consumed during the trials. Smaller quantities of sea urchins and the winkle, *Oxystele sinensis*, were consumed, but no rock lobsters ate the second winkle species, *Turbo cidaris* (Figure 5.6). The frequency with which rock lobsters selected different prey species were significantly different from those expected, as highlighted by the large χ^2 values. Rock lobsters were not offered the ribbed mussel, as previous experiments had already shown a preference for black mussels over ribbed mussels of similar size (Griffiths & Seiderer 1980).

The gut contents of rock lobsters collected from the West Coast (Chapter 4) were mirrored in the selection of prey in these laboratory experiments. By far the most common prey item in the gut contents of rock lobsters was mussels, particularly *C. meridionalis*. Black mussels do have the highest calorific value of the four prey species offered (Table 5.2) - perhaps selection was based on this criterion. Sea urchins are often under-represented in the natural diet (relative to their abundance in the benthos) and in these experiments although they were the second most preferred prey species, very few individuals were consumed when compared to mussels. The same is

true of the winkles. They were almost untouched in the choice experiments, and they contribute minimally to gut contents of rock lobsters on the West Coast.

When offered a choice between sea urchins and abalone, rock lobsters again showed prey selectivity. In nearly all cases, whether in the presence or absence of kelp, rock lobsters ate most of the juvenile abalone offered, but few sea urchins were consumed (Figure 5.7). Hence, despite the fact that juvenile abalone live almost exclusively under sea urchins in the field (Day 1998) and that their foraging trips from beneath sea urchins are less frequent and of shorter duration when kelp is present (Day 1998), rock lobsters actively selected juvenile abalone in these experiments despite sea urchins being available to shelter them. In selecting abalone over sea urchins, the rock lobsters showed preference for the prey species that was both easier to consume (juvenile abalone have an extremely soft shell, easily crushed between ones fingers) and richer in energy. Compared to sea urchins ($1.1kJ.g^{-1}$), abalone are a much richer energy source ($6.18kJ.g^{-1}$, Table 5.2). These results seem to contradict the fact that in the field sea urchins provide shelter for juvenile abalone. However, in natural systems rock lobsters preferentially feed on particular prey - notably mussels - and may only turn to sea urchins and the associated juvenile abalone in the absence of preferred prey. In the presence of mussels, sea urchins should effectively protect small abalone. Indeed, no juvenile abalone remains have ever been found in the gut contents of rock lobsters collected in the field. In the laboratory experiments, rock lobsters had no alternative prey other than sea urchins and abalone, and in these circumstances the normal protection granted to the juvenile abalone by sea urchins failed.

The results from my laboratory experiment also contradict those of Scott *et al.* (in press). They conducted contemporary experiments on the same species of abalone, sea urchin and rock lobster, but conducted the research at Port Nolloth, 600km North of Cape Town on the West Coast (Figure 1). When Scott *et al.* (in press) presented rock lobsters with both sea urchins and juvenile abalone, they ate very few juvenile abalone and no sea urchins - thus, the sea urchins did provide shelter and protection to the juvenile abalone. When offered only abalone, the rock lobsters consumed them in high numbers. Hence, these two studies on the same species yielded different results,

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despite being done in an almost identical way and with the rock lobsters in the same stage of their annual moult cycle. The only difference was that the surface area of the experimental tank occupied by sea urchins and juvenile abalone was much higher in my experiments than the ones in Port Nolloth. Comparative studies on the behaviour and natural diets of rock lobsters from Port Nolloth and those further South, coupled with studies on the relative susceptibility of sea urchins from the two regions to predation may resolve the reasons for the differences between the two sets of experiments. Behavioural responses of sea urchins to the presence of decapod predators has received attention (Bernstein *et al.* 1981, Vadas *et al.* 1986, Hagen & Mann 1994). Perhaps differences in sea urchins behaviour (brought about by their different densities in the experimental tanks) could account for the disparity between these two sets of results.

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Thus, my laboratory experiments failed to uphold the prediction the sea urchins would protect juvenile abalone from rock lobsters, although there are plausible reasons why the results conflict with field observations. Why I obtained results so different from Scott *et al.* (in press) remains an open question. On the other hand, rock lobsters did show preference for prey rich in energy, both in the laboratory (this chapter) and in the field (Chapter 4), selecting mussels over sea urchins and winkles, and abalone over sea urchins.

Clear prey selection has previously been demonstrated for *J. lalandii*. This species shows a preference for *O. sinensis* over *T. cidaris* (van Zyl *et al.* 1998), and for black over ribbed mussels (Griffiths & Seiderer 1980). In the latter case, ease of removal and the weaker shell strength of *C. meridionalis* are thought to be reasons for the observed preference. Preference for abalone over keyhole limpets (*Fissurella mutabilis*) has also been shown (Scott *et al.* in press). A clear choice of crabs over sea urchins has been demonstrated for *Homarus americanus* (Evans & Mann 1977), and a preference for the urchin, *Strongylocentrotus purpuratus*, over another urchin, *S. franciscanus* has been recorded for *Panulirus interruptus* (Tegner & Levin 1983). Preferences by rock lobsters for particular prey in laboratory choice experiments are

thus the norm, and reinforce the conclusion in Chapter 4, based on field data, that *J. lalandii* is a selective predator.

5. CONCLUSION

Small rock lobsters (< 68mm CL) proved incapable of consuming sea urchins in the laboratory feeding trials, but larger rock lobsters fed on all sizes of sea urchins offered, although they preferentially ate small sea urchins (< 30mm TD). The number of sea urchins consumed per rock lobster per day showed no increase with rock lobster size. However, because larger individuals on average ate larger sea urchins, and completely consumed whole sea urchins rather than partially consuming them, daily calorific intake increased linearly with rock lobster size. Rock lobsters of all sizes preferentially ate black mussels when offered these together with sea urchins and two species of winkle. This mirrored the results for dietary analysis of rock lobsters collected in the field between Elands Bay and Cape Point. In the laboratory, juvenile abalone were consumed in preference to sea urchins whether in the presence or absence of kelp. This result differs intriguingly to that obtained from similar experiments conducted elsewhere on the same suite of species. These results provide supporting evidence for the selectivity of prey by rock lobsters in the field, as presented in Chapter 4. They are also an important backdrop and input into assessing the influence of rock lobsters on sea urchins (and hence juvenile abalone) in the field. It is this relationship between sea urchins, rock lobsters and juvenile abalone that forms the focus of the next chapter.

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Chapter 6

Inter-relations among rock lobsters, sea urchins, winkles and juvenile abalone: implications for community management.

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1. INTRODUCTION

The fishery for South African abalone (*Haliotis midae*) is currently of immense economic importance to many people along the Cape South Coast. It was valued at about US$15 million in 1993 (Oakes & Ponte 1996) and has an annual total allowable catch (TAC) in the region of 600 tons. In addition, about 30 000 people harvest abalone recreationally (Sea Fisheries Research Institute (SFRI), unpublished data) and the species is targeted by a burgeoning poaching community. The main fishing area ranges between Cape Hangklip and Danger Point (Figure 6.1, Tarr 1992).

There has been a recent suggestion of a strong link between juvenile abalone and sea urchins (*Parechinus angulosus*). Field surveys since 1988 (Tarr *et al.* 1996), and both large- and small-scale field and laboratory experiments (Day 1998) have confirmed the dependence of juvenile abalone on sea urchins - for both shelter and food - and highlighted a delicate ecological balance. The relationship between these two species appears most intimate when sea urchins have access to kelp debris. They then trap and feed on it and consequently move around less, thus providing a more secure shelter and also a source of food for juvenile abalone hiding beneath them.

It has been suggested (Tarr *et al.* 1996) that a massive increase in the abundance of rock lobsters has occurred since the late 1980's in the area stretching about 150km to the East of Cape Hangklip (abbreviated here as EOCH). West Coast rock lobsters (*Jasus lalandii*) clearly consume sea urchins in the field (Chapters 3 & 4) and, once they reach sufficient size, can consume large quantities of sea urchins of all sizes (Chapter 5). It has been postulated that this massive increase in rock lobster density EOCH has reduced the local sea urchin population below the level critical for the successful recruitment of abalone (Tarr *et al.* 1996). In view of recent declines in the spawner biomass (Moloney 1997, Plagányi & Butterworth 1997) of abalone (due mainly to heavy poaching), any further inroads into future spawning stock by reductions in the survival of juveniles becomes critical.

However, hard data on both the putative increase in rock lobsters in the area EOCH, and their ability to control sea urchin populations are lacking. It is with this in mind

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that four questions are addressed here. Firstly, has the abundance of rock lobsters increased substantially in the area EOCH? Secondly, are there any detectable changes in the populations of other species there? Thirdly, and if so, can these changes be attributed to rock lobsters? Fourthly, if rock lobsters are having a measurable impact, what are the ecological implications (*e.g.* for abalone) and what future management options exist?

Rock lobsters have long been suspected of controlling sea urchin populations. It has been suggested that overfishing of the American lobster (*Homarus americanus*) led to rapid increases in the density of urchins (*Strongylocentrotus droebachiensis*), their formation of 'feeding fronts', and the creation of urchin barrens (Mann & Breen 1972, Mann 1982). This has also been demonstrated experimentally (Breen & Mann 1976b). It was later concluded that spiny lobsters control population densities of the sea urchins *Strongylocentrotus purpuratus* and *Strongylocentrotus franciscanus* (Tegner & Levin 1983), supporting the suggestion of Paine (1969) that rock lobsters are 'keystone' species. These ideas were disputed by Miller (1985) and later Elner & Vadas (1990); consequently few people now accept the original hypothesis proposed. More recently, a negative association between the density of New Zealand lobsters (*Jasus edwardsii*) and urchins (*Evechinus chloroticus*) has been demonstrated (Andrew & MacDiarmid 1991).

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Predation by other species also seems capable of controlling urchin abundances (Sheibling & Hamm 1991). These include starfish, *Pycnopodia helianthoides* (Duggins 1983), fish (Sala & Zabala 1996) and otters (Estes *et al.* 1989). There is, however, some disagreement over whether or not otters do regulate the urchin *S. droebachiensis* (Himmelman & Steele 1971), as field surveys suggest that otters and urchins can live in equilibrium - albeit with the density of urchins lower than in areas without otters (Lowry & Pearse 1973).

Two research groups outside South Africa have mentioned a positive link between the densities of sea urchins and those of juvenile abalone, the first working on *S. franciscanus* and *Haliotis* spp. (Tegner & Dayton 1977) and the second on

Anthocidaris crasspina and *H. discus discus* (Kojima 1981). In South Africa, Tarr *et al.* (1996) and Day (1998) have both suggested a comparable positive association between *P. angulosus* and *H. midae*. Both these researchers suggested that juvenile abalone gain food and shelter by living under the spiny canopy of sea urchins. Other reports usually deal only with adults of urchins and abalone and nearly all suggest that there is a negative association between them. This has been shown in California for two abalone, *Haliotis rufescens* and *Haliotis walallensis,* and the urchin *S. purpuratus* (Lowry & Pearse 1973); and in Australia for *Haliotis rubra* and *Centrostephanus rodgersii* (Andrew and Underwood 1992). In the latter case, segregation seems based on competition for food as a result of their different grazing selectivities (Shepherd 1973). For certain other invertebrate species, sea urchins have also been shown to be beneficial. Removals of *C. rodgersii* led to an initial increase in the abundance of juvenile limpets - which then subsequently declined to levels lower than those in adjacent areas with urchins (Fletcher 1987). Perhaps when they are very small, the juvenile limpets are eaten by the sea urchins - other urchin species (*e.g. S. droebachiensis*) do contain small gastropods, mussels and barnacles as components of their gut contents (Himmelman & Steele 1971). This might explain the initial increase in juvenile limpets in the absence of urchins. Once they supersede the size range in which they are vulnerable to incidental ingestion by urchins, they may become dependent on the urchins. This can be inferred from the work of Ayling (1981) who showed that removal of urchins (*Evechinus*) led to reductions in the number of herbivorous gastropods and a subsequent increase in macroalgal growth.

Ecological implications of increases or reductions in sea urchin densities have received a lot of attention, particularly in California where harvesting of kelp is a valuable industry worth US$50 million in 1988 (Booth 1988). Massive increases in kelp bed area were recorded after both large-scale natural urchin mortality (Pearse & Hines 1979, Scheibling 1986) and experimental removals from selected areas (*e.g.* North & Pearce 1970, Paine & Vadas 1969, Duggins 1983). Exactly the reverse occurs when urchin numbers are very high, as observed under both natural

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circumstances (Breen & Mann 1976a, Dean *et al.* 1984) and when induced experimentally (*e.g.* Ebert 1977, Dean *et al.* 1988, Estes *et al.* 1989).

This Chapter addresses the questions posed earlier. In addition, and based on the literature reviewed above, evidence is sought to test the predictions that: (1) rock-lobster numbers in the area EOCH have increased over the last 5 to 10 years; (2) rock-lobster gut contents from this area will contain few or no sea urchin remains (because sea urchins have already been largely eliminated (Tarr *et al.* 1996)); (3) because large rock lobsters (CL > 68mm) eat sea urchins and are able to do so voraciously and with few limitations, densities of sea urchins will be strongly negatively correlated with those of large rock lobsters; (4) few (if any) juvenile abalone will be found outside of the spine canopies of sea urchins and (5) juvenile abalone densities will be strongly positively correlated with sea urchin density, and will be low in areas where urchins have been eliminated or substantially reduced by rock-lobster predation.

Three approaches were used. First, questionnaire surveys of the opinions of experienced recreational rock-lobster fishermen were sought to test if their observations support the idea that the rock lobsters have recently increased in number EOCH. Second, field surveys were undertaken to explore correlations between sea urchins and rock lobsters and to determine if community structure had changed EOCH since the putative increase in rock lobsters. Finally, using these data and results from earlier chapters, the potential impacts of rock lobsters on sea urchins (and hence on juvenile abalone) were modelled.

2. METHODS

2.1 Estimation of changes in rock lobster abundance EOCH

Only a single survey of rock-lobster abundance has been undertaken in the area EOCH prior to the putative increase (Field *et al.* 1980). In addition to making direct comparisons with this, three indirect methods of estimating relative changes in abundance were employed.

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2.1.1. Interviews of recreational fishermen

A total of 311 interviews of the skippers of recreational rock-lobster fishing boats were carried out between Cape Hangklip and Hermanus during the 1995/1996 (n = 77) and the 1996/1997 (n = 234) fishing seasons (Figure 6.1). All the interviews were presented in the same way, and the questions asked as shown in Figure 6.2. The data gathered included demographic information required for other purposes but irrelevant here and therefore not considered any further. Only the information received from respondents who had been fishing EOCH for at least four years was used in the analyses because only experienced fishermen could reliably provide accurate perceptions about changes in rock lobster abundance over time. Responses were divided into four categories: those who felt that rock lobster density had (1) increased over the last five years; (2) declined over the last five years; (3) not changed and (4) those who did not know or were not prepared to answer the question.

In an attempt to interview more people, 300 questionnaires were placed on the windscreens of vehicles at the four main launching sites between Cape Hangklip and Hermanus (Maasbaai, Stony Point, Onrus & Hermanus) on the two weekends either side of Christmas during the 1995/1996 fishing season (the busiest fishing period). A shortened, 'cut-out' version of the questionnaire was also published in the *Hermanus Times*, a local newspaper.

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2.1.2 Recreational landings EOCH

When each questionnaire was completed, the number of permits carried by the boat was recorded (the maximum legally permitted is 4) and, where possible, the entire catch was measured (to 1mm) and sexed. During the two seasons, 3448 rock lobsters were measured. Information on the number of permits sold annually and the landings of rock lobsters EOCH were obtained from Cockcroft & MacKenzie (1997) and the SFRI (unpublished data).

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Figure 6.1: Map of the study area showing the location of the sites sampled.

CRAYFISH CATCH QUESTIONNAIRE

THIS IS A SURVEY OF RECREATIONAL CRAYFISH FISHERMEN BEING CONDUCTED BY THE UNIVERSITY OF CAPE TOWN. IF YOU CATCH CRAYFISH, PLEASE COULD YOU COMPLETE THIS CARD AND RETURN IT TO US. IF NOT, PLEASE PASS IT ON TO SOMEONE YOU KNOW WHO DOES.

IN WHICH TOWN DO YOU LIVE? .... AGE? .... SEX? ....

HOW MANY YEARS HAVE YOU BEEN CATCHING CRAYFISH? ....

WHERE DO YOU NORMALLY COLLECT CRAYFISH (BE SPECIFIC)? ....

FROM WHERE DID YOU CATCH YOUR LAST CRAYFISH (BE SPECIFIC) ....

HOW DO YOU COLLECT CRAYFISH? | HOOPNET | DIVING | OTHER (SPECIFY)................. |

ON WHAT PERCENTAGE OF TRIPS DO YOU OBTAIN YOUR QUOTA? | <10% | 10-30% | 30-80% | >80% |

DO YOU THINK THE NUMBERS OF CRAYFISH BETWEEN CAPE HANGKLIP AND HERMANUS HAS CHANGED IN RECENT YEARS? | INCREASED | DECREASED | NO CHANGE | DON'T KNOW |

REASONS? ....

DO YOU THINK THE AVERAGE SIZE OF CRAYFISH BETWEEN CAPE HANGKLIP AND HERMANUS HAS CHANGED IN RECENT YEARS? | INCREASED | DECREASED | NO CHANGE | DON'T KNOW |

WHAT WOULD YOU FEEL ABOUT A COMMERCIAL FISHERY FOR CRAYFISH BETWEEN CAPE HANGKLIP AND HERMANUS? | DEFINITELY NOT | NO PROBLEM | NO COMMENT |

REASONS? ....

ARE YOU HAPPY WITH THE RECREATIONAL CRAYFISHING REGULATIONS? BAG LIMIT? | Y | N | SIZE LIMIT? | Y | N |

DO YOU THINK THESE ARE EFFECTIVE MEASURES FOR THE RESOURCE? | Y | N |

REASONS? ....

ANY OTHER COMMENTS?

THANKYOU VERY MUCH FOR YOUR TIME

Figure 6.2: Rock-lobster questionnaire upon which all the interviews were based.

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2.1.3 Boat launches

Data on the number of boat launches taking place at Hermanus between 8am and 4pm during December weekends for the period 1987 to 1997 were obtained from the local Sea Fisheries inspector (Mr C. Duvenhage).

2.1.4 Direct comparisons with earlier data

Direct comparisons could be made with an earlier survey in the area, which included measures of abundance of foliar algae, rock lobsters, sea urchins and winkles in 1980 at Bettys Bay (Field *et al.* 1980). This information was compared with my own recent surveys of rock lobsters and sea urchins; estimates of current winkle densities EOCH (Dr A Pulfrich, University of Cape Town (UCT), unpublished data); and a measure of current macroalgal abundance in Bettys Bay (Dr G Levitt, SFRI, unpublished data).

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2.2 Estimating rock lobster, mussel and sea urchin density and size frequency

Paired deep (12 - 17m) and shallow (< 5m) reefs at 16 sites from Dassen Island to Danger Point (Figure 6.1) were sampled once between December 1996 and January 1998. Jasus lalandii is known to move inshore and offshore at different times of the year and sampling was confined to summer, when rock lobsters are concentrated inshore, thus ensuring that their migratory patterns did not confound the results. Additional non-paired samples were also obtained from Kleinmond (deep) and Stony Point (shallow). Sites West of Cape Hangklip were included because preliminary dives indicated few sea urchins EOCH, and it was essential to have sites with abundant sea urchins to evaluate relationships between rock lobster and sea urchin densities.

Each survey of each site consisted of two components, carried out using SCUBA. First, nine transect lines 26m in length were swum in a haphazard direction and all sea urchins and rock lobsters within 50cm either side of the line were counted, and the presence or absence of mussels recorded. Each of these counts thus covered an area of 26m^2. Second, in excess of 100 individuals of sea urchins, mussels and rock lobsters (when present) were collected at random and measured to an accuracy of 1mm (rock

lobsters: carapace length (CL); sea urchins: test diameter (TD); mussels shell length (SL)).

2.3 Estimates of total rock lobster and sea urchin numbers

The average density of rock lobsters and sea urchins EOCH was determined using all the transect count information. The rock-lobster size frequency distributions for all sites EOCH were combined and the proportion of rock lobsters comprising each size class calculated. An estimate of the total area of reef between 0 and 15m water depth from Cape Hangklip to Danger Point was obtained from van Zyl *et al.* (1998). This value multiplied by the average density of rock lobsters and sea urchins on reefs and was used as an estimate of the total number of these two species between 0 and 15m EOCH. Using this value relies on the assumption that the rock lobsters are relatively resident and would not have migrated between sites over the time span of the survey. Data from tagging experiments in other areas suggest very limited or no movement between fishing grounds (Goosen & Cockcroft 1995), so this assumption is reasonable.

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2.4 Rock lobster wet weight - carapace length regression

Twelve rock lobsters between 65mm and 91mm CL were measured (CL, to 0.1mm) and then weighed (to 0.01g). The resulting regression was used to calculate both calorific standing stocks (using the conversions of Field *et al.* (1980)) and rock lobster biomass EOCH. Rock lobster carapace length and wet weight were significantly linearly correlated over this size range ($r = 0.98$, df = 11, $P < 0.001$) and described by the equation:

$$\text{Wet weight (g)} = 8.72 * \text{carapace length (mm)} - 414.19 \quad \ldots\ldots (1)$$

2.5 Rock lobster diet EOCH

From the sites surveyed EOCH, up to 30 male rock lobsters (70 - 80mm CL) were retained for gut content analysis. Rock lobster stomachs were extracted and processed as already described in Chapters 3 & 4. Briefly, the rock lobsters were anaesthetised by submersion in an ice-cold freshwater bath, exsanguinated and the stomachs

removed and frozen (-20°C) for further analysis. To determine the diet, stomachs were rapidly defrosted, and the contents flushed into a Petri dish. Gut contents were assessed visually under a dissecting microscope (8 X magnification) and the percentage contribution of each prey species to the total gut volume was estimated (after Hyslop 1980, Williams 1981). The frequency of occurrence of each prey item (*i*) was calculated after Berg (1979):

$$\frac{\text{\# stomachs with item } i}{\text{\# stomachs examined}} \times 100 \quad \ldots\ldots (2)$$

The dietary data from all sites EOCH were combined for the analyses, separated only with respect to depth, as from many sites few rock lobster stomachs were obtained thus preventing between site diet analyses.

2.6 Interactions between juvenile abalone and sea urchins

At sites surveyed between November and March estimates of the relationship between the densities of sea urchins and those of juvenile abalone (defined as having a shell length (SL) between 3 and 20mm) were obtained using both large ($0.25m^2$) quadrats and short (3.5m long) transect lines. This time period was selected because most abalone spawn in spring (September, A MacKenzie, SFRI, pers. comm.) and after reaching about 2mm in size they move under sea urchins. As their size increases, their density decreases to such an extent that selecting a longer time window could have biased the results obtained. In each case, the total number of sea urchins was counted. They were then all removed from the survey area and all juvenile abalone found beneath them counted. Additional data for Mudge Point, Bettys Bay and Danger Point were obtained for the period November 1989 to March 1993 from R. Tarr (SFRI, unpublished data); again, only data collected between November and March were used in the analysis (for the reason given above). Values of sea urchin densities per sample were plotted against juvenile abalone density. The resultant plot was analysed in three ways. First, the running mean for successive blocks of 10 data points was calculated (this eliminated the bias for greater spread of data in regions of the plot with denser data points, and gave the pattern of mean abalone densities relative to sea urchin density). Second, to determine the theoretical density of sea urchins below

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which no juvenile abalone should be detected, only data points with a sea urchin density less than $25m^{-2}$ were used. Below this value, densities of sea urchins and juvenile abalone were linearly related; above it juvenile abalone densities varied tremendously and tended to plateau with increasing sea urchin density. Third, the maximum number of juvenile abalone concealed by sea urchins was determined for the entire density range of the sea urchins (after Blackburn *et al.* 1992). Briefly, the sea urchin densities were separated into 14 size classes and the maximum density of juvenile abalone found within each size class was regressed against the midpoint of that size class. Confidence limits (95%) were calculated after Zar (1984).

To determine the degree to which juvenile abalone are found only under sea urchins in the area between Cape Hangklip and Danger Point, a minimum of 42 $0.25m^2$ quadrats were counted at each of 10 sites, at less than 4m depth (juvenile abalone being concentrated in the shallows). For each quadrat, the numbers of sea urchins, the numbers of exposed juvenile abalone (*i.e.* abalone not under sea urchins) and the numbers of protected juvenile abalone (*i.e.* abalone found under sea urchins) were recorded. Additional data were obtained from Day (1998).

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3 RESULTS

3.1 Estimates of changes in rock lobster abundance

3.1.1 Interviews of recreational fishermen EOCH

From interviews of the recreational fishermen (n = 311), 183 (almost 60%) of the respondents had been fishing for more than 4 years EOCH (Figure 6.3). Of these, 106 (58%) said that over the preceding five years rock lobster numbers in the area between Cape Hangklip and Hermanus had increased. In contrast, 23 (13%) responded in the negative. Forty-four (24%) respondents had observed no change and 5% of those interviewed were uncertain. These figures were consistent between the 1995/1996 and the 1996/1997 fishing seasons.

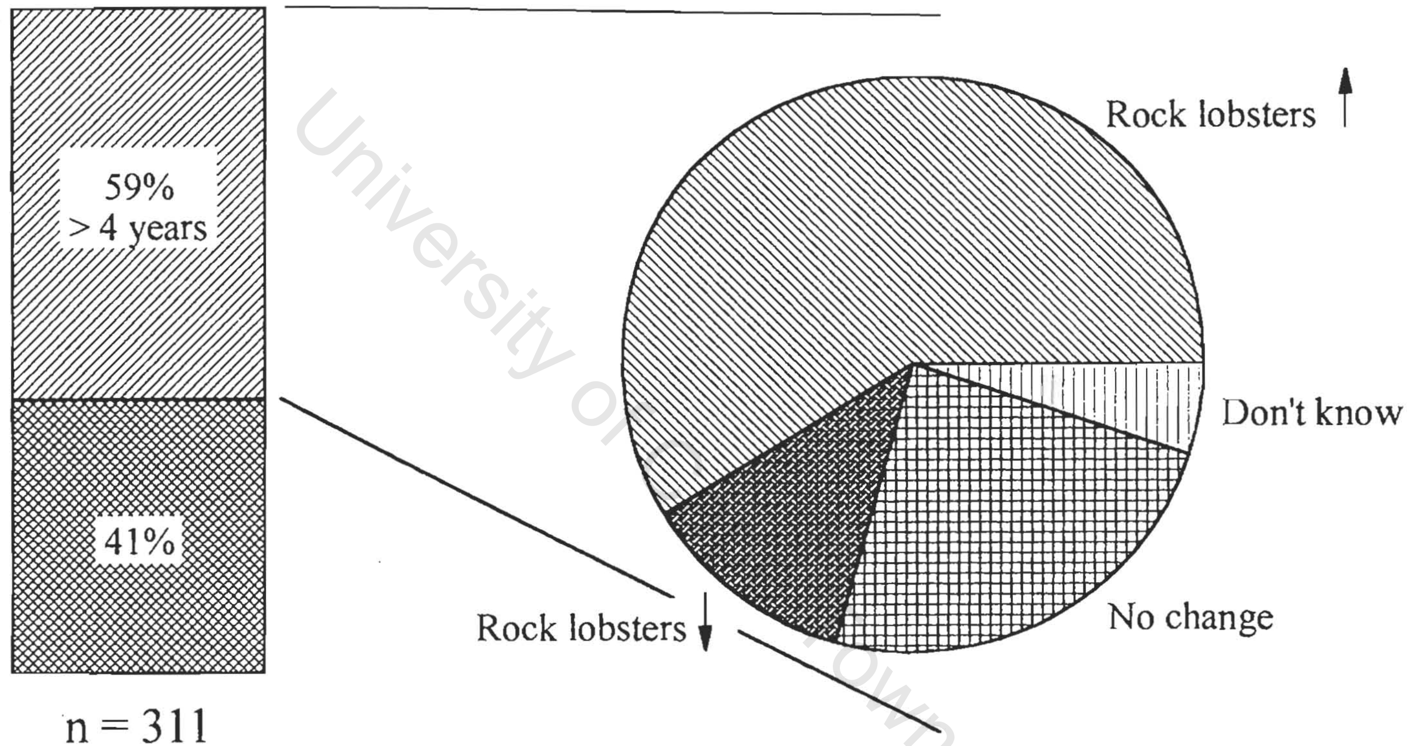

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Figure 6.3: Breakdown of the interviewees responses based on (1) the number of years they had been fishing, and (2) their response to the question 'Do you think the numbers of rock lobster between Cape Hangklip and Hermanus has changed in recent years?'

Forty-one (14%) of the 300 questionnaires distributed onto motor vehicles at the four launching sites were returned. Over 70% of respondents had been fishing for more than 4 years - and 72% of these stated that the density of rock lobsters in the area had increased in recent years. Only 17% said numbers had decreased, while 6.8% and 3.4% suggested respectively that there had been no change or they were uncertain. Setting aside the 'don't know' respondents, and on the null hypothesis that no change in rock-lobster abundance has occurred, both sets of data yield significant support for the view that abundance has increased ($\chi^2 = 64.22$, $P < 0.001$ and $\chi^2 = 21$, $P < 0.001$).

The return from the newspaper questionnaire was low. Only 11 questionnaires were returned, of which half the respondents felt the numbers had increased. Four respondents thought the density had declined - all citing poaching as the reason for this.

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3.1.2 Recreational landings

A breakdown of the size composition of the recreational catch EOCH from the two seasons is presented in Table 6.1. The proportion of the catch from each size class was similar in the two seasons. Although just under 4% of the rock lobsters measured were undersize, about 40% of the rock lobsters landed were larger than 89mm CL (the current minimum legal size (MLS) for recreational fishermen is 80mm CL - having been reduced from 89mm CL in the 1994/1995 season). Only a small proportion of the catch comprised females (< 5%).

3.1.3 Boat launches

Recreational boat launchings (a measure of fishing effort) at Hermanus during December increased 10 fold from about 10 boats per weekend day to 100 between 1987 and 1997 (Figure 6.4). From my surveys during the 1995/1996 season, the average number of permits per boat per launching was 3.4 (n = 277). As each permit is legally valid for four rock lobsters per day, and as less than 1% of fishermen fail to fill their bag limit, this equates to about 14 rock lobsters per boat per day. Thus, on an average weekend day, about 1400 rock lobsters get landed in Hermanus. Boat launches between Cape Hangklip and Danger Point can exceed 400 per day (pers.

Table 6.1: Size frequency of the recreational catch of rock lobsters by size class (males and females combined) for the 1995/1996 and the 1996/1997 fishing season EOCH.

Rock lobster	1995/1996		1996/1997	
size class (mm)	Number	Percentage	Number	Percentage
<80	24	3.5	107	3.8
80-84	215	31.6	896	32.4
85-88	135	19.9	681	24.6
89-94	155	22.8	626	22.6
95-99	63	9.3	235	8.4
100-110	78	11.5	184	6.6
>110	10	1.5	39	1.4
Totals	680		2768	
% of females (1996/1997) = 4.6%				

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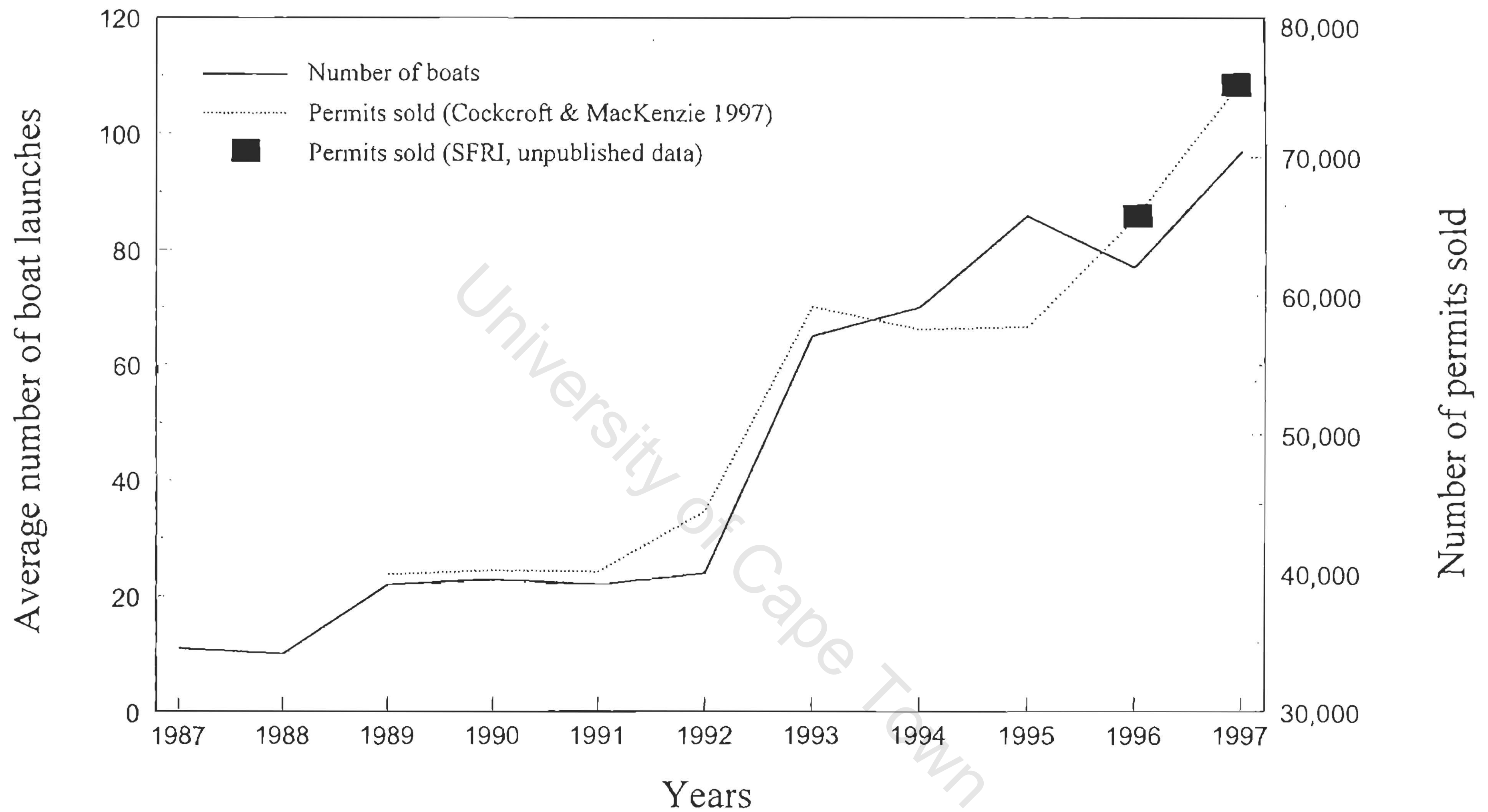

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Figure 6.4: Average number of boat launches per weekend day at Hermanus during December, and the number of recreational rock lobster fishing permits sold from 1987 to 1997.

obs.). Multiplying up, this is the equivalent of nearly 5500 rock lobsters landed daily (*ca.* 1.75 tons). The total numbers of recreational permits sold (although not specifically limited to the area EOCH) mirrored the pattern of boat launches (Figure 6.4).

3.1.4 Rock-lobster size composition

From the diver surveys, sizes of rock lobsters EOCH were large in comparison with other areas (Figure 6.5). At Bettys Bay and Palmiet (both EOCH), modal sizes were respectively 61 and 71mm CL, and 27% & 44% of the rock lobsters were over the commercial minimum size of 75mm. By comparison, data for Sea Point and the Knol show modal sizes of 31 and 36mm CL, with zero and 9% respectively being over 75mm CL.

3.2 Density of rock lobsters, mussels, and sea urchins

Very few mussels were observed on the transect lines, and then only at sites West of Cape Hangklip (Figure 6.6a,b). Considerable differences in the densities of rock lobsters and sea urchins were observed between the 18 sites sampled. This was especially evident for the shallow sites (Figure 6.6a), but less obvious at the deeper depth (Figure 6.6b). From both depth zones, with four exceptions (Knol, Oudekraal, Robben Island and Sea Point), all areas with high sea urchin densities also had low densities of rock lobsters. However, at all but one of these exceptions (Oudekraal deep), sea urchins were abundant only if large rock lobsters were scarce or absent. This was even more apparent when both deep and shallow depths were combined and the density of sea urchins regressed against the density of large rock lobsters (CL > 68mm, Figure 6.7). The decline in the density of sea urchins was defined by a power curve as rock lobster density increased ($r = -0.498$, df = 45, $P < 0.001$), and was described by the equation:

$$\text{Density of sea urchins} = 3.01 * \text{density of large rock lobsters}^{-0.197} \quad ......(3)$$

The relationships between the density of all sizes of rock lobsters, or of small rock lobsters only, when plotted against sea urchin density, yielded much lower r^2 values and were statistically insignificant (Table 6.2).

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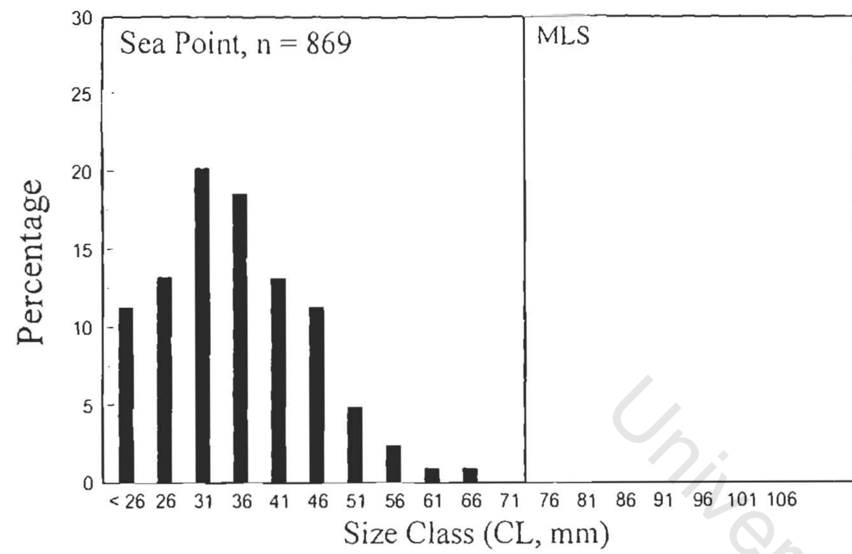

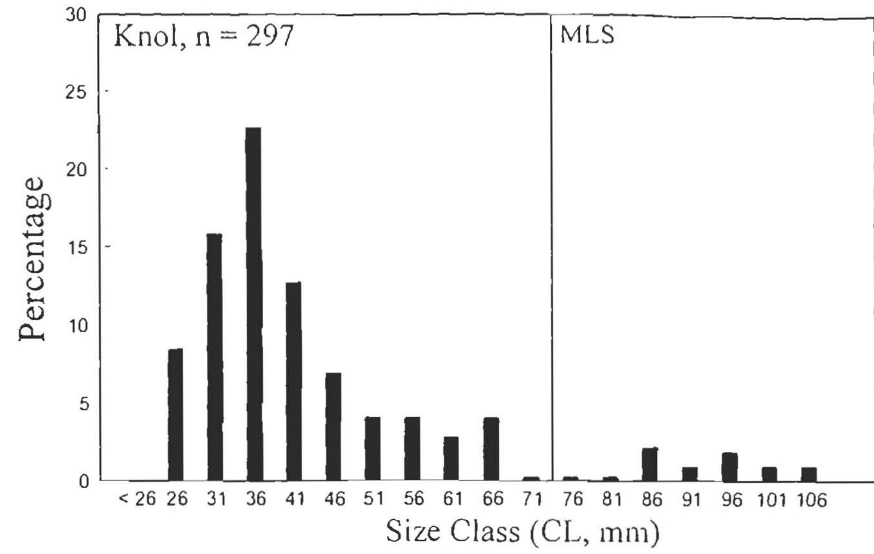

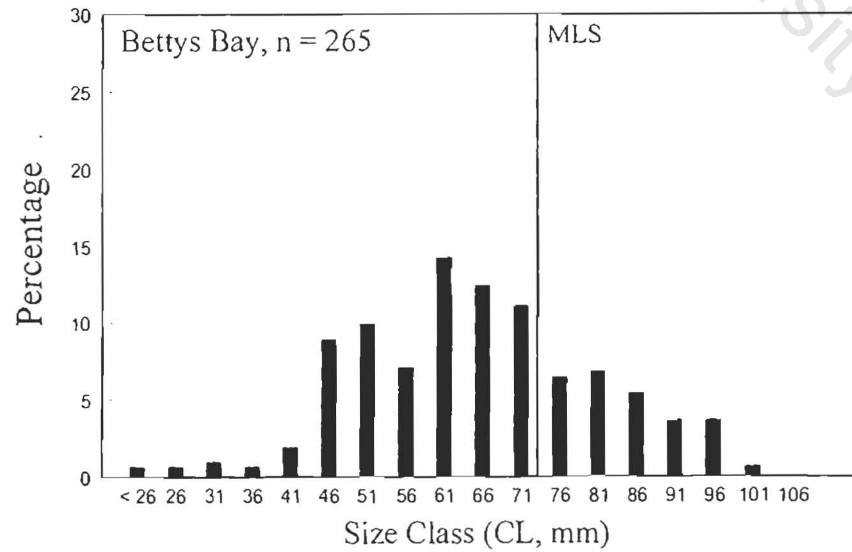

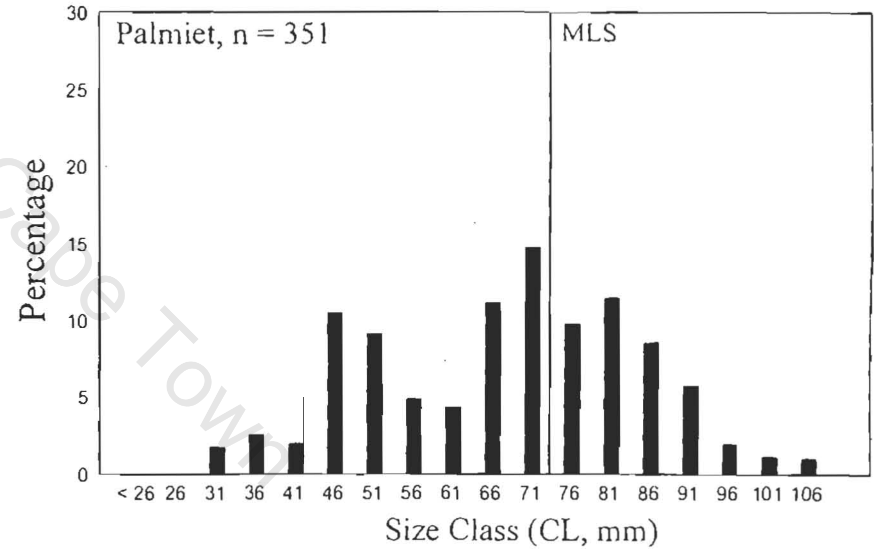

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Figure 6.5: Size frequency distribution of rock lobsters (both sexes combined) for four of the sites sampled.

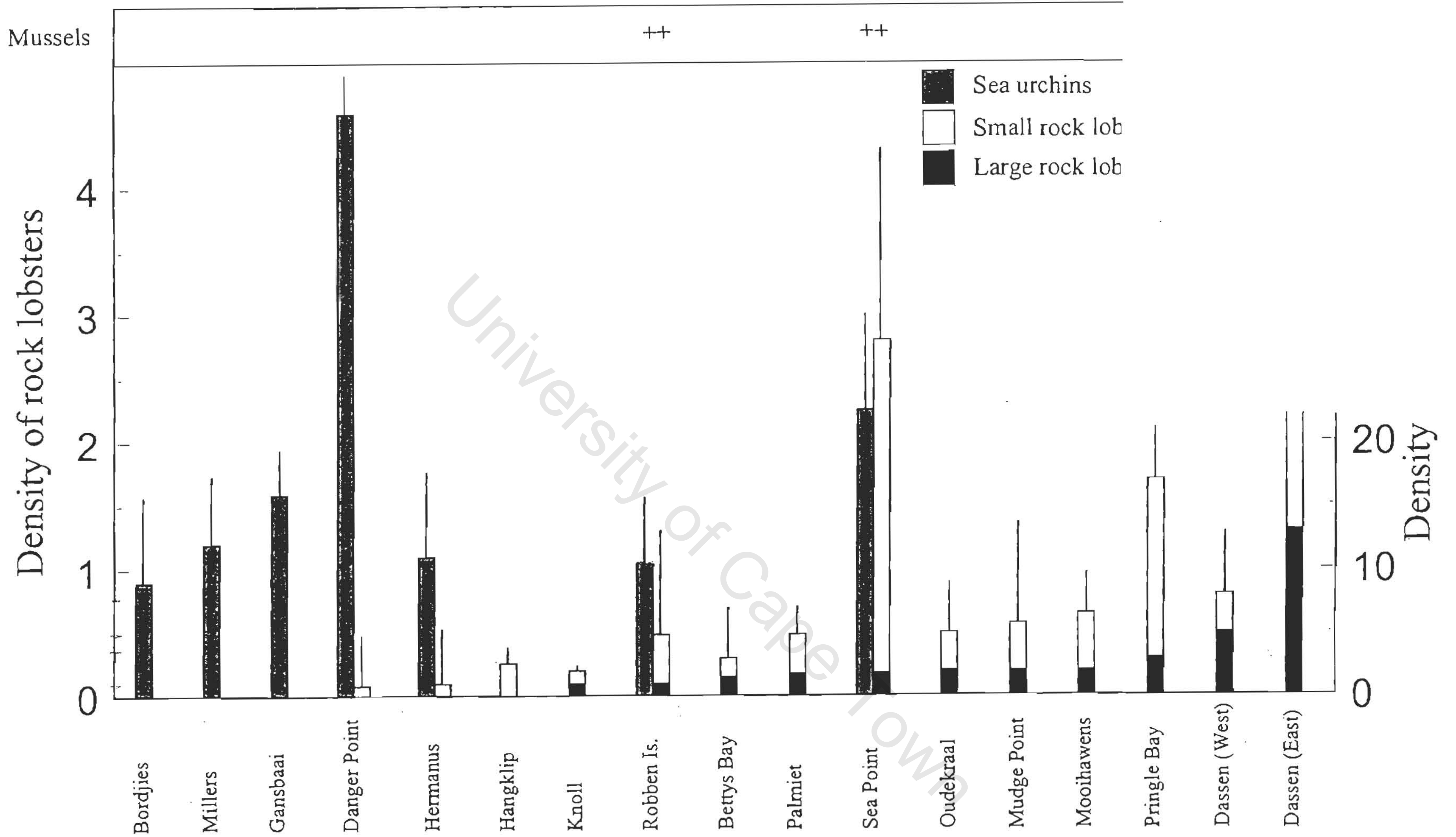

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Figure 6.6a: Density (numbers.m^{-2}) and standard error (vertical lines) of sea urchins and rock lobsters, and presence or absence of mussels at the shallow depth for the 16 sites with paired shallow and deep stations.

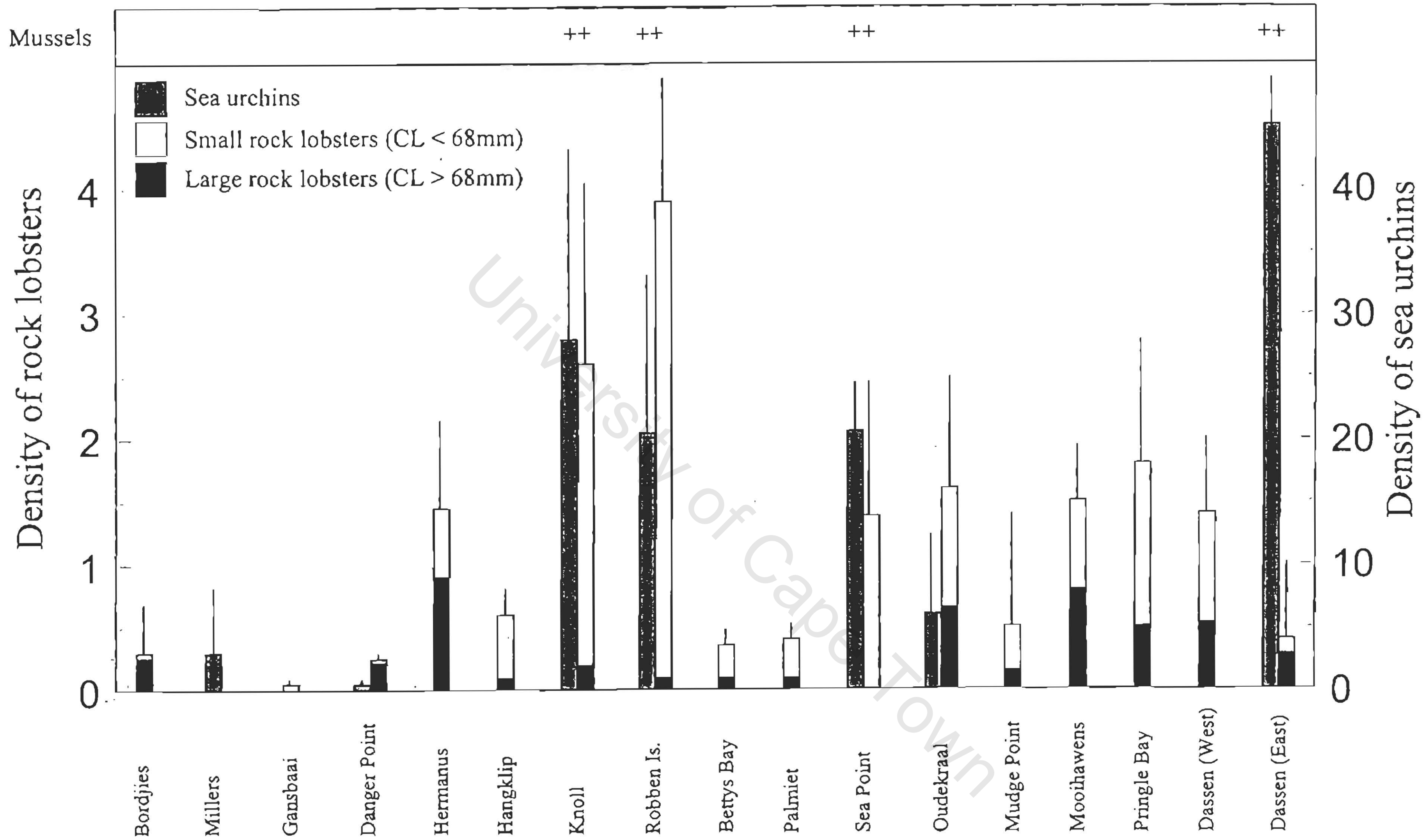

Figure 6.6b: Density (numbers.m^{-2}) and standard error (vertical lines) of sea urchins and rock lobsters, and presence or absence of mussels at the deeper depth for the 16 sites with paired shallow and deep stations.

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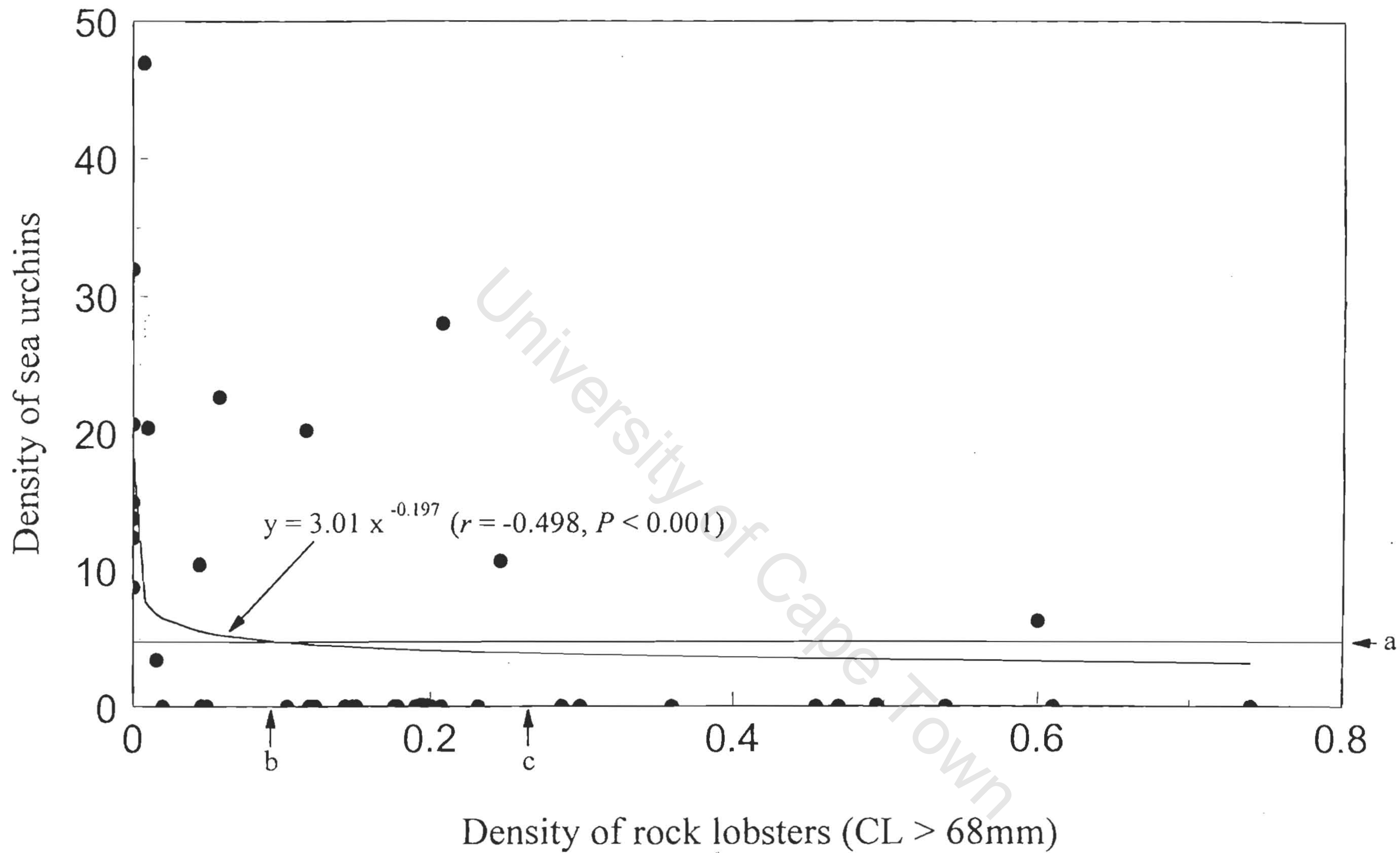

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Figure 6.7: Regression between the density of sea urchins (numbers.m^{-2}) and the density of large rock lobsters (CL > 68mm, numbers.m^{-2}). 'a' refers to the density of sea urchins below which no juvenile abalone will exist. 'b' refers to the density of large rock lobsters at which the density of sea urchins will equal this value. 'C' refers to the current large rock-lobster density EOCH.

Table 6.2: Values for parameters a and b, degrees of freedom (df), r^2 and P values for the relationship ($y = a * x^b$) relating densities of sea urchins to those of rock lobsters of all sizes, small rock lobsters only (< 68mm CL) and large rock lobsters only (> 68mm CL).

	a	b	df	r^2	P
All sizes	9.23	-0.061	45	0.01	> 0.1
CL < 68	5.24	-0.057	45	0.014	> 0.1
CL > 68	3.01	-0.197	45	0.248	< 0.001

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A similar pattern was obtained when comparing the density of the winkle *Turbo cidaris* and rock lobsters (Figure 6.8) - again, the density of winkles declined rapidly (r = -0.332, df = 36, $P < 0.05$) as the density of rock lobsters increased. In this case rock lobsters of all sizes were included in the analysis as van Zyl *et al.* (1998) showed that even small rock lobsters can consume *T. cidaris*. The relationship was described by the equation:

$$\text{Density of winkles} = 0.535 * \text{density of rock lobsters}^{-0.314} \quad ......(4)$$

3.3 Comparisons with earlier surveys

Large changes in the abundance of rock lobsters, sea urchins, winkles and foliar algae at Bettys Bay were evident between 1980 and 1997 (Table 6.3). In 1980, rock lobsters and algae were absent or rare while winkles and sea urchins were abundant. However, in 1997, exactly the reverse situation existed - winkles and sea urchins were almost non-existent while rock lobsters had increased dramatically. The most striking change was that of foliar algae, which showed an increase from about $10 kJ.m^{-2}$ in 1980 to nearly $8000 kJ.m^{-2}$ in 1997 (Dr G Levitt, SFRI, unpublished data). This is an 800-fold increase. No change in the abundance of mussels was observed.

3.4 Rock-lobster diet East of Cape Hangklip

The most common prey item consumed by rock lobsters captured at the deep sites was sponge remains which were found in nearly 65% of stomachs examined (Figure 6.9). Rock-lobster and sponge remains were almost equally almost abundant as the most important prey items in the gut contents of rock lobsters living in less than five metres water depth. Coralline algae, winkles, mussels and sea urchins contributed smaller but roughly similar proportions at both depths. The high occurrence of sea urchins in the diet (*ca.* 25% of stomachs) is of interest as urchins apparently co-existed with rock lobsters at very few of the sites sampled EOCH. Mussels were not recorded on the rock lobster grounds there, but were clearly being located and consumed by the rock lobsters in spite of being scarce - further evidence of selective feeding on them.

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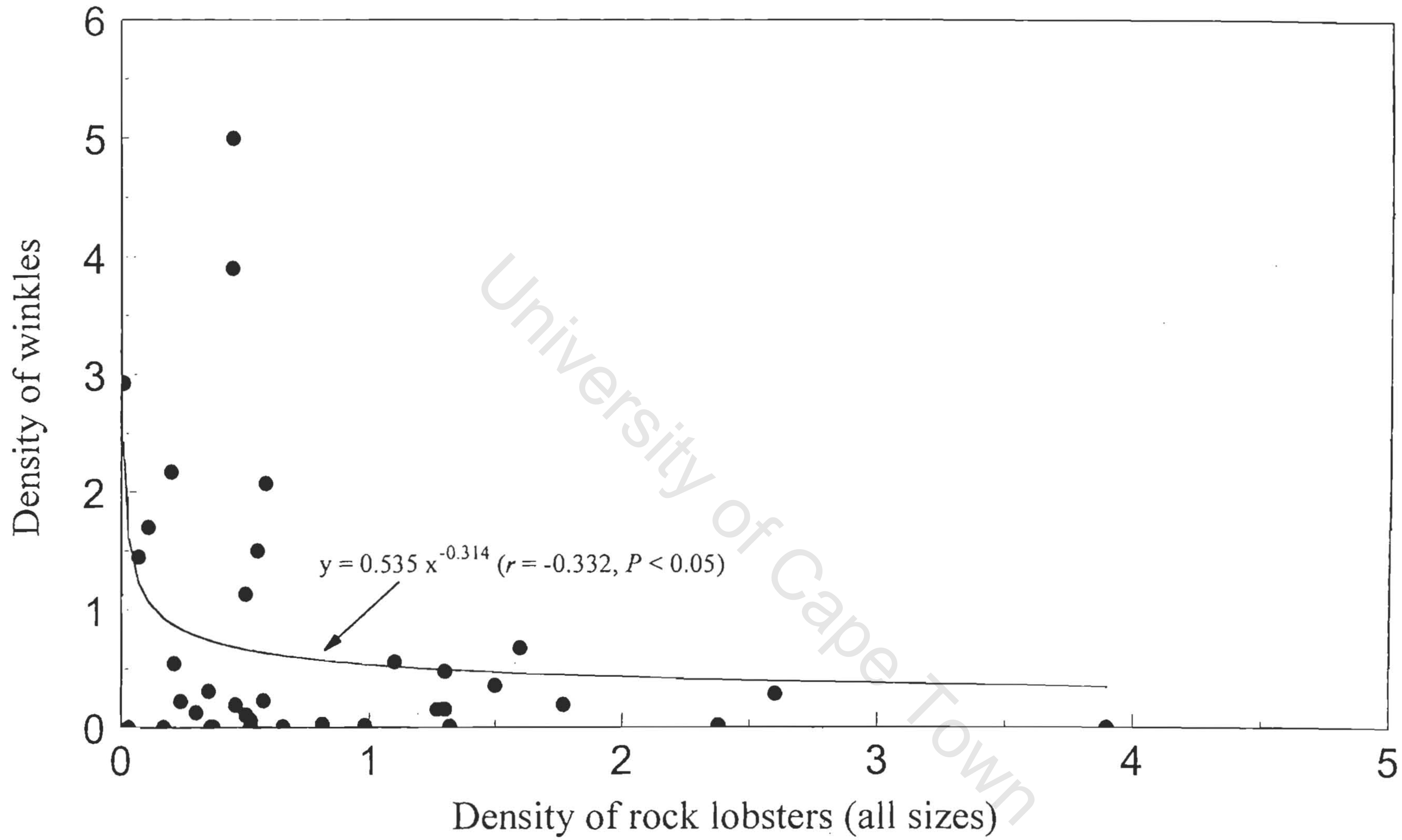

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Figure 6.8: Regression between the density of winkles, *Turbo cidaris* (numbers.m^{-2}) and the density of rock lobsters of all sizes (numbers.m^{-2}). Data on the density of winkles were obtained from Dr A Pulfrich (UCT).

Table 6.3: Calorific standing stocks for mussels, rock lobsters, sea urchins, winkles and foliar algae at Bettys Bay during 1980 and 1997.

	1980		1997	
Species	$kJ.m^{-2}$	Source	$kJ.m^{-2}$	Source
A. ater	0	Field *et al*. (1980)	0	This Chapter
J. lalandii	0	Field *et al*. (1980)	652	This Chapter
P. angulosus	118	Field *et al*. (1980)	6.5	This Chapter
T. cidaris	94.75	Field *et al*. (1980)	9.1	Dr A. Pulfrich, UCT, unpublished data
Foliar algae	9.8	Field *et al*. (1980)	7917	Dr G. Levitt, SFRI, unpublished data

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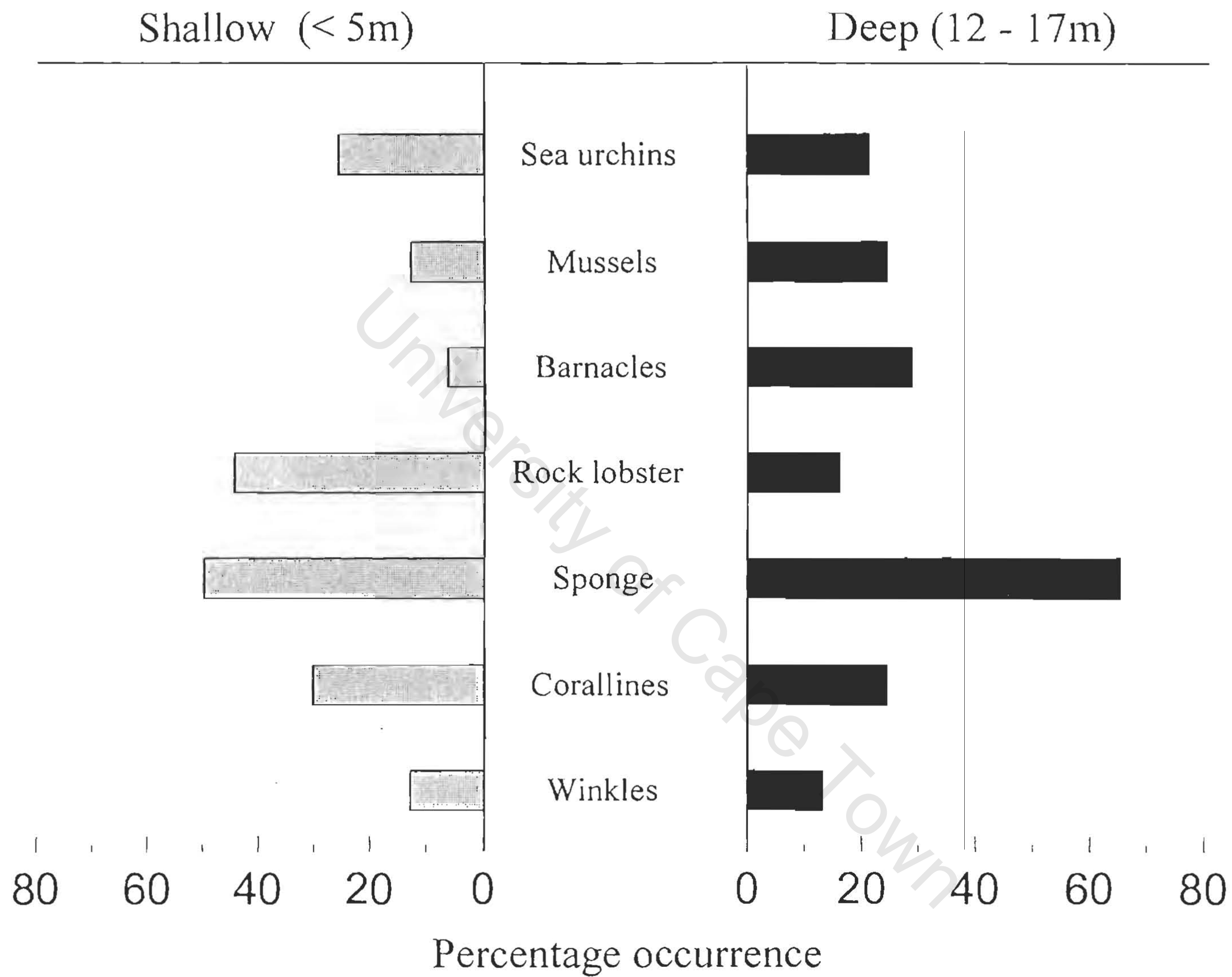

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Figure 6.9: Diet of male West Coast rock lobsters between Cape Hangklip and Danger Point (all sites combined) for deep (12 - 17m) and shallow (< 5m) stations. Corallines refers to coralline algae.

3.5 Relationships between juvenile abalone and sea urchins

The relationship between the density of sea urchins and juvenile abalone is shown in Figure 6.10. The data are dispersed below a line defining a 1:1 relationship - thus the density of juvenile abalone was always below that of sea urchins. The running mean for abalone density suggested that (1) at low sea urchin densities (< 25 - 30 sea urchins.m^{-2}) the mean density of juvenile abalone increased with increasing sea urchin density, and (2) that above a density of *ca.* 30 sea urchins.m^{-2} mean density of abalone is variable but plateaus and does not increase with increasing sea urchin density. At low sea urchin densities (< 25 sea urchins.m^{-2}) the mean density of juvenile abalone increased significantly with increasing density of sea urchins (r = 0.697, df = 15, $P < 0.01$) and was described by the equation:

Density of juvenile abalone = 0.373 * density of sea urchins - 1.6 ......(5)

This line has an x intercept at 4.29 urchins.m^{-2} which suggests that below a sea urchin density of about 5.m^{-2}, juvenile abalone will be rare or absent (using a 95% confidence interval, their density under this condition was estimated to be 0.27 ± 1.92 juvenile abalone.m^{-2}). The maximum density of juvenile abalone per sea urchin density size class increased significantly (r = 0.958, df = 13, $P < 0.001$) with increasing sea urchin density and was described by the equation:

Maximum density of juvenile abalone = 0.78 * density of sea urchins - 4.75 ......(6)

If, instead of plotting the data for all years combined (as in Figure 6.10), each year for the period 1989 - 1991 (for which there is sufficient density information for the period 5 - 7 months following spawning) is separated, then a significant relationship between juvenile abalone and sea urchin densities was only detected in the single year of high abalone recruitment (Table 6.4). This implies that high densities of sea urchins becomes critical in years of good recruitment.

Distribution of juvenile abalone in the area EOCH was strongly linked to that of sea urchins. From 435 quadrats surveyed between Cape Hangklip and Danger Point, 3801 sea urchins were removed, and a total of 198 juvenile abalone observed (Table 6.5). Of these juvenile abalone, only 3 (1.5%) were found outside the spine umbrella of sea

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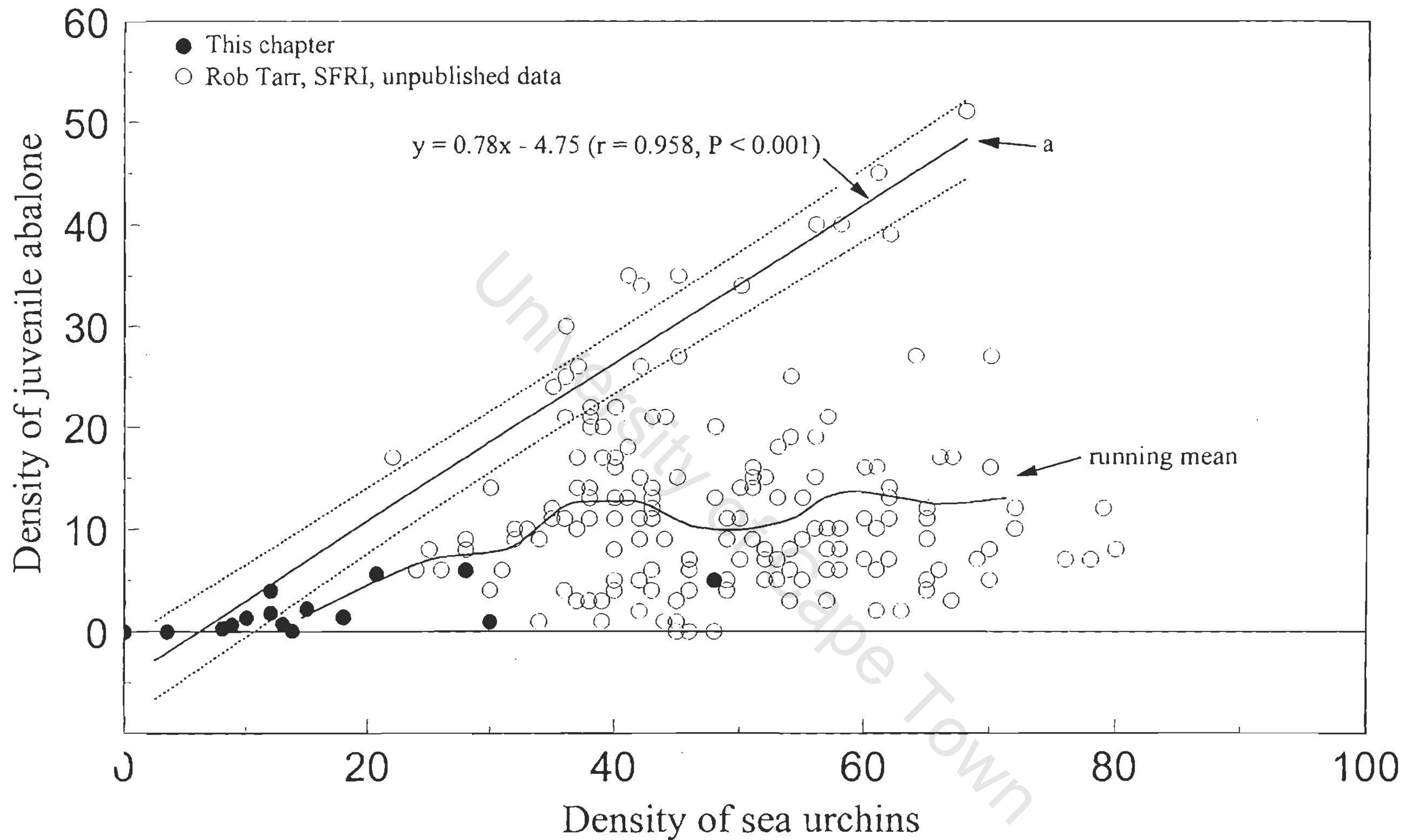

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Figure 6.10: Relationship between the density of juvenile abalone (numbers.m^{-2}) and the density of sea urchins (numbers.m^{-2}). Line 'a' refers to the maximum expected density of juvenile abalone if settlement were to be high. Dotted lines are the 95% confidence interval. The running mean is based on the average number of abalone juveniles for consecutive blocks of 10 data points (see methods). Data obtained from R Tarr (SFRI, unpublished) and covers the years 1989 - 1993.

Table 6.4: Relationship between juvenile abalone and sea urchin density (based on the equation y = ax + b) for the period March to May in each year following spawning the previous September. Recruitment level was taken as 'good' or 'poor'. Data were obtained from R Tarr (SFRI, unpublished data).

Year	Recruitment level in previous year	a	b	df	*r*	*P*
1990	GOOD	1.24	-32.88	9	0.838	< 0.01
1989	POOR	0.98	-40.36	9	0.516	> 0.1
1991	POOR	0.003	17.14	9	0.008	> 0.1

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Table 6.5: Sites sampled, mean number of sea urchins per site, standard error (bold), total number of exposed juvenile abalone (not under sea urchins) and total number of protected juvenile abalone (under sea urchins) for 10 sites EOCH and 6 sites (pooled) in False Bay. [1] indicates data from Day (1998). ND = no data.

Site	Number of $0.25m^2$ quadrats	Avg no. of sea urchins per quadrat	Std error	Total number of exposed abalone	Total number of protected abalone
Cape Hangklip (West)	45	0	-	0	0
Cape Hangklip (East)	45	0	-	0	0
Maasbaai	40	0	-	0	0
Bettys Bay	45	0	-	0	0
Mudge Point	45	0	-	0	0
Onrus	43	0.14	**0.09**	0	0
Hermanus	46	9.35	**12.9**	0	77
Hermanus (Town)	45	31.67	**4.6**	0	1
Gansbaai	39	14.53	**2.1**	2	11
Danger Point	42	32.81	**2.7**	1	106
Totals	435	3801		3	195
% of total				1.52	**98.48**
False Bay[1]	162	N.D.		40	1532
% of total				2.54	**97.46**

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urchins. Sites with few or no sea urchins also had no juvenile abalone. Data for False Bay (West of Cape Hangklip) confirm that the vast majority (97.4%) of juvenile abalone occur under sea urchins.

4. DISCUSSION

This Chapter is based on four distinct and interleading questions. To recapitulate, these are: (1) Have the rock-lobster numbers increased in the area EOCH? (2) Are there any detectable changes in the populations of other species there? (3) If so, can these changes be attributed to rock lobsters? (4) What are the ecological implications and management options?

4.1 Changes in abundance of rock lobsters EOCH

Five lines of evidence exist to suggest that rock-lobster numbers (and particularly those in the large size class) have increased and remain high EOCH. These include the interviews conducted, number of boat launches, the catch rate and catch size frequency of rock lobsters caught by recreational fishers, and a direct estimate of the density and population size frequency obtained by random sampling. The most convincing evidence, however, remains a direct comparison between current and 1980 rock-lobster abundances in Bettys Bay.

Taken collectively, the questionnaire respondents and posted returns indicated that the majority of interviewees (60%) felt that the rock lobster numbers had increased EOCH over the last 5 to 10 years (Figure 6.3). Very few respondents (13%) suggested that the numbers had declined and about 22% felt that the numbers were unchanged and 5% were unable to comment. Although no negative (or reverse) questions were asked, and results from the posted interviews are probably highly biased, the results still remain conclusive given the high number of respondents (n = 352).

Further indirect evidence was also provided when the number of rock-lobster boats launched on an average weekend day during December of each year were analysed

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(Figure 6.4). This figure gives an indication of the recreational effort applied to fishing this stretch of coastline, and can reasonably be expected to be high when the returns (*i.e.* catch of rock lobsters) are high relative to both costs and to returns in other fishing areas. It is unfortunate that Hermanus is the only launching site of seven in the area for which reasonably reliable data are available. Even these data are not complete, because although the launching site is open 24hr per day, skippers only need to purchase a launching ticket during the hours the office is open - 8am to 4pm. For many years (up to about 1992) the number of boat launches was reasonably constant - at around 20 per weekend day. This number increased dramatically in 1993 to about 70 boats per weekend day and since then has continued to increase. Today, upwards of 200 boats often launch from just this one slipway on a single day.

The number of recreational rock-lobster permits sold increased at the same rate as the number of boat launches which could imply that as more permits are sold, more people go fishing in all areas and thus the increase in the number of boat launches at Hermanus may just reflect a general increase in recreational activity. However, additional comparative information shows that the proportion of the recreational catch being landed EOCH as compared to other areas has increased from about 19% in 1991/1992 to nearly 45% in the 1994/1995 season (Cockcroft & MacKenzie 1997). This was associated with a reduction in the percentage of the recreational catch landed in the other main fishing areas (Cockcroft & MacKenzie 1997) which lie between the Knol and Cape Point (Figure 6.1).

The size frequency of the catch of recreational fishermen EOCH is shown in Table 6.1. Although a small fraction (*c.a.* 4%) of the rock lobsters landed were undersize (< 80mm CL), on average, 42% of the rock lobsters landed were larger than the old (pre 1994/1995) size limit of 89mm CL. Furthermore, the catch rate is much higher than between the Knol and Cape Point. EOCH, 80% of nets hauled in November 1997 contained at least one legal-sized rock lobster (Dr D Schoeman, SFRI, unpublished data), and most boats caught their 16 rock lobsters in less than 1hr (pers. obs.). By way of comparison, at Kommetjie about 1 in 10 nets contained a legal-sized rock lobster, and the average fishing time to catch 16 rock lobsters ranges between 2.5 and

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3hr (Mrs C Maree, Secretary, Kommetjie Boat Club, pers. comm.). This provides an indication of just how productive EOCH is for recreational fishermen, as not only were they catching larger specimens, but they were able to do so quickly.

Although not directly a measure of change in rock-lobster abundance, but definitely relevant to the impact of rock lobsters on sea urchins, a comparison was made of the size frequencies of the rock lobster populations sampled by divers on the West Coast (Sea Point and the Knol) and those EOCH (Bettys Bay and Palmiet, Figure 6.5). Of these four sites, only Palmiet lies outside a rock lobster sanctuary, yet the two West Coast sites had left-skewed size-frequency distributions and hence very few legal-sized rock lobsters (average 8.7%). Sites EOCH had a more normally distributed size-frequency distribution, with abundant legal-sized rock lobsters (average 29%).

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Perhaps, however, the most convincing evidence of an increase in rock-lobster density EOCH is obtained by comparing the abundance of rock lobsters currently there to their abundance in 1980 (the only available previous data). Unfortunately the early data were recorded only as calorific values of rock lobsters, so no direct comparison of numbers is possible. Nevertheless, in 1980, the calorific value for *J. lalandii* within 200m of the shoreline was so low as to be recorded as zero (Table 6.3). In 1997, based on the density estimates from the transect lines, random-sample size-frequency distributions and the rock-lobster length-weight regression (equation 1), rock lobsters achieved a standing stock of $652kJ.m^{-2}$.

Taken individually, these five lines of information, *i.e.* the interview responses, the boat launches (and their associated success rates), the current rock lobster population size frequency and the comparisons of abundance between 1980 and 1997 are insufficient to conclusively answer the first question posed. However, all these lines of information are complementary and corroborative. Taken collectively, they enable the confident conclusion to be drawn that rock-lobster numbers have increased substantially EOCH over the last decade. Thus, the initial prediction based on the hypothesis originally proposed by Tarr *et al.* (1996) was upheld by these five lines of evidence.

Briefly, it is necessary to consider why rock-lobster densities should have increased EOCH. Initially it was thought that the observed increase was due to an eastward migration only - but data collected here on the size structure (Figure 6.5) clearly show recruitment of juveniles is taking place. Perhaps the initial migration was due to food requirements - the numbers increased EOCH during the period when growth rates on the West Coast began to plummet. However, this explanation is unlikely as the preferred food source, mussels, is (and always seems to have been) scarce subtidally EOCH (Field *et al.* 1980). Perhaps the initial migration was more a response to large scale changes in climatic and environmental conditions as brought about by the 1990/1991 *El Niño* event. However, as tagging studies have shown limited large-scale migrations an alternative explanation could be that there were a few years of either good recruitment or high survival (or both) of rock lobsters EOCH. The ultimate cause of the change is thus an open question.

4.2. Detectable changes in other species EOCH

Some information on the previous structure of the benthic community at Bettys Bay is available (Field *et al.* 1980). However, since that survey, little other work on community structure has been conducted in this area, until 1996 when the current project was initiated. Concurrently, information on winkles and foliar algae was obtained by other researchers (Dr A Pulfrich, UCT & Dr G Levitt, SFRI). Thus the only community comparisons that can be made are between the species and groups examined in 1980 and then again recently. Table 6.3 lists the changes in their abundance (based on calorific values). In 1980, sea urchins and winkles were common at Bettys Bay, but in 1997 they were almost non-existent. In contrast, rock lobster and foliar algal abundance had escalated to 652 $kJ.m^{-2}$ and 7917 $kJ.m^{-2}$ in 1997 respectively - the latter being almost an 800 fold increase. There was no change in the abundance of the ribbed mussel *Aulacomya ater*, which was absent from the benthos during both the 1980 and current surveys. Given that all of these species and 'groups' are key organisms capable of modifying community structure and functioning, this is powerful evidence that at least over the last 16 - 18 years there have been substantial changes in the benthic community at Bettys Bay.

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4.3. Are the changes attributable to rock lobsters?

Thus far it has been shown that rock-lobster numbers have changed (increased dramatically) EOCH in the last decade or so, and that during the same time period, there have been changes observed in benthic community structure. However, whether or not the rock lobsters are responsible for these changes must now be considered. The first thing that needs evaluation is the diet of rock lobsters EOCH.

Sponges were by far the most common prey item found in the gut contents of rock lobsters collected EOCH (Figure 6.9) - a complete contrast to the diet of specimens collected on the West Coast, where mussel remains were overwhelmingly dominant (Chapter 4, Figure 4.4). Coralline algae and rock lobster remains were also common EOCH, while barnacles were rarely consumed compared with the situation on the West Coast. Of further interest here though, is that almost 15% of stomachs examined contained winkle remains (either *Turbo cidaris* or *Oxystele sinensis*). Although *J. lalandii* is capable of feeding on these winkles in captivity (van Zyl *et al.* 1998) this is the first time that winkle remains have been reported in the natural diet of this rock lobster species. Possibly it reflects their greater abundance EOCH relative to the West Coast (Anderson *et al.* 1997).

Sea urchin and mussel remains were common in the gut contents of rock lobsters from both shallow and deep stations sampled, occurring in almost 30% and 20% of stomachs examined respectively. Furthermore, they were ubiquitous in the gut contents across all the sites. This is in complete contrast with what was predicted, given that at most of the sites sampled, sea urchins (and mussels) were rare to non-existent (Figure 6.6a,b). During their diving surveys in early 1995, Tarr *et al.* (1996) reported finding only 20 sea urchins at Mudge Point - 10 of them in the claws of rock lobsters. Rock lobsters have already been shown to be selective foragers (Chapters 4 & 5) and the results obtained here support that conclusion - even in the near absence of sea urchins and mussels, these prey are still being eaten frequently. The question remains though, as to where rock lobsters are obtaining sea urchins and mussels when they are apparently so rare in the benthic communities. Sea urchins (and juvenile abalone) are still common in very shallow wave-exposed areas (and low intertidal

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rock pools) EOCH (pers. obs. & Tarr *et al.* 1996). This is probably because such areas are usually unsuitable for foraging rock lobsters (Zoutendyk 1988a,b) due to underwater turbulence. However, as most of the sampling coincided with calm sea conditions under which rock lobsters could have foraged in the very shallow subtidal (< 1m), rock lobsters may have been moving inshore at night during high tide to feed on the few remaining sea urchins which are now confined to the relative safety of turbulent waters. Given that rock lobsters EOCH naturally consume winkles and sea urchins, it is now possible to examine rock lobster effects on their population distribution.

When considering the densities of sea urchins and rock lobsters at the deep and shallow sites there was an underlying negative association between the density of rock lobsters (all sizes) and sea urchins (Figure 6.6a,b). Areas with a high density of rock lobsters usually had a low density of sea urchins, and *vice versa*. There were, however, four exceptions (The Knol, Oudekraal, Sea point and Robben Island) and the reasons why these sites contrasted with the general pattern will be explained later. Such a negative association between lobsters and urchins has, to my knowledge, only once previously been reported with substantial data - namely between *Jasus edwardsii* and *Evechinus chloroticus* (Andrew & MacDiarmid 1991). Other authors have, however, reported almost anecdotally that high predator abundance is correlated with low urchin numbers, with the reverse (*i.e.* low predator, high prey) also being true for both lobsters (*e.g.* Mann & Breen 1972, Breen & Mann 1976a, Elner & Vadas 1990) and otters (Lowry & Pearse 1973).

However, the question of whether rock lobsters are in reality having a significant effect on sea urchins still remains unanswered. In laboratory experiments, only *Jasus lalandii* larger than 68mm CL killed any sea urchins, although above this size rock lobsters were able to kill sea urchins of all sizes (Chapter 5, Figure 5.1). Bearing this in mind, the analysis of the relative densities of sea urchins and rock lobsters was done using the density values for *large* rock lobsters (CL > 68mm) only. Using these parameters, the density of sea urchins declined along a power curve relationship (equation 3) as the density of large rock lobsters increased. When this analysis was

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repeated using all sizes of rock lobsters or small rock lobsters only, insignificant results were obtained (Table 6.2). Furthermore, the relationship with large rock lobsters suggests that above a density of roughly $0.2m^{-2}$ (*i.e.* 1 large rock lobster per $5m^2$ of reef), very few (if any) sea urchins will survive (Figure 6.7). The question mentioned earlier of why Robben Island, Sea Point, Oudekraal and the Knol are different to the general trend can now easily be answered - except for Oudekraal (deep reef) these sites have extremely low densities of large rock lobsters. Perhaps more importantly, at all of these sites, mussels are still available as a food source (Figure 6.6a,b). Mussels have already been shown to be the preferred prey source both from gut content analyses (Chapters 3 & 4) and in laboratory feeding trials (Chapter 5).

Several assumptions are inherent in this sea urchin - rock lobster density relationship. The first of these is that sea urchin and rock-lobster densities are related to one another, and not to some other (unmeasured) factor. It is appreciated that the negative correlation between rock lobsters and sea urchins does not necessarily imply a cause and effect. Elner & Vadas (1990) have previously disproved a putative link between lobsters, urchins and kelp along the North American Atlantic coastline and have cautioned against correlations as proof of relationship. However, in the present case there are several lines of evidence supporting this assumption. Rock lobsters do feed on sea urchins in the wild as indicated by their gut contents. My laboratory experiments show that rock lobsters readily consume sea urchins provided they are above the critical size (68mm CL). The rate of consumption, when combined with the density of rock lobsters EOCH suggests that they are easily capable of decimating sea urchin populations. Finally, the experimental work of Barkai & Branch (1988b) has shown that, when it occurs at high densities, *J. lalandii* is capable of completely transforming benthic community structure including the near elimination of mussels and sea urchins. The negative correlation demonstrated here between the two species could be pursued experimentally by the use of field exclusion and inclusion cages. Fisheries scientists are independently planning to do this.

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The second assumption is that the laboratory studies can be extrapolated to the field - this seems reasonable in the light of the fact that rock lobsters do regularly feed on sea urchins in the field. The final assumption is that the relationship described in Figure 6.7 is a general one. As this curve is based on numerous data points lying on the axes, this third assumption is not entirely robust, but the relationship still provides strong qualitative evidence for the relationship even if its quantitative capabilities are limited.

There is no doubt that rock lobsters are predators of sea urchins, and on the basis of the negative correlation between the two, it seems that large rock lobsters are having a profound and controlling impact on sea urchin distribution and abundance EOCH. Thus the prediction made earlier concerning the relationship between sea urchin density and large rock-lobster density was upheld. Further, although not demonstrating cause and effect, the relative abundances of sea urchins and rock lobsters are, suggestively, the reverse today of what they were in 1980 (Table 6.3).

The density of *T. cidaris* also declined as the density of rock lobsters of all sizes increased, in a relation described again by a power curve (Figure 6.8). As with the relationship between sea urchins and large rock lobsters, the density of winkles was extremely low above a certain rock-lobster density - in this case about 1 rock lobster.m^{-2}. The abundance of these grazers is clearly negatively associated with the abundance of rock lobsters. Again this curve has limited predictive capabilities for the same reasons as highlighted above - but, as before, it provides good qualitative evidence of a negative relationship.

Thus, the third question has been answered. The changes in population structure (of at least sea urchins and winkles) can very probably be attributed to rock lobsters. They are negatively correlated; rock lobsters do consume both sea urchins and winkles (in the field and in the laboratory); and the increase in rock lobsters EOCH has coincided with declines in both sea urchins and winkles. Reductions in the density of grazers (of which sea urchins and winkles are the most important in the subtidal benthos) are very likely to have led to the proliferation of foliar algae. In rigorous field experiments,

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Day (1998) has shown that exclusion of benthic invertebrate grazers enhances foliar algae whilst leading to a reduction in encrusting corallines.

What then are the associated ecological implications, and how can this situation best be managed?

4.4 Ecological implications and management options

There is a strong relationship between juvenile abalone and sea urchins. Rigorous field experiments have demonstrated that removal of sea urchins leads to the complete collapse of juvenile abalone (Day 1998). Furthermore, in surveys of 162 quadrats in False Bay between Cape Point and Millers Point in 1995, Day (1998) observed almost no juvenile abalone (< 3%) outside the protection of the spiny canopy of sea urchins (Table 6.5). The data collected here support these findings. In the 435 quadrats sampled between Danger Point and the western edge of Cape Hangklip, only 3 (1.5%) of the 198 juvenile abalone were found anywhere other than under the spines of sea urchins (Table 6.5) - and all three were completely surrounded by sea urchins. Furthermore, sites with no sea urchins also had no juvenile abalone. Sea urchins were only observed East of Onrus - an area that has a lower rock-lobster density than the region between Onrus and Cape Hangklip (Figure 6.6a,b). Thus the prediction was upheld - few juvenile abalone were found outside the spine canopy of the sea urchins.

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There was no relationship between the mean density of juvenile abalone and the density of sea urchins once the sea urchins exceeded about 25 - 30.m^{-2} (Figure 6.10). Above this, there was no further increase in the density of juvenile abalone with increasing sea urchin density (average 12.3 juvenile abalone.m^{-2}). However, the density of sea urchins was always considerably greater than that of juvenile abalone (the data lie well below a 1:1 relationship). In other words, each sea urchin sheltered, on average, less than one juvenile abalone. High densities of sea urchins will probably be particularly important in years of good abalone recruitment because the availability of sea urchins may well then be the factor limiting juvenile survival. Line 'a' on Figure 6.10 reflects the relationship expected in years of excellent settlement. In years of poor abalone recruitment, urchin density is probably irrelevant to survival of

juvenile abalone provided it is above the threshold necessary to meet their needs. This situation is reflected by the line showing the average running mean in Figure 6.10. In years when abalone recruitment is high, however, the correlation between the two species extends over the full range of sea urchin densities and becomes much more significant (Table 6.4), supporting the initial prediction.

Even if one pools the data to obtain an overall picture for several years, juvenile abalone densities remain correlated with sea urchin density, but only at densities less than 25 - 30 sea urchins.m^{-2} (equation 5) thus still upholding the initial prediction. Solving equation 5 for y = 0 (*i.e.* density of juvenile abalone equals zero), yields an x value of 4.29 sea urchins.m^{-2}. This value of 4.29 corresponds to the point 'a' on Figure 6.7. Rounding this up to 5 sea urchins.m^{-2}, equates to 0.27 ± 1.92 juvenile abalone.m^{-2} (thus a range from 0 to 2.2 juvenile abalone.m^{-2}, 95% confidence interval). Substituting 5 for y in equation 3, indicates that this minimum density of sea urchins occurs at a density of large rock lobsters (>68mm CL) of 0.08.m^{-2}. Thus, it is predicted that at rock-lobster densities greater than this, there will probably be insufficient sea urchins to support even a single juvenile abalone. Of course other factors need to be considered - sea urchins may not be the only limiting factor (*i.e.* survival of juvenile abalone is almost undoubtedly dependant on at least one other factor), nor are rock lobsters likely to be the only predators on sea urchins. Both of these complications will, however, only further reduce survivorship of juvenile abalone.

It is common knowledge that the survival rate of recruits into juveniles and juveniles into adults for most species is extremely low. For *H. midae*, natural mortality (M) has been tentatively estimated at 0.05yr^{-1} (Moloney 1997). Juvenile abalone require about 8 years to reach harvestable size (Newman 1968, Tarr 1995). Substituting M = 0.05, t = 8 and N_0 = 0.27 into the logistic decay function ($N_t = N_0 * exp^{-Mt}$, where N_0 = number at start, N_t = number at time t and t is the time interval), predicts that after 8 years, the initial number of 0.27 juvenile abalone.m^{-2} will have declined to 0.18 adult abalone.m^{-2}. This is a higher value than the density of legal-sized abalone in currently commercially exploited areas (0.15 abalone.m^{-2}, SFRI, unpublished data).

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However, adult stocks are in continual decline with poaching adding to the problem (A. MacKenzie, SFRI, pers. comm.).

Juvenile abalone occur predominantly in shallow water, usually less than 5m (Day 1998). Based on the transect line data (Figure 6.6a,b), the current mean sea urchin density in the depth zone 0 - 5m between Cape Hangklip and Gansbaai is only 1.65 urchins.m^{-2} (n = 106 transects; ~ 2700m^2). This is half the minimum sea urchin density (4.29 sea urchins.m^{-2}) required to support any juvenile abalone and about three times smaller than the estimated density of sea urchins needed to maintain the current adult stocks. The prognosis is, however, much better East of Hermanus, where in shallow waters (< 5m) the mean density of sea urchins is 30.5 sea urchins.m^{-2}. The importance of sea urchins to juvenile abalone is further confirmed by analysis of the field data - sites at Hermanus and eastwards, where rock lobsters are scarce in the shallows and sea urchins still common, were the only ones EOCH at which juvenile abalone were found (Table 6.4).

Even if settlement of abalone larvae may be high between Cape Hangklip and Gansbaai because the brood-stock is concentrated there, given that there are currently few sea urchins there, it is likely that the survival rate of the resultant abalone juveniles will be extremely low. Low sea urchin densities and failure of abalone recruitment became a feature of this area in about early 1995 (Tarr *et al.* 1996). As abalone take approximately 8 years to attain the minimum legal size of 114mm shell length, it is probable that there will be very few abalone of harvestable size available to the commercial industry in about the year 2004. Even this may be an optimistic prediction - with the current high poaching levels, the increasing recreational sector and the additional harvesting that may attend the introduction of 'subsistence' fishing permits, adult stocks and even pre-reproductive stocks may be reduced rapidly, and will not be replaced. Fewer adults leads to fewer larvae, and with a low probability of survival of both settlers (see Day 1998) and juveniles in the absence of sea urchins, abalone may soon become severely threatened.

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However, the scenario could get even worse. The average density of sea urchins between Cape Hangklip and Danger Point was estimated at 4.2 sea urchins.m^{-2}. As the total area of reef is 48km^2 (van Zyl *et al.* 1998), the total number of sea urchins in this area is estimated to be 2 x 10^8. The total number of large rock lobsters in the same area was estimated to be 16.3 x 10^6. Rock lobsters consumed on average 6.1 sea urchins.rock $lobster^{-1}$.day^{-1} during the laboratory experiments (Chapter 5). Thus if rock lobsters were to focus only on sea urchins, the total stock of sea urchins could be eliminated in just 12 days. Of course, a proportion of the sea urchins will remain unavailable to rock lobsters in turbulent areas. The point is that all sea urchins below 1m water depth could easily be eliminated - and, like the abalone, not replaced.

From the running average presented in Figure 6.10, perhaps the minimum density of sea urchins needed to achieve *replenishment* of adult and sub-adult abalone stocks is at least 30 sea urchins.m^{-2}. Beyond this value, no increase of juvenile abalone density with increasing sea urchin density was recorded under average conditions of recruitment.

What are the management options? One possibility is to re-supply the area with sea urchins, or sea urchins together with juvenile abalone. Another approach could entail the harvesting of abalone and rock lobsters simultaneously, including the possibility of introducing commercial harvesting into this area (which is currently closed to this sector). Finally, the system could simply be left alone in the hope that the balance will naturally swing back again.

Stock enhancement has been considered for the Californian red sea urchin (*Strongylocentrotus franciscanus*), which was diminished by overfishing (Tegner 1989). Abalone re-seeding has long been investigated in South Africa (Cook & Sweijd in press), Japan (Saito 1979, Kojima 1981) and in many other areas of the world (see Emmett & Jamieson 1989, Schiel 1992, Gaffney *et al.* 1996). Most attempts have yielded limited success, but recent re-seeding of juvenile abalone in South Africa has been promising (Cook & Sweijd in press).

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However, before stock enhancement of sea urchins and/or abalone even becomes an option EOCH, the rock lobster density will need to be reduced - given both their consumption rates of sea urchins and their selection of small abalone in preference to sea urchins (Chapter 5). The predicted maximum density of large rock lobsters that will allow the survival of even a few (maximum 2.2) juvenile abalone.m^{-2} was 0.08 rock lobsters.m^{-2} (equation 3 and Figure 6.7). Based on the transect lines, the current rock lobster density (CL > 68mm) between Cape Hangklip and Gansbaai is 0.23 rock lobsters.m^{-2} (< 5m depth). Thus, for a re-seeding programme to have even minimal effect, the current density of large rock lobsters must be more than halved. This would require an estimated harvest of 8.1 million large rock lobsters, the equivalent of around 2450 tons. However, even halving the density of large rock lobsters and re-seeding the area with sea urchins and abalone will have little effect on improving the future of the abalone industry, because very much more than the minimum levels of juveniles are needed to facilitate stock recovery. The abalone industry will only benefit if the density of large rock lobsters is reduced to a level low enough to enable sea urchin densities to increase to about 30 sea urchins.m^{-2}. The predictive capabilities of equation 3 are too limited to make this calculation precise. However, what is obvious is that more than 2450 tons of rock lobsters would need to be harvested initially. Bearing in mind the limitations of the equations already highlighted, several possible scenarios are presented in Table 6.6. The most striking thing to emerge is that for a wide range of possible densities of abalone juveniles, reductions of between 4381 and 4860 tons of rock lobsters will need to be removed. To achieve this may not only be impractical, but it would also be a very questionable goal. The rock lobsters themselves are a valuable resource that should be harvested sustainably, and not simply slashed by the radical amounts that seem necessary to allow recovery of the abalone stocks. Until more accurate estimates are available for the rock-lobster stock EOCH, it will be impossible to set a meaningful TAC.

Perhaps the most appropriate approach will be to harvest abalone and rock lobsters simultaneously. As South Africa is entering a new era in marine fisheries management, the urchin-abalone-lobster scenario EOCH may provide a useful test case for the future. A new fisheries policy has just been drafted into law (The Marine

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Table 6.6: Scenarios for the minimum initial harvests of rock lobsters (tons) required to ensure survival of different densities of juvenile abalone. (1) Possible initial densities of juvenile abalone.m^{-2}. (2) resultant number of abalone.m^{-2} surviving after 8 years of growth (based on the logistic decay function); (3) Minimum densities of sea urchins.m^{-2} required to support the juvenile abalone specified in (1), as obtained from Equation 6. (4) Maximal densities of rock lobsters that may occur in the area without reducing the sea urchins below the level in (3), calculated from Equation 3. (5) levels to which the present rock-lobster population (0.23.m^{-2}) needs to be reduced to achieve the densities given in (4). (6) Minimum numbers of rock lobsters (expressed in millions) that will need to be harvested to achieve this reduction, based on the approximation that the current population is 16.2 million. (7) Equivalent biomass (tons) that will need to be harvested assuming that the average mass of a 'large' legal-sized rock lobster is 300g. The current density of legal-sized abalone is underlined (SFRI, unpublished data).

University of Cape Town

(1) maximum # of juvenile abalone	(2) estimated # of legal-sized abalone.m^{-2}	(3) minimum # of sea urchins required	(4) maximum rock lobster density	(5) reduction required	(6) minimum harvest millions	(7) minimum harvest tons
0.2	0.13	6.35	0.0227	1/10	14.6	4381
0.22	**0.15**	6.37	0.0222	1/10	14.6	4391
0.4	0.27	6.60	0.0185	1/12	14.9	4468
0.6	0.40	6.86	0.0153	1/15	15.1	4537
0.8	0.54	7.12	0.0127	1/18	15.3	4592
1	0.67	7.37	0.0106	1/22	15.5	4636
2	1.34	8.65	0.0047	1/49	15.9	4761
5	3.35	12.50	0.0007	1/317	16.1	4845
10	6.70	18.91	0.0001	1/2589	16.2	4858
20	13.41	31.73	0.0000	1/35825	16.2	4860
40	26.81	57.37	0.0000	1/724202	16.2	4860

Living Resources Bill), and the goals include (1) redistribution of resources to broaden access to new entrants, (2) consideration of 'subsistence' fisheries and (3) resource co-management. All of these things could be addressed here. There is no commercial fishery for rock lobster EOCH. Thus the door is wide open for the resource to be allocated for commercial harvest by the local communities, many of which are poverty stricken and have subsisted on fishing for decades. However, to achieve successful management of resources needs not only a consideration of social and economic demands, but biological demands as well (Langton *et al.* 1996). A package deal with quotas for abalone and rock lobsters seems appropriate - with TAC's set at an as yet undetermined but sustainable level. Organisation of the communities into groups, co-operation between these groups, and the establishment of a system of co-management between these communities and the Sea Fisheries Research Institute could go a long way to stabilising harvests, reducing poaching and developing legitimate trade and controls. This will be especially true if the package is sufficiently attractive and penalties for poaching are made stricter and (most importantly) enforced.

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A final possibility is just to leave the situation as it is and to monitor it closely. This is of particular relevance given the fact that even if the rock lobster numbers are reduced and re-seeding is an option, there is *no* guarantee that the situation will return to the community structure of the 1980's. The current increase in rock lobsters EOCH is not necessarily a new phenomenon. Hermanus was the port for a lucrative commercial rock-lobster fishery in the 1960's (Dr D Schoeman, SFRI, pers. comm.). Furthermore, there is no evidence to suggest that the current influx of rock lobsters is anything but natural.

On a different note, a brief consideration of the winkles is required. Winkle densities, like those of the sea urchins, were negatively associated with rock-lobster densities. Low winkle abundances (< 1 winkles.m^{-2}) occurred at (total) rock lobster densities greater than 0.7.m^{-2}. The reason underlying such a strong relationship between these two species is evident in results from laboratory feeding experiments. Extrapolations from these data suggest that if the existing population of *J. lalandii* between

Hermanus and Cape Hangklip focused on winkles as their sole prey item, they would consume the existing standing stocks of *Turbo cidaris* and *Oxystele sinensis* in 7.1 and 2.2 days respectively (van Zyl *et al.* 1998). In the field, rock lobsters are major predators of winkles (this chapter) and they clearly have the potential to strongly influence their population structure.

The issuing of an experimental permit (Dr A Pulfrich, UCT, pers. comm.) for the exploitation of *T. cidaris* between Mooihawens and Danger Point may have been premature. Apart from the potential for rock lobsters to consume the entire standing stock of this species in a few days, the current rock lobster density (considering all sizes, and both depth zones) in this area is 0.54 rock lobsters.m^{-2}. Equation 4 predicts that this will be associated with a winkle density below 0.8 winkles.m^{-2}. This is an extremely low density of winkles, and any commercial harvest would lead rapidly to economic overexploitation. This becomes even more serious when one considers that the winkles have a clumped distribution, with the overall density being about 0.06 winkles.m^{-2} (Dr A Pulfrich, UCT, unpublished data). In addition, the removal of large numbers of these important grazers, coupled with reductions in sea urchin densities, could have important side effects on foliar algae and kelp. Already in areas where grazer densities are low, a massive increase in the abundance of foliar algae has occurred, for example at Bettys Bay (Table 6.3). Here, foliar algae increased from about 10 to nearly 8000kJ.m^{-2} between 1980 and 1997. At other sites which have low grazer abundances, the growth of foliar algae was not quite so prolific, but an unidentified microalgal 'fuzz' growing on the reefs and the sloughing of large slabs of encrusting coralline algae are consistent features of the substratum (pers. obs.). These changes are not unique - many authors have documented rapid algal growth in the absence of grazers (*e.g.* North & Pearse 1970, Paine & Vadas 1969, Pearse & Hines 1979, Scheibling 1986, Duggins 1983), usually with succession back to mature kelp beds (Paine & Vadas 1969, Scheibling 1986). Perhaps, then, the kelp beds EOCH will show rapid expansion in the next few years, as reported from California after the decline of sea urchins (Pearse & Hines 1979). However, of more serious consequence here is that these grazers (especially winkles) are important for maintaining encrusting coralline algae free of foliar algal overgrowth (Day 1998). 'Clean' coralline algae are

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essential for the successful settlement of abalone larvae (Tarr 1989). In the light of this evidence, it seems to have been a sensible move for the SFRI to have recently repealed this permit.

Conclusion

Several lines of qualitative and quantitative evidence indicate that rock-lobster numbers EOCH have increased over the last 5 - 10 years, supporting the proposal of Tarr *et al.* (1996). This evidence was obtained from rock-lobster density and size frequency estimates, questionnaires, an index of fishing effort and comparison with an earlier survey. At the densities now recorded EOCH, rock lobsters have the potential to profoundly influence the population abundances of both sea urchins and winkles. The abundance of juvenile abalone was tightly linked to that of sea urchins and it was estimated that a sea urchin density below about 4.29 sea urchins.m^{-2} would lead to the demise of most juvenile abalone. This will occur when large rock lobsters exceed a density of 0.08.m^{-2}, a value well below half that of the current density EOCH. However, to allow recovery of sea urchins to a density that will support a sufficient number of juvenile abalone to promote recovery of abalone stocks, even further reductions in the rock-lobster density will be required. Low densities of grazers are probably resulting in the large increases of macro-algae observed at Bettys Bay and both the development of microalgal 'fuzz' and mass die-offs of coralline algae at this and other sites with high rock-lobster densities. This further contributes to the plight of the abalone, as 'clean' encrusting algae are essential for abalone settlement (Day 1998). It is recommended that rock lobsters and abalone EOCH be commercially harvested sustainably, and preferably together, primarily by local communities in an arrangement of co-management with the Sea Fisheries Research Institute.

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General conclusions

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GENERAL CONCLUSIONS

This thesis has focused on two aspects of rock-lobster predation. In the first instance, the relationships between rock-lobster diet, growth rates and food availability were investigated. In the second, the influence of rock lobsters on sea urchin population density and the ripple effects through to juvenile abalone were elucidated and modelled.

Both of these thrusts required an accurate knowledge of rock-lobster diet. It was for this reason that the first part of the thesis encompassed a review of techniques available for diet analysis and the development and testing of a new non-visual technique to determine the diet of *Jasus lalandii*. This ELISA approach based on immunoassay techniques appeared promising from the literature review and from the early experiments. Species-specific antisera were developed against 26 different prey species, and cross-reactions reduced below 4%. Despite this early success, application of the technique to determining the diet of *J. lalandii* failed, principally because high quantities of digestive enzymes prohibited binding between the antigen and antibody in the micro wells of the ELISA plates. This finding, while preventing the development of a new, potentially more accurate tool for the assessment of the diet of rock lobsters, is of scientific interest in its own right.

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Because studies linking growth rate, rock-lobster diet and food availability require standardisation for concrete conclusions to be drawn, factors which could have influenced those results were examined. Although rock-lobster diet varied considerably with rock-lobster size - from a diet comprising mostly mineral constituents to one comprising mainly proteinacious constituents, limited differences in diet were recorded over the moult cycle or at different capture depths.

Rock lobsters captured at eight sites (5 slow-growth, 3 fast-growth) were collectively consuming similar prey species in similar proportions. Further analysis showed few differences between fast- and slow-growth sites on the basis of gut fullness or the proportion of the population feeding. A more intense survey focusing on just four sites

(two for juvenile and two for adult rock lobsters) was conducted. At each site, rock-lobster diet and food availability were determined simultaneously. The results of this analysis showed rock lobsters to be consuming similar proportions of the same prey despite vast differences in the benthic community composition. Hence, it was concluded that relative availability of prey in the benthos was ~~most~~ the most likely cause of the current slow growth rate. While dietary composition and gut fullness may be similar in areas of fast and slow growth, the availability of profitable food is substantially less in areas of slow growth. Possibly the time and energy devoted to obtaining food is correspondingly greater, and the consequent reduction in profit may cause slow growth.

Rock lobster predation on sea urchins in the laboratory showed (1) that small rock lobsters ate no sea urchins, and (2) that all large rock lobsters (CL > 68mm) were capable of at least partially consuming all sizes of sea urchins - but they all preferentially ate small sea urchins when given a choice. Average size of sea urchin consumed increased with rock-lobster size, but the average number of sea urchins consumed per rock lobster per day did not vary with rock-lobster size. Rock lobsters showed a preference for mussels over all other prey tested, and for juvenile abalone over sea urchins.

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Rock-lobster numbers were shown to have increased substantially over the last 10 years East of Cape Hangklip (EOCH). In this area, and over the same time period, the numbers of winkles, sea urchins and juvenile abalone have declined while the abundance of algae has increased dramatically. Rock lobsters were shown to be capable of influencing the population density of sea urchins and winkles. Juvenile abalone rely on sea urchins for shelter. The minimum density of sea urchins required to support 1 juvenile abalone.m^{-2} was estimated to be 5 sea urchins.m^{-2}. It was estimated that this required a large rock lobster density of less than 0.08.m^{-2}. The current large rock lobster density EOCH is 0.23 rock lobsters.m^{-2}. Thus to ensure survival of abalone, the current rock-lobster density needs to be more than halved. To enhance the currently depauperate commercially exploitable abalone stocks, a much greater reduction in rock-lobster density will be required.

It was recommended that rights for commercial exploitation of rock lobsters (in conjunction with abalone) be offered to poverty-stricken local communities along the Cape South Coast. This approach should be successful if (1) the rewards are high; (2) poaching is reduced (by increasing and issuing fines) and (3) community participation and co-management can be implemented with the Sea Fisheries Research Institute. Future avenues which should be explored include (1) determining the diet of *J. lalandii* from depths greater than 50m; (2) assessing the diet of small (CL < 40mm) and female rock lobsters (both completely ignored here) and (3) long-term laboratory feeding experiments to test the hypothesis developed here that different levels of time and energy expenditure can result in observed differences in growth rate.

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