RHODES UNIVERSITY

THE GROWTH AND REPRODUCTION OF *PATELLA GRANULARIS* (MOLLUSCA: PATELLOGASTROPODA) ON THE SOUTH-EAST COAST OF SOUTH AFRICA

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ABSTRACT

Aspects of the biology and ecology of *Patella granularis* were investigated along a 130km stretch of the south-east coast of South Africa. Distribution, biomass, density and population structure were investigated at seven localities. In addition, a more detailed study of the growth rate and reproductive biology of populations inhabiting three different substrata (aeolianite, quartzitic sandstone and mussel shells) was conducted. The genetic relationships between these three populations was also examined, as was the foraging behaviour of the limpets inhabiting an aeolianite and a quartzitic sandstone shore. Finally, differences in food availability on the different substrata were studied.

On the south-east coast, *P. granularis* has a wide intertidal distribution, occurring from the upper Balanoid zone through to the Cochlear zone, where it is a common inhabitant of mussel shells. The mean shell length of *P. granularis* was found to decrease down the shore. The largest limpets (46.6 mm shell length) were found on an offshore island in Algoa Bay. At most localities investigated, the sex ratio deviated from a 1:1 ratio with more males than females being recorded on five shores. Both limpet density and biomass were lower on the south-east coast when compared to data published for west coast populations. On the south-east coast, both density and dry biomass were highest in the lower Balanoid zone.

Allozyme electrophoresis indicated that *P. granularis* inhabiting aeolianite, quartzitic sandstone and mussel shells are all part of a single population. Extremely high genetic identity values (0.998), low levels of heterozygosities (0.035 - 0.061), low levels of polymorphisms (25% - 31%) and low F_{ST} values (0.021) all suggest that the three populations of *P. granularis* form a common breeding group, despite the high levels of phenotypic plasticity observed.

On all shores, *P. granularis* was found to grow allometrically, increasing in shell height more rapidly than shell length. Estimation of the growth rate (determined by the Von Bertalanffy growth model) of *P. granularis* suggested that limpets inhabiting the mussel shells grew more slowly, and attained a smaller maximum size, than those inhabiting both the aeolianite and the quartzite (K = 0.25, 0.32 and 0.33 respectively; 27.12 mm, 31.89 mm and 32.96 mm respectively). Previous work has shown that west coast *P. granularis* grow more quickly (K = 0.7) and reach a greater size (. 40 mm). Translocation of limpets among sites suggested that limpet size in the mussel beds was spatially constrained. Shell microgrowth bands were deposited tidally, but could not be used for aging limpets due to shell erosion.

Limpets from the aeolianite had the greatest reproductive fitness, producing more eggs (.366 000/limpet) than those inhabiting quartzite (.119 500/limpet) or mussel shell limpets (.85 800/limpet). Aeolianite limpets also spawned throughout the year, whereas those from the quartzite and mussel shells spawned twice a year (once in winter and once in summer) although a great deal of interannual variability was observed. The onset of sexual maturity occurred at a similar age in all limpets (1 - 2 years) and is probably genetically entrenched.

P. granularis inhabiting both an aeolianite and a quartzitic shore were active during nocturnal low tides. All limpets returned to a home scar after foraging. Whilst foraging, limpets inhabiting the aeolianite shore moved shorter distances (.17 cm) at a slower rate than those from quartzite (.30 cm). Limpets that were translocated from one substratum to the other initially moved similar distances to the source group, but after a maximum period of one week, moved distances that mirrored those moved by the resident limpets. Neither season nor tidal phase influenced the distances foraged. No directionality in foraging was found. Wear of radula teeth, particularly the pluricuspid tooth, was greater in limpets from the quartzite.

It is hypothesised that the observed differences in life-history parameters and foraging behaviour of limpets both within the south-east coast and between the west and south-east coasts are related to food abundance. Chlorophyll-*a*, and hence microalgal biomass, was consistently higher on aeolianite (.2.5 times) than on both quartzite and mussel shells. Estimates of chlorophyll-*a* were higher (although not significantly) in winter. Previous studies determined that primary productivity is also higher along the west coast.

Finally, the lack of evidence for migration of limpets from the low-shore to high-shore in south-east coast *P. granularis* is discussed. It is suggested that this species settles opportunistically within its physiological tolerances and responds morphologically to localised environmental conditions.

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Chapter 1

GENERAL INTRODUCTION

Patellogastropods are abundant and conspicuous on rocky shores around the world (Powell, 1973). Although they are considered to be the most primitive group of gastropods (Haszprunar, 1988; Lindberg, 1988), patellogastropods are of great ecological importance as they can have a marked influence on the structure of intertidal communities (Hawkins, 1981b,c; Hawkins & Hartnoll, 1983; Branch, 1984; Hawkins *et al.*, 1992). The patellids reach their greatest diversity on the shores of southern Africa (S. Ridgway *et al.*, 1997), however, their distribution, zonation and feeding habits are sufficiently different to reduce competition amongst the species (Branch, 1971).

The systematics and evolutionary relationship of the group has received a great deal of attention recently (Hodgson & Bernard, 1988; Jamieson *et al.*, 1991; Côrte-Real *et al.*, 1996a,b; Hodgson *et al.*, 1996; S. Ridgway *et al.*, 1997; Weber *et al.*, 1997). Cladistic analysis of morphological characters of 37 patellid species has suggested that 18 species in three genera, *Cymbula, Helcion* and *Scutellastra*, are found along the coast of South Africa (S. Ridgway *et al.*, 1997). Since this study, an additional two species (*Patella* = *Scutellastra natalensis* and *Patella* = *Cymbula sanguinans*) have been described (T. Ridgway *et al.*, 1998; in press), bringing the total number of South African patellid species to 20. According to the cladistic phylogeny of S. Ridgway *et al.* (1997), the genus traditionally recognised as *Patella* (as described by Powell, 1973) is divided into four clades, with the genus *Patella* being restricted to North Atlantic species. In South Africa, representatives of the traditional *Patella* genus are now divided between the genera of *Cymbula* and *Scutellastra* (see Table 1.1), although recent molecular analysis of the Patellidae suggest that *Scutellastra* may not be monophyletic (Koufopanou *et al.*, 1999).

Despite the new phylogenic analyses, recent work, while recognising the proposed clades, has persisted in referring to the newly reassigned species of *Scutellastra* and *Cymbula* by their original names i.e. *P. granularis, P. natalensis, P. miniata* and *P. sanguinans* (see T. Ridgway *et al.*, 1998; 1999 and in press). Until the phylogeny and systematics of the Patellidae is fully resolved, and to facilitate comparisons of species traditionally placed in the genus *Patella*, all representatives of the *Cymbula* and *Scutellastra* genera will, throughout the course of this thesis, be referred to as *Patella*.

Table 1.1:

List of recognised patellid species inhabiting the coast of South Africa (after S. Ridgway *et al.*, 1997, T. Ridgway *et al.*, 1998 and T. Ridgway *et al.*, in press).

Genus	Species	Genus	Species	Genus	Species
Scutellastra	*S. aphanes*	***Cymbula***	*C. adansonii*	***Helcion***	*H. concolor*
	S. argenvillei		*C. compressa*		*H. dunkeri*
	S. barbara		*C. granatina*		*H. pectunculus*
	S. cochlear		*C. miniata*		*H.pruinosis*
	S. granularis		*C. oculus*		
	S. exusta		*C. sanguinans*		
	S. longicosta				
	S. obtecta				
	S. tabularis				
	S. natalensis				

Due to the abundance and diversity of patellid limpets around the coast of South Africa, a number of studies investigating aspects of their biology and ecology have been undertaken. However, until recently, the majority of these studies were concentrated on shores of the Cape peninsula and the west coast (Branch, 1971; 1974a,b; 1975a,b; 1976; 1979). The “west coast” of South Africa is the name generally given to the stretch of coastline that extends west of Cape Town to Namibia, while the “south coast” extends east of Cape Town as far as East London. The area of coastline running north from East London to Maputo is known as the “east coast”, and is mainly subtropical (Branch & Branch, 1988). For the purposes of this study, these definitions of the west and east coasts will remain unaltered. However, a distinction between south and south-east coasts will be made. During the course of this thesis, the south coast of South Africa will refer to the coastline between Cape Town and Port Elizabeth, while the south-east coast will be used to distinguish that section of coast between Port Elizabeth and East London (Figure 1.1), where this study was focussed.

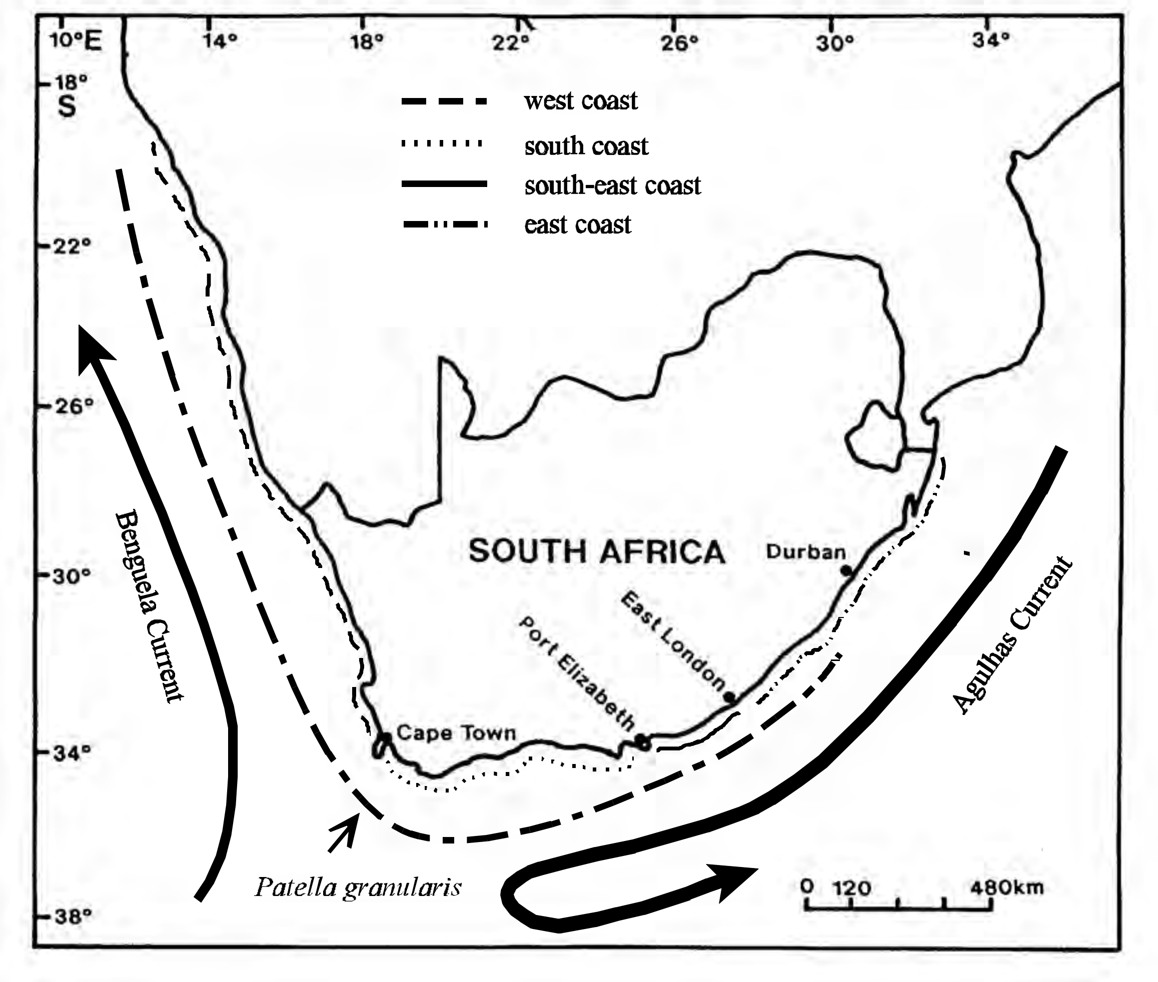


Figure 1.1:

Map of southern Africa showing the major oceanic currents and the four coastal regions referred to during the course of this study. Also shown is the distribution of *P. granularis* around the southern African coast (After Branch & Branch, 1988; Branch *et al.*, 1994).

The coastline of South Africa is influenced by two major oceanic currents which in turn result in differing oceanographic regimes. The Agulhas Current brings warm tropical water down the east coast. In contrast, the west coast receives the Benguela Current which is fed by the cold water South Atlantic circulation (Figure 1.1; Branch & Branch, 1988). In addition, the west coast is also subject to seasonal upwelling of nutrient rich water which results in a much higher degree of primary productivity on this coast than the south and east coasts (Branch & Branch, 1988; Bustamante *et al.*, 1995b).

Rocky intertidal shores are highly variable both temporally and spatially, mainly as a result of community interactions (Dayton, 1971; Menge, 1976). However, in addition to this natural variability, many of the South African marine resources are under increasing pressure from human activities (Bosman & Hockey, 1986; Van Erkom Schurink and Griffiths, 1990; Griffiths & Branch, 1997). The south and east coasts are particularly heavily exploited for their shellfish resources (Lasiak & Dye, 1989; Lasiak, 1991; Tomalin & Kyle, 1998). While mussels, specifically *Perna perna*, are preferred by shellfish gatherers (Siegfried *et al.*, 1985; Lasiak & Dye, 1989; Lasiak, 1993), limpets are also prone to human exploitation (Dye *et al.*, 1997). Along the former Transkei coast, exploitation accounted for an 80% decrease in the abundance of patellid limpets, with this population taking seven years to return to its original abundance (Dye *et al.*, 1997). Furthermore, removal of mussels often results in the inadvertent removal of co-occurring limpets. Many studies have shown that the removal of dominant filter feeders and grazers is followed by an increase in both micro- and macroalgae (Oliva & Castilla, 1986; Farrell, 1988; Dye *et al.*, 1997). In the further absence of grazers, these macroalgae often form dense turfs which preclude the settlement of organisms onto the rock surface. This results in a decrease in adult standing stock, which in turn reduces reproductive output and recruitment resulting in continuous depletion of intertidal species abundance and diversity (Dye *et al.*, 1997). The management of the south-east coast littoral environment can only be sound if it is based on current knowledge of the longevity, reproductive biology and ecology of the organisms inhabiting this region.

P. granularis is one of the most widespread of the 20 patellid species currently recognised along the South African coast (Figure 1.1; Branch *et al.*, 1994). This species is also ubiquitous on shores of differing geomorphologies (pers. obs.) and has a widespread intertidal distribution, ranging from the upper Balanoid zone to the Cochlear zone (Branch, 1971). As already mentioned, most of the information about the ecology and biology of *P. granularis* has been from studies conducted along the Cape west coast and the Cape peninsula. The few investigations which have included south and east coast *P. granularis* have, however, reported some differences, particularly in size and shell morphology, between these limpets and those inhabiting the cooler west coast (Bustamante *et al.*, 1995b; T. Ridgway *et al.*, 1998).

The paucity of information on south-east coast limpets, and the factors which may affect their growth and reproduction in particular, prompted an in-depth study into some aspects of the biology and ecology of *P. granularis.* One aim was to compare the results of such a study with the information available for west coast limpets. Furthermore, *P. granularis* is abundant on shores of differing geomorphologies. This provided an opportunity to investigate how life-history parameters such as growth and reproduction might vary in populations inhabiting different substrata. This information in turn would be used to comment on some of the disadvantages of single site studies. As a result of the parallel motives of the current study, a distinction has been made throughout this dissertation between the study sites and the substratum types selected for the investigations. The current study refers to the rocky shores investigated as “localities”, while the term “site” is used to describe the three substrata chosen for the south-east coast comparative studies.

The first part of this thesis (Chapter 2) examines the intertidal distribution, density and biomass of *P. granularis* at seven south-east coast localities with one of two different geomorphologies. An attempt was made to restrict the range of the study, so all seven localities investigated were situated between Port Elizabeth and Three Sisters (Figure 2.1). The only offshore locality, Bird Island, lies approximately 8 km south-west of Woody Cape, midway between Port Elizabeth and Kenton-on-Sea (Figure 2.1).

T. Ridgway *et al.* (1998; 1999 and in press) have described two new species of *Patella*, *P. natalensis* and *P. sanguinans*. These were traditionally considered to be ecomorphs of *P. granularis* and *P. miniata* respectively. *P. granularis*, as with many limpet species, exhibits a great deal of morphological plasticity which is often related to localised environmental conditions (Powell, 1973; T. Ridgway *et al.*, 1998). In light of this, and the morphological differences observed (Chapter 2), allozyme electrophoresis was conducted on *P. granularis* inhabiting three substrata along the south-east coast. This was included in the current investigation to ensure that all specimens studied were members of a single discrete genetic population despite the morphological differences observed.

In addition to describing the choice of study areas (substratum types), Chapter 4 investigates the growth of *P. granularis* at three closely positioned sites. This enabled a comparison of age and growth rates of west and south-east coast limpets to be made.

Furthermore, the study provides a comparison of growth rates of *P. granularis* inhabiting shores of differing geomorphologies.

Chapter 5 presents the results of a study of the reproductive biology of *P. granularis* inhabiting three substrata. Once again, the results were used to compare aspects of the reproductive biology of west and south-east coast limpets. In an attempt to explain some of the differences observed in the growth and reproduction of *P. granularis* on a more localised scale, the activity rhythms and foraging behaviour of *P. granularis* inhabiting an aeolianite and a quartzitic sandstone shore were investigated (Chapter 6). As the results from this study suggested that the foraging patterns of limpets may vary according to substratum, an investigation of the microalgal abundance on the three substrata was conducted in an attempt to provide an explanation for some of the differences observed in the limpets from the three sites (Chapter 7).

Finally, Chapter 8 provides a comparison of the biology and ecology of *P. granularis* from the west and south-east coasts. Factors which may be responsible for differences between populations inhabiting the two coastal regions are discussed in a hypothetical model. In addition, a summary of the differences in the life-history parameters of *P. granularis* inhabiting the three sites along the south-east coast is also provided, and again a hypothetical model addresses the relative effect of the factors influencing these populations. The disadvantages of the present work are discussed and avenues for future research are suggested.

Chapter 2

Chapter 2

POPULATION STRUCTURE, BIOMASS AND DENSITY OF *PATELLA GRANULARIS* ON THE SOUTH-EAST COAST OF SOUTH AFRICA

2.1: INTRODUCTION:

The importance of limpets to many intertidal rocky shores has motivated numerous studies of their biology and ecology (see Branch, 1981 for a review of literature). These organisms live in accessible habitats and are thus relatively easy to sample and manipulate (Creese, 1981). Furthermore, limpets often occur in large numbers and are widely distributed along coastlines (Creese, 1981) thus enabling investigations into, and comparisons of, the effects of a range of environmental conditions on aspects of the biology and ecology of these organisms. This is particularly true for the many species of limpets that inhabit the coast of southern Africa which are exposed to different oceanographic regimes (Branch & Branch, 1988; Branch *et al.*, 1994).

Patellid limpets are particularly abundant on South African rocky shores (Branch & Branch, 1988). *Patella cochlear* can reach densities of 2 600/m^2 (Branch, 1975a) while populations of *P. granatina* and *P. argenvillei* occur in densities of up to 200/m^2 (Bustamante *et al.*, 1995a). *P. granularis*, which is one of the most widespread of the South African patellid species (see Chapter 1) reaches densities along the west coast in excess of 200/m^2 (Bosman & Hockey, 1988a). This species has been reported to display two size gradients (Stephenson, 1937; Branch, 1971; Kilburn & Rippey, 1982). Firstly, a geographical size gradient exists with the mean shell length of populations decreasing from west to east (Stephenson, 1937; Kilburn & Rippey, 1982; Bustamante *et al.*, 1995b). This has been correlated to a gradient of primary productivity which is evident around the South African coast, a result of the upwelling of nutrient rich water which occurs on the west coast (Bustamante *et al.*, 1995b). Secondly, *P. granularis* exhibits an intertidal size gradient, with the mean size of the limpets increasing in an upshore direction (Branch, 1971). This size difference has been attributed to the settlement of juveniles low on the shore and their subsequent migration upshore as they increase in size (Branch, 1971; 1975b). It has been proposed that larger limpets are more tolerant of the harsher physical conditions that are typical of the high shore (Branch, 1975b). However, as with the majority of the other southern African patellids, much of the information available on this species is a result of the work of Branch (e.g. 1971; 1975a,b) along the Cape peninsula and Cape west coast. Due to the large differences in the biogeographic conditions around the South African coast, it is unlikely that the conditions which structure west coast limpet populations will be similar to those that influence south and east coast populations. In recognition of this, and the fact that shellfish populations are becoming progressively exploited, an increasing number of studies of the population structure and distribution of south and east coast species have recently been conducted (Robson, 1986; Lasiak, 1993; Gray, 1996; Henninger, 1998).

South Africa has a long, wave exposed shoreline extending approximately 3 000 km from Namibia in the west to Mozambique in the east. Traditionally, the Eastern Cape coast, which includes the south-east coast as defined in the present study, comprised 350 km and was bounded in the west by Cape St. Francis and in the east by the Kei mouth (Marker, 1988). These shores consist of numerous sandy beaches interspersed with rocky shores, which are generally composed of aeolianite and quartzitic sandstone (Marker, 1988; Dower, 1989). The south-east coast now includes an additional 250 km as a result of the addition of the former Ciskei and Transkei into the Eastern Cape province. The rocky shores of this area are predominantly composed of sandstone, mudstone and shale (Hockey *et al.*, 1988). Both species distribution (Dower, 1989; Foster, 1994) and community structure (McQuaid & Branch, 1985; McGuinness & Underwood, 1986) are influenced by rock type.

The current study aimed to quantify and compare the population structure, biomass and distribution of *P. granularis* at a number of localities comprising two of the five geomorphologies (aeolianite and quartzite) between Port Elizabeth and the Fish river. In addition, this study also aimed to identify a suitable locality/localities which would sustain long-term reproductive (24 months) and growth (approximately 40 months) studies, which in turn would enable a comparison within south-east coast populations and between south-east and west coast populations of *P. granularis*.

2.2: MATERIALS AND METHODS:

2.2.1: Study localities:

The south-east coast rocky shores occurring between Port Elizabeth and East London generally consist of two rock types: consolidated dune (aeolian calcarenites) which form wave cut platforms, and/or hard quartzitic sandstone which forms boulder shores and reefs (Marker, 1988; Dower, 1989). Preliminary inspections along a 100 km stretch of coastline close to Grahamstown indicated that *Patella granularis* occurs on both substrata. To determine the density, distribution and biomass of this species, six rocky shores located between Port Elizabeth and the Fish river were selected for comparative studies (Figure 2.1; Table 2.1). These shores represented the two types of substrata (i.e. aeolianite and quartzite). Three Sisters, Kenton-on-Sea and Kwaaihoek were all situated on aeolianite headlands. Each of these three localities was characterised by a wave-cut platform and cliff (e.g. Figure 2.2a). Aeolianite is a soft, granular rock with a highly irregular surface (pers. obs.; Figure 2.2b). The Port Alfred, Cannon Rocks and Port Elizabeth localities were all quartzitic sandstone shores consisting of boulder-reefs extending perpendicularly from the shore (e.g. Figure 2.3a). In contrast to aeolianite, quartzite is a hard smooth, rock (pers. obs; see Figure 2.3b). All localities were exposed to the prevailing westerly swell. An additional quartzitic boulder shore, Bird Island, was also included in the study (Figure 2.1). This offshore locality is situated within a marine reserve and is home to a large colony of gannets and penguins.

2.2.2: Sampling procedure:

Five distinct zones within the littoral are recognised along the rocky shores of the south and south-east coasts of southern Africa, namely the Littorina, upper Balanoid, lower Balanoid, Cochlear and Infratidal zones (Branch & Branch, 1988; Lubke, 1988). The high-shore Littorina zone is characterised by the snail *Nodilittorina knysnaensis* and represents the high water spring level (HWS). The upper Balanoid zone indicates the upper mid-shore region and is identified by the presence of the barnacles *Chthamalus dentatus*, *Tetraclita serrata* and the limpet *Helcion pectunculus*, as well as the seaweeds *Ulva* spp and *Iyengaria stellata*. The lower Balanoid zone is the area from mean sea level to low water neap tides and is characterised by *Octomeris* *angulosa*, *Balanus* spp, *Patella longicosta*, *Oxystele sinensis*, *Gelidium pristoides* and *Codium lucasii capensis*. The Cochlear zone represents the low-shore from the low water neap to the low water spring tidal level. Indicator species within this zone include *P. cochlear*, *Pyura stolonifera*, *Caulerpa filiformis* and *Plocamium corallorhiza* (Branch & Branch, 1988, Seagrief, 1988). Along the south-east coast, dense beds of mussel (predominantly *Perna perna*) also occur in this zone. Due to intense wave action, the Infratidal region is inaccessible in all but the calmest weather. As a result this zone was omitted from this study.

At each study locality, three transects, which were each 10 metres wide and separated from each other by at least 10 metres, were carried out from the high water spring (HWS) tidal level to the low water spring (LWS) tidal level during low spring tides. Twenty, 20 cm x 20 cm randomly spaced quadrats were sampled from each of the four zones within each transect. As *P. granularis* does not occur in rock pools, all rock pools were ignored during sampling. All *P. granularis* present within each quadrat, with the exception of those from the Bird Island locality, were removed, sexed, counted and their shell parameters (see section 4.2.2 for details) measured to the nearest 0.01 mm with Vernier calipers. Both wet and dry (after drying to constant weight at 60EC) body mass was determined by weighing to the nearest 0.0001 g using an electronic balance. The shell parameters (length, width and height) of the limpets present in the quadrats from Bird Island were measured on site.

2.2.3: Statistical analyses:

As sample counts within each quadrat were not always greater than 20 (particularly in low-shore samples), all data were square root transformed to comply with the requirements of analysis of variance (ANOVA) tests (Fry, 1993). Multi-factor ANOVA (MANOVA) and one-way ANOVA tests were used to determine whether density and biomass differed between the localities and between the zones at each locality (Fry, 1993). Multiple comparisons on significant data were conducted using Scheffe’s multiple range tests (Fry, 1993). All statistical calculations were done using Statgraphics version 7.0 (Statistical Graphics Corporation, USA).

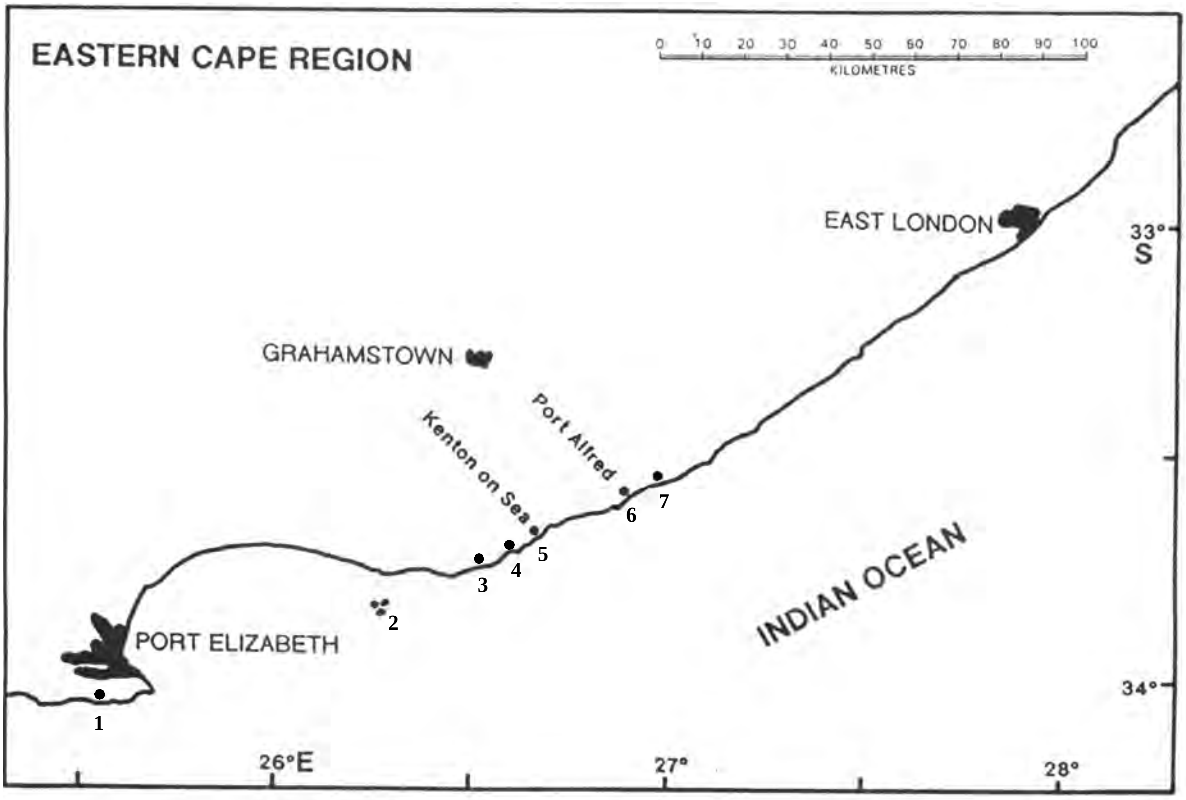


Figure 2.1:

The south-east coast of South Africa showing the seven localities investigated. The name of each locality appears in Table 2.1.

Table 2.1:

The seven localities investigated in the current study listed in geographical order from west to east. The type of substratum present at each locality is also included.

Locality number	Locality	Substratum type
1	Port Elizabeth	Quartzitic sandstone
2	Bird Island	Quartzitic sandstone
3	Cannon Rocks	Quartzitic sandstone
4	Kwaaihoek	Aeolianite
5	Kenton-on-Sea	Aeolianite
6	Port Alfred	Quartzitic sandstone
7	Three Sisters	Aeolianite

Figure 2.2a:

Kenton-on-Sea, an example of an aeolianite wave-cut platform.

Figure 2.2b:

Close-up of aeolianite rock showing pits and indentations. Also visible is the greenish colour of the rock as a result of increased microalgal abundance.

Figure 2.3a:

Cannon Rocks, an example of a quartzitic sandstone boulder beach.

Figure 2.3b:

Close-up of quartzitic sandstone indicating the smoothness of the rock surface.

2.3: RESULTS:

2.3.1: Distribution:

At all study localities, *Patella granularis* was found in the upper Balanoid, lower Balanoid and Cochlear zones (Table 2.2). As no limpets of this species occurred in the Littorina zone, this zone was omitted from further analysis. The biomass of *P. granularis* in the three zones was found to vary among transects at most localities (see Appendix I; Table 1). However, no significant difference in the density of *P. granularis* occurred among the three transects at any locality (Appendix I; Table 2). Despite the differences in biomass, data from each zone of each transect were pooled in order to provide a mean density and biomass per zone for each locality in order to allow inter-locality comparisons to be made. As removal of *P. granularis* from Bird Island was prohibited, it was only possible to collect information about the density and size distribution of these limpets.

Table 2.2:

Mean densities and dry biomass (g/m² ± S.D.) of *P. granularis* in three zones at seven localities. ND = no data collected. As no *P. granularis* was found in the Littorina zone, this zone was omitted from any analysis.

Locality	Shore average		Upper Balanoid Zone		Lower Balanoid Zone		Cochlear Zone	
	Density (Nº/m²)	Dry Biomass	Density (Nº/m²)	Dry Biomass	Density (Nº/m²)	Dry Biomass (g/m²)	Density (Nº/m²)	Dry Biomass (g/m²)
Port	53.61 ± 18.88	5.44 ± 1.23	50.83 ± 19.68	6.84 ± 0.09	65.00 ± 24.74	6.27 ± 0.06	43.75 ± 13.55	3.22 ± 0.07
Bird Island	31.67 ± 14.38	ND	23.75 ± 11.46	ND	54.17 ± 19.47	ND	17.08 ± 8.62	ND
Cannon	65.14 ± 29.29	7.24 ± 0.11	55.42 ± 27.98	7.25 ±	110.83 ± 38.21	13.47 ± 0.11	29.17 ± 5.91	0.50 ± 0.04
Kwaaihoek	83.75 ± 29.57	3.02 ± 2.50	88.75 ± 33.49	6.06 ± 0.31	91.25 ± 34.49	2.00 ± 0.04	71.25 ± 22.69	1.56 ± 0.15
Kenton	59.57 ± 27.01	7.73 ± 0.09	45.42 ± 17.64	6.39 ± 0.08	104.17 ± 37.98	12.06 ± 0.10	29.17 ± 17.83	4.74 ± 0.11
Port Alfred	80.50 ± 53.39	7.61 ± 0.06	60.67 ± 38.59	7.47 ± 0.06	117.08 ± 73.42	12.73 ± 0.06	63.75 ± 38.90	2.63 ± 0.03
Three Sisters	111.25 ± 58.21	6.63 ± 0.29	37.92 ± 15.22	2.04 ± 0.06	177.92 ± 79.07	12.19 ± 1.01	117.92 ± 49.21	5.66 ± 0.380

2.3.2: Density:

The mean density of *P. granularis* differed significantly among the study localities ($p < 0.0001$; Table 2.3). While no differences were found in the densities of limpets on aeolianite and quartzitic shores, when pooled, the former were found to have higher average densities than the latter (. 87/m^2 on aeolianite and . 58/m^2 on quartzite; Table 2.2). The majority of this difference, however, can be attributed to the significantly higher densities recorded at Three Sisters, where the maximum mean shore density of this study was recorded i.e. . 111 limpets/m^2 ($p < 0.0001$; Tables 2.3 and 2.4).

Table 2.3:

Results of a MANOVA to determine whether the density of *P. granularis* differed between localities and zones at seven localities along the south-east coast. (* indicates a significant p - value).

Source of variation	SS	DF	MS	F-ratio	Significance level
Main effects					
A: Locality	72447.317	6	12074.553	31.516	< 0.0001 *
B: Zone	48606.526	2	24303.263	63.435	< 0.0001 *
Interactions					
AB	53482.058	12	4456.838	11.633	< 0.0001 *
Residual	16091.167	42	383.123		
Total (Corrected)	190627.07	62			

Table 2.4:

Scheffe's multiple range test of densities of *P. granularis* at seven localities along the south-east coast. (X's in the same column indicate no significant difference).

	LS Mean	**Homogenous groups**		
Bird Island	38.194	X		
Port Elizabeth	43.611	X		
Cannon Rocks	62.361	X	X	
Kwaaihoek	81.667		X	
Kenton - on - Sea	64.444	X	X	
Port Alfred	57.972	X	X	
Three Sisters	147.306			X

There was a significant difference in the density of *P. granularis* among the three zones and these zonal differences were significantly affected by the locality (Table 2.3). At all localities, the densities of *P. granularis* were highest in the lower Balanoid zone (Tables 2.5 and 2.6), although these results were not significant at Port Elizabeth, Bird Island and Port Alfred. At Kwaaihoek, although a significant difference was found in the densities of *P. granularis* inhabiting the Cochlear zone and both the upper and lower Balanoid zones, the densities of limpets from the upper and lower Balanoid zones were not significantly different (Table 2.6).

Table 2.5:

Scheffe's multiple range test of densities of *P. granularis* in three zones along the south-east coast. (X's in the same column indicate no significant difference).

	LS Mean	**Homogenous groups**	
Upper Balanoid	57.988	X	
Lower Balanoid	109.357		X
Cochlear	45.036	X	

Table 2.6:

Summaries of one-way ANOVA's and Scheffe's multiple range tests performed to determine whether the density of *P. granularis* differed between the zones of a locality, and where the differences occurred between the zones at seven localities along the south-east coast. (X's in the same column indicate no significant difference; * indicate significant p - values).

Locality and zone	**F - ratio**	**One-way ANOVA Sig. level**	**Homogenous groups**		
Cannon Rocks	63.350	p = 0.0001*			
Upper Balanoid				X	
Lower Balanoid					X
Cochlear			X		
Kwaaihoek	47.389	p = 0.0002*			
Upper Balanoid				X	
Lower Balanoid				X	
Cochlear			X		
Kenton-on-Sea	27.674	p = 0.0009*			
Upper Balanoid				X	
Lower Balanoid					X
Cochlear				X	
Three Sisters	34.314	p = 0.0005*			
Upper Balanoid				X	
Lower					X
Cochlear				X	
Port Elizabeth	3.511	p = 0.0978			
Bird Island	3.118	p = 0.1179			
Port Alfred	2.684	p = 0.1471			

2.3.3: Biomass:

The mean dry biomass of *P. granularis* differed significantly among the study localities (p < 0.0001; Table 2.7). Although no discrete pattern was observed, when pooled, biomass was generally higher on quartzitic shores (. 6.7g/m^2) than on aeolianite shores (. 5.8g/m^2; Table 2.2). The maximum mean shore dry biomass of *P. granularis* was recorded at Kenton-on-Sea (7.73g/m^2; Table 2.2).

Table 2.7:

Results of a MANOVA to determine whether the biomass of *P. granularis* differed between localities and zones at seven localities along the south-east coast. (* indicates a significant p - value).

Source of variation	**SS**	**d.f.**	**MS**	**F-ratio**	**Significance level**
Main effects					
Locality	5.333	5	1.067	16.289	< 0.0001 *
Zone	4.657	2	2.329	35.560	< 0.0001 *
Interactions					
AB	5.474	10	0.547	8.360	< 0.001 *
Residual	60.770	928	0.065		
Total (Corrected)	76.234	945			

Table 2.8:

Scheffe's multiple range test of biomass of *P. granularis* at seven localities along the south-east coast. (X's in the same column indicate no significant difference).

	LS Mean	**Homogenous groups**		
Port Elizabeth	0.414	X	X	
Cannon Rocks	0.406	X	X	
Kwaaihoek	0.341	X		
Kenton - on - Sea	0.615			X
Port Alfred	0.482		X	
Three Sisters	0.217	X	X	

The biomass of *P. granularis* inhabiting the three zones differed significantly among the localities studied and this zonal difference was affected by locality (Table 2.7). Generally, mean biomass was significantly higher in the lower Balanoid zone (Table 2.9). No significant difference in mean biomass was found among zones at either Kwaaihoek or Three Sisters (Table 2.10).

Table 2.9:

Scheffe's multiple range test of biomass of *P. granularis* in three zones along the south-east coast. (X's in the same column indicate no significant difference).

	LS Mean	**Homogenous groups**		
Upper Balanoid	0.456		X	
Lower Balanoid	0.536			X
Cochlear	0.352	X		

Table 2.10:

Summaries of one-way ANOVA’s and Scheffe’s multiple range tests performed to determine whether the biomass of *P. granularis* differed between the zones of a locality, and where the differences occurred between the zones at seven localities along the south-east coast. (X’s in same column indicate no significant differences; * indicates a significant p - value).

Locality and zone	F-ratio	One-way ANOVA Sig. level	Homogenous groups		
Port Elizabeth	26.247	p = < 0.0001 *			
Upper Balanoid				X	
Lower Balanoid				X	
Cochlear			X		
Cannon Rocks	58.521	p = < 0.0001 *			
Upper Balanoid				X	
Lower Balanoid					X
Cochlear			X		
Kenton-on-Sea	4.724	p = 0.0104 *			
Upper Balanoid			X		
Lower Balanoid				X	
Cochlear			X	X	
Port Alfred	6.206	p = < 0.0025 *			
Upper Balanoid				X	
Lower Balanoid				X	
Cochlear			X		
Kwaaihoek	0.091	p = 0.914			
Three Sisters	1.055	p = 0.351			

2.3.4: Population structure:

There was a significant difference (MANOVA; $p < 0.0001$; Table 2.11) in the mean size of *P. granularis* inhabiting the seven study localities (Figures 2.4 a-c and 2.5 a-d). Significantly larger limpets occurred at Bird Island (Table 2.12), where individuals with shell lengths larger than 46 mm were found (Table 2.14). Although not present in large numbers, limpets greater than 38 mm were also found at Kwaaihoek (Figure 2.4a; Table 2.14). Limpets in the size class of 36 - 37.9 mm were also found at Port Elizabeth and Cannon Rocks (Figures 2.5a and 2.5b). The mean size of limpets from Three Sisters and Port Alfred was significantly smaller than recorded at the other sites (Table 2.12). The largest size class recorded at both these localities was only 28 - 29.9 mm (Figures 2.4c and 2.5c; Table 2.14).

The mean shell length of *P. granularis* decreased significantly down the shore (MANOVA; $p < 0.0001$; Tables 2.11 and 2.13). No discrete differences in the mean sizes of limpets inhabiting aeolianite or quartzitic sandstone were evident, however, with the exception of the limpets from Bird Island, there was a general west to east decrease in the mean shell length of *P. granularis* along the coastline investigated (Table 2.15).

Table 2.11:

Results of a MANOVA to determine whether the length (maximum shell length) of *P. granularis* differed between localities and zones at seven localities along the south-east coast. (* indicates a significant p - value).

Source of variation	SS	d.f.	MS	F-ratio	Significance level
Main effects					
A: Locality	25459.20	6	4243.20	189.57	< 0.0001 *
B: Zone	9698.00	2	4849.00	216.63	< 0.0001 *
Interactions					
AB	6574.80	12	547.80	24.47	< 0.0001 *
Residual	74245.20	3317	22.40		
Total	121754.30	3337			

Table 2.12:

Scheffe's multiple range test of mean shell length of *P. granularis* at seven localities along the south-east coast. The localities are arranged according to their geographical situation from west to east, with the exception of Bird Island which occurs offshore between Port Elizabeth and Cannon Rocks (See Figure 2.1). (X's in the same column indicate no significant difference).

Locality	LS Mean	Homogenous groups		
Bird Island	26.56			X
Port Elizabeth	19.97		X	
Cannon Rocks	18.79	X	X	
Kwaaihoek	19.33	X	X	
Kenton - on - Sea	18.08	X		

Port Alfred	15.58		X
Three Sisters	14.08	X	

Table 2.13:

Scheffe’s multiple range test of mean shell length of *P. granularis* in three zones along the south-east coast.

Zone	**LS Mean**	**Homogenous groups**		
Upper Balanoid	20.115			X
Lower Balanoid	17.269		X	
Cochlear	15.221	X		

Table 2.14:

Comparative data on the population structure of *P. granularis* at seven localities along the south-east coast.

Locality	**Mean size (mm ± S.D.)**	**Modal size class (mm)**	**Smallest size class (mm)**	**Largest size class (mm)**
Port Elizabeth	19.97 ± 5.06	18 - 19.9	8 - 9.9	36 - 37.9
Bird Island	26.56 ± 6.71	26 - 26.8	12 - 13.9	46 - 47.9
Cannon Rocks	18.79 ± 6.96	18 - 19.9	2 - 3.9	36 - 37.9
Kwaaihoek	19.33 ± 6.75	16 -17.9	4 - 5.9	38 - 39.9
Kenton-on-Sea	18.08 ± 5.28	18 - 19.9	4 - 5.9	32 - 33.9
Port Alfred	15.58 ± 4.10	14 - 15.9	4 - 5.9	28 - 29.9
Three Sisters	14.08 ± 3.10	20 - 21.9	4 - 5.9	28 - 29.9

Table 2.15:

Mean shell lengths (mm ± S.D.) of *P. granularis* inhabiting three zones at each of seven localities.

Locality	Upper Balanoid	Lower Balanoid	Cochlear
Port Elizabeth	22.21 ± 5.94	19.16 ± 4.17	19.27 ± 3.64
Bird Island	32.08 ± 7.11	25.69 ± 6.74	22.42 ± 5.10
Cannon Rocks	20.18 ± 7.62	19.86 ± 6.21	12.86 ± 6.35
Kwaaihoek	25.81 ± 6.12	17.13 ± 3.82	15.58 ± 5.19
Kenton-on-Sea	18.82 ± 4.63	18.17 ± 5.38	18.88 ± 5.82
Port Alfred	16.43 ± 3.94	15.87 ± 4.17	13.50 ± 3.31
Three Sisters	14.94 ± 3.82	13.79 ± 2.93	13.91 ± 2.84

At all localities, except Kenton-on-Sea, the mean shell length of *P. granularis* decreased significantly down the shore (Figures 2.6 - 2.12; Table 2.16). Generally, the size of the limpets found in the lower Balanoid zone was not significantly different from that of limpets occurring in the Cochlear zone, although this was not true at Bird Island (Table 2.16). The largest limpets were found in the upper Balanoid zone at Bird Island, with a mean shell length of 32.08 mm being recorded at this locality. Although large size classes of limpets were present at Kwaaihoek (see Table 2.14), the mean length of limpets recorded in the upper Balanoid zone at this locality was only 25.81 mm (Table 2.15). The smallest limpets occurred at Cannon Rocks, where the mean length of *P. granularis* inhabiting the Cochlear zone was only 12.86 mm (Table 2.15).

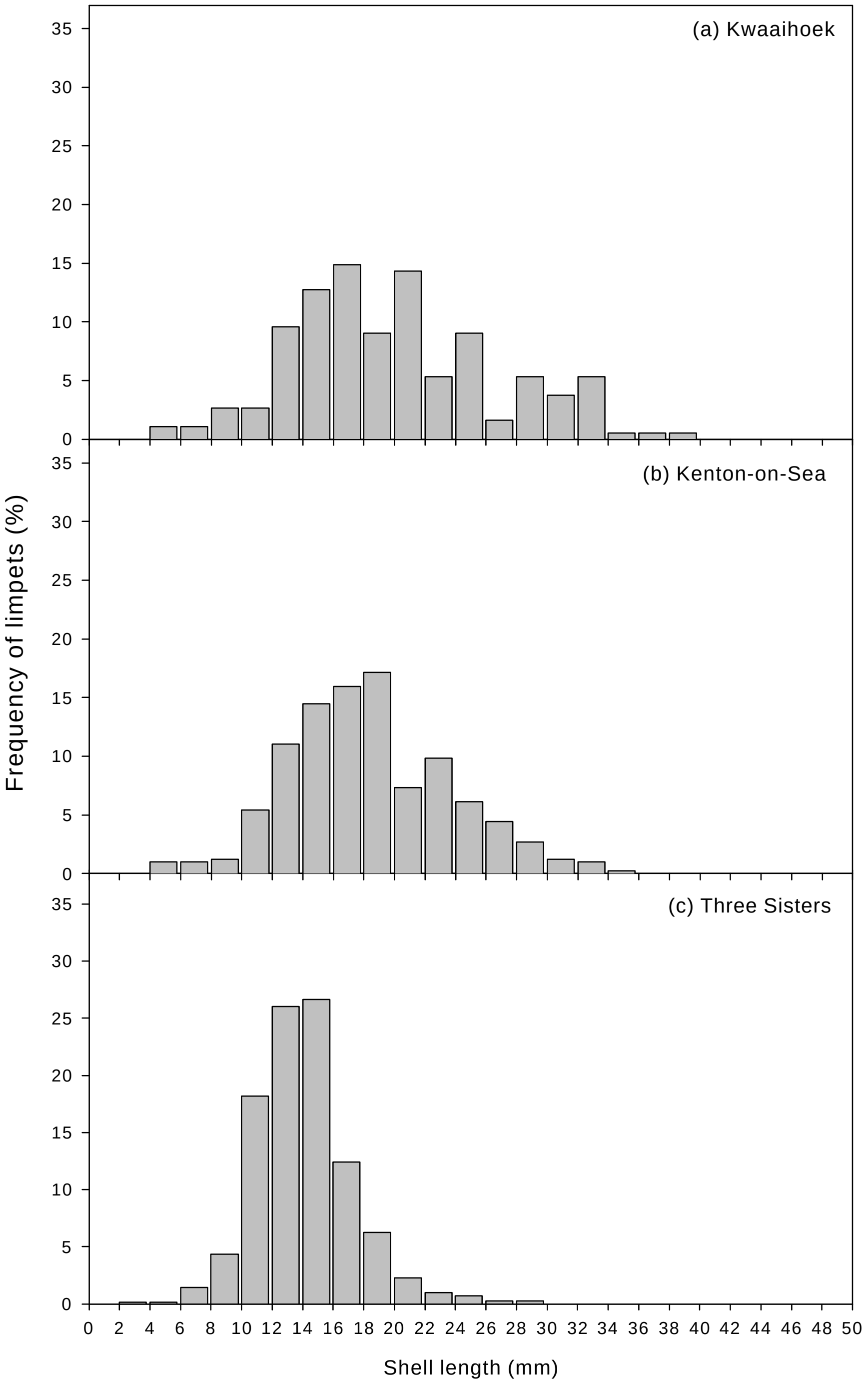


Figure 2.4:

Length-frequency distributions of *P. granularis* inhabiting three aeolianite shores along the south-east coast.

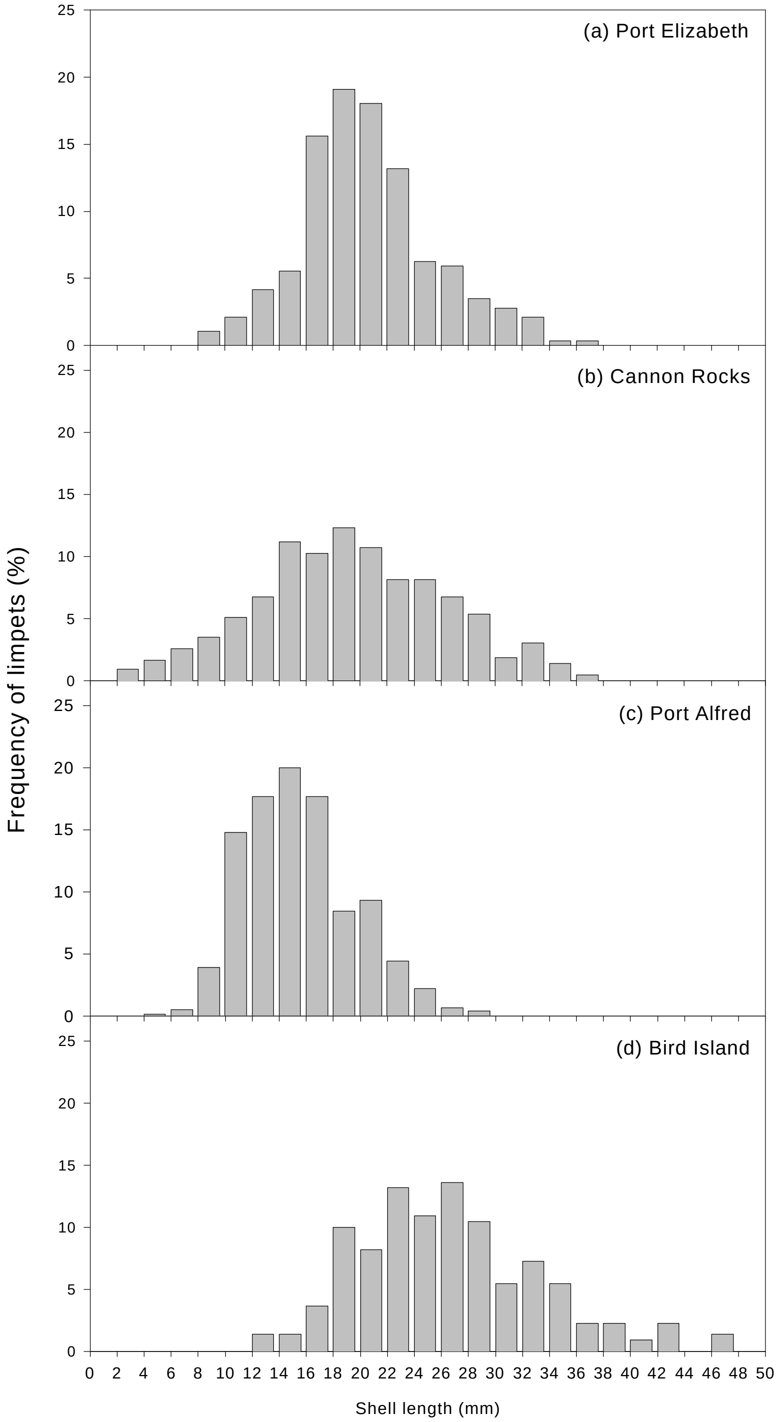


Figure 2.5:

Length-frequency distributions of *P. granularis* inhabiting four quartzitic sandstone shores along the south-east coast.

Table 2.16:

Summaries of one-way ANOVA's and Scheffe's multiple range tests performed to determine whether the mean shell length of *P. granularis* differed between the zones of a locality, and where the differences occurred between the zones at seven localities along the south-east coast. (X's in same column indicate no significant difference; * indicates a significant p - value).

Locality and zone	**F-ratio**	**One-way ANOVA Sig. level**	**Homogenous groups**		
Bird Island	35.744	p = < 0.0001 *			
Upper Balanoid					X
Lower Balanoid				X	
Cochlear			X		
Port Elizabeth	20.407	p = < 0.0001 *			
Upper Balanoid					X
Lower Balanoid				X	
Cochlear				X	
Cannon Rocks	45.229	p = < 0.0001 *			
Upper Balanoid					X
Lower Balanoid				X	
Cochlear				X	
Kwaaihoek	79.668	p = < 0.0001 *			
Upper Balanoid					X
Lower Balanoid				X	
Cochlear				X	
Port Alfred	27.528	p = < 0.0001 *			
Upper Balanoid					X
Lower Balanoid				X	
Cochlear				X	
Three Sisters	26.782	p = < 0.0001 *			
Upper Balanoid					X
Lower Balanoid				X	
Cochlear				X	
Kenton-on-Sea	2.313	p = 0.1002			

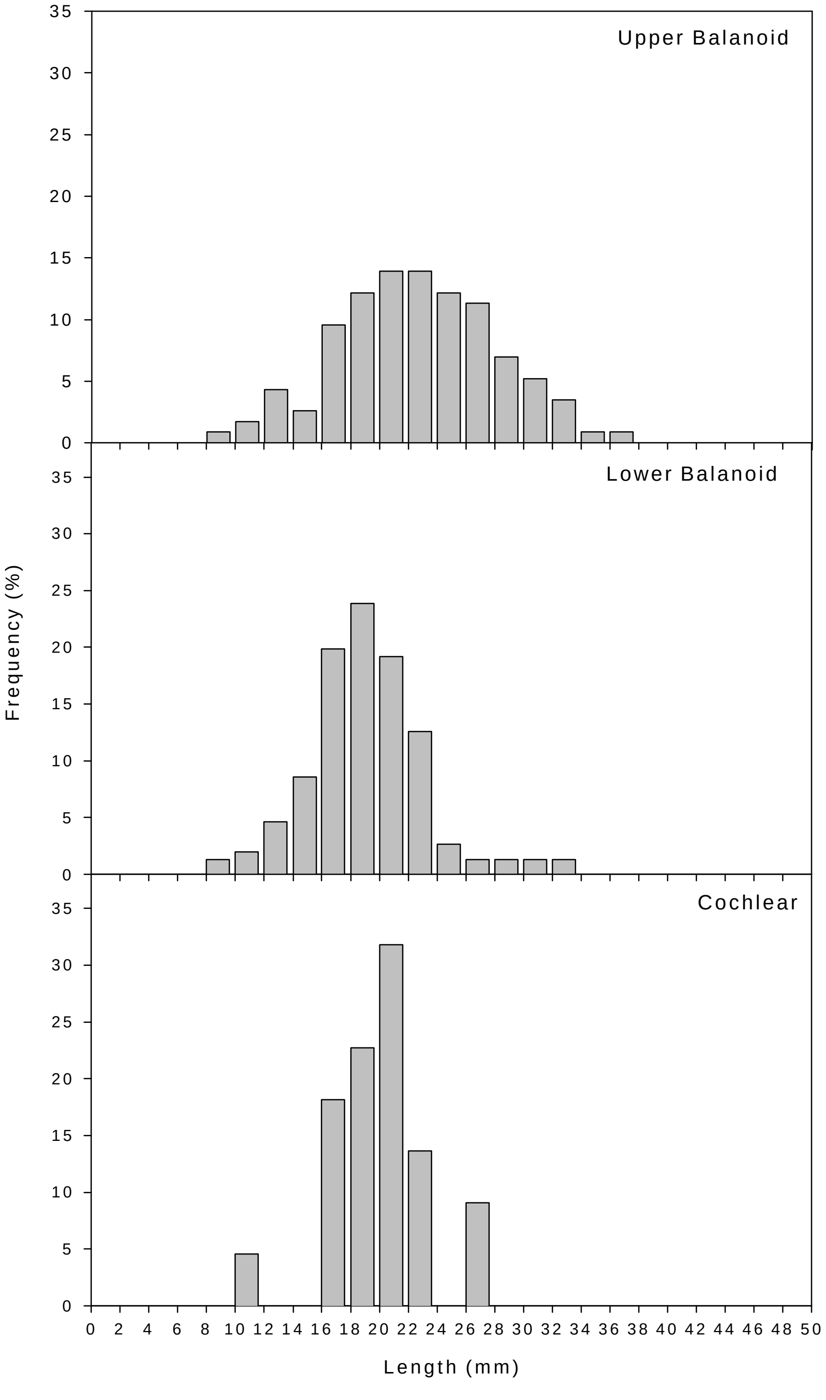


Figure 2.6:

Size-frequency distribution of *P. granularis* inhabiting the three zones at Port Elizabeth.

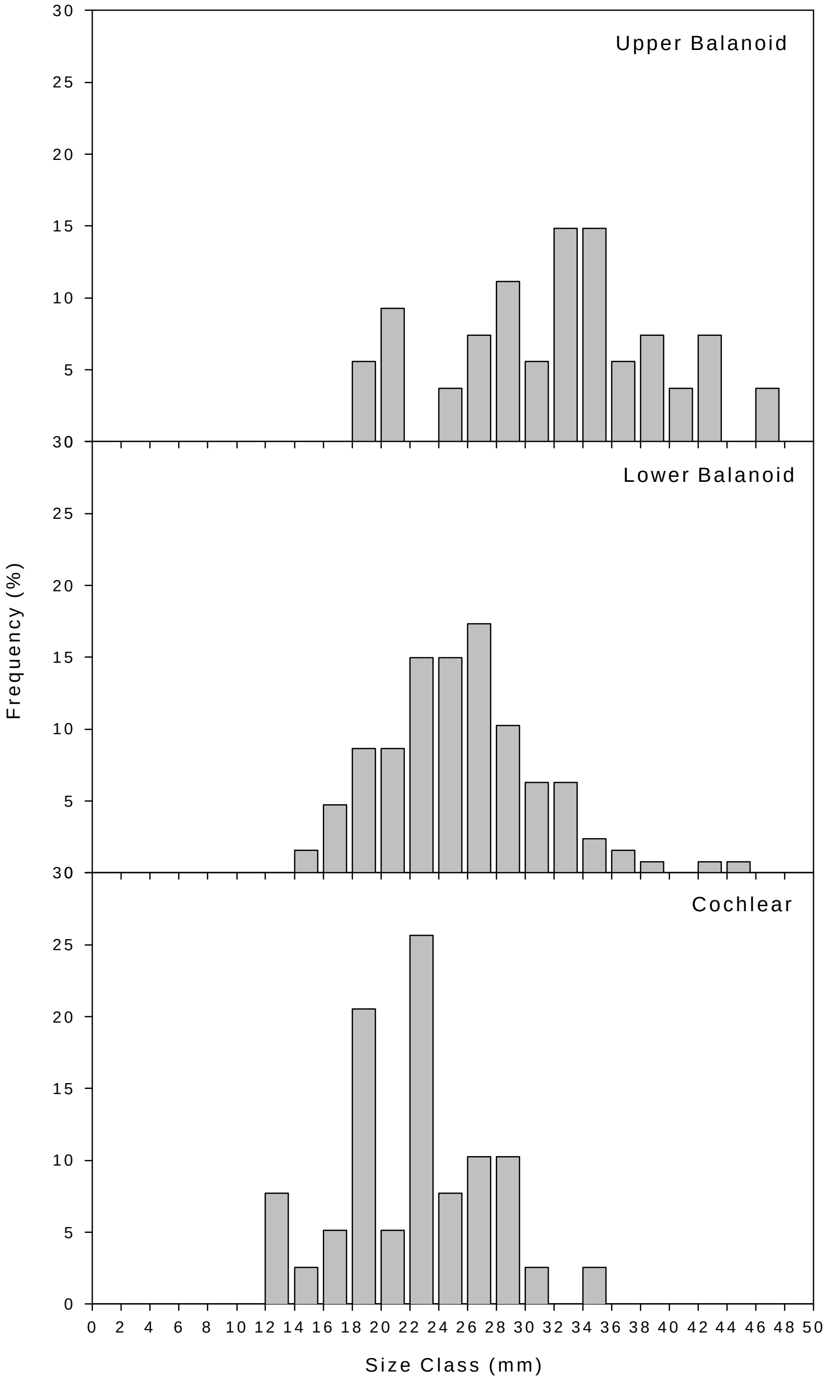


Figure 2.7:

Size-frequency distribution of *P. granularis* inhabiting three zones at Bird Island.

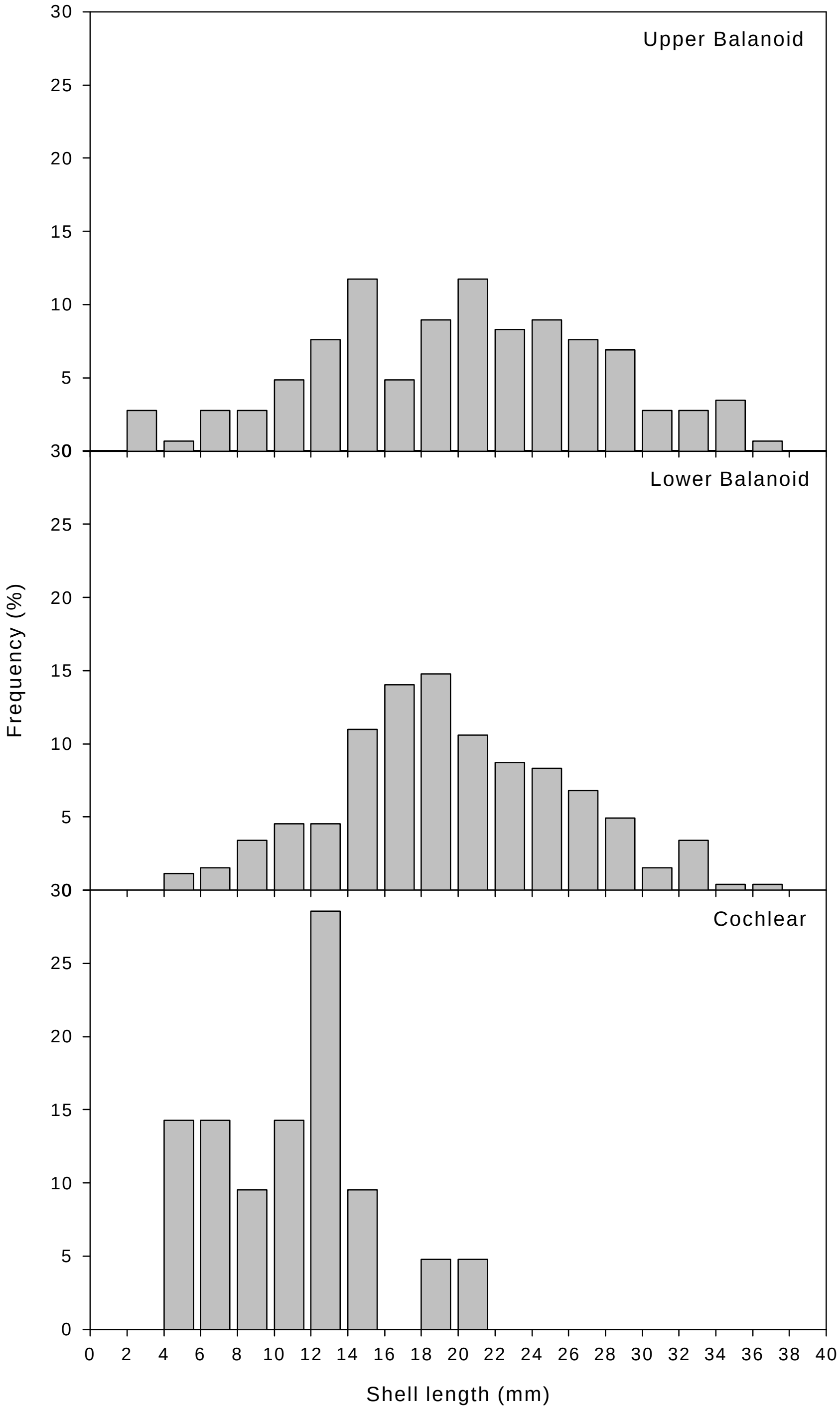


Figure 2.8:

Size-frequency distribution of *P. granularis* inhabiting three zones at Cannon Rocks.

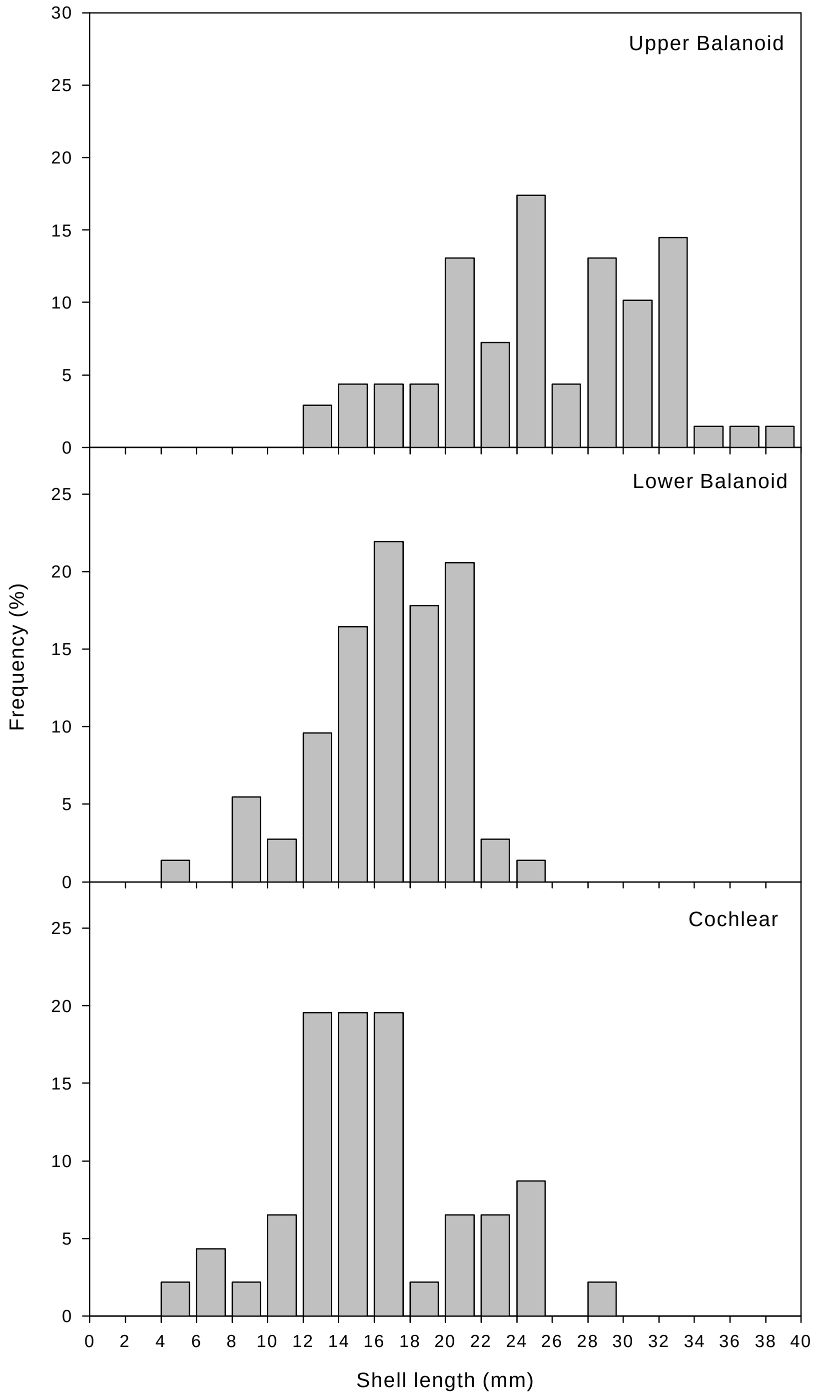


Figure 2.9:

Size-frequency distribution of *P. granularis* inhabiting three zones at Kwaaihoek

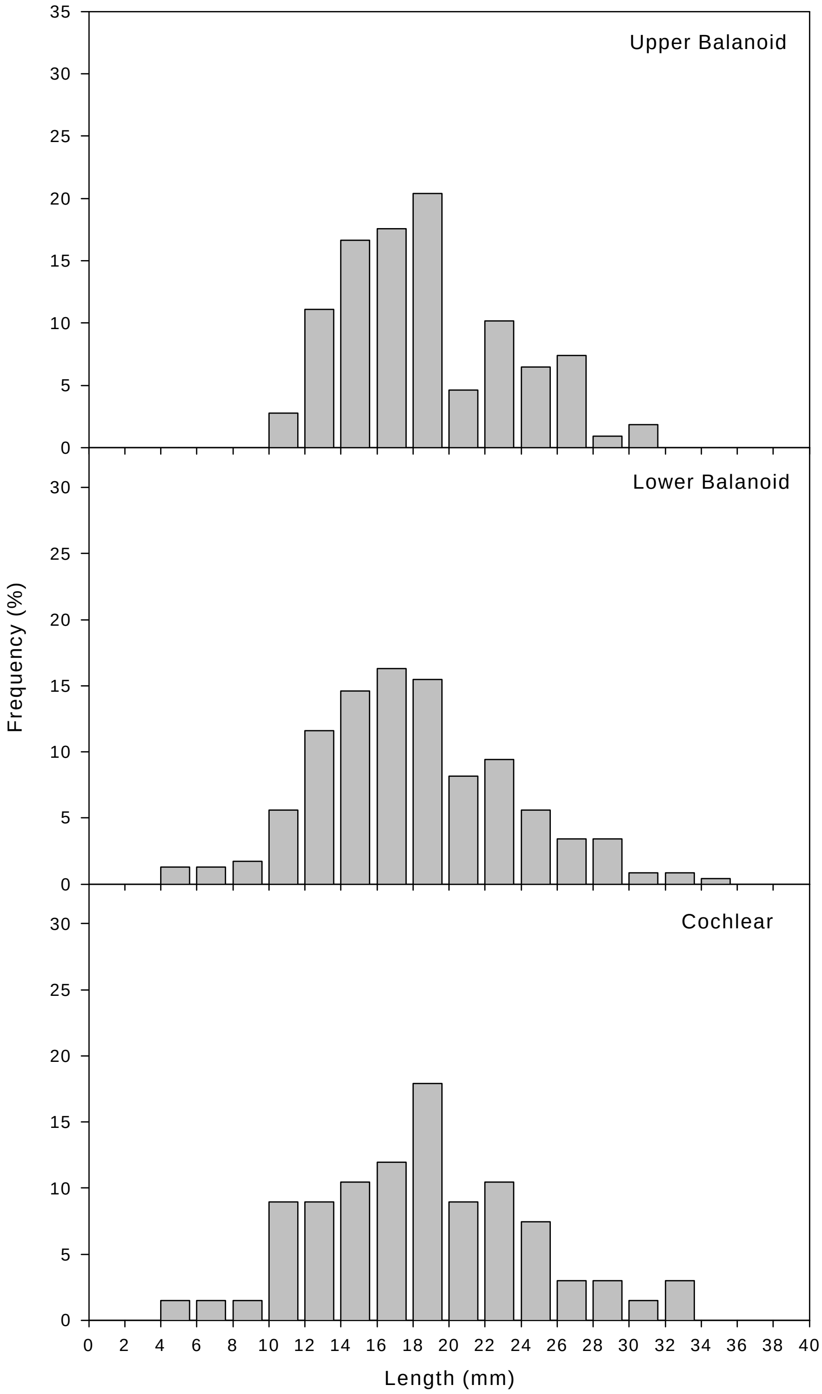


Figure 2.10:

Size-frequency distribution of *P. granularis* inhabiting three zones at Kenton-on-Sea.

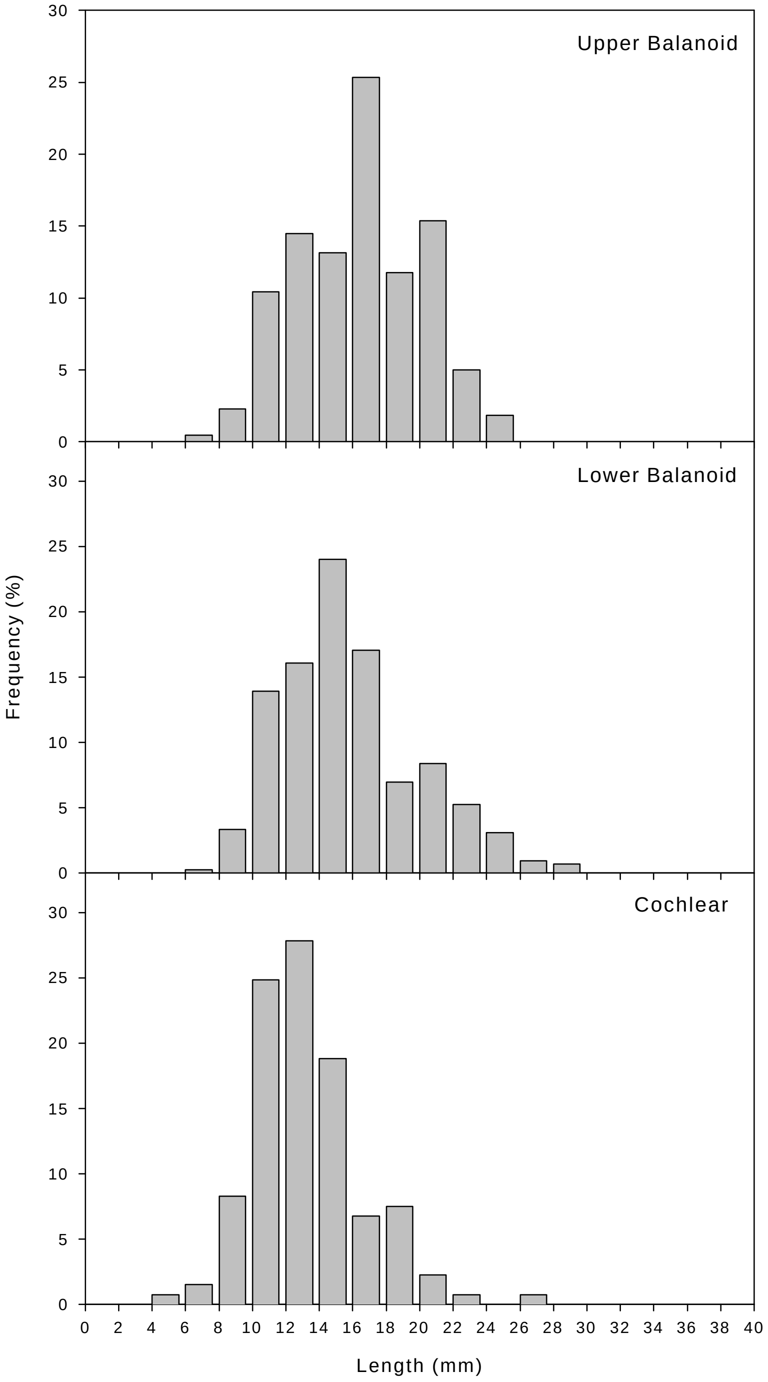


Figure 2.11:

Size-frequency distribution of *P. granularis* inhabiting three zones at Port Alfred.

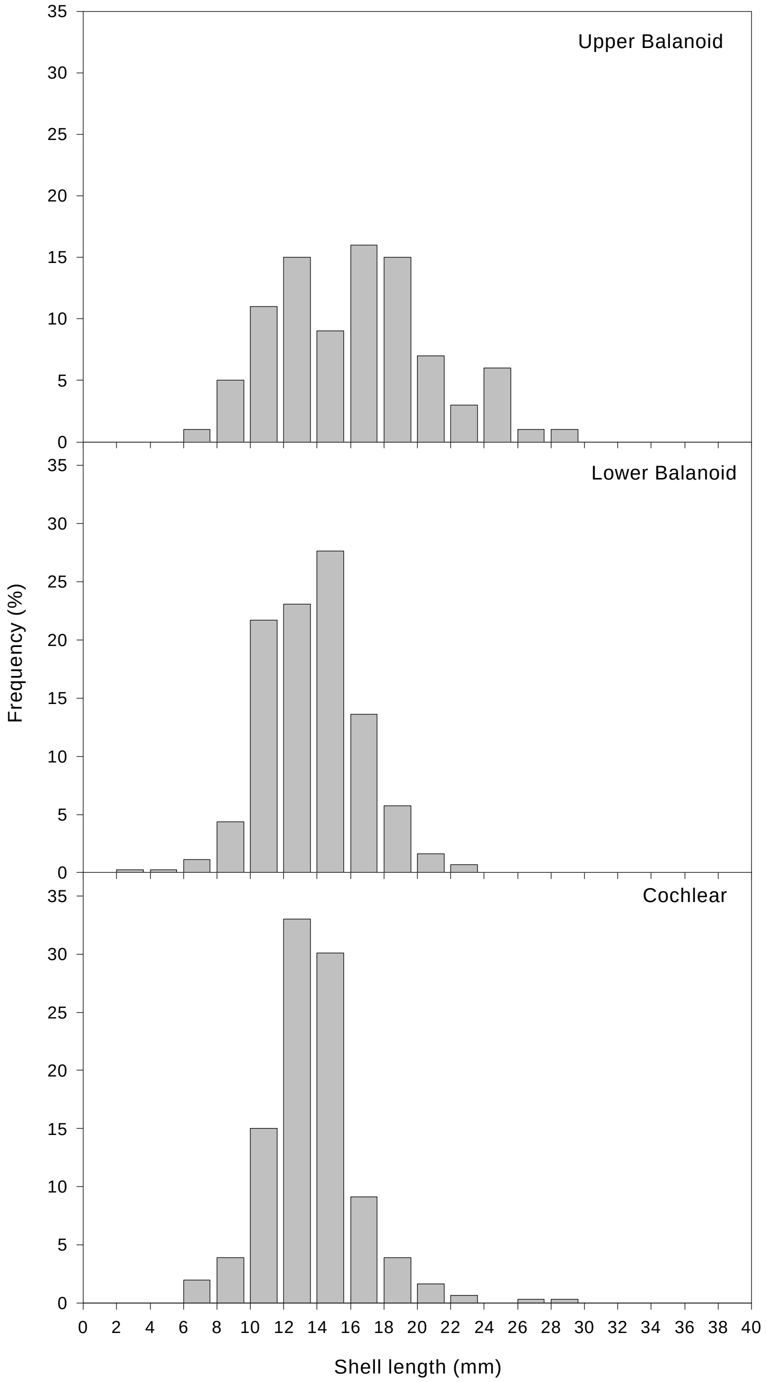


Figure 2.12:

Size-frequency distribution of *P. granularis* inhabiting three zones at Three Sisters.

2.3.5: Sex ratios and size at first sexual maturity:

Male and female *P. granularis* were found in all size classes at all localities (Figures 2.13 and 2.14), suggesting that this species does not change sex. At four of the six localities investigated, the sex ratios of *P. granularis* deviated significantly from a 1:1 ratio (Table 2.17), with males being more numerous on the aeolianite i.e. Kwaaihoek (1 **&** : 1.51 **%**), Kenton (1 **&** : 1.3 **%**) and Three Sisters (1**&** : 1.46 **%**). The sex ratios of *P. granularis* inhabiting quartzitic shores generally complied with a 1:1 ratio, although significantly more females than males were recorded at Port Alfred (1.23**&** : 1**%**; Table 2.17).

The onset of sexual maturity in *P. granularis* at all localities studied generally occurred at a shell length of below 12 mm (Table 2.17). Limpets inhabiting aeolianite reached sexual maturity slightly earlier (6 mm - 10 mm) than those from quartzitic sandstone (8 mm to 12 mm). Earliest sexual maturation was recorded in *P. granularis* from Kwaaihoek, while the latest onset of sexual maturity of *P. granularis* was recorded in the population inhabiting Port Elizabeth (Table 2.17).

Table 2.17:

Frequencies and ratios of male and female *P. granularis* inhabiting seven rocky shores along the south-east coast. Also shown are size at first sexual maturity (shell length when 50% of the population is sexually mature) and p - values obtained from a Chi-squared analysis to determine whether the population differed significantly from a 1:1 sex ratio. (* indicates significant p - values).

		First Sexual Maturity	**Frequency**		**Ratio**		**Chi²**
Locality	**n**		**Female**	**Male**	**Female**	**Male**	**p - value**
Port Elizabeth	311	10 - 12 mm	142	169	1.0	1.19	0.126
Cannon Rocks	397	8 - 10 mm	179	218	1.0	1.22	0.100
Kwaaihoek	609	6 - 8 mm	207	312	1.0	1.51	< 0.0001 *
Kenton-on-Sea	416	8 - 10 mm	181	235	1.0	1.3	0.008 *
Port Alfred	697	8 - 10 mm	384	313	1.23	1.0	0.007 *
Three Sisters	759	8 -10 mm	308	451	1.0	1.46	0.0001 *

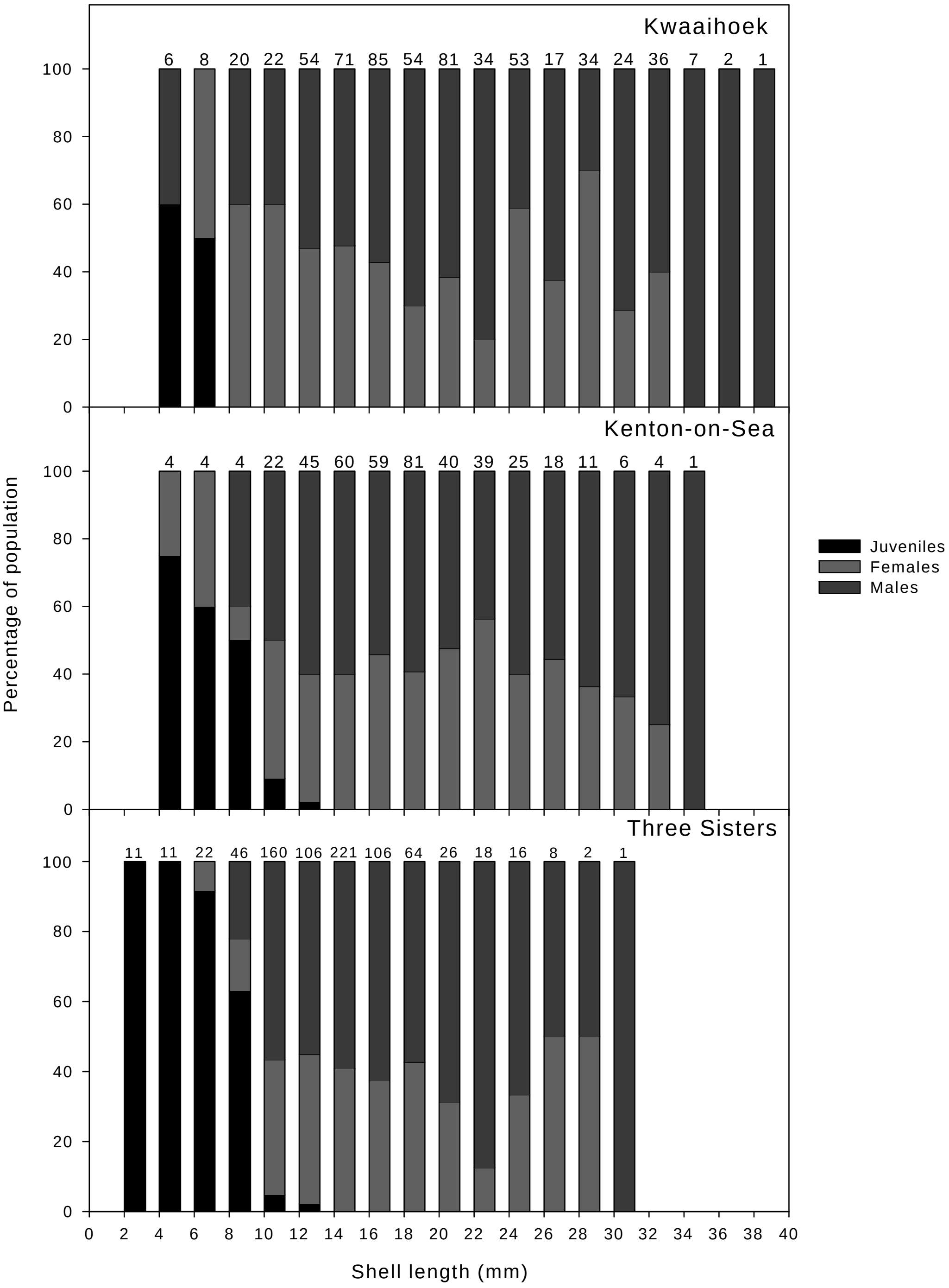


Figure 2.13:

Sex-length distribution of *P. granularis* inhabiting three aeolianite localities showing juvenile, female and male limpets. (Numbers above bars indicate sample sizes).

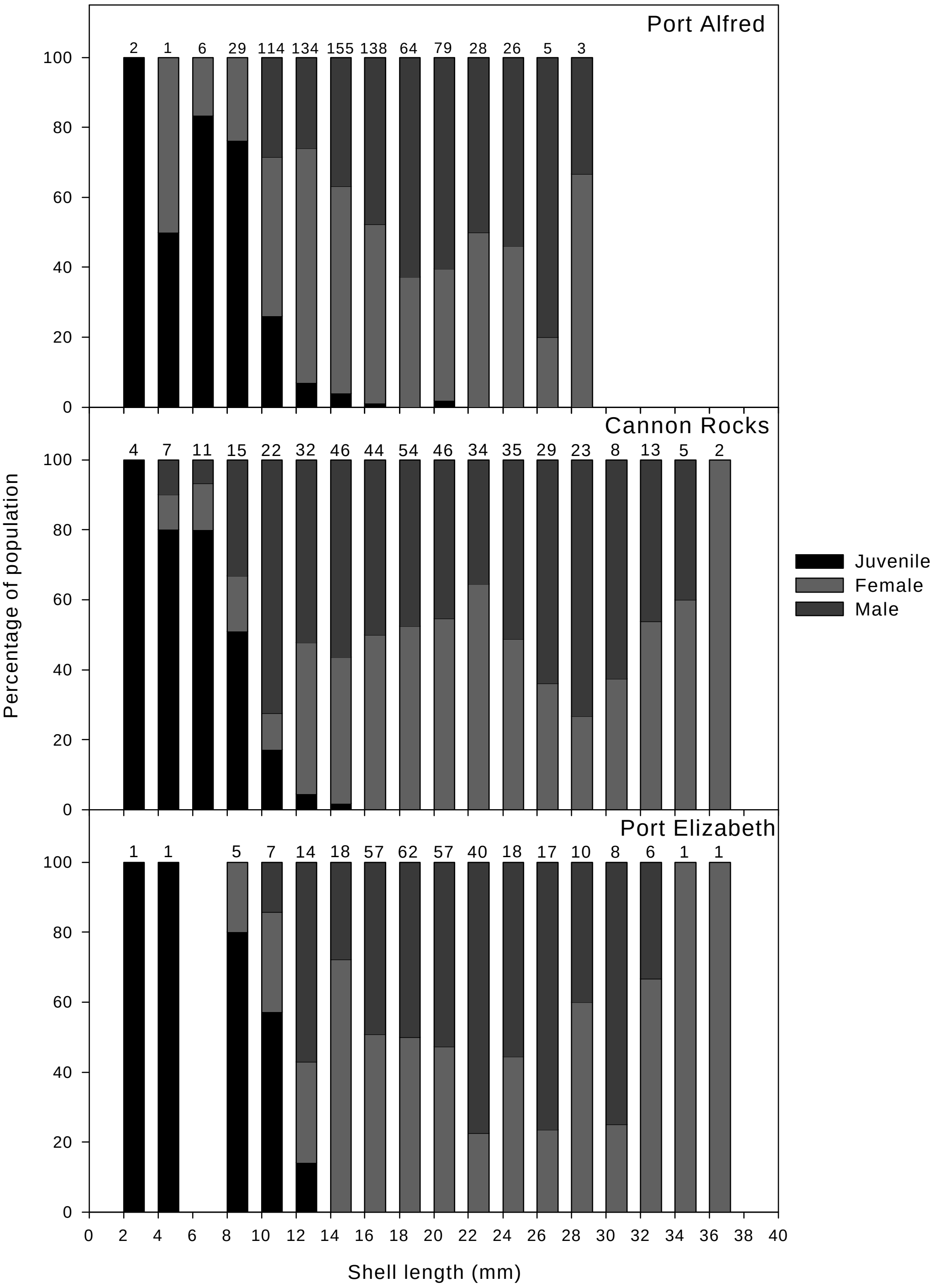


Figure 2.14:

Sex-length distribution of *P. granularis* inhabiting three quartzitic sandstone localities showing juvenile, female and male limpets. (Numbers above bars indicate sample sizes).

2.4: DISCUSSION:

The current study highlights the limitations of inferring the causal mechanisms that structure the population dynamics of intertidal communities from a single, one-off observation of population structure, density and biomass. It is important to realise that the current study, although conducted at a similar period in time at all seven localities, represents only a single moment of the lifespan of the respective populations. As such, the past history of the populations is unknown and thus the factors which structured these communities are similarly unknown. Despite these reservations, the results obtained from this study enable a comparison of the general population structure, density and biomass of *Patella granularis* inhabiting the respective localities to be made. Preliminary information on the population structure of this species from the south-east coast may then be obtained.

P. granularis inhabiting the south-east coast of South Africa has a widespread intertidal distribution, occurring from the upper Balanoid zone to the Cochlear zone. In this latter zone, although limpets can be found on bare rock, they are more common in mussel beds. *P. granularis* is also ubiquitous on both aeolianite and quartzitic sandstone shores.

Both the density and biomass of *P. granularis* were found to differ among the localities investigated. Generally, no positive correlation was found between density and biomass, although a relatively high biomass and density was recorded at both Port Alfred and Cannon Rocks. This is contrary to many studies which show that density and biomass are positively linked (Lasiak & White, 1993; Bustamante *et al.*, 1995a; Keough *et al.*, 1997). The population inhabiting Three Sisters was recorded to have a significantly high density (. 111 limpets/m^2), but this was not associated with a significantly high biomass(. 6.5 g/m^2). No discrete differences were observed in either the density or biomass of *P. granularis* inhabiting shores of different geomorphologies. However, when data were pooled for shores of differing geomorphologies, some differences were observed. The mean density of *P. granularis* was slightly, but not significantly, higher on aeolianite while the mean dry biomass of limpets was found to be higher on quartzitic sandstone. Once again, however, this was not significant. In addition to the observed differences in biomass and density, the size (i.e. mean shell length) of *P. granularis* differed significantly among the localities.

Despite these inter-locality differences, an overall difference in the characteristics of limpet populations exists. Generally, the mean shell length of *P. granularis* decreases around the coast of South Africa, with this species reaching maximum size along the Cape west coast (Stephenson, 1937; Kilburn & Rippey, 1982 ; T. Ridgway *et al.*, 1998). The results of the current study support these findings, with the mean shell length of *P. granularis* inhabiting the south-east coast only reaching approximately 23 mm as compared to the 40 mm previously recorded along the west coast (Hockey & Branch, 1984). This decrease in size is correlated to a decrease in primary productivity along the coast of South Africa (. 29 Fg chlorophyll-*a* cm^{-2} per month on the west coast as compared to . 15 Fg chlorophyll-*a* cm^{-2} per month on the south coast; Bustamante *et al.*, 1995b). The correlation between increased primary productivity and the increased length of *P. granularis* is further supported by the work conducted on *P. granularis* inhabiting west coast islands. The intertidal zones of islands which support large sea bird populations often have enhanced levels of primary productivity as a result of the fertilising effect of dissolved guano (Bosman & Hockey, 1988a). This results in an increased algal productivity on the islands and in turn increases the size of the grazers. (Bosman & Hockey, 1988a). In studies conducted on the west coast, the maximum shell length attained by *P. granularis* inhabiting islands was far greater than that reached by mainland limpets (. 97 mm vs. 47 mm; Hockey & Branch, 1984). A similar result was recorded in the present study, where the maximum size of *P. granularis* was recorded at Bird Island (. 46 mm). This island is home to a large colony of gannets and is also a breeding ground of Jackass penguins (pers. obs). The resultant increase in the productivity of the littoral may explain the increased size attained by *P. granularis* inhabiting this locality. A similar increase in the mean size of *Turbo sarmaticus* was also recorded at Bird Island, although this was also attributed to reduced exploitation of this gastropod on the island (Foster, 1997). Predation by oystercatchers has also been reported to influence the size of *P. granularis* inhabiting offshore islands, as these predators are highly selective when choosing the size of their prey (Hockey & Branch, 1984; Hockey & Underhill, 1984). The increased rate of growth of island-dwelling limpets as a result of increased primary productivity may ensure that an increased number of limpets are too large to be consumed by the oystercatchers. Thus, the size distribution of these limpet populations may be skewed in favour of larger limpets, which in turn results in the larger mean size recorded on the islands (Branch, 1985b).

Associated with the decrease in size between west and south-east coast populations is a decrease in biomass. On the west coast, the highest biomass of *P. granularis* is recorded in the mid-shore (40-120 g/m^2; Bosman & Hockey, 1988a). This is similar to the trends observed in the current study, where a mean of . 9 g/m^2 was recorded in the lower Balanoid zone. The values recorded on the south-east coast are similar to those previously recorded on an exposed shore in Port Elizabeth (9.5 g/m^2; Bustamante *et al.*, 1997). Although the biomass of many marine gastropods has been estimated using equations derived from shell measurements (e.g. Bruton *et al.,* 1991; Foster, 1997), the inter-locality differences of the current study suggested that this would be inappropriate for estimations of the biomass of *P. granularis* from Bird Island. Similarly, estimates of biomass have also been extrapolated from results obtained from a subsample of the population (Bosman & Hockey, 1988a,b). However, as no removal of limpets from this locality was permitted, the biomass of this population could not be determined in this manner. Thus the relationship between density and biomass in the island population is still unknown.

Studies of *P. granularis* conducted along the west coast have recorded a great deal of variability in the density of limpets inhabiting different sites (between 40 and 230 limpets/m^2; Bosman & Hockey, 1988a). Although a high degree of inter-locality variability was also evident in the present study, the density of *P. granularis* along the south-east coast is generally much lower, ranging from . 32 to . 111 limpets/m^2 with a mean density of . 69 limpets/m^2 being recorded for the seven localities examined. It thus appears that the increased primary productivity along the west coast supports a greater density of larger limpets (with an associated increased biomass). It is however, important to note that on both coasts, localities where the greatest shell lengths of *P. granularis* are reached are also the localities where the lowest densities are recorded (95 mm and 94 limpets/m^2 on the west coast - Hockey & Branch, 1984; 46 mm and 32 limpets/m^2 on the south-east coast - current study). Furthermore, on both coasts, these localities are islands. This suggests that on both the west and south-east coasts, populations of *P. granularis* are ultimately influenced by food availability, although biological factors such as a reduction in intraspecific competition may also be important.

Given the apparent importance of food availability in structuring the limpet populations, it is surprising that no trends in the population structure, density and biomass of *P. granularis* inhabiting aeolianite and quartzitic sandstone were evident. As microalgal abundance seems to be higher on aeolianite than quartzite (Chapter 7), an increase in the size of limpets on the aeolianite shores might be expected. However, this was not the case. A number of additional factors may influence intertidal populations. These include wave action (Etter, 1989; Trussel *et al.,* 1993; Bustamante *et al.*, 1997), increased competition (Branch 1975a,b), increased reproductive output which is associated with an decreased growth rate (see Branch, 1981 for examples), temperature (Branch, 1984), differential predation pressure (Marsh, 1986; Trussel *et al.*, 1993) and variable recruitment patterns (Lasiak & Dye, 1989). Although it was beyond the scope of the current study to test the influence of these factors on the populations of *P. granularis* inhabiting the seven localities investigated, the significantly high density and associated small size of limpets inhabiting Three Sisters warrants some discussion. It is possible that the significantly different population characteristics observed at this locality are a reflection of the bias a one-off sampling procedure may have on the results obtained. The small size of limpets may be the result of a particularly successful recruitment that occurred in this area. As such, this population may consist of predominantly younger limpets, however as no aging of limpets from this locality was conducted, this remains speculative. Alternatively, the anomalous situation at Three Sisters may be the result of human interference. Although the access to this area is restricted, it is a popular holiday resort. As a result the number of people utilising the area for recreation increases dramatically during holiday periods (pers. obs.). It is thus possible that the increased traffic at this locality results in an increase in trampling which has been reported to influence intertidal communities (Beauchamp & Gowing, 1982; Povey & Keough, 1991). This may thus influence the size structure of this community, with the resultant decrease in size enabling a reciprocal increase in density.

Marine gastropods often exhibit distinct zonation, with the size of upper-intertidal species increasing in an upshore direction, while the size of lower-intertidal species decreases in an upshore direction (Vermeij, 1972). The present study, in conjunction with observations made by other workers (e.g. Branch, 1971; Branch, 1975b) has shown that *P. granularis* exhibits a size gradient on the shore that is comparable to that of a high-shore species. It has been suggested that the upper limits of populations are limited by physical factors, while lower limits are constrained by biological factors (Paine, 1969; Connell, 1972). Differential growth rates and active migrations have also been proposed to explain size gradients within an intertidal community (Bertness, 1977; McQuaid, 1981a). According to Branch (1975b), the zonation patterns of the South African *Patella* species may be categorised according to the migratory behaviour of respective species. Limpet such as *P. cochlear*, *P. longicosta*, *P. tabularis* and *P. miniata* were considered to be non-migratory. These limpets settled in a narrow section of the shore, where they remained throughout their life. Other species such as *P. granularis*, *P. granatina*, *P. concolor* and *P. oculus* were categorised as migratory limpets which initially settled low on the shore, and migrated progressively upshore as they grew. These species were usually present in high densities on the shore and had a wide intertidal distribution. It was suggested that the migratory nature of these species was related to the increased tolerance of larger limpets to conditions of increasing desiccation stress. The mechanism responsible for this zonation was not investigated in the present study. However, it was noted that at all localities, most of the low-shore *P. granularis* were found inhabiting mussel shells, with very few limpets occurring on bare rock. Furthermore, most of these limpets were reproductively mature (Figures 2.13 and 2.14) and were thus not juveniles as suggested by Branch (1975b). Furthermore, the mean shell length of these limpets increased with an increase in tidal height, suggesting that along the south-east coast, space limitation is the mechanism responsible for the smaller size of *P. granularis* inhabiting the Cochlear zone.

Levels of food availability are also known to vary at different tidal heights (Nicotri, 1977; Underwood, 1984b). This may also be responsible for the zonation patterns evident in the current study as the growth rate of *P. granularis* is increased by enhanced levels of primary productivity (Bosman & Hockey, 1988a,b). Generally, primary productivity is thought to be lower in the high shore (Underwood, 1984a,b), although the current study shows that chlorophyll-*a* values at Cannon Rocks are similar in the lower Balanoid and Cochlear zones (Chapter 7). Although microalgal biomass of the high shore was not investigated in the present study, results from other workers suggest that chlorophyll-*a* levels in the upper Balanoid zones of the south-east coast are higher (Gray, 1996; S. Kaehler, pers. comm.). It is possible that, although greater, the microalgal biomass present at higher tidal levels are just adequate to support the reduced number of larger limpets present in the upper Balanoid zone. Both density and biomass of *P. granularis* peaked in the mid-shore. While the biomass of west coast *P. granularis* is also higher in the mid-shore, densities of these limpets are greatest low on the shore (Bosman & Hockey, 1988a). It is suggested that the trends observed in the present study are directly related to the size distribution of *P. granularis* on the shore, with the reduced densities of larger limpets in the high shore yielding lower biomass estimates, and the higher densities of smaller limpets in the mid-shore being responsible for the higher biomasses recorded in this area.

For each of the shores examined, there was no clear evidence of any size-related differences in the sexes. Thus *P. granularis* probably does not change sex. This supports the conclusions made by Branch (1974b) and is further strengthened by the fact that no hermaphrodites were observed during any of the histological analyses (Chapter 5). No clear trends in the relationship between the number of males and females were evident, with the number of males predominating at three of the localities investigated (Kwaaihoek, Kenton-on-Sea and Three Sisters; see Table 2.17). Females were only found to be numerically dominant at Port Alfred. Generally, a larger number of females is attributed to sex change in the species, differential growth or differential mortality between the sexes (Orton *et al.*, 1956; Branch, 1974b; Gray, 1996). As *P. granularis* does not change sex (Branch, 1974b; Chapter 5, current study), this cannot explain the observed sex ratios at Port Alfred. Similarly, differential growth resulting in a biassed sex ratio was not recorded at any of the other localities investigated, thus is unlikely to be acting on the Port Alfred population. It is possible that the increased number of females present at Port Alfred may be a result of increased mortality of males. However, as this was not tested, this suggestion is speculative. Sperm can be limiting in intertidal populations due to the high wave intensity in this environment (Levitan, 1995). Thus the increased number of males observed during this study is in keeping with this prediction. The size at first sexual maturity was also found to differ among the localities. Generally, the onset of sexual maturity was earlier in *P. granularis* inhabiting aeolianite shores than quartzitic shores (Table 2.15). It has been proposed that in limpets which reach a smaller maximum size, sexual maturity occurs at an earlier age

(Grahame & Branch, 1985), however, in the current study, no direct association between maximum shell length and the size at which sexual maturity occurs was evident. Similarly, no trend between the modal size class or the onset of sexual maturity occurred. As suggested in Chapter 5, it is possible that reproductive development in *P. granularis* is genetically entrenched, with the onset of sexual maturity linked not to size, but age. As the age structure of the populations from these localities was not investigated, it is not known whether this hypothesis could explain the current observations. However, studies linking the population structure of *P. granularis* inhabiting different localities around the South African coast to growth (and thus age) could be particularly enlightening.

As one of the aims of the current study was to identify a suitable site/sites for long-term reproductive and growth monitoring of south-east coast populations of *P. granularis*, the inter-locality variability in population structure, density and biomass observed are particularly relevant. In light of these differences, it was decided to include one aeolianite and one quartzitic sandstone shore in the investigations. Furthermore, as limpets inhabiting the mussel shells were consistently and significantly smaller (Figures 2.6 - 2.12) than those occurring higher up the shore, it was deemed appropriate to also include these limpets in the long-term monitoring. The localities chosen for these studies are discussed in greater detail in Chapter 4.

Although *P. granularis* is widespread both around the coast of South Africa and within the intertidal, it is evident that the factors influencing the abundance, distribution and population structure of this species are complex, and cannot be inferred from the limited studies conducted to date. It is suggested that while *P. granularis* from the west coast may indeed be “migratory”, settling in the low-shore and moving progressively upshore with an increase in size (Branch, 1975b), their counterparts on the south-east coast are not. This hypothesis is tested in later chapters. It appears that limpets inhabiting the south-east coast have adopted different mechanisms for coping with adverse physical conditions, such as differential growth rates and shell shape modifications. The current study emphasizes the localised effect of locality-specific variability in population structure, biomass and density. This suggests that the single locality investigations of the life-history parameters and aspects of population dynamics that have traditionally been conducted along the south-east and east coasts of South Africa (e.g. Gray, 1996;

Foster, 1997; Henninger, 1998) are inadequate. Thus generalisations regarding the factors influencing the population dynamics of widespread species cannot knowingly be made. More detailed analysis on individual populations and their unique conditions are thus required. The results of this study suggest that in order for comparisons of south-east and west coast populations of *P. granularis* to be made, investigations of the growth and reproduction of this species along the south-east coast will need to be conducted at more than one locality.

Chapter 3

Chapter 3

ALLOZYME ELECTROPHORESIS OF THREE POPULATIONS OF *PATELLA GRANULARIS* FROM THE SOUTH-EAST COAST OF SOUTH AFRICA

3.1: INTRODUCTION:

The intertidal habitat is a highly unpredictable one which provides a great deal of variation in the physical environment. As a result, organisms inhabiting this region can develop a wide range of morphological adaptations to the prevailing conditions. Limpets, in particular, exhibit a large degree of morphological plasticity (Powell, 1973; Branch, 1981), with phenotypic traits, although partly under genetic control, being greatly influenced by environmental factors (Janson, 1982). Such factors include tidal height (Lewis & Bowman, 1975), wave action (Thompson, 1979) and food availability (Bosman & Hockey, 1988a,b). Morphological differences and similarities in many intertidal invertebrate species are frequently in conflict with results obtained from molecular studies e.g. *Littorina* (Johannesson & Johannesson, 1989; Mill & Grahame, 1992) and *Patella* (Côrte-Real *et al.*, 1996a,b; T. Ridgway *et al.*, 1998). Morphological characters, on which molluscan taxonomy has traditionally been based, are thus of dubious value when considered in isolation (Côrte-Real *et al.*, 1996a,b).

Marine organisms generally employ one of two reproductive dispersal strategies. Species may have direct developing larvae which disperse by either crawling away from the parents/egg cases or by having a short-lived pelagic phase. Alternatively, species may have long-life planktotrophic or lecithotrophic pelagic larvae (Grahame & Branch, 1985). Species that are reliant on the first mode of reproduction are often restricted on a local scale and as such may be geographically isolated. Genetic differentiation in these species is likely to be a response to local selective pressures (Burton, 1983). Broadcast spawners on the other hand, have a greater potential for dispersal and gene flow (Burton, 1983). Thus the mode of reproductive dispersal is predicted to have a definitive effect on the degree of genetic differentiation amongst populations (Ward, 1990). It is predicted that a planktonic larval stage will increase the homogeneity of populations that may be separated by vast distances, while non-pelagic larvae will restrict gene flow and encourage local differentiation of species (Janson, 1987). This theory is supported in a number of studies particularly with regards to *Littorina* spp. (See Ward, 1990, for a review of literature). A similar correlation between genetic differentiation and dispersal capabilities have also been shown for starfish (Hunt, 1993), limpets (Johnson & Black, 1982; 1984) and sea anemones (Russo *et al.*, 1994). The correlation of genetic variability with dispersal capability is, however, not simple. Contradictory studies, where high genetic heterogeneity occurs in populations with pelagic larvae and increased levels of homogeneity are evident in populations with direct developing larvae, are numerous (e.g. O'Foighil, 1989; Benzie & Williams, 1995).

Localised heterogeneity is also widely influenced by habitat, implying that local populations become adapted to local conditions (Noy *et al.*, 1987). An increase in genetic diversity has been shown to be related to an increase in the ecological niche inhabited by a species (Noy *et al.*, 1987). Furthermore, an increase in heterogeneity is assumed to convey an adaptive advantage to less mobile species as these individuals will then be able to develop different morphs for different habitats. Mobile species, on the other hand, will be exposed to a highly variable environment. Homogeneity for the best overall strategy is an advantage here (Selander & Kaufman, 1973).

Patella granularis is a broadcast spawner (Branch, 1974b) with a distribution extending around the coast of southern Africa (Branch, 1971; Branch *et al.*, 1994). This species shows a marked variation in shell size and morphology throughout its range (Koch, 1949; Kilburn & Rippey, 1982), with the variations being correlated to different oceanic conditions (Bustamante *et al.*, 1995b). It also displays a great deal of shell plasticity in a range of localised habitats (see Chapter 4). Although phenotypic differences may be a result of environmental conditions, they are often a reflection of genetic differences (Cook, 1983; Green *et al.*, 1983). To date, only one study has analysed the population structure of *P. granularis* by combining both morphometric and genetic data (T. Ridgway *et al.*, 1998). This study was conducted on a large-scale, involving populations from around South Africa. The results refuted previous assumptions that *P. granularis* could be divided into distinct groupings according to morphology alone. The genetic analysis did, however, separate *P. natalensis* in Northern Natal from *P. granularis.* Although T. Ridgway *et al.* (1998) showed that the population of *P. granularis* from the rest of the South African coast is genetically homogenous on a large scale, this cannot be taken as an indication that small scale genetic differentiation does not occur. In 1978, Levinton and Suchanek showed that the scale at which studies are conducted can bias results. Given the mode of reproduction of *P. granularis*, one would expect a large degree of genetic homogeneity to occur among populations spread over a large geographical distance. However, considering the morphological differences exhibited by localised populations of *P. granularis* (Chapter 2), it was deemed prudent to investigate whether these phenotypic differences were a result of high heterozygosities.

The aims of the current study were, therefore, to determine whether the observed morphological differences in individuals of *P. granularis* inhabiting three sites with different substrata (i.e. aeolianite, quartzitic sandstone and mussel shells) are a function of morphological adaptation of one species to a localised habitat, or are a phenotypic expression of genetic diversity. For the purposes of this chapter, individuals from each habitat will be considered to be representative of different populations.

3.2: MATERIALS AND METHODS:

3.2.1: Collection of samples:

30 specimens of *Patella granularis* were collected from each site (see Chapter 4 for detailed descriptions of the study sites). The limpets were transported on ice to the South African Museum in Cape Town where they were stored at -80EC pending electrophoretic analysis. All analyses were carried out in the museum's electrophoresis laboratory under the guidance of Dr. Barbara Stewart.

3.2.2: Electrophoresis:

Genetic variation at 16 allozyme loci from each of the three populations of *P. granularis* was examined using standard horizontal starch gel electrophoresis (see Harris & Hopkinson, 1977). Foot muscle and mantle tissue was processed separately for each individual. Preparation for electrophoresis was carried out by roughly mincing the tissues using a scalpel, before homogenisation in cold 0.01M Tris buffer (pH 8.0). The homogenate was then centrifuged at 2500 rpm for five minutes. Three buffer systems were used for the electrophoresis, namely Tris-citrate-lithium hydroxide-borate buffer, pH 8.0 (Ridgeway *et al.*, 1970), Tris-borate-EDTA buffer, pH 8.6 (Markert & Faulhaber, 1965) and Tris-citrate buffer, pH 6.9 (Whitt, 1970). All gels were prepared with 12.5% hydrolysed potato starch and were run at a constant current of 30 mA for between 4 - 5 hours. Each gel was then sliced into 4 slices, which were then stained for the different enzymes using specific histochemical stains as prescribed by Shaw and Prasad (1970) and Harris and Hopkinson (1977). Details of the enzymes screened including abbreviations, enzyme commission numbers (as suggested by Harris and Hopkinson (1977) in line with the International Union of Biochemistry Nomenclature committee 1984), buffer systems used and tissues sampled are shown in Table 3.1, with sample sizes for each locus shown in Table 3.2.

3.2.3: Data analysis:

Analysis on the electrophoretic data was performed using the programme BIOSYS-1 release 1.7 (Swofford & Selander, 1981). Allele frequencies, mean heterozygosities, and levels of polymorphism were calculated for each population. Departures of genotype frequencies from Hardy-Weinberg equilibrium were measured, with the small sample sizes being taken into account by Levene's (1949) test. Fisher's Exact Probability Test was also carried out on the data to confirm any significant deviations from Hardy-Weinberg equilibrium. In this test, deviations are expressed using Selander's (1970) index of homozygote excess (*D*), where negative *D* values indicate a deficiency of heterozygotes and positive values an excess of heterozygotes. The level of genetic differentiation among the three populations was examined using fixation indices. F_{IS}, F_{IT} and F_{ST} (Nei, 1977) were calculated from the genotype data. The level of gene flow among the populations was measured using the following formula:

$$Nm = (1/F_{ST} - 1)/4$$

(Slatkin, 1987).

This value estimates the number of individuals that migrate between populations within one generation. A value that exceeds 1 indicates that gene flow is occurring. Finally, a matrix of Nei's (1978) unbiased genetic identity (*I*) and genetic distance (*D*) was calculated from the allele frequencies.

3.3: RESULTS:

The allele frequencies for the 16 loci detected in the three populations of *P. granularis* analysed are listed in Table 3.2. Eleven of these loci were consistently monomorphic for all populations. Of the remaining five loci, GPI-1, GL-2, LGG-1 and PGM-1 were polymorphic in all populations, with the number of alleles per locus ranging from two to four. The fifth locus, ME-2, only exhibited polymorphism (two alleles) in the population inhabiting aeolianite. The mean number of alleles per locus ranged between 1.3 in the mussel bed to 1.4 at the other two populations, with the highest degree of polymorphism occurring in individuals from the aeolianite platform (31.3%, Table 3.3). The aeolianite population also shows the highest value of mean heterozygosity (0.061).

From the results of tests for Hardy-Weinberg equilibrium, it was clear that of the 13 cases of polymorphism encountered at all loci in all populations, 4 loci exhibited significant deviations ($\div^2$, $p < 0.005$) from Hardy-Weinberg equilibrium (using the 0.99 criterion). In the aeolianite population, the frequencies of ME-2 and PGM-1 deviated significantly from the expected frequencies under both the $\div^2$ (Table 3.4) and the Fisher's Exact Probability Tests (Table 3.5). The population from the mussel shells also exhibited significant deviations in the GL-2 genotype under both tests, but only the frequency of LGG-1 deviated under the $\div^2$ test (Table 3.4). The population inhabiting quartzitic rocks also exhibited deviations in the genotype frequencies of GL-2, however these deviations were only recorded in the $\div^2$ test (Table 3.4). Table 3.4 shows Selander's (1970) index of homozygote excess for the polymorphic loci. From this, it is clear that in all the genotypes that deviate significantly from Hardy-Weinberg equilibrium, these deviations were as a result of a deficiency in the number of heterozygotes.

Nei's (1978) genetic identity (*I*) and distance (*D*) for the three populations are shown in Table 3.6. Overall identity values were very similar ranging from 0.998 to 1.000. Genetic distance values were also very similar in the three populations with the range being from 0.000 to 0.002.

Fixation indices provide a convenient method by which the levels of genetic differentiation among populations can be understood. For the purposes of the current study, the level of genetic differentiation among the populations (F_{ST}) is of the most interest. This value provides a quantitative estimation of the level of variation of allele frequencies among populations. It is evident from the very low mean F_{ST} value (0.021), that the allele frequencies in the three populations of *P. granularis* do not differ much (Table 3.7). This is supported by the values of F_{IS} and F_{IT} which are an order of magnitude higher (0.250 and 0.266 respectively - Table 3.7) illustrating a great deal of interbreeding occurring within the three populations. This assumption is further strengthened by an Nm value equivalent to 11.65.

Table 3.1:

Enzymes, E.C. numbers, locus abbreviations, buffer systems and tissue types used. (a) = Tris-citrate-lithium hydroxide-borate buffer; (b) = Tris-borate-EDTA buffer and (c) = Tris-citrate buffer

Enzyme (Abbreviation)	E.C. number	Locus	Buffer System	Tissue
Arginine kinase (ARK)	2.7.3.3	ARK-2	(a)	Foot
Glucose-6-phosphate (GPI)	5.3.1.9	GPI-1	(a)	Foot
Phosphoglucomutase (PGM)	2.7.5.1	PGM-1	(a)	Foot
Sorbitol dehydrogenase (SDH)	1.1.1.14	SDH-1	(a)	Foot
Superoxide dismutase (SOD)	1.15.11	SOD-1	(b)	Mantle
Malic enzyme (ME)	1.1.1.40	ME-1	(b)	Mantle
		ME-2	(b)	Mantle
Peptidase - Glycyl-leucine (GL) as substrate	3.4.-.-	GL-2	(b)	Foot
Peptidase - Leucyl-glycyl-glycine (LGG) as substrate	3.4.-.-	LGG-1	(b)	Foot
Peptidase - Phenylalanine-proline (PHP) as substrate	3.4.-.-	PHP-1	(b)	Foot
Hexokinase (HEX)	2.7.1.1	HEX-1	(b)	Foot
Isocitrate dehydrogenase (IDH)	1.1.1.42	IDH-2	(c)	Mantle
Aspartate amino transferase (GOT)	2.6.1.1	GOT-1	(c)	Mantle
Malate dehydrogenase (MDH)	1.1.1.37	MDH-1	(c)	Mantle
		MDH-2	(c)	Mantle

Table 3.2:

Distribution of allele frequencies at 16 loci in 3 sub-populations of *P. granularis*. (N) = sample size.

Locus	**Aeolian sandstone**	**Quartzitic sandstone**	**Mussel shells**
ARK-1			
(N)	30	30	30
A	1.000	1.000	1.000
ARK-2			
(N)	30	30	30
A	1.000	1.000	1.000
GPI-1			
(N)	30	30	22
A	0.783	0.900	0.955
B	0.117	0.067	0.000
C	0.000	0.017	0.000
D	0.100	0.017	0.045
SDH-1			
(N)	30	30	30
A	1.000	1.000	1.000
SOD-1			
(N)	30	30	30
A	1.000	1.000	1.000
ME-1			
(N)	30	30	30
A	1.000	1.000	1.000
ME-2			
(N)	30	30	30
A	0.967	1.000	1.000
B	0.033	0.000	0.000
GL-2			
(N)	30	30	25
A	0.983	0.933	0.920
B	0.017	0.067	0.080
LGG-1			
(N)	30	30	25
A	0.917	0.950	0.920
B	0.083	0.050	0.080
PHP-1			
(N)	30	30	30
A	1.000	1.000	1.000
HEX-1			
(N)	30	30	30
A	1.000	1.000	1.000
MDH-1			
(N)	30	30	30
A	1.000	1.000	1.000
MDH-2			
(N)	30	30	30
A	1.000	1.000	1.000
IDH-2			
(N)	30	30	30
A	1.000	1.000	1.000
GOT-1			
(N)	30	30	30
A	1.000	1.000	1.000
PGM-1			
(N)	27	30	28
A	0.796	0.900	0.911
B	0.074	0.017	0.036
C	0.130	0.083	0.054

Table 3.3:

Genetic variability at 16 loci in three populations of *P. granularis*. S.E. are in parentheses.

Population	Mean sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic	Mean Heterozygosities	
				Direct count	HdyWbg expected
Aeolianite	29.8 (0.2)	1.4 (0.2)	31.3	0.049 (0.028)	0.061 (0.031)
Quartzitic	30 (0.0)	1.4 (0.2)	25.0	0.027 (0.013)	0.037 (0.017)
Mussel Shells	28.8 (0.6)	1.3 (0.2)	25.0	0.022 (0.013)	0.035 (0.016)

Table 3.4:

Deviations from Hardy-Weinberg equilibrium measured by Chi-squared test in three populations of *P. granularis*. (No polymorphism was found in ME-2 in either the quartzitic sandstone population or the mussel shell population). (* indicates a significant p - value).

Population	GPI-1	ME-2	GL-2	LGG-1	PGM-1
Aeolianite	0.855	< 0.001*	1.000	0.069	< 0.001*
Quartzitic	0.180	-	0.003 *	0.815	0.193
Mussel shells	0.876	-	< 0.001*	0.008*	0.976

Table 3.5:

Deviations from Hardy-Weinberg equilibrium measured by Fisher's Exact Probability Test and expressed by Selander's (1970) index of homozygote excess in three populations of *P. granularis.* (No polymorphism was found in ME-2 in either the quartzitic sandstone population or the mussel shell population). (* indicates a significant deviation from Hardy-Weinberg equilibrium)

Population	**GPI-1**	**ME-2**	**GL-2**	**LGG-1**	**PGM-1**
Aeolianite	0.084	-1.000 *	0.000	0.073	-0.471 *
Quartzitic	-0.291	-	-0.473	0.035	-0.283
Mussel shells	0.024	-	-1.000 *	-0.467	0.054

Table 3.6:

Matrix of Nei's (1978) genetic identity (above diagonal) and genetic distance (below diagonal) averaged over 16 loci in 3 groups of *P. granularis.*

Group	**1**	**2**	**3**
1 Aeolianite	*****	0.999	0.998
2 Quartzitic sandstone	0.001	*****	1.000
3 Mussel shells	0.002	0.000	*****

Table 3.7:

Summary of F-statistics at all polymorphic loci for three populations of *P. granularis.*

Locus	**$F_{(IS)}$**	**$F_{(IT)}$**	**$F_{(ST)}$**
GPI-1	0.016	0.055	0.039
ME-2	1.000	1.000	0.022
GL-2	0.672	0.676	0.014
LGG-1	0.122	0.125	0.003
PGM-1	0.283	0.295	0.018
Mean	0.250	0.266	0.021

3.4: DISCUSSION:

All three populations of *Patella granularis* are very similar at the gene-enzyme level. The overall genetic identity (*I*) value of 0.998 is particularly high, indicating a high level of genetic similarity amongst the three populations. This falls well within the range previously found for *P. granularis* (0.982 - 1.000; T. Ridgway *et al.*, 1998). The values recorded in this study are higher than both the 0.797 and 0.851 which separate populations of *P. miniata* from *P. sanguinans* and *P. compressa* from *P. miniata* respectively (T. Ridgway *et al.*, in press). Similarly, the *I* value of 0.998 is much greater than the 0.576 found separating *P. candei* from *P. caerulea* and *P. depressa* (Côrte-Real *et al.*, 1996b) and the 0.528 separating *P. granularis* and *P. natalensis* (T. Ridgway *et al.*, 1998). The *I* value of 0.998 is also much higher than the range of 0.35 and 0.85 that Thorpe (1982) suggested was indicative of congeneric species. The three populations inhabiting the different substrata are therefore the same species. A further criterion that is widely used to delineate allopatric species is the presence of fixed allele differences among loci of different populations. This indicates a barrier to dispersal (T. Ridgway *et al.*, 1998). Fixed allele differences between *P. granularis* and *P. natalensis* occurred at the ARK-2, GL-2, PHP-1 and MDH-1 alleles (T. Ridgway *et al.*, 1998). No fixed allele differences were found at any loci in the present study, indicating free gene flow among the three populations of *P. granularis.*

The level of polymorphism among the three populations (25% - 31.3%) is higher than the 17% previously recorded for *P. granularis* (T. Ridgway *et al.*, 1998) but is in keeping with the levels of polymorphism found for other *Patella* species (Côrte-Real *et al.*, 1996a,b). However, the values are very low when compared to other South African limpet species eg *Helcion penctunculus*, *H. pruinosus* and *H. dunkeri* which all showed a level of polymorphism of 81% (Weber *et al.*, 1997). The degree of polymorphism has been shown to be correlated to habitat, with greater polymorphism being found in individuals inhabiting a more variable environment (Cook, 1983). It is unlikely that this theory could explain the low polymorphism observed in the present study as the intertidal environment that the three populations inhabit is variable both temporally and spatially. The low levels of polymorphism are more likely to be a function of the large amount of gene flow occurring between the populations.

The four polymorphic loci (GPI-1, GL-2, LGG-1 and PGM-1) shared by all three populations showed no differences in allele frequencies among the populations. However, an interesting difference was evident in the ME-2 locus with this locus only exhibiting polymorphism in the aeolianite population. According to Black and Johnson (1981), species inhabiting a broad spectrum of habitats may survive in one of three ways. Firstly, genotypes suited to only some specific environments may encounter heavy selective mortality; secondly, genotypes may behaviourally select their preferred habitat either at settlement or following metamorphosis and thirdly, broadly tolerant genes may be selected. The presence of a locus that is polymorphic only in one population suggests that some post-settlement selection is occurring (i.e. strategy 1) at this locus in this particular population. The present study cannot, however, suggest the mechanism of this selection.

Although heterozygote deficiencies are well documented in many bivalves (Tracey, *et al.*, 1975; Mallet *et al.*, 1985), these deficiencies do not occur in the South African brown mussel, *Perna Perna* (Grant *et al.*, 1992) and are uncommon in other marine invertebrates (Ward, 1990). A number of reasons have been proposed for the presence of heterozygote deficiencies including the Wahlund effect, null alleles and non-random mating (Gaffney *et al.*, 1990). Recently, post-settlement selection against heterozygotes has been proposed as the mechanism whereby deficiencies occur (Beaumont, 1981). Although some heterozygote deficiencies were found in the present study, this level is low, with only four of the loci being significantly different to Hardy-Weinberg expectations. This small deficiency is unlikely to be a product of any of the above processes, but is probably only a result of a high level of genetic mixing.

The mean heterozygosities among the three populations of this study are very low (0.035 - 0.061). Values obtained for other *Patella* species range from 0.167 to 0.255 (Cretella *et al.*, 1994) whilst those for *Helcion* species in South Africa vary from 0.202 to 0.341 (Weber *et al.*, 1997). Generally, average heterozygosity is higher in species inhabiting a broad environment (Nevo *et al.*, 1984). This is true for the Tyrrhenian species of *Patella* (Cretella *et al.*, 1994), but does not occur in *P. vulgata* from south-east England (Gaffney, 1980). The low values established in the present study also refute the niche-width hypothesis (an increase in genetic diversity is related to an increase in the ecological niche inhabited - Noy *et al.,* 1987) as *P. granularis* inhabits a wide range of habitats and as such should be expected to display high heterozygosities. The greater mean heterozygosity in the aeolianite population is mainly as a result of the increased heterozygosities of GPI-1 and PGM-1 which are almost double the values from the other two populations. The implications of this observation are, at present, unknown.

The average F_{ST} value calculated for all samples (0.021) further confirms the low level of genetic differentiation amongst these populations. This value is similar to those obtained for other marine invertebrate species with pelagic larvae (Ward, 1990; Watts *et al.*, 1990) being especially close to the value obtained by Hurst and Skibinski (1995) for *P. vulgata* (0.027). The particularly low F_{ST} value obtained in the present study reiterates that there is very little genetic variation among the three populations. The estimate of Nm derived from this low F_{ST} value is also in agreement with the predictions based on the mode of reproduction of *P. granularis.* Although the period of time spent in the water column by the larvae is unknown in this species, the large degree of genetic homogeneity evident along the coast of South Africa suggests a similar time frame as for *P. vulgata* (10 - 14 days, Bowman, 1985). This larval period would provide opportunity for the suggested level of gene flow to occur among populations (**.** 12 individuals per generation).

In 1980, Gaffney proposed a hypothesis whereby morphological differences and genetic similarities in *P. vulgata* could be explained. According to this hypothesis, an increase in morphological variability in a species may result from that species inhabiting a marginal or sub-optimal environment. Although extensive gene flow would prevent changes in gene frequency from occurring, this gene flow would not prevent an increase in morphological variability. Although *P. granularis* is found all along the coast of South Africa, it is well documented that the conditions along the west coast are more favourable for this species, as evidenced by the increase in food availability (Bustamante *et al.*, 1995b) and the increased sizes attained by individuals of *P. granularis* (Bosman & Hockey, 1988b). The current allozyme data conclusively shows that despite the observed morphological differences, the three populations inhabiting the different substrata investigated in the present study are all part of a single population of *P. granularis.*

These three populations form a common breeding group as evidenced by the high genetic identity values, the low levels of heterozygosities and polymorphisms as well as the low F_{ST} values. Under these conditions, it is possible that Gaffney's hypothesis holds true for *P. granularis* as well as *P. vulgata*.

In conclusion, although a great deal of morphological plasticity has been recorded for the populations of *P. granularis* investigated in the current study (see Chapter 4), the results of the allozyme electrophoresis presented here indicates that very little genetic differentiation exists among the populations inhabiting the three substrata. As a result, it is most likely that the observed morphological differences are a habitat adaptation within one species and are not governed by genetic differences.

Chapter 4

Chapter 4

GROWTH RATES OF *PATELLA GRANULARIS* FROM THREE SITES ALONG THE SOUTH-EAST COAST OF SOUTH AFRICA

4.1: INTRODUCTION:

Many marine gastropods show seasonal variation in growth rates, both within and between species (Vermeij, 1980). Although some intraspecific variation may be genetically controlled (Janson, 1982), exogenous factors such as changes in food availability (McQuaid, 1981b; Bosman & Hockey, 1988a,b), wave action (Branch & Marsh, 1978; Thompson, 1979; Brown & Quinn, 1988) and position on the shore (Lewis & Bowman, 1975) greatly influence growth rate. In addition, intra- and interspecific competition can also affect growth (Branch, 1981). The growth rate of shelled marine molluscs is usually measured as an increase in shell length and a variety of methods have been used to estimate the growth rates of limpets. These include periodic monitoring of marked individuals (Branch, 1974a; Gray, 1996; Henninger, 1998), checks in growth as a result of known changes in environmental condition (Vahl, 1971), cohort analysis (Branch, 1974a; Underwood, 1975) and the use of internal shell bands (Ekaratne & Crisp, 1982; Crisp *et al.*, 1990; Gray, 1996; Henninger, 1998). All these methods have their advantages as well as inherent disadvantages.

Shell shape and texture are also important morphological features that are often correlated with physical conditions (Branch, 1985b). Generally, desiccation and wave action appear to exert the most influence on the morphological characteristics of intertidal organisms (Tatarenkov & Johannesson, 1994). Allometric growth, where shell height increases more rapidly than shell length, is common in high-shore limpets (Branch, 1985b). The rate of water loss in these animals is lower than in limpets which grow isometrically (i.e. shell length increases more rapidly than shell height - Branch, 1981; 1985b). Shell texture may also play a role in thermoregulation. Limpets inhabiting areas which are, to a large degree, protected from the sun tend to have smooth shells while those exposed to direct sunlight are sculptured (Vermeij, 1973). Textured shells increase re-radiation of heat and in doing so, reduce heat stress (Branch & Branch, 1988). Morphological features which reduce the effect of turbulence and increase the tenacity of the organism are a result of exposure to stronger wave action (Janson, 1982; Newkirk & Doyle, 1975). A number of biotic factors such as predation (Boulding & Van Alstyne, 1993), parasitism and competition (Sundberg, 1988) can also affect the morphometrics of a population. Morphometric analysis is a tool utilised to describe the form of organisms (James & McCulloch, 1990) and is often used to examine the relationships of populations (Borges, 1995). As intertidal limpets exhibit a great morphological plasticity (Branch, 1981), the use of this technique to investigate morphological differences between limpets occupying a variety of habitats may yield interesting results.

A number of different biogeographic regions occur along the coast of South Africa. The demarcations of these regions are usually governed by the different oceanographic conditions which influence different areas of the coastline (Branch & Branch, 1988; Chapter 2 of this study). Furthermore, these differing oceanographic conditions also result in a higher degree of primary productivity along the west coast than the south and east coasts (Branch & Branch, 1988; Bustamante *et al.* 1995b). Although a number of limpet species occur around the coast of South Africa (Branch, *et al.*, 1994) and thus inhabit all biogeographic regions, very little is know about how these species are influenced by the differing oceanographic conditions. To date, most growth studies have been conducted on the west coast limpet species (e.g. Branch, 1974a; Bosman & Hockey, 1988a,b). It is only recently that the growth rates of south and east coast species have been investigated (see Robson, 1986; Gray, 1996; Henninger, 1998).

Patella granularis is the most widespread of the South African limpets (see Chapter 2). As such, the biology and ecology of this species is likely to be influenced by the different biogeographic regimes that it experiences along the coast of South Africa. Indeed, individuals of this species are documented as being smaller along the south and east coasts when compared to those individuals inhabiting the west coast (Branch, 1971; Bustamante *et al.*, 1995b). Furthermore, *P. granularis* has a widespread intertidal distribution, with the mean size of these limpets increasing up the shore (Branch, 1971; 1975b; Chapter 2). As already discussed (see section 2.4), a number of factors may be responsible for this zonation. Branch (1975b) suggested that this pattern was a result of the migratory behaviour of *P. granularis.* According to this theory, juvenile *P. granularis* settle low on the shore and migrate progressively up shore. This migration results in a size gradient on the shore, with the larger limpets that can tolerate greater desiccation stress occurring higher on the shore. To date, knowledge of the key parameters of the population dynamics of *P. granularis*, including growth, have resulted from studies conducted exclusively along the Cape west coast and the Cape peninsula (Branch, 1974a,b; Bosman & Hockey, 1988a,b).

This study aimed to establish whether the growth rates of *P. granularis* from the south-east coast of South Africa differed to those of the same species inhabiting the west coast (as described by Branch, 1974a). As locality has been shown to affect the population structure, biomass and density of this species (Chapter 2), it was decided to conduct this study on two shores of differing geomorphologies, but with a similar biomass and density of *P. granularis*. In addition to allowing a comparison between west and south-east coast limpets, this would provide baseline information on whether substratum type has an influence on the growth of a limpet species. Furthermore, as the limpets inhabiting the Cochlear zone are generally much smaller than those occurring higher on the shore, it was decided to investigate the growth rate of a group of limpets inhabiting mussel shells. These results could provide insight into whether the observed intertidal zonation patterns of *P. granularis* occur as a result of migration (as proposed by Branch, 1975b) or other factors such as differential growth. Finally, the difference in shell size and shape of *P. granularis* inhabiting both different localities and positions on the shore was investigated using principle component analysis. It was hoped that the results of this study would provide clarification of some aspects that may influence the morphology of this species.

4.2: MATERIALS AND METHODS:

The growth form of *Patella granularis* was investigated at seven localities along the south-east coast (see Chapter 2 for details of localities). From these surveys, two localities, providing three types of substrata (referred to in this chapter as sites) were then selected as suitable for the long term studies of growth rates. Kenton-on-Sea (33E44' S; 26E40' E), is an aeolianite platform and cliff whilst Cannon Rocks (33E44' S; 26E35' E) is a quartzitic sandstone boulder beach. A dense bed of *Perna perna* mussels, which support a high density of *P. granularis*, occurs at the second locality. This site was thus also chosen for the investigation of growth rates.

4.2.1: Shell growth: Allometry vs Isometry:

An investigation into the allometry of *P. granularis* at seven localities along the south-east coast was conducted (see Chapter 2 for details of collection of specimens and descriptions of sites). Although a number of shell parameters were measured, for the purposes of this investigation, only shell height and shell length (in millimetres) were used. The ratio of limpet height (h) and length (L) are related by the function

$$h = cL^{á} \qquad \textbf{Equation 4.1}$$

The constant of allometry (á) = 1 if the two parameters change proportionally, but $á > 1$ if shell height increases faster than shell length (Branch, 1981). A plot of log shell length against log shell height provides an indication of whether growth is allometric or isometric. Regression analysis performed on the plots of log shell length against log shell height yielded the values of á and c (a constant) for each of the seven populations studied (Figures 4.1 - 4.7). These values were substituted into Equation 4.1 to provide the equations of the allometric growth curves (Table 4.1).

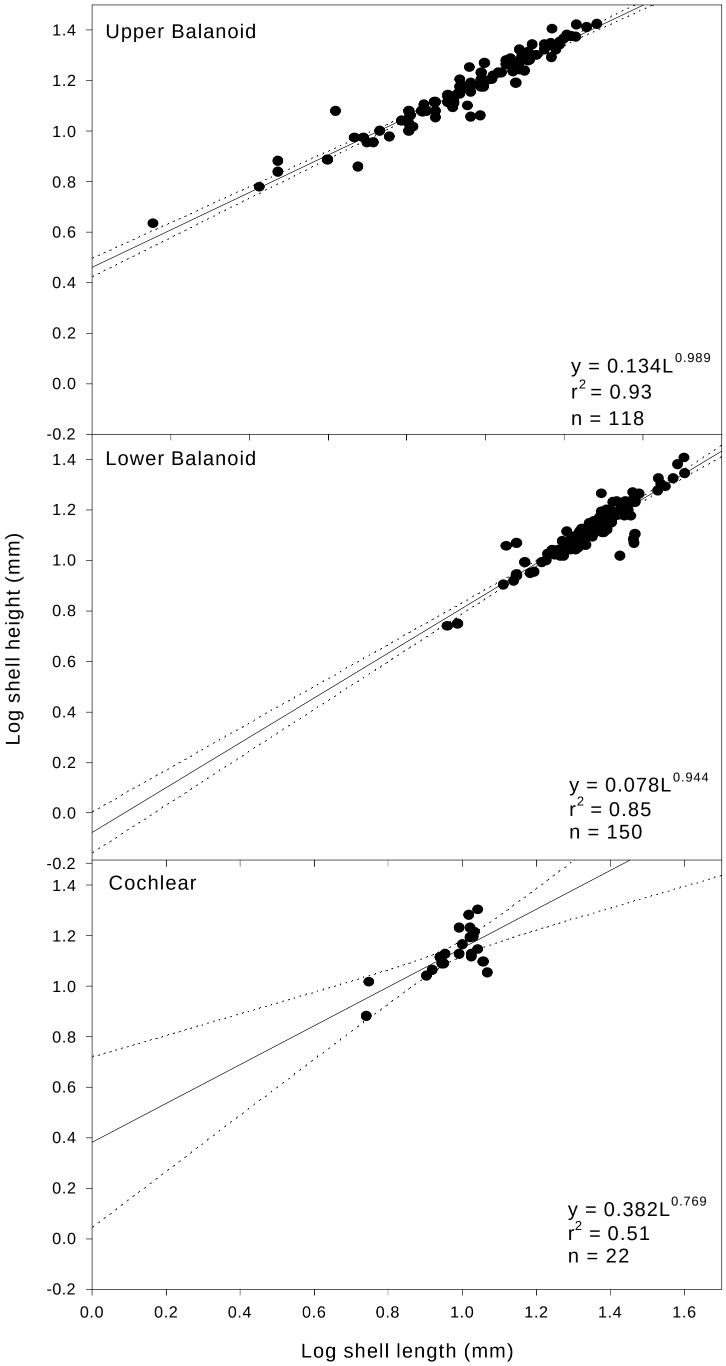


Figure 4.1:

Length to height ratio of *P. granularis* inhabiting three zones at Port Elizabeth. Dotted lines indicate 95% confidence levels. n = sample size.

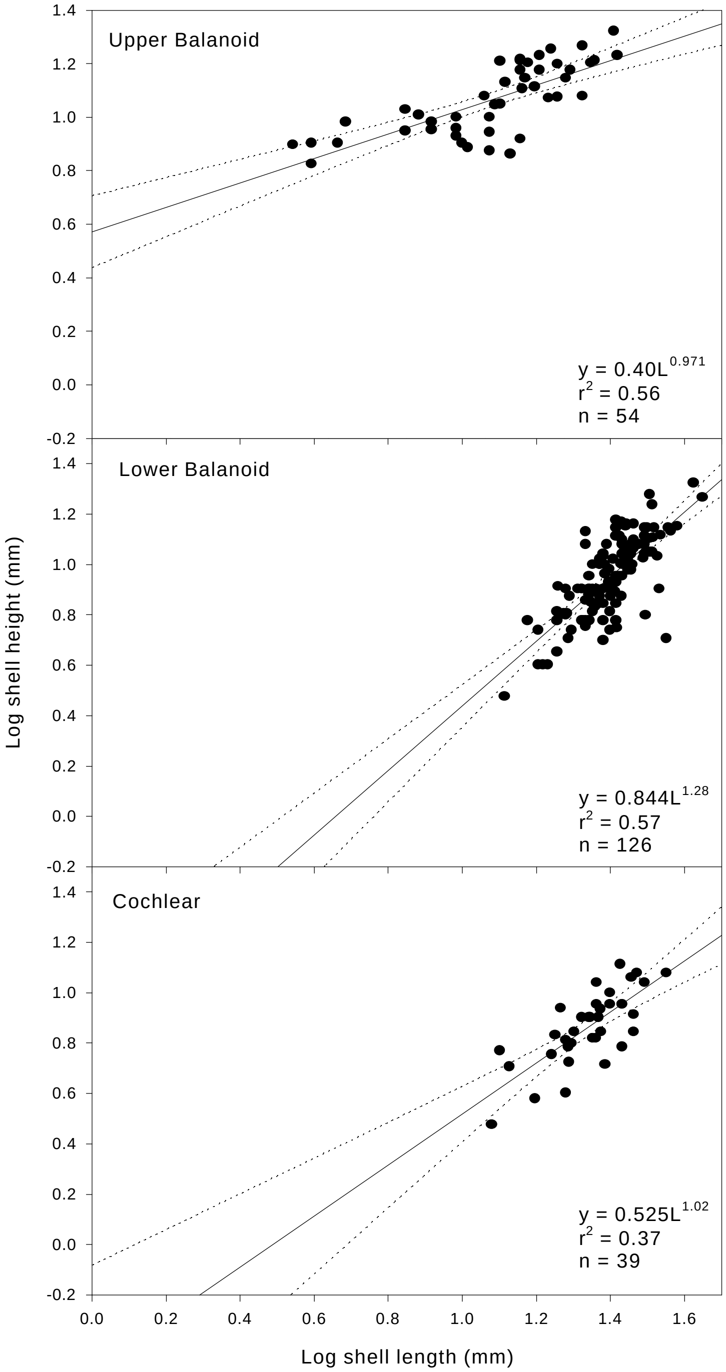


Figure 4.2:

Length to height ratio of *P. granularis* inhabiting three zones at Bird Island. Dotted lines indicate 95% confidence levels. n = sample size.

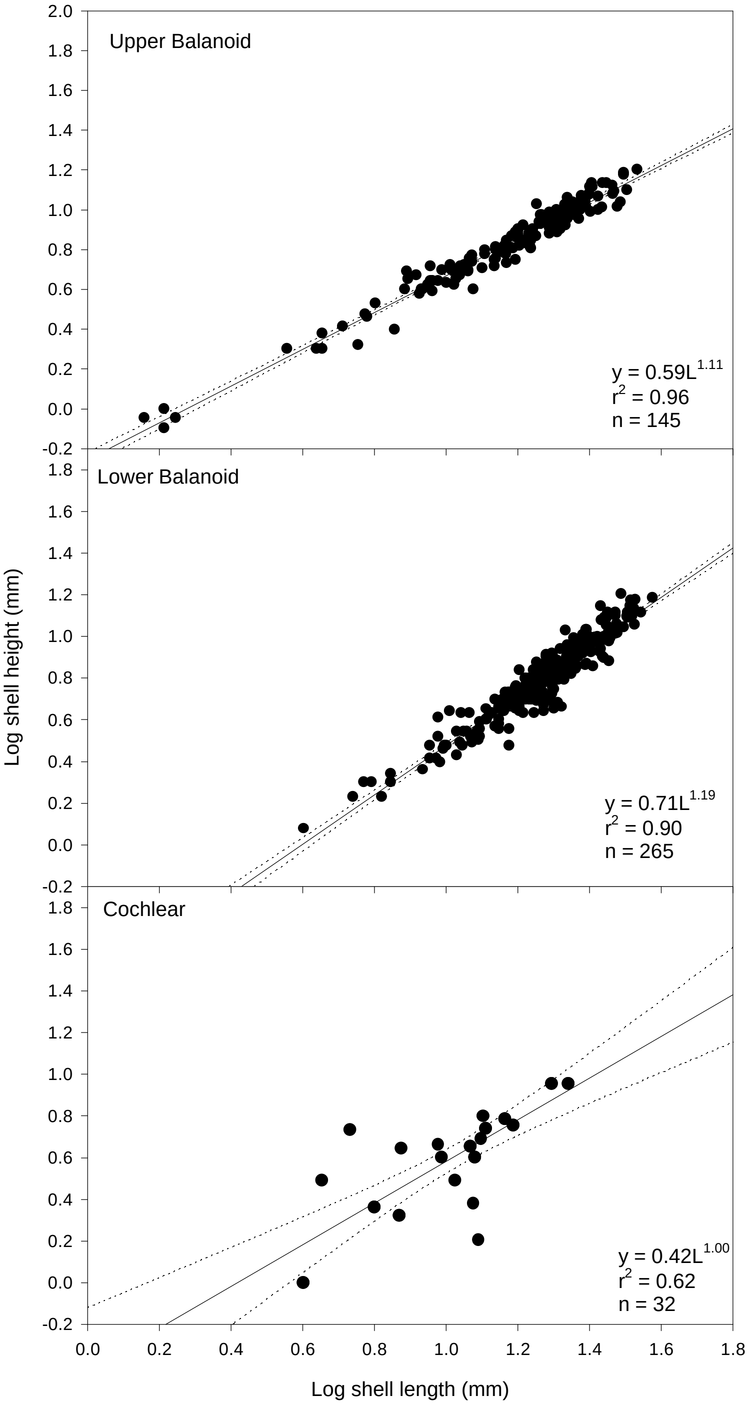


Figure 4.3:

Length to height ratio of *P. granularis* inhabiting three zones at Cannon Rocks. Dotted lines indicate 95% confidence levels. n = sample size.

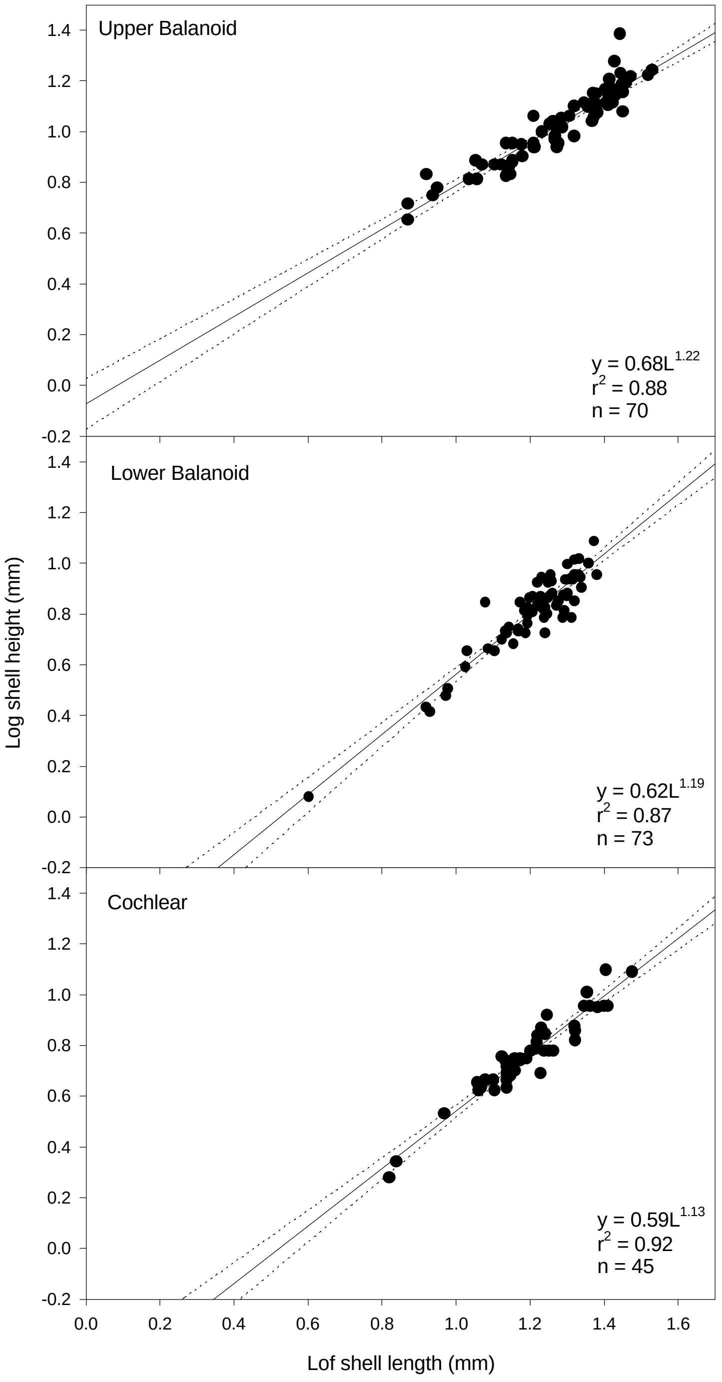


Figure 4.4:

Length to height ratio of *P. granularis* inhabiting three zones at Kwaaihoek. Dotted lines indicate 95% confidence levels. n = sample size.

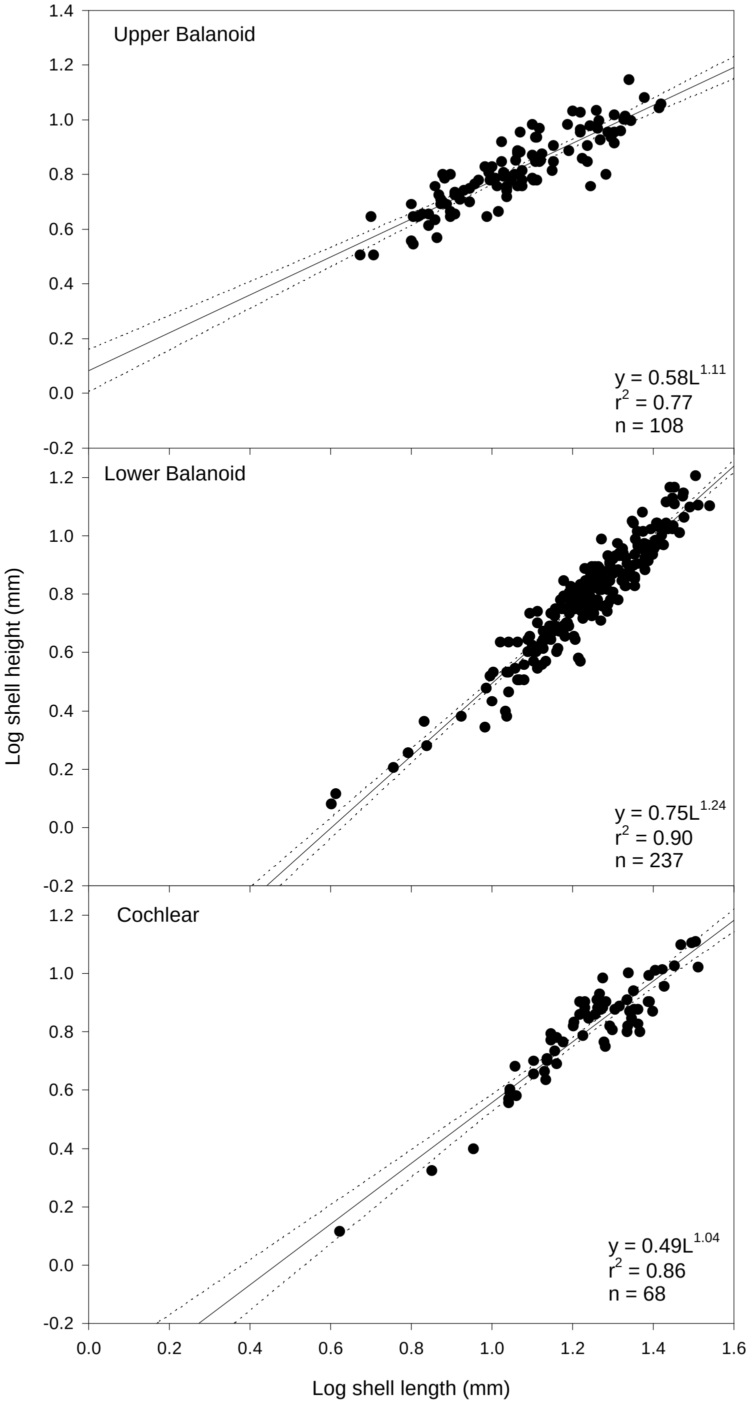


Figure 4.5:

Length to height ratio of *P. granularis* inhabiting three zones at Kenton-on-Sea. Dotted lines indicate 95% confidence levels. n = sample size.

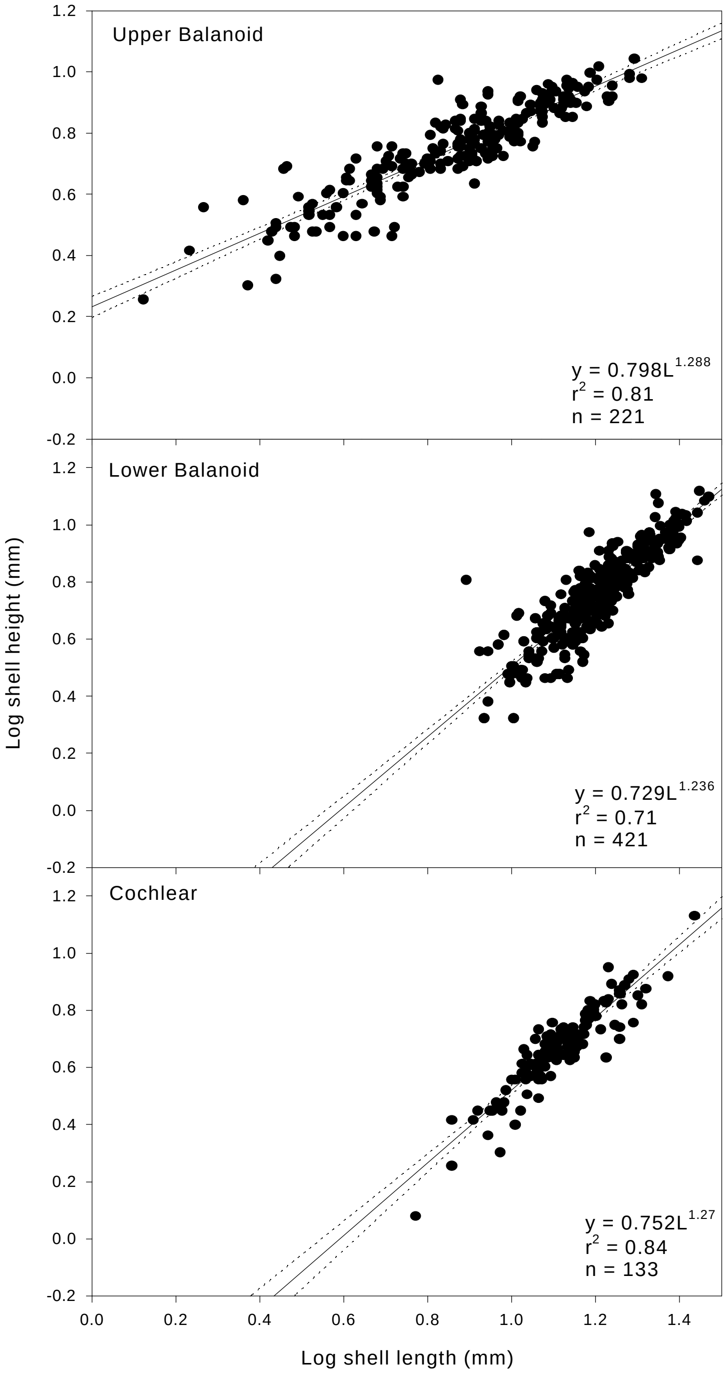


Figure 4.6:

Length to height ratio of *P. granularis* inhabiting three zones at Port Alfred. Dotted lines indicate 95% confidence levels. n = sample size.

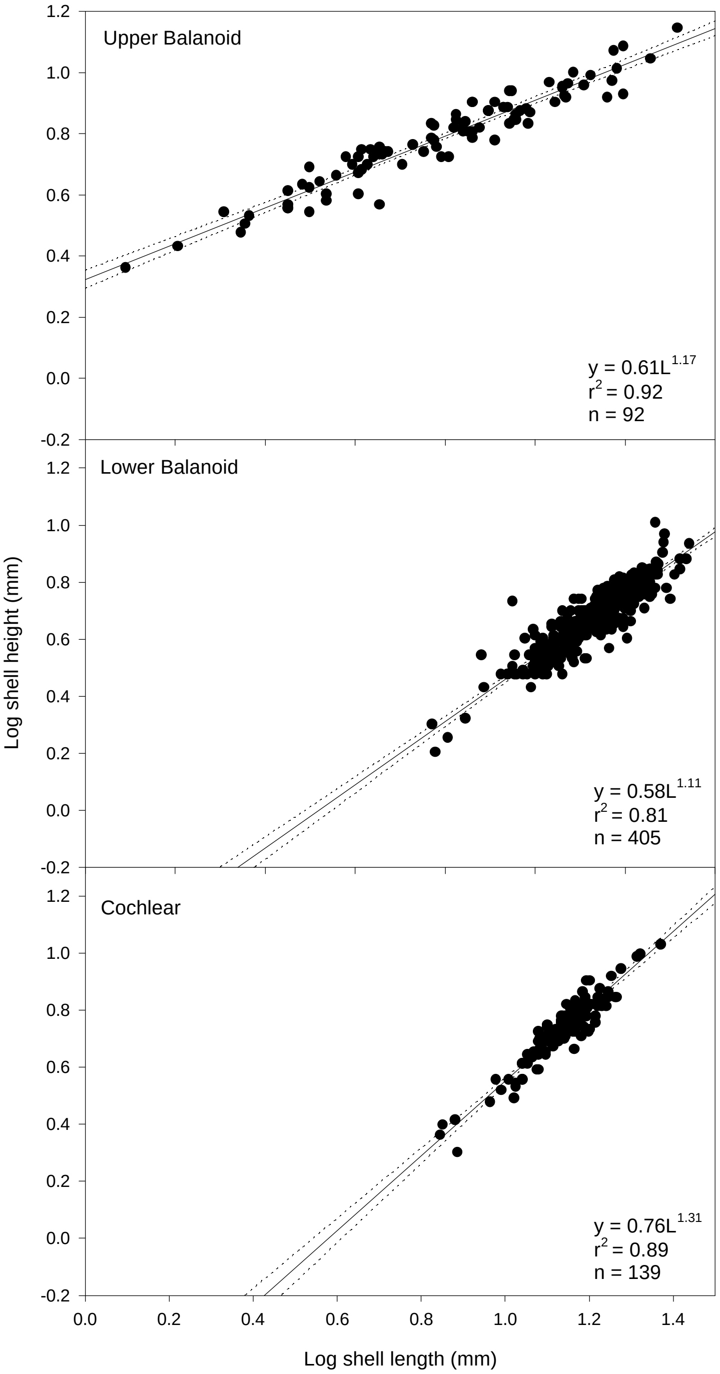


Figure 4.7:

Length to height ratio of *P. granularis* inhabiting three zones at Three Sisters. Dotted lines indicate 95% confidence levels. n = sample size.

4.2.2: Morphometric analysis of shell length and form:

Differences in both the size and shape of *P. granularis* were investigated at each zone of the seven localities. Vernier calipers were used to measure shell length (greatest distance between anterior and posterior ends), shell width (greatest distance perpendicular to the anterior-posterior axis), and shell height (greatest vertical distance from the apex of the shell to the aperture). All data were log-transformed before analysis in order to linearise the allometric effect of size (M. Villet, pers. comm; Boulding & Van Alstyne, 1993). A principal component analysis (PCA - Statistica for Windows, Release 5.1; Statsoft Inc.) was then performed on the transformed data. This method reduces the number of variables in the data set by finding linear combinations of the original variables which then explain most of the existing variability in the form of principle components (James & McClulloch, 1990). This analysis was carried out to investigate whether there was any pattern of morphological variability among the seven populations sampled and whether any morphological variation occurred at a within shore scale (i.e. among zones). Differences between the sampling sites and zones in the measured characters were tested by multi-factor analysis of variance (MANOVA). Where results were significant, the Tukey's procedure was used for *a posteriori* tests of differences among sites and zones (Sokal & Rohlf, 1981).

4.2.3: Estimates of growth-rate using mark-recapture:

Estimates of growth rate were obtained by comparing shell growth increments of *P. granularis* inhabiting the lower Balanoid zone on an aeolianite and a quartzitic sandstone shore and on mussel shells. 200 limpets of a range of sizes were tagged *in situ* at each site using small plastic bee labels (Honey Bee Warehouse, Cape Town). These labels were attached to the shells of the limpets using epoxy putty (Pratley's). This medium is quick setting and long lasting. Furthermore, it is not necessary for the shells to be dry before application, which reduces the application time. To prevent the numbers of the bee labels from either degrading due to exposure to the sun or being eroded, each label was coated with a layer of clear nail polish. The initial shell length of each limpet was measured (along the longest axis) to the nearest 0.1 mm using Vernier calipers. The growth of the labelled limpets was monitored on a monthly basis for one year (May 1996 to April 1997). This was repeated at each site when a further 200 limpets were tagged and monitored from February 1997 to January 1998.

In order to estimate maximum shell lengths and growth rates of *P. granularis* on each substratum type, length data were converted to age data using the Von Bertalanffy growth equation (Equation 4.2; after Beverton & Holt, 1957).

$$L_t = L_{max}\,(1-e^{-K\,(t-t0)}) \qquad \textbf{Equation 4.2}$$

where: L_t = length at age t

L_{max} = theoretical maximum length attained

K = growth coefficient

t = time

t_0 = time at which growth commences from L = 0.

The value of t_0 in this equation holds no biological importance (Ricker, 1975), and as such is often ignored in initial determinations of Von Bertalanffy growth parameters. For the purposes of this study, t_0 was considered to be equal to zero (time of hatching) on all substrata.

A Ford-Walford plot (Beverton & Holt, 1957) was constructed for each site to determine the constants L_{max} and K of the Von Bertalanffy equation. These values were inserted into Equation 4.2 and a growth curve obtained. However, due to the low recapture rates of limpets from the mussel beds and quartzitic sandstone, (Appendix II, Table 1) and the nature of the Ford-Walford plot, which requires a one year time interval between marking and recapture, a Gulland and Holt plot was also used to estimate the parameters of K and L_{max} and produce a growth curve for each site. This plot takes the time interval between marking and recapture into account when calculating L_{max} and K. The data obtained from the labelled limpets at all three sites were used to construct Gulland and Holt plots using the program FiSAT (a FAO stock assessment tool, Gayanilo *et al.*, 1994).

The Gulland and Holt plot is described by the equation

$${}^{a}L/{}^{a}t = a + bL_{(mean)} \qquad \textbf{Equation 4.3}$$

Where: ${}^{a}L = L_{recapture} - L_{marked}$

${}^{a}t = t_r - t_m$

$L_{mean} = (L_r + L_m)/2$

From this: $L_{max} = -a/b$ and

$K = -b.t_{(number\ of\ days)}$

4.2.4: Estimates of seasonal growth rates using similarly sized limpets.

Estimates of growth rate of *P. granularis* inhabiting the three different substrata were also obtained by monitoring growth increments of similarly sized limpets. At each site, 100 limpets (. 15mm shell length, Table 4.12) were labelled using plastic numbered bee labels (see section 4.2.3 for details) in March 1998. Limpets of this size were selected as this size class represents the modal size class of *P. granularis* inhabiting the mussel shells. Furthermore, limpets of this size are between two and three years of age at all sites (see Figure 4.10 a and b). An additional 100 limpets were labelled at each site and transferred to other sites in order to compare growth rates among the sites (see Table 4.12 for details). The shell lengths of all the labelled limpets were recorded monthly for six months. This experiment was repeated from December 1998 to June1999 on a further 200 limpets at each site in order to allow a comparison of seasonal growth.

4.2.5: Age determination using micro-growth band analysis:

Before the age of *P. granularis* could be ascertained by counting the number of growth bands present in each shell, the periodicity of growth band deposition was determined. 60 limpets inhabiting the quartzitic shore (Cannon Rocks) were marked by scraping the growing edge of the shell with a knife. 20 limpets were then sampled at two week intervals for six weeks. Examples of marked limpets are shown in Figure 4.8a.

The shells were prepared using modifications to the method proposed by Richardson *et al.* (1979). Each marked limpet shell was cleaned in 10% sodium hypochlorite solution for ten minutes to remove organic debris. The shell was then rinsed in water and air dried before being embedded in casting resin (Metaserv s.w. resin 137/12742). Each shell was then sectioned along the anterior-posterior axis through the apex of the shell using a diamond saw. The cut surfaces were then smoothed by grinding on wet and dry "Trimite" paper with successively finer grit, before being polished to a blemish-free surface using the household metal polish "Brasso". Each shell was then etched by immersion in cold 0.01M hydrochloric acid for 40 - 45 minutes. Strips of replicating material (Agar Scientific Ltd. no. g255) were immersed for between 45 and 50 seconds in ethyl acetate. These almost molten strips were then applied to the etched shell surface where they were left for five minutes to dry. Once all the ethyl acetate had evaporated, the acetate peels could be removed from the shell surface and placed between two glass slides. The acetate peel replica of the etched surface of the shells was then examined using a phase contrast light microscope (see Figure 4.8b for examples of microgrowth bands). The number of growth bands laid down after the mark were recorded for each animal. A one-sample t-test (Sokal & Rohlf, 1981) was used to correlate the number of growth bands recorded to the number of days and number of tidal cycles that had passed since marking.

Once the periodicity of shell deposition is known, it is possible to determine the age of the individual by counting the total number of growth bands present in the shell. This, in turn, would enable the growth rate of unmarked limpets to be determined. An attempt was also made to age a number of *P. granularis* from each of the three substratum types using micro-growth band analysis.

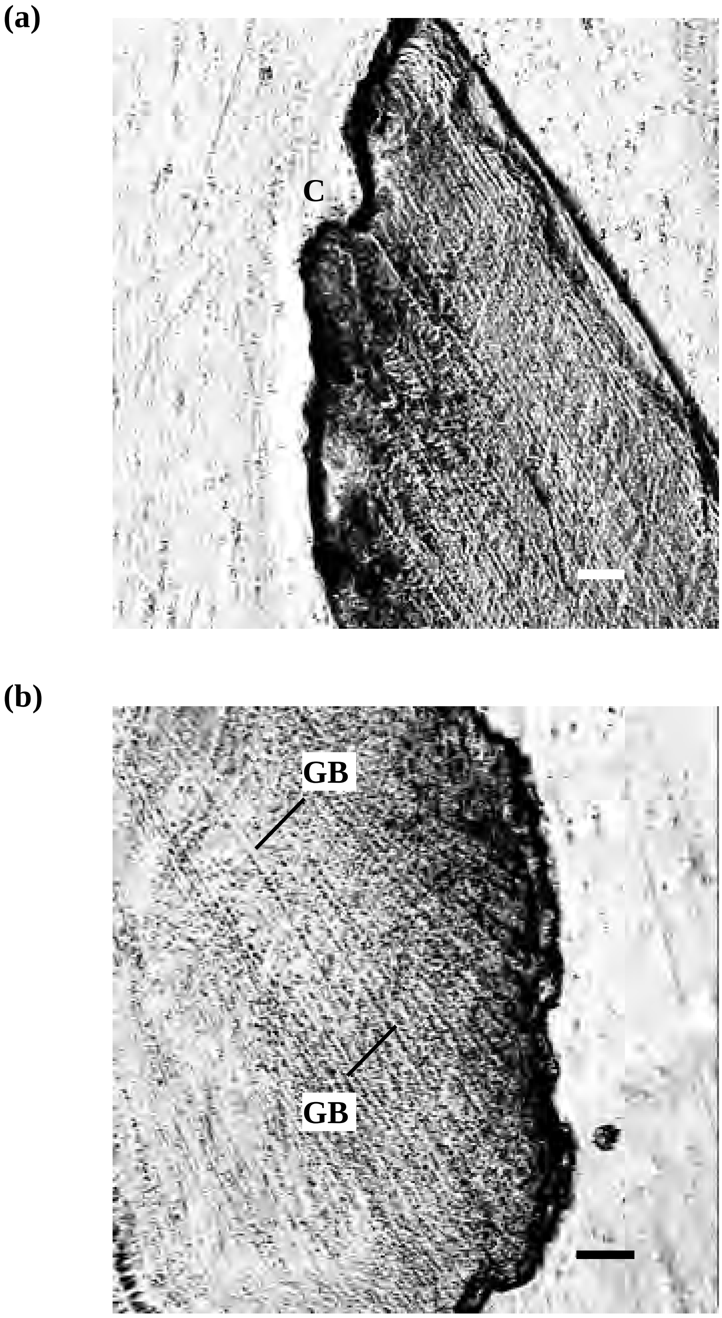


Figure 4.8:

Examples of acetate peel replicas of the etched surface of the shell of *P. granularis* showing (a) the cleft (C) made when marking shells to determine the periodicity of deposition of microgrowth bands (GB) and (b) the microgrowth bands (GB) deposited in the shell. Scale bar = 100 Fm.

4.3: RESULTS:

4.3.1: Allometric vs isometric growth:

Most *P. granularis* inhabiting the south-east coast were found to grow allometrically with shell height increasing faster than shell length (Table 4.1). There was however, a great deal of variability in the degree of allometry both within and among sites, with á varying from 0.77 to 1.31. No trend in the pattern of growth was evident among the zones, although the constant of allometry for limpets inhabiting the mussel beds in the Cochlear zone was lower than that of limpets from the upper Balanoid zone in five of the seven localities (Table 4.1). The majority of *P. granularis* from Port Elizabeth and Bird Island were found to grow isometrically, as the constants of allometry of these limpets were lower than 1. No trends were evident in the degree of allometry of *P. granularis* inhabiting shores with different geomorphologies.

Table 4.1:

The allometric growth equation, á and r² values obtained from the regression analysis performed on plots of log shell length against log shell height for each of the seven localities sampled. UB = upper Balanoid zone; LB = lower Balanoid zone; C = Cochlear zone.

	Zones	Allometric equation	á	r²
Port Elizabeth	UB	$y = 0.13L^{0.99}$	0.99	0.93
(Quartzite)	LB	$y = 0.08L^{0.94}$	0.94	0.85
	C	$y = 0.38L^{0.77}$	0.77	0.51
Bird Island	UB	$y = 0.40L^{0.97}$	0.97	0.56
(Quartzite)	LB	$y = 0.84L^{1.28}$	1.28	0.57
	C	$y = 0.53L^{1.02}$	1.02	0.37
Cannon Rocks	UB	$y = 0.59L^{1.11}$	1.11	0.96
(Quartzite)	LB	$y = 0.71L^{1.19}$	1.19	0.90
	C	$y = 0.42L^{1.00}$	1.00	0.62
Kwaaihoek	UB	$y = 0.68L^{1.22}$	1.22	0.88
(Aeolianite)	LB	$y = 0.62L^{0.94}$		0.87
			1.19	
	C	$y = 0.59L^{0.77}$		0.92
			1.13	
Kenton-on-	UB	$y = 0.58L^{1.11}$	1.11	0.77
Sea				
(Aeolanite)	LB	$y = 0.75L^{1.24}$	1.24	0.90
	C	$y = 0.49L^{1.04}$	1.04	0.86
Port Alfred	UB	$y = 0.80L^{1.29}$	1.29	0.81
(Quartzite)	LB	$y = 0.73L^{1.24}$	1.24	0.71
	C	$y = 0.75L^{1.27}$	1.27	0.84
Three Sisters	UB	$y = 0.61L^{1.17}$	1.17	0.92
(Aeolianite)	LB	$y = 0.58L^{1.11}$	1.11	0.81
	C	$y = 0.76L^{1.31}$	1.31	0.89

4.3.2: Morphometrics:

Principal component analysis carried out on the samples showed only one significant axis ($E_1 = 2.81$; $E_2 = 0.13$; $E_3 = 0.07$) which summarised 93.5% of the data. The second axis explained only an additional 4.3% of the variance while the remaining axis explained 2.2% of the recorded variance. The component weights of the first axis were all similar and correlated significantly with all the original variables (Table 4.2).

Table 4.2:

Correlations of three components of a principal component analysis of limpet shell morphology with each of the morphometric variables. (* indicates significant p - values). Eigenvalues and the percentage variability described by each eigenvector are also included.

Variable	PC1	p	PC2	p	PC3	p
Eigenvalues	2.81	-	0.13	-	0.07	-
Variability (%)	93.51	-	4.9	-	2.20	-
Eigenvectors						
Log_{10} (shell length)	0.985	< 0.0001*	-0.116	< 0.0001*	-0.019	0.312
Log_{10} (shell width)	0.982	< 0.0001*	-0.110	< 0.0001*	0.130	< 0.0001*
Log_{10} (shell height)	0.952	< 0.0001*	0.262	< 0.0001*	-0.017	0.363

Results of a MANOVA performed on the results of the PCA indicate that both locality and zone significantly influence the first principle component (Table 4.3). As the first axis of a principal component analysis is generally an expression of size, it can be seen that there was a trend towards a decrease in the mean shell length of *P. granularis* along the coast from west to east (Table 4.4). Exceptions to this were the limpets inhabiting Bird Island and Kwaaihoek. Similarly, there was a general decrease in the size of the limpets in a downshore direction (Table

4.5). Locality also significantly affected the second principal component which is a size-free expression of shape (Table 4.6). Thus there was a trend towards a taller shell from west to east (Table 4.7). Once again, this was particularly pronounced at Kwaaihoek. Analysis of the trends within a shore indicated no significant difference in the shape of *P. granularis* inhabiting the zones (Table 4.6). The third principal component analysed in the current study is an expression of shell width. This was also affected significantly by both locality and zone (Table 4.8). Generally, shell width increased from west to east. Shells from the upper Balanoid zone were significantly wider than those from both the lower Balanoid and Cochlear zones (p = 0.0197; Table 4.8).

Table 4.3:

Results of a MANOVA performed to investigate whether the first principal component differed significantly among localities or zones. (* indicates a significant p - value).

Source of Variation	SS	df	MS	F-ratio	Sig-level
Main effects					
A: Locality	434.739	6	72.455	104.010	< 0.0001 *
B: Zone	142.879	2	71.440	102.560	< 0.0001 *
Interactions					
AB	127.651	12	10.638	15.270	< 0.001*
Residual	2031.938	2917	0.697		
Total	2737.207	2925			

Table 4.4:

Results of a Tukey's multiple comparison test to investigate whether the first principle component varied among localities. Localities are arranged in geographical order from west to east. (X's in different columns indicates significant differences).

Locality	L.S. Mean	Homogenous groups		
Port Elizabeth	0.358			X
Bird Island	1.149			
		X		
Cannon Rocks	-0.196		X	
Kwaaihoek	0.444			X
Kenton-on-Sea	0.223			X
Port Alfred	-0.332		X	
Three Sisters	-0.372		X	

Table 4.5:

Results of a Tukey's multiple comparison test to investigate whether the first principle component varied among zones. (X's in different columns indicates significant differences).

Zone	L.S. Mean	Homogenous groups		
Upper Balanoid	0.599			
		X		
Lower Balanoid	0.191			X
Cochlear	-0.244		X	

Table 4.6:

Results of a MANOVA performed to investigate whether the second principal component differed significantly among localities or zones. (* indicates a significant p - value).

Source of Variation	SS	df	MS	F-ratio	Sig-level
Main effects					
A: Locality	296.197	6	49.366	57.350	< 0.0001 *
B: Zone	0.956	2	0.478	0.555	0.5740
Interactions					
AB	92.341	12	7.695	8.940	< 0.0001 *
Residual	2510.910	2917			
Total	2900.404	2925			

Table 4.7:

Results of a Tukey's multiple comparison test to investigate whether the second principle component varied among localities. Localities are arranged in geographical order from west to east. (X's in different columns indicates significant differences).

Locality	L.S. Mean	Homogenous groups			
Port Elizabeth	-0.999	X			
Bird Island	-0.677		X		
Cannon Rocks	-0.033			X	
Kwaaihoek	0.451				X
Kenton-on-Sea	-0.042			X	
Port Alfred	0.073			X	
Three Sisters	0.386				X

Table 4.8:

Results of a MANOVA performed to investigate whether the third principal component differed significantly among localities or zones. (* indicates a significant p - value).

Source of Variation	SS	df	MS	F-ratio	Sig-level
Main effects					
A: Locality	16.857	6	2.810	2.837	< 0.0094 *
B: Zone	7.787	2	3.893	3.931	0.0197 *
Interactions					
AB	10.990	12	0.9158	0.9246	0.5210
Residual	2889.270	2917	0.990		
Total	2924.904	2925			

4.3.3: Estimates of growth-rate using mark-recapture:

An estimate of K (the growth coefficient) was obtained for each of the three sites (aeolianite, quartzitic sandstone and mussel shells) from the slopes of the regression lines (k) of the Ford-Walford plots (Figure 4.9) using Equation 4.4,

$$K = -\log_e k \qquad \textbf{Equation 4.4}$$

(see Ricker, 1975; Branch, 1981)

With the exception of limpets from the mussel bed, estimates of K and L_{max} obtained from the Gulland and Holt plots were similar during both years of the study (Table 4.9). Data from both years were thus pooled (M. Villet, pers. comm.) to provide a single K and L_{max} estimate for the aeolanite and quartzite shores. The K and L_{max} estimates obtained were substituted in the Von Bertalanffy growth equation, and a growth curve for each site was constructed (Figure 4.10a). A large difference in the value of K of *P. granularis* from the mussel beds was found between the first and the second year of the study using the Gulland and Holt plot (Table 4.9). As the recovery rate of limpets from this site was so poor in the first year (Appendix II, Table 1), these values have been omitted from the study. The estimates of K and L_{max} obtained during the second year of the study were used to construct Von Bertalanffy growth curves for this site (Figure 4.10a). These low recovery rates necessitated a similar course of action for the data on the mussel shell limpets to be followed with the Ford-Walford plots. Likewise, low recovery rates of limpets inhabiting the quartzitic sandstone were experienced during the first year of the study. Although this did not influence the results of the Gulland and Holt plots for this site, a Ford-Walford plot could not be constructed for limpets monitored on quartzitic sandstone during the first year of the study. As a result only the K and L_{max} values obtained from the Ford-Walford plot for the second year of the study were substituted into the Von Bertalanffy equation to provide an estimated growth curve (Table 4.9; Figure 10b).

Table 4.9:

Estimates of K and L_{max} values from Gulland and Holt and Ford-Walford plots obtained for *P. granularis* inhabiting mussel shells, quartzitic sandstone and aeolianite for two year long study periods. **w** indicates insufficient data to create a plot.

		Gulland and Holt		**Ford-Walford**	
Site		**1st year of study**	**2nd year of study**	**1st year of study**	**2nd year of study**
Mussel shells	n =	13	25	0	25
	K =	0.95	0.25	**w**	0.33
	L_{max} =	24.09	27.12	**w**	24.9
	r^2 =	0.51	0.25	**w**	0.83
Quartzite	n =	23	59	0	71
	K =	0.26	0.22	**w**	0.37
	L_{max} =	35.26	35.97	**w**	31.5
	r^2 =	0.14	0.17	**w**	0.80
Aeolianite	n =	39	47	39	47
	K =	0.23	0.21	0.48	0.42
	L_{max} =	35.81	35.82	29.90	29.1
	r^2 =	0.26	0.16	0.79	0.82

K and L_{max} values of aeolianite limpets were determined for both years of the study using a Ford-Walford plot. Once again, as both the K and L_{max} estimates were similar for both years, data were pooled to provide single values from which a Von Bertalanffy growth curve could be determined (Figure 10b).

Table 4.10:

Comparison of K (Growth coefficient) and L_{max} (theoretical maximum length in mm) for *P. granularis* inhabiting mussel shells, quartzitic sandstone and aeolianite, obtained from pooled data from two year long study periods.

		Gulland and Holt	Ford-Walford
Mussel shells	n =	40	25
	K =	0.25	0.33
	L_{max} =	27.12	24.9
	r^2 =	0.253	0.83
Quartzitic	n =	76	71
	K =	0.33	0.37
	L_{max} =	32.96	31.5
	r^2 =	0.308	0.80
Aeolianite	n =	86	86
	K =	0.32	0.45
	L_{max} =	31.89	29.5
	r^2 =	0.346	0.81

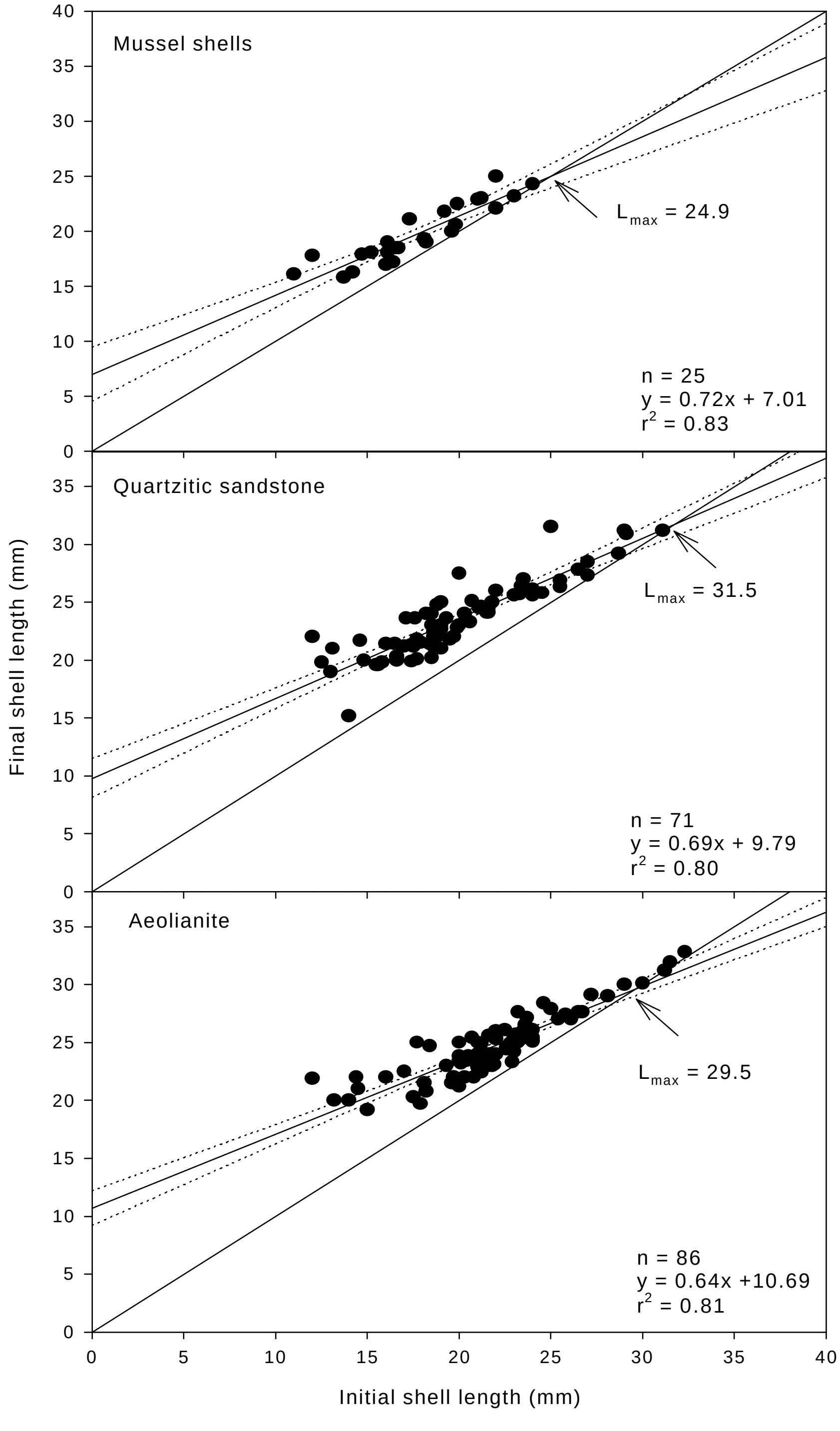


Figure 4.9:

Ford-Walford plots for *P. granularis* inhabiting mussel shells, quartzitic sandstone and aeolianite.

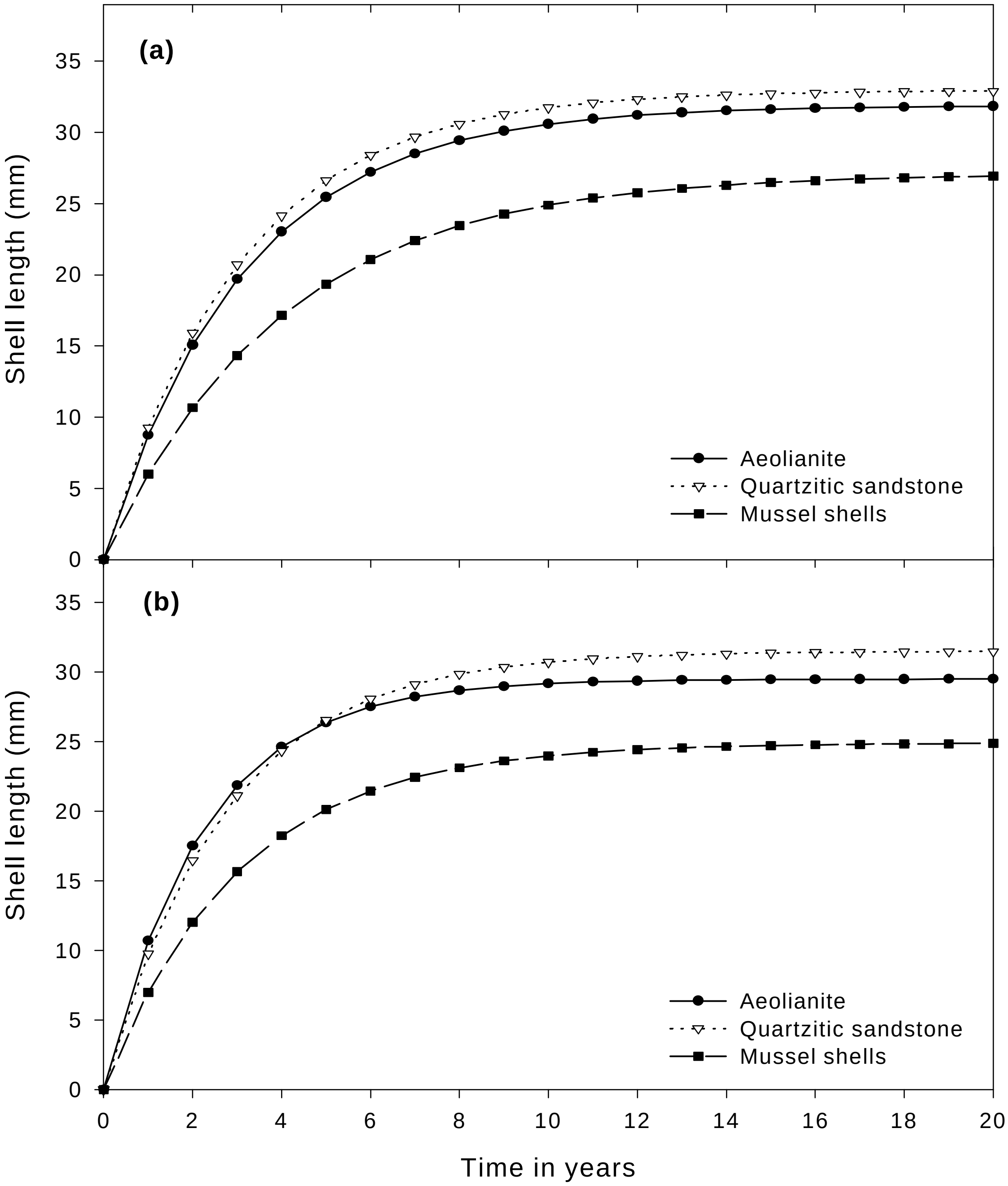


Figure 4.10:

Von Bertalanffy growth curves for *P. granularis* inhabiting aeolianite, quartzitic sandstone and mussel shells. Estimates of L_{max} and K for (a) were obtained from Gulland and Holt plots, while those for (b) were obtained from Ford-Walford plots.

The Von Bertalanffy growth curves constructed with information obtained from both the Gulland and Holt and the Ford-Walford plots described a similar pattern of growth. *P. granularis* inhabiting all three substrata initially grew rapidly. Limpets from the aeolianite and quartzitic shores grew between 8 mm and 12 mm a year until they were approximately 27 mm (Ford-Walford) and 30 mm (Gulland and Holt) in length. Limpets were estimated to be between six and eight years of age at this length (Figures 4.10a and b). Growth of *P. granularis* from both sites is then predicted to slow until it is almost imperceptible. While limpets from the mussel shells also grew relatively rapidly initially, the rate of increase of shell length was only about 5.5 mm a year. Growth in these limpets began to slow down at approximately nine years of age (23 - 25 mm in length; Figures 4.10 a and b).

The growth curves derived for *P. granularis* inhabiting the three sites follow similar trends. Limpets from the mussel shells have a slower growth rate throughout their lifespan than limpets from either the quartzitic sandstone or the aeolianite. Limpets inhabiting the other two shores exhibited a similar growth rate to each other throughout their lives, although both the curves estimate that the maximum length reached by limpets inhabiting the aeolianite will be slightly less than that obtained on the quartzitic shore (Figures 4.10 a and b).

The estimates of L_{max} obtained from both plots compare favourably to the maximum size of limpets found inhabiting aeolianite (Table 4.11). Estimates from both plots are higher than the maximum size of *P. granularis* found inhabiting mussel shells but are lower that the largest limpets occurring on quartzite (Table 4.11). Estimates obtained from the Ford-Walford plots are influenced by the largest animals labelled during the experiment. While these plots provide an estimated growth rate of populations, the results obtained must thus be viewed cautiously (Beverton & Holt, 1957; Ricker, 1975).

Table 4.11:

Comparisons of maximum shell length (mm) of *P. granularis* observed at three sites with estimated maximum shell length using a Gulland and Holt plot and a Ford Walford plot.

Site	Max. shell length observed in field	L_{max} (Gulland and Holt)	L_{max} (Ford Walford)
Mussel shells	21.9	27.12	24.9
Aeolianite	30.7	31.89	29.5
Quartzite	37.7	32.96	31.5

4.3.4: Estimates of growth using similarly sized limpets.

As a significant difference in the mean initial length of *P. granularis* inhabiting the three substrata was evident during both experiments (see Appendix II, Table 2), increases in growth were determined as a percentage of initial length (Table 4.12). During the first experimental period (autumn to late winter; March 1998 - September 1998), *P. granularis* inhabiting the aeolianite grew significantly more rapidly than those inhabiting either quartzitic sandstone or mussel shells (Figure 4.11; F - ratio = 6.643; p = 0.025). Limpets that were translocated from the mussel shells to the quartzitic shore displayed a great increase in growth rate when compared to limpets that remained on the mussel shells (19.20% vs. 11.08%, Table 4.12). The growth of *P. granularis* that were transferred from aeolianite to the quartzitic sandstone was lower than that recorded for resident limpets from the aeolianite shore (12.14% to 19.28%; Table 4.12). Likewise, limpets moved from the quartzitic sandstone to the aeolianite showed an increase in growth to a level that was more similar to the resident limpets from the aeolianite (13.12% to 15.78%; Table 4.12). However, no significant differences in the percentage growth was recorded among the sites for the translocated limpets (Figures 4.11 & 4.12; F-ratio = 1.056; p = 0.364).

Table 4.12: Comparisons of growth estimates of resident and translocated *P. granularis* from three substrata.

		Site	n	Initial Length (mm) 0 ± S.D.	Final Length (mm) 0 ± S.D.	Mean increase (mm)	Mean relative growth (%)
March 1998 - Sept 1998	Resident	**Mussel shells**	7	14.99 ± 0.60	16.67 ± 1.46	1.69 ± 0.95	11.08 ± 5.91
		Quartzitic	18	14.68 ± 0.85	16.58 ± 1.11	1.89 ± 1.12	13.12 ± 8.13
		Aeolianite	10	15.36 ± 0.95	18.31 ± 1.35	2.95 ± 1.01	19.28 ± 6.68
	Translocated	**From mussel shells to quartzite**	10	15.75 ± 0.86	18.79 ± 1.79	3.04 ± 1.29	19.20 ± 7.79
		From aeolianite to quartzite	6	18.17 ± 1.28	20.35 ± 1.64	2.18 ± 1.23	12.14 ± 7.17
		From quartzite to aeolianite	6	15.90 ± 1.78	18.34 ± 2.17	2.44 ± 1.52	15.78 ± 10.76
Dec 1998 - June 1999	Resident	**Mussel shells**	20	14.60 ± 1.50	16.91 ± 1.47	2.31 ± 0.97	16.22 ± 7.46
		Quartzitic	30	15.27 ± 1.01	17.40 ± 1.35	2.12 ± 1.33	14.15 ± 9.06
		Aeolianite	43	14.52 ± 0.95	17.34 ± 1.30	2.81 ± 1.38	19.72 ± 10.02
	Translocated	**From mussel shells to quartzite**	7	15.84 ± 1.24	18.37 ± 1.27	2.53 ± 1.29	16.34 ± 8.23
		From aeolianite to quartzite	15	15.41 ± 0.83	17.49 ± 1.38	2.08 ± 1.43	13.72 ± 9.62
		From quartzite to aeolianite	5	14.8 ± 0.482	17.53 ± 0.53	2.73 ± 0.68	18.51 ± 4.78

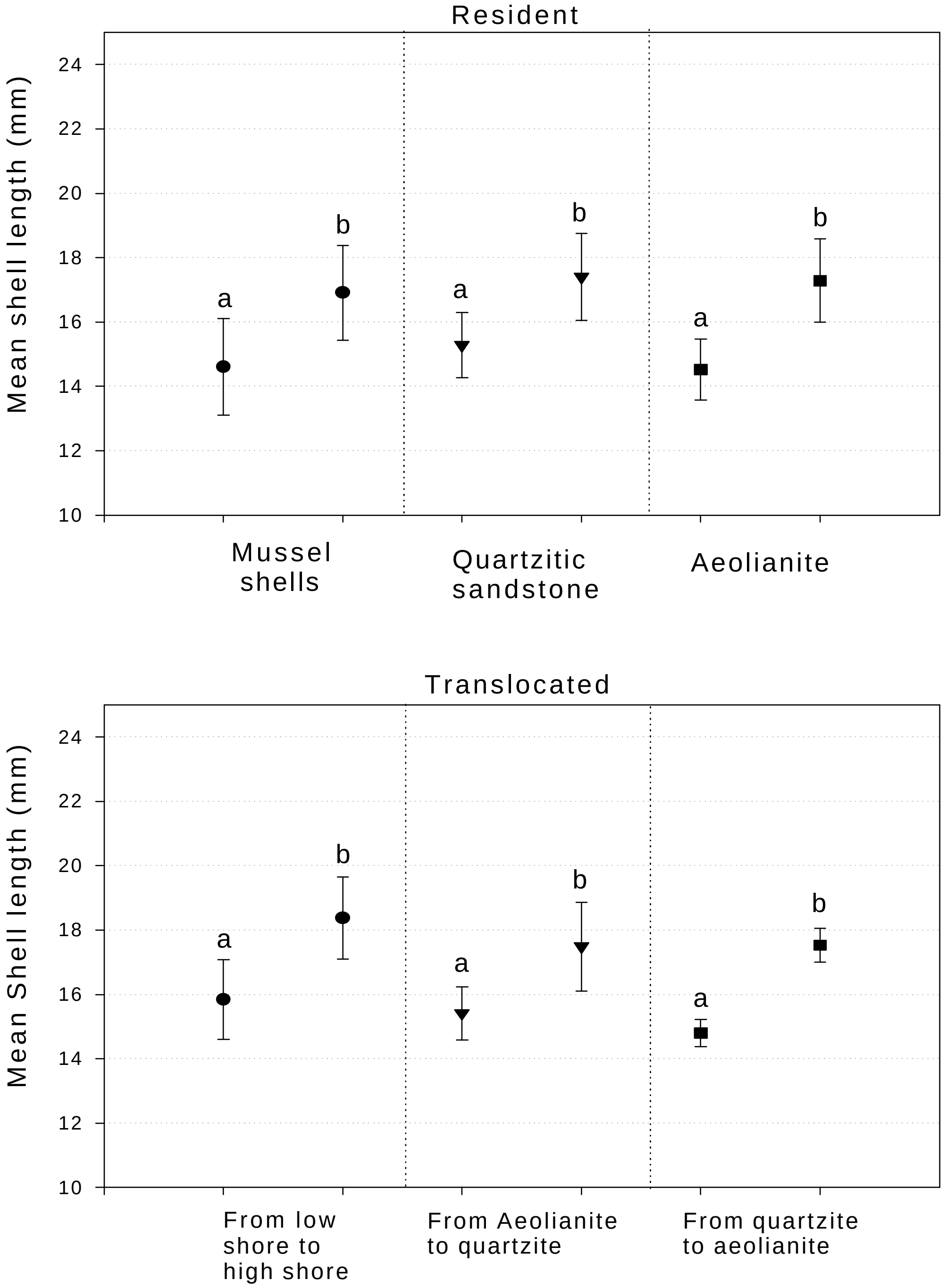


Figure 4.11:

Diagrammatic representation of the difference in growth of resident and translocated *P. granularis* on three substrata from March 1998 to September 1998. a = initial shell length (± S.D.) of limpets; b = final shell length (± S.D.) of limpets.

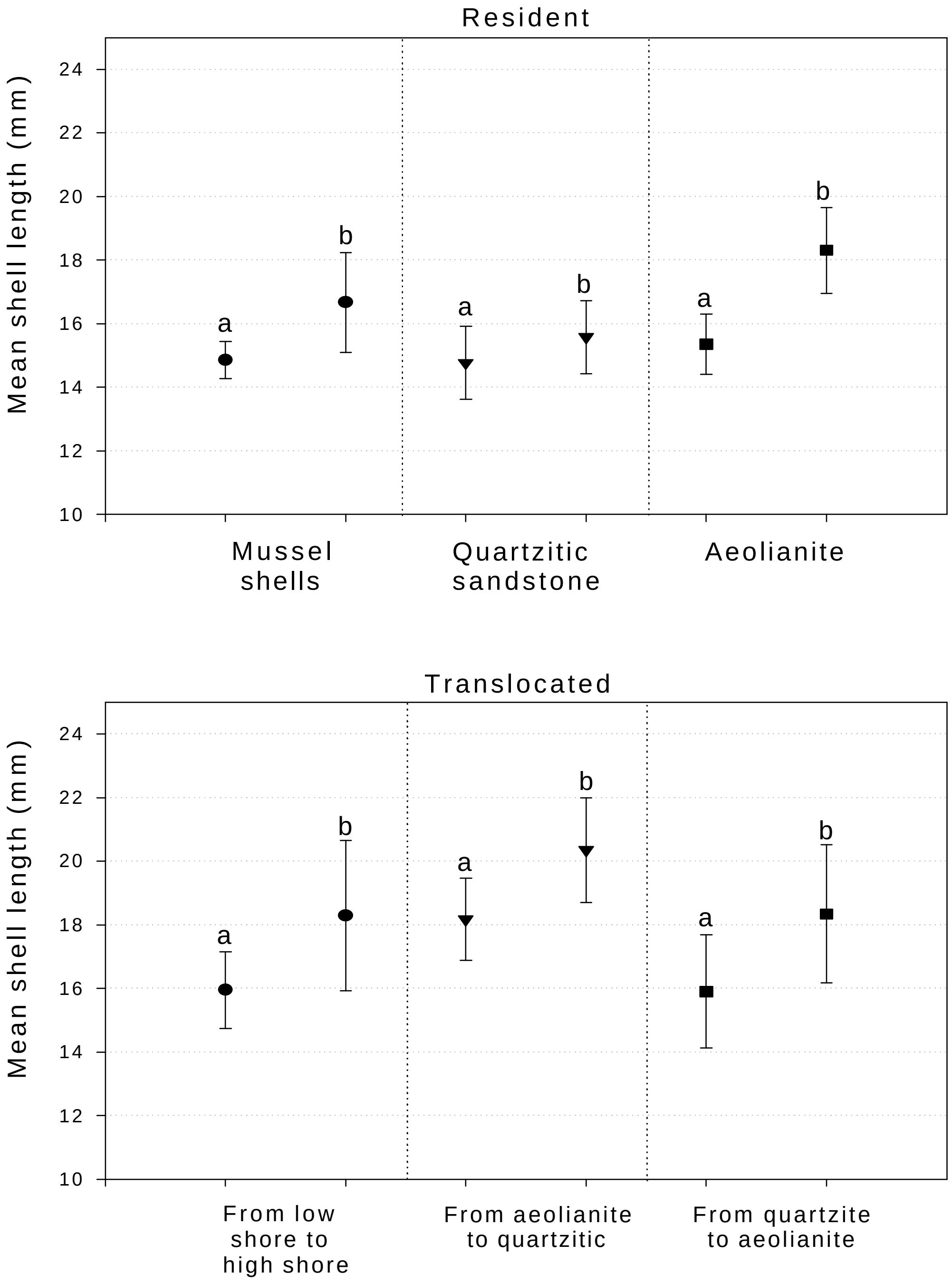
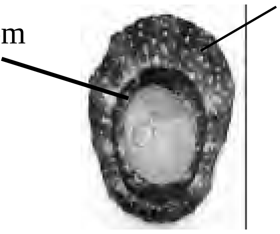


Figure 4.12:

Diagrammatic representation of the difference in growth of resident and translocated *P. granularis* on three substrata from December 1998 to June 1999. a = initial shell length of limpets; b = final shell length of limpets.

During the second experimental period (summer to early winter; December 1998 - June 1999), growth of *P. granularis* was once again significantly higher on aeolianite than the other two substrata (Figure 4.12; Table 4.12; F-ratio = 3.339, p = 0.0398). During this period, *P. granularis* from the mussel shells showed a greater increase in shell length than those from the quartzitic shore (16.22% as compared to 14.15%; Table 4.12). Limpets from the mussel shells again showed an increase in growth when transferred to the quartzite, although the increase in this instance was slight (16.22 % to 16.34 %). Similarly, *P. granularis* moved from the aeolianite to the quartzite showed a decrease in the rate of growth (Table 4.12) and those translocated from the quartzitic sandstone to the aeolianite showed an increase (Table 4.12; Figure 4.12). Once again, no significant difference was found in the growth of translocated *P. granularis* on any substratum (F-ratio = 0.594; p = 0.560). Although growth for the limpets from all three sites varied between the two experimental periods, these differences were never significant (Table 4.13).

In addition to the increase in growth rate observed in limpets that were moved from the mussel shells to the quartzite, a change in shell morphology was observed. The shell of transferred limpets was found to be flatter, lighter in colour and more sculptured than the original shell. In some limpets this change in shell shape was so marked that at first glance, it appeared as though one limpet was attached to the shell of another (see Figure 4.13 for an example). Although this change in shell morphology was observed in limpets that were transferred among the other sites, it was not as conspicuous.



Original shell of limpet from the mussel beds

Growth of shell of limpet after translocation showing the change in colour (lighter) and an increase in sculpturing

Figure 4.13*:*

An example of the changes in the shell morphology of an individual of *P. granularis* moved from the mussel beds to quartzitic sandstone. Both the initial shell and the shell growth that has occurred since translocation are clearly visible. Scale = life-size.

Table 4.13:

Summaries of one-way ANOVA's performed on percentage growth of *P. granularis* during autumn-winter and spring-summer on three substrata.

	Mussel shells		**Aeolianite**		**Quartzitic sandstone**	
	F-ratio	**Sig. level**	**F-ratio**	**Sig. level**	**F-ratio**	**Sig-level**
Resident	2.527	0.125	0.046	0.833	0.151	0.703
Translocated	0.466	0.512	0.257	0.626	0.119	0.738

4.3.5: Age determination using growth bands:

The growth bands produced by *P. granularis* showed no correlation to day/night cycles (p < 0.0001; t-test, Table 4.14). However, the number of bands within the shells did not differ significantly from the number of tidal cycles (Table 4.14) indicating that shell deposition in *P. granularis* occurs on a tidal basis.

Table 4.14:

Results of a one-sample t-test carried out on microgrowth bands obtained from marked *P. granularis* inhabiting quartzitic sandstone.

No. weeks	**n**	**Mean No. of bands**	**Expected No. of bands**	**t-value**	**p-value**
Correlation of day/night cycles					
2	14	28.21	14	25.16	<0.0001
Correlation of tidal cycles					
2	14	28.21	28	0.13	0.90

Ideally, once the periodicity of microgrowth band deposition is known, the age of the limpet can be accurately determined by counting the total number of growth bands present in the shell. Unfortunately, the shells of *P. granularis* from all three sites were found to be heavily eroded, probably by cyanobacterial endoliths (S. Kaehler & C.D. McQuaid, pers. comm). In many cases, the outer prismatic layer was missing from all but the very growing edge of the shell. As such, attempts to age the limpets based on microgrowth bands were impossible.

4.4: DISCUSSION:

Longevity of limpets is generally negatively correlated to growth rate (Ebert, 1975; Branch, 1981). Thus rapid growth is usually associated with early maturation, high mortality and a short life (see Branch, 1981 and Gray, 1996 for reviews). Although the growth rate of *P. granularis* inhabiting the west coast was found to vary among the sites studied (i.e. *P. granularis* grew more quickly at Elands Bay than at Kommetjie; Branch, 1974a), limpets generally grew about 18 mm in the first year, with shell growth ceasing after seven years. The estimated longevity of these limpets is six to eight years (Branch, 1974a). In contrast, the current study has revealed that the growth and longevity of *P. granularis* inhabiting the south-east coast of South Africa is very different. The Von Bertalanffy growth curves estimated that the shell lengths of these limpets increased by only about 5.5 mm - 12 mm in the first year (Figures 10a and b). Similarly, the longevity of the south-east coast *P. granularis* (> 15 years) was found to be almost double that recorded for their counterparts on the west coast (five to seven years, Branch, 1974a). The large difference in the growth rates and resultant estimated longevity recorded for west and south-east coast populations are similar to the differences recorded for populations of *P. cochlear*. The studies conducted on these limpets showed that at two sites within a single locality, a great variability in growth and the estimated maximum size occured. The age of *P. cochlear* at each locality was estimated to range from approximately 15 years to 25 years (Branch, 1974a). Similar differences in longevity have also been recorded in *P. vulgata*, with this species living between 5 and 15 years (Lewis & Bowman, 1975). Thus the growth rates and longevities of south-east coast *P. granularis* estimated in the current study probably reflect the true effect of environmental influences on limpet populations. It therefore appears that *P. granularis* inhabiting the west coast are tending towards a *r*-selected life-strategy, whilst those from the south-east coast may be tending towards a *K*-selected life strategy.

An increase in food availability has been shown to result in an increase in both the growth rate and maximum size attained by *P. granularis* (Bosman & Hockey, 1988a,b). Primary productivity on the west coast is much higher than on the south and east coasts (Bustamante *et al.*, 1995b) and is thus the most likely cause of the different growth rates of rock-dwelling limpets between the two coasts. The reliability of Von Bertalanffy growth curves is hampered by the maximum size of individuals monitored (Ricker, 1975). However, although larger specimens of *P. granularis* occur on the quartzitic shores (38 mm shell length) than were considered in the Von Bertalanffy equations (32 mm shell length), labelled limpets still present on the aeolianite shores after four years were only found to have grown approximately three millimetres during that time (pers. obs.). This suggests that the conclusions reached by the present study regarding longevity and growth rates are correct.

The growth rate of *P. granularis* varied among the three substrata. Although the maximum size attained by this species was greater on quartzitic sandstone boulders than on aeolianite (Chapter 2), the growth curves of limpets from both sites were similar (Figure 4.10a and b). On both the shores, using growth parameters derived from both Gulland and Holt plots and Ford-Walford plots, growth was found to increase rapidly before slowing down at an age of approximately seven years. This type of growth curve is characteristic of many molluscs (Branch, 1974a; Gray, 1996; Foster, 1997; Henninger, 1998; Lindsay, 1998). A number of factors are responsible for decreased rates of shell deposition with age, including a decrease in the carbonic anhydrase activity (Kawai, 1955) and metabolism (Davies, 1966). The onset of sexual maturity has also been associated with a decrease in growth rate (Vahl, 1971), although this does not occur in *P. granularis* from the south-east coast. In these limpets, sexual maturity occurs between six and twelve millimetres in length (between 1 and 2 years of age - Chapters 2 and 5) and is much earlier than the estimated length and age when growth slows down (27 mm - 32 mm; 6 - 8 years).

Morphometric analysis of *P. granularis* inhabiting seven localities along the south-east coast indicates that a gradient in the mean shell length occurs intertidally, with low shore limpets being significantly smaller than those inhabiting higher levels. This supports the observations reported in Chapter 2. Traditionally, the vertical size gradient of this species has been attributed to the settlement of juveniles low on the shore, and subsequent migration up the shore as shell length increased (Branch, 1975b). This migration was thought to be related to increased tolerance of adults to desiccation (Branch, 1975b). Whilst some *P. granularis* which settle on low-shore rock surfaces may well migrate, this study has shown that along the south-east coast, *P. granularis* inhabiting mussel shells do not migrate up the shore. The growth rates of *P. granularis* from the mussel beds were much lower than those estimated for limpets occurring in the mid-shore region (i.e. on aeolianite and quartzitic sandstone). From the Von Bertalanffy growth curves, limpets from the mussel shells were older than similar sized limpets from either of the other two sites. This conclusion is supported by the observation that *P. granularis* inhabiting the mussel shells reached sexual maturity at a smaller size (6 mm - 8 mm), than those of the quartzitic and aeolianite shores (8 mm and 10 mm). It thus appears that the onset of sexual maturity in this species is genetically entrenched (see also Chapter 5).

Intra- and interspecific variations in growth rates of molluscs are the result of many factors. Exposure to wave action (Brown & Quinn, 1988), temperature (Leighton, 1974), genetic differences (Hara, 1990) and density (Sutherland, 1970; Creese & Underwood, 1982; Lasiak & White, 1993; Claereboudt, 1999) are all known to influence growth rates. A decrease in food has often been related to a decrease in growth, but this does not appear to explain the reduced growth rate of *P. granularis* from the mussel shells. Food availability in the mussel beds (as determined by chlorophyll-*a* measurements) was similar to that of the quartzitic boulders (Chapter 7). It is thus suggested that the growth rate of limpets in mussel beds is spatially constrainted. An additional factor that must be considered is the physical nature of the mussel beds which may hamper efficient foraging. Irregular substratum topography is often considered to reduce the foraging efficiency of intertidal organisms (Lewis & Bowman, 1975; Choat, 1977; Hawkins & Hartnoll, 1983).

P. granularis inhabiting the aeolianite shore was found to have a similar growth coefficient to those from the quartzitic sandstone (Table 4.10), despite a far greater food supply present on the aeolianite (Chapter 7). In many molluscs, the partitioning of available energy resources into growth is affected by other biological factors such as reproduction. Growth often ceases during reproduction (Creese, 1980; Chow, 1987). Limpets from the aeolianite had a greater reproductive fitness than those from either the quartzite or the mussel shells and were also found to exhibit prolonged spawning throughout a portion of the study (Chapter 5). As such, the lower maximum size attained on this shore may be associated with an increase in reproductive output. The attainment of a relatively large size (. 30 cm) suggests that the food supply is adequate to maintain some growth at the same time.

Both the Von Bertalanffy growth curves and the estimation of growth rates of similar sized *P. granularis* from the aeolianite and quartzitic sandstone predict that, on both shores, a 15 mm limpet will grow approximately 8 mm in one year. In addition, according to both these methods, the initial growth of *P. granularis* is greater on aeolianite shores than on quartzitic sandstone. However, a large discrepancy was found to exist between the observed growth of a mussel bed limpet with a shell length of 15 mm and the predicted growth. This bias is undoubtably a result of low recovery rates of labelled limpets from the mussel shells.

The growth of translocated limpets quickly (within a month) adjusted to mirror that of the resident limpets. This is particularly clear for *P. granularis* that were moved from the mussel shells to the quartzitic sandstone during autumn (Table 4.12). This rapid morphological adjustment to new circumstances implies that the observed differences in the growth rate of *P. granularis* inhabiting different substratum types are a function of environmentally induced factors and that growth rate of this species is not genetically constrained.

Although it was not possible to detect seasonal differences in the growth rate of *P. granularis* from the Von Bertalanffy curves, estimates of the growth of similarly sized limpets suggested that, although the growth of limpets was greatest during spring and summer on all substrata investigated, no significant seasonal differences in growth occurred. A greater variability in growth rate of *P. granularis* from the different substrata was however evident in autumn and winter. Intraspecific seasonal differences have been recorded for *T. sarmaticus* from the south-east coast (Lombard, 1977; McLaughlan & Lombard, 1981; Yssel, 1989). *H. pruinosus*, which also occurs in the same geographical region, grew twice as quickly in summer than in winter (Henninger, 1998). Although this was possibly attributed to increasing microalgal abundance during this time (Henninger, 1998), studies have shown that on the south-east coast, intertidal microalgal biomass in fact peaks during winter (Bustamante *et al.*, 1995b). In the current study, no seasonality in microalgal abundance was found (Chapter 7). Any seasonal differences in the growth rates observed in these two studies (i.e. Henninger, 1998 and this study) must thus be attributed to other factors such as temperature variability and reproductive cycle (e.g. Blackmore, 1969; Allen *et al.*, 1999).

The shape of the molluscan shell can have a substantial effect on the rate of water loss (Branch, 1981). As a result, high shore limpets usually have a higher, more domed shell than limpets that occur low on the shore (see Branch, 1981 for a review). As *P. granularis* has a widespread vertical distribution on the shore, this species provides an opportunity to investigate the correlation between tidal elevation and the degree of allometry. Limpets inhabiting the upper Balanoid zone would be expected to have a higher á value when compared to low-shore limpets. Although the results of the current study indicated that the shells of high shore *P. granularis* were generally taller (as evidenced by the greater á values), this was not universal for all shores. Low-shore limpets from Bird Island and Port Elizabeth were found to have higher á values than their high-shore counterparts. In addition, limpets from the mussel beds were generally more domed than expected. This may be related to the spatial constraints that are likely to be restricting increases in shell length, resulting in growth in height.

Intraspecific á values have been shown to vary with habitat. *H. pectunculus* inhabiting rocky reefs have higher á values than those that occur on boulder shores of rocky platforms (Gray, 1996). This has been attributed to the fact that limpets from rocky reefs are found higher up on the shore and so need to preserve more water (Gray, 1996). In contrast, *P. granularis* inhabiting quartzitic sandstone were found to have lower á values than those from aeolianite shores. This result was unexpected as aeolianite is a soft substratum that consists of many pits and indentations (see Chapter 2). Quartzitic sandstone, on the other hand, is flat and hard. Although not experimentally tested in the current study, water retention on the aeolianite shore is probably greater than on the quartzite (pers. obs; D. Abbey & A.H. Dye pers. comm.). As such, the need for higher shells in the limpets from the quartzitic sandstone was expected to be greater. *P. granularis* inhabiting the quartzite were however, found to be more heavily eroded, than those from the aeolianite shore. It is thus possible that the results of this investigation were biassed by this.

The texture and colour of the shell of *P. granularis* from different habitats has been ascribed to an attempt at camouflage (Branch & Branch, 1988). Higher shore limpets generally have lighter coloured shells with numerous nodules in order to blend in with the barnacles that occur in this area. Low shore limpets, on the other hand, tend to be darker in colour and have smooth shells (Branch & Branch, 1988). A similar trend was observed in the present study. Lindberg & Pearse (1990) have ascribed differences in shell colouration of limpets to the components of substratum that are involuntarily ingested during foraging. It is thus possible that the darker shell colour of low shore *P. granularis* is a result of the ingestion of particles of the darkly coloured *Perna perna* mussel shells over which they forage. These shell characteristics were also observed to change when the limpets were moved from one substratum type to another, particularly in the limpets that were translocated from the mussel shells to the quartzitic boulders (see Figure 4.13). A similar correlation between these shell characteristics and substratum was also found in the patellid limpets *Lottia asmi* and *L. digitalis* (Lindberg & Pearse, 1990). The shape of limpet shells may also be modified by wave action, as shape profoundly influences drag (Branch & Marsh, 1978, Branch, 1985b). Branch & Marsh (1978) found that *Patella* species that occur in wave beaten areas had shells that were textured with a number of nodules. This is opposite to the findings of the current study. As already mentioned, *P. granularis* from the mussel beds, which are exposed to greater wave activity than either the aeolianite or the quartzite, had smoother shells. It is possible that the irregular nature of the mussel beds shield the limpets from the direct force of the waves by increasing the turbulence of the water (see Branch & Branch, 1988). An additional factor which has been suggested to influence the texture of shells is temperature. Increased sculpturing of shells may increase re-radiation of heat (Branch, 1985b; Branch & Branch, 1988). Limpets that are exposed to direct sunlight are thus often highly sculptured while those inhabiting shady areas are smooth (Vermeij, 1973). In the current study, it appears that this may be the reason for the smooth shells of limpets from the mussel beds. However, *P. granularis* from the aeolianite platform were found to have more highly textured shells than those from the quartzitic sandstone. As water retention of the aeolianite rock is greater than that of the quartzite (D. Abbey & A. H. Dye; pers. comm., pers. obs.), one would expect more sculpturing of the shells of limpets inhabiting quartzitic sandstone. It is possible that, once again, the high degree of erosion of shells from the latter site may have influenced these conclusions.

Principal component analysis has suggested that along the south-east coast, the mean shell length of *P. granularis* decreases from west to east. Associated with this is an increase in shell height and shell width. It must, however, be noted that these analyses were performed on samples collected at a particular period in time, and as such provide only a “snap-shot” of the characteristics of the respective populations. Nevertheless, the results obtained from the morphometric analysis allow an initial investigation into these characteristics and suggest that a number of factors may influence these populations. The factors which may influence the size of *P. granularis* have already been discussed (see Chapter 2). The associated increase in shell height provides additional food for thought. It has been suggested that as sea temperatures increase in an easterly direction along the coast of South Africa, so conditions for larval settlement become more favourable (Gray, 1996). This in turn reduces the restrictions placed on the timing of reproduction (Gray, 1996) and results in south-east coast intertidal species maximising their reproductive effort by spawning either twice a year or having protracted spawning (Robson, 1986; Lasiak, 1987a,b; Gray, 1996; Foster, 1997; Henninger, 1998, present study). Increased reproductive effort may decrease the amount of energy available to channel into growth (see also Chapter 5) resulting in the decreased size of limpets observed at Three Sisters and Port Alfred. In addition, the more favourable environmental conditions experienced with increasing distance eastwards may enhance recruitment success leading to an increased density in these populations. This in turn may reduce the space available for an increase in length, thus forcing the limpets to increase the height of the shell. Alternatively, it is possible that some natural or man-made event may have reduced the number of large limpets present in the easterly localities. This may have been followed by a successful recruitment which would have resulted in an increase in the smaller classes of limpets observed. Although the limpets from this site were not aged, it is possible that these smaller limpets are younger. The shape of limpets are known to change with increasing age (Branch, 1981). Thus it is possible that the smaller, younger limpets naturally have a higher shell than the older limpets and that this has biassed the results obtained in the current study. These suggestions are however speculative and long-term monitoring of these populations would be necessary in order to determine whether the observed trends are indeed real or simply artifacts of the past, unknown history of the populations.

The use of internal shell growth bands for age determination and growth has been used in a number of studies (Crisp & Richardson, 1975; Lutz, 1976; Richardson, 1987; Crisp *et al.*, 1990; Nakaoka & Matsui, 1994; Gray, 1996; Henninger, 1998). Branch's (1974a) statement that no obvious growth bands were present in the shells of *Patella* has subsequently been refuted. *P. vulgata* has been shown to produce growth bands on a tidal basis (Ekaratne & Crisp, 1982). Similarly, the present study conclusively shows that microgrowth bands occur in the shells of *P. granularis*, and that these too are deposited tidally. In addition to being used to accurately establish the age and growth rate of organisms, microgrowth bands can also be used to test the accuracy of a theoretical growth curve (e.g. Von Bertalanffy), as done by Gray (1996) and Henninger (1998) for *Helcion* species. Unfortunately, the high degree of shell erosion in *P. granularis* from all three substrata rendered this method of age and growth estimation unsuitable.

In conclusion, the results of this study indicated that *P. granularis* inhabiting the south-east coast of southern Africa grow at a slower rate and live approximately twice as long as west coast limpets. This difference in growth has been attributed to the greater primary productivity that occurs on the west coast. The growth rate of *P. granularis* on the south-east coast was also found to vary with habitat. Although limpets inhabiting an aeolianite and a quartzitic sandstone shore had similar growth curves and reached similar maximum lengths, *P. granularis* from the mussel shells were much smaller than those from either of the two other sites. This has been attributed to a slower growth rate of the limpets on mussel shells, possibly as a result of spatial constraints, and not to settlement of juveniles which subsequently migrate up-shore as they grow. Furthermore, limpets inhabiting all three substrata were estimated to have a similar longevity. Populations of *P. granularis* were found to generally grow allometrically (i.e. shell height increases faster than shell length), although this was not universal on all shores. Currently, the factors responsible for the different growth rates are not known, although this study offers some suggestions. Future research into the energy apportioned to growth and reproduction of south-east coast *P. granularis* would be helpful in understanding the complex interaction of growth with both biological and environmental factors. Furthermore, the correlation between shell shape, degree of shell erosion and water loss of *P. granularis* would also provide opportunities for future research.

Chapter 5

Chapter 5

REPRODUCTIVE SEASONALITY OF *PATELLA GRANULARIS* FROM THREE SITES ON THE SOUTH-EAST COAST OF SOUTH AFRICA

5.1: INTRODUCTION:

From the many studies on the reproductive biology of prosobranch limpets, two main patterns of spawning have been identified. The majority of patellids have a highly synchronised, clearly defined annual cycle of gamete release, which may or may not be protracted (see Branch, 1981 and Gray, 1996 for reviews). In contrast, several acmaeids have a protracted breeding season (e.g. Underwood, 1974; Creese, 1980; Lasiak, 1987a & 1990) and while synchronisation within a population is rare, it has been recorded in at least one species (Creese & Ballantine, 1983).

Patellogastropods have a simple reproductive system (Branch, 1981) and most are dioecous broadcast spawners (Fretter, 1984) which release mature gametes into the water column where fertilisation occurs (Webber, 1977). The synchronisation of gametogenesis and simultaneous spawning within a population is therefore thought to increase the chances of successful fertilisation (Lasiak, 1990). Increased reproductive success is also dependant upon both the density of mature adults and the extent of the water movement experienced at the time of spawning (Joska & Branch, 1983; Pennington, 1985; Claereboudt, 1999).

Many marine invertebrates which have an annual reproductive cycle show seasonal variation in the timing of gametogenesis and spawning. This variability indicates that reproductive cycles within populations must be synchronised by external environmental factors (Gonor, 1973a; Himmelman, 1979; Stanwell-Smith & Clarke, 1998). Wave action, food availability, temperature and daylength all influence gonadal development (Gonor, 1973a; Giese & Pearse, 1974; Foster and Hodgson, 1995; Vernon *et al.*, 1993). The environmental factors that trigger spawning may, however, be different to those that initiate gonadal growth (Lane & Lawrence, 1979). These cues include temperature (Bowman, 1985), phytoplankton booms (Himmelman, 1975 and 1979), increased wave action and windspeed (Orton & Southward, 1961; Balaparameswara Rao, 1973; Branch, 1974b; Thompson, 1979; Catalan & Yamamoto, 1993), sperm suspension (Himmelman, 1979), lunar cycles (Aliferakis & Berry, 1980), hormones (see Giese & Kanatani, 1987, for a review of literature) and a combination of these (Himmelman 1979; Fretter, 1984).

Both an inter- and intraspecific relationship between the reproductive pattern and the geographic distribution of invertebrates occurs. Generally, species from low latitudes have an extended breeding season, while those from higher latitudes have a restricted one (Fretter, 1984). In South Africa, cool temperate limpet species tend to have only one spawning event a year, while warm temperate to subtropical species either exhibit two spawning events or continuous spawning (see review by Foster, 1997). This pattern also occurs in many North American acmaeid limpets (Fritchman, 1962). Intraspecific differences are also evident around the coast of South Africa, with limpets inhabiting the cool temperate regions spawning in winter whilst their warm temperate counterparts spawn in spring or summer (Branch, 1974b).

Patella granularis is the most widespread of the South African limpet species with its distribution ranging from Namibia in the west, to Natal in the east (Branch, 1971; Branch *et al.*, 1994). Furthermore, this species has an extensive intertidal distribution occurring from the high shore upper Balanoid zone down to the low-shore mussel beds and occurs on shores of differing geomorphologies (Chapter 2). Very little is known about the reproductive biology of *P. granularis* inhabiting the south and east coasts of South Africa although information on the reproductive patterns of this species has been published from the cooler west coast (Branch, 1974b). In addition, although it is well-known that habitat influences many aspects of the biology of intertidal organisms (see Branch, 1981 for a review of literature), to date, no studies have attempted to quantitatively investigate whether there is any variability in the reproductive seasonality of a South African limpet species from different habitats.

The current study therefore aimed to firstly investigate and quantify the reproductive seasonality of *P. granularis* from the south-east coast of South Africa, with a view to comparing the results obtained to those reported by Branch (1974b) for the same species from the west coast. This would in turn enable a comparison of the reproductive biology of a geographically abundant species to be made. Furthermore, the majority of reproductive studies conducted to date have been performed at a single locality. Due to the fact that differences in population structure, biomass and density of *P. granularis* were recorded along the south-east coast (Chapter 2), it was decided to include two localities of differing geomorphologies in the current study. Finally, the limpets inhabiting the mussel beds of the Cochlear zone were much smaller than those inhabiting both the lower and upper Balanoid zones (Chapter 2). Despite their smaller size, limpets inhabiting mussel shells were noted to possess well developed gonads (pers. obs.). Reproductive seasonality and fitness in these limpets was thus also investigated. The inclusion of three groups of limpets inhabiting three types of substrata was also intended to be a preliminary investigation into the influence of one aspect of limpet biology. Finally, an attempt was made to investigate and compare the reproductive fitness (i.e. the number of eggs per female) of south-east coast *P. granularis* inhabiting three substrata.

5.2: MATERIALS AND METHODS:

5.2.1: General procedures:

The reproductive seasonality of *Patella granularis* along the south-east coast was investigated for a period of 24 months, from May 1996 to April 1998. Two localities, providing three types of substrata (also referred to in this chapter as sites), were chosen as they supported large populations of *P. granularis*. The first site, Kenton-on-Sea (33E44' S; 26E40' E), is an aeolianite platform and cliff; the second site, Cannon Rocks (33E44' S; 26E35' E) is a quartzitic sandstone boulder beach. The third type of substratum occurs in the low shore mussel beds *(Perna perna)* at Cannon Rocks and was chosen as a high density of *P. granularis* inhabit the shells of the mussels (see Chapter 2 for a more detailed description of the study sites). On both the aeolianite and quartzitic sandstone shores, specimens were collected from the lower Balanoid zone where they were most numerous (Chapter 2). Limpets from the mussel bed were collected directly from the mussel shells. Sixty specimens were collected on a monthly basis from each site during spring full moon low tides. As no significant difference has been found between dry gonad indices of fresh and preserved limpets (Branch, 1974b; Gray, 1996), specimens were fixed in 4% formal-saline and dissected within 3 days. As a result of the significant difference in the sizes of mature adults from each site (Chapter 2), restrictions were placed on the sizes of animals collected at each site to eliminate any potential effect of size on Gonad Index. Limpets collected from the aeolianite and quartzitic sandstone substrata ranged in size from 20 mm to 30 mm while those inhabiting the mussel beds ranged in size from 10 mm to 20 mm. *P. granularis* were collected from east-facing rock surfaces and mussel beds only, due to the observed differences in foraging activity between limpets inhabiting east- and west-facing slopes (Chapter 6).

The reproductive biology of *P. granularis* inhabiting the three substrata was determined by a variety of methods including Gonad Indices, histological examination and fecundity (see Branch, 1974b and Creese & Ballantine, 1983 for examples of methods). These results were then compared to the observations made on the reproductive biology of *P. granularis* inhabiting the west coast (Branch, 1974b).

5.2.2: Reproductive fitness as determined by Gonad Index:

Gonad Index or GI (Gonor, 1972), which relates gonad weight to total body weight, is often used to determine seasonal changes in gonad activity (Catalan & Yamamoto, 1993; Gray, 1996; Foster, 1997; Henninger, 1998; McClary & Barker, 1998). Spawnings that occur whilst the GI is still increasing may, however, be masked by the continued gonad development (Bowman, 1985). Despite this, GI still provides a quantitative method whereby the overall reproductive patterns of separate populations may be compared. As a result, it was decided to use this method in conjunction with histological analyses of gonads in order to compare the reproductive patterns of *P. granularis* inhabiting different substrata. Mean monthly gonad index (GI) values were determined from 15 males and 15 females from each site using the formula of Gonor (1972) where

$$GI = ((\text{dry gonad weight})/(\text{dry body weight} + \text{dry gonad weight})) * 100.$$

The gonad and somatic tissue of each limpet were weighed to the nearest 0.001 g after drying to constant weight (. three days at 60EC) before the GI was calculated. Macroscopic sexing of the limpets was possible as the mature testis is milky-white in colour while mature ovaries are orange.

5.2.3: Reproductive seasonality as determined by the gametogenic cycle:

As the gross morphology of limpet gonads has previously been described (Dodd, 1956; Orton *et al.*, 1956; Branch, 1974b), only a brief description follows: oocytes and developing sperm are attached to thin sheet-like trabeculae that penetrate the gonad and support a germinal epithelium. Oocytes remain attached to the trabeculae until mature, when they lie loose in the lumen of the ovary. Similarly, spermatocytes that are produced by the germinal epithelium of the trabeculae fill the lumen as they mature (Branch, 1974b). The process of gametogenesis is often divided into a number of stages according to the level of development of the gonad (see Balaparameswara Rao, 1973; Branch, 1974b; Foster, 1997 for examples). Although Branch (1974b) identified six stages within the oogenic cycle of *P. granularis* from the west coast, these stages were not easily identifiable in the limpets of the present study. As such, it was decided to correlate the reproductive pattern identified by the GI to changes in oocyte size frequencies for limpets from each of the three sites. Similarly, the stages of spermatogenesis were difficult to identify as a result of the presence of mature sperm in the testes throughout the experimental period. However, the width of the germinal epithelium changes throughout the gametogenic cycle, with a decrease in width being associated with an increase in the number of mature sperm in the lumen (Branch, 1974b). It was thus decided to correlate these changes to the reproductive pattern identified by the GI.

The gametogenic cycle was determined by histological analyses of five males and five females collected monthly over 12 months (May 1997 to April 1998). The gonad of each limpet collected was fixed in aqueous Bouin's fixative for at least 24 hours. Each gonad was then dehydrated using a graded ethanol series before being embedded in Paraplast. Transverse sections (5 - 7 Fm in thickness) cut through the widest diameter of the gonad using a Leitz rotary microtome were stained with haematoxylin and eosin (Humason, 1967). Each of the subsequent histological slides was then archived using a Jeol Winstor image archiver and analysis system.

Seasonal changes in egg size within the ovary were quantified by calculating the mean monthly oocyte size-frequencies of five females from each site. The diameters of the first 50 oocytes, which had an approximately centrally placed nucleus, were measured from the archived photographs. The data from the five females were pooled (250 oocytes/sample) and used to determine the monthly size range of oocytes. Mean monthly changes in the thickness of the germinal developing layer of the testis were also determined from the photographs. In each male, five measurements were made at intervals of 10 Fm along ten randomly chosen sections of germinal epithelium. All measurements (to the nearest 0.01 Fm) were made from the archived photographs using a digitiser tablet and Summagraphics software.

5.2.4: Sex ratios and size at first sexual maturity:

Sex ratios of *P. granularis* inhabiting the three substrata were determined from limpets collected from transects that were carried out at the two study sites (i.e. Kenton-on-Sea = aeolianite; Cannon Rocks = quartzite and mussel shells; see Chapter 2). All limpets that were collected from the three substrata were examined macroscopically to determine sex (see section 5.2.2). The ratios of males to females were then calculated. Similarly, the size of first sexual maturity was also estimated from the data collected from these transects. The percentage of individuals of *P. granularis* with mature gonads in 2 mm size classes was recorded. The size at which the gonad of 50% of limpets in a given size class contained either spermatozoa or vitellogenic eggs was used as an index of first sexual maturity. This evaluation technique is widely used in fisheries management to determine the onset of sexual maturity in marine molluscs (Pitcher & Hart, 1982; King, 1995; Wood & Buxton, 1996).

5.2.5: Reproductive fitness as determined by fecundity:

The fecundity (i.e. the reproductive capacity - Allaby, 1992) of female *P. granularis* was estimated at each of the three sites. Three females, each of a similar length to the mean length of females analysed for determination of GI, were sampled from each site during the period of peak GI (December for aeolianite and quartzitic shores, January for limpets from mussel shells). The gonad was carefully removed from each limpet and weighed (to the nearest 0.0001 g) using an electronic balance. A subsample (0.001 g) of gonad was then removed from the centre of the gonad and placed in a petri dish containing water. The eggs were then viewed under a dissecting microscope and the number of mature eggs (> 120 Fm) present in the sample counted. The average weight of gonad lost during spawning was then estimated from the decrease in GI for each site. An estimation of the number of eggs lost during spawning could then be made.

5.2.6: Statistical analysis:

All statistical procedures were taken from Fry (1993) and Sokal & Rohlf (1981). Computation of statistical procedures were made using Statgraphics V7.0 (Statistical Graphics

Corporation, USA) Normality of distribution was tested using the Kolmogorov-Smirnoff test and homogeneity of data were tested using Levene's test. As the data conformed to the assumptions of parametric analysis, no transformations were necessary. A two-way ANOVA was used to determine whether the GI differed significantly between males and females at any site, and whether the mean GI differed among sites during the experimental period. Finally, a chi-squared test was performed to determine whether *P. granularis* deviated from a 1:1 sex ratio on any of the substrata.

5.3: RESULTS:

5.3.1: Sex ratio:

Patella granularis was found to be dioecious (no hermaphrodites were observed), and do not exhibit external sexual dimorphism (i.e. neither the shell sizes, nor the external morphology, of males and females differ significantly). *P. granularis* inhabiting the mussel shells did not deviate significantly from a 1:1 sex ratio ($p = 0.574$, Table 5.1). Similarly, although more males than females were recorded in the limpets inhabiting quartzitic sandstone, this difference was not significant ($p = 0.051$; Table 5.1). *P. granularis* from the aeolianite shore did, however, deviate significantly from a 1:1 sex ratio ($p = < 0.0001$), with males predominating.

Table 5.1:

Frequencies and ratios of male to female *P. granularis* inhabiting three substrata.

p - values obtained from chi-squared analysis to determine whether the frequencies deviated from a 1:1 ratio are also shown. (* indicates a significant p - value).

		Frequency		Ratio		Chi-square
Site	**n**	**Females**	**Males**	**Female**	**Male**	**p-value**
Mussel shells	114	60	54	1.11	1.0	0.574
Quartzitic	397	179	218	1.0	1.22	0.051
Aeolianite	416	181	235	1.0	1.30	< 0.0001*

5.3.2: Size at first sexual maturity:

First sexual maturity in *P. granularis* inhabiting both quartzitic and aeolianite shores occurred at a shell length of between 8 mm to 10 mm (Figure 5.1a and b). *P. granularis* from the mussel shells reached sexual maturity at a smaller size with approximately 50% of the population becoming sexually mature at a shell length of between 6 mm and 8 mm (Figure 5.1c).

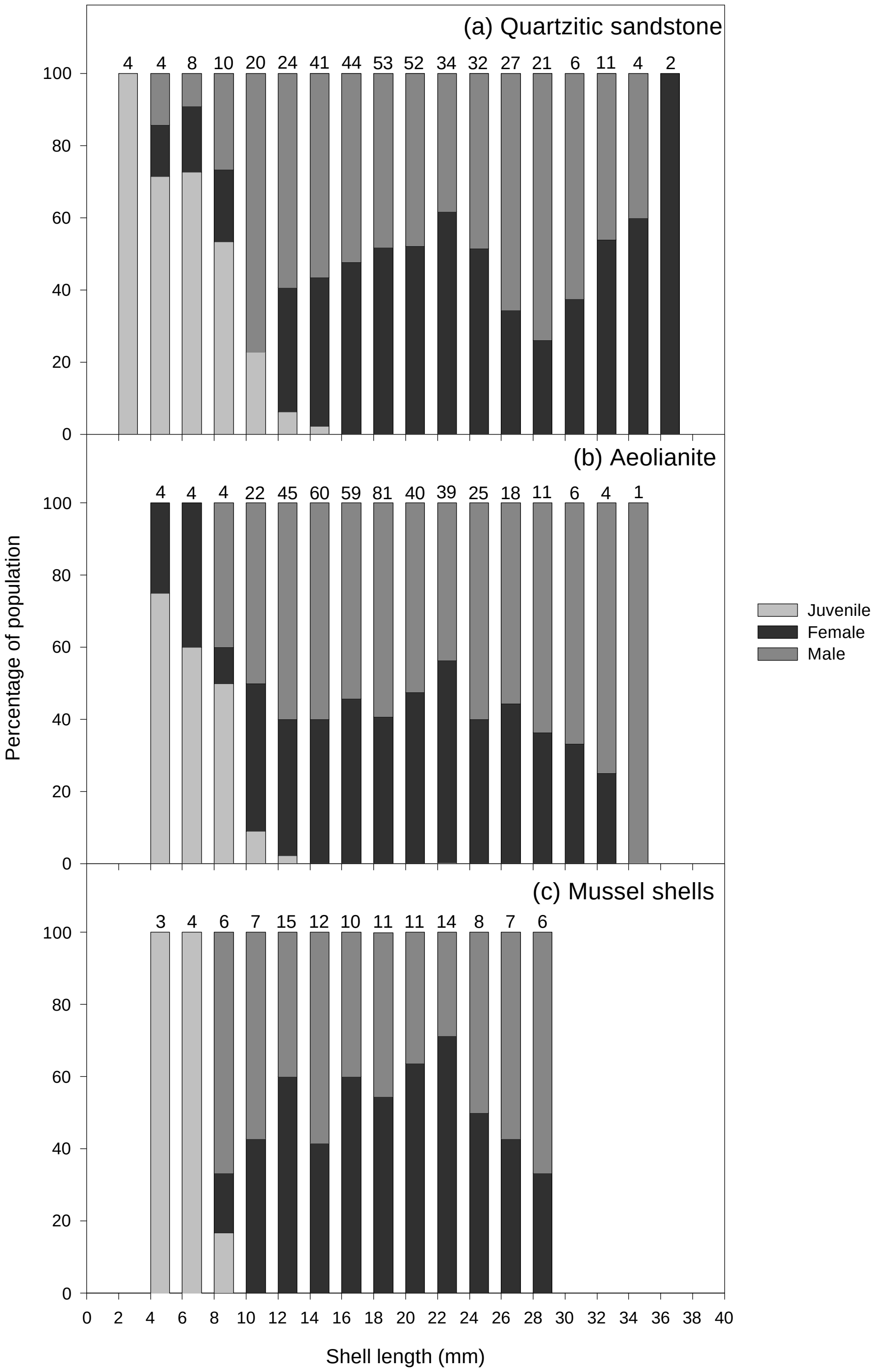


Figure 5.1:

Sex proportions (juvenile, female and male) of *P. granularis* from three substrata. (Numbers above the bars indicate sample sizes).

5.3.3: Reproductive fitness as measured by Gonad Index (GI):

The gonad was present in both sexes at all sites throughout the year. Mean GI values varied between 5% and 25% for individuals on mussel shells and quartzitic sandstone (Figures 5.2 and 5.3), and between 3% and 21% on aeolianite (Figure 5.4). Although there was no significant difference in the mean GI values of males and females at any site during the study period (two-way ANOVA, Table 5.2), a great deal of variability was observed within the monthly samples. Furthermore, the mean GI values did not differ significantly among the three sites (F = 0.313; p = 0.732; Table 5.2). As the mean GI values of males and females were similar (Table 5.2), all subsequent statistical analysis was performed on pooled data for each site.

Table 5.2:

Results of a two-way ANOVA on the effects of sex and site on GI of *P. granularis.*

Source of variation	S.S	d.f.	M.S	F-ratio	Sig. level
Main effects					
Site	12.357	2	6.178	0.313	0.732
Sex	7.382	3	2.461	0.124	0.946
Residual	2727.969	138	19.768		
Total	2747.708	143			
No significant interactions					

The GI suggests that the spawning periods were highly synchronous between the males and females at each site. Although a marked reproductive seasonality was evident in all the limpets studied, the annual reproductive pattern differed among habitats. *P. granularis* living on mussel shells showed the most defined reproductive pattern, with limpets spawning twice a year, as evidenced by the significant decreases in GI values (Table 5.3; Figure 5.2). However, some interannual variability in the duration of the spawning period was observed. Between May 1996 and April 1997, spawning occurred from August to December and again from January to March. In the twelve months from May 1997 to April 1998, however, spawning lasted only from August to October, with the summer spawning beginning slightly earlier in December and continuing until March (Figure 5.2).

Table 5.3:

Results of a one-way ANOVA to determine significant differences in the monthly mean GI of *P. granularis* inhabiting mussel shells.

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
Between groups	13736.203	23	597.226	10.497	< 0.0001
Within groups	33228.135	584	56.897		
Total	46964.338	607			

The reproductive pattern of limpets inhabiting the quartzitic boulder beach differed substantially during the study period. In the first year of the study (May 1996 - April, 1997), a consistent decrease in the GI of females occurred from May to December (Figure 5.3), although a slight increase was recorded in August. This increase was more evident in the males and was followed by a significant drop in the GI in September (Table 5.4; Figure 5.3). A second non-significant increase in the GI of females occurred in January, but was not mirrored in the males. In the second year of the study (May 1997 - April 1998), two peaks in the GI of both males and females were evident. The first, occurring in May and June, was followed by a decrease in GI until September, when significantly low GI values were recorded (females = 9.87; males = 9.83; Figure 5.3; Table 5.4). A second spawning period was evident in summer when the GI dropped significantly from . 19.5 in December to .10 in January and February (Figure 5.3).

Table 5.4:

Results of a one-way ANOVA to determine significant differences in the monthly mean GI of *P. granularis* inhabiting quartzitic sandstone.

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
Between groups	7899.794	23	343.469	7.704	< 0.0001
Within groups	26258.752	589	44.582		
Total	34158.546	612			

The reproductive pattern of *P. granularis* inhabiting the aeolianite shore was more difficult to interpret. From May 1996 to May 1997, these limpets had two well-defined spawning periods, with GI values dropping significantly from May to September, and again from December to February (Table 5.5; Figure 5.4). However, the spawning periods for the rest of the study were less defined. From the GI values obtained for the females, it appears that from June 1997 to April 1998, trickle or partial spawning occurred resulting in GI values of between 10% and 16%. A clearer pattern is evident in the males, with a drop in GI values occurring from August to October and again from February to April, however values were always low, and did not rise above 20%.

Table 5.5:

Results of a one-way ANOVA to determine significant differences in the monthly mean GI of *P. granularis* inhabiting aeolianite.

Source of variation	S.S	d.f.	M.S	F-ratio	Sig. level
Between groups	6457.291	23	280.752	4.532	< 0.0001
Within groups	37291.572	602	61.946		
Total	43748.863	625			

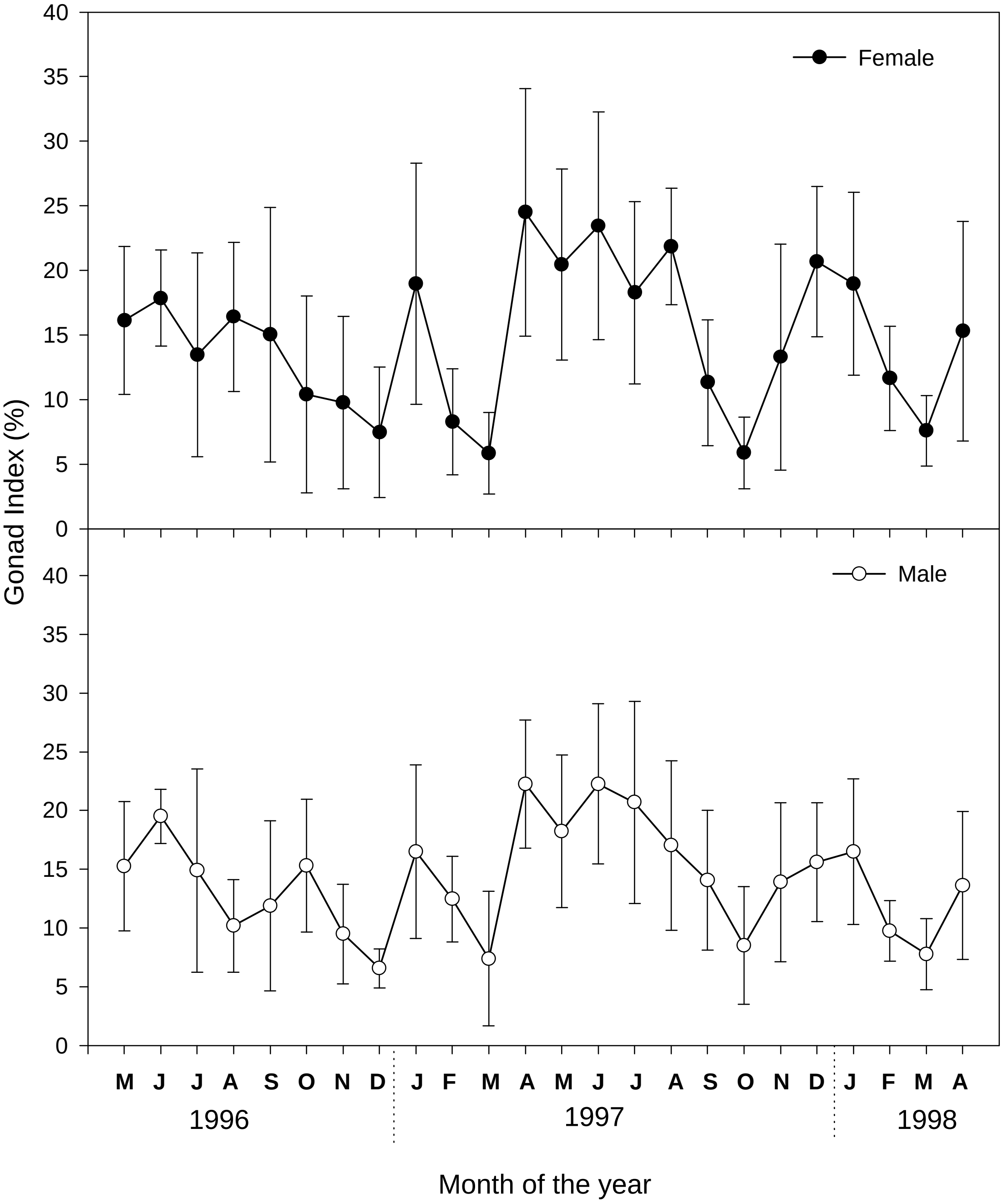


Figure 5.2:

Mean monthly gonad indices (± S.D.) for *P. granularis* inhabiting mussel shells from May 1996 to April 1998. n = 15 limpets of each sex per month.

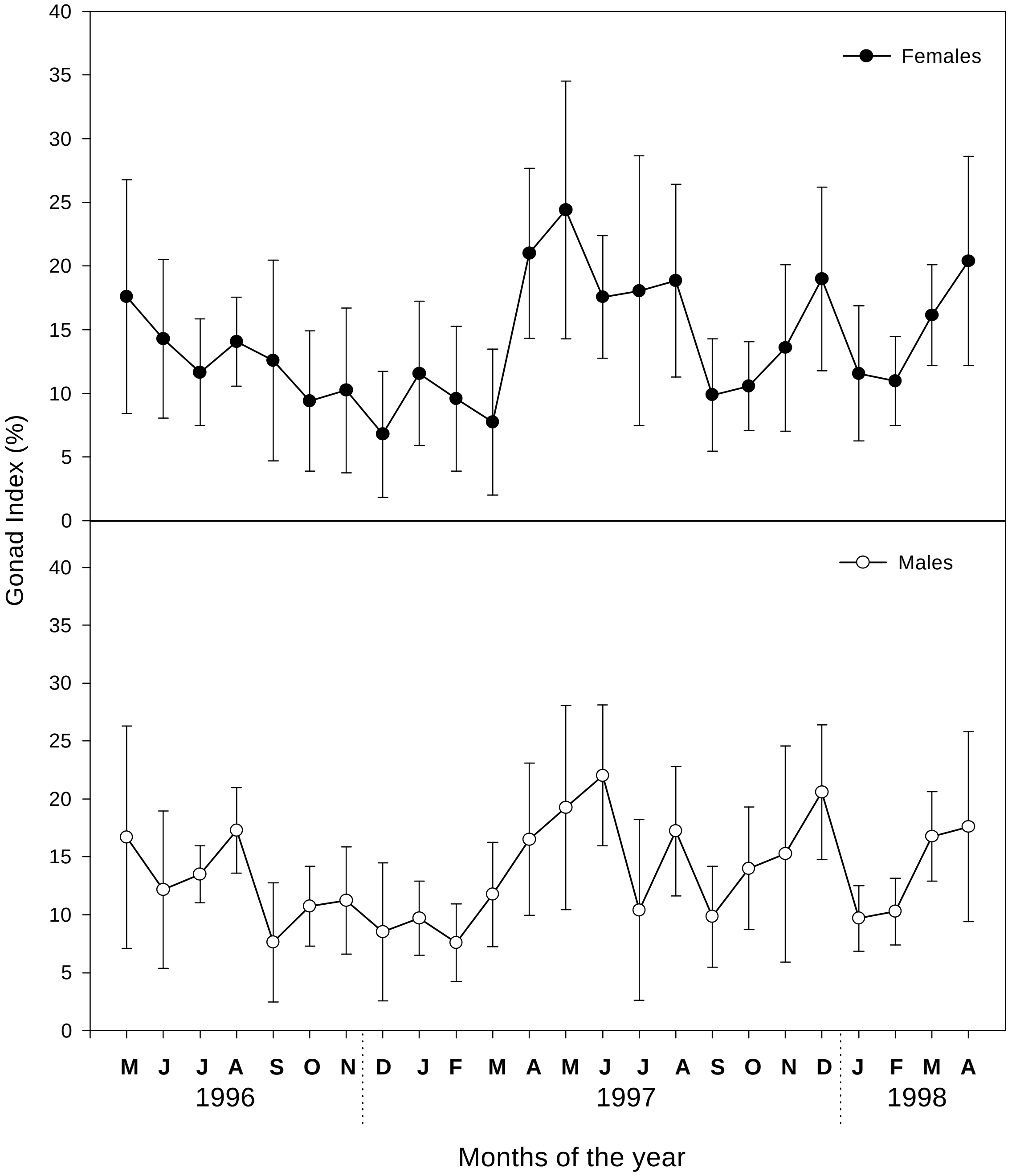


Figure 5.3:

Mean monthly gonad indices (± S.D.) for *P. granularis* inhabiting quartzitic sandstone from May 1996 to April 1998. n = 15 limpets of each sex per month.

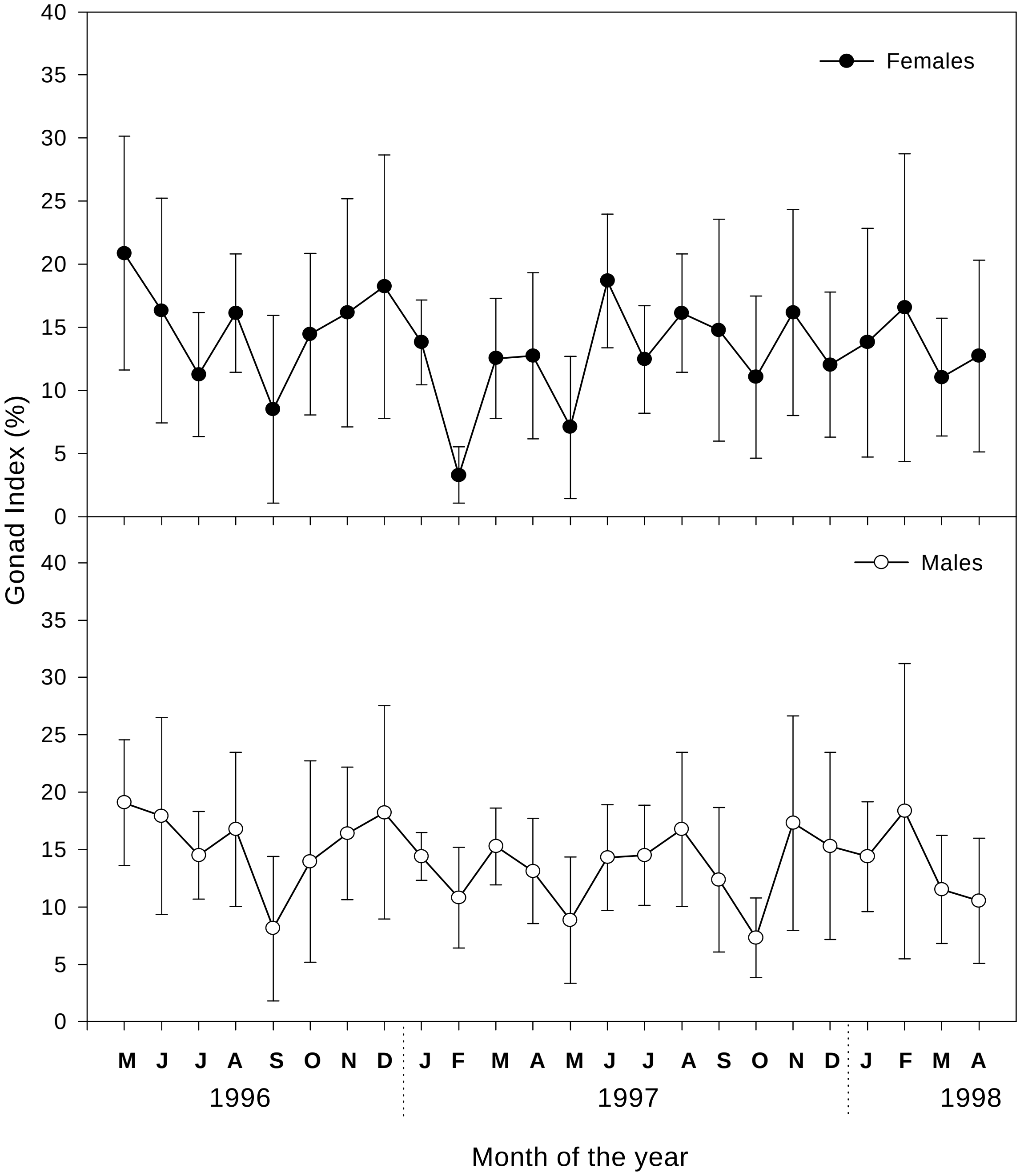


Figure 5.4:

Mean monthly gonad indices (± S.D.) for *P. granularis* inhabiting aeolianite from May 1996 to April 1998. n = 15 limpets of each sex per month.

5.3.4: Reproductive seasonality as determined by the gametogenic cycle:

The reproductive seasonality of *P. granularis* from the three substrata was also determined from an examination of the monthly gametogenic condition. Pre-vitellogenic eggs (< 100 Fm in diameter; e.g. Figure 5.8a) were always present in the limpets inhabiting the mussel shells (Figure 5.5). However, an increase in the numbers of mature vitellogenic eggs (> 125 Fm; e.g. Figure 5.8b) was only found twice in the year, once in August and again in December (Figure 5.5). These times correspond to the periods of peak GI of females. Although not occurring in large numbers, an increase in the size range of the vitellogenic eggs (up to 270 Fm in diameter) was evident in January. A similar trend was also found in the limpets from the quartzitic shore (Figure 5.6). Once again, pre-vitellogenic eggs were always present, with the greatest abundance of this stage of oogenesis occurring in conjunction with a low GI (e.g. September and February). As expected, the number of mature vitellogenic eggs were greatest during two periods of the year, initially in May and again during November and December when the increased frequencies of eggs larger than 125 Fm were particularly obvious. Vitellogenic eggs were present throughout most of the year in the limpets from the aeolianite shore (Figure 5.7). A peak in the number of these eggs was, however, still evident in August. This corresponded to the second highest GI value recorded during this period of the study. Pre-vitellogenic eggs were also always present.

A bimodal distribution of oocyte frequencies was found throughout the year at all sites, with the first peak indicating high numbers of pre-vitellogenic eggs (< 100 Fm), while the second was indicative of large vitellogenic eggs.

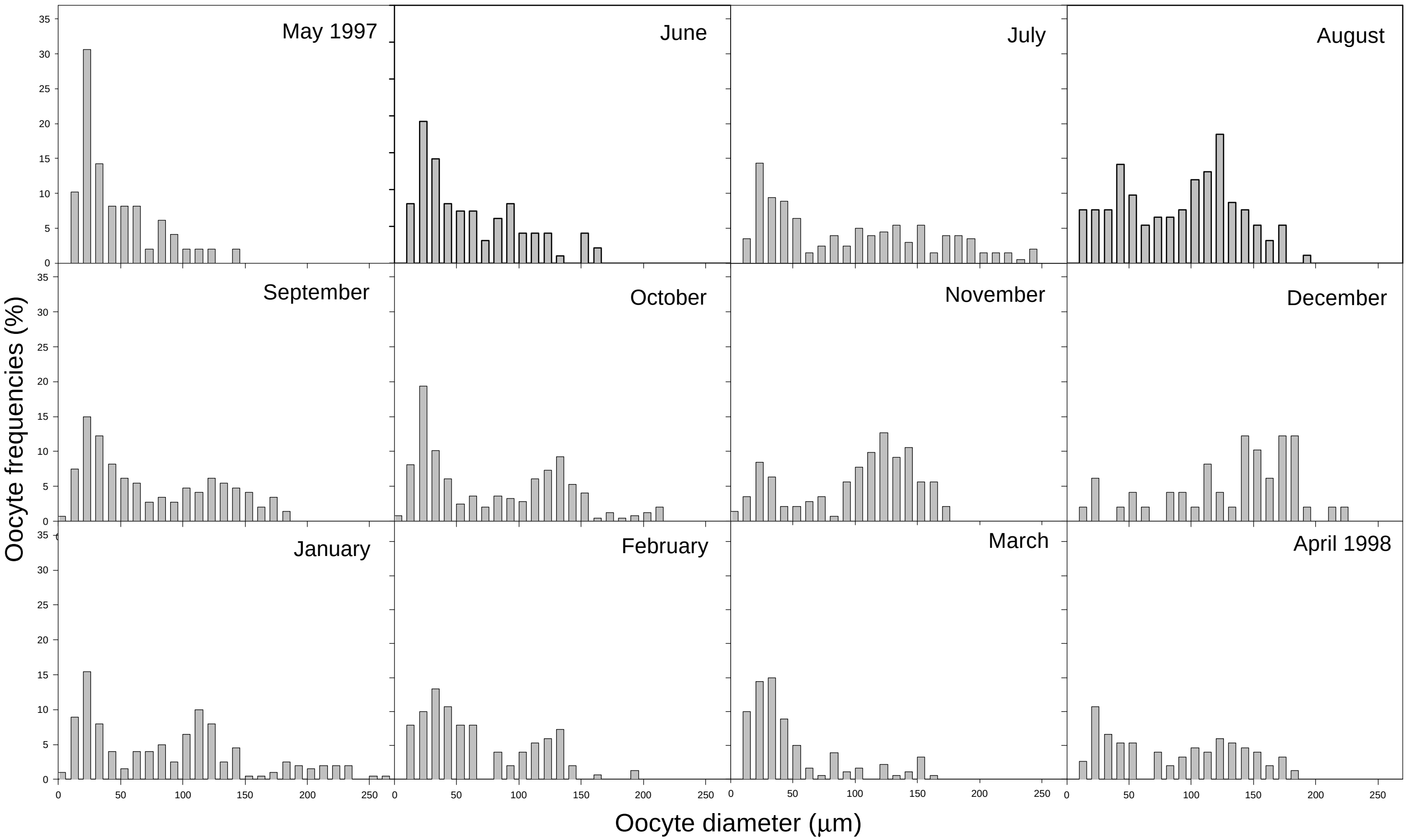


Figure 5.5:

Monthly oocyte-length frequencies of *P. granularis* inhabiting mussel shells.

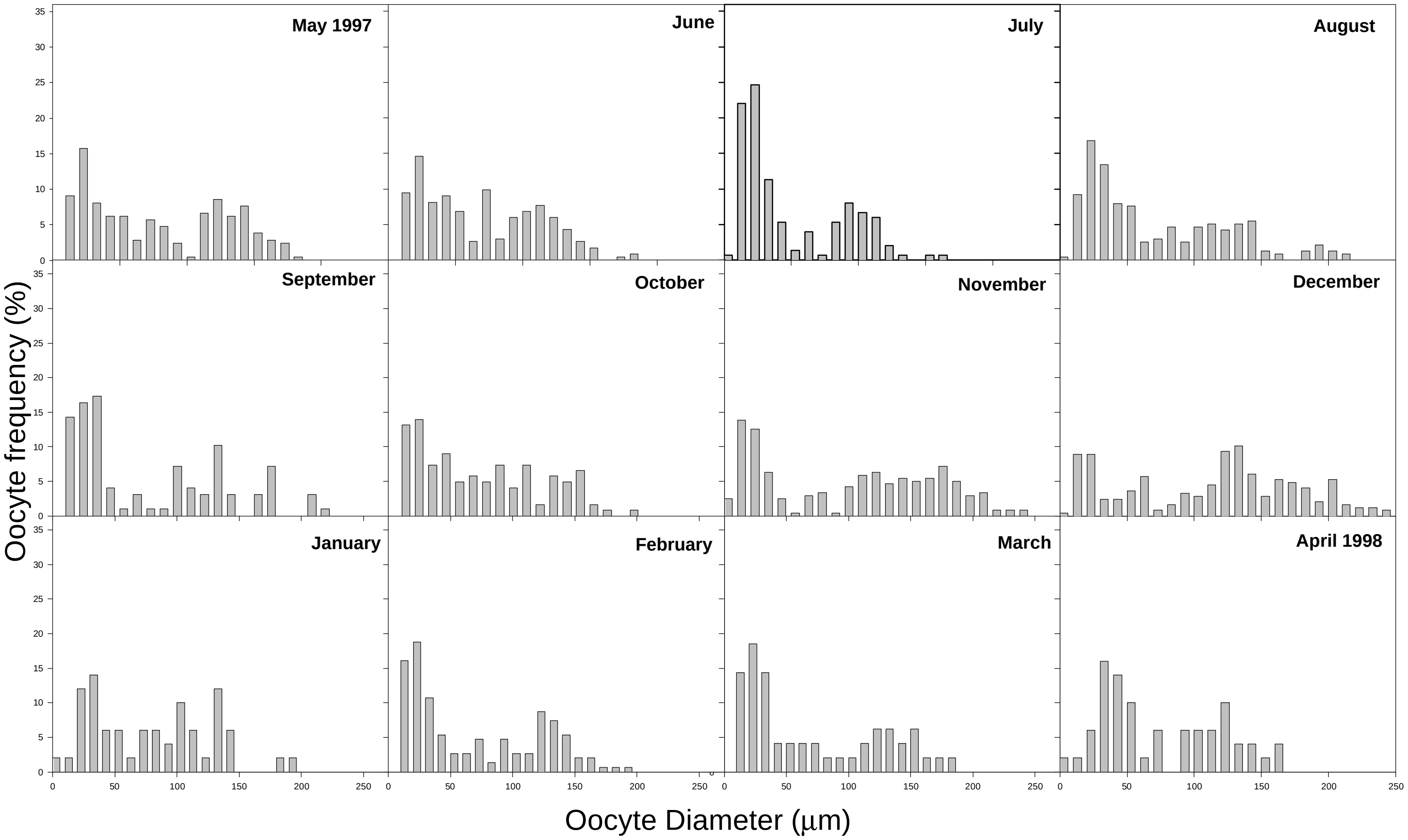


Figure 5.6:

Monthly oocyte-length frequencies of *P. granularis* inhabiting quartzitic sandstone

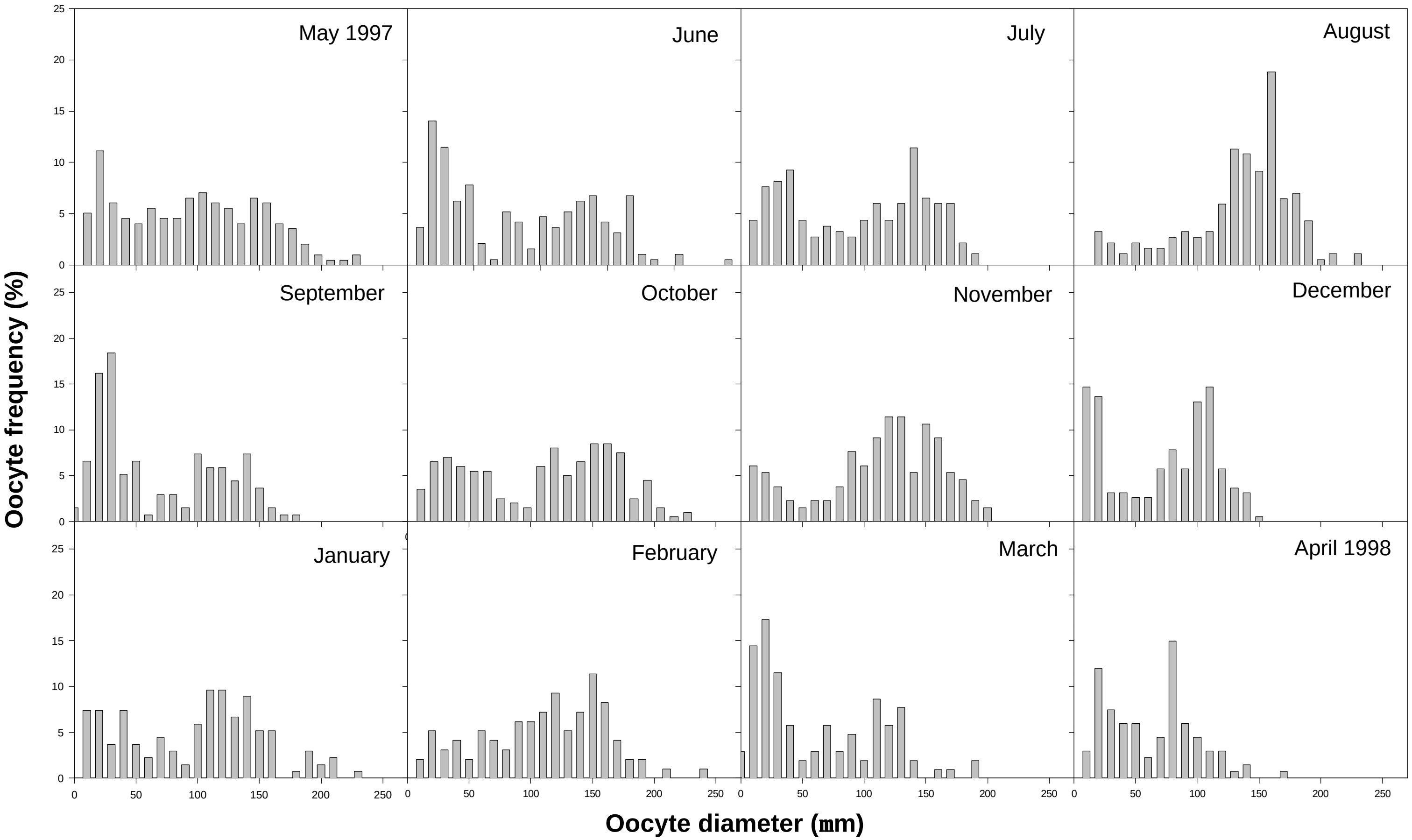


Figure 5.7:

Monthly oocyte-length frequencies of *P. granularis* inhabiting aeolianite.

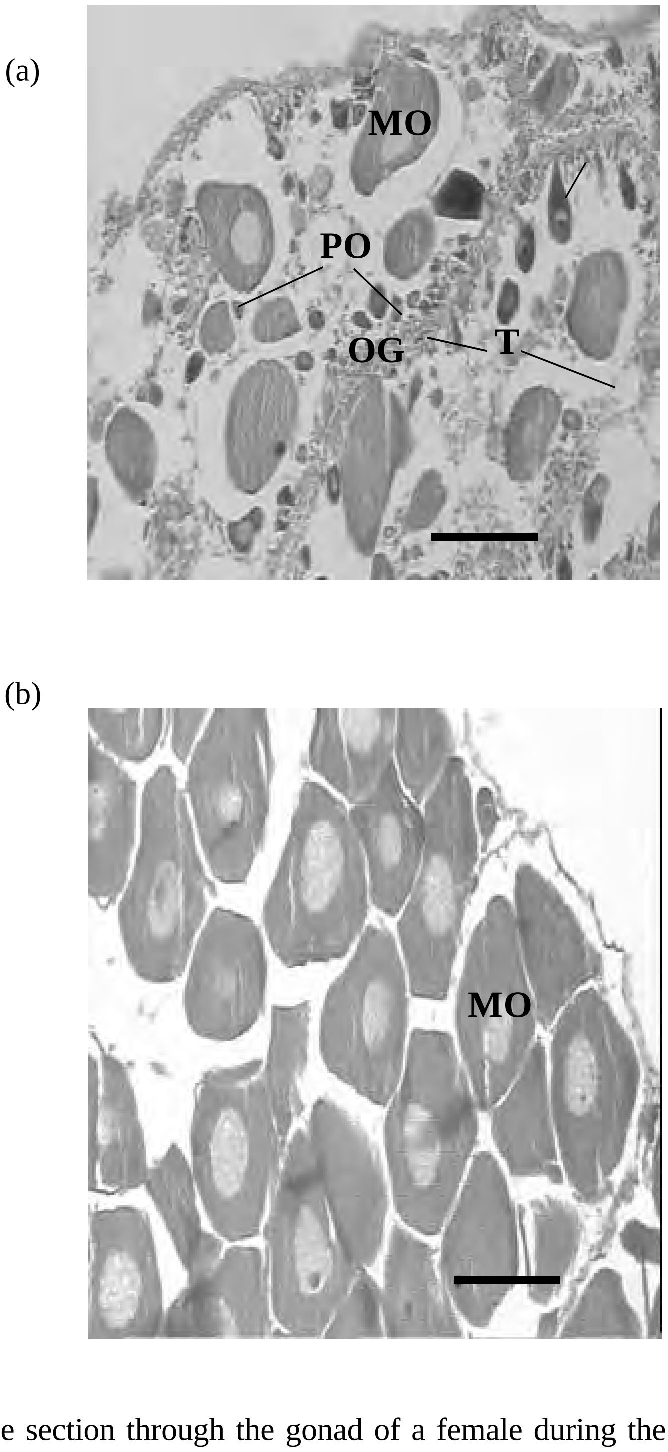


Figure 5.8:

Transverse section through the gonad of a female during the periods of (a) minimum Gonad Index and (b) maximum Gonad Index. OG = oogonia; PO = Pre-vitellogenic oocyte. MO = Mature vitellogenic oocyte; T = Trabeculae; EV = Early vitellogenic oocyte. (Scale bar = 100 μm)

Males inhabiting all three substrata were found to have mature spermatozoa throughout the year, however the timing of spermatogenesis varied among the sites. Limpets from the mussel beds began spermatogenesis in October, after winter spawning, as evidenced by the thick germinal layers of the testes (Figures 5.9a; e.g. 5.10a). The spermatozoan content of the gonads then rapidly increased as reflected by the significant decline in the thickness of the germinal epithelium from a thickness of 80 Fm in October to 30 - 35 Fm between November and January (Tables 5.6 and 5.7; Figure 5.10b). A sharp increase in the width of the germinal epithelium between January and February indicated the commencement of spermatogenesis.

Table 5.6:

Result of a one-way ANOVA to determine whether the mean thickness of the germinal epithelium of male *P. granularis* inhabiting the mussel shells differed on a monthly basis. (* indicates a significant p - value).

Source of variation	S.S	d.f.	M.S	F-ratio	Sig. level
Between groups	7945.832	11	722.349	3.647	0.002 *
Within groups	6337.528	32	198.048		
Total	14283.360	43			

Table 5.7:

Results of a Neuman Keul's multiple range test to determine the months during which the mean thickness of the germinal epithelium differs significantly. (X's in the same column indicate no significant difference)

Month	Homogenous groups	
May	X	
June	X	
July	X	
August	X	
September	X	X
October		X
November	X	
December	X	
January	X	
February	X	X
March	X	X
April	X	X

A well defined seasonal change in the thickness of the germinal epithelium corresponding to the seasonality of the GI values was not evident in *P. granularis* from the quartzitic shore (Table 5.8). The sperm content in these limpets appeared to be high throughout the year, as was evident in the relatively thin germinal epithelium (e.g. Figure 5.9b). A slight increase in the thickness of these layers was found in January and February, coinciding with a low GI. This was then followed by a period of spermatogenesis where the mean thickness of the wall decreased.

Table 5.8:

Result of a one-way ANOVA to determine whether the mean thickness of the germinal epithelium of male *P. granularis* inhabiting quartzitic sandstone differed on a monthly basis.

Source of variation	S.S.	d.f.	M.S	F-ratio	Sig. level
Between groups	4744.625	11	431.330	1.610	0.1416
Within groups	8839.541	33	267.865		
Total	13584.165	44			

The gonads of the limpets inhabiting the aeolianite shore contained an increased abundance of mature spermatozoa between November and February when the germinal epithelium was very thin (30 - 35 Fm thick; Figure 5.9c). A significant increase in the thickness of these layers in March (Tables 5.9c and 5.10) corresponded to spermatogenesis.

Table 5.9:

Result of a one-way ANOVA to determine whether the mean thickness of the germinal epithelium of male *P. granularis* inhabiting aeolianite differed on a monthly basis. (* indicates a significant p - value).

Source of variation	S.S	d.f.	M.S	F-ratio	Sig. level
Between groups	11854.225	11	1077.657	3.503	0.0016
Within groups	12612.194	41	307.615		
Total	24466.419	52			

Table 5.10:

Results of a Neuman Keul's multiple range test to determine the months during which the mean thickness of the germinal epithelium differs significantly. (X in the same column indicate no significant difference)

Month	Homogenous groups	
May	X	X
June	X	X
July	X	X
August	X	X
September	X	X
October	X	X
November	X	
December	X	X
January	X	
February	X	X
March	X	X
April		X

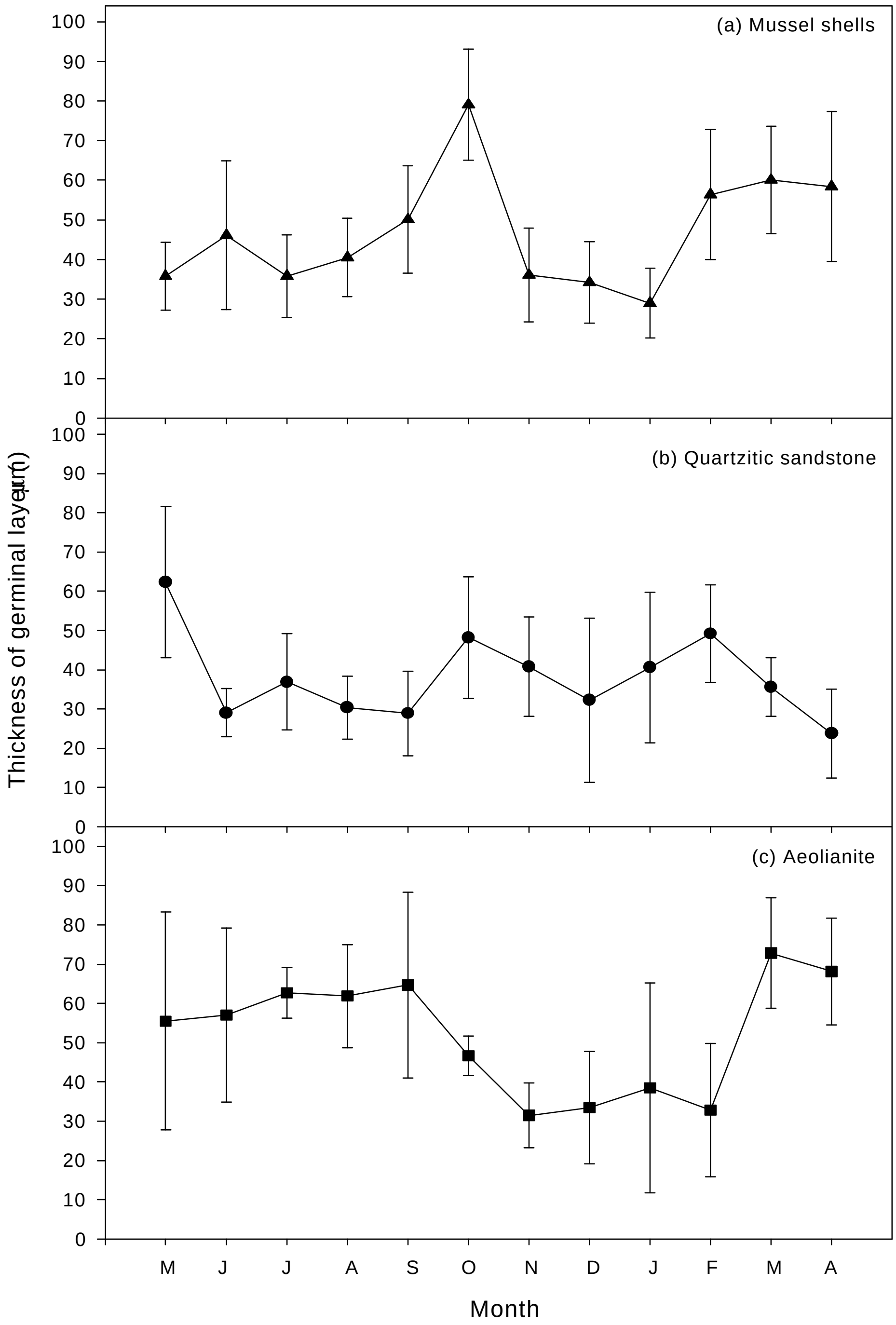


Figure 5.9:

Mean monthly (± S.D.) thickness of germinal layers of *P. granularis* inhabiting mussel shells, quartzitic sandstone and aeolianite. n = 5 at each site.

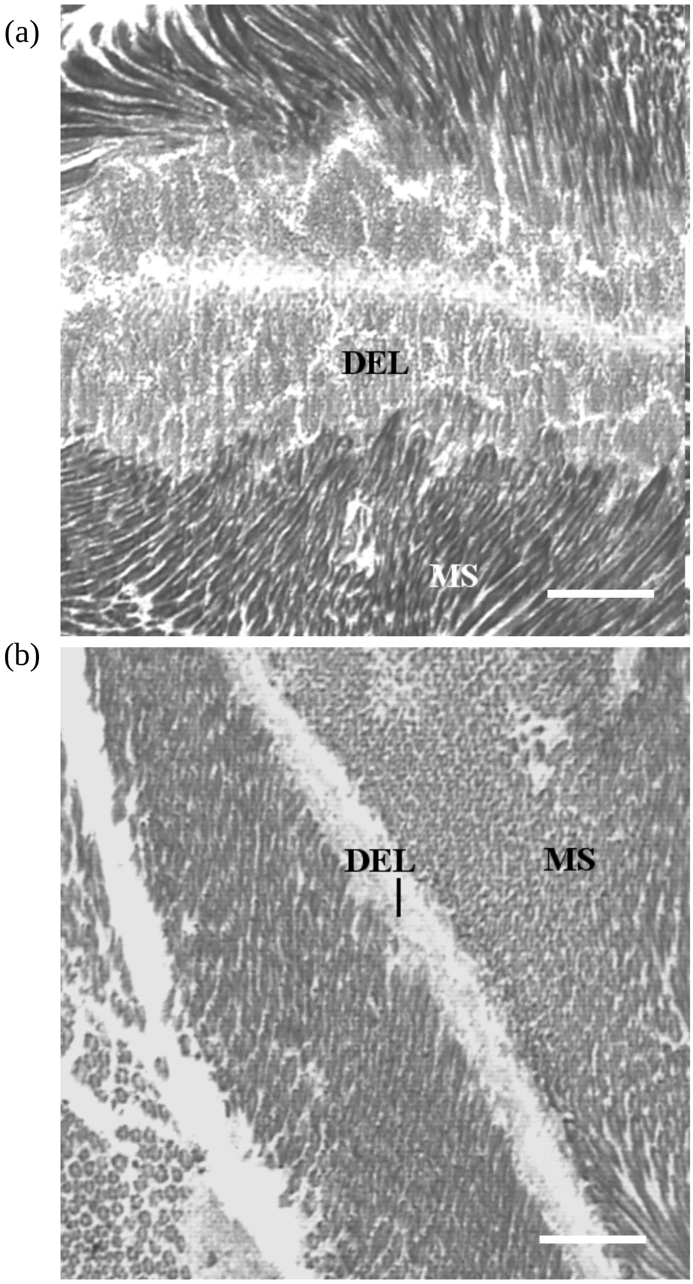


Figure 5.10:

Transverse section through the gonad of a male during periods of (a) minimum Gonad Index; DEL = developing epithelial layer; MS = mature spermatozoa and (b) maximum Gonad Index; DEL = developed epithelial layer; MS = mature spermatozoa. Scale bars = 100Fm.

5.3.5: Reproductive fitness as determined by fecundity:

The estimated number of eggs present in mature ovaries varied among sites. The gonads of *P. granularis* inhabiting mussel shells contained fewer eggs than those of limpets inhabiting either quartzitic or aeolianite shores (Table 5.11), although this difference is not significant (Table 5.12), probably as a result of the high variability among the limpets. This is expected as limpets from the mussel shells are generally much smaller (see Chapters 2 and 4). Although the mean size of *P. granularis* inhabiting quartzitic sandstone shores is slightly larger than those from aeolianite shores, limpets from the aeolianite shores contain a greater number of eggs (Table 5.11). The difference in the number of eggs present is mirrored in the estimated number of eggs released during spawning. A limpet (. 22 mm in length) inhabiting the mussel shells was estimated to release approximately 86 000 eggs in a spawning period, while one (. 26 mm in length) from quartzitic shores released almost 120 000 eggs during spawning (Table 5.9).

Table 5.11:

The estimated number of eggs present in the ovaries of, and released during spawning by *P. granularis* inhabiting three sites.

Site	Mean length of limpet (mm ± S.D.)	Theoretical No. of eggs / gram of ovary	Estimated No. of eggs in mature ovary	Mean decrease in ovary weight	Estimated No. of eggs spawned
Mussel shells	21.75 ± 3.75	180 417	122 684	68.2 %	85 878
Quartzite	26.30 ± 1.69	844 573	282 932	42.2 %	119 510
Aeolianite	24.70 ± 1.84	1 351 371	447 304	81.9 %	366 341

Table 5.12:

Results of a two-way ANOVA to determine whether the estimated number of eggs present in the gonads of *P. granularis* differed significantly among sites. The ANOVA also determined whether there was a significant difference in the estimated number of eggs present in mature and spawned gonads.

Source of variation	S.S	d.f.	M.S.	F-ratio	Sig. level
Main effects:					
Site	7.404	2	3.702	0.960	0.4347
No. of eggs spawned	1.081	1	1.081	2.804	0.1450
Residual	2.313	6	3.855		
Total	4.608	11			
All interactions are non-significant					

5.4: DISCUSSION:

The populations of *Patella granularis* studied on the south-east coast of South Africa were dioecious and did not display external sexual dimorphism. Generally, no sexual dimorphism, other than differences in the structure and colour of the gonad, is evident in most prosobranch molluscs (Webber, 1977; Runham, 1992). In the few South African patellids where there is a difference in size between the sexes (e.g. *P. cochlear, P. oculus, P. longicosta* and to a lesser extent, *P. granatina*), males usually predominate in the smaller size classes (Branch, 1974b). This has been attributed to either an earlier maturation of males or to an increased growth rate of females (Branch, 1974b). In addition, males usually have a higher gonad output than females, which may be a result of more energy being channelled into reproductive effort rather than somatic growth. This in turn would result in the smaller lengths reached by the males (Branch, 1974b).

In other species including *P. oculus*, *P. vulgata* and *Helcion pectunculus*, sexual dimorphism has, however, been attributed to the sex change that occurs in individuals of this species (Orton, 1920; Orton *et al.*, 1956; Branch, 1974b; Gray, 1996).

In many gastropod species, females are more numerous than males (Fretter, 1984; Fretter & Graham, 1994). However, eleven South African patellid limpet species have been reported to have a sex ratio in favour of males with only one of the species studied (*P. barbara*) having a greater number of females than males (Branch, 1974b; Robson, 1986; Gray, 1996; Henninger, 1998). In intertidal species that broadcast their gametes, an abundance of males may counter sperm dilution caused by high turbulence (Glynn, 1970). Additional factors that may affect sex ratios include sampling bias, sex change and differential growth (Branch, 1974b; Shepard & Hearn, 1983). In this study however, the sex ratio of *P. granularis* was found to differ on a localised scale. Two of the three populations studied (i.e. those from quartzite and mussel shells) did not deviate from a 1:1 ratio in any size class examined. Limpets inhabiting the aeolianite shore did however have a significantly greater number of males than females. As no hermaphrodites were found in any samples analysed from this site, it is unlikely that the biassed ratio was a result of sex change. Similarly, as the three populations examined are geographically close (see Chapter 2), they are exposed to similar environmental conditions. Thus the observed predominance of one sex in one population is unlikely to be associated with any environmental conditions. It is possible that the results obtained for the limpets inhabiting aeolianite are an artifact of sampling bias.

The onset of sexual maturity in *P. granularis*, although occurring in limpets of different sizes on different substrata (8 mm - 10 mm on aeolianite and quartzite and 6 mm - 8 mm on mussel shells) occurred at approximately the same age i.e. 1 to 1½ years of age (calculated from growth rates presented in Chapter 4). Although *P. granularis* from the west coast have a more rapid growth rate and a lower longevity (Branch, 1974a), these limpets also achieve sexual maturity at a similar age to their south-east coast counterparts (1 - 2 years, Branch, 1974b). Furthermore, other molluscs inhabiting the coast of South Africa reach sexual maturity between one and two years of age (Gray, 1996; Foster, 1997). The age at which marine invertebrates reach sexual maturity may be influenced by a number of factors. Sexual maturity of the bivalve *Phacosoma japonicum* (Sato, 1999) is strongly influenced by food availability which in turn is related to localised temperature regimes. Latitude may also affect the age of first maturation. Populations of the clam *Venus striatula* from higher latitudes mature later than those inhabiting lower latitudes (Gaspar & Monteiro, 1998). Due to the biogeographic differences between the west and south-east coasts of South Africa, a similar relationship to that described by Sato (1999) could be expected between populations of *P. granularis* inhabiting the two coasts. As sexual maturity is achieved in limpets of similar ages from all habitats investigated, with no influence of size, it is suggested that the onset of sexual maturity is firmly genetically entrenched in this species.

In most of the South African *Patella* species, the gonad size and gamete output (as determined by Gonad Index) of males exceeds that of females (Branch, 1974b). A similar result has been recorded for a number of other gastropod species including *H. pectunculus* (Gray, 1996), *H. pruinosis* (Henninger, 1998), *Cellana capensis* (Lasiak, 1990) and *T. sarmaticus* (Foster, 1997). However, in *P. granularis* inhabiting mussel shells, aeolianite and quartzitic sandstone, GI did not indicate a significant difference in the size of male and female gonads relative to body size.

The reproductive cycle incorporates the events from the time of activation, through growth, gametogenesis, spawning, recession of gonadal activity and the resting period between cycles (Giese, 1959). In South Africa, the marked differences in the oceanographic conditions between the west, south-east and east coasts appear to influence the reproductive seasonality of marine invertebrates. Most limpets from the west coast spawn only once a year, while those from the south and east coasts either spawn twice a year or have a protracted spawning period (Branch, 1981). Two exceptions to this are *H. pectunculus* and *H. pruinosis* which spawn twice yearly on both the west and south-east coasts (Gray, 1996; Henninger, 1998). As with most patellids, *P. granularis* inhabiting both the west and the south-east coasts has a marked reproductive cycle. Limpets inhabiting the west coast spawn once a year in winter followed by a long resting phase (Branch, 1974b). From both the Gonad Indices and detailed histological examination, it appears as though the limpets from the south-east cost spawn twice a year, once in summer and once in winter. Mature oocytes were present for most of the year at all three sites investigated. Thus a resting phase in these limpets is unlikely. Similarly, mature sperm was present throughout the year in all three populations. Although artificial fertilisation of *P. granularis* from the west coast could not be achieved during the resting phase of the reproductive cycle (August to December - Branch, 1974b), the presence of both mature eggs and sperm in south-east coast limpets indicates that these animals may be capable of reproducing at any time of the year. Laboratory experiments are, however, needed to test this hypothesis. A high degree of synchronicity in the timing of spawning between males and females was also evident. As already mentioned, this distinction in the breeding patterns of west and south-east coast limpets appears to be a widespread phenomenon around the coast of South Africa. It is possible that the environmental conditions on the south and east coasts do not fluctuate as greatly as those on the west coast, allowing the south and east coast limpets to spawn twice a year (Gray, 1996). Alternatively, the differences observed in the spawning patterns of limpets from the two biogeographical regions may be associated with the generally smaller sizes attained by limpets from the south and east coasts. It is possible that the smaller size of the east and south coast limpets results in the production of fewer eggs. Thus in order to maintain reproductive fitness at a level similar to the west coast limpets, twice yearly or protracted spawning may be necessary.

Although the fundamental pattern of reproduction of *P. granularis* from the south-east coast differs to that of limpets from the west coast, there is some modification of reproductive seasonality and reproductive fitness (as determined by the Gonad Index and fecundity of limpets) on a localised scale. *P. granularis* inhabiting mussel shells spawned twice annually in both years of the study. Limpets from the aeolianite and quartzitic sandstone shores, however, displayed interannual variation in both the timing and number of spawning periods. In the first year of the study, a general decrease in GI of limpets from the quartzitic sandstone shore indicated a gradual release of gametes, whilst in the second year, two well-defined spawning periods were recorded. *P. granularis* inhabiting aeolianite, on the other hand, displayed two well-defined spawning periods in the first year of the study, while no marked decreases in GI were evident in the second year. Despite these differences, the GI values obtained in the current study fall within the range previously obtained for other limpet species (e.g. *H. pectunculus* - 5% and 35%, Gray, 1996; *H. pruinosus*; 10% and 25%, Henninger, 1998). While the use of GI as a method to determine reproductive seasonality may miss spawning events that are masked by continued gonad development (Bowman, 1985), the use of this technique in conjunction with other quantitative methods should negate such concerns. It has been proposed that oocyte size-frequency distributions can provide more information on the reproductive periodicity of marine invertebrates than Gonad Indices alone (Grant & Tyler, 1986). Analysis of the gametogenic cycles of limpets from all three sites correspond to the developmental stages evident from the GI. As such, it can be assumed that the patterns observed are indeed real and not a function of infrequent, or mis-timed sampling.

Although a number of factors are known to influence gametogenesis (see reviews by Branch, 1981; Grahame & Branch, 1985), food availability has been identified as having a profound influence on observed intraspecific differences in reproductive patterns (Workman, 1983; Fletcher, 1984; Quinn, 1988). In the present study, a consistently greater amount of food is available on the aeolianite shore (Chapter 7). As the limpets inhabiting this site do not grow as large as those from the quartzitic shore (Chapter 4), they are likely to channel more energy into reproduction. Borcherding (1995) found that an increase in food availability did not only result in an increase in the gonad volume of the freshwater mussel *Dreissena polymorpha*, but also resulted in an increase in the size of the oocytes produced. However, this was accompanied by a decrease in the number of oocytes produced. Although the number of eggs spawned by *P. granularis* inhabiting the west coast (> 1 million/limpet, Branch, 1974b) is much greater than the estimates made in the current study, (85 000/limpet - 366 000/limpet), the values obtained for the east coast limpets are similar to those obtained for *P. cochlear* (. 150 000/limpet, Branch, 1974b) but higher than those reported for *Cellana radians* (45 000/limpet, Creese & Ballantine, 1983). Although the current study has estimated the number of eggs produced per gram of ovary to be higher on the south-east coast (180 000 - > 1 million) than on the west coast (300 000 - 840 000; Branch, 1974b), these results are misleading. The gonads of south-east coast *P. granularis* are always less than 1 gram of wet weight. Thus the values estimated in the current study are hypothetical. Therefore, in absolute terms, the number of eggs produced by west coast limpets remains higher than the numbers produced by *P. granularis* from the south-east coast. In the present study, *P. granularis* from the aeolianite shore was found to produce a greater number of eggs than at either of the other sites, but this was not accompanied by a decrease in the size of the oocytes. The increase in food availability to the limpets from this site may also be responsible for the indistinct pattern observed in the second year of the study as this consistent food supply may enable the consistent production of gametes and subsequent continuous spawning. Spawning in *P. vulgata* can occur early in the season, while the GI is still increasing. This results in low peak GI values (Bowman, 1985). As the GI values of *P. granularis* inhabiting aeolianite seldom increased beyond 19% during the second year of study, continuous spawning of small numbers of gametes could have occurred. Trickle or partial spawning, where individual limpets spawn only a part of its gonad at once (Balaparameswara Rao, 1973; Underwood, 1974) is often reported in limpets with protracted breeding seasons (Creese & Ballantine, 1983). Lasiak (1990) proposed that the protracted and asynchronous breeding of *Cellana capensis* may reduce competition for scarce food resources. However, given the increased microalgal biomass on the aeolianite, this is unlikely to be the cause of the trickle spawning observed. It can thus be concluded, as evidenced by the continual development and spawning of mature gametes, that although GI values of *P. granularis* inhabiting aeolianite were the lowest recorded in this study, the reproductive fitness of these limpets was relatively high.

Both the quartzitic shore and the mussel shells were found to have a reduced food availability (Chapter 7) in comparison to aeolianite. The continued decrease in the GI of *P. granularis* inhabiting the quartzitic sandstone shore during the first year of study may have been related to a severe limitation in food during this time. However, as microalgal biomass was not investigated during this time, this suggestion is speculative. Limpets from this site are generally larger than those from either the aeolianite shore and the mussel shells (Chapter 4). Gonad output in *P. granularis* has been shown to be positively correlated to the size of the limpet (Branch, 1981; Bosman & Hockey, 1988a,b). However, in the present study, limpets from this site were found to produce fewer eggs than similarly sized individuals from the aeolianite shore, even though GI values were proportionately similar on both shores. Limpets transferred from the mussel shells to the mid shore region of this site show a marked increase in growth rate and a rapid change in shell shape (Chapter 4). Shell shape is known to offer some protection against the threat of desiccation (Branch & Branch, 1988). Although the physical variables of temperature and humidity were not investigated in the present study, it is suggested that the hard, smooth nature of the quartzite boulders is not conducive to water retention and that desiccation on this shore is much higher than on the aeolianite shore, or in the mussel shells. As such, the channelling of energy into shell growth is probably of great importance to the limpets of this site.

This would, in turn, reduce the amount of energy available for reproduction, and is evident in the reduction of the number of eggs present when compared to individuals of a similar size from aeolianite shores. Thus the overall reproductive fitness of these limpets is lower than their counterparts from the aeolianite shore.

P. granularis from the mussel shells are much smaller than limpets inhabiting aeolianite and quartzite (Chapters 2 and 4). Although the actual size of the gonads of mussel-dwelling limpets are smaller than those from other substrata, proportionally no significant difference in gonad size was found (i.e. the GI was the same). This was unexpected as a smaller size is usually associated with a lower GI (Himmelman, 1979). These limpets do not display a great degree of interannual variation in spawning pattern, although some variation in the timing of spawning periods was found from one year to another. Although the amount of food present at this site is also low (Chapter 7), no seasonal change in microalgal biomass was evident. The availability of a consistent food supply (i.e. no seasonal variability) has been associated with a lack of seasonality in the GI (Sutherland, 1970; Quinn, 1988). Although the availability of food was only studied for one year of the study, no distinct seasonal correlation was evident during the course of that year. This may explain the well-defined reproductive pattern observed in the limpets from the mussel shells during both years of the study. Furthermore, the environmental conditions of the mussel beds are generally much more predictable than those of mid- and high shore zones, and as such may reduce the variability in reproductive patterns.

Throughout the study period, unlike *P. granularis* from the west coast (Branch, 1974b), no completely spent limpets were found at any site. Similarly, no evidence of degeneration or reabsorption of gametes was found, although this process occurs in other prosobranchs (e.g. *T. sarmaticus*, Foster, 1997) and allows all valuable components of superfluous gametes to be re-used for metabolic purposes (de Jong-Brink *et al.*, 1983). It is suggested that the continuous (although low) food supply along the south and east coast of South Africa facilitates ongoing gamete production. The presence of mature oocytes and sperm throughout the year may enable *P. granularis* from this area to reproduce whenever conditions are favourable.

The distribution of oocytes, as determined by oocyte-size frequencies, may be unimodal, with a sudden increase in the growth rate of oocytes (Gonor, 1973b) or bimodal, with a gradual increase in the peak of larger oocytes resulting from the vitellogenesis of increasing numbers of immature oocytes (Creese, 1980). In the present study, histological analysis indicates that in females from all three substrata, a bimodal distribution of oocytes occurs. The first peak corresponds to a large number of immature oocytes, while the second peak is associated with greater numbers of vitellogenic oocytes. No gradual increase in the second peak is evident. Instead, the number of mature oocytes seems to increase rapidly. This has also been observed in *H. pectunculus* from both the west and south-east coasts of South Africa (Gray, 1996). This appears to be related to the fact that vitellogenesis in these species is rapid, resulting in few oocytes of the middle size classes being observed (K. Eckelbarger and A.N. Hodgson, pers. comm.)

Spawning in marine invertebrates can also be triggered by a variety of stimuli. The close similarity in the timing of spawning periods both within and between invertebrate species along the coast of South Africa (see summary by Foster, 1997), suggests that mechanisms must exist whereby the reproductive cycles are synchronised by environmental factors. Seasonal variations in reproductive cycles also indicates that this is likely (Gonor, 1973b). Spawning in many invertebrates has been associated with changes in sea temperature (Giese, 1959; Giese & Pearse, 1974; Grahame & Branch, 1985). On the west coast of South Africa, gonad maturity in *P. granularis* occurs as both sea and air temperatures decrease and spawning takes place during winter, when sea temperatures are at their lowest (Branch, 1974b). Despite this correlation, Branch concluded that spawning is independent of temperature although gonad development is geared towards temperature. On the south-east coast of South Africa, gonad maturation of *H. pectunculus* and *T. sarmaticus* was found to occur as sea temperature increased (Gray, 1996; Foster, 1997). Although the correlation between gonad development and environmental variables was not investigated in the present study, the similarity in the general timing of spawning of many species from a similar geographic area (see review by Foster, 1997) suggests that the same stimulus may be affecting all species. The modified reproductive patterns observed at the three sites of this study, do however, imply an additional, more localised stimulus.

A number of patellogastropod species spawn during rough seas including *P. aspera* (Thompson, 1979), *P. depressa* (Orton & Southward, 1961), *P. vulgata* (Orton *et al.*, 1956; Bowman, 1985), *Cellana radians* (Creese & Ballantine, 1983) and a number of South African *Patella* species (Branch, 1974b). Although broadcast spawning into rough seas may seem counter-productive as the increased turbulence in the intertidal zone seems likely to decrease fertilisation success (Shanks, 1998), there are many advantages to spawning in these conditions. Storms are usually associated with strong onshore winds which may prevent larvae from drifting out to sea (Branch, 1974b; Shanks, 1998). In addition, the increased turbulence of the inshore waters reduces the residence time of water, and consequently eggs and larval stages in the surf zone. This in turn may decrease the probability of contact with benthic predators (Shanks, 1998). Despite the obvious association between spawning and rough seas, this correlation must be viewed sceptically. As mentioned by Branch (1981), the gonads of many invertebrates are often so turgid with mature gametes that any mechanical disturbance is likely to result in spawning.

As already discussed, food availability appears to have a profound effect on gametogenesis. Phytoplankton also acts as a stimulus for spawning. Himmelman (1975, 1979) showed that a number of invertebrates spawned during phytoplankton blooms, enabling the larvae to capitalise on the abundant food source. Spawning in the Antarctic limpet, *Nacella concinna,* was also found to be cued by the spring phytoplankton blooms and not by variations in sea temperatures (Stanwell-Smith & Clarke, 1998). *P. granularis* on the south-east coast spawned during summer and late autumn, a period coinciding with peak chlorophyll-*a* levels in the near-shore waters (de Villiers, 1998). Although patellogastropod larvae do not feed in the plankton (Hadfield *et al.*, 1997), molluscan veliger larvae can absorb dissolved organic matter from the water column (Welborn & Manahan, 1990). Spawning that coincides with an increase in nutrients may thus be important for larval development. The amount of primary productivity present in the intertidal region is also closely correlated to the nutrient status of the near-shore waters (Brown, 1992). Spawning that occurs simultaneously with a phytoplankton bloom may thus also construe an advantage on the settling larvae by providing an abundant food source and decreasing intraspecific competition between adults and juveniles.

Additional factors that have also been shown to influence spawning include photoperiod (Gonor, 1973a; Vernon *et al.*, 1993), salinity (McLusky & Allan, 1976) and lunar periodicity (Aliferakis & Berry, 1980). However, it is more likely that a combination of factors is responsible for triggering spawning (Foster & Hodgson, 1995; Shanks, 1998).

In conclusion, populations of *P. granularis* inhabiting the south-east coast exhibited no external sexual dimorphism but did show a localised difference in sex ratio, with the limpet population from the aeolianite shore investigated having a greater number of males, while those populations studied from quartzite and mussel shells did not deviate from a 1:1 sex ratio. Generally, south-east coast limpets spawn twice a year (in winter and summer), in contrast to *P. granularis* from the west coast which spawn once in winter. The reproductive pattern of this species was found to be modified by habitat and a varying degree of interannual variability in both the timing and duration of spawning periods was recorded at the three sites investigated. However, before unequivocal conclusions can be drawn about the influence of substrata on reproduction, variability between limpet populations inhabiting similar substrata on closely positioned localities needs to be investigated. The present study suggests that the main factor regulating gametogenesis in these populations is food availability, with an increase in food being associated with an increase in gamete production. Similarly, it appears that an increase in food availability results in a protracted breeding season. Although the mechanisms initiating spawning were not investigated, it has previously been suggested that both phytoplankton blooms and temperature may be responsible. Laboratory experiments on both trigger mechanisms and the effect of food availability on gametogenesis are needed to validate these suggestions. Finally, this study has emphasised the need for long-term (i.e. more than one year) multi-locality studies on the reproductive biology of intertidal species to be undertaken before any conclusions on the reproductive status and seasonality of these organisms can be made.

Chapter 6
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Chapter 6

ACTIVITY PATTERNS AND FORAGING BEHAVIOUR OF *PATELLA GRANULARIS* ON TWO SHORES WITH DIFFERENT GEOMORPHOLOGIES

6.1: INTRODUCTION:

Most marine gastropod molluscs which inhabit intertidal rocky shores are grazers, feeding by rasping algae from the rock surface (Underwood, 1979; Hawkins & Hartnoll, 1983). As a result, gastropods play a pivotal role in structuring intertidal communities and this has motivated a number of studies of their biology and ecology, including detailed investigations into activity rhythms and foraging behaviour (see Branch, 1981; Hawkins & Hartnoll, 1983; Little, 1989; Lui, 1993 and Hodgson, 1999 for reviews of literature). These studies have shown that a great variety of activity patterns are employed by intertidal grazers. Foraging activity may be classified according to the relationship of the activity period to tidal and day/night cycles. Some species forage while submerged (during both day and night), others are active only when splashed by the rising and/or falling tides, while some species feed during low tides only (Branch, 1981; Hawkins & Hartnoll, 1983; Hodgson, 1999). These activity patterns are probably controlled by endogenous rhythms (Funke, 1968; Della Santina & Naylor, 1993; Gray & Hodgson, 1999).

Another feature of limpet activity is homing, which has been well documented in many species including the Siphonariidae (Thomas, 1973; Cook & Cook, 1975; Hodgson, 1999) the Acmaeidae (Hewatt, 1940; Galbraith, 1965) as well as the Patellidae (Cook *et al.*, 1969, Gray, 1996). In 1971, Cook suggested four hypotheses to explain the mechanisms by which limpets home. Firstly, limpets return to their home scars by following distant clues such as the plane of polarization of light from the sky; secondly, kinaesthetic information is used to navigate by a reverse-displacement or dead reckoning system; thirdly, limpets use topographic memory and finally, clues that the limpets themselves lay down on the rock, such as mucous trails or rasping paths, are used.

Most studies of foraging activity and homing behaviour of intertidal prosobranch limpets have been carried out on northern hemisphere species inhabiting sheltered shores (Gray, 1996). The majority of the South African rocky intertidal shores however, are exposed shores that are home to a diverse limpet fauna (Branch, 1971). This is particularly true for the Patellogastropoda. Currently, twenty species of patellid limpets (representing the genera *Cymbula, Helcion,* *Scutellastra* and *Patella*) occur along the southern African coast (S. Ridgway *et al.*, 1997). Although much work has been carried out on the biology and ecology of the South African patellid limpets (see Branch, 1981; Gray 1996 for reviews of literature), it is only recently that quantitative studies of their foraging behaviour have been undertaken. To date, such studies include observations on *Helcion pectunculus*, *Patella granularis* (Gray, 1996; Gray & Hodgson, 1997) and *H. pruinosus* (Henninger, 1998). Some quantitative studies have also been undertaken on siphonariid limpets, which although distantly related to the patellids, display similar behavioural patterns while foraging (Branch & Cherry, 1985; Gray & Hodgson, 1997).

P. granularis is the most widespread of the South African species of patellid limpet, with its distribution ranging from Namibia in the west to Natal in the east (Branch, 1971; Branch *et al.*, 1994). It is also ubiquitous on shores of differing geomorphologies (A.N. Hodgson, pers.comm.; Chapter 2). Furthermore, *P. granularis* has a widespread vertical distribution on the shore, occurring from the upper Balanoid zone down to the Cochlear zone (Branch, 1971; Chapter 2, this study) where it is common in mussel beds. This species displays a marked decrease in the size of individuals from the west coast to the east coast (Stephenson, 1937; Stephenson, 1939; T. Ridgway *et al.*, 1998), with the increased size of west coast populations being correlated to increased primary productivity on this coast (Branch, 1971; Bustamante *et al.*, 1995b).

It has been shown that habitat can influence the timing of the foraging activity of limpets (Branch, 1971; Branch & Cherry, 1985; Little, 1989; Gray & Naylor, 1996). To date, no studies of the foraging activity of South African limpets have taken into account the rock type of the shore, although Gray and Hodgson (1997) did consider how the aspect of the rock in relation to the sun could affect limpet foraging. Studies on the foraging behaviour of *Acanthochitona garnoti* on the shores of the Eastern Cape province of South Africa indicated that chitons inhabiting aeolianite travelled shorter distances than those on quartzitic sandstone although a seasonal effect on the distance travelled was not ruled out (Cretchley *et al.*, 1997). Gray (1996) showed that aspect influenced the foraging behaviour of *H. pectunculus*. While Branch (1971) and Gray & Hodgson (1997) observed *P. granularis* to be active twice a day during low tides, Stephenson (1936) recorded the activity of this species to be restricted to nocturnal low tides. It is possible that these differences may be related to the habitats occupied by *P. granularis* in the respective studies.

P. granularis which inhabit different shores have different densities, biomasses, growth rates and reproductive patterns (see Chapters 2, 4 and 5). It is possible that the observed differences may be related to the food availability on these shores and as such be indirectly affected by the foraging behaviour of the limpets. This study therefore aimed to observe and quantify the foraging activity of *P. granularis* on two east facing, exposed shores of different geomorphologies along the south-east coast; and secondly, to investigate whether the foraging patterns of this species differ on the two shores. Foraging activity on both shores was also compared in two seasons.

Finally, limpets may modify the amount of food they consume during foraging by adjusting the rate of radula movement (Newell, 1979). Limpets exhibit a large degree of morphological plasticity and characteristics such as radula proportions can reflect different habitats and responses to localised stresses (Powell, 1973). Although the patellid radula is extremely hard (5 on the Moh’s scale, Branch, 1981), radula tooth wear may be different in animals grazing on different substrata. It was therefore decided to investigate whether there was any difference in the wear of the radula teeth in *P. granularis* inhabiting an aeolianite and a quartzitic sandstone shore.

6.2: MATERIALS AND METHODS:

6.2.1: Study Site:

Observations of the foraging activity of *Patella granularis* were made on two shores with differing geomorphologies on the south-east coast of South Africa; Cannon Rocks (33E44' S; 26E35'E) which is a quartzitic sandstone shore and Kenton-on-Sea (33E44' S; 26E40'E) which is an aeolianite shore. A more detailed description of both sites can be found in Chapter 2. For the purposes of this study, observations were limited to mid-shore limpets. Activity studies of *P. granularis* inhabiting mussel beds were not undertaken due to the high wave intensity experienced on the lower shore. Furthermore, as the mussel beds are located in the lower intertidal, this would have introduced an additional variable of tidal height to the analysis of activity behaviour. During day-time low tides, *P. granularis* can be found in a wide range of habitats, from crevices and vertical slopes to exposed boulders. As the incline of the rock surface may affect limpet activity, observations were restricted to limpets inhabiting vertical east-facing rock surfaces at both sites.

6.2.2: Preparation of limpets for observation:

Twelve hours before the onset of observations, limpets of approximately 22 mm shell length (see Table 6.1) were labelled with plastic, numbered bee labels (Honey Bee Warehouse, Cape Town). These were attached to the shells of the limpets with epoxy putty (Pratley's). Due to the possibility of erosion of the numbers from the bee labels, each label was coated with a layer of clear nail polish. At each site, three groups of limpets (10 individuals per replicate) were labelled on separate but adjacent east facing rocks that were approximately one metre apart. A further 40 labelled individuals were translocated from the aeolianite shore to the quartzitic shore and *vice versa* in order to determine whether any observed differences in foraging activity were a function of habitat (10 limpets per replicate plus an additional 10 limpets in an attempt to compensate for losses due to mortality). During the first transplant experiment (spring, 1998), an additional 10 limpets (also approximately 22 mm in length, Table 6.1) at each site were labelled, lifted from the rock and then replaced back on their home scars. This was done to control for any effect that disturbing the limpets (in the process of moving them from one site to another) may have had on their foraging activity. As disturbance from the rocks had no significant effect (Multi-factor ANOVA; $p > 0.005$) on any aspect of the activity of the limpets (timing of activity, homing and distance travelled; see Figure 6.2, Tables 6.2 and 6.3) at either site, this was not repeated in the autumn 1999 experiments.

In order to determine whether limpets returned to a fixed scar after foraging and had a consistent orientation on their scar, the shell of each limpet was painted with a line of cellulose paint. This line was continued onto the surface of the rock so that when the limpet was "home" and in the correct orientation, the line from shell to rock was continuous. In all observations, the position of each limpet on the rock was determined by the method of triangulation (Cook *et al.*, 1969). The position of the limpets from the reference points was measured to the nearest 0.5 cm.

Table 6.1:

Mean lengths (mm ± S.D.) of *P. granularis* observed during spring and autumn. ND = no data collected. There were no significant differences in the lengths of limpets observed (MANOVA; p > 0.05).

	Aeolianite			Quartzitic sandstone		
	Resident (undisturbed)	Resident (disturbed)	Translocated	Resident (undisturbed)	Resident (disturbed)	Translocated
Spring	22.22 ± 2.58	22.88 ± 2.72	21.27 ± 2.70	22.90 ± 2.99	21.90 ± 3.02	22.24 ± 3.44
Autumn	23.33 ± 2.22	ND	23.00 ± 2.48	23.15 ± 2.01	ND	23.22 ± 2.43

6.2.3: Observations of foraging behaviour:

The foraging activity of *P. granularis* was observed for two consecutive nights on spring new moon tides in August 1998 (spring) and April 1999 (autumn). Measurements were carried out simultaneously at both sites, at hourly intervals, by two groups of researchers from the time the limpets were emersed to the time that they were once again covered by the incoming tide. Measurements of limpet activity were discontinued during high tide as intense wave activity rendered underwater observations dangerous. It was assumed that limpets remained inactive during high tides due to the fact that they returned to a home scar before being immersed completely. Previous observations support this assumption (Gray & Hodgson, 1997). Further observations on the same limpets took place one week after the initial observations (during neap tides) to determine whether the translocated animals had established home scars and made any further adjustments to their foraging behaviour.

At night, observations were made using red light only as it has previously been shown that white light causes premature homing in *Patella* species (Cook *et al.*, 1969). Individuals were considered to be active when away from their home scar and "at home " when they were positioned on the home scar.

All analysis of data was carried out using Statgraphics version 7 (Statistical Graphics Corporation, USA). Further details on the statistical tests performed are provided in the relevant results sections. A summary of relevant non-significant results can be found in Appendix III.

6.2.4: Orientation of foraging excursions:

In order to determine whether *P. granularis* displayed any directional preference while foraging, a "foraging angle" for each marked limpet on each observation period was calculated. This was done by plotting a line from the home scar to the furthest point reached by the limpet during the excursion and measuring the angle of the line relative to the vertical. The mean vector (r) of foraging directions of the limpets on both substratum types and for all observations was then calculated, irrespective of the maximum distance travelled (Mardia, 1972; Batschelet, 1981).

6.2.5: Radula tooth wear:

As the anterior portion of the radula is used during feeding, the greatest wear occurs in this area. Thus, the anterior portions of the radulae of five adult *P. granularis* inhabiting aeolianite and quartzite (mean shell length = 23.2 mm and 22.7 mm respectively) were removed and placed in a 10% sodium hypochlorite solution for 30 minutes. During this time they were periodically ultrasonicated. The radulae were then dehydrated in a graded alcohol series (70%, 80% and 100% for one hour each). Each radula was air-dried before being mounted onto scanning electron microscopy (SEM) stubs and gold coated. The radulae were then viewed in a Jeol JSM 840 scanning electron microscope. Photographs of the images were then taken and the teeth of each radula measured using a digitiser tablet and Summagraphics software. For each radula, measurements were taken from the four lateral teeth (labelled as 1-4) and one of the large plusicuspid teeth, the cusps of which are numbered 1 to 4 from the outside to the centre of the radula (Figure 6.1). The following measurements were quantified: (a) - (d) = ratio of the length of lateral tooth to width of the same tooth (Figure 6.1a), (e) = ratio of the length of cusp 2 to the width of cusp 2, (f) = ratio of the length of cusp 3 to the width of cusp 3 and (g) = ratio of the length of cusp 4 to the width of cusp 4 (Figure 6.1b).

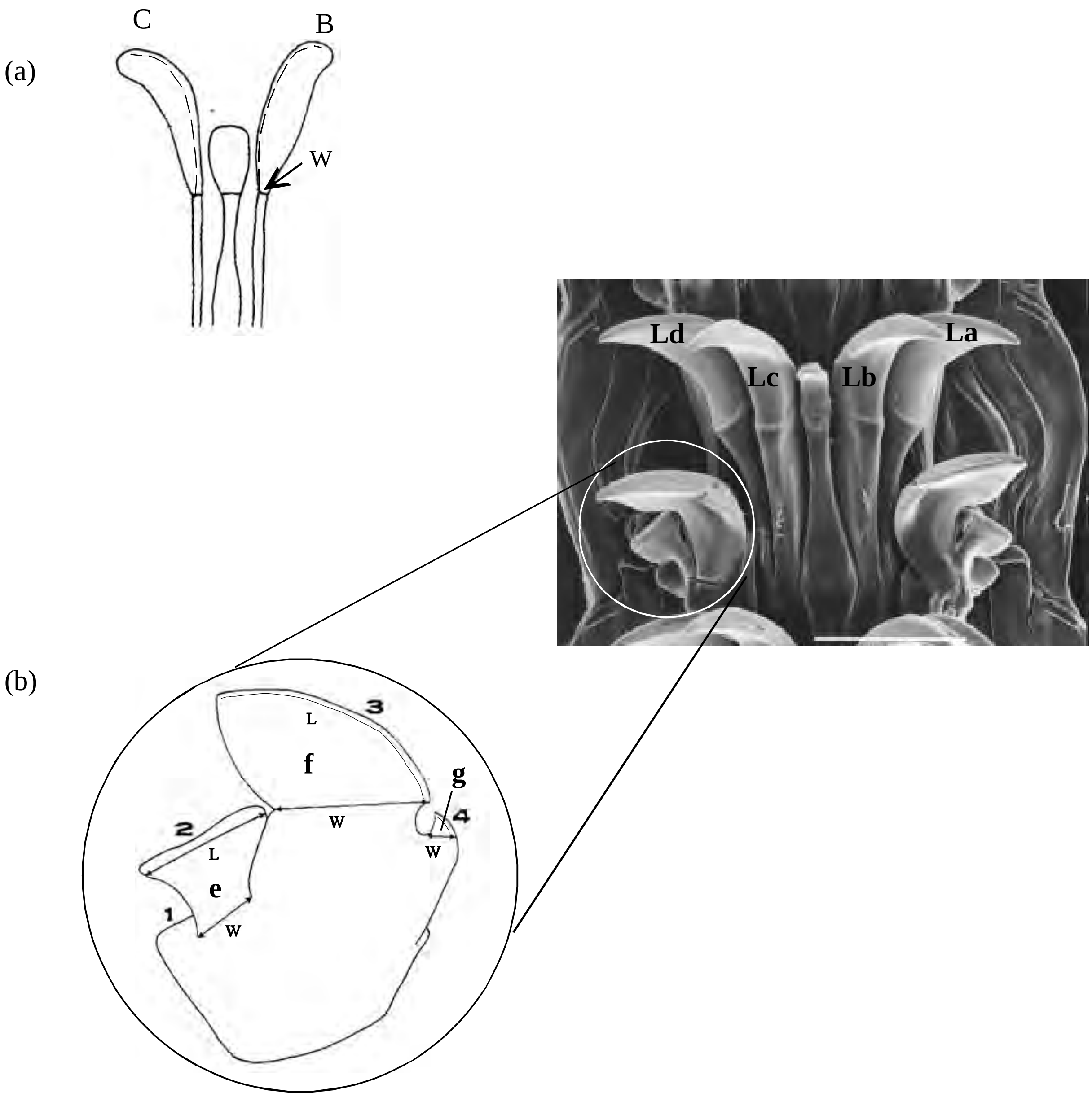


Figure 6.1:

Scanning electron micrograph of the radular morphology (La - Ld = lateral teeth) of *P. granularis* with diagrammatic representations of (a) two of the lateral teeth and (b) the pluricuspid tooth showing the dimensions measured. See text for details on the measurements. Scale bar = 100 Fm.

6.3: RESULTS:

6.3.1: Mortality of limpets:

Although every attempt was made to standardise the number of *Patella granularis* observed during both the spring and autumn experiments, a number of limpets were lost during the course of the experiments. This loss was attributed to mortality of the limpets as no marked limpets were found on the adjacent rocks, thus excluding movement away from the study site as a cause of the reduction in numbers. Although some loss of resident *P. granularis* was recorded, mortality was higher in translocated limpets on both shore types (see Table 6.3). Furthermore, a greater number of limpets translocated from quartzitic to aeolianite were lost (Table 6.3). This is possibly related to the texture of the aeolianite which is more uneven than that of the quartzitic sandstone (see Chapter 2). This may reduce the grip of limpets which have not yet established home scars on the rock.

The number of *P. granularis* observed on different nights varied (Table 6.3). Limpets were observed to sometimes disappear into crevices in the course of their foraging excursion, and were observed again only when they returned to their starting point. The number of limpets that were observed for the entire duration of their foraging excursion were thus considered to be the total number of limpets present within each replicate. This explains, in part, the greater number of individuals which were observed during the second and third observation periods as compared to the first (Table 6.3).

6.3.2: Activity rhythms of resident limpets:

No significant difference was found in the number of *P. granularis* active in each replicate at either site during any observation period ($p > 0.05$, Chi^2 test, Appendix III, Table 1). Data from each replicate were thus pooled to provide a total number of limpets active during each observational period.

P. granularis which inhabited east facing rocks foraged mainly during nocturnal low tides and where low tides occurred around dusk or dawn. Activity began as the limpets were being washed by the waves of the ebbing tide and continued until they were being immersed by the waves of the incoming tide (Figures 6.2 and 6.3). During all observation periods, > 68% of the marked limpets on aeolianite and > 85% on quartzitic rock were active, although the duration of activity on aeolianite was one to two hours longer than that recorded on quartzitic boulders for 4 of the 6 nights observed (Figures 6.2 and 6.3). The number of *P. granularis* active on both shores did not differ significantly between spring and autumn (p = 0.429; Appendix III, Table 2), but more individuals from aeolianite shores were active during spring than autumn. In addition, on the nights where 100% activity was recorded, fewer limpets were active during the following evening. This was evident in both seasons on aeolianite, but only during spring on quartzitic rocks (Figure 6.2, aeolianite and quartzitic; Figure 6.3, only aeolianite).

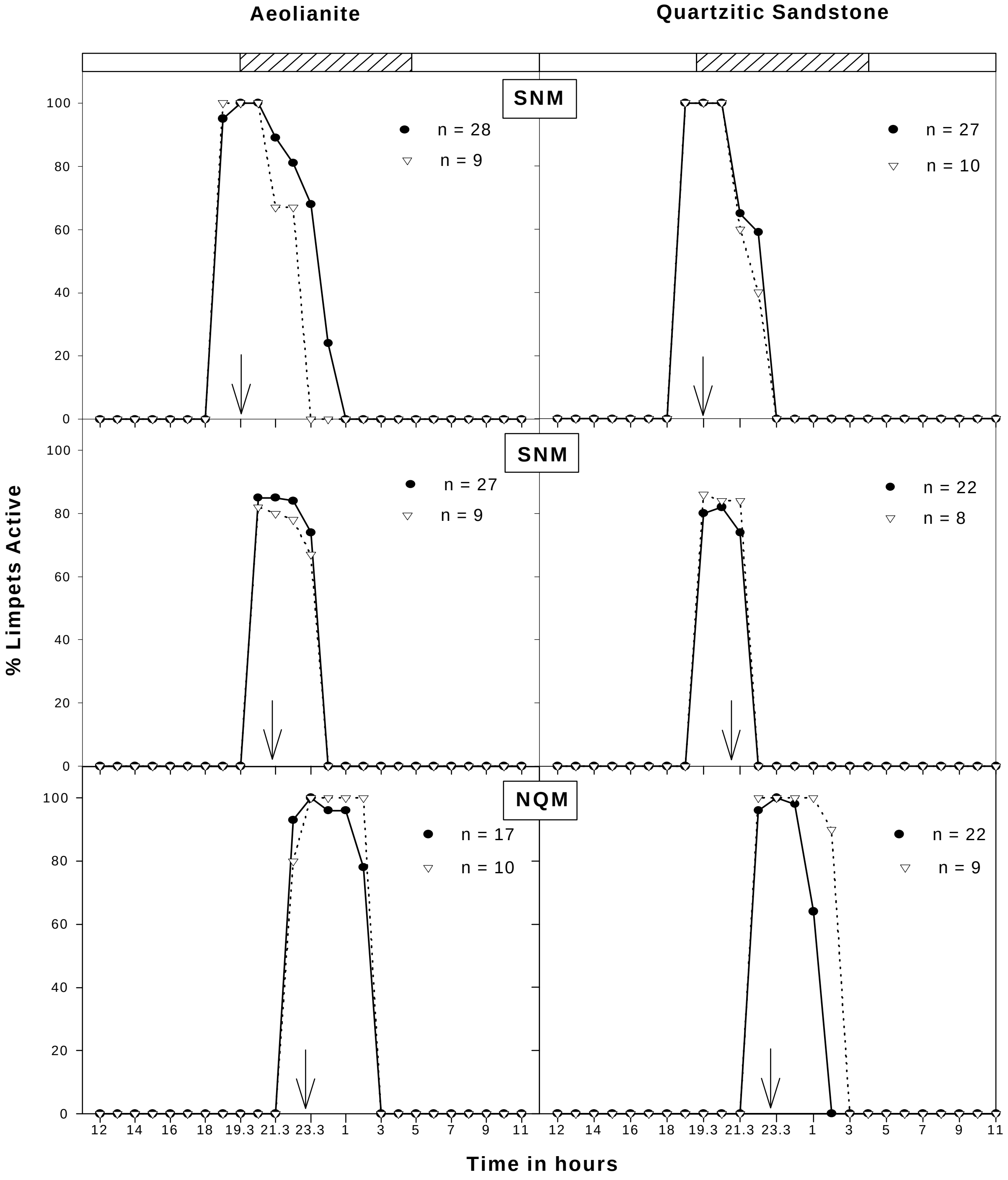


Figure 6.2:

The percentage of resident *P. granularis* active over 24 hours during 3 spring observation periods on an aeolianite and a quartzitic shore. Arrows indicate low tide; shaded bars indicate periods of darkness. n = sample size. SNM = Spring new moon, NQM = Neap quarter moon. ● = resident undisturbed limpets; L = resident disturbed limpets.

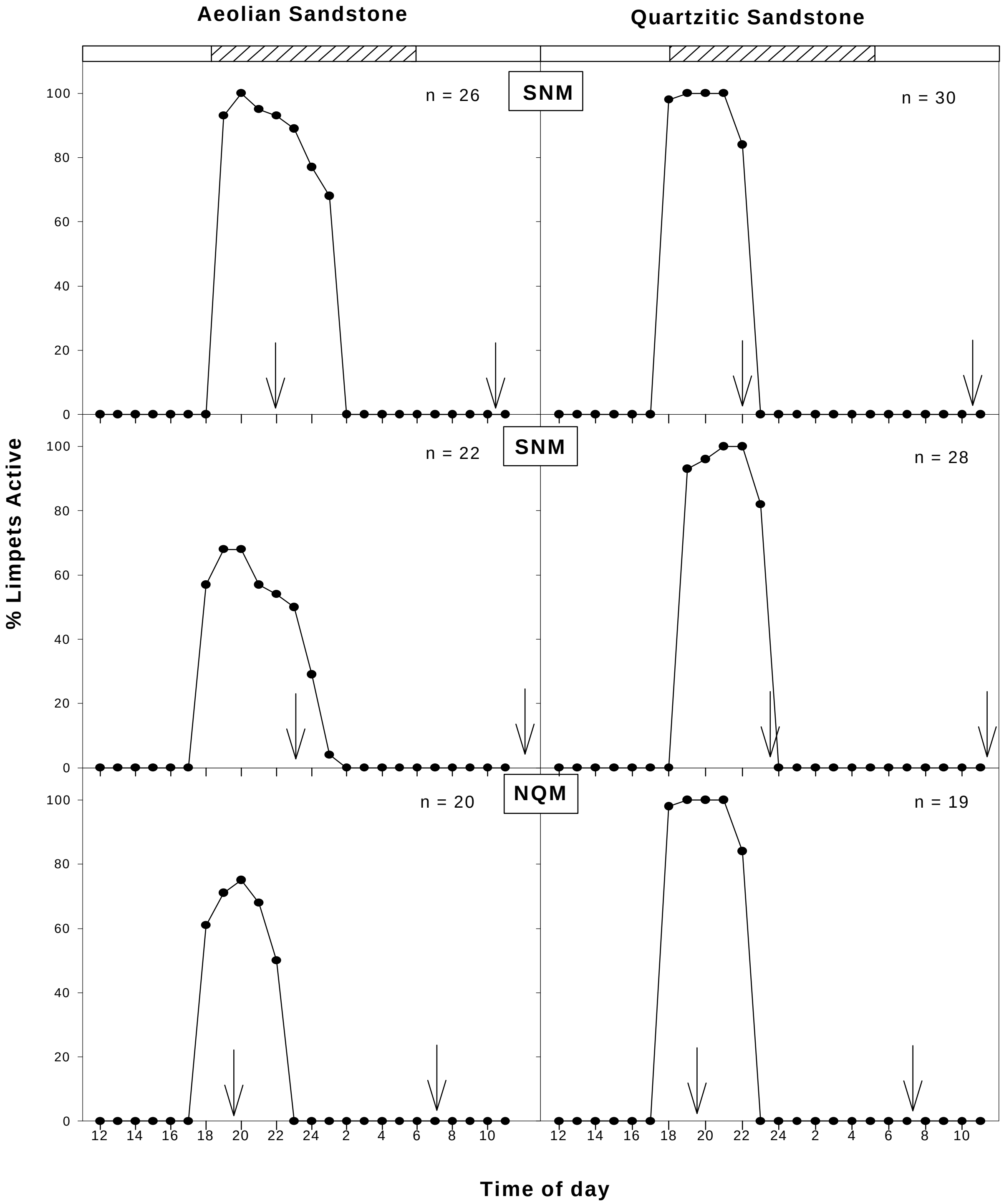


Figure 6.3:

The percentage of resident *P. granularis* active over 24 hours during 3 autumn observation periods on an aeolianite and a quartzitic shore. Arrows indicate low tide; shaded bars indicate periods of darkness. n = sample size. SNM = Spring new moon, NQM = Neap quarter moon. ● = resident undisturbed limpets.

6.3.3: Homing behaviour:

The number of resident *P. granularis* homing successfully (returning to the starting point after their foraging excursion) did not differ among replicates at either site during any of the observation periods ($p > 0.05$, Chi² test; Appendix III, Table 3). Once again, the data were pooled to provide a total number of limpets returning to a home scar at each site for each observation period.

On both aeolianite and quartzitic rocks, *P. granularis* homed to a fixed scar throughout the experimental periods. Resident limpets on both types of substrata had a homing success rate of between 86% and 100% (Table 6.2), with limpets from the quartzitic shore homing more rigidly than limpets from the aeolianite shores, although this difference was not statistically significant ($p > 0.05$, two-way ANOVA). Furthermore, limpets from both shores showed a slightly greater degree of homing success during spring than autumn (Table 6.2) although this difference was not significant ($p = 0.2041$ - aeolianite; $p = 0.1819$ - quartzite).

The homing behaviour of translocated *P. granularis* in spring and autumn was similar at both sites. 24 hours after being transferred, only 50% to 64% of the limpets returned to where they had been placed on the rock. 48 hours after translocation, between 57% and 86% of limpets returned to their starting position after foraging. After seven days, the majority of translocated limpets had established home scars with between 83% and 100% of these limpets homing successfully (Table 6.2).

During the course of the experiment, a number of the resident *P. granularis* changed home scars. This was evident throughout the experimental period, although occurred slightly more often in the limpets inhabiting the aeolianite shore. 15 limpets (22%) from the aeolianite platform changed scars (10 during spring, 5 during autumn), while 11 (19%) from the quartzitic boulders changed home scars (5 during spring and 6 during autumn). No limpets at either site returned to their home scars via their outward paths, although trail crossing did occur. When limpets returned home, they orientated themselves so that they consistently faced the same direction, resulting in the paint marks on the shells forming a continuous line with the painted mark on the rock.

Table 6.2.

The number (and percentage) of *P. granularis* homing successfully on two types of substratum during spring and autumn.

ND = no data collected.

		Aeolianite			Quartzitic Sandstone		
		Resident (undisturbed)	Resident (disturbed)	Translocated (from quartzitic sandstone)	Resident (undisturbed)	Resident (disturbed)	Translocated (from aeolianite)
Spring	SNM (21/08/1998)	27 (96%)	8 (89%)	6 (55%)	27 (100%)	9 (90%)	9 (64%)
	SNM (22/08/1998)	27 (100%)	9 (100%)	8 (73%)	26 (100%)	8 (100%)	12 (86%)
	NQM (28/08/1998)	17 (100%)	10 (100%)	10 (83%)	22 (100%)	9 (100%)	8 (89%)
Autumn	SNM (16/04/1999)	24 (92%)	ND	4 (50%)	28 (93%)	ND	11 (52%)
	SNM (17/04/1999)	19 (86%)	ND	4 (57%)	26 (93%)	ND	12 (75%)
	NQM (25/04/1999)	19 (95%)	ND	10 (91%)	19 (100%)	ND	7 (100%)

6.3.4: Distance moved by resident and translocated limpets:

No significant difference was found in the mean distance travelled by resident and translocated *P. granularis* in each replicate at either site (p> 0.05, Multi-factor ANOVA; see Appendix III, Tables 4 & 5). As a result, the data for these replicates were pooled to provide a mean distance travelled by resident and translocated limpets at each site during each observation period.

In order to determine whether the average distances travelled by *P. granularis* were affected by site, season or tidal phase (spring or neap), a multifactor ANOVA (MANOVA) was used. Resident limpets inhabiting aeolianite travelled significantly shorter distances during both seasons (.17 cm) than those inhabiting quartzitic rocks (.30 cm, Tables 6.3 and 6.4). Neither season nor tidal phase was found to have a significant effect on the mean distances travelled by the limpets at each site (p = 0.804, Table 6.4). An additional MANOVA was run in order to interpret whether the distances travelled by *P. granularis* differed during the observation periods. This additional MANOVA was necessary due to a correlation between tidal phase and observation periods which creates logical zeros in the data set. No statistical analysis can deal with this scenario (Sokal & Rohlf, 1981). It was found that although the limpets foraged further during the first observation period of each season than the following two experimental periods, the differences were not significant (F = 1.590; p = 0.2049) .

The distances travelled by the translocated limpets in both seasons initially mirrored those of the source populations (Table 6.3). Although these distances were often very different to those travelled by the resident limpets (eg 16/04/1999, quartzitic sandstone), the differences were not significant due to the high variability in the distances travelled by the limpets within each experimental group (F = 0.579; p = 0.4551, ANOVA). After a maximum period of one week, the transferred limpets were found to travel similar distances to the resident limpets at both sites (Table 6.3).

Table 6.3:

The mean distances (with standard deviations) travelled by *P. granularis* on 3 nights in spring and autumn. Distances travelled were measured in cm. n = sample sizes. ND = no data collected.

		Aeolianite						**Quartzitic Sandstone**					
		Resident (undisturbed)	**n**	**Resident (Disturbed)**	**n**	**Translocated (from quartzite)**	**n**	**Resident (undisturbed)**	**n**	**Resident (disturbed)**		**Translocated (from aeolianite)**	**n**
Spring	**SNM (21/08/1998)**	22.32 ± 12.03	28	29.50 ± 8.62	9	30.14 ± 12.95	11	30.80 ± 22.02	27	28.55± 15.34	10	21.91 ± 11.44	14
	SNM (22/08/1998)	15.39 ± 11.75	27	15.28 ±	9	21.95 ± 12.92	11	26.92 ± 15.29	26	26.75± 18.71	8	20.62 ± 19.05	13
	NQM (28/08/1998)	12.53 ± 7.96	17	14.75 ± 8.79	10	18.88 ± 12.59	12	27.81 ± 13.11	22	27.94 ± 11.90	9	32.83 ± 16.00	9
	0 Distance	16.75 ± 11.58	72	19.66 ± 11.85	28	23.71 ± 13.79	34	28.58 ± 17.58	75	27.81± 15.45	27	23.74 ± 16.45	37
Autumn	**SNM (16/04/1999)**	20.03 ± 12.68	26	ND		37.31 ± 15.24	8	31.20 ± 20.46	30	ND		18.71 ± 17.82	21
	SNM (17/04/1999)	16.72 ± 12.76	22	ND		23.86 ± 11.67	7	34.74 ± 18.06	28	ND		24.64 ± 19.67	16
	NQM (25/04/1999)	17.55 ± 10.42	20	ND		14.15 ± 13.40	11	27.42 ± 21.14	19	ND		38.21 ± 23.64	7
	0 Distance	18.23 ± 11.82	68	ND		23.89 ± 16.36	26	31.55 ± 19.72	77	ND		24.42 ± 20.42	44

Table 6.4 :

Multifactor Analysis of variance on distances travelled by *P. granularis*.

Source of variation	SS	d.f.	MS	F - ratio	Sig. level
Main effects					
Site	23596.93	1	23596.93	87.108	< 0.0001
Season	5	1	5	0.063	0.804
Tidal Phase	17.143	1	17.145	1.184	0.277
Residuals	320.680	328	320.680		
	88852.764		270.893		
All interactions are non-significant					

Results of Newman-Keuls multiple range analysis for distance travelled			
Site	Aeolianite		Quartzitic
	16.70cm	<	29.17cm
Season	Spring		Autumn
	21.91cm	=	23.96
Tidal Phase	Spring		Neap
	24.48cm	=	21.39cm

6.3.5: Speed of movement of resident *P. granularis*:

The foraging speed of each limpet was calculated on an hourly basis for the entire duration of each grazing excursion. Speeds travelled during each hour by resident limpets were not significantly different between replicates ($p > 0.05$, one-way ANOVA; Appendix III, Table 7), and data were pooled. From this, the mean hourly speeds travelled by the limpets during each observation period could be determined (Table 6.5). A MANOVA was applied to the data from both sites separately in order to determine whether there was any seasonal, tidal or hourly effect on the mean speeds travelled. Whereas a MANOVA showed that neither season nor tidal phase had any influence on the speeds recorded for the limpets from the quartzitic rocks ($p = 8.365$; Table 6.6), *P. granularis* inhabiting aeolianite travelled significantly quicker during autumn ($p = 0.0035$; Table 6.7). These limpets also travelled significantly quicker during spring tides ($p = 0.0394$; Table 6.7). At both sites, the initial movement away from the home scar was significantly quicker than that recorded for any of the subsequent hours (quartzite: Figure 6.5, Table 6.6; aeolianite: Figure 6.4, Table 6.7). The limpets then slowed down and remained in the same area for a period of between two and three hours. This was followed by an increase in the speed travelled with the second fastest speeds of the excursion being reached in the final hour (Figures 6.4 and 6.5) . This phase of activity resulted in the individual correctly orientating itself on the home scar, after which activity ceased (Table 6.5, Figures 6.4 and 6.5). The mean speed of resident limpets inhabiting quartzitic rocks (8.07 cm/hr) was significantly greater than those from aeolianite (3.90 cm/hr)($F = 145.112$; $p = < 0.0001$, One-way ANOVA).

Table 6.5:

Mean speed of movement (cm/hr ± S.D.) for resident *P. granularis* on two types of substrata during each hour of the foraging excursion. n = sample size. Blank cells indicate hours when 100% of the limpets are "at home".

				Hour of excursion						
			n	**1**	**2**	**3**	**4**	**5**	**6**	**7**
aeolianite	Spring	**SNM (21/08/1998)**	28	6.96 ± 5.76	4.32 ± 4.65	2.4 2 ± 1.86	3.00 ± 2.03	2.15 ± 1.60	5.98 ± 3.97	
		SNM (22/08/1998)	27	6.78 ± 4.98	4.17 ± 3.27	1.72 ± 1.17	7.02 ± 5.13			
		NQM	17	4.28 ± 3.60	1.59 ± 1.23	1.78 ± 1.31	1.74 ± 1.40	5.41 ± 4.02		
	Autumn	**SNM (16/04/1999)**	26	3.31 ± 2.93	2.45 ± 1.58	2.82 ± 2.23	2.32 ± 2.08	3.25 ± 2.46	2.95 ± 2.87	3.51 ± 3.41
		SNM (17/04/1999)	22	7.28 ± 4.25	2.95 ± 2.15	2.34 ± 2.14	1.63 ± 1.75	1.63± 1.75	7.21± 3.99	
		NQM	20	7.76 ± 6.22	1.65 ± 1.25	1.91 ± 1.65	2.8 ± 3.64	2.28 ±1.83	3.34 ± 4.25	6.31 ± 4.80
Quartzitic sandstone	Spring	**SNM (21/08/1998)**	27	11.47 ± 8.76	3.79 ± 3.36	8.30 ± 5.09	14.88±			
		SNM (22/08/1998)	26	12.75 ± 6.39	3.56 ± 2.56	2.75 ± 2.28	11.12			
		NQM	22	11.62 ± 7.60	4.62 ± 5.83	4.38 ± 2.66	6.01 ± 5.01	10.49 ± 5.69		
	Autumn	**SNM (16/04/1999)**	30	11.58 ± 8.57	4.60 ± 3.87	3.63 ± 3.98	4.44 ± 4.79	3.84 ± 5.02	9.79 ± 7.08	
		SNM (17/04/1999)	28	12.56 ± 9.04	6.58 ±	3.95 ± 3.07	5.63 ± 5.77	12.72 ± 9.97		
		NQM	19	14.27 ±14.64	2.00 ± 1.54	15.43 ±				

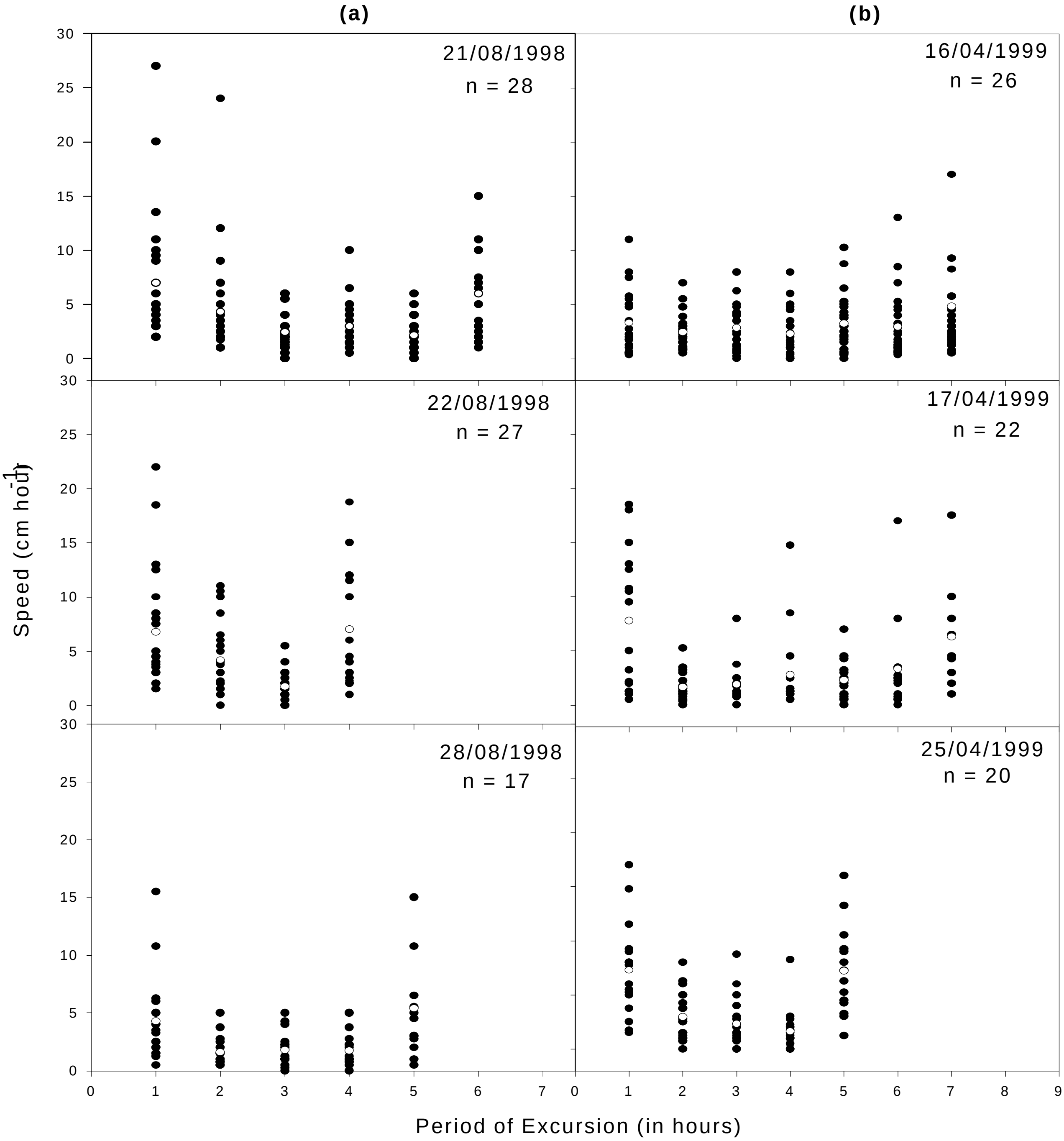


Figure 6.4:

Speed of *P. granularis* inhabiting aeolianite plotted against phase of excursion for three observation periods in (a) spring and (b) autumn. Open symbols = mean speeds. n = sample size.

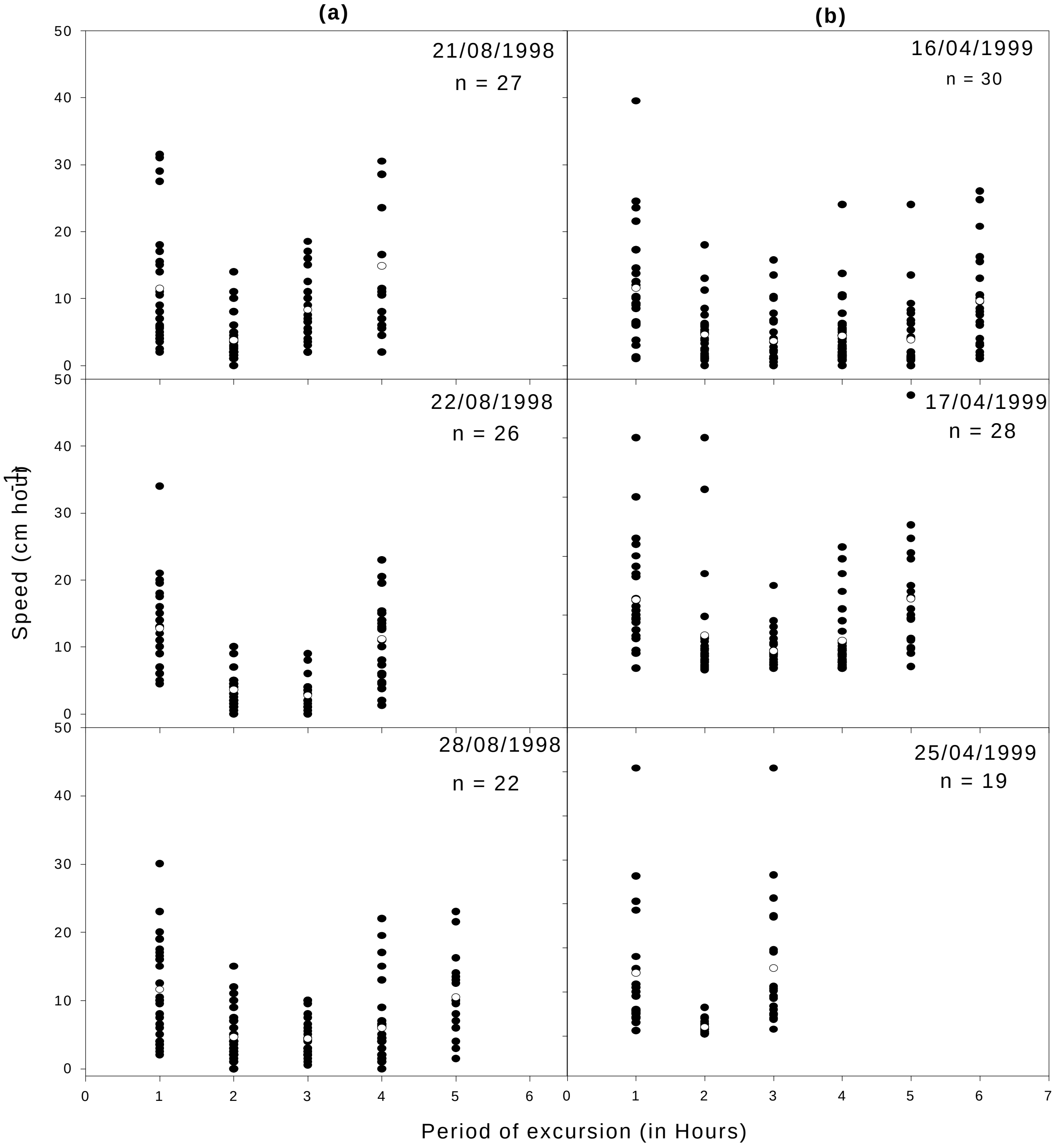


Figure 6.5:

Speed of *P. granularis* inhabiting quartzitic rock plotted against hour of excursion for three observation periods in (a) spring and (b) autumn. Open symbols = mean speeds. n = sample size.

Table 6.6:

MANOVA of hourly speeds of movement of *P. granularis* inhabiting quartzitic sandstone on spring and neap tides during both seasons. (* indicates a significant p - value).

Source of variation	df	MS	F	P
Main effects				
Season	1	2.421	0.044	8.365
Tide	1	52.470	0.950	0.341
Hour	5	1031.0	18.66	0.0001
Residual	634	7	6	*
		55.24		
All interactions are non-significant				

Scheffe's tests for the above speeds			
Season	Spring	Autumn	Scheffe's
	8.45 cm/hr^{1}	8.58 cm/hr^{1}	Spring = Autumn
Tide	Spring	Neap	Scheffe's
	8.19 cm/hr^{1}	8.84 cm/hr^{1}	Spring = Neap
Hour of excursion	1	2,3,4,5,6,7	
	12.64 cm/hr^{1}	3.53 ± 0.88 cm/hr^{1}	Hour 1 > rest of excursion

Table 6.7:

MANOVA of hourly speeds of movement of *P. granularis* inhabiting aeolianite on spring and neap tides during both seasons. (* indicates a significant p - value).

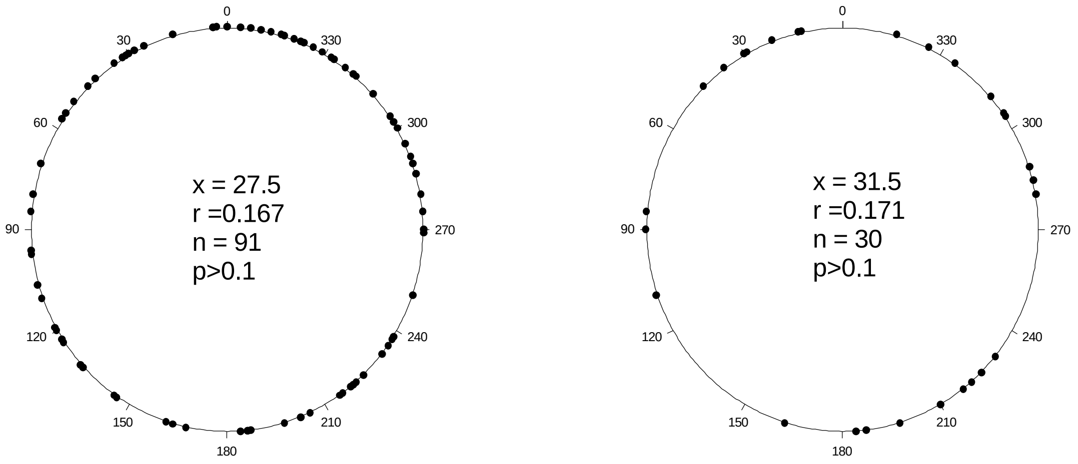
Source of variation	d.f.	MS	F-ratio	Sig. level
Main effects				
Season	1	96.9	8.628	0.0035 *
Tide	1	2	4.262	0.0394 *
Hour	6	47.8	7.665	0.0001 *
Residual	544	8		
		86.1		
		0		
		11.2		
		3		
All interactions are non-significant				

Scheffe's tests for the above speeds			
Season	Spring	Autumn	Scheffe's
	3.48 cm/hr^{1}	4.38 cm/hr^{1}	Spring < Autumn
Tide	Spring	Neap	Scheffe's
	4.28 cm/hr^{1}	3.58 cm/hr^{1}	Spring > Neap
Hour of excursion	1	2,3,4,5,6,7	
	6.27 cm/hr^{1}	3.53 ± 0.88 cm/hr^{1}	Hour 1 > rest of excursion

6.3.6: Orientation of foraging activity:

Significant differences in the mean directions travelled by *P. granularis* from the replicates observed on each night could not be determined as the assumptions of the Watson-Williams F test were not fulfilled. In each case the mean vector was less than the minimum vector length necessary i.e. the parameter of concentration (ê) Ý 2 (Batschelet, 1981). Even when results from each replicate were pooled to give nightly and seasonal foraging directions for each site, the assumptions of this test were not fulfilled.

The directionality of movement of limpets within all replicates observed was indeed found to be random ($p > 0.1$; Rayleigh test). Pooling of data to provide nightly foraging directions also showed that limpets moved randomly. After pooling nightly data, the seasonal directionality of movement could be assessed. *P. granularis* on both substrata exhibited random movement during both seasons ($p > 0.1$; Rayleigh test; Figures 6.6 and 6.7). The only exception was evident in the limpets translocated from aeolianite to quartzitic rock during spring. These limpets showed directionality in foraging with their excursions taking them up the rock face (Figure 6.6d).



(a) Aeolianite (Resident)

(b) Aeolianite (Translocated)

(c) Quartzitic (Resident)

(d) Quartzitic (Translocated)

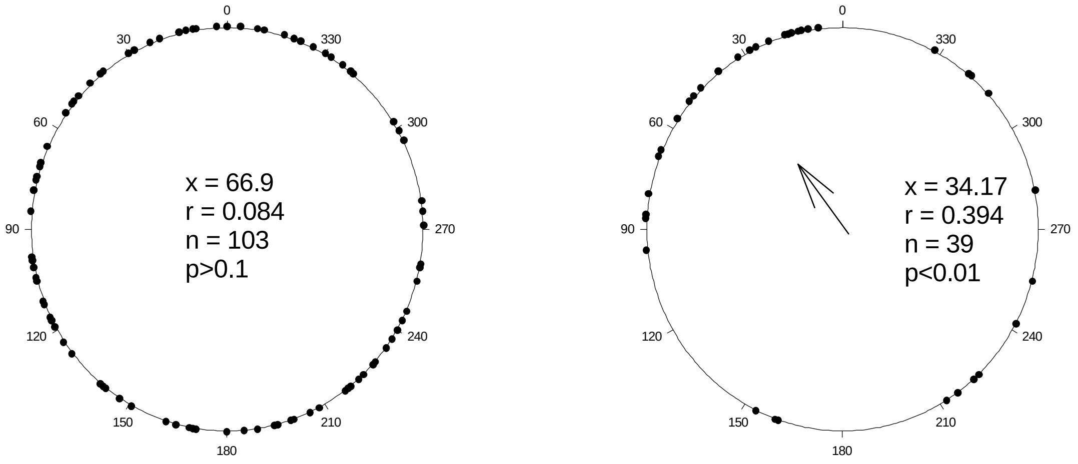
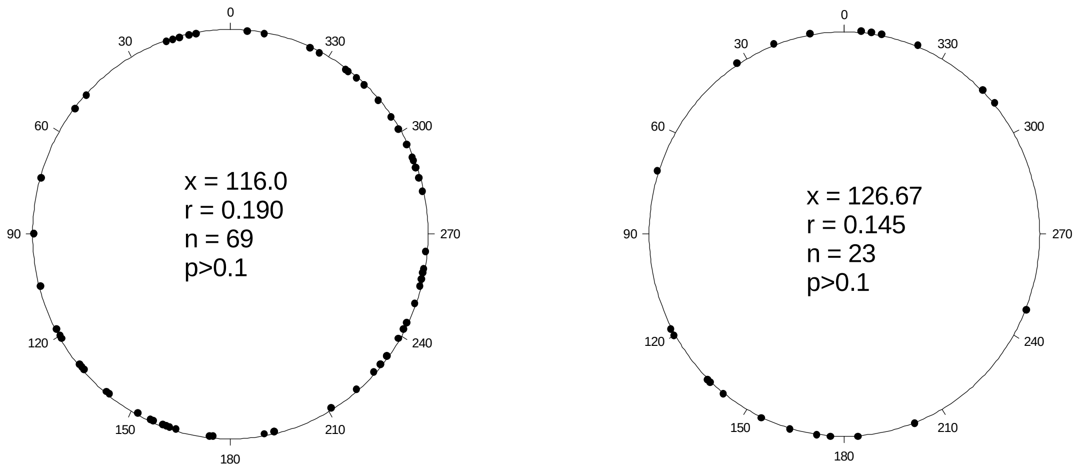


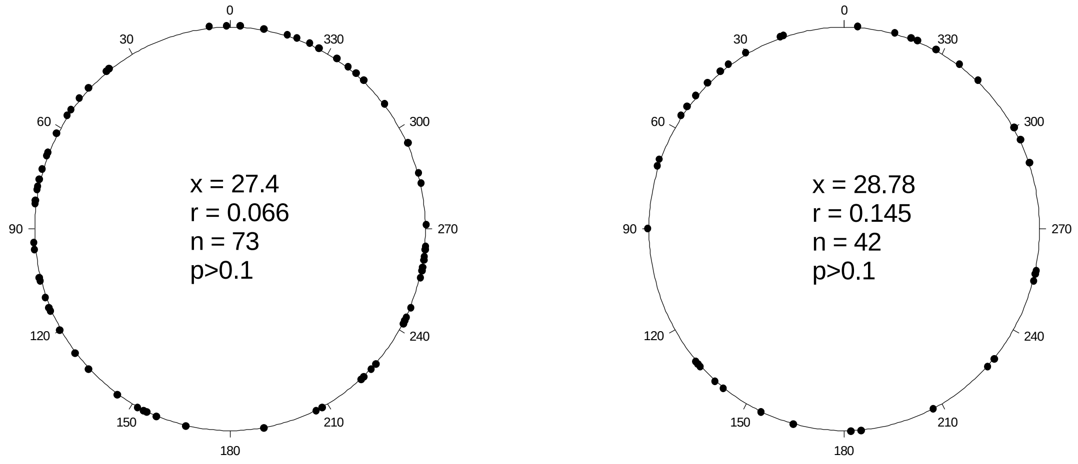
Figure 6.6:

Foraging directions of *P. granularis* on aeolianite and quartzitic sandstone using pooled data recorded during spring 1998. The arrow in (d) indicates the direction in which a significant proportion of the limpets moved.



(a) Aeolianite (Resident)

(b) Aeolianite (translocated)



(c) Quartzitic (Resident)

(d) Quartzitic (Translocated)

Figure 6.7:

Foraging directions of *P. granularis* on aeolianite and quartzitic sandstone using pooled data recorded during autumn 1999.

6.3.7: Radula tooth wear:

Results of a MANOVA performed comparing the mean size of the radula teeth of *P. granularis* inhabiting the two shores showed no significant difference in the degree of tooth wear between the sites (F = 0.421; p = 5.335; Table 6.9). However, the wear of the pluricuspid teeth was significantly greater on quartzitic sandstone than on aeolianite (p = 0.0045; Table 6.10). This is also evident when comparing the ratio of cusp length to cusp width (Table 6.8). The wear of the second cusp (f) appears to be particularly great in limpets inhabiting quartzite when compared to those from the aeolianite (p = 0.0376; Tables 6.8 and 6.10). However, no significant difference in the wear of the lateral teeth of limpets inhabiting different substrata was determined (p = 0.0522; Table 6.11), although at both sites, the ratio of length to width of the first lateral tooth was significantly less than measured for any of the other teeth (p = 0.0239; Table 6.11). These differences in tooth wear are clearly visible in Figure 6.8a and b.

Table 6.8:

Mean ratios obtained of cusp length/cusp width (a - d correspond to the lateral teeth identified in the text) and of tooth length/tooth width (e - g correspond to the cusps identified in the text).

Ratio	Aeolianite	S.D.	Quartzite	S.D.
a	3.381	0.764	2.441	0.684
b	4.232	1.421	5.424	2.953
c	5.117	1.114	5.680	0.886
d	5.720	0.306	5.743	4.307
e	4.218	1.137	2.443	0.095
f	8.353	2.245	3.287	0.845
g	1.500	0.188	1.279	0.221

Table 6.9:

Results of a MANOVA performed on the ratios of tooth length/tooth width performed on the lateral and plusricuspid teeth of *P. granularis* inhabiting aeolianite and quartzitic sandstone. (* indicates a significant p - value).

Source of variation	**SS**	**d.f.**	**MS**	**F - ratio**	**Sig. level**
Main effects					
A: Site	1.216	1	1.216	0.421	0.5335
B: Limpet	15.539	5	7.769	2.695	0.1023
C: Tooth	184.685	6	26.384	9.150	0.0003 *
Residual	40.366	35	2.883		
Total	334.709	51			
AC = Significant interaction (p = 0.0276)					

Table 6.10:

Results of a MANOVA performed on the ratios of cusp length/cusp width performed on the plusricuspid teeth of *P. granularis* inhabiting aeolianite and quartzitic sandstone.
(* indicates a significant p - value).

Source of variation	**SS**	**d.f.**	**MS**	**F -ratio**	**Sig. level**
Main effects					
A: Site	24.384	1	24.384	19.407	0.0045 *
B: Limpet	5.553	5	2.777	2.210	0.1910
C: Tooth	60.886	2	20.295	16.153	0.0028 *
Residual	7.539	15	1.256		
Total	127.706	23			
AC = Significant interaction (p = 0.0376)					

Table 6.11:

Results of a MANOVA performed on the ratios of tooth length/tooth width performed on the lateral teeth of *P. granularis* inhabiting aeolianite and quartzitic sandstone. (* indicates a significant p - value).

Source of variation	**SS**	**d.f.**	**MS**	**F - ratio**	**Sig. level**
Main effects					
A: Site	11.418	1	11.418	5.834	0.0522
B: Limpet	11.943	5	5.972	3.051	0.1219
C: Tooth	39.512	3	13.171	6.729	0.0239 *
Residual	11.743	15	1.957		
Total	122.716	23			
AB = Significant interaction (p = 0.0235)					

Scheffe's test for differences in tooth wear			
Tooth	**LS Mean**	**Homogenous groups**	
Lateral tooth 1 (a)	8.170	X	
Lateral tooth 2 (b)	4.828		X
Lateral tooth 3 (c)	5.399	X	X
Lateral tooth 4 (d)	5.623	X	X

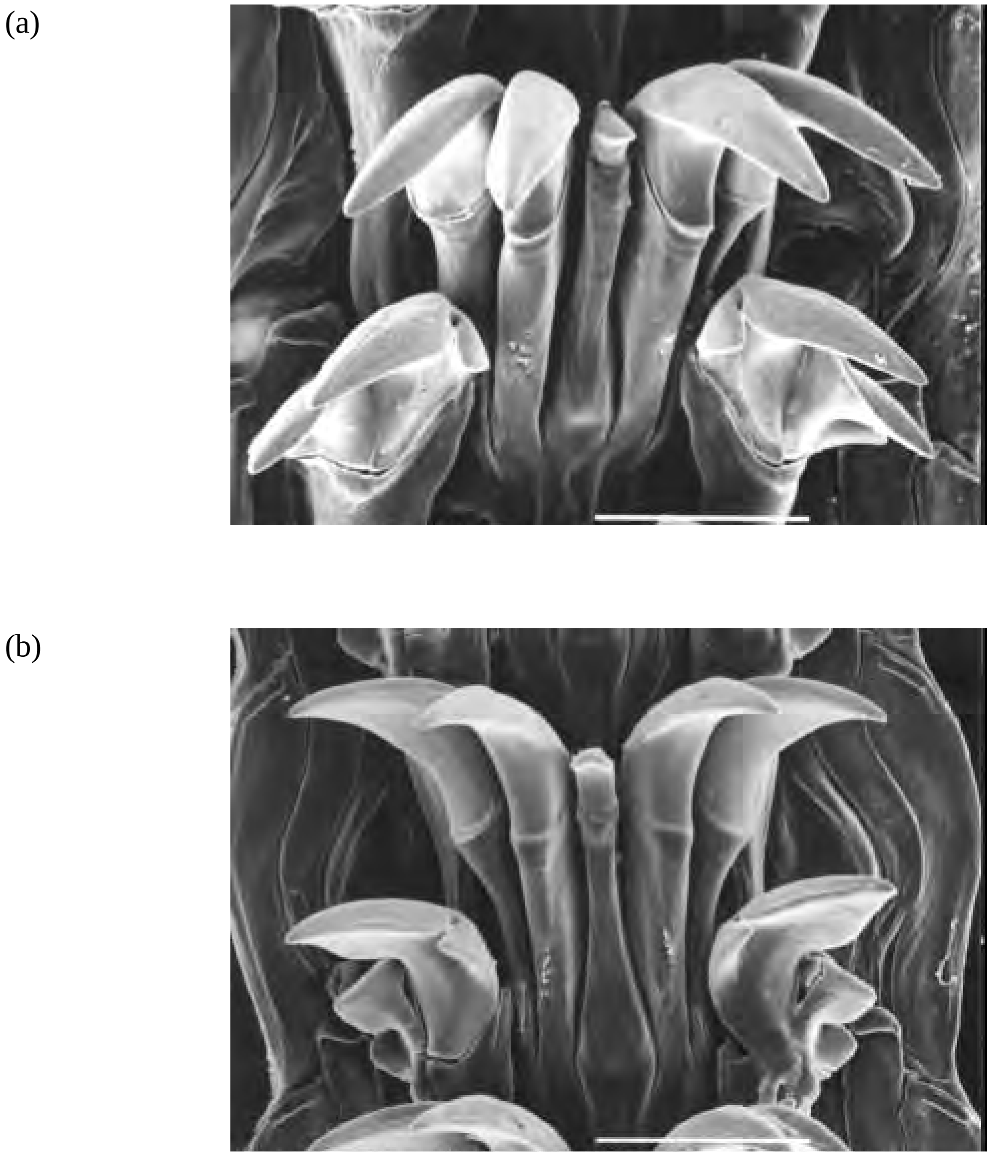


Figure 6.8:

Comparative electron micrographs of radulae representative of *P. granularis* inhabiting (a) aeolianite and (b) quartzite showing the increased tooth wear in limpets from the latter substratum. Scale bar = 100 μm.

6.4: DISCUSSION:

To date, a variety of factors have been reported to influence the timing of foraging activity in intertidal limpets. Although the number of quantitative studies on the foraging activity of *Patella granularis* along the South African coast have been limited, qualitative studies have shown that tidal height greatly modifies the timing of foraging in this species. In 1936, Stevenson observed that mid-shore limpets were active only during nocturnal low tides. More recently though, *P. granularis* from a False Bay shore were reported to be active both whilst submerged and uncovered, and during both the day and night. Furthermore, activity was more pronounced in the high-shore limpets than those from the low-shore (Branch, 1971). Mid-shore *P. granularis* inhabiting an exposed south-east coast shore exhibited a further modification in the timing of their activity. These limpets were active at low water during both the day and night (Gray & Hodgson, 1997). Although only the latter study was quantitative in nature, as no underwater observations were made during this study, the inactivity of *P. granularis* whilst they were submerged was assumed. As such, it is purely speculative that limpets inhabiting the south-east coast are only active during low tides. However, similar variability in the timing of foraging activity has been found for the well-studied limpet, *P. vulgata*. This species has been shown to forage whilst submerged during the day (Hartnoll & Wright, 1977; Hawkins & Hartnoll, 1982), whilst emerged during both the day and night, (Hawkins & Hartnoll, 1982; Little & Stirling, 1985) and during nocturnal low tides (Little & Stirling, 1985; Gray & Naylor, 1996). The observed differences in activity patterns within a single species suggests that habitat plays an important role in modifying the behaviour of individuals. This has been suggested by a variety of authors including Chelazzi (1982), Little *et al.* (1988) and Gray (1996).

In the current study, it was found that on east-facing rocks of both an aeolianite platform and a quartzitic reef, *P. granularis* foraged only during nocturnal low tides or when low tide occurred around dusk or dawn. Activity began as the limpets were uncovered by the ebbing tide and continued until they were covered by the incoming tide, regardless of substratum type. However, the same limitations may be placed on these observations as were aimed at Gray and Hodgson's (1997) reports regarding foraging during high tides. Despite this it is evident that this species may modify its timing of activity according to localised habitat. East-facing limpets, that are exposed to the sun for the majority of the day, limit excursions to nocturnal low tides (this study), whilst those inhabiting west facing rocks make use of the shady conditions in the morning to increase the number of foraging excursions undertaken (Gray and Hodgson, 1997).

The pattern of behaviour exhibited by *P. granularis* inhabiting east-facing slopes offers these limpets a number of advantages. It is generally accepted that restricting activity to periods of low water reduces the chances of being washed away by strong waves (Cook & Cook, 1978; Branch & Cherry, 1985; Gray, 1996; Gray & Hodgson, 1997). However, *P. granularis* is known to be highly tenacious (Branch & Marsh, 1978) and has been found to be active under strong wave action (Gray & Hodgson, 1997). This pattern of behaviour, therefore, is more likely to be a function of both predator and desiccation avoidance.

It has been suggested that some species of limpets remain inactive at high tide in order to avoid marine predators (Wells, 1980; Branch, 1981; Garrity & Levings, 1983; Branch, 1985a,). *P. granularis* is a major prey item of the gastropod *Thais dubia,* making up 28% of its diet (Branch, 1973). This predator feeds on *P. granularis* by inserting its siphon under the shell and feeding directly. However, *T. dubia* is inactive during exposure, preferring to retreat into crevices. *P. granularis* is also preyed upon (to a lesser degree) by the starfish, *Marthasterias glacialis* (Branch, 1973) and the giant clingfish, *Chorisochismus dentex*, (Branch, 1973). All of these predators occur along the south-east coast of South Africa (Branch *et al.*, 1994), thus it is probable that by restricting activity to periods of low tides, *P. granularis* would reduce the risk of predation.

P. granularis is also a major contributor to the diet of the African Black Oystercatcher with up to 2.8 million limpets being consumed by birds on the guano rich islands on the west coast (Hockey & Branch, 1984). By limiting foraging activity to periods of nocturnal low tides, consumption by birds may be reduced. *P. granularis,* as with other *Patella* species, does not have the added protection of a mucous that repels possible predators as is the case with many siphonariid limpets (Branch & Cherry, 1985). A behavioural aspect of predator reduction in this species is thus most important. However, the number of oystercatchers present on the west coast islands is far greater (78/m of coastline; Hockey, 1983) than the number recorded along the section of coast investigated in the present study (31 adult oystercatchers, A. Stephenson, pers. comm.). It would thus seem that the timing of foraging activity in *P. granularis* from the south-east coast is either genetically entrenched or is strongly influenced by the physical conditions of the intertidal shore.

P. granularis can lose up to 34% of tissue water when exposed to air, tolerating this level of desiccation for up to 89 hours (Marshall & McQuaid, 1992). These values are considerably lower than those found for the pulmonate limpet *Siphonaria oculus* which tolerate a loss of 59% of tissue water for a period of 158 hours (Marshall & McQuaid, 1992). Gastropods which restrict movement to nocturnal periods minimise the threat of desiccation (Wolcott, 1973; Branch, 1981; Little & Stirling, 1985), and this is likely to be a major factor controlling the foraging activity of *P. granularis*. This is evident by the fact that the patterns of activity exhibited by this species are flexible within a set of environmental parameters. As already mentioned, *P. granularis* inhabiting west-facing slopes, that receive only afternoon sun, display a biphasic pattern of activity (Gray & Hodgson, 1997), while limpets inhabiting east-facing slopes at the same site, exhibit activity that is restricted to nocturnal low tides (this study). The east-facing rock surfaces receive both morning and afternoon sun. Furthermore, *P. granularis* at this site, on both east- and west-facing slopes, have been observed to be active during day-time low tides on foggy days (pers. obs.). This is presumably due to an increase in the relative humidity.

P. granularis inhabiting east-facing rocks exhibited strong homing tendencies, regardless of substratum type, with between 86% and 100% of the limpets returning to a fixed scar. A variety of factors such as the texture and stability of the substratum and the availability of food influence the rigidity of homing behaviour (Branch, 1981). In the present study, it is evident that the development of a home scar on both aeolianite and quartzitic sandstone shores is important, as translocated individuals homed to a fixed scar after a maximum acclimation period of one week. Returning to a home scar after foraging is thought to be an adaptive feature developed to reduce the chances of being dislodged by wave action and is widely evident in the siphonariid limpets (Branch & Cherry, 1985). However, for the reasons already discussed, this is unlikely to have been the driving force behind the development of homing in *P. granularis*.

Returning to, and remaining on, a home scar during daylight hours reduces desiccation in many limpets (Wolcott, 1973; Verderber *et al.*, 1983; Branch & Cherry, 1985; Little & Stirling, 1985). *P. granularis* exhibits a wide range of homing rigidity according to habitat and height on the shore. Low shore limpets from the Western Cape coast lack a home scar whilst higher shore limpets exhibit homing behaviour and return to a scar (Branch, 1971). *P. granularis* inhabiting west-facing slopes on the south-east coast also do not home and lack a fixed scar (Gray & Hodgson, 1997). It seems plausible, therefore, that the different levels of homing success are strongly related to the threat of desiccation in different microhabitats. This is supported by the fact that the homing rigidity of *P. granularis* in the present study was stronger during spring than autumn on both substrata. It is suggested that this is likely to be the result of an increased rate of desiccation during the warmer spring months. Furthermore, homing was more rigid on quartzitic rock than aeolianite, particularly in autumn. aeolianite rock retains moisture for longer than quartzitic sandstone (D. Abbey & A.H. Dye, pers. comm.). It is thus possible that the increased moisture on aeolianite reduces home scar dependancy of *P. granularis* on this rock type.

A number of limpet species exhibit highly directional foraging excursions that are thought to result in optimal foraging conditions (e.g. *Helcion pectunculus* that consistently moves onto areas of rock that have the highest microalgal biomass, Gray & Hodgson, 1998). The directionality of foraging excursions in *P. vulgata* in a sea-lough was, however, correlated to the height on the shore, with mid-shore limpets moving in a random fashion when compared to the highly directional movements displayed by both low- and high-shore individuals (Little *et al.*, 1988). Mid-shore *P. granularis* on both substratum types in the current study also exhibited a random pattern of movement. It has been suggested that the presence of barnacles adversely affects the foraging behaviour of limpets (Underwood, 1979; Hawkins & Hartnoll, 1982). As barnacles can be found co-existing with *P. granularis* at both of the sites used in the present study, it is possible that the limpets move in a random manner in order to utilise clear patches of rock to the greatest potential. *P. granularis* do not move in the same direction on two consecutive nights (pers. obs.), agreeing with the suggestion by Cook & Cook (1981) that this may maximise the utilisation of food resources.

According to Cook and Cook (1975), the directionality of homing may be based on some property of the mucous trail left by the limpets on their outward path. This is unlikely to be the mechanism by which individuals of *P. granularis* home, as no limpets on either rock type were observed to follow their outward trails home. This was also true for *P. granularis* inhabiting west-facing slopes (Gray & Hodgson, 1997). However, trail crossing was recorded in both studies, which may provide the limpets with adequate information for correct orientation (Gray & Hodgson,
1997).

P. granularis inhabiting both east facing aeolianite and quartzitic sandstone exhibited a pattern of activity that could be divided into three phases: a rapid outward phase, a slower middle phase and rapid return trip. This foraging pattern has also been documented for *Helcion pectunculus* (Gray, 1996), *P. vulgata* (Little *et al.*, 1988; Williams & Morritt, 1991; Chelazzi *et al.*, 1994), *Siphonaria concinna* and west-facing *P. granularis* (Gray & Hodgson, 1997). Hartnoll and Wright (1977) considered moving and feeding to be separate activities, however, this has since been disproved in *P. vulgata*. This species feeds continuously during foraging excursions with greatest foraging activity recorded during the middle "foraging phase" (Little & Stirling, 1985; Santini *et al.* 1995). A similar result was recorded for *Cellana toreuma* which was observed to display continuous radula movement during foraging (Hirano, 1979). At present, it is not known if *P. granularis* feeds for the full duration of its foraging excursion.

The current study suggests that substratum type influences the rate of pluricuspid tooth wear in *P. granularis,* although the wear of the lateral teeth was not affected by substratum. The cusps of the pluricuspid teeth of limpets inhabiting aeolianite exhibited less tooth wear than those from quartzitic sandstone. This was expected as aeolianite is a soft, granular rock. Movement of the radula across this rock surface is likely to remove rock particles with the minimum of effort.

The distance moved whilst foraging was significantly greater in *P. granularis* inhabiting quartzitic sandstone compared to limpets inhabiting aeolianite. This is also the case for the chiton, *Acanthochitona garnoti*, at the same sites (Cretchley *et al.* 1997). *P. vulgata* exhibits a difference in the distance travelled during foraging excursions that can be correlated to exposure (Della

Santina *et al.*, 1994), and height on the shore (Little *et al.*, 1990; Della Santina *et al.*, 1995). In all of these studies, the differences in distances travelled is suggested to be related to food availability. In the present study, microalgal biomass (chlorophyll-*a*) is always higher on the aeolianite platform (. 3 - 15 Fg chlorophyll-*a* cm$^{-1)}$ than on the quartzitic shore (. 1 - 5 Fg chlorophyll-*a* cm^{-1}; Chapter 7). This would indicate that limpets inhabiting the aeolianite shore do not have to travel as far as individuals inhabiting the quartzitic rocks in order to accumulate the same amount of food. The translocation experiments of the current study support this theory with transferred limpets adjusting the distances travelled whilst foraging to distances similar to those exhibited by the resident population. This adjustment occurs over a maximum period of one week. Furthermore, it was found that the speed at which *P. granularis* travels while foraging is approximately 48% lower on aeolianite than on quartzitic sandstone (see section 6.3.5). This suggests that the limpets on the aeolianite platform also do not need to expend as much energy in the acquisition of food as those on the quartzitic rocks.

Limpets that undergo long feeding excursions one night, are less likely to forage the next night (Branch & Cherry, 1985). *P. granularis* on both substrata exhibit a modification of this behaviour. Although activity is not suspended on the second night, it is reduced. Thus these limpets do not take advantage of all conditions that are favourable for foraging, leading to the assumption that activity is indeed not a function of the need to maximise energy gain, but rather a function of the need to minimise the time spent away from the home scar (Evans & Williams, 1991).

P. granularis inhabiting west-facing quartzitic rock show a decrease in the distance travelled on neap tides in comparison to spring tides (Gray & Hodgson, 1997). This is also true for limpets inhabiting east-facing rock, irrespective of substratum type. A similar pattern has been found in the limpets *S. capensis* (Branch & Cherry, 1985), *S. concinna* (Gray & Hodgson, 1997) and *H. pectunculus* (Gray & Hodgson, 1998). Although the distances travelled by *P. granularis* on east-facing quartzitic rocks are similar to those recorded by Gray and Hodgson (1997) for spring new moon tides (. 30 cm), they are higher than those recorded for neap tides (. 27 cm, present study; . 13 cm, Gray & Hodgson, 1997). As such, the influence of tidal phase in the present study is not as marked as previously observed. The activity of siphonariid limpets is thought to be limited by exposure to air, with individuals returning to home before the incoming tide. This results in the shorter distances travelled during neap tides (Branch & Cherry, 1985; Branch, 1988). This is unlikely to be the reason for differences observed in the present study as *P. granularis* has been shown to remain active, although near home, whilst being covered by the waves of the incoming tide (Gray & Hodgson, 1997). It is more likely that previous studies have omitted to take nightly differences into consideration when calculating tidal influences. The duration of activity and the total distances travelled are also affected by the size of the limpets, with smaller limpets travelling shorter distances as a result of lower food requirements (Little *et al.*, 1988; Della Santina *et al.*, 1995). The differences observed in the present study can not be explained by this, however, as similar sized individuals were observed at each site. *P. granularis* has also been shown to travel further during summer than winter (Gray & Hodgson, 1997), but no seasonal differences were observed in the present study. It is suggested that the observed differences are a direct result of the increased food availability on aeolianite shores. As a result, *P. granularis* from the aeolianite platform do not need to travel as far as those from the quartzitic shore to obtain the same amount of food.

In conclusion, it is clear that the foraging activity of *P. granularis* can be highly variable. The current study suggests that this variability is correlated to food availability which was different on the aeolianite and quartzitic sandstone shores studied (Chapter 7). However, before firm conclusions can be drawn about the influence of habitat (in the form of rock type) on any aspect of the foraging behaviour of *P. granularis*, replicate experiments on closely positioned shores of similar geomorphologies must be undertaken to confirm whether the limpet behaviour observed in the current study are typical for the particular substrata. Nevertheless, the present investigation is useful as a comparative study of the foraging behaviour of a limpet on two different shores. Limpets inhabiting aeolianite do not travel as far, nor as quickly, as those from quartzitic sandstone. Furthermore, limpets transferred from one shore type to the other quickly (within one week) adapted to the new conditions and modified their foraging activity to mirror that of the resident population. It is suggested that the observed differences are a direct result of the increased food availability on aeolianite shores. As a result, *P. granularis* from the aeolianite platform do not need to travel as far as those from the quartzitic shore to obtain the same amount of food.

Chapter 7

ESTIMATES OF FOOD AVAILABILITY ON THREE SUBSTRATA ON THE SOUTH-EAST COAST OF SOUTH AFRICA

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7.1: INTRODUCTION:

The population characteristics of limpets are influenced by a number of factors including competition (both intra- and interspecific), tidal elevation, wave action and food availability (Branch, 1981). The contribution of both food quantity and quality to the growth and reproductive fitness of macrophageous marine herbivores has been increasingly studied, mainly as a result of the aquaculture industry (e.g Sato & Notoya, 1988; Hahn, 1989; Peck, 1989; Foster *et al.*, 1999). In contrast, despite the fact that limpets are often numerically dominant in the intertidal region, very little is currently known about the relationship between food source and the life history characteristics of these organisms. Most limpets are generalist grazers, feeding on diatoms, algal spores and detritus (Branch, 1981). Although there is some evidence that microbial films affect both the mortality and growth of limpets (Underwood, 1979; Branch, 1981; Hawkins & Hartnoll, 1983), practical difficulties in the measurement of the settlement and availability of intertidal microalgae have limited the number of quantitative studies in this field (Bosman & Hockey, 1988a; Hawkins *et al.*, 1989).

A gradient of primary productivity exists around the coast of southern Africa as a result of strong upwelling which occurs on the west coast (Bustamante *et al.*, 1995b). This gradient also extends to the nearshore waters, with the north-west coast being an order of magnitude more productive than the south-east coast (Brown, 1992). Associated with this west-east decrease in primary productivity is a decrease in grazer biomass and body size, as is epitomised by the limpet *Patella granularis* (Stephenson, 1937; Bustamante *et al.* 1995b; T. Ridgway *et al.*, 1998). The different oceanographic conditions existing between the Atlantic and Indian Ocean coasts may also affect the reproductive strategies of limpets: those inhabiting the cooler waters of the west coast spawning once a year, while those from the warmer south and east coasts either spawn twice a year or exhibit protracted spawning (Branch, 1974b; Chapter 5; see Foster 1997 for review).

Although a generalised trend in the life history characteristics of limpet species may be identified around the coast of South Africa, the localised microhabitat of these organisms also appears to influence these strategies. *P. granularis* inhabiting an aeolianite and a quartzitic sandstone shore have an increased growth rate when compared to those inhabiting low shore mussels (Chapter 4). In addition, there is some variability in the annual reproductive patterns of animals inhabiting these sites (Chapter 5). Although the establishment of different life history patterns in populations inhabiting a variety of environments is likely to be the result of a combination of factors, investigation of individual factors are a step towards understanding the complexity of the situation. It is for this reason that it was decided to investigate whether the observed differences in growth rate and reproductive pattern of *P. granularis* inhabiting three different substrata (i.e. aeolianite, quartzitic sandstone and mussel shells) could be correlated to differences in the availability of the microbial food resource.

A number of methods have been proposed for the sampling of epilithic microalgae. Brushing and scraping the surface of the rock (Underwood, 1984a,b), grinding rock chips (Dye & White, 1991), attaching settlement strips to the rock (Bosman & Hockey, 1988a,b) and removing intact rock chips (Hill & Hawkins, 1990,1991; Williams, 1994a,b; Gray, 1996; Nagarkar & Williams, 1997) have all been used to collect biofilms for analysis. Chlorophyll-*a* has been extracted using a number of solvents including acetone, ethanol, a 2:1 chloroform/methanol mixture and hot methanol (Nicotri, 1977; Dye & White, 1991; Hill & Hawkins, 1991; Takada, 1993; Gray, 1996; Nagarkar & Williams, 1997). Despite the widespread use of chlorophyll-*a* as an indication of microbial biomass, there are several objections to the procedure. The chlorophyll-*a* content of the cells varies seasonally and daily (Ryther, 1956; Bustamante *et al.*, 1995b); the presence of other pigments such as chlorophylls *b* and *c* may interfere with the estimation of chlorophyll-*a* (Nicotri, 1977), the distribution of microalgae is spatially patchy (Hawkins *et al.*, 1989) and the comparative value of results is limited due to the variety of sampling techniques (Nagarkar & Williams, 1997). However, the advantages of this procedure, e.g. ease of sampling in the field, speed of determination of algal quantities (Underwood, 1984a) and the exclusion of rock debris from analysis (Castenholz, 1961) generally outweigh the drawbacks.

P. granularis, as with most patellid limpets, has a simple docoglossan radula (Fretter & Graham, 1994) which is capable of gouging deeply into the substratum and dislodging large objects such as barnacle shells (Hawkins *et al.*, 1989). It was thus decided to remove chips of rock for analysis as this would provide a more accurate assessment of the total food available to the limpets.

7.2: MATERIALS AND METHODS:

7.2.1: Study sites and sampling procedure:

Microalgal standing stock was sampled on a monthly basis for a period of 12 months (March 1997 - February 1998) from each of the three substrata (aeolianite, quartzitic sandstone and mussel shells - see Chapter 2 for site details). Each month, 20 rock chips (. 2 cm^2) were randomly collected from an approximately 2 m^2 rock surface on both the aeolianite and quartzitic shores, while 20 mussel shells (. 2 cm^2) were removed from the mussel bed. Rock was sampled to a depth of at least 2 mm and all samples were collected during spring low tides on calm, warm days. No estimates of chlorophyll-*a* were possible during November 1997 due to inclement weather during the spring low tides.

7.2.2: Chlorophyll-*a* determination:

Each sample was separately placed in a sealed vial containing 10 ml of methanol (100%) and transported to the laboratory at Rhodes University where it was stored overnight in the dark at -10EC. The monthly microalgal standing stock at each site was analysed using the hot methanol extraction procedure (HMSO, 1986) with modifications to the procedure being made based on the findings of Nagarkar & Williams (1997). The extraction of chlorophyll-*a* using hot methanol has been shown to be more efficient than using either ethanol or acetone (Nagarkar and Williams, 1997). In the present study, the methanol containing the rock chips or mussel shells was heated for two minutes and cooled for three hours in accordance with the recommendations of Nagarkar and Williams (1997). Although cooling for twelve hours would have extracted 100% of the chlorophyll-*a*, the large number of samples rendered this procedure impractical. After cooling, the methanol was centrifuged at 2 500 rpm for five minutes to remove any particulate matter. The absorbance of the chlorophyll-*a* containing supernatant was measured at wavelengths of 665 nm (maximum absorbance of chlorophyll-*a*) and 750 nm (compensation for background turbidity) using a spectrophotometer.

The surface area of each rock chip or mussel shell was then calculated. This was done by moulding a piece of aluminium foil to the surface of each sample, thereby taking into account the irregular shape of the sample. The area of each piece of foil was then measured using a digitiser tablet and Summagraphics software. The chlorophyll-*a* concentration (in Fg cm^{-2}) of each sample was then determined using the equation

$$[\text{Chl-}a] = (A \times V \times 13.9)/a \times 1.0$$

where A = (absorbance at 665 nm - absorbance at 750 nm), V = volume of methanol, 13.9 = spectrophotometric constant , a = area (cm^2) and 1.0 = pathlength of microcuvette (1cm).

7.2.3: Data analysis:

A Kolmogorov-Smirnoff test was performed on all chlorophyll-*a* data prior to analysis in order to determine whether the data conformed to the assumptions of parametric statistics (Sokal & Rohlf, 1981). No transformation of data was necessary. A two-way analysis of variance (ANOVA) was performed to investigate whether mean monthly chlorophyll-*a* values differed between sites. Monthly and seasonal variability within sites was investigated using a one-way ANOVA. Similarly, a one-way ANOVA was used to determine seasonal differences. All statistical analyses were performed using the Statgraphics software package (version7.0).

7.3: RESULTS:

7.3.1: Chlorophyll-*a* determinations:

Estimates of microalgal biomass, as indicated by the concentrations of chlorophyll-*a*, were consistently significantly greater on aeolianite rocks than both quartzitic sandstone and mussel shells ($p < 0.0001$, Table 7.1; Figure 7.1a - c). A great deal of variability within monthly samples was found at all sites (Figure 7.1a - c). Similarly, mean monthly estimates varied considerably at all sites throughout the experimental period (Figure 7.1a - c). Significantly low (F-ratio = 12.950, $p < 0.0001$, Table 7.2) values of chlorophyll-*a* from aeolianite rock were recorded in May (2.99 Fg chl-*a* cm^{-2}; Figure 7.1a) whilst the highest were recorded in August (14.9 Fg chl-*a* cm^{-2}; Figure 7.1a). Lowest microalgal biomass on quartzitic sandstone also occurred in May (Figure 7.1b). Values of chlorophyll-*a* were significantly greater in June and October (F-ratio = 13.795, $p < 0.0001$, Table 7.2). However, monthly values were similar with the range between highest and lowest values being 4.03 Fg chl-*a* cm^{-2} (Figure 7.1b). The mussel shells also showed a lower degree of variability between monthly samples, yet values were significantly higher in April and June (5.5 Fg chl-*a* cm^{-2} and 5.12 Fg chl-*a* cm^{-2} respectively; Figure 7.1c , Table 7.2). Lowest values were recorded in March (1.2 Fg chl-*a* cm^{-2}).

Table 7.1:

Results of a two-way ANOVA on mean monthly chlorophyll-*a* concentrations from three sites with differing substratum types. (* indicates significant p - values).

Source of variation	S.S.	d.f.	M.S.	F-ratio	Sig. Level
Main effects					
Site	2.722	2	1.361	26.219	< 0.0001 *
Month	0.548	10	0.055	1.056	0.4365
Residual	1.038	20	0.052		
Total	4.309	32			

Table 7.2:

Summaries of one-way ANOVA's performed to determine whether mean chlorophyll-*a* values varied on a monthly basis at any site. (* indicates a significant p - value).

Source of Variation	S.S.	d.f.	M.S.	F-ratio	Sig. Level
Aeolianite					
Between groups	6.954	10	0.695	11.265	< 0.0001 *
Within groups	10.494	170	0.062		
Total	17.448	180			
Quartzitic sandstone					
Between groups	346.059	10	34.606	13.795	< 0.0001 *
Within groups	476.695	190	2.509		
Total	822.754	200			
Mussel shells					
Between groups	407.946	10	40.795	17.903	< 0.0001 *
Within groups	426.095	187	2.279		
Total	834.041	197			

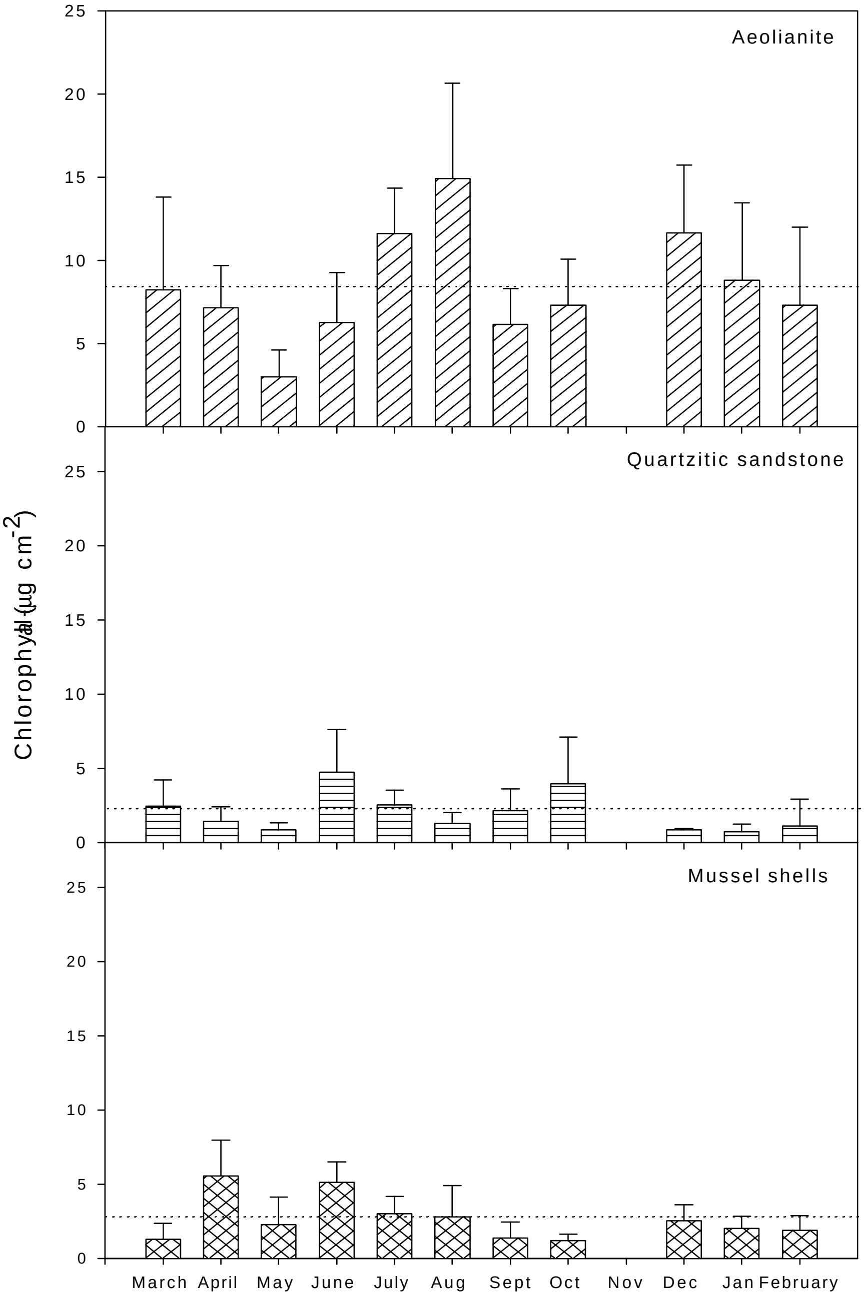


Figure 7.1:

Mean monthly chlorophyll-*a* concentrations (+ S.D.) on three substrata. n = 20. No values were obtained during November due to inclement weather. Horizontal dotted lines indicate mean values for each site.

Although greater microalgal biomass was recorded during winter on aeolianite, this difference was not significant and as such no seasonal differences in chlorophyll-*a* values occurred (Table 7.3). Likewise, no seasonality of microalgal biomass was found on either the quartzitic sandstone or mussel shells (Table 7.3). A similar trend was found when seasonal chlorophyll-*a* values from each site were pooled to provide single seasonal estimates. Although microalgal biomass was greater during winter (Figure 7.2), this value was not significantly different to estimates calculated for the other seasons (p = 0.795; Table 7.4).

Table 7.3:

Summaries of one-way ANOVA's performed on seasonal chlorophyll-*a* data from three substratum types.

Source of Variation	S.S.	d.f.	M.S.	F-ratio	Sig. Level
Aeolianite					
Between groups	44.487	3	14.829	3.340	0.086
Within groups	31.076	7	4.439		
Total	75.564	10			
Quartzitic sandstone					
Between groups	8.496	3	2.832	2.152	0.182
Within groups	9.212	7	1.316		
Total	17.708	10			
Mussel shells					
Between groups	7.852	3	2.617	1.360	0.331
Within groups	13.470	7	1.924		
Total	21.322	10			

Table 7.4:

Results of a one-way ANOVA performed on pooled seasonal chlorophyll-*a* data from three substratum types.

Source of Variation	S.S.	d.f.	M.S.	F-ratio	Sig. Level
Between groups	17.876	3	5.959	0.344	0.795
Within groups	138.637	8	17.330		
Total	156.513	11			

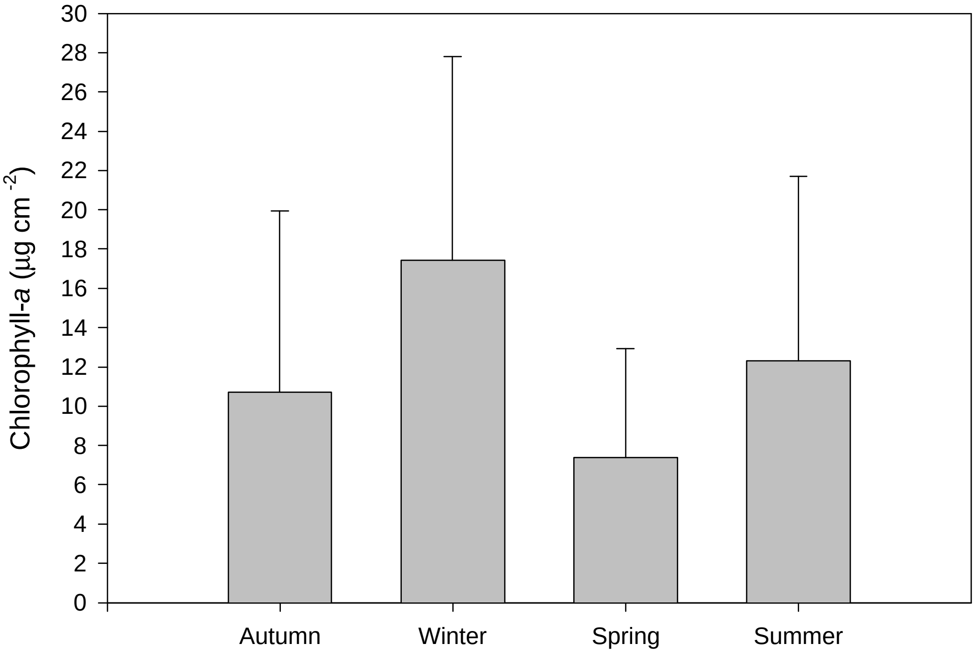


Figure 7.2:

Mean seasonal chlorophyll-*a* concentrations (+ S.D.) obtained by pooling data from three sites. (Autumn = March, April and May; Winter = June, July and August; Spring = September and October; Summer = December, January and February).

7.4: DISCUSSION:

Microalgal films of temperate shores are dominated by diatoms and patches of blue-green algae. These occur in a mucopolysacharide matrix which provides a site for the attachment for macroalgal propagules (Hill & Hawkins, 1991). Analysis of the chlorophyll-*a* levels of this microbial film allows an accurate estimate of food availability in an intertidal community (Underwood, 1984b). Bustamante *et al.* (1995b) determined that mean chlorophyll-*a* values from the south and east coasts of South Africa were 15 Fg cm^{-2} and 4 Fg cm^{-2} respectively. The current study, which was undertaken along the south-east coast, yielded similar results with mean monthly chlorophyll-*a* values ranging from 0.73 Fg cm^{-2} to 14.91 Fg cm^{-2}. However, the estimates of chlorophyll-*a* that were made on quartzitic sandstone from Cannon Rocks were lower than those made by Gray (1996) at the same site. In his study, estimated chlorophyll-*a* concentrations on quartzitic sandstone ranged between 5 Fg cm^{-2} and 35 Fg cm^{-2}. Although a great deal of variability was recorded within samples collected from Cannon Rocks in the present study, estimates were never found to increase beyond 5 Fg cm^{-2}. A number of reasons may account for this discrepancy. Microalgal distribution has been shown to vary with tidal height with microalgal abundance generally decreasing with increasing tidal height (Castenholtz, 1963; Nicotri, 1977; Underwood, 1984a,b). This trend is also evident on South African shores (Bosman & Hockey, 1988a ; Dye & White, 1991). This vertical change in abundance is usually ascribed to the increasing physical conditions which occur higher on the shore (Castenholtz, 1961, 1963). However, Gray's (1996) study was conducted in the upper Balanoid zone of this site, whereas the present study was carried out in the mid-shore (lower Balanoid zone). As such, these observations seem to be anomalous to expectations. Recent observations at Port Alfred (see Figure 2.1 for proximity to current study site) show a similar situation where chlorophyll-*a* levels of the high-shore Littorina zone are greater than the estimates of the present study (S. Kaehler, pers. comm.). It is possible that the lower values recorded in the mid-shore lower Balanoid zone are a function of increasing grazer biomass lower on the shore (see Chapter 2) which decreases the microalgal abundance (S. Kaehler, pers. comm.). Although a number of studies have shown that there is no correlation between grazer biomass and microalgal abundance (Underwood, 1984b; Dye & White, 1991), others have reported that grazer biomass profoundly influences primary productivity (Branch & Branch, 1988; Mak & Williams, 1999).

Chlorophyll-*a* extraction procedures significantly affect estimates of microbial abundance (Hill & Hawkins, 1990). It is thus possible that the extraction procedures used in these studies are responsible for the different results obtained. The solvent used during extraction is species specific (Nagarkar & Williams, 1997). Generally, the mid-shore region of South African coasts are dominated by diatoms, while the microalgal community of the higher, drier zones predominantly consist of blue-green algae (S. Kaehler, pers. comm.). However, in order to allow for comparison of data from different studies, extraction procedures are often standardised regardless of the microalgal species composition. The same procedure was followed in both the present study and that by Gray (1996). Despite the fact that the use of hot methanol is well-documented to be a more efficient extraction solvent (Nagarkar & Williams, 1997), S. Kaehler (pers. comm.) made use of acetone in his study and still recorded higher chlorophyll-*a* concentrations than the current study. Furthermore, a recently proposed protocol suggests that although hot methanol is the most efficient extraction solvent, rehydration of the samples before analysis increases the estimates of chlorophyll-*a* (Thompson *et al.*, 1999). This was not performed in the current study indicating that the absolute values obtained are indeed an underestimate of microalgal abundance and thus food availability. However, to date, no other studies of microalgal abundance along the coast of South Africa have included this step in the analysis (Dye & White, 1991; Bustamante *et al.*, 1995b; Gray, 1996). Thus the current results should be comparable to other studies.

Because a standard extraction technique was used, the results obtained in the current study allow a comparison of food availability at the three sites to be made. Chlorophyll-*a* values are consistently greater on aeolianite shores than on both quartzitic sandstone and mussel shells. Although generally, microalgal abundance is higher on mussel shells than quartzite, this difference is not significant. The increased abundance on aeolianite is likely to be affected by the qualities of the rock surface. Aeolianite is a soft rock that has numerous indentations and pits which may increase the sites available for the recruitment of microalgae. Although this site also supports a greater number of grazers (see Chapter 2), it is probable that the uneven nature of the rock offers microalgae some protection from grazing which in turn results in an increase in biomass. Furthermore, an increase in physical conditions e.g. higher temperatures and dry conditions, decrease chlorophyll-*a* levels (Mak & Williams, 1999). The greater number of pits and indentations on the aeolianite may increase moisture retention at this site (D. Abbey & A.H. Dye, pers. comm.), resulting in increased chlorophyll-*a* levels compared to the other two sites. However, given this argument, higher chlorophyll-*a* levels than were recorded would have been expected from the mussel shells due to the reduced exposure to air. The increased biomass of filter feeders (i.e. mussels) in this zone may reduce the microparticle concentrations in the water thereby reducing the number of microorganisms available to settle on the substrata. It has also been shown that when physical factors reduce the rate of algal production, grazers are able to remove all accessible algae (Underwood & Jernakoff, 1984). It is suggested that the increased physical conditions of the quartzitic rock result in the reduction of algal production at this site which ultimately leads to an increased level of removal of microalgae by grazers.

The abundance of microalgae often varies seasonally, with a greater abundance being recorded in winter when cooler conditions enhance growth (Bustamante *et al.*, 1995b). Although not significant, chlorophyll-*a* values on aeolianite was estimated to peak during July and August (Figure 7.1). A similar trend was, however, not observed at either of the other two sites studied. Similarly, no seasonal differences were observed by Bustamante *et al.* (1995b) until values for sites from specific biogeographical regions were pooled. However, even pooling data from the separate sites of the current study failed to show any significant seasonality in chlorophyll-*a* concentrations (see Figure 7.2) although the estimated biomass was higher in winter. In this regard, the current study shows similar trends in primary productivity as were observed for the east coast (Bustamante *et al.*, 1995b). It was suggested that the lack of seasonality evident on the east coast was associated with a lack of upwelling in this area (Bustamante *et al.*, 1995b). However, some localised upwelling does occur along the south coast of South Africa where the present study was conducted (see Schumann *et al.*, 1982; de Villiers, 1998). This may be responsible for slightly higher values recorded during part of the year which in turn swamps any seasonal differences. However, this explanation is unlikely, as no uniform increases in chlorophyll-

a were evident at the same time for all sites. Furthermore, intertidal primary productivity is known to be patchy (Hill & Hawkins, 1989). Although this was not included in the experimental design of the current study, spatial differences in microalgal abundance were evident by the great monthly variability in chlorophyll-*a* concentrations recorded (see Figure 7.1). Microalgal patchiness on the quartzitic rock was also observed by Gray (1996). It is probable that the increased chlorophyll-*a* values recorded during the winter months are indeed a result of cooler conditions which allow increased growth (Bustamante *et al.*, 1995b). Additional factors such as increased foraging activity by intertidal grazers as a result of the cooler conditions, may also be responsible for the lower than expected microalgal biomass during this time. However, further experiments are required to satisfactorily answer this question.

In conclusion, although the food availability (as determined by chlorophyll-*a* concentrations) is clearly greater on aeolianite shore than on either quartzitic sandstone or mussel shells, the results of this study have given rise to a number of questions which may form the basis for future studies. Although it is generally accepted that a gradient of primary productivity exists around the coast of South Africa, this study shows that localised environmental conditions, such as substratum type, greatly influence microalgal biomass. As primary productivity is closely linked to grazer biomass and size distribution (Bosman & Hockey, 1988a,b), future studies into the relationship between microalgal abundance and environmental conditions are needed in order to further the understanding of the complex interactions of intertidal communities. This has implications for the management of intertidal resources. Furthermore, recent studies show that the procedures previously utilised in order to quantitatively estimate microalgal biomass along the coast of South Africa have more than likely underestimated the primary productivity of these shores (Thompson *et al.*, 1999). A large scale investigation, utilising new protocols (as suggested by Thompson *et al.*, 1999) would probably result in more accurate estimations of the primary productivity gradient around the South African coast. Finally, it is suggested that investigations into the feeding of intertidal limpet species such as *P. granularis* would provide important information on the utilisation of the food resources available in intertidal rocky shores.

Chapter 8

GENERAL DISCUSSION

Patella granularis is one of the most successful of the South African patellid limpets. This success is reflected in its broad geographical distribution which ranges from Namibia to southern Natal (Branch *et al.*, 1994; T. Ridgway *et al.*, 1998). The wide geographical distribution means that different populations of *P. granularis* are exposed to different oceanographic conditions (Figure 2.1), with the most notable environmental difference occurring between the cool temperate west coast and the warm temperate south and east coasts (Branch & Branch, 1988). The success of *P. granularis* is further reflected in its wide intertidal distribution. On all types of rocky shores it can be found from the upper Balanoid zone to the low-shore Cochlear zone (Branch, 1971; present study). Finally, this species is very abundant (90 limpets/m^2 to 116 limpets/m^2; Bosman & Hockey, 1988a,b; this study) throughout its geographic and intertidal range. The success of *P. granularis* must not only be due to its physiological tolerances (Branch & Newell, 1978; Marshall & McQuaid, 1992), but also to an ability to adjust aspects of its life history to different environmental conditions. Some of these adaptations will be further elaborated upon during the course of this general discussion.

While many aspects of the biology and ecology of west and south-east coast *P. granularis* are similar (Table 8.1), differences in key life-history characteristics such as growth, longevity and reproductive seasonality exist. As there are no genetic differences between west and south-east coast populations of *P. granularis* (T. Ridgway *et al.*, 1998), it is suggested that these life-history differences are phenotypic adaptations to environmental conditions. The results of the current investigation, together with those of previous studies (Branch, 1971; Branch, 1974a, b; Bosman & Hockey, 1988a, b), allow some speculation on how populations of *P. granularis* respond to the different environmental conditions of the west and south-east coast. This is presented as a hypothetical model (Figure 8.1) which is further discussed below.

The west coast of South Africa has a higher primary productivity than the south-east coast (Bustamante *et al.*, 1995b). It is thus hypothesised that the increased primary productivity on the west coast results in an increase in the energy available for both growth and reproduction. This enables west coast *P. granularis* to capitalise on this rich food supply. This in turn results in a rapid growth rate and

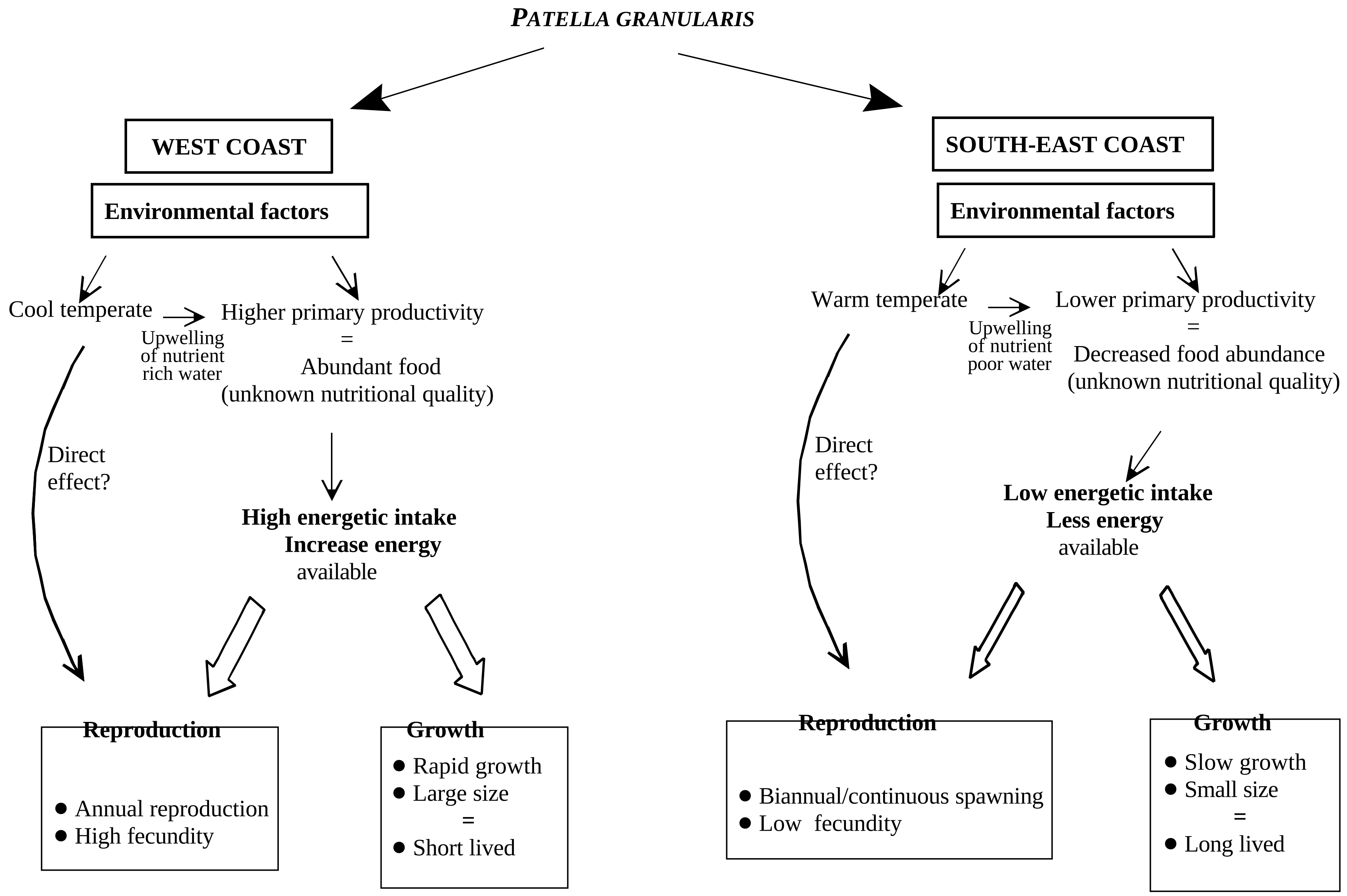
Table 8.1:

Comparison of aspects of the biology of *P. granularis* from the west coast and the south-east coast of South Africa. (Data from Branch, 1974a,b; Bosman & Hockey, 1988a,b; this study).

	South-east coast	**West coast**
Position on the shore	Upper Balanoid to the Cochlear zone, but most numerous in the lower Balanoid zone	
Habitat	Occurs on shores of differing geomorphologies.	
Habits	Found predominantly on the side of rocks. Often found co-occurring with barnacles. Generalised grazer, feeding on microalgae.	
Activity	Shows variability in the timing of foraging; but is active primarily during nocturnal low tides. Also shows variability in distances foraged and rigidity of homing.	
Density and biomass	Mean: . 116 /m^2; . 12 g dry weight /m^2 Max: . 177 /m^2; . 25 g dry weight /m^2	Mean: . 90.3/m^2; . 38 g dry weight/m^2 Max: . 160 /m^2; . 70 g dry weight/m^2
Mean length and (Maximum length)	Mainland: 17.6 mm (38.5 mm) Island: 26.6 mm (46 mm)	Mainland: 48.8 mm (58.2 mm) Island: . 55 mm (97 mm)
Growth form	Generally grows allometrically, increasing in shell length faster than shell height	
Growth rate	Slow growing . 8 mm a year K = 0.25 - 0.32	Rapid growth . 18 mm a year K = 0.6 - 0.8
Longevity	. 15 years	. 6 - 8 years
Sex characteristics	Dioecious	
Fecundity	. 190 000 eggs/limpet	. > 1 million eggs/limpet
Onset of sexual maturity	1-2 years	
Sex ratio	Variable, but generally more males than females	Generally more males than females
Seasonality of Spawning	Twice a year (once in summer and once in winter) or continuous trickle spawning	Once a year (June to September). Resting phase from September to December.

Figure 8.1:

A hypothetical model of the factors influencing the apportionment of energy to the life-history characteristics such as growth and reproduction of *P. granularis* from the west and south-east coasts of South Africa. The thickness of the open arrows (⇨) indicates the relative amount of energy that may be channelled in to growth and reproduction.


PATELLA GRANULARIS
WEST COAST
Environmental factors
Cool temperate
Upwelling of nutrient rich water
Higher primary productivity
=
Abundant food
(unknown nutritional quality)
Direct effect?
High energetic intake
Increase energy available
Reproduction
Annual reproduction
High fecundity
Growth
Rapid growth
Large size
=
Short lived
SOUTH-EAST COAST
Environmental factors
Warm temperate
Upwelling of nutrient poor water
Lower primary productivity
=
Decreased food abundance
(unknown nutritional quality)
Direct effect?
Low energetic intake
Less energy available
Reproduction
Biannual/continuous spawning
Low fecundity
Growth
Slow growth
Small size
=
Long lived

a large maximum size which is attained at a relatively young age (see Table 8.1). The consequence of this is short longevity. In contrast, the decreased food availability on the south-east coast means that there is less energy available for growth and reproduction. These limpets therefore have a slower growth rate, reach a smaller maximum size and consequently live approximately twice as long as their west coast counterparts (Figure 8.1). This fits the general negative relationship between growth rate and longevity in limpets (reviewed by Branch, 1981; discussed in more detail in Chapter 5).

It is possible that increased food supply is not the only factor responsible for the differences observed. In addition to readily available food, the nutritional quality of that food must also be important. The assimilation efficiencies of marine invertebrates are known to be influenced considerably by the quality of the food consumed (Paine & Vadas, 1969; Lawrence, 1975; Hawkins, 1981a; Hawkins & Hartnoll, 1983; Semura, 1995). While much research has been undertaken to determine the nutritional quality of food resources exploited by macrophageous intertidal invertebrates (see Foster, 1997 for a review of literature), very little is known about the nutritional quality of the microalgal resources utilised by limpets. At present, it is not known whether the abundant food supply of the west coast is also of an improved nutritional quality, and what effect this may have on life-history parameters.

The difference in reproductive seasonality between west (single winter spawning period) and south-east (biannual or continuous spawning) coast limpets is probably a result of differing climatic conditions. The differences in water temperatures between winter and summer is more marked along the west coast than the south-east coast (Branch, 1973), therefore the west coast limpets have a defined annual reproductive cycle (Branch, 1974b). Whether the effect of water temperature on reproductive seasonality is direct or indirect (by affecting food quantity and quality) is not currently known (Figure 8.1). This restriction in the timing of spawning of west coast limpets is suggested to result in a high fecundity, which in turn has led to the proposal that west coast *P. granularis* are *r*-strategists (Branch, 1975a).

Although growth rate, maximum size, longevity and reproductive seasonality are all affected by exogenous factors, the current study suggests that the onset of sexual maturity is controlled endogenously. Populations of *P. granularis* from both the west coast and the south-east coasts become sexually mature at approximately 1-2 years of age (Branch, 1974b; current study), implying that this is genetically programmed.

On both the west and south-east coasts, the mean shell length of *P. granularis* increases up the shore (Branch, 1971; Chapter 2, current study). This observation led Branch (1975b) to suggest that *P. granularis* has a migratory “life-strategy”. Limpets which fall into this classification tend to settle low on the shore and migrate to higher areas as they age and become more tolerant of the increased physical stress which characterises higher zones. Other migratory *Patella* species include *P. granatina*, *P. concolor* and *P. oculus*. This upward migration was suggested to reduce competition between adults and juveniles of the same species and generally occurs in species that reach high densities and have a wide intertidal distribution (Branch, 1975b). Non-migratory species include *P. cochlear, P. longicosta, P. tabularis, P. miniata, P. compressa* and *P. argenvillei* (Branch, 1975b). These limpets settle and remain in a narrow zone on the shore where food availability is generally higher (Branch, 1975b, Bustamante *et al.*, 1995b). As movement of these limpets from the area of settlement is restricted, most non-migratory species have developed specialised diets or inhabit specialised niches (e.g. *P. compressa*) in order to reduce competition. Many of these species are territorial and maintain their food source by gardening (Branch, 1975b). In 1996, Gray suggested that an additional group of limpets is present. He classed these as “specialised non-migratory” species which neither migrate, nor garden or exhibit territoriality. These limpets exploit a particular niche in the intertidal environment and thereby reduce interspecific competition. *H. pectunculus, P. compressa* and *H. pruinosus* are examples of specialised non-migratory limpets (Gray, 1996; Henninger, 1998) .

While *P. granularis* inhabiting the west coast may possess a migratory lifestyle, the current study offers no evidence that south-east coast *P. granularis* migrate up the shore. On the contrary, *P. granularis* which inhabit mussel shells in the Cochlear zone do not migrate from this habitat and their smaller size is a result of a slow growth rate. This in turn, has been suggested to result from the spatial constraints of a mussel bed. Furthermore, limpets that were labelled in both the lower Balanoid zone and the mussel beds did not move from their respective zones in 24 months of study. Finally, in the current study, limpets inhabiting the mussel shells were sexually mature and thus not juveniles as suggested by Branch (1975b). Thus the populations of *P. granularis* investigated in the present study are not migratory. During the current study, *P. granularis* were observed to be generalist non-territorial grazers. Furthermore, these limpets did not occupy a particular niche that was not exploited by other limpet species. It thus appears that on the south-east coast, this species can not be classed as either a non-migratory (and territorial) (Branch, 1975b) or a specialised non-migratory (Gray, 1996) limpet. Instead it is proposed that *P. granularis* is an opportunistic limpet species which can exploit a variety of habitats within its physiological tolerances, with some of its life-history characteristics responding to the prevailing environmental conditions.

The results from the current study have shown that *P. granularis* not only successfully inhabits different regions of the intertidal, but is also abundant on different types of hard substrata. It is proposed that different substrata have subtly different environmental conditions which in turn affect the energetics and life-history parameters of *P. granularis* (and possibly other intertidal species). A hypothesis, in the form of a model, is proposed whereby the differences in growth and reproduction of populations of *P. granularis* inhabiting three substrata (aeolianite, quartzite and mussel shells) are explained (Table 8.2). Although the results of the current study were limited to one site of each substratum type only, for the purposes of the model, it is assumed that the findings of these single-site investigations are representative of the respective substrata on the south-east coast of South Africa.

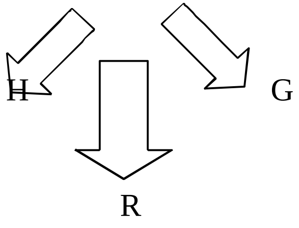
One of the main factors which must influence the energy budgets of *P. granularis* inhabiting aeolianite and quartzitic sandstone shores is food abundance. Microalgal biomass (and presumably primary productivity) is higher on aeolianite than quartzite. It is thus hypothesised that the population from the aeolianite has a higher energy intake than those limpets inhabiting quartzite (Table 8.2). Limpets inhabiting aeolianite most probably do not expend as much energy during foraging (they move shorter distances at a slower rate) in order to obtain a similar amount of food as the quartzitic population. The uneven texture of the aeolianite, and its porous nature, enable it to retain moisture for longer than the quartzitic sandstone (D. Abbey & A.H. Dye, pers. comm.). This must reduce the threat of desiccation for *P. granularis* from this site. In contrast, the hard, smooth and dry nature of quartzite

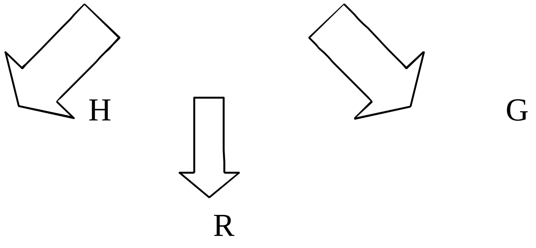
Table 8.2.

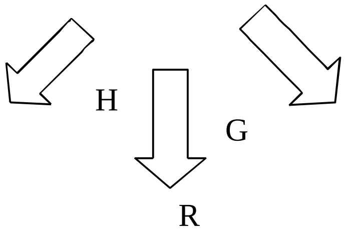
Table of hypothetical factors influencing the apportionment of energy for growth and reproduction in populations of *P. granularis* from three different substrata (aeolianite, quartzite and mussel shells) from the south-east coast of South Africa. The thickness of the open arrows (⇨) indicate the relative amounts of energy being channelled into growth (G), reproduction (R) and homeostasis (H).

Factors influencing energy budget	**AEOLIANITE**	**QUARTZITIC SANDSTONE**	**MUSSEL SHELLS**
Food Availability (quality unknown)	High: more energy consumed	Lower: less energy consumed	Lower: less energy consumed
Respiration during foraging	Move shorter distances at slower speeds - lower respiratory rate	Move further, at higher speeds, to obtain food - higher respiratory rate	Move shorter distances (speculative) - lower respiratory rate
Substratum	Rough: expend energy in adhesion during high tide	Relatively smooth: expend less energy during high tide	Relatively smooth: but expend more energy during high tide due to high wave activity
Desiccation stress	Lower: rocks retain moisture for longer	Higher: rocks do not retain moisture for long	Lower: low shore, thus reduced exposure time
Mucous production during foraging	Relatively low because do not move far	Relatively high because move further	Relatively low because do not move far
Spatial constraints	None	None	Not much space in mussel beds.

Relative apportionment of energy within a site







may result in increased desiccation pressure for these limpets. Such increased physiological pressure may be energetically expensive. Furthermore, as large limpets lose water at a slower rate than smaller limpets (Branch, 1981), it is suggested that *P. granularis* inhabiting the quartzite need to channel most energy into growth, rather than reproduction, in order to reduce water loss. As *P. granularis* inhabiting the quartzitic sandstone move greater distances while foraging, they must produce more mucus than their aeolianite counterparts. Mucous production is energetically costly (Davies & Hawkins, 1998; Nui *et al.*, 1998). Substratum type also influences the tenacity of limpets, however the extent to which this affects the limpets depends on the tackiness of the mucus (Grenon & Walker, 19 81). It is possible that due to the uneven nature of the rock surface, *P. granularis* from the aeolianite expend more energy whilst attempting to remain on the rock during high tide, than the limpets inhabiting the smoother quartzitic sandstone. Given these suggested metabolic energy costs, and the similar growth rates of *P. granularis* on both the aeolianite and quartzitic shores, it is suggested that the amount of energy remaining for reproduction is less on quartzite (Table 8.2).

The population of *P. granularis* inhabiting the mussel shells has some additional factors influencing its energetics. While food availability, and hence the amount of energy consumed, is low in this area, limpets inhabiting the mussel shells are exposed to decreased desiccation pressure. Furthermore, it is hypothesised that energy expenditure during foraging is reduced as a result of both the reduced exposure time, and reduced mucous production. Energy expenditure during high tide may however be costly due to the increased wave action which results in an increase in the effort needed to remain attached to the substratum. Despite this, the proposed energy budget of *P. granularis* inhabiting the mussel shells suggests that a relatively high proportion of energy should be available for growth and reproduction. However, *P. granularis* inhabiting the mussel shells must be constrained spatially within the mussel beds. A decrease in space has often been reported to adversely affect the growth of intertidal organisms (Sutherland, 1970; Creese, 1980; Creese & Underwood, 1982; Lasiak & White, 1993). It appears that this factor overrides the advantages of increased moisture and smooth substratum. Limpets from the mussel shells have a lower growth rate than those from either the aeolianite or the quartzitic sandstone shores. It might be predicted that, due to the reduced amount of energy utilised for growth, there would be an increased amount of energy available for reproduction. The fecundity of these limpets, however, was the lowest recorded in the present study. It is possible that this is a result of the reduced body size, which in turn reduces the space available for egg storage.

It is thus evident that in addition to large-scale environmental factors, more localised differences also influence some of the life-history characteristics of *P. granularis.* Food availability is particularly important on a broad geographical scale and it is suggested that this also operates at a more localised scale. The models proposed contain a number of hypotheses that now require testing. Firstly, the conclusions of the current study are limited as a result of the single-site studies. It is therefore important that the experiments on which the models are based are replicated in order to determine whether the results are indeed representative of the substrata investigated. Secondly, it is possible that *P. granularis* is more selective in its food choice than is currently believed. If so, the algal suite present on the respective substrata may play a more important role in influencing life-history characteristics than food abundance alone. Temporal variability in the nutritional value of the microalgae exploited by *P. granularis* would also affect aspects of it biology. Thirdly, the energy intake and assimilation efficiency of *P. granularis* from different habitats needs to be investigated in order to determine whether the energy available for growth and reproduction are adequately represented by the models. As the energetic costs of these life-history parameters have been well studied (Newell, 1979; see review by Branch, 1981), this knowledge would allow the reproductive and growth costs of *P. granularis* to be accurately determined. Finally, the energetic cost of foraging on different substrata is not known. The current observations provided baseline information on the effect of substratum on the activity of intertidal invertebrates. However, extensive experimental manipulations of populations inhabiting shores of similar substrata are needed to verify the suggestions of the models.

Some of the results of the current study have implications for the management of intertidal organisms. The intertidal resources of the south-east coast of South Africa are increasingly exploited (Siegfried *et al.*, 1994). Although shellfish gatherers have traditionally been selective in terms of both species and size classes collected (Bigalke, 1973; Siegfried *et al.*, 1985; Hockey and Bosman, 1986), increasing pressure on the resources has resulted in an increase in indiscriminate collection of intertidal species (Griffiths & Branch, 1997; pers. obs.). Shellfish collecting has a number of direct as well as indirect effects. Size-specific removal of animals results in the reduction of abundance and mean size of individuals in a population (Branch, 1975b; Hockey & Bosman, 1986; Keough *et al.*, 1993). Although along the south-east coast *P. granularis* is not a target species for collection due to its small size, it is indirectly affected by the collection of other species. Mussels are one of the preferred shellfish harvested along the South African coast (Lasiak & Dye, 1989), providing an estimated 16% of the annual protein intake of the coastal inhabitants of the Transkei (Siegfried *et al.*, 1985). Due to the fact that mussel beds are home to *P. granularis*, removal of the mussels, and resultant disturbance of the mussel beds, is likely to have a significant impact on this species. Removal of grazers often results in an increase in its food source (Moreno *et al.*, 1984; Dye, 1993), or an increase in the abundance of other species (Moreno *et al.*, 1984). Along the south-east coast, the extensive removal of mussels has resulted in an increase in coralline algae (Dye, 1992). This in turn reduces the space available for the recruitment of future mussel populations as most settlement occurs onto existing adult mussels (Dye, 1992). The increased algal growth thus has an effect on the subsequent recruitment of *P. granularis* into low-shore areas. Additional indirect effects include those of trampling, which has been documented to adversely influence intertidal populations (Beauchamp & Gowing, 1982; Povey & Keough, 1991).

The slow growth of *P. granularis* from the south-east coast has further implications for the recovery of these populations from either direct or indirect exploitation. Limpets from this area are predicted to require in excess of 15 years to reach maximum size (Chapter 4). Thus populations of *P. granularis* are likely to take several years to recover from any adverse effects on their community structure. One advantage that this species does have over this however, is the fact that the onset in sexual maturity occurs at a young age (one - two years). Thus reproduction may still occur in populations with a significantly reduced size structure. The efficiency of recruitment of this species may however be adversely affected by the reduced size structure of intertidal populations and the irregular nature of the south-east coastline. Both the size of the adult population and the topography of the coast influences the recruitment of barnacles with rocky headlands limiting larval dispersal (Dye, 1992; Gaines & Bertness, 1992; Dye, 1993). In addition, recruitment is both variable and patchy (McQuaid, 1992; Harris *et al.*, 1998). The current study therefore emphasises the disadvantages of implementing management strategies that are based on information collected from geographically restricted studies. In the case of *P. granularis*, the great differences in both the ecology and biology of west and south-east coast populations necessitates different management strategies for the respective coasts. Similarly, even within a region, data obtained from single-site studies are probably inadequate for the formation of responsible management plans.

Finally, in addition to the necessary testing of the proposed models, the results of the current work have raised a number of questions regarding the biology and ecology of *P. granularis* and provided a number of avenues for future research. These are mentioned briefly below.

1). Detailed examination of the larval life-history of *P. granularis* including dispersal, recruitment and settlement of this species.

2). Long-term monitoring of populations of *P. granularis* from the south-east coasts of South Africa are needed to increase the understanding of intertidal communities.

3). Laboratory manipulations of limpets may provide a more accurate assessment of the influence of particular environmental factors such as temperature, food availability and spatial constraints on life-history parameters.

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APPENDICES

Table 1:

Summaries of MANOVA's performed on the biomass of *P. granularis* inhabiting three zones of three transects at six localities along the south-east coast.

Source of variation	S.S.	d.f.	M.S.	F-ratio	Sig. level
Port Elizabeth					
Main effects					
Transect	0.295	2	0.147	15.597	< 0.0001
Zone	0.225	2	0.112	11.885	< 0.0001
Interactions					
AB	0.200	4	0.050	5.273	0.0004
Residual	3.282	347	0.010		
Total	4.061	355			
Cannon Rocks					
Main effects					
Transect	0.036	2	0.018	0.750	0.4732
Zone	0.780	2	0.390	16.232	< 0.0001
Interactions					
AB	0.267	4	0.067	2.776	0.0267
Residual	10.26	427	0.024		
Total	11.34	435			
Kwaaihoek					
Main effects					
Transect	0.012	2	0.006	0.339	0.7126
Zone	2.760	2	1.380	75.408	< 0.0001
Residual	3.404	186	0.018		
Total	6.191	194			
No Significant interactions					

Table 1 cont.:

Summaries of MANOVA's performed on the biomass of *P. granularis* inhabiting three zones of three transects at six localities along the south-east coast.

Kenton-on-Sea					
Main effects					
Transect	0.36	2	0.18	11.08	<
Zone	0.13	2	0.06	4.093	<
Interactions					
AB	1.13	4	0.28	17.06	<
Residual	6.40	386	0.01		
Total	7.88	394			
Port Alfred					
Main effects					
Transect	0.53	2	0.26	31.29	<
Zone	0.37	2	0.19	22.30	<
Interactions					
AB	0.68	4	0.17	20.26	<
Residual	7.53	886	0.00		
Total	9.37	894			
Three Sisters					
Main effects					
Transect	0.13	2	0.06	6.792	0.0012
Zone	0.21	2	0.10	10.94	<
Interactions					
AB	0.36	4	0.09	9.269	<
Residual	8.26	838	0.01		
Total	9.09	846			

Table 2:

Summaries of MANOVA's performed on the density of *P. granularis* inhabiting three zones of three transects at each of seven localities along the south-east coast of South Africa.

Source of variation	S.S.	d.f.	M.S.	F-ratio	Sig. level
Bird Island					
Main effects					
Transect	147.632	2	73.811	0.327	0.725
Zone	1003.91	2	501.960	2.225	1.370
Residual	4061.25	18	225.626		
Total	5581.18	26			
	No significant interactions				
Port Elizabeth					
Main effects					
Transect	16.204	2	8.1021	0.017	0.986
Zone	312.936	2	306.468	0.625	0.546
Residual	8824.45	18	490.248		
Total	9619.35	26			
	No significant interactions				
Cannon Rocks					
Main effects					
Transect	90.238	2	45.119	0.048	0.953
Zone	4826.62	2	2413.31	2.579	0.104
Residual	16842.5	18	985.695		
Total	23167.2	26			
	No significant interactions				

Table 2 cont.:

Summaries of MANOVA's performed on the density of *P. granularis* inhabiting three zones of three transects at each of seven localities along the south-east coast of South Africa.

Kwaaihoek					
Main effects					
Transect	973671	2	48.836	0.03	0.962
Zone	908.177	2	454.238	0.36	0.701
Residual	16842.51	18	985.695		
Total	23167.21	26			
	No significant interactions				
Kenton-on-					
Main effects					
Transect	226.913	2	113.456	0.12	0.883
Zone	3047.361	2	1525.68	1.06	0.213
Residual	16237.94	18	45.900		
Total	19696.09	26			
	No significant interactions				
Port Alfred					
Main effects					
Transect	11505.29	2	5752.64	1.87	0.182
Zone	8099.284	2	4049.64	1.32	0.291
Residual	5516.657	18	3062.03		
Total	76977.33	26			
	No significant interactions				
Three Sisters					
Main effects					
Transect	956.792	2	478.396	0.12	0.881
Zone	20051.75	2	10025.8	2.67	0.096
Residual	67401.86	18	3744.54		
Total	91495.15	26			
	No significant interactions				

Table 1:

Numbers of labelled *P. granularis* recovered from three substrata (mussel shells, aeolianite and quartzitic sandstone) during two experimental periods.

Month	**May 1996 - May 1997**			**Month**	**February 1997 - February 1998**		
	Mussel shells	**Aeolianite**	**Quartzite**		**Mussel shells**	**Aeolianite**	**Quartzite**
May	181	199	165	Feb.	192	200	128
June	112	131	107	March	148	121	177
July	87	151	110	April	120	125	163
Aug.	43	139	98	May	109	137	155
Sept.	28	114	74	June	110	76	133
Oct.	20	97	63	July	89	107	127
Nov.	9	72	56	Aug.	73	81	137
Dec.				Sept.	65	101	126
Jan.	0	70	17	Oct.	39	96	94
Feb.	0	50	14	Nov.	40	93	114
March	0	47	6	Dec.	31	84	95
April	0	45	0	Jan.	21	94	83
May	0	40	0	Feb.	24	90	71

Table 2:

Summary of one-way ANOVA's performed to see whether the initial shell length of resident *P. granularis* differed among three substrata in two experimental periods. (* indicates a significant p - value).

	Source of variation	**S.S.**	**d.f.**	**M.S.**	**F - ratio**	**Sig - level**
March 1998 - Sept. 1998	**Between groups**	5.621	2	2.811	3.410	0.039*
	Within groups	49.975	57	0.824		
	Total	52.597				
Dec. 1998 - June 1999	**Between groups**	11.265	2	5.632	4.484	0.014*
	Within groups	116.81	93	1.256		
	Total	128.078	95			

Table 1:

Summaries of chi² tests performed to determine whether the number of *P. granularis* active in each replicate were similar.

		Aeolianite		**Quartzitic Sandstone**	
		Resident (undisturbed)	**Translocated (from quartzite)**	**Resident (undisturbed)**	**Translocated (from aeolianite)**
		Chi²; sig-level	**Chi²; sig-level**	**Chi²; sig-level**	**Chi²; sig-level**
Spring	**SNM (21/08/1998)**	0.08; 0.96	0.72; 0.69	0.22; 0.89	0.17; 0.92
	SNM (22/08/1998)	0.50; 0.78	0.18; 0.91	0.33; 0.85	0.61; 0.74
	NQM	0.14; 0.93	0.58; 0.75	2.33; 0.31	2.83; 0.24
Autumn	**SNM (16/04/1999)**	0.90; 0.64	0.33; 0.85	0.80; 0.67	0.17; 0.92
	SNM (17/04/1999)	4.63; 0.10	0.67; 0.72	0.90; 0.64	0.12; 0.54
	NQM	0.90; 0.64	0.73; 0.69	4.10; 0.14	0.30; 0.86

Table 2:

Two-way Analysis of variance on the number of *P. granularis* active between sites during spring and autumn

Source of variation	**S.S.**	**d.f.**	**M.S.**	**F-ratio**	**Sig-level**
Main Effect					
Site	21.333	1	21.333	0.712	0.429
Season	3.000	1	3.000	0.100	0.762
Residuals		9	29.96		
All interactions are non-significant					

Table 3:

Summaries of chi^2 tests performed to see whether the number of *P. granularis* homing successfully varied among replicates for each observation period.

		Aeolianite		**Quartzitic Sandstone**	
		Resident (undisturbed)	**Translocated (from quartzite)**	**Resident (undisturbed)**	**Translocated (from aeolianite)**
		Chi^2; sig-level	**Chi^2; sig-level**	**Chi^2; sig-level**	**Chi^2; sig-level**
Spring	**SNM (21/08/1998)**	1.71; 0.42	0.50; 0.78	2.88; 0.24	0.30; 0.86
	SNM (22/08/1998)	2.88; 0.24	0.37; 0.21	2.77; 0.32	2.70; 0.26
	NQM (28/08/1998)	0.73; 0.69	1.1; 0.58	1.66; 0.44	1.10; 0.58
Autumn	**SNM (16/04/1999)**	1.04; 0.59	1.90; 0.39	2.66; 0.32	1.90; 0.39
	SNM (17/04/1999)	0.42; 0.81	2.70; 0.26	4.73; 0.09	0.30; 0.86
	NQM (25/04/1999)	0.5; 0.78	5.10; 0.78	0.42; 0.81	0.5; 0.78

Table 4:

Summary of one-way ANOVA’s performed to determine whether the mean distance moved by resident *P. granularis* differed among replicates during any observation period at either site. (* indicates a significant p - value).

Source of variation	**S.S.**	**d.f.**	**M.S.**	**F - ratio**	**Sig-level**
Aeolianite					
Between groups	9557.521	17	562.207	4.814	< 0.0001 *
Within groups	17116.761	147	116.781		
Total	26724.282	164			
Quartzitic sandstone					
Between groups	7002.364	17	411.904	1.231	0.2474
Within groups	48505.822	145	334.523		
Total	55508.186	162			

Although a significant difference was recorded in limpets inhabiting the aeolianite shore, a Scheffe’s multiple range test (Table 6) confirmed that these differences did not occur among replicates observed during a single night, but rather in replicates observed on different nights.

Table 5:

Summary of one-way ANOVA's performed to determine whether the mean distance moved by translocated *P. granularis* differed among replicates during any observation period at either site. (* indicates a significant p - value).

Source of variation	S.S.	d.f.	M.S.	F - ratio	Sig-level
Aeolianite					
Between groups	12409.217	17	827.281	4.542	< 0.0001 *
Within groups	11658.145	64	182.156		
Total	24067.362	81			
Quartzitic sandstone					
Between groups	14997.668	17	882.215	2.636	0.0024 *
Within groups	23092.026	69	334.667		
Total	38089.694	86			

Once again, although a significant difference was recorded in translocated limpets from both shore, Scheffe's multiple range tests (Table 6) confirmed that these differences did not occur among replicates observed during a single night, but rather in replicates observed on different nights.

Table 6:

Results of a Scheffe's test performed to determine whether resident limpets in different quadrats travveled significantly different distances from each other on either aeolianite or quartzite. (X's in the same column indicate no significant differences).

	Resident		Translocated	
Observation periods	Aeolianite	Quartzite	Aeolianite	Quartzite
21/08/98	X X	X X	X	X X
	X X	X X	X X	X X
	X X	X X	X X	X X
22/08/98	X X	X X	X X	X X
	X X	X X	X X	X X
	X X	X X	X X	X X
28/08/98	X X	X X	X X	X X
	X X	X X	X X	X X
	X	X	X X	X X
16/04/99	X X	X X	X X	X X
	X X	X X	X X	X X
	X	X X	X X	X X
17/04/99	X X	X X	X X	X
	X X	X	X X	X
	X X	X X	X X	X X
25/4/99	X X	X X	X	X X
	X X	X X	X X	X X
	X X	X X	X X	X X

Table 7:

Summary of one-way ANOVA's performed to determine whether the speeds travelled by *P. granularis* differed among replicates during any observation period at either site. (* indicates a significant p - value).

Source of variation	S.S.	d.f.	M.S.	F - ratio	Sig-level
Aeolianite					
Between groups	710.155	17	41.774	2.969	< 0.0001 *
Within groups	8325.594	592	14.069		
Total	9038.748	609			
Quartzitic sandstone					
Between groups	1868.287	17	109.899	1.791	0.0258 *
Within groups	36808.731	600	61.348		
Total	38677.018	617			

Although significant differences were recorded in the mean speeds travelled by *P. granularis* inhabiting both shores, Scheffe's multiple range tests indicate that this difference did not occur among replicates observed during single observation periods but rather in replicates observed during different observation periods (Table 8).

Table 8:

Results of Scheffe's test to determine whether or not the speed of resident *P. granularis* differed significantly among replicates during each observation period. (X's in the same column indicate no significant differences).

	Resident	
Observation periods	Aeolianite	Quartzite
21/08/98	X X	X X
	X X	X X
	X X	X X
22/08/98	X X	X X
	X X	X X
	X X	X X
28/08/98	X X	X X
	X X	X X
	X X	X X
16/04/99	X X	X
	X X	X X
	X	X X
17/04/99	X X	X X
	X	X X
	X X	X X
25/4/99	X X	X
	X X	X X
	X X	X X