

Aus dem Institut für Polarökologie
der Christian-Albrechts-Universität zu Kiel

**ECOLOGICAL STUDIES ON THE TROPICAL LIMPET *Cellana testudinaria* (Linnaeus, 1758):
INFLUENCE OF ENVIRONMENTAL FACTORS ON THE ROCKY SHORE BENTHOS
OF THE BIG KAI ISLAND, SOUTHEAST MOLLUCCAS, INDONESIA**

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Vorgelegt von

Abraham Seumel Khouw

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Referent: **Priv. –Doz. Dr. D. Piepenburg**

Korreferent: **Prof. Dr. M. Spindler**

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Dedication

This thesis is dedicated to :

*my beloved wife Costavina Litamahuputty and
our son Winfield Bierhoff Khouw*

*Da ist das Meer – so unendlich groß und weit,
unzählbar sind die Tiere darin, große wie kleine
Psalm 104, 25*

*Es lobe IHN Himmel und Erde,
die Meere mit allem, was sich darin regt.
Psalm 69, 35*

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SUMMARY / ZUSAMMENFASSUNG**SUMMARY**

The present study addresses the effects of **environmental factors** (seawater temperature, salinity, humidity, precipitation, insolation, air temperature, wave action, and tidal cycle) on the **ecology** of the patellid limpet *Cellana testudinaria* at the intertidal rocky shore of Ohoiwait (Big Kai Island, Southeast Molluccas, Indonesia). Sampling was conducted once each month at three different shore levels from October 2001 to September 2002.

Average ambient seawater temperature ranged from 29.3 °C in August to 32.2 °C in October (31.2 ± 1.0 °C), and salinity fluctuated from 30.3 in August to 34.0 in October (31.9 ± 1.1). The highest insolation (76 %) occurred in September, the lowest (28 %) during December (54.3 ± 18.0 %). The mean monthly air temperature varied between 26.2 °C in August and 28 °C in October (27.1 ± 0.6 °C). Air humidity and precipitation ranged from 81 to 90 % (85.9 ± 3.3 %) and from 51 to 547 mm (251.8 ± 159.1 mm), respectively. The tides were of the 'mixed prevailing diurnal' type. The average spring range was 1.5 m, the neap range was 1 m. The monthly tidal excursion ranged from 80 hrs in June to 145 hrs in October and November (117.1 ± 25.2 hrs), while the wave heights varied between 0.5 m in September and October and 3 m in March and April (1.6 ± 0.9 m).

Population structure and dynamics

The **distribution, abundance and biomass** of the intertidal limpet *C. testudinaria* are described separately for the high, middle and low shore levels. A total of 2402 limpets were obtained in 12 monthly collections. The mean density over the whole period was 11.12 ± 4.51 ind.m⁻². The density tended to be higher at the high (15.79 ± 7.54 ind.m⁻²) and the middle (14.67 ± 13.99 ind.m⁻²) than at the low shore level (2.90 ± 2.44 ind.m⁻²). Analysis of variance showed significant density differences among shore levels, months, as well as a significant interaction between shore levels and months. The small-scale dispersion patterns did not show any seasonal variability. They were strongly clumped throughout the year and at each shore level.

The conversion factor between body wet weight (WW) and ash-free dry weight (AFDW) was 0.19. The average energy content of the limpets was 21.59 kJ.g⁻¹ AFDW. The power function describing the relationship between body size (shell length L, in mm) and body weight (B, in mg AFDW) was $B = 0.03236 L^{2.7703}$.

The mean annual population biomass was estimated to be 1013 ± 748 mg AFDW.m⁻² (21.8 kJ.m⁻²). The highest biomass (3236 mg AFDW.m⁻² or 69.9 kJ.m⁻²) occurred in September 2002, whilst the lowest (544 mg AFDW.m⁻² or 11.7 kJ.m⁻²) was in March 2002. The monthly mean biomass (619 mg AFDW.m⁻² or 13.4 kJ.m⁻²) of the rainy season was lower than that (899 mg AFDW.m⁻² or 19.4 kJ.m⁻²) of the dry season.

The average relative condition factor (CF) was higher (1.27) for limpets at the middle shore level than for those at the high (1.25) and the low shore (1.22) levels.

Monthly shell-length frequency distributions were used to analyse the **size and age structure** of the population. The lengths of the collected specimens ranged from 8.0 to 31.8 mm. The analysis of the successive frequency distributions suggested that the population consisted of 4 to 5 distinct age groups (cohorts) at any given time, and that two new cohorts recruited during the one-year investigation period.

An analysis to determine the **growth** pattern using the FiSAT software showed that the longevity of *C. testudinaria* extended up to 2 years. The values of von Bertalanffy growth parameters (L_{∞} , K and t_0), estimated from size-frequency distributions, were 33.1 mm, 1.4 yr⁻¹, and 0.09, respectively. The highest growth increments were 25 % and 18 % of the asymptotic length during the first 3 and 6 months, respectively. The effects of environmental variables on the growth rates showed important seasonal variations, with the highest increment of 2.6 mm/month during the dry season.

The present study also highlights the fact that the **recruitment** of *C. testudinaria* was highly variable between months and shore levels. Both methods used to determine recruitment (direct counting and FiSAT) yielded similar results, which indicated that recruitment was highest during the transition season (March/April).

The mean **mortality** of *C. testudinaria* was estimated, using three different methods, to be 94 % per year ($Z = 2.81$). Highest monthly mortality rates, determined with the Ault-Ehrhardt approach, were observed in October 2001 and April 2002, when desiccation stress and wave action, respectively, were highest. However, there was no significant linear correlation between mortality and environmental variables.

A study on the **reproduction** of *C. testudinaria* indicated that the population consisted of 50 % neuters (incl. juveniles as well as adults with resting gonads and, hence, doubtful sex), 30 % adult males and 20 % adult females. Spawning took place in July and August. Males spawned earlier than females. Both sexes had mature gonads from May to August. The mean gonad index (GI) and the gonad condition (MGC) of males were higher than that of females. Male gonads accounted for a maximum of 23 % of the total body weight in July while females invested a maximum of 22 % of their body weight in gonad tissue in August.

The annual population **production** of *C. testudinaria* was estimated by the mass specific growth rate method to be 2.81 g.m⁻² or 60.7 kJ.m⁻².yr⁻¹. The annual biomass turnover ratio (production to biomass ratio, P/B) was 2.77 yr⁻¹.

Shell shape variations

An examination of the **body dimensions** of *C. testudinaria* indicated that specimens collected from the three different shore levels were significantly different in shell length, width, height, distance from apex to anterior margin (AA), distance from apex to posterior margin (AP), and body weight. The overall relationships between body dimensions and shell length were negatively allometric. The relationships of shell length to AA and AP suggest that the apex of limpets living at the low shore level are shifted backward in individuals less than 18 mm in shell length, while it is shifted forward in larger limpets. Morphometric studies on the ratio of shell length to shell width and height and the ratio of shell circumference to volume provided similar results as the analyses of the body dimensions, implying that limpets inhabiting the low shore level were broader and taller and had more acute, backward shifted apex than those living at the high and middle shore levels.

ZUSAMMENFASSUNG

Die vorliegende Studie befasst sich mit den Effekten der **Umweltfaktoren** Seewassertemperatur, Salzgehalt, Feuchtigkeit, Niederschlag, Insolation, Lufttemperatur, Wellenbewegung und Gezeitenzyklus auf die **Ökologie** der eulitoralen Napfschnecke *Cellana testudinaria* an der felsigen Gezeitenküste der „Großen Kai Insel“ der Südöstlichen Mollukken, Indonesien. Die Proben wurden einmal monatlich von Oktober 2001 bis September 2002 in drei verschiedenen Niveaus der Gezeitenzone genommen.

Die durchschnittliche Seewassertemperatur lag zwischen 29,3 °C im August und 32,2 °C im Oktober ($31,2 \pm 1,0$ °C) und der Salzgehalt schwankte zwischen 30,3 im August und 34,0 im Oktober ($31,9 \pm 1,1$). Die Zeit der stärksten Insolation mit 76 % kam im September vor, und die geringste Insolation wurde mit 28 % im Dezember gemessen ($54,3 \pm 18,0$ %). Die monatlichen Mittelwerte der Lufttemperatur lagen zwischen 26,6 °C im August und 28 °C im Oktober ($27,1 \pm 0,6$ °C), die relative Luftfeuchte und die Niederschlagsmengen schwankten zwischen 81 und 90 % ($85,9 \pm 3,3$ %) bzw. zwischen 51 und 547 mm ($251,8 \pm 159,1$ mm). Die Tide an der Küste von Ohoiwait ist eine „gemischt halbtägige Gezeit“. Der mittlere Tidenhub lag bei 1,5 m bei Springtide und bei 1 m bei Nipptide), und der monatliche Tidenzyklus dauerte von 80 Stunden im Juni bis zu 145 Stunden im Oktober und November ($117,1 \pm 25,2$ Stunden). Die Wellen erreichen im September und Oktober eine Höhe von 0,5 m und im März und April von 3 m ($1,6 \pm 0,9$ m).

Populationsstruktur und dynamik

Die **Verteilung**, **Abundanz** und **Biomasse** von *C. testudinaria* wurde für die obere, mittlere und untere Gezeitenzone getrennt bestimmt. Insgesamt 2402 Individuen wurden in den 12 monatlichen quantitativen Probennahmen gesammelt. Die Jahres-durchschnittliche Dichte über alle drei Zonen betrug $11,12 \pm 4,51$ ind.m². Die Abundanz war im oberen ($15,79 \pm 7,54$ ind.m⁻²) und mittleren ($14,67 \pm 13,99$ ind.m⁻²) Gezeitenbereich höher als im unteren ($2,90 \pm 2,44$ ind.m⁻²). Die Varianzanalyse der mittleren Dichten zeigte signifikante Unterschiede zwischen den Gezeitenzonen, den Monaten und eine signifikante Interaktion zwischen Zonen und Monaten. Die kleinräumigen Verteilungsmuster zeigten keine zeitlichen und räumlichen Variationen: Über den ganzen Zeitraum der einjährigen Studie und in allen drei Gezeitenzonen waren sie stark geklumpt.

Der mittlere Konversionsfaktor zwischen dem Feuchtgewicht der Napfschnecken und ihrem aschefreien Trockengewicht (AFTG) betrug 0.19; ihr durchschnittlicher Energiegehalt war 21,59 kJ g⁻¹ AFTG. Die Funktion zur Beschreibung der Beziehung zwischen der Körpergröße (Schalenlänge S, in mm) und Körpergewicht (G, in g AFTG) lautete: $G = 0,03236 L^{2,7703}$.

Die mittlere Biomasse der Population im Verlauf eines Jahres lag bei 10123 ± 748 mg AFTG m⁻² (21,8 kJ m⁻²). Die größte Biomasse wurde mit 3236 mg AFTG.m⁻² (69,9 kJ m⁻²) im September gemessen, die geringste Biomasse lag bei 544 mg AFTG m⁻² (11,7 kJ m⁻²) im März

2002. Die durchschnittliche monatliche Biomasse von 619 mg AFTG.m⁻² (13,4 kJ m⁻²) war während der Regenzeit niedriger als die 899 mg AFTG m⁻² (19,4 kJ m⁻²) während der Trockenperiode.

Der durchschnittliche Wert des relativen Konditionsfaktors der Napfschnecken war in der mittleren Gezeitenzone größer (1,27) als im oberen (1,25) und unteren (1,22) Gezeitenbereich.

Die monatlichen Häufigkeitsverteilungen der Schalenlängen wurden für eine Analyse der **Größen- und Altersstruktur** der Population benutzt. Die Längen der quantitativ gesammelten Exemplare lagen zwischen 8,0 und 31,8 mm. Die Analyse der Häufigkeitsverteilungen deutet darauf hin, dass die Population zu einem gegebenen Zeitpunkt aus 4 bis 5 verschiedenen Altersgruppen (Kohorten) besteht und dass während des einjährigen Untersuchungszeitraums 2 neue Kohorten rekrutiert wurden.

Zur Bestimmung der **Wachstumsmuster** von *C. testudinaria* wurde die „FiSAT Software“ benutzt. Dabei wurde eine Lebenserwartung von zwei Jahren bestimmt. Die Werte der von-Bertalanffy-Wachstumsparameter (L_{∞} , K und t_0), abgeschätzt mit Hilfe einer Analyse der monatlichen Größen-Häufigkeits-Verteilungen, waren 33,1 mm, 1,4 Jahr⁻¹ und 0,09. Das größte Wachstum lag bei 25 % bzw. 18 % der asymptotischen Länge während der ersten 3 bzw. 6 Monate. Die Analyse der Effekte der Umwelteinflüsse auf die Wachstumsraten deutete auf saisonale Variationen hin, wobei das Wachstum mit 2,6 mm/Monat während der Trockenzeit am höchsten war.

Die Studie zeigte, dass die **Rekrutierung** von *C. testudinaria* deutlich zwischen Monaten und Gezeitenzonen variierte. Beide zur Abschätzung der Rekrutierungsraten genutzten Methoden (direkte Zählung von rekrutieren Individuen, Bestimmung der Raten mittels FiSAT) lieferten ähnliche Resultate, die auf eine maximale Rekrutierung während der Übergangszeit zwischen NW- und SO-Monsun (März/April) hindeuten.

Die mittlere **Sterblichkeit** von *C. testudinaria* wurde mittels drei verschiedener Methoden abgeschätzt. Sie betrug 94 % pro Jahr ($Z = 2.81$). Die höchsten monatlichen Sterblichkeitsraten, bestimmt mit Hilfe der Ault-Ehrhardt-Methode, wurden im Oktober 2001 bzw. April 2002 beobachtet, wenn der Trockenstress bzw. die Wellenexposition am höchsten waren. Eine signifikante lineare Korrelation zwischen der Sterblichkeit und den Umweltvariablen wurde aber nicht festgestellt.

Eine Untersuchung der **Reproduktion** von *C. testudinaria* zeigte, dass die Population zu 50% aus Juvenilen (inkl. solchen Tieren, deren Geschlecht wegen des geringen Reifegrades der Gonaden nicht bestimmbar war), zu 30% aus geschlechtsreifen Männchen und zu 20% aus

geschlechtsreifen Weibchen bestand. Das Abläichen geschah nur im Juli und August, wobei männliche Tiere früher laichten als weibliche. Beide Geschlechter hatten reife Gonaden von Mai bis August. Der mittlere Gonaden-Index (GI) und der Gonaden-Zustand (MGC) von männlichen Tieren war immer höher als von weiblichen. Männliche Gonaden machten im Juli bis zu 23 % des Körpergewichts aus, während die Weibchen im August bis zu 22 % ihres Körpergewichts in Gonaden-Gewebe investierten.

Die jährliche **Produktion** der Population wurde durch die „Mass Specific Growth Rate Method“ mit $2.81 \text{ g m}^{-2} \text{ Jahr}^{-1}$ oder $60.7 \text{ kJ.m}^{-2} \text{ Jahr}^{-1}$ geschätzt. Der jährliche Biomassen-Umsatz, d.h. das Verhältnis zwischen jährlicher Produktion und mittlerer Biomasse (P/B), betrug 2,77.

Variation der Schalenform

Eine Untersuchung der **Körperproportionen** von *C. testudinaria* ergab, dass die in den drei verschiedenen Gezeitenzonen gesammelten Individuen sich signifikant in Schalen-Länge, Breite, Höhe, Entfernung von der Spitze bis zum vorderen Rand (AA), Entfernung von der Spitze bis zum hinteren Rand (AP), und Körpergewicht unterschieden. Die gesamten Beziehungen zwischen Körperdimensionen und Schalen-Länge waren negativ allometrisch. Die Beziehungen der Schalen-Länge zu AA und AP zeigten an, dass in der unteren Gezeitenzone die Schalenspitzen von kleinen (< 18 mm) Napfschnecken hinter der Schalenmitte lagen, während sich bei größeren Exemplaren die Spitzen vor der Schalenmitte befand. Morphometrische Studien der Verhältnisse zwischen Schalen-Länge, -Breite und -Höhe bzw. zwischen Schalen-Umfang und -Volumen ergaben ein ähnliches Ergebnis wie die Analyse der Körperdimensionen: Napfschnecken der unteren Gezeitenzone waren breiter und höher und hatten eine ausgeprägte und weiter hinten gelegene Spitze als Exemplare der oberen und mittleren Gezeitenzone.

ABBREVIATIONS

AA	Distance from A pex to A nterior margin
AFDW	A sh F ree D ry W eight
ANCOVA	A nalysis of C ovariance
ANOVA	A nalysis of V ariance
AP	Distance from A pex to P osterior margin
ASP	A vailable S um of P eaks
B	B iomass
BW	B ody W eight
CF	C ondition F actor and also C ircumference
CL	C onfidence L evel
D	D ensity
df	d egrees of f reedom
e	e xponential
EHWST	E xtrême H igh W ater S pring T ide
ELEFAN	E lectronic L ength F requency A nalysis
ELWST	E xtrême L ow W ater S pring T ide
ESP	E xplained S um of P eaks
FiSAT	F AO- I CLARM S tock A ssessment T ools
g	g ram
GI	G onosomatic I ndex
H	H eight
ID	I ndex D ispersion
IM	I ndex M orisita
kJ	k ilo J oule
L	L ength
L_{max}	M aximum L ength
L_t	L ength at time t
L_∞	A symptotic L ength
M	M ortality
MANOVA	M ultivariate A nalysis of V ariance
mg	m illigram
MGC	M ean G onad C ondition
MLE	M aximum L ength E stimation
MPA	M odal class P rogression A nalysis
MR	M ean R ank

MS	Mean Square
MSGRM	Mass Specific Growth Rate Method
N	Number(s)
NORMSEP	Normally Separation
P	Probability and also Production
P/B	Turnover ratio (Production/Biomass)
PCA	Principle Component Analysis
PRIMER	Plymouth Routines in Multivariate Ecological Research
SD	Standard Deviation
SE	Standard Error
SI	Somatic Index
SNK	Student Newman Keuls
SPSS	Statistical Package for Social Science
SS	Sum of Squares
TNI AL	Tentara Nasional Indonesia Angkatan Laut
V	Volume
VBGF	Von Bertalanffy Growth Formula
W	Width
WW	Wet Weight
W_{cal.}	Calculated Weight
WI	Wave Index
W_{pred.}	Predicted Weight
W_t	Weight at time t
W_∞	Asymptotic Weight

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CURRICULUM VITAE	
ERKLÄRUNG	

Chapter 1**GENERAL INTRODUCTION**

We should have to go to the tropics to meet with conditions that would not completely upset the assumption of an about constant physical environment, as it lies at the root of most deterministic population models

P.J. den Boer (1981)

1.1. Motivation

One major problem in long-term monitoring programs of marine environments is to explain any change discernible in the data sets. It has been argued strongly that this will only be feasible when there is a fundamental understanding of the ecology of the species or communities, which are used as the focal point of the programs. The determination of the biological characteristics of a species is the essential requirement for any ecological studies (Fletcher, 1987). Basic knowledge of growth, mortality, recruitment and reproduction are invaluable in any analyses of the structure and dynamics of biological communities (Underwood, 1979; Creese, 1981; Underwood et al., 1983). Furthermore, such studies have been, in part, responsible for the burgeoning number of theories accounting for the different patterns found both among and within a species (Fletcher, 1987). These “trade-offs” will, supposedly, be suggested that the level of successful offspring an individual produces should be maximised by some combination of present *versus* future investigations.

The structure and dynamics of biological communities cannot be understood without considerable background information about the ecology of the component species (Underwood, 1979). The choice of the right species and the emphasis upon their role in the environments were justified as the most economical means of gaining the ability to “explain” major changes in the general character of rocky shore communities and make predictions about natural events to detect regional or temporal consequences of man-made changes in their environmental quality (Bowman & Lewis, 1977). Demographic studies of key species are also important for understanding the dynamics of intertidal communities (Dunmore & Schiel, 2000).

Intertidal organisms are suitable for such direct experimentation because of the ease of access to intertidal areas, and because of the relatively sessile nature and great abundance of many of the organisms (see reviews by Connell, 1974, 1975). Limpets are “foundation species” of marine organisms as defined by Dayton (1972). They can be used as suitable objects for environmental monitoring, since they have a key role in the community and are especially sensitive to the environmental parameters of concern. They have a well-known physiology and have predictable responses to environmental variables (Jones & Baxter, 1985).

Intertidal limpets are a convenient group to examine population dynamics because they live in a readily accessible habitat, often occur in large numbers, and are easy to sample, mark and relocate (Creese, 1981). Populations of limpets exhibit great plasticity, and environmental conditions on a very local scale often influence their dynamics (Ward, 1967; Giesel, 1969; Sutherland, 1970; Lewis & Bowman, 1975; Bowman & Lewis, 1977). As the dominant grazers of many intertidal shores (Dunmore & Schiel, 2000) and herbivores at both temperate and tropical latitudes (Liu, 1994), limpets are important not only because of their relative abundance on most hard shores and in shallow waters, but also because of the significant effect they may have on the ecosystem. Their role in structuring rocky shore communities have attracted the attention of scientists for many years and have been well documented by many authors (for review see Blackmore, 1969a; Branch, 1981). Their widespread distribution has undoubtedly been responsible for the high number of studies already devoted to this taxon (reviews by Underwood, 1979; Branch, 1981).

The patellid limpet *Cellana testudinaria* (Linnaeus, 1758) is the most conspicuous and ecologically most important component of the community of the intertidal rocky shores of Ohoiwait, Big Kai Island, Indonesia. The species is typically found on the volcanic rock in exposed situations, mainly near and below low tide marks. It is common in tropical regions (Powell, 1973). For coastal populations in Ohoiwait, they constitute a source of food and income. Consequently, the high intensity of traditional collection (picking by hand upon encounter) and commercial exploitation may easily cause over-fishing. Therefore, conservation issues are currently of increasing importance in the region. However, relatively few studies have been published with regards to this limpet species. Most of these reports and observations focused on taxonomy and distribution (Schepman, 1908; Oostingh, 1925; Habe, 1964; Powell, 1973; Wilson, 1993) whilst their biological aspects and the ecological factors affecting them have been often overlooked. No detailed studies of the population dynamics or life history characteristics of intertidal limpets in Indonesian have been documented. In other geographic areas, comparative studies of congeneric limpets inhabiting similar habitats have demonstrated that they are strongly influenced by environmental factors which vary considerably (Balaparameswara Rao & Ganapati, 1971a; Underwood, 1974; Creese, 1981). It is, therefore, not possible to apply these findings to the full distribution range of this limpet species and its natural habitats, except in very general terms. This is a major obstacle to providing a sound ecological understanding of limpet populations in tropical regions. The range covered here is the largest studied for tropical limpet *C. testudinaria* and should, therefore, provide a powerful test for variations among populations of this species. The results will be compared with previous work on the biology of limpets living in temperate regions and with similar work on other species.

1.2. Previous work and further research needs

Numerous studies in the past twenty years have yielded a wealth of information on limpet populations, which focus on their biology and ecological interactions, e.g. population dynamics (Lewis & Bowman, 1975; Thompson, 1980; Creese, 1981; Fletcher, 1984; Lohse, 1993; Liu, 1994), larval development (Orton, 1946; Balaparameswara Rao, 1973; Bowman & Lewis, 1977; Baxter, 1983; Creese & Ballantine, 1983; McGrath, 1992), factors affecting variability of foraging (Little & Stirling, 1985; Chelazzi et al., 1998; Gray & Hodgson, 1998), impact of pollution (Liu & Morton, 1998; Glegg et al., 1999), and the interplay of physical and biological factors in maintaining assemblages on rocky coasts (Benedetti-Cecchi et al., 2000). Several physical aspects, such as tenacity (Grenon & Walker, 1981), activity due to tidal amplitude (Cook & Cook, 1978), attachment mechanisms in the field (Smith, 1992), have also been investigated. The existing literature on the biology of limpets, physical factors and ecological interactions (overview in Underwood, 1979; Branch, 1981) and shell shape variations due to the effect of environmental variables (Balaparameswara Rao & Ganapati, 1971b; Hobday, 1995) are surprisingly uniform with regard to the approaches adopted. Interpretations of these phenomena are predominantly based on observations, data resulted from field experiments, and measurements of selected physiological traits.

A part of observations and data analysis was facilitated to extract growth parameters from length frequency data (Sparre & Venema, 1998). This approach was used since the previous methods are limited. Requirements of physiological traits, such as life-cycle stages and migratory from upper to lower or from lower to upper shore levels, are likely to be important in explaining observed recruitment patterns, distribution and abundance but studies regarding this topic are largely lacking. Hence, to enhance our understanding of population dynamics of tropical limpets as well as our ability to predict the consequences of environmental changes (Bowman & Lewis, 1986), an expansion and improvement of experimental work related to the phenomena is necessary, including experimental manipulation in the field (Lotze, 1998). Underwood (1979) pointed out that experimental manipulations of natural populations in field situations are often the most profitable method of determining the factors, which affect the distribution and abundance of species.

1.3. Questions

In this study, I attempted to answer the following main questions:

- *Are population dynamics (e.g. growth, recruitment, mortality) of Cellana testudinaria affected by the environment in which the individual and/or population is found?*
- *How is the shell shape of limpet affected by environmental variables?*

1.4. Solution approach

To answer these questions, I chose an approach integrating ecological studies on the tropical limpet *Cellana testudinaria* on the rocky shore benthos of the Big Kai Island, Southeast Molluccas, Indonesia.

Ecology is the study of interactions between the community of species and the factors regulating them, which determine distribution and abundance of species in space and time (Krebs, 1994). This definition bears the phenomena among several authors, and brings it into the debate. Many ecologists believed that community ecology is primarily influenced by the interaction of the organisms with the physical environment while others believed that biological factors play the most important role. However, today it is accepted that physical and biological factors interact in determining population and community structure (Krebs, 1994). In this study, I will argue that knowledge of environmental constraints on a limpet population is required to gain a comprehensive understanding of the ecological control of recruitment, growth, mortality, and life cycle of intertidal limpets.

Some characteristics of limpets are considerably affected by environmental conditions. Variations in the form of the shell of *C. testudinaria* within the intertidal rocky shore can be related to the tidal cycle, wave action, and desiccation. It is already well known that limpets, which spend a large part of their time in holding tightly to the substratum, develop an elevated shell (Moore, 1934; Balaparameswara Rao & Ganapati, 1971b). Presumably both wave action and exposure to the air cause limpets to hold on tightly (Ebling et al., 1962; Grenon & Walker, 1981; Denny, 1985). In this study, I documented the shell shape pattern along the tidal gradient to investigate the effects of different environments upon structural shell features.

1.5. The objectives of this study

There have been a number of previous studies on the ecology of limpets. However, most of them have focused on limpets living in temperate regions, and few were in the tropical regions. Therefore, the broad aim of this study is to provide information on the ecology of the limpet *Cellana testudinaria* living at the intertidal of the rocky shore of Ohoiwait, Big Kai Island, Southeast Molluccas, Indonesia. Two main themes are dealt with, and both are related to each other. They are:

- *Population dynamics of tropical limpet Cellana testudinaria at the rocky shore of Ohoiwait, Big Kai Island, Southeast Molluccas, Indonesia: Abundance, population structure, recruitment, growth, mortality, and stock assessment*
- *Shell shape variation exhibited by this intertidal limpet in relation to their zonal distribution*

1.6. Outline of thesis

This study addresses two main issues, which both are focused on the ecology of a selected limpet species in relation to environmental factors influencing the community on the rocky shore. First, I will describe the population dynamics of the tropical limpet *C. testudinaria* on the rocky shore of the Big Kai Islands. Pattern of species distribution, abundance and biomass at different shore levels and times are compared in Chapter 3. Chapter 4 provides a determination of growth by analysing the sizes of the cohorts in monthly size-frequency distributions, by calculating the longevity and describing the growth pattern using the von Bertalanffy growth formula (VBGF). Hypotheses of the role of environmental variables as factors that control recruitment and mortality are explored in Chapter 5 and Chapter 6. Moreover, the gonad condition of different life-cycle stages is investigated (Chapter 7), and the population production of this species is estimated (Chapter 8). Furthermore, I will provide a description of the shell shape variation exhibited by this intertidal limpet in relation to the zonal distribution. Shell dimensions from the three different shore levels (high, middle, and low) and the factors influencing them are treated in Chapter 9. A general discussion and conclusion integrating the results of this and other studies and an outlook on further studies are provided in Chapter 10.

Chapter 2

STUDY SITE AND DESCRIPTION OF SPECIES

2.1. Study site

The study was carried out on the intertidal rocky shore of Ohoiwait (latitude $5^{\circ}45'15''$ S, longitude $132^{\circ}57'20''$ E), Big Kai Island, Southeast Mollucas, Indonesia (Fig. 2.1). The intertidal region investigated is about 0.25 km^2 (1 km long and 0.25 km wide). Thus, it is small enough that atmospheric conditions may assumed, for most purposes, to be uniform over the whole area. The shore consisted of shingles, pebbles, medium and big boulders. The physical conditions in the tidal zones are quite different; the higher shore is wetted almost exclusively by tidal sea level rise, but the lower shore receives considerable wave action.

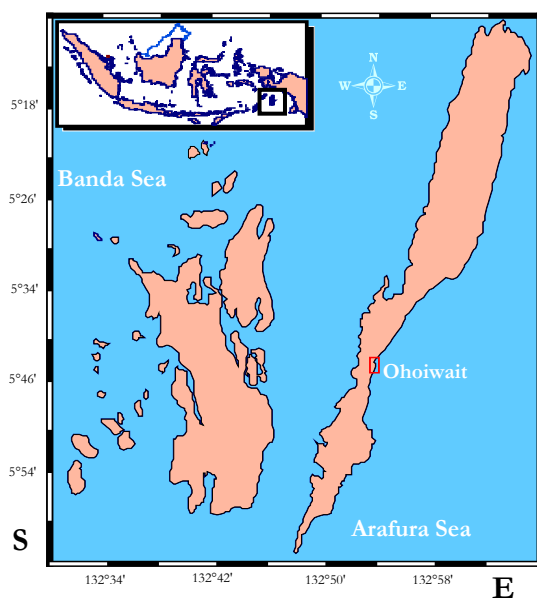


Figure 2.1. Map of study site of Ohoiwait located at the Big Kai Island. Below: profile of the rocky shore intertidal.



The climate and environmental features are strongly influenced by the monsoons. Distinct “dry” and “rainy” seasons with periods of transition between these two extremes occur over most of the region. By June - August the south monsoon, which is characterized of high insolation (Fig. 2.2) and low humidity and precipitation (Fig. 2.3), is fully developed. During this dry season, the average humidity and precipitation decrease from about 87 % and 318 mm, respectively, in June to 81 % and 51 mm in August, respectively. The climate is characterized by high insolation (on average 75 %). In contrast, during the north monsoon (December – February), evaporation is suppressed by cool moist air masses. During this rainy season, relative low humidity of 81 % gave rise to net evaporation rates in excess of 100 mm/month (Tual

Meteorology and Geophysics). In the transition period of transition between September and November, mean monthly air temperature slightly dropped from 27.6 °C to about 26.9 °C in the beginning of December, which may be an effect of greatly decreased insolation (28 %), and increased humidity (88 %) and net precipitation (547 mm).

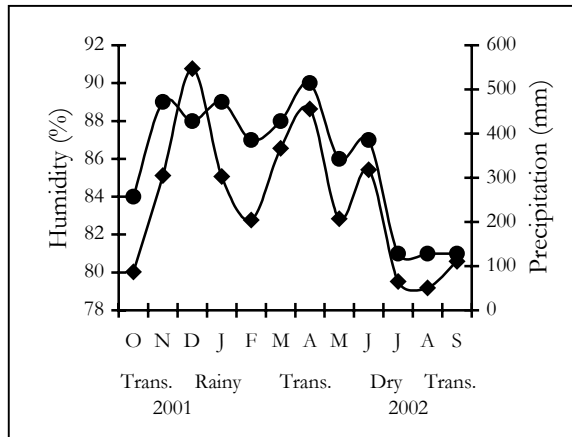


Figure 2.2. Annual variation of the average humidity (●-●) and precipitation (◆-◆).

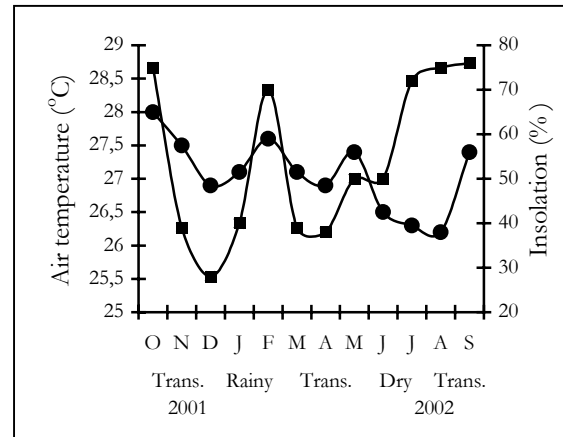


Figure 2.3. Annual variation of the average air temperature (●-●) and insolation (■-■).

In the transition period between March and May, strong winds (average speeds of 17 knots) blow predominantly from the northeastern Arafura Sea. They switch abruptly over Ohoiwait at an angle of 15°, producing short gales of a few days' duration (Tual Meteorology and Geophysics). These winds are considered as the primary source of high wave action during this time of years.

Ohoiwait was chosen as a study site because (1) the benthic community is dominated by limpets, (2) the site is typical of a tropical intertidal rocky shore, (3) it borders a nature reserve inhabited by innumerable *Trochus* sp. and has, therefore, been issued as a conservation region by coastal populations, and (4) so far, no scientific research are conducted in this region, therefore data obtained will contribute new information.

2.2. Water parameters

Seawater temperature. One-liter ambient seawater at *in situ* limpet's habitat was randomly taken for the measurement. Water temperature was measured weekly using hand thermometer to the nearest 0.1 °C. The measurements were used to describe month-to-month variations (Figure 2.4). The water is consistently about 2 °C colder during the dry season than during the rainy season. The paradoxical warming in rainy season may be accidental and within sampling error. The fluctuation of air temperature (Fig. 2.3), however, showed the same trend as seawater temperature, thus it could be assumed that there is a relationship between air and sea water temperature, i.e. an increase in air temperature will be followed by an increase in seawater

temperature. Monthly seawater temperature measured at the time of sampling ranged from 29.3 °C to 32.2 °C. In October, average seawater temperatures that were measured in tide pools ranged from 38.3 °C at the low shore to 39.5 °C at the high shore. The temperatures at the high and low surface substratum were on average 40.8 °C and 41.2 °C, respectively.

Salinity. Salinities were measured, using a refractometer, to the nearest of 0.1. The influence of the monsoons on precipitation gives rise to high and variable rates of surface runoff. This is reflected in the salinity of the ambient waters around the rocky shore of Ohoiwait's intertidal, which shows strong spatial and temporal variability. The monthly distribution of salinity is shown in Figure 2.4.

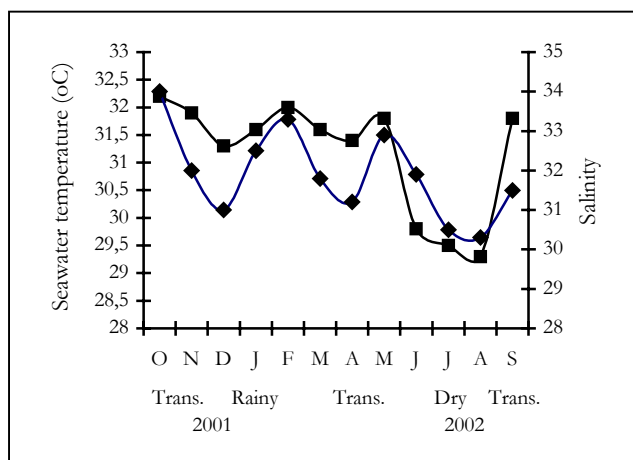


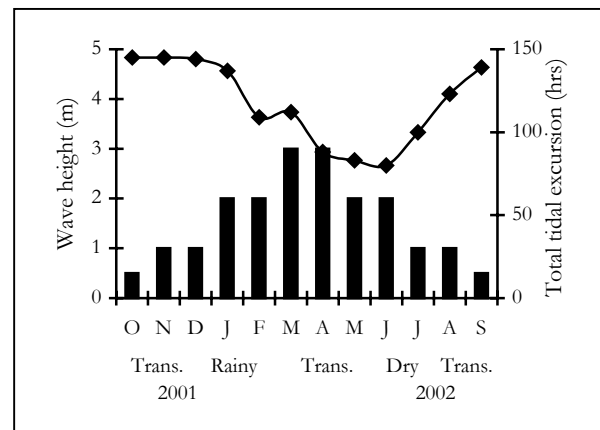
Figure 2.4. Annual variation of the average (■-■) seawater temperature and (◆-◆) salinity.

Because of the monsoons, the mean annual salinity is depressed to 32 over the whole region. In some areas such as around river mouths, substantial fluctuations in salinity coincide with high rainfall and surface runoff. Between this area and the oceanic water mass of Arafura Sea, regions of mixed waters are formed. Subsequently, this mixed waters is transported and immersed throughout the shore by the tidal excursion and wave surf action so that the shore is alternately filled with waters of varying salinity. The relative low salinity of 31 in April and December coincided with high precipitation rates (Figure 2.2). On the other hand, the increase of salinity to 34 in October was very likely caused by high evaporation due to increasing air temperature and decreasing precipitation.

Insolation. Light intensity was continually recorded as %-insolation per day at Tual airport by the meteorological station in Tual. The light intensity was correlated with air temperature and water temperature (Figure 2.3). Water temperature changes followed the major increase and decrease of light intensity. On the period from June to August, contradictory in the trends was probably due to global changing in weather (personal interview with Meteorology and Geophysics staff). The longest insolation of 76 % was recorded in September while the lowest of 28 % was in December.

Wave. Wave force imposed at an exposed site was measured in term of a breaking wave height mounted at intertidal slope (WI; Wave Index) and was defined as an indicator of the degree of wave exposure at each sampling site. Heights of the highest and lowest levels of waves were measured 10-15 times over a period of few minutes using a vertically stood ruler, then, the mean values of the both height were calculated, and WI was represented by the difference between them. The interpretation of one such record was presented as a maximum vertical height of wave appeared in one day.

Figure 2.5. Annual variation of the average (■) wave height and (◆-◆) tidal excursion.



As mentioned above the wave actions that occur at the intertidal of Ohoiwait are strongly dependent on the wind (its duration and the distance of open water over which it can blow), which originated during the monsoons. These waves are often refracted and their form altered as they approach a shore, and their force on the shore itself is governed largely by the slope pattern and detailed configuration of the rocks. By March – April the average maximum of vertical wave height observed at different heights on the shore was 3 m, coinciding with an average of 3 hours per day amplitude of the tidal excursion. The site at 6 m above ELWST and at an angle of about 10° to the surface of the substratum predictably had the highest average maximum force of wave action. Even though it is early to be concluded, this action seems likely as factor-affecting limpets to migrate from the low shore to the middle shore.

Tide. Daily amplitude of the tidal excursion was estimated based on the retention time needed for the first fore coming tide to reach the EHWST until it reached again for the second time. This set of tidal periods was then compared to the periodic tidal table. The tides of the Big Kai Island are co-oscillating tides of the Pacific and Indian oceans. The overall tidal pattern is therefore complex, and each shore has a unique tidal regime. In the eastern part of the island mixed tide prevailing diurnal predominate while the western part is predominated by the mixed tide prevailing semidiurnal (Wyrтки, 1961). The tide of Ohoiwait's intertidal is mixed tide prevailing diurnal with the average spring range 1.5 m and the neap range 1 m. Spring low tides in October occur practically simultaneously with a phase of 12 hours on an average maximum of

145 hours excursion. Hot midday conditions affect the intertidal organisms only when low tides coincide with a high insolation of sunlight.

Statistical analysis. Principle Component Analysis (PCA) was utilized to analyse the overall variance of environmental variables, reflecting the similarity of their incremental changes through time. The Kruskal-Wallis non-parametric ANOVA test (Table 2.1) detected no significant difference ($H = 1.54, P > 0.05$) in the mean levels of environmental gradients with time.

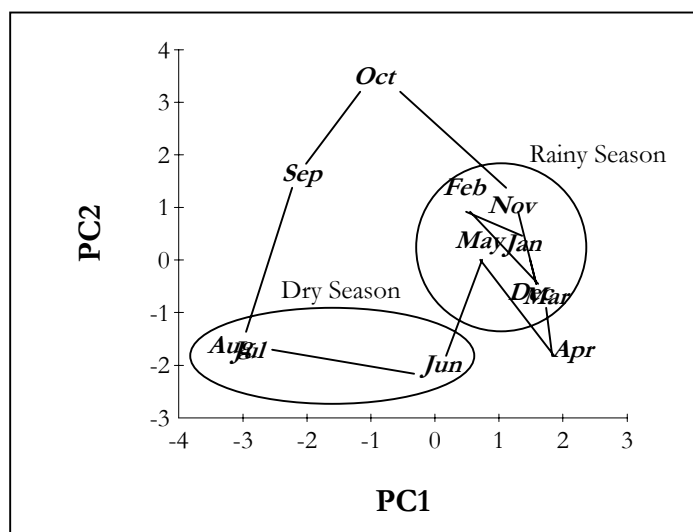


Figure 2.6. Two-dimensional PCA ordination of the 8 environmental variables of Fig.2.2 – Fig.2.5 (normalised) for the 12 months. PC1 (X – axis) and PC2 (Y – axis) together account for 79% of the total sample variability.

The ordination of the monthly environmental observations along the first two axes of the principle component analysis (PCA) are shown in Figure 2.6. The first two components account for 79 % of the total variability, so the 2-dimensional picture provides an accurate summary of the environmental variables relationship. It is clear from the figure that extreme positive values along the first PCA axis (PC1) were in April and March, while extreme negative values were in August and July. These values imply that the environmental parameters observed in April/March and July/August were most variable.

2.3. Biotic environment

Benthic macrophytes were relatively sparse in the study area. *Fucus* sp. and *Sargassum* sp. occupied the upper part of the shore 5 m below Extreme High Water Spring Tide (EHWST), while seagrass primarily occurred 3 m above Extreme Low Water Spring Tide (ELWST). Faunistically, the region was also poor. The only other conspicuous animals present were periwinkles: *Thais lapillus* L. and *Littorina littorea* L., and other limpets such as *Patelloida saccharina* (Linnaeus, 1758), *Collisella striata* (Quoy & Gaimard, 1834), *Cellana radiata* (Born, 1778), and

Cellana radiata subspecies (Reeve, 1854). Although other species such as isopods, amphipods, and echinoderms could be found sporadically, though only in the small numbers.

This study also examined the species composition, distribution, and abundance of the four other limpet species. Sampling utilizing the line transect technique was conducted one each month during low tide, ranging down from EHWST to ELWST. The pure random sampling was not practicable since irregular surface and heterogeneous environments were encountered. All the individual limpets present in one square meter frame were counted. Spatial distribution of the limpets in the habitat was examined by the Morisita Index (Elliott, 1977). The following chi-square test, χ^2 , was used to test for the significant departure of the dispersion index from 1.0 with $P > 0.05$ probability level.

A total of 1080 limpets were obtained in 2 month sampling collections, comprising the 4 species. Their densities at the three shore levels varied greatly around the mean density of 30 ± 13 ind.m⁻². *P. saccharina* was the most abundant species with a mean density of 15 ± 2 ind.m⁻². It predominated at high and middle shore levels (50.9 % of occurrence). *C. radiata* primarily occurred at the high shore with a mean density of 7 ± 3 ind.m⁻², while *C. striata* and *C. radiata* subsp. predominated at middle and low shore levels with mean densities of 5 ± 2 ind.m⁻² and 3 ± 1 ind.m⁻², respectively. The spatial distribution of the four species was highly contagious ($ID > 1$; $P < 0.05$).

Table 2.1. *Cellana testudinaria*. Bartlett-test on environmental variables with an equal number of observations together with the Kruskal-Wallis non-parametric ANOVA test.

BARTLETT-TEST

SUMMARY

Sources	Count	Sum	Average	Variance
Air temperature	12	324.90	27.08	0.30
Seawater temperature	12	374.20	31.18	1.06
Insolation	12	652.00	54.33	323.15
Humidity	12	1031.00	85.92	11.17
Precipitation	12	3021.00	251.75	25312.39
Seawater salinity	12	382.90	31.91	1.25
Tidal Excursion	12	1404.00	117.00	636.68
Wave height	12	18.00	1.50	0.77

COMPUTATION OF BARTLETT'S STATISTIC

Sources	Variance S^2	$\text{Log}_{10}S^2$
Air temperature	0.30	-0.5229
Seawater temperature	1.06	0.0253
Insolation	323.15	2.5096
Humidity	11.17	1.0481
Precipitation	25312.39	4.4033
Seawater salinity	1.25	0.0969
Tidal Excursion	636.68	2.8038
Wave height	0.07	-0.1135
Total	26287.06	10.2506

$$k = 7$$

$$df = 11$$

$$\Sigma S^2/k = 3755.2943$$

$$\log_{10}(\Sigma S^2/k) = 3.5746$$

$$k \log_{10}(\Sigma S^2/k) = 25.0225$$

$$\begin{aligned} \text{Bartlett's statistic (M)} &= 2.3026 * df * [k \log_{10}(\Sigma S^2/k) - \Sigma \log_{10} S^2] \\ &= 2.3026 * 11 * (25.0225 - 10.2506) \\ &= 374.3597 \end{aligned}$$

$$\begin{aligned} \text{Correction factor (C)} &= 1 + [(k + 1)/3k.df] \\ &= 1.0346 \end{aligned}$$

$$\text{Corrected test statistic} = M/C = 361.84$$

(Using M/C instead of M is to lower the test statistic and thereby allow acceptance of some marginal hypotheses which otherwise might be rejected)

$$\text{Tabulated } \chi^2_{0.05(11)} = 19.675$$

KRUSKAL-WALLIS TEST

COMPUTATION OF KRUSKAL-WALLIS STATISTIC

	<i>TIME</i>	<i>N</i>	<i>Mean Rank</i>	<i>MR²</i>
<i>VARIABLE</i>	1	8	51.75	2678.06
	2	8	52.63	2769.92
	3	8	50.88	2588.77
	4	8	48.88	2389.25
	5	8	49.44	2444.31
	6	8	46.81	2191.18
	7	8	41.94	1758.96
	8	8	42.13	1774.94
	9	8	48.63	2364.88
	10	8	50.88	2588.77
	11	8	52.38	2743.66
	12	8	45.69	2087.58
Total		96		28380.29

$$\begin{aligned}
 \text{Kruskal-Wallis statistic (H)} &= [12 / (\sum N)(\sum N + 1)] * (N * \sum MR^2) - 3 * (\sum N + 1) \\
 &= [12 / (96)(96+1)] * (8 * 28380.29) - 3 * (96+1) \\
 &= 1.54
 \end{aligned}$$

Since there were ties, this H value must be corrected by dividing it by:

RESULT

<i>VARIABLE</i>	
Chi-Square	1.54
df	11
Asymp. Sig.	1.000

$$\begin{aligned}
 D &= 1 - [\sum T / (\sum N - 1)(\sum N + 1)] \\
 &= 1.000
 \end{aligned}$$

$$\text{Adjusted H} = (H) / (D)$$

$$\text{Tabulated } \chi^2_{0.05(7)} = 14.1$$

$$= 1.54 / 1.000$$

$$= 1.54$$

2.4. Species description of *Cellana testudinaria*

This study focused on the common turtle limpet *Cellana testudinaria* (Linnaeus, 1758) (Figure 2.7). *Cellana* is truly cosmopolitan genus, which can be found at tropical, temperate and polar rocky shores in both hemispheres (Balaparameswara Rao & Ganapati, 1971a; Underwood, 1975; Fletcher, 1984; Dunmore & Schiel, 2000).



Figure 2.7. *Cellana testudinaria*

Cellana testudinaria is traditionally placed in the Patellid limpets (Family: Patellidae; Order: Archaegastropoda; Sub-class: Prosobranchia; Class: Gastropoda), but some authors have misidentified it as the *Helcioniscus testudinaria* (Linnaeus, 1758), *Cellana rota* (Gmelin, 1791) or *Patella insignis* (Dunker, 1868), because of the occasional occurrence of longitudinal divisions (Powell, 1973; and reference therein). Its morphological characteristics are: Shell semi-translucent, solid, reaching a large size, moderately elevated, with a regular shape; outline rounded-ovate; apex somewhat anterior, at about the anterior 1/3 of shell length; external sculpture rather weak, of numerous and subequal low radial riblets; interior smooth; colour: exterior of shell greenish to yellowish brown, with dark brown radial rays more or less joined concentrically by zigzag patterns or V-shaped marks, within the shell substance; aperture with a continuous brown margin; interior bluish silver, apical region of white to yellowish brown.

C. testudinaria is a sedentary animal, common on exposed hard substrates where they clamp tightly by means of their large foot. Mainly intertidal, often occurring in dense populations and exhibit homing behaviour, excavating a shallow scar to which shell margin conforms exactly and returning to stick fast to the same spot after foraging for food. It grazes on encrusting lichens and algae or scrape tissue from kelp with their powerful radula. Sexes are separate or hermaphroditic. Eggs hatch as free-swimming planktonic larvae. Adults are commonly collected for their edible foot used as food by coastal populations in Southeast Asia and Tropical West Pacific islands (Powell, 1973).

2.5. Life-history

An organism's relationship to its environment can be summarized by its life-history parameters: the average growth rate, size at maturity, clutch size, frequency of reproduction, and event life expectancy of members of its population. As an example, a number of authors have argued that natural selection should favour different life histories for adults that differ in size. Sizes of most organisms are known, so one will be able to predict features of the life history of many organisms when explicit relationships are demonstrated (Sutherland, 1970; Choat & Black, 1979). Life-history patterns vary enormously from species to species, and from population to population within some species. However, these variations seem to follow rules such as characteristics of an organism that might lead to particular life-history features include its morphology and ecological role (Williams, 1966). Since these characteristics are shared among closely related species, one can predict many aspects of a species' life history from observations of related species.

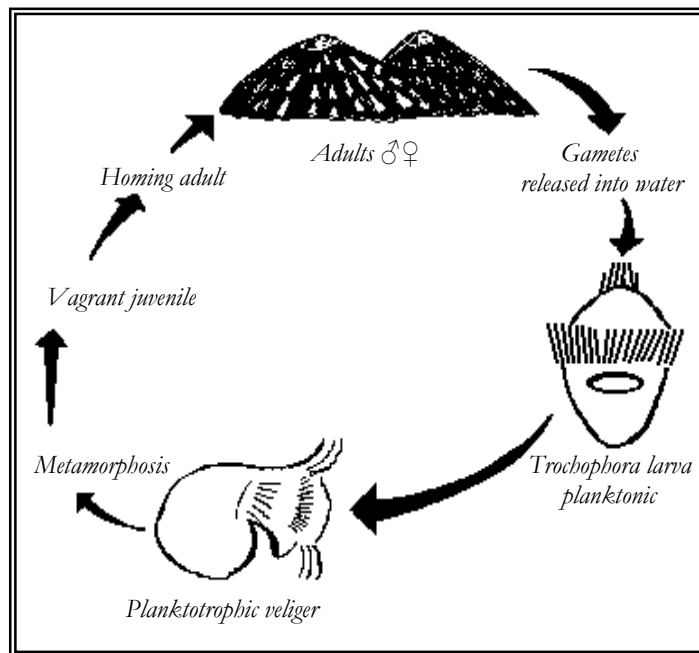


Figure 2.8. Life history of *Cellana testudinaria*

Once each year, from late April to early August 2002 (see Chapter 7), mature males and females of *C. testudinaria* gather on rocky surfaces. The gonads of the males mature earlier than these of the females. These organisms spawn by liberating their eggs and sperm directly into the water (Fig 2.8). At most sites, spawning reaches its maximum in July for males and in August for females, and declines to a minimum by the early rainy season of the following year. The onset of spawning may possibly be correlated with variations of seawater temperature and salinity.

However, the proximate cues involved in stimulating an actual spawning event are still not fully understood but may involve a combination of both endogenous and exogenous factors. Temperature appears to be an extremely important cue for initiating spawning. This is clearly seen from *Patella vulgata* that initiates gametogenesis as sea temperature are falling and then begin spawning as temperatures rise, or vice versa (Bowman & Lewis, 1977). A trochophore stage, which is reached after 24 – 30 hours, is followed by a period of 3 – 4 days as a planktotrophic veliger (Crofts, 1955), before the larva becomes benthic and settles as “spat” on the lower shore or in damp crevices. It metamorphoses and grows, slowly making its way up to higher shore

levels. The velum may be retained for up to 3 weeks (Smith, 1935), and final metamorphosis occurs at a shell length of 0.2 mm (Fretter & Graham, 1962). The most important factors that affect larval recruitment success are (1) suitable environmental conditions, (2) adequate food supply, (3) predation, and (4) location of a suitable settling substratum.

2.6. Diurnal rhythm of behaviour

Understanding the ecology of this species in its natural community requires knowledge of diurnal rhythm that may present the behavioural adaptations of limpet as the responses to the effects of some environmental variables. Williams & Morritt (1995) pointed out that some physical extremes during summer might strongly select for limpet activity rhythms entrained by wave splash and selection of refuges from high temperatures and desiccation when inactive. Numerous species show adaptations to reduce these stresses by hiding in cracks and crevices, sealing their shells with mucus and positioning their shell to minimize exposure to insolation (see Garrity, 1984, for review), and therefore habitat choice seems to play vital role in the survival of many species (Davies, 1969; Wolcott, 1973; Leviten & Kohn, 1980).

Like other tropical limpets, *C. testudinaria* is a homing species, which usually forages in the field whilst awash by the waves. They extend their tentacles and then begin to move actively as the tide rises and waves start to splash them. I found that individuals move rapidly up-shore with the incoming tide, while movement at high tide was slow and appeared to cease until the tide starts to ebb. Some limpets tried to return to their normal shore levels during this immersion, and could move over 1.5 m difference in elevation during a spring tide. During stormy condition, when the shore was awash, brief observations suggested that *C. testudinaria* remains active and tends to move toward each other. Williams & Morritt (1995) reported that *Cellana grata* still remains active when wave splash the shore during stormy conditions, while Hirano (1979) found that *Cellana toreuma* remains active for up to 18 hours. I think that the importance of wave action is a cue factor to stimulate movement of limpet, as has been reported by Wells (1980) on limpet *Collisella limatula*. This behavioural rhythm can be found commonly in tropical species (Garrity, 1984) and temperate limpets such as *Patella vulgata* (Della-Santina & Naylor, 1993). *C. testudinaria* on the upper shore levels was more active to forage during immersion than at the lower shores. This may be related to the higher food abundance on the shore or relatively unused resources of food (Little et al., 1991). *Patella vulgata* living at high water levels reduces their movements in favour of a more intense feeding (Little et al., 1988; Evans & Williams, 1991; Chelazzi et al., 1994).

Chapter 3

DISTRIBUTION, ABUNDANCE AND BIOMASS

3.1. Introduction

Natural assemblages of species are inherently variable (Menconi et al., 1999). Changes in the composition and abundance of organisms occur at several spatial and temporal scales (Andrew & Mapstone, 1987; Barry & Dayton, 1991; Menconi et al., 1999). This variability was primarily explained by the effects of abiotic parameters and resources availability in the environment in relation to physiological traits of the species (Colman, 1933; Doty, 1946; Lewis, 1964; Stephenson & Stephenson, 1972; Underwood, 1979). However, several authors reported an increasing body of evidence for the importance of biotic factors such as competition and predation (Dayton, 1972; Paine, 1974; Underwood, 1979). Recently, there is increasing evidence from different areas of ecology for the notion that patterns in nature are influenced by both abiotic and biotic factors (Underwood, 1979; Levin, 1992; Schneider, 1994; Wu & Loucks, 1995). A number of processes have been invoked to explain natural patterns, including variation in recruitment, the interactive effects of physical and biological factors, behavioural effects and small-scale changes in the topography of the substratum (Underwood & Chapman, 1996, 1998; Benedetti-Cecchi et al., 2000; Benedetti-Cecchi, 2001).

Rocky coastlines are heterogeneous environments supporting variable assemblages of sessile and mobile organisms. A prominent feature of these systems is that species generally occur at some levels on the shore but not at others. Limpets, which occupy both intertidal and intratidal habitats, have a high relative abundance on most hard substrata in shallow (Liu, 1994). Many studies on rocky coasts have focused on the seasonal changes and the factors influencing the distribution and abundance of limpets (see review by Branch, 1981). These factors include physical conditions related to desiccation, temperature, salinity, and light (Evans, 1947; Southward & Orton, 1954; Balapameswara Rao & Ganapati, 1971b; Liu, 1994), wave action (Jones & Demetropoulos, 1968; Denny, 1982, 1985), tidal levels (Stephenson & Stephenson, 1949; Ebling et al., 1962). The vertical patterns in the distribution (zonation) are thought to be primarily caused in response to the major gradient of emersion and desiccation (Stephenson & Stephenson, 1949; Southward, 1958; Lewis, 1964). However, biotic interactions have also been described to affect limpet distribution. A wide range of organisms has been recorded preying on limpets. A large number of predatory gastropods, particularly thaidis, which can drill through shells, feed on limpets (see the review by Branch, 1981). Intra-specific competition may also limit population density by density dependent mortality, by modifying the success of recruitment or by influencing the rate of migration in relation to food availability (Underwood, 1978; Thompson, 1979, 1980; Creese, 1980b).

C. testudinaria as an intertidal limpet ranged freely on all rocky substrata at the intertidal of Ohoiwait. The individuals were abundant on bare rock outcrops and big encrusted boulders, but

they were also found on small stones, gravel, pebbles, and shingles. The number of adults and juveniles on permanent substrata was also reasonably stable throughout the period of the study. Only little quantitative information is, however, available on the distribution and abundance of this species in Indonesia or elsewhere in the tropics (Powell, 1973). Therefore, I investigated these aspects of the ecology of *C. testudinaria* in relation to the effects of different environmental regimes at the intertidal rocky shore of Ohoiwait.

3.2. Materials and Methods

3.2.1. Sampling

Fieldwork was undertaken at the intertidal rocky shore of Ohoiwait between October 2001 and September 2002 (Table 3.1). Three different tide levels (from the extreme high water spring tide EHWST to the extreme low water spring tide ELWST) parallel to the shore line (Fig. 3.1) were defined in relation to heights above the mean low water level predicted by local tide tables (Dinas Hidro-Oceanografi TNI AL, 2001; 2002). These zones were identified using staffs and a spirit-level during calm days. In the following, they are high (1 – 1.5 m), middle (0.5 – 1 m) and low (0 – 0.5 m) shore levels. The width of each shore level ranges from 80 m to 85 m (Fig. 3.1).

As pure random sampling was not feasible due to pronounced environmental heterogeneity within the shore levels, a systematic sampling design was chosen. At each shore level, 6 sampling squares (1 m²), 25 m apart, were installed along a line parallel to the shoreline. Hence, a total of 18 permanent quadrates were placed between the EHWST and the water edge, the first square being randomly defined. Heights of the quadrates above ELWST and the distances between quadrates were calculated from the profiles obtained.

In each quadrate, adults (> 25 mm), juveniles (> 10 mm and < 25 mm), and recruits (< 10 mm) were collected by hand picking or dug out with a spade, and the shells were cleaned from the material covered. Sampled specimens were counted and measured for their shell dimensions using vernier calliper to the nearest 0.1 mm, while their total body wet weights were weighed using an analytical balance to the nearest 0.01 g, as this allowed limpets to be measured *in situ* on the shore. In some cases, the juvenile limpets could not be dug out because of their soft and brittle shell. Therefore, their shell was measured directly by placing the vernier calliper to the shell. After measurements, limpets were released back to their habitats.

Most of *C. testudinaria* live attached to the surface of the substratum, although some of them were also found hiding below the boulders, pebbles, and shingles. Rocks lying within the quadrates, which were movable, were turned over and removed down to bedrock, and the undersides of rocks were searched as far as possible, so that concealed limpets were included in the count. Loose small gravel, a habitat sometimes favoured by small individuals, was also carefully searched.

Sampling was conducted monthly during low tide (Table 3.1) and continued until the samples represent the most complete series of data, so that all settled individuals are represented in accordance with their relative frequency in the population.

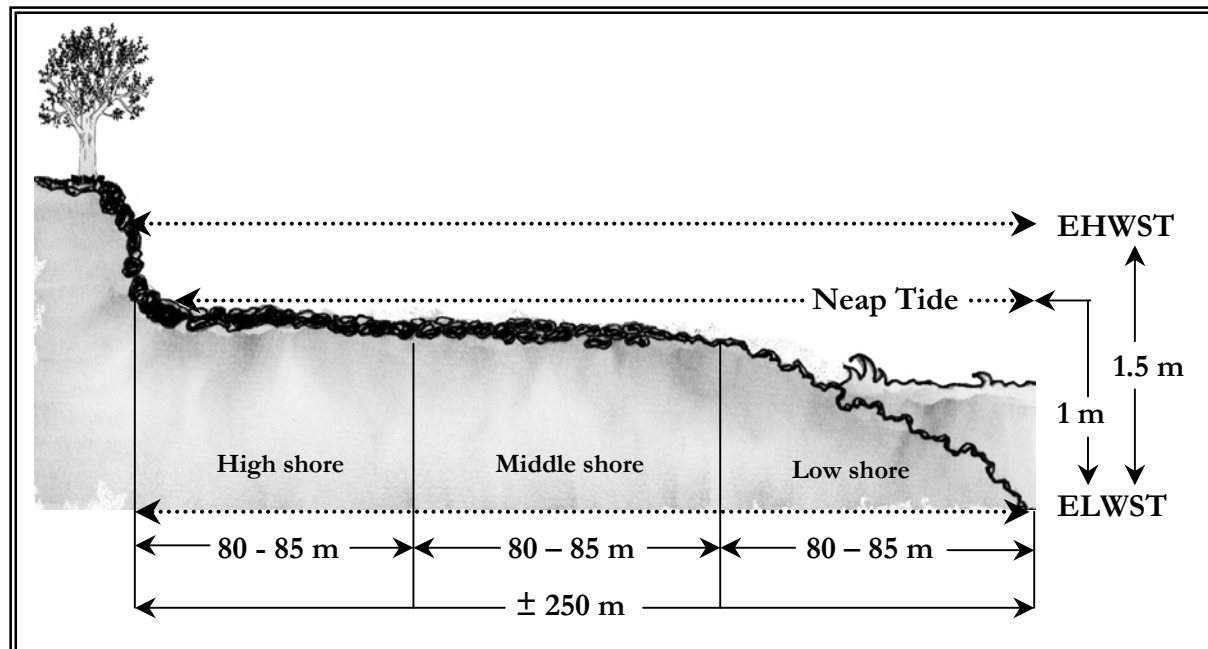


Figure 3.1. Intertidal rocky shore of Ohoiwait with schematic sketch of the tidal range.

Table 3.1. Station list.

* numbers refer to the water level above ELWST (as predicted from tide tables).

Sampling dates	Numbers of limpets	Tidal state *	Moon phases	Season
03 October 2001 (09:00 AM)	394	± 50 cm	Full moon	Transition
03 November 2001 (08:30 AM)	216	± 38 cm	Full moon	Transition
05 December 2001 (11:10 AM)	137	± 23 cm	Last quarter	Rainy
02 January 2002 (09:30 PM)	146	± 23 cm	Last quarter	Rainy
03 February 2002 (10:45 AM)	171	± 45 cm	Last quarter	Rainy
04 March 2002 (11:10 PM)	129	± 45 cm	Last quarter	Transition
03 April 2002 (10:00 AM)	301	± 68 cm	Last quarter	Transition
05 May 2002 (12:10 PM)	161	± 83 cm	Last quarter	Transition
07 June 2002 (17:20 PM)	140	± 68 cm	Last quarter	Dry
05 July 2002 (15:00 PM)	165	± 68 cm	New moon	Dry
05 August 2002 (16:08 PM)	174	± 60 cm	New moon	Dry
05 September 2002 (17:30 PM)	268	± 53 cm	New moon	Transition

In addition to the quantitative samples taken to assess abundances and distribution patterns, 50 specimens of *C. testudinaria* encompassing an even size distribution of limpets within the range of the population (i.e. 15 individual of 8-15 mm length; 20 individuals of 15-25 mm; 15 individuals of >25 mm) were collected monthly for the determination of biomass and energy conversion factors. This standardization of the sample composition minimized the effect produced by “weight” samples on the results of regression analysis (Baxter, 1981). Their shell lengths were measured to 0.1 mm using vernier calliper.

3.2.2. Analyses

3.2.2.1. Distribution and abundance

I used crossed two-way analysis of variance (ANOVA) to test the null hypothesis that “patterns in distribution and abundance of individual limpets did not change significantly between shore levels and months” (Green, 1979; Morrisey et al., 1992; Sokal & Rohlf, 1995; Underwood, 1997). Testing the null hypothesis model, I designed the comparison of vertical and temporal variability using the factors shore level (3 levels) and sampling date (12 levels). This procedure resulted in independent estimates of variability for each level of shore (Searle et al., 1992; Underwood, 1997). The assumption of homogeneity of variances was checked using Hartley’s F_{\max} test (Bakus, 1990; Sokal & Rohlf, 1995). Data transformation of $\log(X+1)$ was used.

The dispersion pattern of limpets in their habitat was assessed using the Morisita Index (Brower and Zar, 1977) $MI = [n(\sum x^2) - n^2]/N(n - 1)$, where n is numbers of quadrates, x is number of individuals per quadrate, and N is total number of individuals. The dispersion can be contagious ($MI > 1$), random ($MI = 1$), or regular ($MI < 1$). This analysis was applied to determine whether environmental factors have any effect on the dispersion of the population, and whether there is no tendency for individuals in population to avoid or to move towards each other.

3.2.2.2. Biomass

The relationship between shell length (L , in mm) and total body wet weight of limpet specimen (WW, in g) was examined by testing the fit of pairs of variables to the allometric equation $Y = A X^b$, or, rewritten in logarithmic form $\ln Y = b \ln X + \ln A$, where y is the total body wet weight and x is the shell length. The constants b and A were estimated by least-squares regression. The variance of b was estimated using the equation $sb^2 = 1/(n-2) * [(y/sy)^2 - b^2]$, where sy and sx are the standard deviations of y and x . Using a value of t from statistical table with $n - 2$ degree of freedom, the 95 % confidence limits were estimated as: $b = t * sb$. Students’ t -test was applied to test the deviation of b from isometry ($b = 3$).

The soft tissues of limpets sampled for determining biomass and energy conversion factors were removed from their shell and dried at about 60°C for 3 days in an oven and finally vacuum-sealed in polythene bags. After transporting them to Ambon, samples were dried to constant weight at 108 °C. The total ash free dry weights (AFDW) of tissues were determined (accuracy 0.1 mg) by incinerating the sample in a muffle furnace at 500 °C for 12 h.

The energy content of the flesh was determined from a representative sample using a Gentry diabatic microbomb calorimeter. For each determination, at least 10 individuals were homogenised and the ash contents of 3 replicate samples and the energy contents of 4 replicate samples were measured.

The relationship between log total body mass (BM, in mg AFDW) and log shell length (L, in mm) were also calculated using least-square method (Sokal & Rohlf, 1995). Using this size-weight relationship in combination with the mean population densities and the mean body sizes, the mean population biomass was computed for each month.

3.2.2.3. Condition Factor (CF)

For each individual, a relative condition factor, CF, can be computed, which measures the influence of various ecological and biological factors that affect the growth rate, reproduction, degree of fitness, and suitability of the environment for the individual (Saad, 1997). King (1995) proposed that the more a limpet weighs for a given length, the greater will be its condition. He suggested that seasonal variations in the condition of a limpet may reflect variations in food abundance and the reproductive stage. CF was calculated as $CF = W_{cal.}/W_{pred.}$, where $W_{cal.}$ is the weight of an individual and $W_{pred.}$ is the weight of this individual predicted from the length-weight relationship. One-way ANOVA was used to test the differences of the monthly mean CF values among the sampling dates.

3.3. Results

3.3.1. Spatial and temporal patterns of abundance

The abundances of the limpet *C. testudinaria* at the three shore levels varied greatly during sampling (Table 3.2). A total of 2402 limpets was obtained in 12 month collections with a mean density for the whole period of study of 11.12 ± 4.51 ind.m⁻². It is evident that densities were significantly greater at the high shore level, with a mean of 15.79 ± 7.54 ind.m⁻², and at the middle shore level, with a mean of 14.67 ± 13.99 ind.m⁻², than at the low shore level, with a mean of 2.90 ± 2.44 ind.m⁻².

Table 3.2. Density of *Cellana testudinaria* at the three shore levels during the period of study. Q - quadrates

Time	High						Middle						Low					
	Q1	Q2	Q3	Q4	Q5	Q6	Q1	Q2	Q3	Q4	Q5	Q6	Q1	Q2	Q3	Q4	Q5	Q6
Oct	15	21	18	27	17	12	45	52	48	36	40	45	3	1	1	5	4	4
Nov	10	13	5	18	6	5	18	30	24	34	15	20	2	5	0	6	3	2
Dec	23	18	22	12	28	12	4	2	2	3	5	2	0	0	2	2	0	0
Jan	21	21	17	20	19	23	1	5	5	7	1	3	1	1	0	0	1	0
Feb	18	25	11	9	30	37	6	3	4	3	2	4	3	2	5	5	3	1
Mar	15	8	22	17	13	30	2	4	3	1	1	5	2	0	1	1	1	3
Apr	17	20	14	22	20	15	31	33	27	18	30	23	5	7	9	3	4	3
May	19	15	18	13	21	38	4	7	3	4	2	5	4	3	0	1	3	1
Jun	11	21	14	27	18	20	3	1	4	3	7	4	2	0	3	0	1	1
Jul	5	3	8	5	13	13	8	20	15	18	18	20	4	5	4	2	2	2
Aug	5	7	7	14	6	6	17	9	19	20	19	15	5	6	5	7	3	4
Sep	8	4	10	11	4	3	43	13	28	12	40	28	3	8	8	11	7	3
Mean	14	15	14	16	16	18	15	15	15	13	15	15	3	3	3	4	3	2

Temporal variability of densities was also very pronounced. Limpets at the high shore level were particularly abundant during the period between December and June. This was caused by seasonal recruitment (see Chapter 6). At the middle shore level, the density did not show the same trend. Significant reductions in density, from 44.33 ind.m⁻² to 3.00 ind.m⁻² and 27.00 ind.m⁻² to 4.17 ind.m⁻², occurred from October to December and from April to May, respectively. On the other hand, pronounced increases occurred following the period of reduction in April, July and September (Fig. 3.2). At the low shore level, the density was generally low. Slight increases were found between the periods of June to September (Fig. 3.2).

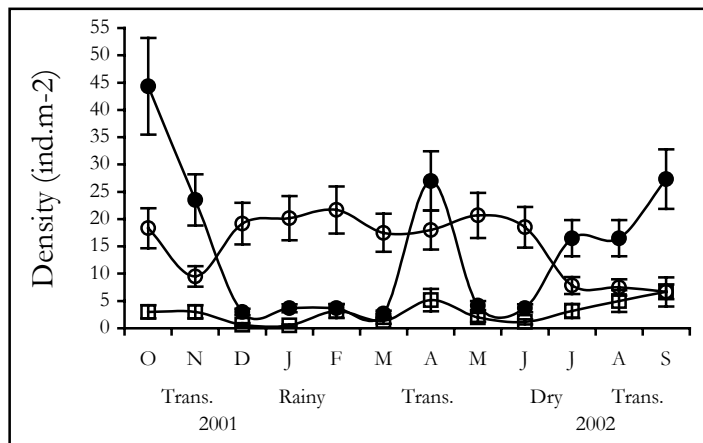


Figure 3.2a. *Cellana testudinaria*. Density (ind.m⁻²) of limpets at different shore levels. o-o High shore level; ●-● Middle shore level; □-□ Low shore level. Vertical lines represent standard deviations (± 1 SD).

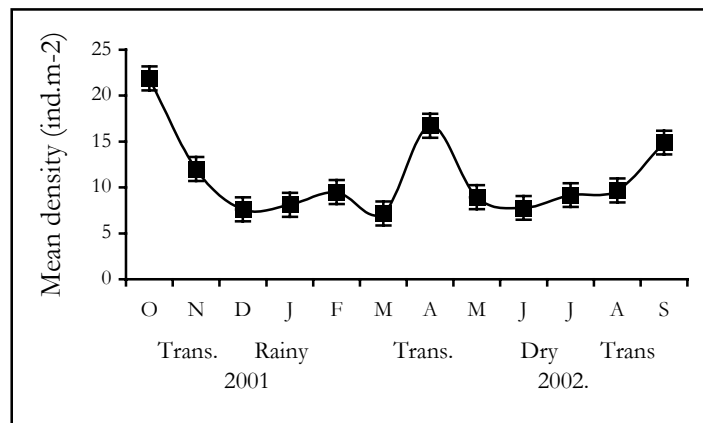


Figure 3.2b. *Cellana testudinaria*. Mean monthly density (ind.m⁻²) of limpets, averaged across shore levels. Vertical lines represent standard error (± 1 SE).

The analysis of variance (ANOVA) of densities of *C. testudinaria* showed significant differences for the shore level, time of sampling, and interaction between shore level and time (Table 3.3). The percentage of the total sum of squares attributable to the parametric effects in the analysis of variance was 86 %. The effects of the shore level and time of sampling removed 44 % and 16 %, respectively, of the total sum of squares. Orthogonal polynomial was calculated on time of sampling using the total densities pooled from the shore levels, and there were significant interactions between time and shore levels. Thus, three distinct outcomes were evident from the analysis of spatio-temporal variation in abundance. The first involved the

variability at small spatial scales, as indicated by the significant differences among the shore levels (Table 3.3). The next two outcomes consisted of the significant main effect of time and of the interaction between time and shore level. Significant differences in density occurred among the months while preliminary analyses indicated that temporal variation might have confounded estimates of spatial variability between sites sampled at different times (Table 3.3). A similar pattern was evident by SNK tests, which could discriminate any specific alternative to the null hypothesis in this case. Exactly the same results were obtained by comparing the variances within the time of sampling, indicating that significant change occurred in abundance of limpets throughout the time. Abundance was greater in October and April significantly more often than in March and December.

Table 3.3. Analysis of variance on mean densities of limpets at the three different shore levels. Hartley's $F_{\max} = 9.48$, $P < 0.05$; values were $\log(x + 1)$ transformed for analysis. SS -sum of squares; df -degrees of freedom; MS -mean square; P -probability; ** highly significant.

Source of variation	SS	df	MS	F	F _{crit}	P
Times of sampling	6.47	11	0.59	18.76	1.84	< 0.05**
Shore levels	17.80	2	8.90	283.70	3.05	< 0.05**
Interaction	10.54	22	0.48	15.27	1.60	< 0.05**
Within	5.65	180	0.03			
Total	40.46	215				

The dispersion of limpet *C. testudinaria* did not follow any seasonal pattern. In all months, the MI values was much greater than 1 (Table. 3.4), implying that the distribution of this species on the three shore levels was highly contagious.

Table 3.4. Dispersion indices of *Cellana testudinaria* together with the chi-square test. Probabilities that are relevant for interpretation of the results are shown. N -total number of individuals; n -number of quadrates; x -number of individuals per quadrates; MI -Morisita Index. χ^2 -chi square.

Month	N	n	Σx^2	MI	χ^2	P
October	394	18	14174	38.09	253.58	< 0.05
November	216	18	4338	21.26	145.37	< 0.05
December	137	18	2479	19.15	188.67	< 0.05
January	146	18	2574	18.66	171.30	< 0.05
February	171	18	3583	22.18	206.03	< 0.05
March	129	18	2203	18.07	178.22	< 0.05
April	301	18	6715	23.62	100.66	< 0.05
May	161	18	3119	20.51	187.88	< 0.05
June	140	18	2326	17.58	158.99	< 0.05
July	165	18	2267	14.54	82.23	< 0.05
August	174	18	2268	13.80	60.58	< 0.05
September	268	18	6360	25.12	196.52	< 0.05

Statistical analysis on the sample dispersions using the Morisita Index (Brower and Zar, 1977) shows a highly significant agreement with the theoretical negative binomial distribution (Table. 3.4), indicating that environmental factors have an effect on the dispersion of the limpets and/or that there was a tendency for individuals to avoid or to move towards each other.

3.3.2. Length-weight relationships

The 2402 collected specimens ranged in length from 8.0 to 31.8 mm with an average of 16.22 ± 5.26 mm. Their total body wet weight varied between 0.11 and 4.93 g with an average of 0.55 ± 0.70 g. The relationships between shell length and total body wet weight for the whole period of study is given in Table 3.5 and presented in Figure 3.5.

Table 3.5. Analysis of variance on the relationship between shell length (L) and total body wet weight (W). *SE*-standard error; *df*-degree of freedom; *SS*-sum of squares; *MS*-mean square.

Regression statistic				
<i>R square</i>		0.85		
<i>SE</i>		0.1507		
<i>Observations</i>		2402		
<i>Mean length</i>		16.22 ± 5.26		
<i>Mean weight</i>		0.55 ± 0.70		

ANOVA				
Sources	df	SS	MS	F
Regression	1	299.8435	299.8435	13194.79
Residual	2400	54.5385	0.0227	
Total	2401	354.3821		

Sources	Coeff.	SE	t-stat
Intercept (A)	- 3.6303	0.0277	- 131.0070
Variable (b)	2.6601	0.0232	114.8686

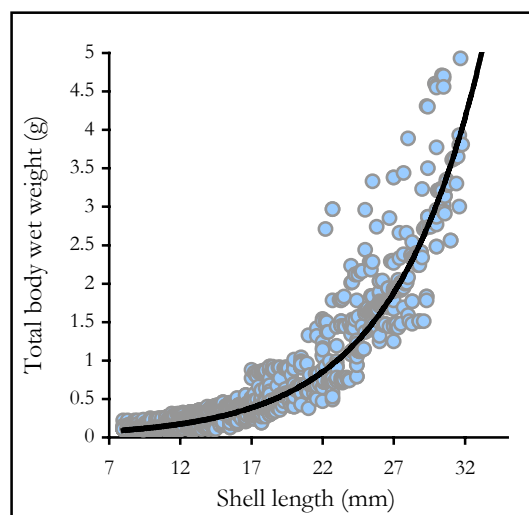


Figure 3.5. *Cellana testudinaria*. Relationship between shell length (L) and total body wet weight (W).

The power regression equation of this relationship is $W = 0.0002343 L^{2.6601}$ ($r = 0.92$). Statistical analysis of the 95 % confidence intervals on the relationship gives the value of $A = \pm 0.000031$ and $b = \pm 0.0454$ (t -test = 27.27, $P < 0.05$).

Table 3.6. Relationship between body mass (BM, AFDW mg) and shell length (L, mm) according to $BM = A * L^b$ for all sizes of *Cellana testudinaria* (N = 600) during monthly collection.

Date	A	b	r
October	0.03475	2.8949	0.98
November	0.02995	2.8456	0.95
December	0.03249	2.3277	0.93
January	0.06663	2.2242	0.92
February	0.06095	2.3168	0.97
March	0.02721	2.5942	0.95
April	0.03871	2.6513	0.95
May	0.02356	2.8165	0.90
June	0.03779	2.9191	0.94
July	0.01623	3.2421	0.93
August	0.00448	3.5274	0.95
September	0.01562	2.8843	0.98
Equations	BM = 0.03236 L ^{2.7703}		

Table 3.6 gives the monthly relationships between body mass (AFDW) and shell length calculated from 600 specimens (50 specimens collected in each month). The overall relationship is $BM = 0.03236 L^{2.7703}$ ($r = 0.95$). The standard deviations (SD) of the intercept-*A* and the variable-*b* of this relationship are 0.0178 and 0.3813, respectively.

3.3.3. Conversion factors

The ash-free body weight (mg AFDW) and total body wet weight (mg WW) of mid-size classes, computed using the equations $W = 0.2343 L^{2.6601}$ and $BM = 0.03236 L^{2.7703}$ were used to estimate a WW-AFDW conversion factor (Table 3.7). The linear relationship is $BM_{(mg\ AFDW)} = -5.1361 + 0.2026 W_{(mg)}$ (Fig.3.6), which is highly significant ($r = 0.99$) (Table 3.8). Ratios of AFDW/WW range from 0.17 to 0.20 with an average of 0.19 ± 0.0018 (Table 3.7), indicating that body weight (mg AFDW) of specimens is on average 19 % of the total body wet weight (mg WW).

Table 3.7. *Cellana testudinaria*. Ratios between ash-free body weight and total body wet weight of the specimens with the midpoints of class interval length of 8.5 – 31 mm. L-midpoints of class interval length; W-total body wet weight (mg); BM-body mass (mg AFDW).

L	WW	AFDW	AFDW/WW
8.5	69.52	12.16	0.17
9.5	93.46	16.54	0.18
10.5	121.97	21.83	0.18
11.5	155.36	28.08	0.18
12.5	193.94	35.38	0.18
13.5	238.00	43.79	0.18
14.5	287.82	53.38	0.19
15.5	343.70	64.21	0.19
16.5	405.88	76.35	0.19
17.5	474.65	89.87	0.19
18.5	550.27	104.82	0.19
19.5	632.98	121.28	0.19
20.5	723.05	139.30	0.19
21.5	820.71	158.95	0.19
22.5	926.22	180.29	0.19
23.5	1039.80	203.37	0.20
24.5	1161.70	228.25	0.20
25.5	1292.14	255.00	0.20
26.5	1431.36	283.68	0.20
27.5	1579.58	314.33	0.20
28.5	1737.02	347.03	0.20
29.5	1903.91	381.82	0.20
30.5	2080.45	418.76	0.20
31.5	2266.88	457.91	0.20

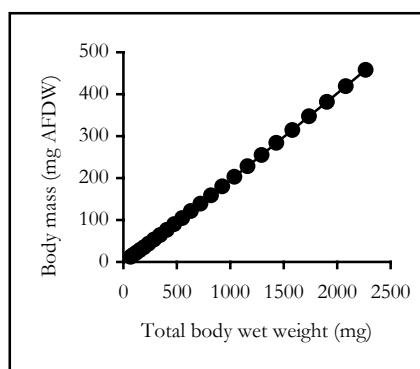


Figure 3.6. *Cellana testudinaria*. Relationship between total body wet weight (mg) and Body mass (mg AFDW).

Table 3.8. Analysis of variance on the relationship between total body wet weight (mg) and body mass (mg AFDW). *SE*-standard error; *df*-degree of freedom; *SS*-sum of squares; *MS*-mean square.

Regression statistic	
<i>R square</i>	0.99
<i>SE</i>	1.96
<i>Observations</i>	24
<i>Mean WW (mg)</i>	855.4 ± 681.6
<i>Mean AFDW (mg)</i>	168.18 ± 138.12

ANOVA				
Sources	df	SS	MS	F
Regression	1	438689.6	438689.6	114738
Residual	22		3.82	
Total	23			

Sources	Coeff.	SE	t-stat
Intercept (A)	- 5.1361	0.6489	- 7.92
Variable (b)	0.2026	0.0006	338.73

The mean energy content of *C. testudinaria* from Ohoiwait is 21.59 kJ.g^{-1} AFDW.

3.3.4. Biomass

The population biomass values were calculated from the mean density of *C. testudinaria* multiplied by the monthly mean AFDW of specimens (Table 3.9). The average biomass was $1012.84 \pm 748.10 \text{ mg AFDW.m}^{-2}$ (Table 3.9).

A loss in population biomass between October 2001 and January 2002 was followed by an increase, reaching the highest value of 3235.75 mg AFDW.m⁻² in September 2002 (Fig. 3.7). The lowest monthly biomass of 544.49 mg AFDW.m⁻² was recorded in March 2002 (Fig. 3.7). The monthly mean biomass during the rainy season (619.08 mg AFDW.m⁻²) was lower than during the dry season (899.01 mg AFDW.m⁻²).

Table 3.9. *Cellana testudinaria*. Monthly mean biomass. Density is derived from Fig. 3.2 and body mass (BM) from the equation $BM_{(mg\ AFDW)} = 0.2026 W_{(mg)} - 5.1365$.

Date	Density (ind.m ⁻²)	BM (mg AFDW)	Biomass (mg AFDW.m ⁻²)
October	21.89	59.07	1293.07
November	12.00	71.27	855.24
December	7.61	86.46	657.95
January	8.11	68.56	556.00
February	9.50	67.71	643.29
March	7.17	75.94	544.49
April	16.72	58.87	984.25
May	8.94	76.85	687.00
June	7.78	74.50	579.59
July	9.17	88.53	811.80
August	9.67	135.02	1305.64
September	14.89	217.31	3235.75
Mean	11.12	90.00	1012.84

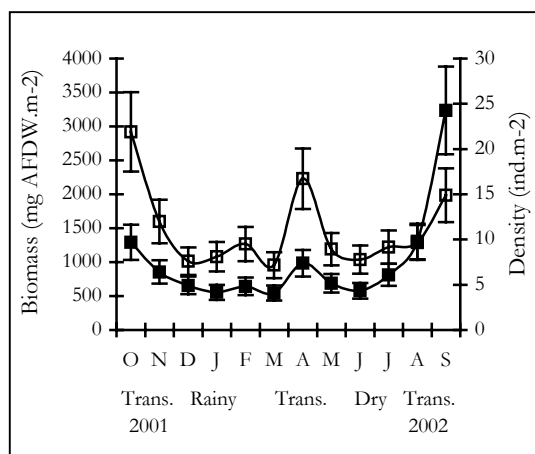


Figure 3.7. *Cellana testudinaria*. Monthly means of biomass (■) and density (□) for all size classes of limpets collected from the three different shore levels. Vertical lines represent standard deviation.

Variations in monthly mean biomass of *C. testudinaria* at the three different shore levels are summarised in Table 3.10.

Table 3.10. Summary of monthly mean biomass of limpet *C. testudinaria* living at the three different shore levels. *D*-density (ind.m⁻²); *BM*-body mass (mg AFDW); *B*-biomass (mg AFDW.m⁻²).

Date	High shore			Middle shore			Low shore		
	D	BM	B	D	BM	B	D	BM	B
October	18.33	60.30	1105.36	44.33	45.92	2035.58	3.00	184.50	553.49
November	9.50	54.23	515.14	23.50	59.70	1402.84	3.00	146.00	438.01
December	19.17	82.59	1583.24	3.00	165.66	496.97	0.67	198.48	132.98
January	20.17	66.38	1338.91	3.67	105.28	386.38	0.50	86.03	43.02
February	21.67	60.71	1315.55	3.67	149.85	549.96	3.17	65.37	207.22
March	17.50	73.47	1285.77	2.67	149.04	397.94	1.33	91.71	121.97
April	18.00	26.67	480.09	27.00	48.55	1310.92	5.17	95.35	492.98
May	20.67	106.50	2201.27	4.17	177.41	739.78	2.00	276.68	553.36
June	18.50	107.51	1988.92	3.67	194.42	713.54	1.17	167.88	196.42
July	7.83	147.83	1157.48	16.50	128.17	2114.88	3.17	170.11	539.26
August	7.50	158.77	1190.75	16.50	120.48	1987.85	5.00	163.02	815.11
September	10.67	235.35	2511.18	27.33	278.91	7622.57	6.67	282.35	1883.29

From Figure 3.8, it can be seen that the biomass of limpets living at the high shore level was higher than at the other shore levels during the rainy season (December – February), whilst during the dry season (June – August), the mean biomass of limpets living at the middle shore level was higher than at the other two shore levels.

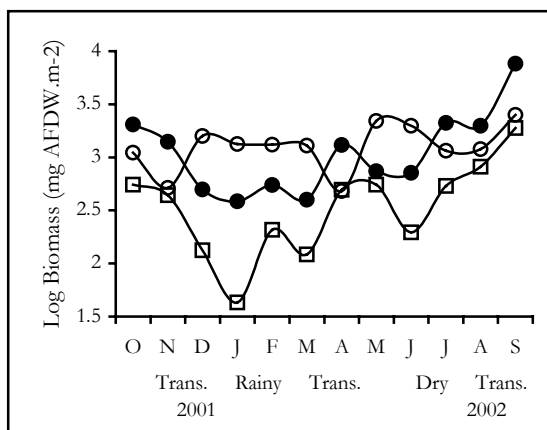


Figure 3.8. Monthly mean biomass of *C. testudinaria* living at the three different shore levels. ○-○ high shore level; ●-● middle shore level; □-□ low shore level.

Two-way ANOVA (Table 3.11) shows that biomass differences among shore levels and months were significant ($F = 2.49$ and 4.61 , $P < 0.05$).

Table 3.11. Analysis of variance on mean biomass of limpets at the three different shore levels. F -values that are relevant for the interpretation of the results are shown in **bold**. SS -sum of squares; df -degrees of freedom; MS -mean square; P -probability; * significant.

Source of Variation	SS	df	MS	F	P-value	F crit
Sampling time	20286431	11	1844221	2.49	$P < 0.05^*$	2.258517
Shore level	6825722	2	3412861	4.61	$P < 0.05^*$	3.443361
Error	16295932	22	740724			
Total	43408084	35				

3.3.5. Condition Factor (CF)

The mean monthly condition factors were calculated for individuals *C. testudinaria* sampled at the three shore levels (Table 3.9). Mean CF values ranged between 1.15 and 1.42, with a grand average of 1.25 ± 0.08 . The overall mean value of 1.25 was higher than 1, indicating that the mean weight of limpets higher than predicted from the length-weight relationship equation. The highest mean CF value was observed in December whilst the lowest was recorded in November 2001 and April 2002. The mean CF value of 1.27 of limpets living at the middle shore level was a bit higher than the other shore levels. Mean CF of 1.30 during the rainy season was slightly higher than the value of 1.25 during the dry season. Analysis of variance of the mean CF showed, however, that there was no significant difference between shore levels and months ($F = 0.99$ and 0.44 , $P > 0.05$).

Table 3.9. *Cellana testudinaria*. Monthly variation of the mean condition factor (CF) for limpets living at the three different shore levels. *H*-high shore level; *M*-middle shore level; *L*-low shore level.

Date	H	M	L	Mean
October	1.12	1.21	1.49	1.27
November	1.10	1.28	1.07	1.15
December	1.38	1.30	1.59	1.42
January	1.32	1.19	1.00	1.17
February	1.30	1.54	1.09	1.31
March	1.31	1.29	1.03	1.21
April	1.02	1.15	1.28	1.15
May	1.23	1.31	1.19	1.24
June	1.27	1.32	1.08	1.22
July	1.30	1.27	1.33	1.30
August	1.34	1.14	1.18	1.22
September	1.28	1.27	1.30	1.28
Mean	1.25	1.27	1.22	1.25

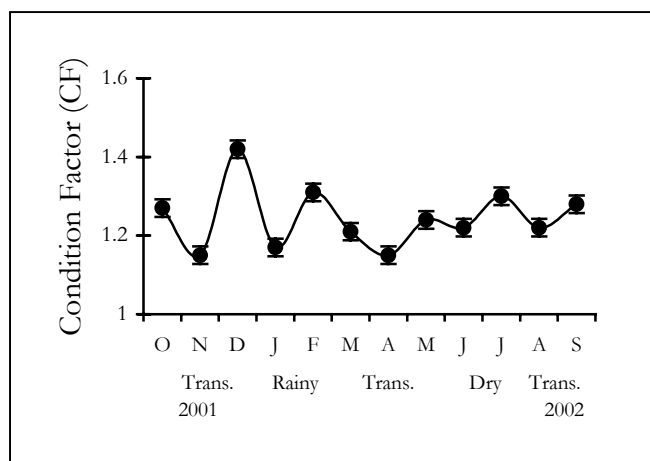


Figure 3.9. *Cellana testudinaria*. Seasonal variation of the mean condition factor (CF). Vertical lines represent standard error.

3.4. Discussion

3.4.1. Spatio-temporal distribution patterns

This study shows that the density of *C. testudinaria* was very variable on the three shore levels examined (Fig. 3.2), with the density of individuals being greatest at the high shore level running down to the lowest one at the low shore level. Similar patterns were also found by Underwood (1975) for *Cellana*, by Sutherland (1970) for *Acmaea scabra*, by Lewis & Bowman (1975) and Thompson (1980) for *Patella vulgata*, and by Creese (1980b) for *Notoacmaea petterdi*. Although Fletcher (1984) reported that population of *Cellana tramoserica* was denser at the mid-shore region than at the high and low intertidal regions of Cape Bank, New South Wales, Australia, and that the abundances of intertidal population were generally higher than those of subtidal populations. In contrast, the abundances of *Patelloida pygmaea* off Wu Kwai Sha, Hong Kong were highest at the lower shore (Liu, 1994), and *Patella flexuosa* occurred only in the lower intertidal zone of the sandstone rocky reef shore near the Seto Marine Biological Laboratory, Japan (Iwasaki, 1998).

Besides the clear spatial pattern, there was also a pronounced seasonal variation. The mean density of *C. testudinaria* was greater at the middle than at high or low shore levels in April and from July to October, while it was greater at the high shore level during rainy season (Fig. 3.2). This resulted from an increase in abundance at the middle shore level from the rainy to the dry season, while a decrease in abundance at the high shore level occurred from the rainy to the dry season. These patterns can be explained as a seasonal response of the animals to physical stress, indicating that insolation is an important factor in tropical intertidal regions, as reported by Moore (1972). The abundance of *C. testudinaria* at the high shore level is reduced during the

dry season because in this season most limpets migrate down to lower levels. Field observations showed that many limpets migrate within the intertidal zone and scatter and regroup at certain places at the middle shore level. These movements have been related to temperature changes (Lewis, 1954) and are probably regulated by the location of rock pools and crevices on the sheltered side of boulders, which protect the limpets, particularly at low tide, from the heavy insolation. Seasonal migrations have been also observed in *C. testudinaria*, which contributes to the clumped distribution of the limpets. The result of sample dispersion analyses was not surprising and corroborated the findings of many other studies on rocky shores, reporting migration as a prominent process maintaining the spatial structure of assemblages (Liu, 1994).

Higher biomass at the middle shore level in October 2001, April and September 2002 (Fig. 3.2 and 3.9) also suggests that limpets migrated to this shore level to avoid desiccation and strong wave action during this month (Fig. 2.5). Small limpets at the middle shore level were proportionally more abundant than large ones in April 2002. By virtue of their smaller size, they can enter shelter from desiccation and wave action. Small-scale migrations occur in many limpet species and have been considered to be a behavioural response to physical stress (reviewed by Branch, 1981; Hartnoll, 1985; Ruiz Sebastián et al., 2002), including avoidance of desiccation (Branch & Cherry, 1985) and survival of wave action (Gray & Hodgson, 1998). Migration patterns can be affected by extrinsic factors including shore height and slope, desiccation and food availability, or by intrinsic factors such as limpet size (Williams et al., 1999; Jenkins & Hartnoll, 2001).

Pattern in assemblages were significantly variable between shore levels. In an analysis of the structure of assemblages on a rock platform in New South Wales (Australia), Underwood (1981) found considerably differences in distribution of organisms from one part of the shore to another and from season to season, in addition to variability among tidal levels. This pattern was related to the effects of spatial physical factors such as slope of the substratum, which is important in maintaining differences between mid-shore and low-shore assemblages (Benedetti-Cecchi, 2001). Therefore, spatio-temporal heterogeneity in the physical features of the habitat could generate spatio-temporal variations in abundance of organisms regardless of the assemblage. This result is consistent with the findings of other studies of rocky shores indicating large differences in the structure of assemblages from top to the bottom of the shore (Southward, 1958; Lewis, 1964), highlighting the importance of physical factors (Benedetti-Cecchi et al., 2000).

Most univariate analyses indicated significant spatial variability and, even more pronounced, temporal variations. These results support a model of spatial distribution of organisms on rocky shores where vertical variability is at least as important as any other scales of variability on an ecosystem (Liu, 1994; Benedetti-Cecchi et al., 1996; Benedetti-Cecchi, 2001). Multivariate analyses produced similar results, which are, however, more complicated in interpretation because of the large number of processes that may potentially regulate the

structure of assemblages and the direct and indirect effects of interactions among organisms. This suggests that patterns were primarily governed by biological processes, which is not lost when examining patterns in assemblages (Wootton, 1993; Menge, 1995; Benedetti-Cecchi, 2000). Therefore, the question why these or any other processes contributing to multivariate variation produced more variability in distribution and abundance of *C. testudinaria* remain still unknown. Nevertheless, meaningful comparisons of spatio-temporal variation in abundance and biomass were possible. Differences from one level to another within any particular shore, as well as inconsistencies in temporal changes, accounted for a proportion of variability in pattern of distribution, which was at least as large as that explained by the differences in environmental gradient.

3.4.2. Effects of environmental variables

The distribution and abundance of intertidal limpets are considerably influenced by environmental conditions. Early studies of rocky shores in temperate regions have explored the responses of vertically separated groups of organisms to presumably steep environmental gradients (Davies, 1969; Sutherland, 1970; Wallace, 1972), which affect the exposure of organisms to the tidal regime (Smith, 1975), and wave action (Stephenson & Stephenson, 1949; Southward, 1958; Lewis, 1964). The response of organisms are thought to reflect their physiological tolerance to physical factors (Benedetti-Cecchi et al., 1999). Several aspects such as height of splash, latitude of locality, timing of low water spring tides, and presence or absence of food were also considered as important factors determining the upper limits of the distribution of the species (Orton, 1920; Evans, 1947; Southward & Orton, 1954). For instance, the upper limit of *Patella aspera* is reported as the mean high water of neap tides (Southward & Orton, 1954). Off Ohoiwait, immerse and splash to the water during tidal excursion are the critical factor that affects *C. testudinaria* more abundant at the middle shore level on the period between September to November (Fig. 2.5 and Fig. 3.2). Unfortunately, it is not evident whether there is any direct physiological necessity for exposure to the wave action or to the water during immersion at some stage in the life of *C. testudinaria*, or whether the effect is an indirect one. Thus, it is important to distinguish between response variations attributable to vertical height on the shore and those attributable to the amount of wave action the shore receives. Nevertheless, the high density in April (Fig 3.2) presumably indicated that wave action is an important factor of the precise nature of the effects, because significant numbers of limpets were found at higher shore levels, coinciding with higher wave action. Branch (1981) pointed out that many limpets occur intertidally and are subject to intermittent wetting and drying during each tidal cycle. Several physical factors, therefore, may potentially be stressful under these conditions, including desiccation, temperature and salinity. These factors are often inter-related so that it is often not possible to separate their effects under field conditions. Although the present study did not investigate causal processes explicitly, the patterns described here may provide some clues on the

causes of the spatial and temporal variation in the distribution of limpets at the intertidal of Ohoiwait, and were successful in identifying the factors which most probably regulated the spatio-temporal heterogeneity.

The structure of intertidal limpet assemblages cannot be predicted reliably on the basis of vertical position on the shore alone. This study has shown that environmental factors may influence patterns of the distribution of intertidal populations both directly, by imposing physiological constraints on their life, and by mediating their activity. In some cases, physical factors may operate additively. This may occur, for example, during the dry season in the upper shore habitats where the annual variation of the average insolation was highest (Fig. 2.3). Desiccation resistance is one physiological response related to the insolation, which may be variable and allow a species to inhabit a variety of intertidal areas. Previous studies of desiccation response have shown that upper intertidal limpets are able to withstand greater loss of body water than lower intertidal conspecifics (Davies, 1969; Wallace, 1972; Branch, 1981).

The patterns of variation in distribution and abundance documented in the present study can be expected to also occur on other rocky shores of the tropical regions. In conclusion, the results of this study indicated that environmental variables are good predictors of the distribution and abundance of limpets in the intertidal regime, even though it is not feasible to determine the single most important factor. The understanding of ecological heterogeneity requires sampling at several spatial and temporal points, which may reveal more about structure of natural assemblages than simply focusing on the most obvious environmental gradients. This suggests that any investigation of the distribution of populations and assemblages along environmental gradients should be integrated in a hierarchical analysis to fully represent the complexity of an ecosystem.

3.4.3. Condition

The average CF value of 1.25 of *C. testudinaria* in this study is similar to the value of 1.23 of *Cellana karachiensis* from the Gulf of Oman and the Arabian Gulf (Emam, 1994) but higher than those of *Cellana radiata* from the Gulf of Agaba, Red Sea (Ismail & Elkarmi, 1999). This difference is probably due to the fact that Ismail & Elkarmi (1999) used dry weight for CF calculations, while in the present study and also Emam (1994) wet weights were used.

A variety of factors controls the condition of limpets. These are environmental factors (temperature, desiccation, waves) exerting physical stress, as well as biotic factors, such as sex, age and stage of maturity, stomach contents and others (Pauly, 1984; King, 1995). Wright & Hartnoll (1981) recorded that lower total soft-body dry weight of *Patella vulgata* in March each year are a result of both reduced feeding activity during winter and the release of gametes which comprise a greater proportion of the total soft-body weight with increasing overall size. Unfortunately, analyses of these factors were not included in this study because of the limited of equipments and time.

Chapter 4

DETERMINATION OF GROWTH

4.1. Introduction

The tropical limpet *C. testudinaria* is reasonably well known from systematic and geographical distribution aspects (Schepman, 1908; Oostingh, 1925; Habe, 1964; Powell, 1973; Wilson, 1993), but there are no published studies regarding its growth or other aspects of its biology. Many investigations about growth rates of various Patellacea that have been carried out, focusing on several factors affecting the growth, such as tidal height (Sutherland, 1970), water temperature (Balaparameswara Rao, 1976), seasonal changes and food availability (Parry, 1978; Brêthes et al., 1994), and using a variety of measurement techniques such as labelling (Ward, 1967; Balaparameswara Rao, 1976; Fletcher, 1987), growth rings (Picken, 1980), and the distribution of size cohorts (Cassie, 1954; Underwood, 1975; Fletcher, 1987; McGrath, 1992). As far as age and growth are concerned, an extensive survey of literature is given, i.e. for *Patella vulgata* (Russell, 1909; Orton, 1928), *Patina pellucida* (Graham & Fretter, 1947), *Cellana radiata* (Balaparameswara Rao, 1976; Ismail & Elkarmi, 1999), *Palloida alticostata* (Fletcher, 1987), *Cellana eucosmia* (Saad, 1997).

A burst of theoretical contributions regarding the estimation of growth parameters from growth data (Ebert, 1973; Yamaguchi, 1975) has led to clarification of the concept of the effects of environmental variables on the growth of limpets (Balaparameswara Rao, 1976; Creese, 1981; Fletcher, 1987). Frank (1969) examined growth rates of some tropical gastropods in an attempt to determine whether life spans were under primary temperature control, as suggested by Levins (1968) in quite a different context, or whether some other feature of the milieu of these animals determined their longevity. He concluded that most of the truly large shelled molluscs inhabit tropical seas; but within species, maximum size usually seems to be attained toward the higher latitudes. In a review of the physiological variation among intertidal molluscs, Newell (1964) concluded that on the whole southern species have a shorter life and attain a smaller final size than northern ones. He cited evidence from the north Atlantic and Pacific coasts of North America indicating that this generalization also holds within species with broad latitudinal distribution. These findings imply that the differences of growth parameters between limpets in different regions, which have some generality, can be explained either by a single primary environmental effect or may result from a combination of interaction between environmental and other factors.

C. testudinaria is an excellent species to study growth, as they are present in high numbers, range freely on all rocky substrata, and the shells of these animals show distinct dimensions, suggesting a possibility for measurements and exact growth determinations. The notion that environmental stress is generally an important factor which directly or indirectly influences the population dynamics of common intertidal animals (Picken, 1980; Fletcher, 1987; Lohse, 1993)

has encouraged me to form the same hypothesis. This chapter, therefore, presents information on the growth rates of *C. testudinaria* and some factors presumably affecting them. Moreover, the results are compared it with those of similar limpets from temperate regions.

4.2. Materials and Methods

4.2.1. Growth measurements

In order to determine size distributions, growth rates and longevity of *C. testudinaria*, the same specimens collected for the study of distribution, abundance and biomass were used. There are three main techniques to estimate the growth parameters. Firstly, by interpretation of annual growth lines, which laid down on the shell of individual animals (Kenny, 1969; Parry, 1978). The formation of growth bands are caused by periods of slower and faster shell growth (Lammens, 1967). It is often been suggested that the shell edge is extended and withdrawn alternatively resulted in faster or slower growth (Fairbridge, 1952). During winter, dark band (slower growth) is formed, and the material would be mainly deposited under the old edge of the shell. If conditions for growth are favourable again (during summer), normal growth of the shell recommences, which denote light band (Picken, 1980). However, this method seems not to be applicable for the animals living in the tropical regions, because the marked bands are very difficult to distinguish between seasonal periods (Pauly, 1982). In the present study, 100 specimens of various sizes at the three shore levels were randomly sampled. Only very few specimens of *C. testudinaria*, however, have such clear growth marks that all consecutive marks can be identified and measured without doubt. It was not possible, therefore, to estimate the growth rate through the direct counting of annual growth on the shells. Especially in older specimens, the earliest growth lines along the apex of the shell become obscured by erosion and by a covering of encrusting algae, and precise aging becomes impossible.

Secondly, growth can be determined by marking individuals and measuring their sizes periodically (Frank, 1965; Seapy, 1966; Ward, 1967; Black, 1977; Fletcher, 1987). I attempted to obtain growth rates using this method by marking individuals with Tipp-Ex (Tipp-Ex GmbH & Co. KG, Frankfurt am Main, Germany). Approximately 50 to 60 of these marked individuals (from the three shore levels) were measured to the nearest 0.1 mm using vernier callipers at monthly interval from October 2001. It was, however, difficult to mark individual *C. testudinaria* especially if they occurred low on the shore in damp microhabitats and were very exposed to wave action. Loss of paint marks occurred frequently during the period of sampling, and all marked individuals had lost their marks in January 2002. Therefore, no estimates of individual growth rates could be assessed.

Finally, a population may be sampled periodically and the mean size of the individuals within distinct age cohorts can be calculated from polymodal size frequency distributions (Ward, 1967; Ebert, 1973; Underwood, 1975; Yamaguchi, 1975; Choat, 1977). Age classes were defined through analysis of length frequencies distributions to decompose monthly size-frequency into

their component normal curves (Brêthes et al., 1994). The estimate of growth used the monthly size frequency data, whereby the mean sizes of cohorts were followed through time. Early attempts in using this method to obtain rates of growth have often been unsuccessful because of the inability to separate the various age classes (Ebert, 1973). The first one or two age classes are often distinct and are easy to separate either by inspection or by using probability paper (Harding, 1949; Cassie, 1954; Blackmore, 1969a), but this is difficult to apply for old individuals, which often form a single mode at the right side of a size distribution (Ebert, 1973). In addition, Frank (1969) and Yamaguchi (1975) stated that the assumptions underlying the estimation of growth from size frequency data is rarely met and hence, they often do not give any conclusive picture in analysing population dynamics of marine sedentary invertebrates, because such animals may tend to grow slowly with long life spans and unpredictable recruitment. Nevertheless, I used this method anyway because it is considered robust and supplementary by most authors, to estimate the parameters of growth, and since the first two methods failed in analysing.

4.2.2. Analyses

Population structure. The population structure of *C. testudinaria* was analysed using the Modal class Progression Analysis, MPA (Gayanillo et al., 1996). This methodology infers growth from the apparent shift of the modes or means in a time series of length frequency samples. It was applied to the frequency histograms of the limpets' shell lengths to divide them into distinctive cohorts. MPA consists of 2 methods: Bhattacharya's method (Bhattacharya, 1967) and SEPARation of the NORMally (NORMSEP) distributed components of size-frequency samples. This analysis involves 3 stages: (1) decomposition of composite distributions into their components to identify means, (2) subjective identification and "linking" of the means perceived to belong to the same cohorts and (3) using the growth increments and size-at-(relative)-age data resulting from the linking to estimate growth parameters. The individual cohorts were separated using both techniques by the aid of FiSAT software (Sparre & Venema, 1998) (Appendix 3). In cases where the individual cohorts of inflection were ambiguous, however, a student t-test automatically tests the significance of the difference between two successive distribution units. In this analysis, the relative age provided by the modal progression analysis will be transformed into any absolute age in years and fractions of years, which are assumed to belong to the same cohorts. Thus, this will arbitrarily set the birthday on January 1st (Brêthes et al., 1994). However, according to Pauly (1982), such assumptions may be "dangerous" if we do not have a preliminary idea about the species' growth. The modal progression analysis should, therefore, have the following assumptions: the growth was reasonable according to the previous works; for the single cohort, the mean length at the end of the year should be close to the mean length of the older cohort one year before; reproduction occurs only once a year (Picken & Allan, 1983).

Growth. A number of equations have been developed to describe growth in limpets, one of the most widely used being that developed by von Bertalanffy (1938) that has come to be known as the Bertalanffy equation:

$$L_t = L_\infty [1 - e^{-K(t - t_0)}]$$

where L_t is the length at age t , L_∞ is the asymptotic length (representing the maximum theoretical length of animal), K the growth coefficient (describing the rate of growth of the animal to its maximum size), and t_0 the theoretical age at length 0 (the start of growth of the settled larva); the intercept between the curve and the t axis. The equation has been used extensively in fisheries biology (Beverton & Holt, 1957; Allen, 1969; Pauly, 1982; Sparre & Venema, 1998) and has also been applied to limpets (Ward, 1967; Brêthes et al., 1994; Saad, 1997; Ismail & Elkarmi, 1999). It gives a good approximation for observed growth rate data obtained from a number of animals with various initial sizes for a particular period, often 1 year (Frank, 1969; Gulland, 1969; Yamaguchi, 1975).

The parameter L_∞ was estimated using ELEFAN I and the Maximum Length Estimation (MLE), while K was estimated from ELEFAN I corresponding to L_∞ . All estimations were carried out using computer program FiSAT (Sparre & Venema, 1998). This work involved the use of three routines available in the program: (1) response surface generator (to identify the best combination of parameters), (2) direct search for the “optimum” combination of growth parameter values, and (3) curve fitting by eye (to fit a growth curve through the structured sample). These three routines use a new index of the goodness of fit (Rn) to avoid getting negative values. Rn is computed as $10^{(ESP/ASP)}/10$, with ESP (Explained Sum of Peaks) over ASP (Available Sum of Peaks) as a measure of goodness of fit.

Statistical analysis. Seasonal variation in the growth of cohorts was assumed to be negligible in the present study, because growth rates calculated at different times could confound an analysis of seasonal variability. As a consequence, I proceeded with the comparison of regression lines resulting from the relationships between shell length and the numbers of months, using an analysis of covariance ANCOVA (Snedecor & Cochran, 1967). This test determines the level of similarity between the samples, and, in case of heterogeneous relationships, obtained the nature of the source of the differences (Baxter, 1983). Bartlett’s test (Snedecor & Cochran, 1967) was used to check the assumption of homogeneity of the residual variances before using ANCOVA.

t_0 and longevity. The computation of t_0 requires the setting of a birth date coinciding with the peak of the breeding season (Lopez Veiga, 1979). When a single birth date is assigned, the quality of the t_0 estimation is surely affected, particularly the variance around t_0 . This problem

occurs mainly for species with a long breeding season (Moreau, 1987). Lopez Veiga (1979) suggested, however, that t_0 can be calculated from L_∞ and K because the von Bertalanffy plot is a more robust method for estimating t_0 than other methods. t_0 was calculated using the formula: $t_0 = 1/K * \ln [(L_\infty - L_t)/L_\infty]$ where K is the growth coefficient, L_∞ is the asymptotic length, and L_t is the length at age $t = 0$ or length of recruits.

The longevity can be defined as the natural life span of a species in which 99% of a cohort would have died if it had been exposed to natural mortality only (Pauly, 1982). This parameter can be estimated as $3/K$ when $L_{max} = 0.95 L_\infty$ (Taylor, 1965).

Asymptotic weight. If the length-weight relationship is approximately cubic, then the asymptotic weight W_∞ can be calculated as $W_\infty = A L_\infty^3$. So, the von Bertalanffy growth formula (VBGF) can be written in the form: $W_t = W_\infty [1 - e^{-K(t-t_0)}]^3$, where W_t is the weight at age t and W_∞ is the asymptotic weight.

4.3. Results

4.3.1. Population structure

The size structure of the population was studied through the analysis of percentage size frequency distribution (Fig. 4.1). The overall specimen sizes ranged from 8.0 to 31.8 mm, representing 6 cohorts. The first cohort consists of juvenile limpets with an average length of < 10.5 mm, representing 13 % of the total population. The second cohort encompasses the limpets with lengths of 10.5 – 15.5 mm, accounting for 48 % of the total population. This sub-population generally presents the group of limpets with the highest growth rates (Russell, 1909). The remaining cohorts consist of limpets with an average length of 15.5 – 20.5 mm, 20.5 – 25.5 mm, 25.5 – 30.5 mm, and > 30.5 mm, accounting for 22 %, 9 %, 7 %, and 1 % of the total population, respectively. Specimens > 25.5 mm are considered to be mature. This is the common length of *Cellana* (Balaparameswara Rao, 1973).

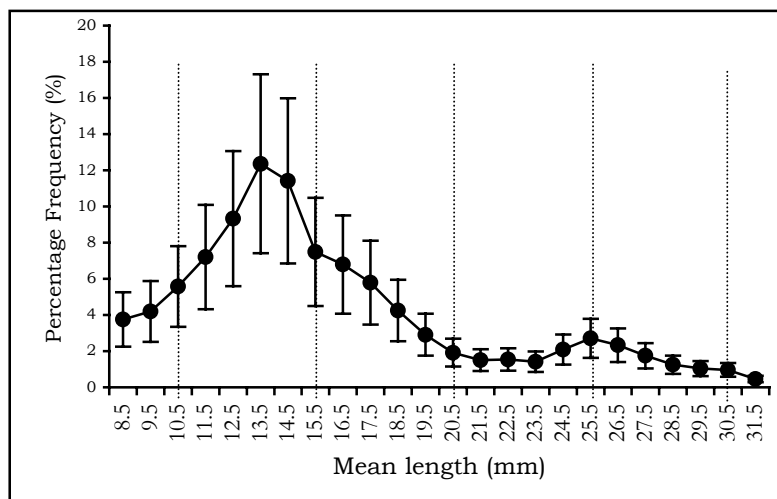


Figure 4.1. *Cellana testudinaria*. Mean percentage frequency of each size class category: Vertical lines represent standard deviation (SD). Dot line drawn at arbitrary delineates 6 cohorts.

4.3.2. Growth

All monthly size-frequency histograms are polymodal; 4 modes were found in October, March, July, August and September, and 5 modes in the remaining months (Fig. 4.2). The presence of several modes in the frequency distributions indicates that more than one age class is present in the population. The overall size-class distribution ranged from 8.5 mm to 31.5 mm (Fig 4.1). The smallest limpets (8.5 – 10.5 mm) are assumed having settled 2 months before being sampled. The larger (and older) cohorts overlapped considerably in size. Overall eight distinct cohorts were indentified (using the Bhattacharya method implemented in the FiSAT software) throughout the 1-year study period. For each cohort, mean length, its standard deviation, and its percentages have of the total population were calculated (Table 4.1).

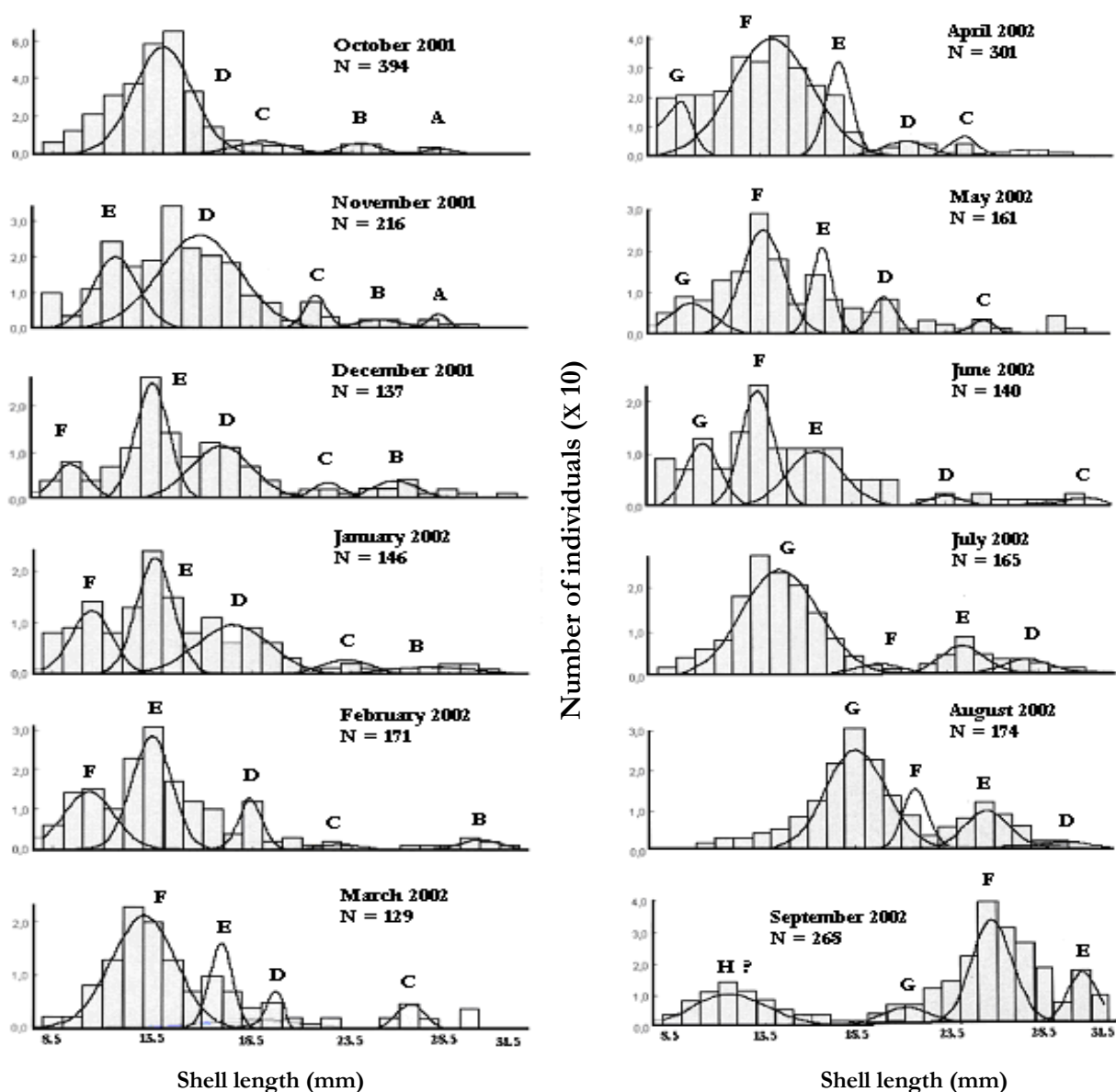


Figure 4.2. Monthly size-frequency histograms of *Cellana testudinaria*. Curves show estimated modal sizes. Numbers refer to cohorts. Analyses used the Bhattacharya method by the FiSAT software.

Table 4.1. Size cohorts of *Cellana testudinaria* summarised from Figure 4.2. *SD*- standard deviation.

Month	Cohort	Mean length (mm) \pm SD	% population
October '01	D	13.98 \pm 1.46	84.49
	C	18.91 \pm 1.42	8.57
	B	23.89 \pm 0.94	5.31
	A	28.00 \pm 0.90	1.63
November '01	E	11.70 \pm 1.10	26.44
	D	15.89 \pm 2.03	62.98
	C	21.77 \pm 0.50	5.77
	B	25.02 \pm 1.19	2.88
December '01	A	27.91 \pm 0.40	1.92
	F	9.50 \pm 0.85	12.60
	E	13.58 \pm 0.82	40.16
	D	17.06 \pm 1.52	34.65
January '02	C	22.45 \pm 0.70	4.72
	B	25.81 \pm 1.07	7.87
	F	10.44 \pm 1.00	21.83
	E	13.62 \pm 0.91	36.62
February '02	D	17.57 \pm 1.84	30.99
	C	23.25 \pm 1.18	5.63
	B	27.68 \pm 2.19	4.93
	F	10.27 \pm 1.26	31.25
March '02	E	13.42 \pm 0.99	49.31
	D	18.38 \pm 0.59	12.50
	C	23.00 \pm 1.20	2.08
	B	30.00 \pm 1.06	4.86
April '02	F	13.04 \pm 1.61	66.15
	E	16.94 \pm 0.58	18.46
	D	19.25 \pm 0.91	8.46
	C	26.51 \pm 0.73	6.92
May '02	G	10.31 \pm 1.94	10.23
	F	14.13 \pm 2.14	62.56
	E	17.71 \pm 0.68	18.71
	D	21.35 \pm 1.21	5.10
June '02	C	28.04 \pm 2.24	3.40
	G	9.83 \pm 1.19	15.83
	F	16.85 \pm 0.55	20.86
	E	20.18 \pm 0.63	10.07
July '02	D	25.50 \pm 0.67	3.60
	C	29.54 \pm 0.56	49.46
	G	13.44 \pm 0.87	40.00
	F	18.40 \pm 1.47	31.67
August '02	E	23.50 \pm 0.85	3.33
	D	27.00 \pm 0.90	22.5
	C	31.00 \pm 1.20	2.50
	G	14.58 \pm 2.05	78.21
September '02	F	19.82 \pm 1.19	4.49
	E	24.34 \pm 1.18	11.54
	D	27.81 \pm 1.23	5.77
	G	18.55 \pm 1.60	65.33
October '02	F	21.75 \pm 0.65	16.00
	E	25.59 \pm 1.14	17.33
	D	30.00 \pm 1.40	1.33
	H?	11.38 \pm 0.53	26.13
November '02	G	21.03 \pm 1.24	10.05
	F	25.72 \pm 1.09	46.73
	E	30.59 \pm 0.77	17.09

were very probably only a few months old and represented cohort-F recruited in the second event in 2001. This cohort attained the majority of the population in March 2002. They grew very rapidly and needed only 3 months to reach a mean length of 13 mm. A new cohort appeared in April 2002 (the mode of 9.32 mm) and was referred to as cohort-G, encompassing the first recruits of the year 2002. After three months, in July 2002, it has attained a mean size of 14.6 mm and accounted for the majority of the population. In September 2002, an eight cohort emerged,

In October 2001, 4 cohorts were identified with average estimated lengths of 13.98 mm, 18.91 mm, 23.89 mm and 28 mm, respectively. These cohorts were assigned successively. The clear majority of limpets (84 % of the total population) belonged to the smallest age group, which is referred to as cohort-D. It probably consists of individuals, which have settled during the second of the two recruitment events of the year 2000. This cohort remained in the study area until August 2002 when they reached a maximum length of about 30 mm. The older cohorts (A, B and C) accounted for only 2 – 9 % of the total population and remained for only 1 – 8 months before they completely disappeared. They probably encompassed limpets, which recruited in the first half of 2000 or joined the population during two recruitment peaks in 1999. In November 2001, a new age group appeared in the samples, attaining a quite high average length of 11.70 mm. It was referred to as cohort-E and consisted probably of limpets settled during the first recruitment event in 2001. Smaller (and younger) specimens were not found in the regular collections. This was presumably because of incomplete sampling. Limpets less than 10 mm in length, even a few specimens of 4 – 5 mm in length were occasionally encountered during free collections on the shore, but such small ones were distinctly rare. During samples collected in December 2001 limpets of < 10 mm length appeared. They

probably representing the settlers of the second recruitment event in 2002. The specimens of this age group are comparatively large (mean shell length of 11.4 mm), indicating that this cohort was missed in the collections of July 2002 and August 2002.

Growth curves (Fig. 4.3) were estimated from the pattern of the modes in the histograms. The oldest cohort-A decreased from a mean size of 28.00 (± 0.90 SD) mm in October 2001 to 27.91 (± 0.40 SD) mm in November 2001. Thus, it was not possible to calculate the growth of this cohort, as there was no increase in the mean size during the period of investigation. The next oldest cohort-B grew at a steady rate of 1.49 mm/month from October 2001 to February 2002 and disappeared from the study area in March 2002. Limpets of cohort-C had virtually disappeared from the population by June 2002, the few remaining individuals presumably having merged with cohort-D. The growth of cohort-E and cohort-F could be followed throughout the period of sampling. Cohort-E increased from a mean size of 11.70 (± 1.10 SD) mm in November 2001 to 30.59 (± 0.77 SD) mm in September 2002, equivalent to an average growth of 1.8 mm/month. Cohort-F grew at 1.77 mm/month during the period between December 2001 and September 2002. The youngest cohort-G entered the population in April 2002 and grew at 2.45 mm/month.

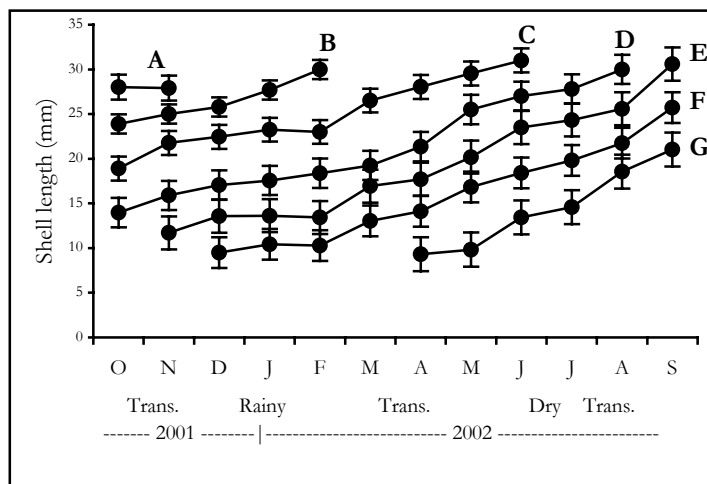


Figure 4.3. Growth curves of *Cellana testudinaria* estimated from monthly size-frequency histograms (Fig. 4.2). Standard error (vertical bars) of the estimated normal distributions of each cohort are shown.

4.3.3. Statistical analyses

For comparison of the growth of the various cohorts, regression lines were fitted to the growth curves (Table 4.2). Analysis of covariance ANCOVA was used to test the difference of growth among all cohorts (Table 4.4). The residual variance of the regressions were found to be homogeneous when compared using Bartlett's test (Table 4.3). ANCOVA results show that there is a highly significant difference between the slopes ($F_{5,51} = 135.71$, $P < 0.05$), indicating that young cohorts grow faster than old ones.

Table 4.2. *Cellana testudinaria*. The regression equations of the relationship between the shell increment and time. Y is the shell length; X is the numbers of year.

Cohort	Equation	r
B	$Y = 1.4880 X + 22.016$	0.98
C	$Y = 1.4352 X + 17.165$	0.98
D	$Y = 1.6039 X + 11.630$	0.98
E	$Y = 1.8005 X + 8.395$	0.97
F	$Y = 1.7679 X + 6.269$	0.98
G	$Y = 2.4529 X + 5.873$	0.98

From the growth equations (Table 4.2), it has been fully confirmed that the rate of growth decreased as the limpet grows older. For example, cohort-G grew at a steady rate of 2.45 mm/month while cohort-B at 1.48 mm/month. However in order to gain a precise knowledge of the manner of the decrease it is necessary to discount the possible influence of time of year and environmental variables upon the rate of growth. This has been done by taking the growth

increments of the shell length for the periods of dry season and rainy season into account. During the period between June and August (dry season) limpets grew more quickly (at an average of 2.61 mm/month) than during the period between December and February (rainy season) (growth at an average of 0.66 mm/month). In the periods of transition, the growth rate was about 1.10 mm/month between September and November and 1.91 mm/month between March and May.

Table 4.3. *Cellana testudinaria*. Bartlett's test on the rate of growth of the cohorts. *df*-degree of freedom; S^2 -variance.

Sources	<i>df</i>	S^2	$\ln S^2$	$df * \ln S^2$	$1/df$	$df * S^2$
Cohort-B	4	5.78	1.75	7.02	0.25	23.12
Cohort-C	8	16.15	2.78	22.26	0.13	129.20
Cohort-D	10	29.56	3.39	33.86	0.10	295.60
Cohort-E	10	37.61	3.63	36.27	0.10	376.10
Cohort-F	9	29.65	3.39	30.51	0.11	266.85
Cohort-G	5	21.76	3.08	15.40	0.20	108.80
Total	46			145.32	0.885	1199.67

$$\begin{aligned} \text{Mean } S^2 &= \Sigma df * S^2 / \Sigma df \\ &= 1199.67/46 \\ &= 26.08 \end{aligned}$$

$$\begin{aligned} Y &= \Sigma df * \ln(\text{Mean } S^2) \\ &= 46 * 3.26 \\ &= 150.01 \end{aligned}$$

$$\begin{aligned} M &= 2.3026 * (Y - \Sigma df * \ln S^2) \\ &= 2.3026 * (150.01 - 145.32) \\ &= 10.81 \end{aligned}$$

$$C = 1.058$$

Corrected test statistic (M/C) = **10.22**

Tabulated $\chi^2_{0.05(5)} = 11.1$

Table 4.4. *Cellana testudinaria*. Analysis of covariance on the relationships of cohorts B – G together with the ANOVA test. *F*-values that are relevant for the interpretation of the results are shown. *df*-degrees of freedom; *SS*-sum of squares; *MS*-mean square

Sources	<i>df</i>	<i>SSy</i>	<i>SPxy</i>	<i>SSx</i>	<i>by.x</i>	<i>SSy'</i>	<i>df</i>	<i>SSy.x</i>	<i>MSy.x</i>
Cohort-B	4	23.12	14.88	10	1.4880	22.14	3	0.98	0.33
Cohort-C	8	129.19	86.11	60	1.4352	123.58	7	5.61	0.80
Cohort-D	10	295.65	176.43	110	1.6039	282.98	9	12.67	1.41
Cohort-E	10	376.14	198.05	110	1.8005	356.58	9	19.56	2.17
Cohort-F	9	266.86	145.85	82.50	1.7679	257.85	8	9.02	1.13
Cohort-G	5	108.81	42.92	17.50	2.4529	105.26	4	3.55	0.89
Sum of groups							40	51.39	1.2848
Among b's							5	17.07	3.4140
Pooled within	46	1199.78	664.24	390	1.7032	1131.32	45	68.46	1.5213
Adjusted means							5	1032.23	206.45
Total	51	1997.35	632.93	446.77			50	1100.69	
Groups	5	797.57	- 31.31	56.77	- 0.55	17.27	4	780.30	195.08

Anova of covariance

Source of variation	df	SS	MS	F	F _{tabulated}
Ajusted means (among a's)	5	1032.23	206.45	135.71***	2.42 (P < 0.05)
Error (deviation from a common slope)	45	68.46	1.52		3.44 (P < 0.01)

- The rate of growth differ very significantly among months
- There is significant heterogeneity of the means around their regression slopes.

4.3.4. Growth parameters and longevity

The theoretical growth parameters, K and L_{∞} for *C. testudinaria* estimated from size-frequency data using ELEFAN I of the FiSAT software (Sparre & Venema, 1998) (Appendix 3) were 1.4 and 33.08 mm, respectively. These two parameters were substituted into the equation with $L_t = 4$ mm (based on the length of the smallest limpets obtained by free collection during the period of study) to give the value of $t_0 = 0.09$ year. The growth can be described by the following von Bertalanffy equation:

$$L_t = 33.08 [1 - e^{-1.4(t + 0.09)}]$$

From Figure 4.3, an estimate of the continuous increase in shell length was calculated by joining cohorts, which have similar sizes in different years with the assumption that growth is equivalent for any cohort (Underwood, 1975). Thus, using the observed increases in size of cohorts B to G for this population gave an estimate of growth for 22.8 months (1.9 years). The continuous growth curve generated by this method was compared with the curve constructed from the estimate of shell growth based on von Bertalanffy equation. The line fitted approximately to the growth curve is superimposed on the von Bertalanffy growth curve to show that both curves gave very similar results, which suggests that either estimate was reliable (Figure. 4.4).

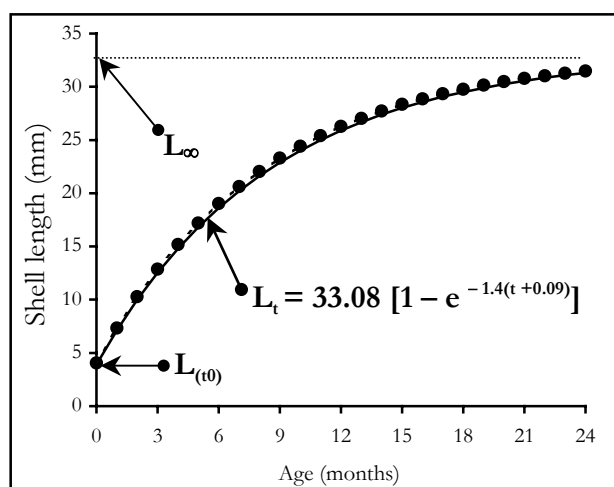


Figure 4.4. *Cellana testudinaria*. Growth model as estimated from the increase in shell length of age cohorts. The points represent the modes defined by the size distributions; the solid line corresponds to the von Bertalanffy growth curve and the dashed line represents the theoretical maximum length (L_{∞}). Only the sizes considered in the growth curve calculation are represented.

Limpets reached a shell length of 25.5 mm after 18 months, which is the approximate size of maturity for this population. Growth continued at a relatively constant rate for further 6 months, and the maximum size of 31.43 mm (computed as $L_{\max} = 0.95 L_{\infty}$, according to Taylor, 1965) was attained after about 25 months. L_{\max} was only a bit shorter than the actual maximum length, 31.8 mm, measured during the period of study. Therefore, it is reasonable to estimate longevity (t_{\max}) as $3/K$, resulting in a value of 25.71 months (2.1 years), which is very similar to the maximum age derived from the analysis of the size distributions.

4.3.5. Asymptotic weight

Combination of the von Bertalanffy growth equation $L_{t(mm)} = 33.08 [1 - e^{-1.4(t+0.09)}]$ and the length-weight relationships of $W_{(mg)} = 0.2343 L^{2.6601}$ and $BM_{(mg\ AFDW)} = 0.03236 L^{2.7703}$ give the equation of $W_{\infty} = 0.2343 L_{\infty}^3$ and $BM_{\infty} = 0.03236 L_{\infty}^3$. Thus, the “weight-based von Bertalanffy equation” becomes $W_t = 8481.42 [1 - e^{-1.4(t+0.09)}]^3$ and $BM_t = 1171.40 [1 - e^{-1.4(t+0.09)}]^3$.

4.4. Discussion

4.4.1. Population structure

The analysis of the monthly size frequency distributions suggests that there are 8 distinct age groups of limpets at the intertidal of Ohoiwait. The population predominantly consists of individuals with shell lengths between 10.5 mm and 20.5 mm. In this regard, the results for *C. testudinaria* are similar to those reported for other limpets, such as *Nacella concinna* (Picken, 1980), *Nipponacmea concinna* and *Nipponacmea nigrans* (Takada, 1997), *Cellana eucosmia* (Saad, 1997), and *Cellana radiata* (Ismail & Elkarmi, 1999), which all were dominated by individuals of 14 – 22 mm in length.

4.4.2. Growth and longevity

The interpretation of polymodal size frequency distributions is problematic of the overlapping of the cohorts, especially the older ones, and there is quite a high uncertainty in the determination of absolute ages. However, a total of 8 distinct cohorts were identified using the modal progression technique. By following these cohorts in the monthly taken size distribution, the growth of each cohort could be assessed. The growth rate of cohorts obtained by applying regression lines to the growth curves (Fig. 4.3) are average rates only. The cohort-G grew at the rate of 2.45 mm/month, whereas the cohort-B grew at the rate of 1.49 mm/month, indicating that the young cohorts grew more rapidly than the older ones (Underwood, 1975; Creese, 1981; Fletcher, 1987; Takada, 1997). Moreover, ANCOVA showed that the rates of growth might vary from year to year for cohorts of corresponding age. This indicates that limpets of similar sizes

had significant differences in rates between years, whereas limpets of the same ages showed considerable variability in growth even over the same period.

In common with most organisms (Bertalanffy, 1957), larger gastropods grow less rapidly than smaller ones of the same species (Laxton, 1970). This phenomenon can be adequately described with a linear equation. When the net annual length increment is plotted as a function of shell length, increments decrease more or less linearly from those of rapidly growing juveniles to those of larger adults (Laxton, 1970; Yamaguchi, 1975). Size-frequency distribution data as shown in Fig. 4.2, which are composed to the continuous growth curve in Fig 4.4, showed that the smallest *C. testudinaria* grew more rapidly at the first 4 months than did those that are 18 to 24 months old. The relationship is curvilinear, since large limpets grow much more slowly than predicted by extrapolating from growth rates of juveniles. Monthly increments in shell length begin decreasing after the first year. It is clear from the estimated lengths at different age that the Bertalanffy equation describes the growth of *C. testudinaria* over the whole of its growth range. Many sedentary invertebrates such as limpets show a sigmoid pattern of growth (Ward, 1967; Yamaguchi, 1975; Fletcher, 1984; Saad, 1997).

The von Bertalanffy growth equation describes individual growth by means of three parameters: L_{∞} , K , and t_0 . The maximum theoretical length L_{∞} and the growth coefficient K are mathematically linked. If the calculated L_{∞} for a species is clearly very different from the largest observed length, the K value is of little significance (Knight, 1968). For *C. testudinaria*, the calculated $L_{\infty} = 33.08$ mm is reasonable, as it is quite close to the length of the largest shells recorded, $L_{max} = 31.8$ mm. It should be noted, however, that Khouw (2002) collected some specimens of *C. testudinaria* as long as 40 mm in the same area. For comparison, the asymptotic length, L_{∞} of other limpet species obtained by previous works are presented in Table 5.2.

Table 4.2. Comparison of L_{∞} (mm) values of *Cellana testudinaria* with values reported for other species.

Species	L_{∞}	Locality	Authors
<i>Cellana testudinaria</i>	33.1	Ohoiwait, Southeast Molluccas, Indonesia	This study
<i>Cellana eucosmia</i>	44.1	Ain Soukhna, Gulf of Suez	Saad, 1997
<i>Cellana radiata</i>	54.0	Gulf of Aqaba, Red Sea	Ismail & Elkarmi, 1999
<i>Nacella delesserti</i>	61.8	Marion Island, Sub-Antarctic	Blankley & Branch, 1985
<i>Patelloida alticostata</i>	48.0	New South Wales, Australia	Fletcher, 1987
<i>Patelloida alticostata</i>	22.3	New South Wales, Australia	Creese, 1981
<i>Patelloida latistrigata</i>	15.1	New South Wales, Australia	Creese, 1981
<i>Montfortula rugosa</i>	22.0	New South Wales, Australia	Creese, 1981
<i>Notoacmea petterdi</i>	19.9	New South Wales, Australia	Creese, 1981
<i>Siphonaria denticulate</i>	21.8	New South Wales, Australia	Creese, 1981
<i>Siphonaria virgulata</i>	18.0	New South Wales, Australia	Creese, 1981
<i>Fissurella barbadosis</i>	33.9	Barbados Island	Ward, 1967
<i>Nacella (Patinigera) concinna</i>	71.4	Esperanza Bay, Antarctic Peninsula	Br�thes et al., 1994
<i>Nacella (Patinigera) concinna</i>	41.0	Signy Island, South Orkney Islands	Picken, 1980

The L_{∞} value estimated for *C. testudinaria* seems realistic, since limpets in the tropical regions grow more quickly but usually reach a smaller maximum size and have a shorter life span than limpets living in temperate or polar regions (Ward, 1967; Frank, 1969). Moreover, it is

known for some gastropods in the northern hemisphere that individuals in a southern population are smaller than those in a northern (Lewis et al., 1982; Smith & Breyer, 1983). This has been considered to be caused by higher water temperatures in the southern habitat. The differences could be explained by geographical variations, but presumably also by the techniques used to estimate age and growth parameters. Pickens (1980) demonstrated that it is generally more precise using the counting of annual growth annuli than modal progression analysis in estimating absolute ages.

It is the growth coefficient K that is the most interesting parameter of the Bertalanffy equation, since it describes the rate at which an organism approaches its maximum size and may be used to make comparisons of the rate of growth of different species or of the same species from different environments (Ebert, 1973; Yamaguchi, 1975). Beverton & Holt (1957) noted that K can be used as an index of “the intrinsic development rate” of a species and has great value in intra- and inter-specific comparisons of growth. It measures the rate of diminution of growth with the increase in age. It tends to increase with the decrease in the life span of the animals (Lewis & Bowman, 1975; Picken, 1980; Fletcher, 1987; Brêthes et al., 1994). The continuous growth curve (Fig. 4.4) indicates that *C. testudinaria* reaches a length of about 25.9 mm in its first year. This is slightly less than the first-year length attained by *Cellana eucosmia*, 27 mm, in the Gulf of Suez (Saad, 1997), but more than that of *Cellana radiata* (10 mm) in the Gulf of Aqaba (Ismail & Elkarmi, 1999). On the other hand, the Antarctic limpet *Nacella concinna* reaches only 5 mm in length during its first year (Picken, 1980). Table 4.3 shows some K values of comparable species reported from different regions. It is evident that limpets in the tropical regions grow more rapidly than in temperate and polar regions.

Table 4.3. Comparison of K values of *Cellana testudinaria* with values for other limpets.

<i>Species</i>	K	<i>Locality</i>	<i>Authors</i>
<i>Cellana testudinaria</i>	1.4	Ohoiwait, Southeast Molluccas, Indonesia	This study
<i>Cellana eucosmia</i>	0.69	Ain Soukhna, Gulf of Suez	Saad, 1997
<i>Cellana radiata</i>	0.19	Gulf of Aqaba, Red Sea	Ismail & Elkarmi, 1999
<i>Patelloida allicostata</i>	0.72 - 0.86	New South Wales, Australia	Fletcher, 1987
<i>Fissurella barbadensis</i>	0.126	Barbados Island	Ward, 1967
<i>Nacella (Patinigera) concinna</i>	0.077	Esperanza Bay, Antarctic Peninsula	Brêthes et al., 1994
<i>Nacella (Patinigera) concinna</i>	0.072	Signy Island, South Orkney Islands	Picken, 1980
<i>Nacella delesserti</i>	0.074	Marion Island, Sub-Antarctic	Blankley & Branch, 1985

The parameter t_0 (the theoretical age at which the length of an animal is zero) determines the accuracy of the estimation of the other growth parameters as well as longevity of the limpets. Observations of newly settled *C. testudinaria* in the field would suggest that t_0 is negligible, i.e. it is unlikely to be greater than 1 months. However, such low t_0 value seems unrealistic because this estimate was based on a minimum length of 4 mm (the size of smallest limpets obtained by free collection during the study) and it was not possible to find living limpets with shell length < 0.2 mm (Smith, 1935). Therefore, it is reasonable to estimate the longevity or life span of *C. testudinaria* to be 2 years. In general, limpets in tropical regions have shorter life spans than those

in temperate and polar regions (Table 4.4). This is probably also due to the different intertidal habitats, which these animals occupy. Several authors noted that the nature of the habitat in the intertidal range and, hence, the exposure to wave action, might influence the growth and shell form of limpets (Balaparameswara Rao & Ganapati, 1971a, 1971b; Ward, 1976).

Table 4.4. Comparison of longevity of *Cellana testudinaria* with values for other limpets.

<i>Species</i>	<i>Longevity (yr)</i>	<i>Locality</i>	<i>Authors</i>
<i>Cellana testudinaria</i>	2	Ohoiwait, Southeast Mollucas, Indonesia	This study
<i>Cellana eucosmia</i>	5	Ain Soukhna, Gulf of Suez	Saad, 1997
<i>Cellana radiata</i>	5	Gulf of Aqaba, Red Sea	Ismail & Elkarmi, 1999
<i>Patelloida alticostata</i>	6 - 7	New South Wales, Australia	Fletcher, 1987
<i>Patelloida alticostata</i>	5 - 6	New South Wales, Australia	Creese, 1981
<i>Patelloida latistrigata</i>	3	New South Wales, Australia	Creese, 1981
<i>Montfortula rugosa</i>	> 3	New South Wales, Australia	Creese, 1981
<i>Notoacmea petterdi</i>	> 10	New South Wales, Australia	Creese, 1981
<i>Siphonaria denticulate</i>	> 6	New South Wales, Australia	Creese, 1981
<i>Siphonaria virgulata</i>	2 - 3	New South Wales, Australia	Creese, 1981
<i>Patella vulgata</i>	10 - 20	Millport, United Kingdom	Russell, 1909
<i>Nacella (Patinigera) concinna</i>	63	Esperanza Bay, Antarctic Peninsula	Brèthes et al., 1994
<i>Nacella (Patinigera) concinna</i>	30 - 40	Signy Island, South Orkney Islands	Picken, 1980

It is clear that the growth rate of *C. testudinaria* was strongly related to environmental conditions. Despite the general problems of data interpretation due to inadequate sampling, a slight seasonal variation in temperature is discernible for the seawater and air at the intertidal of Ohoiwait. During the dry season when seawater and air temperatures are on average 29 °C and 26.5 °C, respectively, the limpets grew faster. This growth acceleration occurred in August, i.e. later than it would be expected if the limpet responded immediately to an increase in temperature. In general, the limpet grew more quickly between September and November than during other times, when seawater and air temperatures were more variable (Fig. 2.4 and Fig. 2.3). This suggests that there is an increase in growth in response to temperature, indicating that the limpets do respond, with regard to their metabolism, to changes in environmental conditions. This has been demonstrated for many limpets in temperate regions where seasonal variations in temperature are far more pronounced than in the tropics (Russell, 1909; Blackmore, 1969b; Picken, 1980). Beside temperature, the availability of food is known to regulate growth. Blackmore (1969b) suggested that an increase in growth of temperate limpet, during summer is related to an increased food supply. However, since I do not have quantitative data on a variation in food availability that might influence the growth performance, temperature is considered to be the primary factor affecting the growth rates of limpet in the study area.

The calculated maximum body weight of 8481 mg wet weight or 1171 mg AFDW of *C. testudinaria* in the present study was smaller than those of *C. eucosmia* (9185 mg wet weight; Saad, 1997), but higher than *C. radiata* (487 mg AFDW; Ismail & Elkarmi). This is probably because the maximum length reached by *C. eucosmia* (44.1 mm) was greater than that of *C. testudinaria* (33.08 mm) and because *C. radiata* has lower ratio between soft-tissue and shell weight (ST/SW = 0.14).

Chapter 5

RECRUITMENT

5.1. Introduction

Recruitment often plays a critical role in controlling the distribution and abundance of marine invertebrate species (Connell, 1985; Butman, 1987), and it has been identified as a major factor (Dayton, 1972; Meadows & Campbell, 1972; Delany et al., 1998), organizing the general character of rocky shore communities (Bowman & Lewis, 1977). The spatial and temporal arrival and subsequent survival of new individuals is a fundamental process shaping community structure in most habitats (Connell, 1985) and is responsible for the supply of individuals to existing assemblages as well as the re-colonisation of denuded areas or newly created space (Lubchenco & Menge, 1978; Sousa, 1984). In practice, recruitment is defined as the abundance of juveniles, which have survived for a certain period of time (Keough & Downes, 1982; Connell, 1985).

Recruitment fluctuations are not simply “noise” but can rather be viewed as an adaptive response to an inherently variable environment. Numerous physical and biological factors play important roles in the arrival and subsequent survival of recruits in intertidal assemblages (Hutchinson & Williams, 2001). These include limited local scale processes such as the impact of predators and benthic grazers (Hawkins & Hartnoll, 1983; Menge & Sutherland, 1987), substrate type and their complexity (Raimondi, 1988), the availability of free space to recruit (Pierron & Huang, 1926; Paine and Levin, 1981), non-local processes including oceanic currents (Crisp, 1974; Roughgarden et al., 1987) and change in climate conditions (Barry et al., 1995).

Understanding the ways in which physical and biological forcing influences recruitment of marine species holds particular importance for predicting the potential effects of global change on these species and system (Fogarty, 2001). Many ecologists have tried to sort out the real effects of the environment on recruitment, but still faced some problems (Myers, 2001). Several authors suggested that the whole enterprise of finding correlations between environment and recruitment should be abandoned (Smith, 1995; Myers, 2001). Nevertheless, in an attempt to identify the causes on the recruitment fluctuations, the consequences of environmental effects should be justified (Denny, 1985; Bowman & Lewis, 1986; Myers & Pepin, 1994; Botsford, 2001).

Spatial variation in the recruitment of sessile marine invertebrates with planktonic larvae generally involves four phases: (1) development; including dispersal as a planktonic form, (2) testing of a habitat for suitability, (3) settlement; including attachment to the substratum and metamorphosis, (4) recruitment of juveniles into the population (survival until the organism is counted by an observer). The last phase may last from hours to months (Scheltema, 1974), but it is not a true life-history stage, merely a reflection of the limitation of the observer (Keough & Downes, 1982). The number of organisms passing to the third phase is termed *settlement*, while

the number passing through the fourth phase, which is a composite of larval and juvenile stages, is termed *recruitment*.

According to Keough & Downes (1982), it is important to distinguish between settlement and recruitment. Failure to do it may lead to misleading inferences in explanation for the spatial distribution of adult organisms, which frequently neglects the importance of recruitment (Underwood & Denley, 1984), and to an overestimation of the importance of interactions between adult organisms and physical factors in limiting these distributions. However, although these phases are differentially susceptible to biotic and abiotic influences, many studies of recruitment have not discriminated between phases, leading to an over- or under-estimation of the influence of certain factors in limiting the subsequent distribution and abundance of adults (Pulfrich, 1995). According to Smith (1935) that newly-settled juveniles of the common patellid limpets are 0.2 mm in size. In this study, it was not possible to sample this size class quantitatively.

The specific aim of this chapter is particularly concerned with how important such local-scale and seasonal environmental impacts are influencing recruitment that may be spatially variable on the three shore levels.

5.2. Materials and Methods

5.2.1. Sample collection

Standard quadrat sampling technique was applied in order to obtain a rough estimate of the monthly patterns of juvenile dispersal and recruitment. A sequence of 9 quadrates (1x1 m) was exposed randomly to the three different shore levels in which 3 quadrates were fixed per shore level. All individuals *C. testudinaria* of < 4 mm length in each frame were counted. Allocation of quadrates was done haphazardly by tossing the sample devices to the shore, since true randomisation by using the random number tables or grids is often difficult to realise in the field. Although this method is not truly random, I could think of no better mechanism by which I would have introduced a subjective bias except that central parts of shore have greater chance to be sampled.

Investigations of the recruitment of juvenile limpet should consider the relative roles of initial settlement patterns and differential post-settlement mortality rates on the distribution and abundance. However, because the organisms are unlikely to be detected immediately, the total numbers of settling could not be determined accurately. Hence, it was generally impossible to measure, and often difficult to classify. Consequently, more precise information on the intensity of recruitment of juveniles was gained from the number of recruits of < 4 mm in length found at any time during the year following the seasonal spawning. These were done by searching regularly and intensively for the presence of juveniles and these specimens were taken as the measure for estimating the actual “recruitment”. This method allows only minimum estimates of the numbers of recruitment because a sampling problem arose from considerable variation in

limpet size. Here, the larger initial size of 4 mm in length of recruits and conspicuous animals was considered, which may be attached or stationary on the substratum. In many cases, this size of recruits produce pelagic dispersal larvae, which settle and metamorphose into juveniles when a suitable substratum is found, or they have direct development to benthic juveniles with no planktonic larvae (Thorson, 1950; Mileikovsky, 1971). Russell (1909) proposed the recruitment size for *Patella vulgata* is < 5 mm in length. The same size was obtained for *Nipponacmea concinna* (Takada, 1997), and *Cellana tramoserica* (Underwood, 1975).

5.2.2. Analyses

The recruitment pattern of *C. testudinaria* populations was estimated by 2 methods: (1) direct counting of juveniles of the size at recruitment (< 4 mm in length), where recruitment means entering the population that can be sampled or caught and not necessarily the size at settling. Spatial and temporal variation in recruitment densities on the three shore levels were analysed using a mixed model ANOVA with the differences in shore level and sampling date as experimental factors (Morrisey et al., 1992; Underwood, 1997). (2) The settlement of the first < 8.5 mm mid-class juveniles perceiving data for the growth study was facilitated by a recruitment-based approach method with the aid of computer software FiSAT (Sparre & Venema, 1998). This routine reconstructs the recruitment pulses from a time series of length-frequency data along a trajectory defined by the von Bertalanffy growth formula (VBGF) to determine the number of pulses per year and the relative strength of each pulse (Appendix 5). The pattern would be decomposed automatically by the integrated program NORMSEP and the peak recruitment fitted with up to two Gaussian distributions (Moreau and Cuende, 1991).

The significant effects of environmental factors on recruitment vary considerably throughout the time, indicating that recruitment is highly dynamic following diverse and complex temporal-spatial processes. Recently, some researchers have realised the importance of examining processes at varying spatial scale in an attempt to determine the process from previous localised models (Underwood & Denley, 1984; Underwood & Petraitis, 1993). The shortage of spatial analyses can now be remedied by using existing information to design new observational systems at defined spatial scales. Potential linkage between the abundance of juvenile limpets recruit to the shore and environmental factors regulating them was examined by relating recruitment success to juvenile density spanning a number of time-series of environmental variables via multiple regression analysis. Rice (1997) established correlation relationship between the environmental variables and the index of juvenile density using the linear version: $\text{Log}(D) = a + b_0X_0 + b_1X_1 + \dots + b_nX_n$ where D is juvenile density, X_0 , X_1 , and X_n are environmental variables. In this study, I only considered the main environmental variables such as water temperature and salinity, tidal cycle, and wave action, which presumably strongly influence dispersal and recruitment of limpets on a range of spatial and temporal scales.

5.2.3. Statistical analyses

A *t*-test of variance was used to test whether the number of recruited limpets estimated by both methods are not different during the whole experimental period of 12 months. Therefore, each pair of data from the monthly sampling was treated as an independent variable. This test has the advantage of having a greater power of detecting a real difference and is useful for small samples where the true variances of the two populations are assumed equal (Wardlaw, 1985; Bakus, 1990).

As the shore is not a uniform environment, the time when conditions favour spat survival must vary with habitat as well as season (Bowman & Lewis, 1986). For hypothesis testing that the recruitment rates may be different between the dry and rainy seasons, the post hoc *R X C G*-test of independence (Sokal and Rohlf, 1995) was chosen. Such a test would examine whether the recruit densities exhibited by the margin totals are independent of the seasons at which the individuals were sampled. Of interest was whether the percentage of recruits changed significantly during the time of observation. I therefore tested whether the proportion of recruitment is independent of the time of collection. Recruit densities of both methods for the three shore levels were summed according to the season; June to August represent the dry season, September to November represent the second transition period, December to February represent rainy season, and March to May represent the first transition period. In this analysis, however, it should be noted that I did not consider the kind of factors affecting the recruit densities itself but only focused on whether the number of recruitment was different among the seasons. Even though this test only tested the differences among the season, it was useful to differentiate the result in which interpretation could be made (Sokal and Rohlf, 1995). Subjecting the result from *R X C G*-test to whether the differences between successive points are significant, the Student-Newman-Keuls (SNK) test was used additionally with the aid of computer program SPSS-10 to give the status of variance according to which analysis is used (Sokal and Rohlf, 1995).

5.3. Results

5.3.1. Estimation of recruitment by direct counting of recruits

The numbers and timing of juvenile *C. testudinaria* recruited at the three shore levels are summarised in Table 5.1 and are shown in Figure 5.1. Judging from the figure, it is suggested that main recruitment events could be observed by following curve trends. New juveniles were observed abundantly on the three shore levels during the periods of transition following higher settlement of juveniles occurring in April ($\pm 4.78 \text{ ind.m}^{-2}$). However, a few small limpets were sampled during each sampling occasion, suggesting that recruitment may occur throughout the year. Although these interpretations cannot be used to define the start and finish of periods of settlement, it is possible to identify periods of recruitment, which is defined as the time when

small limpets appear in large numbers in population. During the period of October to February, the settled population remained relatively stable. However, in the beginning of March, juvenile limpets were almost totally absent. A marked rejuvenation of the limpets at the three shore levels occurred in late February and April, followed by a second, less pronounced, recruitment in June (Fig. 5.1).

Table 5.1. *Cellana testudinaria*. Number of recruits in 9 quadrates on the three shore levels.

Months	Number of recruits			Density (ind.m ⁻²)
	High	Middle	Low	
Oct'01	8	7	3	2.00
Nov'01	7	6	0	1.44
Dec'01	6	4	2	1.33
Jan'02	7	6	4	1.89
Feb'02	8	8	4	2.22
Mar'02	3	1	0	0.44
Apr'02	20	15	8	4.78
May'02	8	5	1	1.56
Jun'02	11	5	0	1.78
Jul'02	4	1	1	0.67
Aug'02	1	1	0	0.22
Sep'02	6	5	1	1.33

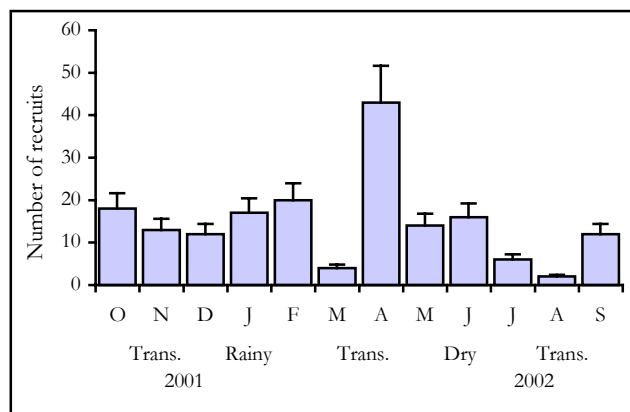


Figure 5.1. *Cellana testudinaria*. Abundance of recruits on the three shore levels. Nine quadrates of 1 m² in size were sampled approximately monthly. Error bar is ± 1 SD.

From regular monitoring of the numbers of juveniles settling at the three shore levels, it was determined that average recruitment observed during the dry season was lower than during the rainy season. Heavy settlements of juvenile limpets were found at the high shore level, those were concentrated on top and below big boulders as well as medium boulders in and around the tide pool. This non-random distribution of limpet settling in an area, suggests that these recruitment pattern were not random or coincidental.

5.3.2. Estimation of recruitment by FiSAT

The monthly percentages of *C. testudinaria* recruits estimated by FiSAT based on the individual lengths of < 8.5 mm are shown in Figure 5.2. A decrease in the recruitment occurred in the period between January and September. Although some rejuvenation of limpets were observed during this period, recruitment levels remained weak throughout the year. The expected settlement peak of 17 % occurred in March rather than in April as estimated by the first method. This reflects the fact that, when considering the parameters L_{∞} , K , and t_0 on the von Bertalanffy equation, then it can be concluded that the population has already 2 – 3 months old on the shore. Hence, if the time is set arbitrarily to January 1st and the recruitment time is counted down, then the estimated time of real recruitment occurs in March. It also can be seen from the figure that the trends of the peak recruitment did not follow any seasonal pattern.

However, the curves represent the period of heavy recruitment, in turn, proposed that the average recruitment on the periods of dry season was lower than on rainy season.

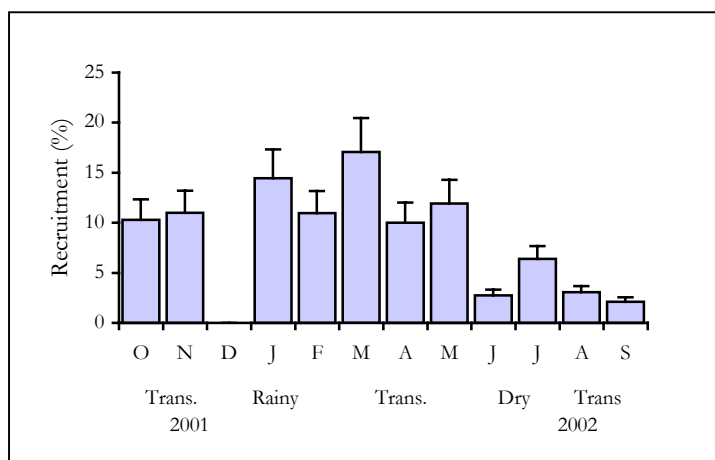


Figure 5.2. *Cellana testudinaria*. Percentage of recruits estimated by FiSAT. Numbers are based on the individuals of < 8.5 mm mid-class of juveniles. Error bar is ± 1 SD.

5.3.3. Recruitment event and the relationship with environmental variables

A consideration of the analyses of regular monthly recruitments indicates how synchronous recruitment pattern is between both techniques (compare the Fig. 5.1 and Fig. 5.2). A *t*-test of variance of the proportion of juveniles in samples both methods showed that there was no significant difference ($t = 0.43$, $P > 0.05$) in the numbers of recruitment per month (Table 5.2). This confirms that either direct-counting method or FiSAT would give the same result in estimating recruitment rates.

Substantial increase and decrease noted in juvenile numbers of limpets following recruitment peaks indicate that recruitment gains are also strongly affected by the conditions on their natural habitat. The juvenile abundance data collected at the three shore levels therefore were found to be different during monthly sampling. The result of *R X C G*-test confirmed that the number of recruitment was strongly dependent on the seasons (*G*-value is highly significant at $P < 0.05$). Subjecting this result to SNK test, confirmed that either the dry season differs from the rainy season or the period of first transition differs from the second transition (Table 5.3).

Table 5.2. *Cellana testudinaria*. *t*-test on the means number of recruits between both methods.

Variables	Samplng	FiSAT	<i>t</i> -test	<i>t</i> _{tab.}
Number of recruits	177	200		
Mean	14.75	16.67	0.43	1.72
Standard deviation	10.5151	11.1546		
Variance	110.5673	124.4251		

Table 5.3. *Cellana testudinaria*. G-test of independence, together with the SNK-test. The calculation of these values are presented below.

Computation:

1. Sum of transforms of the frequencies in the body of contingency table
 $= \sum \sum f \ln f = 49 \ln 49 + 40 \ln 40 + \dots + 62 \ln 62$
 $= 1482.6428$
2. Sum of transforms of the row totals
 $= \sum (\sum f) \ln (\sum f) = 69 \ln 69 + \dots + 105 \ln 105$
 $= 1633.2153$
3. Sum of transforms of the column totals
 $= 177 \ln 177 + 200 \ln 200$
 $= 1975.8420$
4. Transform of the grand total
 $= 377 \ln 377$
 $= 2236.4564$
5. $G = 2 [\text{quantity 1} - \text{quantity 2} - \text{quantity 3} + \text{quantity 4}]$
 $= 2 [1482.6428 - 1633.2153 - 1975.8420 + 2236.4564]$
 $= 220.0838$
6. William's correction
 $q = 1 + [(2+1)(4+1)]/6(377)$
 $= 1.0066$
 $G_{adj} = G/q$
 $= 218.6408$

Since $\chi^2_{0.05(3)} = 7.81$, the G - value is significant at $P \ll 0.05$, that the recruitment pattern is dependent of season.

G - test of independence

Season	by sampling	by FiSAT	Totals
Dry	49	40	69
Transition (Sep-Nov)	61	78	139
Rainy	24	20	44
Transition (Mar-May)	43	62	105
Totals	177	200	377

Student-Newman-Keuls (SNK) - test

Season	N	Subset for alpha = .05
Dry (June - August)	2	44.5
Transition (September - November)	2	69.5
Rainy (December - February)	2	22.0
Transition (March - May)	2	52.5
Sig.		.072

A plot of estimated recruitment and corresponding monthly environmental variables (Fig. 5.3 - 5.6) reveals apparent synchronous trends, with significant scatter indicating that recruitment must be additionally affected by those factors. However, contradictive trends are observed in April for seawater temperature, salinity, and tidal excursion variables, which is interpreted as the variables independent effect on recruitment.

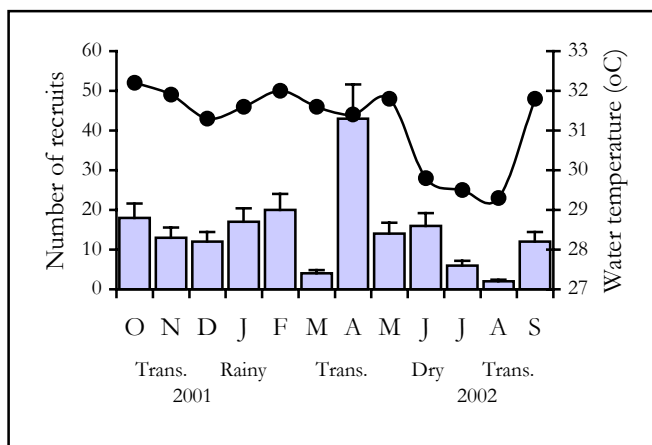


Figure 5.3. *Cellana testudinaria*. The pattern of monthly recruitment variability (\square) versus mean monthly seawater temperature (\bullet). Error bar is $1 \pm$ SD.

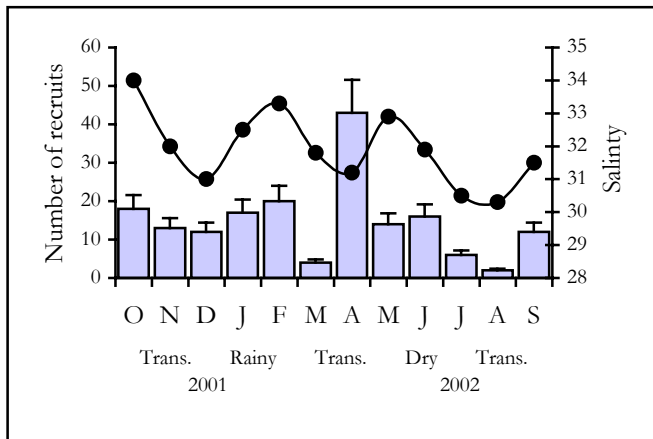


Figure 5.4. *Cellana testudinaria*. The pattern of monthly recruitment variability (\square) versus mean monthly seawater salinity (\bullet). Error bar is $1 \pm$ SD.

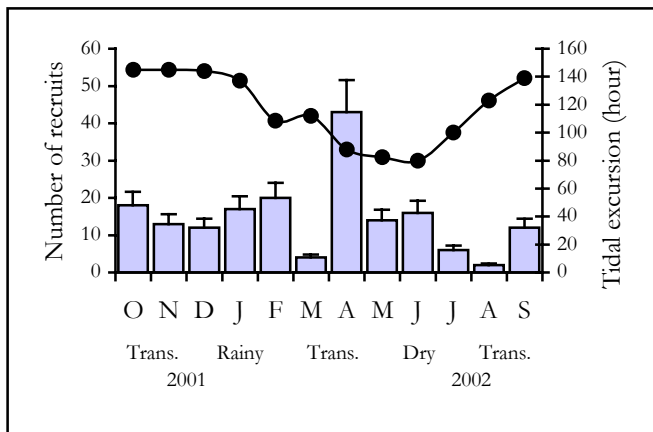


Figure 5.5. *Cellana testudinaria*. The pattern of monthly recruitment variability (\square) versus mean monthly tidal excursion (\bullet). Error bar is $1 \pm$ SD.

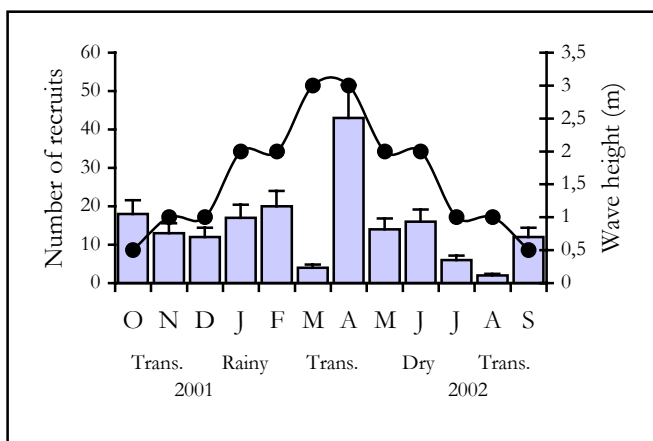


Figure 5.6. *Cellana testudinaria*. The pattern of monthly recruitment variability (\square) versus mean monthly wave height (\bullet). Error bar is $1 \pm$ SD.

The relationships between number of recruits and environmental variables are summarised in Table 5.4. Although the regression coefficients show no significant relationships, seawater temperature and salinity had more effects (i.e. higher slopes) on recruits than tidal excursion and wave height (Table 5.4). The analysis of variances on the regression detected no significant departure from linearity for all variables (Temperature: $F_{(1,11)} = 4.49$, $p > 0.05$; Salinity, $F_{(1,11)} = 3.40$, $p > 0.05$; Tidal excursion, $F_{(1,11)} = 0.18$, $p > 0.05$; Wave height, $F_{(1,11)} = 0.54$, $p > 0.05$), indicating that relationships were polynomial. The regressions confirmed that seawater temperature, salinity, and wave height significantly increased the total recruits density whereas tidal excursion significantly decreased the density (Table 5.4).

Table 5.4. *Cellana testudinaria*. Linear regression of \log_{10} -transformed recruit densities dependent on environmental variables. The table lists the source of environmental variables and the results of the linear regression: intercept, slope with standard error (SE), regression coefficient r , significant levels (*ns*: not significant) and the model of regression.

Sources	Intercept	Slope (SE)	r	Significant	Model
Temperature	- 4.84	0.19 (0.31)	0.56	0.06 <i>ns</i>	Polynomial
Salinity	- 3.98	0.15 (0.32)	0.50	0.09 <i>ns</i>	Polynomial
Tidal excursion	1.28	- 0.002 (0.36)	0.13	0.68 <i>ns</i>	Polynomial
Wave height	0.92	0.09 (0.36)	0.22	0.48 <i>ns</i>	Polynomial

Multiple regression analysis on the relationship between recruits density and the four environmental variables gives a different result compared to partial regressions in which a negative non-linear trend was observed on the wave height. This negative trend indicates that the wave height could decrease the recruitment density. The regression equation of this relationship is: $Y = 0.2525X_1 + 0.0095X_2 - 0.0072X_3 - 0.0685X_4 - 6.1635$ where X_1 is temperature, X_2 is salinity, X_3 is tidal excursion, and X_4 is wave height, respectively. This regression also shows no significant departure from linearity ($F_{(4,11)} = 1.49$, $p > 0.05$).

5.4. Discussion

5.4.1. Some problems in recruitment

Recruitment success or failure may be determined during various distinct phases: gonad development and spawning, planktonic life or the period of settlement, and establishment on the shore (Bowman & Lewis, 1977). Therefore, a more complete understanding of recruitment variation in the species of *C. testudinaria* would require insight into the studies on planktonic larval abundances and subsequent observation on settlement of larvae. In the present study, there are three major impediments to that understanding: (1) links between planktonic larval life-stages, larval settlement to the substratum, and the nearshore mesoscale physical variability on weekly time scales are insufficiently known; (2) biological and physical conditions have not been

sampled at fine scales for long enough, or over large enough areas; (3) equipments needed for field experiment setup are very limited, and hence methods used are sometime ambiguous.

Regular and intensive monitoring is usually necessary to follow seasonal variations in recruitment of marine benthic populations. This seems to be particularly true in case of limpet population in the rocky shore of Ohoiwait, where sporadic bursts of spat settlement are superimposed on continual observations. While I have no information on active settlement choice of limpet recruits, monthly field observations indicate that in fact, large differences in density of < 4 mm juvenile limpets were found on the different shore levels.

Because of the tremendous difficulty in following larval cohorts in the field (if such cohorts even remain as discrete entities), most larval life-spans have been determined either directly from laboratory cultures (Bayne, 1965) or inferred from the difference between spawning date of a local population and subsequent recruitment dates into that same region (Kennedy, 1977; Suchanek, 1981). However, this second approach seems unrealistic in the present study because one needs long-term studies to conduct it and the other ignores the importance of immigration and emigration of larvae (Lewis & Bowman, 1975; Suchanek, 1981). To follow temporal recruitment patterns, subsequent spat settlement of limpet were taken randomly on a prior selected shore levels. These data only considered and integrated settlement and various sources of post-settlement survival as the rate of recruitment. Therefore, the pattern of recent recruitment (Fig. 5.1) cannot reflect an actual annually recurring pattern, since these populations are driven by recruitment without post-recruitment density dependence, because different but constant settlement rates at the three shore levels would merely imply identical size distributions at different densities.

Additional years of sampling presumably would be required to confidently describe the stochastic mechanism of spatio-temporal variability in recruitment to this species. I followed the density of *C. testudinaria* recruits over 12 months on the three different shore levels at intertidal rocky shore. Although, quantitative information of environmental variables measured during the periods of study is limited, an interpretation of the effect of season using the factor “effectiveness of measurement” is permissible. Therefore, the regular sampling designed may well provide estimation of how temporal and seasonal variability of environmental variables affect recruitment throughout a period of 12 months.

5.4.2. Estimation of recruitment

The present study highlights the fact that recruitment of *C. testudinaria* is highly variable within the intertidal rocky shore of Ohoiwait. At any point in time, recruitment to areas of the different shore level may differ in the density of new recruits. Both methods of estimating recruitment (direct counting and size-frequency distribution by FiSAT) yielded similar results. Recruitment patterns varied broadly throughout the three shore levels in dry and rainy seasons, with the greatest percentage cover of recruits occurring in April (estimated by direct counting,

Fig. 5.1) and in March (estimated by FiSAT, Fig. 5.2). The substantial difference in the peak of recruitment between both methods was presumably because of the difference in size at recruitment. Initial size at recruitment used by the first method is the overall individual limpet with shell length of < 4 mm whereas by FiSAT method is 8.5 mm in length. Furthermore, the time of most of the pulses was within 2 to 3 months after the period of spawning activity (see also Chapter 7), which is consistent with the relatively short larval life of 3 months old, estimated using the VBGC (see also Chapter 4).

The availability of recruits at the time when suitable conditions exist for their settlement and survival is paramount (Sousa, 1984). The low rates of recruitment among habitats during the periods of sampling may be either the result of different settlement of larvae into areas, or something killing the settlers before they became visible and could be censused. The last hypothesis seems unlikely because the limpets were observed to recruit at densities of up to 4.78 ind.m⁻² at the three shore levels. It is more likely that some process during the settlement phase was reducing the numbers recruiting to the areas. The observed spatial variation in density of settled spat may result from differential survival (Butman, 1987), but the comparatively low larval abundances and associated with high mortalities suggest that redistribution and accumulation of settlers is more likely to be responsible for observed recruitment patterns (Pulfrich, 1995). The direct effect of differences in the rates of recruitment within different habitats on the population dynamics of a species has also been reported for *Patella vulgata* (Lewis & Bowman, 1975; Thompson, 1980; Workman, 1983), *Acmaea scabra* (Sutherland, 1970), *Notoacmaea petterdi* (Creese, 1980b), and *Cellana tramoserica* (Fletcher, 1984). As with most other studies of marine invertebrates, the phenomenon of settlement was not observed; therefore, I could not distinguish between these two alternatives. The other consideration that may be applied to the population of limpet *C. testudinaria*; it is possible that this species may be came from other populations from different shores and recruited into the areas. However, in order to confirm this assumption, it is important to consider the magnitude and extent of larval exchange between the populations of other shores, but I did not cover it in the present study.

5.4.3. Effect of environmental factors on recruitment

The success and intensity of recruitment at the shore varied seasonally: fewer recruits became juveniles in dry season than during the cooler rainy season. Even though there have been relatively few studies on settlement and recruitment of sessile species in tropical regions (Lawson, 1957; Sutherland & Ortega, 1986; Sutherland, 1987; Walters et al., 1996), previous studies have shown that survival of recruits during summer is low, because some parts have increased levels of physical stress (Kaehler & Williams, 1996). When spat first appeared on the shore in early December, good recruitment usually followed. In poor recruitment periods, spat were not found on the observation shore until the following transition period in March, and were often stunted and atypically coloured. Though there is probably an element of truth in this

explanation, the correlation between variability in seawater temperature and salinity and recruitment success was built up. Determination of the effects of seawater temperature and salinity on the recruitment of *C.testudinaria*, using data available, has been hindered by a succession of poor recruitments, so field experimental studies are still lacking. However, analyses in progress with known differences in seawater temperature and salinity regimes during different seasons are indicating that this species is more susceptible to temperature and salinity variation.

Such a widespread recruitment succession during the rainy season, and its being optimal coincided with the temperature and salinity between ca. 31-32 °C and 33-34, respectively. However, heavy recruitment following the period of transition in April, suggests that recruitment intensity is not governed by temperature alone. Bowman and Lewis (1986) studied the effect of geographical differences in breeding cycles on the recruitment variation of the limpets *Patella vulgata* and *Patella aspera* found that the cause of these differences could be correlated with geographical and annual differences in seawater temperature over the potential breeding periods. They pointed out that the spawning period coincides with the proposed temperature “window” optimal for spat settlement and survival, and it appears that the main spawning time for each region occurs during autumn and winter when mean seawater temperature drops below 12 °C.

Since no experimental work relating the effect of salinity on the recruitment of limpet could be traced in the available literature, the results so far obtained indicate that salinity effecting the recruitment may be coupled with temperature. In the present study, therefore, no conclusion can be drawn as to how succession of recruitment might be related to the salinity variabilities. Arnold (1955) shows the response to different salinities as a function of age; young limpets seem tolerant of reduced salinities, but as they age the high-shore specimens retain this tolerance while low-shore limpets lose this potential.

The idea that physical factors such as tidal cycle and wave action limit the recruitment of limpets is well established. Tidal currents and wave processes are important in transporting larvae to suitable settlement areas, where they may subsequently choose to settle or remain unattached, depending on settlement preferences. Comparatively recently it has been shown that the correlations are not proof that these factors limit the species but they may simply reflect adaptations to prevailing conditions. Regardless of whether tidal cycles and wave forces significantly affect the recruitment pattern, analyses evidence show that an increase in tidal excursion caused a decrease in recruit density while an increase wave height will be followed by an increase in recruit density (Table 6.1).

Recruitment is variable and affected by differences in various physical and biological factors (Kaehler & Williams, 1996; 1997) at broad scales (e.g. tidal height). Of the tidal excursion factor considered in this study, desiccation may reasonably be expect to be the unusual range of tidal cycle related to regularly high levels of mortality events. Of course, it is difficult to separate the effects of desiccation from those of heat stress, as both often act together, and heat may lower the resistance to desiccation. During high tide, juvenile limpets may be flushed

immediately to the higher shore levels and retained in the tide pools for a little bit longer time. Sutherland (1970) proposed the mechanism of tidal cycle, which might have led to greater differences in densities of juveniles at different shore levels. There was gregariousness during settlement of the larvae, the longer period of submersion during which settlement can occur, and the lower rates of mortality due to desiccation.

Most of the effects of wave action are anecdotal. No attempts have been made to simultaneously quantify wave action to measure this effect on recruitment. However, several authors proposed that wave forces could break or dislodge organisms, in the process opening patches of substratum for settlement or invasion (Connell, 1972; Paine & Levin, 1981; Denny et al., 1985). Wave forces are also too severe, herbivores such as limpets, may be unable to forage (Menge, 1978; Lubchenco & Menge, 1978; Wright, 1978). In the present study, even though no correlation was found between the variability of wave height and recruitment rate, it seems that an increase of wave height will be followed by an increase in recruit density (Figure 6.6). Denny (1985) showed that an increase of wave action might increase the probability of settling limpets to the substratum. It is also possible that increased wave exposure is correlated with increased juvenile survival because there is more splash and spray on exposed shores during low tide and therefore, perhaps, less desiccation of juvenile limpets.

It is obvious from the preceding discussion of the available literature that only partial success can be achieved in the fulfilment of the original aims of this chapter. Although recruitment pattern varied between different seasons and shore levels, there was no evidence to suggest that variation in environmental variables within the shore examined at intertidal of Ohoiwait consistently, or predictably, affected juvenile *C. testudinaria* recruitment patterns. Several factors of the life history during the planktonic phase of the species are not yet quantified, and some aspects of limits to their pre- and post-settlement have not been tested by experiments or observations in the field. It is, therefore, impossible to make coherent general statements about many of the factors influencing the recruitment of this species.

Chapter 6

MORTALITY

6.1. Introduction

The determination of the mortality of a species is a fundamental requirement for any analysis of the structure and dynamics of biological communities (Ebert, 1973; Van Sickle, 1977; Underwood, 1979; Creese, 1981; den Boer, 1981; Fletcher, 1984; Takada, 1997). For many years, ecologists believed that it is impossible to have a better understanding of population dynamic of species without knowing a theoretical mortality model for a population (Underwood, 1975; Van Sickle, 1977; Fletcher, 1987). Admittedly only after some years, it became evident that mortality is a vital parameter in analysing the community structures of population.

Many studies have investigated the mortality rate of limpet species, often under different environmental conditions, relating any variations found to differences in their respective regimes of selection. Many authors mention the presence of mortality caused by natural influences, such as heavy storms, strong wave action, and extreme high or low temperatures; these effects are sometimes observed in limpets during their life stage as planktonic larva, juvenile, and adult individuals (Lewis & Bowman, 1975; Blankley & Branch, 1985; Fletcher, 1987). Catastrophic limpet mortality due to temperature extremes in relation to desiccation has been noted in the high intertidal on particularly stressful days (Orton, 1933; Frank, 1965; Sutherland, 1970; Wolcott, 1973). Little mortality is found at high levels on the shore, although occasional “catastrophic” mortality may occur (Creese, 1980b). In addition, sublethal levels of desiccation may be responsible for making limpets more susceptible to predation because of the decreasing of their tenacity (Lewis, 1954; Frank, 1965; Breen, 1972). Such studies on intertidal limpets, have also found that marked inter- and intraspecific variations in the population communities strongly influence mortality rate of species (Sutherland, 1970; Lewis & Bowman, 1975; Choat, 1977; Thompson, 1979; Creese, 1980a, Workman, 1983; Fletcher, 1984). The importance of the spreading of the risk of extinction species over differently fluctuating subpopulations is demonstrated by analysing the density of population in which mortality influences upon them (den Boer, 1981). Workman (1983) investigated two populations of *Patella vulgata* and found that the rates of mortality between the two populations differed although the reproductive effort of the two populations was similar. He concluded that the influences of the environment on the time of fecundity of individuals from different two places must have been different.

In order to establish a basis for the later more extensive mortality analysis in this chapter, the general variation of limpet's density of *C. testudinaria* throughout the sampling period was studied. Therefore in some cases the environmental variables influencing the mortality should also be included. In this study three different methods in estimating mortality rate were carried out.

6.2. Materials and Methods

Each year a proportion of limpets alive at the beginning of the year will die; some by predation, disease, or other natural causes, and some through exploitation, while others will survive until the beginning of the following year. Mathematically, this can be expressed through the relation:

$$N_1 = P + D + O + E + N_2$$

where N_1 and N_2 are the respective abundance at periods 1 and 2, P is annual predation rate, D is annual rate of the death by disease, O is annual rate of death by other causes, E is annual rate of death by exploitation. Unfortunately, this equation cannot be used when there are combined effects of different causes of mortality or mortality happened at an unpredictable time. It is therefore better to consider the instantaneous rates, i.e. the rates applying over a short period of time (e.g. 1 month), during which the numbers in the population do not change significantly. So, the death combining all causes namely total mortality (Z) can be measured as:

$$N_2 = N_1 e^{-Zt}$$

Where t is the elapsed time between N_1 and N_2 .

Data on distribution, abundance, and biomass were used to determine mortality of the limpet *C. testudinaria*. Simulating the fluctuation pattern of population density, three different methods were applied to have direct estimating of mortality rates:

1. *Reduction in the survival of individual size frequency cohorts through time.* The estimate of mortality rates was based on the monthly survival number of individuals, derived from the size frequency of cohorts. This method helps to provide an optimal fit to a size-frequency histogram, and then survival curves of those organisms were estimated. The efficacy of this method is that if the recruitments occur several times along the year, then it is difficult to differentiate the mode of polymodal curves. The survival curve of *C. testudinaria* was constructed by following the cohorts recruited during the period of sampling. The periods used to estimate the rates of mortality were deliberately chosen to correspond to periods when numbers in a particular cohort decreased in an approximately logarithmic way.
2. *Monthly calculation of the Ault and Ehrhardt moment estimate of mortality* (Ault & Ehrhardt, 1991). This model does not assume an infinite life span for the animal of the stock being analysed and thus is applicable to short-lived tropical species (Sparre & Venema, 1998). The formula is:

$$[(L_{\infty} - L_{max}) / (L_{\infty} - L_c)]^{ZK} = [Z(L_c - L_{\infty}) + K(L_{\infty} - L_{\infty})] / [Z(L_{max} - L_{\infty}) + K(L_{\infty} - L_{\infty})]$$

where Z is the instantaneous rate of mortality, L_∞ and K are growth parameters, L_{max} is the maximum length or the largest limpet in the sample, L_x is the mean length of limpet in a sample representing a steady-state population, L_c is the cut-off length or the lower limit of the smallest length class included in the computation (< 8.5 mm). This equation uses the fact that the size frequency distribution of a population is the result of previous growth, mortality, and recruitment (Ebert, 1973).

3. *Length-converted catch curves.* This method was developed by Sparre & Venema (1998) under the computer program FiSAT. The mortalities can be estimated directly from observed size-frequency curves in which individual growth rates are changing with body size, as discussed by Ricker (1958), and growth data without explicitly determining the population's age distribution (Van Sickle, 1977) with the assumptions that the population should fulfil the required stationary or steady-state and of time-invariance in growth and mortality rates (Smith, 1972).

Several physical factors including desiccation, water loss and osmotic equilibrium of the limpet body may potentially cause increasing mortality rate, even though water loss in the field seldom reaches immediately lethal levels in the mid- to low-intertidal (Davies, 1969; Wolcott, 1973). To estimate the mortality rates due to those causes, least-square method (Sokal & Rohlf, 1995) was applied to analyse the relationships between monthly mortality rates (as estimated using Ault-Ehrhardt method) and monthly environmental variables.

6.3. Results

6.3.1. Reduction in the survival of cohorts

Estimations of seasonal and annual mortalities for *C. testudinaria* populations were quantitatively calculated. Logarithms of survival individuals in each cohort ($\text{Log}_{10} N$) were plotted against time (month) to provide the survivorship curves. This produces a straight-line plot, which suggests that the probabilities of death numbers of limpets were evenly distributed throughout the sampling period. Mortality rates were determined from the slope of the regression line. Three different cohorts of cohorts-C, cohort-D, and cohort-E were chosen, which presumably represent the all cohorts from the 12 months of sampling period.

Cohort-C. Survival curve of this cohort is presented in Figure 6.1 and summarised in Table 6.1. Survival limpets decreased rapidly in the first 5 months. A slight increase in individual numbers was observed between December 2001 and January 2002 and from February to April 2002. No clear seasonal trends can be recorded in May 2002. Regression equation is: $\text{Log}_{10}(\text{individual numbers}) = 1.1973 - 0.0699 * \text{Julian date (months)}$, $r = 0.65$, $n = 8$. From this equation the mortality rate was calculated to be 85 % per year ($Z = 1.93$).

Table 6.1. Summary of survival numbers (SN) of cohort-C together with ANOVA test on the relationship. *df*-degrees of freedom; *SS*-sum of squares; *MS*-mean square.

Time	SN
October '01	21
November '01	12
December '01	6
January '02	8
February '02	3
March '02	9
April '02	10
May '02	69
June '02	3
July '02	-
August '02	-
September '02	-

ANOVA				
Sources	df	SS	MS	F
Regression	1	0.24	0.24	4.32
Residual	6	0.34	0.06	
Total	7	0.58		

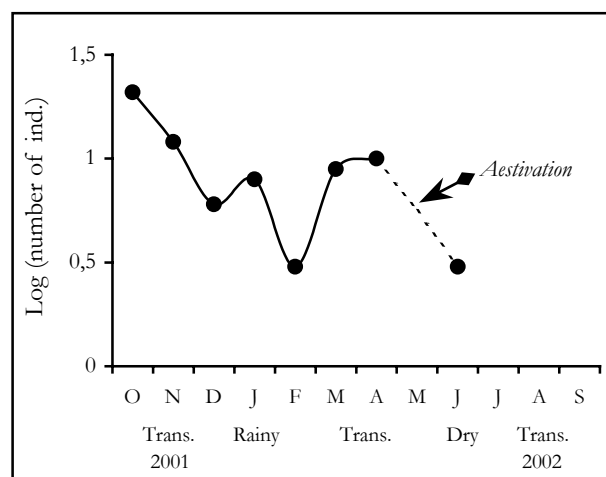


Figure 6.1. *Cellana testudinaria*. Survival curve of cohort-C. There were 21 individuals at the beginning of which 3 remained after 7 months.

Cohort-D. Survival curve of this cohort is presented in Figure 6.2 and summarised in Table 6.2. The numbers of survival decrease slightly and almost linear throughout the period of study, in which 209 individuals marked at the beginning of which only 2 individuals remained after 11 months of sampling. An increase of survival limpets between May and June 2002 was may be partly due to an extended period of recruitment. Regression equation is: $\text{Log}_{10}[\text{number of individuals}] = 2.2755 - 0.1582 \times \text{Julian date (months)}$, $r = 0.88$, $n = 11$. The mortality rate of this cohort is 98.7 % per year ($Z = 4.37$).

Cohort-E. Survival curve of this cohort is presented in Figure 6.3 and summarised in Table 6.3. This cohort has a much steeper survivorship curve. In the first four months and in the last three months of their life, no dying individuals of limpets were encountered. However, this cohort had relatively great mortality rate in the middle period of their life. The greatest decrease in numbers was found between February and June, in which 67 individuals die during this period. Regression equation is: $\text{Log}_{10}[\text{number of individuals}] = 1.9047 - 0.0816 \times \text{Julian date (months)}$, $r = 0.64$, $n = 10$. The mortality rate of this cohort is 90 % per year ($Z = 2.30$).

Table 6.2. Summary of survival numbers (SN) of cohort-D together with ANOVA test on the relationship. *df*-degrees of freedom; *SS*-sum of squares; *MS*-mean square.

Time	SN
October '01	209
November '01	132
December '01	45
January '02	44
February '02	18
March '02	11
April '02	15
May '02	5
June '02	27
July '02	9
August '02	2
September '02	-

ANOVA				
Sources	df	SS	MS	F
Regression	1	2.75	2.75	32.01
Residual	9	0.77	0.09	
Total	10	3.53		

Table 6.3. Summary of survival numbers (SN) of cohort-E together with ANOVA test on the relationship. *df*-degrees of freedom; *SS*-sum of squares; *MS*-mean square.

Time	SN
October '01	-
November '01	55
December '01	51
January '02	52
February '02	71
March '02	24
April '02	55
May '02	14
June '02	4
July '02	18
August '02	26
September '02	-

ANOVA				
Sources	df	SS	MS	F
Regression	1	0.55	0.55	5.67
Residual	8	0.77	0.09	
Total	9	1.32		

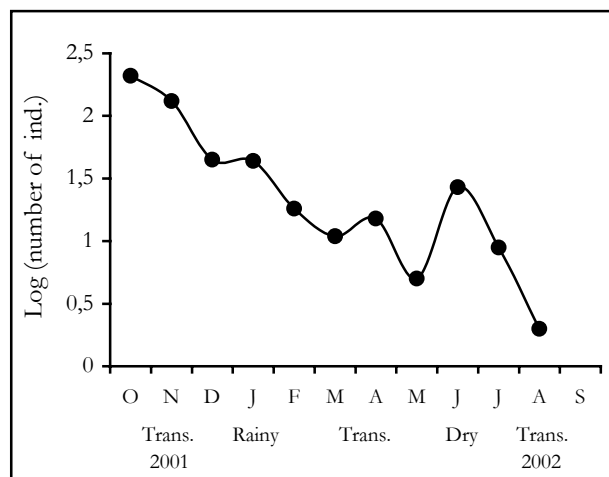


Figure 6.2. *Cellana testudinaria*. Survival curve of cohort-D. There were 209 individuals at the beginning of which 2 remained after 11 months.

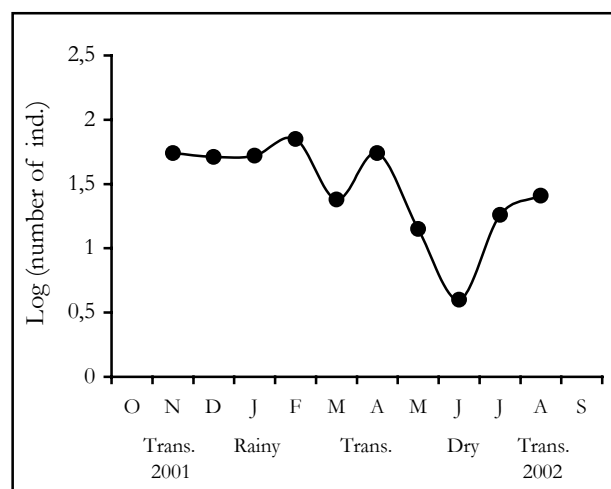


Figure 6.3. *Cellana testudinaria*. Survival curve of cohort-E. There were 55 individuals at the beginning of which 4 remained after 7 months.

6.3.2. Ault-Ehrhardt's method

Monthly mortality rates of limpet *C. testudinaria* are summarised in Table 6.4 and presented in Figure 6.4. An average rate of mortality for this species was 91 % per year ($Z = 2.46 \pm 0.55$ of 95 % confidence level), which has been estimated from the mean length of 17.42 ± 1.99 mm and the mean maximum length of 30.32 ± 1.02 mm.

Table 6.4. Monthly mortality rates of limpet *Cellana testudinaria* based on the Ault-Ehrhardt's equation. $L_{\infty} = 33.08$; $K = 1.4$; $L_h = 8.5$ mm; L_x - the mean length (mm); L_{max} -the largest limpet in the sample (mm); Z -mortality rate.

Time	L_x	L_{max}	Z
October '01	15.58	29.3	3.40
November '01	16.55	29.3	2.72
December '01	18.74	31.8	1.90
January '02	16.48	30.5	2.87
February '02	16.35	31.6	2.97
March '02	17.34	30.0	2.33
April '02	15.22	29.0	3.67
May '02	17.32	30.5	2.40
June '02	16.81	30.5	2.68
July '02	16.60	30.4	2.79
August '02	19.64	29.1	1.03
September '02	22.40	31.8	0.77

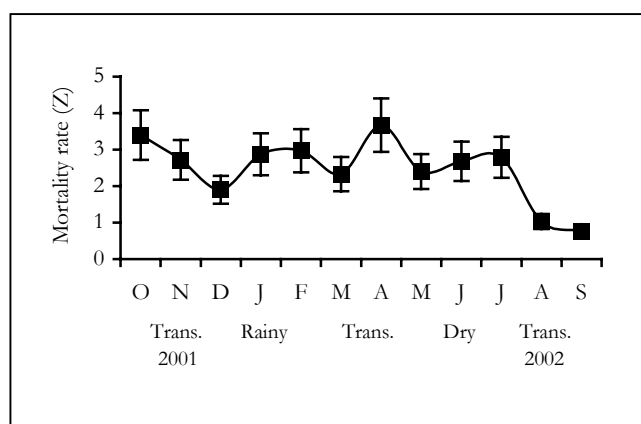


Figure 6.4. *Cellana testudinaria*. Monthly mortality rates calculated using the Ault-Ehrhardt equation within the population. Vertical lines represent standard deviation (± 1 SD).

The greatest mortality rate was calculated for October 2001 and April 2002, while the lowest one was in September 2002. Monthly mean mortality rate of 7.7 % per month ($Z = 2.58$) during the rainy season was higher than 7.4 % per month ($Z = 2.17$) during the dry season. On the other hand, during the transition (September – November), the mean rate of mortality of 7.5 % per month ($Z = 2.30$) was lower than 7.8 % per month ($Z = 2.8$) during the transition (March – May).

6.3.3. Length converted catch curve

Another estimate of the mortality of the limpet *C. testudinaria* was made from the losses of individuals in the size-classes (Table 6.5). These animals were separated into size groups in the interval of 1-mm. The length converted catch curve was constructed by plotting relative age of samples (dt) against natural logarithm of individual numbers in each class ($\ln N/dt$), which is presented in Figure 6.5. Data of the relative age in each class were derived from the von Bertalanffy growth formula (VBGF); $L_t = 33.08 [1 - e^{-1.4(t + 0.09)}]$. The linear regression equation was: $Y = -2.8143 \pm 0.3740 X + 9.5033 \pm 0.3886$. Analysis of variance on this relationship showed that there is a high correlation ($r = -0.96$) and highly significant on linear regression ($F = 243, P < 0.05$) (Table 6.5). The calculated mortality rate was 94 % per year ($Z = 2.81 \pm 0.37$ of 95 % confidence limit).

Table 6.5. Calculation of mortality rate of limpet *Cellana testudinaria* based on the length converted catch curve method together with ANOVA on the relationship. N-number of individuals at size class; dt-relative age. *df*-degrees of freedom; *SS*-sum of squares; *MS*-mean square; *CL*-confidence levels.

Mid-Size	N	dt	Ln(N/dt)
8.50	90	0.302	8.038
9.50	101	0.332	8.112
10.50	134	0.363	8.351
11.50	173	0.395	8.561
12.50	224	0.429	8.772
13.50	297	0.465	9.004
14.50	274	0.502	8.871
15.50	180	0.542	8.396
16.50	163	0.583	8.238
17.50	139	0.628	8.017
18.50	102	0.675	7.641
19.50	70	0.726	7.193
20.50	46	0.781	6.697
21.50	36	0.840	6.369
22.50	37	0.904	6.306
23.50	34	0.975	6.122
24.50	50	1.054	6.397
25.50	65	1.142	6.535
26.50	56	1.243	6.244
27.50	42	1.361	5.791
28.50	30	1.502	5.255
29.50	25	1.678	4.824
30.50	23	1.912	4.407
31.50	11	2.263	3.157

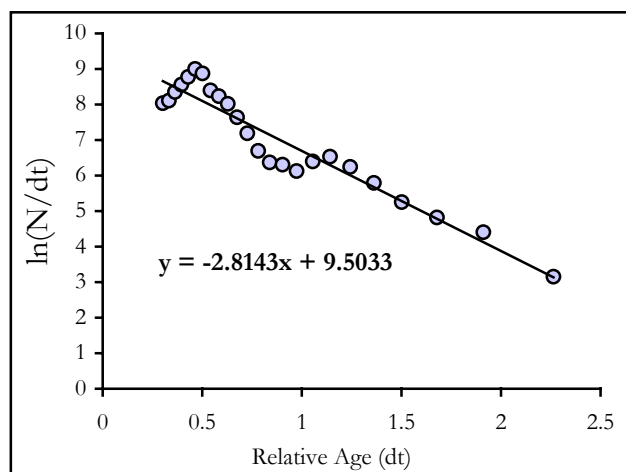


Figure 6.5. Length converted catch curve of *C. Testudinaria*.

ANOVA				
Sources	df	SS	MS	F
Regression	1	51.18	51.18	243.37
Residual	22	4.63	0.21	
Total	23	55.81		
Multiple R	- 0.96		95 % CL	
Intercept (a)	9.5031		0.3886	
Variable (b)	- 2.8140		0.3740	

Analysis of variance on the relationship between mortality rates and environmental variables showed that the relationship has a high correlation ($r = 0.86$) but no significant linear regression ($F = 1.04$, $P > 0.05$).

Table 6.6. *Cellana testudinaria*. Multiple regressions on the relationship between monthly mortality rates (Z) and environmental variables. Mortality rates were derived from Ault-Ehrhardt method. Data of environmental variables were transformed using $\log_{10}(X)$.

Regression statistic	
<i>R square</i>	0.86
<i>SE</i>	0.85
<i>Observations</i>	12

ANOVA				
Sources	df	SS	MS	F
Regression	8	6.0534	0.7567	1.0401
Residual	3	2.1825	0.7275	
Total	11	8.2359		

6.4. Discussion

Despite the problems of data interpretation created by inadequate sampling in the present study, a general pattern of seasonal mortality occurrence of *C. testudinaria* is suggested for the intertidal rocky shore of Ohoiwait. Mortality in this limpet species may be divided into juvenile and adult phases as was pointed out by Lewis & Bowman (1975), whereby juvenile mortality is inextricably bound up with recruitment processes, and is complicated by differences between populations at the time of emergence, and also immigration and emigration. A general distinction, however, could not be differentiated since the numbers of juvenile limpets derived from the reduction in their cohort density in the stage of post-larval settlement were unlikely counted due to recruitment occurrence throughout most of the year, and the cause of these juvenile mortalities is not clear (Creese, 1981). Subject to these limitations, the three different methods were considerably used, so that all limpet sizes in their life stage are possible to be included to provide the right estimation.

Mortality rate of *C. testudinaria* population appeared to be positively related to their density as shown in the reduction of individual size frequency cohorts and length converted catch curve methods, but not to the growth rate (as suggested by Ebert, 1982 and Parry, 1982). This was similar to the most other intra-specific studies of limpets, which have also proved that mortality has a negative correlation with respect to the growth but positively related to the population density (Sutherland, 1970; Underwood, 1976; 1978; Creese, 1980b; Creese & Underwood, 1982; Fletcher, 1984). Similar results in the estimation of mortality rates using these three methods; an average of 91 %, 91 %, and 94 % loss of individuals per year, indicates that these results are realistic and the assumptions behind those methods have been matched. This consideration shows that size-frequency analyses is most applicable to short-lived species (< 5 years, say) with relatively restricted periods of recruitment and rapid enough growth so that age cohorts can be confidently distinguished over long periods of time preferably until they disappear from the population.

It has often been stated that rapid growth is directly related to high mortality rate (Ebert, 1973). Of the species studied here, *C. testudinaria* grows faster ($K = 1.4$) and mortality rate was higher ($Z = 2.81$), thus lives shorter ($t_{\max} = 2$ years). Creese (1981) reported that *Siphonaria virgulata* is the fastest growing limpet that is suffering the greatest mortality, while *Notoacmea petterdi* grows slowest and lives longest (lower mortality).

Many earlier workers have concluded that most adult limpets die of old age, and that longevity is inversely related to the mortality rate (Moore, 1937; Lewis & Bowman, 1975; Thompson, 1980). These observations agree with Ault-Ehrhardt estimation of mortality in the present study; e.g. limpets with shorter maximum length (shorter longevity) have lower mortality than those with longer maximum length (see Table 6.4). An alternative estimation, therefore, can be gained from this method resulting an approximately maximum longevity of this limpet species

of 2 years old. This estimation is also very close to the previous estimation using von Bertalanffy equation (see also Chapter 4, section *t₀ and longevity*).

Catastrophic mortality from either environmental or biological factors, or both, has been documented for several populations of intertidal limpets (Lewis, 1954; Hodgkin, 1960; Frank, 1965; Sutherland, 1970; Creese, 1981; Parry, 1982). I put forward the hypothesis that rate of mortality of *C. testudinaria* is enhanced by environmental conditions. In this study, even though, sources of mortality were not directly determined, it seems to be related to the harsh summer conditions. Since insolation and air temperature data from the intertidal rocky shore of Ohoiwait do not exist, I have assumed that insolation and air temperature in this area is generally correlated with insolation and air temperature in Tual (± 20 km far away) (source from Tual Meteorology and Geophysics), and hence with mortality of limpets in Ohoiwait. From July to August during the dry season, intertidal of Ohoiwait received high insolation, causing an increase of seawater temperature in rock pools. Field observation indicated that temperature of surface rocks and pools might reach 40 °C. Many individuals of juvenile and adult limpets were observed dead on the bare rocks high on the shore and in the pools during this season. Result of ANOVA proves this hypothesis, since mortality is highly positive correlated to the air temperature and insolation (Table 6.6). Fletcher (1987) found that the population of *Patelloida alticostata* living at intertidal and subtidal of New South Wales, Australia, could have been affected by extremes in air temperature, for the density of the adults declined considerably during the summer.

Desiccation is generally considered to be an important environmental stress causing mortality of organisms (see review by Branch, 1981). Many tropical gastropods were found dying due to higher desiccation stress (Garrity, 1984), specifically related to limpet species (Evans, 1947; Southward & Orton, 1954; Lowell, 1984). Desiccation is also more likely to have killed *Kerguelenella lateralis* (Branch, 1981), but this factor could not be blamed for dying sub-Antarctic limpet *Nacella macquariensis* (Simpson, 1976).

Temperature may also be important in enhancing the juvenile survival by affecting the seasonal pattern of algal occurrence on the shore. Hodgkiss (1984) found that during summer, encrusting algae are being bare on the high Hong Kong shores, but a few macroalgae occur on the middle and lower shores. From July to August, macroalgae are impoverished on Ohoiwait rocky shore (direct field observations), the high shore being bare of algae at this time, causing lower numbers of limpets at this shore level (see also density of limpets at different shore levels as presented in Fig. 3.2). This result is similar to the previous work by Parry (1982) who suggested that the rate of mortality of *Cellana* increased when macroalgae was absent. In contrast, Underwood & Jernakoff (1981) found that *Cellana* could not control the amount of macroalgae low on the shore and actually died of starvation when surrounded by macroalgae.

The mortality of limpets was also found to be higher in April 2002. This is presumably because of strongly breaking waves during this period (such caused by higher wave height as shown in Fig. 2.5), which may impose large forces on intertidal organisms. A wave breaking on

the shore could exert very large impact pressure on the organisms present (Jones & Demetropoulos, 1968; Denny, 1982; 1985). Wave action may also affect a number of factors including desiccation, abrasion, crushing, and the danger of detachment (Branch, 1981; Grenon & Walker, 1981). No attempts have been made to simultaneously quantify the effects of wave actions on the mortality of limpets, but direct field observations found that many organisms have been dislodged from the substratum and sporadically breaking shell due to frequent large wave action along the shore on the period of transition (from March to May).

Biological factors, e.g., predation, competition for food, and commercial exploitation by coastal populations can also cause mortality. Predation on *C. testudinaria* on the intertidal of Ohoiwait has seldom been observed. I have no direct observations on predation of this species, but assume that they appear to be an erratic and local influence on limpets. Shells of these limpets were sometimes found drilled by the dogwhelk *Thais lapillus*. This predation has not been included in this study, but is probably similar in principle to the predation of *Nucella lapillus* on *Patella vulgata* (Thompson, 1980). On the rocky shore of Ohoiwait, *Thais lapillus* is abundant but patchily distributed in groups. Nevertheless, although a roaming group of this species could potentially destroy a population of *C. testudinaria*, the proportion of drilled shells was quite low in groups of dead shells. *T. lapillus* appeared to be feeding on limpets only occasionally. Other predators are birds, but they were not responsible for any regular and widespread pattern of adult mortality, because they feed in small flocks and only during spring low tide (in October), and hence could not well be counted for the sudden disappearance of limpets from the intertidal of Ohoiwait.

Mortality is greater at low levels because of increased intra-specific competition, which is a result of greater recruitment to these levels (Creese, 1980b), and overcrowding in a diminished food intake and restricted to a narrow range on the shore (Liu, 1994). In the present study, I could not point out the sources causing mortality on limpet species from the intra-specific competition view, since there were some problems in determining certain factors responsible for it.

Commercial exploitations as the source of food by coastal populations were also not responsible for any regular and widespread pattern of adult mortality, but they could exert catastrophic effects on species groups of limpets. During spring low tides, many coastal populations exploited the limpet species for their daily food, but this is occasionally and in small areas. I did not, therefore, include it in determining mortality rate from these sources.

It is apparent that the present work could best be described as pre-surveillance with an estimated mortality. Since it is impossible to provide really satisfactory results in estimating mortality from available data, summer heat, predation, intra-specific competition for food, and commercial exploitation thus appear to be the major causes of death of limpets on Ohoiwait's intertidal.

Chapter 7

GONAD CONDITION

7.1. Introduction

The concept of gonad condition has assumed a central position in studies of the reproductive cycle of limpet species, since it was first elaborated by Orton (1928). In Orton's original concept, the expenditure of gonad condition in any increase of gonad development stages was associated with reproductive cycle. Now, this concept is an evidence for preliminary and further observations in studying of reproductive cycle of limpet species (Balaparameswara Rao, 1973), and has been developed by using histological observation techniques (Dunmore & Schiel, 2000).

Many studies of intertidal invertebrates have examined reproductive cycles to provide basic information on spawning times and fecundity (see review in Branch, 1981). This knowledge is useful in determining the timing of recruits on the shore, sexual maturity, and turnover rate of individuals in a population (Dunmore & Schiel, 2000), since many limpet species show different reproductive cycles with varying extended breeding seasons, having restricted spawning periods per year, and long resting stages in between.

Extensive investigations have been made on gonad condition of many temperate climate limpets in order to study their reproductive cycles, e.g., *Patella vulgata* (Orton, 1928; 1946; Das & Seshappa, 1947; Orton et al., 1956; Blackmore, 1969a; Bowman & Lewis, 1977; Thompson, 1980), *Patella depressa* (Orton & Southward, 1961), *Patella aspera* (Thompson, 1979), *Nacella concinna* (Picken, 1980; Picken & Allan, 1983; Brêthes et al., 1994). Similar studies on tropical species are scanty, e.g., *Cellana radiata* (Balaparameswara Rao, 1973), *Notoacmea petterdi* (Creese, 1980a; Parry, 1982), *Cellana tramoserica* (Parry, 1982), *Cellana radians* (Creese & Ballantine, 1983), *Patelloida alticostata* (Creese, 1980a; Parry, 1982; Fletcher, 1984), *Patella flexuosa* (Iwasaki, 1998), *Cellana ornata* (Dunmore & Schiel, 2000), and the keyhole limpet *Fissurella barbadensis* (Ward, 1967).

In this chapter, gonad conditions of *C. testudinaria* limpets are studied in detail by examining every gonad development stage (Orton et al., 1956; Balaparameswara Rao, 1973), in order to determine its breeding cycle more accurately. Furthermore, environmental variables, which may influence the spawning time have been investigated.

7.2. Materials and Methods

7.2.1. Sample collection and gonad analyses

Because limpets generally possess no known secondary sexual characters (Blackmore, 1969a), sexing and measurements of gonad activity can be made directly upon the gonads after cutting open the foot (Balaparameswara Rao, 1973). To determine gonad activity, collections were made monthly on three different shore levels of the intertidal of Ohoiwait. Thirty limpets between 15-25 mm and greater than 30 mm shell length were collected. Limpets are reproductively mature at this size (Blackmore, 1969a, b). The specimens were then divided into two size groups, namely those with a shell length between 15-25 mm (15-25 mm group) and those with a shell length greater than 30 mm (>30 mm group). The numbers of females and males are approximately equal, and in each group they were treated separately. These sexes of limpets (Fam. Patellidae) could usually be separated clearly by color (Orton, 1928): the male gonad being yellow or orange, and the female violet brown or dark brown (Balaparameswara Rao, 1973). But sometimes it was doubtful to distinguish the sexes of individuals in the resting phase between spawning and development of the gonad. In *C. testudinaria* the gonad in both sexes lies on the ventral face between the visceral mass and foot. Gonad condition was assessed from morphological examination of the gonad as described by Orton et al. (1956) (Figure A-1, Appendix 1).

7.2.2. Gonad indices

Reproductive activity was described using a gonad index. Gonads may be examined morphologically and classified into various developmental stages. One such classification is suggested by Orton et al. (1956) as is shown in Table A-1 and Figure A-1 (Appendix 1). Although it should be noted that gonad development is continuous and all such staging is somewhat artificial. Whichever classification is used, there is some merit in defining as few stages as possible as the main interest in limpets studies is in determining the time when the majority of the population is in the final developing and ripe, spawning, and resting stages. The classification of gonads into different stages may be possible with detailed histological studies, but this research is necessarily laboratory-based and expensive, and hence it was not included in the present study. In many cases, division into broad stages may be based on simple morphologic criteria such as gonad appearance and colour. Such assessments are useful in the fieldwork, although, the criteria should first be validated by histological studies.

On the basis of size, colour and abundance of ripe (loose) gametes, each gonad was classified as neuter or in one of five stages of development, spawning or re-development, from which a mean gonad condition (MGC) was calculated: $MGC = 0$ if all animals were neuter, $MGC = 5$ if all fully developed. Three methods were used to identify sex ratios and to estimate maturation and spawning time. Firstly, the MGC was determined for each sample by multiplying

the number of limpets in each gonad stage with the number allotted to the stage (Table A-1. Appendix 1) and the values obtained added together. To give the MGC value, the sum of these products are divided by the total number of individuals in the whole sample (Chipperfield, 1953; Orton et al., 1956). Values for males and females were separated. The resultant mean gonad condition varies from 0, when the entire population is resting, to 5 when all individuals are fully ripe or spawning. A steady increase in the MGC indicates gonad development, while a sudden decrease indicates that spawning has commenced. Secondly, the Gonosomatic Index (GI; Ratios of gonad weight to somatic weight): $GI = Mean (G_w / S_w) \times 100 \%$, and finally, Somatic Index (SI): $SI = [Mean(S_w)^{0.5}] / L_{sh}$, where G_w is the gonad weight, S_w is the somatic tissue weight, and L_{sh} is the shell length. The specimens were then placed into a 10 % formalin-seawater solution and left for at least 4 weeks to allow the gonads to harden which aids in their dissection (Underwood, 1974; Creese, 1980a). This process decreased the body weight, but the decrease was assumed to be similar for gonad and somatic tissues (Creese, 1980a), and was therefore ignored. After this preservation, the shell length of each individual was measured and the gonad dissected from the remaining somatic tissue. Each part was wet weighed to the nearest 0.01 g, and consequently the monthly gonad index and somatic index was calculated, using the formula by Fletcher (1987).

7.2.3. Statistical analyses

The significant differences on mean gonad index and gonad condition for the whole year investigation were testified using Mann-Whitney U-test (Sokal & Rohlf, 1995), while the comparison of the monthly gonad condition for both sexes was examined using *t*-test (Sokal & Rohlf, 1995). In addition, I applied least-square regression analysis (Sokal & Rohlf, 1995) to analyse the relationship between gonad weight and somatic weight, and also to describe relationship between monthly gonad condition and the effects of environment variables upon them.

7.3. Results

7.3.1. Gonad Index

Although the gonad index introduced by Orton et al., (1956) was devised for *Patella vulgata*, and *Cellana radiata* (Balaparameswara Rao, 1973), no problems were encountered in applying it to *C. testudinaria*. Males and females followed similar trends in the mean stage of development of gonads along with the breeding cycle of these limpets throughout the year (Fig. 7.1).

The results of the observations made on the reproductive cycle show that *C. testudinaria* had mature gonads from May to August 2002. The mean GI and MGC for males were always higher than that for females from April to July (Table 7.1), and a significant difference in the indices were observed in May 2002 (Mann-Whitney U-test, $P < 0.05$), suggesting that males

matured earlier than females. Comparison of the monthly GI for both sexes using *t*-test (Sokal and Rohlf, 1995) showed that there were no significant differences between the gonad indices of male and female limpets ($t = 0.68$, $P > 0.05$) (Table 7.2).

Table 7.1. *Cellana testudinaria*. Monthly changes in gonad condition. GI-gonad index; MGC-mean gonad condition.

Month	GI (%)		MGC	
	Male	Female	Male	Female
Oct'01	4.92	10.01	0.91	2.01
Nov'01	-	8.99	-	1.80
Dec'01	-	-	-	-
Jan'02	-	-	-	-
Feb'02	-	-	-	-
Mar'02	2.13	2.13	0.43	0.43
Apr'02	3.53	2.51	0.71	0.50
May'02	13.21	6.77	2.64	1.35
Jun'02	22.54	17.53	4.51	3.51
Jul'02	23.56	19.32	4.71	3.86
Aug'02	18.32	22.59	3.66	4.52
Sep'02	9.12	15.04	1.82	3.01

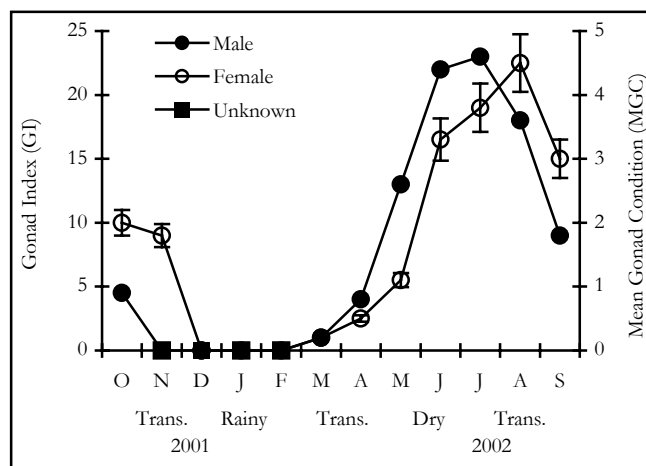


Figure 7.1. *Cellana testudinaria*. Monthly changes in gonad index (GI \pm 1SD) and mean gonad condition (MGC).

Table 7.2. *Cellana testudinaria*. *t*-test on the monthly gonad index (GI) between males and females.

Variables	Male	Female	<i>t</i> -test
Total numbers	168	192	0.68
Mean	8.11	8.74	
Standard deviation	8.70	8.74	
Variance	75.68	76.40	

It is clearly evident also from the figure that there is only one peak in the development of gonads, in July for male and in August for female limpets. The indices increased from March to July for males and from March to August for females. The peak occurred during the dry season (June – August) in both sexes with a quiescent period during the rainy season from December to February. Male gonads exceeded 24 % of body weight in July while females invested 23 % of their body weight in gonad tissue in August. These observations suggest that the gonads develop from March to June or July. The spawning months, which is indicated by dramatic decrease in the gonad indices, begin in July and August and extend up to November or December, and therefore indicates that the main reproductive season of this species was the dry season. After spawning in August 2002, the indices presumably remained low on the period of the transition and rainy seasons until February the next year, when the indices began a marked increase.

In the course of somatic index (SI) determinations, it became apparent that for the same shell length, males were usually lighter in gonad weight than females (Table 7.3). Spawning, as indicated by a significant increase in the proportion of SI-value, obviously occurred in the period

of August – September, while gonad maturity, as indicated by a decrease in the proportion of SI, was found in the period between March and April (Table 7.3).

Table 7.3. Average somatic indices (SI) of 15 – 25 mm and > 30 mm limpets of both sexes on the periods of March – April and August – September.

Period	Male		Female	
	15 – 25 mm	> 30 mm	15 – 25 mm	> 30 mm
March – April	0.12	0.09	0.13	0.12
August - September	0.07	0.08	0.10	0.11

A comparison of the somatic indices of limpets in the 15 – 25 mm and > 30 mm groups shows that the mean somatic indices of females was generally higher than those of males (Table 7.3).

7.3.2. Sex ratio

The actual sex ratios of limpets were determined from the length-frequency compositions of the respective limpet populations. The distribution of neuter, male and female limpets with respect to the shell length is shown in Figure 7.2. Sexes could be distinguished only from 10 mm shell length. In some cases, it is not possible, however, to distinguish the sex by colour of the gonad in a few forms of this size group especially in the range of 10 – 12 mm. Individuals of less than 12.5 mm in length were usually neuter, and rarely fully mature or immature. Males of *C. testudinaria* first mature at a shell length of 15 mm in small proportion, while females at shell length of 21 mm, have a low proportion and gradually increased in abundance. Female *C. testudinaria* were not found at lengths < 21 mm.

The proportion of sex ratio of *C. testudinaria* is rather disproportional, representing approximately 50 % neuter, 30 % male, and 20 % female of the overall population, respectively (Fig 7.2). Male limpets are greatest at about a length of 31 mm, where they represent almost 60 % of sexually mature individuals, while the proportion of females steadily increases from 21 mm to 31 mm shell length, ultimately comprising 40 % of the sexually mature individuals, and it is greatest at about 31 mm length.

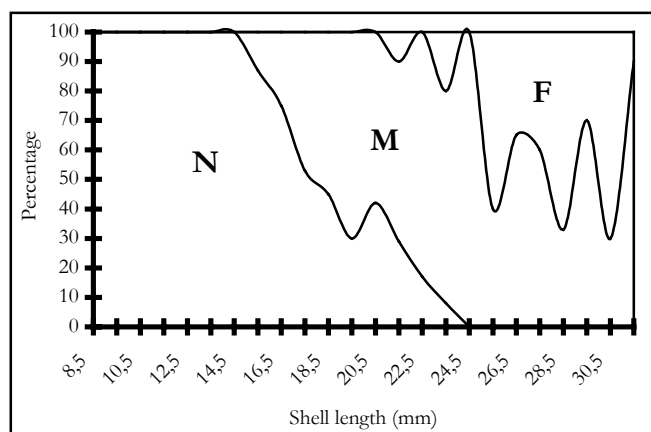


Figure 7.2. *Cellana testudinaria*. Sex proportions as estimated from length-frequency compositions of the respective limpet populations. N is neuter; M is male; F is female.

7.3.3. Gonad condition before and after spawning

The precise events that occur on the periods before and after spawning are examined in more detail by using least-square method on the relationship between gonad weight and somatic weight as are shown in Figure 7.3. The results showed that the relative gonad weight ratio dropped from 0.35 before spawning to 0.10 after spawning.

Table 7.4. Summary of specimen measurements of limpets collected in May 2002 (before spawning) and September 2002 (after spawning).

Spec.	Before spawning		After spawning	
	Gonad (g)	Somatic (g)	Gonad (g)	Somatic (g)
1	0.15	0.02	0.18	0.020
2	0.16	0.04	0.19	0.010
3	0.17	0.07	0.20	0.025
4	0.18	0.08	0.24	0.030
5	0.19	0.06	0.29	0.030
6	0.20	0.05	0.31	0.050
7	0.21	0.06	0.32	0.040
8	0.21	0.08	0.35	0.055
9	0.22	0.08	0.35	0.040
10	0.23	0.09	0.38	0.045
11	0.23	0.07	0.45	0.070
12	0.28	0.11	0.47	0.050
13	0.29	0.10	0.53	0.060
14	0.29	0.09	0.54	0.080
15	0.30	0.10	0.56	0.085
16	0.32	0.09	0.56	0.030
17	0.38	0.15	0.60	0.040
18	0.50	0.15	0.61	0.050
19	0.52	0.19	0.62	0.045
20	0.55	0.25	0.67	0.088
21	0.56	0.25	0.70	0.091
22	0.59	0.13	0.72	0.093
23	0.61	0.15	0.75	0.087
24	0.69	0.20	0.75	0.077
25	0.71	0.31	0.76	0.079
26	0.71	0.22	0.77	0.090
27	0.83	0.25	0.78	0.099
28	0.85	0.27	0.78	0.088
29	0.87	0.33	0.80	0.068
30	0.88	0.32	0.89	0.076

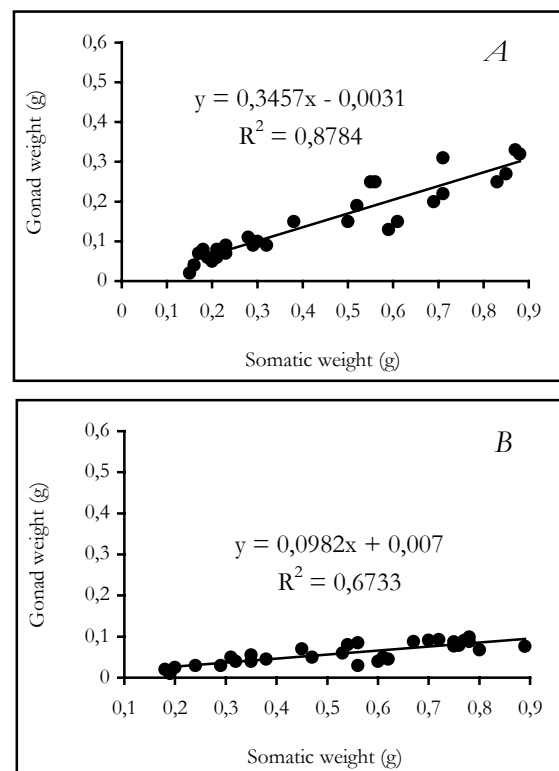


Figure 7.3. *Cellana testudinaria*. Relationship between gonad weight and somatic weight; before (A) and after (B) spawning event.

7.4. Discussion

7.4.1. Appropriate in using the method

Gonad condition can be a useful indicator of the reproductive status of limpet populations (Orton et al., 1956; Balaparameswara Rao, 1973; Bowman & Lewis, 1977; Thompson, 1979; Parry, 1982). However, many methods of measuring the gonad condition described in the literatures require cumbersome, arduous, and event innovation in measurements, and hence are inappropriate for routine and long-term monitoring studies. Because, the principle aim of this study was to determine the overall cyclic nature of gonad activity, an appropriate gonad condition was required that would reflect in gonad index for every step of their stages,

with an adequate resolution to detect significant differences between the sampled populations. Gonad index determinations should ideally be complemented by histological analysis to detect changes in gonad structure through the time. However, the subjective method, such as the one used here, can be carried out rapidly and easily on a large number of specimens in the field by identifying visually their relative size, shape or colour in every gonad stage (Creese & Ballantine, 1983), and can therefore be used reliably to pinpoint spawning events (Orton & Southward, 1961). On the other hand, the objective method (histological technique) requires some treatment of the specimens, and there is an inevitable time lag between the collection of samples and their analyses (Creese & Ballantine, 1983).

7.4.2. Gonad cycle

Most patellid and acmaeid limpets have restricted breeding seasons, often with a single spawning episode each year and a long resting period thereafter (see review by Branch, 1981; Creese & Ballantine, 1983; Dunmore & Schiel, 2000). Relatively few species have extended seasons with multiple spawning episodes and little or no resting stage (Orton et al., 1956; Branch, 1981; Wright & Hartnoll, 1981; Baxter, 1983; Creese & Ballantine, 1983). *Cellana testudinaria*'s annual reproductive cycle has a single, clearly defined spawning period per year followed by a long resting stage. Gametes develop over the transition period before dry season and early summer and limpets spawn in late summer to early rainy season. After spawning gonads are inactive until the end of the rainy season. Such pattern is evident for other *Cellana* species; *C. radiata* (Balaparameswara Rao, 1973), *C. tramoserica* (Underwood, 1974), *C. radians* and *C. denticulate* (Creese & Ballantine, 1983), *C. nigrolineata* (Catalan & Yamamoto, 1993), and *C. ornata* (Dunmore & Schiel, 2000) as well. Observations on the GI and MGC show that these gonad indices largely reduce 2 to 3 months after the peak gonad condition. These reductions indicate spawning had occurred, resulting in a gradual decrease in the gonad indices. The variance of the indices of female limpets was higher for post-spawning samples, implying that each individual spawned almost all part of its gonads. This is characteristic for a low degree of synchrony at spawning (Creese & Ballantine, 1983). In this study, therefore, collections needed to be more frequent (as close as a few days apart) to establish a high degree of synchrony in *C. testudinaria* by pinpointing spawning episodes.

In *C. testudinaria*, the gonads develop from March to June or July for both sexes. Spawning begins in July or August (dry season) and extends up to November or December (the beginning of rainy season). The presence of resting phase in November to February suggests that spawning and redevelopment did not occur throughout the year. Similar pattern has been reported in *Cellana radiata*, where gonads develop from February to May and spawning begins in June, extending up to February or March (Balaparameswara Rao, 1973).

7.4.3. Sex ratio

As a special case of sexual development, a number of limpets may be consecutive hermaphrodites, almost all being protandrous (male first, then female). According to Orton et al. (1956) that during their life cycle, Patellid limpets usually attribute the differences in the sex proportion in the different size groups due to sex changes, but it has rarely been correlated with age (Thompson, 1979). Later more detailed work confirms that in view of the preponderance of males in the smaller size classes and females in the larger size classes (Das & Seshappa, 1947; Orton et al., 1956; Blackmore, 1969a; Thompson, 1980). They state that the limpets first mature as males and later on change their sex at a certain stage in their life history to females. Blackmore (1969a) found that in the first year of their life, *Patella vulgata* was immature and in the second year was all functioning males. Females first appeared in three years old limpets, and about half the males change sex between four and six years of age.

The absence of a predominance of one of the sexes in any size group gives evidence that sex reversal is absent in *C. testudinaria*. Orton & Southward (1961) found a similar condition in *Patella depressa*, and *Cellana radiata* (Balaparameswara Rao, 1973), where there is no indication of sex changes with growth. Furthermore, Ward (1966) also observed that the proportion of the two sexes in *Fissurella barbadensis* of all lengths over 11.5 mm, are relatively constant and no indication of change in sex was found. Results from earlier work (Orton et al., 1956; Blackmore, 1969a; Thompson, 1979), therefore, have been omitted because the data suggest that the smallest limpets are the last to mature in any given year, and animals, which are neuter in November, may be male or female by March. The proportions of neuters, males, and females in each year class of the population as are shown in Fig. 7.2 may be concluded that all limpets were immature in their first year to the third year, but the proportion of immature animals then fell each year, reaching zero in seven-year-old limpets. Male limpets first appeared in three-year-old but only formed 10 % of the total, and in subsequent years the proportion of males increased to reach a maximum of 60 % of the total sexually mature individuals in thirteen-year-old, while females first appeared in five-year-old limpets and reached 40 % of the total sexually mature individuals in their maximum age of thirteen-year. A large number of limpets mature during their first or second year and live about five years with increasing fecundity due to an increase in body size (Blackmore, 1969a; Creese, 1980a; Creese, 1981).

7.4.4. Spawning events

Within *C. testudinaria* species, the size of the gonads of both males and females increases approximately linearly with the size of the limpet. Linear relationships between gonadal and somatic weights as are shown in Fig. 7.3, suggest that the animals before spawning contain proportionally more weight of gonad than does the animals after spawning. Similar results have been reported for *Acmaea limatula* of limpet (Seapy, 1966). Female limpets seem to produce larger

gonads than males of similar sizes (Table 7.2), indicating that larger female limpets produce more ovary prior to spawning than do male individuals. The significant differences in the slopes of the regression lines between times of sampling indicate that spawning is characterised by a sharp decline in the value of gonad weight. The relative gonad weight ratio dropped from 0.35 to 0.10 during intervening many days after spawning implying that spawning occurs faster than the time needed for gonads to mature. Given these relationships, it would appear to be selectively advantageous for limpets to grow as large as possible before beginning to reproduce. Under such a consideration, the strategy for the initiation of maturation of the gonad might be dependent on reaching a certain size in order to maximize reproductive output (Creese, 1980a).

7.4.5. Effect of environmental variables

Although possible environmental factors responsible for the initiation of various stages of the reproductive cycle were not investigated in detail and therefore was insufficient to allow any correlation between spawning activity and environment. This limpet predictably spawns in July or August, a time of dry season when there are often calm seas but seawater temperature and salinity are low, as well as humidity and precipitation.

It has been well established that sea temperature plays an important role in breeding cycles and tropical animals have been known to breed throughout the year (Orton, 1920). The spawning stimulus in littoral limpets has been described as change in temperature (Fritchman, 1962; Picken, 1980). Although two other major parameters, the level of incident light and the amount of phytoplankton (Picken, 1980), are also changing at this time of year. Experiments by Fritchman (1962) showed that a change in temperature was responsible for the final maturation of gametes. It is, therefore, conceivable that *C. testudinaria* requires such suitable environmental conditions to enable it to release its gametes into the water. The present study supports the finding of Balaparameswara Rao (1973), which pointed out that the extended breeding season and the absence of a resting phase between the spawning and redevelopment of the gonads in *Cellana radiata* is probably due to the uniformly high temperature in tropical regions. He added, however, temperature and salinity seem to have no marked influence in inducing spawning of *Cellana radiata*. The absence of spawning when temperature is high and the smaller numbers of spawning gonads when salinity is low suggests that these conditions are probably unfavourable to spawning. Orton et al. (1956) observed while studying the breeding of *Patella vulgata* that the gonads develop from June to September and the peak spawning occurs between October and December, so that there is a suitable temperature for that limpet to spawn. In addition, Orton & Southward (1961) found that *Patella depressa* breeds in summer. A similar breeding behaviour has been reported in the other tropical limpets *Acmaea jamaicensis* and *Fissurella barbadensis* by Lewis (1960) and Ward (1966), respectively. Underwood (1974) noted that *Montfortula rugosa* begins to spawn in September – October when sea temperatures begin to rise. Within the family Patellidae, *Patelloida alticostata* and *P. latistrigata* (Creese, 1980a; Fletcher, 1987), *Patella flexuosa* (Iwasaki, 1998),

and *Cellana ornate* (Dunmore & Schiel, 2000), breed throughout summer. Observation on the reproductive cycle of the Antarctic limpet *Nacella concinna* also showed that temperature is an important factor in stimulating spawning. The spawn of this limpet occurs when the water temperature rises and exceeds -1.4°C (Picken, 1980; Picken & Allan, 1983; Brêthes et al., 1994).

Even though, temperature seems to be responsible for triggering the spawning in *C. testudinaria*, rough sea conditions due to strong wind and wave action appear to be truly responsible for stimulating spawning in many limpets and other archaeogastropods (Orton et al., 1956; Orton & Southward, 1961; Balaparameswara Rao, 1973; Grange, 1976; Creese, 1980a; Creese & Ballantine, 1983). Orton et al. (1956) first suggested violent on-shore storms being a stimulus for spawning and this has been endorsed by a number of other workers (Bowman & Lewis, 1977; Thompson, 1980; Baxter, 1983), although the precise mechanism and causal factors are still unresolved (Balaparameswara Rao, 1973). Creese (1980b) had evidence that *Notoacmea petterdi* spawns in May or June during storms and rough seas, while Balaparameswara Rao (1973) noticed that there is the better correlation between the wind speeds associated with the southwest and northeast monsoons and spawning in limpet *Cellana radiata*. Orton et al. (1956) and Orton & Southward (1961) found that high wind speed induces spawning in *Patella vulgata* and *P. depressa*, respectively. They found that strong onshore winds, which result in rough seas, act as a mechanical trigger in stimulating the spawning in these limpets. Creese & Ballantine (1983) suggested that increased wave action associated with storms is a likely stimulus for spawning in *Cellana radians*.

Chapter 8

PRODUCTION

8.1. Introduction

The production of an animal or group of animals (e.g., a population) is the quantity of matter or energy which is potentially available as food for the next trophic level, i.e. for natural predators or, regarding exploited stocks, for man. Hence, it is that part of an energy budget both the ecologist and the fishery biologist are most interested in.

Production and productivity of a steady-state population are defined by two processes: individual growth and mortality. In terms of the general energy budget production P is defined as that part of the assimilated matter that is turned into body mass (mass per individual per time). In terms of energy flow, it is the change in biomass B with time (mass per area per time). If we relate production P to the biomass B already present, we get an estimate of productivity. Traditionally, benthic ecologists use the production-to-biomass ratio (P/B ; per time), which relates production P to the average biomass B present during the period of investigation.

While there is a wealth of information about production and productivity of temperate and polar benthos species, including patellid gastropods (Sutherland, 1972; Picken, 1980; Wright & Hartnoll, 1981; Workman, 1983; Blankley & Branch, 1985), only little is known about tropical limpets. Here, I provide the first data on production and productivity of *C. testudinaria*.

8.2. Materials and Methods

I used the Mass Specific Growth Rate Method (MSGRM) to estimate the production and productivity of *C. testudinaria* (Brey, 2003). From the definition of the (instantaneous) Mass Specific Growth Rate of an individual, $G_{M,t} = (1/M_t) * dM/dt$, it is obvious that we can compute its production as $P_{ind,t} = dM/dt = G_{M,t} * M_t$. To apply this principle to a population, the following data are required:

- *A size-frequency sample of the population (N_j)*. It should represent the true size distribution, i.e. the individuals should be taken randomly from the population and the overall number taken should be sufficiently high; therefore, it is usually obtained by pooling several samples taken at different times during the observation period (see chapter 4, section 4.3.1; Fig. 4.1).
- *The average body mass (M_j) per size class j* . Provided by applying the size-mass relation: $M_j = a * L_j^b = 0.03236 * L^{2.7703}$ (see chapter 3, section 3.3.3).
- *The mass specific growth rate ($G_{M,j}$) per size class j* . Computed by combining the 1st derivative of the von Bertalanffy length growth function (featuring the parameter $K = 1.4 \text{ yr}^{-1}$ and $L_\infty = 33.08 \text{ mm}$; see chapter 4, section 4.3.4) and the parameter $b (= 2.7703)$ of the size-mass relation: $G_{M,j} = b * K * [(L_\infty/L_j) - 1]$.

The individual production in size class j is computed by $P_{ind,j} = G_{M_j} * M_j$, and the production of a size class j is computed by $P = \sum P_j; j = (1, 2, 3, \dots, m)$ for m size classes. The production-to-biomass ratio (P/B) is computed by dividing production P by the corresponding average biomass B of the observation period.

8.3. Results

Mean annual density (D), mean annual standing crop or population biomass (B) and annual production (P) were calculated to be 11.12 ind.m⁻², 1.01 g AFDW.m⁻² (21.8 kJ.m⁻²), and 2.81 g AFDW.m⁻².yr⁻¹ (60.7 kJ.m⁻².yr⁻¹), respectively. Thus, the annual biomass turnover ratio (production to biomass P/B) was 2.77. Overall, distinct peaks of annual production and mean annual biomass were identified for limpets with shell lengths of 13.5 mm and 25.5 mm (Fig. 8.1).

Table 8.1. Calculation of annual production of *Cellana testudinaria* by the mass specific growth rate method. Annual production based on sets of samples from October '01 to September '02, where N_j abundance per size class (ind.m⁻²); M_j the average body mass per size class j (in g AFDW); G_{M_j} the mass specific growth rate per size class j (in yr⁻¹); B_j standing crop or population biomass per size class j (in g AFDW.m⁻²); P annual production (in g AFDW.m⁻².yr⁻¹).

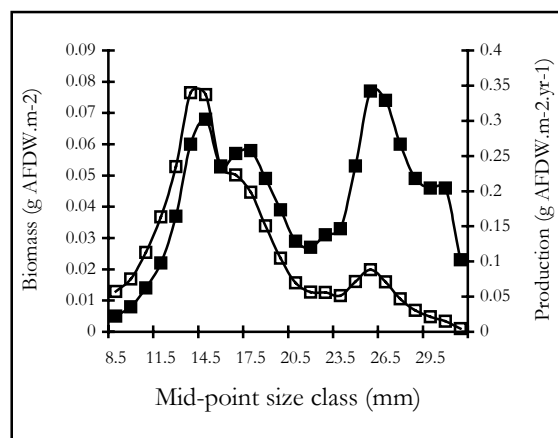


Figure 8.1. *Cellana testudinaria*. Mean annual biomass (■) and annual population production (□) of each size class.

Mid-class interval	N_j	M_j	G_{M_j}	B_j ($N_j * M_j$)	P ($B_j * G_j$)
8.5	0.42	0.012	11.215	0.005	0.0573
9.5	0.47	0.017	9.627	0.008	0.0749
10.5	0.62	0.022	8.340	0.014	0.1129
11.5	0.80	0.028	7.278	0.022	0.1635
12.5	1.04	0.035	6.385	0.037	0.2350
13.5	1.38	0.044	5.625	0.060	0.3400
14.5	1.27	0.053	4.970	0.068	0.3370
15.5	0.83	0.064	4.399	0.053	0.2345
16.5	0.75	0.076	3.897	0.057	0.2232
17.5	0.64	0.090	3.453	0.058	0.1986
18.5	0.47	0.105	3.057	0.049	0.1506
19.5	0.32	0.121	2.701	0.039	0.1048
20.5	0.21	0.139	2.380	0.029	0.0696
21.5	0.17	0.159	2.089	0.027	0.0565
22.5	0.17	0.180	1.824	0.031	0.0559
23.5	0.16	0.203	1.581	0.033	0.0515
24.5	0.23	0.228	1.358	0.053	0.0713
25.5	0.30	0.255	1.153	0.077	0.0882
26.5	0.26	0.284	0.963	0.074	0.0710
27.5	0.19	0.314	0.787	0.060	0.0470
28.5	0.14	0.347	0.623	0.049	0.0303
29.5	0.12	0.382	0.471	0.046	0.0216
30.5	0.11	0.419	0.328	0.046	0.0151
31.5	0.05	0.458	0.195	0.023	0.0045
Total	11.12			1.013	2.8148

8.4. Discussion

8.4.1. Production

Annual production of several limpet species has been reported, i.e. $2.9 \text{ g.m}^{-2}.\text{yr}^{-1}$ for *Nacella concinna* (Picken, 1980), $96.5 \text{ g.m}^{-2}.\text{yr}^{-1}$ or $2003 \text{ kJ.m}^{-2}.\text{yr}^{-1}$ for *Nacella delesserti* (Blankley & Branch, 1985), $430 \text{ kJ.m}^{-2}.\text{yr}^{-1}$ for *Acmaea scabra* (Sutherland, 1972), $163 \text{ kJ.m}^{-2}.\text{yr}^{-1}$ (Wright & Hartnoll, 1981) and between 7.5 and $152 \text{ kJ.m}^{-2}.\text{yr}^{-1}$ (Workman, 1983) for *Patella vulgata*, and $1045.5 \text{ kJ.m}^{-2}.\text{yr}^{-1}$ for *Cellana tramoserica*, $1514.1 \text{ kJ.m}^{-2}.\text{yr}^{-1}$ for *Notoacmea petterdi*, and $196.7 \text{ kJ.m}^{-2}.\text{yr}^{-1}$ for *Patelloida alticostata* (Parry, 1982). The considerably lower estimate of annual production obtained for *C. testudinaria* in the present study ($2.81 \text{ g AFDW.m}^{-2}.\text{yr}^{-1}$ or $60.7 \text{ kJ.m}^{-2}.\text{yr}^{-1}$) is a reflection of relatively low densities of this limpet species.

The P:B ratio of 2.77 of *C. testudinaria* is higher than those obtained for other limpet species living 2 to 5 years, e.g., those reported by Sutherland (1972) for *Acmaea scabra* (1.58), Branch (1975) for *Patella oculus* (1.55), Robertson (1979) for *Siphonaria diemenensis* (1.23). For limpets with longevities of 6 to 15 years, Robertson (1979) obtained the P:B ratios of 0.83, 0.63 and 0.66 for *Cellana tramoserica*, *Notoacmea petterdi* and *Patelloida alticostata*, respectively.

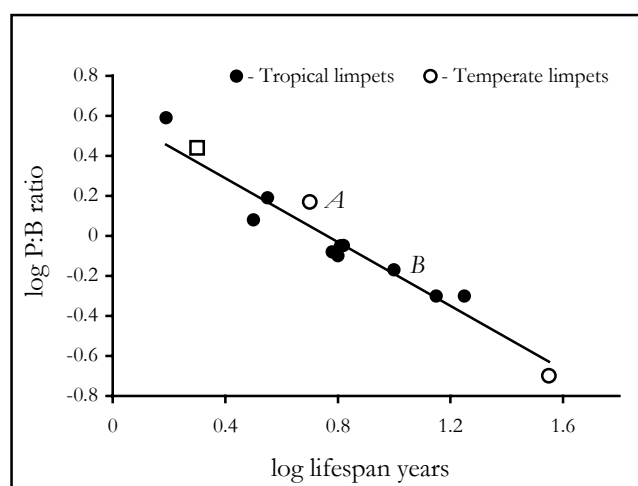


Figure 8.2. Relationship between $\log(P:B)$ and $\log(\text{lifespan})$ for 11 species of limpet including *Cellana testudinaria* (\square) (after Branch, 1981). A, *Acmaea scabra* (Sutherland, 1972); B, *Patelloida alticostata* (Robertson, 1979).

longevity (Robertson, 1979; Branch, 1981). Pattern of low or high turnover in different invertebrate species, and the position of *C. testudinaria* are shown in Figure 8.2. It shows that *C. testudinaria* has a productivity that is substantially greater than that of other limpet species, which is primarily a function of its high growth rate (Robertson, 1979).

In general, there is a strong positive correlation between growth and production. Somatic growth (P_g) can be reduced in favour of reproductive effort (P_r), as it has been showed by Branch (1981) for seven South African *Patella* species. However, overall the growth rate was positively correlated with the reproductive effort. This implies that although energy may be diverted from P_g to P_r (or *vice versa*) when food is short, in general species have “strategies” of high or low turnover (Branch, 1981). This is reflected in the significant negative correlation of the productivity (P:B) and

8.4.2. Energy content

Seasonal changes in the calorific value of the soft tissues of *C. testudinaria* were probably due to changes in the proportion of lipids (energy storage). The relationship between somatic calorific value and gonad index (Fig. 8.2) suggests that lipid is stored in the soft tissues (somatic and gonadal tissues) during the gonad resting phase and then transferred to the gonad during gametogenesis.

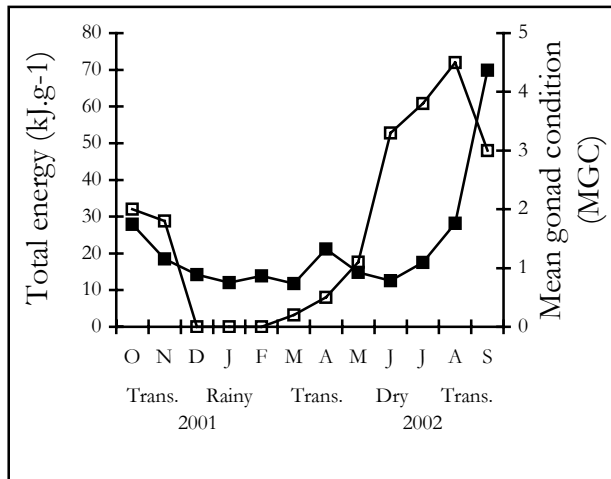


Figure 8.2. *Cellana testudinaria*. Monthly total energy values (■) and mean gonad condition (MGC) indices (□).

In September, however, the maximum of the energy content in the limpets' soft tissues coincided with a decrease in the mean gonad condition index, suggesting a utilisation of lipid stocks at times of spawning in August.

The lower calorific values of soft tissue of immature limpets suggests that an energetic advantage is gained by building a growing individual initially out of "cheap" material until it is capable of making a genetic contribution to the population (Paine, 1971).

Chapter 9

SHELL SHAPE

9.1. Introduction

Many limpets are characteristic inhabitants of the intertidal zone of wave-tide-swept rocky shores, and thus occupy one of the most physically stressful environments on earth. This extended distribution subjects them to an alternating wetting and drying due to the wave exposure and the tidal cycle. At low tide, those limpets are exposed to terrestrial conditions and the concomitant heat and desiccation stresses, while at high tide as waves crash on the shore, water impose large hydrodynamic forces (Hobday, 1995; Denny & Blanchette, 2000), and can push and pull a limpet and threaten to lift it from the rock surface. Not surprisingly biologist and ecologists have speculated that these challenges must have been important in the evolution of these creatures. Surely, they argued, natural selection must have favoured – at least in part – limpets with shells shaped to resist the assault of the deep. Bannister (1975) said that the general structure, decoration and sculpture of a gastropod shell is not entirely unrelated to the environment in which the gastropod lives.

A variety of functional interpretations and evolutionary scenario have been assigned to the differences among limpet shell shape (for reviews, see Branch, 1981; Vermeij, 1993). These include the possibility of the shell shape in response to thermal and desiccation stress in the intertidal zone (Denny, 2000), predation on limpets by crabs (Lowell, 1985; 1986) and birds (Hahn & Denny, 1988), and foraging and territorial behaviour by the limpets itself (Stimson, 1970). An analysis of the shell form in several families of intertidal limpets has revealed the existence of morphological gradients (Russell, 1909; Orton, 1928; Das & Seshappa, 1947; Balaparameswara Rao & Ganapati, 1971; Breen, 1972; Bannister, 1975; see review by Branch, 1981; Lowell, 1984; Hobday, 1995). These gradients are also evident from temperate to tropical latitudes, and may be modified or overridden by exposure to wave action and other factors (Vermeij, 1973). To verify the relationship between dryness of the environment and height of the shells by transplanting the animals from dry rocks to pools, Moore (1934) found that the shells developed shelves and new growth resulted in a flattening of the shell. Although Orton (1928) found that wave action plays only a minor and secondary role in controlling shell height in limpets, he correlated differences in shell height with differences in the degree of exposure to desiccation. Since then, several authors have shown this trend for taller shells to be linked with drier habitats in many species (Balaparameswara Rao & Ganapati, 1971; Bannister, 1975). However, measurements made by Denny (2000) suggest that the evolved shape of limpet shells has not been “fine-tuned” to the flow environment. In addition, several studies have addressed the role of shell shape in determining the hydrodynamic force to which a limpet will be subjected (Warburton, 1976; Branch & Marsh, 1978; Denny, 1985; 1995; Denny & Blanchette, 2000).

Authors (Russell, 1909; Orton, 1928; Moore, 1934; Das & Seshappa, 1947; Evans, 1953; Southward & Orton, 1954; Ebling et al., 1962) have already drawn attention to the variations in

the shape of *Patella vulgata* shells induced by different ecological factors. They had a well marked that inter-specific increase in relative shell height with increasing shore levels. *P. vulgata* from high levels have taller and more domed shell, because in dry desiccation habitats the limpet has to clamp down more tightly, pulling in its mantle so that if the mantle glands deposit the shell while in this position, the shell circumference will be reduced and the shell taller (Russell, 1909; Orton, 1933). Among tropical limpet *Cellana radiata* (Balaparameswara Rao & Ganapati, 1971) and *Cellana toreuma* (Ino, 1935), relative acute height and degree of development of external sculpture generally increase inter-specifically, and often intra-specifically, from low to high shore levels. Vermeij (1993) pointed out that because of the coiling characteristic of most gastropod shells, which is greatly reduced in limpets, results in a conical shell with a large aperture (Denny, 2000). Therefore, some limpets' shells are very high-acute, with a height-to-length ratio that exceeds of 0.2 – 0.6 and mean ratio of 0.53 (Denny & Blanchete, 2000). Furthermore, it is also widely acknowledged that limpets “clamp” or “hunker down” when disturbed in an effort to prevent dislodgement (McAlister & Fisher, 1968; Cook et al., 1969), although no attempt has been done to quantify the clamping response (Ellem et al., 2002). Shell clamping brings the lower rim of the conical shell of a limpet into direct contact with the substratum.

In this chapter, observation was undertaken with a view to studying the effects of environmental variables upon some structural features of the shell of the tropical limpet *Cellana testudinaria* living at the three different shore levels, and to compare it with those of previous work, not only from the same tropical species but also from other temperate climate limpets. An attempt was then made to answer the following questions: (1) *Does environmental variables influence the features of the limpet's shell ?* (2) *Does the morphological shell sculpture generally tend to follow the form and characteristics of the shell such suggested by previous workers ?*

9.2. Materials and Methods

9.2.1. Data collection

Shell shape variation exhibited by intertidal limpet *C. testudinaria* and factors responsible for it were examined at the intertidal rocky shore of Ohoiwait. Data on density and biomass studies were used to confine it.

9.2.2. Shell and body weight measurements

Five main characteristics of the shell: length (L), width (W), height (H), distance from apex to anterior margin (AA) and distance from apex to posterior margin (AP) were measured using vernier callipers to the nearest 0.1 mm, whilst total body wet weight (BW) were weighed using an analytical balance to the nearest 0.01 g. The dimensions utilized in shell measurements are shown in Figure 9.1.

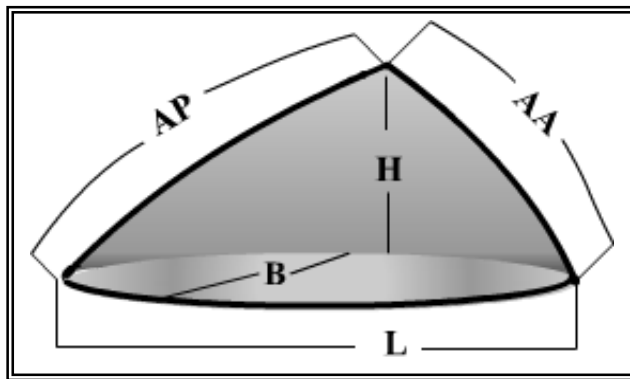


Figure 9.1. Dimensions utilized for shell measurements. AA-distance from apex to anterior margin; AP-distance from apex to posterior margin; H-height; B-width; L-length.

9.2.3. Environmental variables

Not all environmental parameters were included in the analyses, except for seawater temperature, wave height, tidal cycle, air temperature, and precipitation. Seawater temperature, wave height, and tidal cycle were measured *in situ*, while the other parameters were collected from “Tual Meteorology and Geophysics”.

9.2.4. Data analyses

Length of the limpet shell was taken as the main parameter to study the variations of the other 5 variables of the body dimensions. Linear regression equations were calculated separately by the method of least square (Sokal and Rohlf, 1995), to examine the relationship between width and length, height and length, distance from the apex to the anterior margin and length, distance from the apex to the posterior margin and length. The relationship between the total body wet weight and length was described by the exponential function $BW = a L^b$, where BW is the total body wet weight in mg, L is the shell length in mm, and a and b are constants. The constants a and b were calculated by the method of least squares. Regression lines were drawn separately for the above 6 relations at the three shore levels, and the mean values were plotted above them. Using the position of the regression lines, the body dimensions of limpets living at different shore levels were compared.

Multivariate analysis of variance MANOVA (Anderson, 2001) was applied to study interrelations between the 5 characteristics of the shell and the total body wet weight, and to determine if the mean size differed in each shore levels. The Barlett's test of homogeneity of variances was used to test the differences of regression (Snedecor & Cochran, 1967), while the students' t -test was used to calculate the deviation of the shell allometry from the isometric b -value through the equation of $y = b x^\alpha$ where y is body dimensions, x is shell length, b and α are constants, in which for any value of α different from 1, an increase of shell dimensions was considered allometrical. In the total body wet weight and length relationship, α was considered different from 3, when an increase was allometrical. This linear regression was also used to determine if a shell shape change was associated with environmental parameters.

The additional models of height-to-length ($H/(L/2)$) and height-to-width ($H/(W/2)$) ratios, and proportional location of shell apex ($E = AA/(L/2)$) formulated by Denny (2000), and the means of the ratios L/B , $(L+W)/2$, $(L+W)/2H$ and BW/L^3 formulated by Bannister (1975), were calculated in each shore levels, since morphological gradient can best be described in terms of relative shell height over the geometrical mean of length and width of the base (Ward, 1967; Vermeij, 1973). The influence of changing shell shape can be quantified further by the function: $W = c L^\alpha$ and $H = c L^\alpha$ where W is shell width, H is shell height, L is shell length, c and α are constants. If α (the constant of allometry) = 1, then the two factors change proportionally, but $\alpha > 1$ if the shell width or shell height increases faster than length during growth.

The allometric relationship between circumference (CF) and volume (V) of limpet shells was also calculated. The shell was treated as an elliptical cone, thus volume and circumference were estimated by the formula from Lowell (1984): $V = \pi.L.W.H / 12$ and $CF = 2\pi (L^2/8 + W^2/8)^{0.5}$ where V is volume, CF is circumference, L is shell length, W is shell width, and H is shell height. The allometric change in circumference relative to volume was determined by regressing natural logarithm (\ln) CF against $\ln V$ using the major axis method. This method is preferred in morphological analyses when neither variable can be regarded as independent (Gould, 1966). Taking antilogarithms of the \ln - \ln linear functions yielded power functions of the form: $CF = \beta.V^\alpha$ where β and α are constants. α is significantly < 0.33 using a Model II comparison (Clarke, 1980), indicating an average change in shape with increasing size; i.e. volume exhibits positive allometry relative to circumference because the shell becomes more domed as the limpet grows (Lowell, 1984). To separate the effects of shape from the effects of size, a measure of size should be independent from shape. This measure can be obtained by rearranging the power function to obtain $V^\alpha/CF = 1/\beta$. $1/\beta$ is approximately equal to the allometric values of V^α/CF for limpets of all size within a species (Lowell, 1984). Individuals for which $V^\alpha/CF > 1/\beta$ are more highly acute than average for the species, whereas those for which $V^\alpha/CF < 1/\beta$ are flatter than average.

9.3. Results

9.3.1. Body dimensions

An examination of 6 variables of body dimensions in the specimen of *C. testudinaria* collected at the three different shore levels for the whole period of study are summarized in Table 9.1 and are presented as frequency distribution histograms in Figure 9.2 and an average of body dimensions in Figure 9.3.

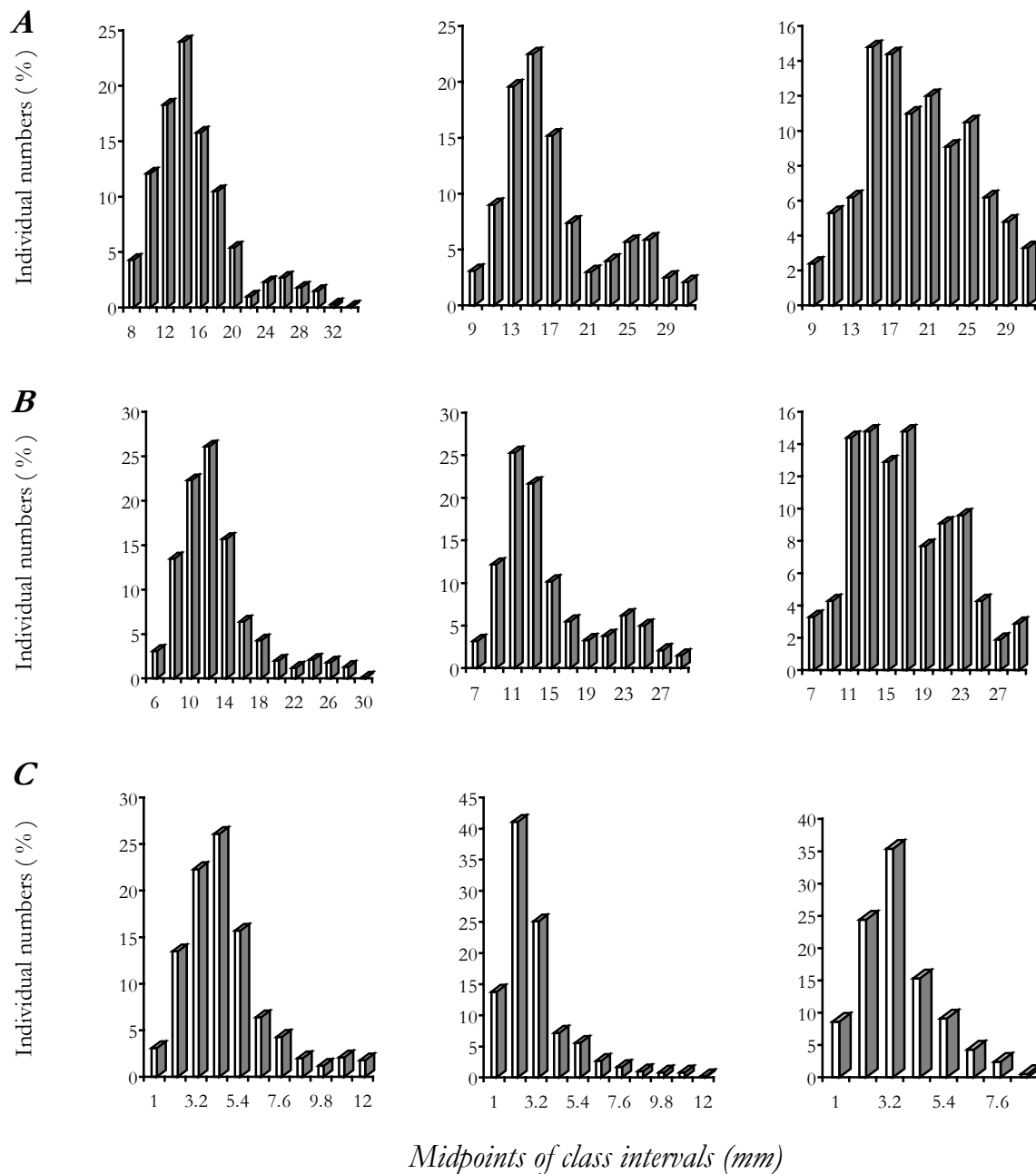
Table 9.1. *Cellana testudinaria*. Summary of shell and total body wet weight measurements of limpets collected from the three shore levels, together with the analysis of variance (ANOVA) on these shell dimensions and total body wet weight. *SS*-sum of squares; *df*-degrees of freedom; *MS*-mean square; *P*-probability; ** highly significant; *ns*-not significant.

Time	N	Average \pm Standard Deviation (SD)					
		Length (mm)	Width (mm)	Height (mm)	AP (mm)	AA (mm)	Body weight (mg)
Oct	110	14.77 \pm 3.11	12.31 \pm 2.89	2.38 \pm 0.80	11.72 \pm 2.62	4.13 \pm 1.19	323 \pm 267
Nov	57	14.43 \pm 3.60	12.03 \pm 3.22	2.24 \pm 0.91	11.60 \pm 3.17	3.87 \pm 1.21	293 \pm 159
Dec	115	15.39 \pm 4.59	12.58 \pm 4.13	2.35 \pm 1.30	12.58 \pm 3.67	3.89 \pm 1.83	433 \pm 644
Jan	121	14.18 \pm 4.46	11.61 \pm 4.08	2.04 \pm 1.27	11.70 \pm 4.07	3.43 \pm 1.35	353 \pm 522
Feb	130	13.76 \pm 3.68	11.45 \pm 3.45	1.97 \pm 0.95	11.56 \pm 3.38	3.18 \pm 1.16	325 \pm 534
Mar	105	14.65 \pm 4.25	12.19 \pm 4.04	2.18 \pm 1.24	12.19 \pm 4.04	3.52 \pm 1.25	388 \pm 550
Apr	108	12.17 \pm 2.60	9.93 \pm 2.35	1.75 \pm 0.68	9.98 \pm 2.28	2.96 \pm 0.81	157 \pm 56
May	124	14.70 \pm 4.32	12.21 \pm 3.92	2.11 \pm 1.23	12.16 \pm 3.90	3.51 \pm 1.39	551 \pm 706
Jun	111	14.47 \pm 4.59	11.93 \pm 4.16	2.16 \pm 1.29	11.85 \pm 4.09	3.63 \pm 1.46	556 \pm 718
Jul	47	16.24 \pm 4.88	13.22 \pm 4.28	2.53 \pm 1.51	13.01 \pm 4.50	4.41 \pm 1.50	755 \pm 824
Aug	45	18.89 \pm 4.51	16.22 \pm 4.57	3.20 \pm 1.07	15.73 \pm 4.36	4.79 \pm 0.97	809 \pm 841
Sep	64	21.70 \pm 6.75	19.35 \pm 6.81	4.13 \pm 2.04	19.41 \pm 6.86	5.10 \pm 1.75	1187 \pm 854
Average	95	14.96 \pm 4.72	12.46 \pm 4.44	2.29 \pm 1.31	12.36 \pm 4.36	3.72 \pm 1.45	560 \pm 628
Middle shore level							
Oct	266	14.32 \pm 3.24	11.46 \pm 2.91	1.94 \pm 0.85	11.57 \pm 2.59	3.55 \pm 1.37	252 \pm 275
Nov	141	15.21 \pm 3.89	12.35 \pm 3.39	2.24 \pm 0.93	12.32 \pm 2.95	3.86 \pm 1.67	320 \pm 359
Dec	18	19.74 \pm 5.86	16.56 \pm 5.15	3.62 \pm 1.33	15.56 \pm 4.34	5.90 \pm 2.17	843 \pm 707
Jan	22	17.58 \pm 4.77	15.06 \pm 4.51	3.11 \pm 0.91	14.56 \pm 3.85	4.67 \pm 2.47	545 \pm 466
Feb	22	18.84 \pm 7.44	15.88 \pm 6.63	3.09 \pm 1.87	15.11 \pm 6.25	5.51 \pm 2.87	765 \pm 922
Mar	16	19.25 \pm 5.21	16.29 \pm 4.70	3.48 \pm 1.36	15.73 \pm 4.48	5.30 \pm 1.70	761 \pm 699
Apr	162	14.98 \pm 3.53	12.12 \pm 3.13	2.21 \pm 0.91	12.20 \pm 2.75	3.74 \pm 1.44	265 \pm 261
May	25	17.19 \pm 4.99	14.68 \pm 4.43	3.07 \pm 0.71	14.60 \pm 4.39	4.28 \pm 1.19	901 \pm 1223
Jun	22	17.78 \pm 4.91	15.65 \pm 4.45	3.18 \pm 0.88	15.15 \pm 4.46	4.37 \pm 1.11	985 \pm 1127
Jul	99	15.74 \pm 4.37	12.78 \pm 3.72	2.38 \pm 1.24	12.64 \pm 4.37	4.18 \pm 0.97	658 \pm 726
Aug	99	19.34 \pm 3.67	17.22 \pm 3.67	3.47 \pm 0.94	16.24 \pm 3.62	4.95 \pm 0.77	620 \pm 414
Sep	164	22.83 \pm 6.62	20.73 \pm 6.62	5.25 \pm 2.86	20.76 \pm 6.69	6.06 \pm 2.71	1402 \pm 973
Average	88	16.93 \pm 5.38	14.27 \pm 5.32	2.87 \pm 1.84	14.15 \pm 5.16	4.37 \pm 1.93	581 \pm 721
Low shore level							
Oct	18	17.65 \pm 5.71	14.05 \pm 4.81	3.36 \pm 0.87	11.96 \pm 4.45	7.12 \pm 2.05	936 \pm 901
Nov	18	20.03 \pm 2.89	16.75 \pm 2.81	2.94 \pm 0.82	15.54 \pm 2.54	5.71 \pm 1.89	746 \pm 333
Dec	4	21.10 \pm 7.57	13.25 \pm 5.50	3.43 \pm 2.41	15.73 \pm 4.77	6.74 \pm 4.11	1005 \pm 1340
Jan	3	17.67 \pm 2.57	14.87 \pm 2.98	3.13 \pm 0.75	14.87 \pm 2.98	4.49 \pm 0.53	450 \pm 92
Feb	19	16.46 \pm 3.03	13.47 \pm 2.58	2.32 \pm 0.69	12.83 \pm 2.44	4.53 \pm 1.16	348 \pm 182
Mar	8	18.13 \pm 2.52	15.46 \pm 2.21	2.75 \pm 0.54	15.11 \pm 2.28	4.30 \pm 1.13	478 \pm 160
Apr	31	18.52 \pm 4.86	15.04 \pm 4.60	2.85 \pm 1.18	14.79 \pm 3.62	4.97 \pm 1.92	496 \pm 492
May	12	20.08 \pm 3.47	16.18 \pm 2.74	3.83 \pm 0.99	16.32 \pm 2.90	5.74 \pm 1.62	1391 \pm 980
Jun	7	18.19 \pm 3.30	15.06 \pm 2.73	3.11 \pm 1.00	15.20 \pm 3.20	4.62 \pm 0.95	854 \pm 464
Jul	19	17.82 \pm 6.76	14.07 \pm 5.23	2.84 \pm 2.03	14.73 \pm 6.74	4.59 \pm 1.63	865 \pm 829
Aug	30	20.69 \pm 4.72	18.28 \pm 4.65	3.37 \pm 1.23	17.46 \pm 4.81	4.99 \pm 0.90	830 \pm 536
Sep	40	22.66 \pm 6.93	20.31 \pm 7.15	4.35 \pm 2.21	20.12 \pm 7.14	5.47 \pm 1.84	1419 \pm 1143
Average	17	19.53 \pm 5.44	16.39 \pm 5.31	3.29 \pm 1.55	15.96 \pm 5.38	5.28 \pm 1.81	878 \pm 823

Anova

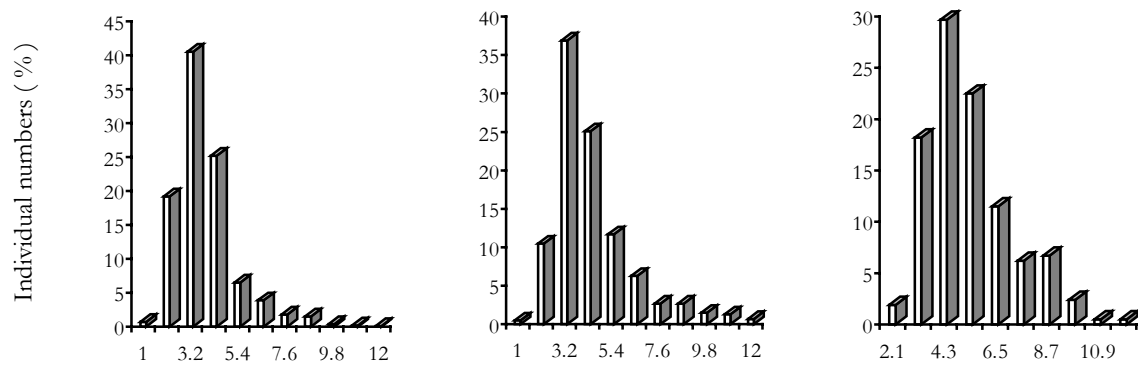
Source of Variation	SS	df	MS	F	P-value	F crit
Shore levels	151.9704	2	75.9852	22.6216	0.05**	3.0415
Body dimensions	9034.9260	5	1806.9850	537.9586	0.05**	2.2597
Interaction	43.9898	10	4.3990	1.3096	0.05 <i>ns</i>	1.8788
Within	665.0754	198	3.3590			
Total	9895.9610	215				

The size frequency distributions of the 6-body dimensions measured are essentially unimodal, indicating that the individuals measured belonged to the same size group and may be vitiated by the differences of growth. The differences in shell sizes due to different stages of growth can be overcome by a comparison of shell form or shape using various dimensional ratios (Bannister, 1975). Individual limpets with 15 mm midpoint of class intervals of shell length at the three shore levels are more abundant than others size classes (Fig. 9.2 – A), indicating that most limpets sampled were 4 - 5 months old (estimated using Fig. 4.4). The other shell dimensions also show the same trends in size frequency distribution to the shell length, implying that the measurements of shell dimensions are exactly related.

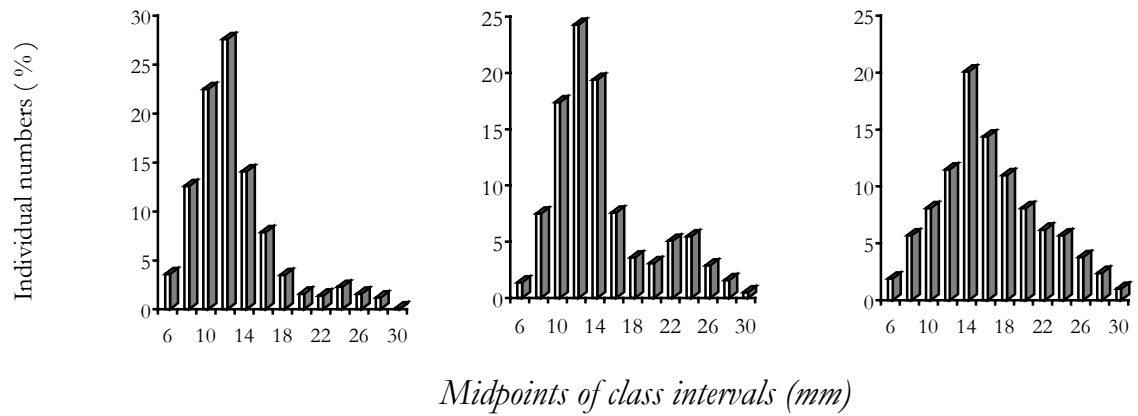


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D



E



F

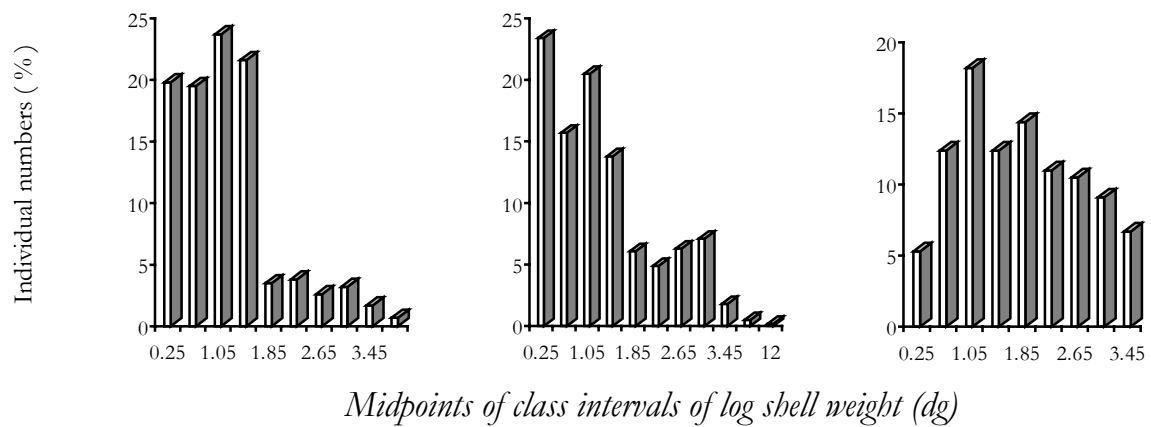


Figure 9.2. *Cellana testudinaria*. Frequency histograms of **A** – shell length; **B** – shell width; **C** – shell height; **D** – distance from apex to anterior margin; **E** – distance from apex to posterior margin; **F** – total body wet weight.

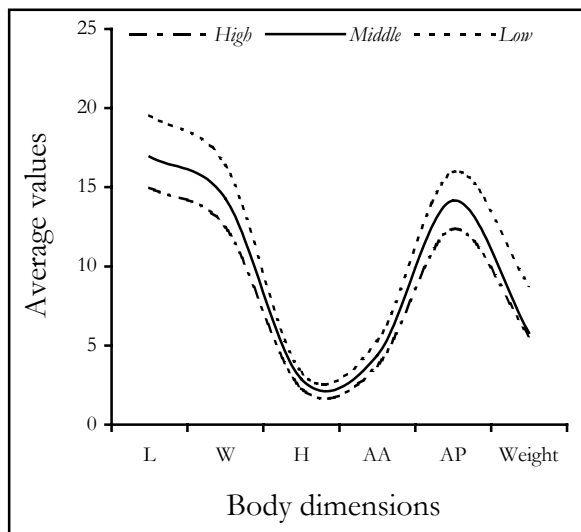


Figure 9.3. *Cellana testudinaria*. Average of body dimension for limpets living at the three different shore levels. L-length; W-width; H-height; AA-apex to anterior; AP-apex to posterior.

The comparison of the overall body dimensions due to different shore levels show that limpets living at the low shore level are significantly longer, broader, taller and heavier than those living at the high and middle shore levels (Figure 9.3). Further, it may be seen that the average of shell sizes of limpets collected on September from the three shore levels are higher than other months, except for AA; distance from apex to anterior margin of shell, collected on October, November, December and May at low shore level (Table 9.1). Two-way without replication of ANOVA test on the body dimensions for the three shore levels shows highly significant differences between the shore levels (Table 9.1).

9.3.1.1. Shell width

One-way ANOVA test on the means shell width shows that there are highly significant differences between limpet's shell width for the three shore levels (Table 9.2), indicating that an average of shell width of limpets living at the low shore level was wider than the other two shore levels (Fig. 9.3).

The relationships between shell width and shell length of limpets living at the three different shore levels are presented in Figure 9.4. The following regression equations were obtained: the high shore level $W = 0.9271 L - 1.4106$ ($r = 0.98$, $n = 1137$), the middle shore level $W = 0.9752 L - 2.2400$ ($r = 0.99$, $n = 1056$), and the low shore level $W = 0.9404 L - 1.9762$ ($r = 0.96$, $n = 209$), respectively.

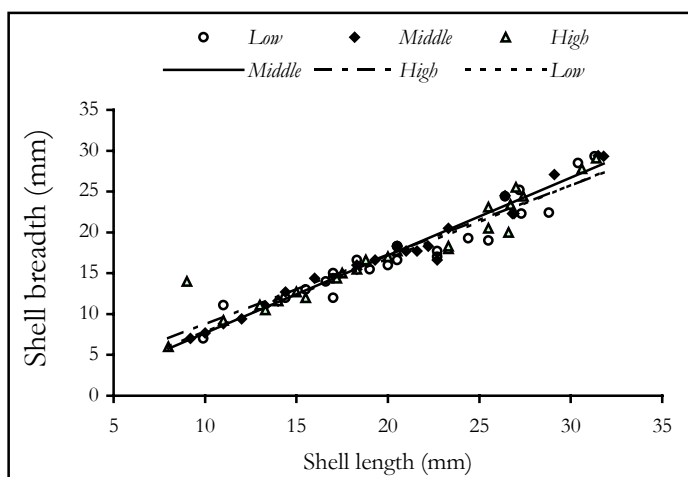


Figure 9.4. *Cellana testudinaria*. Relationship between shell width (W) and shell length (L) for limpets living at the three different shore levels. Since the mean values when plotted were found to interfere with regression lines, they were not plotted to show clearly the position of the lines. No all data were plotted in this figure, because the point of each datum overlapped each other, therefore, could not be separated.

Table 9.2. Analysis of variance on the shell width for the three shore levels during the study periods. *SS*-sum of squares; *df*-degrees of freedom; *MS*-mean square; *F*-probability.

Source of variation	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>F_{crit}</i>	<i>P</i>
Between groups	81.15	2	40.57	7.77	3.28	< 0.05
Within groups	172.30	33	5.22			
Total	253.43	35				

low shore level are broader than those limpets living at the other shore levels. Further, when they grow to reach the shell length of > 18 mm, limpets at the high and middle shore levels are broader than those at the low shore level. The scatter distribution of limpets with shell length ranges from 15 – 25 mm around the regression lines (Fig. 9.4), implying that the relationships are highly significant linear for limpets at those sizes living at the three different shore levels.

Table 9.3. *Cellana testudinaria*. Test for deviation from isometry ($b = 1$) on the 95 % confidence limits of b together with the Bartlett's test. *df*-degrees of freedom; S^2 -variance of samples; *-negatively allometric.

t-test

Shore levels	<i>b</i> at 95 %	<i>t</i> -test	<i>P</i>
High	± 0.0097	187	< 0.05*
Middle	± 0.0097	198	< 0.05*
Low	± 0.0360	51	< 0.05*

Bartlett's test

Shore levels	<i>df</i>	S^2	$\ln S^2$	$df * \ln S^2$	$1/df$	$df * S^2$
High	1136	19.71	2.98	3385.28	0.0009	22390.56
Middle	1055	28.30	3.34	3523.70	0.0009	29856.50
Low	208	28.20	3.34	694.72	0.0048	5865.60
Total	2399			7603.70	0.0066	58112.66

$$M = 2.3026 * [(\sum df * \ln(\sum df * S^2 / \sum df)) - (\sum df * \ln S^2)] = 112.61$$

It was clear evidence from the position of regression lines that those lines for limpets at the high and middle shore levels overlap each other, while the regression line for limpets at the low shore level crosses the other both lines at the shell length of 18 mm. This indicates that limpets with shell length of < 18 mm living at the

The results of the statistical analysis carried out to test the deviations of the shell allometry from the isometric b -value showed that the relationships were negatively allometric for the all entire shore levels (Table 9.3), reflecting an increase in the shell width is approximately slower than an increase in shell length as the limpet grew in size for the three different shore levels. In addition, the residual variances analysed by Bartlett's test showed that the slopes of lines to be heterogeneous ($M = 112.61$, $P < 0.05$; Table 9.3), indicating that a change in emphasis of shell length and width during the study periods among the shore levels were significant different.

9.3.1.2. Shell height

One-way ANOVA test shows that there were highly significant differences between limpet's shell heights at the three shore levels (Table 9.4), indicating that the average shell height of limpets living at the low shore level was taller than at the other two shore levels (Fig. 9.3).

Table 9.4. Analysis of variance on the shell height for the three shore levels during the study periods. *SS*-sum of squares; *df*-degrees of freedom; *MS*-mean square; *F*-probability.

Source of variation	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>F_{crit}</i>	<i>P</i>
Between groups	4.19	2	2.10	4.24	3.28	< 0.05
Within groups	16.31	33	0.49			
Total	20.50	35				

Simple observation of the data showed that an apparent linearity of the relations, but taking a possible allometric phenomenon, it was decided to analyse the data in greater detail. The analysis gave the best method of plotting to be a straight line, and every regression line seems to intersect each other consecutively (Fig. 9.5). These trends, indicating that limpets grow in terms of increasing shell height are similar to an increasing of shell length. It is also clearly evidence from these equations of the regression lines that, when the shell height is plotted against the shell length, the regression line for limpets with the shell length of > 20 mm living at the high shore level lies below those of the middle and low shore levels (Fig. 9.5). Individual limpets being growing to reach 18 mm in shell length presumably would have the same sizes of shell height for the entire three shore levels. The overlapping of regression lines between the middle and low shore levels indicated that the slopes of the regression lines were found not to be significantly different between these two levels of shore.

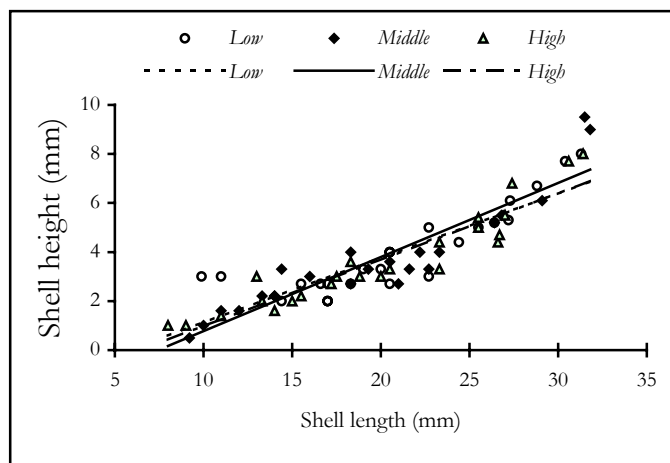


Figure 9.5. *Cellana testudinaria*.

Relationship between shell height (*H*) and shell length (*L*) for the three shore levels. Dot line drawn at the arbitrary of the shell length delineates the crossing of regression lines. Since the mean values when plotted were found to interfere with regression lines, they were not plotted to show clearly the position of the lines. No all data were plotted in this figure, because the point of each datum overlapped each other, therefore, could not be separated.

t-test on the deviation of the shell allometry from isometric showed that the relationships between both variables for the three different shore levels were negatively allometric (Table 9.5), indicating that an increase in shell height was slower than an increase in length when limpets grew in size. These calculations gave a clear idea of allometry in an increase of shell height.

Table 9.5. *Cellana testudinaria*. Test for deviation from isometry ($b = 1$) on the 95 % confidence limits of b together with the Bartlett's test. df -degrees of freedom; S^2 -variance of samples; *-negatively allometric.

t-test

Shore levels	b at 95 % t-test P		
High	± 0.0053	97	< 0.05*
Middle	± 0.0085	17	< 0.05*
Low	± 0.0165	31	< 0.05*

Bartlett's test

Shore levels	df	S ²	ln S ²	df*ln S ²	1/df	df*S ²
High	1136	1.72	0.54	613.98	0.0009	1953.92
Middle	1055	3.39	1.22	1287.10	0.0009	3576.45
Low	208	2.40	0.88	183.04	0.0048	499.20
Total	2399			2084.12	0.0066	6029.57

$$M = 2.3026 * [(\sum df * \ln(\sum df * S^2 / \sum df)) - (\sum df * \ln S^2)] = 292.11$$

9.3.1.3. Position of the apex

One-way ANOVA test on the means distance from apex to anterior margin and distance from apex to posterior margin show that there are highly significant differences between the distance from apex to anterior and posterior margins for the three shore levels (Table 9.6), indicating that an average distance of shell apex to anterior and posterior margins of limpets living at the low shore level was longer than the other two shore levels (Fig. 9.3).

The relationships between the distance from the apex to the anterior margin and shell length, and the distance from the apex to the posterior margin and shell length, are shown in Figure 9.6 and Figure 9.7, respectively.

But when the theoretical value of the means shell height corresponding to the given shell length of each range was calculated, it was observed that they increased according to the size range of the sample up to a length of 18 mm, remaining constant for the smaller sizes. It is, therefore, possible to infer that at least, *C. testudinaria* presents characteristic allometric features up to a size of 18 mm, and from then on tends to be allometric.

The residual variances analysed using the Bartlett's test showed that the slopes of lines to be heterogeneous at the three shore levels for all relationships ($M = 292.11$, $P < 0.05$; Table 9.5), indicating that those relationships were highly significant different among the shore levels.

Table 9.6. Analysis of variance on the distance from apex to anterior and posterior margins for the three shore levels during the study periods. SS -sum of squares; df -degrees of freedom; MS -mean square; P -probability.

The distance from apex to anterior margin

Source of variation	SS	df	MS	F	F _{crit}	P
Between groups	11.96	2	5.98	9.14	3.28	< 0.05
Within groups	21.59	33	0.65			
Total	33.55	35				

The distance from apex to posterior margin

Source of variation	SS	df	MS	F	F _{crit}	P
Between groups	43.50	2	21.75	3.95	3.28	< 0.05
Within groups	181.77	33	5.51			
Total	225.27	35				

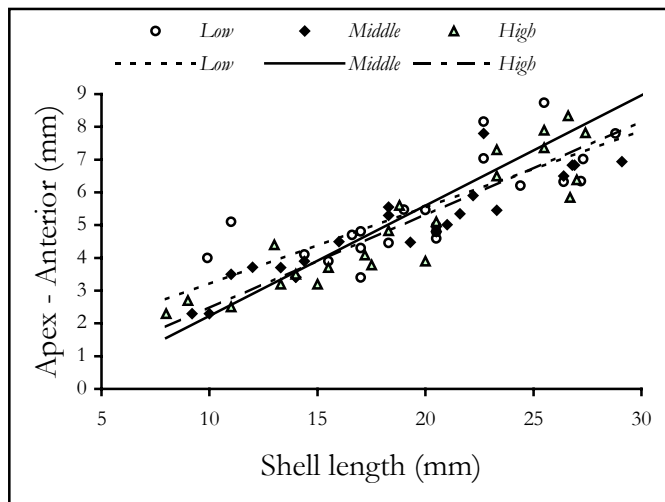


Figure 9.6. *Cellana testudinaria*.

Relationship between the distance from apex to anterior margin (AA) and shell length (L) for the three shore levels. No all data were plotted in this figure, because the point of each datum overlapped each other, therefore, could not be separated.

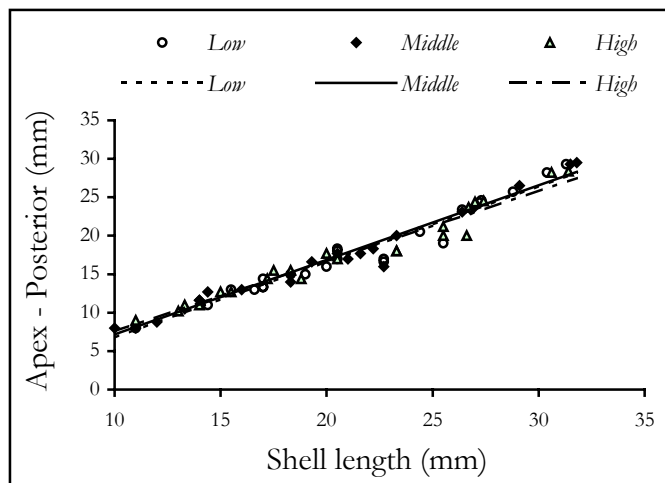


Figure 9.7. *Cellana testudinaria*. Relationship between the distance from apex to posterior margin (AP) and shell length (L) for the three shore levels. Dot line drawn at the arbitrary of the shell length delineates the crossing of regression lines. Since the mean values when plotted were found to interfere with regression lines, they were not plotted to show clearly the position of the lines. No all data were plotted in this figure, because the point of each datum overlapped each other, therefore, could not be separated.

The following regression equations were obtained: the high shore level $AA = 0.2773 L - 0.4289$ ($r = 0.90$, $n = 1137$) and $AP = 0.9081 L - 1.2180$ ($r = 0.98$, $n = 1137$), the middle shore level $AA = 0.3168 L - 0.9974$ ($r = 0.88$, $n = 1056$) and $AP = 0.9370 L - 1.7210$ ($r = 0.98$, $n = 1056$), and the low shore level $AA = 0.2469 L + 0.4543$ ($r = 0.74$, $n = 209$) and $AP = 0.9523 L - 2.6345$ ($r = 0.96$, $n = 209$).

It may be seen from Fig. 9.6 that when the distance from apex to the anterior margin (AA) is plotted against the shell length, the regression line for limpets with shell length of < 18 mm living at the low shore level lies above those live at the high and the middle shore levels. This indicates that, for the same shell length up to 18 mm, the distance from the apex to the anterior margin is greater in limpets at the low shore level than those at the high shore levels. In addition, no comparison, however, can be done for the distance from the apex to the posterior margin (AP) of limpets living at the three different shore levels, since the regression lines of the

relationships are approximately more or less parallel to each other, and that the distance between the lines is not very marked (Fig. 9.7).

Table 9.7. *Cellana testudinaria*. Test for deviation from isometry ($b = 1$) on the 95 % confidence limits of b together with the Bartlett's test. df -degrees of freedom; S^2 -variance of samples; *-negatively allometric.

t-test

Shore levels	b at 95 % <i>t</i> -test	P
High	± 0.0078	70 < 0.05*
Middle	± 0.0101	61 < 0.05*
Low	± 0.0304	16 < 0.05*
<i>Apex to posterior margin</i>		
High	± 0.0099	179 < 0.05*
Middle	± 0.0123	150 < 0.05*
Low	± 0.0368	51 < 0.05*

Bartlett's test

Shore levels	df	S^2	$\ln S^2$	$df^2 \ln S^2$	$1/df$	$df^2 S^2$
High	1136	2.10	0.74	840.64	0.0009	2385.60
Middle	1055	3.72	1.32	1392.60	0.0009	3924.60
Low	208	3.28	1.19	247.52	0.0048	682.24
Total	2399			2480.76	0.0066	6992.44
<i>Apex to posterior margin</i>						
High	1136	19.01	2.94	3339.84	0.0009	21595.36
Middle	1055	26.63	3.28	3460.40	0.0009	28094.65
Low	208	28.94	3.37	700.96	0.0048	6019.52
Total	2399			7501.20	0.0066	55709.53

$$M = 2.3026 * [(\sum df * \ln(\sum df * S^2 / \sum df)) - (\sum df * \ln S^2)]$$

$$M_{AA} = 197.19$$

$$M = 2.3026 * [(\sum df * \ln(\sum df * S^2 / \sum df)) - (\sum df * \ln S^2)]$$

$$M_{AP} = 101.07$$

t-test on the deviation of the shell allometry from isometric showed that the relationships between both variables for the three different shore levels were negatively allometric (Table 9.7), indicating that an increase in the distance from apex to anterior and posterior margins were slower than an increase in length when limpets grew in size. The Bartlett's tests clearly show that there is a high significant difference on the slopes of regression lines for the three different shore levels during the periods of sampling (Table 9.7).

The above observations indicate that the apex of the shell is shifted more backwards in individual *C. testudinaria* of less than 18 mm in shell length living at the low shore level and more forward in limpets living at the high and the middle shore levels. However, when limpets grow bigger and the shell length increases longer than 18 mm, then the apex of those limpets living at the high shore level is shifted more backwards. A diagrammatic representation of the shape of the shell of the limpets inhabiting the high and low shore levels is shown in Figure 9.8.

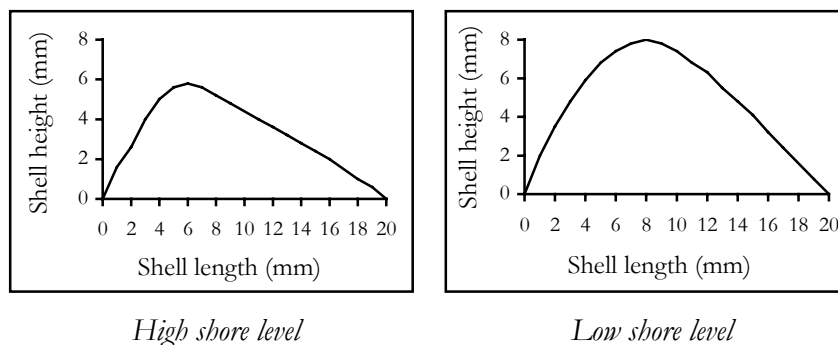


Figure 9.8. *Cellana testudinaria*. Shell shape of limpets inhabiting high and low shore levels.

9.3.1.4. Total body wet weight

Shell length and total body wet weight of each specimen were measured in order to find out if there were any definite relationships between them. By using the data obtained over the 12 months period, an attempt was made to discover the general length-weight relationships as are shown in Figure 9.9. The power regression equations of these relationships are: $BW = 0.4171 L^{2.4765}$, ($r = 0.92$, $n = 1137$) for the high shore level, $BW = 0.1047 L^{2.9140}$ ($r = 0.93$, $n = 1056$) for the middle shore level, and $BW = 0.1121 L^{2.9188}$ ($r = 0.93$, $n = 209$) for the low shore level, respectively. (It should be noted here that the back-transformation from logarithms introduces a bias, which is beyond the scope of this study). From these equations, when comparisons are done for the same shell length, the total body wet weight was greater in limpets living at the high shore level than those at the lower ones.

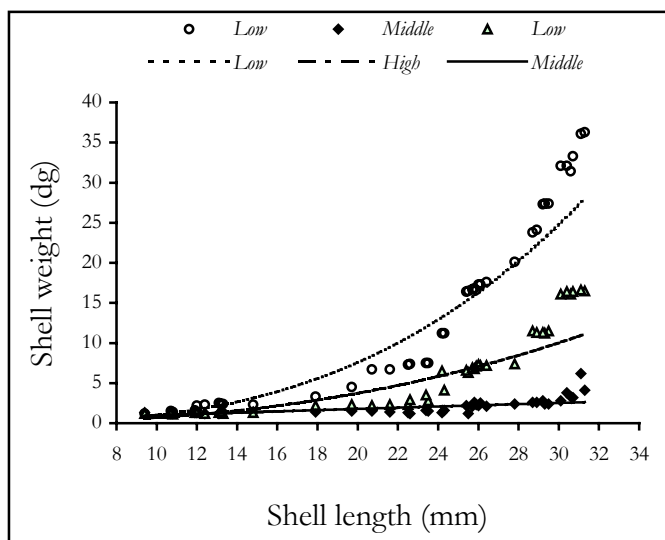


Figure 9.9. *Cellana testudinaria*. Relationship between total body wet weight (W) and shell length (L) for the three shore levels. No all data were plotted in this figure, because the point of each datum overlapped each other, therefore, could not be separated.

t -test was applied to determine the deviation from allometry, and the results showed that all relationships for the different shore levels were negative allometry (mean t -test = 15.07, $P < 0.05$), indicating that an increase in total body wet weight of limpets living at these shore levels was slower than an increase in shell length. By using the Bartlett's test on their residual variances gave the result that there is highly significant differences of slopes of regression lines among the shore levels ($M = 86.80$, $P < 0.05$).

9.3.2. Shell shape variation

9.3.2.1. Changing in shell dimension

Means of the 7-ratios of the body dimensions and the significant differences among them (one-way ANOVA test) are given in Table 9.8. Ratio of L/W indicates that the shells are slightly broader in limpets living at the middle shore level than those at the other levels, although, one-

way ANOVA test shows that there is no significant differences among shore levels. In addition, the other ratio of $(L+W)/2$ indicates that the “mean” of length and width is significantly higher in the shells of limpets living at the low shore level than the other levels. The result of ANOVA test also showed that there was highly significant difference among shore levels ($P < 0.001$). The ratio $(L+W)/2H$ indicates that the shells of limpets living at the low shore level are higher than the others living at the high and the middle shore levels, since ANOVA test showed highly significant difference on that ratio among shore levels (Table 9.8). The mean ponderousness indices of BW/L^3 give further evidence of the significant difference in total body wet weight of limpets living at the three shore levels, since the mean ponderousness index of 0.1673 was observed for limpets at the high shore level to be differ significantly from the mean values of 0.1197 and 0.1179 of limpets at the middle and low shore levels ($P < 0.001$). The significantly lower ponderousness indices of limpets living at the middle and low levels seem to be a reflection of significantly greater length in these shells compared with those living at the high shore level (Fig.9.3).

The models of $H/(L/2)$, $H/(W/2)$, and $E = AA/(L/2)$ ratios developed by Denny (2000) show that there are also highly significant differences (one-way ANOVA test, $P < 0.001$) on the mean values of shell among shore levels (Table 9.8). The α -values of the three ratios are higher for the limpets living at the low shore level than those at the other shore level, indicating that limpets living to the lower shore levels are taller, broader, and the apex of the shell are shifted more backwards (Figure 9.8). In addition, the proportional location of the shell apex values (E-value) for limpets at the three different shore levels are less than 1 (Table 9.8), implies that the apex is upstream of the centre of the shell form (Denny, 2000).

An analysis on the relationships between shell width (W) and shell height (H) on shell length (L) using the function of changing shell shape, gives the mean constant of allometry α -values of less than 1 ($\alpha < 1$) for the three shore levels, indicates that shell width or shell height changes impropotionally to the shell length. Thus, an increase in both factors is slower than an increase in the shell length during growth.

Table 9.8. *Cellana testudinaria*. Means of body dimensional ratios at the three shore levels. *L*: length (mm); *W*: width (mm); *H*: height (mm); *BW*: total body wet weight (mg); *AA*: apex to anterior (mm). *F*-Statistical significant differences between means (one way – ANOVA test); *ns*- not significant.

Shell dimensional ratio	High shore level	Middle shore level	Low shore level	F
<i>L/W</i>	1.2164 ± 0.0791	1.2084 ± 0.0886	1.2141 ± 0.1424	<i>ns</i>
$(L+W)/2$	13.7053 ± 4.5620	15.6038 ± 5.3331	17.9591 ± 5.3273	$P < 0.001$
$(L+W)/2H$	6.8384 ± 2.3475	6.3019 ± 1.9177	5.9930 ± 1.5165	$P < 0.001$
BW/L^3	0.1673 ± 5.9724	0.1197 ± 4.6301	0.1179 ± 5.1150	$P < 0.001$
$H/(L/2)$	0.2898 ± 0.0755	0.3163 ± 0.0975	0.3245 ± 0.0805	$P < 0.001$
$H/(W/2)$	0.3500 ± 0.0842	0.3774 ± 0.1001	0.3914 ± 0.0971	$P < 0.001$
$E = AA/(L/2)$	0.4936 ± 0.0815	0.5077 ± 0.0977	0.5471 ± 0.1426	$P < 0.001$

The comparison of shell forms using $(L+W)/2$ and H as described by Orton (1928) can be made further wider in scope by studying the regression of $(L+W)/2$ on H for the three different shore levels.

Table 9.9. *Cellana testudinaria*. Analysis of variance on the relationship between $(L+W)/2$ and H as described by Orton (1928).

Regression Statistics		High shore levels			
Multiple R	0.95				
Standard Error	1.45				
Observations	1137				
Sources	df	SS	MS	F	
Regression	1	21270.52	21270.52	10178.66	
Residual	1135	2371.83	2.09		
Total	1136	23642.35			
Variables	Coeff.	SE	t Stat	Lower 95%	Upper 95%
Intercept	6.1008	0.086714	70.36	5.9307	6.2709
X Variable 1	3.3142	0.03285	100.89	3.2497	3.3786

Regression Statistics		Middle shore levels			
Multiple R	0.91				
Standard Error	2.10				
Observations	1056				
Sources	df	SS	MS	F	
Regression	1	25349.55	25349.55	5737.91	
Residual	1054	4656.48	4.42		
Total	1055	30006.03			
Variables	Coeff.	SE	t Stat	Lower 95%	Upper 95%
Intercept	7.9429	0.1201	66.16	7.7074	8.1785
X Variable 1	2.6673	0.0352	75.75	2.5982	2.7364

Regression Statistics		Low shore levels			
Multiple R	0.91				
Standard Error	2.23				
Observations	209				
Sources	df	SS	MS	F	
Regression	1	4873.87	4873.87	980.33	
Residual	207	1029.14	4.9717		
Total	208	5903.01			
Variables	Coeff.	SE	t Stat	Lower 95%	Upper 95%
Intercept	7.7062	0.3620	21.2900	6.9926	8.4198
X Variable 1	3.1160	0.0995	31.3102	2.9198	3.3122

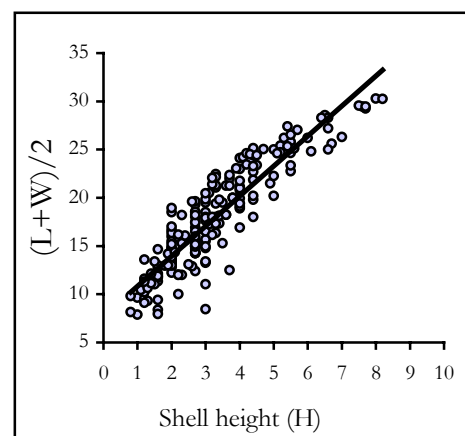
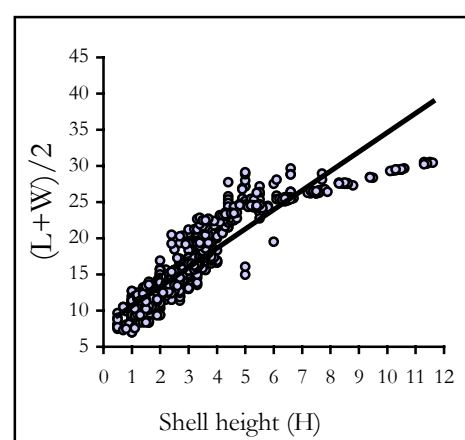
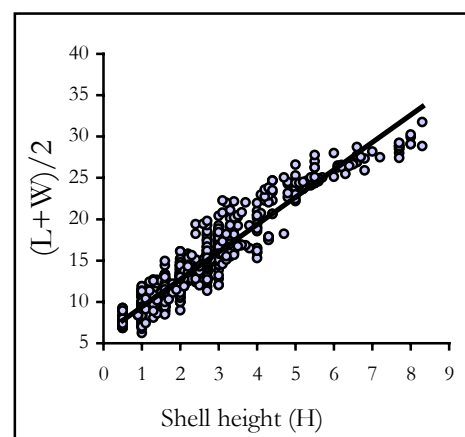


Figure 9.10. *Cellana testudinaria*. Relationship between $(L+W)/2$ and H for limpets living at the three shore levels.

The following results of regression equations are obtained:

- High shore level: $(L+B)/2 = 6.1008 + 3.3142 H$
- Middle shore level: $(L+B)/2 = 7.9429 + 2.6673 H$
- Low shore level: $(L+B)/2 = 7.7062 + 3.1160 H$

Standard error (SE) of the estimate (or sample standard deviation from regression; Sokal & Rohlf, 1995) was found to be 1.4456 for the high shore level, 2.1019 for the middle shore level, and 2.2297 for the low shore level, respectively. The calculated linear correlations among those relationships were highly significant for the three different shore levels with determination coefficients of 0.95 ($P < 0.001$) for the high shore level, 0.91 ($P < 0.001$) for the middle shore level, and 0.91 ($P < 0.001$) for the low shore level (Table 9.9). The coefficients of the slopes of these regression equations were 3.3142 ± 0.0329 (SE) for the high shore level, 2.6673 ± 0.0352 (SE) for the middle shore level, and 3.1160 ± 0.0995 (SE) for the low shore level, and the difference between those was found to be highly significant (t -test, $P < 0.001$). The higher regression coefficient (i.e. slope of regression line) for the high shore level reflects a more pronounced increase in length and width with increasing height of the shell in limpets living at this shore level than those at the other shore levels (Table 9.4).

The statistical domains of the regressions of $(L+W)/2$ on H in the limpets living at the three shore levels have been plotted in Figure 9.11 to include 95 % (mean \pm SD) of the sample populations in terms of $(L+W)/2$ and H values. The domains show some overlap: 67 % of the domain of the limpets at the high shore level is overlapped by that of limpets at low shore level, and almost the whole domain (98 %) by limpets at the middle level. Further, those limpets at the middle shore level approximately overlap 95 % of the domain of limpets at the low shore level.

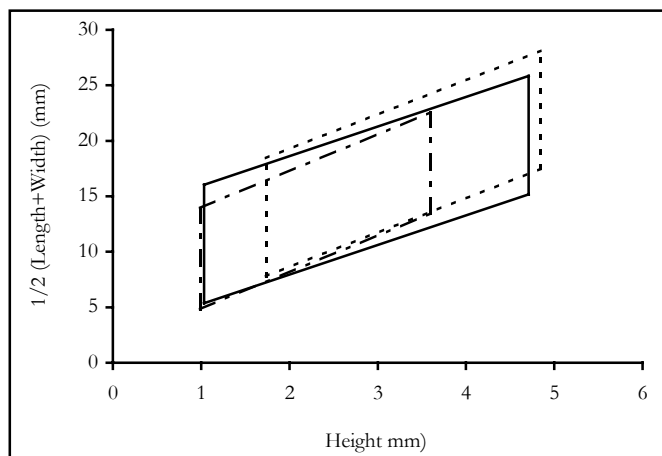


Figure 9.11. *Cellana testudinaria*.

Statistical domain of regression $(L+B)/2$ on H in limpet shells living at high shore level (---), middle shore level (—), and low shore level (-.-). Slopes have been drawn to standard deviations of estimate on either side of regression line, and vertical boundaries to standard deviations on either side of mean height.

The statistical domains as are shown in Fig. 9.11 relate to shell shape. If the differences in shell shape between the limpets live at the different shore levels are regarded due to morphologically adaptation, then it could be considered to reflect the differences and overlap in their distribution on the shore.

9.3.2.2. Changing in shell circumference and volume

The allometric relationships between circumference (CF) and volume (V) for the three different shore levels are shown in Figure 9.12. The regression equations are:

- High shore level: $\ln CF = 2.4035 + 0.2892 \ln V$
- Middle shore level: $\ln CF = 2.5595 + 0.2585 \ln V$
- Low shore level: $\ln CF = 2.4860 + 0.2754 \ln V$

Shell volume increases as the cube of linear morphological measurements of shell circumference for an isometrically growing limpet, due to simple geometric considerations.

The result of t -test on the deviations from isometry ($\alpha < 0.33$) shows that shell volume of limpets living at the three shore levels exhibits highly significant positive allometry relative to the shell circumference ($P < 0.001$, t -test), as is the case described above, volume increases even more rapidly relative to circumference as the animals grows. In addition, α is significantly smaller for limpets living at the low shore level than for those living at the high shore level. Thus, limpets living at the low shore level show a greater average change in shape with increasing size.

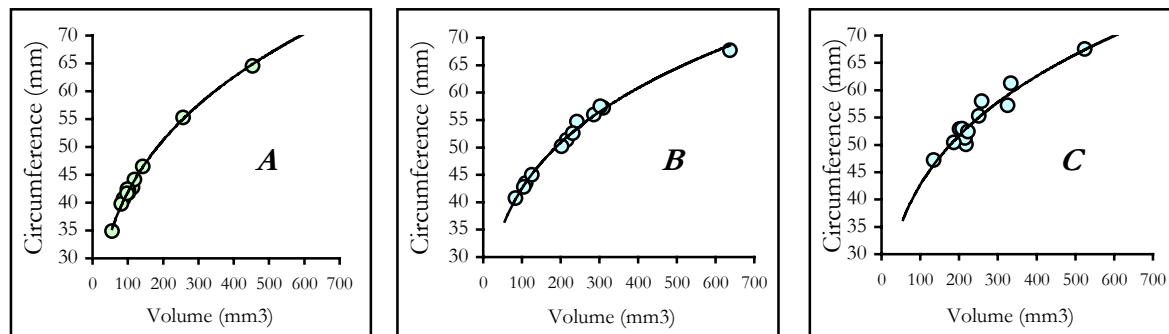


Figure 9.12. *Cellana testudinaria*. Monthly circumference (CF) of shell aperture versus monthly shell volume (V): curves drawn from power functions derived from reduced major axis regressions of $\ln CF$ against $\ln V$; A, the high shore level, $CF = 11.062V^{0.2892}$, $r = 0.99$, $n = 12$; B, the middle shore level, $CF = 12.929V^{0.2585}$, $r = 0.99$, $n = 12$; C, the low shore level, $CF = 12.013V^{0.2754}$, $r = 0.96$, $n = 12$; r , correlation coefficient; n , sample size.

One-way ANOVA tests on the mean shell circumference and volume for the three different shore levels show that there is significant difference in the shell circumference, but no significant difference is obtained for the shell volume (Table 9.10). The mean circumference of limpets living at the low shore level (54.7269 ± 5.6025 mm) is higher than at the high (44.7136 ± 7.8626 mm) and the middle (51.6117 ± 7.7658 mm) shore levels. This may indicate that when limpets exhibiting the same shell volume are compared, those limpets living at low shore level are taller than those living to the higher level, otherwise the shell of limpets living at the high shore levels becomes more domed as the limpets grow.

Table 9.10. Analysis of variance on the mean of the shell circumference (CF) and the shell volume (V) for the three shore levels during the study periods. SS -sum of squares; df -degrees of freedom; MS -mean square; P -probability; * significant; ns not significant.

Shore level	SS		df	MS		F		P	F crit
	CF	V		CF	V	CF	V		
Between Groups	630.2	89656.2	2	315.1	44828.1	6.16*	3.03 <i>ns</i>	0.005	3.28
Within Groups	1688.7	488056.6	33	51.2	14789.6				
Total	2318.9	577712.8	35						

The results of separating the effects of shape from the effects of size by rearranging the power function to obtain the ratio: $V^\alpha/CF = 1/\beta$, give the ratio values of (0.090405 : 0.0904) for the high shore level, (0.077351 : 0.077346) for the middle shore level, and (0.083269 : 0.083243) for the low shore level, indicating that limpets live at the low shore level are more highly acute than those living to the higher shore levels. Regression of V^α/C against V yielded non-significant correlation coefficients ($r < 0.30$, $P > 0.05$), demonstrating that this measure of shape is indeed independent of size.

9.4. Discussion

9.4.1. Body dimensions

The results of the present study have shown that the shell characteristics vary with the habitat of *C. testudinaria*. Two extreme forms have been described: high shells with a remarkable apex curvature, and flat shells only slightly curved. Those limpets inhabit the low shore level are generally larger in size (Fig. 9.3), with a high acute and with the apex of the shell shifted more towards to the posterior margin (Fig. 9.8). Previous workers found that the average shell length of *Patella vulgata* (Brian & Owen, 1952), and *Lottia digitalis* (Wootton, 1993) at lower shore level is greater than those limpets at higher levels. They attempted to explain that limpets live to the lower shore levels, which inhabit damp locations, reduce their activity of the shell muscle, and so the consequent absence of any constraint on the extension of the shell. Segal (1956) observed that shells of high intertidal *Acmaea limatula* have a smaller volume than shells of low intertidal individuals. Khouw (2002) independently reported that several species of limpets such as *Cellana radiata* and *Colisella striata* inhabiting the lower shore levels are generally larger in size, with a high acute and wide based shells. The similar patterns were also found for *Patella* living at low-level, in which *Patella oculus*, *Patella argenvillei*, and *Patella barbara*, have very tall shells (Branch, 1975). In contrast to the pattern found in this study, a greater shell of limpets in a high shore level than in a damp habitat has been found by Orton (1928) in *Patella vulgata*, by Sutherland (1970) in *Macclintockia scabra*, by Balaparameswara Rao & Ganapati (1971) in *Cellana radiata*, and by Bannister (1975) in *Patella lusitanica*. Davies (1969) has shown that in British and Mediterranean *Patella* species, the shell is tall and conical in upper shore species and progressively flatter in lower shore and subtidal species. Orton (1928) and Branch (1975) attributed the higher acute and narrow based shells at the limpets living at higher shore levels as the result of the attempt on the part of the limpets to contract the shell down more tightly to the substrate in order to avoid desiccation under exposed conditions. Whereas, several authors (Lowell, 1984; Denny & Blanchette, 2000) concluded that the flat shell of limpets is an adaptation to the lower shore levels due to the high degree of water turbulence. It is, therefore, usually thought that, in *Cellana*, as in other patelliform molluscs, the type with a high shell is found where the limpets are exposed to drought and slight water movements, whereas the flat varieties occur where there is a

high degree humidity and water turbulence (Orton, 1933; Moore, 1934; Lowell, 1986; Denny, 2000; Denny & Blanchette, 2000).

The influence of environmental conditions on the morphometrics of *C. testudinaria* shells is probably partly related to an increased tolerance of desiccation. Several authors have considered the “extravisceral” space between the body and the shell to be adaptive in terms of resistance to desiccation. Segal (1956) showed that although high-level *Acmaea limatula* have thicker shells (and hence smaller internal volume) their smaller body sizes result in a larger extravisceral space, presumed to ease the stress of desiccation. Unfortunately, no exactly description can be given in this study related to the desiccation as the major factor determining the morphometrics of shell, since many high-shore animals with a mean smaller body size were also collected in April 2002 (Table 9.1). This period notably represents the period with higher humidity and precipitation, lower air temperature and insolation, and extremely wave action (Fig. 2.2, 2.3, and 2.5).

Based on my field observation found that at the high shell type habitat (the high shore level) and, although these occurred with certain degree of variation, none of the acute-shelled type ever appeared. As these limpets were never exposed to desiccation for several longer tidal periods (see Fig. 2.5), humidity was obviously not a determination factor for high or flat shells; thus a reasonable explanation is that the high-flat shell varieties inhabit regions such as the intertidal rocky shore of Ohoiwait where the water is sometimes calm with longer periods of tidal cycle (in September and October), and or a high degree of turbulence (in March and April) (see Fig. 2.5). High or flat shells, therefore, appear to be not an adaptation either to the desiccation or to resist the fierce onslaught of waves in turbulent areas. The results of the analysis on the differences in the characteristics of the shell suggest that the influence of desiccation is less significant pronounced than that of the other factors. The pattern of an increase in average limpet shell dimensions associated to the lower shore level could also be the result of ontogenetic migration across the level of shore due to the availability of food (Liu, 1994; Hobday, 1995; Benedetti-Cecchi, 2001). With more splash, food abundance may occur at higher tidal levels, resulting in more small limpets migrate to the high shore levels. An alternative hypothesis to explain upward migration of small limpets comes from the observations by Stimson (1970) and Marshall & Keough (1994) that small limpets, with smaller radulae, are more efficient grazers and can remove the algae closer to the rock, and thus have superior competitive ability in food gathering over larger conspecifics. On the other hand, it is, therefore, an alternative implication that large limpets by migrating downwards or keeping in lower residents, can avoid competition with superior foragers and still feed at optimum times. I documented also an increase in total density of small limpets, attributable to the higher shore level (Fig. 3.1 and 4.1). Another reason for the large limpets in shell dimensions to occupy the low shore level may be attributed to their behaviour as an active role in the clamped mechanism (adhesion mechanism) in response to the environmental conditions. Ellem et al. (2002) found that limpets

clamp sharply for several times in response to single disturbances such as to lifting forces applied to the shell by a highly dynamic wave profile. They showed that the force of clamping could be implicated on the shell dimensions as a result of activity of the shell muscle, causing any constraint on the extension of the shell. Thus, shell becomes taller.

The limpet shell measurements may also be used for interspecific differentiation. The ratio of the distance from the apex to the posterior margin (AP) to the distance from the apex to the anterior margin (AA) of the present specimen *C. testudinaria* were 3.32 for the high shore level, 3.24 for the middle shore level, and 3.02 for the low shore level with the mean of 3.19, which appeared higher than those reported by Saad (1997) for *Cellana encosmia* (1.50), by Emam (1994) for *Cellana karachiensis* (1.21), and by Khouw (2002) for *Cellana radiata* (1.16) and *Colisella striata* (1.79). This indicates that the shell of *C. testudinaria* found in the present study is more acute than those reported by previous researchers. In general this ratio decreased slightly as the animal grew in size (Emam, 1994). Although the shell of limpets living at the low shore level are more shifted backwards than those living at the other shore levels, the overall pattern showed that it was more shifted forwards compared to the other species reported by Russell (1909) and Orton (1928) for *Patella vulgata*, by Balaparameswara Rao & Ganapati (1971b) for *Cellana radiata*, by Emam (1994) for *Cellana karachiensis*, and by Saad (1997) for *Cellana encosmia*.

It can be attributed to the differences in total body wet weight for limpets living at the three different shore levels that the low shore limpets were heavier than those at the high and middle shore levels (Fig 9.3). The best implication may be related to the relative coefficient of condition factors (for explanation see chapter 3; section 3.4.4). *C. testudinaria* settled high on the shore where food is relatively scarce (Liu, 1994); consequently, growth rates are low. As the limpets grow, they migrate down the shore into progressively better food conditions (Liu, 1994). This size-specific migration would increase the possibility to have more abundant in food available. Consequently, it would produce a composite increment of the total body wet weight with an increment in shell. The mean *b*-values from the power regression equations (Table 9.9) prove that these increments of limpets living at the low shore level were higher than those at higher levels. These results are a little bit lower compared with those obtained by previous other worker for various limpet species; *Cellana encosmia* of 3.0066 (Saad, 1997), *Cellana radiata* of 3.0900 (Ismail & Elkarmi, 1999), *Notoacmea schrenckii boninensis* of 3.3288 (Asakura & Nishihama, 1987), and *Patella vulgata* of 3.2520 (Baxter, 1983). The significant results of *b*-values indicated that limpets living between different shore levels might possess distinct physiological responses for the particular situation in which they were growing.

Although shell morphometrics of *C. testudinaria* from the intertidal rocky shore of Ohoiwait are slightly different from those of *Cellana radiata* from India (Balaparameswara Rao & Ganapati, 1971b) and from the Gulf of Agaba, *Cellana encosmia* from the Gulf of Suez (Saad, 1997), patterns of the changes in the shell shape with growth are generally similar. The relationships of shell length to shell width, height, apex to anterior, and apex to posterior are

linear, while the relationships of shell length to shell weight are curvilinear. Similar results were reported for *Cellana karachiensis* from the Arabian Gulf and Gulf of Oman (Emam, 1994), and for *Cellana radia* and *Colisella striata* from the intertidal of Ohoiwait (Khouw, 2002). The shell of *C. testudinaria* was proportional during growth as the relationships of length to width and height were isometric.

9.4.2. Shell shape

In general, the results of this study on actual limpet shells reinforce the conclusion on the basis of conical models. For the symmetrical models, Denny (2000) found that the lowest risk of dislodgement by wave action for a limpet is associated with a shell having a central apex (roughly one to one; 1.06) and a height-to-length ratio of approximately 0.53. The average ratio of shell height to radius ($H/(L/2)$) for a selection of limpets that Denny (2000) chose at random from every continent was 0.68 with the span ratios among these animals fairly evenly distributed in a range from 0.37 to 1.27. However, *C. testudinaria* limpets rarely have shells with these dimensions. The ratios calculated for those limpets from the three shore levels were 0.49 and 0.35 for the high shore level, 0.51 and 0.38 for the middle shore level, and 0.55 and 0.39 for the low shore level, with the mean ratio of 0.52 and 0.37, respectively. This indicates that most limpets have an apex that is well anterior of the centre of the shell, and slightly domed. These values are slightly lower than those found in *Patella vulgata* from Europe (0.75 and 0.92), *Notoacmea schrenkii* from Japan (0.39 and 0.78), *Cellana tramoserica* from Australia (0.95 and 0.75), and *Patella argenvillei* from South Africa (0.95 and 0.85), reported by Denny (2000). The significantly higher value of the ratios in *C. testudinaria* living at the low shore level (Table 9.4) has been found to be associated with a higher resistance to wave action in this limpet in comparison to those at the higher shore levels. An analogous situation is seen when the data of Denny (2000) are taken into account, in which shell shape of limpets at the low shore level is more proportional, and hence more stable to the higher degree of wave action than those limpets at the higher shore levels. In addition, when the data of the ratio ($E = AA/(L/2)$) from April 2002 (representing the period of higher degree of wave action) and from September 2002 (representing the period of lower degree of wave action) are compared, the mean ratio in April (0.50) is higher than in September (0.49), implying that the shell shape of limpets collected in April is more proportional than limpets in September (Table 9.6). This shell shape allows the limpets living to the lower shore level to have “good enough” interaction with flowing water caused by wave action, and at the same time that it was being fine-tuned by other selective pressure to act as an effective bulldozer (Denny & Blanchette, 2000).

Table 9.6. *Cellana testudinaria*. Comparison of the $AA/(L/2)$ ratio of sample collected in April 2002 and September 2002 for the three different shore levels, together with *t*-test on the mean sample.

Shore levels	April	September	<i>t</i> -test
High	0.4844 ± 0.0629	0.4736 ± 0.0667	P < 0.05
Middle	0.4922 ± 0.0844	0.5169 ± 0.1126	P < 0.05
Low	0.5241 ± 0.0783	0.4894 ± 0.0904	P < 0.05
Average	0.5002 ± 0.0752	0.4933 ± 0.0899	

Variation in shape about the allometric average in the terms of the shell circumference and the shell volume may be compared with variation in shape among limpets live at different shore levels. In *C. testudinaria* live at the intertidal rocky shore of Ohoiwait, known to fit the power function of $CF = 11.062V^{0.2892}$, $CF = 12.929V^{0.2585}$, and $CF = 12.013V^{0.2754}$, ranges from the high to the lower shore levels. Standardising allometrically average limpets from their shell volume to one size yields a circumference difference of 15.8% for the high shore level, 17.58% for the middle shore level, and 16.86% for the low shore level, respectively. Tentatively, the ratio of V^α/C to $1/\beta$ can suggest that lower limpets are more acute than average for those higher limpets. Clearly those limpets conform to the results obtained by analysing the shell dimension. Therefore, the average change in shape due to allometry could potentially have at least as great an effect on variation in shape about the allometric average. This potential effect may be primarily or partially related to developmental responses to the variation in environmental conditions. For instance, development differences may be an adaptational response to selective pressure such as hydrodynamic forces (Hobday, 1995; Denny & Blanchette, 2000), desiccation (for review see Branch, 1981), predation (Lowell, 1986; Hahn & Denny, 1988), or the behaviour by the limpet itself (Stimson, 1970). Possible morphological constraints on the shell shape can also be affected by the muscle attachment (Branch & Marsh, 1978), or may be due to the genetic factors as has been shown to be the case in other gastropods (Struhsaker, 1968).

From the above explanation, it can be, therefore, concluded that the shell form of *C. testudinaria* occupies the intertidal rocky shore of Ohoiwait, exhibiting a significant tendency for basal dimensions of the shell to decrease interspecifically from low to high shore levels. The same trend is apparent from temperate and tropical latitudes in at least the species *Patella vulgata* (Brian & Owens, 1952) and *Lottia digitalis* (Wootton, 1993), *Patella argenvillei* (Branch, 1975), *Cellana radiata* and *Colisella striata* (Khouw, 2002). Temperature effect in terms of desiccation stress seems less pronounced to be an important factor in determining the shell shape changing, since the analysis on the relationship between shell circumference and volume showed that the lower limpets are taller than the higher ones. Finally, the environmental conditions in the intertidal rocky shore of Ohoiwait and the characteristics of the shell resulting from the analyses, made it possible to demonstrate the factors, which really condition the form of the *C. testudinaria* shells, thus disproving or disagreeing the opinions expressed by other authors in this subject.

Chapter 10

GENERAL DISCUSSION AND CONCLUSION

10.1. Conservation issues

The warm, humid tropical climate of the Southeast Molluccas favours the proliferation of diverse marine and terrestrial communities. Extensive coral reefs flourish along the region's coastlines, some of which are vitally important to valuable species, including those of commercial importance. Consequently, the ecosystems are utilized by local coastal populations as fishery resources of vital economic importance, and this is reflected in rival territorial claims with respect to fishing grounds. Many such disputes have been resolved by the formation of local economic treaties. One important example was the traditional "interim arrangement" between two coastal populations, concerning the commercial exploitation of reef fishes, *Trochus* sp., and limpet species including *Cellana testudinaria*.

Because of the economic benefits, which arise from the exploitation of such rich and diverse ecosystems, the coastal zone of Ohoiwait is densely populated. The total coastal population, depending directly on the exploitation of coastal resources, is around 1 thousand people. Consequently, environmental pressures, most notably as a result of increased terrestrial and marine pollution, have compounded the problems faced by the region in the last decade.

Future economic growth of this area relies upon sensible use of the natural resource base, and therefore the marine and coastal environments must be managed to facilitate sustainable use of available marine resources. However, in order to succeed such an approach requires cooperation, but this has the potential of creating tensions and conflicts between policies for the development of marine and coastal resources on the one hand, and their conservation on the other. Sensitive resource aspects requiring particularly careful consideration include the intensity and methods of fishing, habitat preservation and marine pollution control. Currently, the local government agency on nature conservation formulated an action plan for the future management of the natural resources of the Ohoiwait's coastal region.

From those increasingly pressing issues of conservation related to the problems faced on the intertidal rocky shore of Ohoiwait, it could be concluded that further investigations on the population dynamics of valuable species, such as *Cellana testudinaria*, are needed.

10.2. Population dynamics

10.2.1. Distribution, abundance and biomass

A common feature of the distribution, abundance and biomass of the limpet *C. testudinaria* living at the intertidal rocky shore of Ohoiwait is largely correlated to the strong seasonal patterns of environmental conditions. During the south monsoon or the dry season (June – August) and the transition period afterwards (September – November), the mean density of individual limpets at middle and low shore levels increases steadily from $3.67 \pm 1.80 \text{ ind.m}^{-2}$ in June to $44.33 \pm 5.19 \text{ ind.m}^{-2}$ in October, and from $1.17 \pm 1.08 \text{ ind.m}^{-2}$ in June to $6.67 \pm 2.87 \text{ ind.m}^{-2}$ in September (Fig. 3.1). The high shore limpets show the inverse trend with mean densities decreasing from $18.50 \pm 5.12 \text{ ind.m}^{-2}$ in June to $7.50 \pm 2.99 \text{ ind.m}^{-2}$ in August. Conversely, during the north monsoon or the rainy season (December – February), the mean density of limpets at middle and low shore levels decreases to the average of $3.67 \pm 1.25 \text{ ind.m}^{-2}$ and $3.17 \pm 1.46 \text{ ind.m}^{-2}$, whilst it increases to the average of $21.67 \pm 10.03 \text{ ind.m}^{-2}$ for limpets at the high shore.

Early work established that such patterns of distribution and abundance existed in relation to exposure to wave action and tide level (Das & Seshappa, 1947; Southward & Orton, 1954; Menconi et al., 1999). Ebling et al. (1962) observed that *Patella aspera* extends on the open rock up to high water of spring tides at Carrigathorna, and from mid-tide level downwards it provides the most abundant limpet. Southward & Crisp (1954) found that at wave-exposed places on the Irish coast, *Patella vulgata* seldom occurs below high water of neap tides. Blackmore (1969) described that *Patella vulgata* was found to be abundant at the low shore of Robin Hood's Bay in July and August. Moreover, Liu (1994) when studied the ecology of the Hong Kong limpets *Cellana grata* and *Patelloida pygmaea* at Cape d'Aguilar and Wu Kwai Sha found that those limpets were most abundant at the lower shore in summer between May and July. Fluctuations in density of limpets may also be linked to desiccation (Davies, 1969; Wallace, 1972; Branch, 1981), which is related to insolation and tide level. Liu (1994) reported a correlation between temperature and vertical distribution and abundance of the population of *Cellana grata* and *Patelloida pygmaea*. In summer, these species were more abundant at the low shore.

The data on *C. testudinaria* suggest that the relatively higher densities at the low shore during the dry season characterized by high wave action are in agreement with the results of other authors, but understanding of them was severely limited by the lack of a comprehensive scheme for relating the populations to their natural habitats. Even though the mean seasonal density presents convincing evidence for the interpretation, it was not possible to arrange the results of the present study adequately without taking account of the other environmental variables. Therefore, to study the distribution, abundance and biomass of this limpet species more accurately, more *in situ* data of the environmental variables and their effects are needed.

10.2.2. Growth

The frequency distribution of length groups of *C. testudinaria* indicates the presence of 5 cohorts (Fig. 4.2), with the mean observed lengths of these cohorts being 10.2, 13.3, 17.6, 23.4, and 27.7 mm. These six cohorts are similar to the results obtained for *Cellana eucosmia* from the Gulf of Suez (Saad, 1997) and *Cellana radiata* from the Gulf of Agaba (Ismail & Elkarmi, 1999).

There is a striking inverse correlation between growth coefficient K and longevity, suggesting that species with higher growth rates have lower longevity. This is a relationship that has been pointed out by several authors, particularly in comparisons of species from different habitats (Lewis & Bowman, 1975; Thompson, 1979; 1980). Growth rate and longevity of *C. testudinaria* limpets fit well into this inverse relationship (Fig. 10.1).

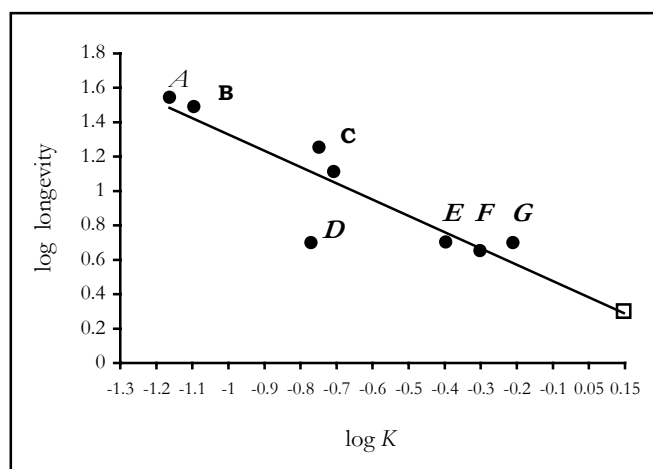


Figure 10.1. Correlation between longevity and growth coefficient for 9 species of limpet including *Cellana testudinaria* (\square). A, *Nacella concinna* (Br thes et al., 1994); B, *Notoacmea petterdi*; C, *Patella alticostata* (Parry, 1978); D, *Cellana radiata* (Ismail & Elkarmi, 1999); E, *Collisella digitalis* (Frank, 1965); F, *Cellana tramoserica* (Fletcher, 1984); G, *Cellana eucosmia* (Saad, 1997).

There are several ways of looking at this inverse relationship. Some species have a low or high probability of survival because of the nature of their environment. Choat & Balck (1979) have argued that *Collisella digitalis* grows rapidly to sexual maturity and can survive the instability of its habitat by continual recruitment. Creese (1980a) suggested that some species may be compelled by shortage of space to remain small, so that growth beyond sexual reproduction is futile and an annual longevity is a more obvious adaptation. The converse applies in situations where food may be predictably in short supply. Picken (1980) found that the very low growth, low metabolic rate, and delayed sexual maturity of *Nacella concinna* as adaptations to the conditions in the Antarctic where food availability is low for most of the year. Thus, for different reasons in different species it is possible to view each growth pattern as an adaptation to particular circumstances.

From the simple explanation above, it may be concluded that the similarities and differences in growth of various species of limpets including *Cellana testudinaria* can be attributed to the nature of their habitats and the influence of the environmental conditions in those habitats.

10.2.3. Recruitment

It is not until 1 – 2 months after spawning that limpet spat (< 4 mm in length) can be detected under natural conditions. However, early recruits cannot be determined accurately because of the lack of data obtained. Mortality rates by environmental stresses and predation were doubtless very high during the planktonic phase, and continues to play an important and variable role immediately after settlement (Bowman & Lewis, 1977). The fact that the greatest percentage of recruits occurred during the period of transition (in March and April) indicates that recruitment is dependent on season. The possible explanation is that significantly higher wave action during this season caused a higher spat supply.

In the present study, however, an attempt to estimate the recruitment rate of *C. testudinaria* using the available methods still faces some problems. As it has been described in the previous discussion (see Chapter 5, section 5.4.1), the determination of the recruitment patterns of this species requires accurate methods and detailed analyses in its various phases of life stages (Keough & Downes, 1982).

10.2.4. Mortality

The techniques used in the present study to estimate the mortality of limpet *C. testudinaria* are most useful when size distributions data are available. The first two methods (the survival of individual size frequency cohorts and the monthly calculation of the Ault-Ehrhardt moment estimate) gave the same values of 91 % per year ($Z = 2.46$). This estimate was only a little lower than that of 94 % per year obtained by the direct estimation from size distribution and growth rate (length converted catch curve). The mortality of *C. testudinaria* is higher than that of several other species (Table 10.1).

The natural mortality of this species seems to be correlated with the environmental conditions. Seasonal fluctuations of mortality calculated by the Ault-Ehrhardt method suggest that the highest mortality rates occurred mainly in April and October. This indicates that wave action and temperature may play the major role in enhancing survival of this limpet species. Additionally, I recorded the highest temperature measured on the substratum and in tide pools (40 °C in October 2001 and the transition periods afterwards), which suggests that the effect of temperature on this limpet species is the primary proximate factor causing higher mortality.

In summary, this study suggests that the high mortality of *C. testudinaria* is clearly affected by the environmental conditions.

Table 10.1. Comparison of mortality rate of *Cellana testudinaria* with values for other limpets.

<i>Species</i>	<i>Mortality rate</i> (% ind. loss per month)	<i>Locality</i>	<i>Authors</i>
<i>Cellana testudinaria</i>	7.6	Ohoiwait, Southeast Molluccas, Indonesia	This study
<i>Cellana tramoserica</i>	5.2	Botany Bay, New South Wales, Australia	Fletcher, 1984
<i>Cellana grata</i>	7.3	Cape d'Aguilar and Wu Kwai Sha	Liu, 1994
<i>Patelloida pygmaea</i>	7.7	Cape d'Aguilar and Wu Kwai Sha	Liu, 1994
<i>Nacella concinna</i>	7.7	Esperanza Bay, Antarctic Peninsula	Brêthes et al., 1994
<i>Patelloida alticostata</i>	3.0	Botany Bay, New South Wales, Australia	Fletcher, 1987
<i>Patella vulgata</i>	5.8 – 6.7	Bantry Bay, United Kingdom	Thompson, 1980
<i>Nipponacmea teramachii</i>	5.0	Boulder shore in Amakusa, Japan	Takada, 1997
<i>Nipponacmea nigrans</i>	2.3	Boulder shore in Amakusa, Japan	Takada, 1997
<i>Nacella delesserti</i>	6.3	Marion Island, Sub-Antarctic southern Ocean	Blankley & Branch, 1985
<i>Acmaea digitalis</i>	4.3	Ellwood Beach, Santa Barbara, USA	Choat & Black, 1979
<i>Acmaea insessa</i>	20	Ellwood Beach, Santa Barbara, USA	Choat & Black, 1979

10.2.5. Gonad condition

C. testudinaria has a single breeding episode within a year with a clearly recognizable spawning event and a long resting stage thereafter. All individuals large enough to be sexually mature had visible and sexable gonads throughout most of the year. There is evidence from my study that synchronous spawning occurs in this limpet species. Sometimes, however, some individuals in a population spawn at a particular time, but others do not (Creese, 1980). Such a situation results in a mean gonad index after spawning having a very large variance (see Fig. 7.1), which implies a low degree of synchrony at spawning. But I believe that events in the breeding cycles of other species of *Cellana*, like *C. radiata* (Balaparameswara Rao, 1973), *C. radians* (Creese & Ballantine, 1983), *C. tramoserica* (Underwood, 1974), and *C. ornata* (Dunmore & Schiel, 2000), could have been similar to those reported here for *C. testudinaria*, i.e. that spawning is synchronous for virtually the whole population. Such a conclusion can only be reached if frequent samples are taken around the time of spawning (Balaparameswara Rao, 1973; Creese & Ballantine, 1983). Monthly samples, as taken in this study, are not frequent enough to establish how synchronous spawning is, but good enough to study the gonad condition related to population dynamics.

Seasonal changes in the time of spawning and gonad development of *C. testudinaria* coincided with changing environmental conditions (see section 7.4.5).

Therefore, I conclude that the reproductive cycle of *C. testudinaria* is strongly influenced by environmental variables at the intertidal rocky shore of Ohoiwait.

10.2.6. Production

Production of *C. testudinaria* in this study was estimated from its soft tissues (somatic tissue + gonad tissue). The total annual production from October 2001 to September 2002 was estimated to be $60.67 \text{ kJ.m}^{-2}.\text{yr}^{-1}$. It is lower than that of many limpet populations such as *Nacella concinna* (Picken, 1980), *Acmaea scabra* (Sutherland, 1972), *Patella vulgata* (Wright & Hartnoll, 1981; Workman, 1983), *Patelloida alticostata* (Parry, 1982), *Nacella delesserti* (Blankley & Branch, 1985), and *Cellana tramoserica* and *Notoacmea petterdi* (Parry, 1982). These conflicting reports may be due to the different methodologies used to determine production. For instance, production can be estimated as the energy content which included organisms lost from the population due to mortality (Parry, 1982), or from the variation in epilithic organic matter ingested by limpets (Workman, 1983). However, the higher production of the other species was very probably primarily due to the higher densities. The P/B ratio of *C. testudinaria* was 2.77. This value is in the upper range of values reported for other species of limpets, indicating that this species is more productive than the other species, which had a higher absolute production.

The assessment of the energy content calculated for the limpet *C. testudinaria*, gives a value of 21.59 kJ.g^{-1} AFDW. This value was considerably higher than that of many temperate limpet species, such as *Patella vulgata* (19.3 kJ.g^{-1} AFDW) (Workman, 1983), and *Nacella delesserti* (20 kJ.g^{-1}) (Blankley & Branch, 1985), but similar to that of other tropical limpet species such as *Cellana tramoserica* (22.59 kJ.g^{-1}), *Notoacmaea petterdi* (21.63 kJ.g^{-1}), *Patella peroni* (22.30 kJ.g^{-1}), and *Patelloida alticostata* (22.93 kJ.g^{-1}) (Parry, 1982). It is known that the quantity and quality of energy content of soft tissue differ among limpet species living at different shore levels (Paine, 1971), or at neighbouring sites (Hughes, 1971), or even in the same area because of seasonal variation in food availability (Blankley & Branch, 1985).

10.3. Shell shape variation

The shell dimensions and forms of *C. testudinaria* show distinct differences among the shore levels, which reflect the adaptation of this species to the environmental conditions in the different habitats. If desiccation was considered as the most important physical stress, then shell morphometrics in terms of shell height and the position of apex tends to increase interspecifically from low to high shore levels (Balaparameswara Rao & Ganapati, 1971; Vermeij, 1973; Bannister, 1975; Branch, 1975). Clearly, the shore-level gradients in the morphology of intertidal limpets are as much a reflection of adaptation to increased temperature and desiccation stress in higher shore levels. However, the morphological gradients, i.e. a well-marked decrease in relative shell height with increasing shore level, observed in this study appear to be not the result of an adaptation to the dominant physical stress factors in the environment. The fact that the low shore limpets with high acute shells and with the apex shifted backwards are more abundant in September and October. An increasing of insolation and air temperature suggests

that there should be other dominant factors influencing this limpet species. Unfortunately, I could not differentiate these factors.

Physiological factors such as the ontogenetic migration across different shore zones due to the availability of food (Liu, 1994; Hobday, 1995; Benedetti-Cecchi, 2001) may also affect the morphological gradient in the shells of *C. testudinaria*, but I did not design a field experiment specifically related to this topic.

From the above discussions, it can be concluded that further research focused on a detailed analysis of the dominant factors affecting the shell morphometrics of *C. testudinaria* living at the intertidal rocky shore of Ohoiwait is needed.

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APPENDICES

Appendix 1. Gonad Condition

 Table A-1. Arbitrary scheme of classification of gonad stages used in the present work. (*Source* : Orton et al., 1956).

Stage	Brief description of gonad
Developing	
0 (or N)	Inactive or neuter, either rudimentary virgin stage or resting after discharge of gametes
1	Beginning to develop and sex detectable but only slight increase in size
2	Developing to one-third of full size
3	Between one-third of full size
4	Two-third of full size
5	Fully developed
Spawning	
5+	Full, but actively discharging gametes
4	Discharging, two-thirds full
3	Discharging, two-thirds to one-third full
2	Discharging, one-third full
1	Almost completely discharged

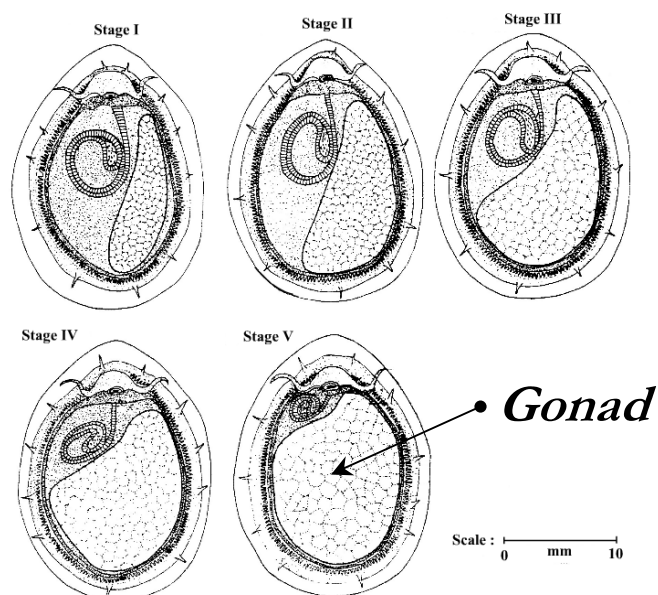


Figure A-1. Diagrammatic representation of progressive increase in the size of the gonad in different stages of development (modified from Balaparameswara Rao, 1973).

Appendix 2. Modal class Progression Analysis (MPA)

BHATTACHARYA'S METHODS

Functions $\ln(N_{i+1}) - \ln(N_i) = a_j + b_j \cdot L_i$

where N_i and N_{i+1} are successive frequencies of the same component of a group of fish in a sample (i.e., representing age group j) and where L_i is the upper class limit of N_i .

From this, the mean of the normal distribution is

$$\bar{L}_j = -a_j/b_j$$

while its standard deviation (σ) is

$$\sigma_j = (-\Delta L/b_j)^{1/2}$$

where ΔL is the constant class size.

Also, a separation index (SI) is computed.

$$SI = \frac{\Delta \bar{L}_j}{\Delta \sigma_j}$$

where $\Delta \bar{L}_j$ is the difference between two successive means, and $\Delta \sigma_j$ is the difference between their estimated standard deviations.

The separation of length-frequency samples into their component is an iterative process in that every identified component is subtracted from the remainder of the sample using the Gaussian function,

$$N_{2i+1} = N_{i+} - \left\{ \left(\frac{1}{\sigma_j \sqrt{2\pi}} \right) \cdot \text{EXP} \left(- \left(\frac{(L_i - \bar{L}_j)^2}{2\sigma_j^2} \right) \right) \right\}$$

where N_{i+} refers to the previous set of frequencies and N_{2i+1} is the new set of frequencies, less the component identified so far. Also, the user can backtrack and redo any step of the analysis without losing intermediate results.

Outputs Mean lengths, population sizes (in numbers), standard deviations and separation index (SI) for the age groups identified. These results are automatically saved to disk as a "mean and standard deviation" file, using the same filename as the input.

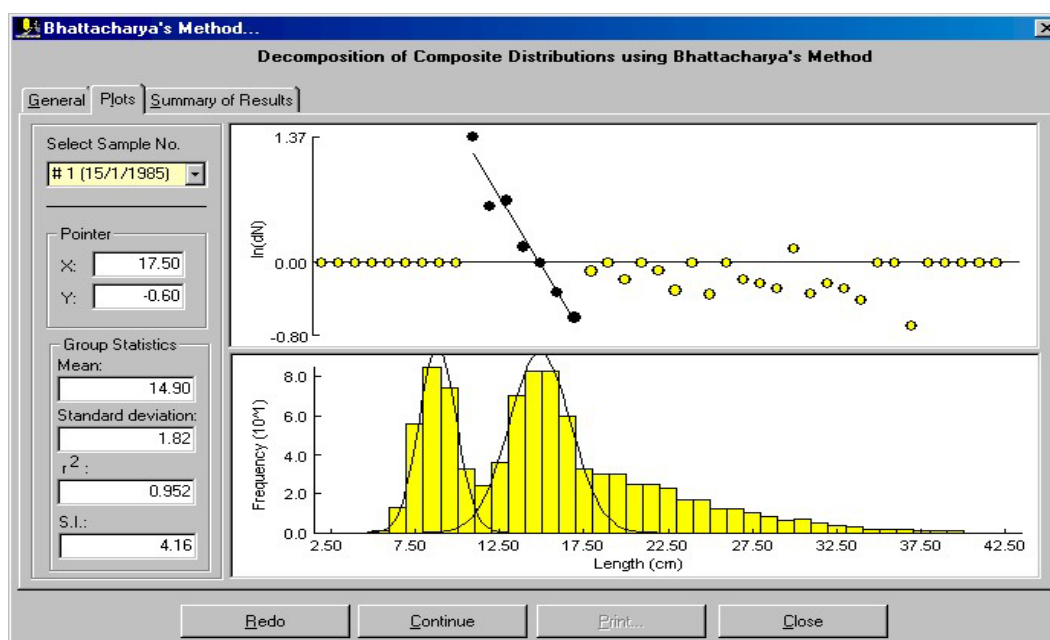


Fig. A-2. Model of the Bhattacharya's method in FiSAT.

Remarks Up to 10 age groups can be identified per sampling period, but separation is generally unreliable when the SI value is below 2. Also, note that the standard deviations usually increase with lengths.

NORMSEP

This method applies the maximum likelihood concept to SEparation of the NORMally distributed components of size-frequency samples.

Input parameters The expected number of age groups (maximum of ten) and their expected mean lengths. [The results from [Bhattacharya's routine](#) are used as initial inputs when available].

Functions (Refer to suggested readings for the maximum likelihood method)

Outputs Mean lengths, population sizes (in numbers), standard deviations and separation index (SI) for the groups identified.

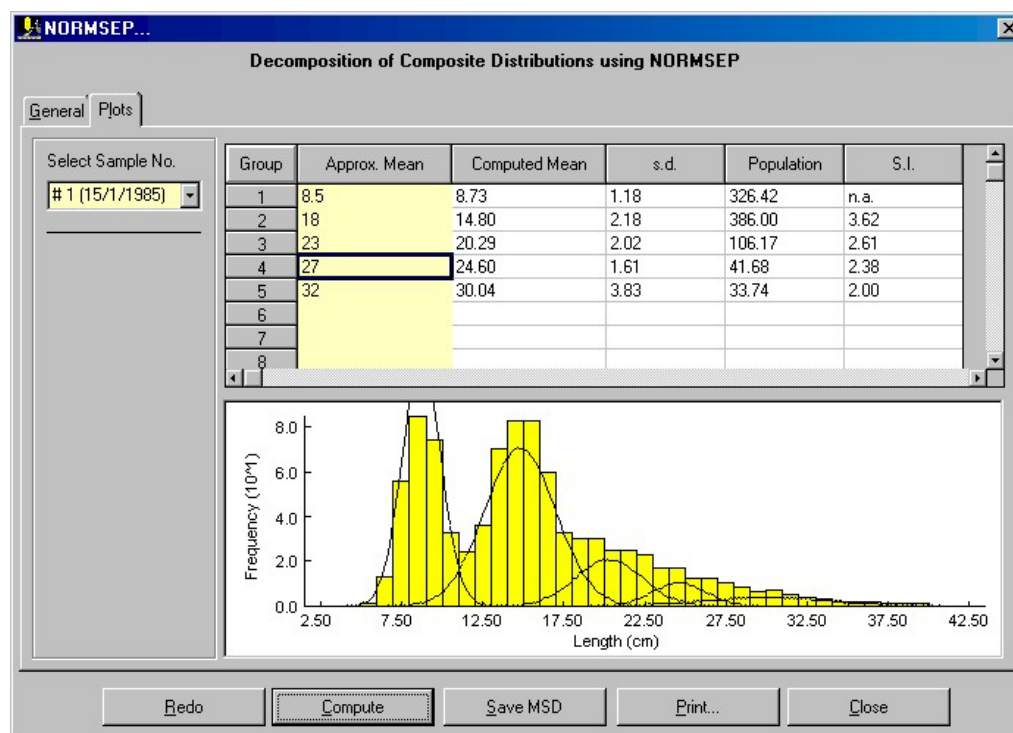


Fig. A-3. Model of NORMSEP in FiSAT.

Remarks It is advisable to use Bhattacharya's method to obtain initial guesses and to refine the results using NORMSEP.

Appendix 3. Estimation of growth

ELEFAN I

ELEFAN I is a routine that can be used to identify the (seasonally oscillating) growth curve that "best" fits a set of length-frequency data, using the value of R_n as a criterion (Gavanilo and Pauly 1997). FiSAT provides four options to the user to identify that "best" growth curve: (1) curve fitting by eye, (2) response surface analysis, (3) scan of K-values, (4) Automatic search routine; a routine is also available whose only function is to output the results.

Input parameters **Option 1:** Scanning of K-values

Fixed value of L_∞ , C and WP and, as an option, a fixed starting point.

Option 2: Response surface analysis

Range of values for two parameters (e.g., L_∞ and K), fixed value for the two others (e.g., C and WP), and, as an option, a fixed starting point.

Functions

In ELEFAN I, data are reconstructed to generate "peaks" and "troughs", and the goodness of fit index (R_n) is defined by $R_n = 10ESP/ASP/10$

where the ASP ("Available Sum of Peaks") is computed by adding the 'best' values of the available 'peaks' and the ESP ("Explained Sum of Peaks") is computed by summing all the peaks and troughs "hit" by a growth curve of the form, $L_t = L_\infty (1 - \text{EXP}(-K(t - t_0) + St_s + St_o))$

where

$$St_s = (CK/2\pi) \cdot \sin(2\pi(t-t_s)),$$

$$St_o = (CK/2\pi) \cdot \sin(2\pi(t_0-t_s)), \text{ and}$$

L_t is the length at time t .

Outputs

Option 1: Scanning of K-values

This plots R_n values for a range of K values (0.10 to 10) on a log-scale. We recommend use of this plot for all growth analyses, if only to assess how reliable an estimate of K is.

Option 2: Response surface analysis

This outputs an 11 by 11 matrix showing R_n values and in which the 10 best values are highlighted, thus enabling selection of the "best" combination of growth parameters.

User Interface

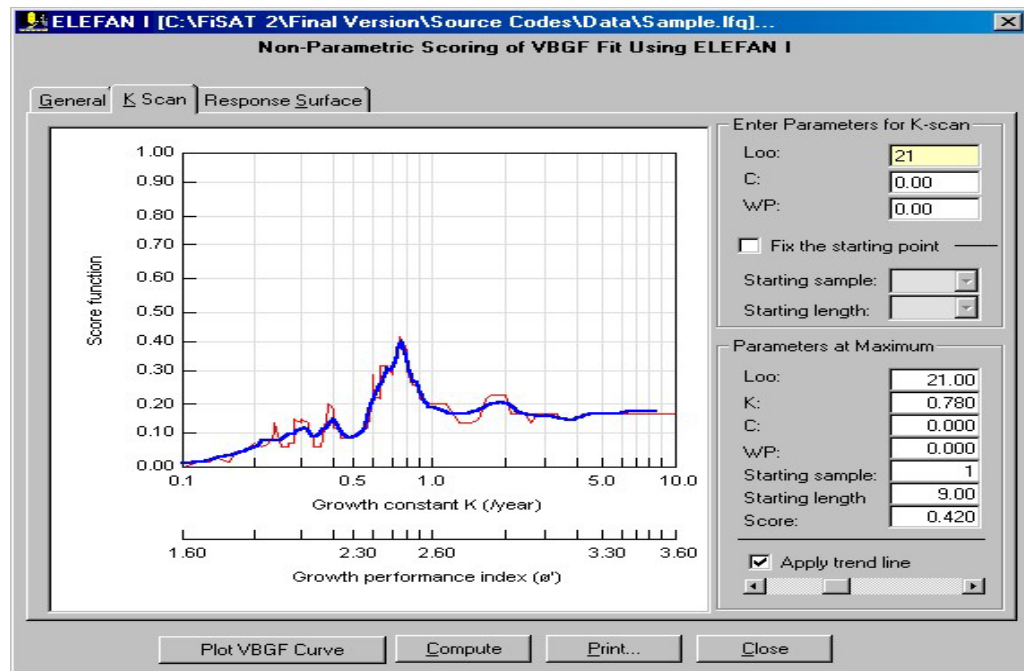


Fig. A-4. Model of ELEFAN I in FiSAT.

Appendix 4. RECRUITMENT

FISAT

Required file Length-frequency data with constant class size.

Input parameters Growth parameters: L_{∞} , K, (C, WP), and t_0 if available.

Functions Backward projection, along a trajectory defined by the VBGF; of the frequencies onto the time axis of a time-series of samples (one single, but well-structured sample may also be used). Two options are provided; use of the length-frequency data and **Option 2**, use of restructured data.

Output Plot showing the seasonal pattern of recruitment.

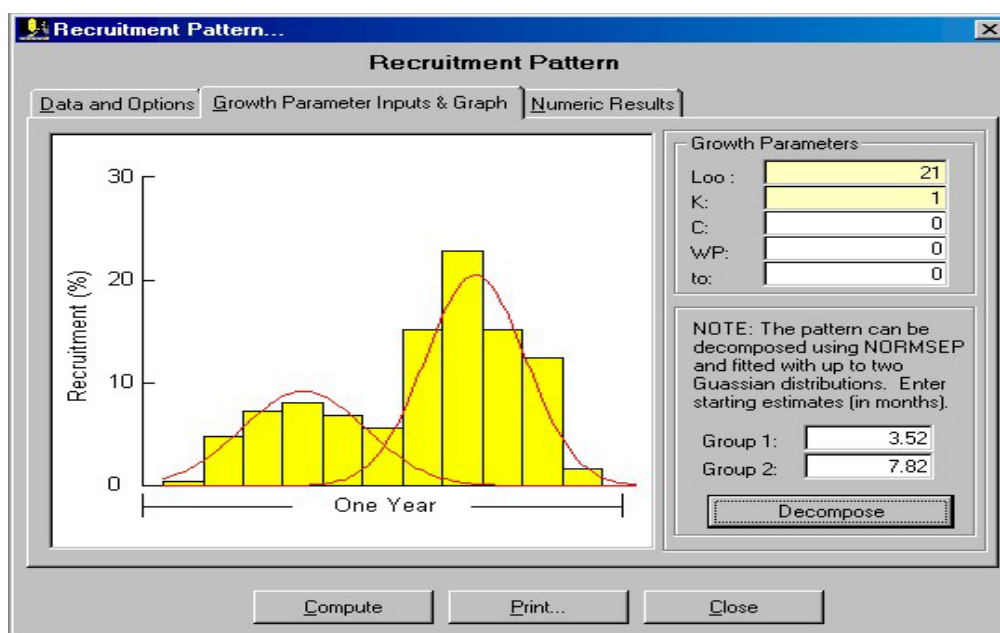


Fig. A-5. Estimate the recruitment pulses in FISAT II.

CURRICULUM VITAE

- Name** : Abraham Seumel Khouw
- Place and Date of Birth** : Haruku, 31 August 1965
- Citizenship** : Indonesian
- Occupation** : Academic staff at the Faculty of Fisheries
Pattimura University, Indonesia
- Present address** : Steenbeker Weg 10 EOH Zim. 05
24106 Kiel
Phone (HP) 0162 – 7387225
- Office address** : Department of Aquatic Resources Management
Faculty of Fisheries
Pattimura University, Ambon, Indonesia
E – mail : askhouw@web.de
- Marital Status** : Married
Costavina Litamahuputty (wife)
Winfield Bierhoff Khouw (son, 5 yrs old)
- Education** :
- 1984 Graduated from High School in Ambon, Indonesia.
- 1988 Graduated from the Faculty of Fisheries, Pattimura University,
Ambon. B.Sc. thesis: The influences of sun height, transparency,
and cloudiness on the light penetration in Maluku Waters.
- 1990 - Education staff member of the Faculty of Fisheries, Pattimura
University (UNPATII), Ambon, with following past activities:
(1) Lecturing and supervising the students, and (2) Research and
cooperation with other Institutions.
- 1995 Master of Philosophy in Tropical Coastal Management from the
University of Newcastle Upon Tyne, England. M.Phil thesis:
Aspects of physical and chemical oceanography with particular
reference to the oceanography of Southeast Asia and regional
denitrification studies along the North East UK Coast.
- 2000 Postgraduate student (Doktorand) at the “Institute für Polar-
ökologie an der Christian-Albrechts-Universität zu Kiel”.

Training

- 1989** Training course on Physical Sciences at Pattimura University, Ambon. The program of the Canadian International Development Agency (CIDA).
- Training course on Thermodynamic in Engineering at Pattimura University, Ambon. The program of the Canadian International Development Agency (CIDA).
- 1990** Training course on Mathematics at Pattimura University, Ambon. The program of the Canadian International Development Agency (CIDA).
- Basic English Language Training Course (BELT) at Pattimura University, Ambon. The program of the Canadian International Development Agency (CIDA).
- 1991** Training course on Computer Programmer at Pattimura University, Ambon. The program of the Canadian International Development Agency (CIDA).
- Course on English for Academic Purposes (EAP) at IALF, Bali. The program of Indonesia-Australia Language Foundation (IALF).
- 1995** Workshop on Statistical Consideration and the Design of Oceanography Surveys and Laboratory/Field Experiments at Pattimura University, Ambon. The program of MSEP.
- Workshop on Collecting Good Data: Protocols for Quality Assessment and Quality Control (QA/QC) at Pattimura University, Ambon. The program of MSEP.
- Training course on Oceanographic Data Management at Japan Oceanographic Data Centre (JODC), Tokyo. The program of WESTPAC, the Intergovernmental Oceanographic Commission.
- 1996** Training course on the use of Meiofauna as biological indicators of Pollution at Dove Marine Laboratory, Newcastle Upon Tyne, UK. The program of Pattimura-Newcastle University Academic Link.
- 1999** DAAD fellowship: "Deutsch" language training course at Goethe Institut Jakarta (ZdAf Certificate).
- 2000** DAAD fellowship: "Deutsch" language training course at Goethe Institute in Bremen (M-2 Certificate).



ERKLÄRUNG

Hiermit erkläre ich, dass die vorliegende Dissertation – abgesehen von der Beratung durch meine akademischen Lehrer – nach Inhalt und Form meine eigene Arbeit ist. Sie wurde keiner anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt. Dies ist mein erster Promotionsversuch.

Kiel, den 3 November 2003

Abraham Seumel Khouw