STUDIES OF THE BIOLOGY AND ECOLOGY OF THE HIGH SHORE SOUTH AFRICAN LIMPET, *HELCION PECTUNCULUS* (MOLLUSCA: PATELLOGASTROPODA).

THESIS
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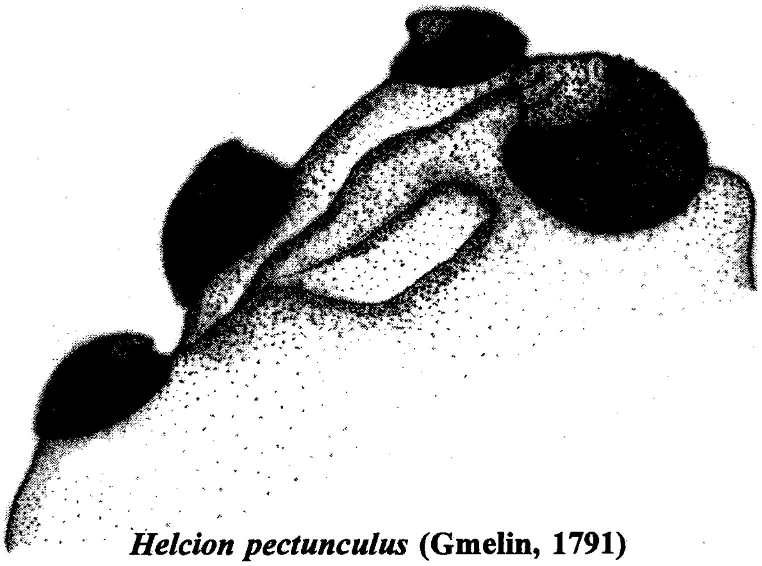

Helcion pectunculus (Gmelin, 1791)

CLAMP THE MIGHTY LIMPET

I am Clamp the Mighty Limpet
I am solid, I am stuck
I am welded to the rockface
With my superhuman suck.
I live along the waterline
And in the dreary caves.
I am Clamp the Mighty Limpet!
I am ruler of the waves.

What care I for the shingle,
For the dragging of the tide,
With my unrelenting sucker
And my granite underside?
There's only one reward
For those who come and prise at me
And that's to watch their fingernails
As they go floating out to sea.

Don't cross me, I'm a limpet,
Though it's plankton I devour.
Be very, very careful
I can move an inch an hour!
Don't poke me or prod me
For I warn you - if you do
You stand there for a fortnight
And I'll come and stick on you!

Pam Ayers.

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Abstract

Investigations were carried out into aspects of the biology and ecology of *Helcion pectunculus* along the coast of South Africa. These included studies of the distribution, density and biomass of the limpet at six sites along the east coast plus one site on the west coast; the growth of *H. pectunculus* on both west and east coasts; a comparison of the reproductive biology of the east and west coast populations; the foraging activity and feeding behaviour of *H. pectunculus* and the driving forces behind the rhythmic behaviour of this limpet; the importance of the crevice environment in the biology and ecology of *H. pectunculus.*

Helcion pectunculus has a restricted zonation, with the majority of animals residing in crevices in the upper Balanoid zone during diurnal lowtides, although individuals were occasionally found in the lower Balanoid zone on shores with a gently sloping aspect. This limpet occurs in higher densities (50-100 individuals/m^2) on shores which have large numbers of crevices and boulders *i.e.* quartzitic sandstone shores. On most shores, the ratio of males to females differed significantly from a 1:1 ratio with the highest ratio being obtained on the west coast (3 males : 1 female). At all sites, the populations of *H. pectunculus* exhibited strong sexual dimorphism. Males and females were always found to differ in size, with individuals of < 20 mm shell length generally being male whilst limpets with a shell length of > 22 mm were generally female.

Helcion pectunculus grows allometrically, increasing in height faster than length, which is expected of a high shore gastropod mollusc attempting to reduce evaporative water loss. Growth rates were similar on both east and west coasts regardless of the differing oceanographic conditions. The theoretical values of L_{max} were also similar being 30.86 mm and 30.71 mm respectively. Micro-growth bands are laid down within its' shell which have the same periodicity as the tidal cycle and these enabled age estimates to be made. Younger individuals were male whilst older animals were female, suggesting that *H. pectunculus* is a protandrous hermaphrodite.

Histological examination proved, unequivocally, that this limpet undergoes a protandric sex change, changing from male to female when they are about 2 years old. Both east and west coast populations had a marked reproductive cycle, exhibiting two spawning periods a year, one in April and another in November. The possibility that the reproductive pattern exhibited is now phylogenetically constrained is discussed. It is suggested that *H. pectunculus* has evolved a reproductive cycle which will allow its planktonic larvae to utilise the valuable phytoplankton bloom food source whilst using onshore winds to ensure that larvae are not transported out to sea and lost. The number of foraging excursions carried out by individuals of *H. pectunculus* was found to have a significant effect on Gonad Index and hence potential reproductive output.

The activity pattern of *H. pectunculus* varied depending upon micro-habitat; animals inhabiting both east and west facing rock surfaces are active during nocturnal low tides whilst animals on west facing rock surfaces are also active during daytime low tides whilst in the shade. Limpets travel further during foraging excursions in winter ($\bar{x}$ = 85.53 cm) than in either spring ($\bar{x}$ = 55.7 cm) or summer ($\bar{x}$ = 48.8 cm) and also during spring low tides ($\bar{x}$ = 89.8 cm) compared with neaps ($\bar{x}$ = 40.9 cm). This limpet exhibits rigid homing to a fixed scar within a crevice and feeding excursions were found to consist of three distinct phases, a rapid outward phase, a slower foraging phase and a rapid homeward phase. Foraging was always highly directional, with a mean vector which took limpets onto an area of the rockface with the highest microalgal biomass and also the smoothest rock surface.

Helcion pectunculus exhibits a free-running endogenous rhythm of locomotor activity with both circadian and circatidal components and it is suggested that this rhythm plays a role in allowing the limpet to avoid unfavourable environmental conditions. The exogenous entrainment factor of this endogenous rhythm was the time of exposure to air in the field.

There was found to be an organized distribution of limpets within crevices with smaller, younger limpets being towards the back of the crevice and larger, older limpets towards the crevice mouth. It is hypothesized that juvenile limpets of this species actively select and settle at the backs of crevices responding to chemical cues of adult conspecifics. The crevice refuge supplies the limpets with a stable and buffered environment with higher relative humidities ($\bar{x}$ = 72.3%) and lower rock surface temperatures ($\bar{x}$ = 19.7°C) than adjacent exposed rock surfaces ($\bar{x}$ = 64.5%; $\bar{x}$ = 22.9°C). Limpet body temperatures were significantly lower in crevice refuges compared to limpets on exposed rock surfaces. Body temperatures never exceeded the rock surface temperatures. It is suggested that this is the result of morphological adaptations such as shell ornamentation and allometric growth. Light levels above 1000 $\mu E.m^{-2}.s^{-1}$ inhibited foraging activity in *H. pectunculus* whilst limpets subjected to 30-50% shade foraged even during daytime lowtides. This limpet is one of the least tenacious (2.75 ± 0.13 $kg.cm^{-2}$) of all South African limpets and the possibility that wave activity governs both the activity patterns and homing behaviour of this limpet is discussed. Limpets deprived of a crevice refuge experienced extremely high mortalities, with 45% of the limpets being lost during the first high tide period.

A hypothetical model of the hierarchy of exogenous factors controlling limpet foraging activity is introduced and discussed in relation to the results of this study. Finally, it is suggested that in addition to the "migratory" and "non-migratory" groups of limpets present on southern African shores a third group of limpets seem to be present which may be classed as "specialized non-migratory" species. These are species that do not migrate, garden or aggressively fight off like conspecifics. They have overcome the competition for space and food on intertidal rocky shores by adapting to a particular habitat which is exclusive to them alone. From the combined results of these studies, it can be stated that *H. pectunculus* has adapted physiologically, morphologically and behaviourally to successfully survive the extreme conditions in the upper Balanoid zone.

Chapter 1:

General Introduction

1.1 General Introduction

Prosobranch limpets (Patellogastropoda) are some of the most familiar animals on rocky shores, with an almost worldwide distribution (Powell, 1973). They are believed to be one of the most primitive groups of gastropods, and are probably the first branch of the "Archaeogastropoda" (Lindberg, 1988; Haszprunar, 1990). According to a recent classification (Lindberg, 1988), the Patellogastropoda contains three superfamilies, Patelloidea, Nacelloidea and Acmaeoidea. The Patelloidea includes the family Patellidae which, under present classifications, contains only two genera, *Patella* and *Helcion* (Ridgway, 1994). The majority of species of both *Patella* and *Helcion* occur in two geographic areas, the northeastern Atlantic Ocean/Mediterranean and southern Africa.

At present about 36 species of *Patella* are recognized, 14 of which are found on the shores of South Africa (Jamieson, Hodgson & Bernard, 1991). The genus *Helcion* was recognised as being distinct from *Patella* as early as 1810, the distinction being based mainly on shell characters (Ridgway, 1994). The type species of *Helcion* is *Helcion pectunculus* (Gmelin, 1791) from South Africa. The names *Ansates* Sowerby, 1839 and *Patina* Gray, 1847, have been extensively used in the literature (Graham & Fretter, 1947; Kain & Svendsen 1969; Vahl, 1971, 1972, 1983; Fretter & Graham, 1976; Warburton, 1976) as both have the European *H. pellucidum* (Linnaeus, 1758) as the type species, but it is now generally accepted that the name *Helcion* has priority (Powell, 1973).

Helcion, in which four species are recognized, has a somewhat anomalous distribution. One species, *H. pellucidum*, is found in northern Europe and the Mediterranean Sea, while the remaining three species, *H. pectunculus*, *H. pruinosus* and *H. dunkeri* are restricted to southern Africa (Powell, 1973). A recent study (Weber *et al.*, in press; see appendix I) which used electrophoretic procedures to determine the genetic divergence between these species, found that the degree of genetic divergence found within the South African branch of *Helcion* was characteristic for congeneric species whilst the genetic divergence found between *H. pellucidum* with its South African congeners showed values previously attributed in the literature for non-congeneric species. Other work comparing sperm morphology of the patellid limpets suggests that *H. pellucidum* is more closely related to the northern *Patella* species than to the southern species of *Helcion* (Hodgson, pers. comm.). Therefore the monophyly of the genus *Helcion* is uncertain and its taxonomic status is still an area for debate (Weber *et al.*, in press).

Many aspects of the biology of patellids have been studied including their foraging activity and feeding behaviour (*e.g.* Cook *et al.*, 1969; Branch, 1971; Hartnoll & Wright, 1977; Hawkins & Hartnoll, 1982, 1983; Little & Stirling, 1985; Little *et al.*, 1988, 1990, 1991; Evans & Williams, 1991; Della Santina & Naylor, 1993; Chelazzi *et al.*, 1994; Gray & Naylor, 1996; Gray & Hodgson, in press), reproductive cycles (Orton, 1920, 1928; Das & Seshappa, 1948; Orton *et al.*, 1956; Choquet, 1966, 1967; Blackmore, 1969; Branch, 1974a; Robson, 1986) and growth (Vahl, 1971; Branch, 1974b; Bannister, 1975; Ekaratne & Crisp, 1982; Robson, 1986)(see Branch, 1981, 1985 for reviews). Much of our understanding of the ecology of patellids is a result of the work of Branch (1971, 1974a, 1974b, 1975a, 1975b, 1976, 1979) on South African species. This work formed a foundation for many other studies (Hockey & Bosman, 1986; Bosman & Hockey, 1988a, 1988b; Lasiak, 1987, 1990, 1991, 1992, 1993; Lasiak & White, 1993; Gray & Hodgson, in press) some of which have concentrated on the species inhabiting the eastern coast of South Africa. One of the end products of all this work has been to demonstrate how, in both the northern and southern Atlantic, limpets are ecologically important, and interact with barnacles and seaweeds to determine community structure (Hawkins, 1981; Hawkins & Hartnoll, 1983; Bustamante *et al.*, 1995). For this reason it is imperative that we gain an insight into the life histories, life strategies and population dynamics of these ecological "keystone" species and understand the biological interactions taking place between the limpets and the other species cohabiting the rocky intertidal. Despite the extensive work on South African patellids, all these studies have omitted any detailed work on any species of *Helcion*. These species, in certain areas, can occur in large numbers (see chapter 2) and so an understanding of the role *Helcion* plays in the ecological web of the rocky shore would be beneficial.

Of the three species of *Helcion* which occur in South Africa two, *H. pectunculus* and *H. pruinosus*, are particularly common intertidal inhabitants of quartzitic sandstone shores along the eastern cape coast (region from Cape St. Francis (34°12'S/24°52'E) to Kei Mouth (32°41'S/28°23'E); Lubke, 1988) of South Africa (pers. obs.). The third species *H. dunkeri*, although supposedly occurring from southern Natal to Namibia (Kilburn & Rippey, 1982) is very scarce along the eastern Cape coast. During the period of this study *H. dunkeri* was found only at one specific site in the eastern Cape from which specimens were collected for electrophoretic analysis (Weber *et al.*, in press). Kilburn & Rippey (1982) suggest that the three *Helcion* species are sympatric in their distribution from the North-western Cape coast to the eastern Transkei (Figure 1.1). *Helcion pectunculus* has previously been recorded to

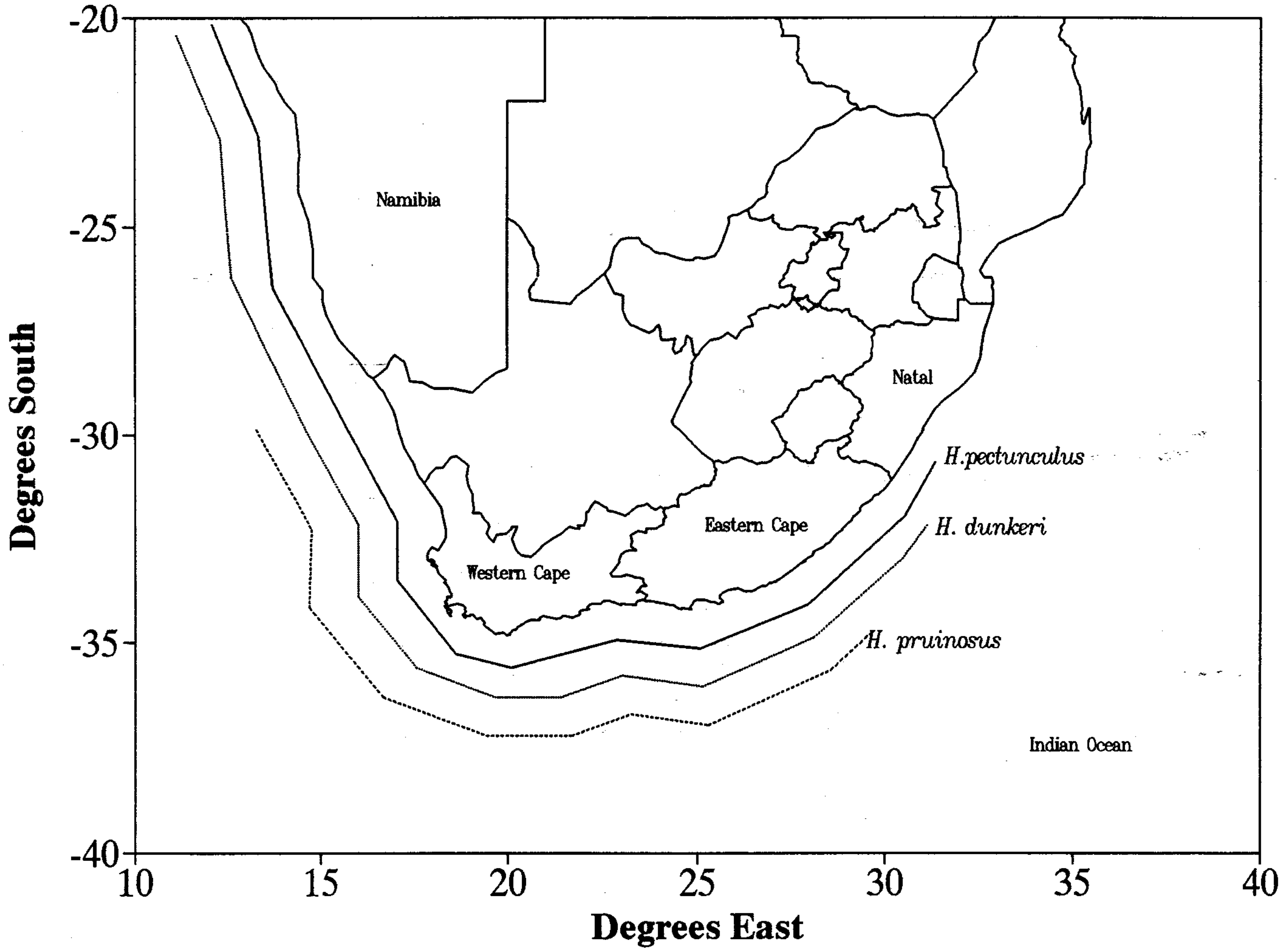

Figure 1.1:

The distribution of the three South African *Helcion* species, *H. pectunculus, H. pruinosus* and *H. dunkeri* along the coast of southern Africa as documented by Kilburn & Rippey (1982).

attain shell lengths of up to 33 mm, *H. pruinosus* attains a maximum shell length of 35 mm whilst *H. dunkeri* has seldom been recorded over 25 mm in shell length (Kilburn & Rippey, 1982; Branch *et al.*, 1994). All three species occur in highly specific micro-habitats on the shore (pers. obs.), *H. pectunculus* occurring under boulders and in crevices in the upper regions of the shore, both *H. pruinosus* and *H. dunkeri* are found inhabiting the undersides of sheltered boulders, often in colonies, in shallow water and in pools, from about low-water neap-tide level to low-water springs. These habitats tend to make studying these animals extremely difficult and this may well be the reason that, to date, their biology has not been examined in any detail.

Helcion pectunculus can be regarded as one of the most successful of all southern African limpets. It is highly abundant in the upper reaches of rocky shores and has a wider geographic distribution than any other South African patellid limpet (Kilburn & Rippey, 1982). The extremely high densities attained by *H. pectunculus* on shores within the eastern Cape (pers. obs.) and the important role limpets are known to play in the rocky shore ecosystem (Branch, 1981, 1985) prompted an investigation into aspects of the biology and ecology of this species. The primary aim of this study is to improve our knowledge of the biology of *Helcion pectunculus*, which in turn is hoped, will lead towards an even greater understanding of the role that this high shore herbivorous grazer plays in rocky intertidal ecosystems.

The first part of this thesis (chapter 2) examines the intertidal distribution, density and biomass of *Helcion pectunculus* at six sites along the eastern Cape coast and one site on the west coast. Following on from this, chapter 3 investigates the growth of this animal on both east and west coasts of South Africa whilst a comparison of the reproductive biology (primarily seasonality of reproduction and possibility of protandrous hermaphroditism) of the east and west coast populations of *H. pectunculus* is dealt with in chapter 4. Chapter 5 presents the results of a study into the foraging activity and feeding behaviour of *H. pectunculus* in the field whilst chapter 6 delves into the driving forces behind the rhythmic behaviour exhibited by this limpet on the shore. Chapter 7 documents the results of a number of experimental manipulations carried out on the shore to determine the importance of the crevice environment in the biology and ecology of *Helcion pectunculus*. Finally, chapter 8 presents a general discussion, and suggests avenues for future work.

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Chapter 2:

Distribution, density and biomass of *Helcion pectunculus* along the eastern Cape coast of South Africa

2.1 Introduction

To understand the structure and function of an intertidal ecosystem, an adequate knowledge of the community composition and the abundance of organisms at each trophic level is crucial (Odum, 1971). Limpets are some of the most dominant inhabitants of intertidal rocky shores (Branch, 1981, 1985). These herbivorous grazers are generally found in readily accessible habitats and often occur in large numbers, *e.g.* on the Natal coast of South Africa *Patella aphanes* often occurs in densities exceeding 7600 individuals per m^2 (Robson, 1986). The habitat of limpets also promotes easy sampling (Creese, 1981) thus making them a convenient group in which the complex theories on factors controlling the population dynamics and community composition of species on an intertidal rocky shore can be examined.

Early studies of limpet population biology centred mainly on the northern hemisphere patellid limpet *Patella vulgata*. Orton (1914, 1928, 1929, 1933 & 1946) determined the intertidal habitats of *P. vulgata* at Plymouth, England in relation to tidal zones, and zoned the habitats of *P. vulgata* by correlating them with some common shore organisms. These studies also showed *P. vulgata* to be a protandrous hermaphrodite, which was later confirmed by Das & Sesheppa (1947). Thompson (1979) carried out further studies into the distribution and population dynamics of U.K. limpets with work on *P. aspera.* The distribution of the Antarctic limpet, *Nacella (Patinigera) concinna*, has also been studied in detail (Picken, 1980). This work also discussed the abundance of the limpet in terms of biomass enabling comparisons with earlier studies (Hedgpeth, 1969; Walker, 1972) resulting in an overview of how the population structure varied with time. Biomass values of 69 g flesh wt/m^2 were recorded which were found to be towards the lower end of the range of biomass estimates (13-282 g flesh wt/m^2) given by Lewis & Bowman (1975) for *P. vulgata* at Robin Hood's Bay, England. Thompson (1979) stated that an accurate understanding of limpet population biology offers the best hope of detecting long term ecological changes in the intertidal community arising from catastrophic events such as oil pollution (Southward & Southward, 1978). Such studies have also been suggested to be of significant value in terms of littoral monitoring in general (Lewis, 1976; Bowman, 1978).

Patellid limpets are extremely abundant on rocky shores along the South African coast (Branch & Branch, 1981). Much of our knowledge of the population structure and distribution of South African limpets comes from the early papers by Branch (1971, 1975a, 1975b) on the genus *Patella*. More recent work includes studies of *P. aphanes* (Robson, 1986), *P. granularis* (Bosman & Hockey, 1988a, 1988b) and *Cellana capensis* (Lasiak, 1993). There are fourteen species of *Patella* occurring along the South African coast, up to ten of which may co-habit the same shore. However, Branch (1971) found that their zonation, distribution and feeding habits are generally sufficiently different to reduce competition between the species. Branch (1971) proposed that patellid limpets on South African shores can be divided into two distinct groups; those that settle low down on the shore and migrate upwards as they increase in size *i.e.* "migratory" (*e.g. P. granularis*, *P. granatina*, *P. oculus*, *P. concolor* and *P. barbara*) and those that settle and stay in one place whilst displaying territorial behaviour and producing algal "gardens" *i.e.* "non-migratory" (*e.g. P. longicosta*, *P. tabularis*, *P. cochlear*, *P. compressa* and *P. miniata*). These were strategies for reducing interspecific competition.

A recent study (Bustamante *et al.*, 1995) has discovered that the dense populations of *P. granatina* and *P. argenvillei* which occur in the low- to mid-zones of the southwestern rocky intertidal shore of South Africa, are only possible due to the evolution of highly specialised feeding mechanisms by these limpets which are normally regarded as generalist grazers (Branch, 1973). Both species occur in densities of up to 200/m^2 on these shores and they have been shown to utilise kelp fronds to supplement their diets.

There are numerous different types of rocky shore along the southern African coastline including mixed shores (neither homogenous rock or sand) subject to frequent sand scour or inundation (Dower, 1989). Previous studies have shown that species distribution is often influenced by rock type (Dower, 1989; Foster, 1994). Although it is known that *Helcion pectunculus* occurs in crevices and under boulders in the upper reaches of rocky shores (Kilburn & Rippey, 1982; Branch *et al.*, 1994) there have been no quantitative surveys of the density or distribution of this species. This study aimed to establish and compare the population structure, biomass, density and distribution of *H. pectunculus* between various sites with different geomorphologies along the eastern Cape coast, as well as one site on the west coast of South Africa. Inter-zonal differences in biomass and density would also be investigated within the individual sites. The west coast site, Bloubergstrand, was chosen due to the fact that it was one of the sites at which a study on reproductive seasonality (chapter 4) and growth (chapter 3) in *H. pectunculus* was carried out. The results would also form the basis of future work on the growth, reproduction and foraging behaviour of *H. pectunculus.*

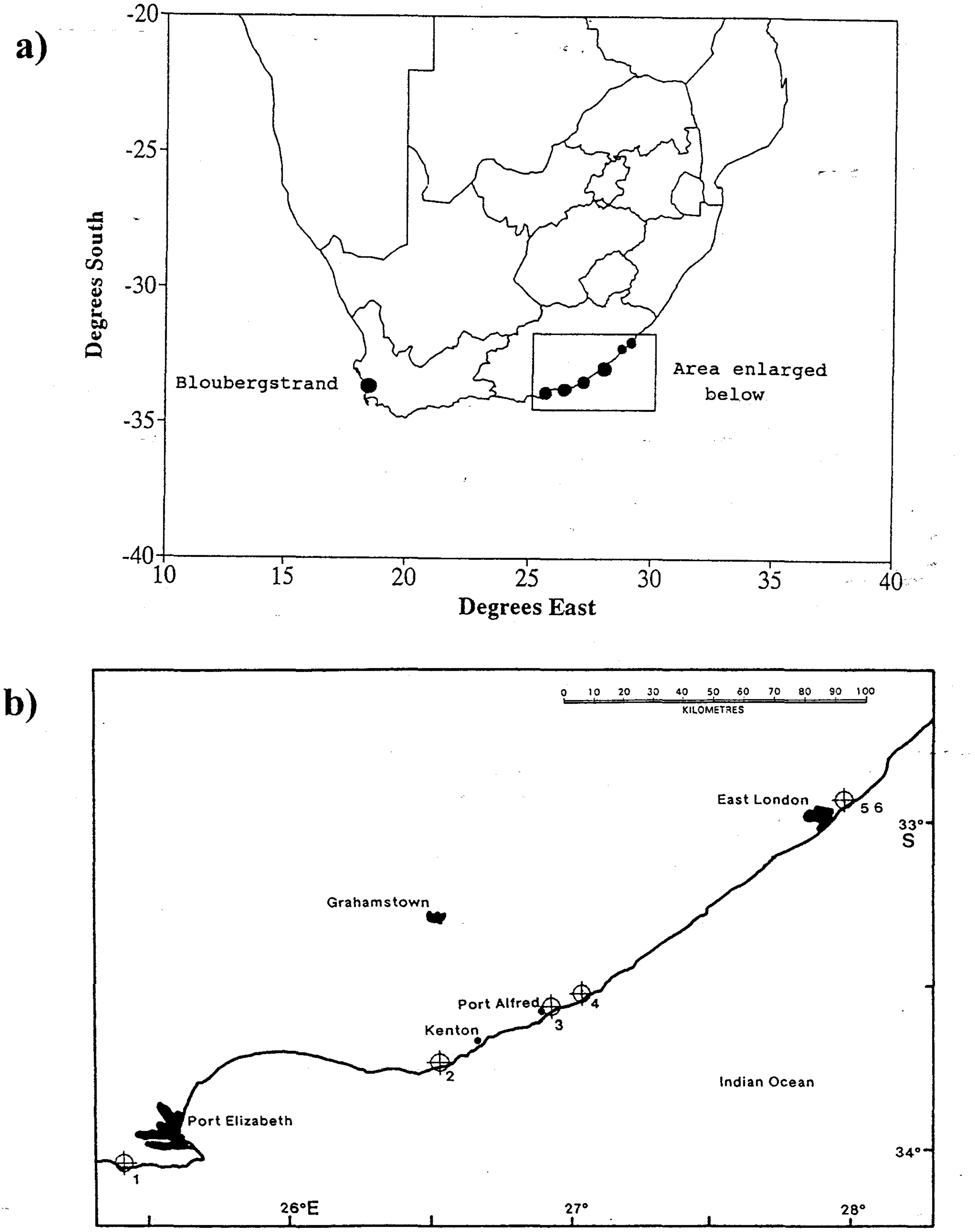
2.2 Materials & Methods

2.2.1 Study sites

To determine the intertidal distribution, density and biomass of *Helcion pectunculus* along the eastern Cape coast, six rocky shores with different geomorphologies were examined from Port Elizabeth to just north of East London (figure 2.1a & b). All six of these sites lay within the boundaries delimiting the "eastern Cape region" of South Africa (Lubke, 1988a). The seventh site studied, Bloubergstrand, was situated on the west coast.

The eastern Cape rocky shores are generally made up of one of two rock types; consolidated dune (aeolian calcarenites) and/or hard quartzitic sandstone (Marker, 1988). Preliminary inspections along the coastline revealed that *H. pectunculus* occurs predominantly on quartzitic sandstone shores and hence all of the sites sampled consisted of such shores. The six sites sampled in the eastern Cape were (from west to east) (figure 2.1):- Port Elizabeth (33°58'S/25°38'E), Cannon Rocks (33°44'S/26°35'E), Port Alfred (33°36'S/26°54'E), Riet River Mouth (33°34'S/27°01'E), Gonubie - rocky platform and boulder shore (32°56'S/28°02'E). All sites are exposed to the prevailing westerly swell.

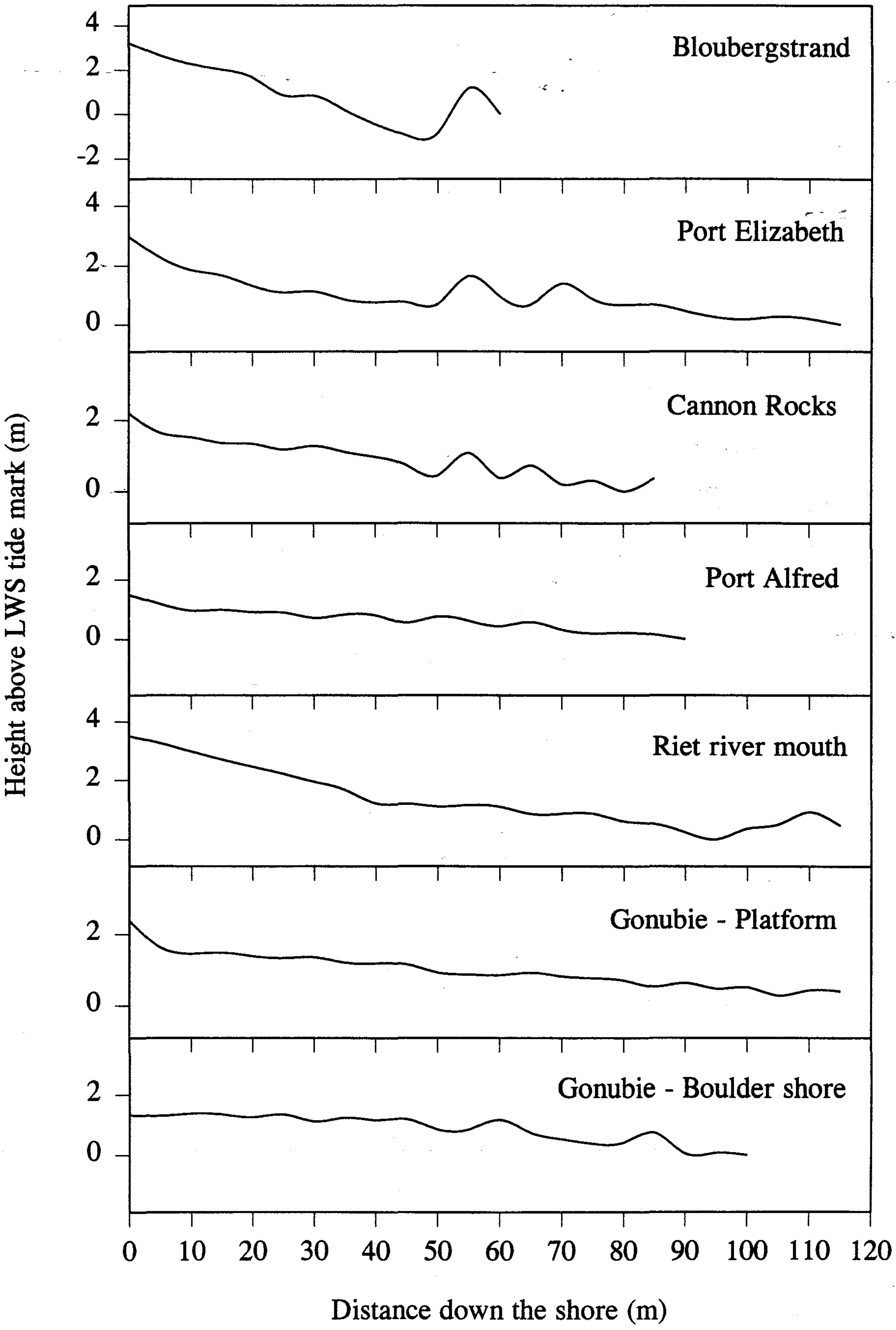
The seventh site, Bloubergstrand (33°48'S/18°27'E) (grid references obtained from Skead, 1973), was chosen due to the fact that it was one of the sites at which a study on reproductive seasonality and growth of *H. pectunculus* was carried out. It was decided to establish the distribution, density and biomass at this shore.


a)
-20
-25
-30
-35
-40
Degrees South
10
15
20
25
30
35
40
Degrees East
Bloubergstrand
Area enlarged below
b)
0 10 20 30 40 50 60 70 80 90 100
KILOMETRES
East London
5 6
33°
S
Grahamstown
Port Alfred
4
3
Kenton
2
Indian Ocean
Port Elizabeth
34°
1
26°E
27°
28°

The Port Elizabeth site was characterised by ridges of rocky outcrops containing many crevices. Between the rock ridges the gullies contained numerous boulders of various sizes (≈10-150 cm in diameter). The shore was subject to minimal sand inundation and had a gentle gradient of 1:38 m (figure 2.2). Cannon Rocks consisted of low-lying rock outcrops with few boulders between the ridges and was heavily inundated with sand, the shore gradient being 1:43 m. Port Alfred had a shallow shore gradient of 1:60 m and was characterized by ridges of rocky outcrops, similar to those at Port Elizabeth, with sand filled gullies containing some boulders (≈50-80 cm in diameter). The shore at Riet River Mouth had a gradient of 1:31 m and consisted of numerous boulders of various sizes (≈10-120 cm in diameter) as was the boulder shore at Gonubie, which had a very shallow gradient of 1:66 m. The rocky platform at Gonubie was a wave cut platform (with relatively few crevices) and a gradient of 1:46 m, which can mainly be attributed to a rather sharp incline in the upper Balanoid and Littorina zones. Finally the site at Bloubergstrand consisted of both rocky ridges and boulders, some of which were extremely large in size (> 200 cm in diameter), and had a more severe gradient of 1:14 m (figure 2.2).

2.2.2 Sampling procedure

Due to inadequate bench-marks and the complications of extrapolating the mean low water spring tide levels from empirical values of tide levels published by the South African Naval Hydrographer, characteristic indicator species were used to determine the high-, mid- and low-shore regions. Five distinct zones are recognised on rocky shores of the southern Cape, these are; the Littorina zone, the upper Balanoid zone, the lower Balanoid zone, the Cochlear zone and the Infratidal zone (Branch & Branch, 1981). The Littorina zone represents


Bloubergstrand
Port Elizabeth
Cannon Rocks
Port Alfred
Riet river mouth
Gonubie - Platform
Gonubie - Boulder shore
Height above LWS tide mark (m)
Distance down the shore (m)
4
2
0
-2
0
10
20
30
40
50
60
70
80
90
100
110
120

the high water spring tide level, the Balanoid zones the mid-shore, whilst the Cochlear zone represents the low water spring level (Branch & Branch, 1981). The pattern of the eastern Cape coastline closely resembles that of the southern Cape (Lubke, 1988b).

At each study site, a transect 10 metres wide was carried out from the high water spring tide level to the low water spring tide level of the shore during monthly spring tides. Transect profiles were obtained using surveying equipment (theodolite and 5 m measuring staff) and height measurements were taken at 5 metre intervals down the shore from the high water spring tide mark. Due to the fact that *H. pectunculus* reside in crevices and under boulders during daytime low tides and periods of immersion, sampling was carried out during nocturnal low tides to ensure that the majority of the population was mobile. This should have also ensured reasonably accurate estimates of density and biomass. At every site, twenty 0.5 m^2 quadrats were sampled in each of the Littorina, upper and lower Balanoid and Cochlear zones. This sampling method enabled a multifactor analysis of variance to be used to compare statistically the density and biomass of *H. pectunculus* between the seven geomorphologically different shores and between the four different zones within each shore. All *H. pectunculus* in each quadrat were removed, sexed, counted and their shell parameters (length, height and width) measured to the nearest 0.01 mm with Vernier callipers and weighed to the nearest 0.001 g using an electronic balance. All shell parameters were recorded for later analysis during a study of growth in *H. pectunculus* (chapter 3). Dry weights were also obtained by drying the limpets to constant weight at 60°C.

2.3 Results

2.3.1 Distribution, density and biomass

At all sites, the density and biomass of *Helcion pectunculus* was greatest in the upper Balanoid zone, with fewer individuals in the lower Balanoid zone on shores with a gently sloping aspect *e.g.* Port Elizabeth, Port Alfred and Gonubie (figure 2.2). *Helcion pectunculus* was virtually absent from the Littorina zone and completely absent from the Cochlear zones (table 2.1). Multifactor analysis of variance on the biomass data from the various zones on all the shores shows that both zone and study site have a significant effect on the biomass of *H. pectunculus* (table 2.2). Due to the fact that counts of animals within quadrats were often far greater than 20, the density and biomass data did not require square root transformation (Fry, 1993). Scheffe's multiple range test (table 2.3) verified the fact that significantly greater biomasses were present in the upper Balanoid zone than any other zone.

Of all the sites studied, the biomass of the limpet was significantly greater (site biomass = 0.8 - 1.59 g dry wt/m^2; Scheffe's multiple range test) at Port Elizabeth, Cannon Rocks and Gonubie-platform than any of the other sites (table 2.4). The biomass of limpets in the upper Balanoid zone was also greatest at the above 3 sites (2.45 - 5.88 g dry wt/m^2; table 2.1). Interestingly, these three sites consisted of rocky ridges with numerous crevice environments. The maximum density recorded for *H. pectunculus* was 212 individuals/m^2 at the boulder shore, Gonubie, whilst the maximum biomass recorded was 18.1 g dry wt/m^2 at Port Elizabeth. Densities were also high at both Port Elizabeth and the platform at Gonubie (table 2.1). Although a density of limpets of 62.8 individuals/m^2 was recorded from the upper Balanoid zone at Gonubie (Boulder shore) with a biomass of 1.21 g dry wt/m^2, at Port

Table 2.1

Average densities and dry biomasses of *Helcion pectunculus* from transects carried out at seven rocky shore sites along the South African coast.

	Littorina zone		**Upper Balanoid zone**		**Lower Balanoid zone**		**Cochlear zone**	
Site	Density (m^{-2}) x (±SD)	Biomass (g/m^2) x (±SD)	Density (m^{-2}) x (±SD)	Biomass (g/m^2) x (±SD)	Density (m^{-2}) x (±SD)	Biomass (g/m^2) x (±SD)	Density (m^{-2}) x (±SD)	Biomass (g/m^2) x (±SD)
Cape Town	0.0 (0.0)	0.0 (0.0)	20.0 (18.1)	1.855 (2.332)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Port Elizabeth	0.8 (3.5)	0.075 (0.336)	52.8 (56.9)	5.884 (5.533)	3.0 (8.09)	0.424 (1.200)	0.0 (0.0)	0.0 (0.0)
Cannon Rocks	0.0 (0.0)	0.0 (0.0)	18.6 (29.6)	3.33 (4.78)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Port Alfred	0.0 (0.0)	0.0 (0.0)	15.4 (16.6)	2.168 (2.491)	2.0 (4.82)	0.248 (0.607)	0.0 (0.0)	0.0 (0.0)
Riet River	0.0 (0.0)	0.0 (0.0)	8.2 (15.44)	0.984 (1.84)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Gonubie Platform	0.0 (0.0)	0.0 (0.0)	44.8 (30.5)	2.45 (1.67)	7.0 (8.84)	0.753 (0.953)	0.0 (0.0)	0.0 (0.0)
Gonubie Boulder	0.0 (0.0)	0.0 (0.0)	62.8 (64.7)	1.212 (1.750)	2.0 (6.5)	0.01 (0.00)	0.0 (0.0)	0.0 (0.0)

Table 2.2

Results of a Multifactor analysis of variance on biomass data obtained from seven rocky shores along the South African coast.

Source of Variation	SS	d.f.	MS	F value	p-value
Zone	674.065	3	224.688	79.452	0.0000
Study site	96.415	6	16.069	5.682	0.0000
Interaction Zone - Study site	231.527	18	12.862	4.548	0.0580
Residual	1504.489	532	2.8279		
Total (Corrected)	2506.498	559			

Table 2.3

Results of Scheffe's multiple range test showing the effect zone has on biomass.

Zone	Count	LS Mean	Homogeneous groups	
Cochlear	140	0.000000	X	
Littorina	140	0.0107429	X	
Lower Balanoid	140	0.2035143	X	
Upper Balanoid	140	2.5982143	X	Significantly different

Table 2.4

Results of Scheffe's multiple range test showing the effect the study site has on shore biomass.

Study site	Count	LS Mean	Homogeneous groups
Reit River	80	0.2460000	X
Gonubie Boulder	80	0.3779500	X
Cape Town	80	0.4637500	X
Port Alfred	80	0.6042000	X
Gonubie Platform	80	0.8019500	XX
Cannon Rocks	80	0.8321000	XX
Port Elizabeth	80	1.5958750	X

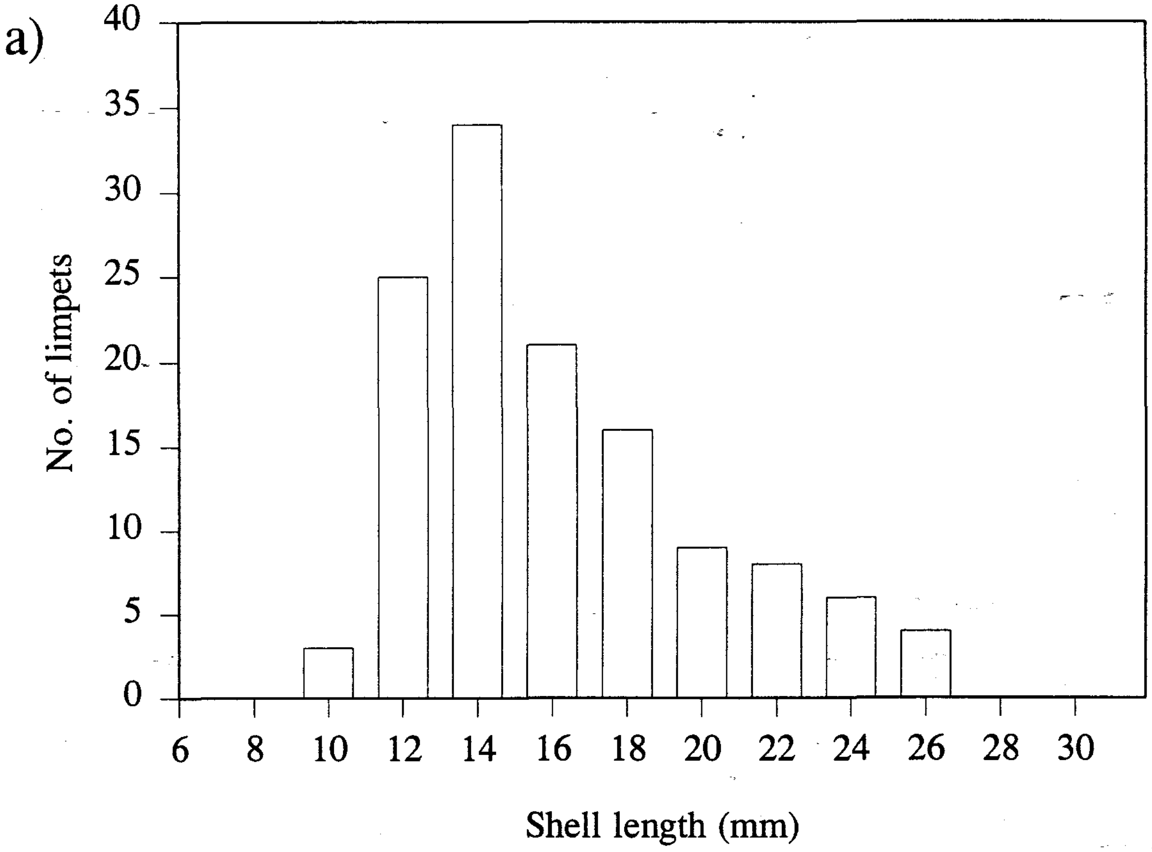
Elizabeth the biomass of *H. pectunculus* was much greater (5.88 g dry wt/m^2) despite the lower density (52.8 individuals/m^2). This indicates that although the boulder shore at Gonubie had more limpets per m^2 they were smaller than those at Port Elizabeth, hence the reduced biomass. This size difference is reflected in the size frequency distributions for these shores (compare figure 2.4 and 2.8).

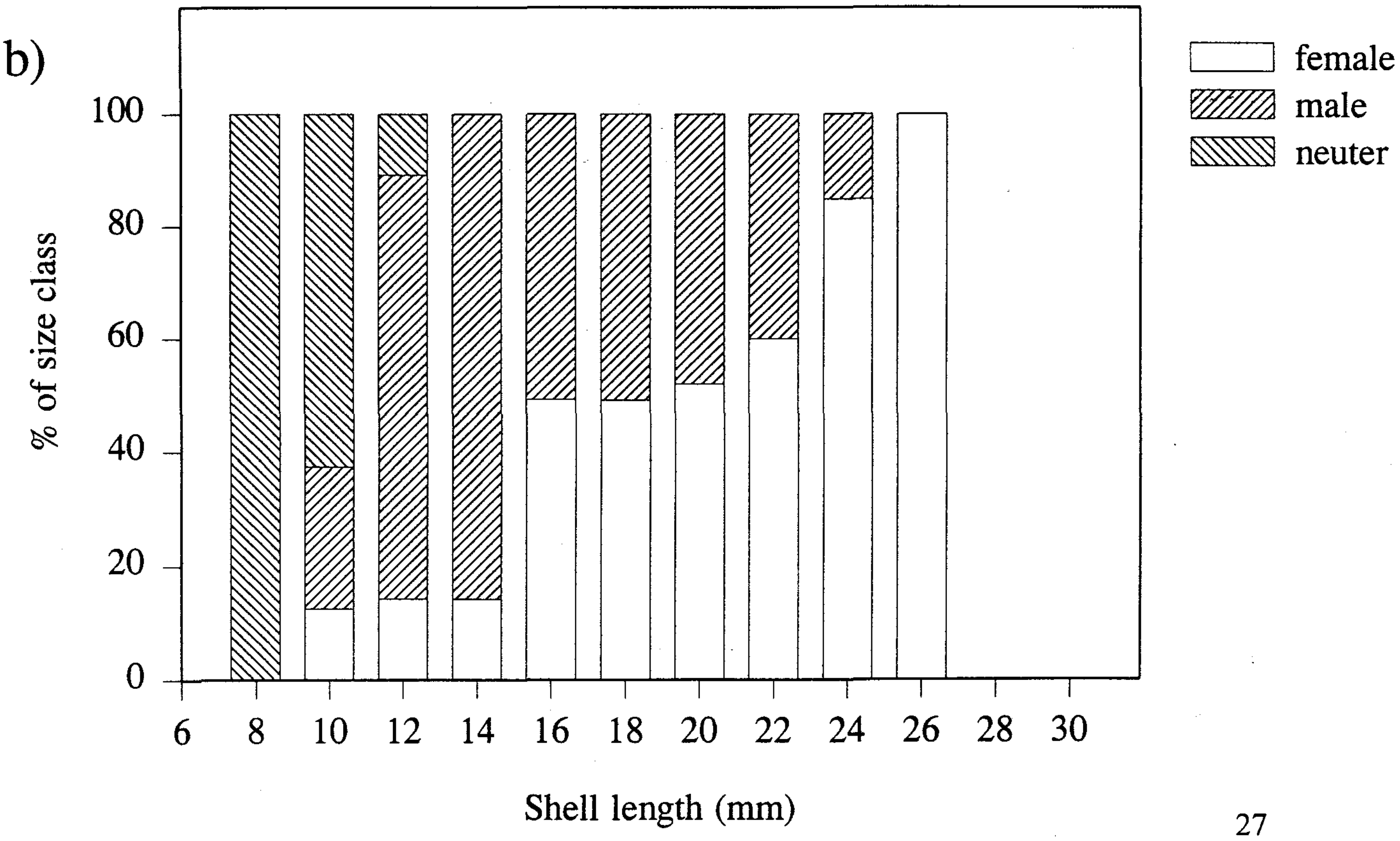
Due to the very narrow distribution of *H. pectunculus* up the shore (only occurring in the upper Balanoid) it is difficult to compare size distributions of *H. pectunculus* between the lower and upper shore areas. The size distributions illustrated (figures 2.3a - 2.9a) therefore include all animals sampled at each site.

An examination of the size frequency distributions at the different sites (figures 2.3a - 2.9a) shows that the largest animals collected occurred at Port Elizabeth (30 mm maximum shell length), Cannon Rocks (34 mm maximum shell length) and Port Alfred (28 mm maximum shell length), all of which are sites which consisted of rocky outcrops and reefs with many crevice refuges for the limpets. The other sites did not yield many large individuals and had modal shell lengths of between 12 mm (Gonubie - boulder shore) and 18 mm (Gonubie - platform). The sample obtained from Riet river mouth is somewhat anomalous in the fact that it does not contain any small individuals (< 16.0 mm). The fact that few very small individuals (< 6.0 mm) were present at any of the sites is also noteworthy.

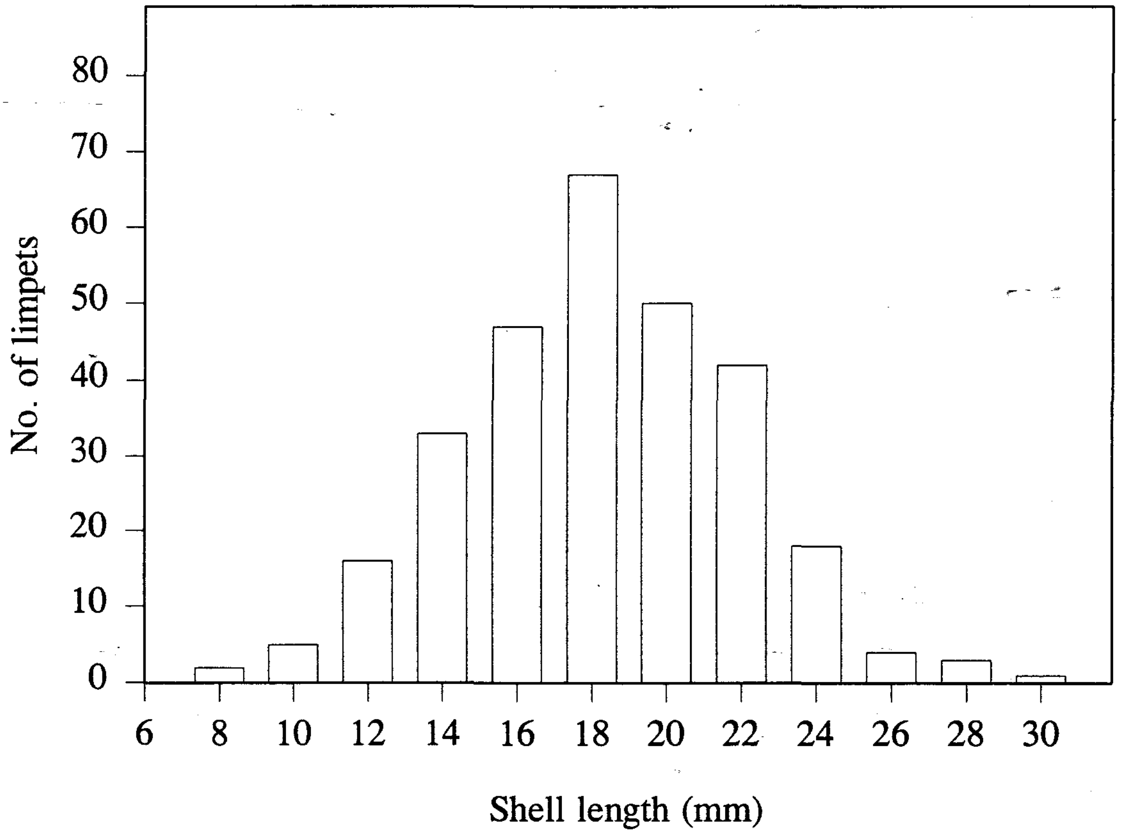
2.3.2 Sex ratios and sex/size distribution

The relationship between limpet size (shell length) and sex within the populations at

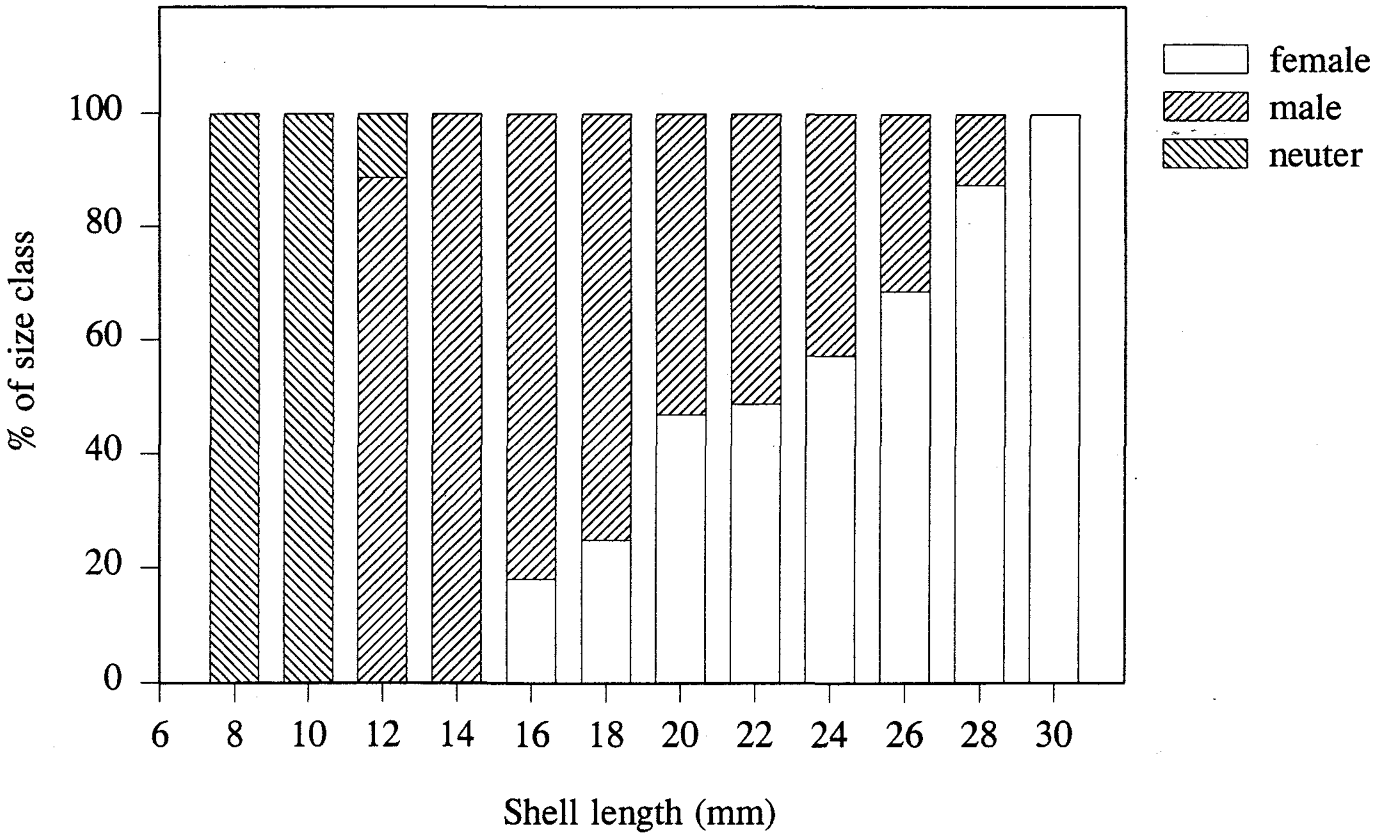

a)
No. of limpets
0
5
10
15
20
25
30
35
40
6
8
10
12
14
16
18
20
22
24
26
28
30
Shell length (mm)


b)
% of size class
0
20
40
60
80
100
6
8
10
12
14
16
18
20
22
24
26
28
30
Shell length (mm)
female
male
neuter

a)


80
70
60
50
40
30
20
10
0
No. of limpets
6
8
10
12
14
16
18
20
22
24
26
28
30
Shell length (mm)

b)


100
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0
% of size class
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12
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20
22
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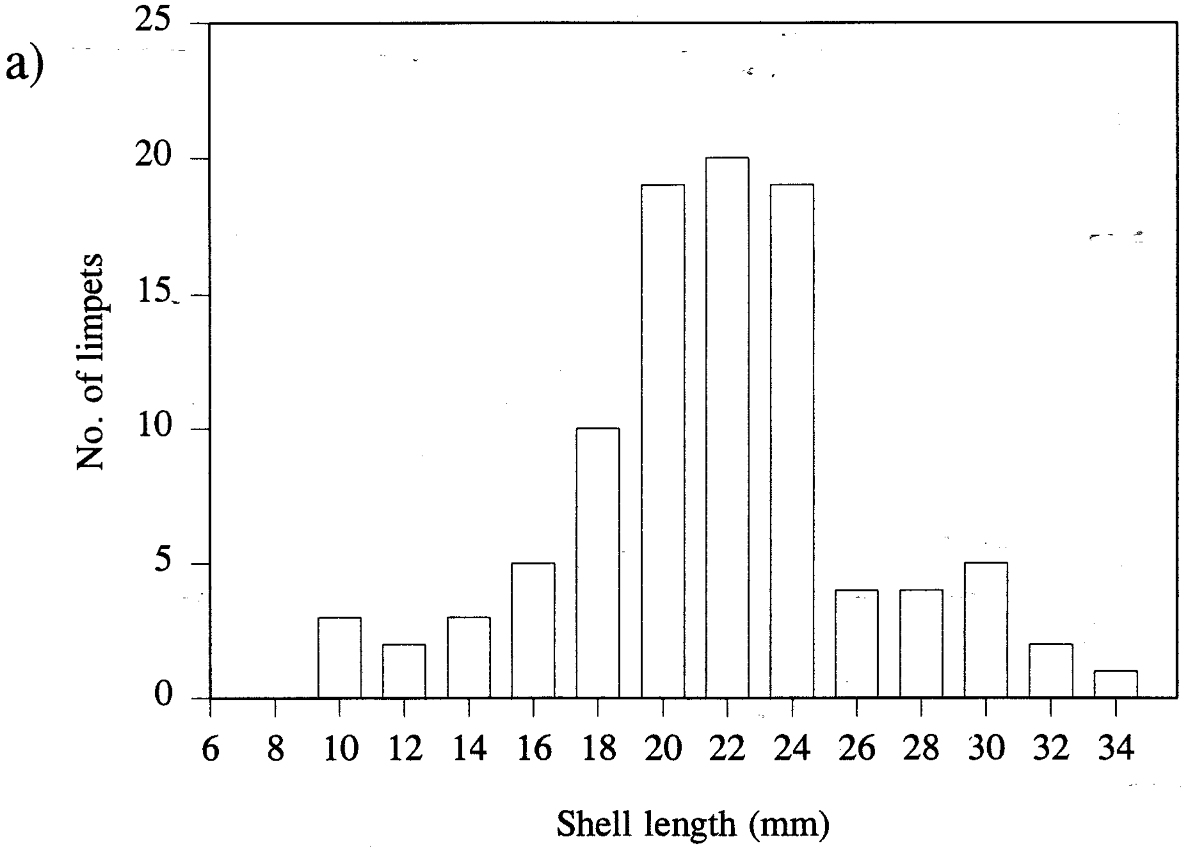
28
30
Shell length (mm)
female
male
neuter


a)
No. of limpets
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15
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8
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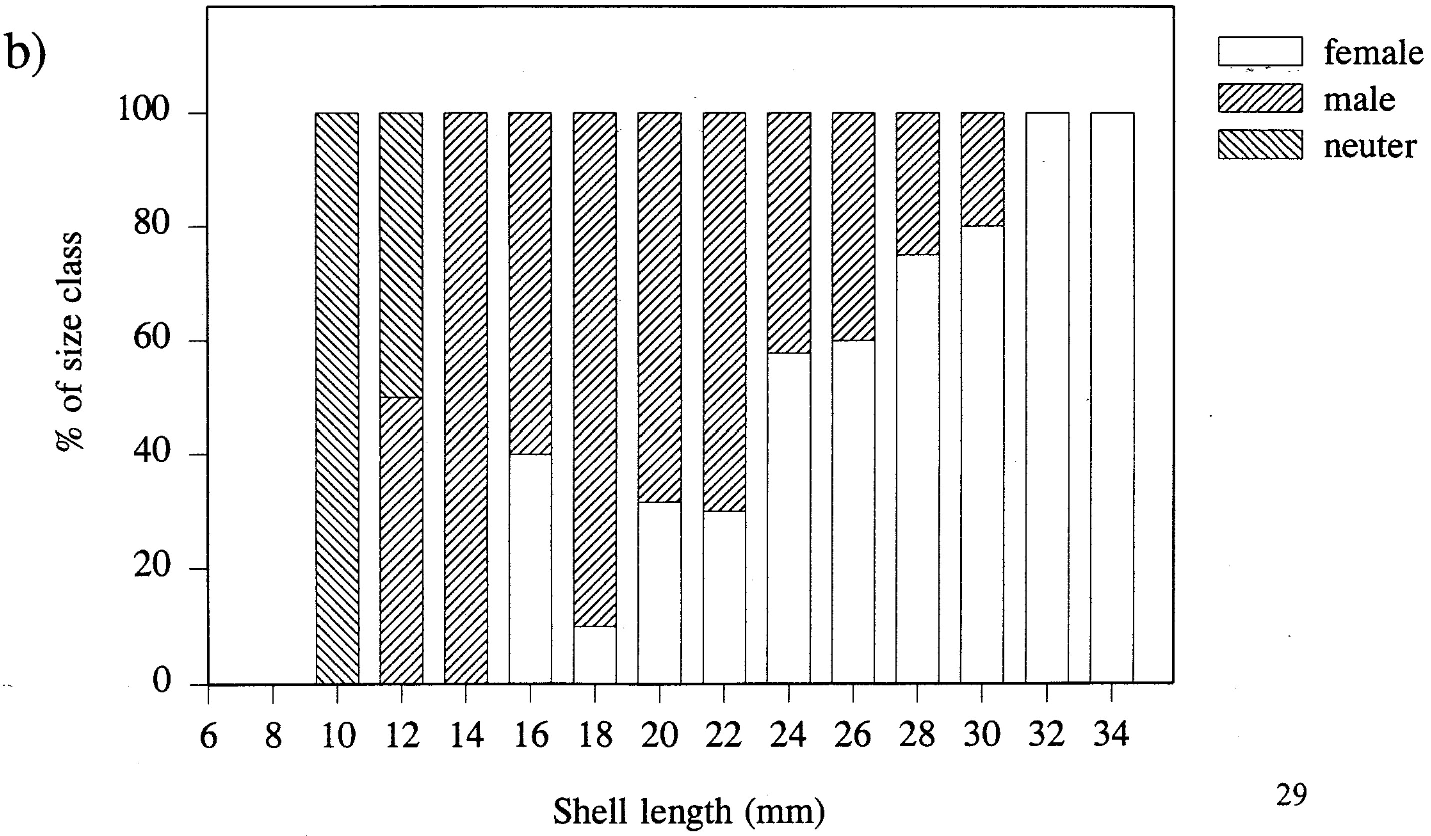
14
16
18
20
22
24
26
28
30
32
34
Shell length (mm)


b)
% of size class
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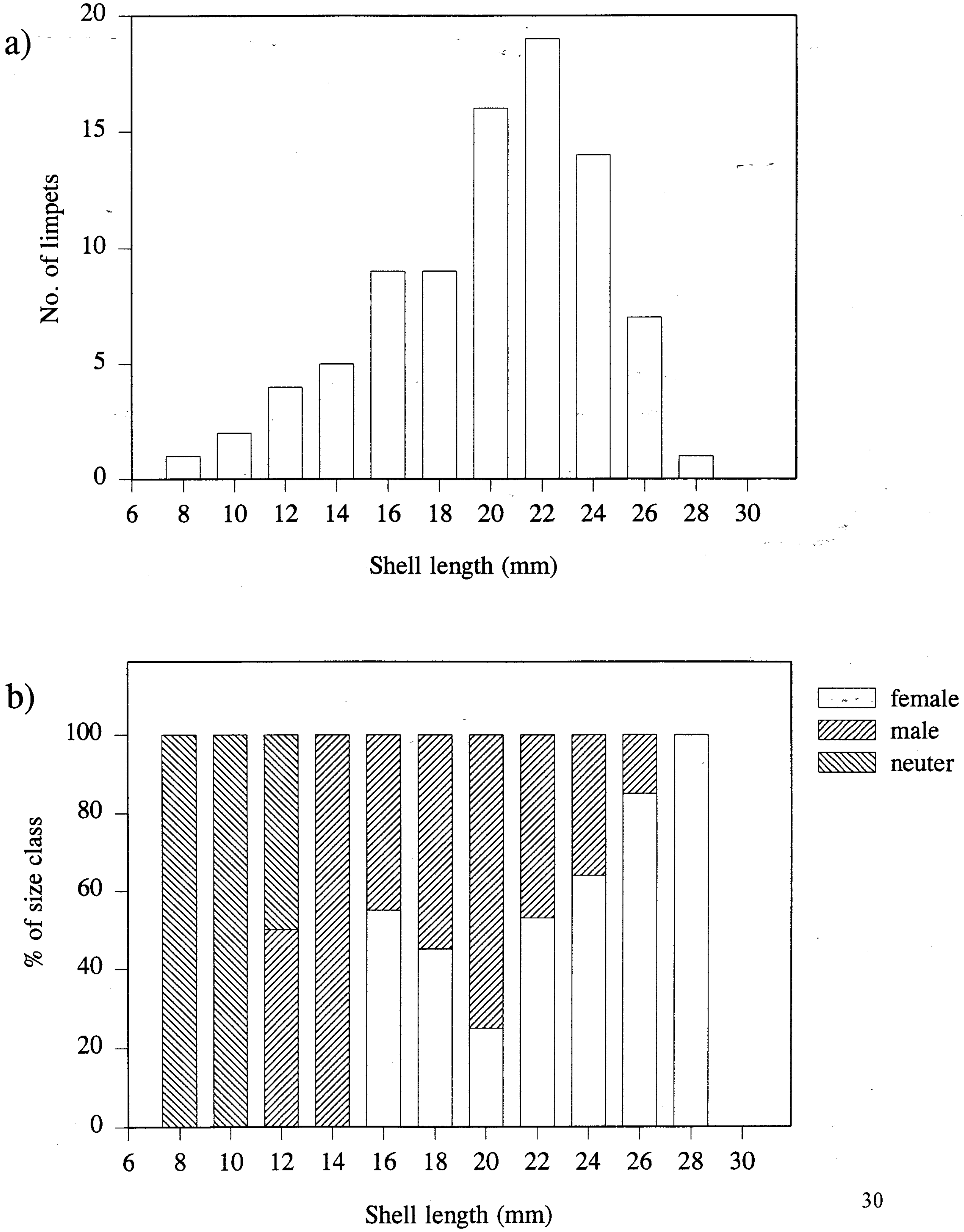
26
28
30
32
34
Shell length (mm)
female
male
neuter


a)
No. of limpets
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Shell length (mm)
b)
% of size class
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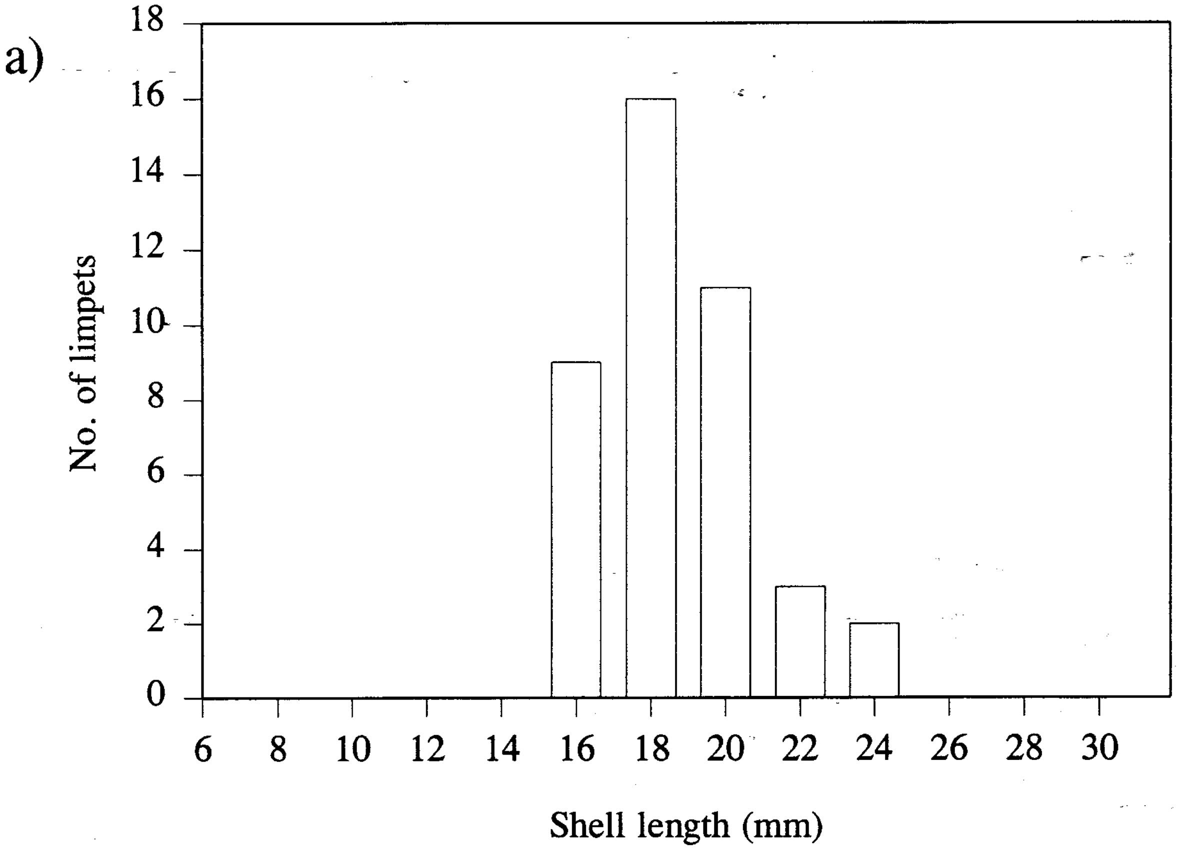
Shell length (mm)
female
male
neuter


a)
No. of limpets
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8
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Shell length (mm)
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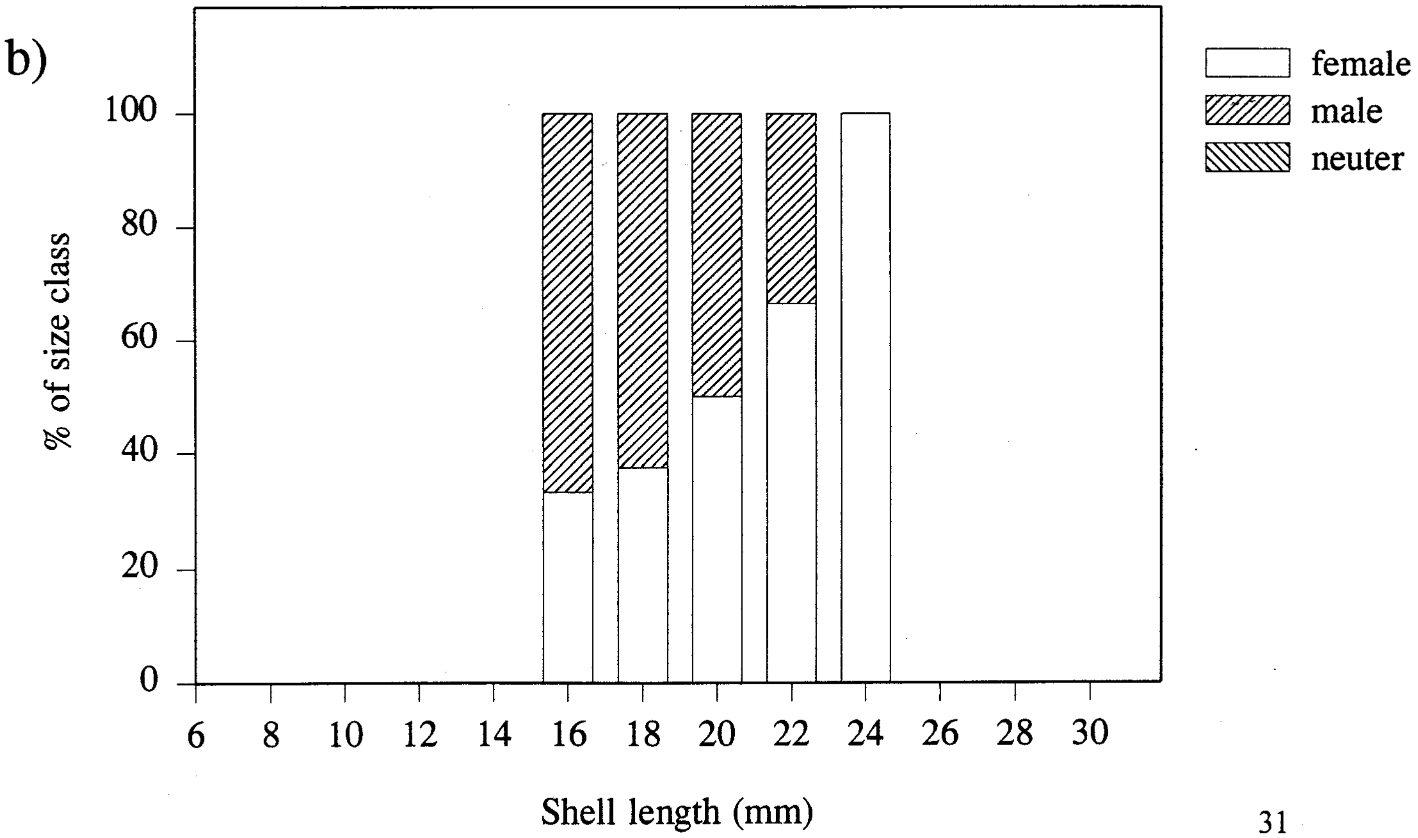
30


b)
% of size class
0
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Shell length (mm)
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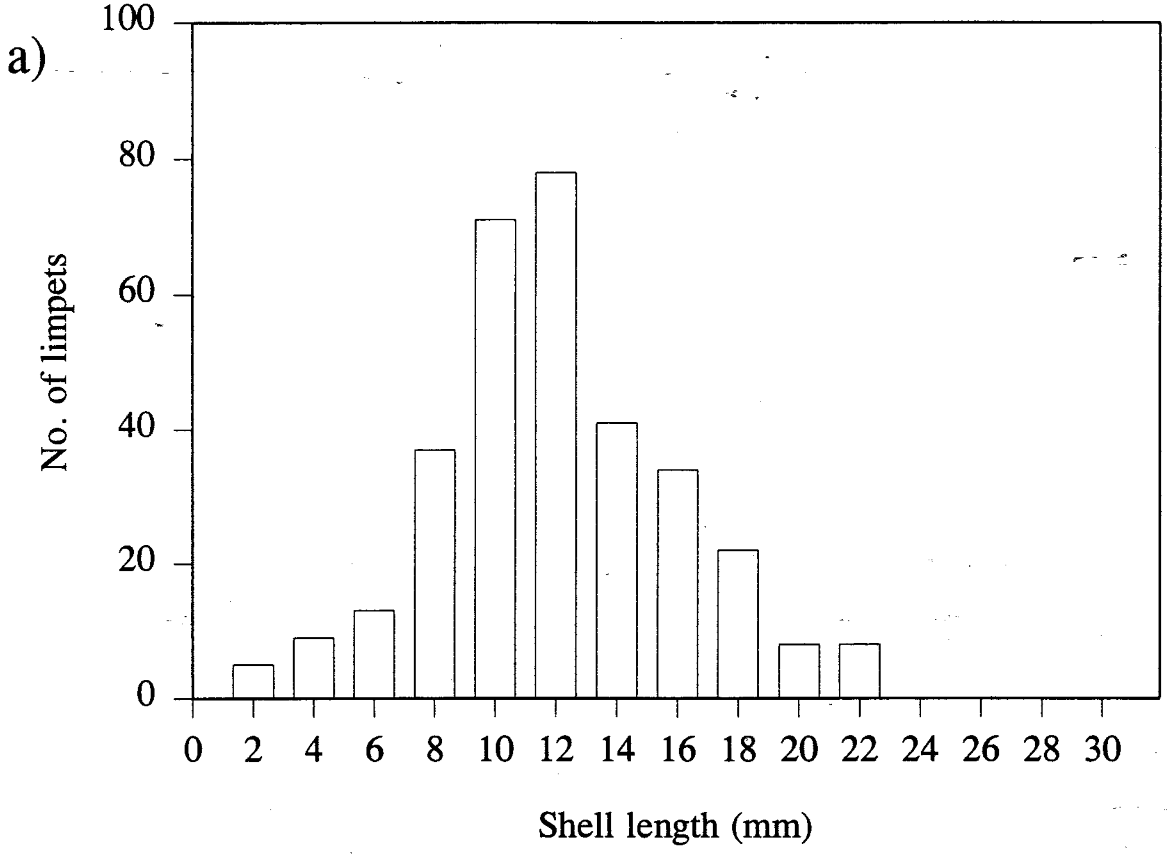
28
30
female
male
neuter


a)
No. of limpets
0
20
40
60
80
100
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4
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22
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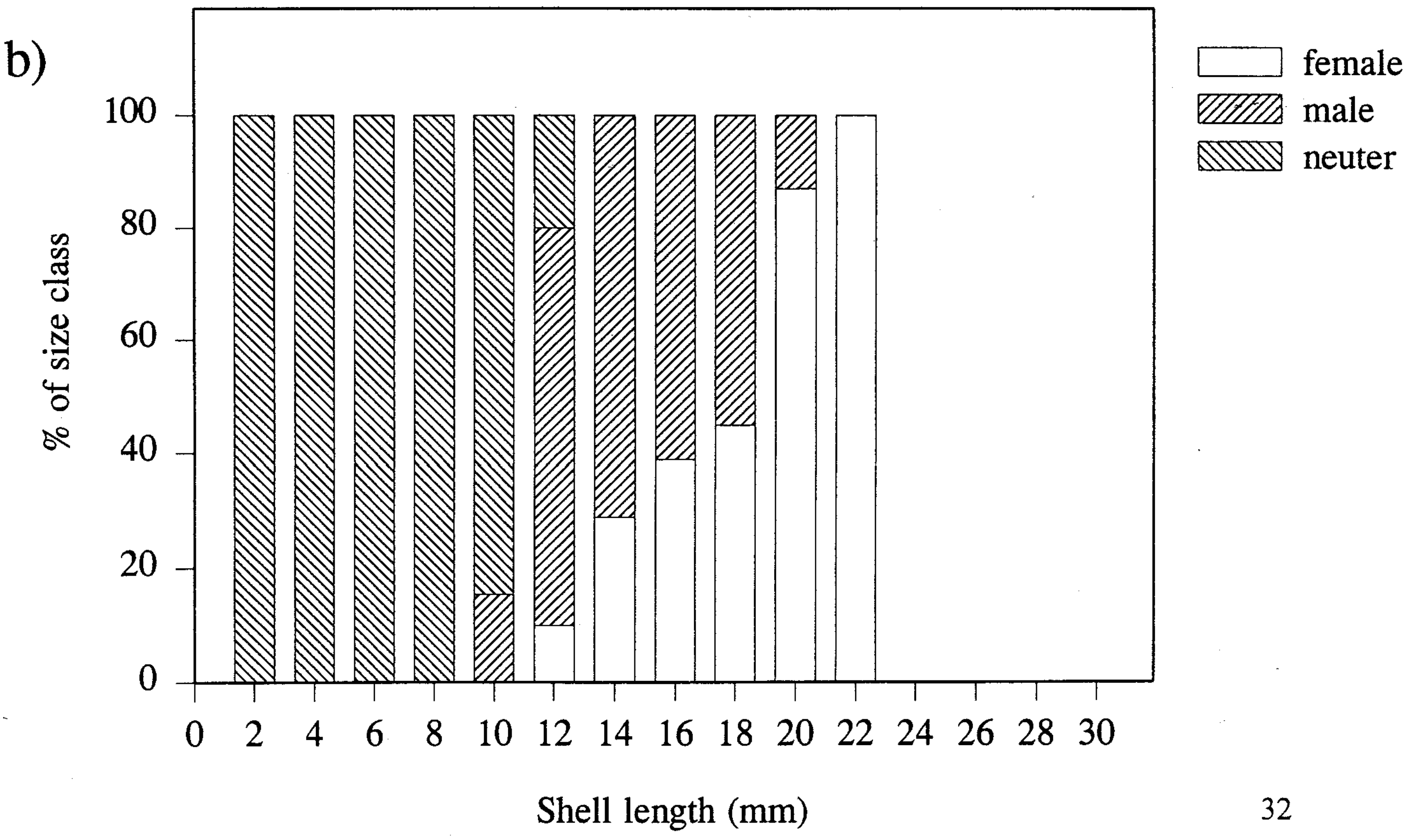
28
30
Shell length (mm)


b)
% of size class
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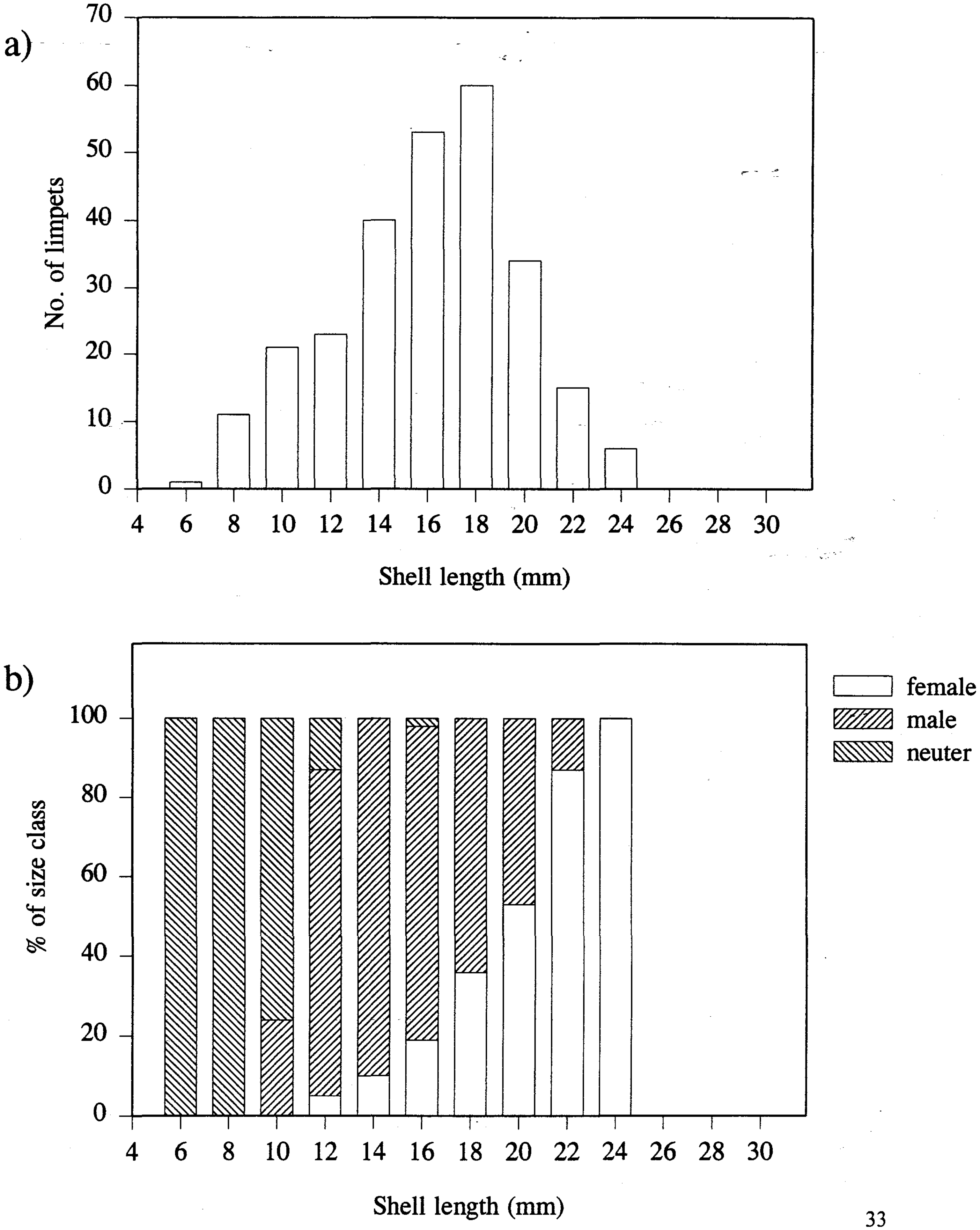
Shell length (mm)
female
male
neuter


a)
No. of limpets
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20
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50
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70
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Shell length (mm)
b)
% of size class
0
20
40
60
80
100
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Shell length (mm)
female
male
neuter

the seven sites is illustrated in figures 2.3b - 2.9b. At all sites, males and females were found to differ in size, with individuals of < 20 mm shell length generally being male whilst limpets with a shell length of > 22 mm shell length were generally female. Table 2.5 provides data on the frequencies and ratios of males to females at each of the transect sites. Chi-squared analyses showed that on most shores the ratios of males to females differed significantly from a 1:1 ratio (table 2.5). On all shores there were more *H. pectunculus* males, with the highest male to female ratio obtained from Bloubergstrand on the west coast, where there are 3 males to every female.

2.4 Discussion

The intertidal rocky shore is generally regarded as one of the most stressful physical environments (Branch & Branch, 1981). Upper limits of a species distribution are usually determined by physical conditions, whilst the lower limits by biological interactions (Underwood, 1979). This study has not only confirmed that *Helcion pectunculus* lives very high up on the shore but has shown that it is restricted in its distribution, being absent from the Littorina and Cochlear zones. The highest densities occur in the upper Balanoid zone. Even though *H. pectunculus* has a critical temperature (at which oxygen consumption peaks) of >35.0° (Branch, 1981), which makes it more tolerant to thermal stress than any other South African limpet, it resides in crevices and under boulders during day time low tides. This behaviour presumably reduces desiccation and thermal stress from the high temperatures experienced during the summer months (see chapter 7).

It is well known that high-shore limpets are more tolerant to physical stress than are

Table 2.5

Frequencies and ratios of male to female individuals of *Helcion pectunculus* at seven rocky shore sites along the coast of South Africa. Also shown are p-values obtained from Chi-squared analysis on the frequencies to determine whether they differ significantly from 1 : 1.

	Frequency		**Ratio**		**Chi-squared**
Site	**Male**	**Female**	**Male**	**Female**	**p-value**
Cape Town	75	25	3	1	5.73×10^{-7}
Port Elizabeth	170	94	1.8	1	2.9×10^{-6}
Cannon Rocks	56	33	1.7	1	1.1×10^{-2}
Reit River	23	18	1.3	1	0.527
Port Alfred	49	35	1.4	1	0.126
Gonubie - Boulder	130	57	2.3	1	5.76×10^{-8}
Gonubie - Platform	137	90	1.5	1	1.47×10^{-3}

low-shore species (Branch, 1985). Zonation patterns are correlated with the evolution of physiological adaptations fitting each species to its own particular habitat. Wolcott (1973) showed that acmaeid species, that live at successively higher levels on the shore, have an increased tolerance to desiccation. It has also been shown that many high shore limpets have altered their shell shape by adding ornamentation to increase convective heat loss. By producing highly domed shells, high shore limpets are able to minimise the area over which evaporative water loss may occur in response to desiccation pressure (see Vermeij, 1973 for review) and this includes *H. pectunculus* which has the most highly domed shell of all South African limpets (see chapter 3). The question then arises as to why limpets live so high up on the shore if desiccation and thermal stress are a constant problem to the extent that shell shape and physiology must adapt to ensure survival?

The answer may well lie in the fact that lower limits of zonation are set by biological factors such as food availability and competition (Branch, 1976, 1984). South African shores have a rich and diverse group of intertidal grazers of which the limpets make up a large number. Up to ten species of *Patella* may coexist on the same shore (Branch, 1971). For this reason, their zonation, distribution and feeding habits have had to evolve to be different enough to reduce competition between species. This has given rise to what are termed as "specialists" (Branch, 1976). Specialization by a species reduces overlaps of niches and thus reduces interspecific competition for space and food. *Helcion pectunculus*, in adapting to the extreme high shore environment is able to utilise food and space resources unavailable to most other limpet species, although some overlap does occur with *P. granularis* in the lower reaches of the distribution of *H. pectunculus*.

Helcion pectunculus is found in higher densities on shores which have large numbers of crevices and boulders in and under which limpets may seek shelter from the extreme conditions of the upper Balanoid zone. Crevices are known to aid in reducing limpet desiccation, with juveniles of many species being restricted to the low-shore or damp crevices (Branch, 1976). However, in the case of *H. pectunculus*, aggregating in crevices may be adaptive in several respects:-

1) Crevice dwelling may be a strategy for avoiding strong wave activity. Dislodgement of a limpet via wave action is most likely if a limpet is sheared off with a pull parallel to the substratum (Jones & Demetropoulos, 1968; Miller, 1974). This would only generally occur whilst a limpet was moving. Some limpets have evolved rhythms of activity which ensure that they are not off their home scars when wave action can dislodge them *i.e.* many species feed only at low tide (see chapter 5, table 5.7). *Helcion pectunculus* not only feeds at low tide but homes to the same crevice before being covered by the incoming tide. Tenacity measurements carried out in the field also show that *H. pectunculus* is one of the least tenacious of South African limpets (see chapter 7).

2) Aggregating in crevices may allow the limpets to exploit areas of the rocky shore which would normally be unhabitable for single individuals due to long aerial exposure and desiccation at low tides. Clustering may result in the retention of water within the group thus increasing the humidity within the crevice allowing them to survive long aerial exposure at low tides. Clustering has been observed in other gastropods *e.g. P. granularis* (Branch, 1975b), *Siphonaria concinna* (Gray & Hodgson, in press), *Cerithium moniliferum* (Rohde &

Sandland, 1975) and *Nodilittorina africana* (Branch *et al.*, 1994). It also occurs in other intertidal animals including the Holothuroidea (Rutherford, 1973; Foster, 1994). Although it could be argued that by evolving a crevice escape from extreme physical conditions *H. pectunculus* have, as a consequence, been forced to cluster/aggregate in the few suitable refuges available to them.

3) Living in close proximity within a crevice or under a boulder may be a strategy to ensure reproductive success. Freire *et al.* (1992) suggest that in species that rely on external fertilization, such as *H. pectunculus* (see chapter 4), living in close proximity or "clumping" would be advantageous, allowing for synchronous spawning and the release of gametes in close proximity to one another, increasing reproductive efficiency. Levitan (1995) suggests that in exposed habitats, both increased dilution due to turbulence and increased shear forces on gametes can disrupt sperm-egg interactions and thus reduce fertilization efficiency, whilst a more sheltered habitat would slow down the process of gamete dilution and increase the chances of fertilization taking place.

It was not possible to determine whether juvenile *H. pectunculus* settle preferentially on the shore in a particular habitat, or whether they settle haphazardly over the whole shore and then suffer differential mortality so that the juveniles are only found in one particular habitat (Underwood, 1979). It is suggested, however, that the juveniles settle at the back of crevices which would make them very difficult to locate. By the time they become visible and large enough to identify *i.e.* 2 mm shell length, they will have been on the shore for approximately 2-3 months (chapter 3). For this reason Creese (1980) stated that the factors operating during these first few months are impossible to investigate experimentally. Small individuals of *H. pectunculus*, with shell lengths ranging from 2-4 mm, were only ever found to be present with adult limpets and never in places where adults did not occur thus ruling out migration. This suggests that *H. pectunculus* larvae exhibit selective settlement or that by the time larvae reach 2-4 mm in length, most of those that did not settle in conducive areas have already been removed by natural processes.

The lack of individuals < 16 mm in shell length in the population at the Riet River site could be due to a number of reasons. Firstly, this particular site is prone to sand scour during stormy weather which may well cause high mortality in juvenile limpets, or juveniles may not venture out of their crevices during foraging excursions and so were not visible during the sampling period. A third possibility is that, for some reason, recruitment to this particular site was either poor or failed completely at some time prior to the sampling period. Very few juveniles were found at any of the study sites and during two and a half years of field work (1994-1996) I have never observed a sudden increase in juvenile numbers. However, the large numbers of limpets of 18-24 mm shell length on most shores are presumably a result of a successful period of recruitment at some time (approximately 18 months, chapter 3) prior to sampling. Since this recruitment event(s), only a few juveniles have successfully settled on the shore, hence the low numbers. Clearly, a more detailed study of recruitment is now required.

The size distribution (shell lengths) of males and females within populations of *H. pectunculus* suggests that either males and females exhibit differential growth rates, with females growing at a significantly faster rate than males, or *H. pectunculus* is what is termed as a consecutive hermaphrodite (Fretter, 1984). A number of limpet species are thought to be consecutive hermaphrodites with almost all of them being protandrous. Orton (1920; 1928) first suggested that this is the case for *P. vulgata* in view of the larger numbers of males in the smaller size classes and females in the larger size classes and later, more detailed work confirmed this (Das & Seshappa, 1947; Dodd, 1956; Orton *et al.*, 1956 see chapter 4 for a full review). For similar reasons, other limpet species may also be protandric (Branch, 1981). However, in many cases, the only reason for suggesting protandry is the change of sex ratio with age.

There are other explanations for this effect within a population. Another possibility is that females may suffer a higher mortality rate. In one species of limpet, *P. oculus*, the phenomenon of protandry is unequivocal. This species has been shown to live rarely longer than two years and all juveniles are neuter for at least six months (Branch, 1974). The limpets then become male in their first year and female in their second year with oocytes developing in the gonad after spawning which eventually replace any remnants of sperm left behind (Branch, 1974). Robson (1986) obtained similar results studying *P. aphanes* on the Natal coast of South Africa, skewed sex ratios, differential sizes of male and females and histological examination also revealed individuals undergoing sex change.

The densities and biomasses of *H. pectunculus* varied considerably both between study sites and also within sites. The differences between the sites may be attributed to differences in shore geomorphology. The greatest densities and biomasses obtained during the study were recorded at Port Elizabeth, Cannon Rocks and the rocky platform and boulder shores at Gonubie. These sites provide numerous refugia under boulders, ledges and in crevices, and had minimal amounts of sand inundation. The reduced numbers of *H. pectunculus* at the other sites may be attributed to various factors, from shore geomorphology to human impact. For example, boulder shores are subjected to high human impact during the holiday seasons with people constantly turning the intertidal boulders in search of bait (pers. obs.). Shores can also be heavily inundated with sand at certain times of the year, which will probably result in sand scouring and an unstable substratum, both of which are factors that can reduce species richness and abundance (Coetzee, 1991). The variability in limpet numbers within the upper Balanoid zone at each site may be attributed to the fact that sampling occurred at lowtide at night whilst *H. pectunculus* was foraging. It is known that *H. pectunculus* is highly selective in where it feeds (chapter 5) and exhibits "grazer clumping behaviour". The variability could be due to the fact that certain areas of the shore, with optimal foraging conditions, would be densely populated with limpets at the time of sampling. Despite the variability in limpet density, the densities of *H. pectunculus* recorded in this study are similar to those of other limpet species on South African shores (Branch, 1971; Robson, 1986).

Branch (1973) suggested that patellid limpets along the South African coast fall into two main categories: migratory and non-migratory. He found that migratory species such as *P. granularis*, *P. granatina*, *P. concolor* and *P. oculus* settle low on the shore and migrate progressively upwards, thus occupying a wider range on the shore and reducing competition. These limpets were found to be present on the shore in high densities (*e.g. P. granularis* between 200-500 individuals/m^2) but were widely distributed amongst the zones.

Non-migratory species include *P. cochlear*, *P. longicosta*, *P. tabularis*, *P. miniata*, *P. argenvillei* and *P. compressa*. *Patella compressa* is only found on the kelp *Ecklonia maxima*. These species are known to settle in a narrow zone on the shore and remain there throughout life. Within this zone they can again be found in large densities (*P. argenvillei* up to 200/m^2, *P. cochlear* between 100-200/m^2). It is interesting to note that all of the non-migratory species found by Branch (1973) were found low down on the shore and none were found to prefer the upper shore regions.

Helcion pectunculus appears, therefore, to fit into the "non-migratory" group. Although its distribution is high up on the shore there is no evidence to suggest that a migration of limpets from low to high shore occurs. This limpet also homes rigidly to a fixed scar (see chapter 5) and is dispersed on the shore which are also criteria put forward by Branch (1973) for non-migratory species. However, *H. pectunculus* does not act in a territorial way, nor does it maintain and defend established food "gardens" or its home scar as the other species in this group do, suggesting that perhaps a third group of limpets are present on South African shores.

Branch (1973) went on to suggest that the development of migratory and non-migratory tendencies within species is graded, and most intense in high density species such as *P. granularis* and *P. concolor*. *Patella barbara* have very low densities on the shore (in the order of 1 individual/m^2) and so conforms to this theory. *Helcion pectunculus*, with densities of between 50-100 individuals/m^2 in places, may not. It is therefore proposed that a third group of limpets seems to be present which are "specialized non-migratory species".

These are species that do not migrate but neither do they garden or aggressively fight off conspecifics. They have overcome competition for space and food on the intertidal rocky shore by specializing to a particular habitat which is exclusive to them alone. This group may well include species such as *P. compressa* which is highly specialised for life on kelp stipes (Branch, 1973); *Helcion pellucidum* occurs only on the stipes and blades of *Laminaria hyperborea* although this species does undergo a migration along the algal frond as it gets older (Graham & Fretter, 1947; Vahl, 1971, 1972, 1983; McGrath, 1992). *Helcion pruinosus* also has a rather specialized habitat, occurring only in low-shore rock pools and under boulders (A. Henninger, Rhodes University, pers. comm.). *Patella barbara* is another possibility but it has not needed to specialize to a particular habitat due to the fact of its sparsity on the shore making intra-specific confrontations very unlikely, although it may be argued that this, in itself, is a specialist adaptation.

It is therefore apparent that more detailed analysis is required to understand the complex factors at work which influence limpet distribution in the intertidal zone. It is suggested that the parallel evolution of behavioural traits put forward by Branch (1973) exist but are not so clear cut as "migratory" and "non-migratory". *Helcion pectunculus* is a high-shore limpet which has clearly evolved, both physiologically and morphologically, to survive the extreme conditions in the upper Balanoid zone. This it has done with great success, as the densities and biomasses obtained in this study show that this patellid is a major grazer in the upper intertidal. The only other species which occurs in similar densities in the upper Balanoid is the chiton, *Acanthochitona garnoti* which can reach densities of 300 individuals/m^2 (P. Kuun, Rhodes University, pers. comm). The question of whether selective settlement or natural mortality drives the distribution of *H .pectunculus* on the rocky shore is not known and more detailed field-based experimentation is required to verify this.

2.5 Summary

1. *Helcion pectunculus* is always found residing within rock crevices or under boulders during daytime lowtides, and it is suggested that this is a behavioural adaptation to reduce desiccation, thermal stress and avoid wave activity.
2. *Helcion pectunculus* has a restricted zonation, with the majority of animals being found in the upper Balanoid zone. Individuals were occasionally found in the lower Balanoid zone on shores with a gently sloping aspect.
3. *Helcion pectunculus* occurs in higher densities on shores which have large numbers of crevices and boulders, in and under which, limpets may seek shelter from extreme physical conditions.
4. At all sites, males and females were always found to differ in size, with individuals of < 20 mm shell length generally being male whilst limpets with a shell length of > 22 mm were generally female. This phenomenon suggests either males and females grow at different rates or that *H. pectunculus* exhibits protandric hermaphroditism.
5. From the results of this study it is suggested that, along with the "migratory" and "non-migratory" groups of limpets present on South African shores (Branch, 1971), a third group exists; the "specialized non-migratory species". These species have overcome the fierce competition for space and food on intertidal rocky shores by specializing to a particular habitat which is exclusive to them alone.

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Chapter 3:

Shell morphology, growth and longevity of the high shore limpet, *Helcion pectunculus*.

3.1 Introduction

In any analysis of the structure and dynamics of a biological community data on growth, mortality, recruitment and reproduction are invaluable (Underwood, 1979). Lack of detailed empirical data is still a major drawback in improving the predictions of the present diffuse theories about population dynamics.

Intertidal limpets are a convenient group in which to examine population dynamics and life history parameters such as growth, mortality and reproductive strategies (Creese, 1981). They generally live in a readily accessible habitat, often occurring in large numbers whilst being easy to sample, mark and relocate. Populations of limpets exhibit great plasticity (Branch, 1981), and environmental conditions on a very local scale often influence their growth and dynamics (Ward, 1967; Giesel, 1969; Sutherland, 1970; Lewis & Bowman, 1975; Bowman & Lewis, 1977). Comparative studies of a group of congeneric species inhabiting the same shore have revealed great variability in growth and mortality (Branch, 1974a, 1974b; Choat, 1977). Attempts have been made to compare the population dynamics of co-existing limpets from different families (Parry, 1978; Creese, 1981) to determine whether inter-specific differences in growth and reproduction are present when experiencing similar environmental parameters.

Rapid growth in invertebrates has been found to be directly related to rapid mortality (Ebert, 1973, 1975; Branch, 1981), and that longevity is inversely proportional to the rate of growth for several limpet species (Frank, 1965a, 1965b; Lewis & Bowman, 1975; Creese, 1981). Branch (1981) discusses this phenomenon in detail and suggests that some limpet species will have a low probability of survival because of the nature of the environment in which they live. These species will grow rapidly to sexual maturity and can thus survive the instability of the environment by continual recruitment.

Growth rates of limpets can be determined in a number of ways, including arithmetic, geometric or total growth (Branch, 1974b), or as mean specific growth (Wilbur & Owen, 1964). In the case of limpets, shell dimensions are the only parameters which can be measured periodically without damaging or disturbing the animal. A variety of methods have been utilised in order to gain estimates of limpet's growth, including periodic measurement of labelled animals (Branch, 1974b), checks in growth produced by known changes of environmental conditions (Vahl, 1971), annual growth rings (Picken, 1980) and periodic measurements of mean shell length of cohorts on the shore (Blackmore, 1969; Branch, 1974a; Underwood, 1975). More recently the procedure of micro-growth band analysis has been successfully adopted which yields data on both the growth and age of the limpet (Ekaratne & Crisp; 1982; Crisp *et al.*, 1990).

A number of factors have been found to alter the growth rate and shell shape of limpets (Branch, 1981). Increase in tidal height results in an intra-specific decrease in growth rate (Sutherland, 1970; Lewis & Bowman, 1975). Changes in the availability of food and seasonal fluctuations in temperature are also known to decrease limpet growth (Frank, 1965a; Parry, 1978; Picken, 1980; Bosman & Hockey, 1988a, 1988b; Lasiak, 1993). Bosman and Hockey (1988a) found that the growth rate of *Patella granularis* was closely correlated to the rate of algal production on a particular shore. Intra-specific differences in the size structures of populations of *P. granularis* from algal enriched and unenriched shores were evident. However, changes in growth rate may not always be due to algal standing stocks. Sutherland (1970) found that growth in high shore *Collisella scabra* is significantly reduced in summer as the algae die off, but low shore individuals grow at a slower rate in winter when the densities of limpets rise due to recruitment of juveniles.

Limpet growth strategies may also be modified by exposure to wave action. Thompson (1979) found that *Patella aspera* dominated highly exposed shores with strong wave activity while *P. vulgata* occurs in large numbers on shores with more moderate wave action. It has been suggested (Thompson, 1979) that the growth strategy of *P. aspera* is not very flexible and so it is outcompeted by the faster-growing *P. vulgata* under more favourable conditions, whilst under conditions of extreme wave activity the growth rate of *P. vulgata* declines while *P. aspera* maintains a constant growth rate. Inter-specific differences in limpet growth rate and mortality cover a considerable range. *Nacella concinna* has been shown to grow only a few millimetres a year, reaching sexual maturity at the age of seven years, whilst living to be 30 (Picken, 1980). Branch (1974b) found that *Patella oculus* is particularly fast growing, becoming sexually mature after a year of growth (6-7 cm shell length) but only surviving for about 3 years.

Southern Africa exhibits an extreme contrast in oceanographic conditions between its east and west coastlines. The Agulhas Current, one of the most powerful currents in the world, sweeps warm water from the subtropical regions down the east coast. Conditions on the west coast are quite different. The waters are chilled by northward drifting cold water.

Wind blows the surface waters off-shore, and deep water upwells near the coast to replace it. This water is not only cold but also rich in nutrients making both phytoplankton and macrophytes far more productive on the west coast than on the south and east coasts (Branch *et al.*, 1994). These vastly differing oceanographic conditions may well affect the growth of the many species occurring along both coastlines. Therefore, it was decided to attempt to gain an estimate of growth for *Helcion pectunculus* along the east and west coasts of South Africa.

Helcion pectunculus is a crevice dwelling limpet inhabiting the upper balanoid zone of most rocky shores along the South African coast (see chapter 2). Being a high shore species, it experiences extreme conditions which may well affect its population dynamics. This study aimed to establish whether limpet populations from the east and west coasts exhibit similar rates of growth, and whether the harsh environment in which these animals live affects their morphology, longevity and general life histories.

3.2 Materials & Methods

3.2.1 Allometric vs Isometric growth

Limpet shell height and length are known to be related by the function

$$h = cL^{\alpha}$$

where α (the constant of allometry) = 1 if the two factors change proportionally, but $\alpha > 1$ if shell height increases faster than length during growth (Branch, 1981). Plotting log shell length against log shell height will show whether *Helcion pectunculus*, occurring along the coast of South Africa, grows allometrically as opposed to isometrically and whether the allometry is positive or negative in function. Transects were carried out at various sites along the South African coast ranging from East London on the east coast through to Bloubergstrand on the west coast (see chapter 2 for details). Shell length, width and height were measured and sex recorded for each individual at each site. Linear measurements were made to an accuracy of 0.01 mm using Vernier callipers, and mass values, determined with an electronic balance, were measured to 0.001 g for consistency.

3.2.2 Mark-recapture study

Two main techniques are used to investigate the growth rates of gastropod molluscs. Large numbers of marked individuals may be released for recapture at regular intervals and direct measurements of their growth rates thus obtained (Sutherland, 1970; Branch, 1974b). Alternatively, the population may be sampled at intervals and a measure of growth of the population obtained by changes in the mean size of cohorts determined from polymodal size-frequency distributions (Sutherland, 1970; Underwood, 1975; Robson, 1986).

Arithmetic changes in shell length were used in this study as the most convenient measure of limpet growth, and determined at monthly intervals for one year (from September 1994 to August 1995) at Cannon Rocks (33°44'S/26°35'E) in the eastern Cape. Two hundred individual limpets were tagged using small plastic labels manufactured for labelling bees (Thorn Bee Hive Works, Rugby, Lincoln, U.K.). Pattex epoxy putty was used to attach the labels to the animals and proved to be a quick setting and very strong adhesive, provided it was applied to dry shells.

One problem encountered during the study was that continuous erosion/abrasion, via wave action, rubbed the numbers off the labels. To overcome this, all labels on animals were covered with clear nail varnish.

The Von Bertalanffy growth equation was chosen to describe the growth of *Helcion pectunculus* for this study. The function has various forms, with that for length being

$$L_t = L_{max} (1 - e^{-K (t-t0)})$$

After Beverton & Holt (1957) where:

L_t = length at age t

L_{max} = theoretical maximum length attained in the field

K = growth coefficient

t = time

t_0 = time at which growth commences from L = 0

Because values of t_0 do not materially influence the other Von Bertalanffy growth parameters (Beverton & Holt, 1959), values of t_0 are often not estimated in initial determinations of Von Bertalanffy growth parameters. It has also previously been stated that the value of t_0 holds no biological importance whatsoever and is present purely as a result of the mathematical modelling procedure (Ricker, 1975). For these reasons, values of t_0 were not calculated during this study and t_0 was taken to equal 0 (zero) at all study sites.

Walford (1946) proposed a graphical method, known as a Ford-Walford plot (Beverton

& Holt, 1957), that gives a straight line with transformation of length-frequency data. A plot of $L_{(t+1)}$ against L_t should approximate a straight line from which values of K and L_{max}, of the Von Bertalanffy growth equation, could be estimated from mark-recapture as well as length-frequency data. The data obtained from the labelled animals at Cannon Rocks were used to plot a Ford-Walford plot. From this, values of L_{max} and K could then be estimated. These values were then inserted into the Von Bertalanffy growth equation and a growth curve obtained.

Monthly measurements of labelled individuals were not possible for west coast animals as the distance between Grahamstown and Bloubergstrand is about 800 km. Therefore, another method had to be utilised in order to obtain a growth model for west coast individuals. The method chosen was micro-growth band analysis. Many molluscs have been shown to lay their shells down in a rhythmic fashion, including most intertidal bivalves (Richardson *et al.*, 1979; Richardson, 1987, 1989; Richardson *et al.*, 1990) and many gastropods (Ekaratne & Crisp, 1982). It is possible, if the period during which the bands are laid down is known, to count the number of bands in a shell and gain an estimate of the animals age. Shells of individuals had been collected during the reproductive section of this study (see Chapter 4) from Bloubergstrand and so these were available for microgrowth band analysis.

3.2.3 Age determination of individuals using micro-growth band analysis

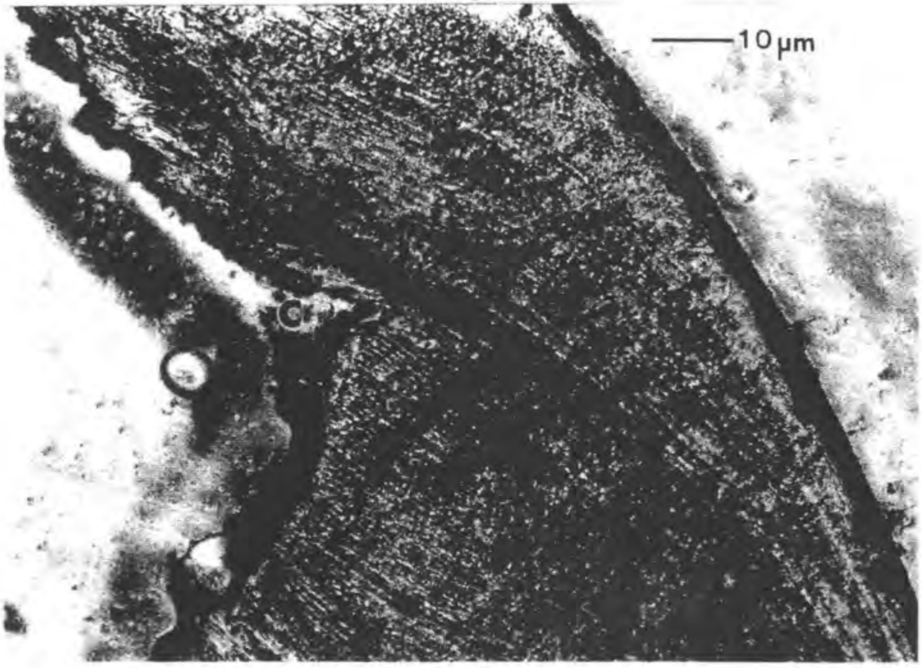
Marked limpet shells were cleaned in 10% sodium hypochlorite solution for 10 minutes to remove any organic debris. The shell was then air dried and embedded in casting resin (Metaserv s.w. resin 137/12742) and was sectioned in a plane from the anterior to the posterior through the centre of the apex of the shell. The cut surfaces were then ground on successively finer grit, wet and dry "Trimite" paper, polished with a cloth soaked in Brasso for 30 seconds and etched for a period of 40-45 min with cold 0.01 M Hydrochloric acid. Acetate peel replicas of the polished and etched surfaces were then prepared by allowing small strips of replicating material (Agar Scientific Ltd. No. G255) to become almost molten after 50 seconds in ethyl acetate. The strips were then applied to the etched shell surface and after 5 minute,s when all of the ethyl acetate had evaporated, the peel could be removed and "sandwiched" between two glass slides for examination using phase contrast microscopy.

3.2.4 Determination of the periodicity of shell micro-band deposition

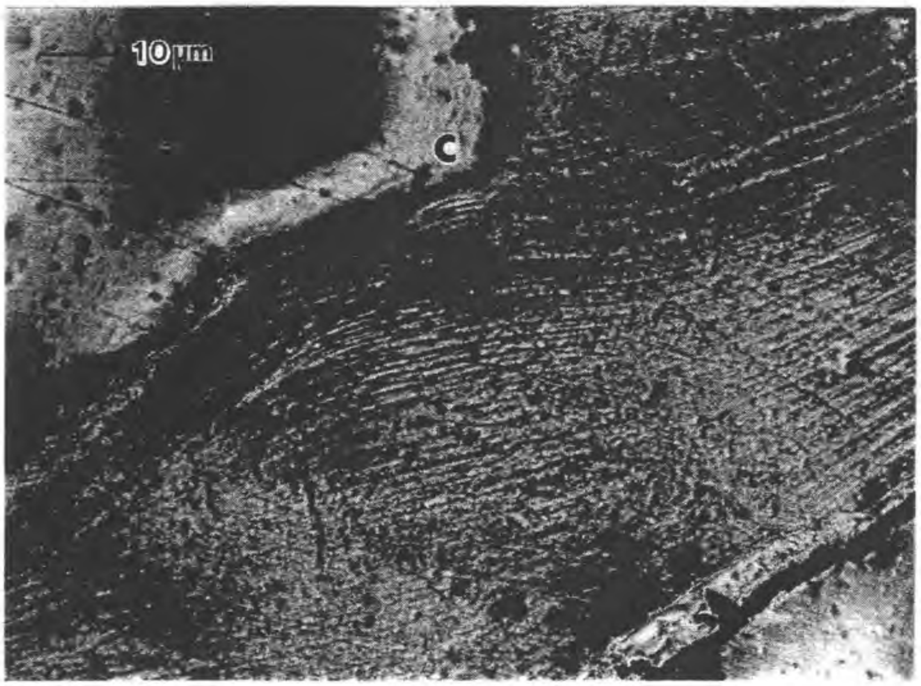
To determine the periodicity of growth band deposition in the shells of *Helcion pectunculus* 60 animals from Cannon Rocks were marked by scraping the growing edge of the shell with a scalpel blade (Ekaratne & Crisp, 1982). Twenty marked limpets were then sampled every 2 weeks for six weeks, their shells set in resin and the bands analyzed as in section 3.2.3. Examples of marked individuals are shown in figures 3.1a and b. The mark was generally found to be quite distinct as were the growth bands (figures 3.2a & b).

The number of growth bands after the mark was recorded for each animal and using a one-sample t-test, the results were correlated to rhythmic external influences such as number of days and number of tidal cycles. Knowing the periodicity of growth band deposition would then enable ageing of individuals from Bloubergstrand and thus an estimate of growth of *Helcion pectunculus* on the west coast could by obtained. Five juveniles, 5 males and 5 females, from both west and east coast populations, were analyzed using micro-growth band

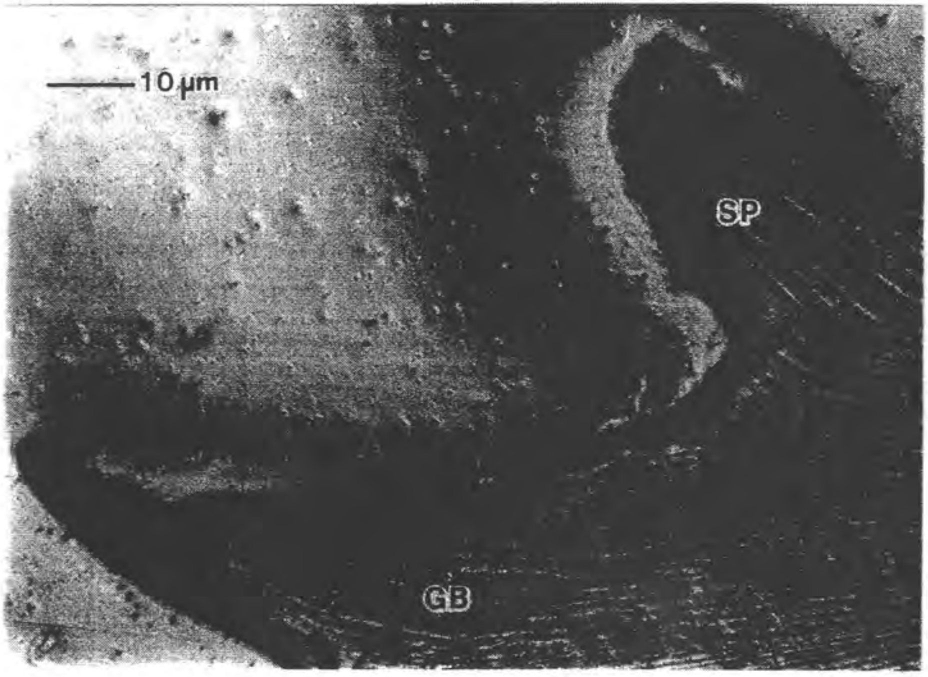
a)



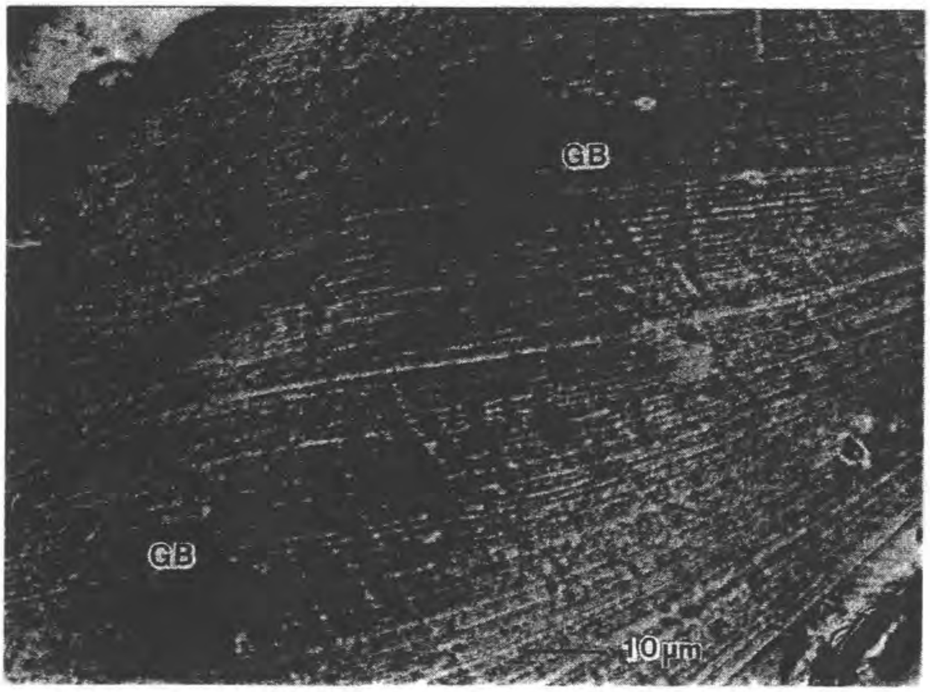
b)



a)



b)



analysis.

3.3 Results

3.3.1 Allometric vs Isometric growth

Plots of log shell height against log shell length for populations of *Helcion pectunculus* occurring at various sites along the South African coast are shown in figures 3.3a & b, 3.4a & b, 3.5a & b and 3.6. Regression analysis on the data obtained yields values of α and c (a constant) which can then be substituted into the equation:

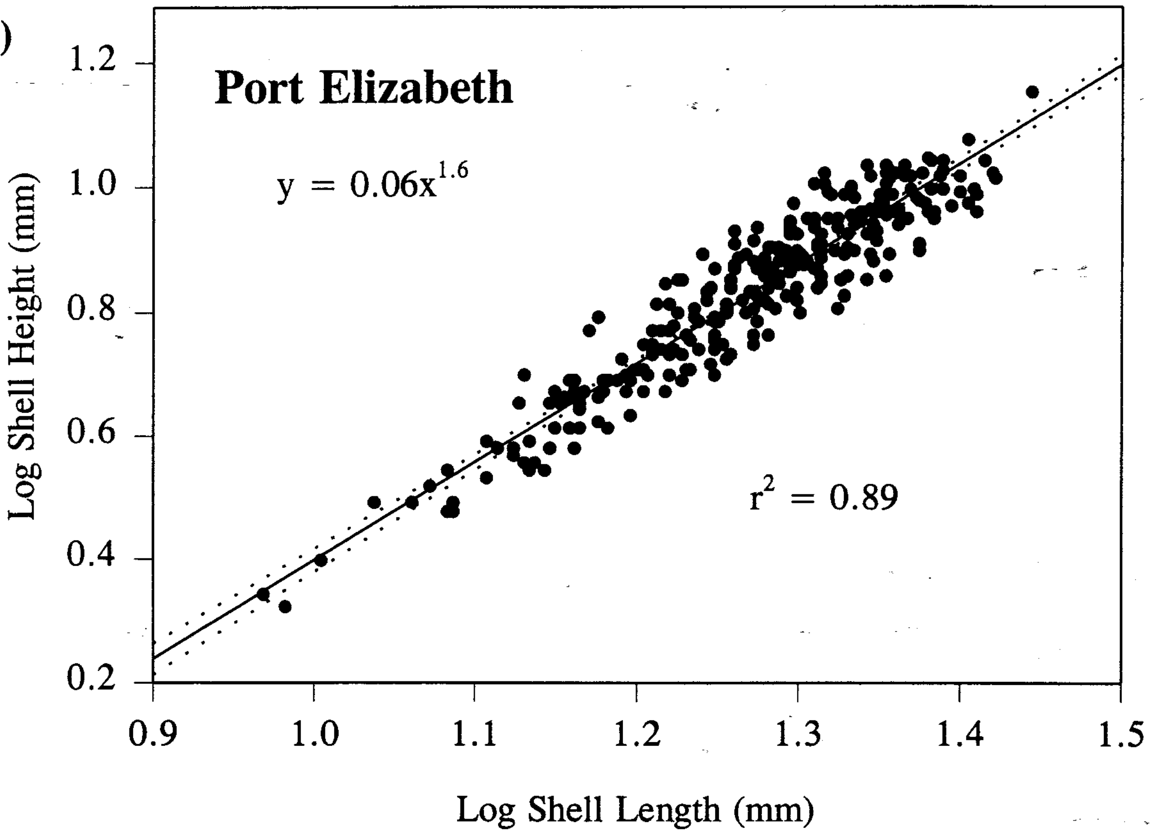
Equation 1 $$h = cL^{\alpha}$$

which in the case of the study populations of *H. pectunculus* gives the following equations:

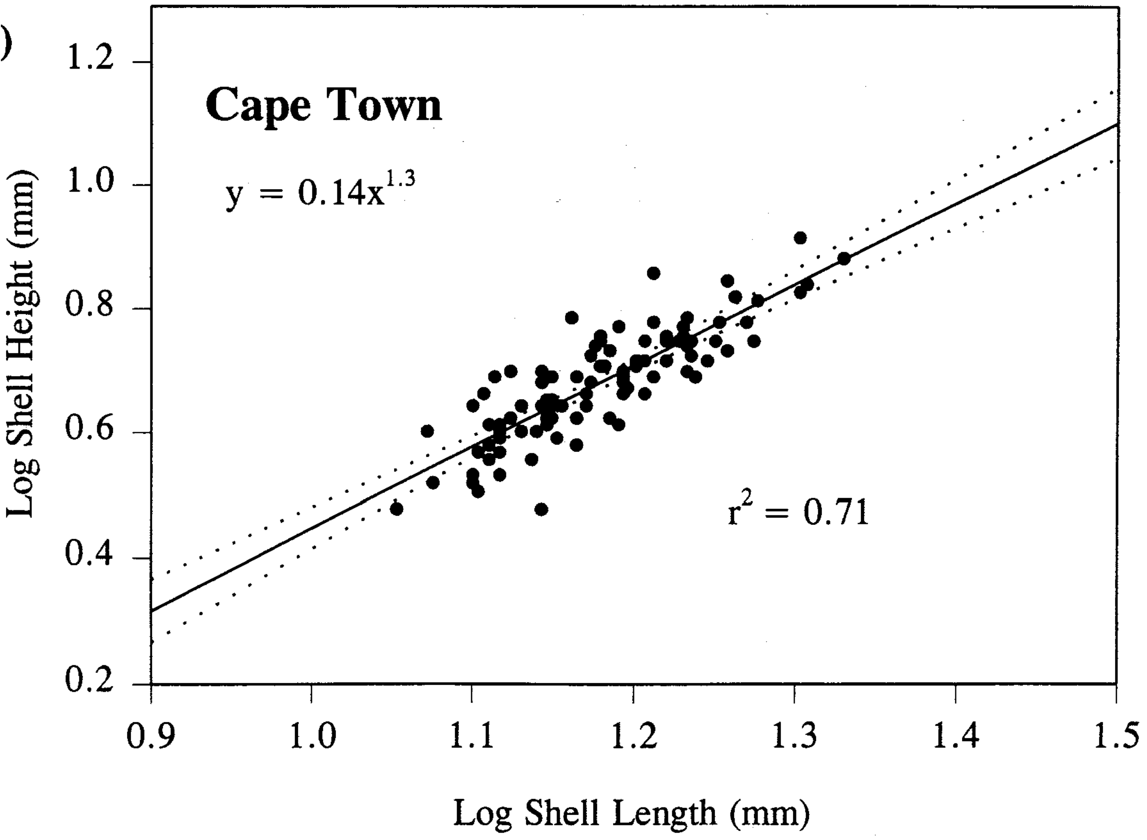
Port Elizabeth	$y = 0.06L^{1.6}$	$\alpha = 1.6$	$r^2 = 0.89$
Bloubergstrand	$y = 0.14L^{1.3}$	$\alpha = 1.3$	$r^2 = 0.71$
Cannon Rocks	$y = 0.08L^{1.4}$	$\alpha = 1.4$	$r^2 = 0.91$
Port Alfred	$y = 0.05L^{1.6}$	$\alpha = 1.6$	$r^2 = 0.90$
Riet River mouth	$y = 0.09L^{1.44}$	$\alpha = 1.44$	$r^2 = 0.72$
Gonubie - rocky platform	$y = 0.08L^{1.45}$	$\alpha = 1.45$	$r^2 = 0.94$
Gonubie - boulder shore	$y = 0.12L^{1.31}$	$\alpha = 1.31$	$r^2 = 0.95$

Individuals from both west (Bloubergstrand) and east (Port Elizabeth) coast populations therefore grow allometrically, increasing in shell height faster than shell length with constants

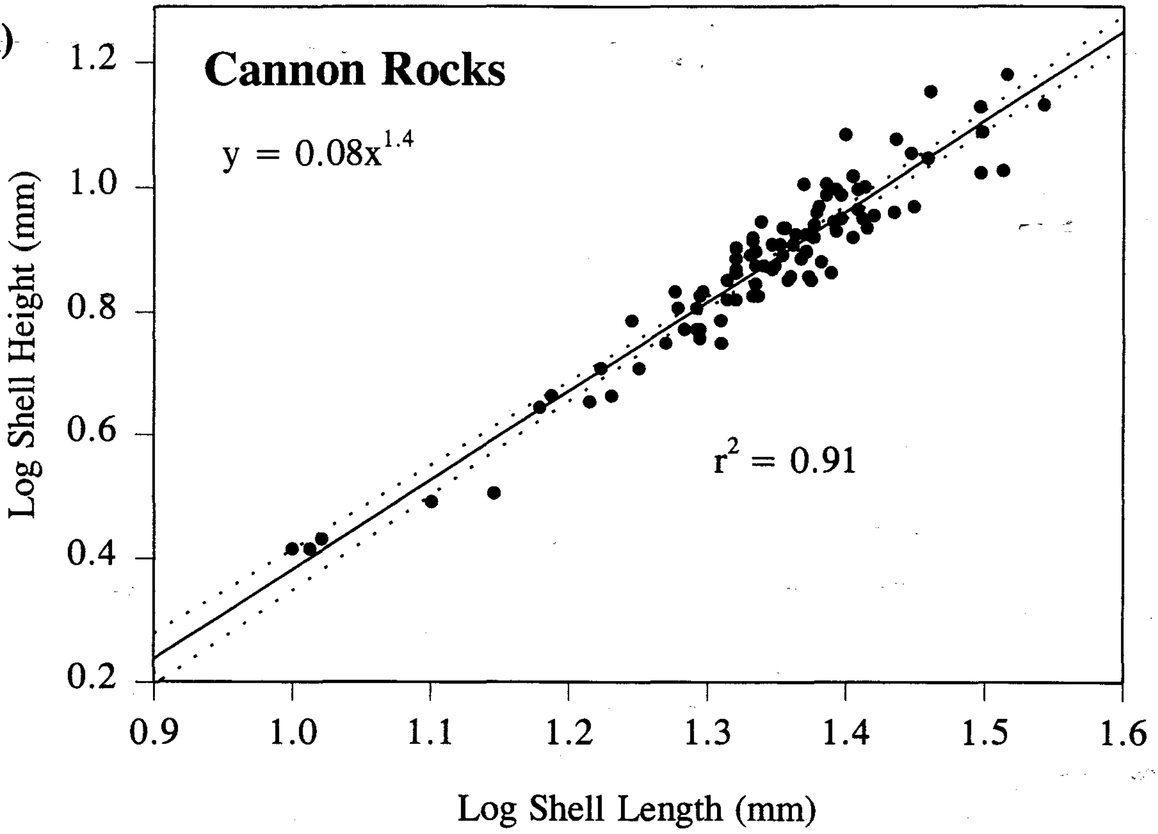
a)


Port Elizabeth
y = 0.06x^1.6
r^2 = 0.89
Log Shell Height (mm)
Log Shell Length (mm)
0.2
0.4
0.6
0.8
1.0
1.2
0.9
1.0
1.1
1.2
1.3
1.4
1.5

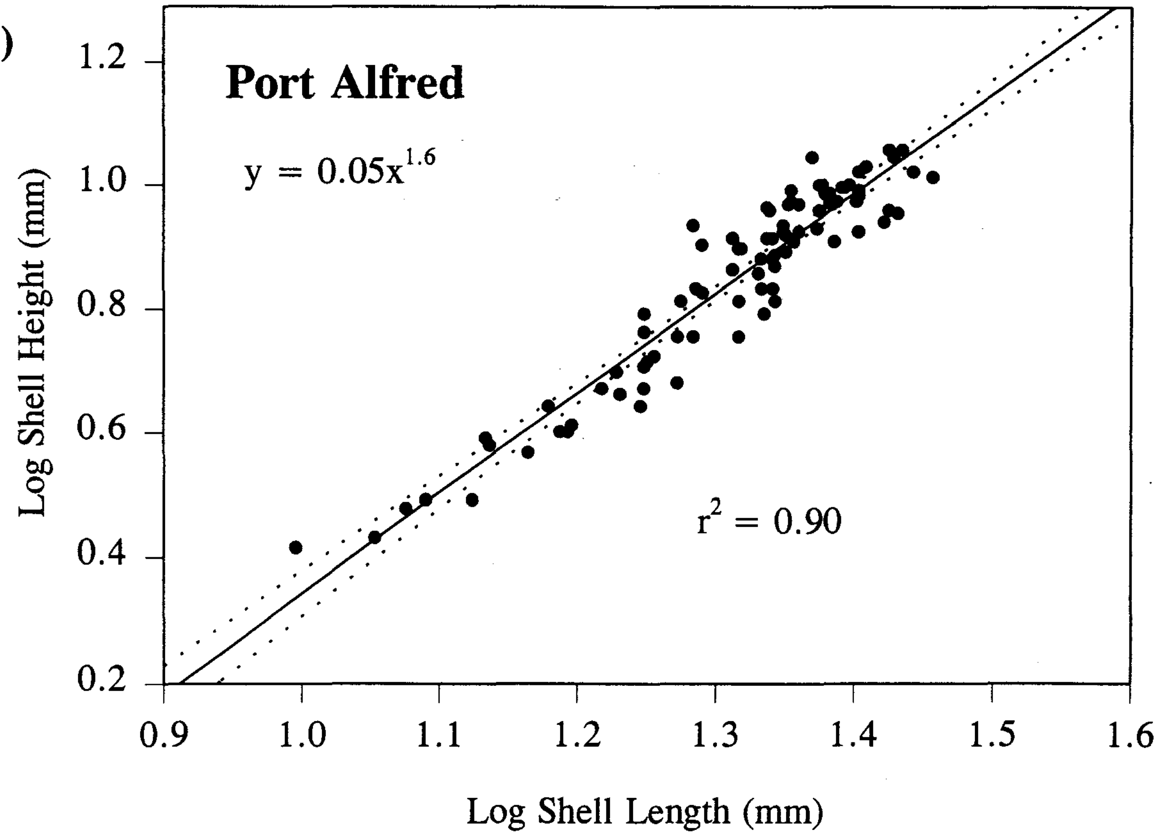
b)


Cape Town
y = 0.14x^1.3
r^2 = 0.71
Log Shell Height (mm)
Log Shell Length (mm)
0.2
0.4
0.6
0.8
1.0
1.2
0.9
1.0
1.1
1.2
1.3
1.4
1.5

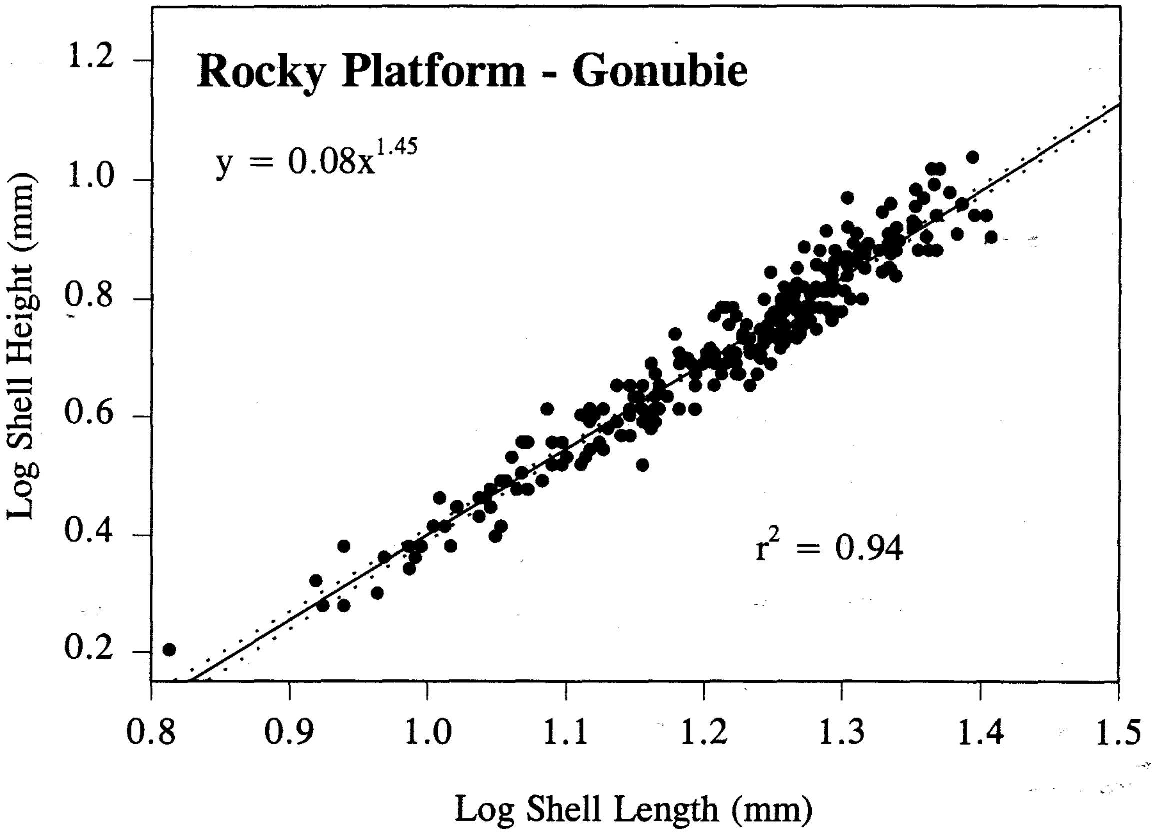
a)


Cannon Rocks
y = 0.08x^1.4
r^2 = 0.91
Log Shell Height (mm)
Log Shell Length (mm)
1.2
1.0
0.8
0.6
0.4
0.2
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6

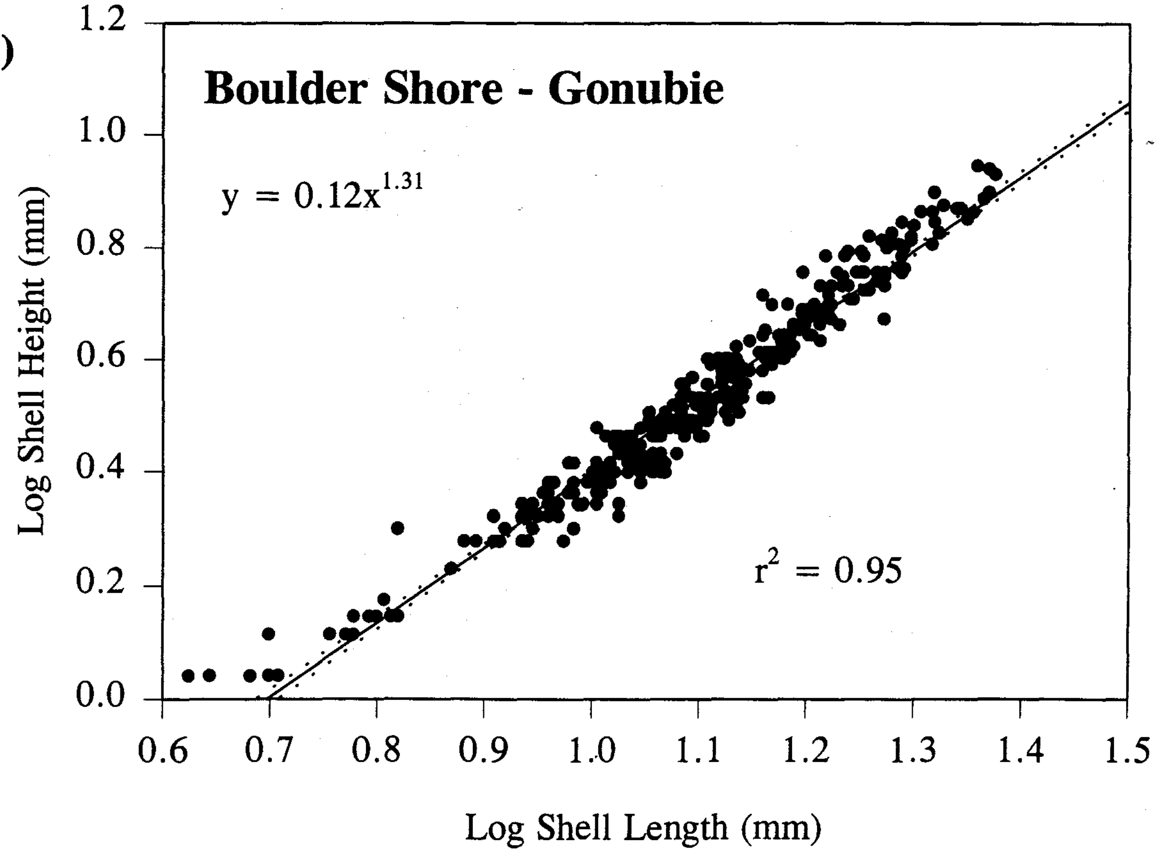
b)

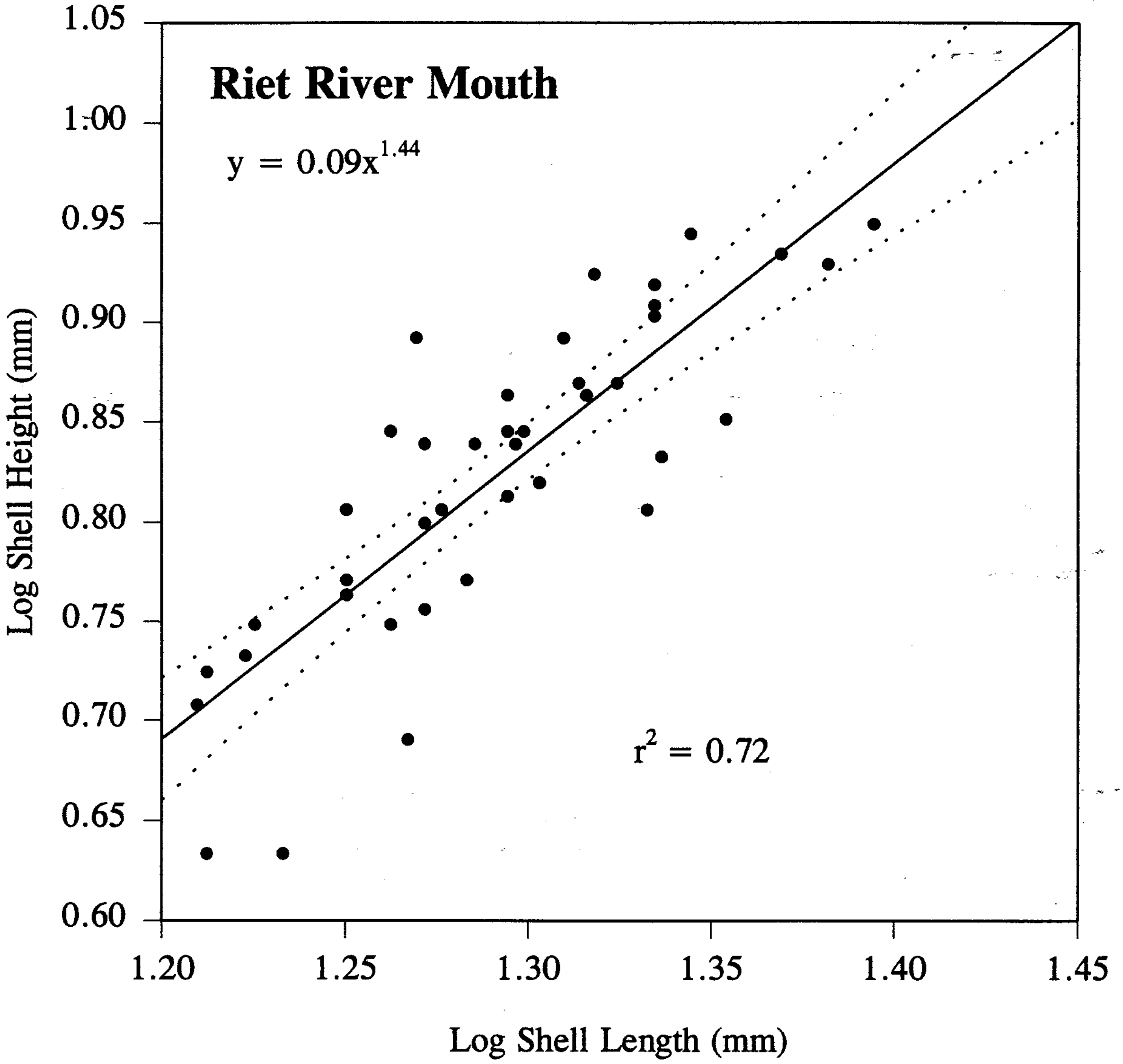

Port Alfred
y = 0.05x^1.6
r^2 = 0.90
Log Shell Height (mm)
Log Shell Length (mm)
1.2
1.0
0.8
0.6
0.4
0.2
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6

a)


Rocky Platform - Gonubie
$y = 0.08x^{1.45}$
$r^2 = 0.94$
Log Shell Height (mm)
1.2
1.0
0.8
0.6
0.4
0.2
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
Log Shell Length (mm)

b)


Boulder Shore - Gonubie
$y = 0.12x^{1.31}$
$r^2 = 0.95$
Log Shell Height (mm)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
1.5
Log Shell Length (mm)


Riet River Mouth
y = 0.09x^1.44
r^2 = 0.72
1.05
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
1.20
1.25
1.30
1.35
1.40
1.45
Log Shell Height (mm)
Log Shell Length (mm)

of allometry equalling 1.3 and 1.6 respectively. Some variability in the degree of allometry was found between the seven sites, with values ranging from 1.3 to 1.6.

3.3.2 Mark-recapture study

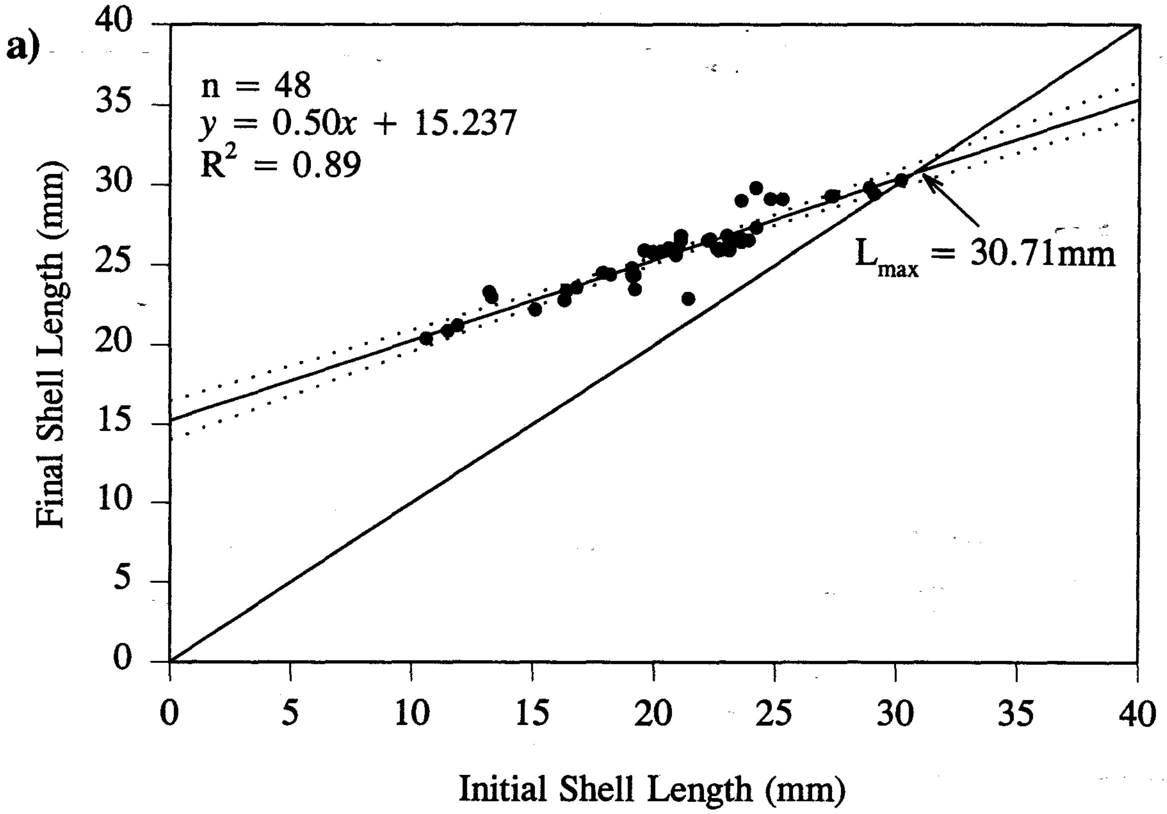
The Ford-Walford plot obtained by plotting L_t against $L_{(t+1)}$ for marked individuals of *Helcion pectunculus* from Cannon rocks is shown in figure 3.7a. If the slope of the regression line plotted in figure 3.7a is given the label *k*, then an estimate of K (the growth coefficient) can be derived as follows:

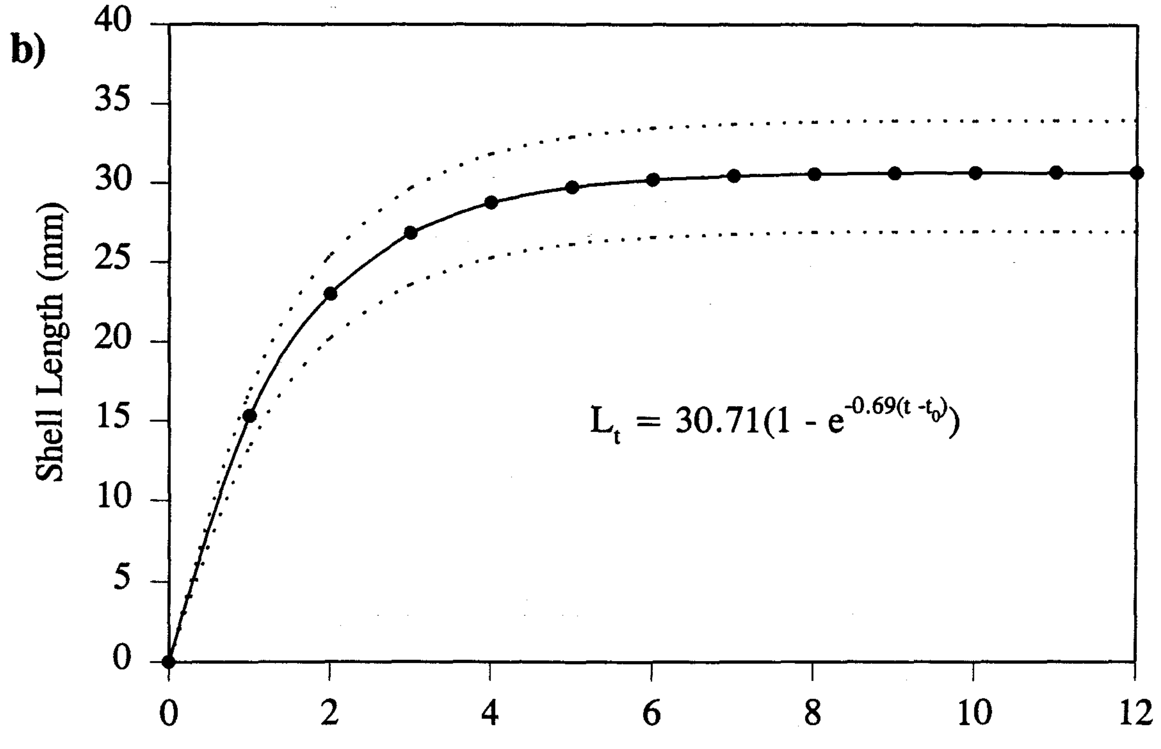
Equation 2 $$K = -\log_e k$$ (Ricker, 1975; Branch, 1981)

An estimate for K of 0.69 is obtained from the Ford-Walford plot along with an estimated value of 30.71 mm for L_{max} which corresponds closely with the maximum size observed in the field during this study (34.6 mm shell length). Estimates obtained from Ford-Walford plots must be used with caution, however, due to the fact that the largest size of animal labelled in the field will bias the final outcome (Beverton & Holt, 1957; Ricker, 1975).

These values of K and L_{max} were then inserted into the Von Bertalanffy growth equation and a growth curve obtained for the population of *Helcion pectunculus* at Cannon Rocks (figure 3.7b). The equation has the form:

Equation 3 $$L_t = 30.71(1 - e^{-0.69(t)})$$


a)
n = 48
$y = 0.50x + 15.237$
$R^2 = 0.89$
$L_{max} = 30.71$mm
Final Shell Length (mm)
Initial Shell Length (mm)
0
5
10
15
20
25
30
35
40


b)
$L_t = 30.71(1 - e^{-0.69(t - t_0)})$
Shell Length (mm)
Time in Years
0
5
10
15
20
25
30
35
40
2
4
6
8
10
12

This growth curve indicates a fairly rapid rate of growth (5-6 mm a year) up to the age of about 5-6 years.

3.3.3 Age determination of individuals using microgrowth band analysis

To determine the periodicity of growth band deposition within the shell of *Helcion pectunculus*, a one-sample t-test was used to correlate the number of bands within a set time interval to rhythmic external influences *i.e.* number of day/night cycles and number of tidal cycles (Sokal & Rohlf, 1981). The test was carried out using the computer software package Statgraphics. The number of bands within the shells did not differ significantly from the number of tidal cycles (table 3.1).

Figure 3.8 plots the results of micro-growth band analysis on the shells obtained from Port Elizabeth superimposed upon the growth model obtained from the Von Bertalanffy growth equation. Estimates of limpet ages based on shell bands follow the growth model fairly well allowing for errors in counting. The model does seem to under-estimate growth in juvenile individuals. The under representation of juveniles in modelling limpet growth on field observations has also been found by Branch (1974b). This could be a result of juveniles being under-represented in the initial mark-recapture study.

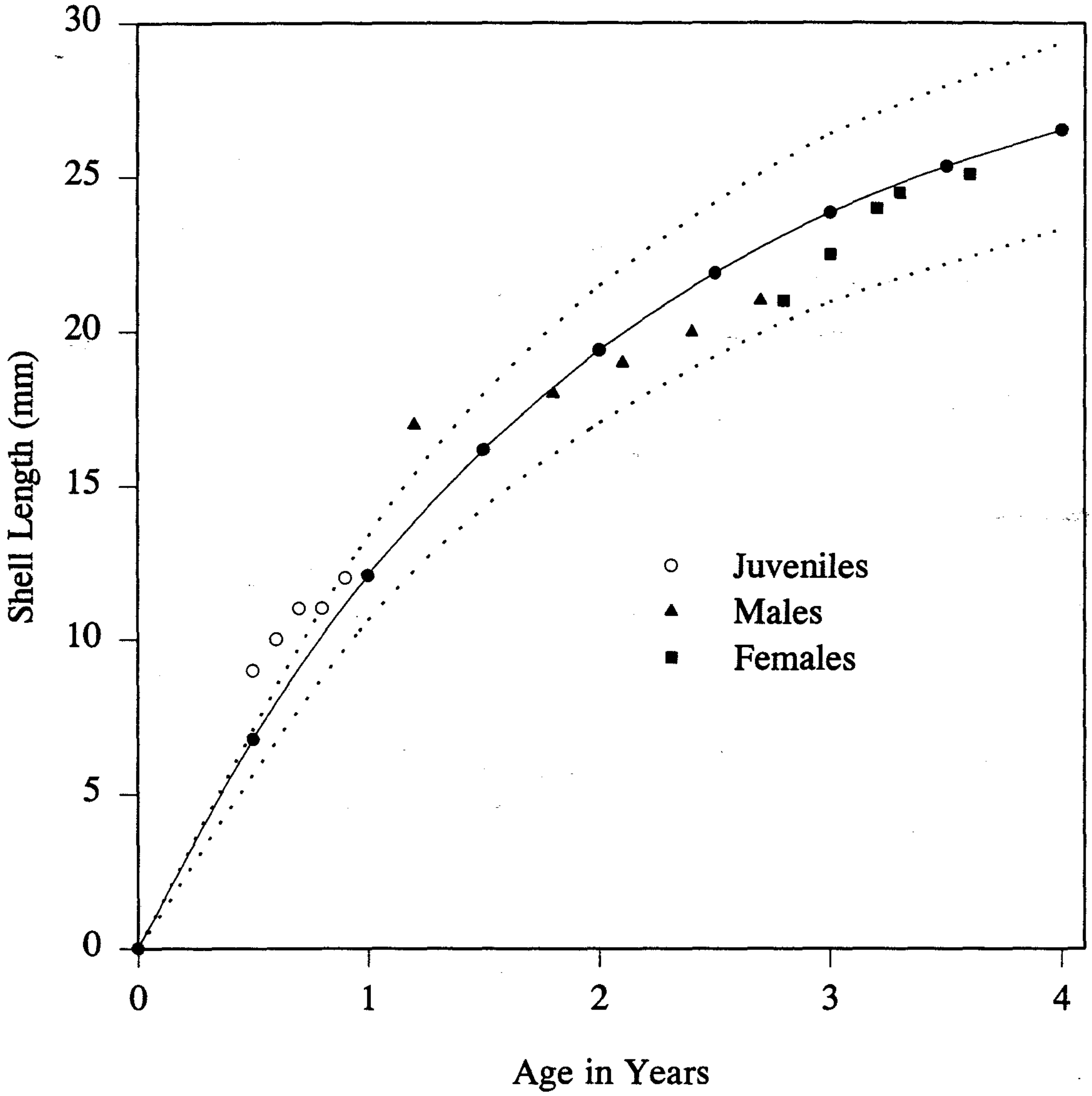
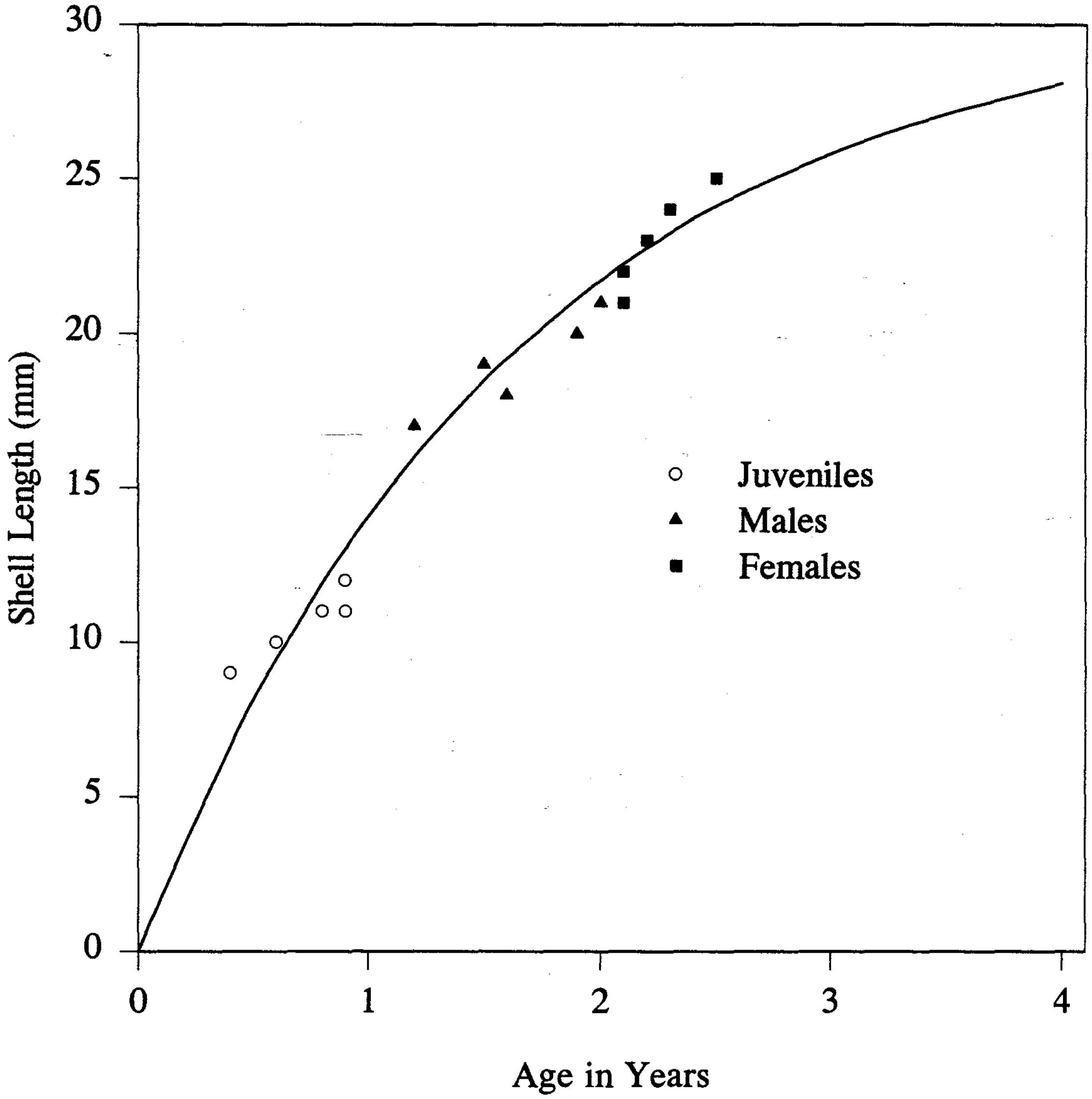

Shell Length (mm)
0
5
10
15
20
25
30
Age in Years
0
1
2
3
4
○ Juveniles
▲ Males
■ Females

Table 3.1

Results of a one-sample t-test carried out on data obtained from marked individuals of *Helcion pectunculus* at Cannon Rocks.

No. Weeks	n	mean No. of bands	Expected No. of bands	t-value	p-value
Correlation of day/night cycles:					
2	20	27.65	14	49.799	<0.001
4	20	55.85	28	68.644	<0.001
6	20	111.2	56	67.623	<0.001
Correlation of tidal cycles:					
2	20	27.65	28	-1.276	0.217
4	20	55.85	56	-0.369	0.715
6	20	111.2	112	-0.980	0.339

A similar plot was obtained for the individuals from the west coast whose shells were also analyzed using micro-growth band analysis (figure 3.9). A curve was fitted to this data using the method of least squares and the computer software package Sigmaplot (Jandell Scientific). A value of 30.86 was estimated for L_{max} whilst a value of 0.61 was given as an


Shell Length (mm)
0
5
10
15
20
25
30
Age in Years
0
1
2
3
4
○ Juveniles
▲ Males
■ Females

estimate of K. The curve has the form:

Equation 4 $L_t = 30.86\ (1 - e^{-0.61(t)})$

This curve can be taken to be an estimate of the growth model for west coast individuals of *Helcion pectunculus*. A comparison between the growth curves obtained for the east (equation 3) and west (equation 4) coast populations of *H. pectunculus* is plotted in figure 3.10. Both curves are very similar and do not differ significantly with the Cape Town growth curve lying within the 95% confidence limits of the Port Elizabeth growth curve.

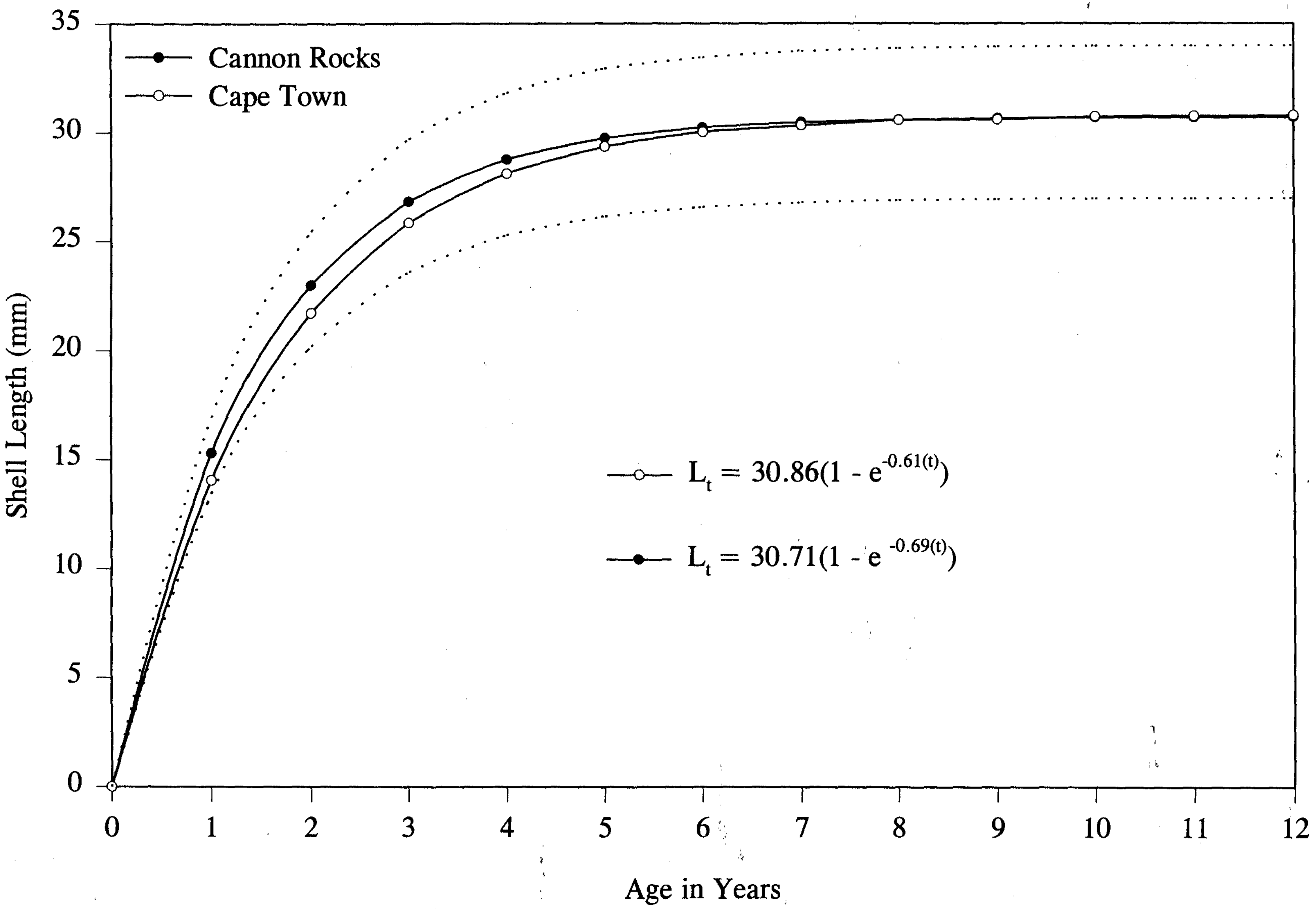
Micro-growth band analysis proves, unequivocally, that males are younger (1 - 2 years old) than female limpets which are generally 2 years old or older (figures 3.8 & 3.9).

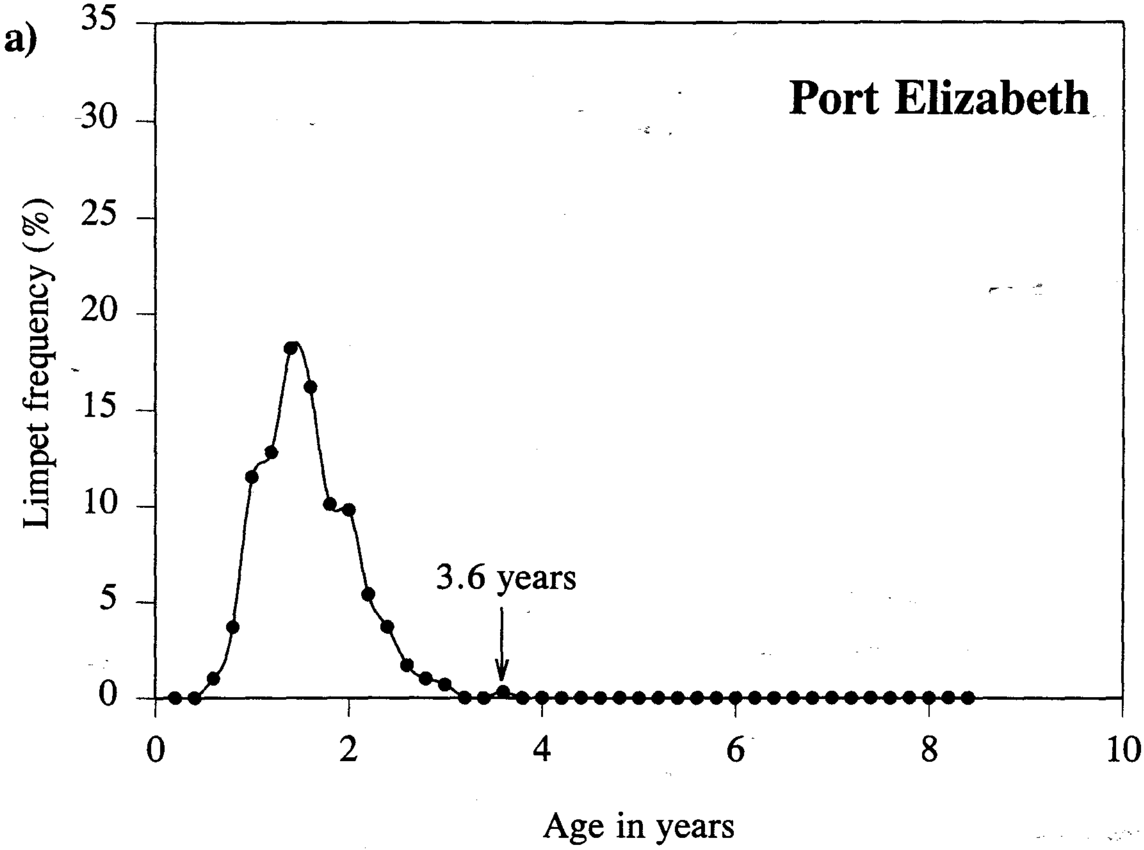
3.3.4 Limpet longevity

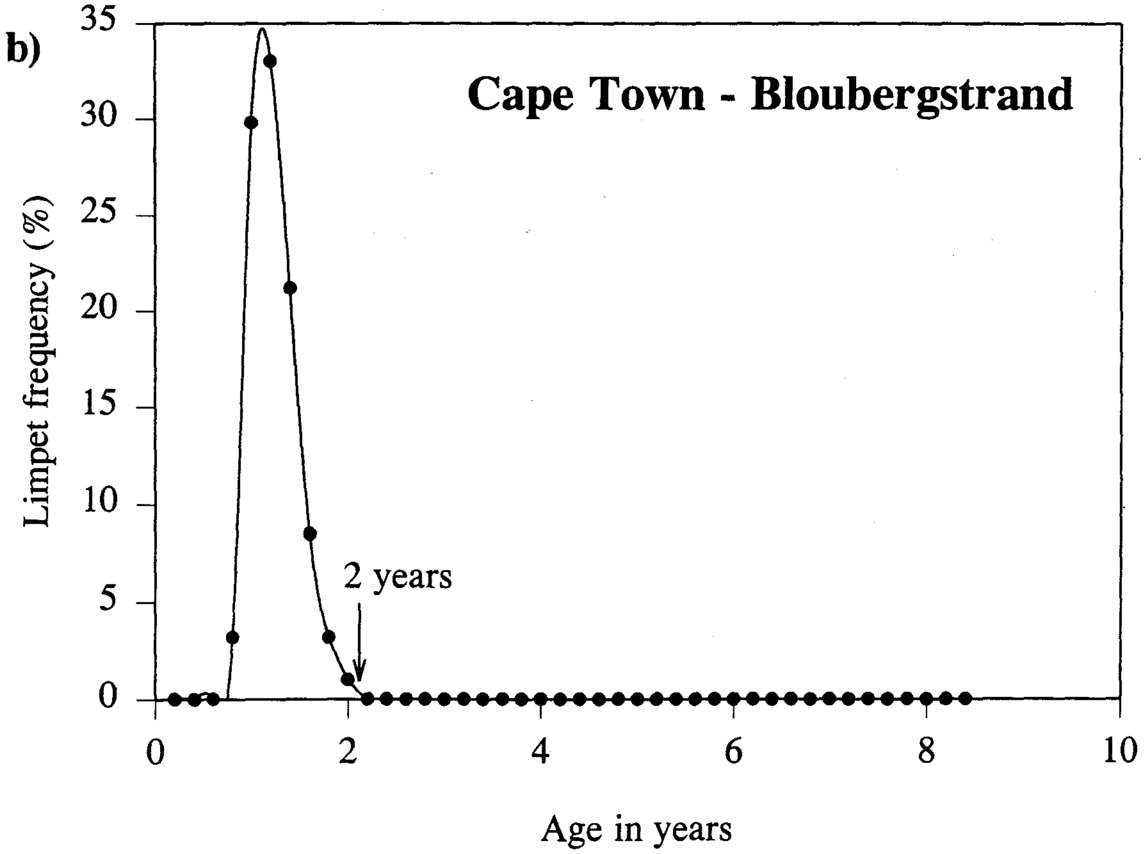
The Von Bertalanffy growth equation is usually used in the form shown in equations 3 and 4. However it can also be transformed with respect to time rather than length.

Equation 5 $t = [\ -\ (\ln(1 - (L_t\ /\ L_{max}))\ /\ K)]$

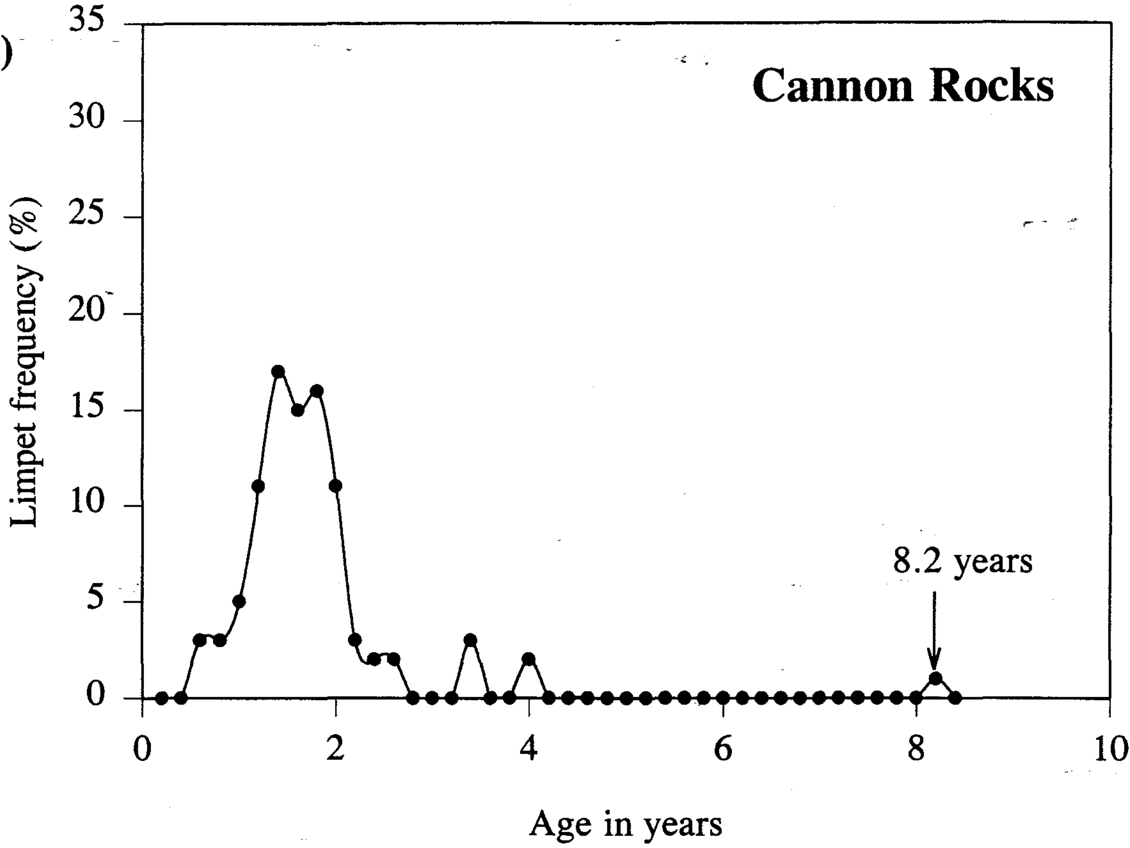
Using this equation (5) it is possible to obtain survivorship curves from the length-frequency data obtained in chapter 2 and thus gain an estimate of the longevity of *H. pectunculus* at the various study sites. The survival curves obtained are shown in figures 3.11, 3.12, 3.13 and 3.14.


Cannon Rocks
Cape Town
$L_t = 30.86(1 - e^{-0.61(t)})$
$L_t = 30.71(1 - e^{-0.69(t)})$
Shell Length (mm)
0
5
10
15
20
25
30
35
0
1
2
3
4
5
6
7
8
9
10
11
12
Age in Years

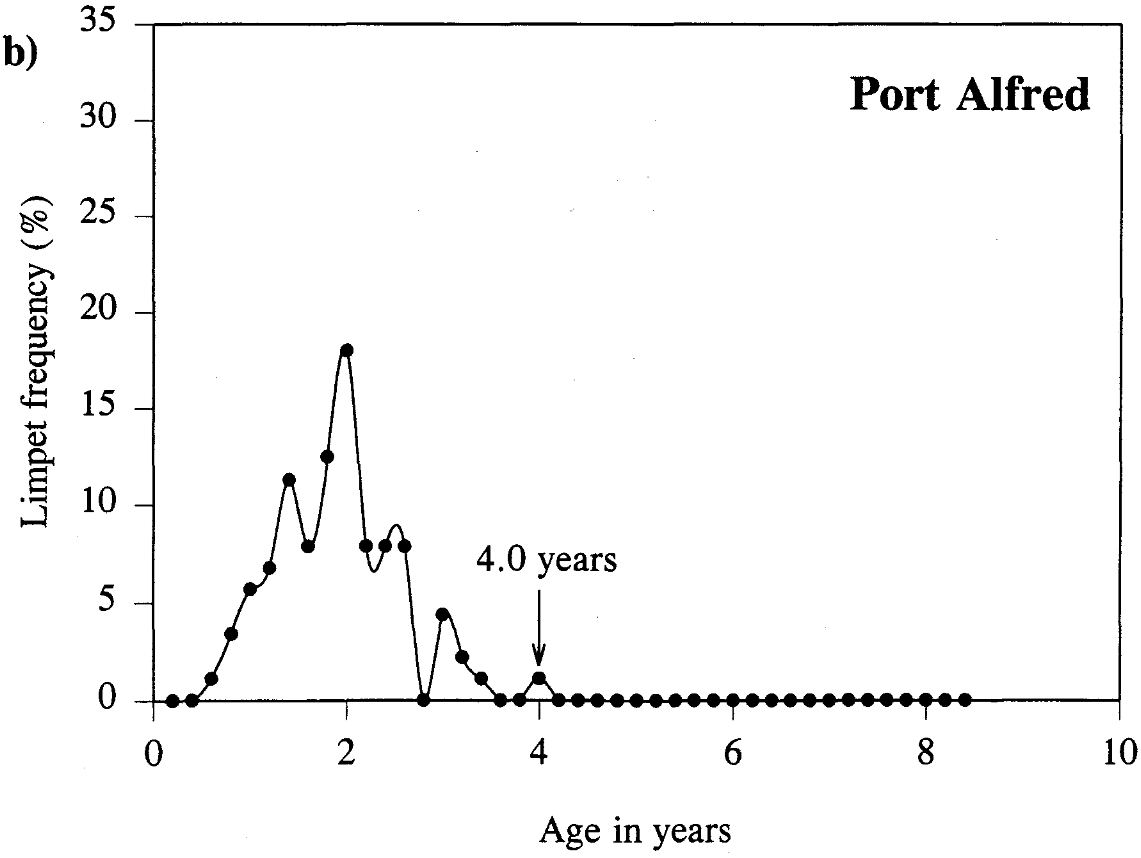

a)
Port Elizabeth
Limpet frequency (%)
35
30
25
20
15
10
5
0
3.6 years
0
2
4
6
8
10
Age in years


b)
Cape Town - Bloubergstrand
Limpet frequency (%)
35
30
25
20
15
10
5
0
2 years
0
2
4
6
8
10
Age in years

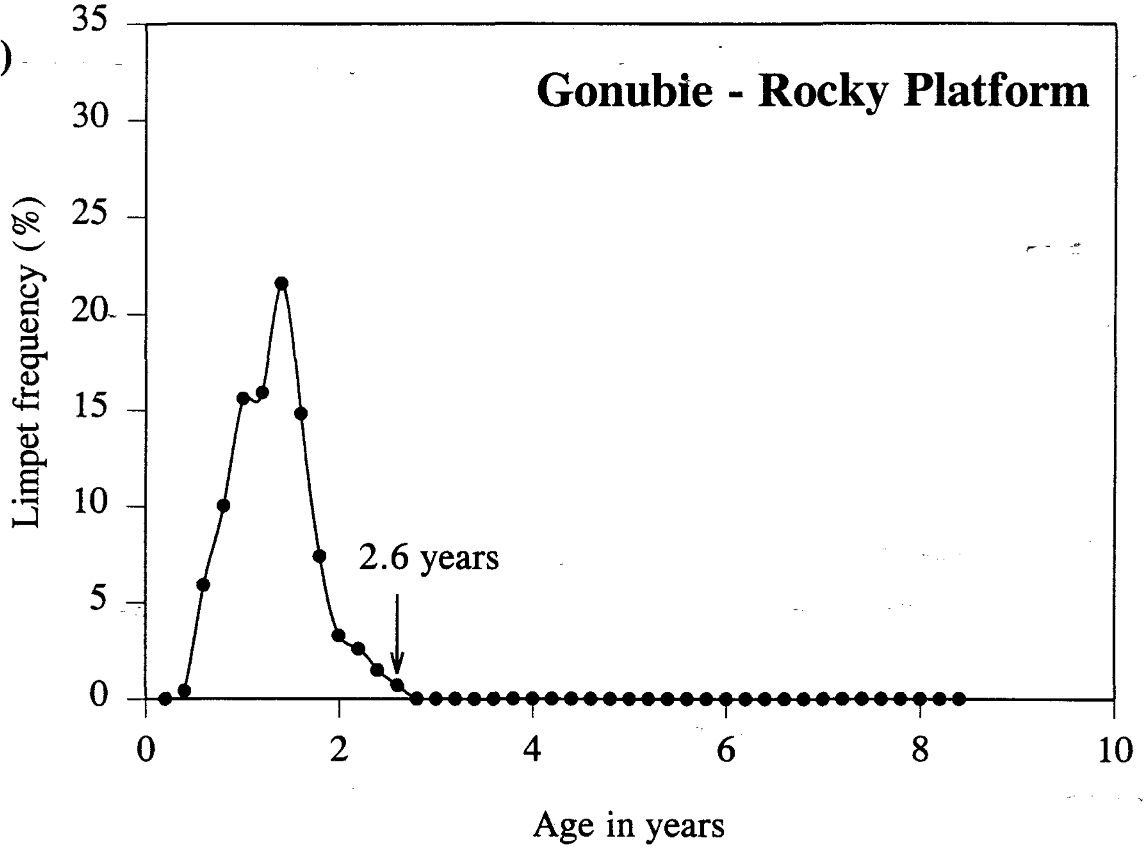
a)


Cannon Rocks
Limpet frequency (%)
35
30
25
20
15
10
5
0
0
2
4
6
8
10
Age in years
8.2 years

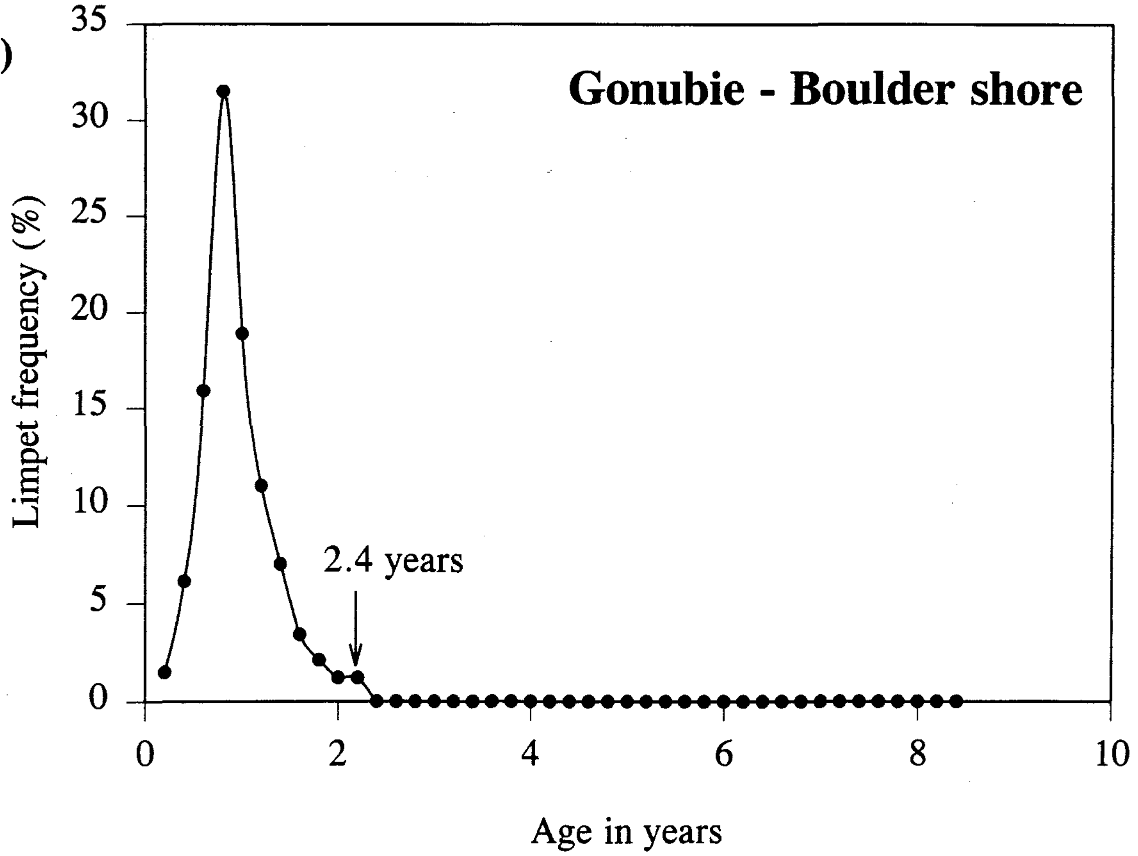
b)


Port Alfred
Limpet frequency (%)
35
30
25
20
15
10
5
0
0
2
4
6
8
10
Age in years
4.0 years

a)


Gonubie - Rocky Platform
Limpet frequency (%)
0
5
10
15
20
25
30
35
Age in years
0
2
4
6
8
10
2.6 years

b)


Gonubie - Boulder shore
Limpet frequency (%)
0
5
10
15
20
25
30
35
Age in years
0
2
4
6
8
10
2.4 years

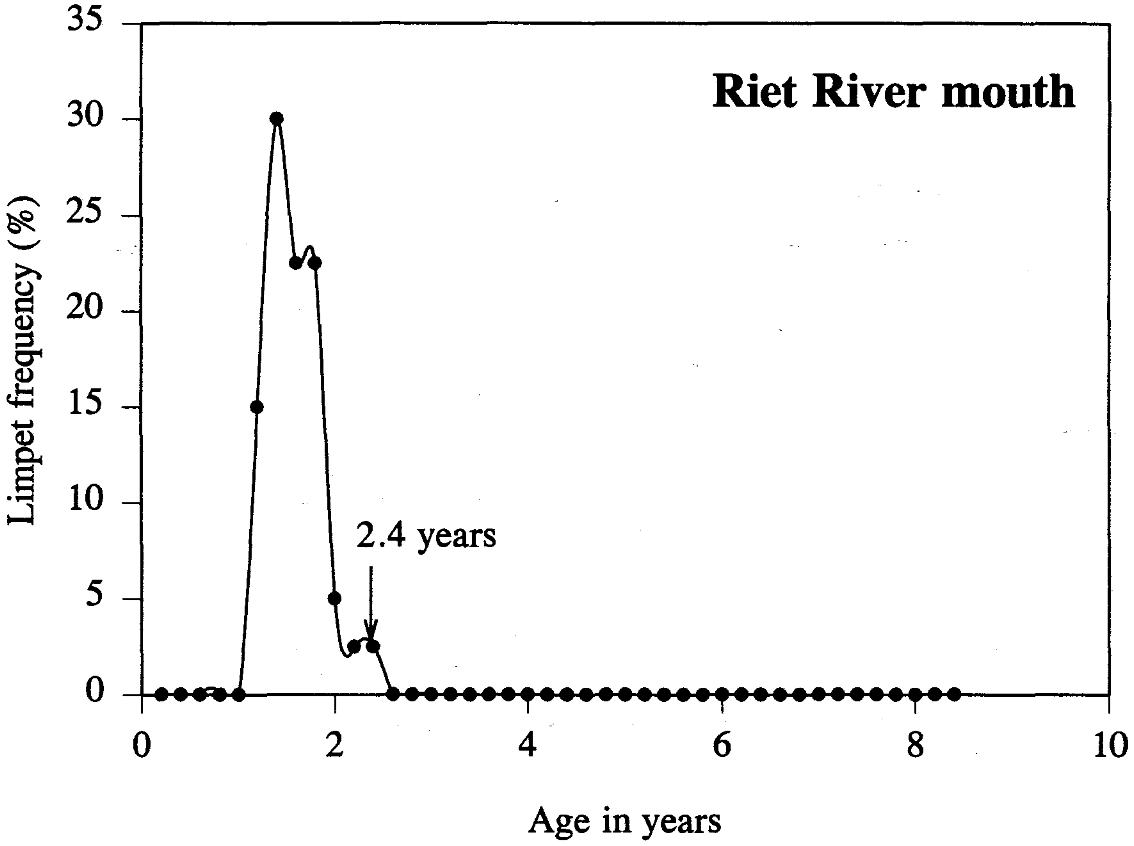

Riet River mouth
Limpet frequency (%)
Age in years
2.4 years
0
5
10
15
20
25
30
35
0
2
4
6
8
10

Table 3.2

Maximum shell sizes and relative ages of limpets found during the main biomass study transects (See chapter 2).

Study Site	Max. shell length (mm)	Maximum Age
Cape Town	21.4	3.6 years
Port Elizabeth	27.8	2.0 years
Cannon Rocks	34.9	8.2 years
Port Alfred	28.6	4.0 years
Gonubie - platform	25.5	2.6 years
Gonubie - boulder shore	23.7	2.4 years
Riet River Mouth	24.8	2.4 years

It is therefore apparent that very few animals live longer than about 3 years on the shore and that the theoretical maximum shell length is very rarely obtained.

3.4 Discussion

It has been suggested that shell shape in molluscs can have a substantial influence on the rate of water loss experienced by the individual (Branch, 1981). Orton (1933) suggested that individuals of *Patella vulgata* from high levels of the shore have taller, domed shells due to the fact that in dry desiccating habitats the limpet has to clamp down more tightly, pulling in its mantle so that, if the mantle glands deposit the shell while in this position, the shell circumference will be reduced and the shell taller in shape. Since this study, a great deal of research has been carried out on this topic which has shown a trend for taller shells to be linked to drier habitats (see Branch, 1981 for a review). Spencer Davies (1969) related this trend to desiccation rates. He suggested that surface area, from which evaporative water loss occurs, varied with the power of -0.33 of the volume (proportional to the body weight) so that:

$$\text{Rate of water loss} \quad \alpha \quad \text{Body weight}^{-0.33}$$

The animals change shape as they grow, becoming taller and hence increasing their body weights more than their surface areas from which water loss occurs. Higher on the shore, *P. vulgata* has a taller shell. Similar intraspecific differences exist in the Mediterranean *Patella* species (Bannister, 1975).

The shell of *H. pectunculus* also increases in height faster than shell length which, according to Branch (1981), is expected of high shore gastropod molluscs attempting to reduce evaporative water loss. Branch (1975) documented values of α for seven South African *Patella* species (see table 3.3). Vermeij (1973) reviewed interspecific differences in shell proportions and texture in limpets, neritids and littorinids, relative to their zonation and geographic distribution. Amongst the limpets a trend was apparent for high-shore species to have taller shells than lower shore species. Branch (1981) states that the South African species of *Patella* studied by Branch (1975) do not seem to follow the trend.

Table 3.3

The relationship between α, the constant of allometry, and height on the shore for seven species of *Patella* (After Branch, 1975) and *Helcion pectunculus*.

Species	α	Upper zonation limit (m above LWS)
P. granatina	1.36	1.46
P. granularis	1.34	2.12
P. argenvillei	1.31	0.32
P. barbara	1.09	0.46
P. oculus	1.03	1.57
P. cochlear	0.97	0.27
P. longicosta	0.91	0.97
H. pectunculus (C.T.)	1.30	1.43
H. pectunculus (P.E.)	1.60	2.38

Those individuals of *H. pectunculus* found on rock reef shores, such as Port Elizabeth and Port Alfred, tend to have higher values of α than animals inhabiting boulder shores (Cannon Rocks, Riet River, Gonubie) and rocky platforms (Gonubie - platform). This could be a function of the upper limit of distribution on these shores. Limpets inhabiting rocky reefs tend to be found higher up the shore than limpets on boulder or platform shores and so would need to preserve water to a greater extent. The individuals from the west coast were also found on a rocky reef shore, but were not found higher than 1.43 m above LWS tide mark so the need for allometry is not so great, thus the smaller α value of 1.3.

Microgrowth bands with a tidal periodicity have been established in certain barnacles (Bourget & Crisp, 1975a, 1975b; Crisp & Richardson, 1975), bivalves (Evans, 1972; Richardson *et al.*, 1979, 1980a, 1980b, 1981, 1990; Richardson, 1987, 1989), Polyplacophora (Jones & Crisp, 1985) and various gastropods (Ekaratne & Crisp, 1982). All of these animals inhabit the intertidal zone and so experience cyclical fluctuations in temperature and exposure to air. Crisp (1989) reviewing the phenomenon, gave various lines of evidence to suggest that harder and more perfectly crystalline parts of the shell comprised the bands and that these formed when the body fluids were temporarily at a lower pH due to accumulation of carbon dioxide and perhaps organic acids during emersion. All shell-secreting invertebrates exposed to the air and closed temporarily to avoid water loss, would be likely to experience acidosis and thus would slow down or prevent secretion of calcium carbonate.

As far as limpets are concerned, Branch (1974b) stated that in the case of *Patella*, no obvious growth rings occur within the shell and that growth and age could not be directly obtained from the shells of individuals. Ekaratne & Crisp (1982) subsequently carried out a series of studies on four intertidal gastropods including *Patella vulgata* and showed that not only were growth bands present within the shell of *Patella vulgata* but that they were laid down with a tidal periodicity. Crisp *et al.* (1990) then carried out a study on the pulmonate limpet *Siphonaria gigas*, counting growth rings and working out growth rates of limpets at two different sites. Again it was shown that the bands within the shells were laid down in synchrony with tidal emersion due to acidosis.

Helcion pectunculus also lays down bands within its shell which have the same periodicity as the tidal cycle. It is thus possible to count these bands and gain an estimate of growth of populations where mark-recapture studies are not practically possible. This was the case for the study populations situated on the west coast. However it must be stated that this procedure is very time consuming and it is only possible to count bands in individuals up to the age of about 4 years due to the fact that by this stage the bands are getting so close together *i.e.* growth of the shell has slowed down to such an extent that one band is difficult to discern from another. It has recently been established that both *P. granularis* and *H. pruinosus* also exhibit micro-growth bands in their shell microstructure (A. Henninger & L. Moxham, Rhodes University, pers. comm.) but more work is required to determine the periodicity of growth band deposition in these limpets.

Helcion pectunculus grows at a similar rate at the study sites on both the east and west coasts regardless of the differing oceanographic conditions. It must, however, be stated that the results obtained for the west coast are only from one site and it is suggested that more sites must be examined on the west coast in order to get a more complete picture of the growth of *H. pectunculus* along this section of the South African coast.

The theoretical values of L_{max} were similar for Bloubergstrand and for Port Elizabeth (30.86 mm and 30.71 mm respectively) as were the growth coefficients. The K value (growth coefficient) obtained from the Von Bertalanffy growth equation is a useful comparative index of growth within limpet species as it is not dependent upon the size of the animal concerned (Branch, 1981). Figure 3.15 is a modification of a figure first published by Branch (1981) and later, Robson (1986). It shows that both inter- and intraspecifically there is an inverse correlation between K and longevity. Some limpet species have a low probability of survival because of the nature of their environment (Branch, 1981). Vahl (1971) and Choat and Black (1979), have found that species living on essentially annual algal hosts were very unlikely to survive for more than one year because the host plant was prone to being torn free even before the year was complete. It is argued that growth patterns of such species living under harsh and extreme conditions would adapt their life histories to counter the high mortality by growing rapidly to sexual maturity and thus allowing continuous recruitment into the environment.

On the west coast of southern Africa *P. granatina* and *P. argenvillei* reach biomasses greater than reported for any intertidal grazer anywhere in the world (Eekhout *et al.*, 1992). This is only possible due to the fact that these two limpets subsidise their food intake with macroalgae (Bustamante *et al.*, 1995). *P. granatina* traps drifting kelp and seaweed debris whilst *P. argenvillei* actively prunes nearby plants. This food supplement will probably not be available to *H. pectunculus* living on the west coast due to it being a high shore species. Thus the growth rates of west coast *H. pectunculus* will not be affected by subsidized energetic input. Bosman and Hockey (1988a, 1988b) have also found that microalgal production has a significant effect on the growth rate of *P. granularis*. Those limpets observed on microalgal enriched shores exhibited significantly higher growth rates than those inhabiting unenriched shores. It is therefore suggested that in future studies of limpet growth, researchers

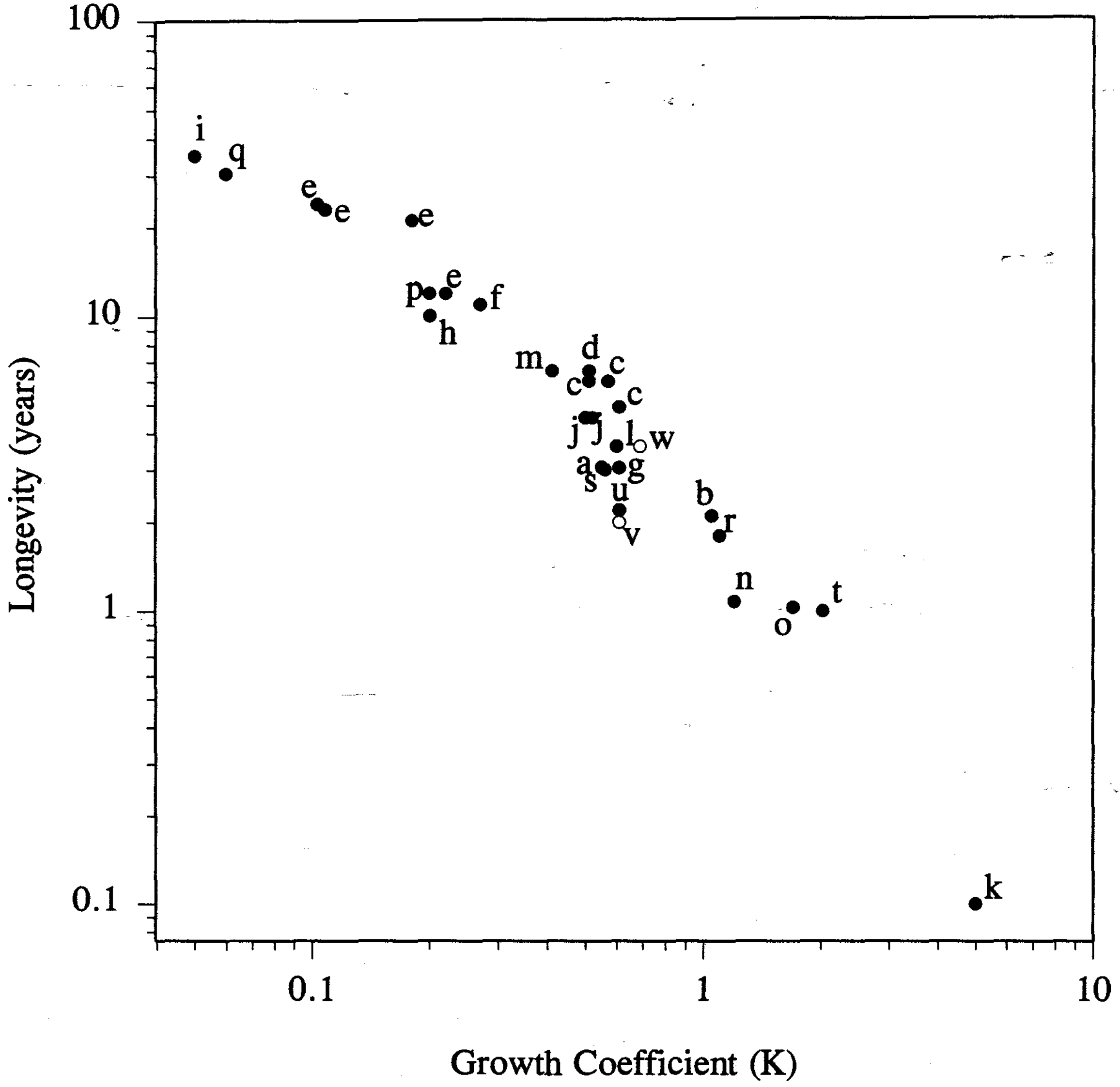


Figure 3.15

Correlation between longevity and growth coefficient in prosobranch limpets: a) *Patella compressa*; b) *P. barbara*; c) *P. granularis*; d) *P. granatina*; e) *P. cochlear*; f) *P. longicosta*; g) *P. oculus*; h) *P. vulgata*; i) *Nacella concinna*; j) *Collisella digitalis*; k) *Notoacmea insessa*; l) *C. strigatella*; m) *Notoacmea persona*; n) *Patelloida mimula*; o) *P. insignis*; p) *P. alticostata*; q) *Notoacmea petterdi*; r) *Cellana radiata*; s) *C. tramoserica*; t) *Helcion pellucidus* (after Branch, 1981, Pg 303); u) *P. aphanes* (Robson, 1986); v) *Helcion pectunculus*, west coast; w) *Helcion pectunculus*, east coast (present study).

consider food availability along with other environmental influences.

Helcion pectunculus live very high up on the rocky shore and thus experience extreme conditions both daily and seasonally. It is feasible that these conditions exert a high mortality upon individuals and have forced *H. pectunculus* to adapt its life history strategy accordingly. In growing at a fairly rapid rate in its first year the limpet attains sexual maturity quickly and so increases recruitment back into the environment, guaranteeing the continuation of the population. *Helcion pectunculus* lives to be about 3 years old, maturing between 10 to 12 months to become an active male and then a year later changing sex to become female. *Helcion pectunculus* exhibits two periods of spawning per year (chapter 4) and so a limpet will theoretically reproduce 4 times in a life time, twice as a male and twice as a female. However, the fact that *H. pectunculus* changes sex (chapter 4) complicates things somewhat in that the time period required to change from an active male to an active female is not yet known.

The microgrowth band analysis carried out on individuals from both east and west coast populations show that younger animals tend to be male whilst older animals are female. This hints towards *H. pectunculus* being a protandrous hermaphrodite, changing sex from male to female when it is between 2 and 3 years old (see chapter 4).

It has been suggested that changing sex from male to female is advantageous because larger females may hold more eggs (Hoagland, 1978; see chapter 4 for a review). However, from the curves obtained by plotting limpet frequency against age (figures 3.6, 3.7, 3.8 & 3.9) it becomes apparent that limpets very rarely survive to live beyond 4 years of age. This then becomes somewhat of a paradox; it is advantageous for females to be larger so that they may produce and release more eggs, however there is a greatly reduced chance that they will live to become female. *Helcion pectunculus* exhibits a 2:1 male to female sex ratio on most shores (chapter 2). The question then arises as to whether a greater number of smaller females would produce and release more eggs than fewer, larger females. Although this is a theoretical problem, it does require answering if we are to understand the complex interactions between growth and reproduction in protandrous molluscs.

3.5 Summary

1) *Helcion pectunculus* grows allometrically, increasing in height faster than it does length, which is expected of a high shore gastropod mollusc attempting to reduce evaporative water loss.
2) *H. pectunculus* lays down micro-growth bands within its shell which have the same periodicity as the tidal cycle. It is thus possible to count these bands and gain an estimate of the age of animals.
3) *H. pectunculus* grows at a similar rate on both the east coast and the west coast site at Bloubergstrand regardless of the differing oceanographic conditions. The theoretical values of L_{max} were similar being 30.86 mm for Bloubergstrand and 30.71 mm for Port Elizabeth.
4) Younger individuals tend to be male whilst older animals are female. This hints towards *H. pectunculus* being a protandrous hermaphrodite, changing sex from male to female when it is about 2 years old.

5) It is suggested that in future studies of limpet growth researchers consider food availability along with environmental influences.

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Chapter 4:

The reproductive biology of the high shore South African limpet, *Helcion pectunculus*.

4.1 Introduction

Most patellid limpets are dioecious broadcast spawners (Fretter, 1984). Mature gametes are released via the right renal duct into the mantle cavity and thence into the external environment where fertilization takes place (Webber, 1977). In patellids, the chances of successful fertilization are generally maximised by synchronization of gametogenesis within the population followed by the simultaneous release of gametes by males and females (Lasiak, 1990). In contrast, most acmaeids spawn almost continually (Fritchman 1961a & b, 1962a & b), although they may achieve local synchronization within a population (Creese & Ballantine, 1983).

Reproductive studies are well documented for several patellid and cellanid limpets. Gonad development has been described for numerous species of *Cellana* (Rao, 1973; Underwood, 1974; Kay & Magruder, 1977; Creese & Ballantine, 1983; Fletcher, 1984; Lasiak, 1987a & b, 1990; Catalan & Yamamoto, 1993; Liu, 1994), *Patella vulgata* and other North Atlantic species including *Helcion pellucidum* (Orton, 1920, 1928; Lebour, 1937; Orton *et al.*, 1956; Orton & Southward, 1961; Choquet, 1967; Blackmore, 1969; Bowman & Lewis, 1977; Bowman, 1985) and ten South African *Patella* species (Branch, 1974; Robson, 1986; Lasiak, 1987a). Most of these studies, which investigated seasonal variations in gonadal indices and histology of gonads, revealed that limpets have a marked annual reproductive cycle with definite spawning events.

Orton *et al.* (1956), who reported that *Patella vulgata* from various localities in Great Britain spawn in winter (October to March), considered wave-action and on-shore winds as potential factors triggering spawning. If this hypothesis holds true, then limpets on exposed and highly wind swept shores should exhibit highly cyclic periods of reproductive activity. In the Hawaiian limpet *Cellana exarata*, which does experience strong wave activity, spawning occurs from April to June and from September to November (Ito, 1981). Throughout each spawning season, a fraction of both sexes undergoes gametogenesis and/or spawning, and gametogenesis within the gonad of any individual is synchronous. Branch (1974) found that limpets on the west coast of South Africa, which also experience strong wave activity and periods of stormy weather, all exhibit marked reproductive cycles with fixed spawning periods. Creese and Ballantine (1983) reported that *Cellana radians*, inhabiting a sheltered reef, spawned at least three times a year whilst Lasiak (1987a) found that both *Cellana capensis* and *Patella concolor*, on the subtropical east coast of South Africa, exhibited protracted spawning periods with several peaks of spawning activity. Protracted spawning periods have been reported more frequently in acmaeid and fissurellid limpets (Ward, 1966; Underwood, 1974; Creese, 1980; Bretos *et al.*, 1983). All studies on the reproductive biology of limpets, to date, have been single biogeographic or single site studies. This is mainly due to the fact that many limpet species have a fairly narrow geographic distribution. Most studies on the reproductive biology of patellid limpets which inhabit the rocky shores of southern Africa have been carried out in the cooler waters surrounding the Cape Peninsula (Branch, 1974; 7 species), although a few studies have been carried out in the warmer waters of the east coast (Lasiak, 1987a, 1990; 2 species; Robson, 1986; 1 species).

The west and south/east coasts of South Africa have very different oceanographic conditions (Branch & Branch, 1981). The Agulhas Current, one of the most powerful currents in the world, sweeps warm water from the subtropics down the east coast. In the region of East London, the continental shelf widens so that the Agulhas Current is further off shore and the coastal waters become slightly cooler. Eventually the Agulhas Current swings back on its tracks to flow eastwards once more. Conditions on the west coast are quite different. It is chilled by the northward drifting cold water. Wind blows the surface waters offshore, and deep nutrient rich water upwells near the coast to replace it. Reaching the photic zone (sunlit waters), these nutrient rich waters fertilise phytoplankton. Both phytoplankton and macroalgae are far more productive on the west coast than on the south or east coasts. Despite the differing oceanographic conditions, many species inhabit both geographic regions (Branch *et al.*, 1994). It is possible that the different environmental conditions would influence the reproductive patterns of such organisms, but few studies have addressed this issue.

Helcion pectunculus has a very large geographic distribution, ranging from southern Natal up to the northern coast of Namibia (Kilburn & Rippey, 1982), making it a perfect model species in which to study possible intraspecific biogeographic differences in reproductive strategy. The study aimed to examine the reproductive biology of populations of *Helcion pectunculus* from both the east and west coasts of southern Africa in relation to possible external spawning stimuli and synchrony of reproductive cycles both within and between the populations.

4.2 Materials & Methods

4.2.1 General procedures

The reproductive cycles of *Helcion pectunculus* on both the east and west coasts of South Africa were studied for 24 months between August 1993 & August 1995 for the eastern coast population and for 18 months between January 1994 & August 1995 for the western coast population. The study sites, chosen for their large populations of *H. pectunculus*, were the Willows (west of Cape Receife) near Port Elizabeth (33° 58' S / 25° 38' E) and Bloubergstrand (33° 48' S / 18° 27' E) near Cape Town. All specimens were collected from crevices within the upper Balanoid zone. Fifty specimens were collected from each site, monthly, at full moon spring low tides. All animals from the east coast site were brought back to the laboratory, stored at 4°C and dissected within 48 hours. Those specimens from the west coast were stored in 4% formal-saline and transported to Grahamstown for dissection. Storage in formal-saline was not found to significantly affect dry weight gonad index ($p < 0.05$; two sample t-test between formal-saline stored limpets and fresh limpets). Shell length, width and height were measured to the nearest 0.01 mm with Vernier callipers and shell weight was measured to the nearest 0.001 g. Attempts were made to restrict the size range of limpets collected, thus avoiding any potential influence of size on the gonadal index. This, however, proved difficult due to the difference in size of limpets from the east and west coasts. Hence, limpets collected each month were limited to a minimum size of 20 mm from the east coast and 12 mm from the west coast. Limpets from both east and west coasts were collected from rock surfaces which had an east facing aspect due to known differences in foraging habits between east and west facing groups of *H. pectunculus* (chapter 5).

A variety of methods can be used to determine the reproductive cycle and spawning season of gastropods, and marine invertebrates in general (Giese, 1959; Giese & Pearse, 1974). Ideally, histological studies in conjunction with gonad index values are recommended. Both of these, together with several other parameters, were examined in this study.

4.2.2 Gonad index and other reproductive parameters

The mean monthly gonad index (GI) values for *Helcion pectunculus* from both the east and west coasts were calculated from 15 males and 15 females and are expressed as a percentage of gonad weight to entire body weight (excluding shell). The formula used is as follows:-

G.I. = ((dry gonad weight) / (dry gonad weight + dry body weight)) * 100

(after Gonor, 1972)

All masses were recorded to the nearest 0.001 g and dry masses were determined after drying to constant weight at 60°C.

4.2.3 Gametogenic cycle

Each month, from August 1994 to July 1995, for both Port Elizabeth and Cape Town populations, gonads from 5 males and 5 females were fixed for at least 24 hours in aqueous Bouin's fixative. After fixation, gonads were dehydrated using a graded ethanol series, embedded in Paraplast, and where possible, transverse sections (5-7 μm in thickness) were cut on a Leitz rotary microtome. Sections were then stained with haematoxylin and eosin (Humason, 1967) and histological slides prepared. Examination of the sections revealed stages of maturity, oogenesis and spermatogenesis.

Seasonal changes in the oocyte diameters and the thickness of the germinal developing layer in the gonad of the males were observed for each month from August 1994 to July 1995 for both sites. For oocyte diameters, oocytes were measured from histological slides using a Nikon compound microscope, fitted with a Nikon Filar optical Micrometer eye-piece. Data were collected from 5 females each month and the diameters of the first 50 oocytes encountered across the widest diameter of the ovary section were measured (250 oocytes/sample). The thickness of the developing layer (acini wall) present in the male gonads during the same time period was measured in a similar fashion from histological slides. Ten layers were chosen at random from 5 males each month and 5 measurements were made at intervals of 10 μm along each layer. A mean was then calculated by pooling the data from the five individuals for each month. Where necessary, slides were photographed with a Nikon Optiphot compound light microscope.

4.2.4 Sex ratios

Transects were carried out at various sites along the South African coast ranging from East London through to Bloubergstrand on the west coast (see chapter 2). At each site, individuals were sexed macroscopically and the ratio of males to females was calculated.

4.2.5 Size at first sexual maturity

The size at first sexual maturity was obtained by recording the percentage of individuals of *H. pectunculus* with mature gonads in 2 mm size classes. Ten individuals in each size class were collected during the peak reproductive period (= maximum gonad index) and examined macroscopically to determine sex. An individual was classed as being sexually mature if the gonad contained spermatozoa or vitellogenic oocytes. The size at which 50% of the individuals in a given size class were mature has previously been used as an index of first sexual maturity (Foster, 1994) and was adopted in this study.

4.2.6 Effect of number of foraging excursions on gonad index

It has been shown that individuals of *Helcion pectunculus* which inhabit west facing rock surfaces have two foraging excursions a day due to the fact that they are shaded from direct sunlight in the early morning (see chapter 5). Those animals inhabiting east facing rock surfaces only forage once. If algal biomass is the same on both east and west facing rock surfaces, those animals on west facing surfaces have a distinct advantage and would have a greater energy intake to those on east surfaces. It could therefore be hypothesized that animals from west facing rock surfaces have a greater reproductive output to limpets on east facing rock surfaces due to this extra energy intake. To test this hypothesis 300 individuals of similar size (20-22 mm shell length) from both east and west facing rock surfaces were collected from Port Elizabeth during a period whilst Gonad Index was increasing, their gonads removed and their Gonad Index calculated. The Gonad Indices obtained were then compared using an ANOVA.

4.2.7 Effect of boring algae on relative gonad size

It was observed that the shells of many specimens of *H. pectunculus* were severely damaged by a species of alga[1] which bores into the shell surface. In many instances the shells were very severely pitted and in extreme cases penetrated. To combat this "erosion", and to prevent penetration of the shell, the individual would have to continually lay down shell (Kohlmeyer, 1969; Bergman *et al.*, 1982; Webb & Korrubel, 1994). This process possibly carries an energetic cost and so would be detrimental to the individual, *e.g.* the reproductive output of the animal may suffer accordingly. To test this hypothesis 300 animals, of similar size (20-22 mm shell length), which had varying degrees of algal infestation on their shell surface were collected during the peak reproductive period, their gonads were removed and their Gonad Index values obtained. Algal infestation was measured using a scale of percentage infestation, that is the percentage of the shell surface covered by the alga. Thirty individuals were collected from each "infestation class" ranging from 0% up to 100%. These were compared using a two-way ANOVA to test for any significant effects of the algal infestation and limpet sex on Gonad Index.

4.2.8 Correlations between environmental conditions and reproductive seasonality

Daylength (at time of animal collection) was calculated from the sunrise and sunset times (S.A. Navy Tide Tables, 1993, 1994, 1995). Mean monthly sea temperature readings were obtained from the Port Elizabeth municipality who take daily readings at Humewood beaches, and Koeberg environmental centre, where readings were taken daily at Melkbosstrand which is close to the collection site at Bloubergstrand. These readings were taken to indicate annual sea temperature fluctuations at the two study sites which, in both cases, was only a few kilometres away. As it has been suggested that on shore wave action and storms may stimulate

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[1] Specimens of the alga have been sent to Prof. J. Bolton, University of Cape Town for identification.

spawning in intertidal limpets (Orton *et al.*, 1956), mean monthly wind speeds and directions were also collected from the respective meteorological offices.

4.2.9 Statistical Analysis

All statistical procedures were taken from Sokal and Rohlf (1981). Multiple-factor analysis of variance were used to determine whether the gonad index values of male and female *H. pectunculus* varied significantly over the study period and between east and west coasts.

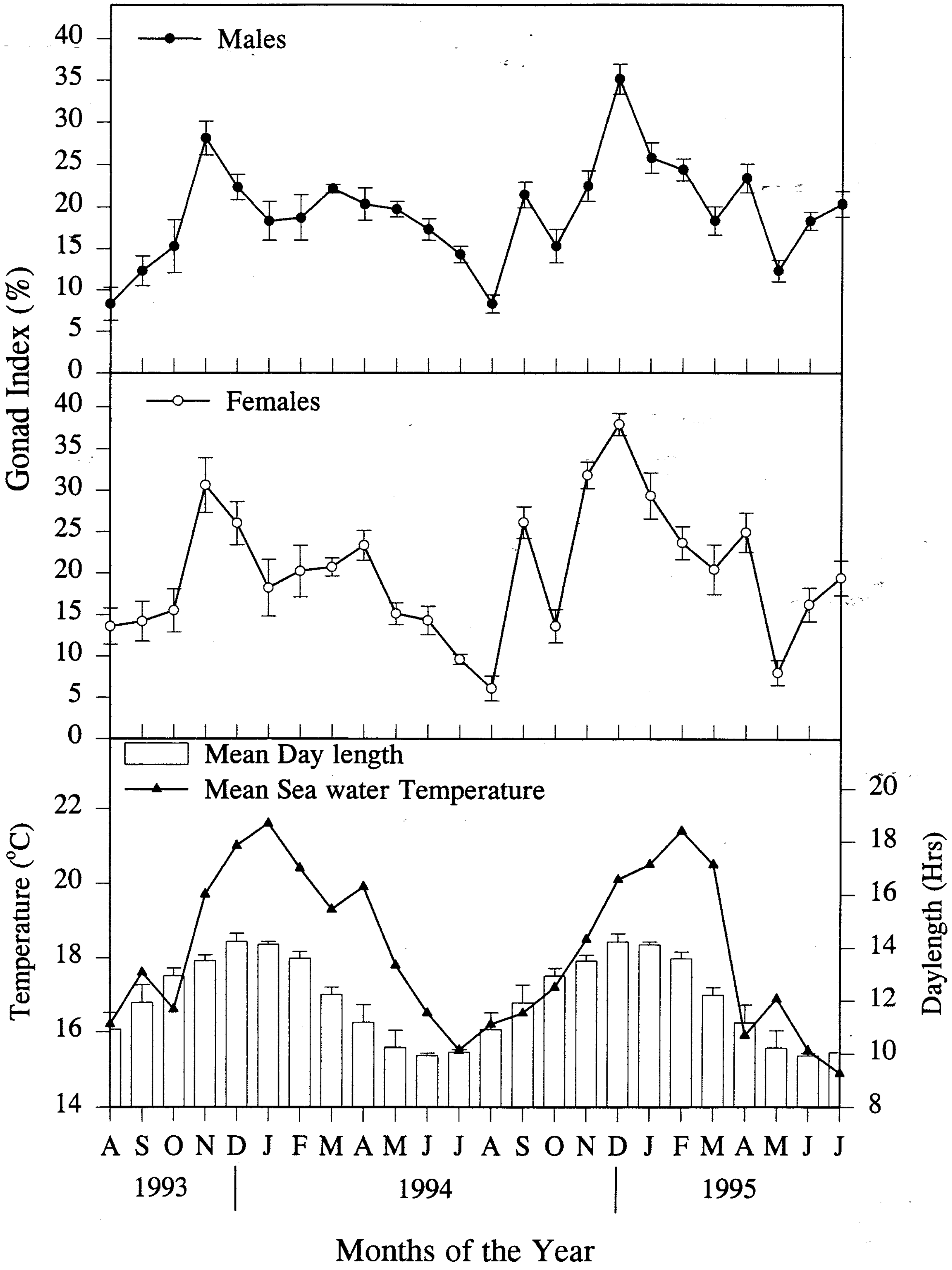
4.3 Results

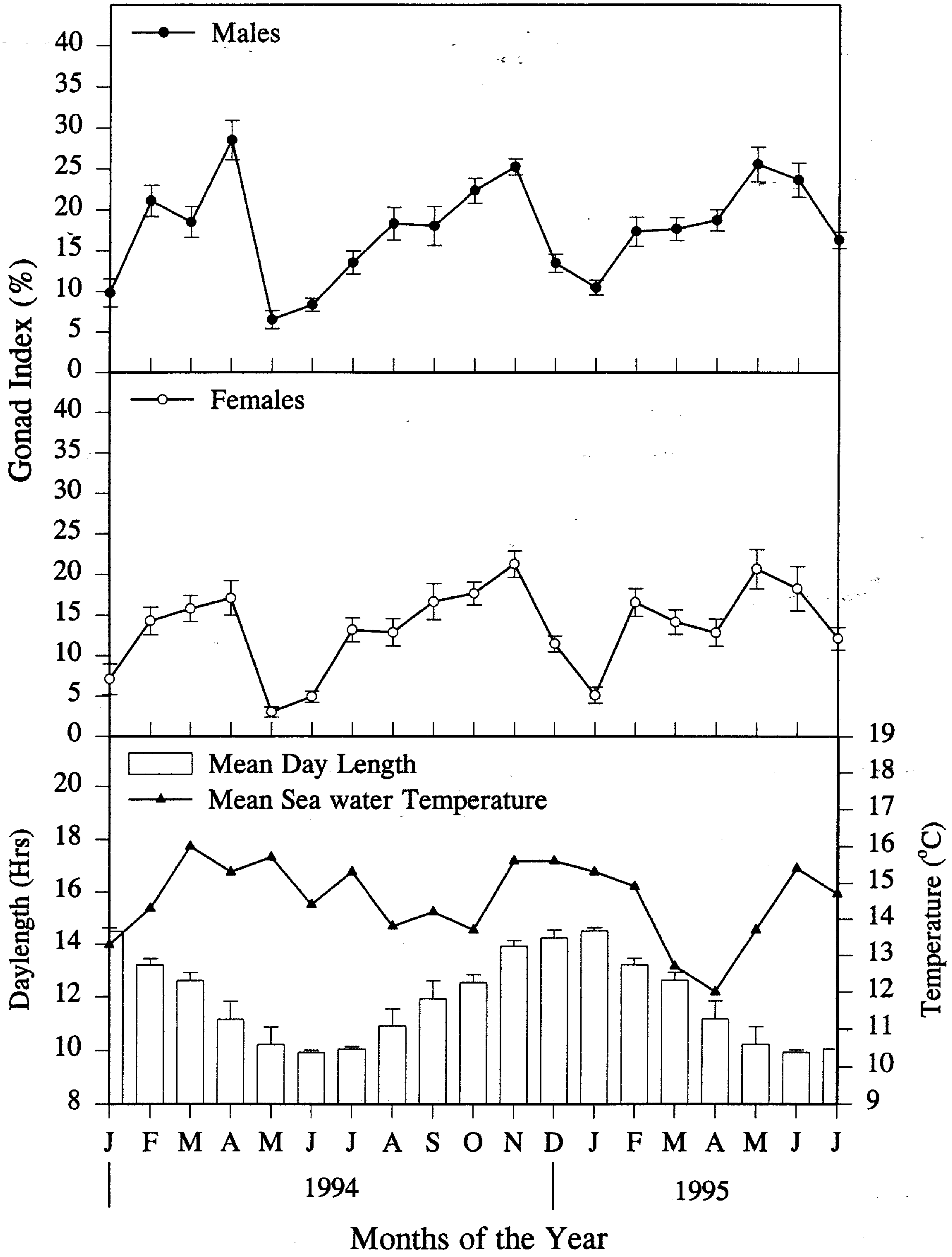
4.3.1 General gonad morphology

Males and females on both east and west coasts were always found to differ in size. Individuals of < 20 mm shell length were generally male, whilst limpets with a shell length of > 22 mm shell length were generally female (see chapter 3). In both sexes the gonad forms a discrete solid body which lies dorsally to the foot and below the visceral mass, its uppermost surface being interdigitated slightly with the digestive glands. The gonad of mature males are milky-white in colouration, whilst those of mature females are yellow.

4.3.2 Gonad index

In *H. pectunculus* from both east and west coast populations gonads were present throughout the year and showed an 8 and 6 fold increase and decrease in GI over time respectively (figures 4.1 & 4.2). There was no significant difference in GI values between males and females from Port Elizabeth over the entire study period. However, there was a


Males
Females
Gonad Index (%)
0
5
10
15
20
25
30
35
40
Mean Day length
Mean Sea water Temperature
Temperature (°C)
14
16
18
20
22
Daylength (Hrs)
8
10
12
14
16
18
20
A S O N D J F M A M J J A S O N D J F M A M J J
1993
1994
1995
Months of the Year


Males
Females
Gonad Index (%)
0
5
10
15
20
25
30
35
40
Mean Day Length
Mean Sea water Temperature
Daylength (Hrs)
8
10
12
14
16
18
20
Temperature (°C)
9
10
11
12
13
14
15
16
17
18
19
J F M A M J J A S O N D J F M A M J J
1994
1995
Months of the Year

significant difference between males and females from Cape Town ($F = 29.5$; $p < 0.000$) with the GI's of males always being approximately 5% greater (figure 4.2).

Table 4.1

Results of a two-way ANOVA on the effects of sex and location on Gonad Index of *Helcion pectunculus.*

Source of Variation	SS	df	MS	F	P value
Main effects:					
Sex	0.7789	3	0.2596	29.5	<0.0001
Location	0.0000	1	0.00	0.00	0.95
(No significant interactions)					
Residual	11.2389	1277	0.0088		
Total (corrected)	12.1556	1281			

Results of a multiple range analysis (Newman-Keuls) on the effect sex has on Gonad Index

West coast Females < West coast males = East coast males & females

For this reason the GI values for males and females from each site have been plotted separately. The Gonad Index values obtained from both Port Elizabeth and Cape Town were not found to differ significantly (F = 0.000; p = 0.995) *i.e.* location did not have a significant effect on Gonad Index.

The Gonad Index of *Helcion pectunculus* (male and female) from the east coast reached a maximum of 30-38% in November and December with smaller peaks (20-25%) occurring in April (figure 4.1). After April 1994, the Gonad Index then declined to less than 5% in August of that year. The west coast population also exhibited two peaks of Gonad Index a year, occurring in April 1994, November 1994 and April-May 1995 with males reaching maximum G.I.'s of 25-30% whilst females obtained maximum gonad indices of 17-20%. After each peak in Gonad Index, the gonad of both males and females declined rapidly to between 5 and 7% the following month (figure 4.2).

4.3.3 Gametogenic condition

The gametogenic (cycles) conditions of both the west and east coast populations mirrored that of Gonad Index (figures 4.1 & 4.2). The frequency of different size oocytes on a month by month basis for Port Elizabeth are shown in figure 4.3. Pre-vitellogenic eggs were always found to be present but large vitellogenic eggs (> 190 μm in diameter) were only present in large numbers during November/December. No further peaks of large vitellogenic eggs occurred between February and July. By contrast west coast females (figure 4.4) had two periods during which large numbers of mature oocytes were present, one in November/ December with newly developing eggs (pre-vitellogenic) appearing in January and another

Figure 4.3

The following two pages illustrate the frequency of different size oocytes from female individuals of *Helcion pectunculus* collected from Port Elizabeth on the eastern Cape coast on a month by month basis from August 1994 to July 1995.

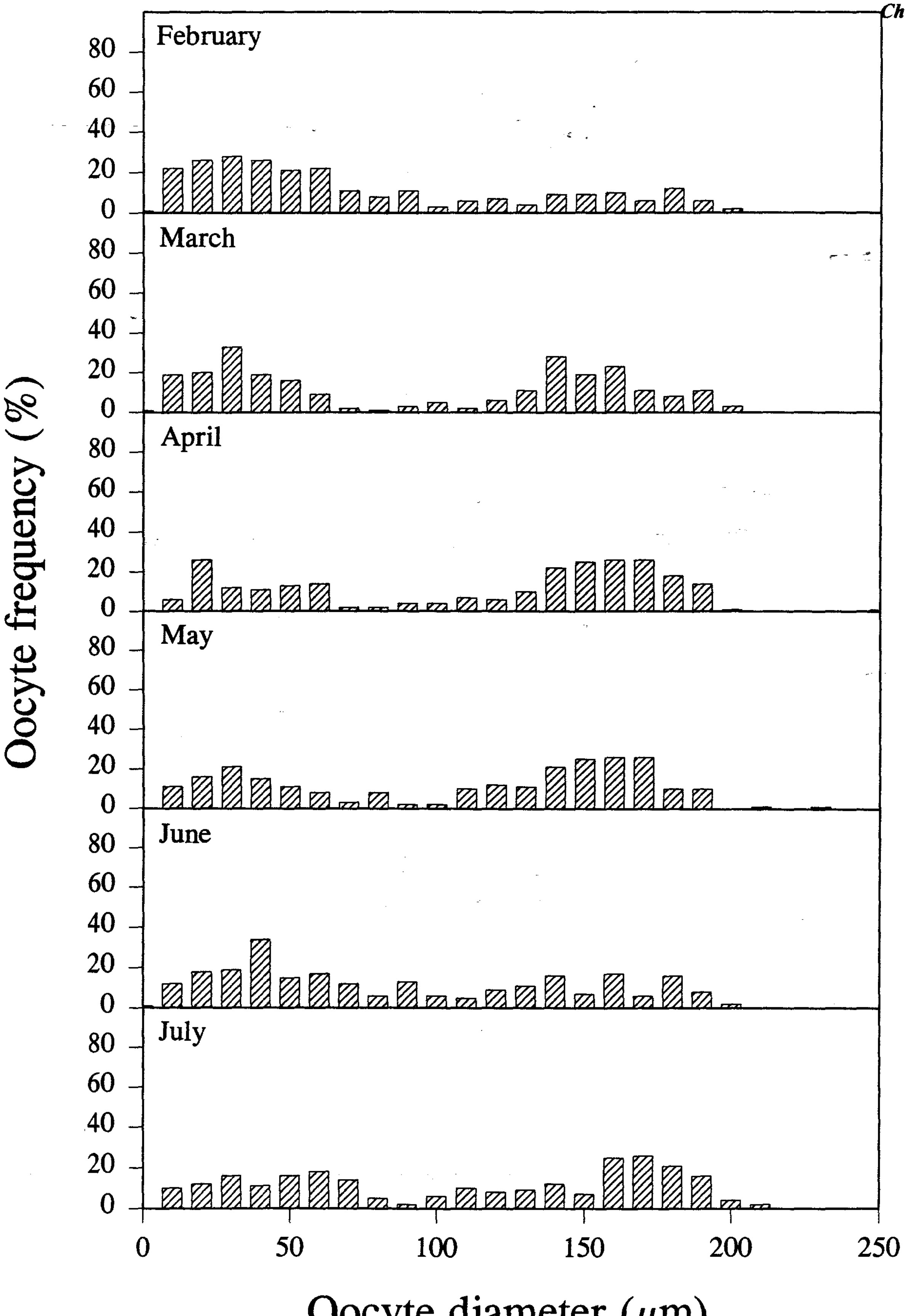
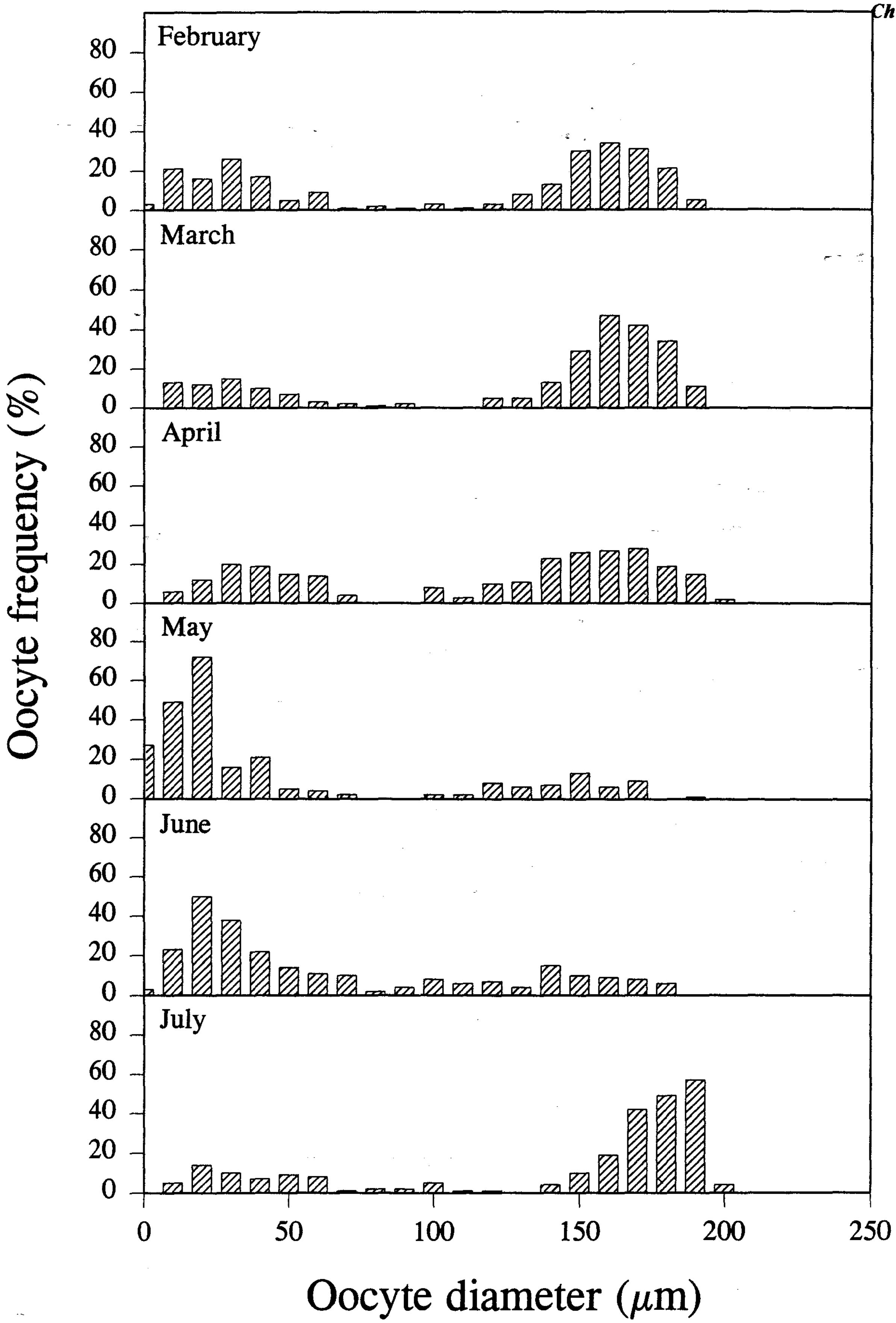

February
March
April
May
June
July
80
60
40
20
0
Oocyte frequency (%)
0
50
100
150
200
250
Oocyte diameter (μm)

Figure 4.4

The following two pages illustrate the frequency of different size oocytes from female individuals of *Helcion pectunculus* collected from Bloubergstrand on the west coast on a month by month basis from August 1994 to July 1995.


February
March
April
May
June
July
Oocyte frequency (%)
0
20
40
60
80
Oocyte diameter (μm)
0
50
100
150
200
250

peak in March with newly developing eggs appearing in May. In April 1994 (autumn) both populations exhibited a peak in GI which corresponds to their gonads being mature. The gonads of west coast females were packed with mature vitellogenic oocytes (figure 4.5) whilst the east coast females exhibited only a partial spawning (figure 4.6). By August (mid-winter) the GI values had fallen significantly ($p < 0.0001$) in the east coast population and the gonads contained large numbers of previtellogenic oocytes plus a few residual vitellogenic eggs (figure 4.7), signifying a spawning event had taken place. The west coast females reached their lowest GI value in May with large numbers of pre-vitellogenic eggs being present.

Males from both east and west coast populations were observed to have mature spermatozoa throughout the year, however spermatogenesis appeared to commence in April after the spawning event in February/March when the acini walls were very thin (30-40 µm thick) and their gonads still contain mature spermatozoa (figure 4.8). An increase in the thickness of the acini wall as early spermatogenesis occurred continued until August (figures 4.9, 4.10a & b).

A second peak in Gonad Index was also present in both coastal populations around the months of November/December. During these months, gonads from females from both populations were packed with mature vitellogenic eggs whilst the males possessed gonads with very thin acini walls (30-35 µm) and packed with mature spermatozoa (figure 4.11). Both populations then showed a decrease in GI values and by February both had spawned. Sperm is produced for the rest of the year.

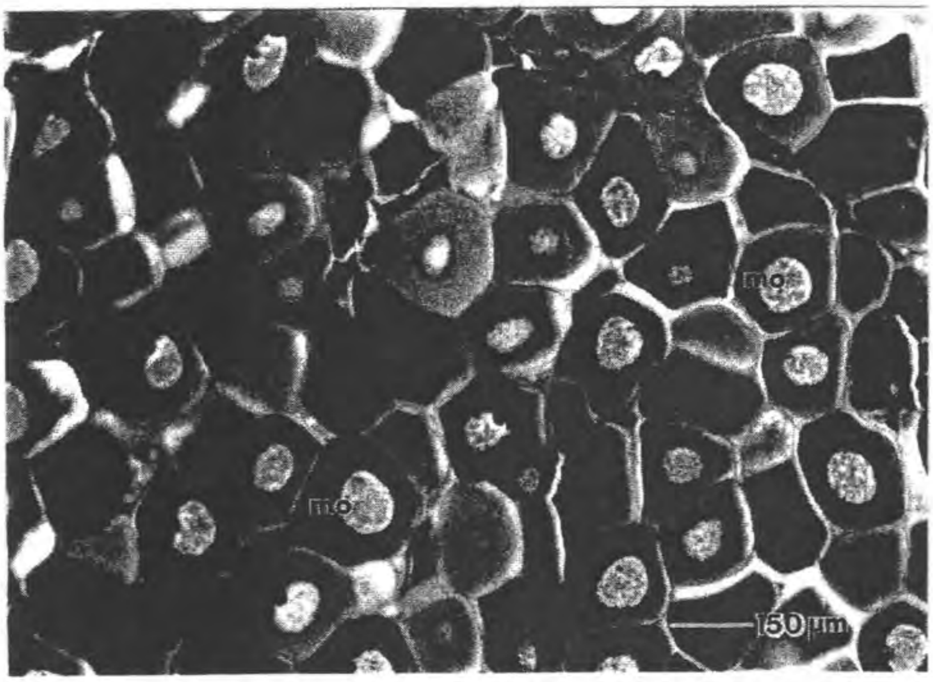


Figure 4.5

Transverse section through the gonad of a west coast female during the April period of maximum Gonad Index. (mo) Mature vitellogenic oocyte.

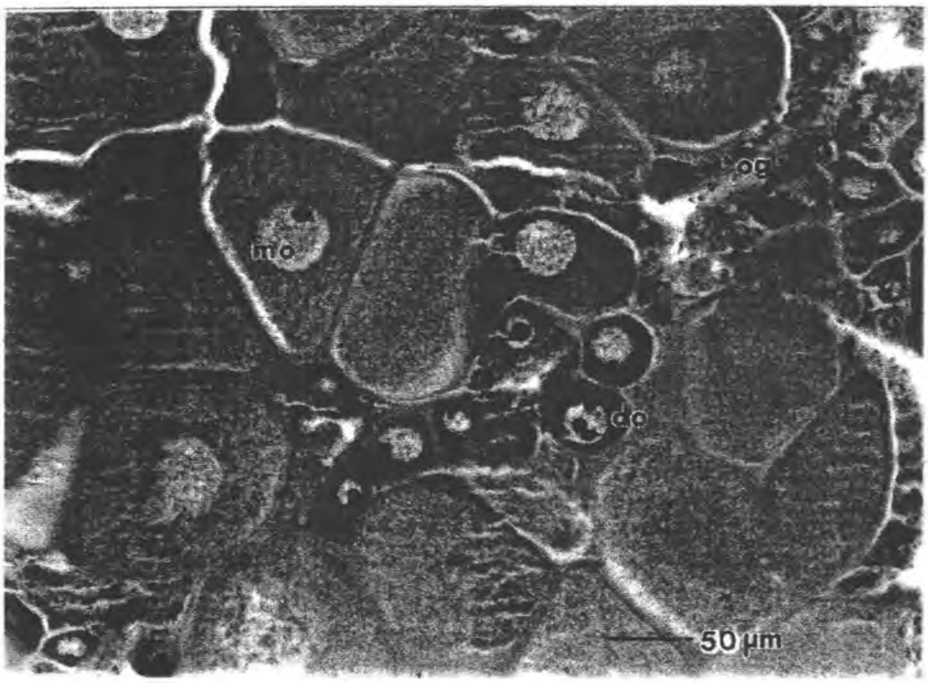


Figure 4.6

Transverse section through the gonad of an east coast female in May during the period of declining Gonad Index. (mo) Mature vitellogenic oocyte; (do) Developing oocyte; (og) Oogonia.

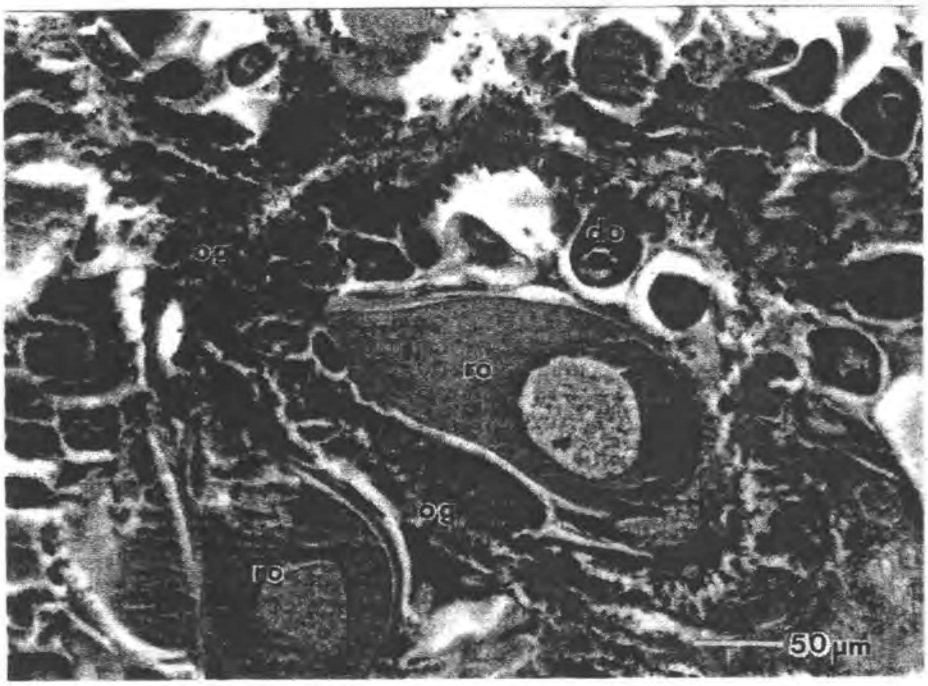


Figure 4.7

Transverse section through the gonad of an east coast female in August during the period of minimum Gonad Index. (ro) Residual vitellogenic oocyte; (do) Developing oocyte; (og) Oogonia.

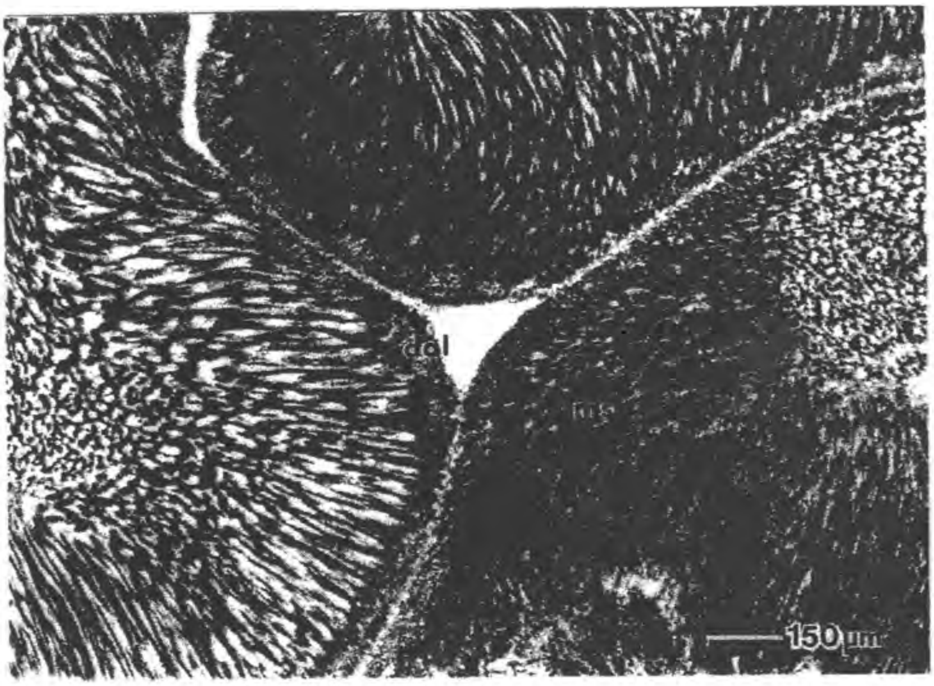


Figure 4.8

Transverse section through the gonad of a male in February as Gonad Index is increasing. (ms) Mature spermatozoa; (dal) developed acini layer.

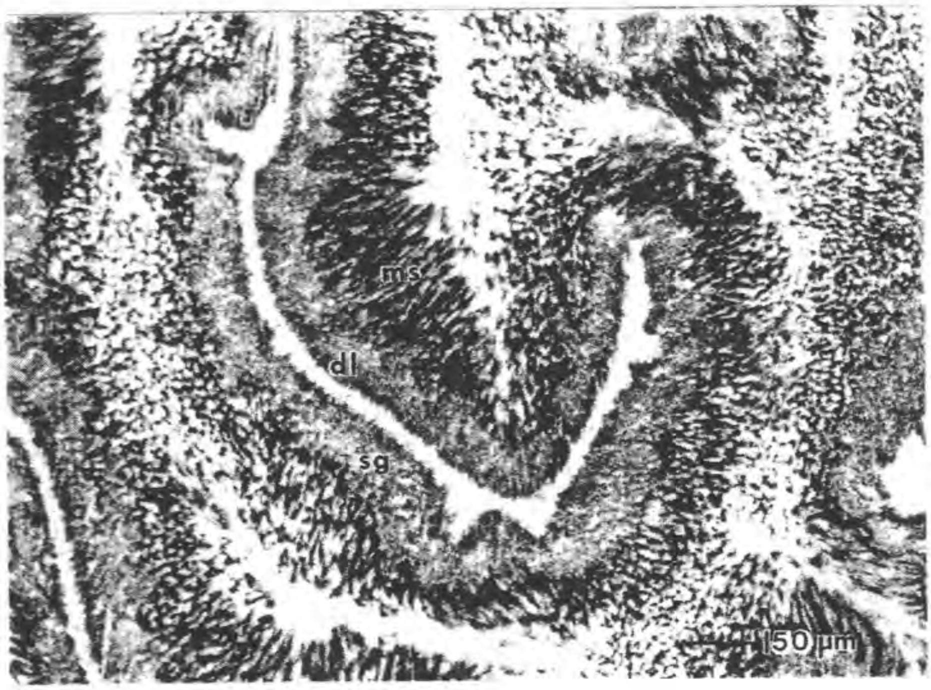
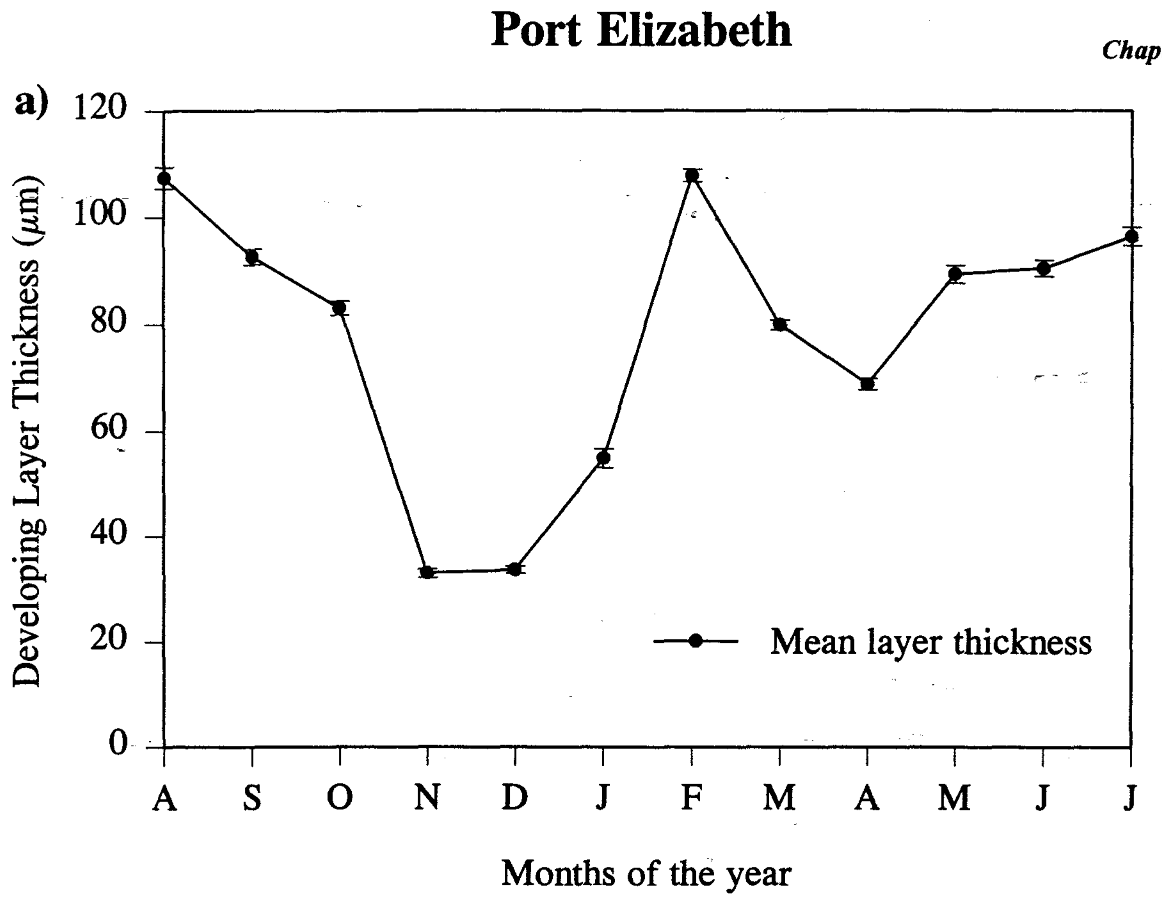
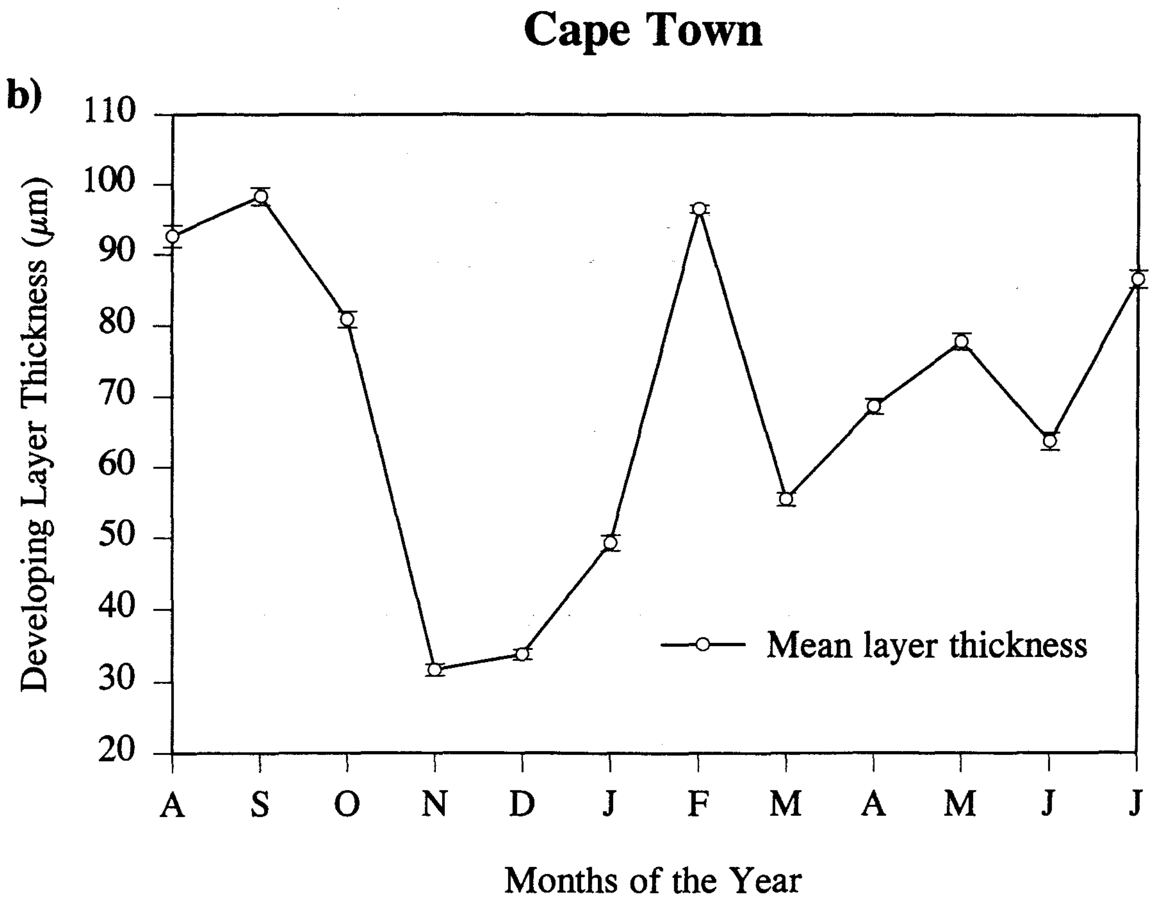


Figure 4.9

Transverse section through the gonad of a male in August during the period of minimum Gonad Index. (ms) Mature spermatozoa; (dl) Developing acini layer; (sg) Spermatogonia.



Port Elizabeth



Cape Town

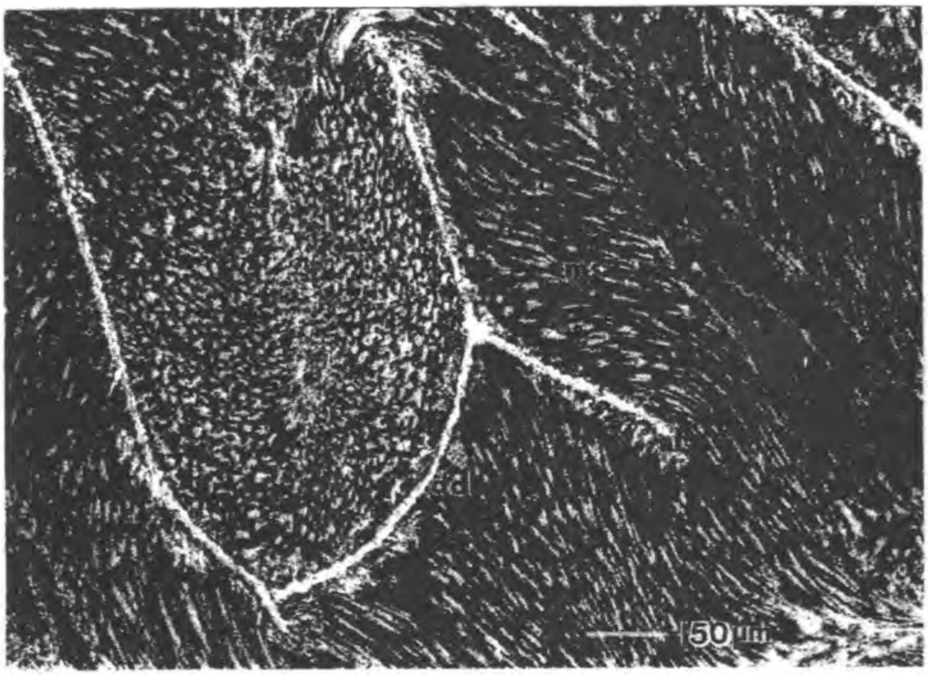


Figure 4.11

Transverse section through the gonad of a male in December during the period of maximum Gonad Index. (ms) Mature spermatozoa; (dal) Developed acini layer.

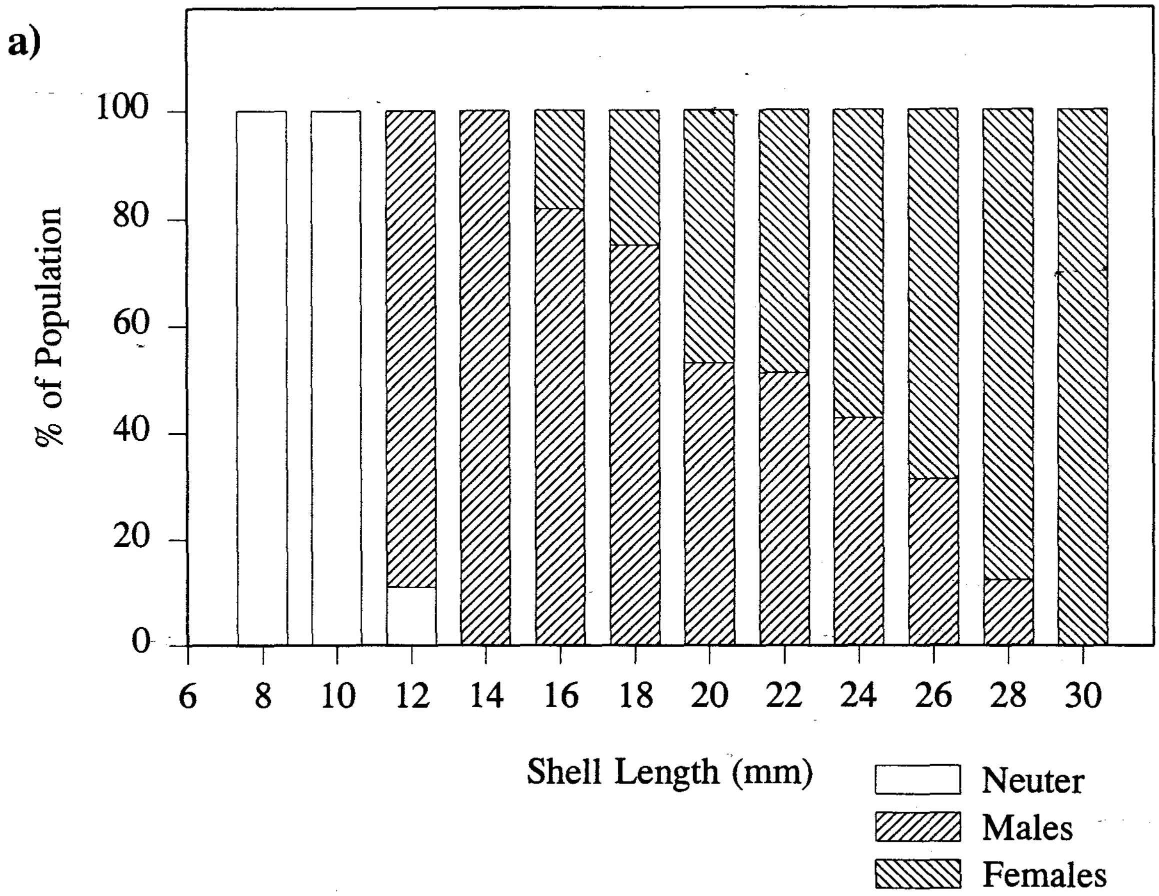
Another interesting point is the bimodal size frequency distribution of oocytes for both east and west coast populations. The first peak signifies eggs that have not undergone the process of vitellogenesis with vitellogenic eggs usually being > 120 µm in diameter (figures 4.3 & 4.4).

4.3.4 Size at first sexual maturity and hermaphroditism

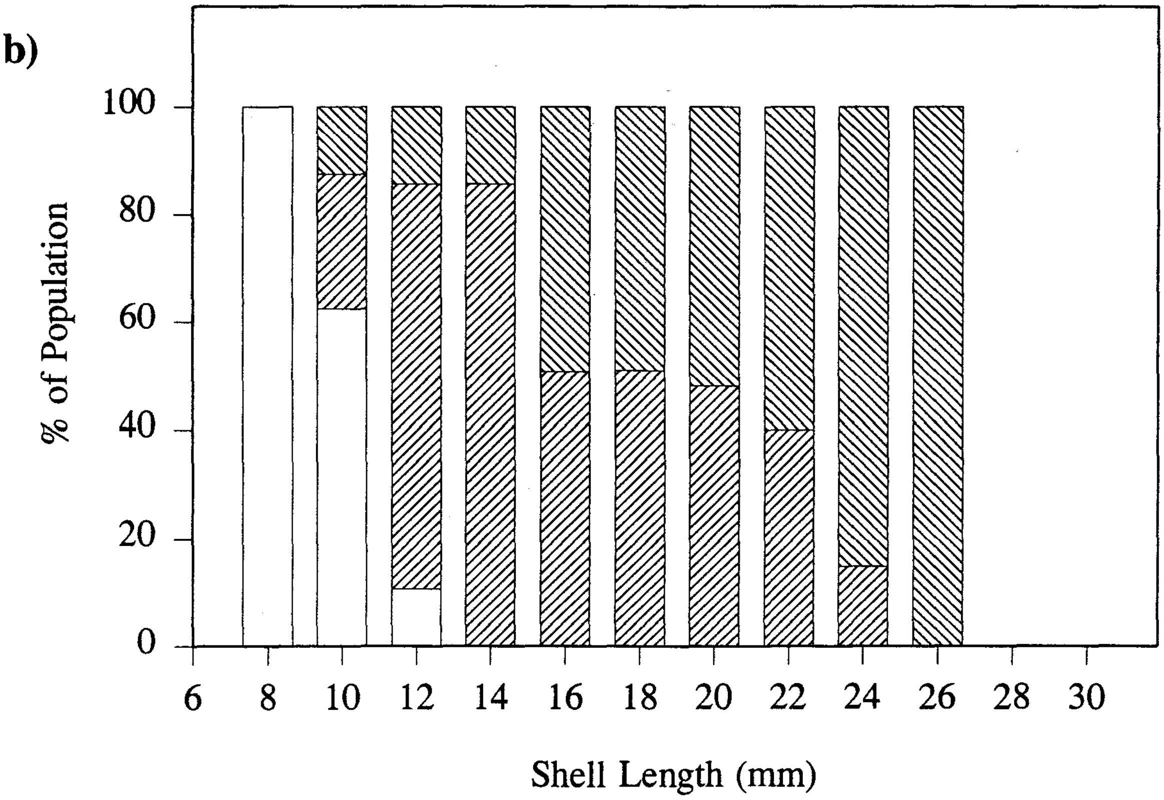
Analyses of the ratios of males to females within the populations at both Port Elizabeth (n = 283 limpets) and Bloubergstrand (n = 164 limpets) showed very similar trends. A 2:1 sex ratio of males to females was found for both west and east coast populations. Males and females were also found to differ in size with individuals of < 20 mm shell length generally being male whilst limpets with a shell length of > 22 mm were generally female (figure 4.12a & b). Some hermaphrodites were found to be present in the histological samples every month from both east and west coasts. These hermaphrodites had the external appearance of a male *i.e.* their gonads had a milky white colouration but histological examination revealed that they were hermaphrodites (figure 4.13a & b).

In both east and west coast populations, the onset of first sexual maturity occurred in individuals of about 10-12 mm in shell length (figure 4.12a & b) and all individuals below this size were identified as immature. Immature individuals lacked any visible gonad, even under microscopical examination. The suggested sex change seems to occur from about 16 mm shell length in individuals from the east coast whilst females were found to be present in the west coast population from about 12 mm shell length upwards.

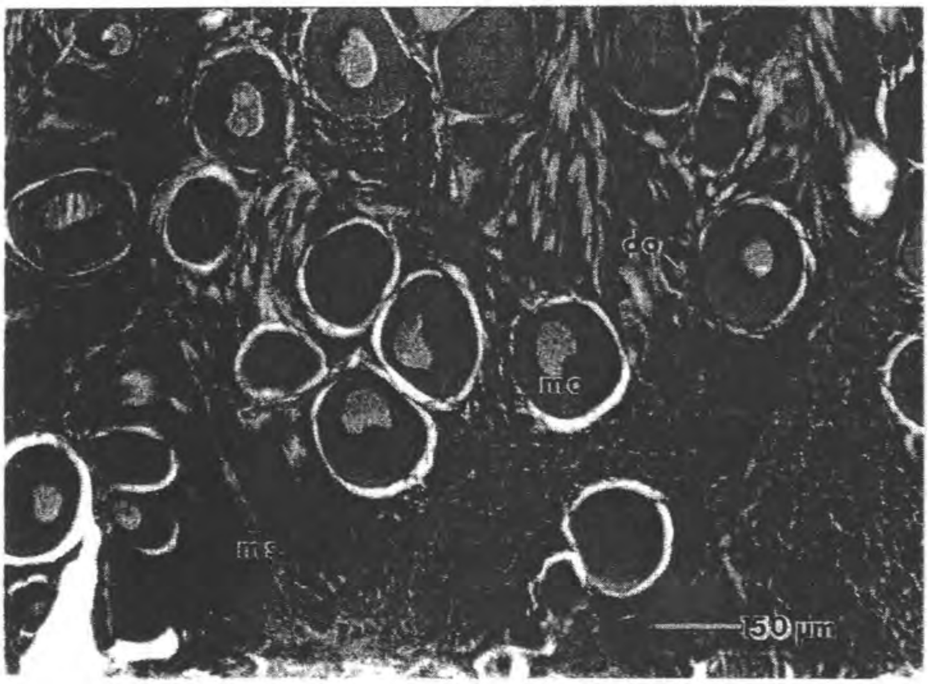
Port Elizabeth



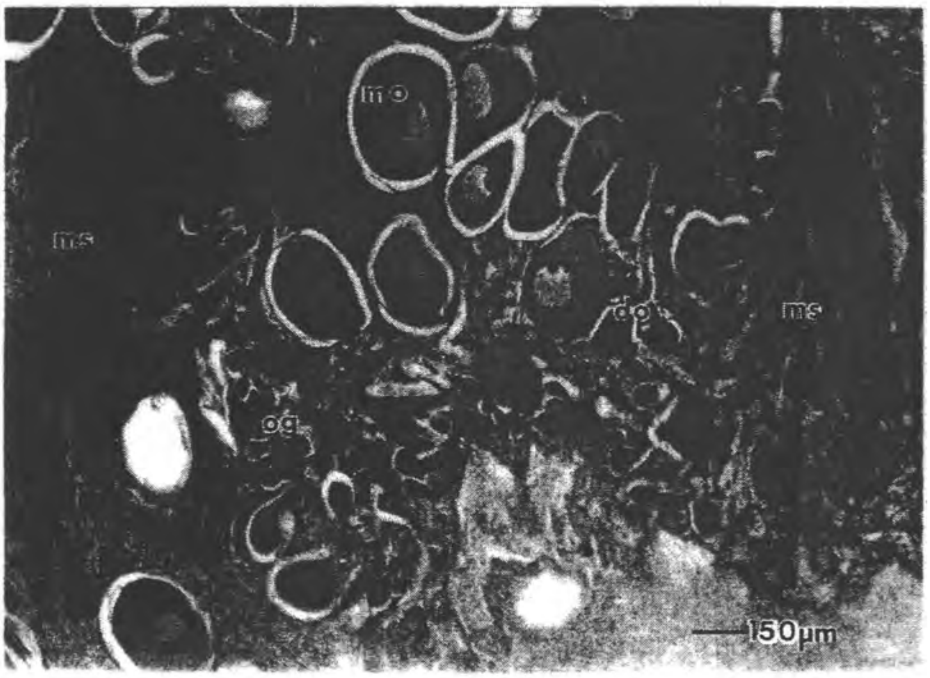
Cape Town



a)



b)



4.3.5 Effect of frequency of foraging excursions on gonad index

Limpets inhabiting west facing rock surfaces at Port Elizabeth were found to have significantly larger Gonad Indices (about 4% larger) than individuals of a similar size from east facing surfaces. The sex of the limpets did not, however, have any effect with the gonads of male and female individuals being of the same size (table 4.2).

Table 4.2

Results of a two-way ANOVA on the effect sex and rock aspect have on Gonad Index during the maximum Gonad Index period.

Source of variation	SS	df	MS	F-ratio	P
Main effects:					
Sex	0.0159	1	0.01598	1.938	0.1645
Rock aspect	0.2536	1	0.25360	30.73	10.0000
Residual	4.8606	589	0.00825		
Total (corrected)	5.1349	591			

East Facing Rock	<	West Facing Rock
13%	<	17%

4.3.6 Effect of boring algae on relative gonad size

The presence of a boring alga was found to have no significant effect on the gonad index *i.e.* the potential reproductive output of an individual which has the alga boring into its shell (mean G.I. = 28.5% ± 9.8%) does not differ significantly from animals whose shells are free of the alga (mean G.I. = 27.1% ± 10.7%) (table 4.3).

Table 4.3

Results of a two-way ANOVA to test the effects of sex and degree of algal infestation on gonad index in *Helcion pectunculus.*

Source of variation	SS	df	MS	F-ratio	P
Main effects:					
Algal infestation	0.14138	9	0.00942	1.114	0.3432
Sex of limpet	0.0106	1	0.01060	1.253	0.2640
Residual	2.32669	275	0.00846		
Total (corrected)	2.48010	291			

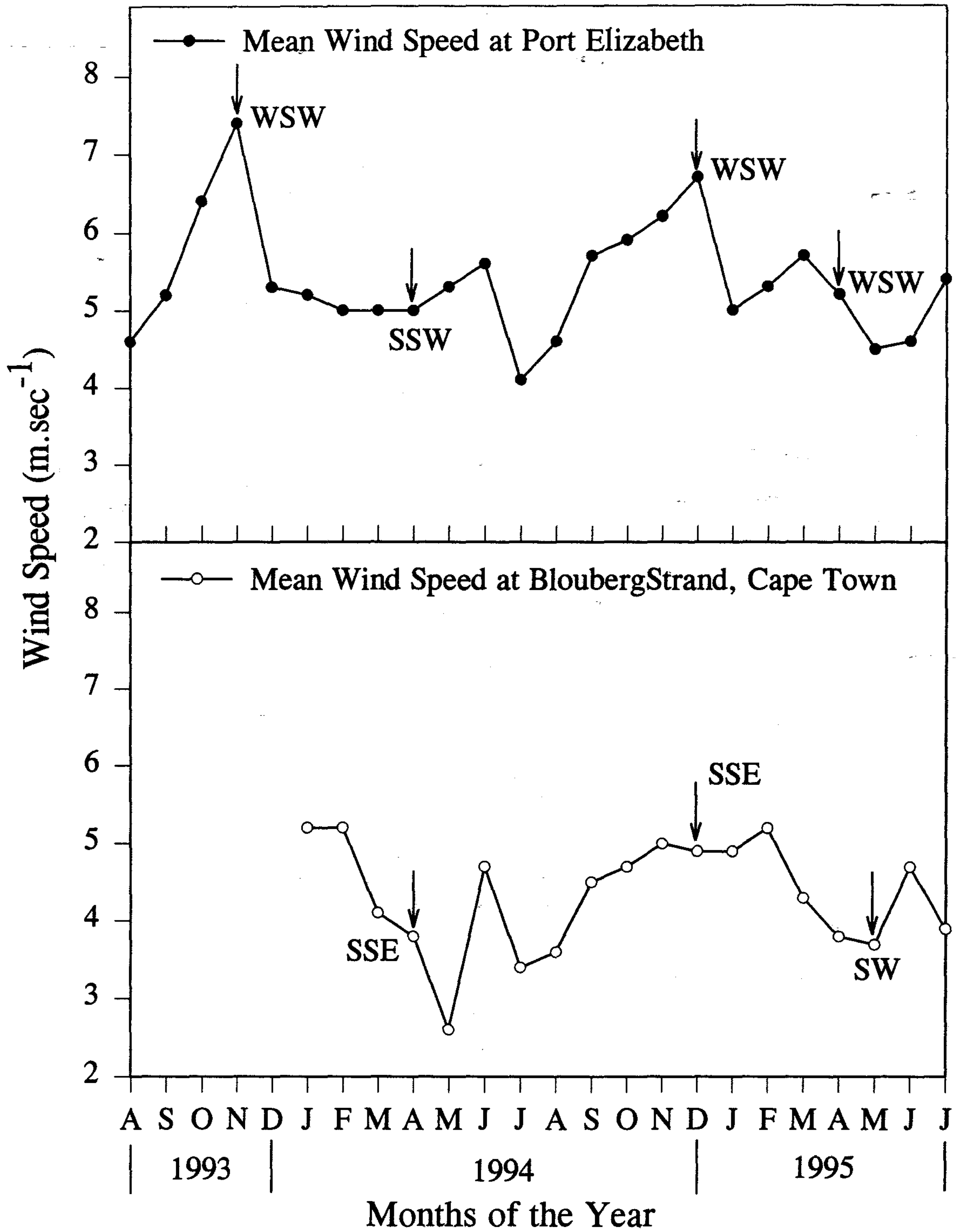
4.3.7 Environmental factors

The seasonal variations in sea temperature and daylength during the study periods for Port Elizabeth and Bloubergstrand are illustrated in figures 4.1 & 4.2 respectively. Maximum monthly mean sea temperatures of 21.6°C and 21.35°C were recorded at Port Elizabeth in January 1994 and February 1995, respectively, whilst at Bloubergstrand maximum monthly mean sea water temperatures of 16.0°C, 15.5°C and 15.4°C were recorded in March 1994, November/December 1994 and June 1995. Monthly mean sea water temperature minima at Port Elizabeth of 15.5°C and 14.8°C were recorded in July 1994 and July 1995 , respectively, whilst at Bloubergstrand minimum monthly mean sea water temperatures dropped to 13.7°C and 12.0°C in October 1994 and April 1995, respectively.

Maximum daylengths at Port Elizabeth were recorded in December for both years (1994 - 863 minutes, 1995 - 857 minutes), while at Bloubergstrand maximum daylength was in January 1995 (850 minutes). Minimum daylengths at Port Elizabeth were recorded in June for both years (1994 - 607 minutes, 1995 - 597 minutes). Bloubergstrand also experienced minimum daylength in June 1994 (also 607 minutes).

Maximum windspeeds at both sites were also collected and are illustrated in figure 4.14. Maximum windspeeds were recorded at Port Elizabeth during the months of November 1993 and December 1994. During these months the wind direction was predominantly onshore. At Bloubergstrand maximum onshore winds were recorded in January 1994 and February 1995.

No correlation was found between Gonad Index and sea water temperature at either Port Elizabeth ($r^2 = 0.14$) or Bloubergstrand ($r^2 = 0.09$), although spawning does occur as sea water temperatures are increasing. There was also no correlation between Gonad Index and


Mean Wind Speed at Port Elizabeth
WSW
WSW
WSW
SSW
Mean Wind Speed at BloubergStrand, Cape Town
SSE
SSE
SW
Wind Speed (m.sec^{-1})
2
3
4
5
6
7
8
A S O N D J F M A M J J A S O N D J F M A M J J
1993
1994
1995
Months of the Year

daylength for either population (east coast $r^2 = 0.11$; west coast $r^2 = 0.13$) although spawning again takes place whilst daylength is increasing. No relationship exists between wind speed and Gonad Index for either east or west coast populations (P.E. $r^2 = 0.08$; Bloubergstrand $r^2 = 0.03$) and spawning seems to take place during times of both high and low wind speeds. It therefore appears that in both coastal populations, spawning coincided with increasing sea temperature and daylength in both spring and Autumn seasons.

4.4 Discussion

The populations of *Helcion pectunculus* studied on the eastern Cape (Port Elizabeth) and western Cape (Bloubergstrand) coasts of South Africa exhibited strong sexual dimorphism with females generally having a larger shell than that of males, although males and females grow at the same rate (chapter 3). Differences between male and female prosobranchs of the same species are generally not pronounced (Webber, 1977) and this result appears somewhat as an anomaly. Both populations also exhibited a sex ratio of males to females of approximately 2:1. In a number of gastropod species, females have been reported to be more numerous than males (Fretter, 1984). Moore (1937) found differences in *Littorina* only in individuals having a shell length greater than 25 mm. Smaller animals had a ratio of 1:1. Moore (1937) suggested that the frequency difference in larger animals may be due to a more rapid rate of growth in female individuals. Although another hypothesis is that individuals are changing sex from male to female after reaching 25 mm shell length. Branch (1974) found that females of *Patella oculus* were very much larger than males and were much less common. In fact the scarcity of females in the population resulted in inadequate sample sizes during gravimetric analysis. *Patella aphanes* also exhibits a similar distribution of sexes (2 males : 1 female) within populations (Robson, 1986) with small individuals predominantly being neuter, medium sized individuals being male, whilst only the largest of limpets were female.

Differences between the sexes in average size is more common (Heller, 1993). In species exhibiting size dimorphism, most often females are larger than males, although Edwards (1968) found males of *Olivella biplicata* larger than females. In *Strombus spp.* (Abbott, 1960) females were found to grow faster than males, and this results in the size difference. Chi and Wagner (1957) found equal growth rates for small *Oncomelania spp.* but, after approximately 11 weeks, females grew more rapidly than males. In *Tegula funebralis*, Paine (1969) felt that the preponderance of females was not due to differential growth but instead to a selective predation on males. Again another possibility is that individuals start out life as a male and then, at a predetermined size, change sex to become female, *i.e.* are protandric hermaphrodites. This certainly appears to be the case for *Helcion pectunculus*.

Protandric hermaphrodites may occur in only some species of a genus (Webber, 1977). In *Patella*, *P. vulgata* (Orton, 1920, 1928; Das & Seshappa, 1948; Dodd, 1956; Orton *et al.*, 1956; Choquet, 1966), *P. caerulea* (Bacci, 1953), *P. oculus* (Branch, 1974) and *P. aphanes* (Robson, 1986) are all protandric hermaphrodites, while *P. aspera* and *P. depressa* (Dodd, 1956) are generally dioecious, although a proportion of the population do tend to change sex. As far as the acmaeids are concerned very few species have been found to be hermaphroditic. *Acmaea fragilis* exhibits protandric hermaphroditism (Fretter, 1984) whilst in a study of 11 *Acmaea* species, Fritchman (1961a, b, 1962a, b) found no evidence of a sex change in any of the species. Among the South African limpets, only *Patella oculus*, *P. aphanes* and *Helcion pectunculus* undergo protandric sex change. This form of hermaphroditism has been suggested to be advantageous to the animal with it being an advantage for females to be larger in size, as mature eggs take up much more room than spermatozoa. This mode of reproduction has been discussed in detail by Hoagland (1978) and has been termed the "size advantage hypothesis". In the case of *H. pectunculus* it seems that any "size advantage" may well be offset by the reduced numbers of females present in the population.

As in other invertebrates, the reproductive season of limpets is related to the geographic origin of the species and its specific reproductive strategy. In general, those of low latitudes have an extended season, and those of high latitudes have a restricted one (Fretter, 1984). South Africa is somewhat unique in the fact that it has remarkably different oceanographic conditions on its west and south/east coasts and yet many species inhabit both geographic regions including *Helcion pectunculus*. Few studies have addressed the issue of comparing reproductive cycles within a species under different environmental conditions within such a relatively short distance of coastline.

Helcion pectunculus, as with most patellids (Creese & Ballantine, 1983), has a marked reproductive cycle. It exhibits two spawning events a year, one in April and another in November/December. The Gonad Index shows a well-defined annual cycle for both sexes from both east and west coasts. The greatest abundance of mature gametes coincides with the greatest GI values. There is also a high degree of synchrony between the sexes. Previous studies on the reproductive biology of South African patellid limpets have been restricted to animals from the cooler waters of the western cape (Branch, 1974), although Lasiak (1987a) has carried out a study of *Patella concolor* occurring in the subtropical waters of the east coast and Robson (1986) studied *P. aphanes* occurring in mussel beds on the coast of Natal (table 4.4). Limpet species occurring in the cooler waters of the west coast exhibit one spawning period a year whilst those species studied on the warmer east coast exhibit two spawning events. It is possible that the environmental conditions on the east coast fluctuate to a far lesser degree than on the west coast and so limpets take advantage of this by spawning twice a year.

From both GI values and detailed histological examination it appears as though *Helcion pectunculus* has similar reproductive patterns on both east and west coasts despite the different environmental conditions. However, although the fundamental pattern is similar there does appear to be a degree of modification *e.g.* the autumn peak in GI was found to be suppressed in the population occurring on the east coast. The conditions under which this particular reproductive pattern evolved are not known, but the fact that both east and west coast populations exhibit the same fundamental pattern suggests that this reproductive pattern is now phylogenetically constrained. This is also thought to be the case for some deep sea molluscs (Eckelbarger & Watling, 1995) which, although unaffected by seasonal environmental fluctuations, still maintain a cyclical reproductive pattern similar to those exhibited by their shallow sea counterparts.

A variety of factors have been reported to trigger spawning of gametes in gastropod molluscs including temperature, mechanical stimulation, genetic and hormonal factors

Table 4.4

Summary of records of spawning periods of molluscs along the South African coast.

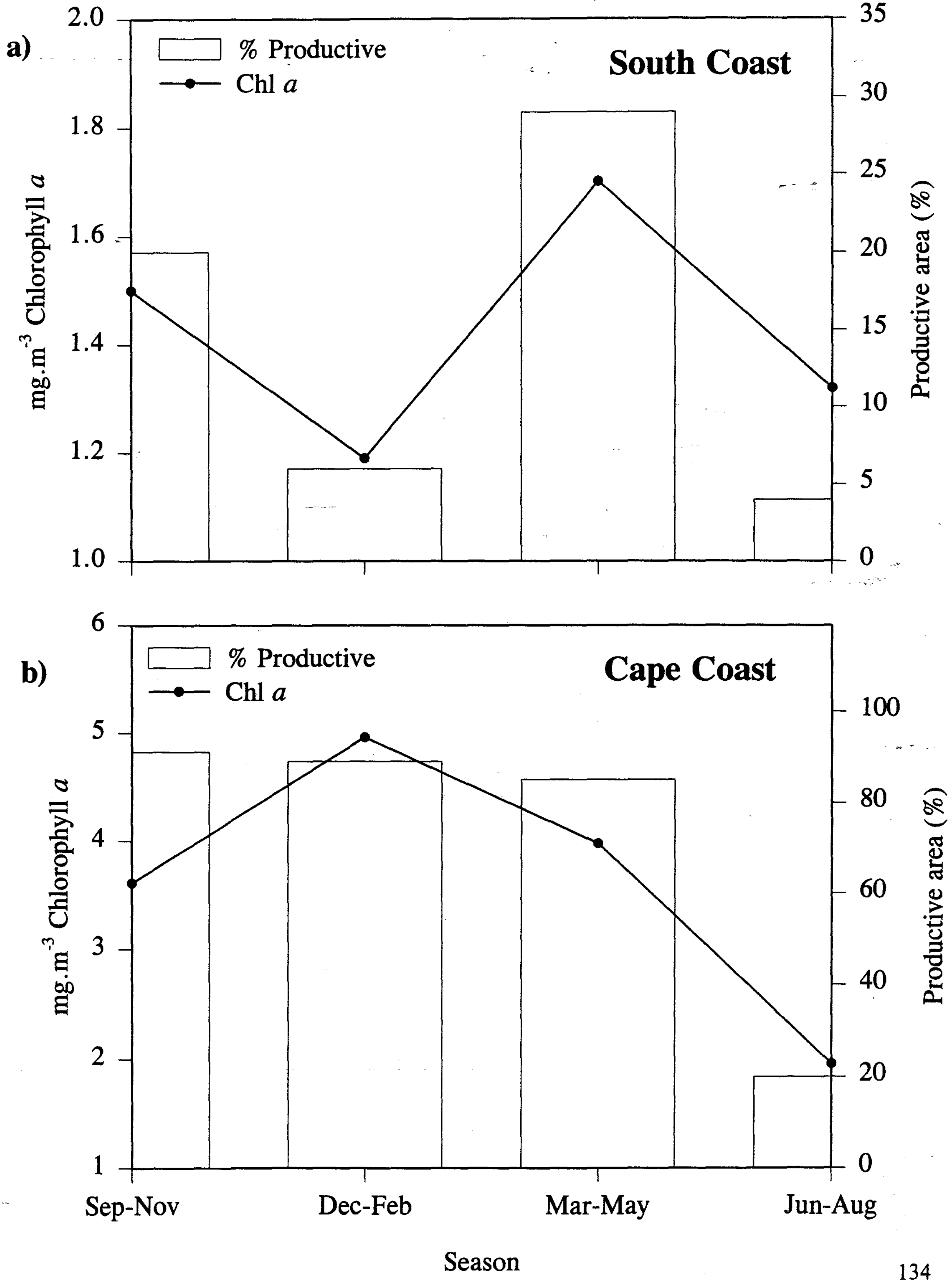
Species	Location	Spawning period	Reference
Limpets:			
Patella concolor	Transkei	Sept-Nov / Feb-Mar	Lasiak, 1987a
P. aphanes	Natal	Jan-Feb / Apr-Jun	Robson, 1986
P. argenvillei	Kommetjie	May-June	Branch, 1974
P. barbara	Kommetjie	May-June	Branch, 1974
P. granatina	Kommetjie	May-June	Branch, 1974
P. oculus	Kalk Bay	September	Branch, 1974
P. longicosta	Kalk Bay	Oct-Nov	Branch, 1974
P. granularis	Komme/Kalk Bay	May-June	Branch, 1974
P. cochlear	Komme/Kalk Bay	May-June	Branch, 1974
Helcion pectunculus	Bloubergstrand	Apr-May / Nov-Dec	Gray, 1996
	Port Elizabeth	Apr-May / Nov-Dec	Gray, 1996
Cellana capensis	Transkei	Sept-Oct / Feb-Apr	Lasiak, 1987a
Siphonaria concinna	Waterloo Bay	Nov-Dec	Chambers, 1994
Other Gastropods:			
Oxystele tabularis	Transkei	continuous	Lasiak, 1987b
O. variegata	Transkei	continuous	Lasiak, 1987b
Turbo coronatus	Transkei	Dec-Feb	Lasiak, 1986
Littorina kraussi	Transkei	Dec-Mar	Lasiak, 1987c

(Orton, 1920; Linke, 1933; Gabe, 1951, 1953; Hancock, 1960; Kessel, 1964; Berry & Chew, 1973; Alifierakis & Berry, 1980), as well as combinations of these factors (Fretter, 1984). Although no correlation between GI and temperature was found in the present study, spawning in *H. pectunculus* on both east and west coast populations occurred as both sea water temperature and daylength were on the increase indicating that an increase in sea water temperature may initiate spawning. However, if temperature varies seasonally, it is almost inevitable that breeding can be associated with some change of temperature, and other cues may be overlooked (Grahame & Branch, 1985).

Previous studies have observed patellid limpets to spawn after being stimulated mechanically by wave action during stormy weather (Branch, 1974; Catalan & Yamamoto, 1993). Branch (1974) emphasized the advantages of spawning during storms, since these reduce desiccation of newly settled limpets, and accompanying onshore winds may prevent larvae from being washed out to sea. Unfortunately there is no information on the length of the limpet larval phase at present. Spawning events in *H. pectunculus* do seem to be correlated to wind activity to a degree. The spawning event during November/December on both coasts occurs at a time when wind speeds are at their greatest and in an onshore direction. This is also the time at which most storms take place. However, the hypothesis does not explain the second pronounced peak of reproductive activity in April on both coasts, indicating that mechanical stimulation may play a part in the onset of spawning, as well as an increase in sea temperature but they are not the primary driving factors. Perhaps food availability for larvae, whilst in the water column, may be a more important cue than temperature (Grahame & Branch, 1985).

Himmelman (1975, 1979) has shown that a number of invertebrates spawn at the time of the spring phytoplankton bloom to allow their larvae to capitalize on this rich, but transient food source. When transferred to the laboratory, the animals failed to spawn in spite of both temperature and light conditions being manipulated. Only when phytoplankton was introduced did spawning immediately follow. *Helcion pectunculus* exhibits spawning events in both spring and autumn. In a study of chlorophyll distribution in the photic zone of inshore waters around the South African coastline, Brown (1992) found the highest mean concentrations of chlorophyll *a* along the south coast to be in Spring and Autumn, with patches of 2-4 $mg.m^{-3}$ being fairly widespread. Whilst on the west coast high values were obtained for Spring, Summer and Autumn with chlorophyll *a* levels falling in winter (see figure 4.15). This then suggests that *H. pectunculus* has evolved a reproductive cycle which will allow its planktonic larvae to utilise the valuable phytoplankton bloom food source, whilst using high onshore winds to ensure that larvae are not transported out to sea and lost. This hypothesis also explains the east/west coast divide which is present in the reproductive strategies of limpets along the South African coast. The east/south coast only experiences high concentrations of phytoplankton twice a year (Spring and Autumn) and it is during these periods that spawning in east/south coast species occurs. The west coast experiences high levels of phytoplankton for most of the year but physical factors such as wave activity and temperature may well be the limiting factors forcing the limpets into only one spawning event.

Histological examination of the gonads of female individuals from both east and west coast populations of *H. pectunculus* show a bimodal size distribution of oocytes within the ovary (figures 4.3, 4.4, 4.5 & 4.6). Detailed histological studies of other prosobranchs (*e.g.*


a)
% Productive
Chl *a*
South Coast
mg.m^{-3} Chlorophyll *a*
2.0
1.8
1.6
1.4
1.2
1.0
Productive area (%)
35
30
25
20
15
10
5
0
b)
% Productive
Chl *a*
Cape Coast
mg.m^{-3} Chlorophyll *a*
6
5
4
3
2
1
Productive area (%)
100
80
60
40
20
0
Sep-Nov
Dec-Feb
Mar-May
Jun-Aug
Season

Creese, 1980; Garwood & Kendall, 1985) suggest that as the gametogenic cycle progresses, oocyte growth results in the establishment of a bimodal distribution of oocyte sizes within the ovary. This is followed by a gradual increase in peak size of the larger mode as more and more immature oocytes undergo vitellogenesis. This gradual increase in the size of the second mode was not observed in *H. pectunculus*. Instead, oocytes appear to undergo rapid vitellogenesis with the second peak becoming large and spawning occurring within the space of a couple of months. A detailed cytological investigation is required to examine this phenomenon further.

The presence of a microalga boring into the shell of *Helcion pectunculus* does not have a significant effect on reproductive output. Many studies have been carried out on species which infest and weaken the shells or outer casing of other animals (see chapter 7 for a review) but none of these examined the effect the infesting species had on the reproductive output of the organism concerned. Bergman *et al.* (1982) suggested that high infestations of *Polydora ciliata* weaken the shells of the sea scallop, *Pactopecten magellanicus*, making them more vulnerable to predation by predatory decapods. The alga found on the shell of *H. pectunculus* also substantially weakens the shell (pers. obs) but no effect on reproductive output was found.

Finally, the number of foraging excursions carried out by individuals of *H.pectunculus* was found to have a significant effect on GI and hence potential reproductive output. Animals inhabiting west facing rock surfaces were able to carry out two foraging excursions to every one carried out by limpets on east facing rock surfaces. This extra energy intake obviously gives them an added advantage and it appears that much of the extra energy is placed into gamete production. No studies, to date, have compared reproductive output on such a small scale and it is suggested that this phenomenon may not be limited to *H. pectunculus* and that other neighbouring grazing species which are open to the same external factors may also be utilizing extra potential foraging excursions for energy gain and hence greater reproductive output.

4.5 Summary

1) The populations of *H. pectunculus* from both the east and west coasts of South Africa exhibited strong sexual dimorphism with females generally having a larger shell than males. Both populations also exhibited a sex ratio of 2 males to 1 female.
2) Histological examination proves unequivocally that *H. pectunculus* undergoes a protandric sex change, changing from male to female when they are about 2 years old.
3) *Helcion pectunculus* from both east and west coasts have a marked reproductive cycle. It exhibits two spawning periods a year, one in April and another in November, with a high degree of synchrony between the sexes. It is suggested that the reproductive pattern exhibited is now phylogenetically constrained.
4) *Helcion pectunculus* has evolved a reproductive cycle which, it is suggested, will allow its planktonic larvae to utilise the valuable phytoplankton bloom source whilst using onshore winds to ensure that larvae are not transported out to sea and lost.
5) The number of foraging excursions carried out by individuals of *H. pectunculus* was found to have a significant effect on Gonad Index and hence potential reproductive output.

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Chapter 5:

Foraging and homing behaviour in the high shore South African limpet, *Helcion pectunculus*

5.1 Introduction

The majority of mobile molluscs in the marine intertidal zone are herbivores. They include the chitons, many prosobranchs such as the "true" limpets, trochids, neritids, littorinids and hydrobiids, the pulmonate limpets and onchidiids, and some opisthobranchs (Little, 1989). Within these taxa, there are a wide variety of foraging patterns in relation to external factors such as tidal cycles, day/night cycles, and other less predictable factors such as wave action and rainfall (see Branch, 1981; Hawkins & Hartnoll, 1983 for reviews of the literature).

The patterns of foraging activity observed in these molluscs have been classified into 3 main groups based on their relation to tidal and day/night cycles (Branch, 1981); species that feed while submerged (both day and night); those that move while awash during the rising and falling tides but not while exposed or completely covered (also during day and night); and those that feed only at night and at low tide. Hawkins & Hartnoll (1983) have since emphasized that rigid classification is impossible due to the labile nature of these patterns. The most common activity pattern described in herbivorous intertidal marine molluscs is to be active while submerged.

Prosobranch limpets can be some of the most dominant organisms on exposed rocky shores and therefore play a major role in the structuring of intertidal communities through their grazing activities (Branch, 1985; Branch & Barkai, 1988). The importance of limpets in the ecology of rocky shores has prompted numerous studies on their activity and foraging behaviour, although the majority of such studies have centred on northern hemisphere species (see Branch, 1981; Hartnoll, 1986; Liu, 1993 for reviews). Much of the work on limpet activity has involved limpet populations from sheltered shores, such as Menai Bridge, U.K. (Della Santina & Chelazzi, 1991; Chelazzi *et al.*, 1994; Della Santina, 1994; Della Santina *et al.*, 1994, 1995; Gray & Naylor, 1996) and Port Erin, Isle of Man, U.K. (Hartnoll & Wright, 1977; Hawkins & Hartnoll, 1982). Lough Hyne, Southern Ireland, which has been the location of many limpet behavioural studies (Little & Stirling, 1985; Little *et al.*, 1988, 1990) not only is a sheltered site but also exhibits an abnormal tidal regime (Little *et al.*, 1991). A study to compare limpet activity inside and outside of the Lough found a significant difference in their behaviour (Little *et al.*, 1991). The foraging activity and homing behaviour of northern hemisphere acmaeid limpets have also been studied (Craig, 1968; Jessee, 1968; McKendree Eaton, 1968; Miller, 1968; Rogers, 1968; Ross. 1968) but again these were carried out on sheltered shores. What little work has been carried out on exposed shores has been on the Hong Kong limpet, *Cellana grata* (Williams, 1993, 1994a, 1994b; Williams & Morritt, 1995). Foraging activity in this limpet was stimulated by sea-spray and could be brought on artificially by spraying limpets with a hose-pipe (Williams, pers. comm.).

Many of the prosobranch and pulmonate limpets found on rocky shores exhibit "homing" behaviour *i.e.* they have a fixed "home" site on the rock face to which they return after each foraging excursion (Underwood, 1979; Branch, 1981 for reviews). Underwood (1979) stated that homing was seldom defined adequately, and that while in one population of the limpet *Cellana tramoserica* 85 % of individuals homed, in another population only 15 % homed. It has been shown in previous studies that *Patella vulgata* is an obligate homing species (Cook *et al.*, 1969; Hartnoll & Wright, 1977; Little & Stirling, 1985; Little *et al.*, 1988, 1990; Gray & Naylor, 1996), however, at least one population on smooth rock has been shown not to home (Lewis, 1954). Even in homing populations, individuals change their home scars over periods of months or even years (Little, 1989). It has been suggested that homing to a fixed scar where the shell margin exactly matches the contours of the rock surface reduces desiccation (Branch, 1981), however, it is unlikely that homing has a single function due to it being present in low-shore species and individuals occurring in tidal pools.

The exposed rocky shores of South Africa have a particularly diverse limpet fauna, with some species occurring in large densities (Branch, 1971). Except for the work of Branch & Cherry (1985) on the pulmonate limpet *Siphonaria capensis* and more recently Gray & Hodgson (in press) on *Patella granularis* and *Siphonaria concinna*, very few quantitative studies have been carried out on the foraging behaviour of South African limpets, although results from numerous qualitative observations (*i.e.* documenting when animals are active in relation to the tide) of west coast limpets and those inhabiting False Bay (Cape Town) have been published (Branch, 1971, 1981). These observations were made during one season only and so do not reveal anything about long term (inter-seasonal) or short term (*e.g.* effects of spring or neap tides) variation in foraging behaviour. However, a recent study on the foraging behaviour of *Siphonaria concinna* and *Patella granularis* (Gray & Hodgson, in press), two South African limpets occurring at a similar height on the shore, showed that differences in behaviour were apparent between spring and neap tides and also seasonally.

All studies on limpet foraging have centred on species occurring on open rock surfaces and have not taken into account either the aspect of the limpet on the shore or the micro-environment in which the limpet is found. *Helcion pectunculus* can be regarded as one of the most successful of all southern African limpets. It has a wide geographic distribution, ranging from Natal to Namibia, although it has a restricted range on the shore, occurring only in the upper Balanoid zone (chapter 2). The aims of this study were, therefore, to examine and quantify the foraging activity of *H. pectunculus* (Prosobranchia), a high-shore crevice dwelling limpet on an exposed shore on the eastern Cape coast of South Africa on both east and west facing rock surfaces. Foraging activity was compared during spring and neap tides during all 4 seasons.

5.2 Materials & Methods

5.2.1 Study site

Observations were made at Cannon Rocks in the Eastern Cape (33° 44' S; 26° 35' E). This site consisted of an exposed boulder beach, the substratum being composed of quartzitic sandstone, which experienced semi-diurnal tides. The tidal range at Cannon Rocks is 1.9 m above chart datum on mean spring tides, and 0.9 m above chart datum on mean neap tides with highest spring tides phased around 0400 and 1600 hrs (S.A. Navy tide tables, 1993, 1994, 1995). The site was chosen for easy access and the fact that it is relatively free of human activity even during peak holiday times. The rock face had no macrophytes except at the low water level and in pools.

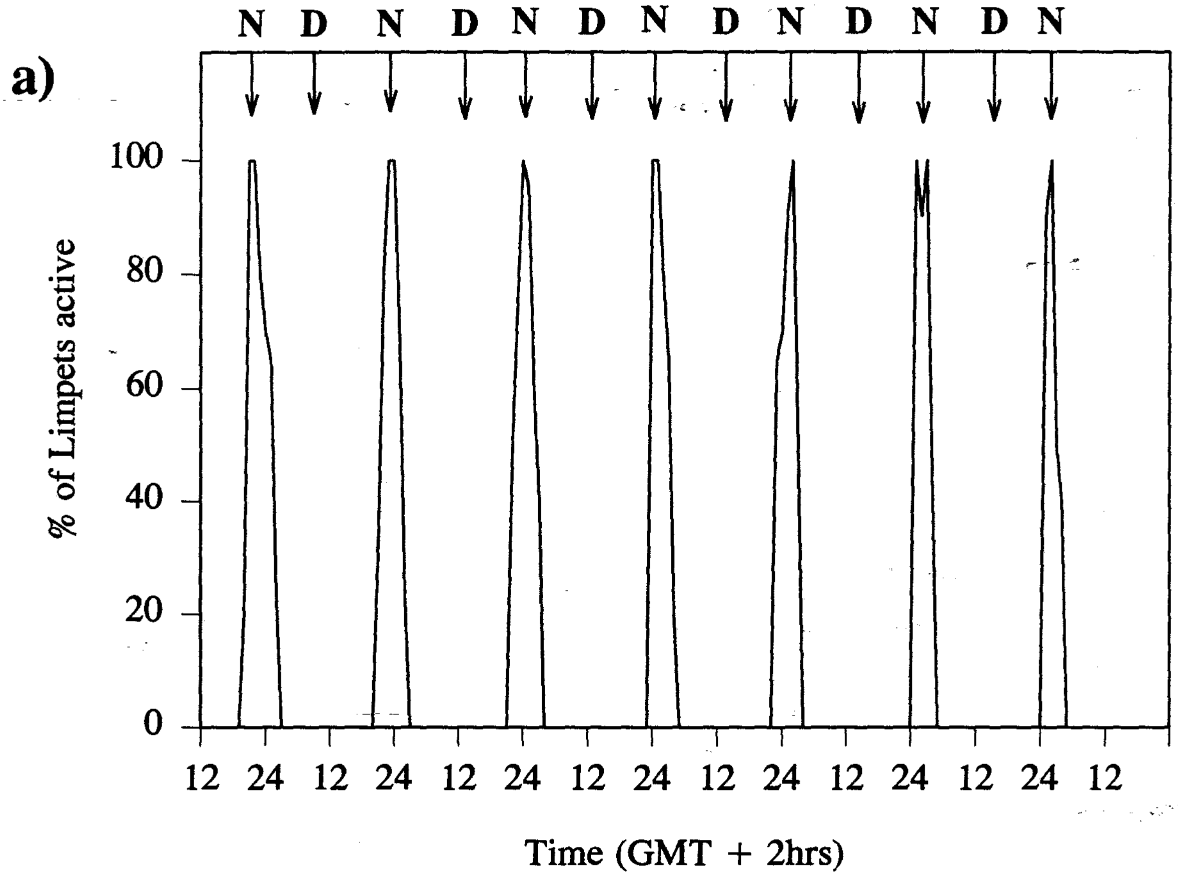
During day-time low tides *Helcion pectunculus* was found in crevices and under boulders in the upper-balanoid zone. Individuals occurred in densities of 18.6 ± 29.6 individuals per m^2 (see chapter 2). The limpets were sheltered from wave action whilst in their crevice but as soon as a limpet moved out of the crevice on to the adjacent rock surface, it was not sheltered in any way.

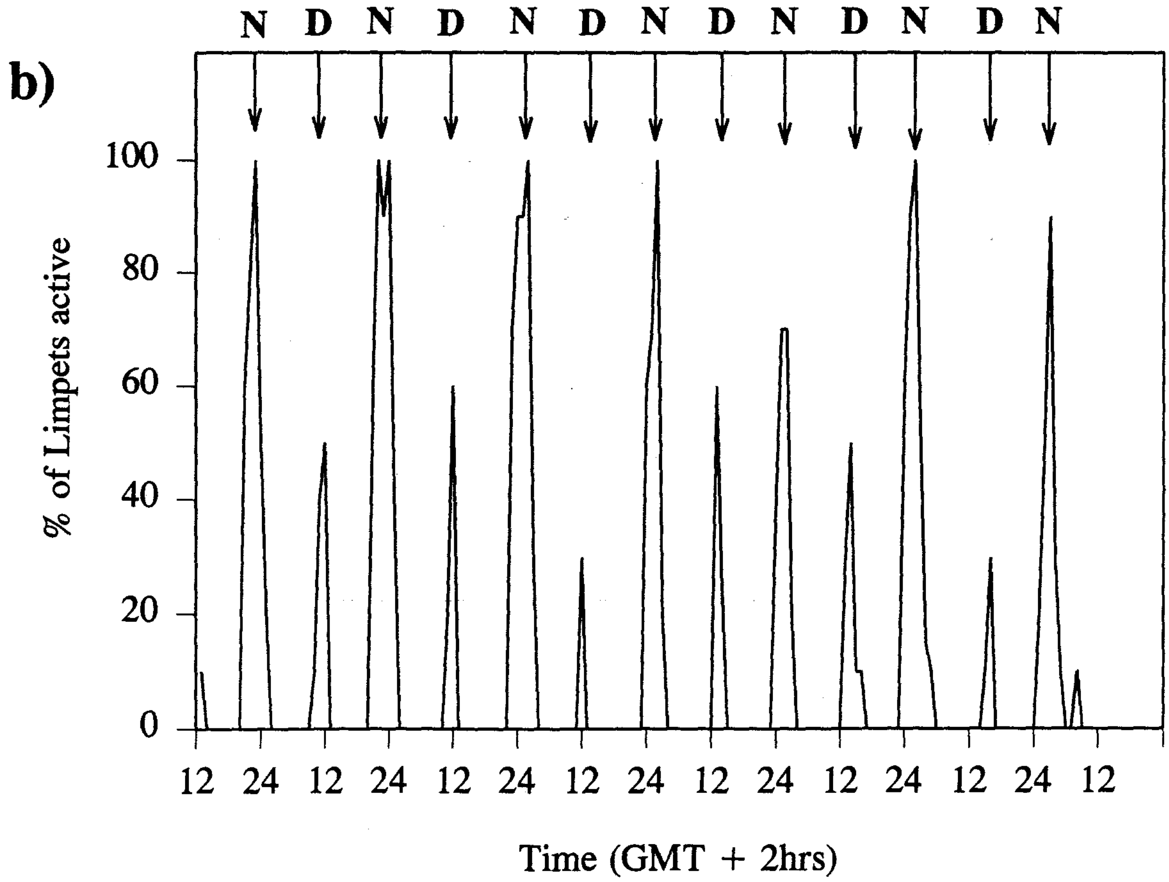
5.2.2 General Methods of observation

Twelve hours prior to recordings, 20 limpets with shell lengths of approximately 20 mm, were marked with small plastic numbers (Dymo tape) attached to the shell with epoxy resin (Cook *et al.*, 1969; Gray & Naylor, 1996). This was most successfully achieved after removing all encrusting material from the surface of the shell and blotting off all excess water. In order to determine whether limpets had a consistent orientation on a home scar, individuals were marked with a line of cellulose paint from the apex to the margin of the shell (Little & Stirling, 1985). The line was extended on to the adjacent rock face so that when the limpet was "home", the line was continuous. A number was painted on the rock next to each limpet. To determine the position of any individual at any given time, the method of triangulation (Cook *et al.*, 1969) was adopted. Three crosses equidistant from each other (100 cm) were painted on the rock. Using these as reference points, the path followed by each limpet could be plotted.

5.2.3 Preliminary observations of foraging activity:

Preliminary field observations suggested that *H. pectunculus* on the shaded sides of boulders (= east facing) were generally active during day-time low tides whilst in the shade (Gray, pers. obs). Observations on limpet foraging were therefore carried out on 20 labelled limpets on both east and west facing rock surfaces. Limpets were observed continuously for 7 days at Cannon Rocks (figure 5.1 a & b). The activity data obtained were analyzed using


a)
N D N D N D N D N D N D N
100
80
60
40
20
0
% of Limpets active
12 24 12 24 12 24 12 24 12 24 12 24 12 24 12
Time (GMT + 2hrs)

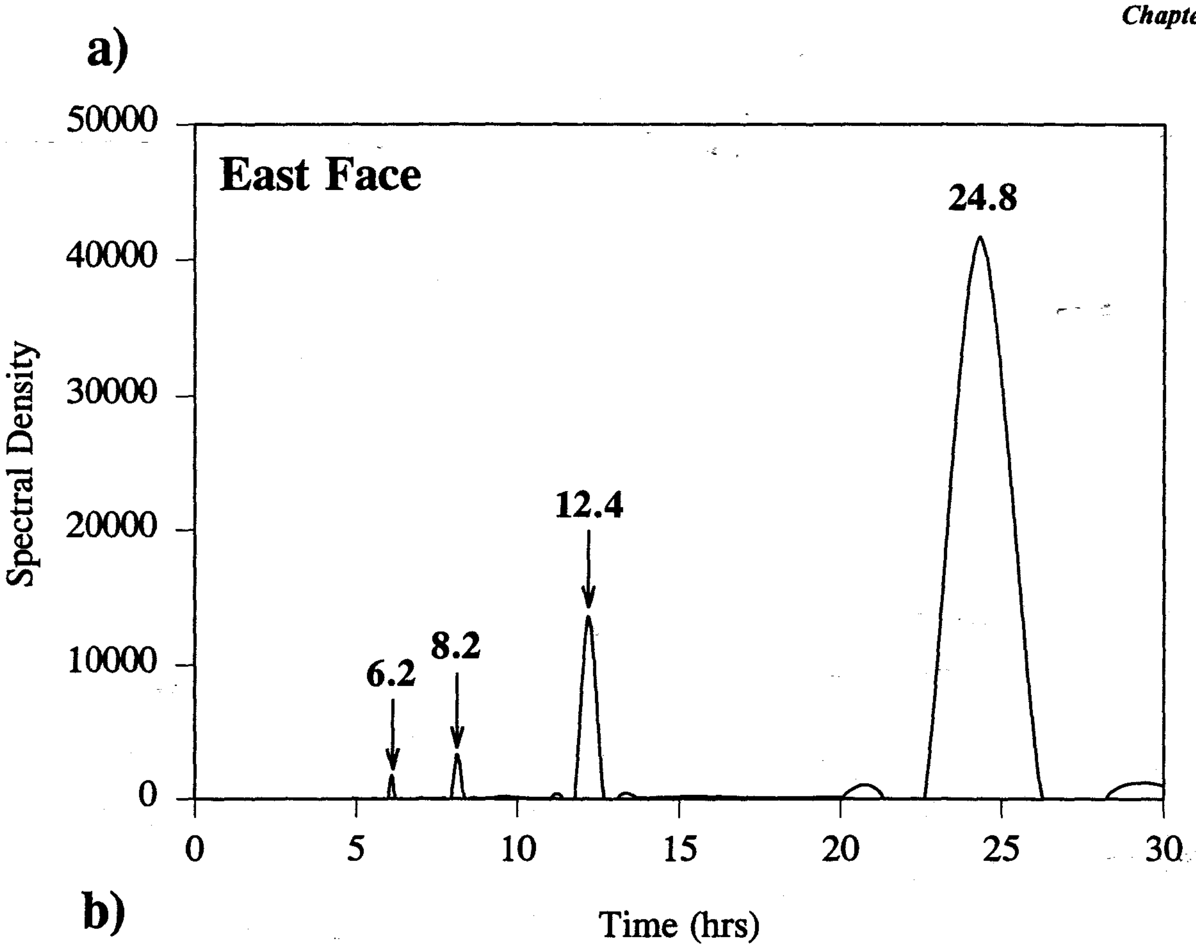

b)
N D N D N D N D N D N D N
100
80
60
40
20
0
% of Limpets active
12 24 12 24 12 24 12 24 12 24 12 24 12 24 12
Time (GMT + 2hrs)

Maximum Entropy Spectral Analysis (MESA) (Dowse & Ringo, 1989; Little *et al.*, 1991) to estimate periodicities of activity for east and west facing populations. The MESA results are shown in figure 5.2a & b.

In both east and west populations the major time interval between bouts of activity was around 24 hrs. A second peak occurred at around 12.4 hours but this is quite possibly a simple submultiple of the 24 hour peak. The west population of limpets also have a 13.5 hr peak, which could suggest a tidal component. Although several other small peaks appeared in the analysis, they have been ignored because they were represented at less than 20% of that with maximum period. From these results it was decided to observe *H. pectunculus* from both east and west rock faces due to the fact that their activity periods seem to differ in the field.

5.2.4 Observations of foraging behaviour

Helcion pectunculus was observed on both east and west facing rock surfaces on spring and neap tides in all 4 seasons (see table 5.1). Measurements were carried out at hourly intervals on twenty labelled individuals (see section 5.2.2.) from when the limpets were uncovered to a time when they were re-submerged. Measurements were not taken during high tide due to the intense wave activity in the intertidal zone making underwater observations highly dangerous. However, it was assumed that the limpets remained inactive during immersion due to the fact that they returned to a home scar or site before being covered by water. It has also been found that submergence in water inhibits locomotor activity in *H. pectunculus* (see chapter 6), and that limpets cannot survive wave action (chapter 7).


a)
East Face
Spectral Density
50000
40000
30000
20000
10000
0
24.8
12.4
8.2
6.2
0
5
10
15
20
25
30
Time (hrs)

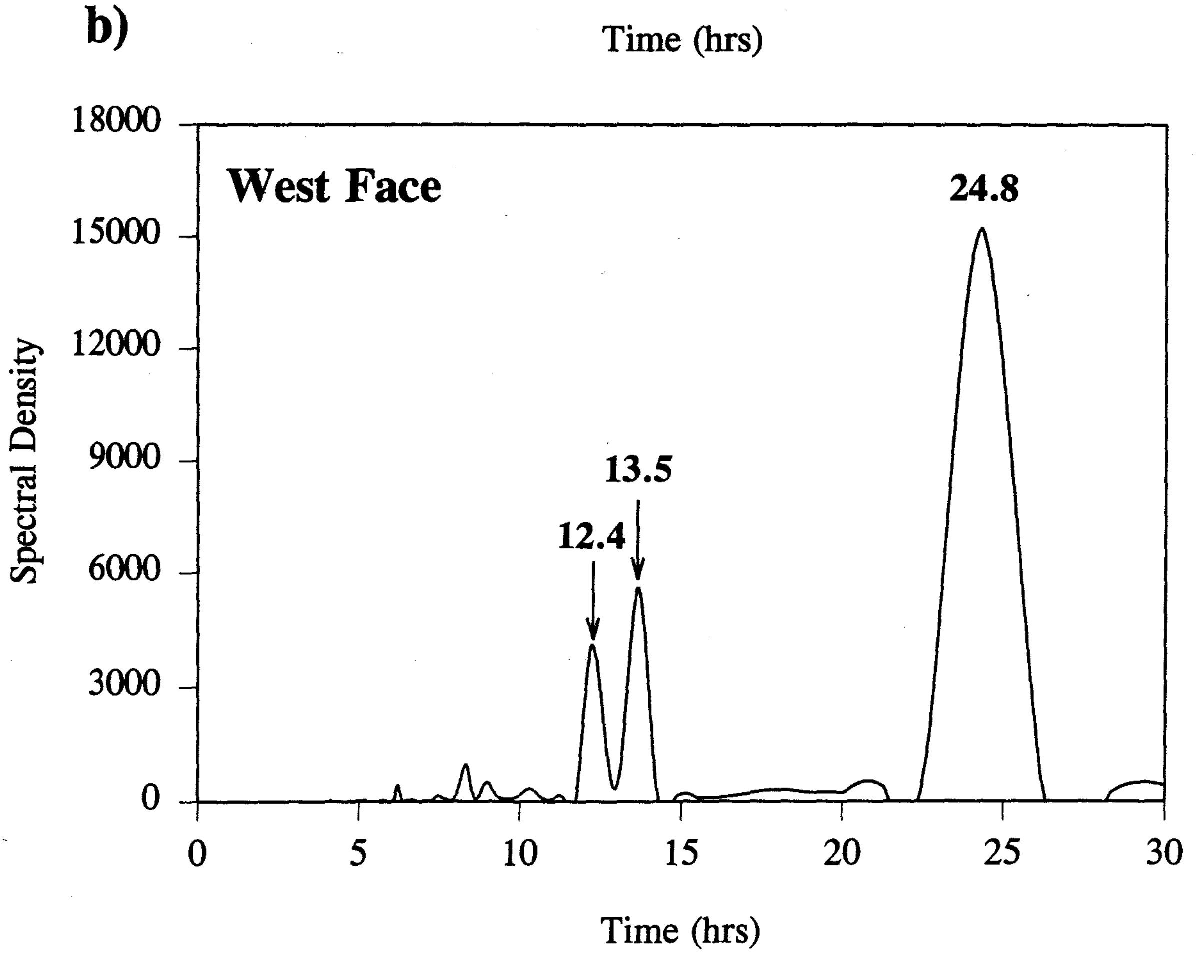

b)
West Face
Spectral Density
18000
15000
12000
9000
6000
3000
0
24.8
13.5
12.4
0
5
10
15
20
25
30
Time (hrs)

Table 5.1:

Dates of observation periods for *Helcion pectunculus* at Cannon Rocks showing the phase of the tide and the season.

Date	Tidal Phase	Season
01.11.94	Spring New Moon	Spring
11.11.94	Neap Quarter Moon	Spring
16.11.94	Spring Full Moon	Spring
17.03.95	Spring Full Moon	Summer
25.03.95	Neap Quarter Moon	Summer
02.03.95	Spring New Moon	Summer
14.07.95	Spring Full Moon	Autumn
20.07.95	Neap Quarter Moon	Autumn
26.07.95	Spring New Moon	Autumn
09.09.95	Spring Full Moon	Winter
16.09.95	Neap Quarter Moon	Winter
23.09.95	Spring New Moon	Winter

Limpets were recorded as 'at home' when they were on their home scar and as 'active' when away from their home scar. At night, observations were made using red light only since previous studies have shown that white light causes limpets to clamp down and cease foraging (Cook *et al.*, 1969; Gray & Naylor, 1996). This was also found to be the case for *H. pectunculus*.

During the periods of observation, physical variables were measured hourly whilst limpets were exposed. Measurements included rock and air temperature (Hanna instruments HI 9040 microcomputer thermometer), relative humidity (Hygrocheck relative humidity probe) and light intensity in $\mu E.m^{-2}.s^{-1}$ (Integrating Quantum/radiometer/photometer, model LI-188B by Li-cor inc.). Hourly notes were made on the weather and on the condition of the sea. Sea conditions were arbitrarily termed calm if no waves reached more than 25 cm up the rock face. Reference to wave action, if any, therefore refers to vertical movement greater than 25 cm.

5.2.5 Orientation of foraging movements in relation to micro-algal abundance

To determine whether or not *H. pectunculus* forages optimally *i.e.* travels directly to an area of high algal productivity, a 'foraging angle' was calculated for each excursion of the limpets by plotting a line through the home scar and the furthest point reached by the limpet during that excursion and measuring the angle in a clock-wise direction from magnetic North. The mean vector (r) of foraging directions of the samples, irrespective of the maximum distance travelled, were calculated (Mardia, 1972; Batschelet, 1981). Rock chippings (n = 5) were taken on a 1 metre radius circle from the crevice every 45° from magnetic north. Rock was sampled to a depth of at least 2 mm and was analyzed for Chlorophyll-*a* concentrations using the standard hot methanol extraction method (H.M.S.O., 1986) as used by Hill & Hawkins (1990) and Bustamante *et al.* (1995). At each site, where rock chippings were removed, the rock surface was graded for roughness following the Powers roughness scale (Powers, 1953; see figure 5.3).

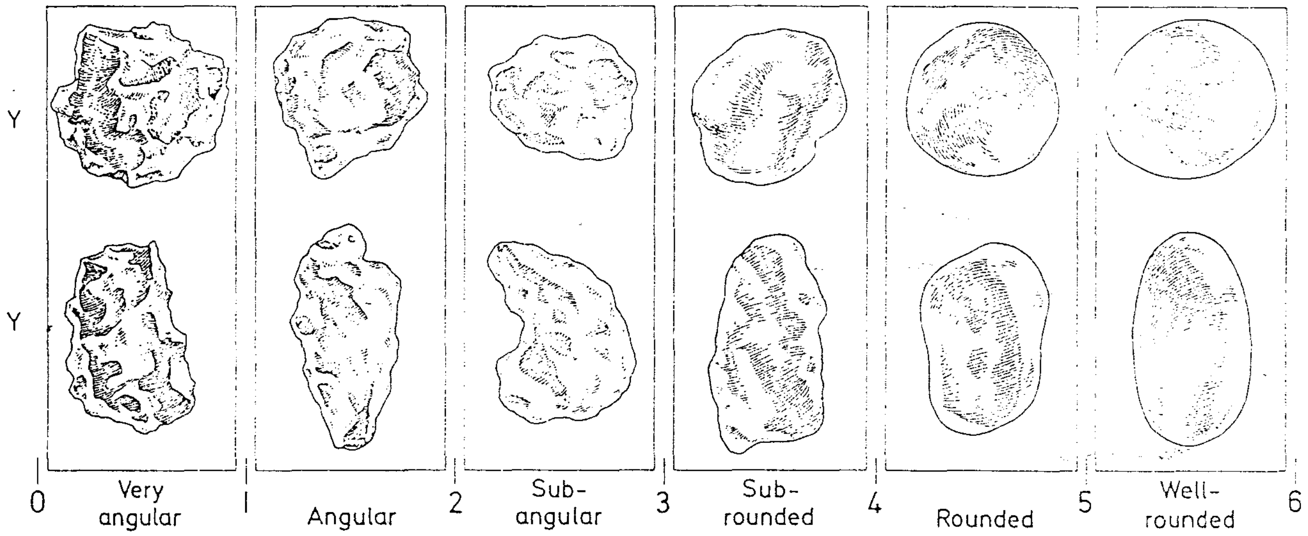
5.3 Results

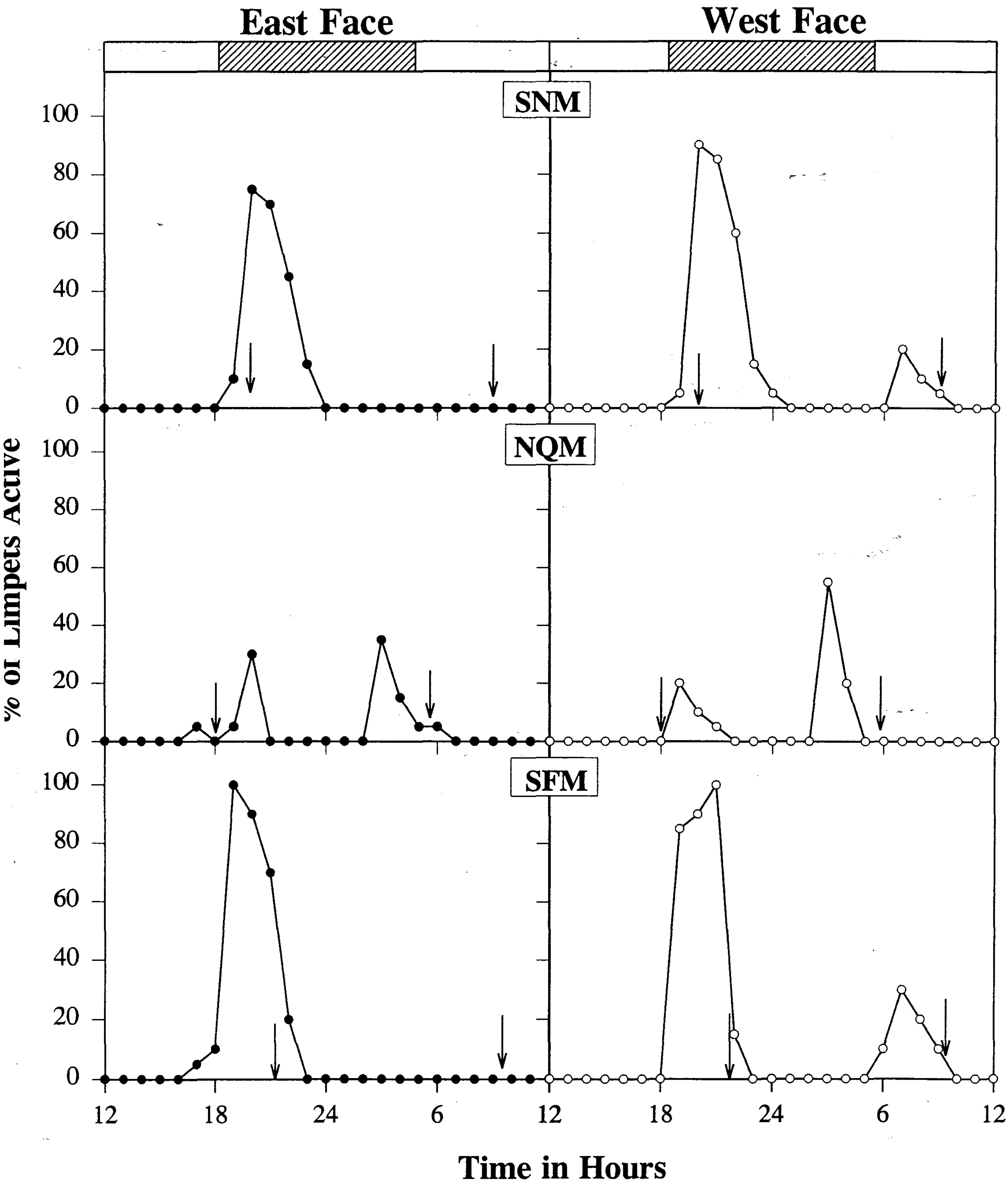
5.3.1 Activity rhythms

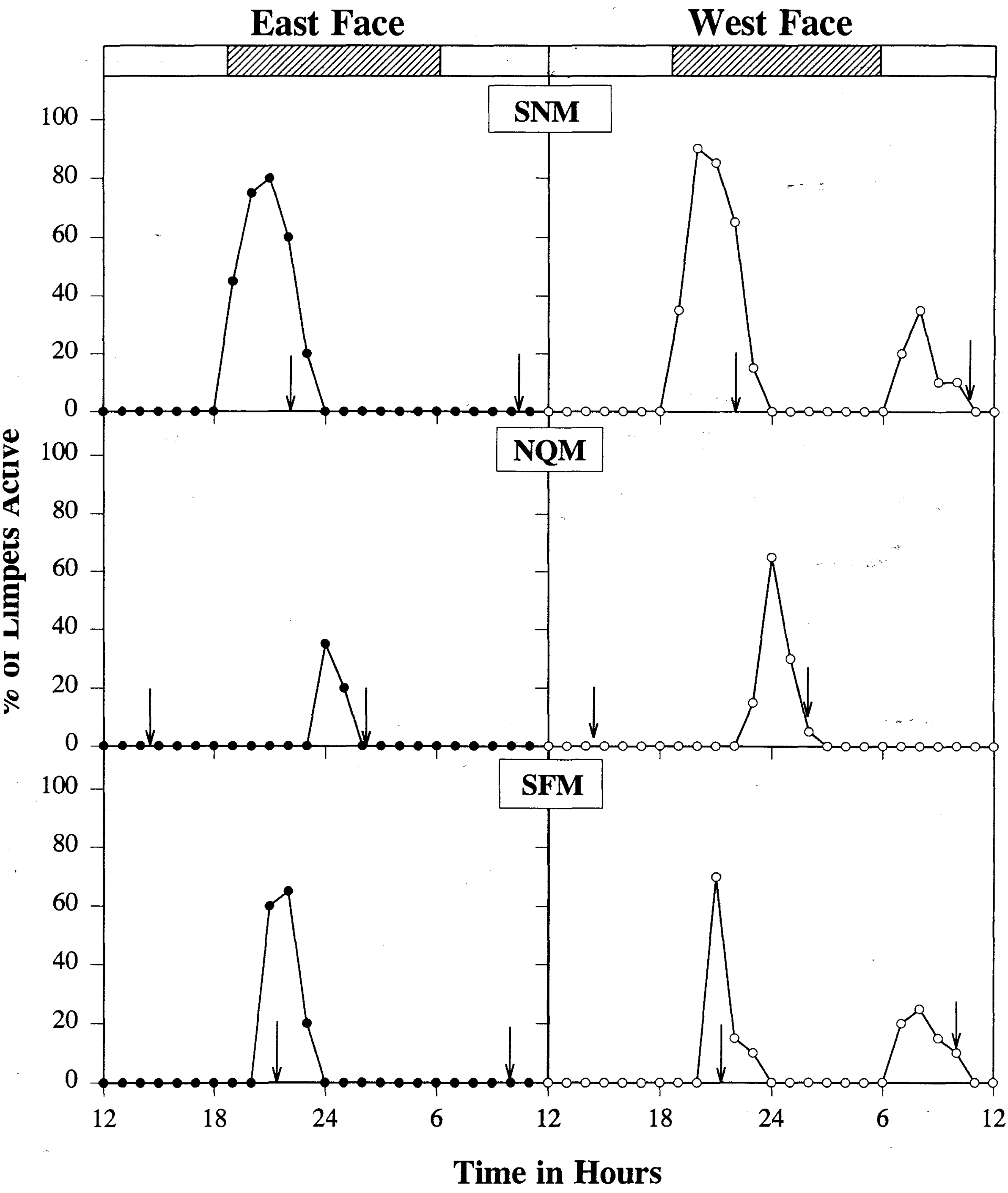
Individuals foraged primarily during low tide which occurred at night or around dusk and dawn. However, those *H. pectunculus* from west facing rocks also exhibited periods of activity during day time low tides whilst in the shade (898.5 ± 236.4 $\mu E.m^{-2}.s^{-1}$) of early morning throughout all seasons (figures 5.4, 5.5, 5.6 & 5.7). Activity commenced soon after emersion and all limpets were back at their home scar within the crevice prior to immersion by the next flood tide (about 7 hours on a spring tide and 5 hours on a neap tide). The number of limpets active throughout the study varied from < 20% to 100%. It is interesting to note that no activity was recorded on 20/7/95 on either east or west rock faces. On this occasion wave action was very strong, the waves covering the limpets regularly throughout the low tide period.

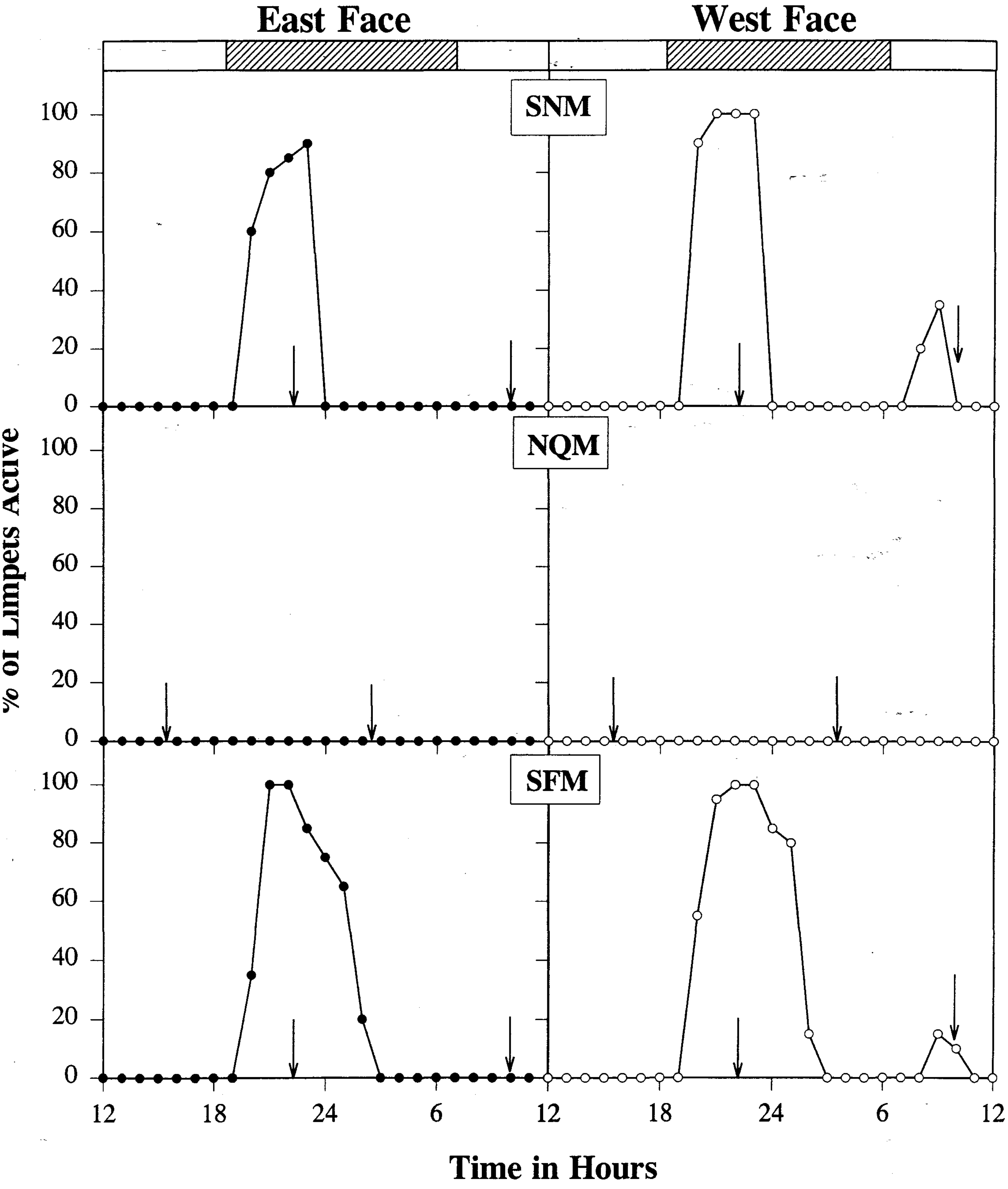
5.3.2 Homing behaviour

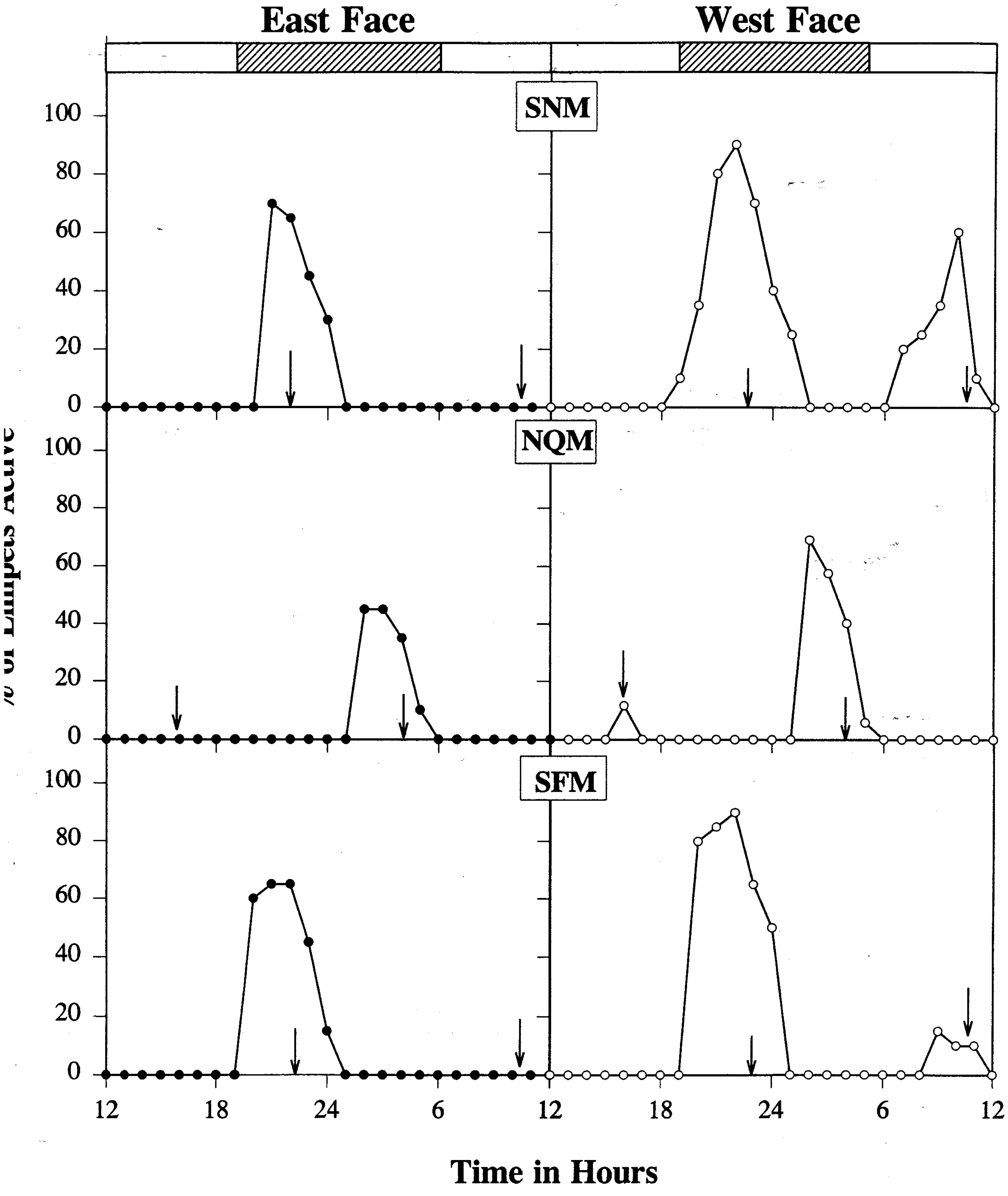
Helcion pectunculus exhibited rigid homing behaviour, homing to a fixed scar within the crevice. Throughout the period of the study 100% successful homing was recorded on both east and west rock faces. No scar swapping was observed and the limpets orientated


Y
Y
0
Very
angular
1
Angular
2
Sub-
angular
3
Sub-
rounded
4
Rounded
5
Well-
rounded
6


East Face
West Face
SNM
NQM
SFM
% of Limpets Active
0
20
40
60
80
100
12
18
24
6
12
18
24
6
12
Time in Hours


East Face
West Face
SNM
NQM
SFM
% of Limpets Active
0
20
40
60
80
100
12
18
24
6
12
18
24
6
12
Time in Hours


East Face
West Face
SNM
NQM
SFM
% of Limpets Active
100
80
60
40
20
0
12
18
24
6
12
18
24
6
12
Time in Hours


East Face
West Face
SNM
NQM
SFM
100
80
60
40
20
0
12
18
24
6
12
18
24
6
12
Time in Hours

themselves exactly on their scars so that their heads were facing into the crevice. None of the individuals observed during the study returned to their home scar via their outward path. Trail crossing did occur but no attempt was made by individuals to change direction and follow the previous trail.

5.3.3 Distance moved and speed of movement

The average distances travelled by *H. pectunculus* are given in table 5.2. To interpret the influence of spring or neap tides, phase of the moon (Full moon, quarter moon & New moon light intensities) and season, on the various aspects of limpet movement, a two-way ANOVA was used twice (Sokal & Rohlf, 1981). Firstly season and tide were analyzed and then season and phase of the moon. Two separate analyses were carried out because of the correlation between phase of the moon and tidal phase which causes logical zeros in the data set and there is no statistical technique that can deal with this scenario.

Helcion pectunculus was found to forage significantly further on spring tides than on neap tides (twice as far on spring tides compared to neaps) (table 5.3). There was also a difference in the distances travelled in different seasons, with individuals travelling twice as far in winter than in spring or summer months. Distances travelled by the limpets during Autumn did not differ significantly from the other seasons.

Table 5.2:

Mean foraging distances, speed of movement ($\bar{x}$ ± S.D.) for *H.pectunculus* during outward, foraging & homeward phases of foraging excursions.

	Mean displacement ± S.D. (cm)	**Outward Speed (cm.min^{-1})**	**Foraging Speed (cm.min^{-1})**	**Homeward Speed (cm.min^{-1})**	**Duration of Activity (Min)**
Summer					
SFM (17.03.95)	63.0 ± 63.68	0.58 ± 0.31	0.14 ± 0.17	0.6 ± 0.28	132 ± 86.1
NQM (25.03.95)	17.2 ± 32.62	0.42 ± 0.30	0.03 ± 0.08	0.38 ± 0.23	36 ± 62.7
SNM (02.03.95)	90.4 ± 51.0	0.48 ± 0.3	0.06 ± 0.06	0.39 ± 0.27	171 ± 68.2
Autumn					
SFM (14.07.95)	109.8 ± 51.2	0.72 ± 0.47	0.11 ± 0.14	0.68 ± 0.46	288 ± 99.7
NQM (20.07.95)	No Movement Recorded				
SNM (26.08.95)	110.1 ± 57.8	0.65 ± 0.45	0.08 ± 0.04	0.52 ± 0.36	210 ± 56.7
Winter					
SFM (09.09.95)	107.6 ± 103.4	0.56 ± 0.41	0.11 ± 0.12	0.68 ± 0.36	123 ± 79.0
NQM (16.09.95)	45.0 ± 48.1	0.48 ± 0.29	0.12 ± 0.1	0.51 ± 0.28	105 ± 79.9
SNM (23.09.95)	86.3 ± 61.3	1.0 ± 0.72	0.1 ± 0.07	0.79 ± 0.59	144 ± 101
Spring					
SFM (16.11.95)	72.5 ± 69.8	0.58 ± 0.38	0.09 ± 0.13	0.63 ± 0.34	90 ± 66.0
NQM (11.11.95)	40.5 ± 59.7	0.94 ± 0.31	0.03 ± 0.05	0.93 ± 0.32	37.9 ± 57.3
SNM (01.11.95)	78.65 ± 48.2	0.65 ± 0.49	0.07 ± 0.11	0.7 ± 0.48	174 ± 93.1

Table 5.3

Two-way Analysis of variance on distances travelled by *H. pectunculus*

Source of variation	df	ms	F	P
Tidal Phase	1	95355	24.361	<0.001
Season	3	12639	3.229	<0.05
Residual	215	3914		
(All interactions nonsignificant)				

Results of multiple range analysis (Newman-Keuls) for Tidal phase & Season:

Spring tides		Neap tides		
89.8cm	>	40.9cm		
Winter	>	Spring	=	Summer
85.53cm		55.7cm		48.8cm

A significant difference was also found to be present between phases of the moon, with limpets travelling further on full moons and new moons than on quarter moons in all seasons. This result however is correlated strongly with phase of the tide due to the fact that phase of the moon causes the differences in tidal phase (*i.e.* full and new moons occurring on spring tides and quarter moons falling on neap tides).

Foraging speed during active periods was also determined. On all occasions *H. pectunculus* moved rapidly away from their home scar as activity commenced (table 5.2). They then slowed down by up to 75% upon reaching a particular area, where they remained for some time (1-3 hours). Movement back to the home scar or site was also rapid, often equalling the outward speed and after the correct orientation on the scar was reached, activity ceased. To analyse differences in rates of movement, excursion periods were divided into three equal phases. These were termed the "outward phase", "foraging phase" and "return phase". Three-way ANOVA (Sokal & Rohlf, 1981) showed that there was no significant effect on these speeds of tidal phase (spring or neap tide) or of season (table 5.4). There was, however, a significant difference between the phases of excursions. Scheffe's tests showed that the mean speed of movement during the "foraging phase" was significantly lower than during either the outward or return phases (table 5.4).

Table 5.4

Three-way ANOVA of speeds of movement on outward, foraging and homeward phases on spring and neap tides during all four seasons.

Source of variation	df	MS	F	P
Phase of journey	2	40.1	7.17	0.0008
Phase of tide	2	4.7	0.835	0.4344
Season	3	3.9	0.695	0.5551
Residual	604	5.59		

(All two- and three-way interactions non-significant)

Scheffe's tests for above speeds

Speed (cm/min^{-1})

Outward	Middle	Return	Scheffe
0.603	0.072	0.927	Middle<return = outward

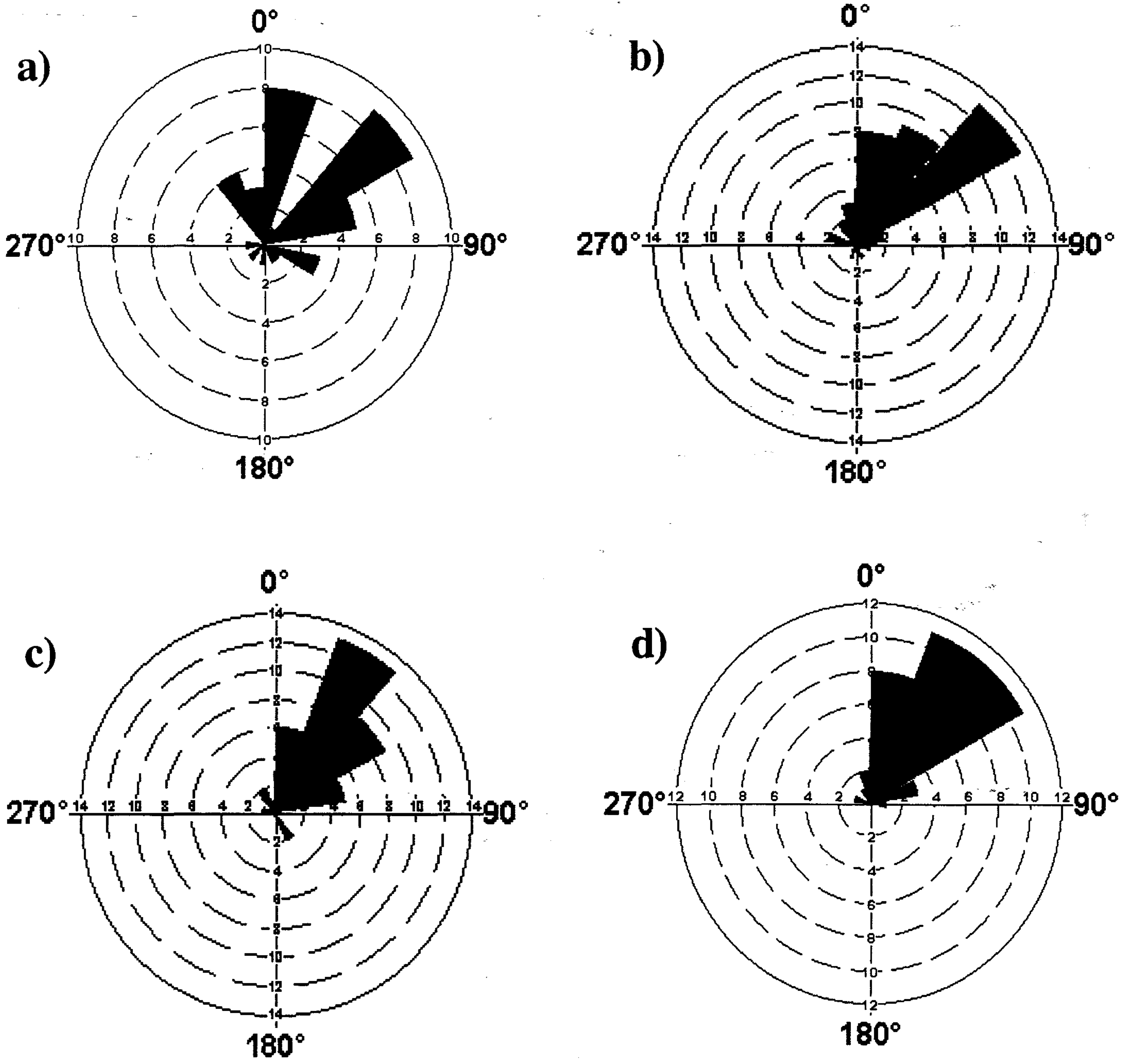
5.3.4 Orientation of foraging behaviour

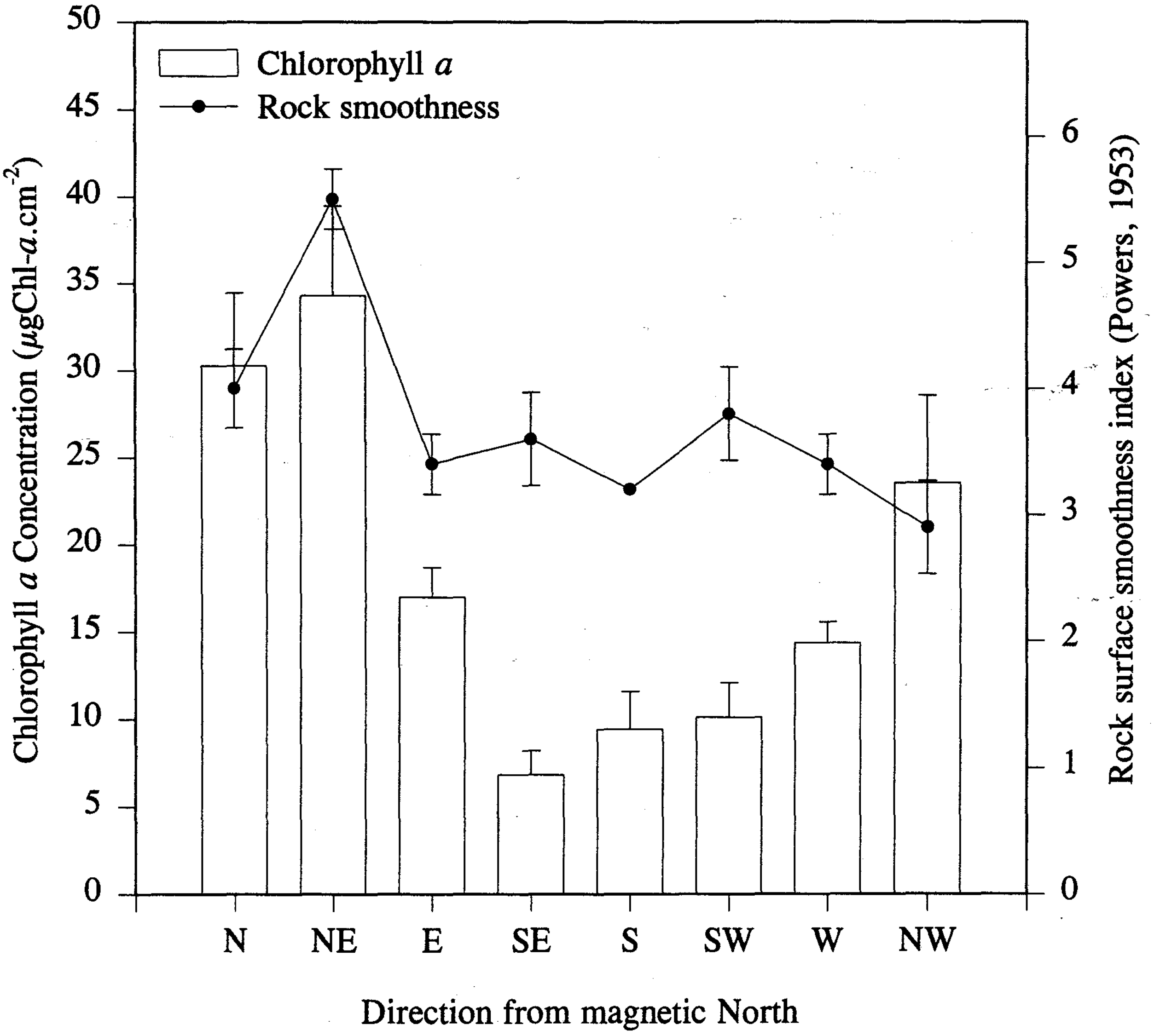
The foraging directions of *H. pectunculus* are plotted in figure 5.8. Due to the fact that there were no significantly different directions between spring and neap tides ($p >> 0.05$; Watson-Williams F test) the results for these dates have been pooled to give a monthly foraging direction. The results show that the foraging activity of *Helcion pectunculus* is highly directional. The direction followed took individuals onto an area of the rock surface which had the highest chlorophyll-*a* concentration (= 30-33 μg Chl-*a*.cm^{-2}) (indicating higher algal biomass) and also exhibited the smoothest rock surface (figure 5.9). No significant difference was found between direction travelled by *H. pectunculus* in the different seasons.

Table 5.5:

Results of Watsons pairwise F-test for circular means carried out on seasonal directional data for *H. pectunculus*.

Sample	d.f.	F-value	P-value
November vs March	78	0.65	0.42
November vs July	75	0.08	0.78
November vs September	74	0.33	0.57
March vs July	79	0.39	0.54
March vs September	78	0.12	0.73
July vs September	75	0.15	0.70


a)
0°
90°
180°
270°
2
4
6
8
10
b)
0°
90°
180°
270°
2
4
6
8
10
12
14
c)
0°
90°
180°
270°
2
4
6
8
10
12
14
d)
0°
90°
180°
270°
2
4
6
8
10
12


Chlorophyll *a*
Rock smoothness
Chlorophyll *a* Concentration (μgChl-*a*.cm^{-2})
Rock surface smoothness index (Powers, 1953)
Direction from magnetic North
0
5
10
15
20
25
30
35
40
45
50
0
1
2
3
4
5
6
N
NE
E
SE
S
SW
W
NW

5.3.5 Physical variables

Recordings of the environmental conditions for summer and winter months are summarized in table 5.6. During summer low tides, daytime air and rock temperatures reached 24.2 ± 2.9 and 27.1 ± 5.2 °C respectively. The temperature of the rock surface was generally 1-3 °C warmer than the air temperature during the day. At night the air and rock temperatures were very similar. Relative humidity of the air was found to be 20-40% higher at night than during the day. In general, temperatures (both air and rock surface) were lower during the winter periods of observation than those recorded during the summer.

5.4 Discussion

In the present study, quantitative data has been obtained on the foraging activity of the high-shore crevice dwelling limpet *Helcion pectunculus*. The intention was to establish whether these limpets showed similar behaviour to other species studied on South African shores (Branch, 1971; Branch & Cherry, 1985; Gray & Hodgson, in press), and to limpets living on open rock faces in more sheltered environments (Hartnoll & Wright, 1977; Hawkins & Hartnoll, 1982; Little & Stirling, 1985; Little *et al.*, 1988, 1990, 1991; Gray & Naylor, 1996). It is possible that a specific habitat may control and restrict the activity period and foraging behaviour of limpets. Table 5.7 shows examples of limpets displaying the three main activity patterns suggested by Branch (1981). This table also indicates the specific habitats in which the limpets were studied. The data in the table is not exhaustive due to the fact that many of the studies of limpet foraging activity do not give any detail about the habitat of the animals.

Table 5.6:

Air and rock surface temperature ranges (°C) and the range of relative humidity measurements recorded during activity observations of *H.pectunculus* during both summer and winter.

		Range of Air Temp. (°C)	**Range of Rock Temp. (°C)**	**Range of Rel. Humidity (%)**
Summer				
17.03.95	Day	21.1 - 27.1	19.6 - 32.6	54.8 - 70.3
	Night	16.0 - 19.7	17.2 - 18.2	80.4 - 91.0
25.03.95	Day	18.9 - 21.9	20.2 - 25.6	68.5 - 89.6
	Night	17.1 - 18.1	17.7 - 17.8	90.3 - 93.3
02.03.95	Day	21.2 - 24.2	18.3 - 29.2	42.8 - 73.2
	Night	19.1 - 20.5	19.3 - 21.0	92.2 - 99.7
Winter				
09.09.95	Day	18.5 - 20.4	19.8 - 26.2	52.1 - 56.2
	Night	12.2 - 17.3	13.1 - 15.3	70.8 - 82.7
16.09.95	Day	16.4 - 17.6	18.3 - 22.6	29.6 - 58.6
	Night	16.0 - 17.3	14.0 - 14.6	71.6 - 86.7
23.09.95	Day	13.2 - 18.6	13.4 - 20.3	40.6 - 61.8
	Night	13.5 - 16.8	14.4 - 17.4	69.1 - 75.3

Table 5.7:

Patterns of foraging behaviour exhibited by limpets occurring in differing micro-habitats.

Species	Timing of activity	Habitat	References
1) Foraging activity whilst submerged			
Patella vulgata	Day	Horizontal rock	Hartnoll & Wright, 1977
P. vulgata	Day	Horizontal rock	Hawkins & Hartnoll, 1982
Cellana ornata	Day & night	rock	Boyden & Zeldis, 1979
C. radiata	Day & night	rock	Hulings, 1985
Siphonaria laciniosa	Night	rock	Hulings, 1985
2) Foraging activity whilst emersed			
P. vulgata	Day & night	Vertical rock	Hawkins & Hartnoll, 1982
	Night	High shore rock	Little & Stirling, 1985
	Day & night	Low shore rock	Little & Stirling, 1985
	Night	Vertical rock	Gray & Naylor, 1996
P. granularis	Day & night	Vertical rock	Gray & Hodgson, in press
Helcion pectunculus	Night	High exposed rock	Gray, present study
	Day & night	High shaded rock	Gray, present study
Siphonaria capensis	Day & night	Rock pools	Branch & Cherry, 1985
	Night	Rock	Branch & Cherry, 1985
S. concinna	Night	Horizontal rock	Gray & Hodgson, in press
S. gigas	Night	Rock	Levings & Garrity, 1984
S. thersites	Night (winter)	High algal beds	Branch, 1988
	Day (summer)	High algal beds	Branch, 1988
3) Foraging activity whilst awash			
Cellana toreuma	Day & night	Rock	Hirano, 1979
C. grata	Day & night	Rock	Williams & Morritt, 1995
Collisella scabra	Day	Rock	Wells, 1980

Firstly, limpets which forage whilst submerged do so during both the day and night probably due to the fact that desiccation is not a problem. Many limpet species have also been found to be active during tidal emersion (table 5.7). Once again, species were active both at night and in the day, but with different patterns in different microhabitats. *Siphonaria capensis* was active by day and night in pools, but only at night when living on bare rock (Branch & Cherry, 1985). *Patella vulgata* was active only at night when studied at high tide levels, but in both day and night near low water levels (Little & Stirling, 1985).

Helcion pectunculus on west facing rock exhibited a biphasic activity pattern being active during both day and night low tide periods, whilst limpets on east facing rock only foraged during nocturnal low tides. Many other species of grazing mollusc have exhibited both nocturnal and diurnal foraging bouts, but with different patterns in different microhabitats. *Siphonaria capensis*, for example, was found to be active by day and night whilst in pools, but only at night when living on a bare rock face (Branch & Cherry, 1985). *Patella granularis*, on South African shores, has been shown to exhibit two bouts of activity a day, whilst *Siphonaria concinna* are only active during nocturnal low tides (Gray & Hodgson, in press). *Patella vulgata* has been shown to be active only during nocturnal emersion when at high tide levels, but in both night and day near low-water level (Little & Stirling, 1985; Little *et al.*, 1988; Williams & Morritt, 1991).

The activity pattern of *H. pectunculus* may have several driving factors. Garrity & Levings (1983) suggested that limpets remain inactive at high tide to avoid marine predators. A study of the feeding habits of the giant clingfish, *Chorisochismus dentex*, has shown that

H. pectunculus makes up a substantial part of this fish's diet, and that the clingfish actively searches for particular limpet species (Stobbs, 1980). Another explanation is that by being active at low tide, *H. pectunculus* reduces the chance of being washed away by wave action as they have a very low tenacity in relation to other patellid limpets (see chapter 7). This also explains the lack of foraging activity on 20/07/95 when the wave action was extremely strong and the limpets were continually being covered by sea water even at the time of low tide. By contrast, *P. granularis* have been found to be active under strong wave action (Gray & Hodgson, in press) and are known to be highly tenacious (Branch & Marsh, 1978). It is therefore suggested that both avoiding predators, such as the clingfish, and being washed away by wave activity determines the activity pattern of *H. pectunculus* as being active at low tide.

Little *et al.* (1988) found that there is a general reduction in limpet feeding activity on neap tides in comparison with spring tides, especially in upper shore individuals. This is also true for *H. pectunculus*, with limpets moving significantly shorter distances during neap tides than springs. This has also proved to be the case for other South African limpets, *S. capensis* (Branch & Cherry, 1985), *P. granularis* and *S. concinna* (Gray & Hodgson, in press). The distance travelled by a limpet during an excursion maybe a function of the time the limpet is exposed to air *i.e.* the time available for foraging. *Helcion pectunculus* is limited to foraging whilst exposed, presumably due to predator avoidance and low tenacity, and so foraging excursions will be both shorter in time and distance on neap tides, thus enabling the limpet to return home before being covered by the incoming tide.

Although many behavioural studies have been carried out on limpets around the world, few examples have been examined in sufficient detail to decide whether the activity rhythms are endogenous, or are purely triggered by external events (Little, 1989). Della Santina & Naylor (1994) showed that *P. vulgata* did exhibit an endogenous rhythm of activity and furthermore, the time of emersion appears to be the effective environmental synchronizer of the rhythm. *Helcion pectunculus* has also been shown to have an endogenous rhythm of locomotor activity (see chapter 6) and this may well account for the difference in distance travelled between spring and neap tides *i.e.* the rhythm gives the limpet the ability to predict when the tide will be returning and so the limpet is able to be back on its home scar by the time it is submerged.

There was significant seasonal variation in the distance travelled by *H. pectunculus*, with individuals travelling nearly twice as far in winter than in either spring or summer. *Patella granularis*, however, has been shown to exhibit a behavioural pattern which is opposite to that of *H. pectunculus* in that foraging excursions are significantly longer in summer (Gray & Hodgson, in press). There could be a number of reasons for this difference in foraging strategy between two limpets which have overlapping distributions on the shore. Firstly it has been found that epilithic algal production (in the form of Chlorophyll *a*) per month peaks during the winter months along the south coast of South Africa (Bustamante *et al.*, 1995) and so it is possible that *P. granularis* need not travel as far to obtain their quota of algae. Cubit (1984) found this to be true for high shore *Collisella digitalis* (on the west coast of America). However, this hypothesis does not explain the behaviour of *H. pectunculus*. Another possibility is a reduction in activity due to the cold temperatures in winter compared with those of the spring/summer months suppressing the limpets metabolism, thus their need for food is less or they cannot physically move as fast due to a reduction in basic bodily functions (Marshall, 1991). This, however, is not the case with *H. pectunculus* due to the fact that there is no significant difference in the speed travelled by individuals between the different seasons and the limpets are travelling further during the cold winter months. Another possible reason for this discrepancy in the behaviour of these two limpets relates to tenacity. *Patella granularis* is one of the most tenacious limpets occurring along the South African coast whilst *H. pectunculus* exhibits a very weak tenacity. It is therefore quite possible that wave action severely limits foraging excursions of *H. pectunculus* during the stormy winter months, whilst not having any effect on the stronger limpet. *Helcion pectunculus* would then be forced, when favourable conditions arose, to forage further and for the maximum time possible to obtain enough food to sustain individuals until the next available foraging excursion.

Helcion pectunculus exhibited rigid homing to a fixed scar within a crevice. No scar swapping was observed throughout the study period. Homing has been observed in most species of limpets (Branch, 1971; Creese & Underwood, 1982; Garrity, 1984) and has been shown to reduce desiccation (Verderber *et al.*, 1983; Branch & Cherry, 1985; Kunz & Conner, 1986). There are often great differences between species, and even within species the rigidity of homing behaviour, or the proportion of the population homing can be influenced by a number of factors *i.e.* size of the animals, the texture and stability of the rock, availability of food and the amount of desiccation (Branch, 1981). However, *H. pectunculus* was found to home on both east and west facing rock faces.

The actual mechanism of homing to the scar is still much debated. *Helcion pectunculus* was never observed to follow outward paths back to their home scar. Cook (1971) showed that the limpet *S. alternata* can home without using either distant clues, reverse-displacement or topographic memory, and indicated that limpets were capable of following mucous trails. Karow, *et al.* (1993) found that the freshwater pulmonate snail *Physia parkeri* responds both to con- and heterospecific mucous trails, whilst a recent study on the terrestrial carnivorous snail, *Haplotrema concavum* (Pearce & Gaertner, 1996), showed that the snail was able to use information from mucous trails to forage optimally. Mucous trail following does not, however, seem to be the homing mechanism utilised by *H. pectunculus* or by other South African limpets, namely *S. concinna* and *P. granularis* (Gray & Hodgson, in press). This, therefore raises the subject of how limpets home to a fixed scar? Although further work is required in this area of limpet foraging behaviour, it is possible that individuals follow mucous trails laid during previous foraging excursions. According to Cook (1971), *S. alternata* can follow trails after the trails have been soaked for 48-49 hours in sea water but do not follow trails soaked for 68-72 hours. The fact that *H. pectunculus* cross their own trails without actually following them may give the individual sufficient information for correct orientation and successful homing. However, it is also quite possible that different species of limpet have evolved different homing methods depending upon their environment and situation.

Present observations of *H. pectunculus* show that feeding excursions could be divided into 3 distinct phases, a relatively rapid outward phase travelling away from the home scar, a slower foraging phase and, finally, a rapid homeward phase. Such behaviour has been recorded for *P. vulgata* (Hartnoll & Wright, 1977; Little *et al.*, 1988; Chelazzi *et al.*, 1994) and for *S. concinna* and *P. granularis* (Gray & Hodgson, in press). Although *P. vulgata* was shown to feed for the entire activity cycle, grazing was most intense during the middle phase of the excursion (Little & Stirling, 1985; Evans & Williams, 1991). It is not known whether *H. pectunculus* feeds throughout its activity cycle, but the fact that it exhibits a rapid outward phase seems to suggest that the individual knows where it is going to feed and so raises the possibility of a learning component *i.e.* the individual is returning to optimal feeding areas. This has previously been suggested to be the case in *P. vulgata* (Gray & Naylor, 1996).

In *H. pectunculus*, foraging excursions were highly directional, with a mean vector which took the limpets onto an area of the rock face which exhibited the highest microalgal biomass and also the smoothest rock surface. *Siphonaria concinna* has also been shown to forage in a direction which took them into an area of rock which had a smooth flat surface compared to the rough, pitted area within which their home scars were situated (Gray & Hodgson, in press). It has been suggested that barnacles affect limpets adversely because their rough and irregular topography hinders foraging behaviour (Lewis & Bowman, 1975; Underwood, 1979; Hawkins & Hartnoll, 1982). It is possible that these limpets are selecting the smoother rock surfaces to optimise foraging effort. Barnacles are not generally found co-habitating with *H. pectunculus* and so do not influence foraging activity. In the case of *H. pectunculus*, optimal foraging is even more plausible due to the fact that individuals selectively forage in the area of highest algal productivity. *Patella vulgata* has also been shown to exhibit directionality during foraging excursions (Little *et al.*, 1988; Gray & Naylor, 1996) and several other patellids show vertical movements in relation to tidal rise and fall (Hirano, 1979; Williams & Morritt, 1995). However, in this study the dominant foraging directions were not in the vertical plane but horizontally across a rock surface. This then rules out the suggestion of a tidal influence on foraging direction (Hirano, 1979) in the case of *H. pectunculus* and again points towards the possibility that certain limpet species are able to learn the position of optimal feeding patches. Evans & Williams (1991) have argued that limpets do not need to maximise energy intake, given as foraging opportunities are predictable in certain environments. This does not appear to be the case for *H. pectunculus* during the stormy winter months, with individuals utilising periods of favourable conditions to forage further distances and for longer thus gaining sustenance until the next, unpredictable, foraging opportunity.

It is therefore apparent that *H. pectunculus* has adapted a behavioural pattern to minimize the effects of the extreme physical conditions of the high intertidal. By being active at night, desiccation is no longer a major problem and by being active at low tide, certain predators are avoided. It is suggested that the behavioural patterns exhibited by many limpet species are defined by the micro-environment the limpet inhabits and this gives rise to the, often large, intraspecific differences in behaviour at different localities. Whether *H. pectunculus* exhibits different behavioural patterns on shores of differing geomorphologies is not known and requires further work.

5.5 Summary

1) The activity pattern exhibited by *H. pectunculus* varied depending upon micro-habitat; animals inhabiting both east and west facing rock surfaces are active during nocturnal low tides whilst animals on west facing rock surfaces are also active during daytime

low tides whilst in the shade.

2) *H. pectunculus* travelled further during foraging excursions in winter than in either spring or summer and also during spring low tides compared to neaps.

3) *H. pectunculus* exhibited rigid homing to a fixed scar within a crevice.

4) Feeding excursions of *H. pectunculus* can be divided into 3 distinct phases, a relatively rapid outward phase, a slower foraging phase and a rapid homeward phase.

5) The foraging excursions of *H. pectunculus* were highly directional, with a mean vector which took the limpets onto an area of the rock face which exhibited the highest microalgal biomass and also the smoothest rock surface.

6) It is suggested that *H. pectunculus* has adapted a behavioural pattern to minimize the effects of the extreme physical conditions of the high shore and that behavioural patterns exhibited by many limpet species are defined by the micro-environment the limpet inhabits.

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Chapter 6:

Rhythms of locomotor activity in the high shore limpet *Helcion pectunculus*

6.1 Introduction

The behavioural patterns of intertidal animals often consist of rhythmic sequences of movements which are correlated with environmental variables of daily or tidal periodicity (Naylor, 1988). Many such rhythms are driven solely by environmental variables and hence are responses to exogenous stimuli, but others free-run in constant conditions under the control of internal physiological pacemakers. The latter are often expressed at periodicities which approximate to those of geophysical variables defined, for example, as circatidal, circadian and circasemilunar rhythms (Palmer, 1995). Such endogenous rhythmicity appears to be a general feature amongst eukaryotes (Brady, 1982) and most evidence suggests that it is innate and therefore a true genetic adaptation (Naylor, 1989).

Circatidal and circadian rhythmicity of the behaviour and physiology of many mobile intertidal animals are well documented (see Naylor, 1988 for a review). Many chronobiological studies have included work on the rhythmic foraging activity of intertidal grazers and predators (Naylor, 1958; Zann, 1973; Petpiroon & Morgan, 1983). This temporal organisation of foraging activity of intertidal animals is adaptively synchronised with major cyclical environmental variables including photoperiod and tidal cycles, providing optimal resource utilization and stress avoidance (Naylor, 1988, 1989). In the intertidal zone of rocky shores slow-moving herbivorous gastropods, such as limpets, adopt a pattern of exploitation of this resource-rich environment involving rhythmic bursts of grazing around their home scars to which they return after every excursion. Evidence of such rhythmic activity recorded in the field in various species of limpets is long established (Orton, 1929; Branch, 1971; Hartnoll & Wright, 1977; Branch, 1981; Little & Stirling, 1985; Little, 1989).

Extensive recent studies have reported activity of the limpet *Patella vulgata* under various field conditions including nocturnal low tide (Cook *et al.*, 1969; Little *et al.*, 1988) during daytime high tide (Gray & Naylor, 1996) or during immersion (Hawkins & Hartnoll, 1983; Gray & Naylor, 1996). Whilst several of these studies speculate upon whether limpet foraging is driven by a biological clock, apart from some rather equivocal experiments (Funke, 1968) there is, so far, only one published piece of work which indicates that the rhythmic foraging activity of limpets recorded in the field is endogenously controlled (Della Santina & Naylor, 1993). Furthermore, clear endogenous rhythms have been reported in only a few species of intertidal molluscs including both bivalves (Beentjes & Williams, 1986; Akumfi & Naylor, 1987) and gastropods (Zann, 1973; Petpiroon & Morgan, 1983), possibly due to experimental difficulties in trying to work with these animals in the laboratory. However, Della Santina & Naylor (1993) succeeded in working with *P. vulgata* in the laboratory using a continuous fine spray of sea water during times when limpets were emersed.

Helcion pectunculus forages during nocturnal low tides and during daytime low tides whilst in the shade (chapter 5). Whether the foraging activity exhibited by this high shore limpet is controlled by an internal clock or purely by exogenous stimuli such as light levels and tidal phase is not known. The present work, therefore, aimed to establish whether the rhythms of locomotor activity exhibited by *Helcion pectunculus* in the field have an endogenous component and if so, which of the many external environmental variables present within the intertidal zone re-entrains the rhythm and so acts as zeitgeber (time-giver) to the limpet.

6.2 Materials & Methods

6.2.1 Collection and maintenance of study animals

Prior to each experiment, twenty freshly collected limpets (shell length approximately 20 mm) were transferred, with minimal disturbance, on their natural substrata (*i.e.* boulders with their home scars) to the laboratory within 1 hour of collection. All limpets used during experiments were collected from Port Alfred (33°36'S/26°54'E). They were placed in different combinations of light and tidal regimes whilst temperature (25°C ± 1°C) and relative humidity (90%) remained constant. The temperature was kept at 25°C throughout the study as this was calculated to be the mean annual temperature experienced by limpets during diurnal low tides in the field. Both the limpets and their respective home scars were marked and they were observed every hour using dim red light. Any limpet found away from its home scar was recorded as active.

Certain experiments required the animals to experience a light regime of continuous darkness and so the constant environment room used throughout this study was equipped with a light trap to prevent any light from entering the room as the door was opened or closed. As *H.pectunculus* is a high shore animal, which experiences long periods of dry conditions and exposure, the fine spray of sea water used by Della Santina & Naylor (1993) to maintain *P. vulgata* was not needed. The high relative humidity in the constant environment room also prevented the animals from drying out too quickly. When an experiment required the animals to be immersed in sea water for any length of time, freshly collected water from Port Alfred was used (salinity 35 ‰). Once water had been used, it was discarded and fresh water was collected. The animals were not given food directly but were found to graze on algae present on their home rocks.

6.2.2 Preliminary study; choice of light regime:

A preliminary study was carried out to determine whether red light had any effect on the activity of limpets maintained under constant conditions. Field work had revealed that dim red light had no effect on limpet foraging behaviour whilst white light caused limpets to clamp down and cease all locomotor activity (chapter 5). Twenty limpets were maintained under the conditions described in section 6.2.1 but instead of continuous darkness, continuous dim red light was used. The limpets were constantly exposed for 72 hours and activity was recorded at hourly intervals. A further twenty limpets were then subjected to exactly the same conditions but instead of continuous dim red light, they experienced continuous darkness and a dim red light was used to observe the limpets every hour.

The results of these two experiments were analyzed and it was found that both methods yielded the same result. It was therefore decided to adopt the continuous darkness light regime as this most closely related to the conditions experienced by limpets during their usual foraging periods on the shore.

6.2.3 Continuous darkness and continuous exposure:

To determine whether *H. pectunculus* exhibits a rhythm of locomotor activity when placed in isolation from day/night cycles, tidal cycles and daily temperature fluctuations, twenty freshly collected limpets (date of collection: 25/2/94) were maintained under the conditions detailed in section 6.2.1. The animals were exposed for a period of 72 hours under conditions of constant darkness. The number of limpets active (out of 20) was recorded every hour throughout the experiment.

To isolate possible exogenous entrainment factors for the rhythm exhibited by *H. pectunculus* under constant conditions, further experiments were carried out. The first set of experiments were designed to determine whether the rhythm of activity could still be distinguished whilst limpets were constantly immersed (section 6.2.4), and if not, whether the rhythm was re-established upon re-exposure *i.e.* does the internal clock of the limpet continue running even if locomotor activity is inhibited?

6.2.4 Continuous darkness, immersion then exposure:

Twenty freshly collected limpets (date of collection: 10/1/95) were kept in a constant environment (see 6.2.1.). After a period of 24 hours immersion, the animals were exposed to air, for 24 hours, beginning at a time in phase with the time of expected ebbing tide. Recordings of limpet activity were made at hourly intervals throughout the experiment.

6.2.5 Continuous darkness, exposure then immersion:

Twenty freshly collected limpets (date of collection: 10/1/95) were also initially exposed to moist air for a period of 24 hours and subsequently immersed in sea water for a further 24 hours. Constant temperature and humidity were maintained as in 6.2.1. Recordings of limpet activity were made at hourly intervals throughout the experiment.

To determine whether the time of exposure of the limpets to air is the exogenous factor which re-entrains the endogenous rhythm, experiments were carried out in which the time of expected low tide was shifted. By moving the time of exposure to air forward by approximately six hours the rhythm should also undergo a "phase shift" and all peaks of activity should also be shifted forward by 6 hours.

6.2.6 Continuous darkness, phase shift:

In a further experiment, 20 freshly collected limpets (date of collection: 10/1/95) were subjected to 24 hours of immersion during which time they were kept under constant conditions (see 6.2.1) followed by a 24 hour period of exposure beginning at a time when on the shore they would have experienced submergence. Limpet activity was recorded at hourly intervals throughout.

Light is known to inhibit foraging activity in herbivorous grazers on the shore (Gray & Hodgson, in press). The following experiment tested whether light also inhibits the endogenous rhythm of locomotor activity exhibited by *H. pectunculus*.

6.2.7 Continuous light, exposure then immersion:

Twenty freshly collected limpets (date of collection: 25/1/95) were maintained in constant light (1500 $\mu E.m^{-2}.s^{-1}$) and continuous exposure for 24 hours. They were then submerged at a time corresponding to submergence on the shore for a further 24 hours. All other conditions were kept constant as detailed in section 6.2.1. Limpet activity was recorded at hourly intervals throughout. A further twenty limpets were collected (25/1/95) and were maintained in constant light and continuous submergence for 24 hours and then exposed at a time corresponding to the ebbing tide on the shore for a further 24 hours. All other conditions were kept constant as described in section 6.2.1. Limpet activity was recorded at hourly intervals throughout.

6.2.9 Statistical Analysis:

There are several sophisticated statistical techniques which attempt to decipher the noisy data of tidal rhythms (Palmer, 1995). Each has its strengths and weaknesses. The chief programs most widely used at present are: periodogram analysis (Whittaker & Robinson, 1944; modified by Williams & Naylor, 1978); autocorrelation; Biomedical & Dental Package, Univariate and Bivariate Spectral Analysis (BMDP-1T); and MESA (Maximum Entropy Spectral Analysis).

These methods were reviewed and tested on a number of "tidal models" by Palmer (1995). The results indicate that MESA, employed in combination with autocorrelation is a powerful tool for finding periodicities and testing their significance. MESA is a spectral analysis technique that applies an autoregressive model to the data vector, and subsequently extracts information on rhythmicity by Fourier analysis (Ables, 1974; Dowse & Ringo, 1989). MESA is a relative newcomer to chronobiology, and has proven itself to be very useful (Palmer, 1995). It has a tendency to produce low-amplitude, near-submultiples, but their reality is easily checked with autocorrelation. In addition to its accuracy, it is unquestionably the easiest to read of the many time-series analysis programs that are available. MESA is an excellent way to examine noisy data. The program does not indicate the significance of periods it finds, but the more power it indicates (*i.e.*, the greater the amplitude of its spikes), the more likely the importance of the spikes. In practice, it is always employed in combination with autocorrelation. The copy of MESA used during this study was obtained via e-mail from Professor Harold. B. Dowse, Department of Zoology, University of Maine, U.S.A. (dowse@maine.maine.edu for more information).

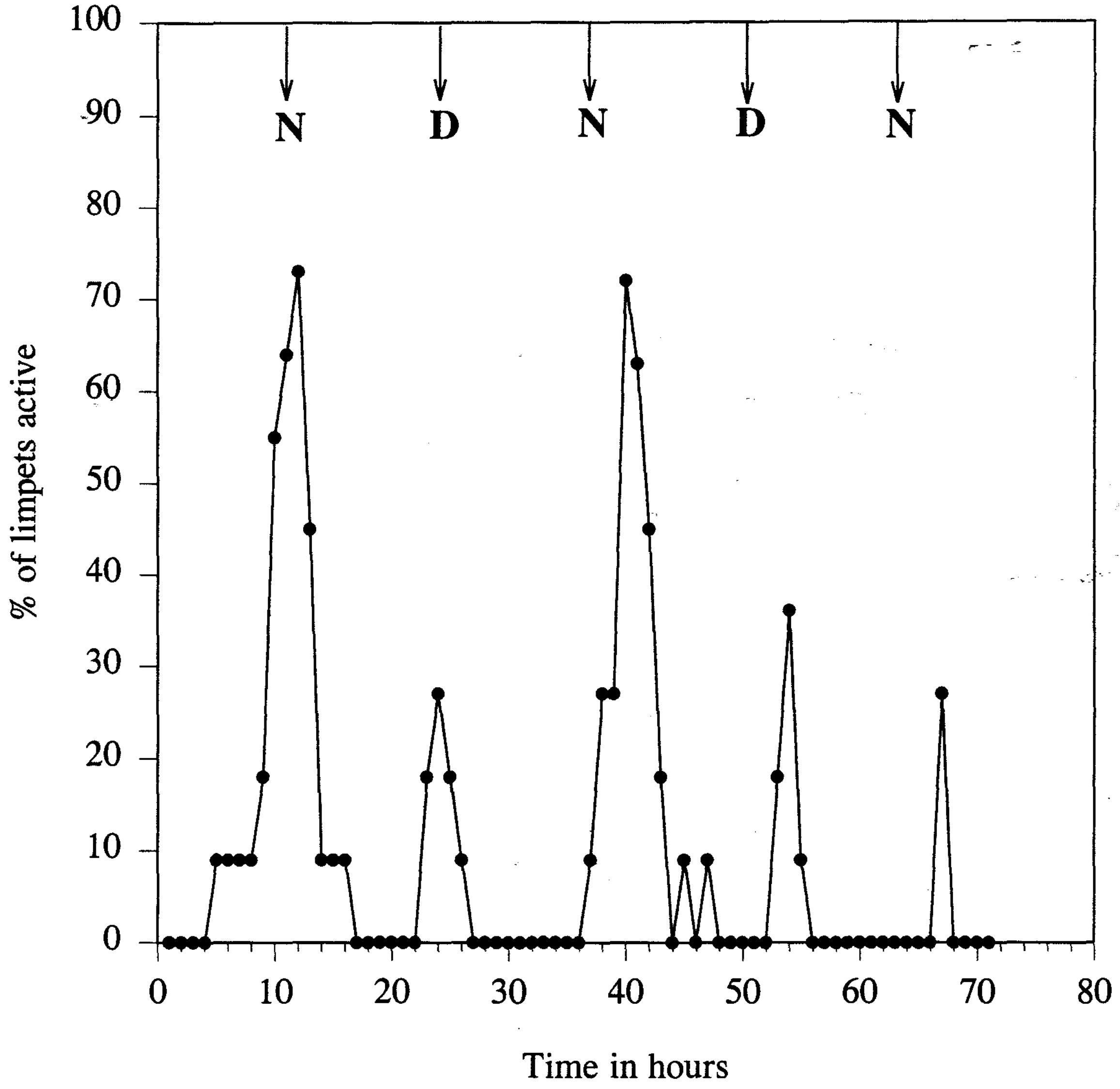
The tests used during this study were periodogram analysis plus MESA in conjunction with autocorrelation. Two tests were used to determine the exact periodicity of any rhythm present and to separate circatidal components from circadian components of the data.

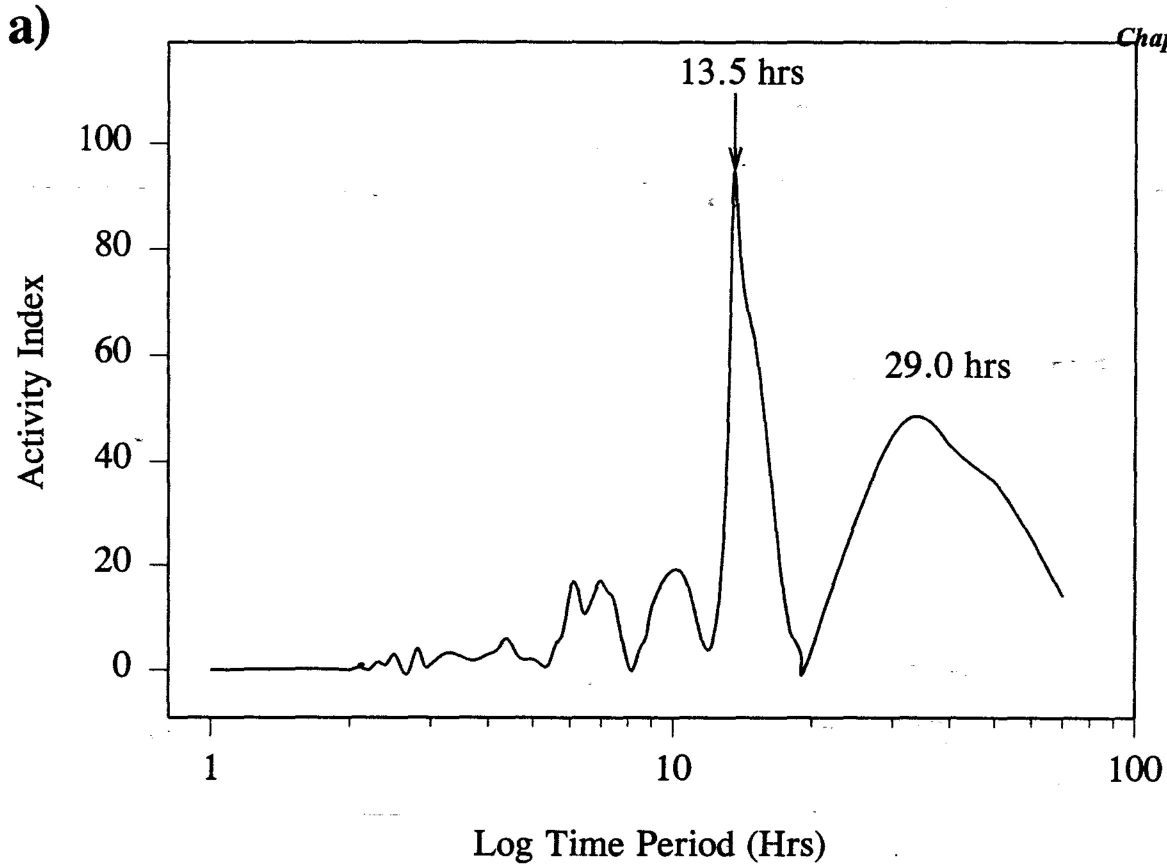
6.3 Results

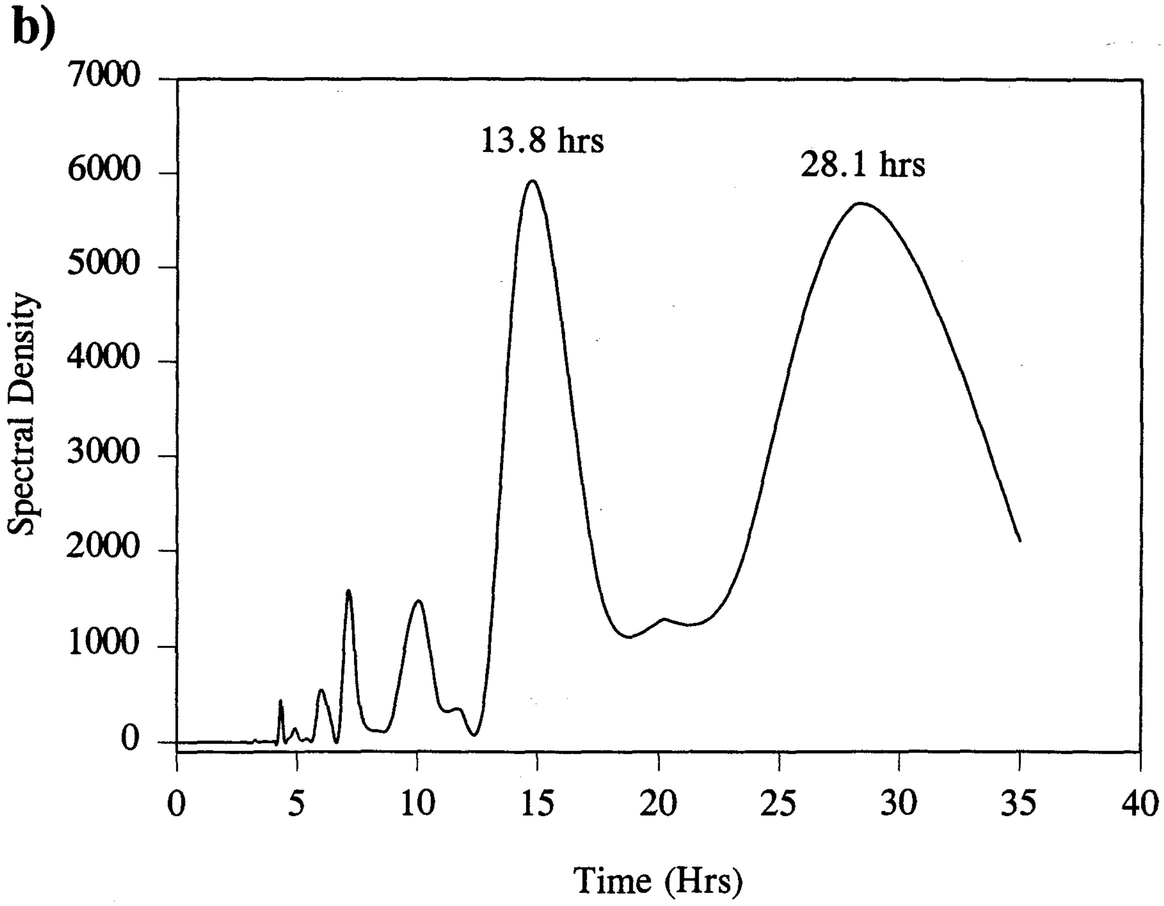
6.3.1 Continuous darkness and exposure:

Figure 6.1 illustrates the percentage of limpets which were active every hour throughout 3 days of continuous observation under conditions of constant darkness and exposure. Activity was found to be rhythmical throughout the experiment. Most limpets were active during times of expected low tide at night with smaller peaks of activity occurring during times of expected low tides during the day. This indicates the presence of both circatidal and circadian components of endogenous locomotor activity.

A periodogram analysis of this activity data (figure 6.2a) shows a definite activity peak occurring at 13.5 hours signifying a circatidal component with a further peak of activity


100
90
80
70
60
50
40
30
20
10
0
% of limpets active
N
D
N
D
N
0
10
20
30
40
50
60
70
80
Time in hours


a)
13.5 hrs
29.0 hrs
Activity Index
100
80
60
40
20
0
1
10
100
Log Time Period (Hrs)


b)
13.8 hrs
28.1 hrs
Spectral Density
7000
6000
5000
4000
3000
2000
1000
0
0
5
10
15
20
25
30
35
40
Time (Hrs)

at about 29 hours, but due to the log scale this is difficult to verify. Figure 6.2b shows the results of the MESA test and two peaks are present, one at 13.8 hours and the other at 28.1 hours. Autocorrelation (figure 6.3) shows that peaks do occur at 13.5 hours and 28.1 hours and that the peaks at these times are statistically significant, passing above the 95% confidence limits. It is also interesting to note that the rhythm of locomotor activity exhibited by *Helcion pectunculus* becomes supressed with time. After about 60 hours the peaks of activity are not as distinct as previously observed.

6.3.2 Continuous darkness, immersion then exposure:

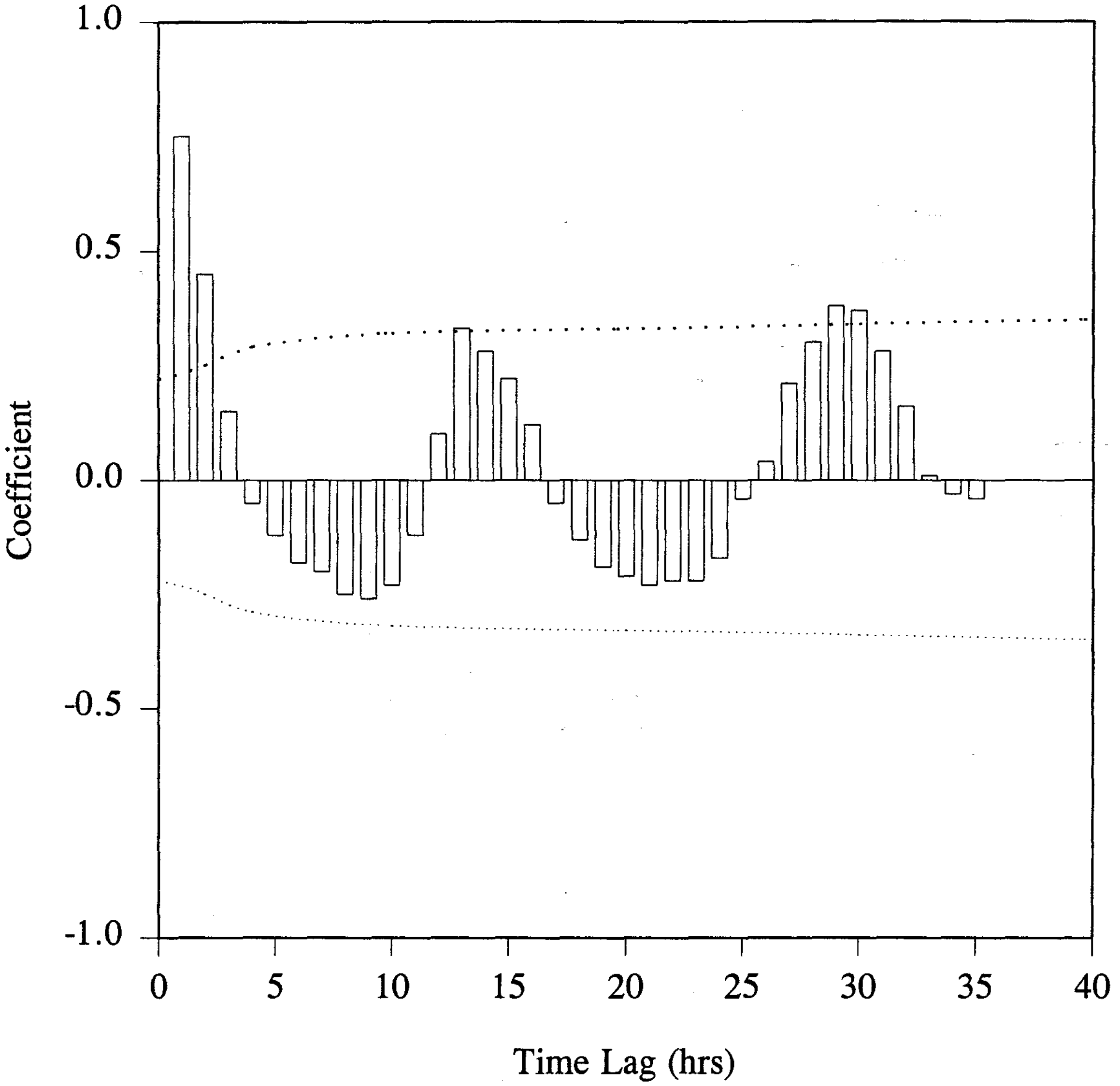
Foraging activity whilst immersed was virtually suppressed, with only 10-20% of the limpets being active around times of expected low tide (figure 6.4a). However, after exposure, foraging activity showed peaks (70% of limpets active) in synchrony with the expected time of the ebbing tide.

6.3.3 Continuous darkness, exposure then immersion:

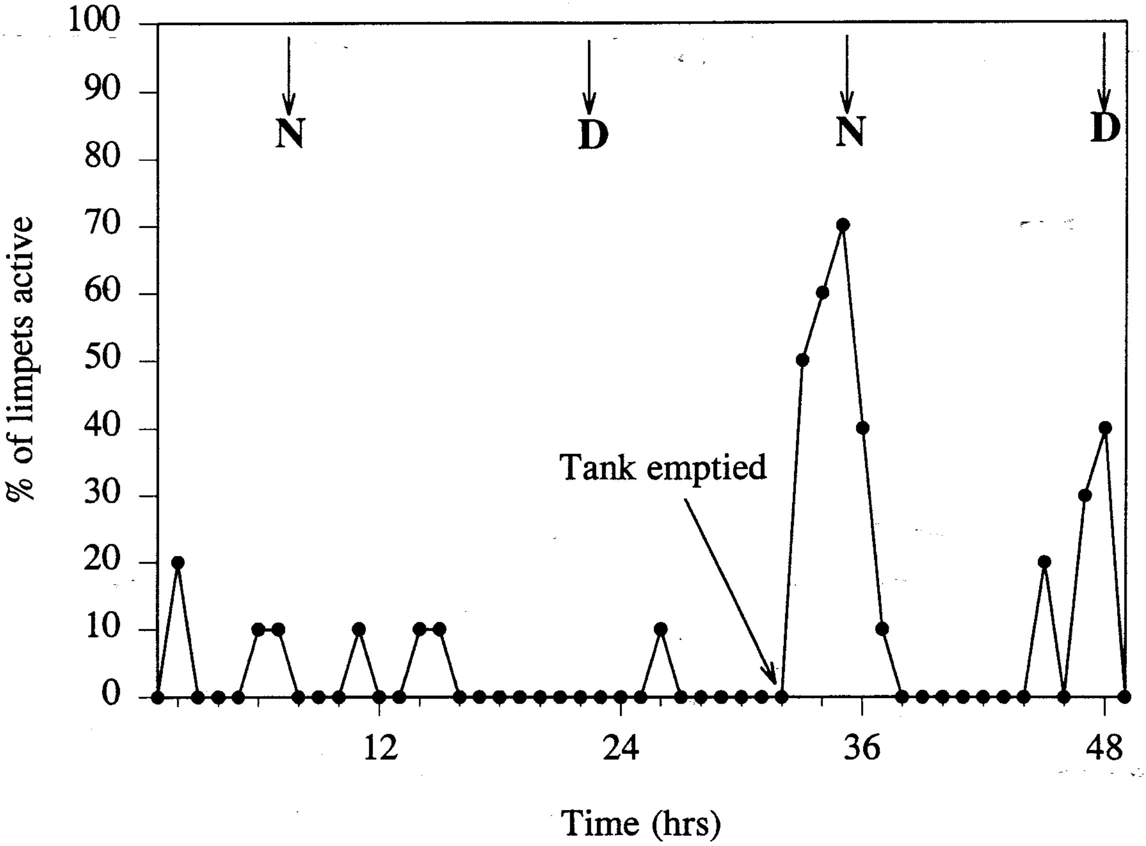
During constant exposure for 24 hours there were peaks in limpet activity at the times of expected low tides (80%). Once continuously immersed, the limpets displayed little activity (10-20%) (figure 6.4b).

6.3.4 Continuous darkness, phase shift:

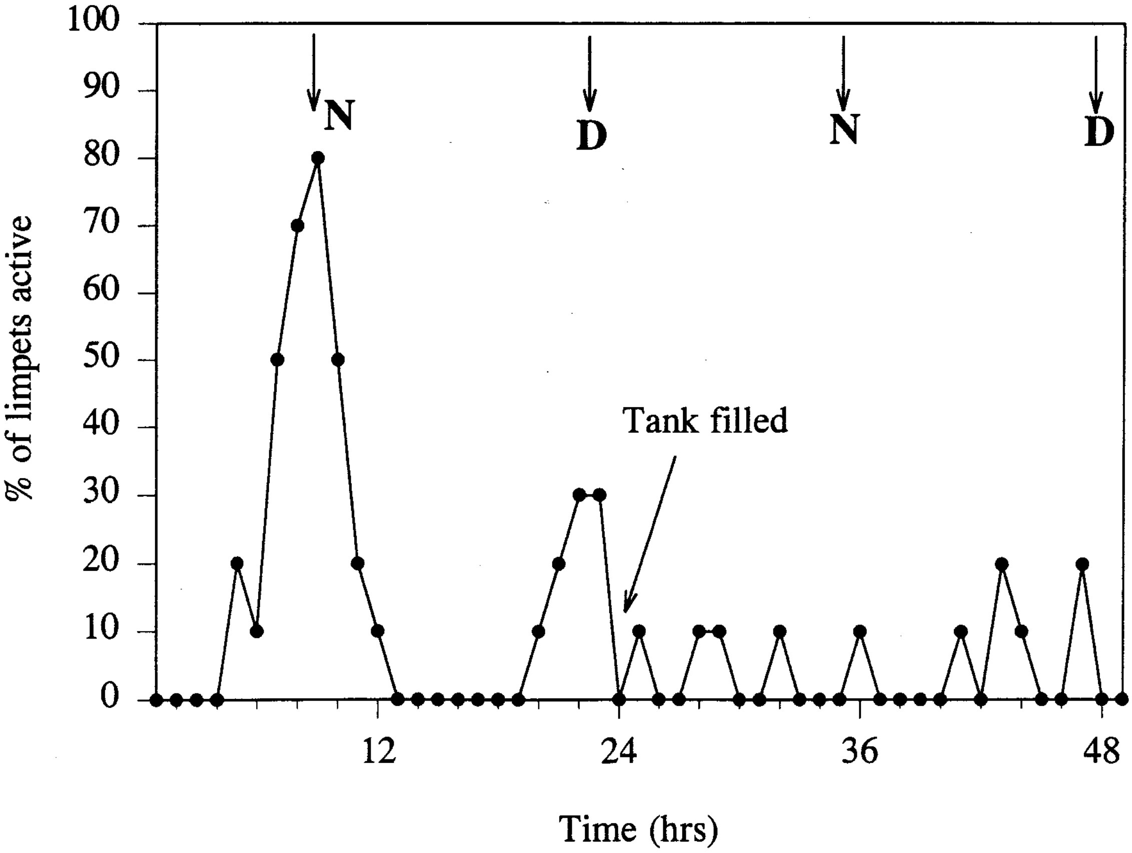
Whilst submerged, the limpets displayed very little activity. However, upon exposure, 70% of the limpets became active (figure 6.5). Also, the subsequent circatidal peaks of locomotor activity showed slight phase advances (approximately 6 hours) in relation to times


1.0
0.5
0.0
-0.5
-1.0
Coefficient
0
5
10
15
20
25
30
35
40
Time Lag (hrs)

a)


N
D
N
D
Tank emptied
% of limpets active
0
10
20
30
40
50
60
70
80
90
100
12
24
36
48
Time (hrs)

b)


N
D
N
D
Tank filled
% of limpets active
0
10
20
30
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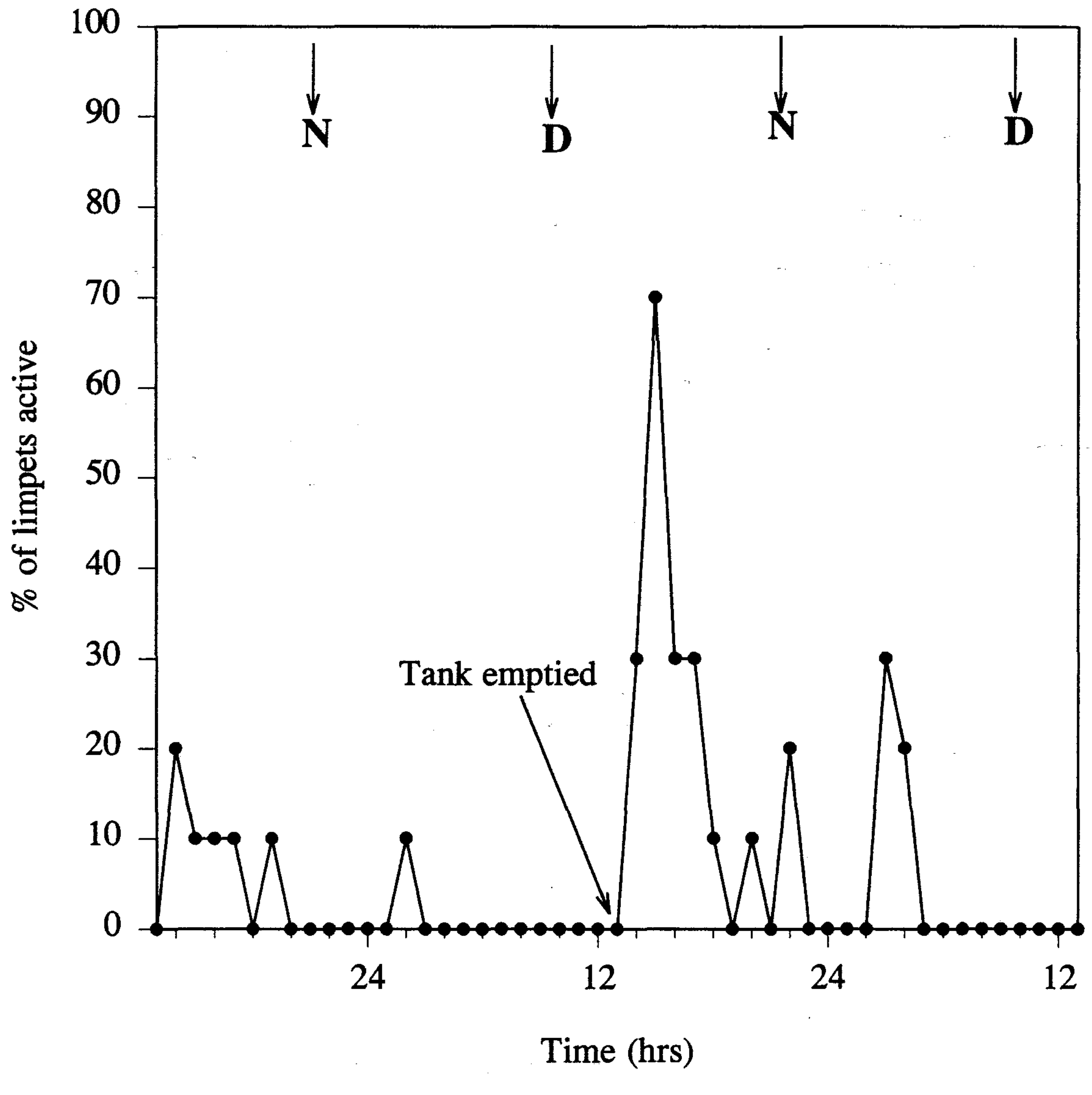
90
100
12
24
36
48
Time (hrs)


100
90
80
70
60
50
40
30
20
10
0
% of limpets active
N
D
N
D
Tank emptied
24
12
24
12
Time (hrs)

of expected low tide.

6.3.5 Continuous light, exposure then immersion:

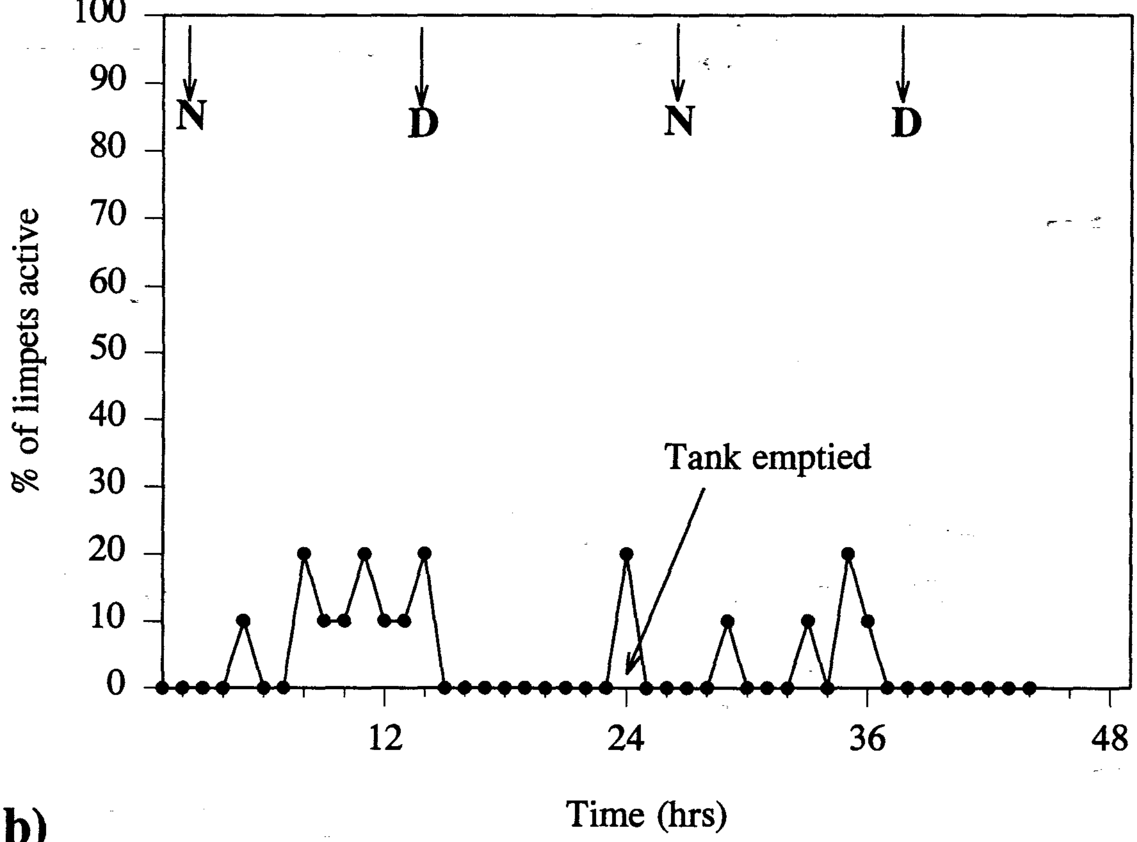
Limpets kept in constant light showed that foraging activity around times of expected low tide times was suppressed significantly, whilst exposed (10-20%) (figure 6.6a). The same results were obtained for limpets kept in constant light and continuously immersed for 24 hours and then exposed (figure 6.6b), significant suppression of locomotor activity *i.e.* no more than 10-20% of the limpets were ever active.

6.4 Discussion

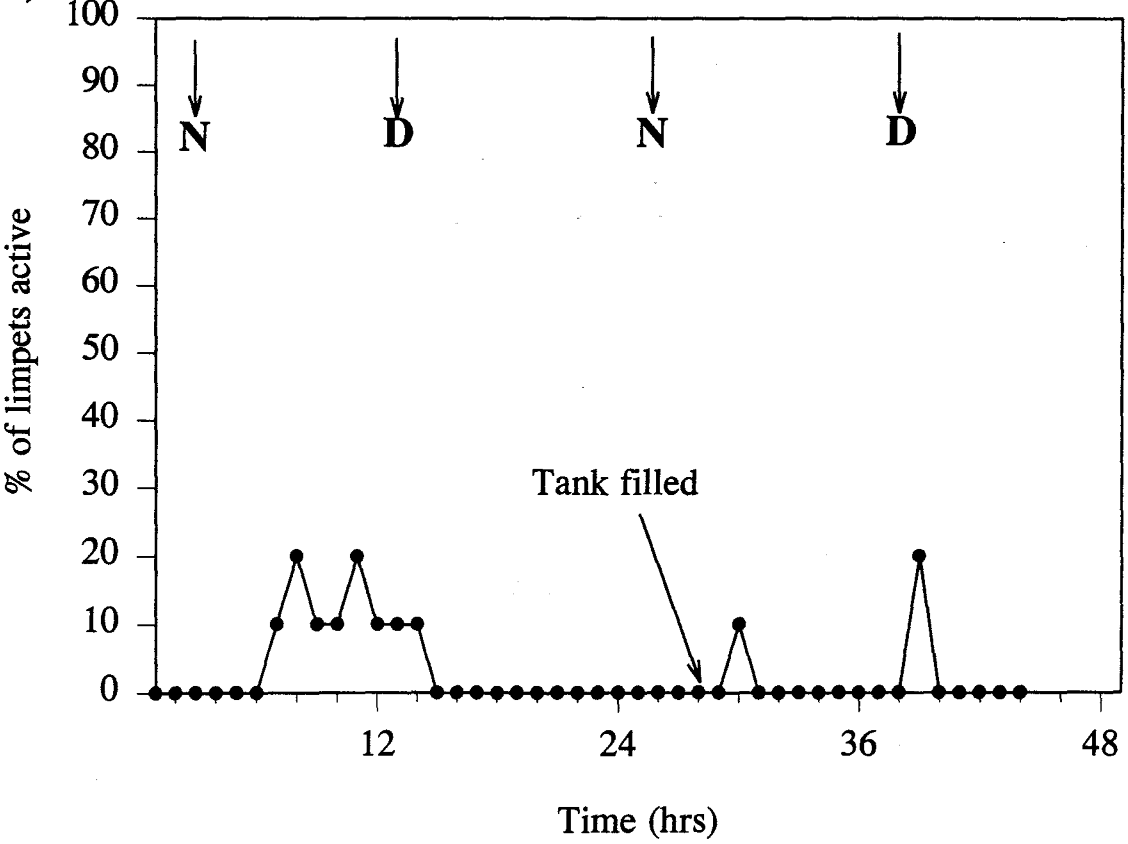
Casual observations on marine shores with extensive tidal oscillations suggest that many animals are active at one phase of the tidal cycle and inactive at the antiphase (Naylor, 1988). The European shore crab *Carcinus maenas*, which remains inactive beneath stones at low tide, has been observed by divers to forage actively between tidemarks at high tide (Naylor, 1958). In contrast, the North American fiddler crab *Uca pugilator* is active mainly at low tide and retires to a burrow at high tide (Barnwell, 1968). The field observations of these two crabs have been confirmed by laboratory studies which indicate that their respective tidally-rhythmic behaviour patterns are biological clock-controlled (Naylor, 1958; Barnwell, 1968; Atkinson & Naylor, 1973).

The simplest hypothesis to explain the rhythmic behaviour in intertidal animals is that they respond to tidal and daily variables, and this appears to be the case for some sessile species such as barnacles (Sommer, 1972) and mussels (Akumfi & Naylor, 1987). In *Mytilus*

a)


100
90
80
70
60
50
40
30
20
10
0
% of limpets active
N
D
N
D
Tank emptied
12
24
36
48
Time (hrs)

b)


100
90
80
70
60
50
40
30
20
10
0
% of limpets active
N
D
N
D
Tank filled
12
24
36
48
Time (hrs)

edulis, kept continuously immersed in sea water in constant conditions in the laboratory, there are spontaneous changes in shell gape (Akumfi & Naylor, 1987), but these changes are not circatidal and appear largely random, with only a very weak circadian component.

In contrast, it has been found that many mobile species exhibit tidally, daily or lunar-phased locomotor activity patterns which are often highly persistent without reinforcement in constant conditions in the laboratory *e.g. Talitrus saltator* (Bregazzi & Naylor, 1972). This certainly appears to be the case for *H. pectunculus* which exhibited a free-running rhythm of locomotor activity with both circatidal and circadian components.

It has been suggested that endogenous locomotor activity patterns may, in some circumstances, play a role in achieving avoidance of unfavourable environmental conditions (Naylor, 1988). The endogenous rhythm exhibited by *Helcion pectunculus* seems to support this hypothesis. The circatidal component of the rhythm ensures that the individual is only active at low tide and also gives the limpets the ability to predict the time of immersion on the flowing tide, thus preventing the animal from being active whilst being immersed. As the tenacity of *H. pectunculus* is particularly weak (chapter 7), this rhythm would aid in preventing the limpets from being swept off the rock surface by wave activity. A further benefit of this rhythm would be the avoidance of predators which feed during high tide. A study of the feeding habits of the giant clingfish, *Chorisochismus dentex* (Stobbs, 1980) showed that *H. pectunculus* made up a substantial part of this fish's diet. It would therefore be preferential for *H. pectunculus* to be active during low tides to avoid this efficient predator.

The circadian component gives the individual information regarding the light and dark cycle. This enables the limpet to be able to gauge its foraging excursions so that it forages for longer and further during nocturnal low tides. Thus, the limpet limits its activity to times when environmental conditions are optimal. Light also seems to inhibit foraging activity in *H. pectunculus* in the laboratory as well as in the field (chapter 5). This suggests that a hierarchy of exogenous factors may well control the endogenous rhythm of activity which is coupled to an internal clock which continues to run regardless, thus allowing the limpet to forage at the right time as soon as the opportunity arises.

The results of this study agree with those found by Della Santina & Naylor (1993) for *Patella vulgata*. The population of *P. vulgata* studied by Della Santina and Naylor exhibits the same activity pattern as *H. pectunculus* and so it is possible that the same driving factors are acting on both species. It is also apparent that in both species the exogenous entrainment factor of the endogenous free running rhythm is the time of exposure to air, although light above a threshold intensity inhibits locomotor activity in *H. pectunculus* (chapter 7). This may also be the case for *P. vulgata* but as yet, has not been proven. It is also interesting that *P. vulgata* also exhibited supression of the exhibited rhythm of locomotor activity after a prolonged length of time (Della Santina & Naylor, 1993), a phenomenon observed in *H. pectunculus* during this study. This is probably due to the lack of any zeitgeber being present, thus the animals were unable to re-entrain the rhythm . The individuals' rhythms become out of phase with one another and so locomotor activity becomes less rhythmic (Palmer, 1995).

The endogenous rhythms of *H. pectunculus* appear to be correlated with the avoidance of unfavourable foraging conditions, and also gives the individual an anticipatory capacity allowing prediction of certain rhythmic influences such as the time of immersion.

6.5 Summary

1. *Helcion pectunculus* exhibits a free-running endogenous rhythm of locomotor activity with both circadian and circatidal components and it is suggested that this rhythm plays a role in allowing individuals to avoid unfavourable environmental conditions.
2. The exogenous entrainment factor of this endogenous rhythm in *H. pectunculus* is the time of exposure to air.
3. Light, above a threshold intensity, may well inhibit locomotor activity in *Helcion pectunculus*.

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Chapter 7:

The importance of the crevice environment in the biology and ecology of *Helcion pectunculus*

7.1 Introduction

A number of physical factors influence the ecology of intertidal organisms (Moore, 1972; Wolcott, 1973; Branch, 1981; McMahon, 1990). The upper limits of a species distribution, especially sessile species such as barnacles (Connell, 1961) and algae (Schonbeck & Norton, 1978) are thought to be controlled by temperature and desiccation extremes. The upper limits of limpet distribution have also been shown to be affected by physical extremes (Wolcott, 1973; Newell, 1976, 1979 for reviews; Williams & Morritt, 1995). High shore species, in particular, often experience high temperatures with associated thermal and desiccation stresses (Moore, 1972). Such species usually have increased physiological tolerances (Newell, 1976, 1979). However, numerous species show both morphological and behavioural adaptations to reduce physiological stress. Morphological adaptations include shell ornamentation (Branch & Branch, 1981), alteration of shell shape (Vermeij, 1973) and allometric growth (Branch, 1981). Some limpets have been shown to adapt their behavioural patterns by hiding in refuges *i.e.* cracks and crevices (Williams & Morritt, 1995), sealing their shells with mucus (Garrity, 1984) and positioning their shells to minimise exposure to insolation and maximise evaporative cooling (Garrity, 1984; Williams & Morritt, 1995).

Habitat choice plays a vital role in the survival of many species (Davies, 1969; Wolcott, 1973; Leviten & Kohn, 1980). To ensure prolonged survival, sessile species have to settle in areas of the shore that will provide shelter from physical extremes (Raimondi, 1990). Mobile intertidal organisms, such as gastropods, which use refugia, if prevented from returning to home sites after foraging, desiccate and die (Garrity, 1984; Williams & Morritt, 1995).

Limpets are often the most dominant mobile herbivores on temperate and tropical shores (Branch, 1981). Different species exhibit a wide range of behavioural rhythms, foraging whilst submerged or exposed during the day or night and returning to a fixed scar whilst other species select suitable non-permanent resting sites (Branch, 1981; Little, 1989). It has been hypothesized that wave activity may limit limpet distribution on the shore and that many of the behavioural traits exhibited by limpet species also ensure that the limpets are safe from dislodgement by strong wave activity (Branch & Marsh, 1978). The choice of resting site and period of activity have been suggested to be imposed by physical limitations on the physiological tolerances of the species concerned (Little, 1989), although shelter from predation and access to available food are also important factors.

If a non-homing limpet should settle in the wrong habitat or move at the wrong time of day, then physical factors may exceed the tolerance limits of the species resulting in physiological stress, and often mortality (Wolcott, 1973). Numerous workers have investigated the physiological tolerances of limpets by measuring limpet body temperature, corresponding water loss and ionic concentrations of the haemolymph or extravisceral water (Segal & Dehnel, 1962; Davies, 1970; Wolcott, 1973; Verderber *et al.*, 1983; Lowell, 1984; Branch & Cherry, 1985; Williams & Morritt, 1995). Limpets have been shown to be remarkably resilient to such extremes (Wolcott, 1973) and their physiological limits measured in the laboratory are rarely exceeded under field conditions. The success of *H. pectunculus* in colonizing the high shore may, in part, be due to its behavioural strategy of returning to a crevice refuge during daytime low tides (chapter 5).

This study aimed to investigate the relationship between the high shore South African limpet, *Helcion pectunculus*, and its physical environment. Differences between habitat (*e.g.* crevices and exposed rock surfaces) and limpet body temperatures were monitored during diurnal emersion periods, along with tenacity measurements and these physical parameters are related to the behaviour and distribution of *Helcion pectunculus* on the rocky intertidal shore. Investigations were also carried out into factors affecting the foraging pattern of *H. pectunculus* to determine whether or not this pattern has evolved to reduce thermal stress and desiccation thus allowing this limpet to colonise an extremely inhospitable area of the shore which is relatively free from other grazers.

7.2 Materials & Methods

7.2.1 Size distribution of *Helcion pectunculus* within crevices

During diurnal low tides, *H. pectunculus* resides on a home scar within a crevice. To determine whether *H. pectunculus* are arranged randomly within crevices, 25 crevices, all of which had an approximate depth of 100 mm, were chosen for study at Port Elizabeth in the Eastern Cape. All limpets were removed from each crevice starting at the outer lip and moving towards the back of the crevice. Different "rows" of limpets were recognised and the limpets within these rows had various shell parameters measured including shell length, height and width. Using the Von Bertalanffy growth equation obtained in chapter 3, it was possible to work out the average age of limpets within each row and determine whether there is a gradient of size/age of limpets from the front to the back of a crevice.

7.2.2 The crevice habitat as a refuge

Physical factors within and outside the crevice

In order to determine whether the crevice provides *H. pectunculus* with a stable and buffered environment, air and horizontal (<45°) rock surface temperatures were taken at hourly intervals during periods of emersion on a spring-full moon tide, a neap-quarter moon tide and a spring-new moon tide in spring, summer, autumn and winter using a hand held thermometer (Hanna instruments HI 9040 microcomputer thermometer ± 0.1 °C) both inside and outside of a typical crevice (depth ≈ 100 mm). Relative humidity was also recorded from both inside and outside of the crevice using a Hygrocheck relative humidity probe (± 2%). Readings were taken hourly for both humidity and air and rock surface temperatures. Light intensity (in $\mu E.m^{-2}.s^{-1}$) readings were also taken both within and outside of the crevice using an integrating quantum/ radiometer/ photometer, model LI-188B (Li-cor Inc.).

Rock surface and crevice temperatures were measured by placing the tip of the temperature probe in direct contact with the rock surface. The probe was insulated from the surrounding air using thick (5 cm) polystyrene. Air temperature was measured by placing the temperature probe, unshaded, between 2.0 and 4.0 cm above the rock surface. Humidity measurements were taken by placing the humidity probe into the crevice until it was between 2.0 and 4.0 cm from the back, air humidity readings were taken between 2.0 and 4.0 cm above the rock surface.

Limpet body temperatures

Body temperatures of limpets were measured using a thermocouple inserted onto the foot by pushing between the shell and the rock surface (Wolcott, 1973; Williams & Morritt, 1995). There was no evidence (100% recapture of marked individuals) that inserting a probe under the shell of limpets had any negative effect on them. The body temperatures of marked limpets were measured hourly during emersion on a particularly hot spring-tide day in summer (maximum air temperature = 32°C). Twenty limpets were measured whilst "at home" in their crevice, whilst another twenty were measured having had their crevice destroyed *i.e.* the limpets were fully exposed to sunlight during emersion.

As individual specimens were monitored at hourly intervals during the day, statistical analysis of diurnal changes is inappropriate because readings from individuals are not independent. Diurnal changes are thus presented graphically and statistical analysis was carried out at the hottest part of the day.

Removal of the crevice habitat

To determine whether *H. pectunculus* is able to survive high up on the shore without using a crevice refuge, actively foraging marked individuals (n = 97) were prevented from retreating back to their home scars within crevices, as the tide came in, by filling the crevices with "Styrafoam". The foam, once hard (approximately 1 hour), was filed down and smoothed to follow the contours of the rock and prevent the limpets from finding any form of refuge. At the next low tide, the position of the marked limpets was recorded as *i)* missing, *ii)* adjacent to their home crevice, *iii)* in a new crevice within 1 m distance of their old crevice, *iv)* in a new crevice greater than 1 m but less than 2 m away from old crevice, and *v)* in a new crevice greater than 2 m distance from old crevice. The limpets were observed at every low tide for a week and then at monthly intervals for a period of 6 months.

Effect of shade on foraging behaviour

Helcion pectunculus has been shown to forage during early morning low tides whilst being shaded from direct sunlight *i.e.* residing on west facing rock surfaces (chapter 5). Experimental manipulations were carried out to investigate the effect of shading as a driving factor behind the timing of foraging activity and the threshold light intensity at which foraging activity is inhibited (see chapters 5 & 6). As the tide receded during early morning on a summer spring tide (19/2/96), 20 limpets on east facing rock surfaces, which are usually inactive, were shaded (approximately 2 m^2) with varying degrees of shade netting (10%, 20%, 30% and 50%). Twenty limpets on the west facing rock surfaces, which were usually shaded and active, were subjected to sunlight by reflecting sunlight onto the rock using a large (3 m x 2 m) wooden board covered with aluminium foil (date of study 19/2/96). The number of limpets active every hour was recorded under all conditions. Light intensity readings ($\mu E.m^{-2}.s^{-1}$) were also taken under all experimental conditions.

7.2.3 Tenacity of *H. pectunculus*

The tenacity of 30 limpets, in their natural habitat, was measured using two loops of fishing line slipped under the anterior and posterior edges of the limpet shell and spaced in such a way that force would be equally distributed to the two loops. The fishing line was then attached to a 25 kg Salter spring balance modified to give a permanent record of the maximum force, normal to the substratum, required to detach each limpet. As duration of force effects tenacity, force was exerted for approximately 5 seconds in all cases. As the regression between force of attachment and surface area of foot results in a high r^2 value, it is unlikely that any variation resulting from differing initial pull strengths are important.

Forces required to detach limpets from the substratum were measured whilst 30 animals were on their home scars. No attempt was made to record the force required to detach limpets whilst they were mobile due to the fact that a soon as the initial pull is felt, the limpet clamps down onto the rock and thus a measurement of "mobile tenacity" is not gained. Once detached from the substratum, the limpet was allowed to reattach to a sheet of transparent acetate. Once it had reattached firmly, the foot area was drawn around with water proof marker pen. Once back in the laboratory, the area of the foot could then be acquired by superimposing the acetate sheet onto graph paper and counting the number of squares within the area of the foot.

7.3 Results

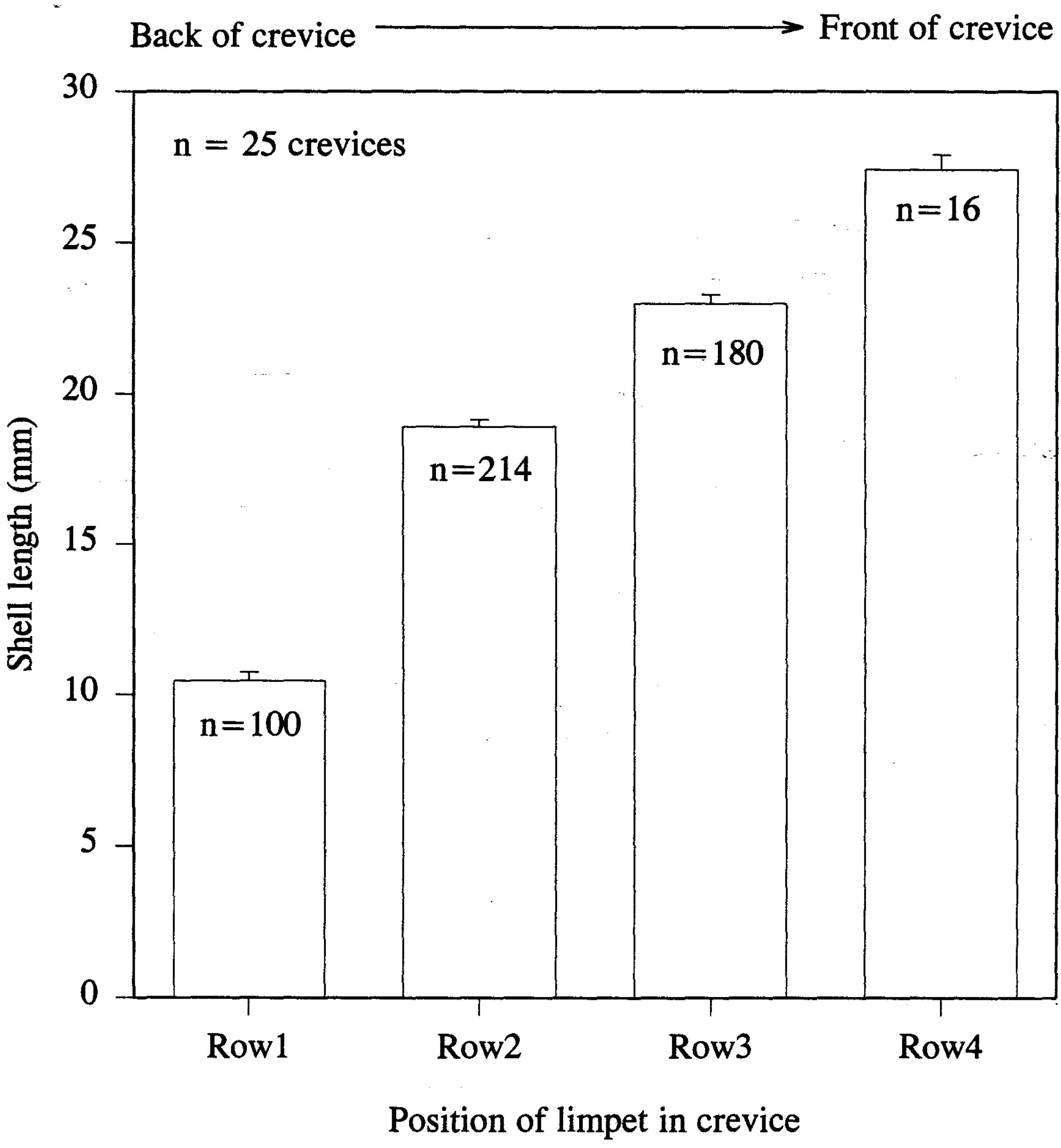
7.3.1 Size distribution of *Helcion pectunculus* within crevices

The smallest individuals ($\bar{x}$ = 11.2 mm shell length) were found at the rear of the crevice (row 1, figure 7.1). Limpets progressively increased in size towards the front of the crevice with the largest animals (≈ 25 mm shell length, figure 7.1) in the outer row (row 4).

7.3.2 The crevice habitat as a refuge

Physical factors within and outside the crevice

During all low tide periods in all seasons, the relative humidity within the crevice refuge was significantly higher (tables 7.1-7.3) than the relative humidity outside of the


Back of crevice
Front of crevice
n = 25 crevices
n=100
n=214
n=180
n=16
Shell length (mm)
0
5
10
15
20
25
30
Row1
Row2
Row3
Row4
Position of limpet in crevice

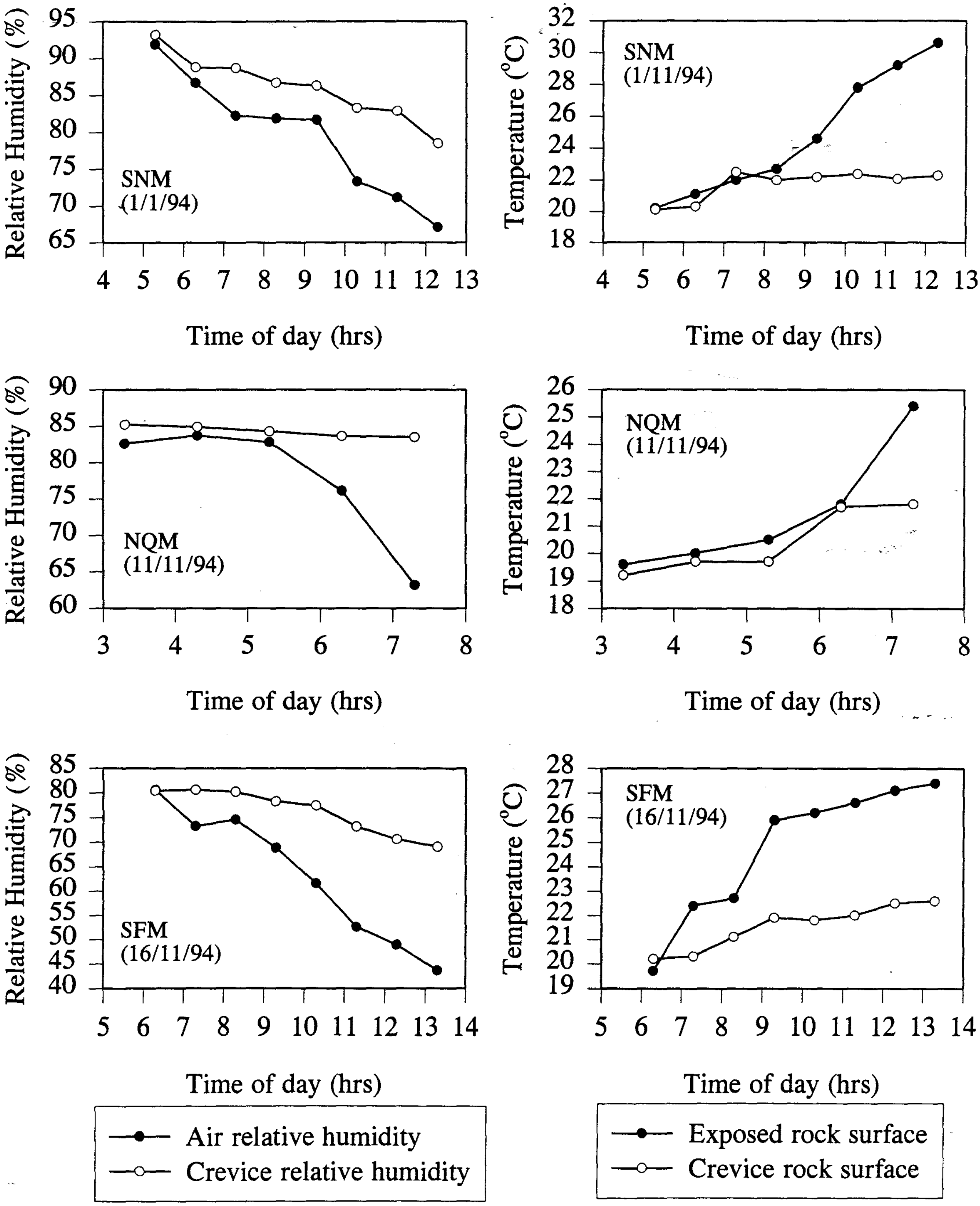
crevice (figures 7.2, 7.3, 7.4 & 7.5). The humidity within the crevice never dropped below 73% whereas outside the crevice the humidity reached as low as 42%. Rock surface temperature was also always found to be significantly lower within crevices compared to exposed rock surfaces (tables 7.4 - 7.6). Rock surface temperatures within the crevice refuge were never recorded above 22°C whist outside the crevice rock surface temperatures of up to 31°C were recorded.

Table 7.1

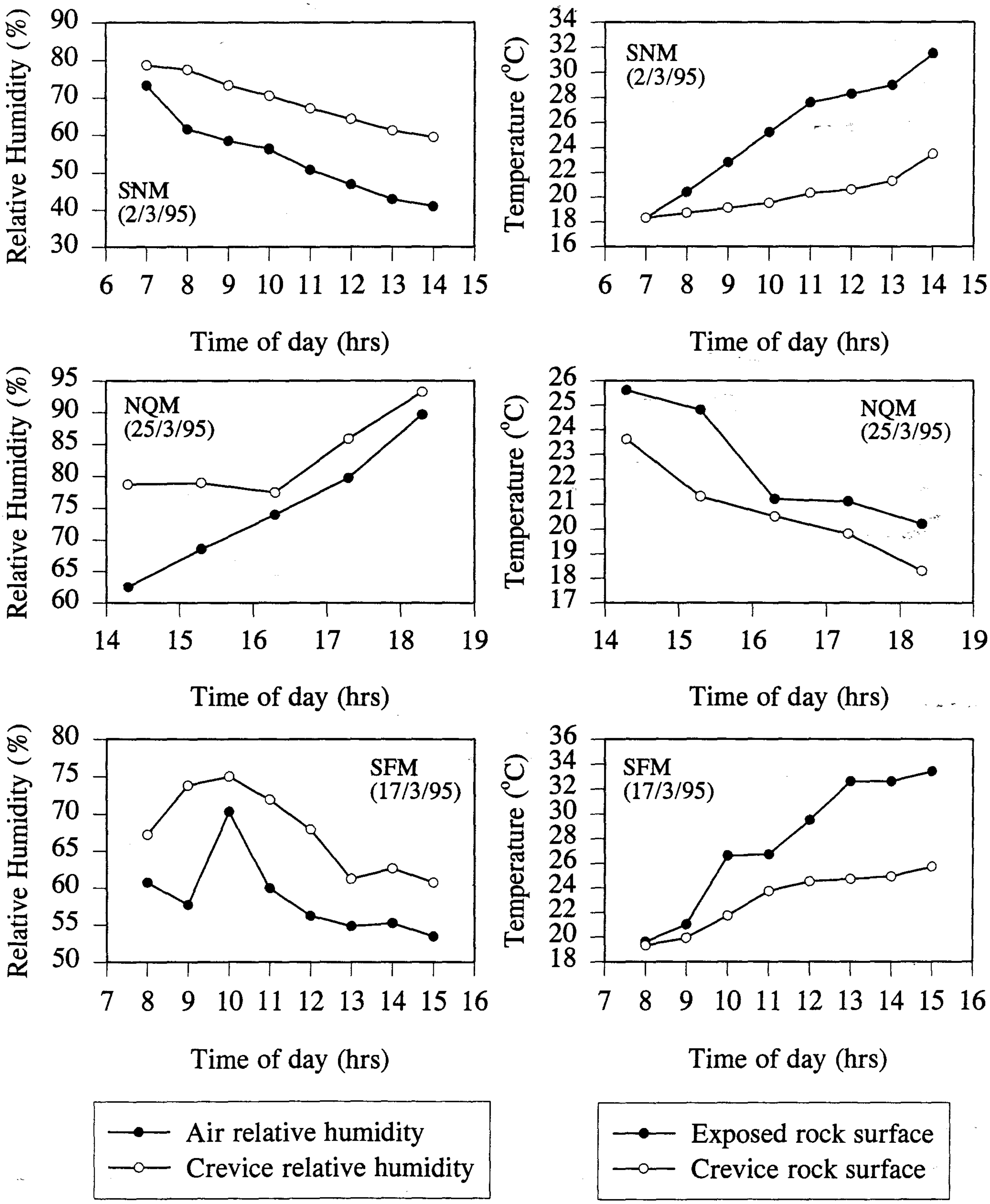
Results of a multiple analysis of variance to determine the effect of microenvironment and season on relative humidity.

Source of variation	SS	d.f.	MS	F-ratio	Sig.level
Main effects					
Micro-environment	2136.079	1	2136.079	26.129	0.0000
Season	9701.750	3	3233.916	39.558	0.0000
Residual	11118.159	136	81.751173		
Total (corrected)	23191.847	143			

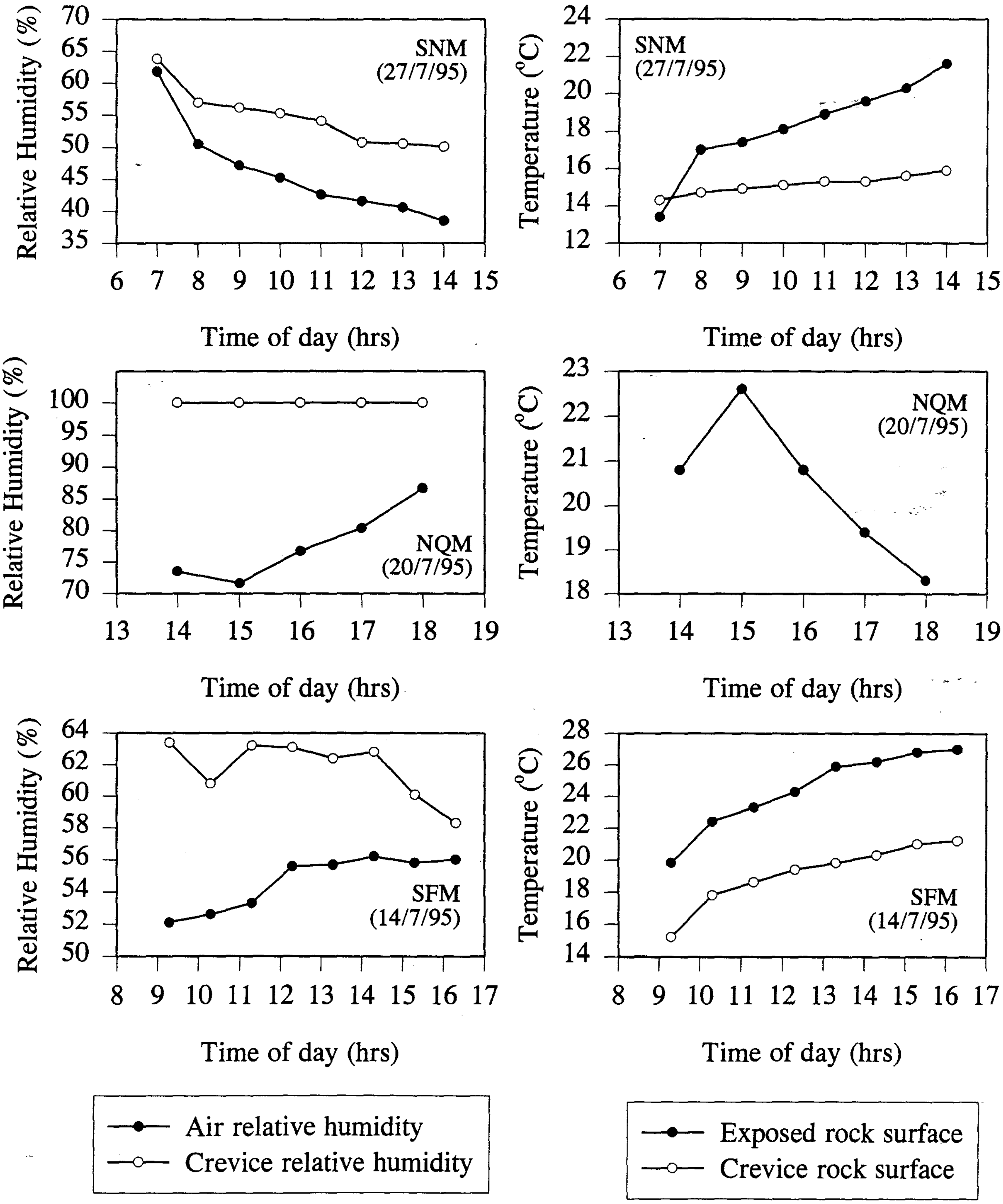
Spring - 1994


Relative Humidity (%)
95
90
85
80
75
70
65
SNM
(1/1/94)
4 5 6 7 8 9 10 11 12 13
Time of day (hrs)
Temperature (°C)
32
30
28
26
24
22
20
18
SNM
(1/11/94)
4 5 6 7 8 9 10 11 12 13
Time of day (hrs)
Relative Humidity (%)
90
85
80
75
70
65
60
NQM
(11/11/94)
3 4 5 6 7 8
Time of day (hrs)
Temperature (°C)
26
25
24
23
22
21
20
19
18
NQM
(11/11/94)
3 4 5 6 7 8
Time of day (hrs)
Relative Humidity (%)
85
80
75
70
65
60
55
50
45
40
SFM
(16/11/94)
5 6 7 8 9 10 11 12 13 14
Time of day (hrs)
Temperature (°C)
28
27
26
25
24
23
22
21
20
19
SFM
(16/11/94)
5 6 7 8 9 10 11 12 13 14
Time of day (hrs)
● Air relative humidity
○ Crevice relative humidity
● Exposed rock surface
○ Crevice rock surface

Summer - 1995


Relative Humidity (%)
90
80
70
60
50
40
30
SNM
(2/3/95)
6 7 8 9 10 11 12 13 14 15
Time of day (hrs)
Temperature (°C)
34
32
30
28
26
24
22
20
18
16
SNM
(2/3/95)
6 7 8 9 10 11 12 13 14 15
Time of day (hrs)
Relative Humidity (%)
95
90
85
80
75
70
65
60
NQM
(25/3/95)
14 15 16 17 18 19
Time of day (hrs)
Temperature (°C)
26
25
24
23
22
21
20
19
18
17
NQM
(25/3/95)
14 15 16 17 18 19
Time of day (hrs)
Relative Humidity (%)
80
75
70
65
60
55
50
SFM
(17/3/95)
7 8 9 10 11 12 13 14 15 16
Time of day (hrs)
Temperature (°C)
36
34
32
30
28
26
24
22
20
18
SFM
(17/3/95)
7 8 9 10 11 12 13 14 15 16
Time of day (hrs)
Air relative humidity
Crevice relative humidity
Exposed rock surface
Crevice rock surface

Autumn - 1995


Relative Humidity (%)
70
65
60
55
50
45
40
35
6 7 8 9 10 11 12 13 14 15
Time of day (hrs)
SNM
(27/7/95)
Temperature (°C)
24
22
20
18
16
14
12
6 7 8 9 10 11 12 13 14 15
Time of day (hrs)
SNM
(27/7/95)
Relative Humidity (%)
100
95
90
85
80
75
70
13 14 15 16 17 18 19
Time of day (hrs)
NQM
(20/7/95)
Temperature (°C)
23
22
21
20
19
18
13 14 15 16 17 18 19
Time of day (hrs)
NQM
(20/7/95)
Relative Humidity (%)
64
62
60
58
56
54
52
50
8 9 10 11 12 13 14 15 16 17
Time of day (hrs)
SFM
(14/7/95)
Temperature (°C)
28
26
24
22
20
18
16
14
8 9 10 11 12 13 14 15 16 17
Time of day (hrs)
SFM
(14/7/95)
Air relative humidity
Crevice relative humidity
Exposed rock surface
Crevice rock surface

Winter - 1995

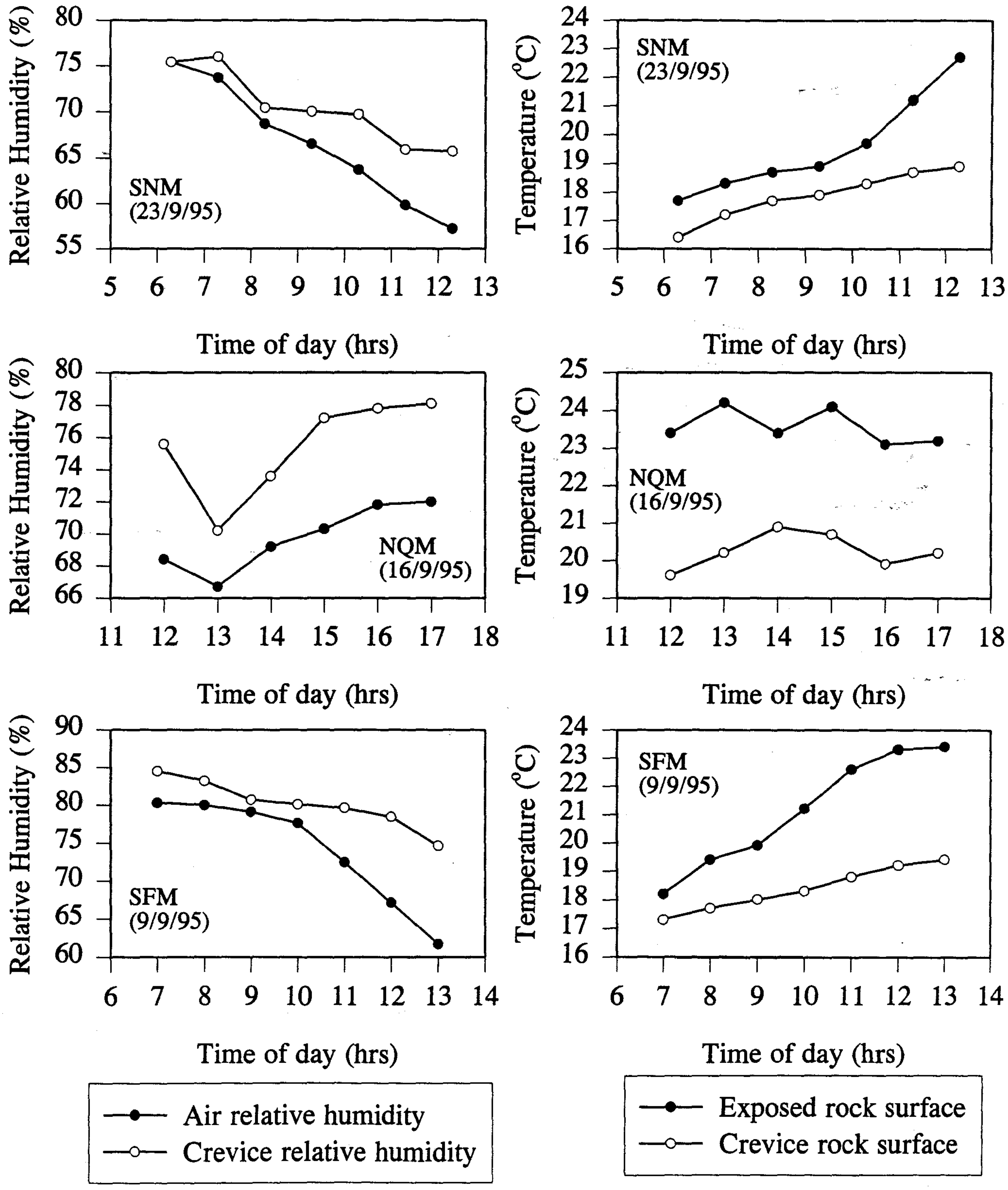

Relative Humidity (%)
80
75
70
65
60
55
SNM
(23/9/95)
5 6 7 8 9 10 11 12 13
Time of day (hrs)
Temperature (°C)
24
23
22
21
20
19
18
17
16
SNM
(23/9/95)
5 6 7 8 9 10 11 12 13
Time of day (hrs)
Relative Humidity (%)
80
78
76
74
72
70
68
66
NQM
(16/9/95)
11 12 13 14 15 16 17 18
Time of day (hrs)
Temperature (°C)
25
24
23
22
21
20
19
NQM
(16/9/95)
11 12 13 14 15 16 17 18
Time of day (hrs)
Relative Humidity (%)
90
85
80
75
70
65
60
SFM
(9/9/95)
6 7 8 9 10 11 12 13 14
Time of day (hrs)
Temperature (°C)
24
23
22
21
20
19
18
17
16
SFM
(9/9/95)
6 7 8 9 10 11 12 13 14
Time of day (hrs)
Air relative humidity
Crevice relative humidity
Exposed rock surface
Crevice rock surface

Table 7.2

Results of a Scheffe's multiple range test to determine any significant differences between relative humidities recorded in the different micro-environments.

Level	Count	LS Mean	Homogeneous Groups
Exposed	72	64.516	X
Crevice	72	72.348	X

Where X is in the same column, no significant difference was found between the treatments/groups

Table 7.3

Results of a Scheffe's multiple range test to determine any significant differences between relative humidity recorded in the four seasons.

Level	Count	LS Mean	Homogeneous Groups
Spring	42	78.238095	X
Summer	38	68.765789	X
Autumn	26	54.138462	X
Winter	38	72.584211	XX

Where X is in the same column, no significant difference was found between the treatments/groups.

The crevice environment always had a higher relative humidity compared to exposed habitats (table 7.2). Relative humidity also varied significantly with season with the highest values being obtained in Spring (78%) and lowest in Autumn (54%).

Table 7.4

Results of a multiple analysis of variance to determine the effect of microenvironment and season on rock surface temperature.

Source of variation	SS	d.f.	MS	F-ratio	Sig.level
Main effects					
Micro-environment	335.73	1	335.73	43.517	<0.0001
Season	609.88	3	203.29	26.350	<0.0001
Residual	1049.24	136	7.71	7.71	
Total (corrected)	1998.76	143			

Table 7.5

Results of a Scheffe's multiple range test to determine any significant differences between rock surface temperatures taken within a crevice and on exposed rock surfaces.

Level	Count	LS Mean	Homogeneous Groups	
Crevice	72	19.798337	X	
Exposed rock	72	22.903865		X

Table 7.6

Results of a Scheffe's multiple range test to determine any significant differences between rock surface temperatures recorded in the four seasons.

Level	Count	LS Mean	Homogeneous Groups	
Autumn	26	18.573077	X	
Winter	38	19.960526	X	
Summer	38	23.418421		X
Spring	42	23.452381		X

Rock surface temperatures were always found to be significantly lower within crevices compared to exposed rock surfaces (table 7.5). Rock surface temperature also differed significantly with season. Higher values being obtained in spring and summer to those of autumn and winter (table 7.6) which is to be expected due to longer sunshine hours and warmer temperatures in general.

Limpet body temperatures

Rock temperatures and limpet body temperatures were significantly cooler (t-statistic = -130.089, $p < 0.001$) by up to 12°C in crevice habitats compared with exposed habitats (figure 7.6). Exposed limpet body temperatures were found to be approximately 2°C lower than the substratum on which they were situated. Body temperatures of limpets located in crevices were found to follow the temperature of the substratum much more closely (figure 7.6).

Removal of the crevice habitat

On returning to their crevice and finding it filled with "styrofoam" limpets actively sought out a new crevice refuge. Of 97 limpets labelled, 33 settled immediately adjacent to their old crevice (table 7.7) whilst 20 found new crevices close by. Forty four limpets were lost during the first high tide period after the crevice was filled. After 1 month, 83 limpets had been lost and after 6 months, 94 of the original 97 limpets were no longer present, 2 limpets still maintained their position adjacent to their old crevice whilst 1 limpet had managed to find a new crevice refuge over 2 metres away from its original "home" crevice.

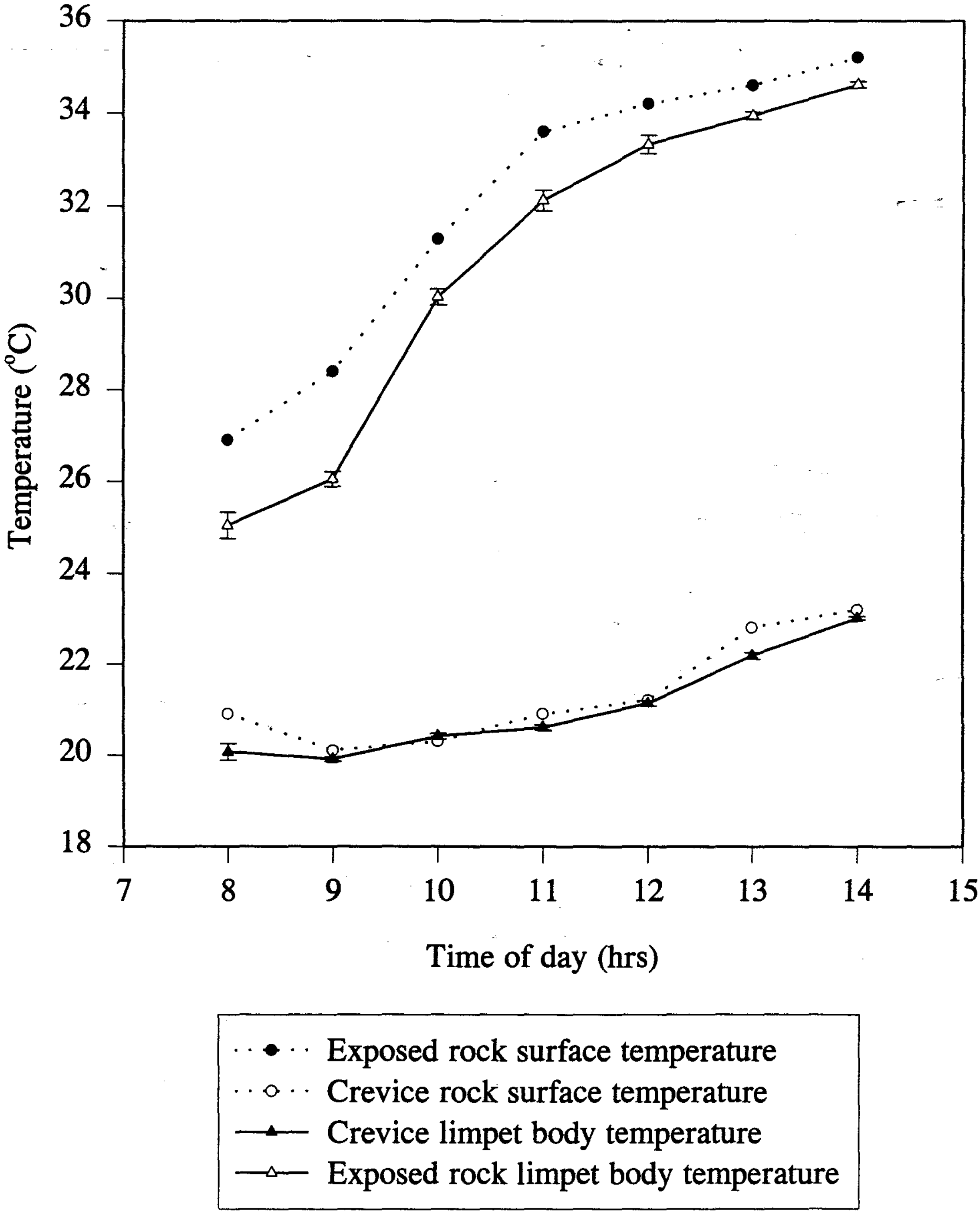

36
34
32
30
28
26
24
22
20
18
Temperature (°C)
7
8
9
10
11
12
13
14
15
Time of day (hrs)
Exposed rock surface temperature
Crevice rock surface temperature
Crevice limpet body temperature
Exposed rock limpet body temperature

Table 7.7

Numbers and location of marked limpets recorded at regular intervals after having removed their crevice refuge whilst they were actively foraging.

Date	No. in crevice	No. immediately adjacent	New Crevice < 1 m	New crevice < 2 m	New crevice > 2 m	Gone
18/2/96	97	0	0	0	0	0
19/2/96	0	33	20	0	0	44
20/2/96	0	17	18	1	0	61
21/2/96	0	11	23	1	0	62
22/2/96	0	11	9	1	1	75
23/2/96	0	11	6	1	1	78
24/2/96	0	11	3	1	2	80
1 month	0	11	0	1	2	83
3 months	0	4	0	0	2	91
6 months	0	2	0	0	1	94

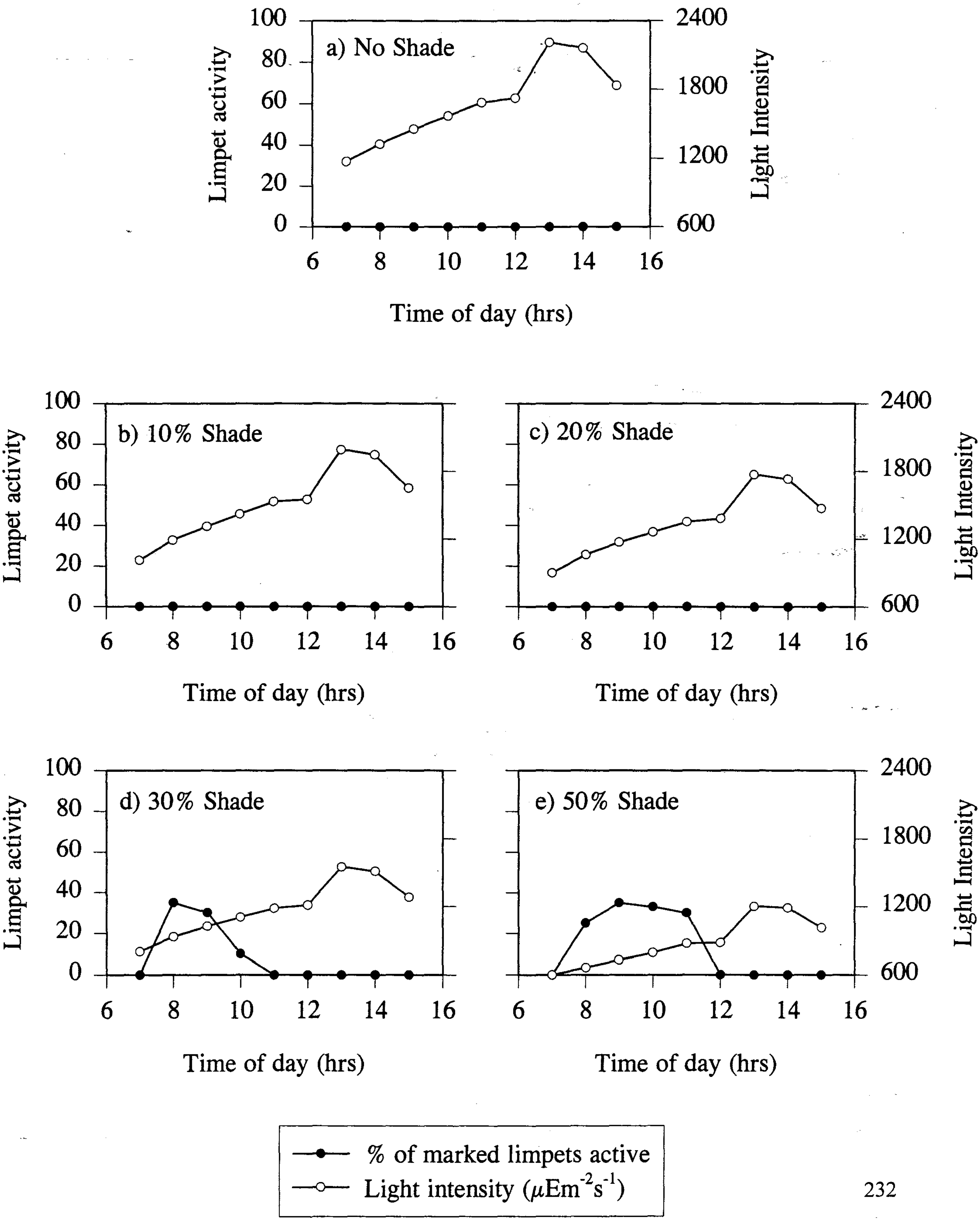
Effect of shade on foraging behaviour

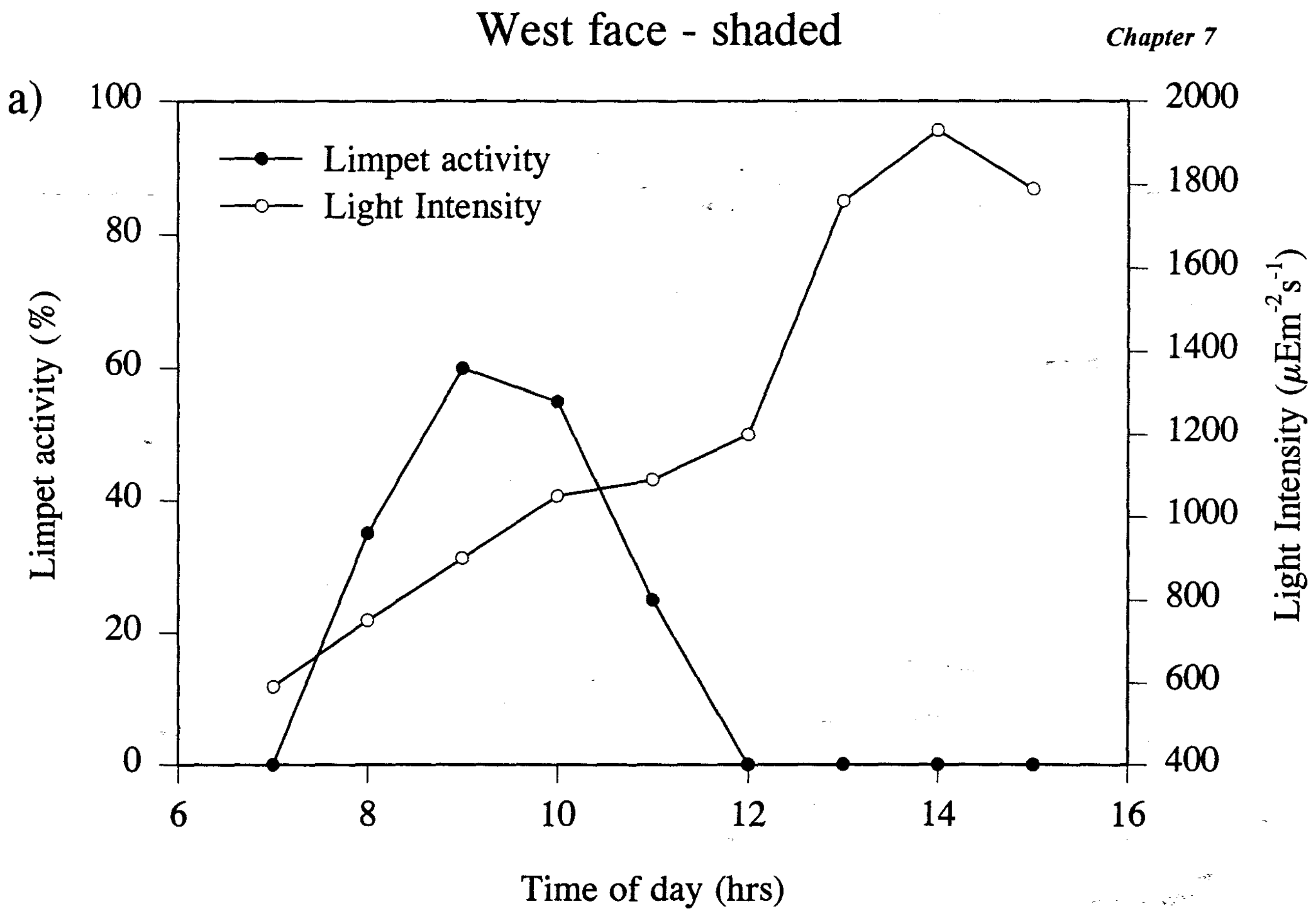
Some of the *Helcion pectunculus* (40%), which would have normally been inactive, exhibited foraging behaviour when subjected to 30%-50% shading. However, as the light intensity reached approximately 1000 $\mu E.m^{-2}.s^{-1}$, foraging activity ceased (figure 7.7d & e). Under conditions of 50% shade, light intensity never went above 1200 $\mu E.m^{-2}.s^{-1}$ and those limpets foraging (40% of the animals in area) were active until the tide was about to cover them. No foraging activity was observed under either 0% (control), 10% or 20% shade.

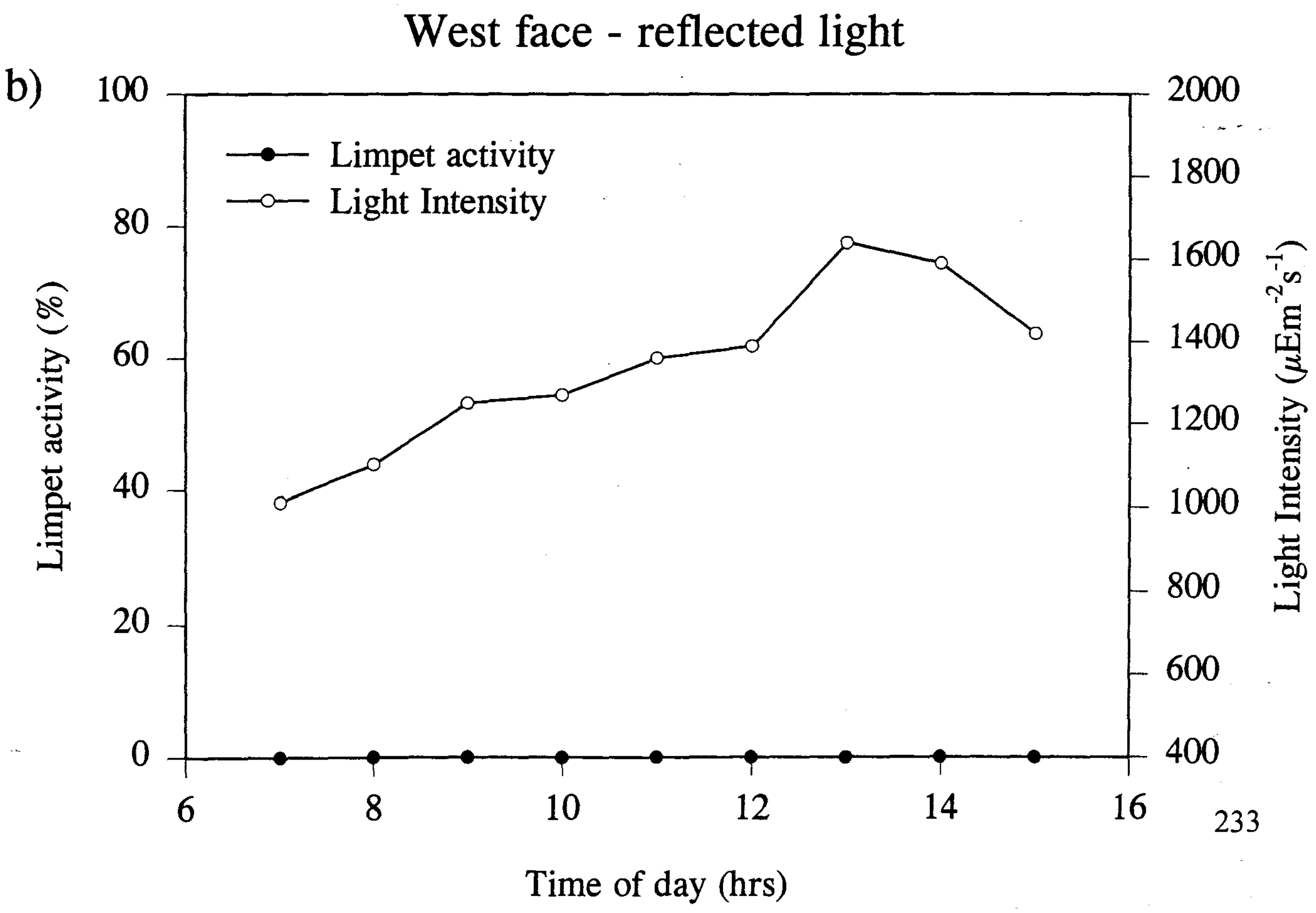
Limpets on the west facing rock surface, which usually forage during morning low tides whilst in the shade exhibited no foraging activity whilst sunlight was reflected onto them (figure 7.8). Limpets on a nearby west facing rock surface which did not experience reflected sunlight did undergo usual foraging activity.

7.3.3 Field tenacity measurements

Figure 7.9 shows the forces of attachment in relation to surface area of foot for *Helcion pectunculus*. The mean tenacity value of *H. pectunculus* is shown in table 7.8 along with the values obtained for the six South African *Patella* species studied by Branch & Marsh (1978). A linear relationship was found between tenacity and foot area (cm^2) using linear regression ($r^2 = 0.92$, see figure 7.9).


a) No Shade
b) 10% Shade
c) 20% Shade
d) 30% Shade
e) 50% Shade
Limpet activity
Light Intensity
Time of day (hrs)
0
20
40
60
80
100
600
1200
1800
2400
6
8
10
12
14
16
% of marked limpets active
Light intensity ($\mu Em^{-2}s^{-1}$)


West face - shaded
a)
Limpet activity
Light Intensity
Limpet activity (%)
0
20
40
60
80
100
Light Intensity (μEm-2s-1)
400
600
800
1000
1200
1400
1600
1800
2000
6
8
10
12
14
16
Time of day (hrs)


West face - reflected light
b)
Limpet activity
Light Intensity
Limpet activity (%)
0
20
40
60
80
100
Light Intensity (μEm-2s-1)
400
600
800
1000
1200
1400
1600
1800
2000
6
8
10
12
14
16
Time of day (hrs)

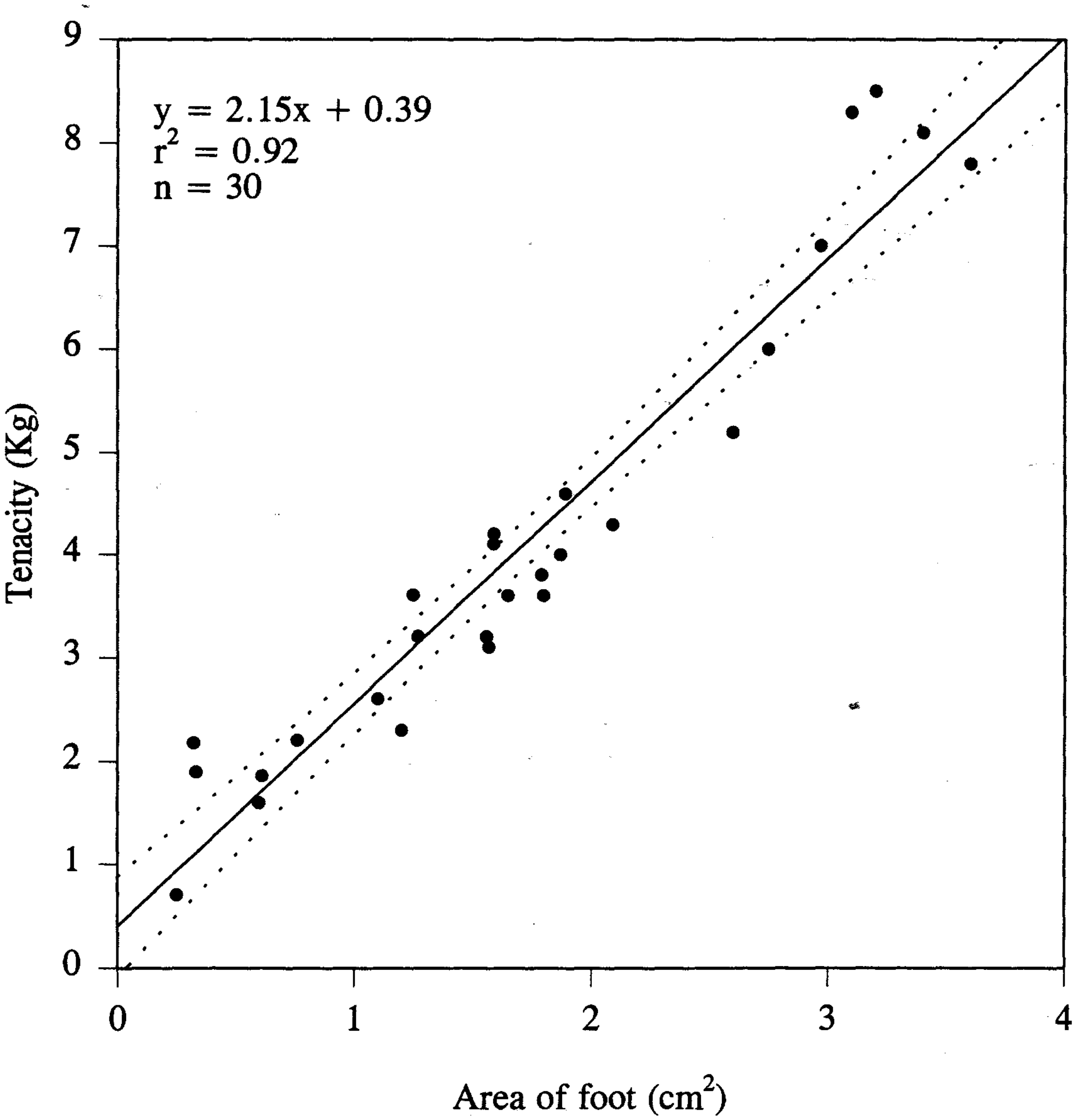

y = 2.15x + 0.39
$r^2 = 0.92$
n = 30
Tenacity (Kg)
Area of foot (cm²)
0
1
2
3
4
5
6
7
8
9
0
1
2
3
4

Table 7.8

Mean forces of tenacity (± S.E.) for *Helcion pectunculus* (n = 30) along with six species of South African *Patella* studied by Branch & Marsh (1978).

Species	**Tenacity (kg.cm^{-2})**	**Position on the shore**
P. cochlear	5.18 ± 0.20	Low shore
P. argenvillei	4.67 ± 0.20	Low shore
P. longicosta	4.40 ± 0.27	Low shore
P. granularis	3.25 ± 0.16	Mid shore
P. granatina	2.71 ± 0.18	Mid/high shore
P. oculus	1.95 ± 0.12	Mid shore
H. pectunculus	2.75 ± 0.13	High shore

7.4 Discussion

Crevice refuges are known to aid in reducing desiccation, and juveniles of many limpet species are restricted to low shore or damp crevices (Branch, 1976). During high tides and diurnal low tides *Helcion pectunculus* resides in crevices and under boulders in the upper balanoid zone of the intertidal rocky shore (chapter 2). The size gradient of *H. pectunculus* within crevices is to be expected as only small individuals will be able to fit at the back of crevices. However, the fact that small individuals are always at the rear of a crevice suggests that this is a site of settlement of juveniles.

Using the growth equation for *Helcion pectunculus* on the eastern Cape coast (chapter 3) the ages of limpets within crevices can be calculated. The smallest limpets found within crevices had a mean shell length of 11 mm which, therefore, have an average age of approximately 7 months, the smallest limpet found had a shell length of 6.1 mm which means it was only 3.8 months old. The largest individuals in the crevices had a mean shell length of 27 mm which converts to an age of 3 years. Thus, as limpets grow in size and age, they must migrate towards the mouth of the crevice due to the spatial constraint imposed by the crevice size.

Within one year of settlement, *H. pectunculus* will grow to be approximately 15-20 mm in shell length. These individuals will have moved from the back of the crevice into the "second row", leaving space for the next recruits to settle at the back of the crevice. Clearly, the number of rows present in a crevice will depend largely on the depth of the crevice. All crevices used during this study had a depth of about 100 mm which allowed a maximum of 4 rows of limpets to be present. It is not known whether *H. pectunculus* larvae settle selectively and actively search out the backs of crevices. However, many larvae respond in a gregarious fashion to chemical cues (see Chia, 1989 for a review) and it is suggested that juveniles of *H. pectunculus* act in the same way to chemical cues released from adult limpets.

The measurements of rock surface temperature and relative humidity show that limpets within crevice refuges experience a stable and buffered environment compared to adjacent exposed rock surfaces in the high shore. During diurnal low tides, crevices are significantly more humid and have lower rock surface temperatures than adjacent exposed rock surfaces.

Other species of limpet *e.g. Cellana grata* on Hong Kong shores, actively search out damp or shaded habitats or refuges on hot summer days (Williams & Morritt, 1995). However, rock surface temperature is also dependent on the aspect of the rock surface and the inclination to the sun (Davies, 1969; Wolcott, 1973). Williams (1994) suggested that duration of emersion and rock aspect are probably the most important factors affecting individual survival in *C. grata*. These factors also appear to play a major part in the life of *H. pectunculus*.

Limpet body temperatures were never found to exceed the rock surface temperatures which conflicts with the results of Williams & Morritt (1995) for *C. grata*, *Fissurella* (Lewis, 1963) and *Siphonaria* spp (Vermeij, 1971). Williams & Morritt (1995) suggest that the higher limpet body temperature may be due to the rock surface being cooled evaporatively by wind action whilst the sole of the limpet's foot prevents this. The limpet may, therefore, heat up via radiation from the sun and conduction from the rock (Davies, 1970) while not experiencing the same degree of convective heat loss as the open rock surfaces. *Helcion pectunculus* has a highly prickled shell surface (Kilburn & Rippey, 1982) which may well increase the amount of re-radiation of heat back into the air (Branch, 1981) and may also create a turbulent air flow over the shell, increasing convective heat loss to the air. *Helcion pectunculus* also exhibits strong allometry with its shell increasing in height faster than length *i.e.* the animals change shape as they grow, becoming taller and hence increasing their body weights more than the surface area from which water loss occurs and over which conduction from the rock surface takes place. These morphological adaptations may well be the reason why the body temperature of *H. pectunculus* seldom rises above that of the rock surface on which it is attached.

Figure 7.6 shows that limpet body temperatures within crevices are significantly lower than those attached to rock surfaces which are fully exposed to the sun's radiation. The maximum body temperature of crevice inhabiting *H. pectunculus* was 23°C as compared to 34°C in limpets not in a crevice. Crevice refuges high up on the shore therefore reduce thermal stress on *H. pectunculus* and may be why this limpet is able to survive on the high shore. In a study examining the thermal tolerance of another high shore limpet, Williams & Morritt (1995) found that during diurnal low tides *C. grata* occupied areas of the shore which were significantly cooler than adjacent habitats. They found that vertical rock surfaces shaded from direct sunlight were cooler compared to horizontal surfaces, and that surfaces facing west heated up greatly during the latter part of the day. *C. grata* was also found to exhibit clustering behaviour at rock pool interfaces, a behaviour also found to be present in *P. granularis* (Branch, 1975). Branch (1975) suggested that *P. granularis* uses the pool interfaces as means of evaporative cooling.

Helcion pectunculus therefore reside in environments, in the form of crevices, which remain humid during diurnal low tides whilst keeping the limpets body temperature relatively low. *Helcion pectunculus* also exhibits other adaptations to tolerate temperature and desiccation stress. Individuals home to a fixed scar which has been shown to be an adaptation to temperature stress in both *Siphonaria* (Verderber *et al.*, 1983; Branch & Cherry, 1985) and *Patella* (see Branch, 1981 for a review) and has been shown, experimentally, to reduce desiccation (Branch, 1973). *Helcion pectunculus* does not, however, form a mucus sheet to reduce water loss as do some species of *Collisella* (*e.g. C. digitalis*, Wolcott, 1973; Gallien, 1985 and *C. scabra*, Collins, 1979).

A further adaptation of *H. pectunculus* to reduce thermal stress is being active during nocturnal low tides and diurnal low tides whilst in the shade. This behaviour is also found in many other patellid limpets (see chapter 5 for a full review). During this study foraging behaviour of *H. pectunculus* was found to be stimulated by artificial shade (30-50% shade cloth) when light intensity never exceeded 1200 $\mu E.m^{-2}.s^{-1}$. *Helcion pectunculus* on west facing rock faces which normally forage whilst in the shade were prevented from doing so when sunlight was directed onto the rock face on which they were situated. This points towards the fact that *Helcion pectunculus*, although driven by an endogenous rhythm (chapter 6) will only become active if certain criteria are met. In this case, the light intensity needs to be below 1200 $\mu E.m^{-2}.s^{-1}$ for foraging to take place. As soon as the light levels rise above this threshold level, the limpets return to their home scars within their crevices to avoid the high temperatures and desiccation associated with a high shore existence.

Cellana grata is another limpet species which exhibits behavioural adaptations to reduce thermal stress (Williams & Morritt, 1995). *C. grata* "shuttles" up and down the shore whilst awash with waves. As the tide comes in, individuals move up the shore to feed on the unexploited food source, then as the tide goes out, they move back down the shore to their usual tidal height where they find a refuge to "sit out" low tide.

Finally, field tenacity measurements for *Helcion pectunculus* show that it is one of the least tenacious limpets occurring along the South African coast. There is a linear relationship between force of attachment and foot area (cm^2). Branch & Marsh (1978) found that limpets which grow allometrically (*e.g. Patella argenvillei* and *P. granatina*) exhibit a curvilinear relationship between tenacity and foot area. This, they suggest, is due to the fact that as size increases, force per unit area of foot also increases, hence the curvilinear relationship. Shell growth in *Helcion pectunculus* is strongly allometric and a curvilinear relationship would be expected, but this is not the case. A possible reason for this is that *H. pectunculus* is a small limpet compared to other South African limpets with its largest recorded size being approximately 35 mm in length.

Several authors have attempted to measure the powers of adhesion in limpets (Aubin, 1892; Menke, 1911; Thomas, 1948; Miller, 1974; Branch & Marsh, 1978). The results of these studies are highly variable between the species but much of this variability can be put down to the crude methods used. Grenon & Walker (1981) used a much more sophisticated approach and thus obtained highly accurate tenacity values for *Patella vulgata.*

Wave action is probably a major agent determining the selective value of tenacity (Branch & Marsh, 1978). Miller (1974) measured wave force at various sites in northern and central America, recording a maximum value of 232 g/cm^2. None of these readings were taken during stormy periods and so were not totally representative of conditions on the rocky shore. Jones & Demetropoulos (1968) recorded forces in excess of 1 kg/cm^2 along the coast of North Wales (U.K.). Such forces would usually be acting parallel to the substratum, and remembering that tenacity against a shear force is about one quarter of that against an upward pull (Miller, 1974), could easily limit the distribution of limpets on the rocky shore.

Branch & Marsh (1978) have suggested that a relationship was present, in the six patellids they studied, between tenacity and wave action experienced by the limpets. *P. cochlear* has a high tenacity (5.18 ± 0.20 kg/cm^2) and experiences high wave activity. Whilst *P. oculus* has a low tenacity value (1.95 ± 0.12 kg/cm^2), experiences low wave activity but has a very large foot surface area. It is suggested that the same does not hold true for *H. pectunculus*. Individuals of *H. pectunculus*, although occurring high up on the shore, experience very strong wave activity especially during the stormy season mainly due to the geomorphology of the shores they inhabit. It is therefore strange that *H. pectunculus* has a very low tenacity and a small foot surface area. Removal of the crevice refuge is catastrophic to *H. pectunculus*. A large majority of limpets deprived of their refuge are lost during the first high tide (45%) and after the first week without a crevice 82% of the limpets have been lost.

In summary, the crevice environment fulfils a number of functions for *H. pectunculus*. Firstly it acts as a buffer, the more equitable rock surface temperatures and humidities reduce thermal stress and desiccation and result in lower limpet body temperatures. However, the crevice also acts as a refuge from wave activity, reducing the chance of the limpets being washed away by the incoming tide. It has previously been stated (chapter 5) that all limpets are back on their home scars by the time they are covered again by the incoming tide. Why is this so? It is suggested that *H. pectunculus* has evolved a number of behavioural adaptations to reduce the chance of being stranded on the exposed rock surface whilst the tide comes in. It is therefore clear that selection favours both crevice habitation and a strong behavioural rhythm to reduce both the thermal stresses of the high shore and the chance of being dislodged by the strong wave action experienced on the rocky reefs inhabited by *H. pectunculus*. Such behavioural adaptations will allow this limpet to live higher up on the shore than other herbivorous grazers and utilise a food source which is otherwise scarcely touched.

7.5 Summary

1) *Helcion pectunculus* have an organized distribution within crevices with smaller, younger limpets being towards the back of the crevice and larger, older limpets towards the crevice mouth. It is hypothesized that *H. pectunculus* juveniles actively settle at the backs of crevices responding to chemical cues of adult conspecifics.

2) The crevice refuge supplies *H. pectunculus* with a stable and buffered environment with higher relative humidities and lower rock surface temperatures than adjacent exposed rock surfaces.

3) Limpet body temperatures were significantly lower in crevice refuges compared to limpets on adjacent exposed rock surfaces. Body temperatures never exceeded the rock surface temperatures. It is suggested that this is a result of morphological adaptations such as shell ornamentation and allometric growth.

4) Light, above 1200 $\mu E.m^{-2}.s^{-1}$, was found to inhibit foraging activity in *H. pectunculus* whilst limpets subjected to 30-50% shade foraged even during daytime low tides. This behavioural adaptation enables the limpets to avoid the high temperatures and desiccation associated with the high shore.

5) *Helcion pectunculus* is one of the least tenacious limpets occurring along the South African coast and it is suggested that wave action is a major factor in governing both the activity patterns and homing behaviour of this limpet.

6) Limpets deprived of a crevice refuge experienced extremely high mortalities, with 45% of limpets being lost during the first high tide period.

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Chapter 8:

General Discussion

8.1 General Discussion

The intertidal rocky shore is generally regarded as one of the most stressful physical environments (Branch & Branch, 1981). The upper limits of zonation of many, if not all species on such shores are determined by physical conditions whilst the lower limits are controlled by biological interactions (Underwood, 1979). Species richness on rocky shores has been found to be particularly high in zones with a high biomass of macroalgae or filter-feeders which offer many microhabitats for small species, but declines from the bottom of the shore to the top as physical conditions become far more severe (McQuaid, 1985). Thus, few marine species have been able to adapt to the upper shore habitat due to the physical stresses and the low food availability (Newell, 1976; Barnes & Hughes, 1982).

This study has not only shown that *Helcion pectunculus* lives very high up on the shore, but has also shown that its presence is restricted to the upper Balanoid zone, being absent from the Cochlear and Littorina zones (chapter 2). *Helcion pectunculus* can be regarded as one of the most successful of all southern African limpets. It is highly abundant in the upper reaches of rocky shores (chapter 2) and has a wider geographic distribution than any other southern African patellid limpet, occurring from southern Natal to the Namibian coast (Kilburn & Rippey, 1982), a distance of some 3000 km. Not only has *H. pectunculus* adapted to the extreme and highly demanding conditions of the high shore, but also to the very different oceanographic conditions occurring on the west and east coasts of southern Africa (Branch, *et al.*, 1994). Being a high shore limpet species, *H. pectunculus* occupies a niche which ensures that it has very few competitors, both for space and for food, due to the fact that most herbivorous grazers occur lower down on the shore (Newell, 1979; Branch, 1985).

Success in an upper shore environment could be achieved by four main evolutionary/adaptational routes. Firstly, species could adapt morphologically to the extreme conditions experienced in the upper Balanoid zone, including high rock surface and air temperatures, low relative humidity, long periods of emersion and, on certain shores, very strong wave activity. Secondly, animals could evolve behavioural traits which would minimize the effects of these and other physical factors. The third route would be to adapt physiologically. Finally, a combination of the above routes could be adopted. Which particular avenue of evolutionary change did *H. pectunculus* follow and just how efficient are the results? It is suggested that *H. pectunculus* uses a combination of morphological, behavioural and physiological adaptations with some being more influential than others. At present it is not possible to state with any certainty which adaptations carry "more weight" due to the lack of information on the physiology of the animal and this area requires further detailed study. However, both morphological and behavioural adaptations do seem to play an important role in the survival of *H. pectunculus* on the upper shore.

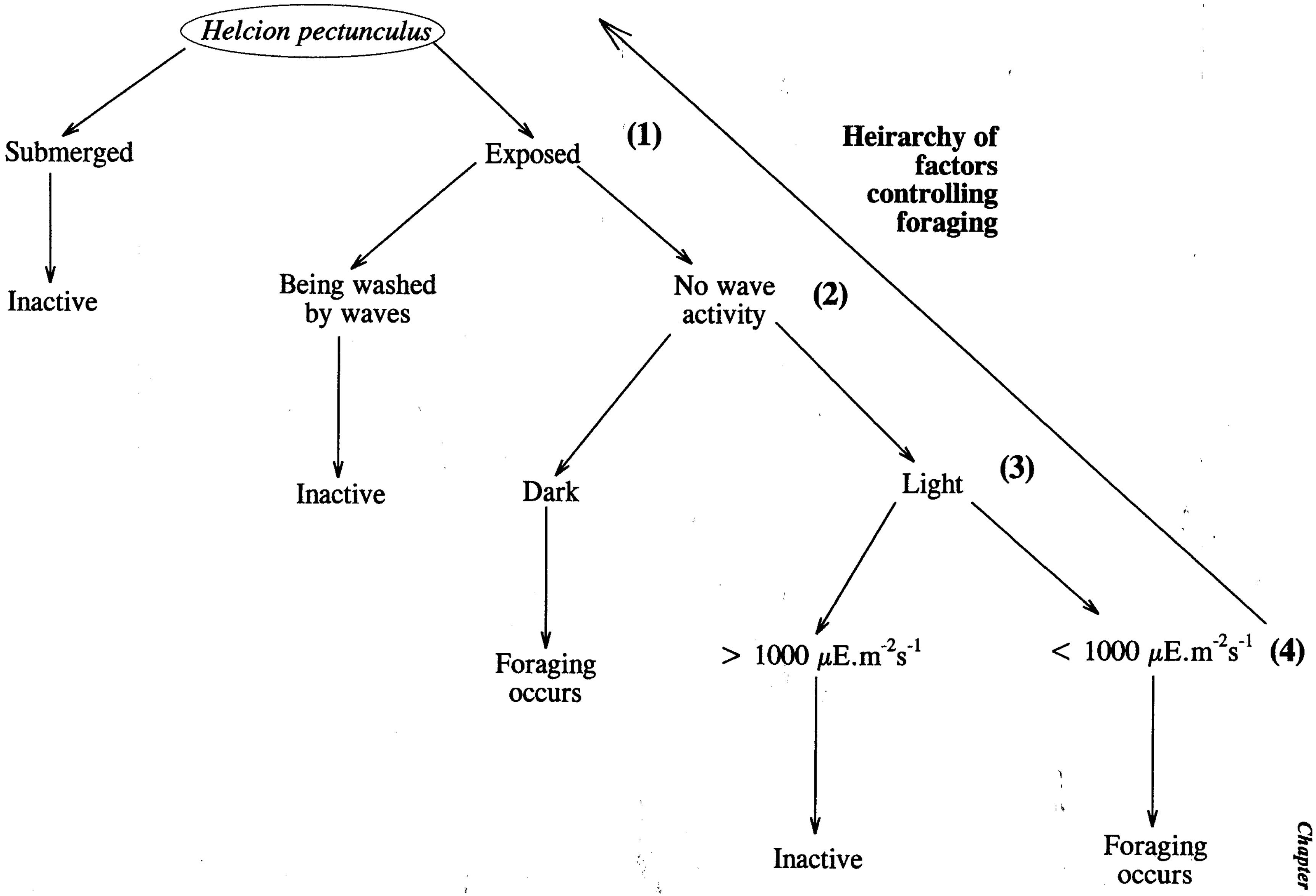
Morphological adaptations can come in many different forms. While many prosobranch gastropods have an operculum which is used to close the aperture of the shell to reduce water loss, limpets have a thick impermeable shell which can be clamped tightly against the substratum and so prevent water loss (Branch, 1981). Many high shore gastropods, including limpets, have altered their shell shape in response to desiccation pressure (Vermeij, 1973 reviews this). It is also known that limpets high up on the shore will exhibit allometric shell growth, with their shells increasing in height faster than length which, according to Branch (1981) is expected of high shore gastropod molluscs attempting to reduce evaporative water loss. The shell of *H. pectunculus* is rather remarkable in appearance. It is generally a tall domed shell, the surface of which is covered with radiating bands which are covered with small spines. This type of ornamentation on shell surfaces has been shown to increase heat loss via re-radiation (Vermeij, 1973; Branch & Branch, 1981). Upper shore gastropod species living in tropical environments have been found to have a globose shell which is strongly sculptured and these specialized species tend to disappear as one moves away from the tropics towards higher latitudes (Vermeij, 1973). An increase in shell sculpturing would provide an effective means both of reduction of heat gain by radiation and increase of heat loss by convection.

Heat gain on the high shore can be minimised by one or more of the following strategies; a reduction in the total surface area to volume ratio of the organism, since this will reduce heat gain by radiation; a reduction in the surface area in contact with the substratum, since this will reduce conductive heat gain; a reduction in the metabolic rate (Newell, 1976). In the case of limpets, an increase in tissue volume compared with the basal parameter through which evaporation occurs would require a substantial increase in the relative shell height (allometric growth). *Helcion pectunculus* grows allometrically, in fact it exhibits the greatest degree of allometry ever recorded for a South African limpet (chapter 3). Growing allometrically increases the limpet's body volume whilst retaining a relatively small area of the body in contact with the rock surface. Previous work has also shown that *H. pectunculus* has a critical temperature (at which oxygen consumption peaks) of greater than 35°C (Branch, 1981) making it one of the most temperature tolerant limpets on the South African coast. *Helcion pectunculus* has also been found to forage further distances in winter than in summer.

This has been attributed to the unpredictable nature of optimal foraging conditions occurring during winter making it necessary for the limpet to forage maximally when conditions allow. Another possibility is that the limpet's metabolism has been reduced during summer to minimise heat gain. This would then insinuate that *H. pectunculus* has also adapted physiologically but this requires further experimental verification.

Behavioural adaptations to its position on the shore also contribute to the success of this limpet. *Helcion pectunculus* exhibits rigid homing to a fixed scar within a crevice (no scar swapping was ever observed). Homing has been observed in many species of limpet (Branch, 1971; Creese & Underwood, 1982; Garrity, 1984), behaviour which has been shown to reduce desiccation significantly (Verderber *et al.*, 1983; Branch & Cherry, 1985; Kunz & Conner, 1986). *Helcion pectunculus* also exhibits a fixed pattern of foraging activity, being active only during periods of least physiological stress *i.e.* nocturnal low tides and during early morning low tides whilst in the shade (chapter 4).

The activity pattern exhibited by *H. pectunculus* may have a number of driving factors. Firstly, the pattern exhibited may well be a form of behavioural thermoregulation. Foraging at night or whilst in the shade, heat gain by conductance from the rock surface, and radiation from the sun are greatly reduced (Newell, 1979). Garrity & Levings (1983) suggested that some limpets remain inactive at high tide to avoid marine predators. *Helcion pectunculus* has been found to make up a substantial part of the diet of the giant clingfish, *Chorisochismus dentex* (Stobbs, 1980), so this may well be a valid reason for the observed inactivity at high tide. Another plausible explanation is that being active at low tide, *H. pectunculus* reduces the chance of being washed away by wave action as they have a very low tenacity in relation to other patellid limpets (chapter 7) which may well also account for the large numbers eaten by clingfish. This work has also shown that this pattern of activity is controlled by an endogenous rhythm which appears to be correlated with the avoidance of unfavourable foraging conditions and also gives the individual an anticipatory capacity allowing prediction of certain rhythmic influences such as time of immersion (chapter 6). It is proposed that a hierarchy of factors control whether or not *H. pectunculus* forages. The endogenous rhythm gives the animal information about when foraging should occur assuming optimal conditions. Whether or not the limpet actually forages depends on an environmental "check-list" to determine whether conditions are optimal. Figure 8.1 presents a hypothetical model of the hierarchy of exogenous factors controlling limpet activity. There are 4 levels of control and locomotor activity may be inhibited at any level. Level 1 determines whether or not the limpet is submerged, foraging activity does not occur whilst under water. If the limpet is exposed, then level 2 becomes the next controlling factor; is the limpet being washed by waves? *Helcion pectunculus* has a very low tenacity and does not forage whilst subjected to wave activity. If there is no wave activity then control shifts to level 3. *Helcion pectunculus* is known to forage whilst in the dark or in the shade *i.e.* in light intensities of 1000 $\mu E.m^{-2}.s^{-1}$. If light levels are below this threshold intensity then foraging occurs, if not then foraging activity is inhibited until such time that all of the above criteria are met. This model is based purely on behavioural adaptations but should be expanded to include physiological factors and thresholds as soon as this information is available.


Helcion pectunculus
Submerged
Inactive
Exposed
(1)
Being washed by waves
Inactive
No wave activity
(2)
Dark
Foraging occurs
Light
(3)
> 1000 μE.m-2s-1
Inactive
< 1000 μE.m-2s-1
(4)
Foraging occurs
Heirarchy of factors controlling foraging

Living within a crevice, in itself, could be classed as a behavioural adaptation, although it is not known whether *H. pectunculus* "chose" to live in crevices or is forced to do so by the extreme conditions it experiences. However, due to the fact that individuals return to the same crevice after every foraging excursion it will be classed as an adaptation for discussion purposes. Crevices are known to aid in reducing desiccation with juveniles of many limpet species being restricted to low shore or damp crevices (Branch, 1976). The crevice habitat is also known to offer shelter from the rigours of the intertidal zone (Newell, 1979). However, in the case of *Helcion pectunculus*, aggregating in crevices may well be adaptive in several respects.

It may well be a strategy for avoiding strong wave activity. Dislodgement of a limpet via wave action is most likely if a limpet is sheared off with a pull parallel to the substratum (Jones & Demetropoulos, 1968; Miller, 1974). This would only generally occur whilst a limpet was moving. Some limpets, including *H. pectunculus*, have evolved rhythms of activity which ensure they are not off their home scars when wave action can dislodge them (chapter 5). Residing in crevices may also allow the limpets to inhabit areas of the shore which would otherwise be unsuitable due to the extreme physical conditions. The crevice environment produces an "oasis" of low-shore physical parameters in an otherwise inhospitable area of the shore. In some ways crevices cause anomalies in the otherwise rigid zonation pattern of the rocky intertidal. Due to the lower temperatures and higher humidities present within crevices animals are capable of living higher up on the shore. However, if *H. pectunculus* is representative of this group of animals then the crevice environment does not appear to be enough and certain other adaptations must be made by the species in order to survive.

The fact that the limpets are forced to aggregate within crevices may well be advantageous in itself. Clustering may result in the retention of water within the group thus increasing the humidity within the crevice allowing the limpets to survive long aerial exposure at low tides. Clustering has also bee observed in *Siphonaria concinna* (Gray & Hodgson, in press), *Cerithium moniliferum* (Rohde & Sandland, 1975) and *Patella granularis* (Branch, 1975b). There is only circumstantial evidence that clustering behaviour relates to desiccation stress (Branch, 1981) and it could well be argued that by evolving a behavioural response that causes *H. pectunculus* to seek out crevices refuges in the high shore, they are being forced to cluster and so this may well only be an artifact rather than the preliminary result, even though it may be beneficial.

Living in close proximity within a crevice or under boulders may be a strategy to ensure reproductive success. Freire *et al.* (1992) suggested that in species that rely on external fertilization, such as *Helcion pectunculus* (see chapter 4), living in close proximity or "clustering" would be advantageous, allowing for synchronous spawning and the release of female and male gametes in close proximity to one another, increasing reproductive efficiency. This, although beneficial to the animal, probably is not an evolved behavioural adaptation but an artifact of the "crevice escape" mechanism employed by *H. pectunculus.*

Most patellid limpets exhibit a marked reproductive cycle (Creese & Ballantine, 1983), and *H. pectunculus* is no exception, spawning twice a year, once in April/May and again in December/January on both east and west coasts of South Africa. A variety of factors have been reported to trigger spawning in gastropods including temperature, mechanical stimulation, genetic and hormonal factors (Orton, 1920; Linke, 1933; Gabe, 1951, 1953; Hancock, 1960; Kessel, 1964; Berry & Chew, 1973; Alifierakis & Berry, 1980), as well as combinations of these factors (Fretter, 1984). The present study suggests that *H. pectunculus* has evolved a reproductive cycle which will allow its planktonic larvae to utilise the valuable phytoplankton bloom food source which is present in the inshore waters of South Africa during summer whilst using high onshore winds to ensure that larvae are not washed out to sea and lost. However, this hypothesis assumes that *H. pectunculus* produces feeding larvae, a fact not yet proved, and so should be treated with caution.

This study has determined that *Helcion pectunculus* is a protandrous hermaphrodite, changing sex from male to female when it is between 2 and 3 years old (chapters 3 & 4). Previous to this study *Patella oculus* and *P. aphanes* were thought exceptional amongst the South African limpets as being protandrous. It has been suggested that changing sex from male to female is advantageous because larger females may hold more eggs (Hoagland, 1978; see chapter 4 for a review). However, it is now apparent that individuals of *H. pectunculus* rarely survive to live beyond 4 years of age. This then becomes somewhat of a paradox; it is advantageous for females to be larger so that they may produce and release more eggs, however there is a greatly reduced chance that they will live to become female. Whether a greater number of smaller females would produce and release more eggs than fewer, larger females is not known and poses a theoretical problem which requires answering if we are to understand the highly complex interactions between adaptation and constraint in high shore gastropod molluscs.

According to Branch (1973), two basic "life strategies" have evolved in the Patellidae to reduce intraspecific competition. The first group of limpets migrate up the shore as they age and become more tolerant of physical stress, these were termed "migratory species". The second group do not migrate and so were classed as "non-migratory". The former group of limpets were found to all be mid- to high shore species, which may reduce intraspecific competition by their migration. Up shore movement is usually seasonal, occurring when physical stress is reduced and when food availability should be increasing (Branch, 1981). Migratory species such as *P. granularis*, *P. granatina*, *P.concolor* and *P. oculus* were found to be present on the shore in large densities but were widely distributed among the zones. All are known to rapidly exploit their food source, and as far as is known all have high growth rates, high mortality, low longevity (Branch, 1974a) and very large gonads (Branch, 1974b, 1975b). Branch (1975a) classes all of these species as "r-strategists", channelling their resources mainly into reproductive activity and high replacement rates (figure 8.2).

The non-migratory species are mostly low-shore and sub-tidal species. On the low shore, food is abundant and more constantly available than higher up on the shore and there are many macroalgae which are used to supplement the diet (Bustamante *et al.*, 1995). Non-migratory species include *P. cochlear*, *P.longicosta*, *P.tabularis*, *P.miniata*, *P.argenvillei* and *P. compressa*. These species are known to settle in a narrow zone on the shore and remain there throughout life. If a species is to occupy practically the same position throughout life, it cannot escape intraspecific competition by moving away. On the other hand, the low shore does provide opportunities for specialization and many of the species have specialized diets or are epiphytic on large algae. These limpets are territorial and have relatively small gonads

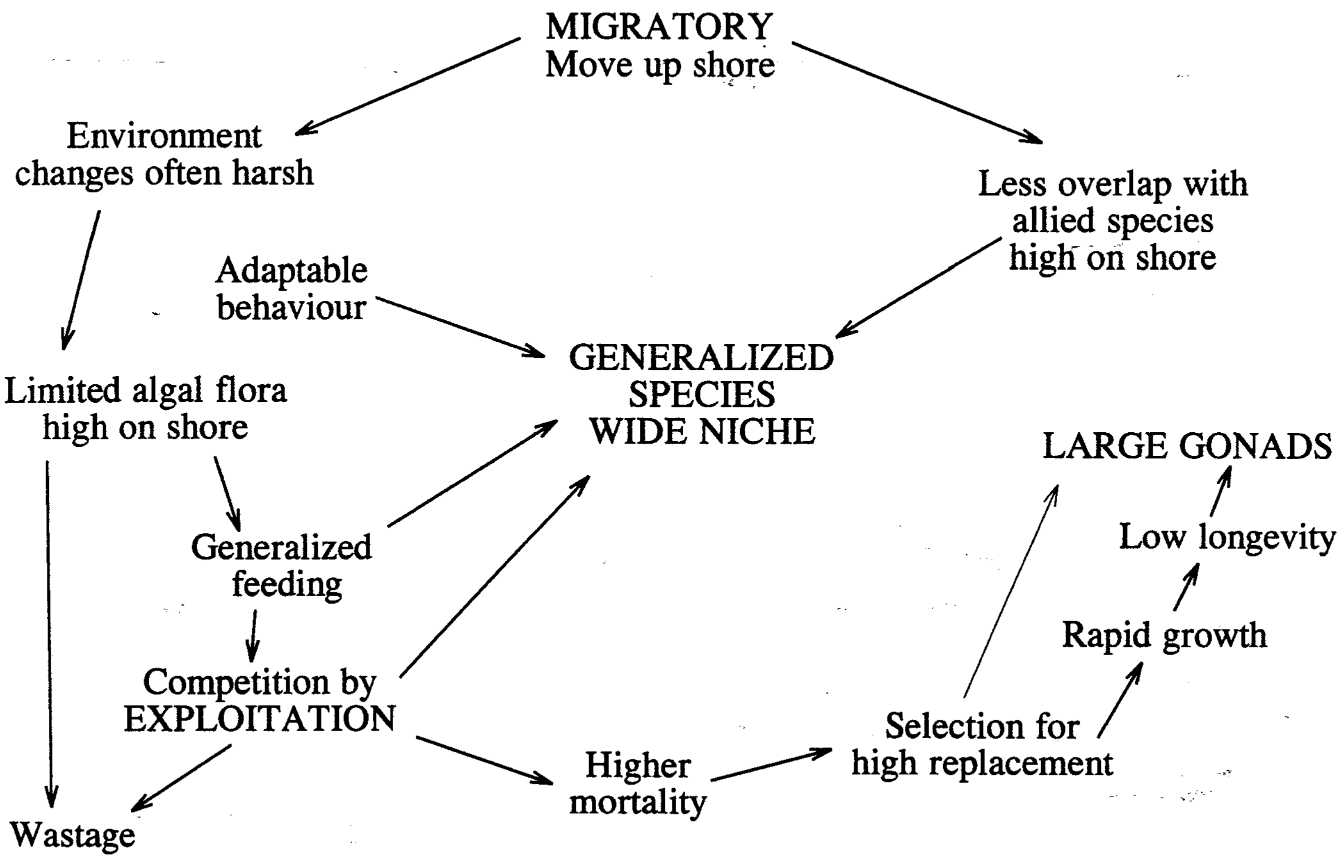


Figure 8.2

Features which are characteristic of the "migratory limpet species" put forward by Branch (1973).

(Branch, 1974a). All of these species are probably "k-strategists", devoting more resources to non-reproductive activities with increased survival and greater competitive ability (figure 8.3).

At first glance then, *H. pectunculus* appears to fit rather nicely into the "non-migratory" group of limpets suggested by Branch (1973). Although its distribution is high up on the shore there is no evidence to suggest that a migration of limpets from low shore to high shore occurs. *Helcion pectunculus* seems to start out life, and end life at roughly the same position on the shore. *H. pectunculus* does not act in a territorial way, nor does it maintain and defend established food "gardens" as the other species in this group do and its gonads are of a medium size suggesting perhaps a third group of limpets to be present on South African shores.

Branch (1973) suggested that the development of migratory and non-migratory tendencies within species is graded, and most intense in high density species. *H. pectunculus*, in places, does exhibit high densities (chapter 2) and so does not conform to the non-migratory theory. It is therefore suggested that a third group of limpets seem to be present which may be classed as "specialized non-migratory" species. These are species that do not migrate but neither do they garden or aggressively fight off like species. They have overcome the fierce competition for space and food on intertidal rocky shores by adapting, both physiologically and behaviourally, to a particular habitat which is exclusive to them alone (figure 8.4). This group may well include species such as *P. compressa* which is highly specialised for life on kelp stipes (Branch, 1973); *Helcion pellucidum* which, although does carry out a migration during its short life, occurs only on the stipes and blades of *Laminaria*

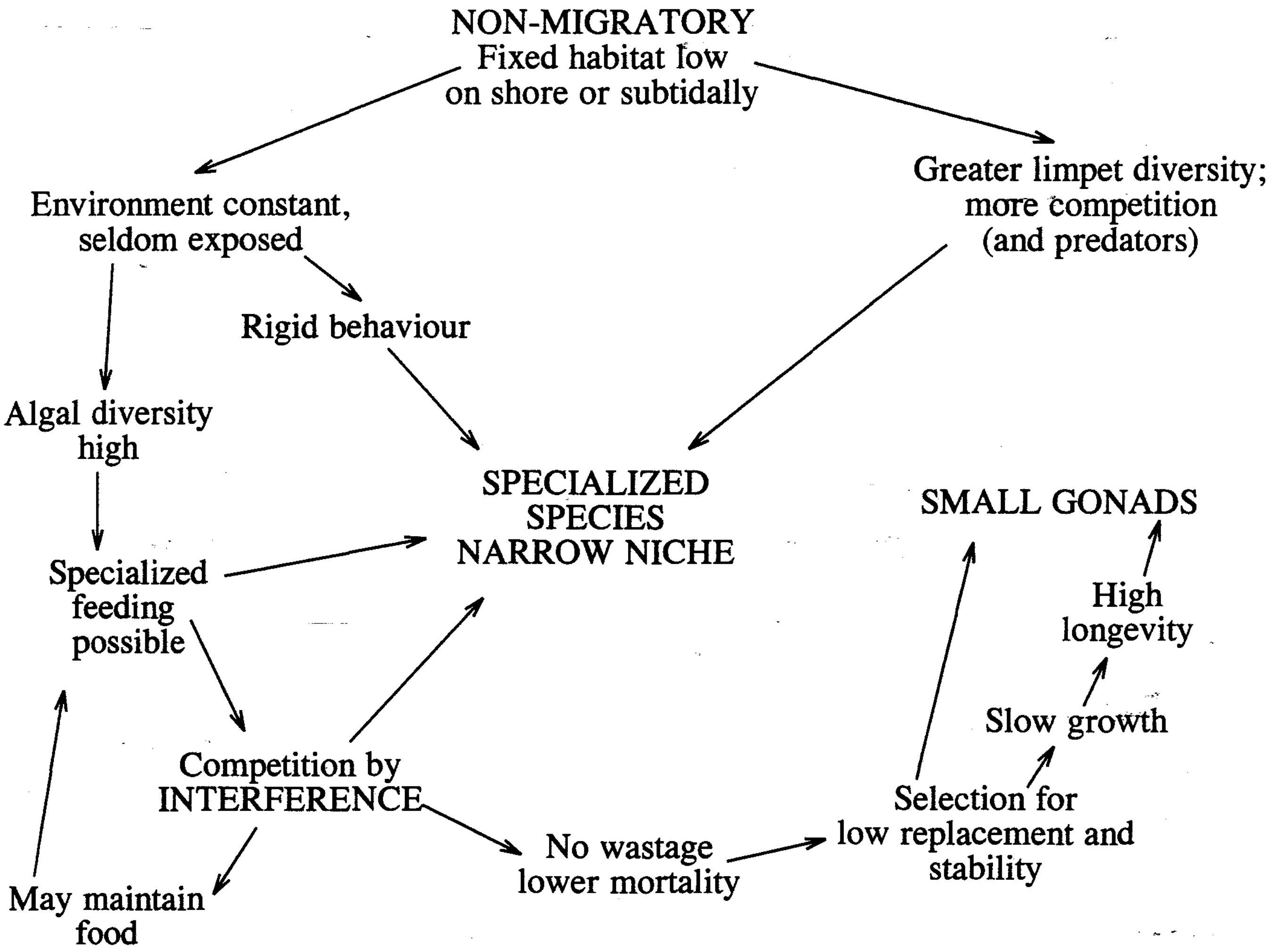


Figure 8.3

Features which are characteristic of the "non-migratory limpet species" put forward by Branch (1973).

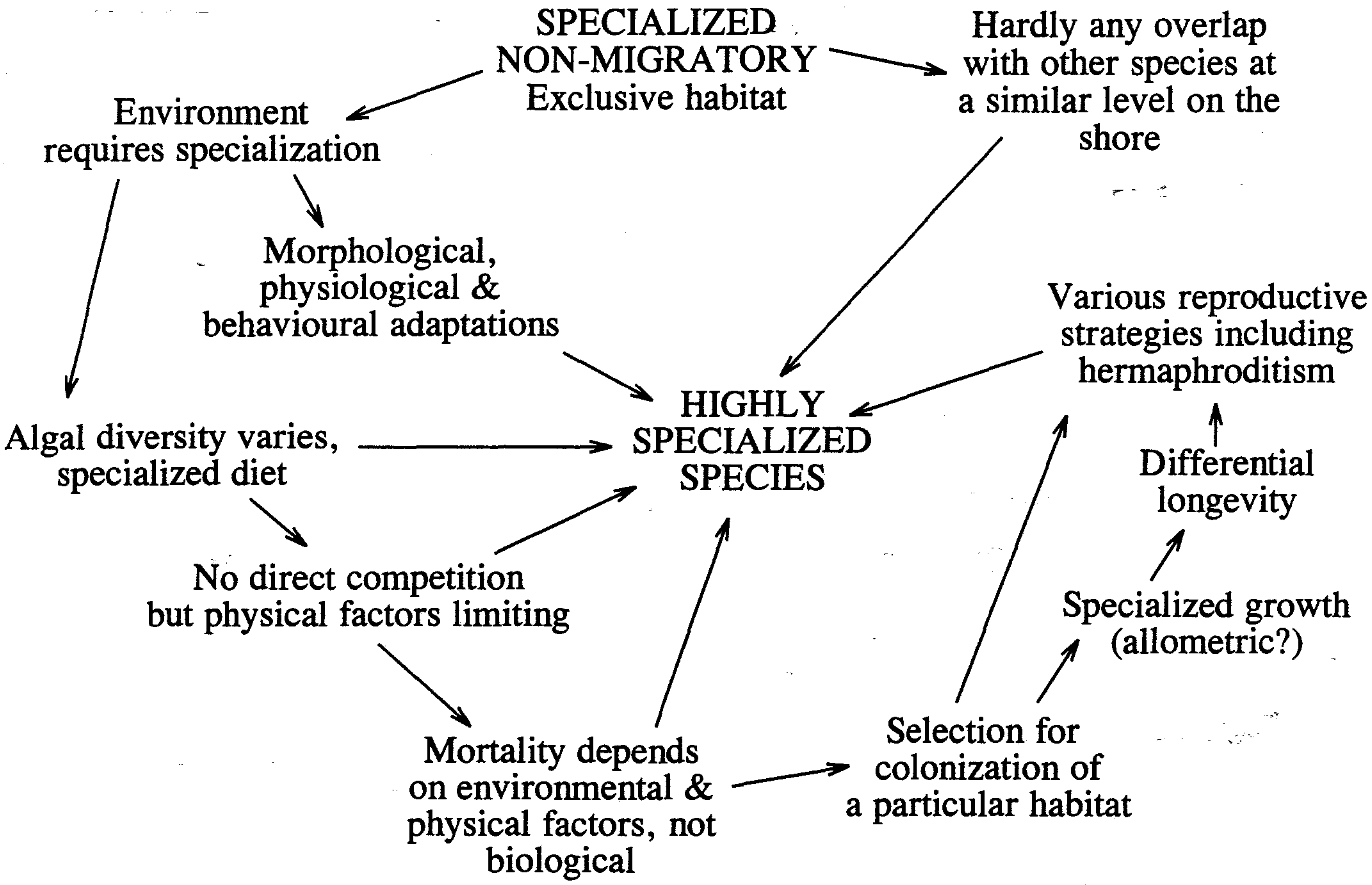


Figure 8.4

Characteristic features of the specialized, non-migratory group of limpets put forward in this study.

hyperborea (Graham & Fretter, 1947; Vahl, 1971, 1972, 1983; McGrath, 1992). *Helcion pruinosus* also has a rather specialised habitat, occurring only under boulders in low shore rock pools (A. Henninger, Rhodes University, pers. comm.). *Patella barbara* is another possibility but it has not needed to specialise to a particular habitat due to the fact of its sparsity on the shore making intra-specific confrontations very unlikely, although it could be argued that this in itself is a specialist adaptation.

Helcion pectunculus has successfully adapted to life in the high shore by means of morphological and behavioural specializations. However, it also exhibits one of the largest geographical distributions of all South African limpets. How is this so? By adapting to the high shore environment, *H. pectunculus* has been able to utilise a niche on the shore which guarantees minimal competition from other grazing species (McQuaid, 1985) but this is not enough. It is suggested that the reproductive strategy of *H. pectunculus* may be a major contribution to its phenomenal success. *Helcion pectunculus* exhibits two spawning events a year on both the east and west coast making it exceptional amongst southern African limpets. These spawnings may allow a double recruitment to occur every year at a time when phytoplankton biomass is at its maximum. *Helcion pectunculus* also undergoes a sex change from male to female. Living for three years it spends nearly 10 months as a juvenile then one year as a male and one as a female (chapter 4). This strategy, although seeming remarkably inefficient, in that very few limpets actually live to become female, may be the answer to the geographical success of *H. pectunculus* and further analysis is required to determine whether this is so.

It is therefore apparent that more detailed analysis is required to understand the complex factors at work which influence limpet distribution, both intertidally and geographically, and thus also drive behavioural traits such as foraging activity, homing behaviour and seasonal reproductive cycles which have been the centre of so many studies. Perhaps, rather than just looking at certain types of behaviour, we should now centre our attention more on looking at the behaviour of an animal as a whole and thus gain an insight into controlling factors which may well be missed when the whole picture is ignored. *Helcion pectunculus* is a high shore limpet which has clearly evolved both morphologically and behaviourally, to survive the extreme conditions in the upper Balanoid zone. It must be stressed however that it is not known whether these adaptations are enough to ensure the limpets survival at the height on the shore where it occurs and that physiological adaptations, which have not been examined during the present study, may well be playing a major role and thus further work on the physiology of this animal is essential.

8.2 Future research directions

1) A detailed examination of the larval life-history of *Helcion pectunculus.*

2) A comprehensive study to determine the exact time of recruitment of juveniles of *H. pectunculus* onto the shore, and where they settle.

3) Optimal foraging theory: does *H. pectunculus* forage on the same patch of algae all year round, or does this limpet use a form of "crop rotation"?

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Appendix I:

GENETIC DIVERGENCE BETWEEN SOUTH AFRICAN *HELCION* SPECIES AND NORTH-EAST ATLANTIC *H. PELLUCIDUM* (MOLLUSCA: PATELLOGASTROPODA).

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ABSTRACT

Allozyme electrophoresis was used to estimate the genetic divergence between 3 species of South African *Helcion* and the North-east Atlantic *H. pellucidum* (Patellidae). At a finer level the genetic relationships among South African *Helcion* were explored. Twenty-one loci were resolved for all the species. High values of expected heterozygosities (0.202-0.341) were found for the genus. Phenetic analysis (UPGMA and Distance Wagner Procedure) and cladistic parsimony methods (alleles as binary characters and loci as multistate unordered characters) agreed in showing the same topology for the tree which represents the genetic relationships among *Helcion* species. The North-east Atlantic *H. pellucidum* was the most divergent species showing high values of Nei's genetic distances (1.423 to 1.654) with its South African congeners. In the literature such values of genetic divergence have been associated with non-congeneric species. The degree of genetic divergence (0.351 to 0.615) found within the South African branch was characteristic for congeneric species. The results of this study supported the monophyletic condition of this branch, and showed *H. pectunculus* and *H. pruinosus* to be more closely related to each other than to *H. dunkeri*. Nevertheless, the monophyly of the genus *Helcion* is uncertain and therefore its taxonomic status was questioned. A possible independent origin for northern and southern hemisphere species of *Helcion* is considered.

INTRODUCTION

The genus *Helcion* Montfort (1810) was assigned to the family Patellidae Rafinesque (1815), on the basis of radular morphology, but unlike the genus *Patella* it was described as having a gill cordon interrupted anteriorly by the head. Five species of *Helcion* have been described. One of them, *H. tella* (Bergh, 1871) is an uncertain species from the Sargasso Sea described from soft parts only, the shell having been detached and lost (Powell, 1973). Three are well recognised South African species. *H. pectunculus* (Gmelin, 1791), is an upper-intertidal crevice-dwelling species, with a distribution extending from Namibia to Natal (Powell, 1973; Kilburn & Rippey, 1982) . *H. pruinosus* (Krauss, 1848), is a lower-intertidal species that hides beneath boulders of rather exposed shores, being found from the north-western Cape coast to eastern Transkei (Kilburn & Rippey, 1982). *H. dunkeri* (Krauss, 1848), lives on the undersides of sheltered rocks, in shallow water and in pools from about low water neap tides (LWNT) to low water spring tides (LWST), with a distribution ranging from Namibia to Natal (Kilburn & Rippey, 1982). The fifth species of the genus is the North-east Atlantic *H.pellucidum* (Linnaeus, 1758), formerly known as *Patina pellucida* (L.). It lives mainly on laminarian seaweeds from LWST to 27m (Vahl, 1971; Fretter & Graham, 1976), being found in places with considerable water movements but not in extreme exposure (Ebling *et al.*, 1948). The geographical distribution of this species is broader and more complex than its congenerics, ocurring on Icelandic shores, all British and Irish coasts, the Lofoten Islands, and from Norway to Portugal, but absent from Belgium, Holland, the east coast of Denmark and the Baltic Sea (Fretter & Graham, 1976; Hayward & Ryland, 1990). It has also been recorded from the western coasts of the Mediterranean Sea (Thorson, 1941; D'Angelo & Gargiullo, 1979).

The biology and ecology of the South African species of *Helcion* are not well known, and to date studies have concentrated on *H. pellucidum*, probably due to its interesting association with macrophytes, with which its life history is correlated. Therefore studies on its life history (Graham & Fretter, 1947), behaviour (Kain & Svendsen, 1969), growth and density (Vahl, 1971), and recruitment and growth (McGrath, 1992) have been published.

Recently the potential relationships between *Helcion* spp. as well as between *Helcion* and members of the Patellidae have been explored using sperm morphology (Hodgson & Bernard, 1988; Jamieson *et al.*, 1991; Hodgson *et al.*, in press). *H. pectunculus* and *H. pruinosus* were found to have sperm with similar morphologies (termed Type IV by Hodgson & Bernard, 1988) and distinct from species of *Patella*. *H. dunkeri* however had a sperm which was similar to species of *Patella* with Type III sperm (Hodgson *et al.*, in press). Sousa & Oliveira (1994) suggested that the sperm of *H. pellucidum* was similar to other North Atlantic and Mediterranean species whereas Hodgson *et al.* (in press) proposed that the affinity of this species was uncertain, but on the basis of acrosomal morphology aligned it with species with Type III sperm. In a spermiocladistic analysis (which excluded *H. dunkeri* and *H. pellucidum*) Jamieson *et al.* (1991) found that *H. pectunculus* and *H. pruinosus* clustered together in a heterogeneous group with other species of *Patella*, *Cellana* and *Nacella*.

Thus the relationships within the genus *Helcion* and between *Helcion* and other patellids remains unresolved. This together with the interesting geographic distribution of *Helcion* prompted this investigation.

Allozyme electrophoresis was chosen as a means to further evaluate the relationships between these species as well as assessing whether or not they constitute a monophyletic group. This technique has proven to be a valuable tool to assess the degree of genetic divergence between species (Thorpe, 1982, 1989; Buth, 1984; Richardson *et al.*, 1986); it has been widely used in invertebrate systematics (see Thorpe & Solé-Cava, 1994); and in addition has been successfully used to solve species problems and assess relationships within *Patella* (Gaffney, 1980; Sella & Badino, 1980; Côrte -Real, 1992; Sella *et al.*, 1993; Cretella *et al.*, 1994).

MATERIALS AND METHODS

Collection of Samples

Samples of *Helcion pellucidum* (L.) were obtained during LWST at Port Erin Bay (54° 05'N-4° 45'W) (Isle of Man) on March 1994 from the inside of holdfasts of *Laminaria hyperborea* (Gunnerus). Samples of *H. pruinosus* and *H. pectunculus* were collected from rocky shores at Port Elizabeth (33° 58'S-25° 38'E), South Africa on May 1994, and samples of *H. dunkeri* were obtained from the same locality on August 1994. All limpets were sent alive to the Port Erin Marine Laboratory of the University of Liverpool, Isle of Man, where they were frozen at -70°C until required for electrophoresis.

Electrophoretic Analysis

Enzyme extracts were prepared using the foot muscle of each animal, which was macerated in 0.02M Tris-HCl, pH 8.0 buffer and centrifuged at 10.000 rpm for 4 min. The standard horizontal 12.5% starch-gel (Sigma Chemicals Co.) electrophoresis was carried out using the following buffer systems:

I. Tris-citrate, pH 8.0 (Siciliano & Shaw, 1976); II. Tris-citrate, pH 7.0 (Siciliano & Shaw, 1976); III. Discontinuous borate-tris-citrate, pH 8.2-8.7 (Poulik, 1957); IV. Tris-citric-boric-LiOH, pH 8.26-8.31 (Redfield and Salini, 1980); V. Tris-boric EDTA, pH 9.0 (Ayala *et al.*, 1974).

The enzymes resolved with their codes, EC numbers and names (based on IUBNC, 1984 and Shaklee *et al.*, 1990), buffer systems used, and number of loci analysed are listed in Table 1. The enzyme-specific staining procedure followed the techniques of Brewer (1970), Shaw & Prasad (1970), Harris & Hopkinson (1976) and Murphy *et al.* (1990).

Statistical parameters such as allele frequencies, variability measures, unbiased genetic identities and distances (Nei, 1978) were obtained using the BIOSYS-I Program (Swofford & Selander, 1981). The same program was used for constructing phenetic trees by means of Cluster Analysis (UPGMA) and Distance Wagner Procedure. DISPAN (Genetic Distance and

Phylogenetic Analysis) version 1.1 of 1993, supplied by T. Ota and The Pennsylvania State University, was used to obtain bootstrap proportions for clades after 1000 replications of UPGMA analysis.

The PAUP (Phylogenetic analysis using parsimony) program, version 2.4 for IBM-PC (Swofford, 1985) was used for obtaining cladograms of the taxa. This program was used mainly for clarifying relationships within the South African branch. An exhaustive search for the most parsimonious tree(s) (shortest) over all possible topologies was performed by Branch and Bound methods (Hendy & Penny, 1982), using 1. Alleles as binary characters, 2. Loci as multistate unordered characters. *H. pellucidum* was used as outgroup for both analyses. All alleles were included as characters in the first analysis. For the second one, autapomorphic alleles for single taxa were excluded for coding states and *H. pellucidum* was assumed to have state A (Table 2).

No attempt to order states in any of the characters was made due to the small sample sizes that were restricted by the availability of *H. dunkeri* individuals. Many transitions of states that could be described in this study would be based on rare alleles. It is known that the detection of rare alleles (of very low frequency) is dependent on sample size. The only recognition of states at loci as multistate characters will be more vulnerable to sample errors if sample sizes are small or different (see Buth, 1984). Therefore, any ordering of states would be even more liable to this type of error, excepting for taxa with very low variability, which was not the case for *Helcion.*

RESULTS

The allele frequencies for the twenty-one loci analysed for *Helcion* spp. as well as measures of genetic variability are given in Table 3. All species showed the same percentage of polymorphic loci (81%) at a maximum frequency of 0.99 for the most common allele. This value was higher than those given in the literature (Sella *et al.*, 1993; Cretella *et al.*, 1994) for some species of the genus *Patella*. The range of expected heterozygosity for *Helcion* spp. was 0.202-0.341, slightly higher than the range (0.167-0.280) that we derived from the literature (Sella *et al.*, 1993; Cretella *et al.*, 1994; Hurst & Skibinski, 1995) for some species of *Patella*.

Phenetic Analysis. The genetic relationship among *Helcion* species can be visualised by the UPGMA (Figure 1A) and by the Wagner tree (Figure 1B). Both phenetic trees agreed in showing a separate branch for the South African species, which are more closely related to each other than to the North-east Atlantic *H. pellucidum*. The Nei's genetic identity (I) within the South African group ranged from 0.541 to 0.704, where the most closely related species were *H. pectunculus* and *H. pruinosus*, and the most distant ones were *H. pectunculus* and *H. dunkeri* (Table 4). The degree of genetic divergence within the South African branch correspond to a level that has been characterised in the literature for congeneric species (Thorpe, 1982). The phenetic analysis showed a very low genetic identity between *H. pellucidum* and its South African congeners, with a range of I values of 0.191-0.241, which are lower than the critical level of identity value (I=0.35) which distinguishes between genera (Thorpe, 1982). The highest distance (D= -lnI = 1.654) was observed between *H. pellucidum* and *H. pruinosus*.

Cladistic Analysis. After an exhaustive search with the Branch and Bound method, one shortest (most parsimonious) tree was found for each analysis. The cladogram obtained when considering alleles as binary characters (presence-absence) (Figure 2A) showed the same topology as when loci were considered as unordered multistate characters (Figure 2B). Both cladograms were consistent with the results of the phenetic analysis (Figure 1), showing *H. pectunculus* to be more closely related to *H. pruinosus* than to *H. dunkeri*.

DISCUSSION

Studies of sperm morphology of *Helcion* spp. suggested that *H. pellucidum* is distantly related to the 3 Southern African species (Hodgson *et al.*, in press). The high degree of genetic divergence found in the present study between *H. pellucidum* and the South African group is more characteristic of non-congeneric species (Thorpe, 1982; Nei, 1987). Only two electromorphs (see Table 3: Pgm^C, Pgm^D) out of seven that could have characterised *Helcion* were found not to be plesiomorphic after comparison with some North-east Atlantic and Mediterranean limpets of the genus *Patella* (Weber *et al.*, in prep.). In the same work, *H. pellucidum* was grouped with the North-east Atlantic and Mediterranean species of *Patella* while the South African *Helcion* were clustered together with the only African species (*P. safiana*) included in the analysis. This evidence suggests that *Helcion* may not be a monophyletic group and that *H. pellucidum* and the South African branch might have arisen independently, perhaps from different patellid ancestors. However a cladistic analysis based on sperm, radula, shell and soft part morphology has suggested that *Helcion* is monophyletic (Ridgway, 1994). Thus it is difficult to propose an origin of *Helcion* while its monophyly is uncertain and therefore its status as a genus might not be justified. The taxonomic characterisation of the genus *Helcion* does not help on this matter. *Helcion* is only distinguished from *Patella* by the pallial circle of gills which are interrupted anteriorly by the head. However, *H. pruinosus* has been observed as having a complete pallial cordon (Powell, 1973); and the development of the anterior portion of the pallial gills has been described as a character that can vary within species of limpets (Lindberg & McLean, 1981; Lindberg, 1988). Only further studies using biochemical and molecular genetics, sperm structure, and other conservative characters in all *Helcion* and related patellid species will enable conclusions to be drawn about the monophyletic or non-monophyletic conditions of the genus *Helcion*.

In the present study, the South African branch was well characterised as a monophyletic group by seven electromorphs (see Table 3: $Aat\text{-}2^{D*}$, $Sod\text{-}1^A$, $Est\text{-}3^B$, $Ldh\text{-}1^B$, $Ldh\text{-}2^{D*}$, $Idh\text{-}1^{E*}$, and $Idh\text{-}2^{A*}$). Only four of them (*) were maintained as synapomorphies for the group after comparing *Helcion* species with some North-east Atlantic and Mediterranean limpets of the genus *Patella* (Weber *et al.*, in prep.). *H. dunkeri* was found to be the most distantly related species and therefore confirms those findings based on sperm morphology (Hodgson *et al.*, in press). *H. pectunculus* and *H. pruinosus* were more closely related to each other, being characterised by five synapomorphic electromorphs (see Table 3: Est-1^{L}, Mdh-2^{A}, Mdh-2^{C}, Fbald^{A}, and Pgdh^{B}). Both *H. pectunculus* and *H. pruinosus* were found to be monophyletic in the cladistic analysis by Jamieson *et al.* (1991). The same authors suggest *H. pruinosus* as the apomorph sister-species of *H. pectunculus*, differing from the latter spermatologically in the development of an anterior extension of the acrosome. The longer branch length observed for *H. pruinosus* in both cladograms (Figure 2) is congruent with this observation, suggesting the presence of more autapomorphies for this taxon. Thus these findings do not agree with the taxonomic proposals of Powell (1973) who placed *H. pruinosus* and *H. dunkeri* in the subgenus *Patinastra* and *H. pectunculus* in the subgenus *Helcion*.

The molecular clock hypothesis predicts that amino acid substitution in protein molecules is an approximately regular, but stochastic, process and consequently the number of substitutions occurring between homologous proteins may be related to evolutionary time (Thorpe, 1982). Using the calibration suggested by Thorpe (1989) of a Nei's D of 1.0 equivalent to 18 Myr and the equation for correcting to linearity ($D=0.091+0.289t$; $t=6Myr$) we calculate that the divergence between *H. pellucidum* and the stem species of the South African branch of *Helcion* might have happened around 28 to 30 Myr ago, during the Oligocene Epoch of the Tertiary Period. By the same calibrations *H. dunkeri* might have diverged from the stem species of *H. pruinosus* and *H. pectunculus* by the beginning of the Pleistocene (8.9 to 9.4 Myr), and *H. pruinosus* might have diverged from *H. pectunculus* by the second half of the same epoch (5.4 to 6.3 Myr ago).

It is very important to be cautious in using these estimates, because estimates of time of divergence based on molecular data are compounded by too many sources of error and could be highly misleading (Hillis & Moritz, 1990). All calibrations are for vertebrates, and therefore it is not possible to assign confident limits to estimations derived from any of the possible rates because of the lack of calibrations for the group of interest (Hillis & Moritz, 1990). It is also known that different DNA sequences evolve at markedly different rates, therefore rates of evolution are heterogeneous across nucleotide sites, genes, and genomes within phylogenetic lineages (Gillespie, 1991; Li & Grauer, 1991; Avise, 1994). A more controversial form of rate heterogeneity involves possible differences in evolutionary tempo of homologous DNA sequences across organismal lineages (Avise, 1994), where nucleotide generation time seems to be important for explaining differences in absolute rates of molecular evolution across animal groups (Martin & Palumbi, 1993).

In conclusion, there is some doubt about the monophyletic origin of *Helcion* species and the validity of the genus. Perhaps *Patina* should be retained as a genera name for the North-east Atlantic species. Even if *Helcion* is monophyletic the divergence of *H. pellucidum* was long before the South African species branched. The existence of several South African *Helcion* species compared to one of the North-east Atlantic suggests that speciation happens more readily in the South Atlantic. This resembles the situation in *Patella*.

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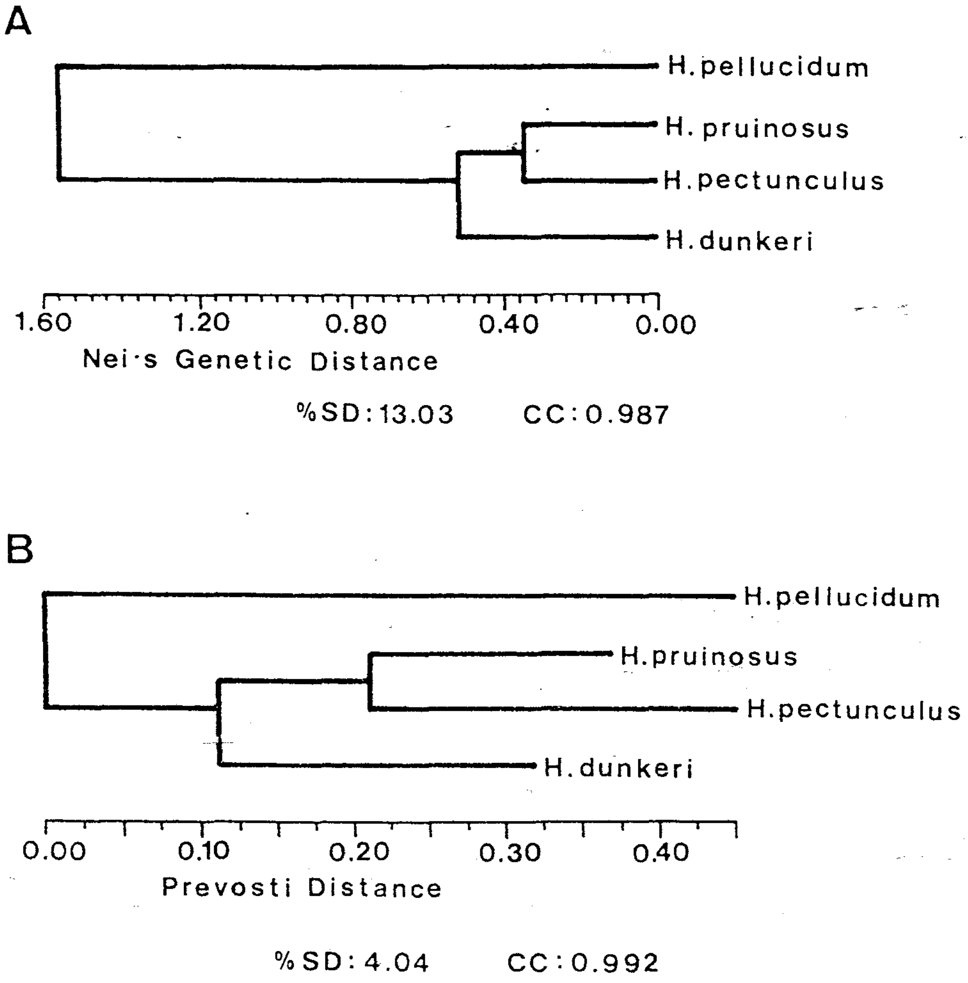

Figure 1: Phenetic trees. A. UPGMA. B. Distance Wagner Procedure. (BP) Bootstrap Proportion in %; (%SD) Percent Standard Deviation; (CC) Cophenetic Correlation.

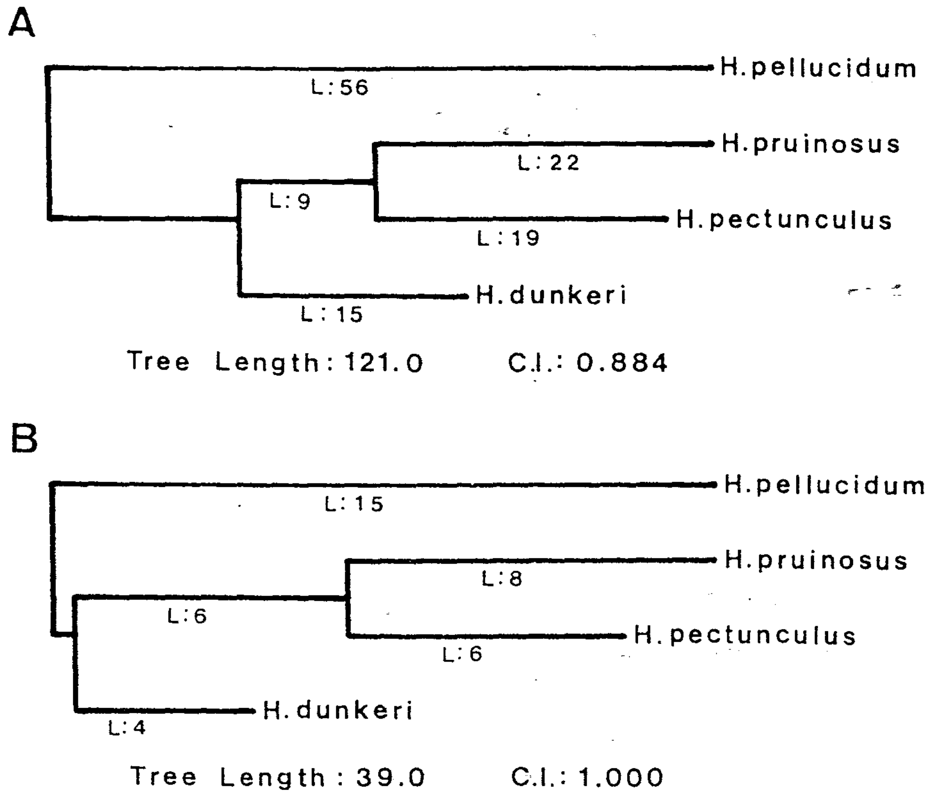

Figure 2: Cladograms. A. Alleles as binary characters. B. Loci as unordered characters. (L) Branch Length; (C.I.) Consistency Index.

Table 1: Names, EC numbers, abbreviations, number of loci and buffer systems used for the enzymes analysed for *Helcion* spp.

Enzyme Name*	EC number*	Abbreviation	Number of loci	Buffer Systems
Aspartate aminotransferase	2.6.1.1	AAT	2	III
Esterase	3.1.1.-	EST	3	III
Fructose-biphosphate aldolase	4.1.2.13	FBALD	1	V
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	GAPDH	1	V
Glutamate dehydrogenase	1.4.1.-	GLUDH	1	V
Glucose-6-phosphate isomerase	5.3.1.9	GPI	1	I
Isocitrate dehydrogenase (NADP+)	1.1.1.42	IDHP	2	I
L-Lactate dehydrogenase	1.1.1.27	LDH	2	II
Malate dehydrogenase	1.1.1.37	MDH	2	IV
Malic Enzyme (NADP+)	1.1.1.40	MEP	2	I
Mannose-6-phosphate isomerase	5.3.1.8	MPI	1	IV
Phosphoglucomutase	5.4.2.2	PGM	1	II
Phosphogluconate dehydrogenase	1.1.1.44	PGDH	1	I
Superoxide dismutase	1.15.1.1	SOD	2	III

*IUBNC (1984); Shaklee *et al.* (1990)

Table 2: Data matrix for *Helcion* spp. when loci were considered unordered multistate characters. Each column shows the state for the character (locus) listed in the same order as in Table 3.

H. pellucidum	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
H. pruinosus	A	B	B	D	C	B	C	A	B	B	B	B	B	B	B	B	B	A	B	B	A
H. pectunculus	A	D	B	B	B	C	B	B	C	B	C	B	C	C	B	D	C	B	C	B	A
H. dunkeri	A	C	A	C	C	D	A	A	C	B	C	B	C	D	C	C	B	C	D	B	A

Table 3: Allele frequencies for *Helcion spp.*

	Population			
Locus	*H. pellucidum*	*H. pruinosus*	*H. pectunculus*	*H. dunkeri*
Aat-1				
(N)	(40)	(27)	(30)	(25)
A	0.000	0.019	0.000	0.000
B	0.000	0.000	0.017	0.000
C	1.000	0.963	0.983	0.960
D	0.000	0.000	0.000	0.040
E	0.000	0.019	0.000	0.000
Aat-2				
(N)	(40)	(27)	(30)	(25)
A	0.050	0.000	0.000	0.000
B	0.000	0.000	0.950	0.040
C	0.950	0.000	0.000	0.000
D	0.000	0.185	0.050	0.520
E	0.000	0.370	0.000	0.360
F	0.000	0.019	0.000	0.060
G	0.000	0.426	0.000	0.000
H	0.000	0.000	0.000	0.020
Est-1				
(N)	(34)	(27)	(30)	(25)
A	0.000	0.000	0.000	0.120
B	0.000	0.000	0.000	0.140
C	0.000	0.000	0.000	0.120
D	0.309	0.000	0.000	0.000
E	0.235	0.000	0.000	0.300
F	0.191	0.000	0.000	0.280
G	0.265	0.000	0.000	0.000
H	0.000	0.000	0.000	0.040
I	0.000	0.074	0.000	0.000
J	0.000	0.148	0.000	0.000
K	0.000	0.407	0.000	0.000
L	0.000	0.370	0.367	0.000
M	0.000	0.000	0.633	0.000
Est-2				
(N)	(36)	(27)	(30)	(24)
A	0.000	0.000	0.000	0.208
B	0.917	0.000	0.067	0.021
C	0.083	0.000	0.883	0.000
D	0.000	0.130	0.050	0.646
E	0.000	0.000	0.000	0.125
F	0.000	0.796	0.000	0.000
G	0.000	0.074	0.000	0.000
Est-3				
(N)	(30)	(27)	(30)	(25)
A	0.000	0.000	0.000	0.040
B	0.000	0.278	0.017	0.520
C	0.000	0.037	0.000	0.300
D	0.900	0.685	0.950	0.140
E	0.100	0.000	0.000	0.000
F	0.000	0.000	0.033	0.000

Continue next page.

Table 3 (Continued).

Fbald				
(N)	(39)	(27)	(30)	(25)
A	0.000	0.130	0.850	0.000
B	0.000	0.000	0.150	0.000
C	0.000	0.852	0.000	1.000
D	1.000	0.019	0.000	0.000
Gapdh				
(N)	(36)	(27)	(30)	(25)
A	0.000	0.222	0.000	0.000
B	0.000	0.000	0.000	0.100
C	0.000	0.778	0.000	0.000
D	0.000	0.000	0.883	0.000
E	0.208	0.000	0.117	0.200
F	0.792	0.000	0.000	0.700
Gludh				
(N)	(38)	(27)	(30)	(25)
A	0.974	0.000	0.000	0.000
B	0.000	0.000	1.000	0.000
C	0.026	1.000	0.000	0.940
D	0.000	0.000	0.000	0.060
Gpi				
(N)	(42)	(27)	(30)	(25)
A	0.095	0.000	0.000	0.000
B	0.905	0.000	0.000	0.000
C	0.000	0.185	0.100	0.400
D	0.000	0.722	0.883	0.560
E	0.000	0.000	0.017	0.040
F	0.000	0.093	0.000	0.000
Idhp-1				
(N)	(37)	(27)	(30)	(25)
A	0.135	0.000	0.000	0.000
B	0.676	0.000	0.000	0.000
C	0.000	0.852	0.883	0.520
D	0.189	0.000	0.000	0.000
E	0.000	0.148	0.100	0.480
F	0.000	0.000	0.017	0.000
Idhp-2				
(N)	(36)	(27)	(30)	(25)
A	0.000	0.019	0.033	0.060
B	0.069	0.074	0.000	0.000
C	0.000	0.907	0.950	0.940
D	0.931	0.000	0.000	0.000
E	0.000	0.000	0.017	0.000
Ldh-1				
(N)	(37)	(27)	(30)	(25)
A	0.000	0.000	0.000	0.420
B	0.000	1.000	0.800	0.580
C	0.919	0.000	0.000	0.000
D	0.000	0.000	0.200	0.000
E	0.081	0.000	0.000	0.000

Continue next page.

Table 3 (Continued)

Sod-1				
(N)	(38)	(27)	(30)	(25)
A	0.000	1.000	1.000	1.000
B	1.000	0.000	0.000	0.000
Sod-2				
(N)	(40)	(27)	(30)	(25)
A	0.075	0.000	0.000	0.000
B	0.925	1.000	1.000	1.000
Ho*	0.197	0.220	0.173	0.230
	(0.042)	(0.048)	(0.036)	(0.041)
He**	0.245	0.256	0.202	0.341
	(0.051)	(0.053)	(0.037)	(0.054)
$P_{0.99}$***	81.0	81.0	81.0	81.0

* Direct-count mean heterozygosity;

** Unbiased estimate (Nei, 1978) of Hardy Weinberg mean expected heterozygosity;

***A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99.

Table 4: Degree of genetic divergence expressed by Nei's (1978) unbiased genetic distance (below the diagonal) and identity (above the diagonal).

Population	*H. pellucidum*	*H. pruinosus*	*H. pectunculus*	*H. dunkeri*
H. pellucidum	*******	0.191	0.199	0.241
H. pruinosus	1.654	*******	0.704	0.652
H. pectunculus	1.615	0.351	*******	0.541
H. dunkeri	1.423	0.427	0.615	*******