ASPECTS OF THE ECOLOGY AND REPRODUCTIVE BIOLOGY OF THE LIMPET, *HELCION PRUINOSUS* (GASTROPODA : PROSOBRANCHIA).

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requirements for the degree of

MASTER OF SCIENCE

OF

RHODES UNIVERSITY

by

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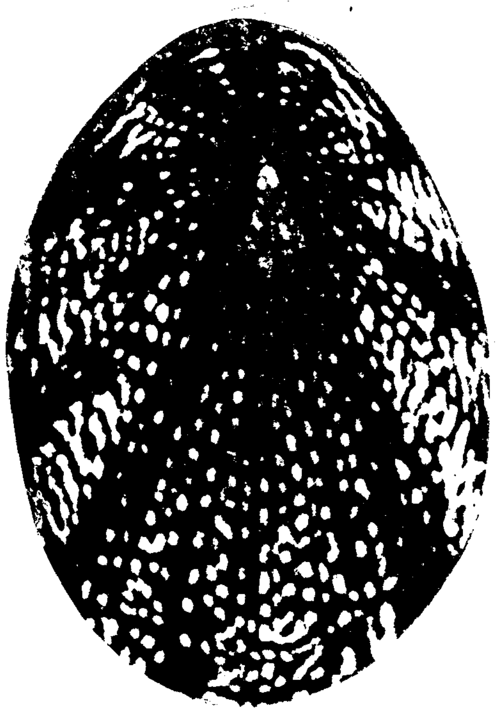
December 1998



Helcion pruinosus
(Krauss, 1848)
[Mag. x 5]

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ABSTRACT

Helcion pruinosus, the rayed limpet, is found in the midshore region of the intertidal zone, (i.e. the Balanoid zone) of rocky shores, from Saldanha Bay to Sodwana Bay along the coast of South Africa. At Gonubie (32°57'S/25°01'E) this species of limpet was found under boulders, (when not feeding), and could reach average densities of $85/\ m^2$. The limpets prefer smooth boulders, avoiding those encrusted by coralline algae or boulders with a rough texture. There was no habitat segregation between adult and juvenile *H. pruinosus*, i.e. there was no sign of up-shore migration by larger individuals. Males out-numbered females by, on average 1.4 : 1, which was a similar sex ratio to that of the congeneric species, *H. pectunculus* (Gray, 1996). Unlike *H. pectunculus*, there were no differences in shell lengths between males and females ($p = 0.946$ at Gonubie, south-east coast and $p = 0.961$ at Kommetjie, south-west coast; t-test). Shell height could also not be used as a criterion to differentiate between the sexes.

The average maximum shell length of *H. pruinosus* at Gonubie and Kommetjie was 25 mm, but the maximum shell lengths found were 30 mm, (one individual in each case), on both the south-east coast and west coast. Growth occurred most rapidly in the first year of life, with individuals reaching 15 mm after 1 year. In the second year limpet growth slowed to only 7 mm. Sexual maturity was reached at a shell length of between 11 to 14 mm (at an age of 8 months to 1 year). The life-span of *H. pruinosus* was determined at 2.55 years on the south-east coast and 2.9 years on the south-west coast. Micro-growth bands were produced tidally. Growth occurred allometrically, i.e. shell length increased at a faster rate than shell height.

The limpets foraged during low tide, at night, presumably feeding on epilithic algae on the boulders. A second smaller peak of limpet activity was often recorded during the day, when low tide coincided with dawn). More limpets (up to ten times more) were active at spring tides compared to neap tides. Limpet activity was greatest during the spring tides of autumn. During all seasons limpet activity peaked 30 minutes before low water, after which numbers decreased rapidly. All limpets had retreated beneath the boulders before being covered by the flooding tide. It was concluded that the limpets were responding to both endogenous and exogenous cues, but the actual stimulus for retreat could not be determined. The limpets did not return to a fixed scar nor did they return rigidly to a home site.

H. pruinosus was found to be gonochoristic. Sexes could be separated, on dissection, according to the colour of their gonads (males had white gonads, whilst that of the females was

olive-green). Gonads were present throughout the year in most individuals, i.e. totally spent animals were never observed.

Reproductive seasonality was similar in west coast and south-east coast animals. The gonad indices of the west coast limpets were higher, (maximum of 35%), compared to that on the south-east coast, (25%). The animals were probably trickle spawners, with some individuals liberating gametes throughout the year. There was however synchrony in gametogenesis between male and female limpets on both the west coast and south-east coast lines. Gonad indices peaked in the summer months (October to December) with a second minor peak in April/May. These peaks occurred before the release of the gametes in spawning events. The gonad index was lowest in winter, (July). The ovaries were full of mature eggs (oocytes having a diameter of 200 to 250 μm) prior to spawning. The acinal wall thickness had been decreasing prior to spawning. After spawning there was an increase in previtellogenic eggs (< 100 μm) in females, and an increase in the acinal wall thickness to indicate spermatogenesis. The two populations are subjected to different environmental conditions and the actual cue for stimulating the release of gametes could not be determined.

Finally, a table of differences between *H. pruinosus* and its sister species, *H. pectunculus* was created to show the life-style of *H. pruinosus*.

CHAPTER 1:

General introduction

CHAPTER 1 - GENERAL INTRODUCTION

Prosobranch limpets of the family Patellidae through their grazing activities play a major role in the structuring of intertidal communities (Hawkins and Hartnoll, 1983). These gastropods, therefore, have been the subjects of numerous investigations (see Branch, 1981, 1985a, 1985b; Hawkins and Hartnoll, 1983; Little, 1989; Gray, 1996 for reviews of literature). A great number of studies on limpets have been on species of *Patella* (Patellinae). By contrast, the biology of the sister genus, *Helcion*, is not well known, although recently Gray (1996) examined aspects of the biology of *Helcion pectunculus*. Although there are several published works on the North Atlantic species of *Helcion*, *H. pellucidum* (Vahl, 1971, 1972, 1983), it has now been shown that this species is distantly related to the three other species (*H. pectunculus, H. pruinosus* and *H. dunkeri*), which are restricted to southern Africa, and should be removed from the genus and placed within *Patella* (Weber et al., 1997; Ridgway et al., 1998).

One of the commonest limpets within the mid-intertidal regions (usually lower Balanoid zone) of South African boulder shores is *H. pruinosus* (Krauss, 1848) (Kilburn and Rippey, 1982; A.N. Hodgson, pers. comm.). Within South Africa it has a broad distribution, being found from Saldanha Bay on the west coast, to Sodwana Bay in KwaZulu-Natal on the east coast (Kilburn and Rippey, 1982; Branch et al., 1994) (Figure 1.1). This limpet, reaching a maximum size of 30 mm, is readily recognised by its shell which bears luminescent green markings (frontispiece), giving it its common name of the rayed-limpet (Branch et al., 1994). These markings are best displayed when the limpet is

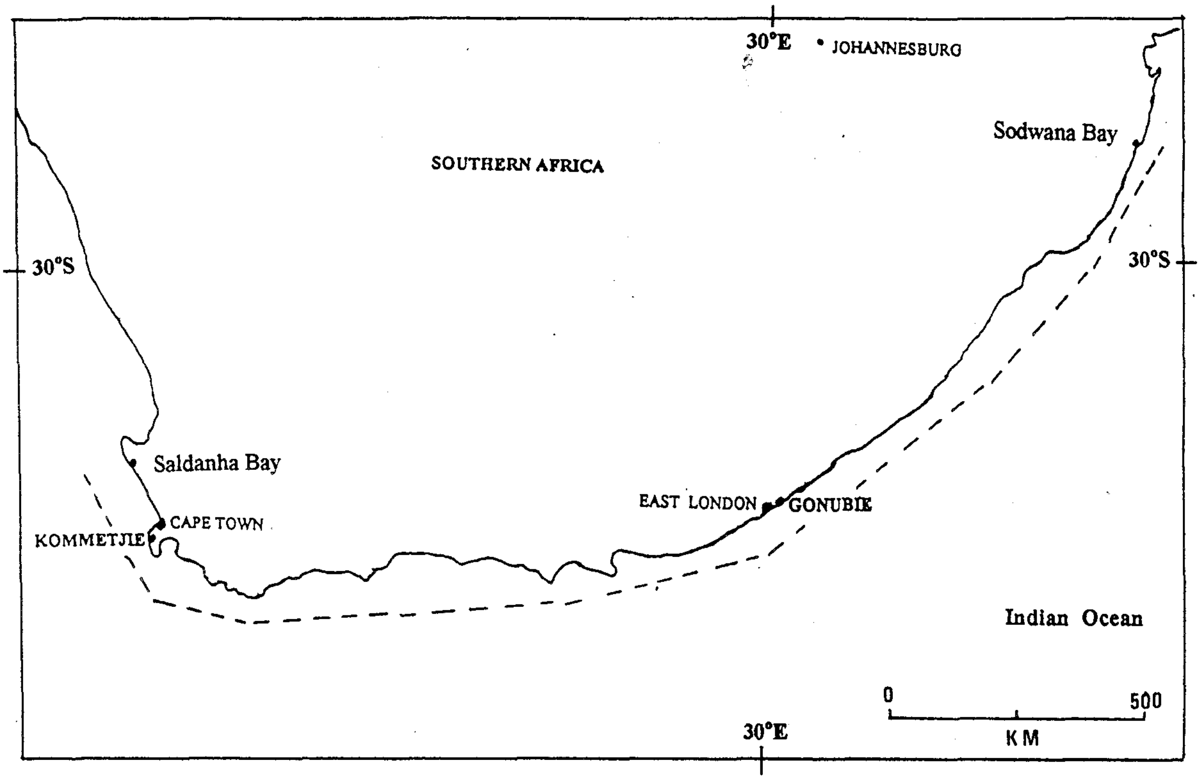

Figure 1.1 Map of southern Africa to show the distribution of *Helcion pruinosus* (shown as dotted line), as well as the location of the two study sites, Kommetjie on the west coast and Gonubie on the south-east coast.

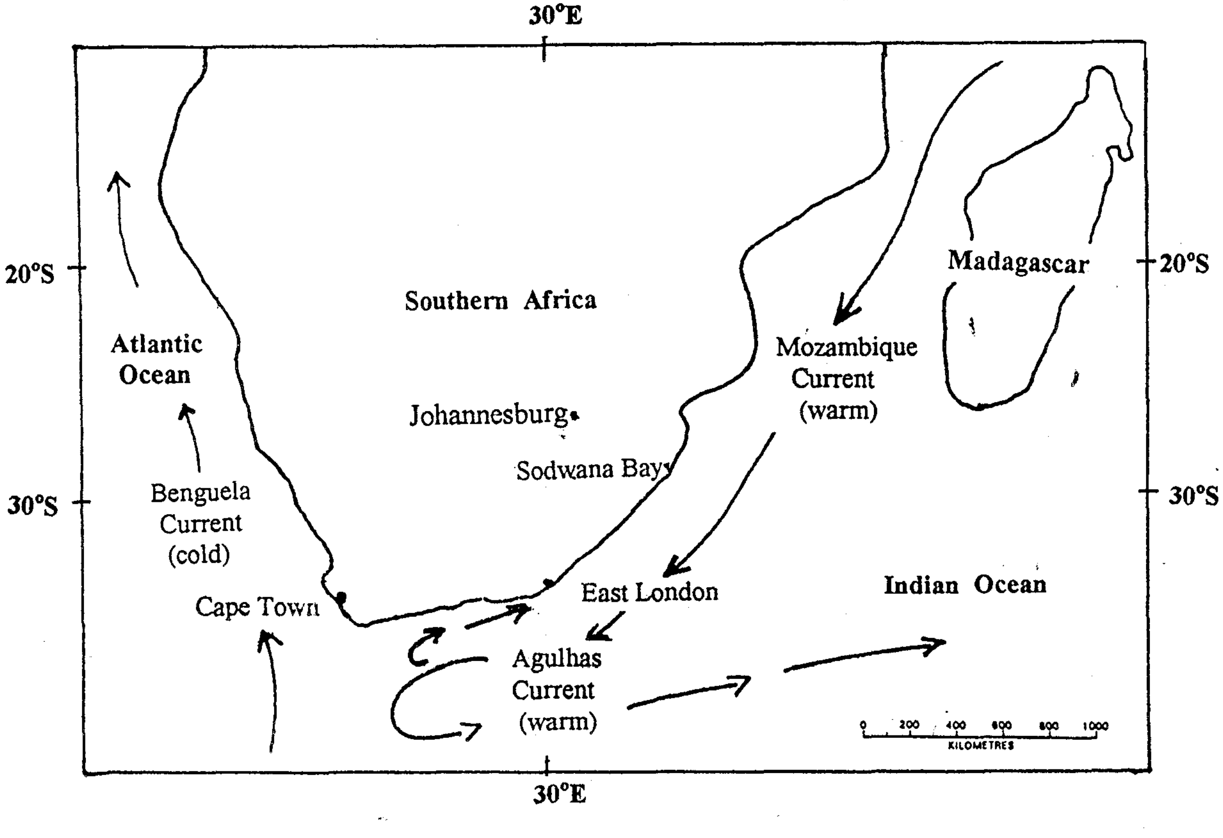

Figure 1.2 Map of southern Africa to show the major ocean currents influencing the two coast lines. (Map is modified from Stone, 1988).

submerged. Despite the apparent abundance of *H. pruinosus* on many shores, there is no published information on the biology of this species except for morphological descriptions of spermatozoa (Hodgson and Bernard, 1988) and pallial tentacles (Hodgson et al., 1987) and the genetic relationships of the species to other members of the genus (Weber et al., 1997).

The abundance of *H. pruinosus* on boulder shores suggests that this species is important to community dynamics within these ecosystems. The main aim of this work, therefore, was to provide some fundamental information on the biology (in particular, the reproductive seasonality) of this limpet. The data would serve as a first step towards understanding the role of *H. pruinosus* on South African rocky shores. The main study site chosen was a stretch of rocky shore, north-east of East London, at Gonubie, (32°57' S/ 28°01' E), on the south-east coast of the Eastern Cape Province of South Africa (Figures 1.1 and 1.2). (This population will be known henceforth as the south-east coast population). However, some reproductive data was collected from a population living on the west coast, near Cape Town, at Kommetjie, (34°04' S/ 18°19'E) on the Cape Peninsula. (This population will be known as the west coast population in the rest of the study). This allowed some comparisons to be made between animals subjected to different oceanographic and climatic conditions.

The average water temperature at Kommetjie (14°C) is 5°C lower than the average experienced at Gonubie (19°C) (Chapter 5). This is due to the prevailing cold Benguela current (Figure 1.2) originating from the southern oceans which is deflected northward by southern Africa. The southern and eastern coastlines are influenced by the warmer

Agulhas current (Figure 1.2). This latter current is part of the Mozambique current which is forced to flow southward from the equator, owing to deflection by the African continent. The Agulhas current is narrow (about 100 km wide) and as it averages a flow of 1 m/ sec, and frequently exceeds 2 m/ sec at its core, it is considered fast flowing (Ross, 1988). Its surface temperature varies seasonally between 22°C and 27°C (Ross, 1988). The current is closest to the South African coast at East London (Stone, 1988) and then veers off the coast at Port Elizabeth, south of East London, where its flow is less concentrated and it becomes less rapid. A counter current develops offshore, due to retroflection and this circulates water eastward once more (Figure 1.2). The presence of such retroflective currents from the Agulhas current can cause rapid changes in sea temperature. In winter a temperature difference of as much as 10°C can be found between the inshore water and the warm Agulhas current further offshore (Stone, 1988).

The amount of solar radiation both research sites receive should theoretically be identical, as both sites are only one degree of latitude apart, (Gonubie at 32°50' south and Kommetjie at 34°08' south). Kommetjie, however, is subjected to misty conditions owing to the cold water meeting warm air subsiding from the land. The amount of nutrients within the water of the south-east coast and west coasts, which would influence the abundance of micro-algae on the rocks differs as the west coast is subjected to regular upwellings of nutrient-laden waters (Bustamante et al., 1995). Such upwellings are not a regular occurrence on the south-east coast.

In Chapter 2 data on the density, biomass and distribution of *H. pruinosus* at the main study site (Gonubie) are presented. Chapter 3 presents information on the growth rate of the limpets. Obtaining food is clearly necessary for growth and reproduction. As active molluscs are susceptible to desiccation and predation, foraging must be timed to maximise survival, and therefore the foraging activity of *H. pruinosus* at Gonubie is examined in Chapter 4. Chapter 5 describes and compares the reproductive cycles of limpets at Gonubie and Kommetjie. Finally, Chapter 6 is a general discussion in which avenues for future research are also proposed.

CHAPTER 2 : Distribution, density and biomass of *Helcion pruinosus* at Gonubie.

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CHAPTER 2 - Distribution, density and biomass of *Helcion pruinosus* at Gonubie

2.1 - Introduction

If the structure and function of an intertidal ecosystem is to be understood, information on the community composition, abundance of organisms at each trophic level and the flow of energy to each trophic level is crucial (Odum, 1971). Limpets are known to play a pivotal role in the structuring of intertidal marine ecosystems (Branch, 1981) and on South African shores they can reach very high densities, e.g. *Patella aphanes* can reach densities of 7600/ m^2 on the Natal coastline (Robson, 1986). Because limpets are so prominent on rocky shores it has been suggested that studies on limpet population biology could help in the detection of long term ecological changes in the intertidal community (Thompson, 1979), especially where changes are caused by anthropogenic disturbances, such as oil spills (Southward and Southward, 1978).

Much of our knowledge of the population structure and distribution of South African limpets has come from the studies of Branch (1971, 1974a, 1974b) on the genus *Patella*. Branch (1971, 1981) has shown how the distribution and feeding habits of the numerous sympatric species of *Patella* are generally sufficiently different to reduce competition between them. More recent information on limpet density, biomass and population structure have been from studies on *P. aphanes* (Robson, 1986), *P. granularis* (Bosman and Hockey, 1988a, 1988b), *Cellana capensis* (Lasiak, 1993) and *Helcion pectunculus* (Gray, 1996). Thus, for the genus *Helcion*, of which there are three species in southern Africa (Kilburn and Rippey, 1982), quantitative information on the abundance and population structure is available for one species only.

Whereas *H. pectunculus* is found mainly in the upper intertidal zone around the coast of southern Africa (Kilburn and Rippey, 1982; Gray, 1996), its sister species *H. pruinosus* lives lower down the shore (Kilburn and Rippey, 1982). There have been no quantitative surveys of the density and distribution of this species. The aim of this study was to document the distribution, density, biomass and population structure of *H. pruinosus* at the main research site in the south-eastern Cape. This information was of particular importance to establish whether the shore could sustain long-term (one to two years) sampling of animals for a study of reproduction. Furthermore, establishing the distribution of these limpets on the shore was crucial to enable a study of their foraging activities.

2.2 Materials and Methods

2.2.1 Sampling Procedure

The shore at Gonubie (32° 57' S/25° 01' E) consists of rock platforms with gullies (containing small boulders) and ridges, adjacent to which are sections of flat boulder shores. It was therefore decided to determine the distribution and population structure on both shore areas (hereinafter referred to as platform shore and boulder shore). Two line transects, one on a region of platform and one on the boulder section of shore, were laid out at low water spring tide in April 1995 between the spring high water and low water marks. The profile of each transect was then surveyed using a theodolite and 5 m measuring staff.

At five metre intervals along the transect lines, four randomly placed 0.5 m^2 quadrats were sampled. Using key indicator species to identify the five recognised zones on southern and eastern Cape shores (Branch and Branch, 1981; Lubke, 1988), the five metre sampling intervals meant that 20 x 0.5 m^2 quadrats were sampled in each of the *Littorina*, upper Balanoid, lower Balanoid and *Cochlear* zones. Owing to the continuous strong wave activity, the subtidal zone could not be sampled. From each quadrat, all specimens of *Helcion pruinosus* were collected and once back at the laboratory stored in a deep freezer until further analysis could be carried out.

In addition to counting all limpets within each quadrat, the dimensions of the limpets' shells (length, height, width) were measured to the nearest 0.001 mm using vernier callipers. Shell length only was used to describe the population size structure, the remaining morphometric data being used for analysis of limpet growth (Chapter 3).

After removal of their shells, animals were blotted dry of excess water and their wet body mass determined (weighed to nearest 0.001 g). Removal of animals from their shells also allowed each limpet to be sexed macroscopically according to the colour of the gonad. Males have a white to cream coloured gonad, whilst the gonad of females is olive-green. Individuals with a shell length of less than 10 mm could not be sexed as they never possessed a discernible gonad. Once sexed the bodies of each animal were dried (at 60 °C) to a constant weight.

Sex ratios were looked at for the population at Gonubie, as well as that of Kommetjie to allow comparison.

2.2.2 Statistical Analysis

ANOVA tests were used to determine if density and biomass differed significantly between the sites and within some of the zones (upper and lower Balanoid - see results for explanation). F- tests were used to compare the slopes of the length to height data (Fry,1993). Chi-square was used to analyse the population sex ratios.All statistical calculations were done using "Sigmastat" (Jandel Scientific) and the statistical package on "Excel" (Version 5.0, Microsoft).

2.3 Results

2.3.1 Study Sites

Both the rock platform and boulder shore at Gonubie have a shallow profile and are exposed to the prevailing westerly swells. The platform shore had a gradient of 1 : 47 m (Figures 2.1.A and 2.1.B) and consisted of a flat wave-cut platform with occasional rocky ridges orientated at 130 degrees to the shoreline. Shallow pools were scattered throughout the platform and the bases of the many shallow gullies contained small boulders. The adjacent boulder shore had a slightly shallower gradient of 1 : 58 m and consisted of rounded to oval rocks lying either on a sandy or stony substratum (Figures 2.2.A and 2.2.B). Large pools were found in amongst the boulders, particularly within the upper Balanoid. Many of the boulders in the pools were covered by coralline algae.

2.3.2 Distribution, Density and Biomass

The distribution of *Helcion pruinosus* was found to be similar along both transects. Limpets were found mainly within the upper and lower Balanoid zones, and were absent from the *Littorina* and *Cochlear* zones. Because of the absence of limpets from these two zones on both shores, comparisons of density and biomass were made for the Balanoid zones only.

The overall density and biomass (dry weight) of limpets from the Balanoid zones were significantly greater ($p = 7.24 \times 10^{-7}$ for the density and $p = 2.08 \times 10^{-6}$ for the biomass, One Way ANOVA) on the platform shore (40.10 ± 4.63 m^2 ; 1.161 ± 1.116 g/m^2) than the boulder shore (11.80 ± 1.89 m^2 ; 0.460 ± 0.059 g/m^2) (mean ± standard

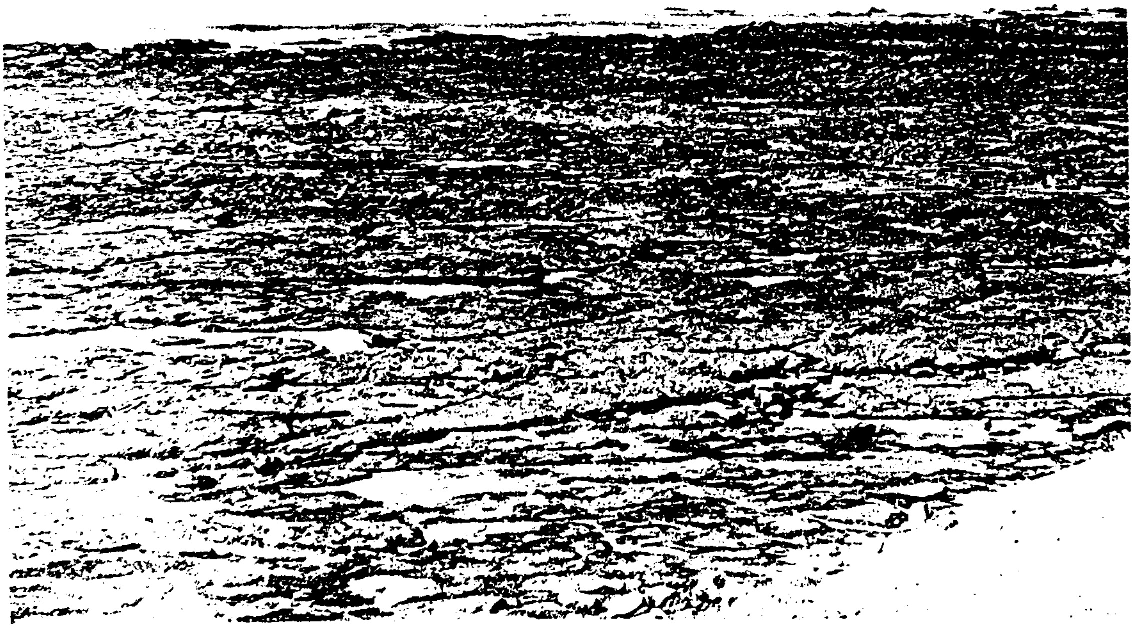

Figure 2.1 A. A section of the platform shore at Gonubie showing general area in which the transect was carried out to determine the distribution, density and biomass of *H. pruinosus.*

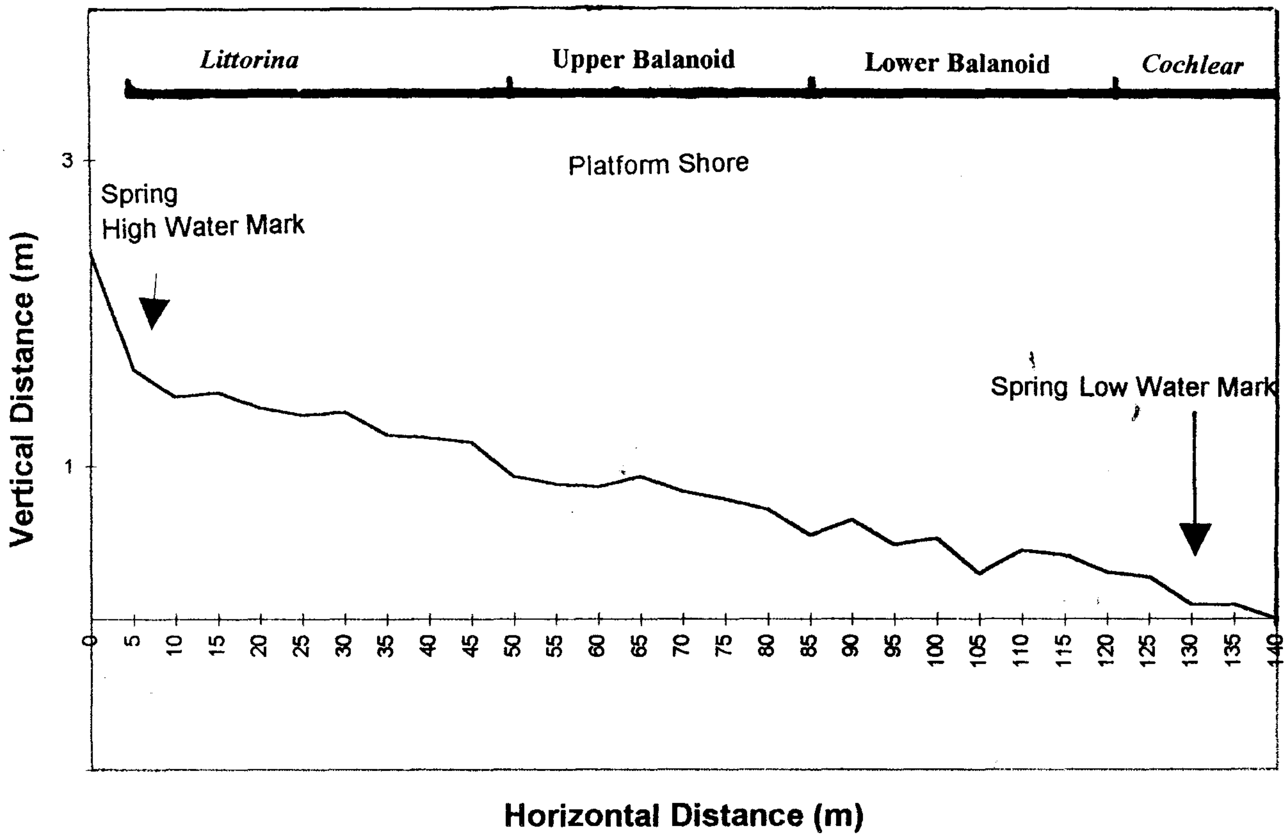

Figure 2.1 B. Transect profile of a section of the platform shore at Gonubie. The bar represents the approximate positions of the intertidal zones.

Figure 2.2.A A section of the boulder shore at Gonubie showing general area in which the transect was carried out to determine the distribution, density and biomass of *H. pruinosus*.

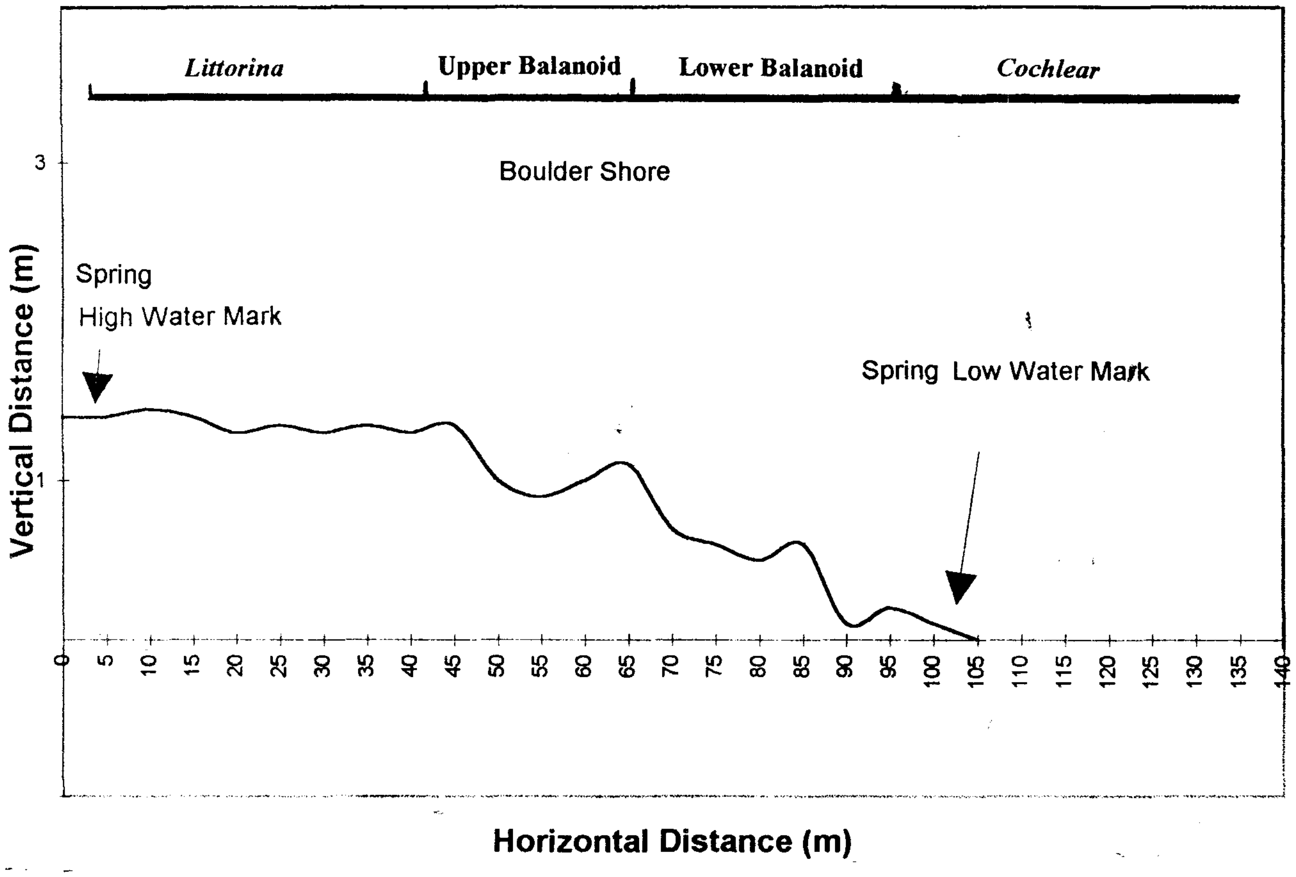

Figure 2.2.B. Transect profile of a section of the boulder shore at Gonubie. The bar represents the approximate positions of the intertidal zones.

error of the means). A comparison of the density and biomass between zones of the different shores revealed that the upper and lower Balanoid zones of the platform shore had a significantly greater density and biomass of limpets than the equivalent zones on the boulder shore (Tables 2.1, 2.2). On the platform shore *H. pruinosus* reached a maximum density of 294 / m^2, but only 72 / m^2 on the boulder shore.

Table 2.1 Distribution, density, biomass and shell length of *Helcion pruinosus* in two zones (upper and lower Balanoid zones), at two sites near Gonubie. Limpets were not found in the *Littorina* and *Cochlear* zones.

	Upper Balanoid Zone			**Lower Balanoid zone**		
	Density (m^{-2}) x (± S.E.)	Biomass (g/m^2) x (± S.E.)	Shell length (mm) x (± S.E.; max and min)	Density (m^{-2}) x (±S.E.)	Biomass (g/m^2) x (±S.E.)	Shell length (mm) x (±S.E.; max and min)
Platform Shore	**49.64** (7.37)	**1.364** (0.251)	**15.81** (0.502; 25.3, 3.7)	**36.57** (5.65)	**1.087** (0.129)	**16.67** (0.469; 28.7, 3.2)
Boulder Shore	**15.21** (3.53)	**0.720** (0.1.33)	**20.72** (0.594; 28.6, 7.7)	**9.23** (1.94)	**0.460** (0.086)	**22.27** (0.590; 28.9, 7.5)

There was no significant difference ($p < 0.05$, Student-Newman-Keuls Method) in the density and biomass of limpets in the upper and lower Balanoid zones of the platform shore, and between these two zones on the boulder shore (Table 2.2).

Table 2.2 Two Way ANOVA of density (A) and biomass (B) of *Helcion pruinosus* on the platform and boulder shore at Gonubie (alpha = 0.050 : 0.9953 and 0.9234). An All Pairwise Multiple Comparison Procedure (Student-Newman-Keuls Method) was also applied to the data. Plat = platform shore; Boul. = boulder shore; U.B. = upper Balanoid zone; L.B. = lower Balanoid zone).

A. Density

Source of variance	Df	SS	Ms
Between sites	44	20804.1	472.8
Between zones	1	18450.0	18450.0
Residual	40	21431.0	535.8
Total	85	59438.4	699.3

Comparison	Diff of Means	p	q
Plat. U.B. vs. Boul. L.B.	35.63	4	5.49
Plat. U.B. vs. Boul. U.B.	31.83	3	4.79
Plat. U.B. vs. Plat. L.B.	7.82	2	1.21
Plat. L.B. vs. Boul. L.B.	27.81	3	5.97
Plat. L.B. vs. Boul. U.B.	24.01	2	4.56
Boul. U.B. vs. Boul. L.B.	3.80	2	0.72

Comparison	$p< 0.05$
Plat. U.B. vs. Boul. L.B.	Yes
Plat. U.B. vs. Boul. U.B.	Yes
Plat. U.B. vs. Plat. L.B.	No
Plat. L.B. vs. Boul. L.B.	Yes
Plat. L.B. vs. Boul. U.B.	Yes
Boul. U.B. vs. Boul. L.B.	No

B. Biomass

Source of variance	Df	SS	Ms
Between sites	51	15.5	0.303
Between zones	1	12.3	12.323
Residual	40	16.0	0.401
Total	85	42.8	0.465

Comparison	**Diff. of Means**	**p**	**q**
Plat. U.B. vs. Boul. L.B.	0.904	4	5.68
Plat. U.B. vs. Boul. U.B.	0.644	3	3.84
Plat. U.B. vs. Plat. L.B.	0.278	2	1.78
Plat. L.B. vs. Boul. L.B.	0.627	3	5.28
Plat. L.B. vs. Boul. U.B.	0.366	2	2.82
Boul. U.B. vs. Boul. L.B	0.260	2	1.95

Comparison	**p<0.05**
Plat. U.B. vs. Boul. L.B.	Yes
Plat. U.B. vs. Boul. U.B.	Yes
Plat. U.B. vs. Plat. L.B.	No
Plat. L.B. vs. Boul. L.B.	Yes
Plat. L.B. vs. Boul. U.B.	Yes
Boul. U.B. vs. Boul. L.B.	No

2.3.2 Population Structure

At Gonubie animals ranged in size (shell length) from 3 to 28 mm on both the platform shore) and the boulder shore (Figures 2.3; 2.4). The mean shell length of limpets was greater on the boulder shore ($\bar{x}$ = 22.27 mm and $\bar{x}$ = 20.72 mm; lower and upper Balanoid zones), when compared to the platform shore ($\bar{x}$ = 16.67 mm and $\bar{x}$ = 15.81 mm) (Table 2.1), and there was no significant difference between zones within a particular shore type (Table 2.3) ($p<0.05$; Student-Newman-Keuls Method). Males and females were found in all size categories (Figures 2.3 B, D; 2.4 B, D), indicating that *H. pruinosus* does not change sex during its life time, and is thus dioecious. The length to height relationship of the shells of males and females was not significantly different (p = 0.951, F-test) when comparing the Gonubie population to that of the Kommetjie population (Figure 2.5). Shell length to height could not be used as a criterion for differentiating between the sexes as could be done for *H. pectunculus* (Gray, 1996).

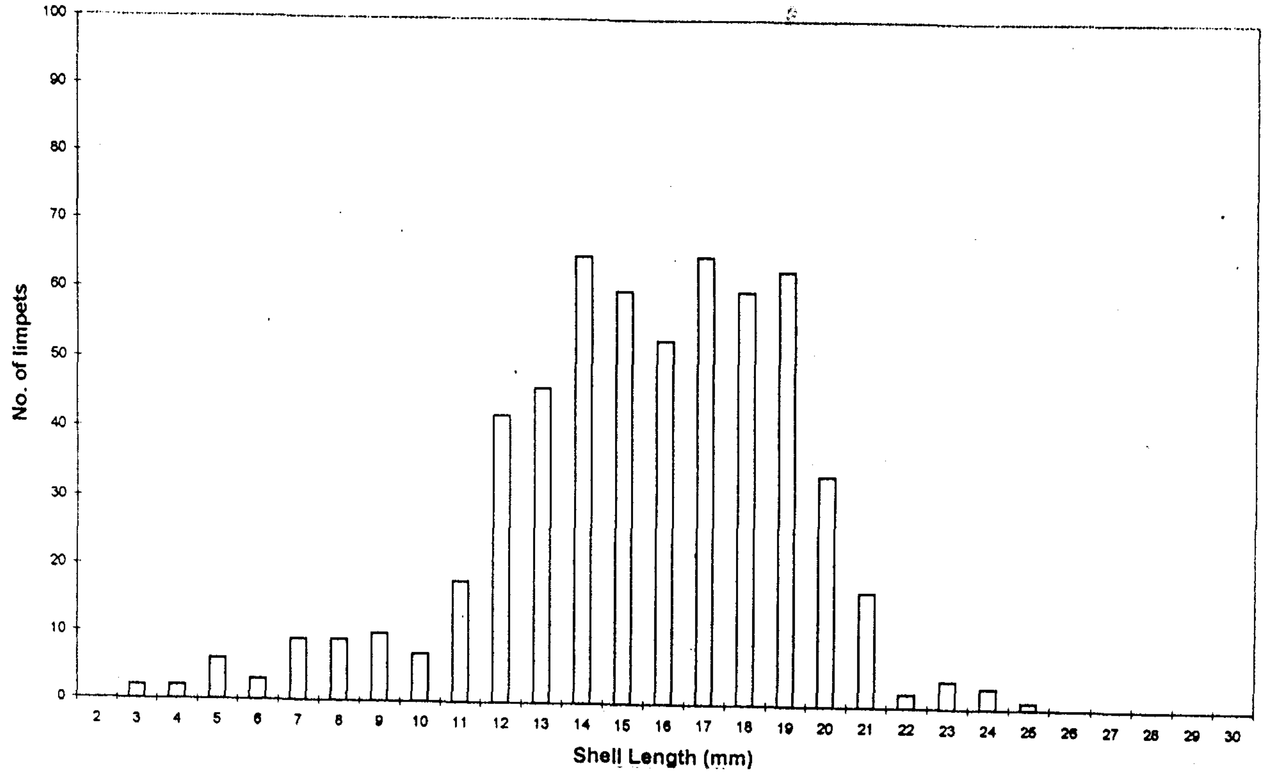

Figure 2.3 A. The size frequency distribution of *Helcion pruinosus* in the upper Balanoid zone of the platform shore near Gonubie.

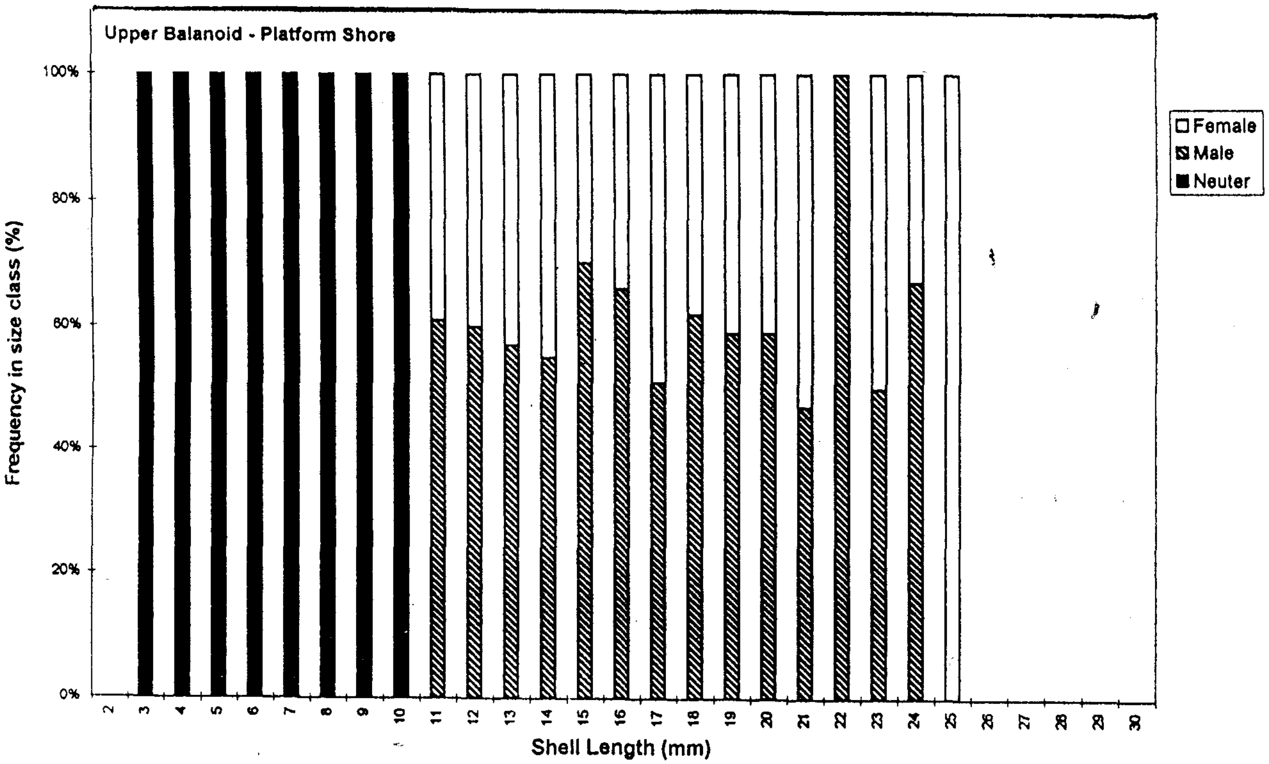

Figure 2.3 B. The percentage of male, female and neuter (indistinguishable gonads) *Helcion pruinosus* in each of the size groups from the upper Balanoid zone from the platform shore near Gonubie.

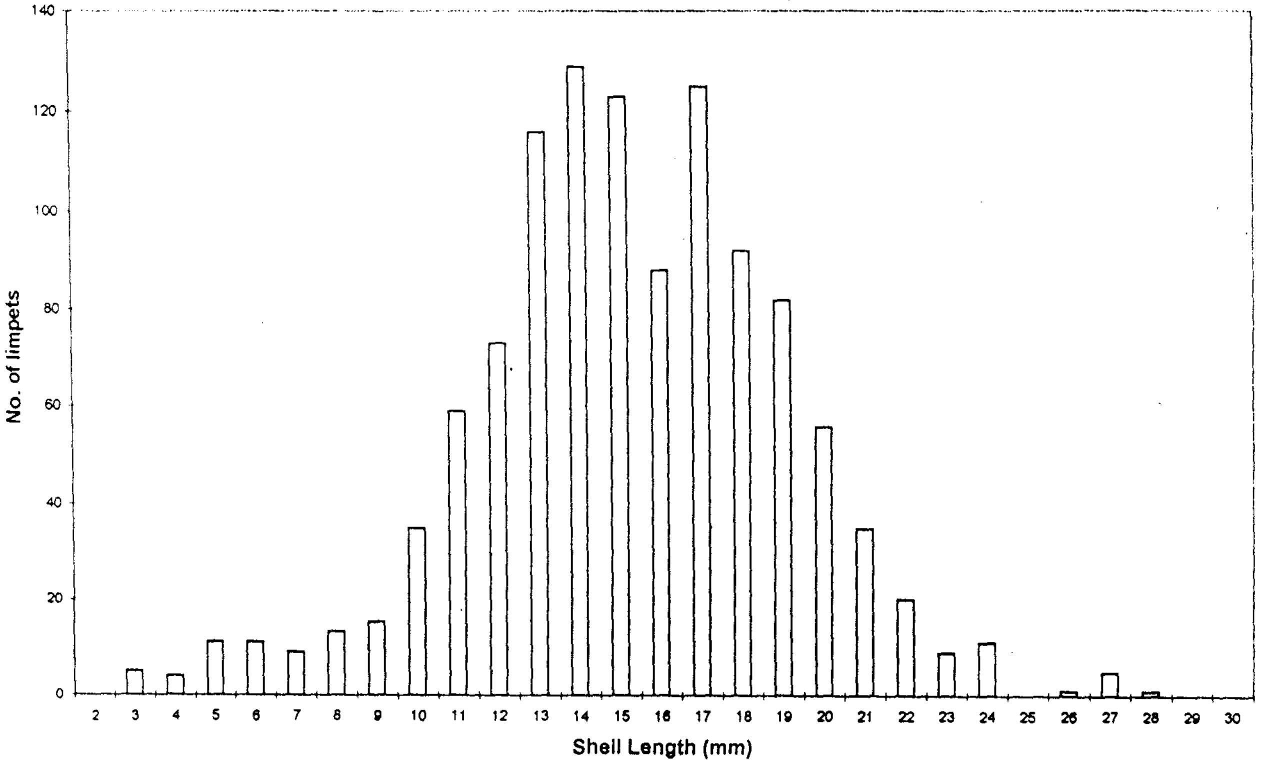

Figure 2.3 C. **The size frequency distribution of *Helcion pruinosus* in the lower Balanoid zone of the platform shore near Gonubie.**

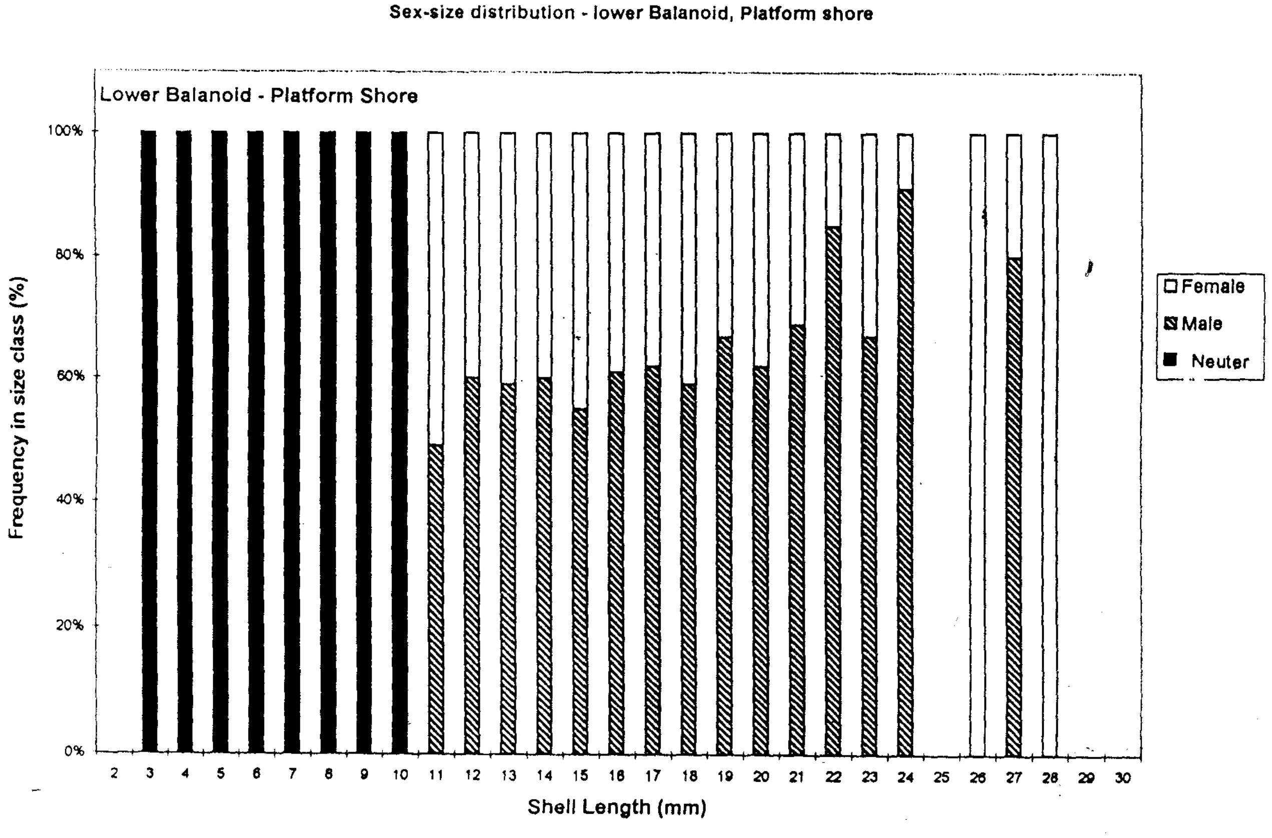

Figure 2.3 D. **The percentage of male, female and neuter (indistinguishable gonads) *Helcion pruinosus* in each of the size groups from the lower Balanoid zone from the platform shore near Gonubie.**

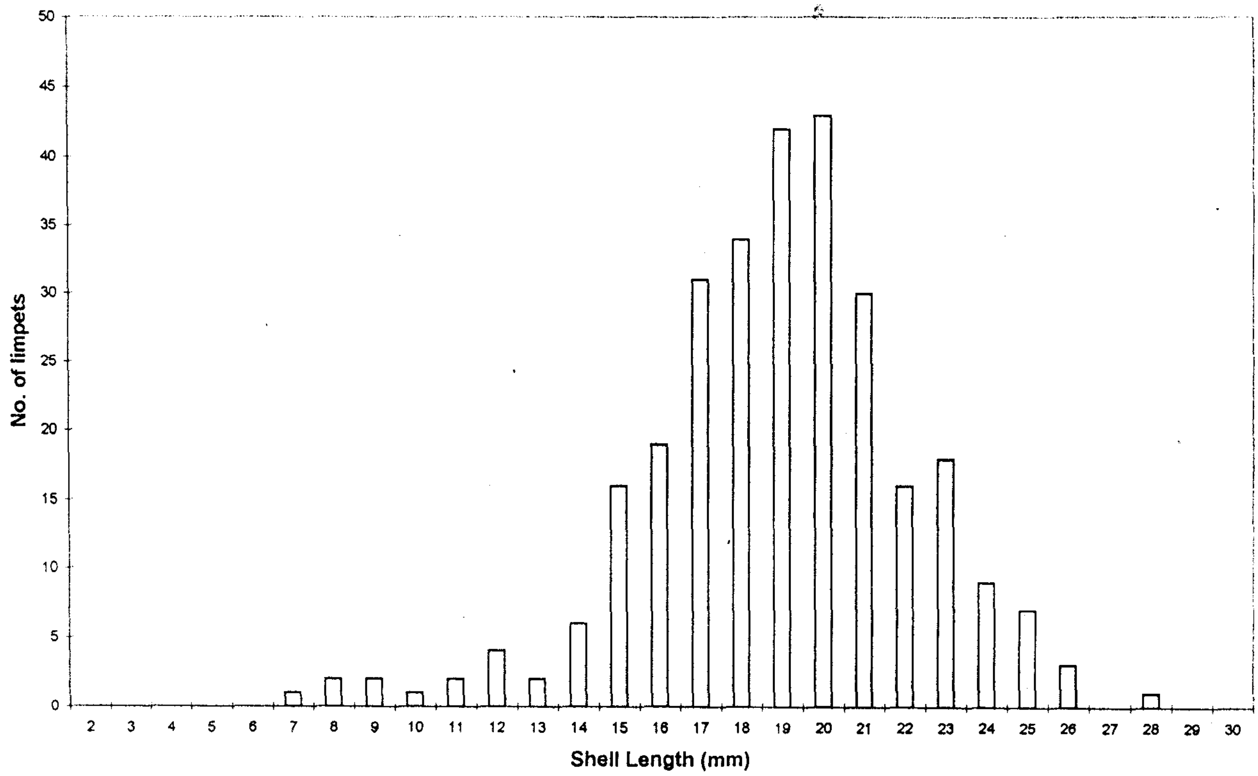

Figure 2.4 A. The size frequency distribution of *Helcion pruinosus* in the upper Balanoid zone of the boulder shore near Gonubie.

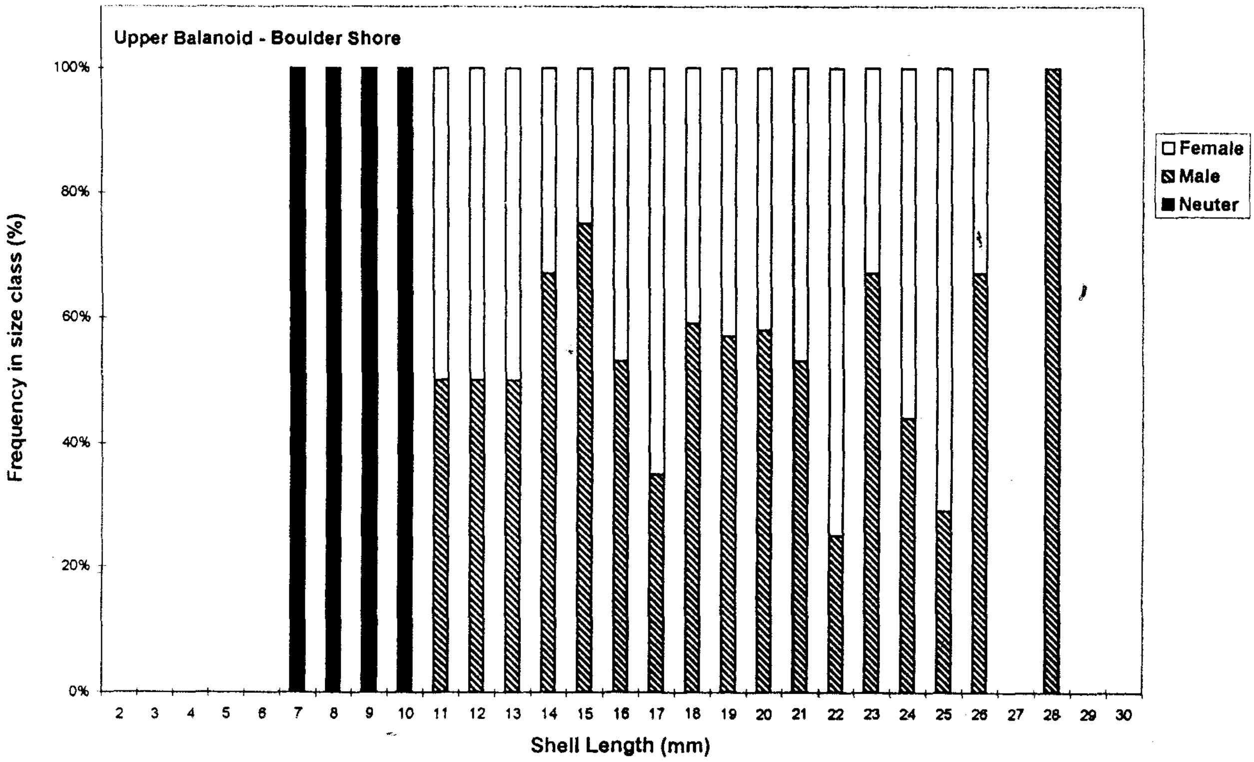

Figure 2.4 B. The percentage of male, female and neuter (indistinguishable gonads) *Helcion pruinosus* in each of the size groups from the upper Balanoid zone from the boulder shore near Gonubie.

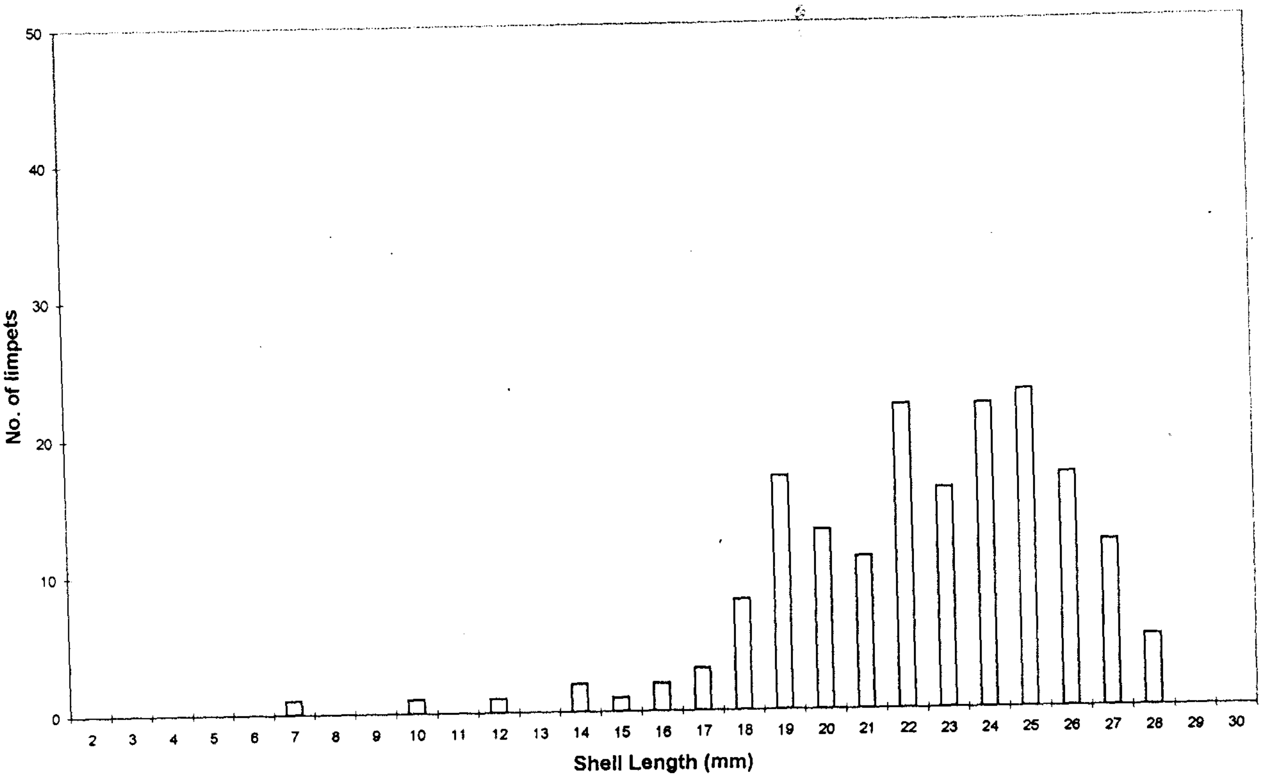

Figure 2.4 C. The size frequency distribution of *Helcion pruinosus* in the lower Balanoid zone of the boulder shore near Gonubie.

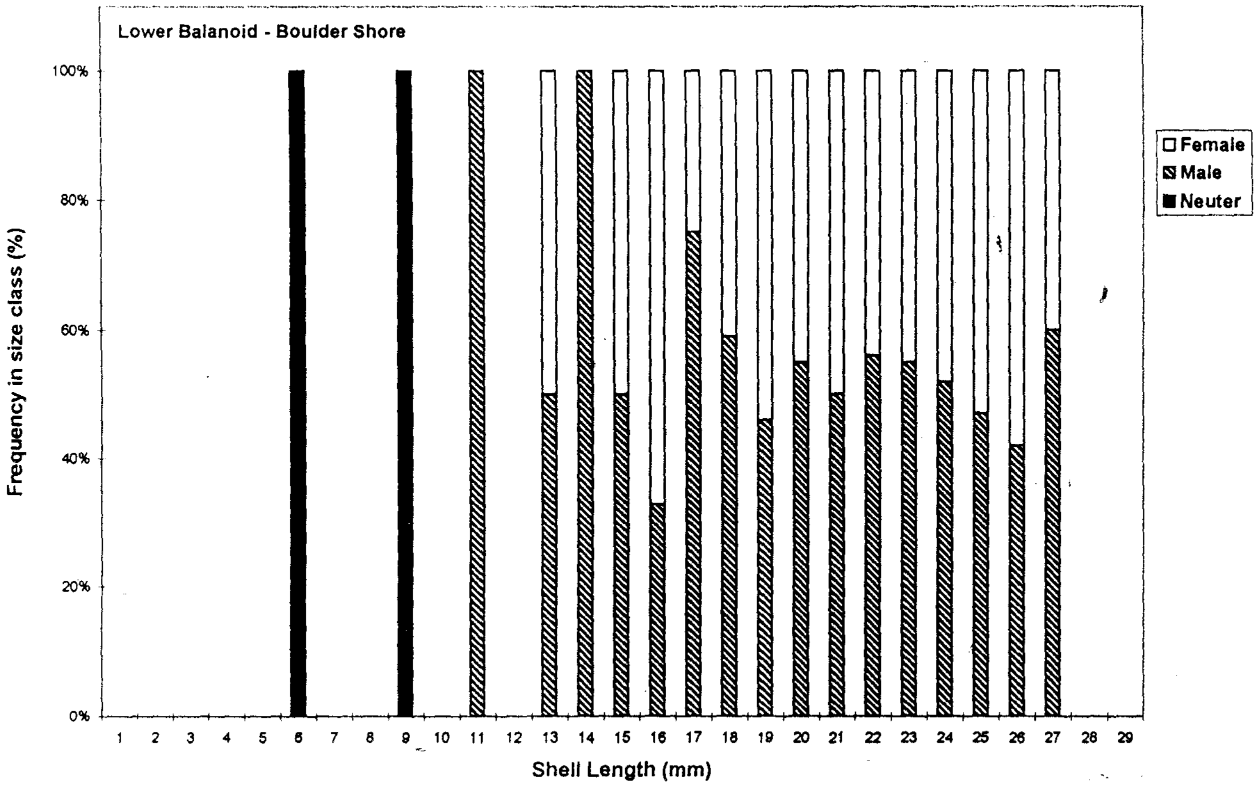

Figure 2.4 D. The percentage of male, female and neuter (indistinguishable gonads) *Helcion pruinosus* in each of the size groups from the lower Balanoid zone from the boulder shore near Gonubie.

Table 2.3 Two Way ANOVA of the size (shell length) of male and female *Helcion pruinosus* in the upper (U.B.) and lower (L.B.) Balanoid on a platform Shore (Plat.) and boulder shore (Boul.) at Gonubie. All pairwise multiple comparison procedures (Student-Newman-Keuls Method) at alpha of 0.05 were performed to compare shell lengths between sites and zones.

Source of variance	**DF**	**SS**	**MS**
Site	1	491.37	491.37
Zone	1	24.97	24.97
Site x zone	1	2.30	2.30
Residual	81	560.90	6.92
Total	84	1156.94	13.77

Comparison	**Diff of Means**	**p**	**q**
Boul. L.B. vs. Plat U.B.	6.434	4	9.29
Boul. L.B. vs. Plat L.B.	5.610	3	11.25
Boul. L.B. vs. Boul. U.B.	1.543	2	2.75
Boul. U.B. vs. Plat. U.B.	4.892	3	6.73
Boul. U.B. vs Plat. L.B.	4.067	2	7.45
Plat. L.B. vs. Plat U.B.	0.825	2	1.21

Comparison	**p<0.05**
Boul. L.B. vs. Plat U.B	yes
Boul. L.B. vs. Plat L.B.	yes
Boul. L.B. vs. Boul. U.B.	no
Boul. U.B. vs. Plat. U.B.	yes
Boul. U.B. vs Plat. L.B.	yes
Plat. L.B. vs. Plat U.B.	no

At Gonubie there was a significantly greater number of males than females in the population on the platform shore (p = 0.271, chi-squared, Figures 2.4 B, D, Table 2.4).

This difference in the number of males and females was, however, not significant on the boulder shore (p = 0.922, Table 2.4).

Table 2.4. Frequencies and ratios of male to female *Helcion pruinosus* at two sites (platform and boulder shores) near Gonubie, and those at Kommetjie. P-values obtained from chi-square analyses are also shown to determine whether the data differ significantly from the expected 1:1 ratio. (* - indicates a significant deviation from the expected 1 : 1 ratio)

Site	Frequency Male	Female	Ratio Male : Female	Chi-squared p-value
Boulder shore (n = 475)	257	218	1.18 : 1	0.922
Platform shore (n =1594)	935	659	1.42 : 1 *	0.271
Gonubie (n = 2851) **(transect and reprod.)**	1739	1112	1.56 : 1 *	0.624
Kommetjie (n = 570) **(Reproduction)**	277	293	0.94 : 1	0.835

The ratios are very different for the samples at Gonubie, but in the total sample for Gonubie, (transect and reproduction results), there was an overall bias towards males. It is interesting to note that as the n value increases so the ratio of males to females also increases. In contrast, at Kommetjie there was a slight bias towards females (0.94 males to 1 female, Table 2.4), but this was not significant (p = 0.835).

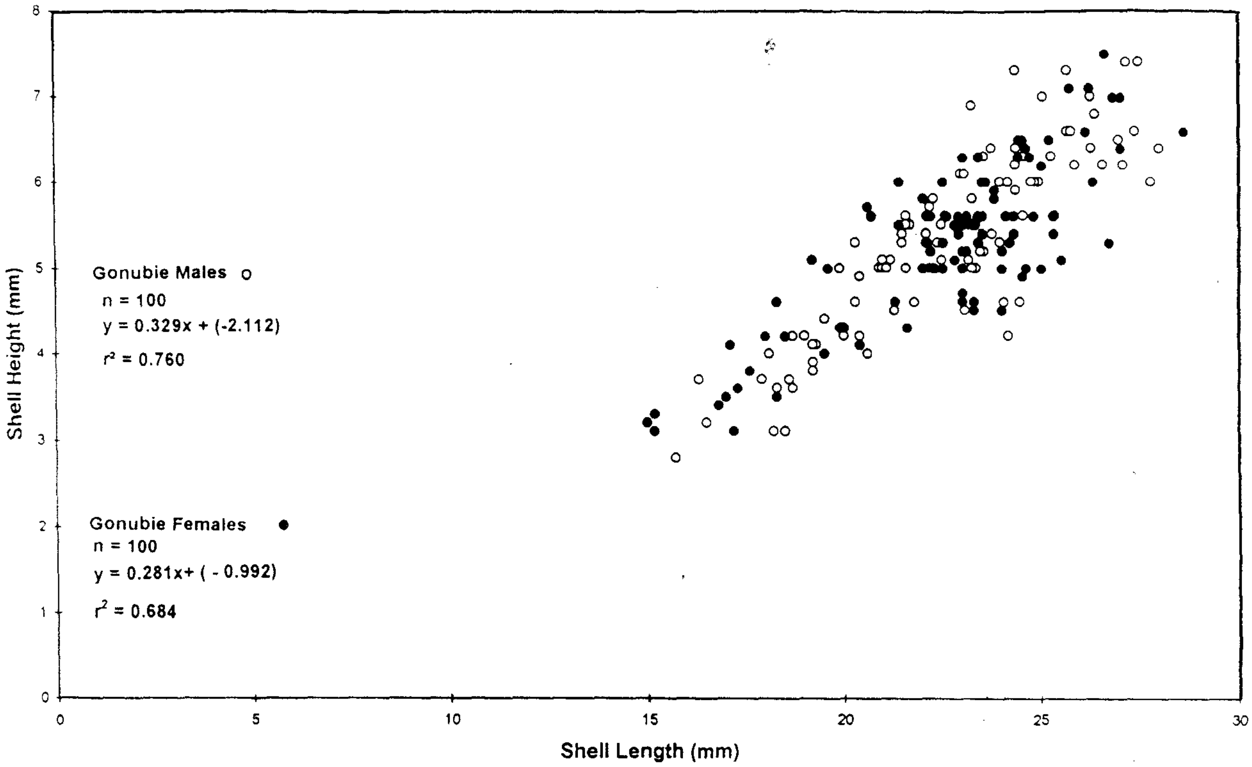

Figure 2.5.A. The ratio of shell length (mm)to shell height (mm) of male (o) and female (•) *Helcion pruinosus* at Gonubie.

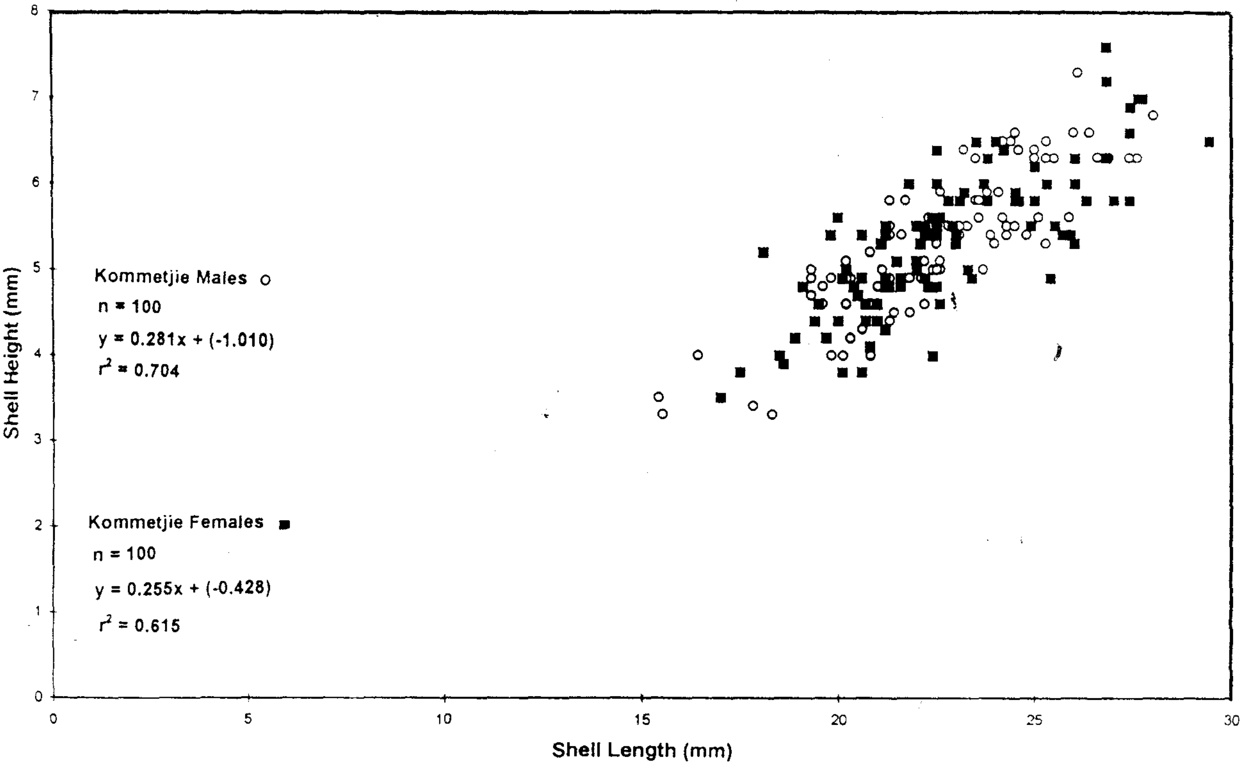

Figure 2.5.B. The ratio of shell length (mm)to shell height (mm) of male (o) and female (■) *Helcion pruinosus* at Kommetjie.

2.4 Discussion

The intertidal rocky shore is one of the most stressful environments (Branch and Branch, 1981) and the distribution of organisms within it is a result of the interactions between physical and biological factors (Branch, 1981). Whereas physical factors (e.g. temperature and desiccation stress) are regarded as being most important in limiting the upper distribution of many intertidal organisms, biological factors (e.g. availability of food and space; predation) are thought to be more important in controlling the lower limits to distribution (Lewis 1964; Newell 1979; Branch 1981; 1984).

Helcion pruinosus is restricted to the upper and lower Balanoid zones at Gonubie, being more abundant in the upper of these two zones. Branch (1981, 1984) has identified temperature and desiccation as being major factors in controlling the distribution of populations of many rocky shore organisms. Although there are no data on the physiological tolerances of *H. pruinosus*, it is likely that this limpet is unable to withstand the physical conditions above the upper Balanoid zone. During daytime emersion *H. pruinosus* avoids exposure to direct solar radiation, remaining hidden beneath boulders. If exposed to sunlight, inactive limpets become highly mobile and attempt to move away from the light. Whether the observed upper limit to the distribution of this limpet is a result of habitat choice (by adults or juveniles) or mortality of individuals which settle in the upper intertidal zone, is not known. What controls the lower limit to the distribution of *H. pruinosus* is also not known. It is likely, however, that the competitive abilities of other grazing limpets such as the territorial limpets *Patella longicosta* and *P. cochlear* (Branch 1974c , 1981), as well as food type and predation must all play a role.

The shell shape of limpets should play a role in the distribution of limpets on the shore, as this can be equated to the ability of the limpets to withstand desiccation and to withstand the force of water surging over them with the movement of the tides. *H pruinosus* has a low shell profile (Chapter 3) and would therefore be well adapted to life on the low shore. There is, however, no conclusive proof of this theory. Houlihan *et al.* (1981), using *Patella vulgata* as an example, worked on the premise that the shape of the shell of limpets living on the high, or low shore, could be correlated to the ability of the limpets to withstand desiccation. They found little evidence between this factor and the amount of emersion the animals experienced. Lowell (1984), on the other hand, concluded that the water loss was dependent on the length of the shell edge, rather than upon the volume of the mantle cavity. *H. pruinosus* having a low shell profile would therefore have a greater shell perimeter for a given volume in comparison to a limpet with a tall shell, e.g. *H. pectunculus* (Gray, 1996), and would therefore be found in greater densities lower down the shore, i.e. within the Balanoid zone.

For each of the shores examined (boulder and platform), there was no clear evidence (Figure 2.4) of any difference in the size frequency distribution of *H. pruinosus* between the upper and lower Balanoid zones. More commonly younger limpets are found lower down the shore after spat settlement, while older (larger) individuals would venture higher up the shore, as larger individuals are more tolerant of desiccation (Bowman, 1981). Young individuals, (shell length <5 mm, were found in most quadrats together with larger limpets (shell length > 20 mm) on the platform shore at Gonubie. On the boulder shore very few small individuals were present with only 11 limpets having a shell length less than 10 mm being found. The limpets were also larger on the boulder shore, and this could be related to food availability, or to the possibility that the boulder shore had an older population., as evidenced by the few small individuals and greater number of larger individuals. The reason for this difference between two adjacent sites is not known.

Herbivorous gastropods often show intraspecific zonation patterns (e.g. Bertness 1977, McQuaid 1981a and b, Cushman 1989, Takada 1993, Foster 1997). Vermeij (1972) proposed that there were two main types of size-specific gradients: Type 1 in which shell size tends to increase in an upshore direction in species that are characteristic of the high shore; Type 2, in which shell size decreases in a downshore direction in species typical of the lower tidal areas. In the type 1 gradient, mortality is caused by physical factors such as high temperatures or desiccation; in the type 2 gradient, mortality is generally caused by predation or other biotic factors. As a mid to low shore species, it might have been expected that *H. pruinosus* would have a type 2 distribution. At Gonubie it does not comply with either type.

The density and biomass of *H. pruinosus* are similar to that recorded for other species of limpet along the South African coastline. *H. pectunculus* reached a maximum density of 212 at Gonubie (Gray,1996), compared to the maximum (294) of *H. pruinosus* on the same shoreline. Average densities were also similar, ranging from 2 m^{-2} to 62.8 m^{-2} for *H. pectunculus* (Gray, 1996) and 9.23 to 49.64 for *H. pruinosus*. The biomass of *H. pectunculus* (Gray, 1996) was higher on both shore types at Gonubie (2.45 g/m^2 on the platform shore and 1.21 g/m^2 on the boulder shore, compared to 1.364 g/m^2 and 0.720 g/m^2, respectively for *H. pruinosus* on the two shores).

H pruinosus seems to be similar to *H. pectunculus* in being a non-migratory species of limpet (Branch, 1971 and Gray, 1996), as there is no clear evidence to support the fact that larger, and therefore older limpets, migrate up the shore to reduce competition. Branch (1971) suggested that limpets should be categorised into two main sub-groups: migratory and non-migratory limpets. He proposed that migratory species of limpets e.g. *Patella granularis, P. granatina, P. concolor* and *P. oculus* initially settle on the low shore and then migrate upshore as they grow in size. Such migratory limpets were widely distributed in all the zones and were usually found in high densities (e.g. between 200 to 500 limpets / m^2 in *P. granularis*) along the shore. Non-migratory limpets include *P. cochlear, P. longicosta, P. tabularis* and *P. miniata*. Such limpets, after settling in only a narrow section of the shore, remain there throughout their life. These limpets can be found in high densities (e.g. between 100 and 200 / m^2 for *P. cochlear*) within their zone. Branch (1971) found that such non-migratory limpets were always found to prefer the low shore. This study supports that of Gray (1996) that *H. pruinosus*, like *H. pectunculus* belongs to a third group of limpets, viz. "specialised non-migratory species". Such species overcome competition for food and living-space by specialising to a particular habitat (living below boulders, often submerged in rock pools) and food source (epilithic algae). Other limpets which could be included within this group include *P. compressa* which is specialised for a life on kelp stipes (Branch, 1971) and *H. pellucidum* found on the stipes and blades of *Laminaria hyperborea* (Vahl, 1971).

Density and biomass were found to be greater on the platform than the boulder shore. There could be a number of reasons for this. Differences in micro-algal primary productivity between the two adjacent sites could be one reason and warrants further investigation. Disturbance could also explain the difference. The very nature of the boulder shore makes it more susceptible to disturbance. If boulders are being disturbed on a regular basis by wave activity (particularly during winter storms) this may reduce the number of stable habitats for the limpets.

The population of *H. pruinosus* at Gonubie contained a greater number of males. This trend has been observed in a number of molluscs (e.g. Stephenson 1934; Sinclair, 1963; Glynn, 1970; Poore, 1973; Pearse, 1978; Wells and Keesing, 1989; Foster, 1997) including South African patellid limpets (Branch 1974a; Gray 1996). Foster (1997) suggests that male abundance may be a strategy to counter sperm dilution in broadcast spawners, although he cautions that sex ratio could be affected by biased sampling, sex changes, differential growth, or selective predation. Gray (1996) in a study of the high shore species *H. pectunculus* found that this species changed sex with age, larger animals being predominantly female. As there were fewer larger animals in the populations studied by Gray (1996) this explained the observed bias towards males. *H. pruinosus* does not undergo a sex change and it is difficult to explain the male bias in the population. The sample size seems to influence the sex ratio of the limpets, as when fewer animals were (e.g. at Kommetjie, or on the boulder shore), the male to female ratio was not significant. The greater the number within the sample, the greater the significant difference between the sexes became.

In conclusion, *H. pruinosus* is an abundant grazer in the mid-low intertidal at Gonubie. Its abundance suggests that it may play an important role in structuring the community within the above region of the intertidal as well as in energy transfer on this shore. It can also be concluded that the Gonubie site is ideal for further studies on the biology of this limpet.

CHAPTER 3 : The growth of *Helcion pruinosus*

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Chapter 3 - Growth of *Helcion pruinosus*

3.1 Introduction

All organisms grow in size as they mature and limpets are no exception, having to reach a certain size before they become sexually mature and are able to reproduce. The micro-algae on the rocks which *H. pruinosus* inhabit provide the nutrients and energy needed for growth. Growth in all shelled molluscs can be defined as an increase in shell size. This occurs by the production of new rings of conchiolin impregnated with calcium carbonate on the edge of the shell (Fretter and Graham, 1994). These secretions are produced by the mantle or pallium and have been studied by several authors, (e.g. Rhoads and Panella, 1970; Branch, 1974b; Lutz, 1976; Richardson, 1988; Crisp *et al.*, 1990; Galinou-Mitsoudi and Sinis, 1995; Gray, 1996; Foster, 1997; Shepherd and Avalos-Borja, 1997).

A limpet shell may grow either isometrically (where length and height do not change in proportion to one another), or allometrically (i.e. one part of the body/shell changing in proportion to another, e.g. shell length changing more rapidly than the shell height or *vice versa*). The growth rate of limpets may be determined by measuring the shell parameters over a period of time or by counting the internal micro-growth bands which are produced either daily or on a tidal basis (see Gray, 1996 for references). The former method relies on large numbers of marked animals remaining within an area for an extended time period, thus enabling their dimensions to be recorded on a regular (e.g. monthly) basis. This should continue for six months or preferably, a year. As the south-east Cape coast is subjected to periodic storms which disturb the boulders on the shore, this may make the locating of marked *H. pruinosus* difficult. Nevertheless estimating the growth of marked limpets was attempted in this study.

Micro-growth bands within shells are visible after preparing acetate peels of impressions of sectioned shells, (e.g. Rhoads and Panella 1970; Lutz 1976; Galinou-Mitsoudi and Sinis, 1995; Gray, 1996). Once the cycle of laying down the growth bands has been established, micro-growth bands can then be counted, in a similar way to annual tree rings, in order to determine the age of the limpet. This technique was also applied in this study.

The aim of this section of the work was to try to establish the age and longevity of *H. pruinosus* at Gonubie on the south-east coast and Kommetjie on the west coast. A further aim was to determine the size at which this species becomes sexually mature.

3.2 Materials and Methods

3.2.1 Estimates of the growth rate by measurement of shell length.

On two occasions *Helcion pruinosus* from the mid-intertidal zone at Gonubie (32°57' S/ 25°01' E), were marked with numbered bee labels. The bee labels were affixed to the shell with epoxy adhesive. The shell length of each numbered individual was measured regularly (monthly) for five months. The rocks under which the animals sheltered were marked with paint enabling the limpets' movements to be monitored as well as identifying the location of the marked limpets. The paint on the boulders had to be renewed every month as it faded in the turbulent conditions. On the first occasion 54 individuals (figure 3.1.A) were labelled on the 9/11/96 and measured monthly for five months until the 6/4/97. The second sample of 100 individuals (figure 3.1.B) was monitored from the 22/2/97 to the 9/7/97.

The measuring of the limpets was carried out at low tide at night, as it was found that turning over the boulders during the day often harmed the animals by damaging the shell. Measurements (shell length) were taken with Vernier Callipers, measuring the shell to the nearest 0.01 mm.

3.2.2 Estimates of the growth rate by micro-growth band analysis.

Initially 30 *H. pruinosus* were selected, marked with paint, and part of the growing edge of the shell notched. The notched area would allow the determination of the number of growth bands produced within a fixed time period. Ten of the animals were harvested after two weeks and 10 more after 5 weeks. The animals were prepared as described by Galinou-Mitsoudi and Sinis (1995). The shells were embedded in polyester resin. The resin blocks were then cut on a diamond circular saw (Geology Department, Rhodes University) to expose the longitudinal axis of the shell. The blocks were sanded until smooth with a series of graded water papers (100 to 600 grit papers). The smooth surfaces were then polished with a metal polish ("Brasso"). Etching in 0.01 M HCl followed, (8 minutes), before creating the acetate peel, (using acetate sheets, Agar Scientific Ltd. No. G255).

Acetone was placed on the etched shell sections and acetone strips were stretched over the blocks and allowed to dry (approximately 5 minutes). The strips were then sandwiched between two glass microscope slides and viewed under a phase contrast light microscope to observe and count the growth bands, after two and five weeks . The fixed time period counts could then be used to determine whether the growth bands were deposited on a daily basis or after exposure and being recovered by the incoming tide, i.e. twice within a 24 hour period. Ten shells ranging in length from 13 to 26 mm, from both Gonubie and Kommetjie were examined to relate shell length to age.

3.3. Results.

3.3.1 Growth of labelled limpets

Of the 54 individuals marked in the first sample (summer growth) only 10 individuals (19% of total marked animals) were recovered after five months (figure 3.2.A). Within this period one individual (initial length = 14.5 mm) grew 6.5 mm in five months (i.e. 1.3 mm/ month) The average growth of the other 9 individuals was 3.1 mm (0.62 mm/ month) with one animal (initial size, 20.3 mm) growing only 1.8 mm in 5 months.

Of the second sample (100 individuals, winter growth) only 8 (or 8%) were recovered after 4½ months (figure 3.2.B). A very severe storm curtailed the study by washing away most of the boulders within the area. Of the animals recovered the mean growth in length was 0.6 mm,(0.13 mm/ month), with a maximum of 1.3 mm (for an individual of initial length of 15 mm) and a minimum of 0.1 mm (initial size of limpet being 19.3 mm) in the 4½ month period from 22/2/97 to the 9/7/97.

The limpets moved around within the study site. Many individuals were found up to 1m away from the rocks on which they were originally located and tagged.

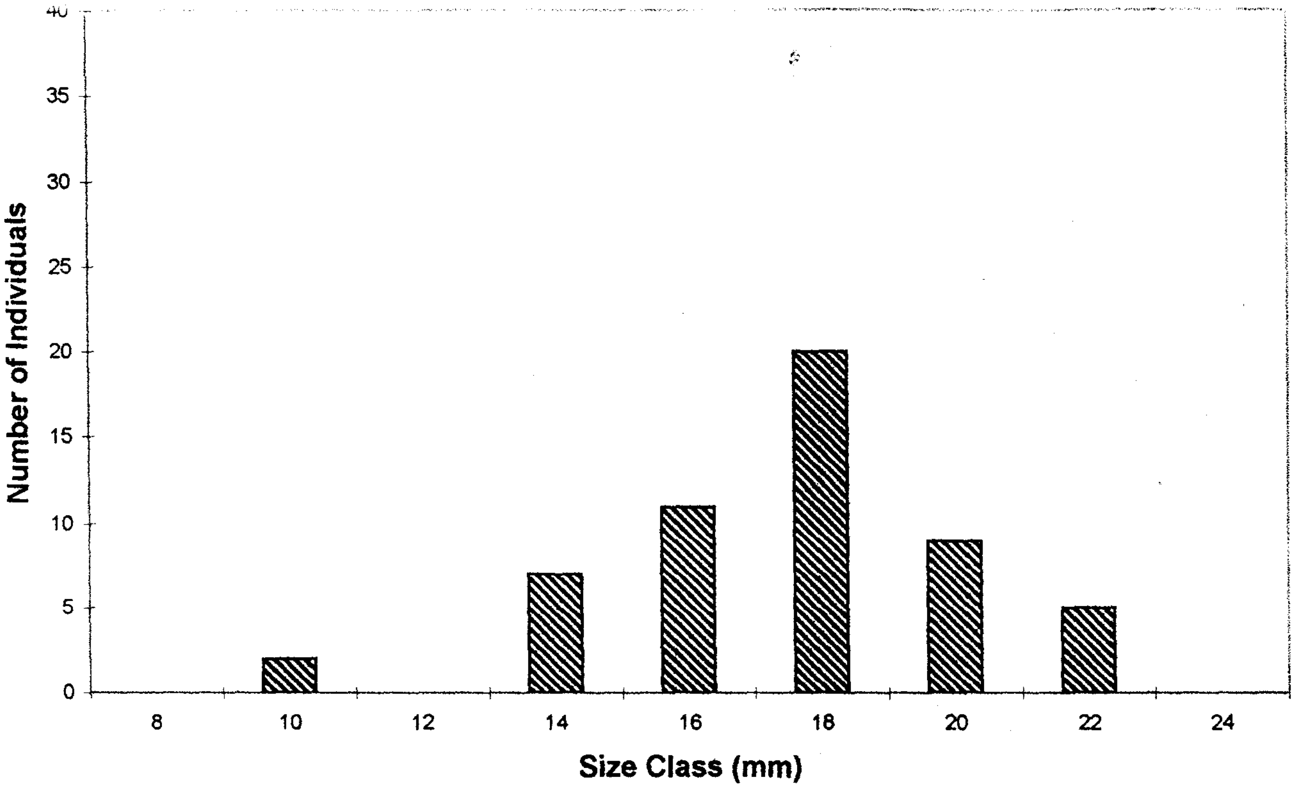

Figure 3.1.A Size-frequency distribution of *Helcion pruinosus* used in the summer growth rate experiment (n = 54) at Gonubie.

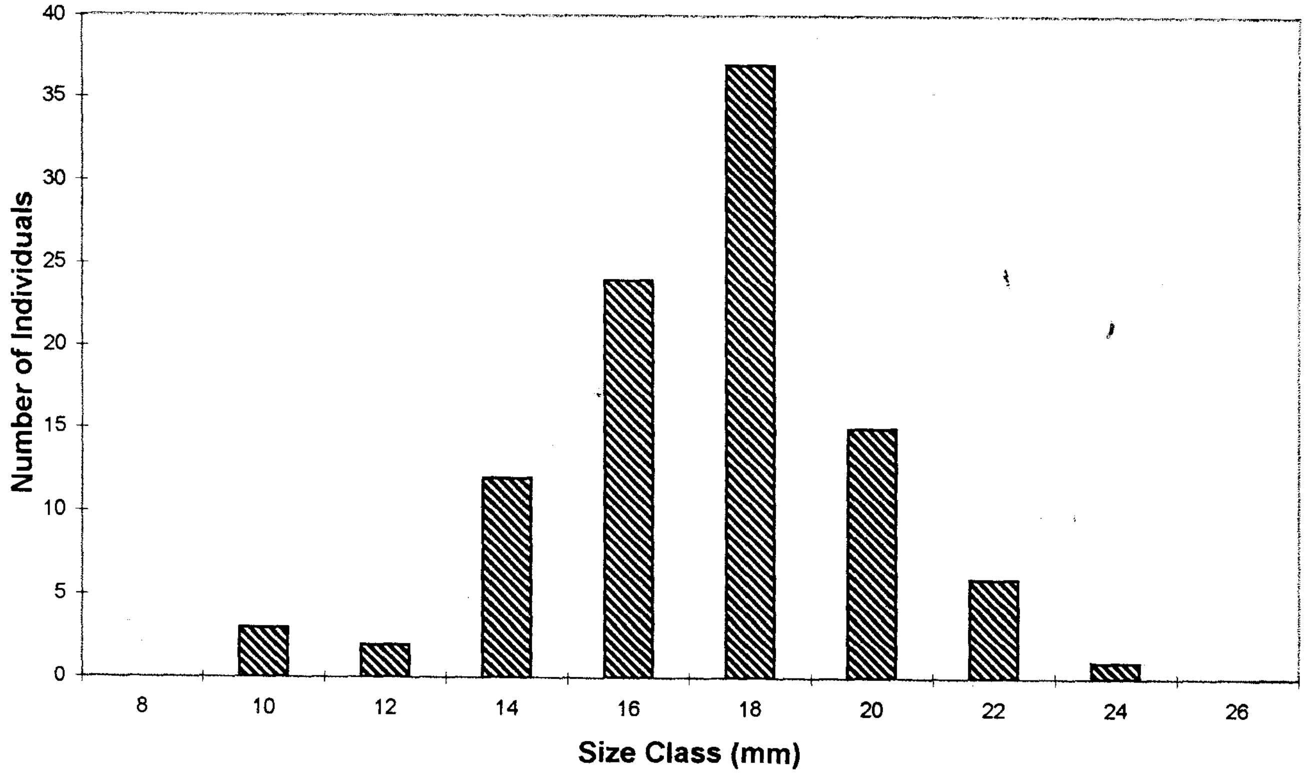

Figure 3.1.B. Size-frequency distribution of *Helcion pruinosus* used in the winter growth rate experiment (n = 100) at Gonubie.

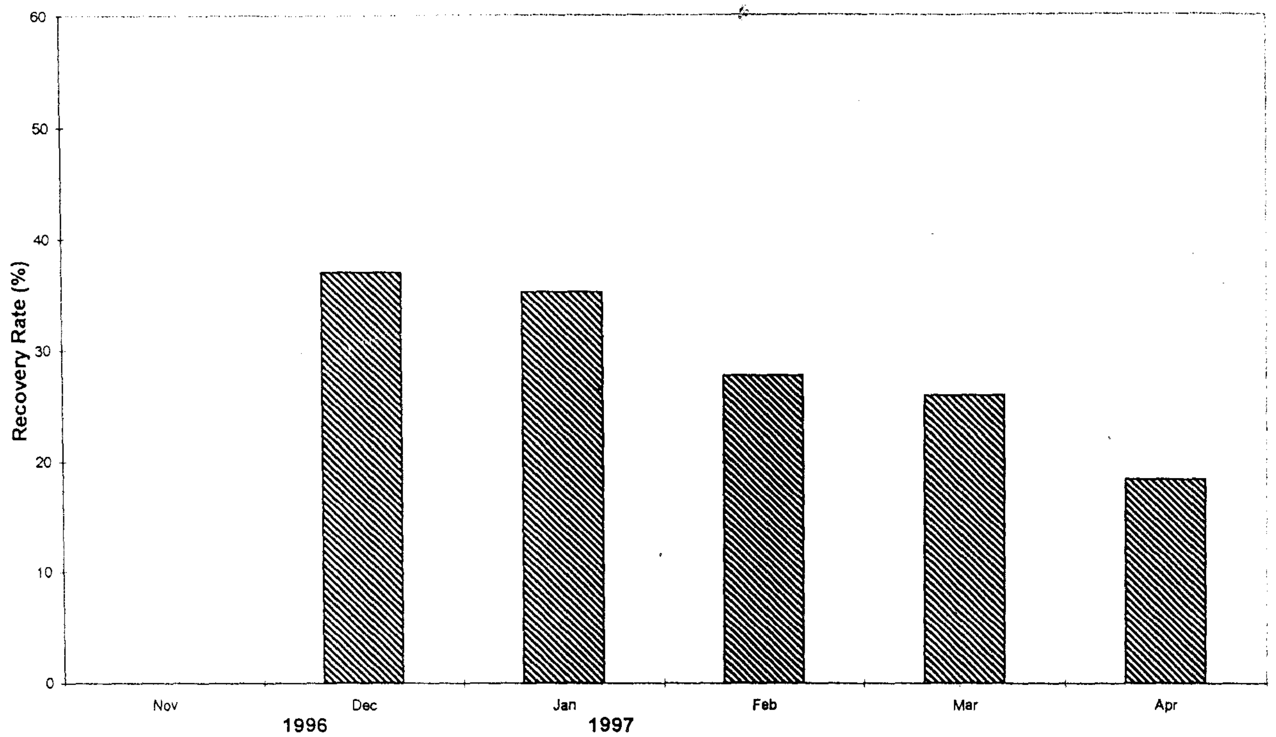

Figure 3.2.A Monthly recovery rates (% of the total marked animals) of *Helcion pruinosus* used in the summer (1996 to 1997) growth rate experiment at Gonubie.

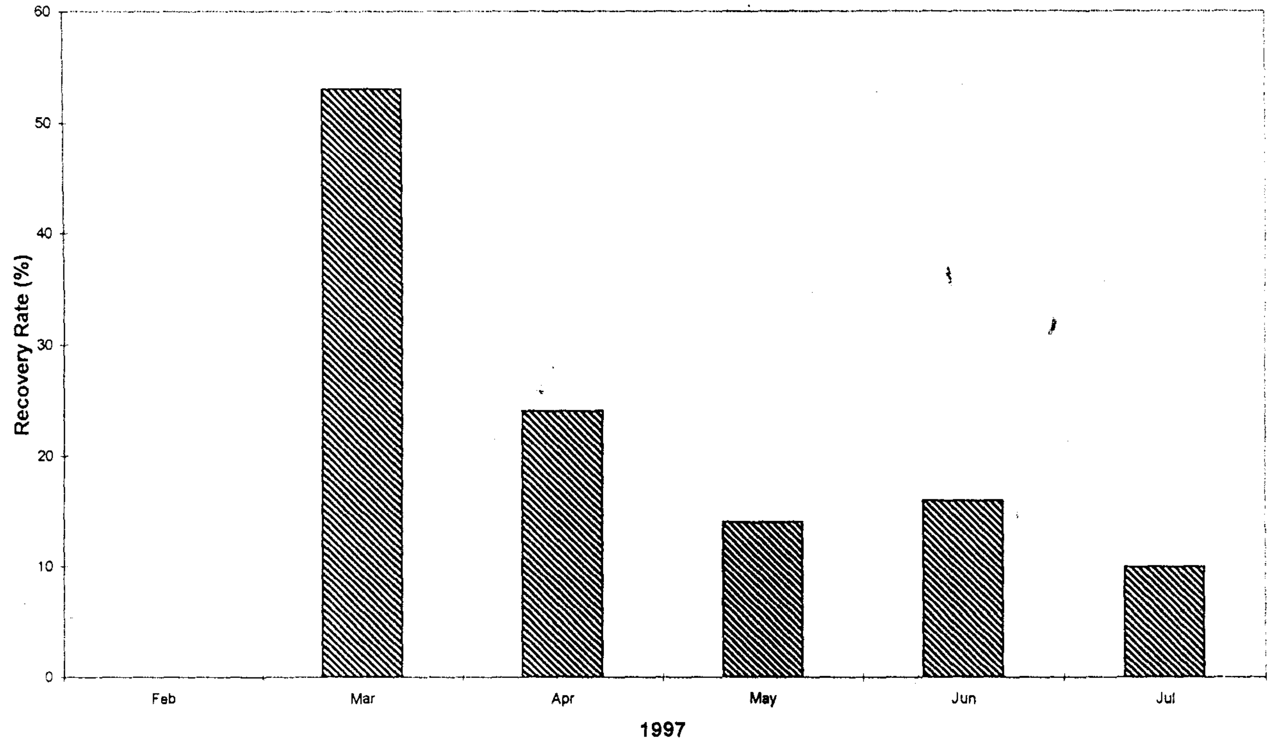

Figure 3.2.B Monthly recovery rates (% of the total marked animals) of *Helcion pruinosus* used in the winter (1997) growth rate experiment at Gonubie.

3.3.2. Micro-Band Growth Analysis

Counting the micro-growth bands from the shells of two-week old animals proved difficult, possibly owing to the very thin shell of *H. pruinosus*. It was also necessary to repeat the procedure, as after the initial period of 2, and 5 week intervals, the etching time in hydrochloric acid had still to be determined and the shell sections were dissolved by the acid (as initial times of etching were 20 to 25 minutes as for similar shells). For the five week period shells, the correct etching time of eight minutes in HCl had been determined which yielded clear micro-growth bands. A comparison of the number of growth-bands with the number of days (Table 3.1) revealed that twice as many growth-bands were laid down, indicating that two bands were produced in 24 hours, i.e. they were laid down tidally.

Table 3.1 - Chi-square comparison of the number of micro-growth bands in marked *Helcion pruinosus* and daily or tidal cycles at Gonubie.

No. Weeks	n of Sample	Mean No. of bands	Expected No. of bands	p-value
A. Correlation between day/night cycles:				
5	10	69.80	35	1.6^{-11}
B. Correlation between tidal cycles:				
5	10	69.80	70	0.820

Micro-growth banding was used to determine the relationship between the age of limpets to shell length (Figure 3.3 for the south-east coast animals and for the west coast animals). The curves were fitted to the data using the method of least squares. The growth rate was initially faster for the west coast limpets, but this rate then declined so that in both populations the larger individuals of the same shell length (22 to 28 mm) were of the same age (Figure 3.3).

Five months of growth data from the labelled individuals did not allow a Ford-Walford plot to be constructed to determine factors such as the maximum shell length. This plot requires a set of growth results based on a year's work. Estimates of length for a given age were therefore derived using the von Bertalanffy equation in the same manner as Robson, (1986) :

$$\mathbf{L_t = L_{max} [1 - e^{K(t - to)}]} \qquad \text{(after Branch, 1981)}$$

where : $\mathbf{L_t}$ is the length of the limpet at age **t** (days),

$\mathbf{L_{max}}$ = the theoretical maximum length attained by the limpet,

K is the growth coefficient, and

$\mathbf{t_o}$ = the time when **L** (length) would theoretically be at zero.

L_{max} is usually approximated by a Ford Walford plot using the formula:

$$\mathbf{L_{max}} = \frac{\mathbf{Intercept}}{\mathbf{1 - slope}}$$ to determine the maximum shell length (L).

$\mathbf{L_{max}}$ was estimated from the maximum size of *H. pruinosus* encountered in the field, based on the maximum size of 29.6 mm observed in the field at Gonubie and 30.4 mm found in the Kommetjie samples. The exact value of $\mathbf{L_{max}}$ could then be determined by plotting **t** against **log e($\mathbf{L_{max}}$ - $\mathbf{L_t}$)**, while varying the value of $\mathbf{L_{max}}$ until a linear regression, obtained by the method of least squares, produced the highest r^2 value. The highest r^2

values (0.976) were obtained at the theoretical values of 28.8 mm for the limpets at Gonubie and 29.6 mm for those at Kommetjie.

The slope of the regression line produced an estimate of the growth coefficient (**K**) for the von Bertalanffy growth equation. **K** values of 0.67 and 0.685 were obtained for *H. pruinosus* from Gonubie and Kommetjie respectively. Growth curves (Figures 3.4.A, 3.5.A) were then produced using these estimates to show the theoretical growth rates of *H. pruinosus* on the two shores. These theoretical growth curves (Figures 3.4A, 3.5A) virtually mirror that of the obtained growth curve for *H. pruinosus* at Kommetjie (Figure 3.3). On the south east coast growth in younger individuals was slower than the theoretical curve, but mirrors the growth of the older individuals. Growth is most rapid in the smaller individuals (growing 15 mm in the first year), slowing down as the limpets mature, (growing only 7.5 mm in the second year). Sexual maturity (i.e. the presence of visible, viable gonads) which is reached in individuals between 10 to 11 mm in length (Chapter 2), makes the limpets 7 to 8 months old at that time. This was corroborated by the observed counts of growth bands (Figure 3.3).

The von Bertalanffy growth equation can be transformed to determine time instead of length, as follows : $\mathbf{t = [\ -(1-(lt/Lmax))/K)]}$

In this manner, an estimate of the animal's survivorship can be determined (Figures 3.4.B, 3.5.B) using the length to frequency data from Chapter 2 for the Gonubie population and from Chapter 5 for the Kommetjie population. At Gonubie *H. pruinosus* is estimated to live for a maximum of 2.5 years, while those at Kommetjie live for 2.9 years.

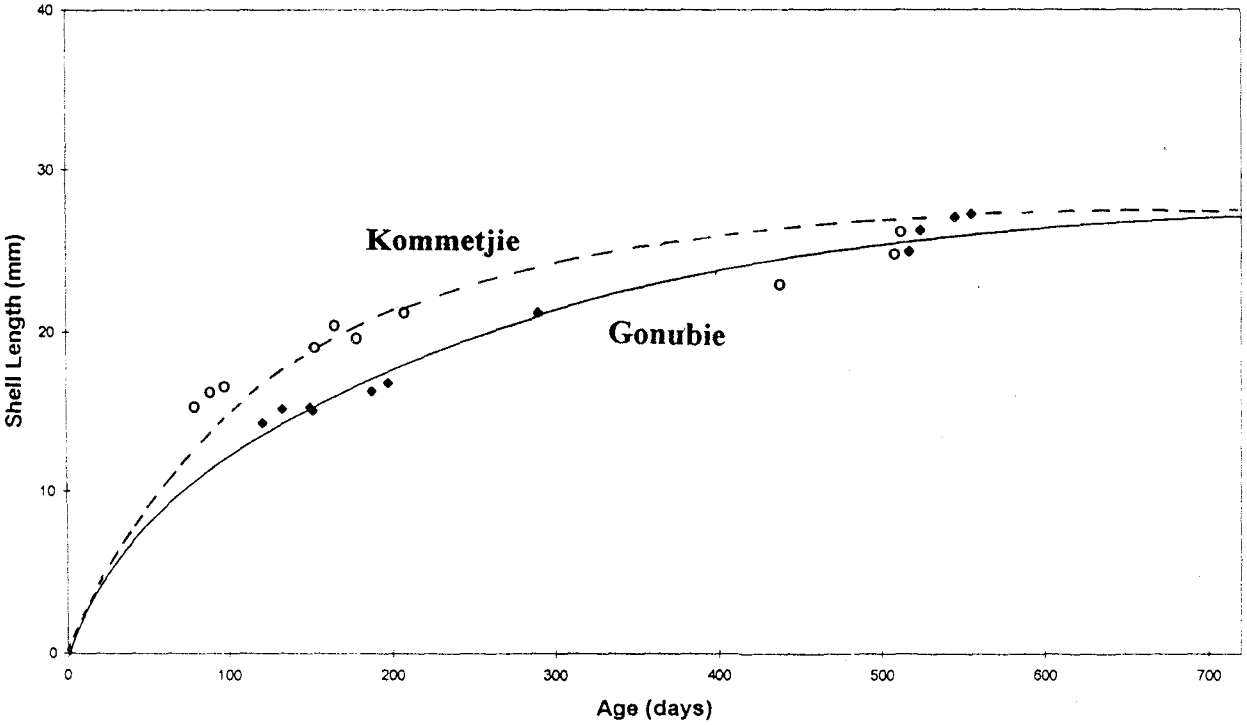

Figure 3.3. Relationship between shell length (mm) and age (days) of *H. pruinosus* at Gonubie, ◆ ,(south-east coast) and Kommetjie, o, (west coast), using the method of least squares. Ages for a given shell length were determined from micro-growth bands.

3.3.3 Allometric versus Isometric Growth.

H. pruinosus grows allometrically, increasing in shell length faster than shell height (Figure 3.6.A and B). Regression analysis yielded values for the constant of allometry (α) and c (a constant) when fitted to the field data from both Gonubie and Kommetjie, using the following equation : $\mathbf{h = cL}$

Gonubie $y = 1.07\ L^{0.4}$ $\alpha = 0.4$ $r^2 = 0.71$

Kommetjie (west coast) $y = 0.92\ L^{0.6}$ $\alpha = 0.6$ $r^2 = 0.78$

Gray (1996) produced the following results for *H. pectunculus:*

Gonubie $y = 0.12L^{1..31}$ $\alpha = 1.31$ $r^2 = 0.95$

Bloubergstrand (west coast) $y = 0.14L^{1..3}$ $\alpha = 1.3$ $r^2 = 0.71$

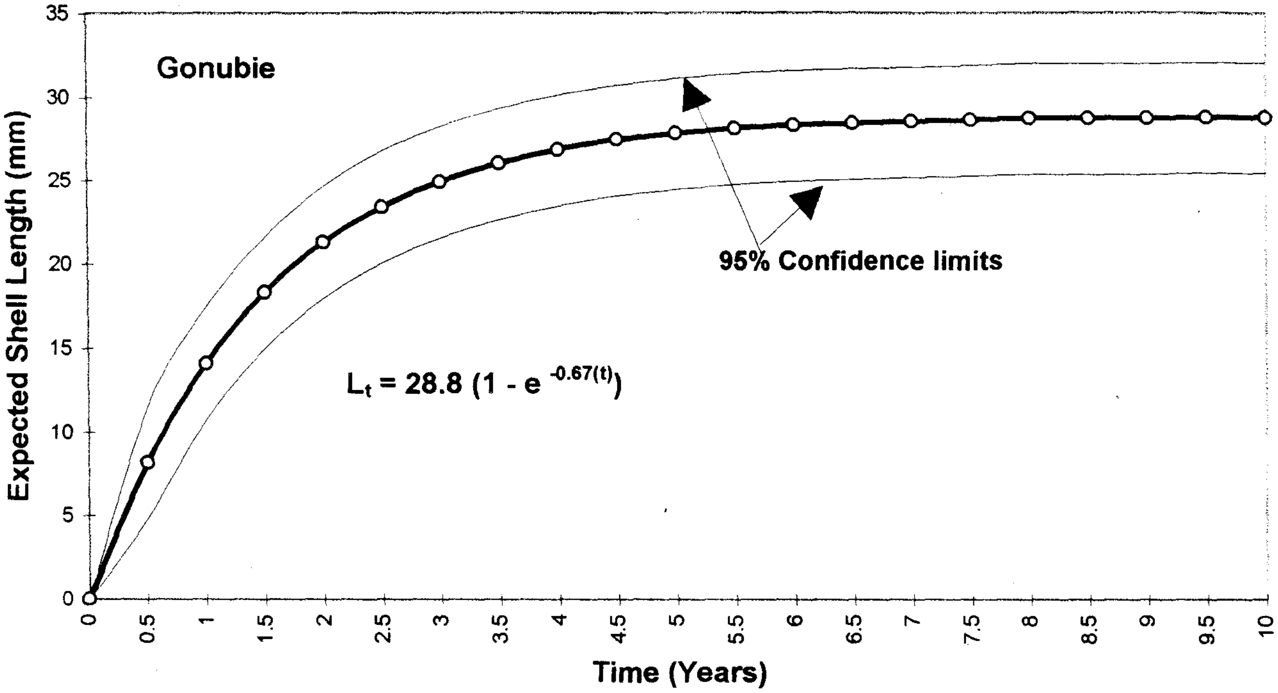

Figure 3.4.A. Theoretical growth curve of *H. pruinosus* at Gonubie using the von Bertalanffy growth equation, (95% Confidence limits are indicated).

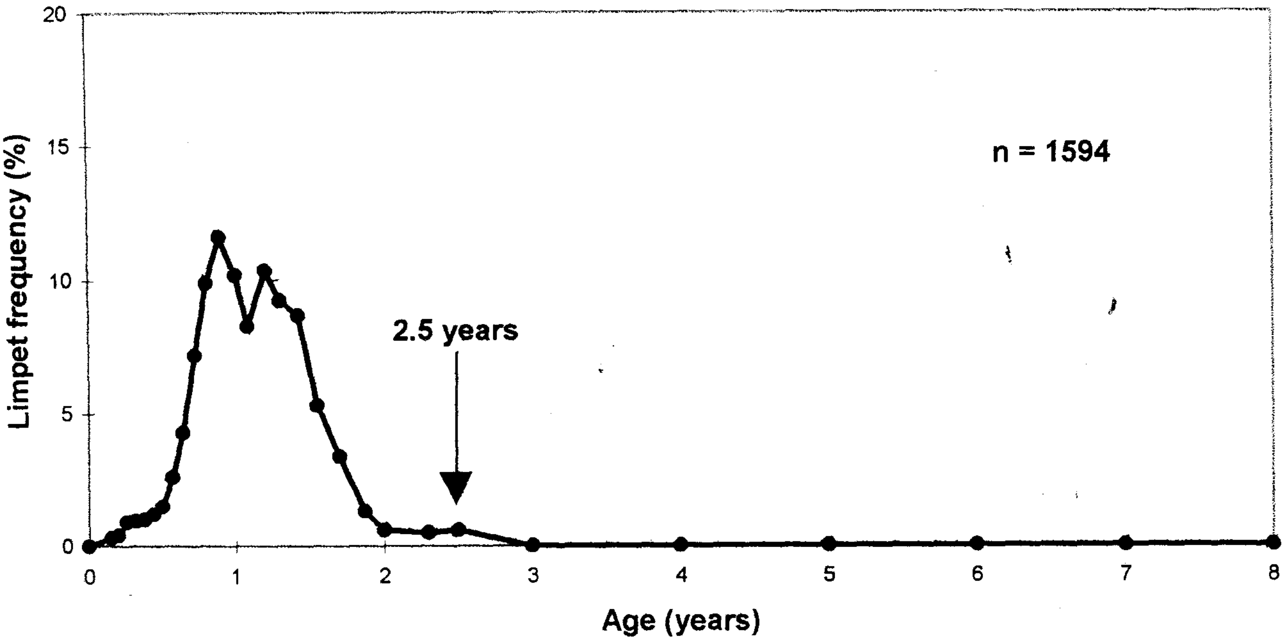

Figure 3.4.B Longevity of *H. pruinosus* based on a survivorship curve obtained from length-to frequency data (Chap. 2) at Gonubie.

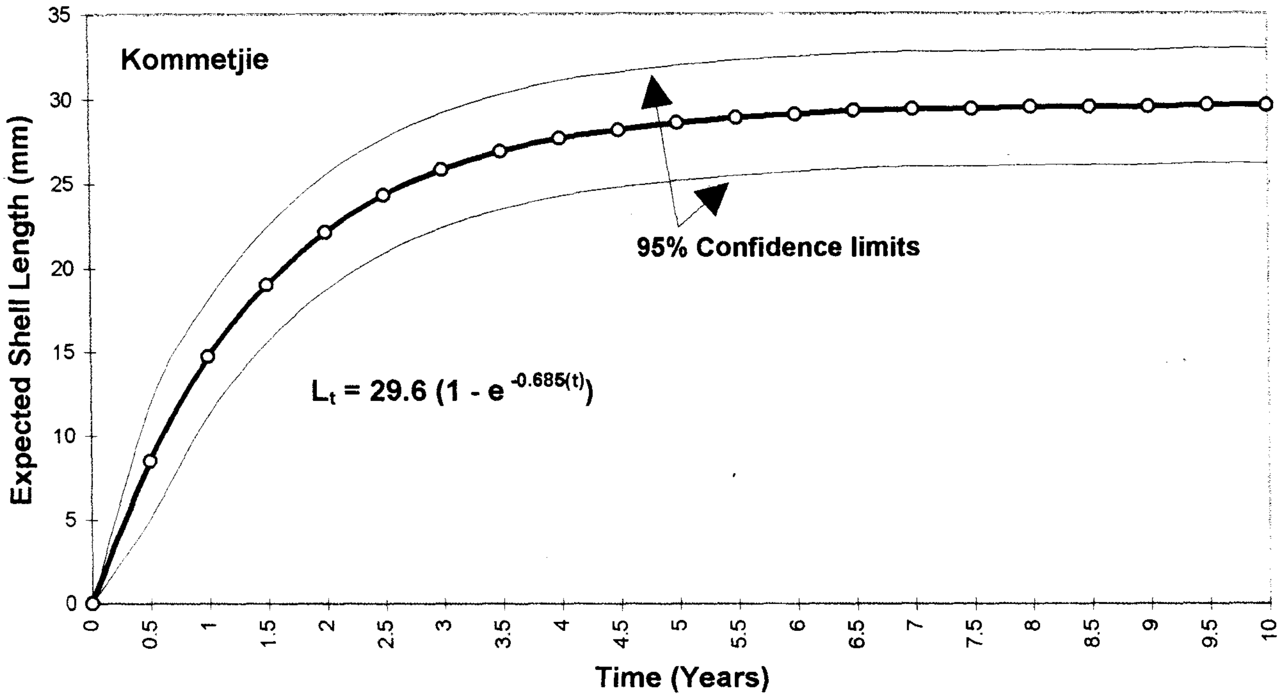

Figure 3.5.A. Theoretical growth curve of *H. pruinosus* at Kommetjie using the von Bertalanffy growth equation, (95% Confidence limits are indicated).

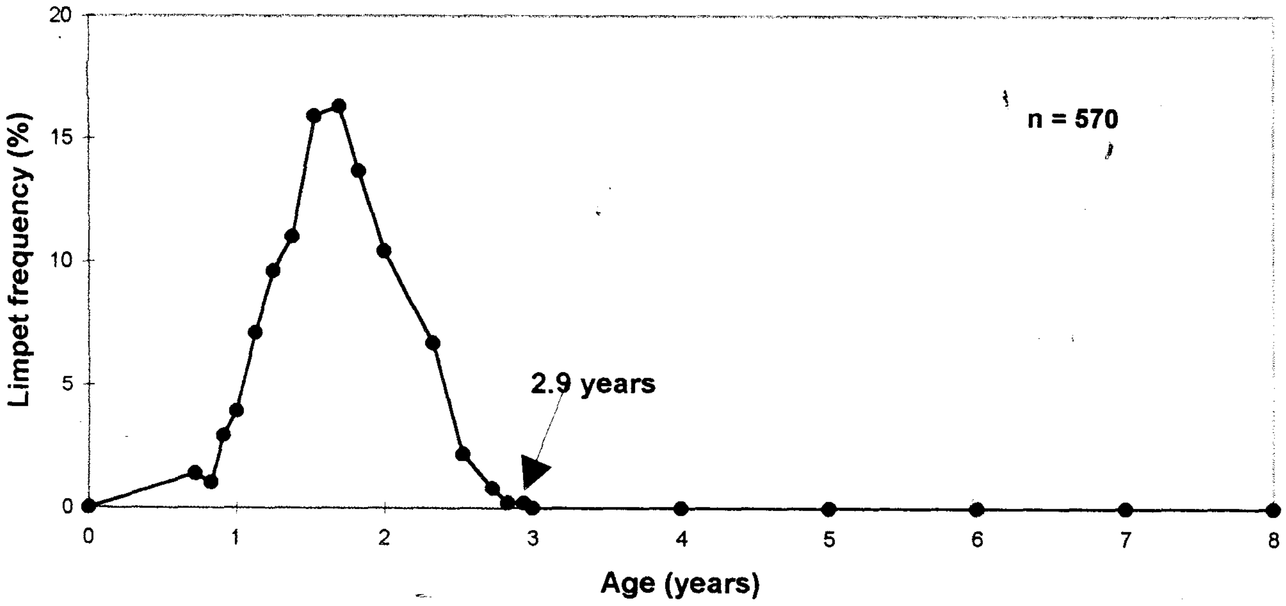

Figure 3.5.B Longevity of *H. pruinosus* based on a survivorhip curve obtained from length to frequency data (Chap. 2) at Kommetjie.

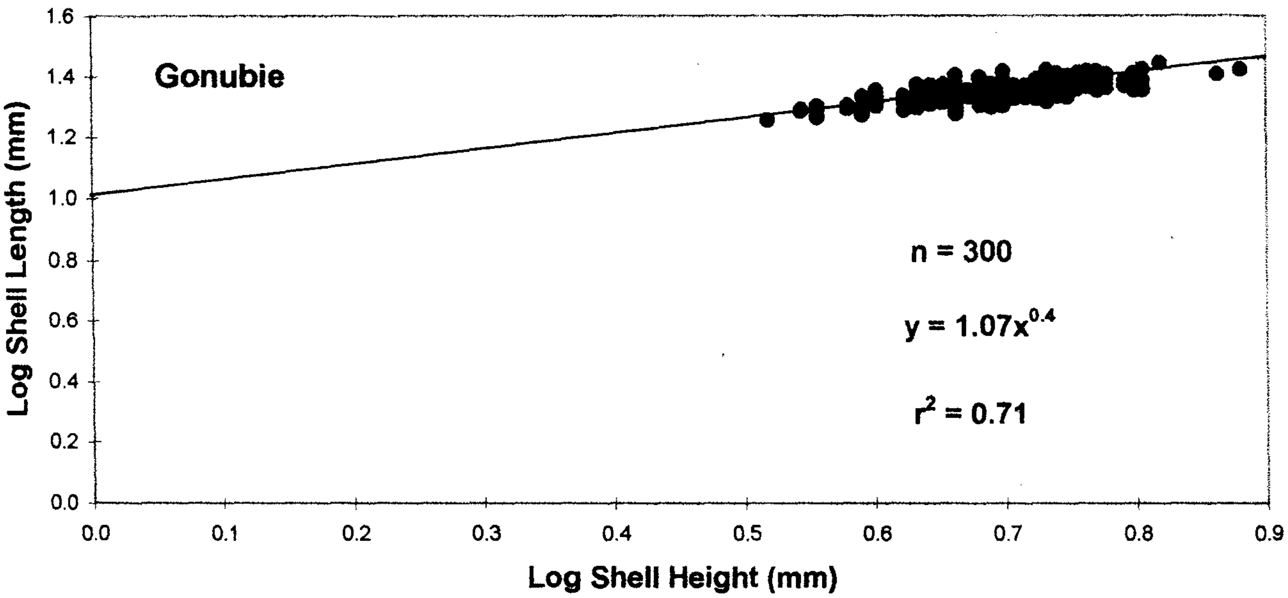

Figure 3.6.A. Log shell height versus log shell length of *H. pruinosus* at Gonubie (south-east coast).

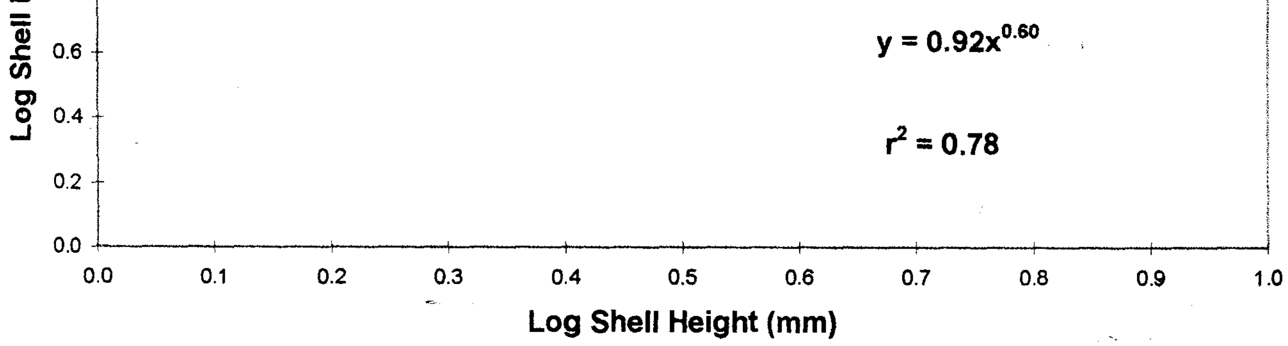

Figure 3.6.B. Log shell height versus log shell length of *H. pruinosus* at Kommetjie (west coast).

3.4 Discussion

There was a low rate of recovery (19% and 8% for the 5 and 4.5 months experiments respectively) of the labelled animals, recovery values which are similar to those recorded by Foster, (1997) for *Turbo sarmaticus* (5% to 14.5%). Gray (1996) in a study of the high shore sister species, *H. pectunculus*, had a high recovery rate (40 to 60%) and L. Vat (pers. comm.) working with *P. granularis* also has had similar high recovery rates of high shore populations, but not of populations living in mussel beds. These results suggest that on South African shores tagging is more successful on high shore animals, but not in mid- to low shore animals, such as *H. pruinosus* or *T. sarmaticus*. Poor recovery is probably a result of intense wave activity which removes the labels. This merely highlights the difficulties of life in the inter-tidal zone of all marine organisms.

There is usually a faster growth increment in younger individuals, which slows as animals age (Branch, 1974b; Shepherd *et al.*, 1995; Gray, 1996; Foster, 1997). The von Bertalanffy model suggests that this occurs in *H. pruinosus*. The attempted tagging study of growth also suggested a faster rate of growth of *Helcion pruinosus* in summer (0.62 mm/ month) compared to winter (0.13 mm/ month). Seasonal variability of growth has also been found in *T. sarmaticus* on south-east Cape shores (McLachlan and Lombard, 1981). There was, however a high variability of growth amongst similar sized limpets. For example, one individual (in the winter sample) with an initial length of 19.2 mm grew 1.1 mm in the 4.5 months of the study, while another individual of 19.1 mm grew only 0.1 mm over the same time period. In summer this varied from 4.0 mm (for an individual of 19.0 mm) to 2 mm for another individual of 19.0 mm. The reasons for such individual variability of growth is difficult to explain, but possibilities include, patchiness of food availability (longer periods of sunlight for micro-algal growth in summer),disturbances by predators or wave activity. Seasonal differences in growth could be ascribed to seasonal differences in food, differences in water temperature and the reproductive effort, or a combination of these and other possible factors (Sakai, 1962; Blackmore, 1969; Dame, 1972 and Arnold *et al.*, 1991). Chapter 5, (Reproduction), shows a release of gametes in May (autumn). This could result in greater effort being placed into gamete production rather than into growth during the autumn and winter months, when food may be scarcer owing to lower light intensities. The growth rate of *H. pruinosus* was twice as high in summer compared to that in winter. The seasonal micro-algae on the south-east coast possibly provide less energy than those on the west coast (Bustamante *et al.*, 1995). Although no attempt was made to determine the seasonality of food availability, it is possible that this influenced the difference in growth rates. Such seasonal variability of algae having a differential growth rate on grazing limpets was also found by Bosman and Hockey (1988 a and b) when studying *Patella granularis*.

The growth rate model as determined by the von Bertalanffy growth equation, was similar to the growth curve estimated from micro-growth bands in the shell. Such growth equations are useful when comparing the growth rates of differently sized animals, but should be used with caution (as the theoretical and observed maxima may be different, and this factor could influence the equation).

The value of 0.67 for the growth coefficient (K) for *H. pruinosus* was similar to that of 0.69 for *H. pectunculus* (Gray, 1996) obtained on the east coast, (at Port Elizabeth) and 0.685 for Kommetjie was also similar to that (0.61) obtained at Blouberg Strand for *H. pectunculus*. This would indicate that these congeneric species have similar growth rates, even though they do not share the same habitat on the shore.

H. pruinosus grows rapidly, reaches sexual maturity at an early age (6 to 9 months) and then lives a relatively short time period. Although *H. pruinosus* can live for as long as 2.4 to 2.9 years, very few limpets older than 1.6 years at Gonubie and 2 years at Kommetjie were found in the populations (Figures 3.4B, 3.5B). Reasons for these differences could be due to predation, habitat disturbances or mortality. Once sexual maturity is attained at 6 to 9 months, the gonads are active throughout the life span of the animal, while the population may trickle-spawn throughout the year (Chapter 5). As reproduction is considered to be energetically expensive (Fretter and Graham, 1994) this factor may limit the final size of the *H. pruinosus*.

The micro-band growth analysis, although time consuming, produced consistent results. Micro-growth banding has been found to be valuable for growth studies in various other marine organisms (see Gray, 1996, for review). These growth bands, according to Crisp (1989) are a result of temporary acidosis as a result of being immersed in water (with the incoming tide). The acidosis (due to an accumulation of CO_2) interrupts the secretion of calcium carbonate in the shell. This resulted in the production of two growth bands per day (two tidal changes).

Rapid growth and short longevity is probably correlated to the habitat of *H. pruinosus*. *H. pruinosus* lives on loose boulders with preferably a rock or solid substratum, avoiding boulders over a sandy substratum. These boulders are subjected to strong wave action and the limpets must have a high probability of being squashed by the rolling boulders as the tides surge, especially during storms. They do not live in cracks within rocks, like the congeneric species *H. pectunculus* (Gray, 1996), nor do they return to a home scar (where they would receive a modicum of protection). *H. pruinosus* thus seems to be an opportunist on the mid- to low shore, having an early initial growth spurt. It probably then puts greater effort into reproduction, reaching a maximum length after approximately 1.6 years and living for a maximum of 2.55 years. This is very similar to *H. pectunculus* (Gray, 1996) which reached its maximum lifespan after 2.6 years (Gonubie).

At Cape Town *H. pruinosus*, like *H. pectunculus* also had a longer lifespan (2.9 and 3.6 years respectively). The food availability will influence the rate of growth to sexual maturity, as found by Vahl (1971), Choat and Black (1979), Branch (1981) and Gray (1996). The rapid attainment of maturity would therefore allow the limpets to replace those lost in the high attrition rate (Figures 3.4B and 3.5B) due to the hazardous life-style within the intertidal zone (Branch, 1981). Such hazards would include competition, (both inter- and intraspecific types), predation, (especially by the rock sucker fish, *Chorisochismus dentax*), and the turbulent nature of the waves.

Information for *H. pruinosus* can be placed in a figure (figure 3.7) originally constructed by Branch (1981) and later modified by both Robson (1986) and Gray (1996), to show the inverse correlation between the growth coefficient (K) and longevity. *H. pruinosus* occupies a similar position to its congeneric species, *H. pectunculus*.

H. pruinosus has a shell with a low profile, with height increasing at a factor of 0.4 at any given time at Gonubie, and 0.6 at Kommetjie. This low shell profile and allometric growth is probably an adaptation of *H. pruinosus* to a low shore existence in the intertidal zone where it is subjected to strong wave action, (eventhough it shelters under boulders as the tide rises).

The aspect of the calorific nature of the micro-algae and its seasonality would be subjects for further investigation as these algae provide the necessary energy for the activity (Chapter 4) and reproduction (Chapter 5) of *H. pruinosus*.

Further studies on the growth of *H. pruinosus* could include the determination of the seasonality of growth using micro-growth banding instead of labelled animals.

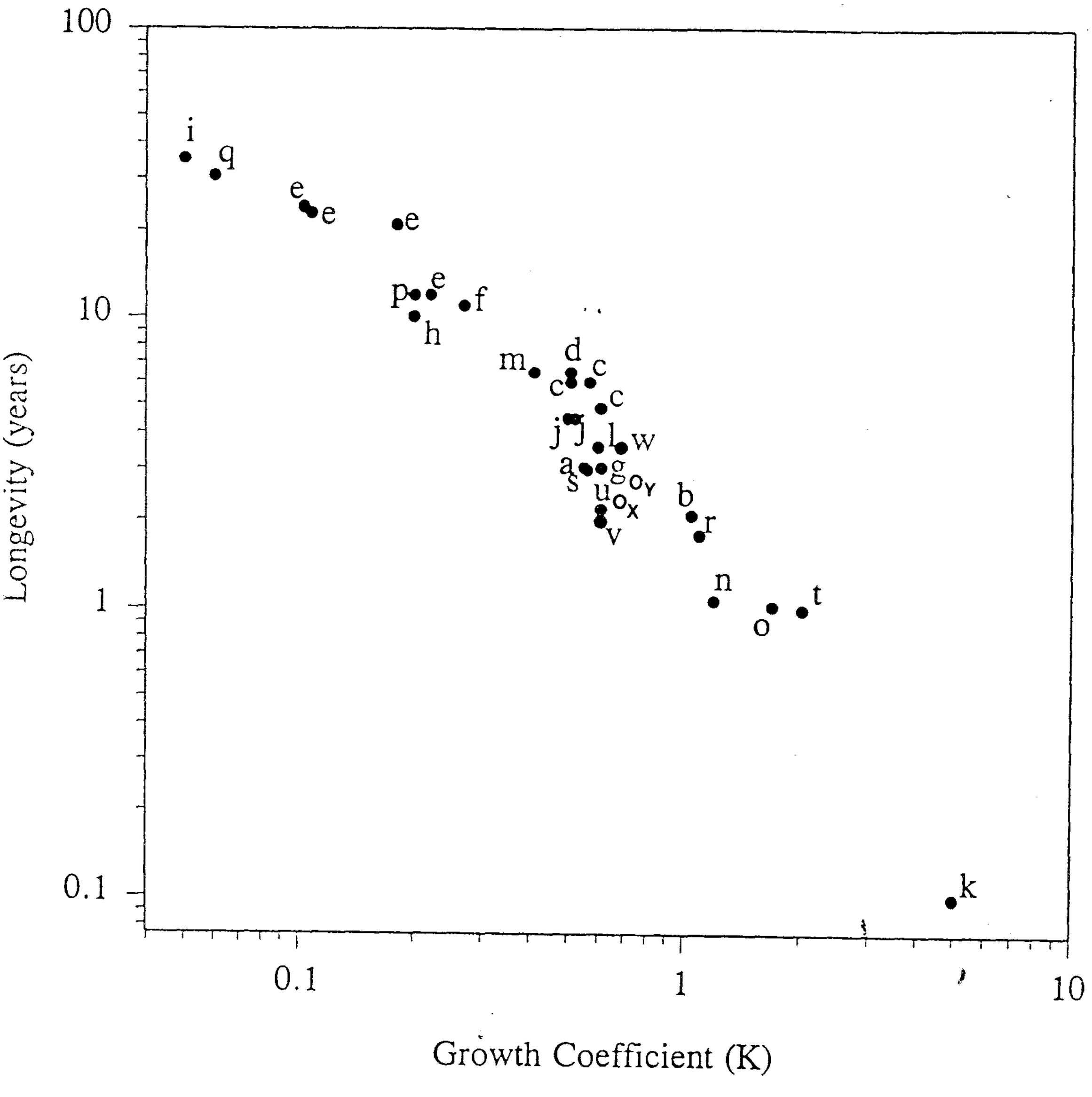

Figure 3.7 The correlation between longevity and the growth coefficient (K) in some prosobranch limpets: (a) *Patella compressa*; (b) *P. barbara*; (c) *P. granularis*; (d) *P. granatina*; (e) *P. cochlear*; (f) *P. longicosta*; (g) *P. oculus*; (h) *P. vulgata*; (I) *Nacella concinna*; (j) *Collisella digitalis*; (k) *Notoaccmea insessa*; (l) *C. strigatella*; (m) *N. persona*; (n) *Patelloida mimula*; (o) *P. insignis*; (p) *P. alticosta*; (q) *N. petterdi*; ® *Cellana radiata*; (s) *C. tramoserica*; (t) *Helcion pellucidus* (after Branch, 1981); *P. aphanes* (Robson, 1986); (v) *H. pectunculus*, west coast; (w)*H. pectunculus*, east coast (Gray, 1996); (x) *Helcion pruinosus*, south-east coast (present study); (y) *H. pruinosus*, west coast (present study).

Modified from Branch (1981), Robson (1986) and Gray (1996).

CHAPTER 4 : Activity rhythms of *Helcion pruinosus* at Gonubie

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Chapter 4 - Activity of *Helcion pruinosus* at Gonubie.

4.1 Introduction

To obtain sufficient energy for growth and reproduction grazers often have to forage considerable distances, and grazing gastropods are no exception. Within the intertidal zone, such foraging excursions by grazers usually occur as periodic rhythmical bursts of activity, alternating with bouts of inactivity (Hawkins and Hartnoll, 1983). Furthermore, most intertidal grazers, including Patellogastropods, are active during a particular phase of the tide and/or day night cycle (see Branch, 1981; Hawkins and Hartnoll, 1983; Little, 1989; Gray, 1996 for reviews of the literature).

Foraging rhythms, which are controlled by both exogenous and endogenous factors, are adaptive (Hawkins and Hartnoll, 1983; Gray, 1996; Gray and Hodgson, 1997). Adaptive reasons proposed for the timing of foraging include the avoidance of predators and strong wave activity, as well as minimising temperature and the stress of desiccation (Little, 1989).

There have been a considerable number of studies on the foraging activity of limpets, because their grazing activities play a major role in the structuring of the intertidal communities (Branch, 1981, 1985a; Hawkins and Hartnoll, 1983). South African rocky shores have a particularly diverse limpet fauna, with some species occurring in large densities (Branch, 1971; Kilburn and Rippey, 1982). Initial studies on the foraging rhythms of South African limpets were primarily qualitative observations on west coast species, aimed at documenting the periods of activity of the animals in relation to the tide and day/night cycle (Branch, 1971,1981). This work revealed that some species, (e.g. *Patella cochlear*) were only active when submerged, whereas others were active when exposed at night (e.g. *P. granularis*). Most of the observations were made during one season only and did not reveal anything about inter-seasonal or shorter term variations (e.g. the effect of spring and neap tides) on foraging behaviour (see Branch, 1981 and Liu, 1990 for references). More recent studies on intertidal grazers such as *Siphonaria capensis*, *S. concinna*, *Patella granularis* and *Helcion pectunculus* (Branch and Cherry, 1985; Gray, 1996; Gray and Hodgson, 1997) have revealed that considerable differences in foraging activity can occur both on a seasonal and on a tidal basis.

The activity of intertidal limpets is usually constrained by one or more physical factors. It might be expected, therefore, that the amount of energy gained for growth (Chapter 3) and reproduction (Chapter 5) during foraging would vary with the season and the phase of the tide. If the energetics of grazing are to be determined, information on the foraging behaviour needs to be established. The aims of this work were to determine when *H. pruinosus* was active with respect to tidal and day/night cycles and to see if there was any variability in activity during different phases of the moon, (i.e. spring and neap tides) and different seasons.

4.2 Materials and Methods

The activity of *H. pruinosus* was studied at a site near Gonubie, 32°57’03”S/ 28°01’07”E, (Magellan GPS 4000XL), just north-east of East London. This was near to the site of collection of animals used for the reproductive study (Chapter 5).

The beach, which consists of exposed flat sandstone rock shelves with loose sandstone boulders, (average diameter 30 to 40 mm), experiences semi-diurnal tides with a tidal range of 1.8 to 2.2m above Chart Datum on a mean spring tide and 0.6 to 0.9m on mean neap tides (Cleo data). Spring tides occur at full and new moon. Highest spring tides at full and new moon are phased between 09:00 and 11:00, and again 21:00 and 23:00.

The waves at the beach have a mean wave height of 1.35m, ranging from 0.5 to 2.20m. The mean wave frequency is 9.26 seconds, having a range of 5.8 to 12.73 seconds (Cleo data). There is thus virtually continuous wave activity at the shore.

Most of the recent quantitative studies on limpet foraging activity have involved the marking of the position of a set number of limpets, numbering each of them (e.g. with paint, Dymo tape or plastic bee labels) and then following their movements at fixed intervals during tidal cycles (Cook, Bamford, Freeman and Teideman, 1969; Little and Stirling, 1985; Chelazzi, Terranova and Della Santina, 1990; Liu, 1990; Gray and Hodgson, 1997, 1999). The precise position of each limpet, relative to its original site, could then be calculated by triangulation (method described in Cook *et al.*, 1969). Attempts to use this method to monitor the movements of *Helcion pruinosus* failed. The fact that *H. pruinosus* has a delicate shell and has a habit of retreating beneath boulders when inactive (see results section) meant that any labels were soon dislodged. Furthermore the 3-dimensional nature of the boulder shore at the research site, made plotting limpet movement by triangulation both difficult and inaccurate. Attempts to monitor the movement of individual limpets were abandoned and instead it was decided to quantify the general activity of a proportion of the limpet population.

Ten permanent quadrats (each 1 m^2) were marked on the rocks in the lower Balanoid zone with non-toxic paint, the paint being re-applied periodically when required. this region of the intertidal zone was chosen to monitor limpet activity because limpets were most numerous there (Chapter 2). Limpet activity within these ten quadrats was monitored on six separate occasions in 1996 and 1997, during spring and neap tides, as well as during different seasons (Table 4.1). The numbers of limpets active, within each quadrat, were counted every half hour for 24 hours from the time the rocks in the quadrats first became visible with the receding tide, until the quadrats were once more awash with the incoming tide. Monitoring was not undertaken at high tide due to intense wave activity in the intertidal zone, which made observations impossible. It was, however, assumed that the limpets remained inactive whilst submerged, as all limpets had retreated beneath small boulders before being covered by water (see results). At night the observations were carried out with red light only, (red cellophane being taped over the torch), since previous studies have shown that ordinary white torch light causes limpets to clamp down and to cease foraging (Cook *et al.*, 1969; Little and Stirling, 1985; Gray and Naylor, 1996, Gray and Hodgson, 1997, 1999).

In addition to monitoring limpet movement, air, rock and water temperature readings, (using mercury thermometers), as well as humidity readings (using an aneroid hygrometer) and wind information (wind strengths were measured with a "Ventimeter" supplied by the CSIR) were taken half-hourly. This data would provide clues as to the effect of the physical conditions on the foraging behaviour of *H. pruinosus*. All the data was analysed by the " Microsoft Excel" version 5.0 spreadsheet and statistical packages, as well as "Sigmastat" (Jandel Scientific).

Table 4.1 Periods of *Helcion pruinosus* activity chosen for the study.

	Phase of the moon		
	New Moon	Quarter Moon	Full Moon
Season			
Spring			X
Summer			X
Autumn	X	X	X
Winter			X

4.3 Results

4.3.1 Activity Rhythms

Helcion pruinosus foraged primarily during low tides, which occurred either at night or around dusk and dawn (Figure 4.1, Table 4.2). On one occasion, summer quarter moon, a light drizzle at dusk coincided a fairly strong south-easterly wind, averaging 19 to 26 kph. The number of limpets active during this evening was reduced compared to the following day.

Table 4.2 Greatest number of *H. pruinosus* active (total of ten 1 m^2 quadrats) during the different phases of the moon and time of the year. Also shown is the maximum time available for foraging at low tide (time exposed) and actual time spent foraging (time active) by the limpet population.

Season and phase of moon	Highest number active		Time exposed (Hours)		Time active (Hours)	
	Night	Day	Night	Day	Night	Day
Winter - Full moon	91	29	3	3.5	3	3.5
Spring - Full moon	370	0	5	4.5	5	0
Summer - Full moon	630	12	5	3.5	5	2
Autumn - Full moon	1076	65	4	4.5	4	3
Autumn - New moon	305	177	2	2.5	2	2.5
Autumn - Quarter moon	116	75	2	2	2	2

Both single and double periods of limpet activity were recorded during the 24 hour observation periods. When limpets had two activity periods in 24 hours, the number active was always greater at night than during the day. During autumn neap tides (Figure 4.1F) few limpets were active during both the night and day. Limpets were never active until they were completely exposed to the air, at which time they would emerge from beneath the boulders to commence foraging. In all cases the maximum number of limpets active coincided with, or just preceded, low tide (Figure 4.1). All the limpets retreated beneath the boulders prior to being splashed with water by the flooding tide. (Figure 4.1).

The maximum observed numbers of limpets active in the ten quadrats differed significantly between the full, new and quarter moon tides in autumn (ANOVA, Table 4.4A). The greatest activity was on the full moon, with significantly fewer limpets active on the new moon and significantly fewer still on the quarter moon (Table 4.4B).

There were significant differences (ANOVA, Table 4.4) in the maximum number of active limpets between full moon spring tides in the different seasons. The greatest number of limpets active (109 ± S.E./ m^2 at the peak of activity) was recorded on an autumn full moon (Table 4.2; Figure 4.1.D). Although limpet activity was high during the summer full moon (66.0 ± S.E./ m^2 at peak activity; figure 4.1C), this was significantly fewer ($p = 0.01$; t-test) than during the autumn. Still fewer limpets were active during the full moon in spring (39 ± S.E./ m^2; Figure 4.1B), although this number was significantly higher ($p = 0.37$; t-test) than the number of *H. pruinosus* active during the winter spring tide (15 ± S.E. /m^2). On spring tides the limpets were active for between 3 to 5 hours per night, whereas on a neap tide their activity was restricted to about half this time (2 hours).

4.3.2 Environmental Conditions

Night time temperatures usually declined to below 15° C except in summer, when nocturnal temperatures did not fall below 19^0 C. As might be expected air and rock temperatures were highest in summer, with rock temperatures always being a few degrees higher than that of the air (Table 4.3). Humidity was always greatest at night, never falling below 75%, but during the day in winter it could drop to as low as 34% (Table 4.3). In winter this region of the South African coastline experiences "berg winds", when air subsides from the dry interior plateau and is heated causing the drop in moisture content. Winds are primarily easterly in summer and from the west in winter (Cleo data).

Table 4.3 Physical conditions, including air, rock surface and water temperature (°C) ranges and the range of relative humidity measured during the activity observations of *Helcion pruinosus* at Gonubie during various seasons and phases of the moon.

	Range of air temp.	Range of rock temp.	Water temp.	Range of humidity (%)
Full Moon				
Winter - Day	19.2 - 21.8	20.4 - 26.1	17	33.9 - 58.3
Night	10.0 - 12.9	9.4 - 11.5	17	75.0 - 84.1
Spring - Day	16.0 - 20.2	20.0 - 28.3	16	48.5 - 75.0
Night	10.5 - 12.0	16.4 - 18.2	16	92.0 - 98.0
Summer - Day	22.6 - 26.5	24.0 - 32.5	22	61.4 - 73.2
Night	19.5 - 21.4	20.1 - 21.7	20	89.0 - 99.7
Autumn - Day	21.0 - 24.0	20.3 - 27.6	19	70.1 - 80.3
Night	13.0 - 15.0	15.0 - 18.0	17	100
New Moon				
Autumn - Day	12.2 - 13.0	14.1 - 14.8	19	100
Night	13.1 - 19.0	16.0 - 22.0	20	100
Quarter Moon				
Autumn - Day	15.0 - 16.0	25.4 - 25.8	18	70.5 - 80.4
Night	10.5 - 12.0	13.5 - 15.0	17	99.2 - 100

Figure 4.1 A to F. Activity of *H. pruinosus* at different phases of the moon and during different seasons of the year. The mean number of limpets (± S.E.) in ten fixed quadrats are shown. Low tides and the periods of darkness (by horizontal bar) are indicated.

Fig 4.1 A

Activity 2/8/96 (Winter - Full Moon)

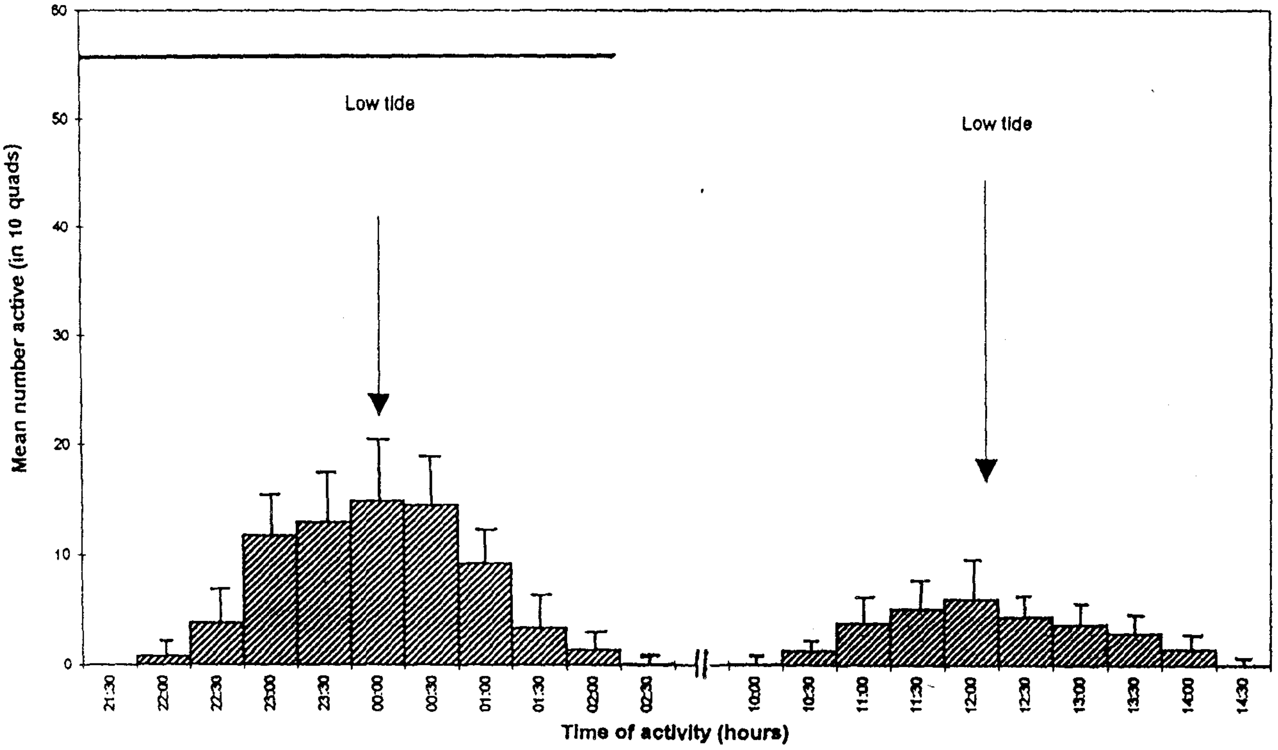

Fig. 4.1 B

Activity 17/10/97 (Spring - Full Moon)

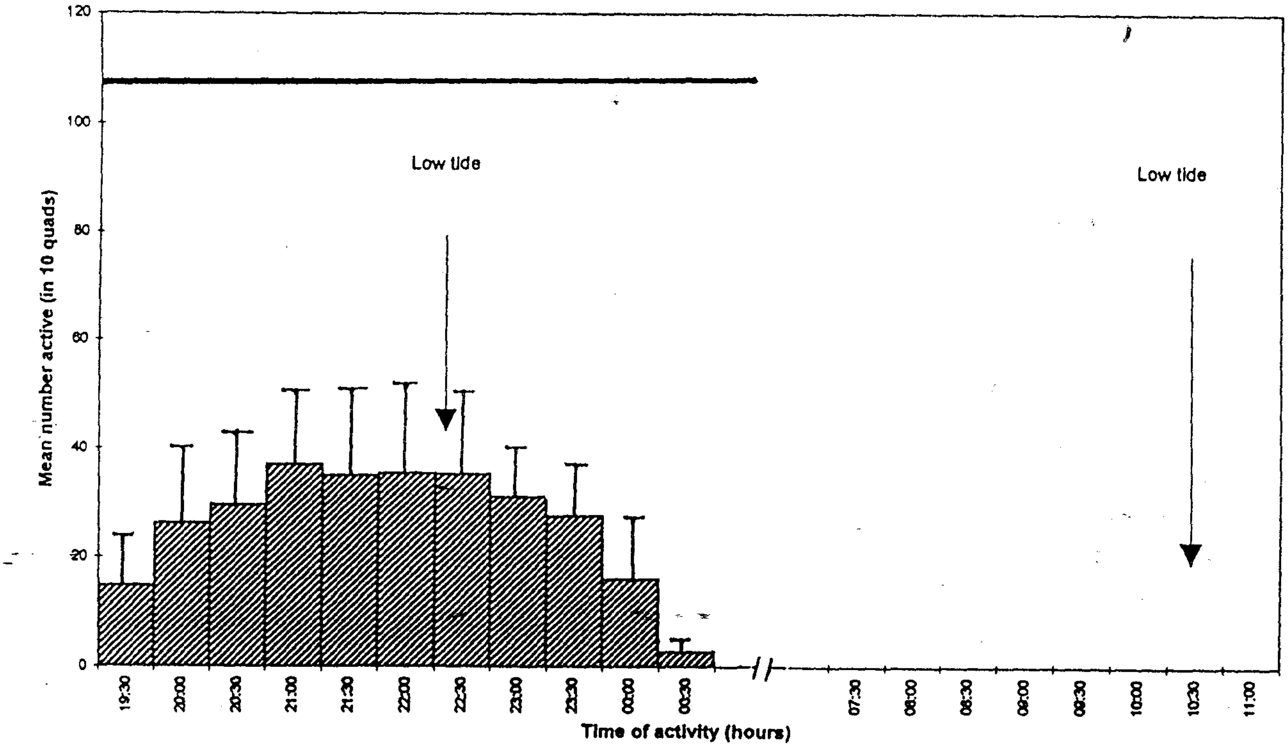

Fig. 4.1 C

Activity 23/12/96 (Summer - Full Moon)

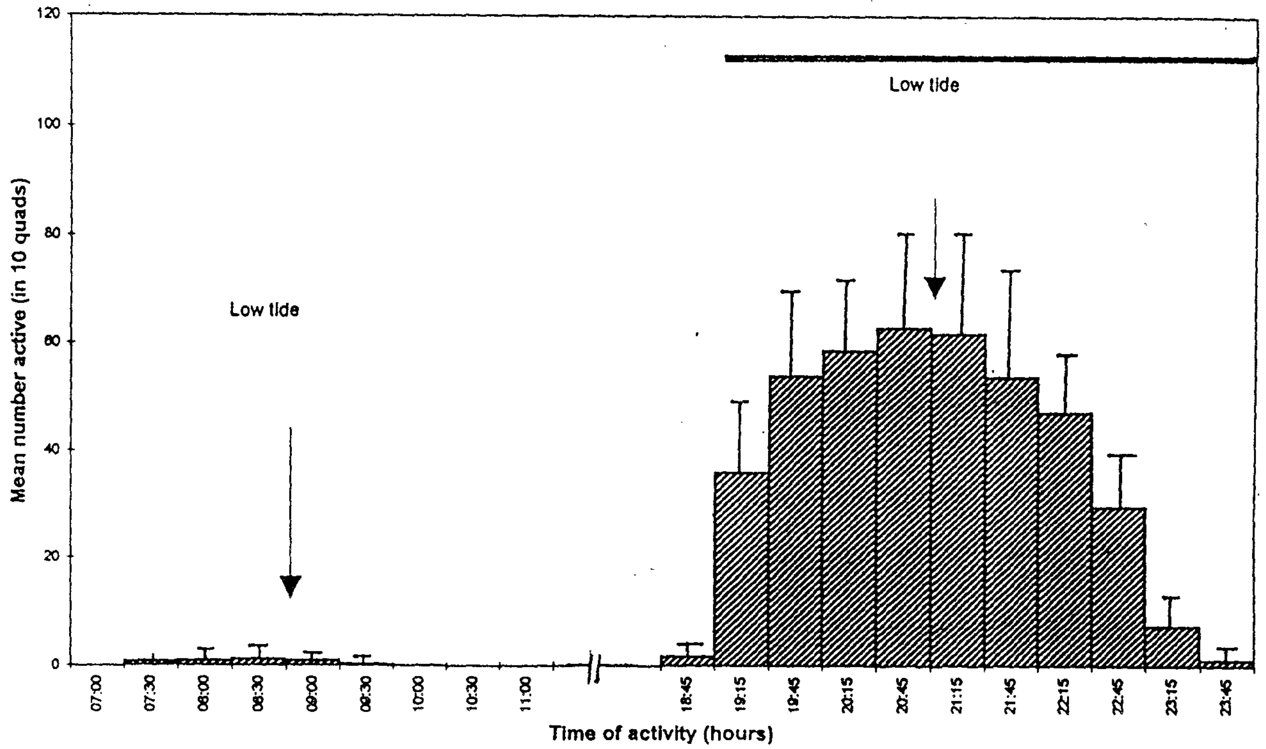

Fig. 4.1 D

Activity 24/5/97 (Autumn - Full Moon)

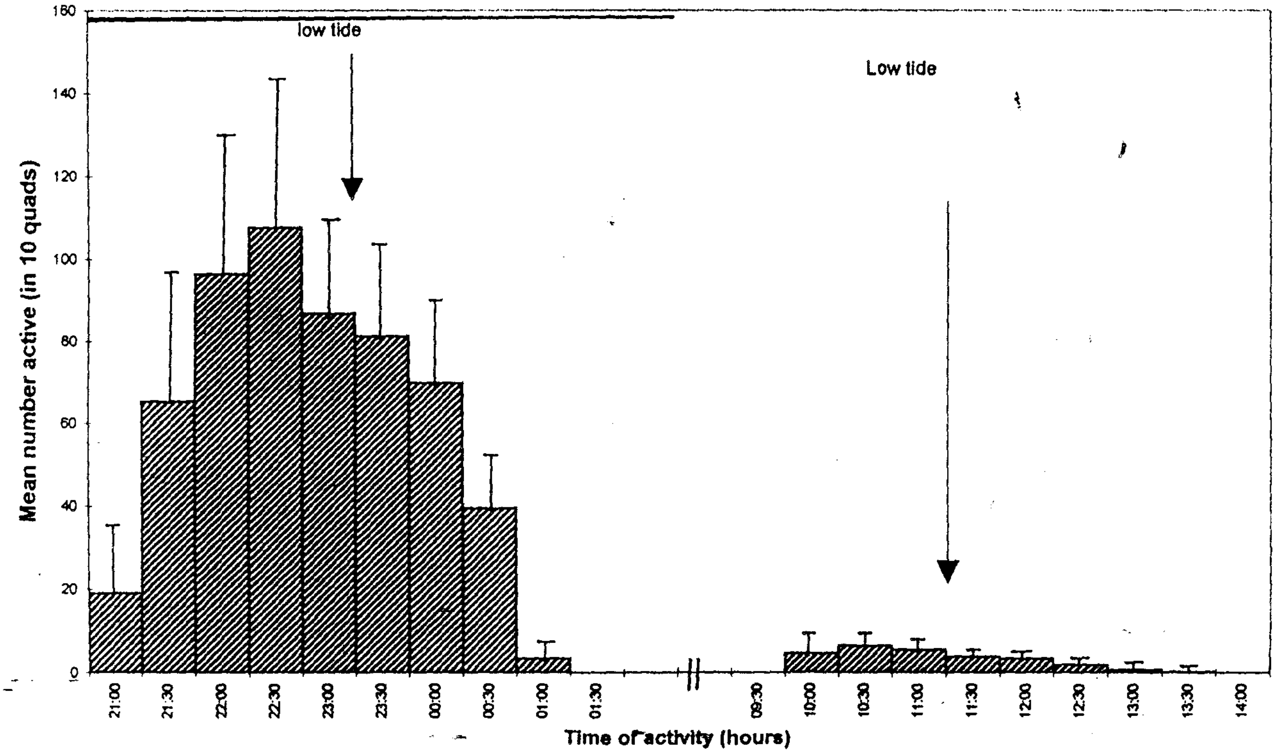

Fig. 4.1 E Activity 2/5/97 (Autumn - New Moon)

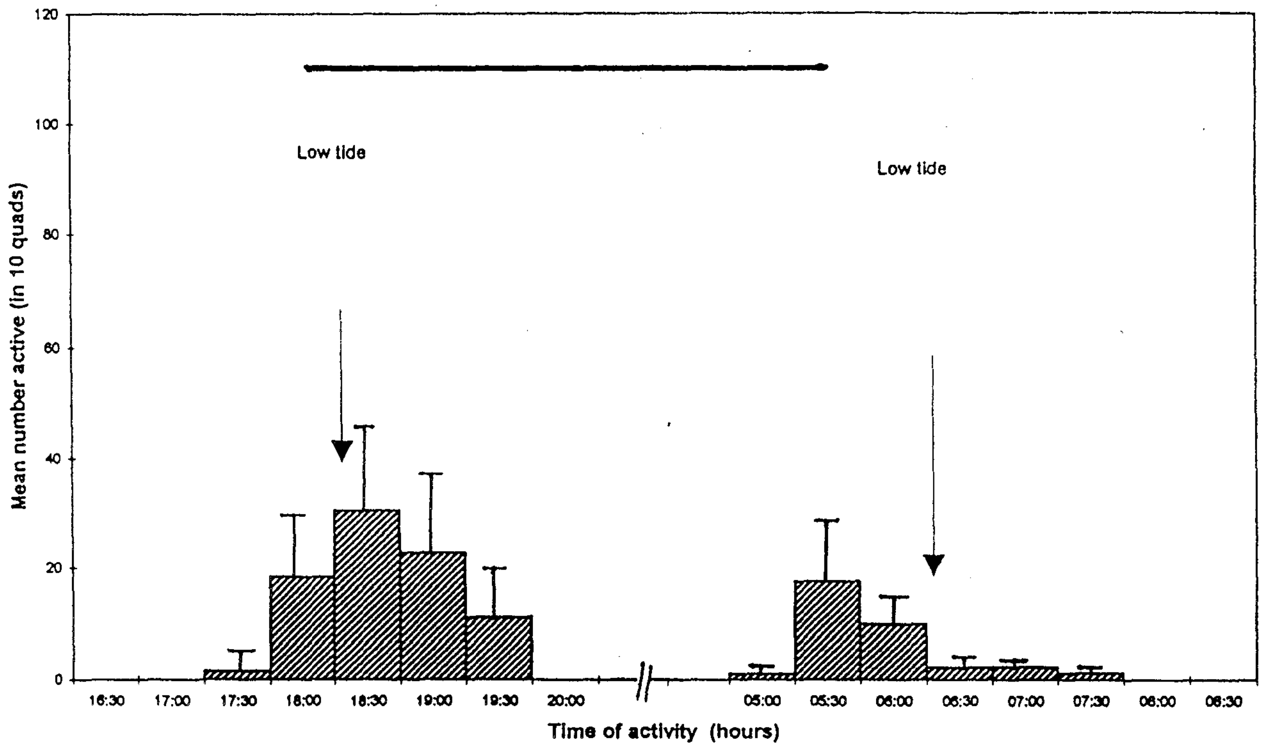

Fig. 4.1 F Activity 27/6/97 (Autumn - Last Quarter)

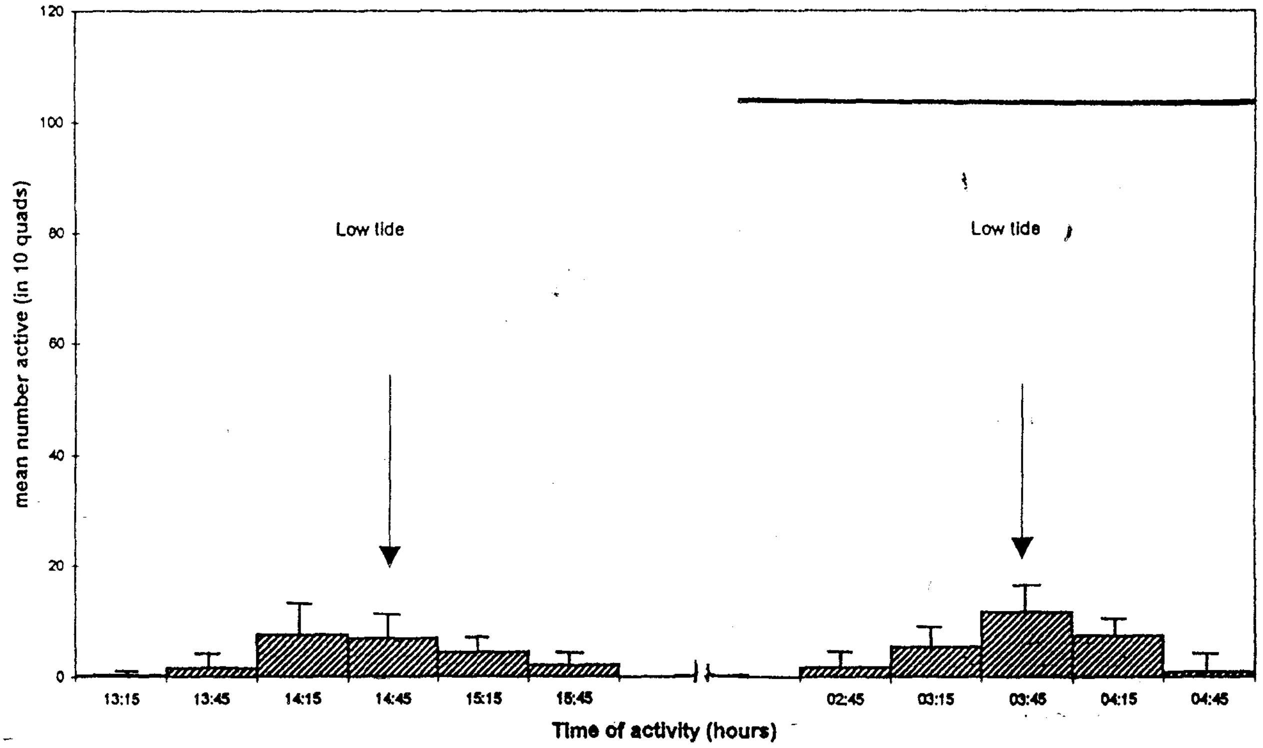

Table 4.4.A. Results of two way ANOVA analysis of variance on activity of *H. pruinosus* obtained from data on activity during different phases of the moon and during different seasons

Source of variation	SS	d.f.	MS	F value	p-value
Phases of the moon	8949.7	9	994.4	24.1	< 0.0001
Seasons	68397.5	5	136797.5	28.3	7.43 x 10^{-13}
Residual	21737.0	45	483.0		
Total (corrected)	99084.2	59			

Table 4.4.B Pairwise Multiple Comparison Procedure (Student-Newman-Keuls Method) between the season of activity and phases of the moon. [Alpha = 0.05]
(f.m. = full moon; q.m. = quarter moon; n.m. = new moon).

Comparison	**Difference of Means**	**p**	**q (test statistic)**
autumn f.m. vs autumn q.m.	98.00	6	14.100
autumn f.m. vs winter f.m.	94.3	5	13.568
autumn f.m. vs autumn n.m.	79.10	4	11.381
autumn f.m. vs spring f.m.	70.50	3	10.144
autumn f.m. vs summer f.m.	44.00	2	6.331
summer f.m. vs autumn q.m.	54.00	5	7.770
summer f.m. vs winter f.m.	50.30	4	7.237
summer f.m. vs autumn n.m.	35.10	3	5.050
summer f.m. vs spring f.m.	26.50	2	3.813
spring f.m. vs autumn q.m.	27.50	4	3.957
spring f.m. vs winter f.m.	23.80	3	3.424
spring f.m. vs autumn n.m.	8.60	2	1.237
autumn n.m. vs autumn q.m.	18.90	3	2.719
autumn n.m. vs winter f.m.	15.20	2	2.187
winter f.m. vs autumn q.m.	3.70	2	0.532

There was significant difference in all of the pairs, except spring full moon vs winter full moon and autumn new moon vs autumn new moon.

4.3.2 Homing Behaviour

Helcion pruinosus remained beneath boulders during the day, when emersed and most probably whilst immersed. Removal of numerous animals from their rocks revealed that they do not form a visible home scar. When retreating beneath a rock the limpets did not appear to follow a mucous trail, nor retrace an outward route back to the underside of the boulders. Although most limpets remained on the same boulders during and after foraging, some limpets were observed to move either into or out of the permanent quadrats (this was also noted during growth experiments of marked limpets in Chapter 3). The majority of limpets, however, remained within a quadrat.

4.3 Discussion

Helcion pruinosus, like many other limpets, e.g. *Patella* spp. (Branch, 1971; Hawkins and Hartnoll, 1983; Little, 1989; Gray and Hodgson, 1997), *Helcion pectunculus*, (Gray, 1996), *Siphonaria concinna* (Gray and Hodgson, 1997), *Cellana grata*, (Liu, 1990) forages mainly during nocturnal low tides, or when low tide coincides with dusk and dawn. There are a number of possible adaptive reasons for this behaviour. By being active at low tide only, the limpets not only avoid the physical stresses of wave activity, but also predation by fish. Of these two factors, fish predation is probably the most important in affecting the timing of activity of *H. pruinosus*, as has been shown for some *Patella* spp. and *Siphonaria* spp. (see Branch 1981, 1985a; Garrity and Levings, 1983; Hodgson in press for reviews). A common intertidal fish on South African shores is the rock-sucker, *Chorisochismus dentex* (Bloch), which specialises in feeding on limpets (Stobbs, 1980). This fish frequently contains whole limpet shells in its gut (Branch, 1981; Branch and Branch, 1981). These predatory fish were observed to be common on the underside of boulders at Gonubie, alongside inactive *H. pruinosus* . Dissection of some of these fish revealed the presence of shells of *H. pruinosus* in their gut contents. Thus, if limpets were active when covered by the tide, loss by the predatory activities by *C. dentex* might be even greater. Crabs and octopus, which have also been found to feed on limpets, could increase predatory pressure at high tide (Little *et al.*, 1990).

By foraging mainly at night, limpets are active when temperatures are lower (Liu, 1993) and the humidity is higher (except for autumn full moon, Table 4.3), which reduces the problem of desiccation (Branch, 1981). During day time exposure, most *H. pruinosus* remain beneath boulders. This behaviour must enable these limpets to avoid the direct effects of the sun's radiation and further reduce desiccation At Gonubie, day time temperatures were usually higher than those at night and nocturnal humidities nearly always exceeded those experienced during the day. Higher humidities seem to be the major factor promoting activity (Figure 4.1.D). For example, no limpets were active throughout the daytime low spring tide during the full moon in spring. On this day a fairly strong easterly wind, (averaging 13.0 kph and gusting up to 29.0 kph), which along with warm temperatures reduced the humidity to 48.5% (Table 4.3).

The differences recorded in the number of limpets active during the different phases of the moon and during different times of the day could be ascribed to the time available for foraging and the environmental conditions experienced at that time. For example, a light drizzle at dusk could possibly be a factor responsible for the reduction in numbers of animals active. Fresh rain water has been observed to reduce the activity of some species of limpets (Arnold, 1957; Gray and Hodgson, 1997; Little and Stirling, 1985; Little *et al.*, 1990).

A number of studies have now shown that the amount of time intertidal grazers can spend foraging may vary with phases of the tide, (i.e. spring or neap tide) and with seasons, (e.g. Branch and Cherry, 1985; Gray and Hodgson, 1997). Significantly more *H. pruinosus* were active on spring tides (especially the full moons) then on a neap tide. This can be explained by the fact that during neap tides, the limpets had a foraging window (i.e. foraging time) which was only half that available on spring tides (Table 4.2) . For example, during spring tide in autumn the limpets were uncovered for 4.5 hours whereas on the neap in the same season they were exposed for only 2 hours (Table 4.2). Furthermore because the tidal range was far less during the neap tide (0.6 to 0.9 metres on neaps vs. 1.8 to 2.2 metres during the spring tides), this meant that the sea virtually covered the limpets for most of the low tide period at this level on the shore.

Similar differences in limpet activity have been recorded for other species inhabiting Eastern Cape shores, and in addition these studies have shown that limpets also foraged over greater distances during spring tides (Gray, 1996; Gray and Hodgson,1997). Unfortunately it was not possible to calculate foraging distances in this study. However, a greater number of limpets were active in the permanent quadrats during spring tide rather than on a neap tide (Table 4.2).

The increase in number of limpets active during a spring tide is more difficult to explain. Whilst no attempts were made to confine the limpets to the permanently marked quadrats, it is unlikely that the increase in the numbers of limpets active during spring tides is a result of significant migrations of limpets into the quadrats during spring tides. Similarly, an exodus of limpets from the quadrats during neap tides is not envisaged. One possible reason may be the factor(s) which stimulate and control foraging in these limpets.

Avoiding wave activity may also be important for some species of limpets to maintain their position on the shore (Branch and Cherry, 1985; Branch, 1988 quoted in Gray and Hodgson, 1997; Hodgson, in press). The maximum number of *H. pruinosus* was always seen at or just before low tide, (Figures 4.1. A to F), with the numbers active rapidly declining to the underside of the boulders as the tide advanced. *Helcion pruinosus*, however, has a relatively smooth shell which has a low profile (Chapter 3). Such a shell would offer little resistance to water movement (Branch and Branch, 1981; Fretter and Graham, 1994), as has been found in limpets with similarly shaped shells (Branch, 1981). The limpets also appear to have an ability to adhere tenaciously to the boulders. Measurements of tenacity were attempted, but these were not very successful. It is suggested that because *H. pruinosus* has a shell with a low profile, this species would be able to withstand high wave activity. The timing of its activity with respect to the tidal cycle is a result of predator avoidance. Quantitative data on tenacity, along with field experiments are now required to test this hypothesis further.

Although limpet activity is influenced by exogenous factors (Branch, 1981, Liu 1990, Della Santina and Naylor, 1993; Gray and Hodgson, 1997), endogenous control of activity may also be important. Funke (1968) proposed that endogenous rhythms might be responsible for homing movements in some species of limpet. Liu (1993) studying the activity of limpets on the shores of Hong Kong found some evidence that found that *Siphonaria sirius and Patelloida saccharina* had endogenous rhythms related to a diurnal cycle. Perhaps the best evidence for endogenous rhythms in Patellid limpets comes from the work of Gray (1996) whose controlled laboratory studies demonstrated an endogenous rhythm in the congeneric species. Endogenous rhythms can therefore not be dismissed as a controlling mechanism for foraging in *H. pruinosus*.

Exogenous factors are probably also very important controlling the commencement and cessation of activity. Exogenous factors which have been suggested in controlling limpet movements, include the detection of changes in hydrostatic pressure, wave activity, fluctuations in temperature and pressure, food supply, light intensity and salinity (Branch, 1981; Liu, 1990) as well as the sensing of vibrations caused by the breaking waves of the incoming tide. The latter is an attractive possibility in explaining why so few *H. pruinosus* are active on nocturnal neap tides. On neap tides the waves do not retreat as far down the shore, which may result in greater vibrations which could be detected by the limpets. This in turn may lead to greater limpet inactivity. This theory does, however, need testing experimentally.

In addition to a greater number of limpets being active on a spring tide, significantly more limpets were active during summer and autumn springs than on spring tides during winter and spring (summer to winter, p = 0.037; summer to spring, p = 0.022; autumn to winter, $p = 1 \times 10^{-4}$; and autumn to spring, $p = 2 \times 10^{-5}$; t-test). Gray and Hodgson, (1997) found that on the Eastern Cape rocky shores *Patella granularis* travelled nearly three times as far whilst foraging in summer when compared to winter movements. They suggested that this may be due to the fact that epilithic algal production is higher in winter than summer (Bustamante *et al.*, 1995) and therefore limpets need not travel as far to obtain enough food. As *H. pruinosus* feeds on the epilithic micro-flora on the boulders, energy gain in summer per foraging excursion would be less forcing limpets to be active more frequently. The south-east coast of South Africa has a lower micro-algal productivity than the west coast (Bustamante *et al.*, 1995), and this would necessitate fairly long foraging activities to graze enough food, or be active more frequently(hence the greater numbers of limpets active in summer). Alternatively the lower winter temperatures may result in a decrease in metabolic rate and therefore the limpets were less active. Both of these possibilities could explain the decreased foraging activites of *H. pruinosus* observed in this study.

Bimodal peaks in feeding activity were observed in *H. pruinosus* and these may be due to the fact that some of the animals had to supplement their food intake by foraging during daylight hours, albeit in lower numbers.

Many species of limpet have been shown to return to a home scar after foraging (Branch, 1981; Branch and Cherry, 1985; Little, 1989; Gray, 1996; Hodgson, in press). *Helcion pruinosus*, like *Patella granularis* (Branch, 1971; Gray and Hodgson, 1997), does not have a home scar, but did return to the underside of the boulders on which they had been feeding. Home scars have been found to fulfil a number of functions including the reduction of desiccation and osmotic stress, as well as protection against predation and wave action (Branch, 1971, 1981, 1988; Garrity and Levings, 1983; Branch and Cherry, 1985; Verderber *et al.*, 1983; Kunz and Conner, 1986; Liu, 1990). Because *H. pruinosus* retreats beneath boulders, which may be moved by wave activity, a home scar is possibly of little value to this limpet. The absence of a home scar could also be related to the smooth nature of the rocks they inhabit, as there are no indentations or irregularities in the rocks to form a home scar. *Helcion pruinosus* does, however, retreat to a refuge, and in this respect resembles its congeneric species, *H. pectunculus*. In the case of *H. pectunculus* this refuge is a crevice in a rock, (Gray, 1996). This different behaviour when compared to species of *Patella*, (which do not retreat to a refuge, although many do have a home scar Branch, 1971, 1981, 1988; Gray and Hodgson, 1997) may indicate a phylogenetic difference between these closely related limpet genera.

Like *H. pectunculus*, *H. pruinosus* conforms to the group of non-migratory limpets as proposed by Branch (1974c), yet it does not respond aggressively to its own kind and there was no differentiation in habitat between adults and juveniles. Observations show that *H. pruinosus* does not follow mucous trails back to the underside of the boulders, often travelling back by a different route to that followed on the outward trail, as in the case of *Patella granularis* and *Siphonaria concinna* (Gray and Hodgson, 1997). Feeding movements seemed to be random, but no actual measurements of individuals were taken in this study.

In conclusion *H. pruinosus*, like many other species of South African limpets, is mainly active at low tide at night, a behaviour which has probably evolved to minimise desiccation and predation. There is considerable variation in the time limpets spent foraging at different phases of the moon and during different seasons. Whilst the amount of time spent active is controlled by the foraging window available (being greater on spring tides than neap tides) the observed differences in numbers of limpet active between seasons remains to be explained satisfactorily.

CHAPTER 5 : Reproductive seasonality in a south-east and a west coast population of *Helcion pruinosus*

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CHAPTER 5. REPRODUCTIVE SEASONALITY IN A SOUTH-EAST AND WEST COAST POPULATION OF *HELCION PRUINOSUS.*

5.1 INTRODUCTION

Annual patterns of reproduction in marine invertebrates are variable and animals may be rhythmic or sporadic reproducers during part or all of the year (Giese, 1959; Giese and Kanatani, 1987). Most shallow water marine invertebrates, however, exhibit seasonality of reproductive events (Giese, 1959; Kinne, 1963; Clark, 1965; Webber, 1977; Fretter, 1984; Fretter and Graham, 1994). One of the simplest reproductive strategies in marine invertebrates is broadcast fertilisation, whereby eggs and sperm are released into the surrounding water column. In species which broadcast their gametes into the water synchronicity of gamete release would seem to be of particular importance if the chances of gametes meeting are to be maximised (Giese, 1959).

Reproductive patterns are controlled by stimuli which may be endogenous or exogenous in origin (Giese, 1959; Giese & Pearse, 1974; Grahame and Branch, 1985; Giese and Kanatani, 1987). The stimulus for such a synchronous spawning may be a change in water temperature, increased wave action, the tides in particular seasons, lunar rhythms, food availability, or an inherent mechanism (under hormonal control) (e.g. Sastry, 1966; Boolootian, 1966; Branch, 1971, 1974a, 1985a, b; Ghosh and Ghose, 1972; Himmelmann, 1975, 1979; Todd, 1977; Newell *et al.*, 1982; Jones *et al.*, 1986; Lasiak, 1990; Hodgson *et al.*, 1991; Catalan and Yamamoto, 1993; Fretter and Graham, 1994; Galinou-Mitsoundi and Sinis, 1994; Goggin, 1994; Liu, 1994b; Qian, 1994; Bustemante *et al.*, 1995; Foster and Hodgson, 1995; Fukui, 1995; McKenny Jr., 1996; Gray, 1996; Foster, 1997).

Synchrony of spawning in the whole population would clearly improve the chances of fertilisation in marine molluscs. There should thus be a marked cycle of gonadal development culminating in a well defined spawning period, (see Branch, 1981, for review). The development of the gonads would be reflected as an increase in the mass of the gonads. This development would be expected to happen at a similar time and rate in males and females so that it could conclude in a mass release of gametes from the whole population to allow fertilisation and larval development at a time beneficial to the larvae. The food availability and conditions are therefore, probably the primary force determining timing of the development and release of the gametes to ensure a good start to larval development and when they settle to become adults.

The chances of reproductive success can be dependent on both the density and proximity of mature adults, as well as on water movements experienced at the time of spawning (Joska and Branch, 1983). In areas of low density, the success of fertilisation would be reduced (Joll, 1980), and in areas of vigorous water movement, such as in the surf zone, the chances of successful fertilisation in broadcast spawners would be further reduced (Lasiak, 1990). The chances of successful fertilisation must therefore be heightened by either complete or partial spawning. "Complete" spawning would imply that most of the large oocytes within the ovary would be liberated, whilst after a "partial" spawning numerous mature oocytes would still persist within the ovary.

In some South African limpets the geographic locality appears to influence the timing of gametogenesis and spawning. The west coast species of limpets spawn mainly in late autumn/early winter (May to June), while those species of the east and south-east coast spawn twice a year, during summer (November/December) and again in autumn to early winter (April to June) time. Branch (1974a) in a study of seven *Patella* spp., from the South African west coast, found a single spawning period each year, occurring simultaneously in both males and females. However, Branch (1974a) did caution that the actual timing of the event could vary from year to year, owing to the trigger mechanism responsible for the event. Studies on the east coast and south-east have revealed two spawning periods (Lasiak, 1987; Gray, 1996), or a single protracted spawning event (Robson, 1986).

Adults presumably have to be well nourished to manufacture gametes (Fretter and Graham, 1994). Although it might be assumed that both sexes put the same effort into gamete production, Branch (1974a) has found that gamete output is generally greater in males, and that males are often numerically dominant in most *Patella* species studied. The mean size of males was also less than that of females, which implies that they mature sexually at a smaller shell size than females. This numerical dominance of males was also reflected by *H. pruinosus* at Gonubie (Chapter 2).

However, synchrony of spawning in South African limpets has not been found in all species. Lasiak (1990) working on *Cellana capensis* found a distinct lack of synchrony in these animals on the South African Transkei coast. This lack of synchrony could be related to the spawning activity, as *C. capensis* could be a partial, rather than complete spawner.

Lasiak (1990) suggested that most early researchers failed to pinpoint the spawning events they described and attributed this to the collection of animals too infrequently. She recommended collecting animals on a more frequent basis, (e.g. weekly), especially during the expected breeding period. Creese and Ballantine (1983) collected limpets on a weekly basis, rather than on the usual monthly basis, during the animals expected breeding period. They found that limpets generally bred throughout the year, but there were two distinct peaks of spawning activity within the year, lasting from two to three months at a time. They could, however, not pinpoint the exact trigger for the spawning. Rao (1973) and Underwood (1974) both attributed such prolonged activity to piecemeal spawning by individual limpets, implying that gametes could be released gradually over a long time period. This is in sharp contrast to the earlier findings of Giese (1959), when he stated that spawning was a single discrete event of short duration.

A variety of methods can be used to determine seasonality of gametogenesis and spawing (Giese, 1959; Boolootian, 1966; Giese and Pearse, 1974; Perez Ruzafa and Marcos Diego, 1985). Gonad activity is usually expressed in terms of a Gonad Index (Gonor, 1972) which relates gonad weight to body weight. This shows a trend of increase, as the gonads develop followed by a sharp decrease as the gonad contents are voided during spawning. Ideally histological examination of the gonads should be used in conjunction with Gonad Indices, as one would find large masses of developing gametes followed by a disappearance of gonadal contents. These events should thus be found to coincide to confirm spawning. Both of these, alonside other parameters were determined in this study.

Helcion pruinosus can be found from Saldanha Bay, on the west coast, to Sodwana Bay, on the east coast of South Africa (Kilburn and Rippey, 1982; Branch *et al.*, 1994). By sampling two populations (Figure 1.1, Chapter 1) of *H. pruinosus* which inhabit different biogeographic provinces of South Africa, i.e. one from the west coast, near Cape Town, and the other from the south east coast near East London, comparisons could be made between the seasonality of reproduction of populations subjected to different oceanographic conditions (Chapter 1).

The aim of this study therefore was to examine and compare the reproductive seasonality of *H. pruinosus* in two populations exposed to differing environmental conditions. This was to be done by monthly sampling to determine the gonad index and by histological examination of the gonads. Few studies, other than that of Gray (1996), have explored geographical differences in reproductive behaviour of marine invertebrates (including limpets) in South Africa. Gray (1996) working on the limpet *H. pectunculus* found two distinct spawning events per annum in west and south-east coast populations, with a high degree of synchrony between the sexes. This study aimed to see if there were any differences in the congeneric species, *H. pruinosus* at the two divergent sites.

5.2. MATERIALS AND METHODS

5.2.1. General Procedures

To compare the reproductive seasonality of *Helcion pruinosus* subjected to different environmental conditions, limpets were collected on a monthly basis from a south-east coast (Gonubie - 32°57'S/28°01'E) and a West coast (Kommetjie - 34°04'S/18°19'E) site (Fig. 1.1, Chapter 1) from October 1994 until April 1996 (19 months). Both sites had large densities of limpets and could therefore sustain exploitation.

At both sites animals were collected from the lower Balanoid zone where they were most numerous (Chapter 2). Thirty animals were sampled from each site on full moon low spring tides. All limpets from Gonubie were dissected within 24 hr of collection. Animals from Kommetjie were fixed and stored in 4% formal saline before transportation to the laboratory in East London for dissection.

5.2.3 Gonad Index

Gonad index (G.I.) of limpets at each site was determined each month from 10 males and 10 females with a shell length of 20-25 mm. These shell lengths were used to eliminate possible size-related variation in the gonad index (Branch, 1974a). Gray (1996) found no significant difference in gonad indices between preserved and unpreserved limpets *(H. pectunculus)* and it was therefore possible to compare the gonad indices of such animals. To determine gonad indices the body and gonad of each animal was dried (at 60°C) to constant weight and the Gonad Index, calculated using the formula of Gonor (1972), i.e.

$$\textbf{Gonad Index} = \frac{\textbf{Dry gonad mass}}{\textbf{(Dry gonad mass + dry body mass)}} \times \frac{\textbf{100}}{\textbf{1}}$$

5.2.3. Gametogenic Cycle

To examine the gametogenic cycle, the gonads of five males and five females per month were prepared for histological sectioning. Animals from Kommetijie were already fixed in formalin. Gonads from Gonubie animals were fixed in aqueous Bouin's for at least 24 hours. Tissues from both sites were then dehydrated in a graded ethanol series and embedded in Paraplast via xylene. Transverse sections (5 µm thick) were cut on an American Optical microtome, and stained in Haematoxylin, and eosin (Humason, 1967).

Some of the ovaries of animals from Kommetjie were brittle and therefore difficult to section (due to storage in 4% formal saline). At the suggestion of Dr. J. Perold (Head Pathologists, Du Biosons Pathology Laboratories, East London) a depilatory cream ("NoHair", Twin products) was applied to the exposed embedded tissue prior to sectioning. The cream was applied and left on for approximately 30 minutes, wiped off and the tissue then sectioned. This facilitated sectioning.

Sections were examined and the various stages of gametogenesis recorded. In many gametogenic studies, five stages of oogenesis (protogonia, previtellogenesis, vitellogenesis, postvitellogenesis, degeneration of gametes) are often recognised (e.g. Chipperfield, 1953, De Gaulejac *et al.*, 1995a, b). It was not possible to identify these stages in *H. pruinosus* and therefore the oogenic categories selected were similar to those proposed by Branch (1974a). In females the stages included: previtellogenic oocytes; vitellogenic oocytes; mature oocytes; spent. In males the thickness of the walls of the gonad acini were used as an indication of the spermatogenic condition, acini wall thickness decreasing and sperm content increasing with maturity (Hodgson *et al.*, 1991; Hodgson, 1993; Gray, 1996). Acini wall thickness of 10 acini per male were measured. The thickness of the developing acinal wall was measured each month from the histological sections of the testes of both south-east and west coast individuals using a Nikon compound microscope fitted with a Nikon filar optical micrometer eye-piece. The walls of the first 10 acini randomly seen were measured for each of the five individuals per month . A monthly mean of the five males could then be determined for each site. Photographs were taken using an Olympus BX50 photomicroscope system, to show the stages of development.

In a similar manner the oocyte diameter was used as an indication of the state of development of the five females per month. Each individual was examined and the first 50 oocytes, which had an approximately centrally placed nucleus were measured. (The centrally placed nucleus was used to indicate whether or not the oocyte was sectioned midway). The results were then pooled to determine a monthly mean and representative photographs were taken.

5.2.4 Environmental Information.

This was obtained from the Weather offices of Cape Town and Pretoria and local CLEO reports. The timing of reproductive spawning in some limpets has been shown to be correlated to environmental factors such as water temperature, wind speed or direction causing surging of water during storms (Orten, Southward and Dodd, 1956). Data were converted into mean monthly averages. Daylengths, (i.e. time from sunrise to sunset), were also calculated and presented as means for each month of the study period.

5.2.5 Statistical Analyses.

All statistics were done using the Microsoft product "Excel" version 5.0 with its statistical package and "Sigmastat" (Jandel Scientific). Statistics included: means, standard deviations, chi-square, t-tests, Pearson's correlation coefficent, analyses of variance (ANOVA) and those mentioned in the text.

5.3 RESULTS

5.3.1 Reproductive cycle.

Although gonads of animals from both sites were present throughout the period of study (G.I. was never less than 10%), some annual pattern in the variation of G.I. and gametogenic condition were apparent. During the reproductive cycle the G.I. of males and females varied significantly with a two fold increase and decrease in G.I. at Gonubie and a three fold increase at Kommetjie (Figures 5.1, 5.2; Table 5.1). Throughout the study males at both sites had a significantly higher G.I. than females (Figures 5.1, 5.2; Table 5.1). Furthermore the G.I. of limpets from Kommetjie were significantly higher than those from Gonubie (Figures 5.1, 5.2; Table 5.1). The maximum G.I (Figures. 5.1, 5.2) attained on the west coast was 35 and 30% for males and females respectively , but only 25 and 22% for males and females at Gonubie on the south-east coast.

Although the reproductive cycle, in terms of gonad index, was more defined in the west coast animals, similar trends were observed at both sites. Gonad indices were lowest from about February, March or April until August/ September (which coincides with late summer to autumn) (Figures 5.1, 5.2). At these times the testes contained fewer mature sperm cells and a greater number of early spermatogenic stages (as indicated by the increased thickness of the acinal walls - Figures 5.3.A, 5.3.B, 5.6.A); the ovaries contained mainly pre- and early vitellogenic oocytes (< 120 μm diameter), although some vitellogenic oocytes (120 to 180 μm diameter) were also present.

Gonad indices began to increase in August/ September and by October the gonads of both sexes were showing signs of late gametogenesis. During this time there was an increase in the number of vitellogenic eggs in the females and in the abundance of mature sperm in males (this was reflected in the decrease in the thickness of the wall of the testicular acini - Figures 5.3.A, 5.3.B, 5.6.B). By the middle of summer (approximately December to February) the gonads started to fill with mature eggs or spematozoa (Figures 5.4, 5.5, 5.7, 5.8). One month after the G.I. was at a maximum, there was a rapid decrease in G.I., probably as a result of spawning. However gonads were never fully spent and both vitellogenic eggs and spermatozoa could be observed after the spawning events (Figures 5.1, 5.2 , 5.3. A, B, 5.4, 5.5, 5.6, 5.7 and 5.8).

Table 5.1 Results of a two-way ANOVA on the Gonad Index of male and female *Helcion pruinosus* located at Gonubie and Kommetjie.

Source of Variation	SS	df	MS	F	P value
Month	11138.0	18	618.8	32.4	< 0.0001
Sex	6548.5	3	2182.8	114.2	< 0.0001
Month x sex	10869.8	54	201.3	10.5	< 0.0001
(Significant differences, p = 3.24E - 079; 6.03E -060; 9.45E -060, respectively).					
Residual	13076.1	684	19.1		
Total (corrected)	41632.4	759	54.9		

Comparisons (alpha = 0.05) [Only those relevant factors which demonstrated significant differences are shown below].

	Diff. of Means	**p**	**q**	**Difference**
Kommetjie Males vs Gonubie Males	5.66	3	17.84	Yes
Kommetjie Females vs Gonubie Females	3.54	3	11.17	Yes
Kommetjie Males vs Kommetjie Females	4.55	2	14.34	Yes
Gonubie Males vs Gonubie Females	2.43	2	7.67	Yes

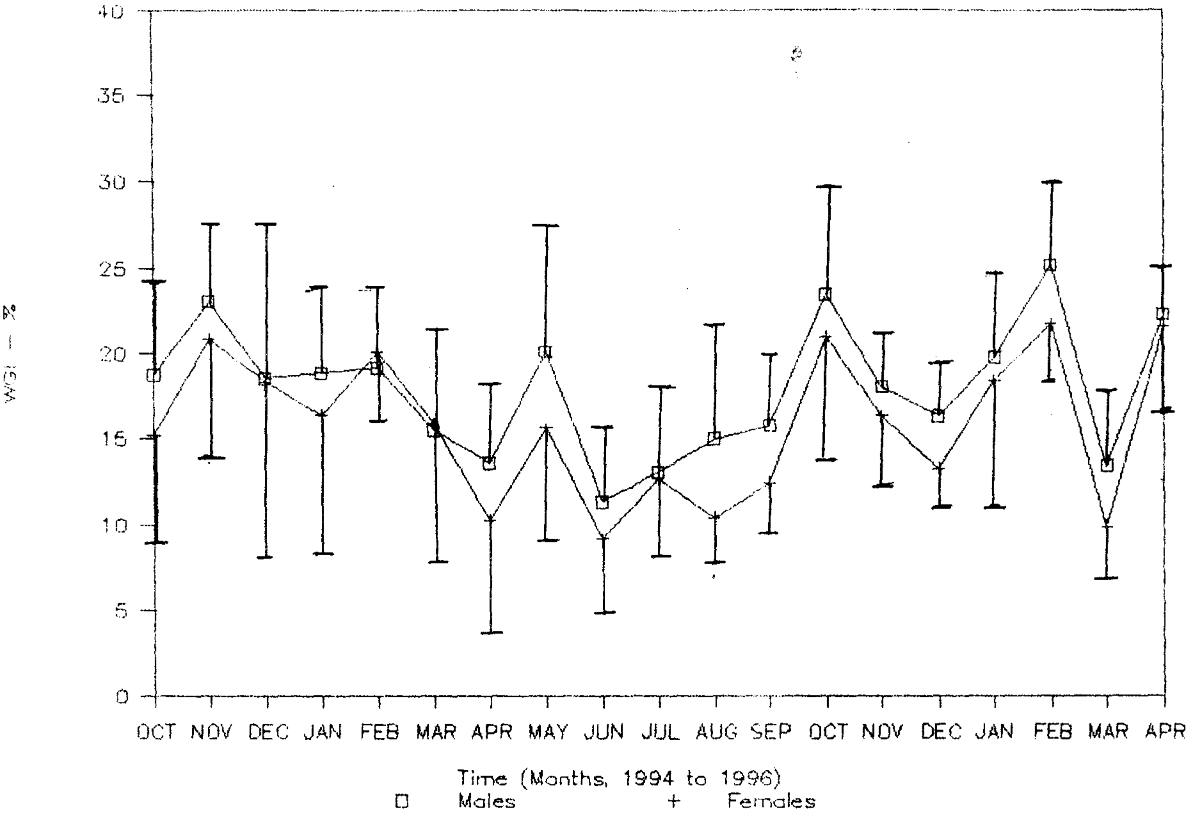


Figure 5.1 Mean wet gonad index of male (□) and female (+) *Helcion pruinosus* at Gonubie for the 19 month period (October 1994 to April 1996). The standard deviation for each month is also indicated.

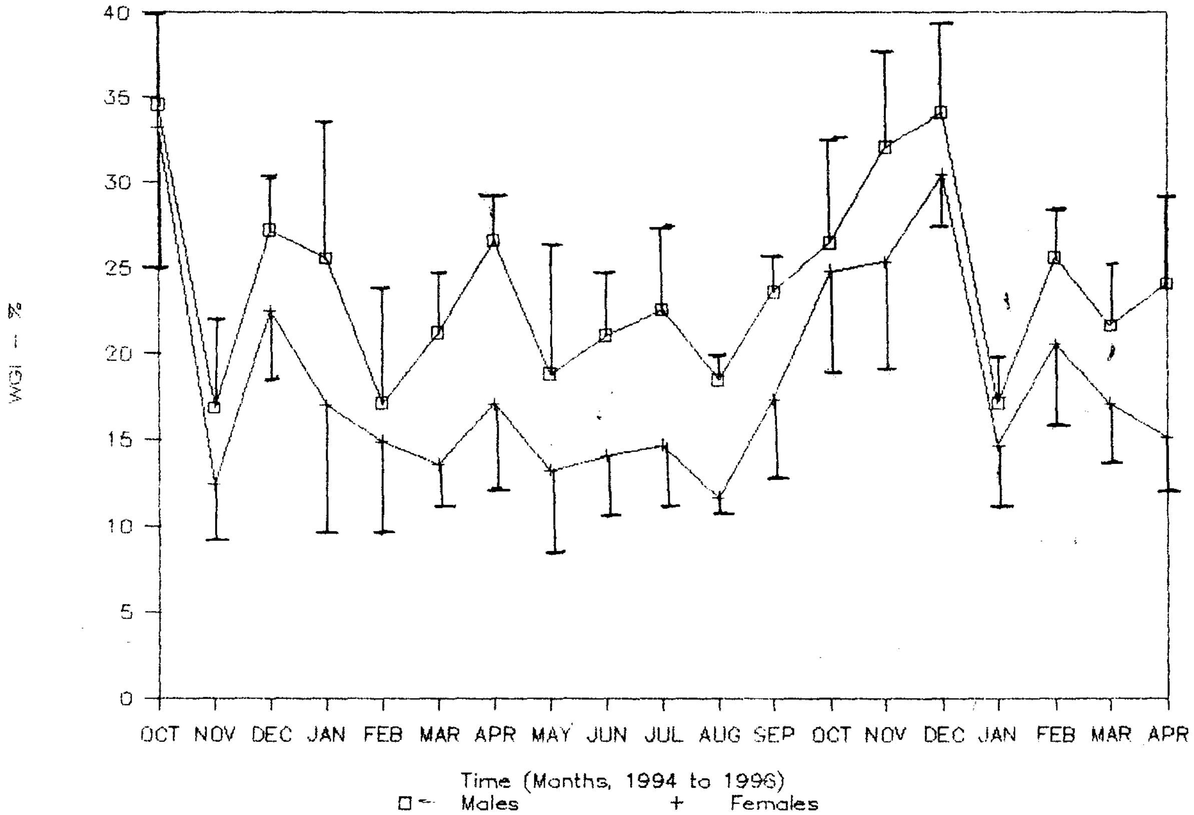


Figure 5.2 Mean wet gonad index of male (□) and female (+) *Helcion pruinosus* at Kommetjie for the 19 month period (October 1994 to April 1996). The standard deviation for each month is also indicated.

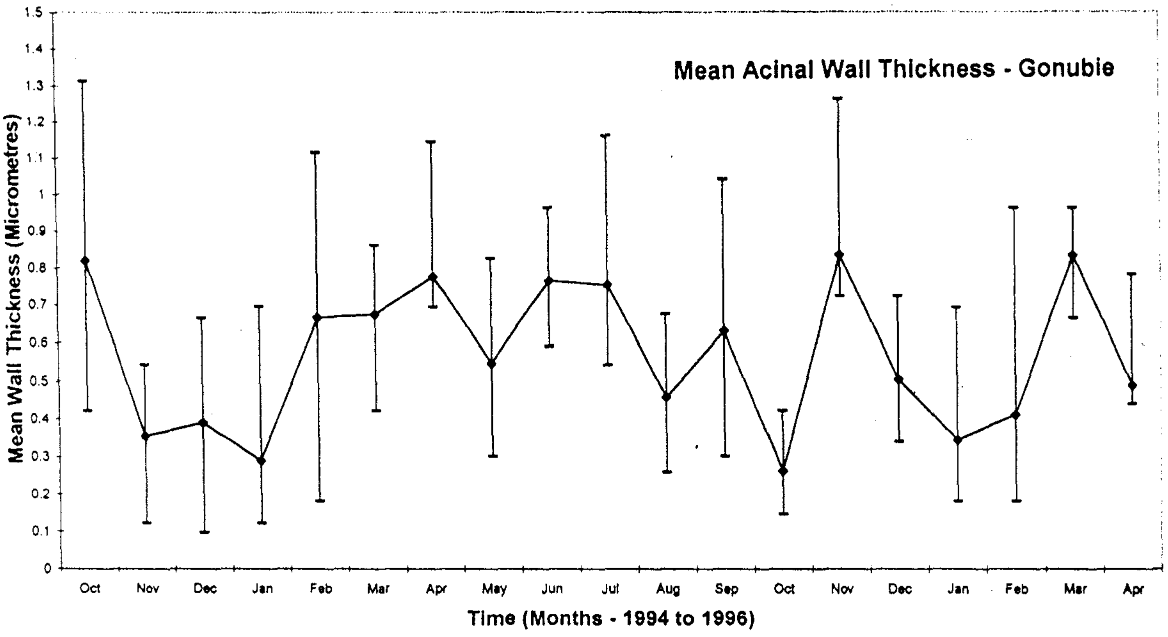


Figure 5.3.A. Mean acinal wall thickness (micrometres) of male *Helcion pruinosus* over a 19 month period (1994 to 1996) at Gonubie. Maximum and minimum sizes are also indicated for each month.

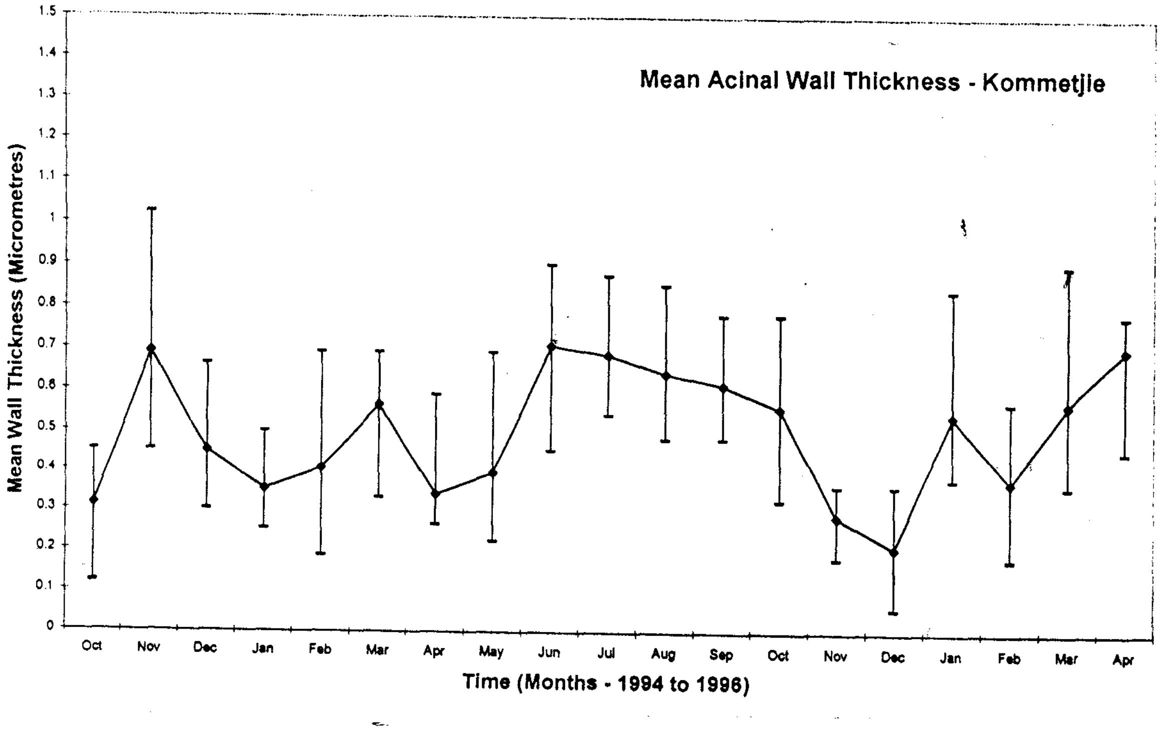
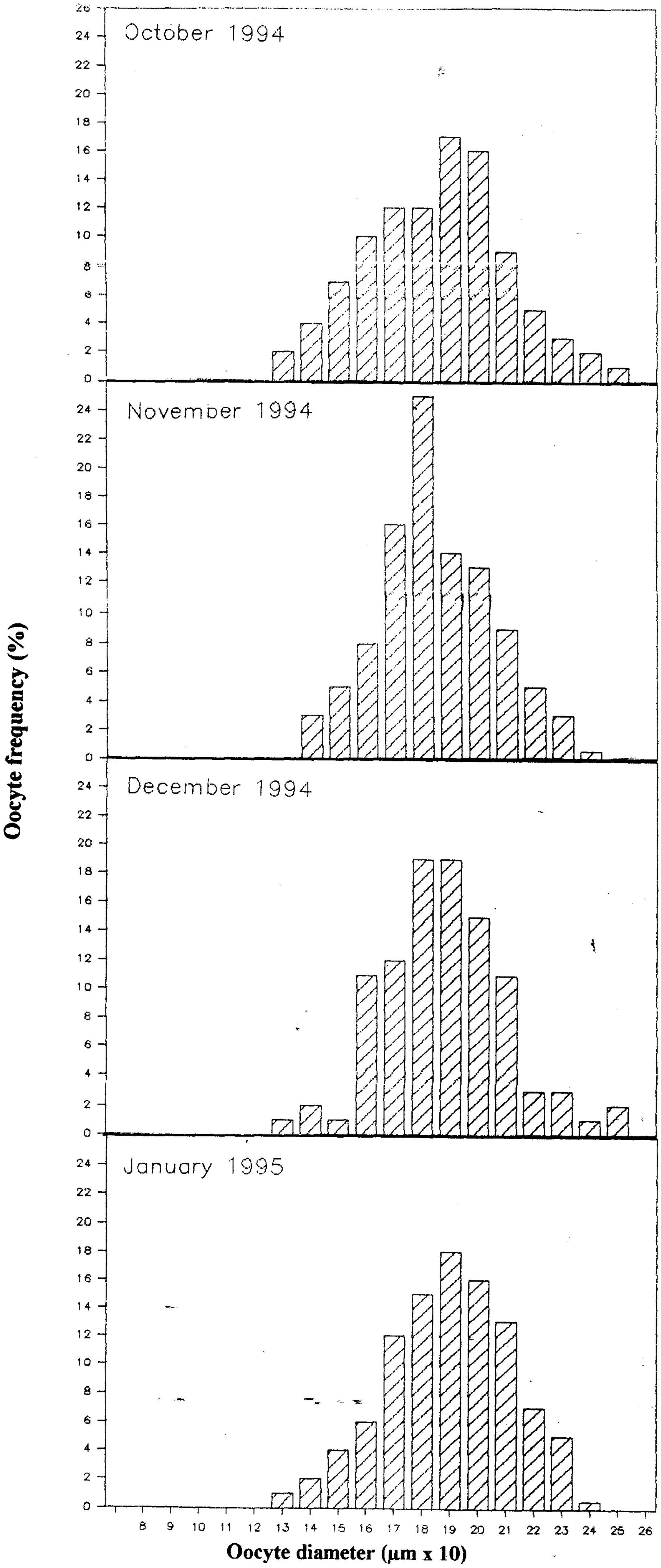
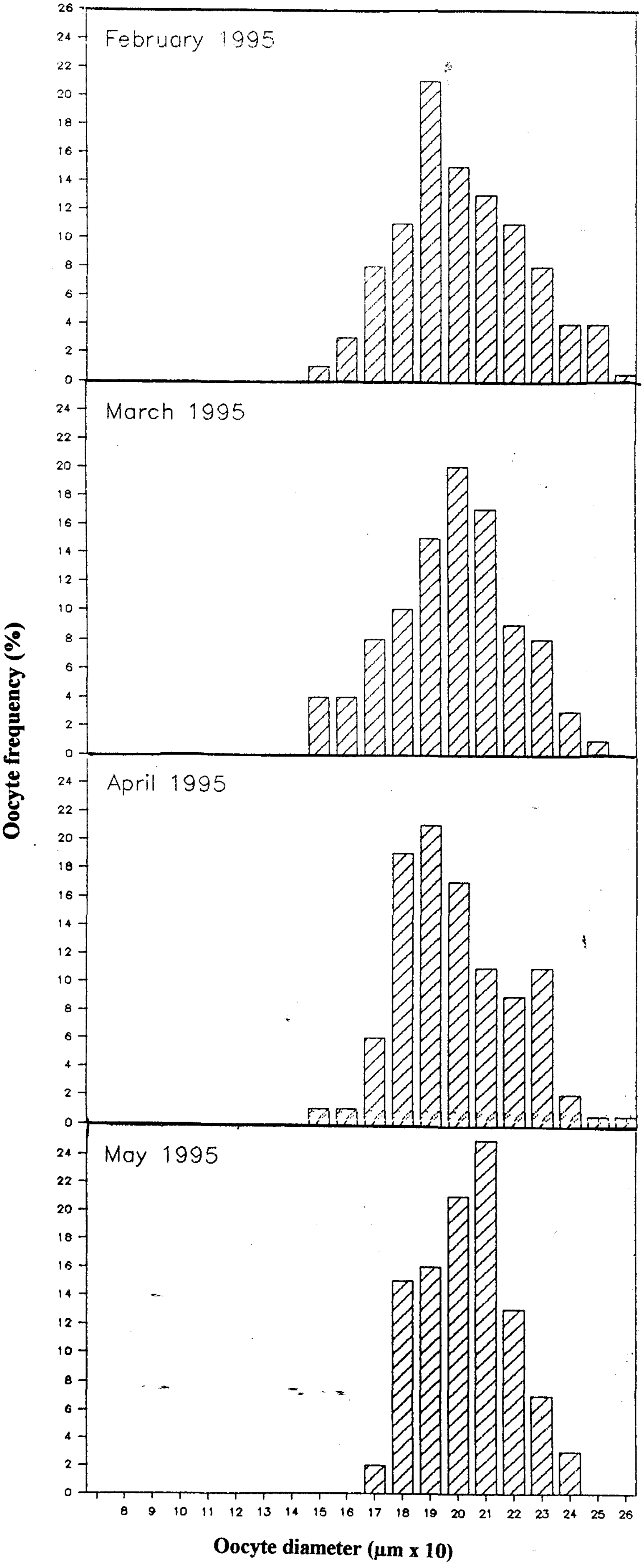


Figure 5.3.B. Mean acinal wall thickness (micrometres) of male *Helcion pruinosus* over a 19 month period (1994 to 1996) at Kommetjie. Maximum and minimum sizes are also indicated for each month.

Figure 5.4. The following 5 pages illustrate the frequency of different sized oocytes from female individuals of *Helcion pruinosus* collected from Gonubie on the south-east coast on a monthly basis from October 1994 to April 1996.


October 1994
November 1994
December 1994
January 1995
Oocyte frequency (%)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
Oocyte diameter (µm x 10)
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26


February 1995
March 1995
April 1995
May 1995
Oocyte frequency (%)
0
2
4
6
8
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18
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24
26
8
9
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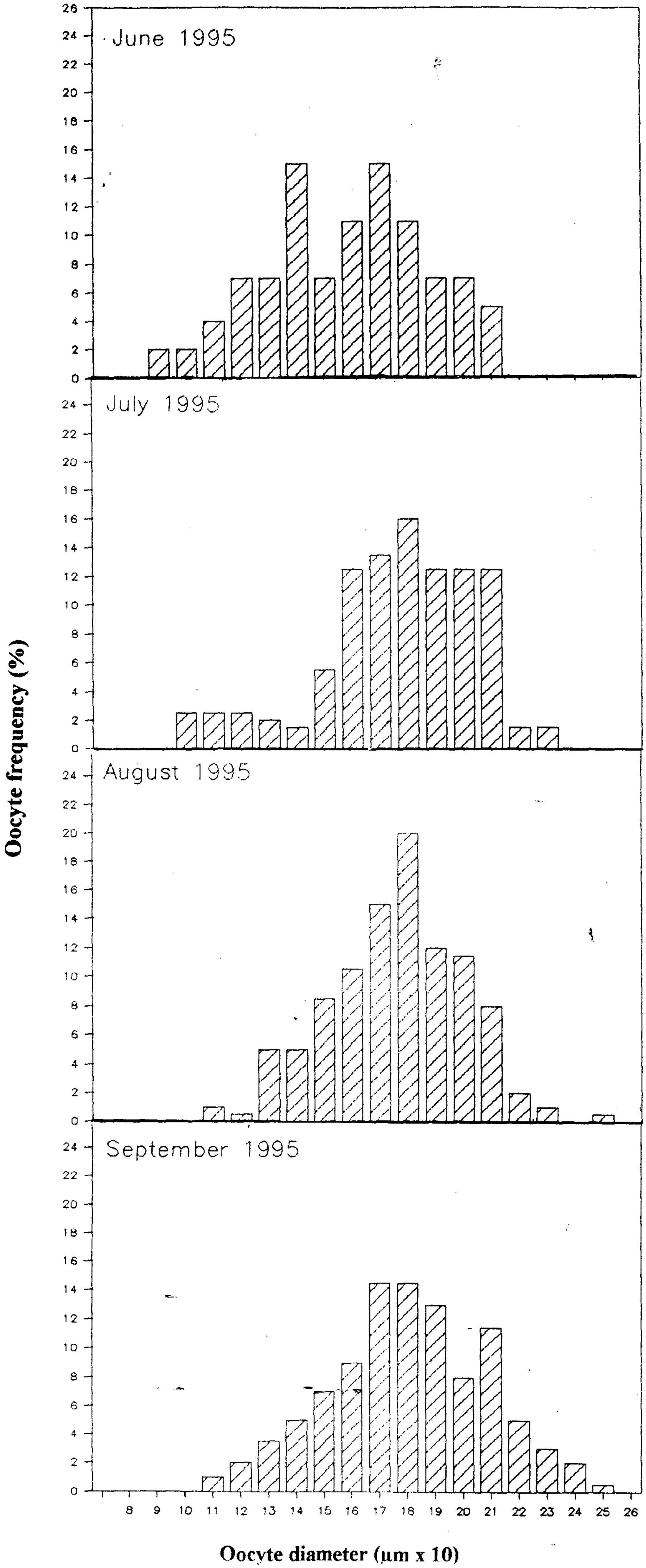
25
26
Oocyte diameter (µm x 10)


June 1995
July 1995
August 1995
September 1995
Oocyte frequency (%)
0
2
4
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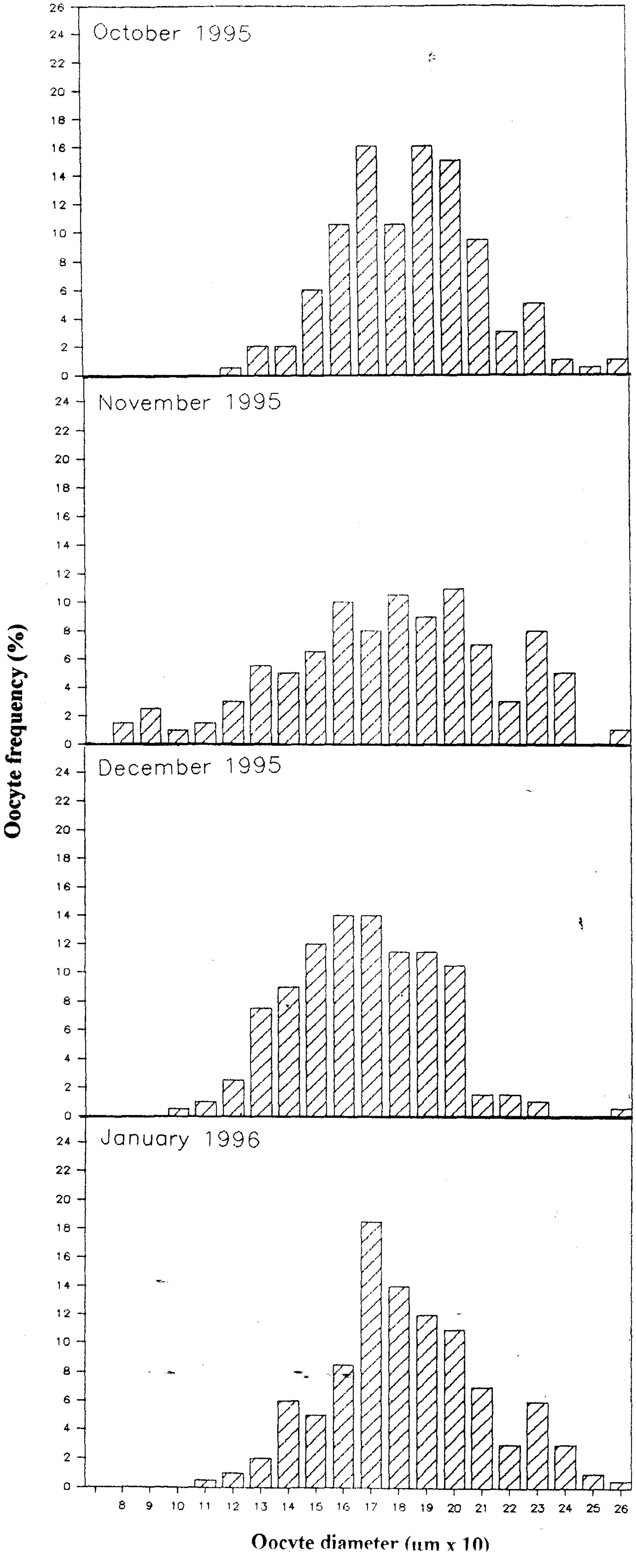
25
26
Oocyte diameter (µm x 10)


October 1995
November 1995
December 1995
January 1996
Oocyte frequency (%)
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Oocyte diameter (um x 10)

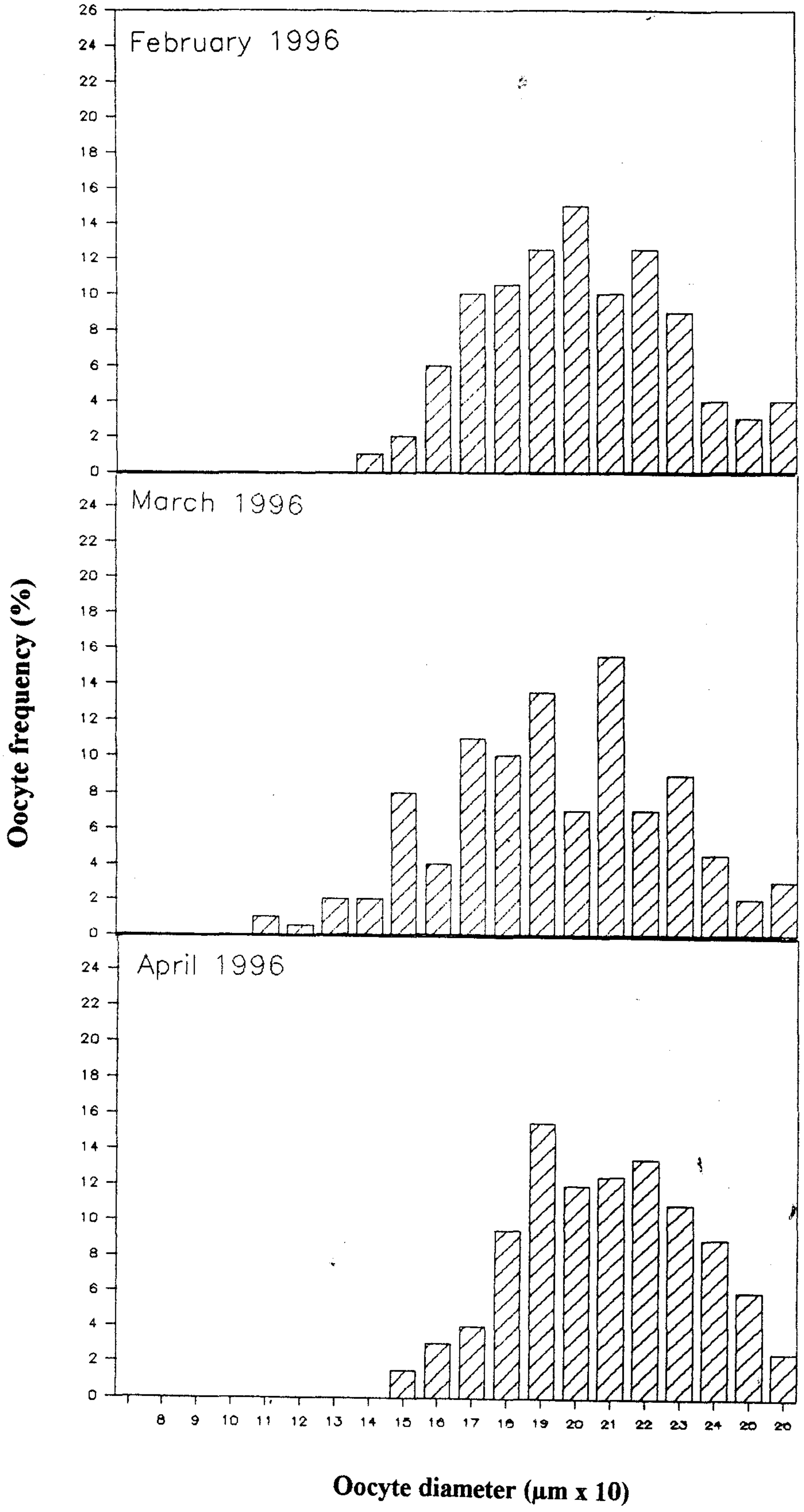
February 1996
March 1996
April 1996
Oocyte frequency (%)
Oocyte diameter (µm x 10)
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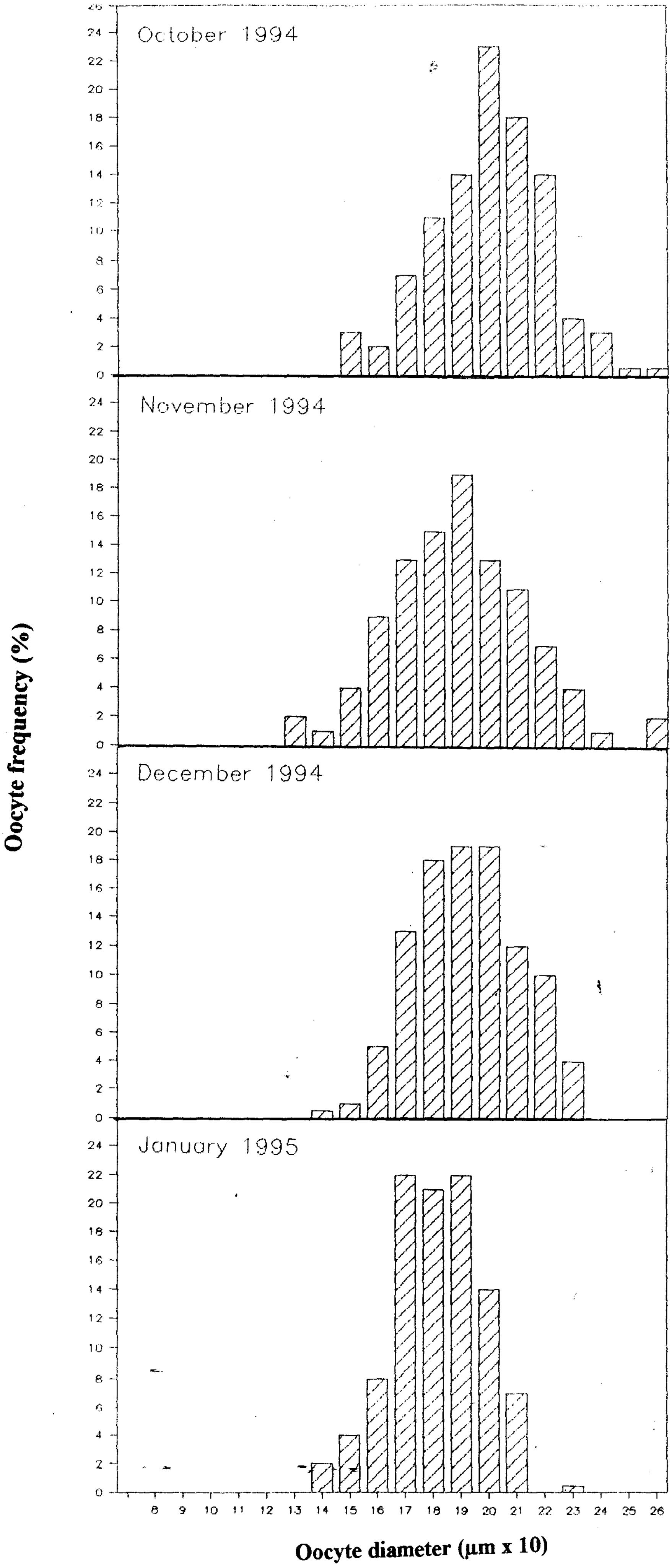
Figure 5.5. The following 5 pages illustrate the frequency of different sized oocytes from female individuals of *Helcion pruinosus* collected from Kommetjie on the south-west coast on a monthly basis from October 1994 to April 1996.


October 1994
November 1994
December 1994
January 1995
Oocyte frequency (%)
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26
8
9
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11
12
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21
22
23
24
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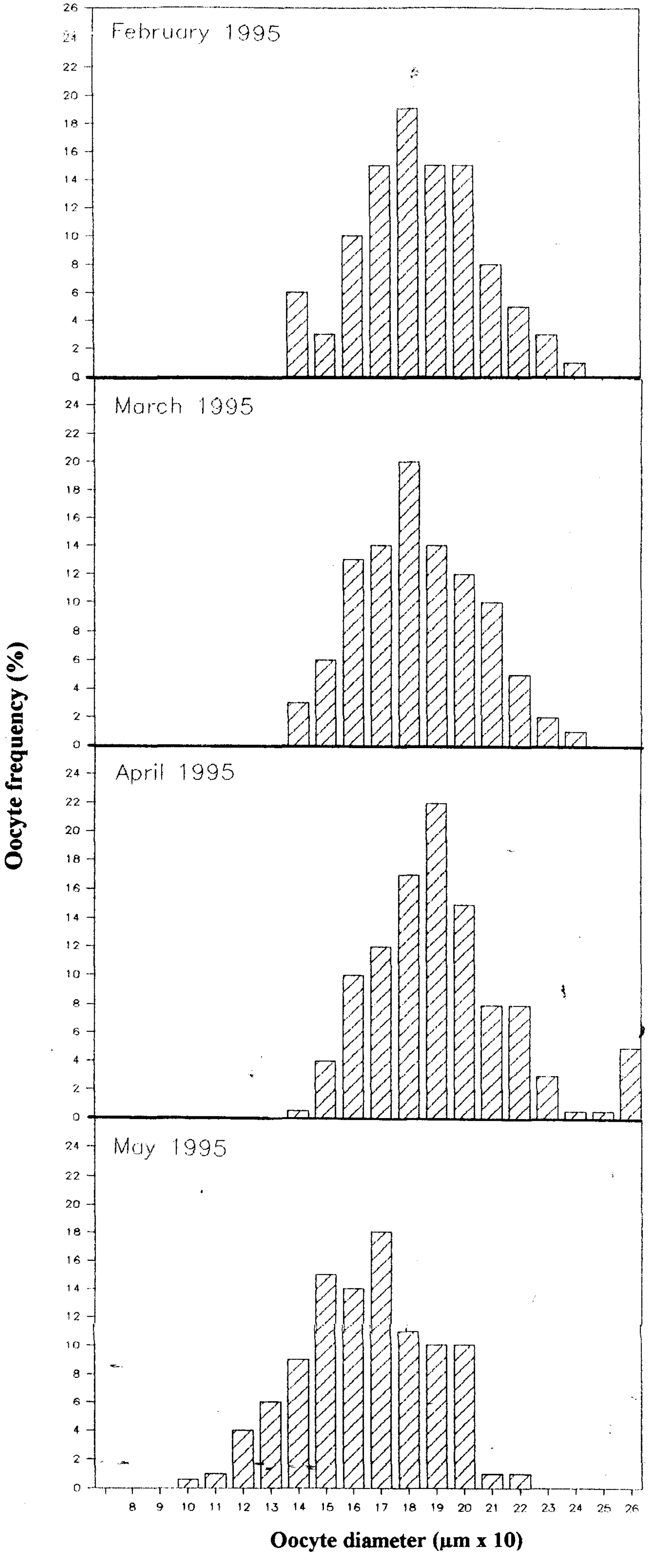
26
Oocyte diameter (µm x 10)


February 1995
March 1995
April 1995
May 1995
Oocyte frequency (%)
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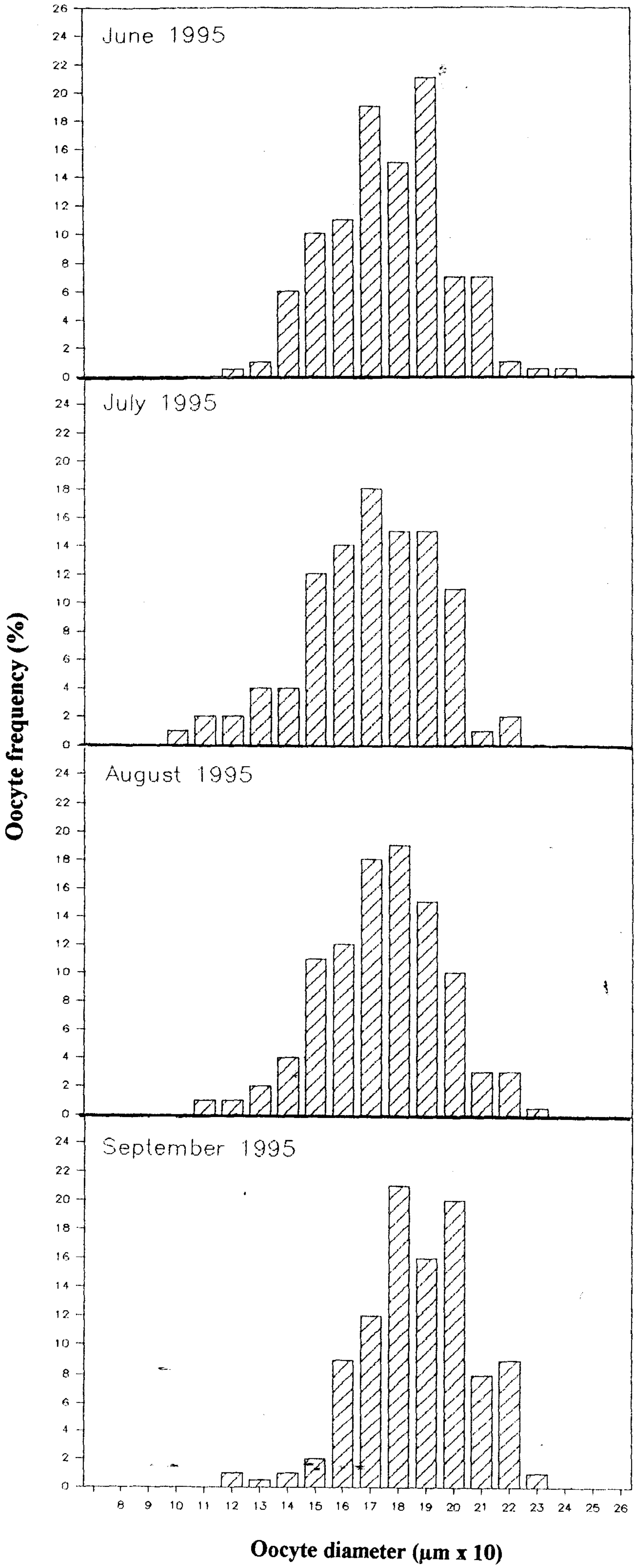
Oocyte diameter (µm x 10)


June 1995
July 1995
August 1995
September 1995
Oocyte frequency (%)
Oocyte diameter (µm x 10)
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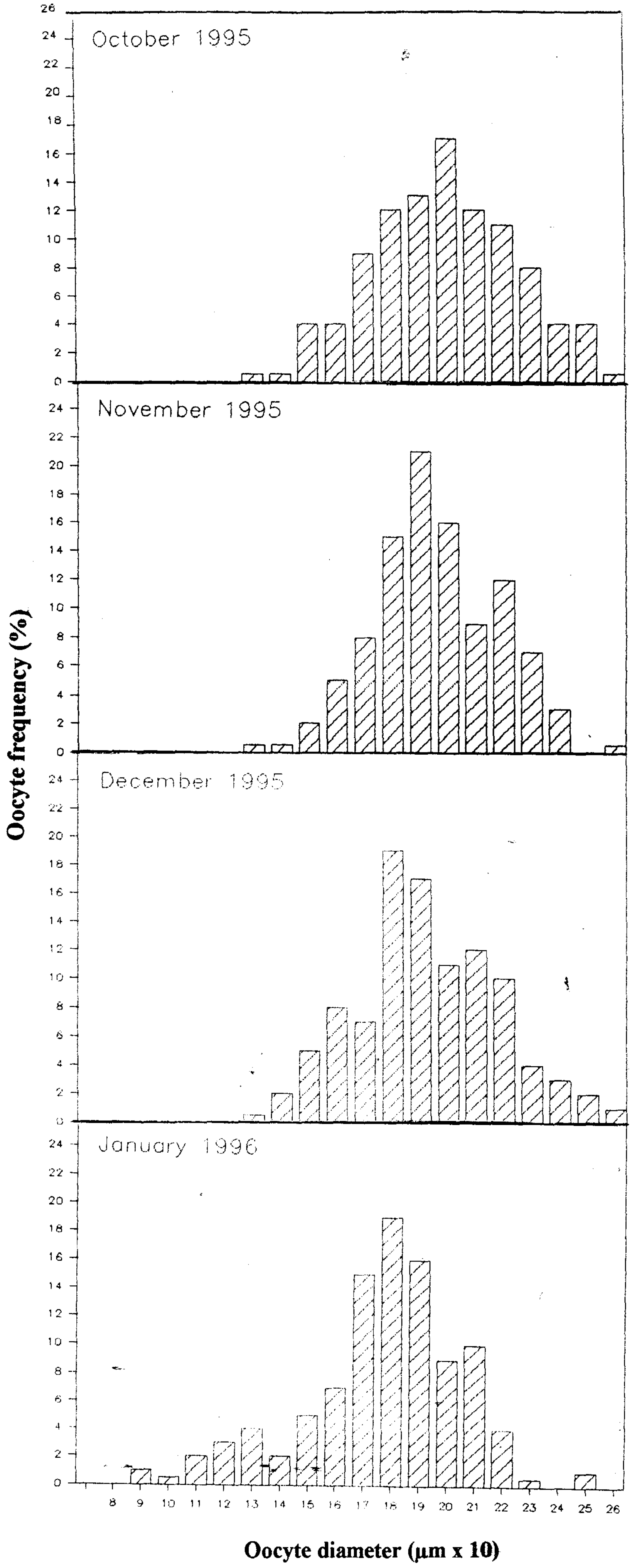
October 1995
November 1995
December 1995
January 1996
Oocyte frequency (%)
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Oocyte diameter (µm x 10)

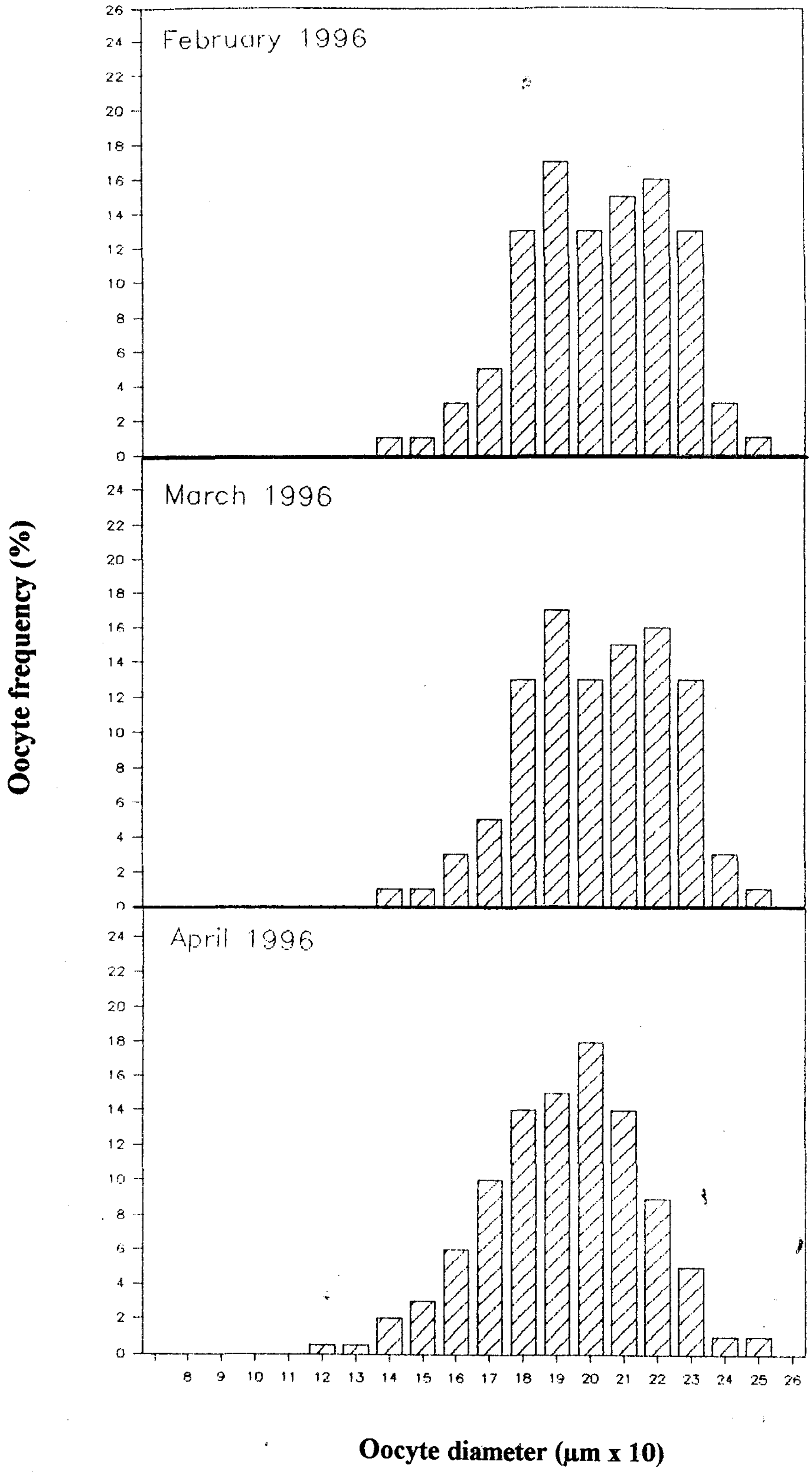
February 1996
March 1996
April 1996
Oocyte frequency (%)
Oocyte diameter (µm x 10)
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Figure 5.6.A

Figure 5.6.B

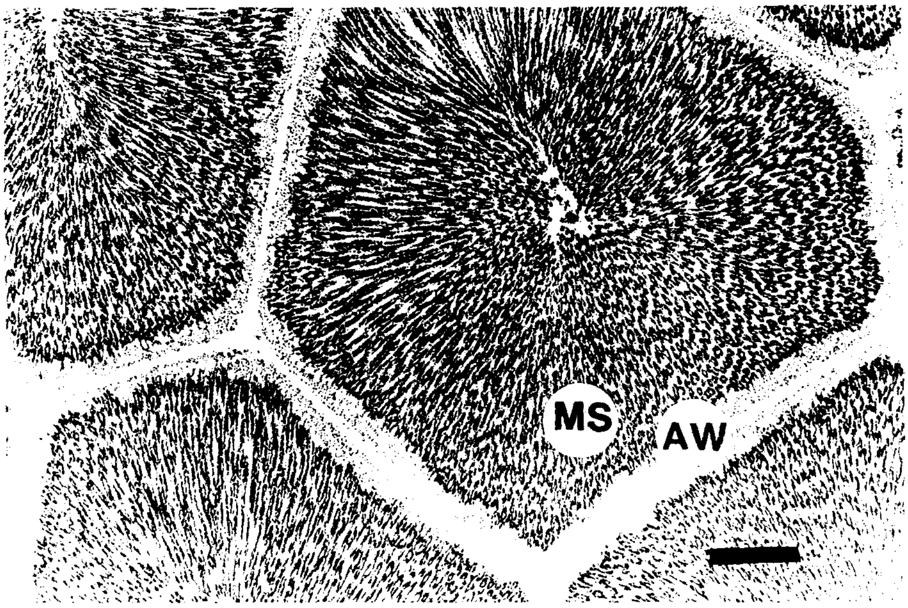

Figure 5.6. A and B. Photomicrographs of acini from west coast males to demonstrate the developmental stages of spermatogenesis within the testes. The thick acinal wall (AW) demonstrates the developing stages of the spermatozoa (DS) within the lumen of the testis as seen in May 1995 (Figure 5.6.A). The thin acinal wall (AW) in Figure 5.6. B. shows the mature spermatozoa (MS) prior to spawning in November 1995. [Bar indicates 150 micrometres].

Figure 5.7.A

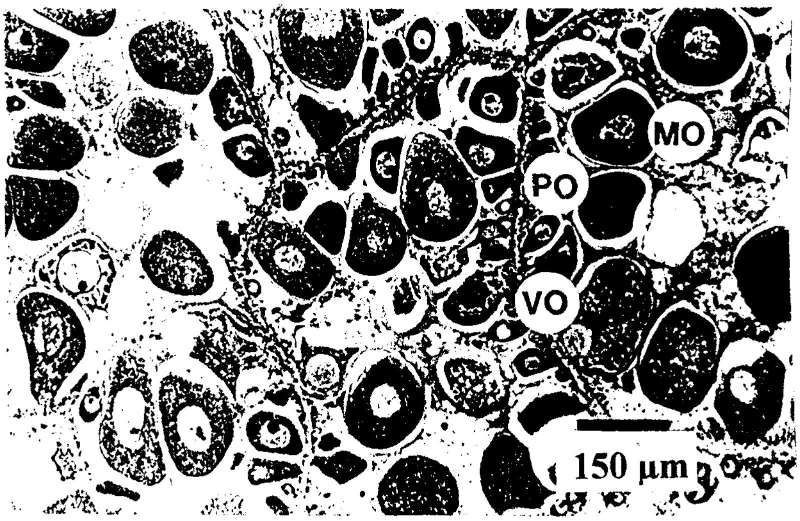

Figure 5.7.B

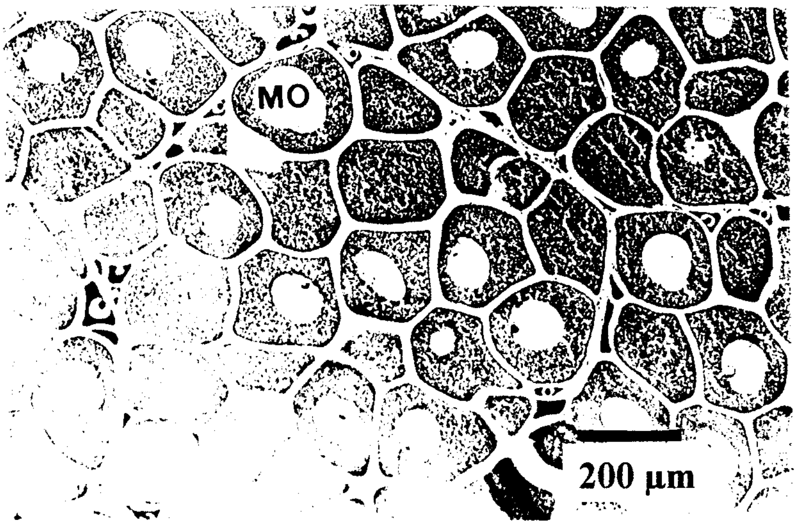

Figure 5.7.C

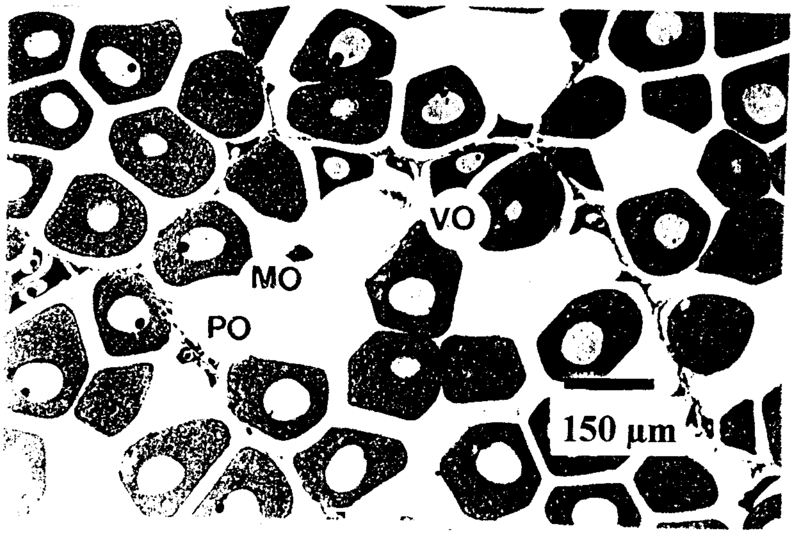

Figure 5.7. A to C. Photomicrographs of sections through the ovaries of *Helcion pruinosus* to demonstrate the stages of oogenesis. A. shows a mixture of previtellogenic oocytes (PO), vitellogenic oocytes (VO) and mature oocutes (MO) as would be expected in a developing ovary.

B. shows an ovary full of large diameter mature ooctyes prior to spawning. C. shows large spaces within the ovary indicating the shedding of ova. There are previtellogenic and vitellogenic oocytes between the mature ones. (A totally spent individual was never observed).

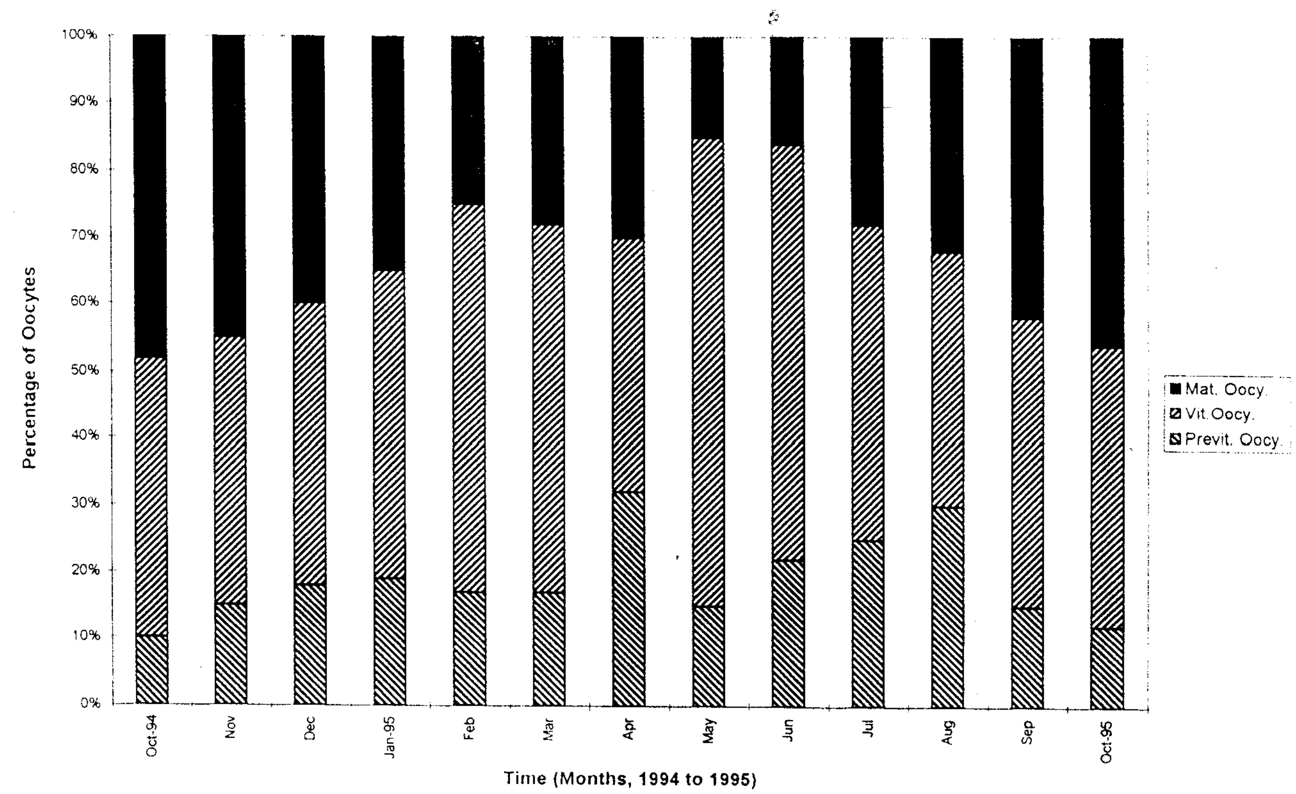

Figure 5.8.A Relative frequency of oogenic stages in female *H. pruinosus* (n = 5) at Gonubie. Oocytes are represented as previtellogenic (Previt., diameter < 120 µm), vitellogenic (Vit., diameter 120 to 180 µm) and mature (Mat., diameter > 200 µm)

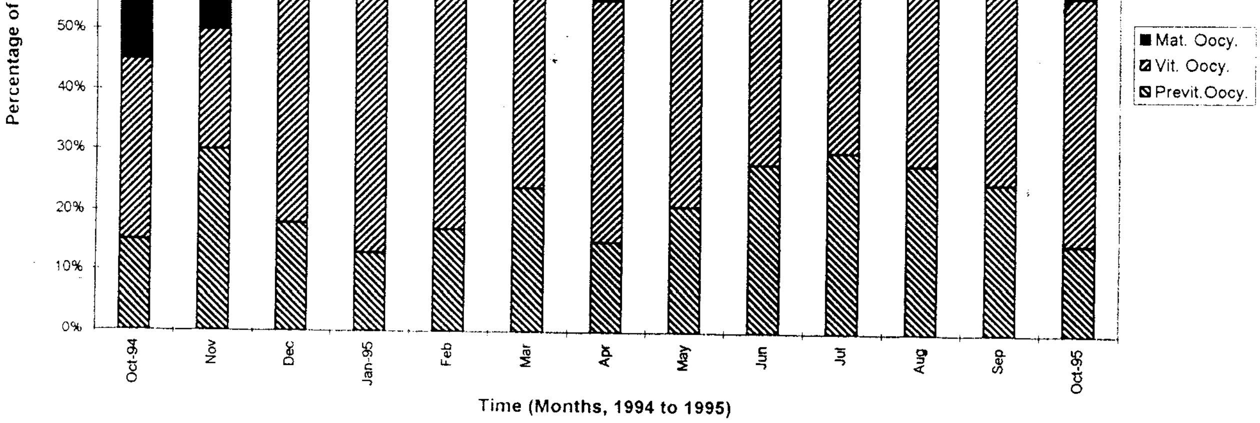

Figure 5.8.B Relative frequency of oogenic stages in female *H. pruinosus* (n = 5) at Kommetjie. Oocytes are represented as previtellogenic (Previt., diameter < 120 µm), vitellogenic (Vit., diameter 120 to 180 µm) and mature (Mat., diameter > 200 µm)

5.3.2 Environmental Results.

The longest day lengths, (maximum 14.19 hours of sunlight), occurred during December, (Fig. 5.11), while the shortest day lengths (minimum of 10.02 hours) were recorded in June. Although day lengths increased from June to December, no significant correlation was found between Gonad Index and day length (south-east coast $r^2 = 0.12$ and west coast $r^2 = 0.14$).

The mean sea temperature was higher on the south-east coast than on the west coast (Fig. 5.9 A and B). throughout the year. At Kommetjie the water reached a maximum of 18°C during January and February 1995 and a minimum of 10°C was maintained from October 1994 until January 1995, and again in May 1995. The greatest extreme in temperature (8°C) was recorded in January 1995 (minimum 10°C and maximum 18°C). On average the water was coldest on the west coast during the summer months (October to December 1994) with a marginal peak in February 1995, (when the mean temperature was 15.8°C).

On the south-east coast the mean water temperature ranged between 17°C and 19.5°C, throughout the year with a mean peak of 21°C in October 1995. Nahoon Beach is approximately 8 km from the study site at Gonubie and therefore temperatures there should not vary greatly from those at Gonubie.

On the west coast there was no strong correlation between water temperature and the G.I. (south-east coast $r^2 = 0.13$, and west coast $r^2 = 0.10$) , as the mean sea temperature stayed relatively stable from June 1995 until April 1996. There was a minor spawning event reflected by the G.I. during February and March 1995 (Fig 5.2), when there were declining water temperatures (Fig. 5.10B).

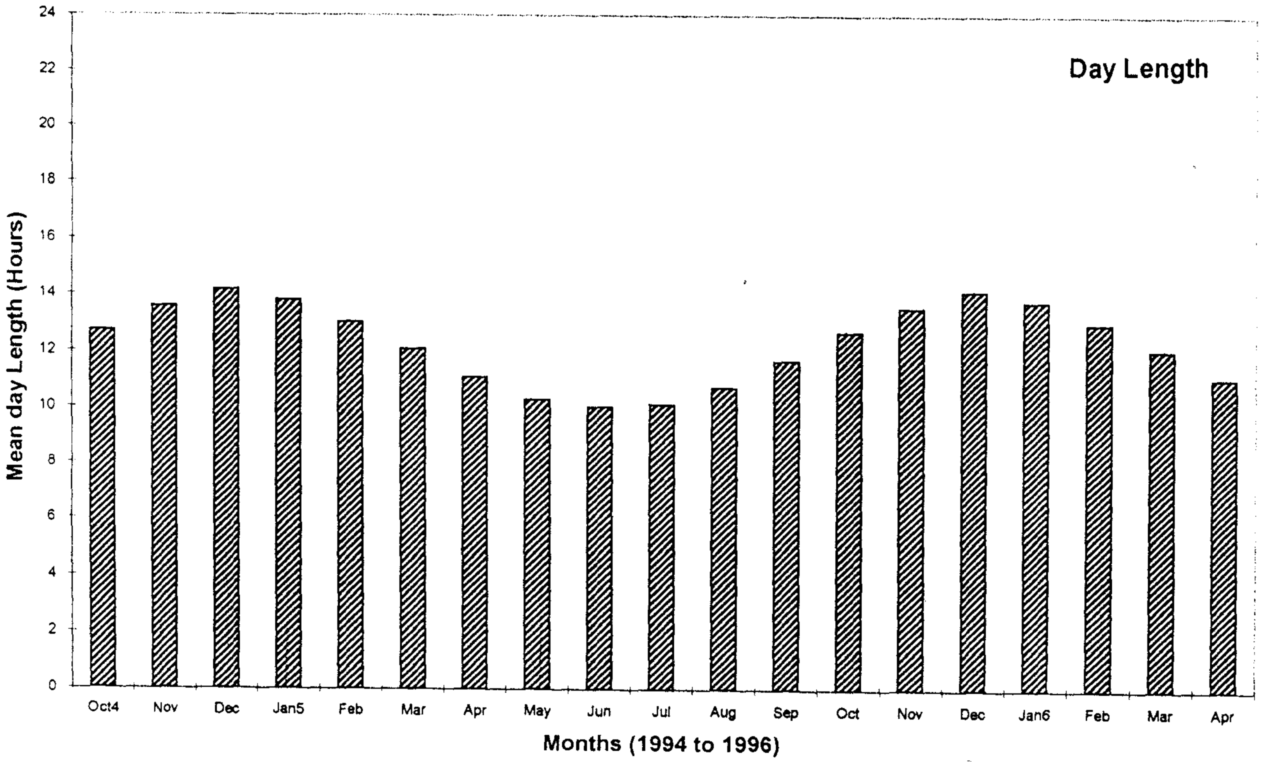

Figure 5.9 Mean day length (hours) in South Africa from October 1994 to April 1996)

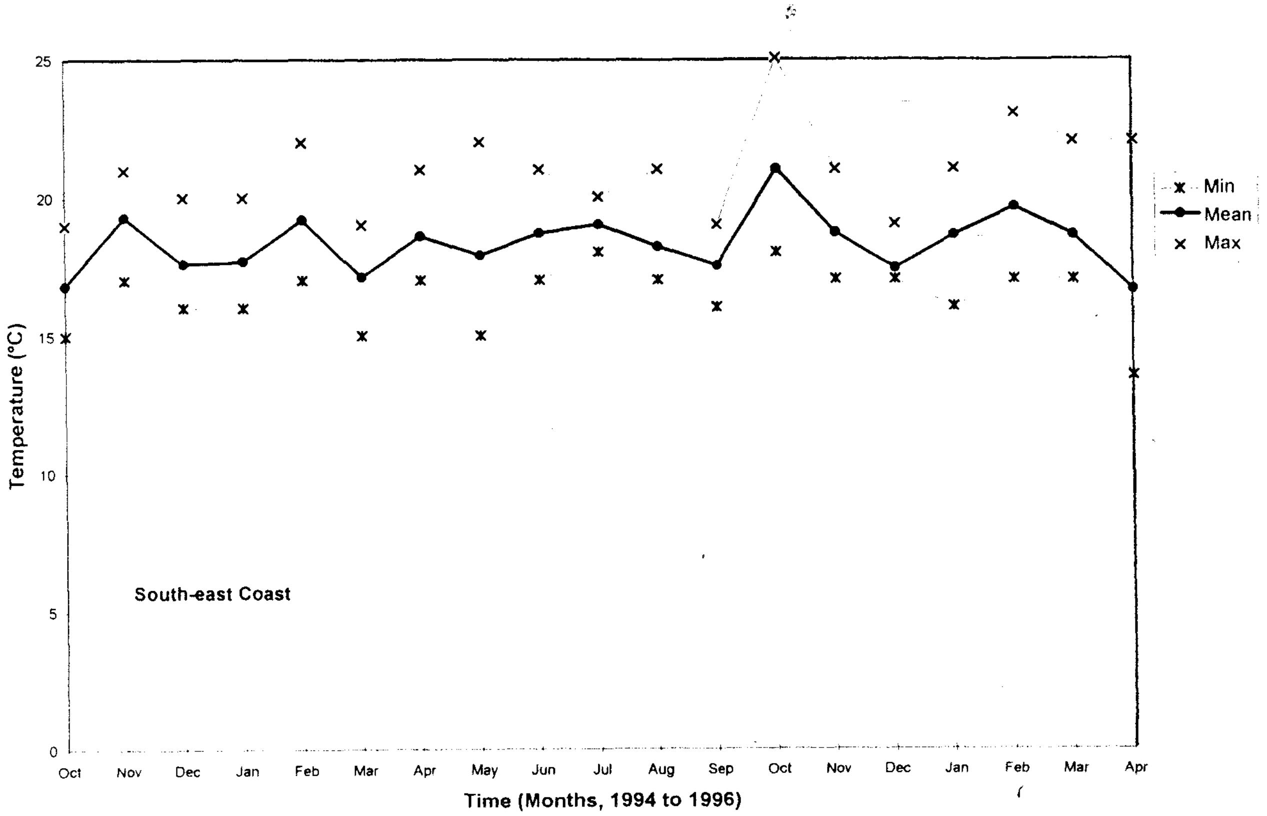

Figure 5.10.A. Mean (•), as well as maximum (x) and minimum (⁎), water temperature (°C) at Nahoon, East London (8 km directly from study site at Gonubie).

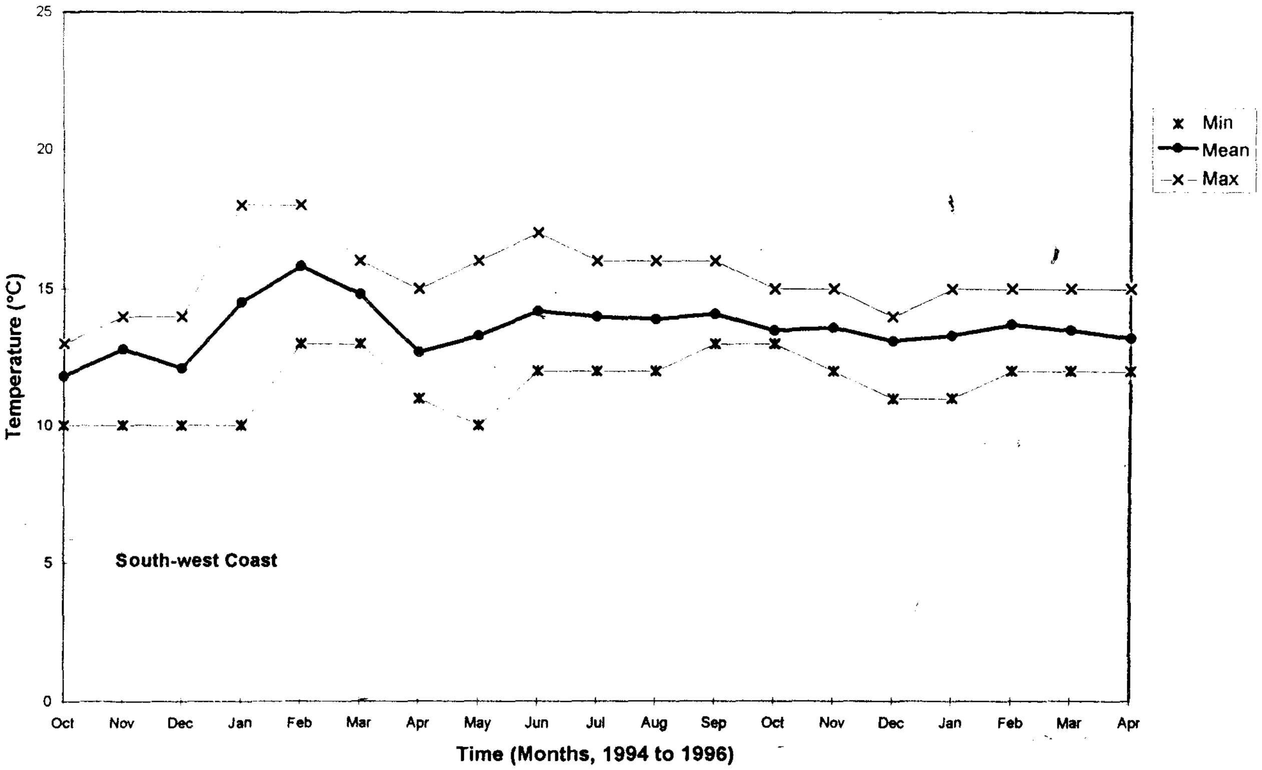

Figure 5.10.B. Mean (•), as well as maximum (x) and minimum (⁎), water temperature (°C) at Kommetjie.

5.4 DISCUSSION

H. pruinosus does not change sex, unlike that reported for the congeneric species, *H. pectunculus* (Gray,1996). In this study only one hermaphroditic animal was found amongst the 3190 animals sampled and thus it can be concluded that hermaphroditism is a rare event in *H. pruinosus*. Only three South African limpets, *H. pectunculus* (Gray, 1996), *Patella oculus* (Branch, 1984) and *P. aphanes* (Robson, 1986) change sex.

Gonochorism, with phenotypically pure male and females, seems to be related to pioneer populations having high densities and uniform size and age compositions, (Dolgov, 1991). Dolgov (1991) proposed that organisms showing simultaneous hermaphroditism could double their opportunity of cross fertilisation over an otherwise gonochoristic population. Such a lifestyle may be advantageous in populations having a low density or in which gametes survive for only a short time (Dolgov, 1991). *H. pruinosus* had a high density at Gonubie and seem to be partial or protracted spawners and this may be the reason for *H. pruinosus* being gonochoristic, while *H. pectunculus* showed a lifestyle including sequential hermaproditism (Gray, 1996).

Branch (1974a) found a higher gonad output in male patellid limpets than in females, (as reflected by the Gonad Index), as this could be due to more energy being used for gonad production, so reducing the somatic growth resulting in the often smaller size of the males. Working in the laboratory, Branch (1974) found that sperm lived for relatively short periods of time in comparison to the viability of eggs, and thus males must put more effort into sperm production, resulting in a higher output to fertilise the relatively fewer eggs.

Results from this study also revealed that males have a significantly higher G.I. than females. This result was also found in *Cellana capensis* (Lasiak,1990), *H. pectunculus* (Gray, 1996), especially in the west coat population and *Turbo sarmaticus* (Foster, 1997). Branch (1974a) and Parry (1982) both concluded that, based on the G. I., male limpets devoted more energy to reproduction than females. This may be due to the testis having a higher moisture content than that of the ovary.

The G.I. was higher on the south-west coast than the south-east coast and this could be related to the higher primary productivity on the west coast compared to the east coast (Bustemante *et al.*, 1995).

A number of factors influence the timing of spawning. Most researchers in South Africa have not identified the environmental factors which actually trigger the spawning events of the animals they have studied. Himmelman (1979) concluded that there was no one single environmental factor responsible for the spawning time of chitons, rather various phases of the annual temperature and photoperiod cycles could be correlated to coincide with particular phases of gametogenesis. Further, according to Himmelman (1979), temperature is most often considered by researchers as the cue for spawning events, even though it is usually not clearly demonstrated by these researchers. The intertidal zone is exposed to diurnal temperature fluctuations during low tide of at least 3° C (Himmelman, 1979). In South Africa this fluctuation can be as great as 14.6° C, during winter (Chapter 4). Loosanoff and Davies (1963) did manage to induce spawning after manipulating a temperature change of between 5° C and 10° C, but such temperature changes do not usually coincide with those in the field (Himmelman, 1979). Himmelman (1975) subjected chitons to different temperature conditions in the lab, but failed to induce spawning. This failure indicates that animals would indeed have to be very sensitive to temperature changes if they were indeed to react to this stimulus.

Sprung (1991) working on the bivalve *Dreissena polymorpha* found that the animal could lose up to 30% of its body mass when spawning, implying that a large amount of effort goes into gamete production. Further, while gametes could develop in autumn and winter, eggs could only be fertilised at a temperature above 10°C. Sprung conceded that according to laboratory observations, only in exceptional cases would a temperature rise alone cause spawning in *Dreissena.* Sprung (1991) links the spawning process to elevated respiration rates at higher temperatures, as possibly enhancing the susceptibility of animals to a spawning stimulus, rather than the actual change in temperature itself

On both the west and south-east coast *Helcion pruinosus* has a bimodal system of spawning, spawning in autumn/winter and again in summer (Table 5.2). A similar result was obtained in *H. pectunculus* (Gray, 1996), (Table 5.2), but the actual cue could also not be determined, and it is assumed that temperature and food availability act as possible trigger mechanisms for the release of gametes.

Table 5.2

Summary of the spawning period of some South African Limpets.

Species	Geographical location	Spawning period	Reference
Patella concolor	Transkei	Sept-Nov / Feb-Mar	Lasiak, 1987
P. aphanes	Natal	Jan-Feb / Apr-Jun	Robson, 1986
P. argenvillei	Kommetjie	May-June	Branch, 1974a
P. barbara	Kommetjie	May-June	Branch, 1974a
P. granatina	Kommetjie	May-June	Branch, 1974a
P. oculus	Kalk Bay	September	Branch, 1974a
P. longicosta	Kalk Bay	Oct-Nov	Branch, 1974a
P. granularis	Kommetjie/Kalk Bay	May-June	Branch, 1974a
P. granularis	Cannon Rocks	May - July/August	L.Vat (Pers. com)
P. cochlear	Kommetjie/Kalk Bay	May-June	Branch, 1974a
Helcion pectunculus	Bloubergstrand	Apr-May/ Nov-Dec	Gray, 1996
	Port Elizabeth	Apr-May/ Nov-Dec	Gray, 1996
H. pruinosus	Kommetjie	May-Aug / Nov-Jan	Henninger, 1998
	Gonubie	Apr-June / Nov-Dec	Henninger, 1998
Cellana capensis	Transkei	Sept-Oct/ Feb-Apr	Lasiak, 1987
Siphonaria concinna	Waterloo Bay	Nov-Dec	Chambers & McQuiad, 1994

Himmelman (1979) correlated the stages of gamete production in chitons to temperature as follows :

(a) increasing temperatures during summer may stimulate gametogenesis,

(b) cooler autumnal temperatures could stimulate the intermediate stages, e.g. vitellogenesis, and

(c) lower winter temperatures, or a change to increasing temperatures could stimulate oocyte maturation.

The changing photoperiod which affects reproductive activities of many insects, birds and mammals has only been suggested as a cue for reproductive activity in marine invertebrates by a few researchers (e.g. Wells and Wells, 1959; Adiyodi and Adiyodi, 1970). Photoperiod could be regarded as a reliable cue as it affects broad geographical areas. Himmelman (1979) found that two species of chiton studied responded to changing photoperiod. The gonads matured at about the time of the winter solstice, (i.e. decreasing and then increasing daylength).

Lunar and tidal cycles have also been looked at as a spawning cue in a number of chitons, (Grave, 1922; Brewin, 1942; Thorpe, 1962; Glynn, 1970), but owing to the fact that most collections were made at monthly intervals, it is difficult to correlate such cycles to the actual spawning events (Himmelman, 1979).

Phytoplankton, especially phytoplankton blooms associated with upwelling of colder water bearing nutrients, may be a reliable cue for synchronous spawning (Himmelman, 1975; Himmelman, 1979). Sea urchins and some chitons could be induced to spawn, in the laboratory, in response to natural phytoplankton at bloom concentrations. (The same animals could not be induced when kept under varying light and temperature conditions). The phytoplankton, or a substance released by the phytoplankton, may alone not be the cue, but it could be linked to changes in water temperatures at the same time as the phytoplankton cue. Thus it may be a combination of factors acting together which stimulate the release of gametes, rather than a single factor. This combination together with the presence of eggs and sperm in the water may then further stimulate the mass release of gametes by others in the population. The presence of the phytoplankton may be crucial for sustaining the planktonic larvae of many marine molluscs. Phytoplankton is, however, unlikely to play a direct role, as the larvae of limpets do not feed. They may, however, gain some indirect benefit through the releases of dissolved organic matter. As no-one has ever worked on the uptake of DOM by limpet larvae, this idea is speculative (Hodgson, pers.comm.). In temperate and arctic seas such blooms are a regular event (Himmelman, 1979) and they occur in spring. The blooms also mark the beginning of warm water temperatures which would be beneficial for larval development.

Large numbers of species spawning, simultaneously, at such times would also lessen the effect of predation. In spring there is also, usually, an increase in primary production in the intertidal and subtidal zones resulting in an abundance of food for all organisms which may further cut down on predation of especially young organisms (Himmelman, 1975).

Phytoplankton blooms do occur on the west coast of South Africa, owing to the presence of the Benguella current and upwellings, and the Kommetjie community would be exposed to increased nutrient levels (Bustamante *et al.*, 1995) due to nearshore upwelling. The south-east coast community is exposed to warm, nutrient depleted water of the Agulhas current, which maintains a low average rate of offshore primary production and also low rates of primary production on the intertidal rocky shores (Bustamante *et al.*, 1995). Phytoplankton blooms could therefore not explain the synchronous release of gametes. Upwellings have, however, been reported for the Transkei coast (MacNae, 1962), further north than the present study site.

The asynchronous or partial spawning behaviour of *H. pruinosus* could benefit the species by creating greater opportunities for increasing genetic variability of the offspring (Lasiak, 1990). According to Lasiak (1990) the Balanoid zone is an area of low algal productivity and supports many generalist grazers. The animals could therefore be responding, individually, to limited food reserves. The asynchronous multiple releases of gametes might therefore reduce competition for scarce food resources amongst the progeny and reduce predation on the young (Lasiak, 1990). A few synchronous spawning events would otherwise heighten the competition.

The boulders which *H. pruinosus* inhabited, at Gonubie, were devoid of visible algae, but micro-algae must be present on the rocks. An investigation of micro-algal concentration was however not carried out to determine the food abundance.

In summary *H. pruinosus* is a partial spawner on both the south-east and west coast, as no totally spent individuals were ever found. Bimodal or trimodal peaks may be a result of gametogenic cycles of development reflected by the varying oocyte sizes in the ovaries of *H. pruinosus*. This would allow the animals to be partial spawners, rather than highly synchronised complete spawners, (Table 5.2). Hannaford-Ellis (1983) found no periodicity in some *Littorina* species, with large numbers of individuals in breeding condition throughout the year. She attributed this to animals having to reach a certain body size or age before maturity is possible and that only then would certain environmental triggers such as daylength or temperature (Orten, 1920) induce the development of the reproductive organs. Hannaford-Ellis (1983) further found that autumn with cooler conditions would not adversely affect the development of eggs, (in jelly cases for *Littorina*). Cooler water temperatures may have been the trigger for *H. pruinosus* as shown in the results.

The main concern of the parent organism seems to be to produce the offspring at a time when the larvae, either planktonic or non-planktonic direct developers, has the best chances of survival.

CHAPTER 6 : General discussion

CHAPTER 6 - GENERAL DISCUSSION

Helcion pruinosus, like its sister-species *H. pectunculus*, is a successful inhabitant of South African intertidal rocky shores. The success of *H. pruinosus* is also reflected in its wide geographical distribution, inhabiting warm and cool temperate waters and even penetrating into sub-tropical areas (Killburn and Rippey, 1982). On some shores (e.g. the platform shore at Gonubie on the south-east coast) it has an abundance which matches that of many other species of limpet (see Gray, 1996 for densities) which are found in the mid- to low shore of our coastline.

The success of *H. pectunculus* and H. *pruinosus* is possibly due to these limpets utilising niches not occupied by other species:

H. pectunculus - being crevice dwelling and living on the high-shore, making it the highest shore species of South African limpet (Gray, 1996).

H. pruinosus - living on the mid-shore under small smooth boulders and stones, in a micro-environment generally avoided by other patellids.

The above major differences between these congeneric species, whose distribution along the coastline overlap, is elaborated upon in Table 6.1 to show how *H. pruinosus* is adapted to its specific habitat.

Table 6.1 Comparison of the biology of two congeneric species of *Helcion*, *H. pruinosus* and *H. pectunculus*, along the South African shore. (Data on *H. pectunculus* is based on the work of Gray, 1996).

	H. pruinosus	*H. pectunculus*
Position on shore	Mid to low-shore. (Absent from Littorina zone)	Mid to high shore. (Sometimes present in Littorina zone).
Distribution	Saldahna Bay to Sodwana Bay.	Walvis Bay to Sodwana Bay.
Habitat	Prefers boulder shores with smooth, medium to small boulders on a non-sandy substratum.	Boulder shores with large boulders with crevices, reef shores.

	H. pruinosus	*H. pectunculus*
Habits	Found beneath boulders when submerged (assumed) or during daylight. Do not home rigidly and there is no home scar. Feed on epilithic micro-algae.	Found in crevices when submerged, (assumed) and during daylight can be found beneath boulders. Home rigidly to a fixed scar. Feed on epilithic micro-algae.
Activity	Forage at night when completely exposed by ebbing tide. Travel over surfaces of boulders foraging on micro-algae before returning to underside of boulders before being submerged by flooding tide.	Forage at night on being exposed by ebbing tide. Forage on micro-algae on exposed rock faces. Return to home scar before being submerged by flooding tide.
Density and biomass	In upper Balanoid zone at Gonubie mean density = 49.6/ m^2 (maximum 294/ m^2). Mean mass = 1.36 g/m^2 (dry weight).	Mean density on E. Cape shores = 62.8/ m^2 (maximum = 212/ m^2). Mean mass =1.21 g/ m^2 (dry weight) in upper Balanoid zone.
Maximum size	30 mm	34 mm
Growth type	Grows allometrically (with shell length exceeding height).	Grows allometrically (with shell height exceeding shell length).
Longevity	2.55 years on east coast; 2.9 years on west coast. Few individuals older than 1.6-2 years.	2.4 to 8.2 years on south coast; 2 years on west coast Few individuals older than 3 years.
Sexual Characteristics	Dioecious. (Only 1 hermaphrodite found).	Protandrous hermaphrodites.
Age at sexual maturity	8 to 10 months	As males = 1 year, before changing into females at 2 years.
Sex ratio	Varied between 1.18 males : 1 female and 1.56 males: 1 female on south-east coast. On west coast = 0.94 males : 1 female.	On south coast varied between 1.3 males : 1 female and 2.3 males : 1 female. On west coast = 3 males : 1 female.
Spawning season and type of spawning	Spawn bimodally on both southern and west coast. Spawn from November. Trickle spawn throughout the year. Is a high degree of synchonicity between males and females.	Spawn bimodally on both southern and west coast. Spawn from November and again in April/May. Trickle spawn throughout the year. There is a high degree of synchronicity between males and females.

All the above characteristics would indicate that *Helcion pruinosus* is a typical "r" strategist. Such animals would have a rapid growth rate, reaching sexual maturity at an early age (size) before putting most effort into reproduction. This strategy would allow a high replacement rate to compensate for the losses incurred by predation, strong wave action (as explained in Chapter 3), and possibly through competition.

One of the reasons for the success of *Helcion* on South African shores may be their capability to reproduce throughout most of the year (animals with mature sperm and eggs are found at all times) in both warm temperate and cold temperate habitats. By contrast many species of *Patella* have a more restricted reproductive seasonality (Branch, 1974a). A second reason may be that recruitment to populations is continuous or high in *Helcion*, or that larvae have good dispersal. Levels of recruitment were not examined in this study, nor in the study of Gray (1996) on *H. pectunculus*. Furthermore, information on the biology of limpet larvae and their dispersal capabilities and recruitment on South African shores is lacking and such investigations would seem crucial if the population dynamics of limpets is to be fully understood. Such data is also necessary for resource management, limpets becoming increasingly exploited by impoverished communities (see Foster, 1997 for references).

H pruinosus has many adaptations which are suitable to a low shore habitat (the lower Balanoid zone of the Balanoid region of the shore). The shell grows allometrically and has a low profile, which is typical of many low shore species of limpet (Branch, 1981).A low shell profile minimises the impact of wave activity and in the case of *H. pruinosus* may facilitate movement beneath boulders. Although desiccation is less of a problem to low shore species of limpet (Branch, 1981), *H. pruinosus* is mainly active during nocturnal low tides. This suggests that these limpets are susceptible to thermal stresses and or desiccation. Of note is the fact that these limpets have a particularly thin and delicate shell (Henninger, pers. obs.) and heat uptake in this species may be particularly rapid. Studies on thermal tolerances and desiccation are required to examine this hypothesis and to determine the ability of *H. pruinosus* to cope with environmental stresses. The fact that this species does not emerge to forage until it is fully exposed at night, and that it retreats to refuge sites before being covered by the tide, suggests that pressure from aquatic predators has been an important driving force in shaping the behaviour of this limpet. The activity of *H. pruinosus* may well be under endogenous control and laboratory experiments such as those carried out recently by Gray and Hodgson (1999) are needed to establish this.

Unlike many species of limpet (see Branch, 1981; Hawkins and Hartnoll, 1983; Little, 1989; Hodgson in press for reviews) which have evolved a home scar to reduce desiccation and help avoid predation, *H. pruinosus* has not done so. by contrast its sister species, *H. pectunculus*, has a home scar.

Food is most abundant lower down the intertidal zone (Branch, 1971,1981; Bustamante *et al.*, 1995). *H. pruinosus* is absent from the lowest regions of the shore possibly because it cannot compete against aggressive territorial limpets, or because juveniles are prevented from becoming established there due to the removal of larvae by the grazing activities of other limpets. Competition with other species of limpet which inhabit the same region of the intertidal zone as *H. pruinosus* is probably avoided because of the more specialised habitat occupied by this limpet. The habitat is occupied by both juvenile and adults. *H. pruinosus* can therefore be described as a specialist limpet (inhabiting a specific microhabitat), but it is a non-territorial generalist grazer. Whether there is any resource partitioning between juveniles and adults is unknown and a more detailed study of the feeding biology of *H. pruinosus* is now required to determine this.

Whereas very few other species of limpet are found together with *H. pruinosus*, other trochid gastropods (e.g. *Oxystele* spp.) were often observed to be feeding on the same rocks. Competition between *H. pruinosus* and *Oxystele* for food resources, however, probably does not occur. Patellid limpets and trochid gastropods have very different radulae (Steneck and Watling, 1982; Hawkins and Hartnoll, 1983; Hawkins *et al.*, 1989). Whereas the docoglossan radula of *Helcion* can excavate and scrape micro-flora from rocks, the rhipidoglossan radula of trochids can only brush the surface of rocks (Hawkins *et al.*, 1989).

In conclusion this study has provided the first insights into the biology of *Helcion pruinosus*, and will hopefully serve as a platform for more detailed research on this common intertidal gatropod mollusc.

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