
THE CONFLICT BETWEEN ADAPTATION AND CONSTRAINT: THE CASE
OF THE SIPHONARIID LIMPETS

Dissertation submitted in fulfilment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY
of
RHODES UNIVERSITY

by

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November, 1994

TABLE OF CONTENTS

PREFACE	i
ACKNOWLEDGEMENTS	ii
DECLARATION	iii
ABSTRACT	iv
1. GENERAL INTRODUCTION	1
1.1 References	5
2. NOTES ON THE TAXONOMY, SPAWN AND LARVAL DEVELOPMENT OF SOUTH AFRICAN SPECIES OF THE INTERTIDAL LIMPET <i>SIPHONARIA</i> (GASTROPODA: PULMONATA)	
2.0 Abstract	7
2.1 Introduction	7
2.2 Material and methods	8
2.3 Taxonomic history of <i>Siphonaria</i> in South Africa	9
2.4 Results	11
2.4.1 <i>Descriptions of species</i>	
2.4.2 <i>Intertidal zonation, spawn and larval development</i>	
2.4.3 <i>Distribution</i>	
2.5 Discussion	19
2.6 References	21
3. A REVIEW OF LARVAL DEVELOPMENT IN THE INTERTIDAL LIMPET <i>SIPHONARIA</i> (GASTROPODA: PULMONATA)	
3.0 Abstract	24
3.1 Introduction	24
3.2 Methods	25
3.3 Results	26
3.3.1 <i>Spawn type and developmental mode</i>	
3.3.2 <i>Egg capsule size</i>	

3.3.3 <i>Intertidal range</i>	
3.3.4 <i>Geographical distribution</i>	
3.3.5 <i>Shell length</i>	
3.3.6 <i>Phylogenetic relationships</i>	
3.4 Discussion	32
3.5 References	35
4. DOES LIFE-HISTORY STRATEGY CORRESPOND TO INTERTIDAL DISTRIBUTION? THE CASE OF THREE SYMPATRIC PULMONATE LIMPETS (GASTROPODA: <i>SIPHONARIA</i>)	
4.0 Abstract	40
4.1 Introduction	42
4.2 Methods	43
4.2.1 <i>Study sites</i>	
4.2.2 <i>Distribution patterns</i>	
4.2.3 <i>Laboratory desiccation experiments</i>	
4.2.4 <i>Field translocation experiments</i>	
4.3 Results	47
4.3.1 <i>Intertidal zonation</i>	
4.3.2 <i>Microhabitat use</i>	
4.3.3 <i>Egg mass desiccation</i>	
4.3.4 <i>Field translocation experiments</i>	
4.4 Discussion	59
4.5 References	64
5. REPRODUCTIVE CYCLES AND ENERGETICS OF TWO SYMPATRIC SPECIES OF <i>SIPHONARIA</i> (GASTROPODA: PULMONATA) WITH DIFFERENT REPRODUCTIVE MODES.	
5.0 Abstract	67
5.1 Introduction	67
5.2 Methods	69
5.2.1 <i>Study site</i>	
5.2.2 <i>Reproductive cycles</i>	
5.2.3 <i>Spawning and recruitment</i>	
5.2.4 <i>Population dynamics</i>	

5.2.5 <i>Fecundity and energetics</i>	
5.3 Results	72
5.3.1 <i>Reproductive cycles</i>	
5.3.2 <i>Spawning and recruitment</i>	
5.3.3 <i>Population dynamics</i>	
5.3.4 <i>Fecundity and energetics</i>	
5.4 Discussion	86
5.5 Results	91
6. DETERMINATION OF GENETIC DIVERSITY OF SOUTHERN AFRICAN INTERTIDAL LIMPETS (GASTROPODA: <i>SIPHONARIA</i>) WITH DIFFERENT REPRODUCTIVE MODES BY POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE) OF TOTAL CELLULAR PROTEINS	
6.0 Abstract	95
6.1 Introduction	95
6.2 Methods	97
6.2.1 <i>Sample collection and preparation</i>	
6.2.2 <i>Electrophoretic analysis of samples</i>	
6.3 Results	98
6.4 Discussion	104
6.5 References	106
7. USE OF DNA FINGERPRINTING (RAPDS) TO DETERMINE THE SYSTEMATIC RELATIONSHIPS AMONG THE INTERTIDAL LIMPETS, <i>SIPHONARIA</i> (PULMONATA: GASTROPODA) WITH IMPLICATIONS FOR THE EVOLUTION OF LARVAL DEVELOPMENT	
7.0 Abstract	109
7.1 Introduction	109
7.2 Methods	111
7.2.1 <i>Sample collection</i>	
7.2.2 <i>DNA extraction</i>	
7.2.3 <i>DNA amplification</i>	
7.2.3 <i>Electrophoresis of samples</i>	
7.3 Results	114
7.4 Discussion	122

7.5 References	124
8. FINAL DISCUSSION	128
8.1 References	132
PUBLICATIONS	

PREFACE

This thesis comprises a series of chapters organised as scientific papers, some of which have been published, others which have been submitted to journals and appear in that format. As a consequence, there is some degree of repetition, particularly in the introductory sections of each chapter, and their respective reference sections.

ACKNOWLEDGEMENTS

My sincere thanks to my supervisor, Christopher McQuaid, for his friendship, support and faith during some difficult times over the last four years.

Numerous others also deserve a mention: Ralph Kirby for his co-supervision and assistance/ loan of a lap-top computer, and the support provided by the staff and students of the Department of Microbiology during this thesis; Tex Reid of Natal for his invaluable information and knowledge of South African *Siphonaria*; David Reid of the British Museum of Natural History for his guidance and many discussions during the first two chapters; Clifford Nxomani and Carlos Bezuidenhout for their patience in teaching me the necessary microbiological skills; David Marshall for assistance during the initiation of this thesis; Terry Butterworth, Neil Cannon & Moira Pogrand for technical assistance; Neil Tarr-Graham for providing a beach cottage during the field research; Anthony Goldstein and the Fish River Sun Hotel for allowing me access to a beautiful research site; Alan Hodgson, Gray Williams and Richard Laubsher for collection of specimens. Many thanks must also go to those who proof-read and commented on sections of this thesis; David Reid, Tex Reid, Dick Kilburn, George Branch, Ric Bernard, and Rehema White.

Financial support, provided by the Foundation for Research and Development, the Joint Research Committee of Rhodes University, and a National Education Research Scholarship, is gratefully acknowledged.

A special thank you to Neil Grange for his comments, friendship and support, and all the other friends I've made in South Africa over the past four years, some of whom accompanied me on field trips, others whose company enabled me to forget about this thesis when I needed to.

Finally, to my parents John and Lynn Chambers. Without their love, encouragement and financial support, this thesis would never have been possible.

DECLARATION

The work described in this thesis was carried out on the Department of Zoology and Entomology and the Department of Microbiology and Biochemistry, Rhodes University under the supervision of Professor Christopher D. McQuaid. These studies represent original work by the author and have not been submitted in any form to another University.

ABSTRACT

The reproductive strategies of marine invertebrates have been related to various aspects of both their ecology, and their phylogenetic history. It has been the purpose of this thesis to try and separate these components among *Siphonaria*, a group of marine pulmonates. The taxonomy of these species is revised and I conclude that nine species are valid. All species deposit benthic egg masses and development may be either direct (*S. anneae*, *S. compressa*, *S. dayi*, *S. nigerrima*, *S. serrata* and *S. tenuicostulata*) or planktonic (*S. capensis*, *S. concinna* and *S. oculus*). Data on distribution and life-history relating to mode of larval development is then presented for 26 species of *Siphonaria* worldwide. Fifteen species are direct developers, nine are planktonic developers and a further two appear to have a dual developmental capacity, retaining both the velar swimming apparatus of a planktonic developer and the crawling foot of a direct developer. Direct developing species hatch from larger egg capsules, and generally occur higher on the shore than planktonic developers. Worldwide, planktonic developers are more widespread than direct developers, and individual planktonic species may have a greater latitudinal range. In most *Siphonaria* subgenera, mode of larval development appears to be constant, although two subgenera (*Patellopsis* and *Sacculosiphonaria*) include both developmental types. Locally, the intertidal zonation of three sympatric species (*S. capensis*, *S. concinna* and *S. serrata*) does not support a model which predicts direct development on the high shore and planktonic development on the low shore. However, distributions do correspond to particular intertidal microhabitats, and while there may be no direct relationship between mode of larval development and intertidal height, the physical structure of egg masses, and the microhabitats used for spawning appear adaptive with regards to desiccation in the intertidal. *S. concinna* (planktonic development) and *S. serrata* (direct development) occur in similar microhabitats and are likely to be under similar

selection pressures. In having different modes of larval development, there appears to be more than one optimal solution in a particular selective regime. In addition, both species seem to apportion similar amounts of energy to reproduction for each spawning episode, and also annually suggesting an optimum allocation of resources to reproduction. Genetic investigations using PolyAcrylamide Gel Electrophoresis (PAGE) confirm the status of the southern African species initially described, and indicate greater genetic variability associated with planktonic developing species than direct developing species. The systematic relationships revealed by DNA fingerprinting support the current classification systems, and also have implications with regards the evolution of larval development: direct development may be the plesiomorphic condition in, and among, some *Siphonaria* groups. There are both phylogenetic and adaptive explanations for the distribution of reproductive mode among benthic marine invertebrates. An evolutionary question, however, is not just a matter of either adaptation or constraint, it is a combination of these. Both contribute to the distribution of developmental mode among *Siphonaria*.

CHAPTER 1

GENERAL INTRODUCTION

"The beauty in nature lies in detail; the message, in generality. Optimal appreciation demands both" wrote Stephen Jay Gould in *Wonderful Life* (1991). Yet in any approach, exploration of this generality requires sufficient detail upon which to base theory. Indeed, Charles Darwin spent over ten years after his famous voyage on the *Beagle* working on the taxonomy of barnacles, a group of marine invertebrates, before finally publishing *The Origin of Species*. This thesis is primarily concerned with adaptation, a concept fundamental to the process of evolution by natural selection. Specifically, the adaptive nature of the life-history strategies of another group of marine invertebrates, the siphonariid limpets. These, like the barnacles, are inhabitants of the rocky intertidal zone on many shores of the world. Before I describe these animals in detail I will return to generality.

In 1966 G.C. Williams considered the central biological problem to be "not survival as such, but design for survival". Evolutionary studies investigate the circumstances which lead to different designs, ecological studies address how such designs are maintained and function in an environment. One of the most fundamental aspects of such design is reproduction and its associated life-history characteristics. S.C. Stearns (1992) identifies four major elements which combine to explain the enormous variation in life-histories demonstrated by related animals. These are (1) Demographic studies; which tell us how selection pressures are distributed across life-history traits, but do not tell us how a population will respond to selection. For this we need (2) Genetic studies; most life-history traits are influenced by many genes of small effect, and quantitative genetics analyses the genetic variation that conditions the response to selection. Studies of physiology and trade-offs (3) have a central role in life-history studies; they evaluate linkages between traits that constrain the simultaneous evolution

of two or more traits (a trade-off exists when a benefit realised through a change in one trait is linked to a cost paid in another). Finally, (4) Linkage specific effects. Taxonomic comparisons tell us how much of a pattern we should attribute to history and design, and how much we should attribute to micro-evolutionary processes that operated within the local population in the recent past.

An adaptionist (Sensu Gould & Lewontin, 1979) would claim that life-history traits are adapted to each other and to local environmental conditions and that optimality models predict the state of traits. This argument rests on the assumption that evolution acts rapidly enough to bring populations into equilibrium with the environmental conditions in which we find them, with sufficient, appropriate, genetic variation available to selection. However, some life-history traits are fixed at higher taxonomic levels, and optimality models must require boundary conditions. Therefore, any evolutionary explanation must have two essential components, those of adaptation and constraint. These will represent two ends of a continuum of biological explanation, although all traits will be a mixture of both (Coddington, 1988; Baum & Larson, 1991; Stearns, 1992; Reeve & Sherman, 1993; Frumhoff & Reeve, 1994).

The siphonariid limpets, more commonly known as the 'false limpets', are common intertidal herbivorous molluscs found on many rocky shores, ranging from the Antarctic circle to tropical coasts (Hubendick, 1947; Berry, 1977; Chambers & McQuaid, 1994). They belong to the pulmonate group of molluscs (which includes the terrestrial slugs and snails) and can be distinguished from patellid limpets by the presence of a shallow furrow on the underside of the shell which leads to a pulmonary chamber. This contains a secondary gill enabling some species to respire both while exposed to the air and submerged in water. Other limpets, for example, the patellid limpets have different evolutionary origins belonging to the more primitive prosobranch group of molluscs. The siphonariid limpets comprise the two genera, *Williamia*, and the far more speciose *Siphonaria* (Hubendick, 1947, 1978; Kilburn & Rippey, 1982). The evolutionary origins and relationships of the Siphonariidae to other molluscan groups are unclear (Hubendick, 1947; Bieler, 1992; Nordsieck, 1992). They had been allied with the Trimusculidae (another family of pulmonate limpets) in the super-family Siphonariacea (Hubendick, 1978, Kilburn & Rippey, 1982), although Haszprunar and Huber (1990) separated the Trimusculidae with some other marine

pulmonates into the new order, the Eupulmonata. However, the allegiance of the Trimusculidae to this new group is questionable (Nordseick, 1990; Ruthensteiner, pers. comm.). In possessing a secondary gill in their air-breathing mantle cavity, the siphonariid limpets are thought to be a group in the process of re-invading the lower intertidal region from either the high-shore or terrestrial habitats (Borland, 1950; Yonge, 1952; Morton, 1955; Solem, 1985). This habitat theoretically brings them into competition with the prosobranch limpets, and emphasises a classic example of parallel evolution in shell form (hence their name, the 'false' limpets) and lends support to adaptionist models proposing optimal designs for a given environment. This thesis concentrates on the reproductive tactics and systematic relationships of the genus *Siphonaria*. Unlike the patellid limpets, which are gonochoristic external fertilisers, the siphonariids are all hermaphroditic internal fertilisers and produce gelatinous egg masses containing encapsulated developing larva. Whilst *Williamia* are reported to have planktonic development (Marshall, 1981; M. Hadfield, pers. comm.), many *Siphonaria* hatch as direct developing larvae passing through the planktonic veliger stage whilst encapsulated (Haven, 1973; Chambers & McQuaid, 1994; pers. obs.). In examining both adaptive and phylogenetic explanations for larval developmental strategy I provide a combined approach to studies of life-history tactics rare in the literature (Baum & Larson, 1991; Stearns, 1992).

Chapter 2, in describing for the first time the full variety of *Siphonaria* species on South African shores (providing new data on the identity, taxonomy and reproductive mode of these species), is an essential prerequisite to the more general ecological and evolutionary considerations which follow. Chapter 3 reviews the available literature containing information on reproduction for *Siphonaria* worldwide, and applies existing adaptive models developed for other marine invertebrates to account for the variation in reproductive mode among *Siphonaria*. Chapter 4 focuses directly on the adaptive nature of development in relation to intertidal distribution and some physiological aspects of life-history stages and egg mass structure. This information on distribution then forms the basis for Chapter 5 which compares a variety of reproductive traits of two synecological congeners with different reproductive modes. The trade-offs between reproductive traits are then considered in the context of optimality theory and adaptive explanations for reproductive mode.

One of the important consequences of planktonic and direct development is that species will have different dispersal capabilities, able to influence population demography and rates of speciation. Chapter 6, using Poly-acrylamide gel electrophoresis (PAGE) of total cellular proteins, investigates levels of genetic heterogeneity among species with different reproductive modes and some information on the higher systematic relationships among South African *Siphonaria* is provided. Chapter 7 takes this attempt further by constructing the phylogenetic relationships of local and some foreign *Siphonaria* using the technique of DNA-fingerprinting. Coupled with software programs which produce nested dendrograms reflecting sequence relationships of the genome, phylogenetic trees constructed from differences in DNA-fingerprints can be postulated and used to consider the evolutionary pathways among the genus.

To conclude, Chapter 8 evaluates both adaptive and phylogenetic explanations of reproductive mode in the genus, considers the general validity of adaptive models of reproductive tactics on the basis of information provided by this thesis, and comments on the evolutionary origins of the marine pulmonates.

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CHAPTER 2

NOTES ON THE TAXONOMY, SPAWN AND LARVAL DEVELOPMENT OF SOUTH AFRICAN SPECIES OF THE INTERTIDAL LIMPET GENUS *SIPHONARIA* (GASTROPODA:PULMONATA).

2.0 ABSTRACT

The taxonomy of South African *Siphonaria* is reviewed. I conclude that nine species are valid. These are: *S. anneae* Tomlin, 1944; *S. capensis* Quoy & Gaimard, 1833; *S. compressa* Allanson, 1959; *S. concinna* Sowerby, 1824; *S. dayi* Allanson, 1959; *S. nigerrima* Smith, 1903; *S. oculus* Krauss, 1848; *S. serrata* (Fischer, 1807) and *S. tenuicostulata* Smith, 1903. Of these, *Siphonaria nigerrima* Smith, 1903, has been incorrectly synonymized with *Siphonaria carbo* Hanley, 1858, which is not present on South African shores. *S. aspera* Krauss, 1848 is reduced to a junior synonym of *S. serrata* (Fischer, 1807). Shell characteristics and mode of larval development are described for these nine South African species. All species deposit benthic egg masses and development may be either planktonic with swimming veliger larvae (*S. capensis*, *S. concinna* and *S. oculus*) or direct, with crawling larvae emerging from the eggs (*S. anneae*, *S. compressa*, *S. dayi*, *S. nigerrima*, *S. serrata* and *S. tenuicostulata*).

2.1 INTRODUCTION

The reproductive strategy employed by a marine invertebrate can be explained by both adaptive and phylogenetic models (Jablonski & Lutz, 1983; Gallardo & Perron, 1982; Grahame & Branch, 1985). This thesis is concerned with examining these alternative hypotheses in the intertidal pulmonate limpet genus *Siphonaria*. Members of this genus are common intertidal herbivores found on rocky shores of tropical and southern latitudes (Yonge, 1952; Hubendick, 1947). All siphonariid limpets are hermaphroditic, internal fertilisers and produce gelatinous egg masses (spawn) which are usually cemented to the substratum, though the egg masses of some species can be pelagic (Creese, 1980; Quinn, 1988).

Mode of larval development has been classified in various ways by different authors (see reviews by Mileikovsky, 1971, 1975; Jablonski & Lutz, 1983; Grahame & Branch, 1985). I distinguish between planktonic development (involving a swimming veliger phase which may be planktotrophic), and non-planktonic, direct development (in which metamorphosed crawling juveniles emerge from the egg mass). Crawling juveniles pass through the veliger stage whilst encapsulated, with the velum regressing prior to hatching (Berry, 1977; Kilburn & Rippey, 1982; Simpson & Harrington, 1985). Kilburn and Rippey (1982) indicate that South African siphonariids demonstrate both modes of larval development, but do not specify which mode each species uses. I review the taxonomic status and distribution of South African *Siphonaria* species and present new data on egg mass morphology, fecundity and mode of larval development for these species.

2.2 MATERIAL AND METHODS

Between 30-40 adults of each of the nine species here recognised were collected from intertidal locations along the South African south, west and east coasts during 1991-1992 and maintained in separate gravel-filter, lighted laboratory aquaria (22°C; 35‰). Shell length was measured along the longest axis. Spawn was collected from laboratory specimens for eight of these species, but none were available for *S. compressa* (information on egg mass form provided by E.T. Reid, pers.comm.). Egg masses produced within three days of capture were removed and cultured in plastic containers at 22°C. Mode of development was determined by observation of hatched larvae from 20 egg masses for each species. Egg capsules are ellipsoid and measurements of mean egg capsule length and breadth, and number (average number of eggs/mm of spawn) were made for each egg mass. Data on distributions beyond South African borders were synthesised from published information.

Voucher shells have been deposited in the Natal Museum, South Africa. Accession numbers are: *anneae*, NMSA V52 (Umhloti), V51 (Umhlanga Rocks); *capensis*, NMSA V46; *compressa*, NMSA V45; *concinna*, NMSA V50; *dayi*, NMSA V47; *nigerrima*, NMSA V49; *oculus*, NMSA V53; *serrata*, NMSA V55 (Waterloo Bay); V56 (Kommetjie), V57 (Umhloti); *tenuicostulata*, NMSA V48.

2.3 TAXONOMIC HISTORY OF *SIPHONARIA* IN SOUTH AFRICA

In 1946 Hubendick published a monograph on the Patelliformia, in which he distinguished eleven 'sectia' of *Siphonaria* (to be regarded as subgenera according to the International Code of Zoological Nomenclature (1985), article 10e) based on the characters of the genitalia and shell. He reported that three of these sub-genera, *Pachysiphonaria*, *Patellopsis* and *Siphonaria*, occur on southern African shores, and comprise thirteen species (Table 1). He made no mention of *S. anneae* Tomlin, 1944. Allanson (1959) further revised the systematic status of South African members of the genus based on shell characteristics and morphology and concluded that only six of Hubendick's species were valid. These were *S. anneae* Tomlin, 1944, *S. aspera* Krauss, 1848, *S. capensis* Quoy & Gaimard, 1833, *S. carbo* Hanley, 1858, *S. deflexa* Helbling in Born, 1780 and *S. oculus* Krauss, 1848. Two new species, *S. dayi* Allanson, 1959 and *S. compressa* Allanson, 1959 and a new variety, *S. aspera* var. *pallida* Allanson, 1959 were also described (Table 1). A further species, *S. tenuicostulata* Smith, 1903, was reduced to a synonym of *S. anneae*. In 1982 Kilburn and Rippey reported seven species present in South Africa, differing from Allanson (1959) in omitting *S. anneae* and *S. dayi*, but including *S. tenuicostulata* (Table 1). In the present study, I differ from Kilburn & Rippey (1982) in the addition of the two species *S. anneae* and *S. dayi*, and in the use of different names for two of the remaining seven species. One of these name changes has been made necessary by the rediscovery of 41 type specimens of molluscs described by Fischer (G. Fischer von Waldheim) in 1807 (Ivanov, Kantor, Sysoev & Egorov, 1993). One of these is a siphonariid named *Patella serrata* (two specimens, ZMUM L-1076, see Ivanov *et al.*, 1993: pl. 2, Figs 3-6,) which corresponds to the species previously known as *S. aspera* Krauss, 1848 (R. Kilburn, G. Branch, pers. comm.). Consequently I consider the name *S. aspera* Krauss to be a junior synonym of the *serrata* (Fischer). Fischer (1807) gave no type locality, but it is likely to be from near the Cape of Good Hope where his collections were made.

The second name change is for the species given by Kilburn & Rippey (1982) as *S. carbo* Hanley, 1858. This name had earlier been used by Hubendick (1946), who gave *S. nigerrima* Smith, 1903 as a synonym based on Smith's (1903) suggestion that his species may have represented juveniles of *S. carbo*.

Table 1. Taxonomic nomenclature applied to South African *Siphonaria*.

Prior to 1946	Hubendick, 1946	Allanson, 1959	Kilbum & Rippey, 1982	This study
<i>Siphonaria concinna</i> Sowerby, 1824	<i>S.(Patellopsis) concinna</i>	<i>S.(Patellopsis) deflexa</i> Helbling in Born, 1780	<i>S.concinna</i>	<i>S.concinna</i>
<i>S.albofasciata</i> Krauss, 1848	<i>S.(Siphonaria) albofasciata</i>	Synonym of <i>S.deflexa</i>	Not discussed	Synonym of <i>S.concinna</i>
<i>S.cyaneomaculata</i> Sowerby, 1906	<i>S.(Siphonaria) cyaneomaculata</i>	Synonym of <i>S.deflexa</i>	Not discussed	Synonym of <i>S.concinna</i>
<i>S.adjacens</i> Turton, 1932	<i>S.(Siphonaria) adjacens</i>	Synonym of <i>S.deflexa</i>	Not discussed	Synonym of <i>S.concinna</i>
<i>S.capensis</i> Quoy & Gaimard, 1833	<i>S.(Patellopsis) capensis</i>	<i>S.(Patellopsis) capensis</i>	<i>S.capensis</i>	<i>S.capensis</i>
<i>S.kowiensis</i> Turton, 1932	<i>S.(Pachysiphonaria) kowiensis</i>	Synonym of <i>S.capensis</i> ?	Not discussed	Not discussed
<i>S.serrata</i> Fischer, 1807	<i>S.(Patellopsis) aspera</i> Krauss, 1848	<i>S.(Siphonaria) aspera</i>	<i>S.aspera</i>	<i>S.serrata</i>
		<i>S.aspera</i> Var. <i>pallida</i> Allanson, 1959	Synonym of <i>S.aspera</i>	Not examined
<i>S.natalensis</i> Krauss, 1848	<i>S.(Patellopsis) natalensis</i>	Synonym of <i>S.aspera</i>	Not discussed	Synonym of <i>S.serrata</i>
<i>S.carbo</i> Hanley, 1858	<i>S.(Patellopsis) carbo</i>	<i>S.(Patellopsis) carbo</i>	<i>S.carbo</i>	Incorrect name (see text)
<i>S.nigerrima</i> Smith, 1903	Synonym: <i>S.carbo</i>	Synonym of <i>S.carbo</i>	Synonym of <i>S.carbo</i>	<i>S.nigerrima</i> (see text)
<i>S.tenuicostulata</i> Smith, 1903	<i>S.(Patellopsis) tenuicostulata</i>	Synonym of <i>S.anneae</i> ?	<i>S.tenuicostulata</i>	<i>S.tenuicostulata</i>
<i>S.anneae</i> Tomlin, 1944	Not discussed	<i>S.(Patellopsis) anneae</i>	Not discussed	<i>S.anneae</i>
<i>S.oculus</i> Krauss, 1848	<i>S.(Patellopsis) oculus</i>	<i>S.(Patellopsis) oculus</i>	<i>S.oculus</i>	<i>S.oculus</i>
<i>S.becki</i> Turton, 1932	<i>S.(Siphonaria) becki</i>	Synonym of <i>S.oculus</i>	Not discussed	Not examined
<i>S.parcicostata</i> Deshayes, 1863	<i>S.(Siphonaria) parvicostata</i>	Records considered doubtful	Not discussed	Not examined
		<i>S.(Sacculosiphonaria) compressa</i> Allanson, 1959	<i>S.compressa</i>	<i>S.compressa</i>
		<i>S.(Patellopsis) dayi</i> Allanson, 1959	Not discussed	<i>S.dayi</i>

The original *S. carbo* was described from the Caribbean (Hanley, 1858). Examination of the holotype (BMHN 19819) reveals a thick, robust shell over 22mm, with a broad, well defined siphon canal, quite unlike *S. nigerrima* as described by Smith (1903) and as collected in the present study. I therefore consider that *S. carbo* does not occur on South African shores, and adopt *S. nigerrima* Smith, 1903 as the correct name for the South African species.

Another nomenclatural problem must be briefly mentioned. Helbling's original description in Born (1780) of *Patella deflexa* is ambiguous; it refers to a siphonariid with a white region dorsal to the muscle scar (as occurs in *S. concinna* Sowerby, 1824), but also mentions the presence of spines on the ribs (as in *S. serrata*). No type of *P. deflexa* has been traced. I therefore consider *deflexa* as an unidentifiable *nomen dubium*, as did Kilburn & Rippey (1982), and use the name *S. concinna* in preference.

2.4 RESULTS

2.4.1 Descriptions of species

Figure 1 (A-N) shows dorsal and ventral views of the shells of nine *Siphonaria* species from South African shores. The characteristics of the shells are listed in Table 2.

2.4.2 Intertidal zonation, spawn and larval development

Table 3 summarises information on the larval development of the nine South African species. Intertidal zonation is defined according to the zonation given by Field & Griffiths (1991) for South African shores: the supra-littoral fringe (high shore), the upper mid-littoral (mid shore) and the lower mid-littoral (low shore). Most species are found in the mid to high shore area and are rare on the lower shore, despite the ability to remain completely submerged for prolonged periods in the laboratory; this may be due to competition with prosobranch limpets on the lower shore (Dower, 1989). *Siphonaria compressa* is thought to be endemic to one locality on the west coast where it occurs only on the sea grass *Zostera capensis* Setchell, on the mid shore. Figure 2A shows a typical planktonic juvenile (*S. concinna*) just prior to hatching; the arrow indicates the bilobed velar apparatus. A typical crawling juvenile of a direct developing species (*S. serrata*) is shown in Figure 2B, also just prior to hatching, and in Figure 2C just after hatching. The arrow indicates the crawling foot. An adjacent planktonic juvenile allows a comparison of the sizes of the two larvae (Figure 2C). Figure 3 gives examples of the three types of egg mass produced by South African siphonariids (Table 3). These are photographed *in situ* and drawn in cross-section. Each egg mass contains strings of ellipsoid egg capsules containing individual larvae distributed evenly throughout the mass.

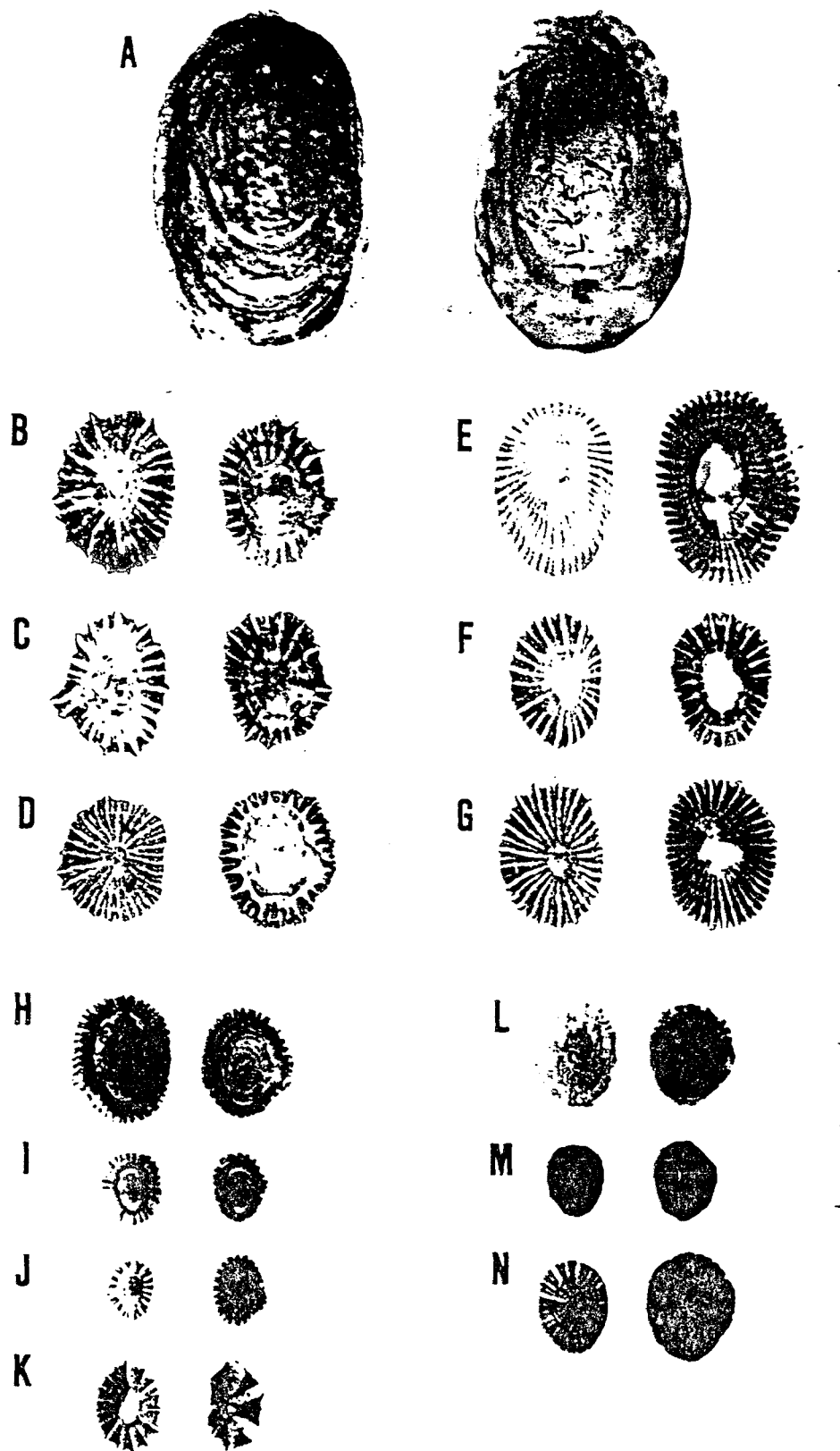


Figure 1. Shells of nine species of South African *Siphonaria*, shown dorsally (left) and ventrally (right) with siphon to the right in ventral view. A: *S.compressa* (Scale bar = 2mm), B: *S.serrata* (South coast), C: *S.serrata* (East coast), D: *S.serrata* (West coast), E: *S.capensis*, F: *S.concinna*, G: *S.oculus*, H: *S.anneae* (large morph), I: *S.anneae* (small morph), J: *S.serrata pallida*, K: Two juvenile forms of *S.concinna*, L: *S.dayi*, M: *S.nigerrima*, N: *S.tenuicostulata*. Scale bar = 2cm (B-G & H-N)

Table 2. Shell characteristics of nine South African *Siphonaria*. Shell length indicates normal size range for adults with maximum shell length in parentheses.

Species	Shell length (mm)	External Colour	Shape and Sculpture	Shell interior	Siphon
<i>S. anneae</i>	12-15 (20)	Pale brown-white, dark bands in between ribs. Paler and eroded at apex and may have a dark ring bordering this region.	30-40 ribs which do not extend to apex, shell low and rounded.	Pale brown-white, dark ventral to muscle line with an even banding of pale stripes corresponding to ribs.	Formed from several fused ribs, slightly raised.
<i>S. serrata</i>	15-25 (40)	Ribs usually pale fawn or grey, darker in between. Algal growth on ribs common.	30-40 ribs, fewer in east coast specimens. Ribs usually visible to a pointed apex. Shell high for west coast specimens, becoming more depressed along south and east coasts. Spines produced from ribs, most common in south coast specimens and hollow in juveniles.	Mottled brown- pale grey, often no obvious muscle scar in south coast specimens. White radiating stripes correspond to ribs.	Distinct, formed from 3-4 fused raised ribs; becoming more prominent in south and particularly east coast specimens.
<i>S. compressa</i>	3-4 (5)	Brown-cream, often radiating stripes of colour from apex.	No distinct ribs; apex left of centre; shell border smooth with growth lines visible.	Smooth, pale translucent.	No groove distinguishable.
<i>S. dayi</i>	15-20 (25)	Pale cream-white; apically eroded, often darker with peripheral brown streaks corresponding to ribs.	40-50 ribs. Low, robust, ovate shell, anterior pointed; apex central. Symmetrical with margin weakly crenulate.	Light brown dorsal to muscle line, white ventrally.	Broad low siphon of 4-5 fused ribs.
<i>S. nigerrima</i>	7-12 (15)	Dark brown-black.	Thin fragile shell with fused ribs, sometimes visible. Growth lines present on uneroded shells, shell border often irregular and eroded	Dark brown with an iridescent golden sheen, adductor muscle line weakly defined.	6-12 fused ribs, canal barely indented.
<i>S. tenuicostulata</i>	10-15 (20)	Variable; four darker coloured segments of shell separated by shades of orange, brown and grey; apex brown-red.	50-60 fine ribs, visible to apex; symmetrical, pyramidal.	Pale brown-white dorsally, darker ventrally but pale at shell margin. Brown stripes correspond to ribs.	Not clearly defined; formed from 4-6 fused, very slightly raised ribs.
<i>S. capensis</i>	15-25 (30)	Broad cream-grey ribs separated by narrow brown channels.	40-50 even ribs; shell low, rounded and often extensively eroded at apex.	Mottled-white dorsal to muscle line, occasionally whole shell interior may be dark brown with a paler apical area. An even banding of brown-grey stripes ventrally corresponds to dorsal ribs.	Only weakly differentiated dorsally.
<i>S. concinna</i>	15-25 (35)	Smooth white oval area apically; ribs pale, darker in between with shorter alternate ribs. Blue flecks visible on some specimens, but more common in juveniles.	30-40 raised ribs, narrow and irregular projecting beyond the shell margin. Shell slightly asymmetric; apex left of centre with left side steep. Edge is crenulate.	Black-dark brown with a distinct white oval area dorsal to adductor muscle corresponding to eroded dorsal apex.	Area depressed, paler canal ventrally.
<i>S. oculus</i>	15-25 (35)	Small white oval area occasionally visible apically; ribs pale brown to white, darker in between. Only alternate ribs reach apex.	30-40 raised, narrow and irregular ribs which extend almost to apex, and project beyond shell margin. Shell steeper on left side, edge is crenulate.	Black-dark brown, darker ventrally, but pale at shell margin. Brown bands correspond to ribs. A narrow white band apically is typical.	Not clearly defined.

The first two types (ribbon and coil) are produced by pelagic developers. Figure 3A is the ribbon produced by *S. capensis*, the only South African species to produce an egg mass of this type (photographed in a high-shore rock pool). This type is flattened, broadly attached to the rock surface and has a high surface area to volume ratio. It is presumably vulnerable to the effects of desiccation if deposited on exposed surfaces. Figure 3B is the coil of *S. concinna*, very similar to the egg mass deposited by *S. oculus*. It is tall and narrow in cross-section, again with a large surface area, though the coils trap water during emersion and so reduce desiccation (unpublished data). Figure 3C shows the third structural type, the collar, which is typical of most direct developing South African siphonariids. In cross section it can be almost square and is of a much firmer consistency than the other two types. Capsules are less densely packed, and a peripheral region without egg capsules near the surface of the egg mass hardens shortly after deposition. Sand and other debris can attach to this surface layer of the egg mass.

Larger capsules (greater than 350µm maximum dimension) are associated with direct development and a longer embryonic period, ranging from 2-4 weeks (Table 3). Locomotion of these crawling juveniles is initially achieved by means of a fleshy foot attached above the operculum (Figure 2C). Planktonic developing species have smaller egg capsules (<300µm) with a correspondingly shorter encapsulated period of 4-6 days (Table 3) and swim using the velum. Growth of the hatched larvae to the normal patelliform shape is from the shell margin in all species.

2.4.3 Distribution

Figure 4 gives the approximate geographic range of the nine siphonariids in southern Africa. The genus is widely distributed with greatest species richness on the east coast (Figures 4B,C). The smallest species, *S. compressa*, occurs only in the sheltered waters of Langebaan lagoon on the west coast (Figure 4A). Elsewhere on the southern west coast only *S. capensis* is found, with isolated populations of *S. serrata* and *S. aspera pallida* (Allanson, 1959) in Langebaan lagoon. *Siphonaria serrata* also occurs further up the west coast from the Orange river northwards (Branch, Eekhout & Bosman, 1991, Figure 4B). Four species; *S. concinna*, *S. oculus*, *S. capensis* and *S. serrata* are ubiquitous along the south and east coasts, extending to the sub-tropical waters of

Durban (Figure 4B), although *S. oculus* is more common in sheltered and estuarine environments (Allanson, 1959; Kilburn and Rippey, 1982). *Siphonaria concinna* and *S. oculus* become rarer east of Durban but isolated populations are found as far east as Kosi Bay (Figure 4C). *Siphonaria anneae*, *S. nigerrima* and *S. tenuicostulata* occur along the sub-tropical Natal and Zululand coasts from Palm Beach to Kosi Bay (Figure 4C), although isolated populations of *S. anneae* and *S. tenuicostulata* can occur as far north as Maputo (R. Kilburn, pers. comm.). *Siphonaria-dayi* extends from Cape Vidal beyond Maputo (Figure 4C).

Table 3: Summary of vertical distribution and developmental characteristics of nine South African *Siphonaria*

Species	Intertidal range	Egg capsule size(µm)	Egg mass form	Fecundity (no. of eggs)	Hatching time (days)	Mode of larval development
<i>S.(Patellopsis) anneae</i>	Mid-high	433 X 307	Collar	350-450	15-20	Direct
<i>S.(Siphonaria) serrata</i>	Mid	484 X 348	Collar	1000-2500	20-28	Direct
<i>S.(Sacculosiphonaria) compressa</i>	Mid	---	Capsule	25-50	---	Direct
<i>S.(Patellopsis) dayi</i>	Mid-high	472 X 349	Collar	400-500	15-20	Direct
<i>S.(Patellopsis) nigerrima</i>	High	440 X 304	Collar	200-300	15-20	Direct
<i>S.(Patellopsis) tenuicostulata</i>	High	474 X 346	Collar	250-350	15-20	Direct
<i>S.(Patellopsis) capensis</i>	Mid-high	200 X 150	Ribbon	20,000	4-5	Planktonic
<i>S.(Patellopsis) concinna</i>	Mid	265 X 198	Coil	>30,000	5-6	Planktonic
<i>S.(Patellopsis) oculus</i>	Mid	259 X 187	Coil	>30,000	5-6	Planktonic

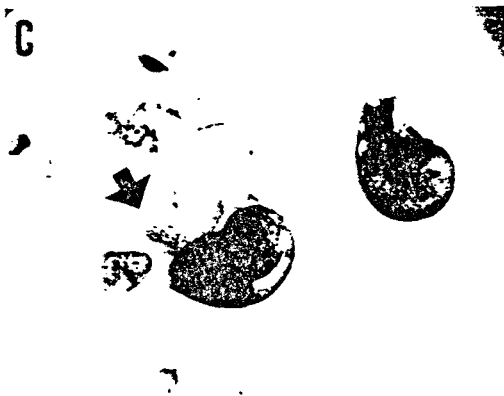
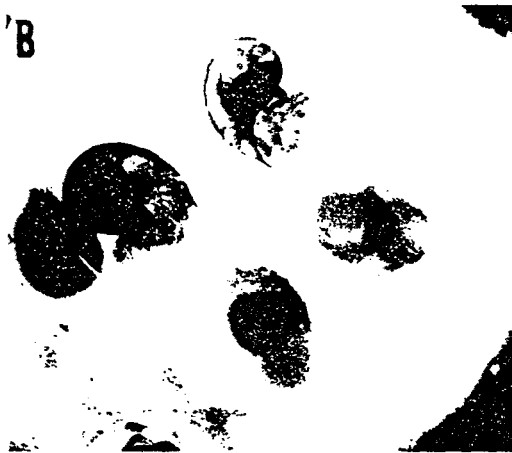
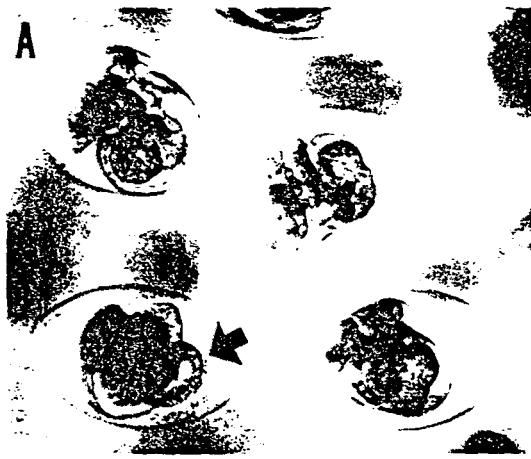


Figure 2. A: Encapsulated planktonic veliger larvae of *S. concinna*, just prior to hatching, arrow indicates velar apparatus. B: Encapsulated non-planktonic (direct developing) juvenile of *S. serrata*. C: Two hatched crawling juvenile of *S. serrata*, with a planktonic veliger of *S. concinna* adjacent, for comparison, arrow indicates foot. Scale bar = 0.25mm.

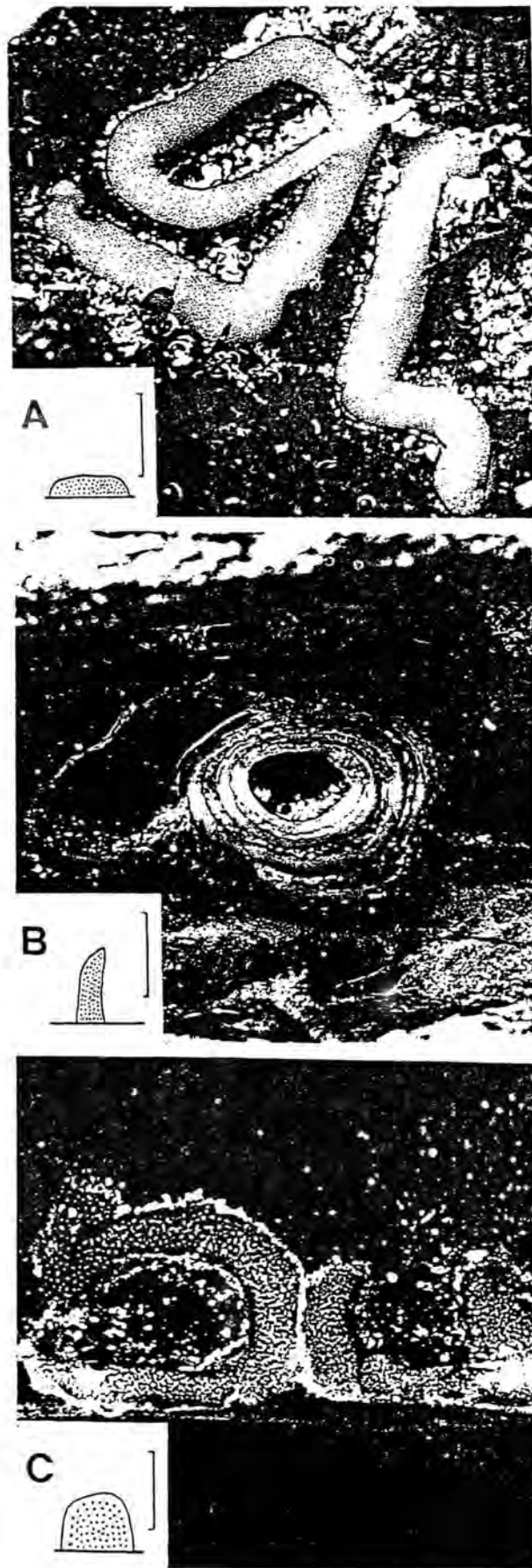


Figure 3. Cross-section and *in situ* view of three types of egg masses produced by South African *Siphonaria*. A: Egg ribbon produced by *S. capensis*, B: Egg coil produced by *S. concinna* (similar to that of *S. oculus*). C: Egg collar produced by *S. anneae* and other South African direct developing species, except *S. compressa* (see text). Scale bar = 5mm.

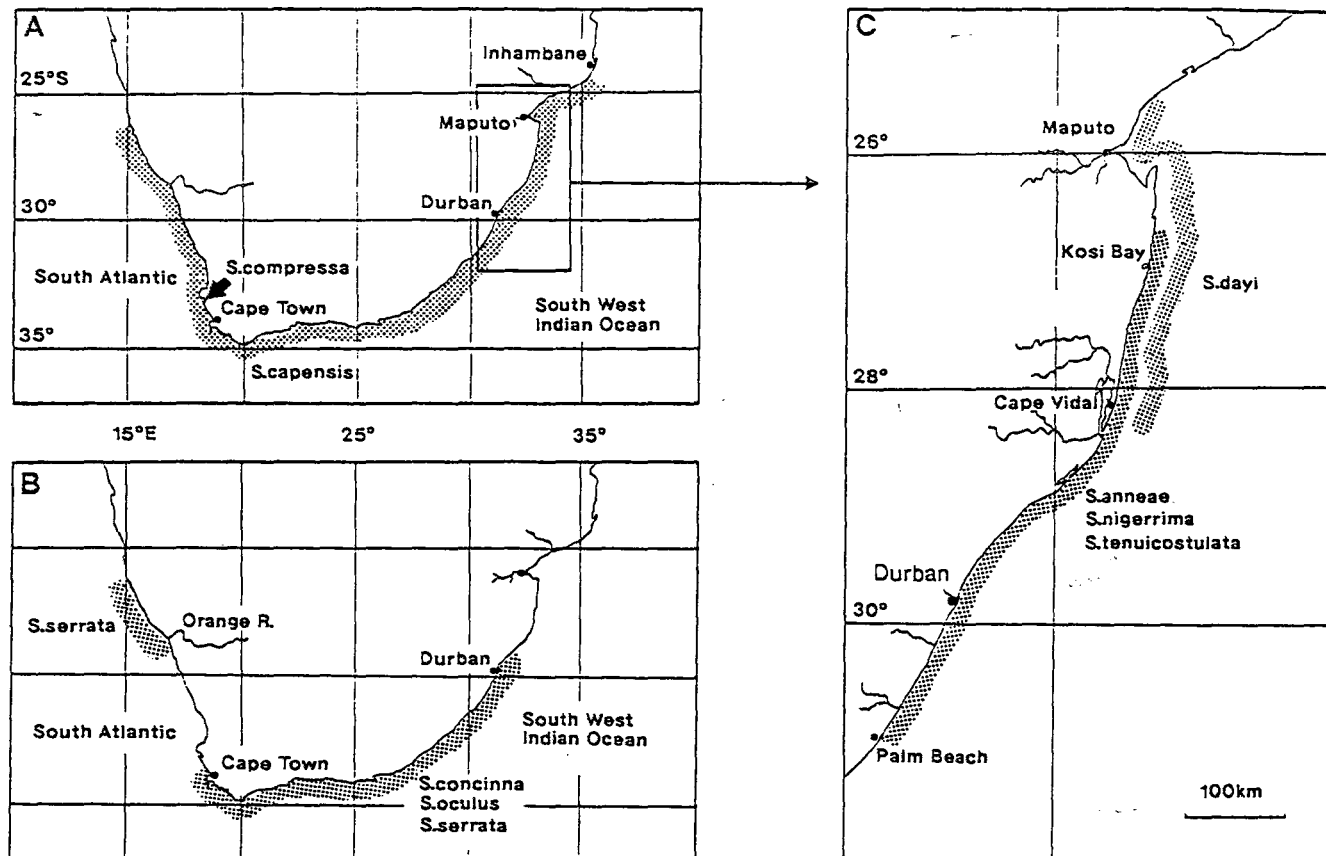


Figure 4. Geographical distribution of South African *Siphonaria*. A: *S. capensis*, arrow indicating Langebaan lagoon where *S. compressa* occurs, B: *S. serrata*, *S. concinna* and *S. oculus*, C: *S. annae*, *S. nigerrima* and *S. tenuicostulata* and *S. dayi*. Based on personal observations, Allanson (1959), E.T. Reid (pers. comm.) and Branch *et al.* (1991).

2.5 DISCUSSION

Many groups of marine organisms show a variety of developmental modes among closely related species. These modes range from the hatching of long-lived planktotrophic larvae, to the hatching of metamorphosed crawling juveniles (see reviews by Thorson, 1950; Mileikovsky, 1975; Underwood, 1979; Jablonski & Lutz, 1983; Grahame & Branch, 1985). Two possible approaches can be taken explain this. Firstly, the adoption of a particular developmental mode has been thought to represent an optimal solution to the ecological circumstances of the animal (Gallardo & Perron, 1982; Strathmann, 1986; Grahame & Branch, 1985; Havenhand, 1991). Mode of larval development may then be correlated with habitat, distribution (both geographical and within shore) and the trade-off between size and number of offspring. The second approach recognises that adaptation is constrained by phylogenetic history: some phylogenetic studies indicate that developmental strategy can have a systematic basis, for example among the littorinid periwinkles (Reid, 1990), so that its adaptive significance must be considered in an historical context and not simply related to the present ecology of the animals.

The phylogenetic relationships of *Siphonaria* remain confused (Jenkins, 1981, 1983). Despite Hubendick's (1946) comprehensive systematic work on the group, their evolutionary origins and their relationships to other groups are still unclear (Yonge, 1952; Morton, 1955; Solem, 1985). However, a systematic review of development based on Hubendick's (1946) and Allanson's (1959) classification suggests that development may be largely clade specific, although the *Patellopsis* and *Sacculosiphonaria* subgenera are exceptional in including both planktonic and direct development species (Chambers & McQuaid, 1994; Table 3).

Among South African *Siphonaria* there are no obvious correlations between egg mass shape and either zonation or geographic distribution, but a strong correlation exists with mode of development. There are two basic types of egg mass, each associated with a particular mode of development. All direct developers (irrespective of subgenus) produce egg masses with a thick outer layer and low surface area to volume ratio (Figure 3C). The egg masses of these species spend a relatively long period on the shore before hatching (up to four weeks). Planktonic developing species produce egg masses which spend a shorter period on the shore (up to one week) and

attachment to the substratum may be broad (Figure 3A), or narrow (Figure 3B). Both have a high surface to volume ratio and this may accelerate the diffusion of respiratory gases, but renders the egg masses vulnerable to desiccation. *Siphonaria capensis* spawn are usually found in intertidal pools where desiccation is not a problem. The spawn of *S. concinna* and *S. oculus* are usually deposited outside pools, but the coiling of the egg mass in conjunction with attachment to the substratum along a narrow border, traps water during low tide and may reduce desiccation.

Within this limited data set there is no clear correlation between developmental mode and habitat, or distribution (intertidal or geographical). Development may be constrained by phylogenetic history (once lost, planktonic larvae are rarely regained; Jablonski & Lutz, 1983; Strathmann, 1986) but the subgenus *Patellopsis* has both developmental types suggesting that a systematic reappraisal may be necessary. The possibility of phylogenetic patterns in development in the genus as a whole will be considered in a future paper.

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CHAPTER 3

A REVIEW OF LARVAL DEVELOPMENT IN THE INTERTIDAL LIMPET GENUS *SIPHONARIA* (GASTROPODA: PULMONATA).

3.0 ABSTRACT

A compilation of distributional and life-history data relating to mode of larval development is presented for 26 species of *Siphonaria*, a genus of intertidal pulmonates. Most species deposit gelatinous egg masses with two species releasing pelagic egg masses. Fifteen species hatch as planktonic-developing larvae, nine hatch as direct developing juveniles and, in a further two, larvae hatch with both the swimming velar apparatus (associated with planktonic development) and a crawling foot (associated with direct development). Data on mode of larval development are interpreted with respect to some adaptive models. Despite important exceptions, there is support for adaptive models based upon egg capsule size (direct developers hatch from larger egg capsules) and intertidal distribution (direct developers generally occur higher on the shore than planktonic developers). Worldwide, planktonic developers are more widespread than direct developing species, and individual planktonic species have a greater mean latitudinal range. The evidence for adaptive models relating latitudinal distribution to developmental mode is equivocal. There appears to be no clear relationship between body size and developmental mode in the genus, although the smallest species has direct development and the largest has planktonic development. In most siphonariid subgenera, developmental mode appears to be constant, but two subgenera contain a mixture of developmental types.

3.1 INTRODUCTION

Siphonariid limpets are unusual in possessing a secondary gill in the mantle cavity (Yonge, 1952, Morton, 1955; Solem, 1985) enabling them to co-occur with other, primitively marine, intertidal molluscs. All are hermaphroditic, internal fertilisers and produce gelatinous egg masses (spawn) which are usually cemented to the substratum. Though the history of the family Siphonariidae is not clear, their ancestors are thought to have been either high shore dwellers, or fully terrestrial species which invaded the rocky intertidal (Borland, 1950; Yonge, 1952; Morton, 1955; Solem, 1985). Many

siphonariids have a planktonic larval stage in their life history, while others produce direct-developing juveniles following intracapsular metamorphosis (Berry, 1977; Kilburn & Rippey, 1982; Simpson & Harrington, 1985; Chambers & McQuaid, 1994). Among intertidal invertebrates the adoption of particular developmental strategies has been thought to represent optimal solutions to various ecological circumstances of the animal (Gallardo & Perron, 1982; Strathmann, 1986; Havenhand, 1991), and may be considered adaptive (Grahame & Branch, 1985). Adaptive models have been proposed based on intertidal zonation (Woodward, 1909; Knox, 1955; Mileikovsky, 1975), latitudinal distribution (Thorson, 1950; Spight, 1977; Clark & Goetzfried, 1978; Perron & Kohn, 1985) and body size (Chia, 1974; Giesel, 1976; Underwood, 1979; Gallardo & Perron, 1982). These models have been developed for many molluscan groups, such as nudibranchs (Todd, 1991; Havenhand, 1991), *Conus* (Perron & Kohn, 1985), Muricacea (Spight, 1976, 1977) and littorinids (Mileikovsky, 1975; but see Reid, 1990). Recently, there has been a call to phrase adaptive questions in the context of a phylogenetic hypothesis (Coddington, 1988; Baum & Larson, 1991), for example, the cladistic work of Reid (1990) on littorinids.

Mode of larval development in *Siphonaria*, a group widely distributed in tropical and southern latitudes (Hubendick, 1947), has received little attention. I review the available published literature on distribution, larval development and systematics for *Siphonaria* in order to produce a data set against which adaptive models relating to developmental mode can be tested.

3.2 METHODS

Authors generally have not distinguished between planktotrophic and lecithotrophic larval development, and I therefore classify development mode as either planktonic development (with a swimming veliger phase, which may or may not be planktotrophic), or non-planktonic, direct development (in which metamorphosed crawling juveniles emerge from the egg mass).

Data on geographical distribution, intertidal range, egg capsule dimensions (length and breadth of an ellipsoid) and mode of larval development (either planktonic or direct) have been compiled from the reviewed literature. The information on shell length has been derived from two sources: the original text of reviewed papers and, where shell

length was not specified, estimates made from plates supplied in Hubendick's (1946) systematic work on the Siphonariidae.

The relationship between intertidal zonation and mode of larval development was tested by scoring each developmental type (direct or planktonic) against two intertidal categories, either low to mid shore (including all species on the mid shore and below), and high shore (including all the mid-high and high shore species). The two species which release pelagic egg masses, those which release swimming-crawling larvae, and *Siphonaria obliquata* which occurs throughout the shore (Table 1) were omitted from the analysis leaving 21 species. These results were then compared with expected frequencies using a 2x2 Chi-squared contingency table. Geographical range was quantified as the number of degrees of latitude occupied by each species, and mean latitudinal range of developmental types was compared using a Student's t-test.

3.3 RESULTS

3.3.1 Spawn type and developmental mode

Of the 26 species reviewed, 24 deposit benthic gelatinous egg masses on the shore, whereas two (*Siphonaria tasmanica* and *S. virgulata*; Creese, 1980; Quinn, 1988) release pelagic spawn masses (Table 1). Fifteen species produce planktonic-developing larvae and nine are direct developers hatching as crawling juveniles. Two species, *Siphonaria hispida* and *Siphonaria alternata* appear intermediate, producing 'swimming-crawling' larvae (Table 1). On hatching, these species possess both the velar swimming apparatus of a planktonic developer and the crawling foot of a direct developer (Marcus & Marcus, 1960; Zischke, 1974). In other direct-developing species the velum regresses prior to hatching.

3.3.2 Egg capsule size

Species with a capsule diameter greater than 300µm show direct development, while below 300µm most larvae hatch as planktonic veligers (Table 1). *Siphonaria alternata* is the only exception, its swimming-crawling larvae hatching from an egg capsule 150µm in diameter (Zischke, 1974). The egg capsules produced by *Siphonaria lateralis* Gould and *S. stewartiana* Powell are exceptionally large compared to other direct-

developing species and this is correlated with a considerably lower fecundity (Simpson & Harrington, 1985).

3.3.3 Intertidal range

There appears to be a broad correlation between developmental mode and intertidal distribution but it is imperfect. A Chi-squared test indicates that mode of larval development is non-randomly distributed on the shore ($P < 0.05$). Most direct-developing species are found on the high shore with planktonic species more common on the mid and low shore. Exceptions do exist, for example the southern African direct developer, *S. serrata* which co-occurs on the mid shore, with the planktonic-developing *S. concinna* (Chambers & McQuaid, 1994). There are three high shore planktonic-developing species. Two, *S. tasmanica* and *S. virgulata*, both release pelagic egg masses (Table 1). A third, *S. capensis*, is found primarily in high shore pools where its egg masses are deposited (see Chapter 4). *Siphonaria obliquata* is reported to occur throughout the shore.

Table 1. Developmental characteristics for 26 species of *Siphonaria*. Either average, or typical range of adult shell length is given (* measurements derived from published figure, ** indicates the release of pelagic egg masses).

Species	Shell length (mm)	Location	Intertidal range	Egg capsule dimensions (µm)	Larval development	Source
<i>Siphonaria (Siphonaria) alternata</i> Say	15	Florida Keys, U.S.A	Mid	150 x ?	Swimming/crawling	Zischke (1974)
<i>S.(Patellopsis) anneae</i> Tomlin	12-15	South Africa	Mid-high	433 x 307	Direct	Chambers & McQuaid (1994)
<i>S.(Sacculosiphonaria) compressa</i> Allanson	2-4	South Africa	Mid	---	Direct	Chambers & McQuaid (1994)
<i>S.(Patellopsis) dayi</i> Allanson	15-20	South Africa	Mid-high	472 x 349	Direct	Chambers & McQuaid (1994)
<i>S.(Siphonaria) hispida</i> Smith	22.5	Brazil	Mid	500 x 300*	Swimming/crawling	Marcus & Marcus (1960)
<i>S.(Siphonaria) kurracheensis</i> Reeve	20	Persian Gulf	High	500 x 380	Direct	Thorson (1940)
<i>S.(Kerguelenella) lateralis</i> (Gould)	20	Macquarie Island, Sub-Antarctic	High	1350 x 2000	Direct	Simpson & Harrington (1985)
<i>S.(Patellopsis) nigerrima</i> Smith	7-15	South Africa	High	440 x 304	Direct	Chambers & McQuaid (1994)
<i>S.(Siphonaria) serrata</i> (Fischer)	15-25	South Africa	Mid	484 x 307	Direct	Chambers & McQuaid (1994)
<i>S.(Kerguelenella) stewartiana</i> (Powell)	15	Stewart Island, Sub-Antarctic	Supra-littoral	1260 x ?	Direct	Knox (1955)
<i>S.(Patellopsis) tenuicostulata</i> Smith	10-15	South Africa	High	474 x 346	Direct	Chambers & McQuaid (1994)
<i>S.(Siphonaria) atra</i> Quoy & Gaimard	20	Palao	Mid	190 x 160	Planktonic	Abe (1941)
<i>S.(Ductosiphonaria) baconi</i> Reeve	15	Victoria, Australia	Mid	206 x 156	Planktonic	Mapstone (1978)
<i>S.(Patellopsis) capensis</i> Quoy & Gaimard	15-25	South Africa	Mid-high	200 x 150	Planktonic	Chambers & McQuaid (1994)
<i>S.(Patellopsis) concinna</i> Sowerby	15-20	South Africa	Mid	265 x 198	Planktonic	Chambers & McQuaid (1994)
<i>S.(?) denticulata</i> Quoy & Gaimard	--	New South Wales, Australia	Low-mid	200 x 160	Planktonic	Creese (1980)
<i>S.(Ductosiphonaria) diemenensis</i> Quoy & Gaimard	25	Victoria, Australia	Mid-high	159 x 125	Planktonic	Mapstone (1978)
<i>S.(Heterosiphonaria) gigas</i> Sowerby	70-80	Panama, Pacific coast	Mid	---	Planktonic	Levings & Garrity (1986)
<i>S.(Sacculosiphonaria) japonica</i> Donovan	20	Northern Japan	Mid	273 x 154	Planktonic	Abe (1940)
<i>S.(Pachysiphonaria) lessoni</i> Gould	22	Buenos Aires, Argentina	---	---	Planktonic	Berry (1977)
<i>S.(Benhamina) obliquata</i> (Sowerby)	25	New Zealand	---	---	Planktonic	Boland (1950); Knox (1955)
<i>S.(Patellopsis) oculus</i> Krauss	15-20	South Africa	Mid	259 x 187	Planktonic	Chambers & McQuaid (1994)
<i>S.(Patellopsis) pectinata</i> Linnaeus	26	Florida Keys, U.S.A.	Mid	190 x 150	Planktonic	Voss (1959); Zischke (1974)
<i>S.(?) sipho</i> Sowerby	25	Persian Gulf	Low-mid	250 x 170	Planktonic	Thorson (1950)
<i>S.(Pachysiphonaria) tasmanica</i> Tenison Woods	12	Victoria, Australia	High	159 x 130	Planktonic**	Quinn (1988)
<i>S.(Pachysiphonaria) virgulata</i> Hedley	22.5	New South Wales, Australia	High	200 x 160	Planktonic**	Creese (1980)

3.3.4 Geographical distribution

Most of the species considered in this review are found in the mid latitudes of the southern hemisphere, particularly between 25-35° south. Three occur in tropical waters and five in the northern hemisphere (Figure 1). Two species, both members of the subgenus *Kerguelenella* (*S. lateralis* and *S. stewartiana*), occupy the highest latitudes and inhabit oceanic islands in sub-antarctic waters (Figure 1). Both have direct development, but since this may have particular adaptive significance on oceanic islands (see Discussion), they are omitted from the following analyses. Considering the remaining southern hemisphere data, direct-developing species occur over a narrower range of latitudes (20-35°S) than their planktonic-developing counterparts (8-45°S). Considering the distribution of all species worldwide, direct-developers occur over a substantially smaller range of latitudes (34°S-30°N) than planktonic developers (48°S-45°N), and are absent from tropical waters (Figure 1). Likewise, a Student's t-test comparing the mean number of degrees of latitude occupied by the species of each developmental type also indicates a significantly larger mean for planktonic species ($P < 0.05$).

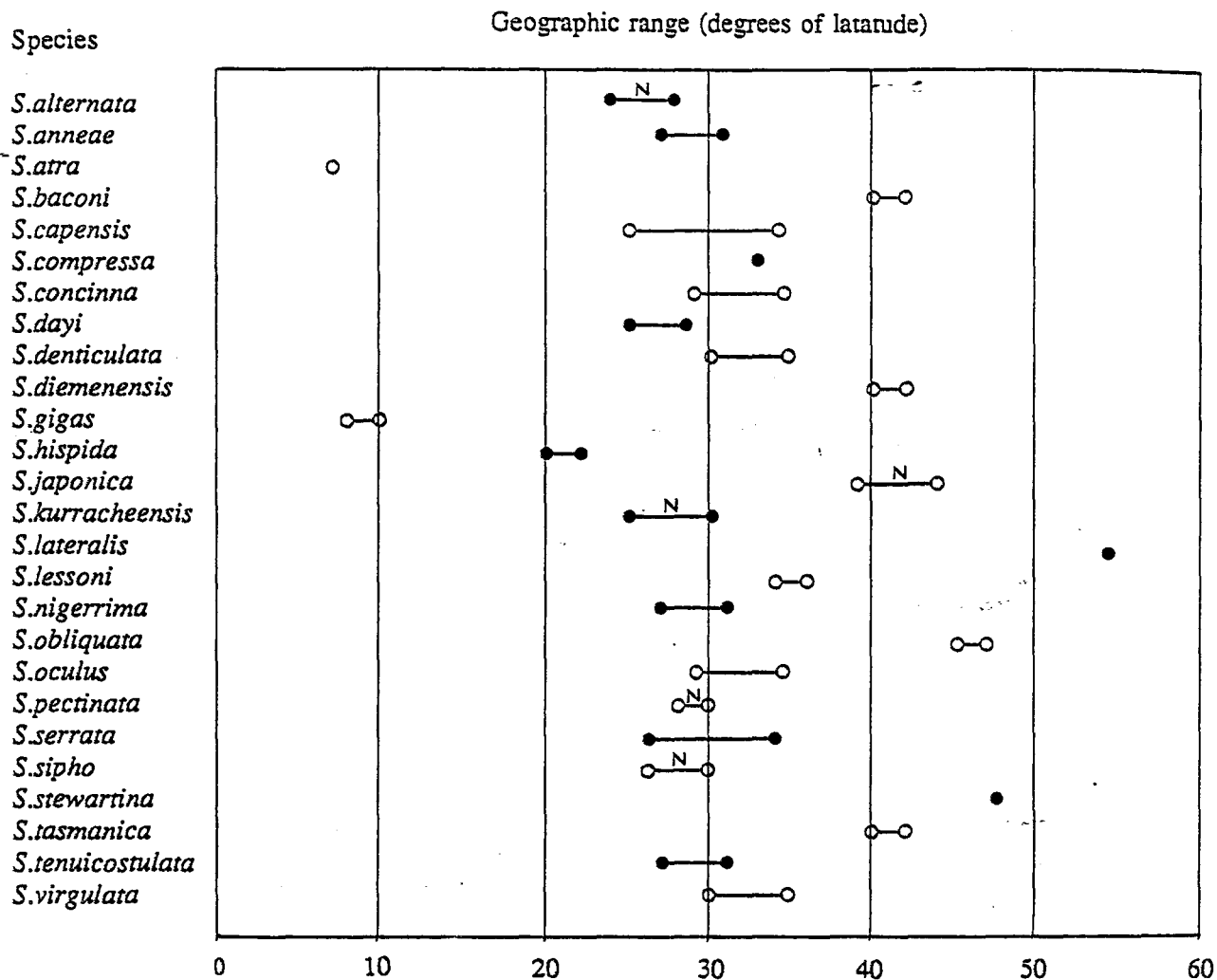


Figure 1. Worldwide latitudinal ranges for 26 species of *Siphonaria* (degrees of latitude). Open circles indicate planktonic development, closed circles indicate direct development (and include species with a dual developmental capacity). Northern hemisphere species are indicated by 'N'.

3.3.5 Shell length

Siphonaria range in body size from the largest, the tropical *S. gigas* (planktonic development), growing up to 80mm (Levings & Garrity, 1986) to the smallest species, *S. compressa* (direct development) which has a maximum length of 4mm and is found exclusively in a southern African lagoon (Allanson, 1959; Kilburn & Rippey, 1982). The remainder are generally in the size range of 10-20mm, although some can grow larger (Table 1).

Using the mean value for shell length to represent body size, and omitting from the analysis the two species with swimming-crawling larvae, a Student's t-test shows that body size for planktonic species is just significantly larger than for direct-developing species (one-tailed test; $P < 0.05$). However, if the two extremes of body size are not included (*S. gigas* and *S. compressa*) there is no significant difference ($P > 0.05$).

3.3.6 Phylogenetic relationships

Hubendick (1946, 1947) separated the genus into two groups, *Siphonaria* and *Liriola*, and considered direct development species to be primitive within the genus. However, phylogenetic relationships among the ten subgenera (Table 1) have yet to be elucidated. Developmental information is available for eight of these subgenera. Mode of larval development appears to have some systematic basis (Table 2), although the subgenera *Patellopsis* and *Sacculosiphonaria* contain species with both direct and planktonic development (Chambers & McQuaid, 1994).

Table 2. Distribution of developmental mode among 8 subgenera of *Siphonaria*. (Subgeneric assignment of *S. sipho* unknown and this species is therefore omitted.)

Subgenus	Number of direct developing species	Number of planktonic developing species
<i>Benhamina</i>	0	1
<i>Ductosiphonaria</i>	0	2
<i>Heterosiphonaria</i>	0	1
<i>Kerguelenella</i>	2	0
<i>Pachysiphonaria</i>	0	3
<i>Patellopsis</i>	4	3
<i>Sacculosiphonaria</i>	1	1
<i>Siphonaria</i>	2	0

3.4 DISCUSSION

The adaptive nature of larval development has been reviewed in some other groups of marine molluscs (Mileikovsky, 1975; Spight, 1977; Todd, 1991; Hickman, 1992), but there has been little discussion of larval development in *Siphonaria*. The present compilation of developmental information allows speculation on the applicability to *Siphonaria* of several adaptive hypotheses.

Among other groups of intertidal molluscs larger egg capsule size generally corresponds to a non-planktonic mode of larval development (Spight, 1976; Perron & Kohn, 1985; Todd, 1991; Hickman, 1992). This has lent support to the proposal that there is a trade-off between mode of development and fecundity. Planktonic development permits greater fecundity, but with a correspondingly smaller egg capsule size (Vance, 1973; Christiansen & Fenchel, 1979; Palmer & Strathmann, 1981). Among South African *Siphonaria*, direct developers are consistently less fecund than their planktonic congeners (Chambers & McQuaid, 1994). Within the genus as a whole, direct developers all hatch from capsules larger than 300µm, with the single exception of *S. alternata*. This size is similar to the size threshold of egg capsules reported in the Littorinidae (Simpson & Harrington, 1985).

Developmental mode has also been related to intertidal zonation, for example among the littorinid periwinkles (Woodward, 1909; Mileikovsky, 1971, 1975). Knox (1955) proposed a similar relationship for *Siphonaria*, suggesting a trend towards direct development on the high shore and planktonic development on the low shore. While obligate relationships between zonation and developmental mode are unlikely (Mileikovsky, 1975), there is some support for Knox's (1955) scheme (Table 1). The Chi-squared test indicated non-random association of mode of larval development with vertical zonation, direct developers generally occurring higher on the shore. This is unexpected as desiccation stress is greater upshore and the egg masses of direct developers have a longer developmental period. As a result, egg masses of these species remain on the shore longer (3-4 weeks) than those of planktonic developers (1 week; Chambers & McQuaid, 1994). Direct-developing species may reduce desiccation of egg masses by a tough protective layer around the egg mass (Chambers & McQuaid, 1994). The larger capsule size associated with direct development will further reduce desiccation stress on embryos due to a lower surface to volume ratio

(Strathmann & Chaffee, 1984). The only high shore planktonic developers avoid desiccation by the release of pelagic egg masses (*S. tasmanica*, *S. virgulata*; Table 1 & Creese, 1980), or by depositing egg masses in high shore pools (*S. capensis*; Chapter 3). Among the species reviewed, there is no indication of seasonal vertical migration associated with reproduction so it is assumed that egg masses are deposited in the intertidal zone inhabited by adults.

A review of geographical range of *Siphonaria* species suggests a greater latitudinal range for individual planktonic species (and for planktonic species as a group). Likewise, planktonic-developing *Conus* species have greater geographical range than direct developers (Perron & Kohn, 1985). This contradicts recent models proposing a greater geographic for direct developing species (Johannesson, 1988; O'Foighil, 1989). However, latitudinal ranges given by authors may in some cases be underestimated.

The evidence for models correlating developmental mode with latitude is equivocal. Perhaps the best known adaptive model of developmental strategy is 'Thorson's rule' (Thorson, 1950), which proposes that the incidence of direct development is at a maximum at high latitudes and a minimum at low latitudes. Spight's (1977) data on the Muricidae supported this, although between the latitudes of 25-30° hatching type can be mixed. A contrary hypothesis has been proposed by Clark & Goetzfried (1978), citing evidence from nudibranchs. They argue that there is greater climatic stability in tropical latitudes, and link this to more stable food supplies, which reduce the need for the wide dispersal associated with planktonic development. So a trend towards direct development in tropical waters is postulated. In *Siphonaria* there is support for Clark & Goetzfried's (1978) hypothesis between 20-45°S. Apart from the sub-antarctic *S. lateralis* and *S. stewartiana* on oceanic islands, no direct developers are reported lower than 35°S. The direct development of the subgenus *Kerguelenella* may have adaptive significance in relation to the problem of planktonic larvae being swept away from isolated islands in ocean currents and failing to maintain the adult population (Christiansen & Fenchel, 1979; Underwood, 1979). Yet worldwide, there is support for Thorson's Rule; the only tropical species are planktonic and the sub-antarctic species are direct developers.

Underwood (1979) has suggested a model whereby a lower size threshold exists in prosobranchs below which the adult cannot channel sufficient energy into reproduction to produce the necessary numbers of pelagic larvae to ensure adequate recruitment. Above this size threshold any strategy may be viable. Body size within the genus *Siphonaria* is generally similar and only the two extremes, the direct developer *S. compressa* and the planktonic *S. gigas*, conform to Underwood's (1979) scheme. If such a scheme is applicable, the threshold size necessitating direct development is probably below the average size of most species (10-20cm).

Different modes of development have not only ecological, but also evolutionary significance as they may influence rates of extinction and speciation (Jablonski & Lutz, 1983). The models discussed above all assume that developmental mode is free to evolve under particular selective conditions. However, this is not necessarily so, and the possibility of phylogenetic constraint must be considered (Coddington, 1988; Reid, 1990). Phylogenetic relationships within the Siphonariidae remain confused (Jenkins, 1981; 1983), but most of the subgenera reviewed exhibit a single developmental mode, although more information is needed to confirm this. If direct development is indeed the primitive condition then we must consider the surprising scenario of planktonic larvae evolving from direct-developing ancestors in *Siphonaria*. In conclusion, more developmental information is certainly required on this group. The phylogenetic relationships within *Siphonaria*, and between this genus and other marine pulmonates, must be ascertained before adaptive models can be considered in a phylogenetic context.

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CHAPTER 4

DOES LIFE-HISTORY STRATEGY CORRESPOND TO INTERTIDAL DISTRIBUTION? THE CASE OF THREE SYMPATRIC PULMONATE LIMPETS (GASTROPODA:*SIPHONARIA*).

4.0 ABSTRACT

Three sympatric species of the intertidal pulmonate limpet genus *Siphonaria* are ubiquitous on the south coast of South Africa. All three lay gelatinous benthic egg masses. *Siphonaria capensis* Quoy & Gaimard and *S. concinna* Sowerby hatch as planktonic larvae. *S. serrata* (Fischer) has direct developing larvae which hatch as crawling juveniles. Intertidal zonation of these species does not support a model which predicts direct development on the high shore and planktonic development on the low shore. On the mid to high shore, *S. concinna* and *S. serrata* reach maximum densities and often co-exist in the same microhabitats. *S. capensis* is more abundant higher on the shore where it is found largely in intertidal pools. Both *S. concinna* and *S. serrata* adults are usually found on bare rock, but deposit egg masses in other microhabitats, particularly in areas where desiccation is reduced. *S. concinna* spawns on bare rock, in pools, crevices and shallow depressions (<10mm deep) which hold water temporarily and usually dry out during low tide. *S. serrata* spawns on patches of foliose algal turf, in crevices and in intertidal pools. *S. capensis* spawns in the high shore pools inhabited by adults. Laboratory desiccation and field translocation experiments indicate that spawning in microhabitats where desiccation is reduced during low tide enhances embryonic survival during development. This is attributed to differences in the physical structure of egg masses and egg capsule size, resulting in trade-offs between the diffusion of respiratory gases and the problems of desiccation. The relationship between mode of larval developmental and intertidal zonation is complex; while there may be no direct relationship between mode of larval development and intertidal height, the physical structure of egg masses and the microhabitats used for spawning appear adaptive with regards to desiccation in the intertidal. In the absence of vertical migration in order to spawn, reproductively successful populations can occur only in zones with microhabitats suitable for egg mass survival.

4.1 INTRODUCTION

Many marine invertebrates hatch as planktonic larvae, while others hatch as fully metamorphosed crawling juveniles. Perhaps the earliest adaptive model proposed to explain the occurrence of different developmental types within groups of intertidal molluscs was a correlation between intertidal height and reproductive mode. Woodward (1909) suggested that, among the littorinid periwinkles, species with direct developing larvae occurred higher on the shore than planktonic developing species. A similar relationship has been proposed for *Siphonaria* (Thorson, 1940; Knox, 1955; Morrison, 1963), a genus of intertidal pulmonate limpets widely distributed on tropical and southern hemisphere shores (Hubendick, 1947; Berry, 1977).

More recently, an obligate relationship between zonation and reproductive mode has been questioned for the littorinid periwinkles (Mileikovsky, 1975) and other adaptive models have been proposed to account for the distribution of larval developmental type within groups of intertidal molluscs (see reviews by Jablonski & Lutz, 1983; Grahame & Branch, 1985; Strathmann, 1986). In a review of larval development among *Siphonaria* (Chambers & McQuaid, 1994^a) the vertical zonation and mode of development was examined for 26 species worldwide. All the siphonariids hatch from gelatinous egg masses, usually deposited in the intertidal (Berry, 1977; Chambers & McQuaid, 1994^a, but see Creese, 1980; Quinn, 1988). Mode of reproduction is non-randomly distributed on the shore, direct development being more common on the high shore and planktonic development on the mid and low shore. Such a relationship is unexpected. Benthic egg masses usually maximise gaseous exchange by a high ratio of surface area to volume (Strathmann & Chaffee, 1984; Chaffee & Strathmann, 1984) and are presumably more susceptible to the effects of desiccation higher on the shore. Furthermore, direct developing egg masses remain longer on the shore than their planktonic counterparts due to a prolonged embryonic period and are therefore exposed to greater desiccation stresses. Successful hatching may then depend on structural adaptations of egg masses which enable them to reduce desiccation during benthic development (Chambers & McQuaid, 1994^b).

Two South African *Siphonaria*, *Siphonaria capensis* and *S. serrata* are exceptions to the correlation between mode of larval development and zonation. *S. capensis* a planktonic developer living on the high shore, often in pools. *S. serrata* (previously

S. aspera: see Chambers & McQuaid, 1994^b) is a direct developer living on fairly exposed rocks from high-water springs to low-water neap tide levels. A further species, another planktonic developer, *S. concinna* Sowerby, 1824, has a similar intertidal to that of *S. serrata* and the three species commonly occur together (Kilburn & Rippey, 1982; Chambers & McQuaid, 1994^b).

Of the nine species of *Siphonaria* reported from South African shores, four (*Siphonaria serrata*, *S. capensis*, *S. concinna* and *S. oculus* Krauss) are ubiquitous along the south coast, although *S. oculus* is more common in estuarine environments (Allanson, 1958, 1959; Kilburn & Rippey, 1982). All reach a similar adult size (shell length usually 15-25cm) and produce gelatinous egg masses attached to the substratum. *S. concinna* and *S. capensis* juveniles hatch as planktonic larvae after approximately one week of encapsulation, from either an egg ribbon (*S. capensis*, Figure 3A, Chapter 2) or a loosely coiled egg mass (*S. concinna*; Figure 3B, Chapter 2). *S. serrata* juveniles develop within the egg capsule for 2-4 weeks and hatch with a crawling foot from a gelatinous egg collar (Figure 3C, Chapter 2) after intracapsular metamorphosis (Chambers & McQuaid, 1994^b).

Sympatric species are likely to be under similar selection pressures and if mode of larval development is closely correlated to intertidal height, then exceptions to expected trends merit investigation. To this end I examine the intertidal distribution and microhabitat use of these three sympatric species of *Siphonaria* on two shores on the south coast of South Africa. I then explore the possible adaptive nature of egg mass structure and egg mass deposition sites by desiccation experiments in the laboratory, and by translocation experiments in the field.

4.2 METHODS

4.2.1 Study sites

The study was conducted at two sites approximately 70km apart on the South African south-east coast. The first, Waterloo Bay (33°29'S, 27°9'E), is an evenly sloping sandstone platform showing clear vertical zonation. The second, Cannon Rocks (33°45'S, 26°33'E), which is steeper, consists of large, irregular sandstone boulders (approximately 3m in diameter) separated by sand. Populations of limpets are found on these boulders and appear to be prevented from migrating between boulders by

areas of sand. Vertical distribution of species at Waterloo Bay is described in terms of four intertidal zones: low shore, mid shore, high shore and littorinid zone (or supralittoral fringe). For Cannon Rocks, where the low shore boulders were submerged in sand, the terms mid shore, high shore, and littorinid zone are employed. Both sites are subject to a simple semi-diurnal tidal regime with a spring tide amplitude between 2 and 2.5m, and a neap tide range of about 1m. Low water neap tides occur in the afternoon between 14.00 and 16.00 and at night between 02.00 and 04.00 hours.

4.2.2 Distribution patterns

Densities of adults, juveniles (defined as being less than 5mm in length) and egg masses were estimated from data collected from 50 X 50cm quadrats thrown in different intertidal zones along two vertical transects. At Waterloo Bay, 20, 50, 50 and 40 quadrats were thrown on the low shore, mid shore, high shore and littorinid zone respectively. At Cannon Rocks, 20, 30 and 20 quadrats were thrown on the mid shore, high shore and the littorinid zone respectively. The microhabitats of individual limpets and egg masses in each quadrat were categorised as follows: 1. Bare rock (no macro-algal cover, but with occasional barnacles); 2. Crevices; 3. Encrusting coralline algae; 4. Algae (foliose algal turfs); 5. Pools with foliose algae; 6. Pools with encrusting coralline algae; 7. Sessile animals (sponges, mussels, barnacles and tube building polychaetes). For *Siphonaria concinna* an additional microhabitat, Rock concavities, was distinguished for the analysis of egg mass deposition. These are shallow depressions (no more than 1-2cm deep, 10-15cm² in area) on the bare rock in which egg masses are frequently observed (pers. obs.). They initially hold water during emersion, but contain no algae and usually dry out during low tide. Mean microhabitat availability (estimated visually as the percentage of total area in each quadrat) and total numbers of adults, juveniles and egg masses for each species in each microhabitat were recorded from each quadrat.

Densities of adults, juveniles and egg masses of each species were then transformed to $\log(x+1)$ and percent covers of algae were transformed by angular transformation to meet the assumptions of normality and homogeneity of variance. An $F_{\max}$ test (Sokal & Rolf, 1981) of the transformed data indicated that the assumption of homoscedasticity was acceptable. This was necessary since the same quadrat was

sampled many times and consequently observations on habitat availability and limpet distribution from a single quadrat were not fully independent (see Ortega, 1987). Microhabitat usage was examined by determining the percentage frequency of each life-history stage of each species in the various microhabitats identified for each intertidal zone.

4.2.3 Laboratory desiccation experiments

To examine the effects of desiccation on survival of developing embryos within egg masses, fresh egg masses of similar size were collected for each species from the shore during neap low tide and maintained in the laboratory for 2-3 hours before experimentation. Each was checked for mortality (live embryos rotate within their egg capsule by beating their velar apparatus), and discarded unless all embryos appeared alive. These egg masses were then subjected to a variety of laboratory desiccation treatments. These treatments involved petri dishes filled with fresh sea water to simulate submerged egg masses and act as a control. Petri dishes without water simulated exposure on bare rock. To investigate if the coiling of *Siphonaria concinna* egg masses reduced desiccation stress, a further treatment tested this hypothesis by uncoiling individual egg masses using a fine scalpel before desiccation. This involved no disturbance of embryos and a control for this treatment, other than a comparison with the coiled egg cases, was considered unnecessary. Field observations indicated that egg masses of *S. serrata* were commonly found on areas of algal turf where desiccation is likely to be reduced. To test this hypothesis an additional treatment for *S. serrata* involved desiccation of egg masses on fresh mats of algal turf (height 5mm, area 40mm²) removed from the shore. For all treatments during desiccation, harsh field conditions were simulated by constant light, high temperature (35°C) and low humidity (45%), with an electric fan (height; 40cm) providing wind. For each treatment, six groups of 20 egg masses of each species were placed in plastic petri dishes initially totalling 180 egg masses per species for each treatment. Egg masses were desiccated for 4 hours to correspond to maximum emersion periods likely to be experienced on the mid-shore. Mean weight loss was determined by weighing individual egg masses initially from a particular group of 20 and then subsequently at 40 minute intervals, totalling seven data points. From each of the 5 laboratory treatments a group of 20 egg

masses of each species was removed at 40 minute intervals and these were assessed for mortality. This was achieved by placing these egg masses in a ventilated sea water aquarium at 22°C for a two hour recovery period, and then examined under a dissecting microscope. Inactive veligers were considered to be dead. Mortality was calculated as the percentage of the group of 20 egg masses which demonstrated widespread (>50%) mortality of embryos within the each egg mass.

4.2.4 Field translocation experiments

To examine the level of hatching success of egg masses in different microhabitats, freshly deposited egg masses of each species were collected following the onset of a neap tide cycle when spawning is greatest for the two planktonic species (unpublished data). During this collection it was clear that egg masses of each species were typically found in particular intertidal microhabitats, these microhabitats were later used as the controls against which data for translocated egg masses were compared. Egg masses were removed from the substratum with a fine scalpel (for *S. capensis* these were usually accompanied with a layer of encrusting algae which minimised disturbance and aided gluing), dried in tissue paper, and groups of 20 egg masses were glued into various microhabitats in the mid shore area using clear silicon adhesive. In each case, one treatment in which egg masses were replaced in the microhabitat they had been most frequently found in the field, acted as a control for each species.

Treatments for *Siphonaria capensis* egg masses were similar to those simulated in the laboratory; pools and bare rock, the pool treatment acting as a control. These pools were drained and then refilled with sea water after the egg masses were glued in position. *S. concinna* egg masses were placed in the microhabitats of bare rock and concavities (coiled and uncoiled groups) and in pools (coiled) comprising five treatments, with the coiled egg masses in concavities acting as a control. *S. serrata* egg masses were placed in four microhabitats; pools, bare rock, concavities, with the control being algal turf in this case. As egg masses could not be stuck onto algal turf, small areas (slightly larger than the egg masses) were cleared within turfs down to the bare rock. The exposed rock was then dried and *S. serrata* egg masses glued in place. To determine level of hatching success, egg masses (or their remains) were later removed from these treatment sites and placed in sea water. They were returned to the

laboratory and mortality and successful hatching were assessed. This was easily determined as egg masses began to fragment as hatching began, and unhatched, dead embryos remained in the egg mass. Thus, absence of embryos in collected egg masses was considered to be evidence of successful hatching.

Egg masses of *Siphonaria capensis* were removed after 5 days, those of *S. concinna* and *S. serrata* after 6 days. For the planktonic developing species this corresponded to the onset of hatching (Chambers & McQuaid, 1994^b). For the direct developing *S. serrata*, removal was much earlier during benthic development, and prior to metamorphosis. Egg mass mortality was evaluated in conjunction with hatching success, and estimated using the method of the laboratory experiments.

4.3 RESULTS

4.3.1 Intertidal zonation

At both sites adults of *Siphonaria concinna* and *S. serrata* were common on the mid and high shore but either rare or absent on the lower shore at Waterloo Bay and in the littorinid zone on both shores (Figure 1). *S. capensis* was also common on the high shore at both locations but extended further upshore into the littorinid zone at Cannon Rocks and was rare or absent on the mid shore at both sites (Figure 1). For each species of limpet, juveniles and egg masses generally co-occurred with adults, but were rare or absent in zones where there were few adults (Figure 1).

There was no obvious relationship between intertidal zonation and mode of larval development. The direct developing *Siphonaria serrata* and the planktonic developing *S. concinna* were sympatric in most zones at each site, and individuals of the two species were often found adjacent to one another in the same microhabitat. The only species common in the littorinid zone was *S. capensis* (a planktonic developer), particularly at Cannon Rocks where it was only found in coralline pools.

4.3.2 Microhabitat use

The two shores showed similar levels of microhabitat heterogeneity with a reduction in habitat diversity up the shore. Macroalgal cover (both in and out of intertidal pools) reduced upshore, while the cover of bare rock and of intertidal pools containing encrusting coralline algae increased (Figure 2). Since the quadrat data were not

collected independently, statistical analyses on microhabitat useage were not possible. However, microhabitat use in each zone on both shores was expressed as percentage of individuals of each species in that habitat is shown in Figures 3-5 (SE of data omitted to avoid confusion). For each species, the distribution of different life-history stages was associated with specific intertidal microhabitats with an increasing range of microhabitats utilised upshore by both *Siphonaria concinna* and *S. serrata*.

Siphonaria capensis adults, juveniles and egg masses were almost exclusively found in association with encrusting coralline algae, usually in encrusting coralline pools (Figure 3), a microhabitat rare on the lower shore (Figure 2). The only exception was on the high shore at Cannon Rocks where water draining from farther upshore kept an area of exposed encrusting coralline algae damp and many juveniles were found there. All egg masses were found on encrusting algae or in coralline pools with the adult *S. capensis*.

Both *Siphonaria concinna* and *S. serrata* adults were abundant on areas of exposed bare rock and, for each species, adults and recruits were often found in similar microhabitats (bare rock, crevices and crustose pools) within the same intertidal zones (Figures 4 & 5). For *S. concinna*, adults and juveniles occurred in the same microhabitat. This was also true for *S. serrata* adults and juveniles on the high shore at Waterloo Bay and the mid shore at Cannon Rocks. However, in the remaining two zones there were microhabitat differences with *S. serrata* recruits tending to occur more often in crevices and crustose pools than with the adults on bare rock.

While egg masses and adults of *Siphonaria capensis* were found in the same microhabitat, there were clear differences between spawning sites and adult microhabitats for both *S. concinna* and *S. serrata* in most cases. *S. concinna* egg masses were deposited in microhabitats not usually occupied by adults, particularly in rock concavities, which were often close to the adult bare rock microhabitat. Bare rock, pools and crevices were also utilised to a lesser extent (Figure 4). Although some egg masses of *S. serrata* were also found in microhabitats occupied by adults, algal turf and intertidal pools (both foliose and coralline) were the primary spawning sites and egg masses were rarely found on bare rock (Figure 5).

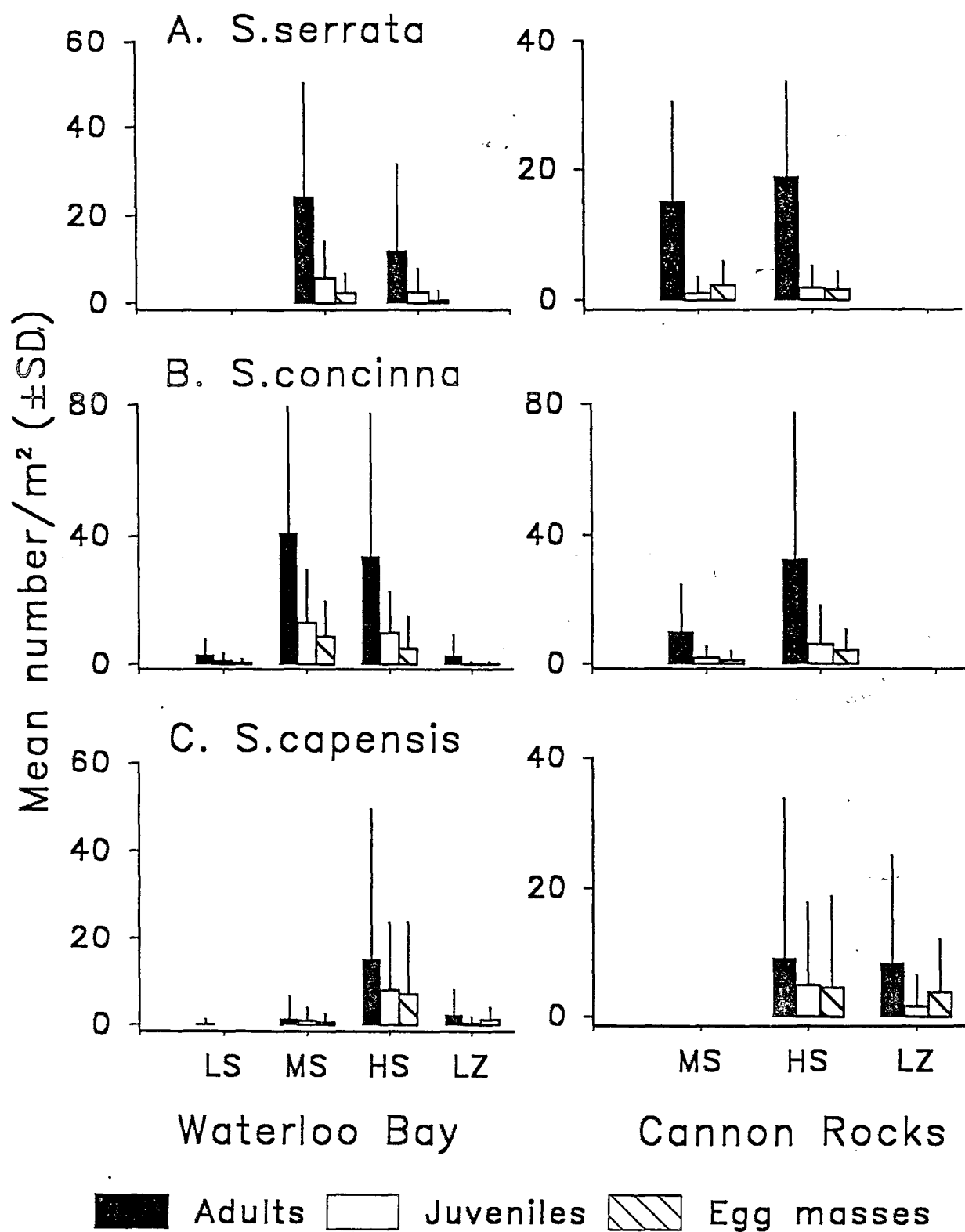


Figure 1. Intertidal densities for A: *S. serrata*, B: *S. concinna*, C: *S. capensis*. LS = Lower Shore, MS = Middle Shore, HS = Upper Shore, LZ = Littorinid Zone.

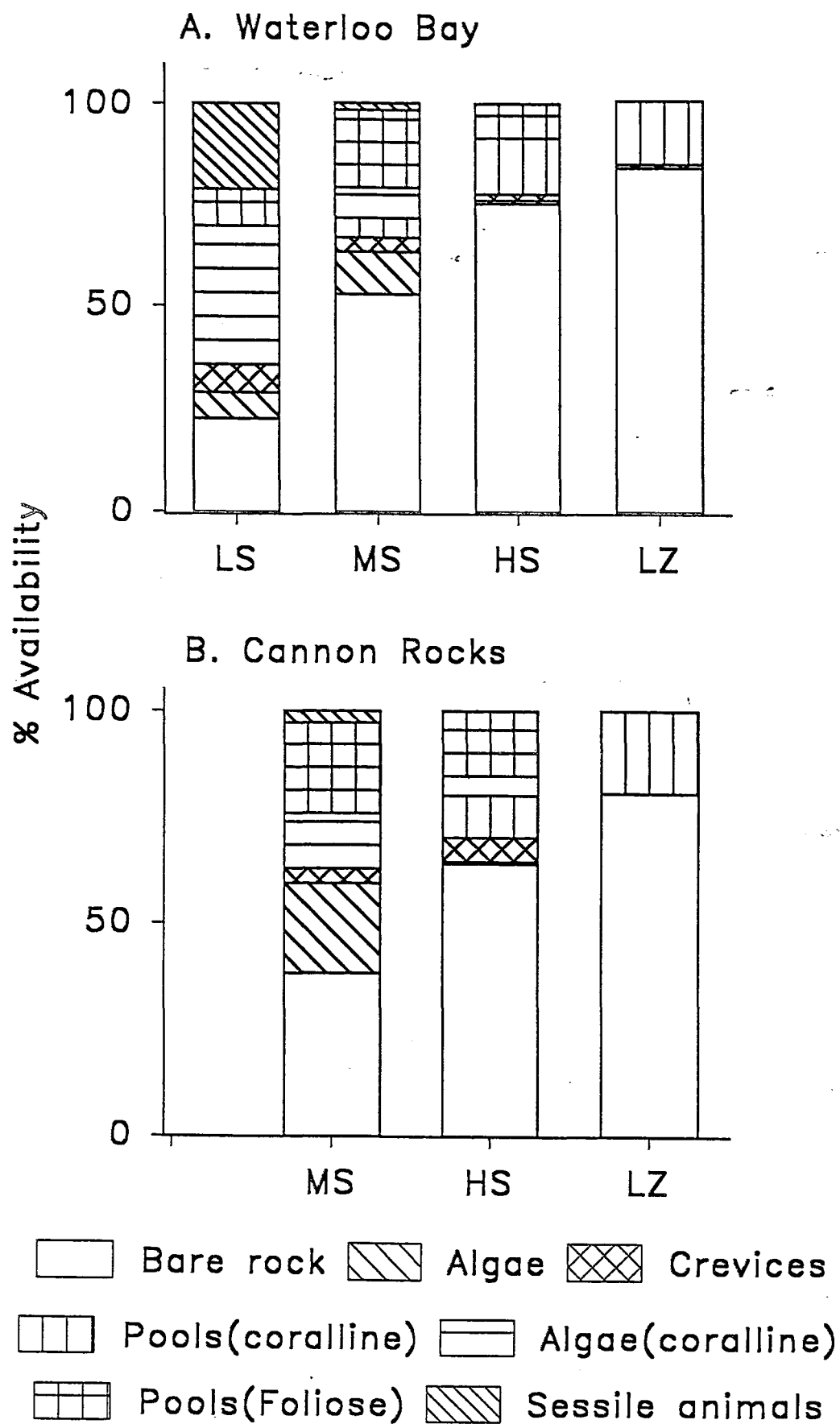


Figure 2. Intertidal microhabitat availability at each site. (Zones abbreviated as in Fig. 1.)

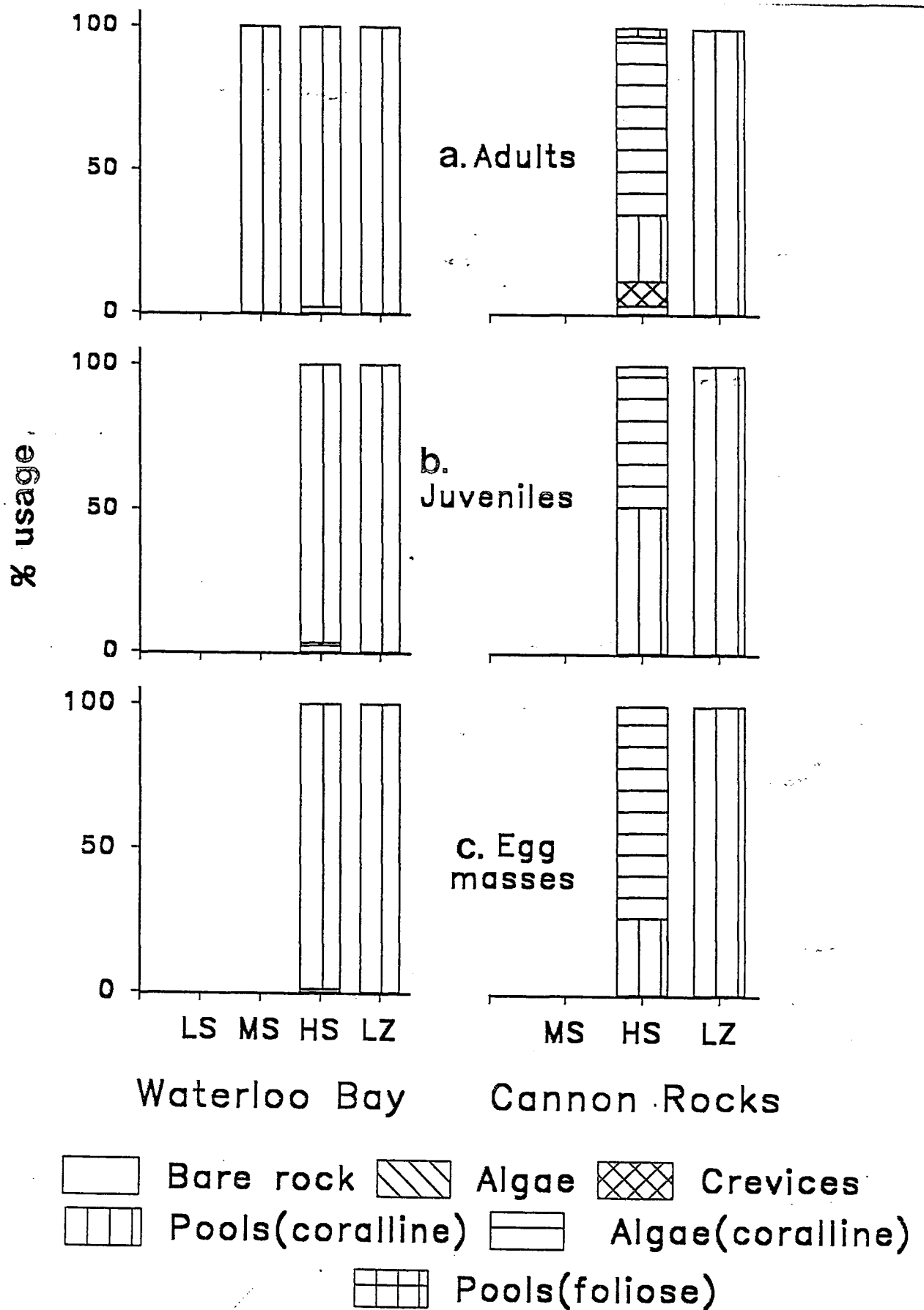


Figure 3. % microhabitat usage in each zone for (a) *S. capensis* adults, (b) juveniles and (c) egg masses. Densities less than 2/m² not shown.

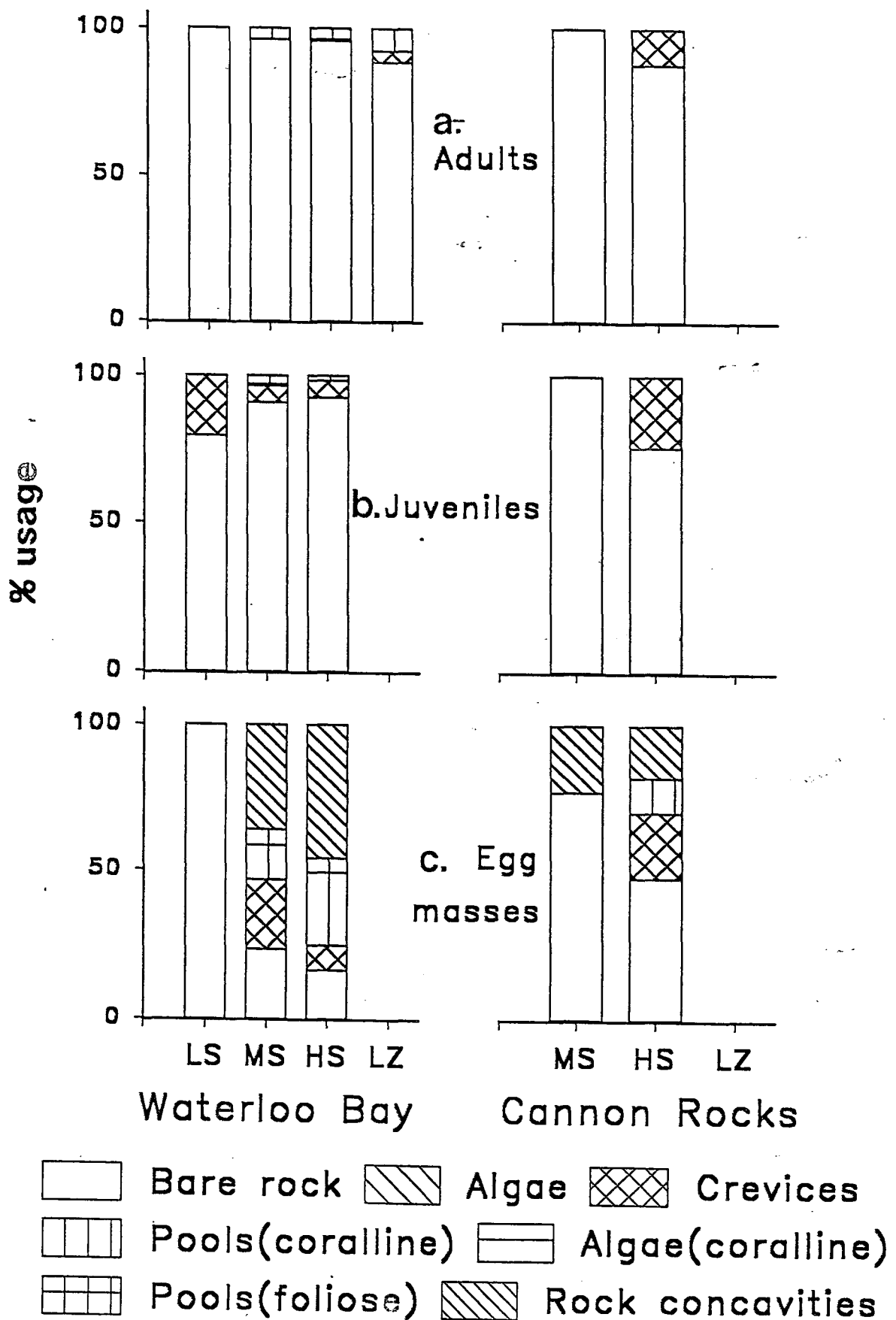


Figure 4. % microhabitat usage in each zone for (a) *S. concinna* adults, (b) juveniles and (c) egg masses. Densities less than 2/m² not shown.

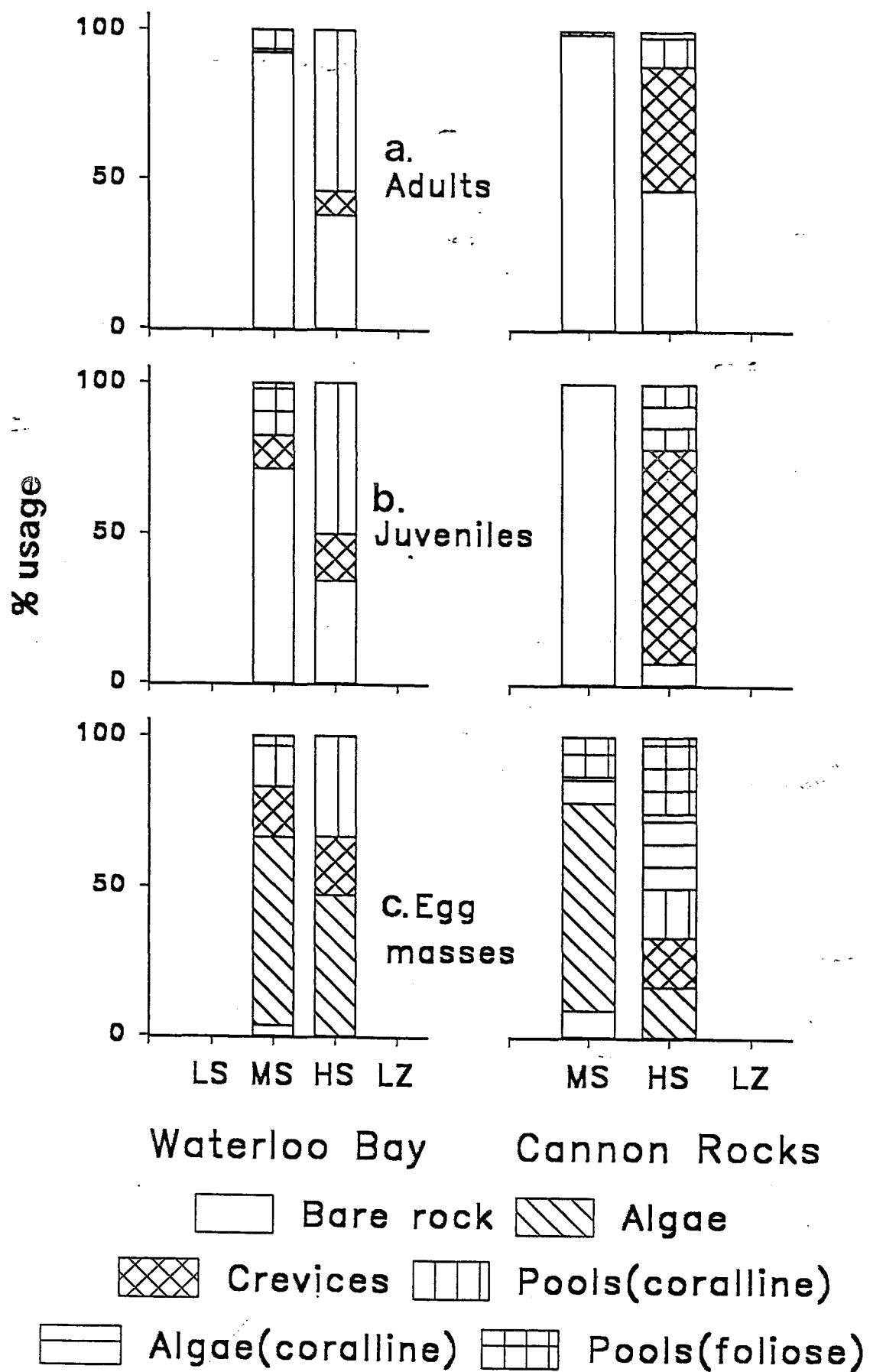


Figure 5. % microhabitat usage in each zone for (a) *S. serrata* adults, (b) juveniles and (c) egg masses. Densities less than 2/m² not shown.

4.3.3 Egg mass desiccation

The results of desiccation experiments (Figures 6-8) were used to estimate values of the time to 50% weight loss of the egg mass and LT_{50} (time to 50% of egg masses showing mortality >50%) given in Table 1.

Table 1. Results of laboratory desiccation experiments. $T_{50}WL$ = time to 50% weight loss (min), LT_{50} = time to 50% embryo mortality (min). For each data point, N = 20 egg masses.

Species	Treatment	$T_{50}WL$	LT_{50}
<i>S. capensis</i>	In sea water (1)	>240	>240
	In petri dish (2)	77 (± 13.5)	113
<i>S. concinna</i>	In sea water (1)	>240	>240
	Coiled in petri dish (2)	152 (± 26.2)	>240
	Uncoiled in petri dish (3)	74 (± 11.6)	125
<i>S. serrata</i>	In sea water (1)	>240	>240
	In petri dish (2)	142 (± 16.6)	>240
	On algal turf (4)	>240	>240

When permanently submerged, all three species experienced no weight loss or mortality over the experimental period. However, there were considerable differences between species when desiccated to simulate exposure on bare rock. The egg masses of *Siphonaria capensis* rapidly lost water; 50% weight loss was reached in just over 1.25 hours and LT_{50} followed 36 minutes later. Exposure for a period of 4 hours resulted in 100% mortality (Figure 6). In comparison, the egg masses of *S. serrata* and *S. concinna* experienced lower rates of water loss (Students t-test; $P < 0.001$ in each case) and demonstrated greater tolerance to desiccation (Figures 7A & 8A). Both reaching 50% water loss after approximately the same period (140-150min, Students t-test; $P > 0.05$; Table 5) and only *S. concinna* egg masses showed any mortality (less than 20%; Figures 7A & 8A) at this time. Neither species' egg masses reached LT_{50} during the experiment, although *S. concinna* egg masses experienced mortality earlier than those of *S. serrata*. Uncoiled, the egg masses of *S. concinna* behaved much like

those of *S. capensis*, both reaching 50% water loss and LT_{50} over similar time periods (Students t-test; $P < 0.05$; Table 1). Likewise, the rate of water loss of desiccated *Siphonaria serrata* egg masses was very similar to that of coiled *S. concinna* egg masses (Table 1), again with limited mortality (approximately 10% over 4 hours). The desiccation of *S. serrata* egg masses on algal turf simulated the most common mid shore deposition site for *S. serrata* (Figures 9A & 9B). Water loss (50% over 240 minutes) was less than in petri dishes (70%) with no mortality during the experimental period (Table 1).

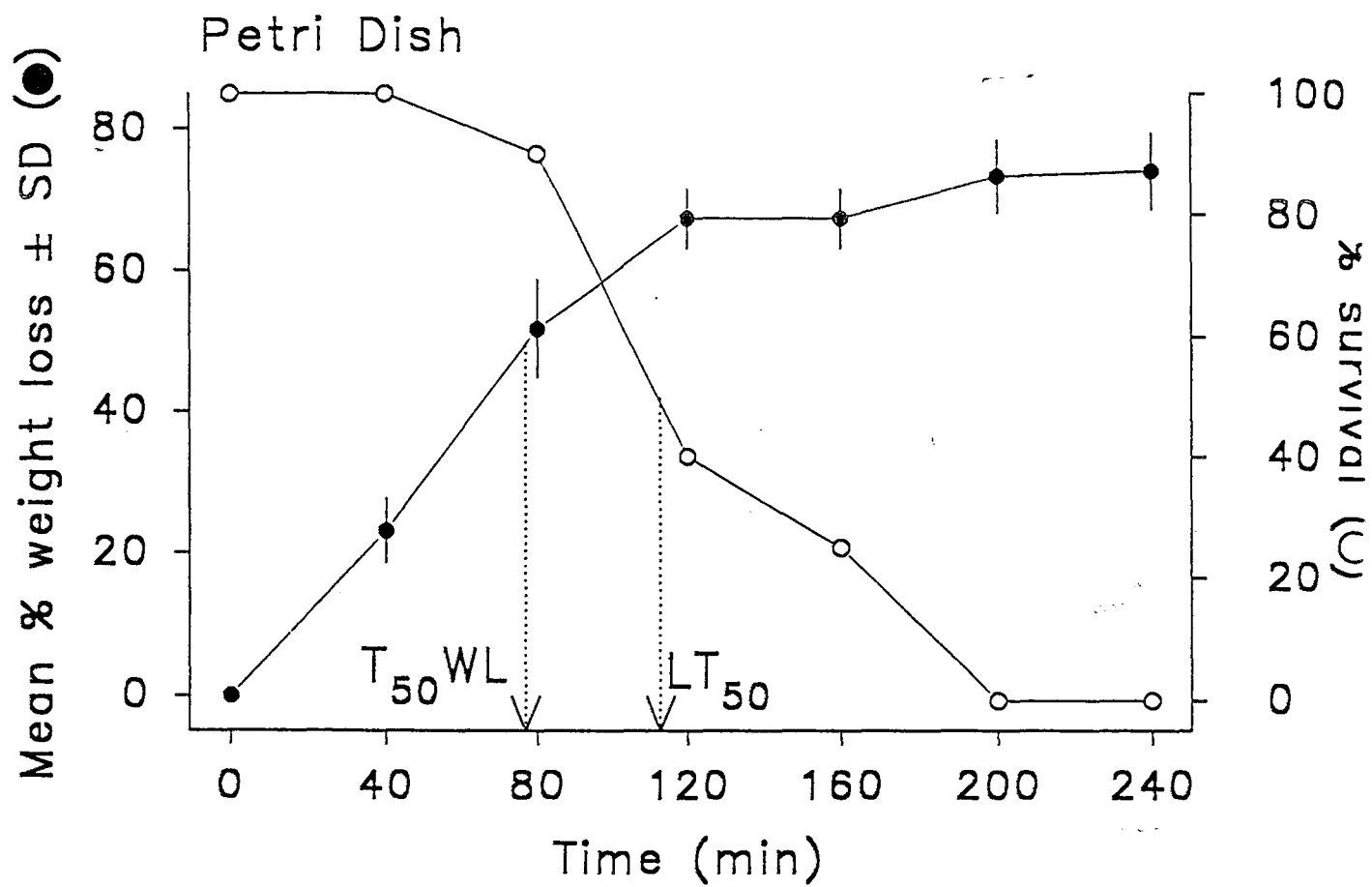


Figure 6. % weight loss (closed circles) and survival (open circles) of *S. capensis* egg masses in petri dish.

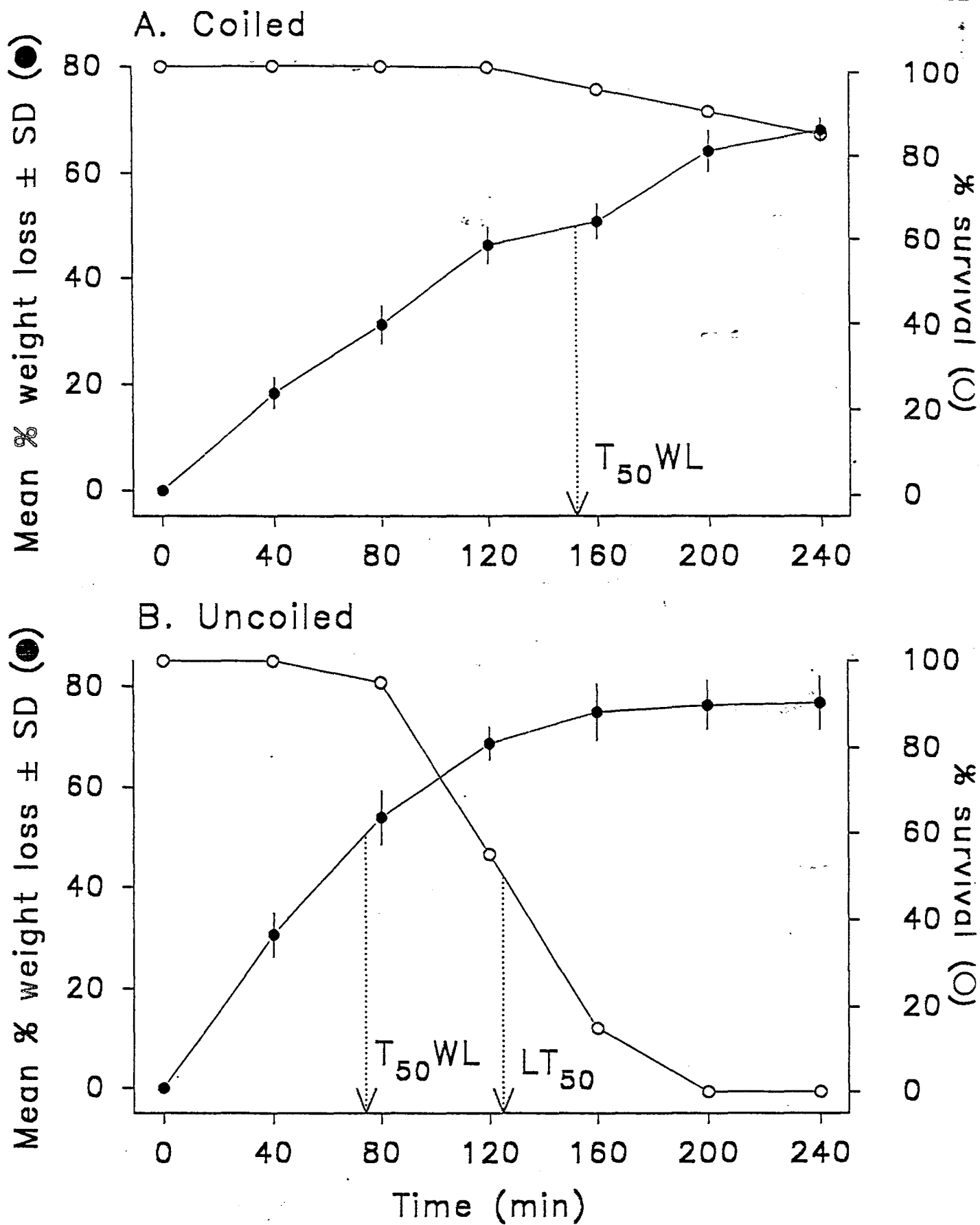


Figure 7. % weight loss (closed circles) and survival (open circles) of *S. concinna* egg masses in petri dish. A: coiled, B: uncoiled.

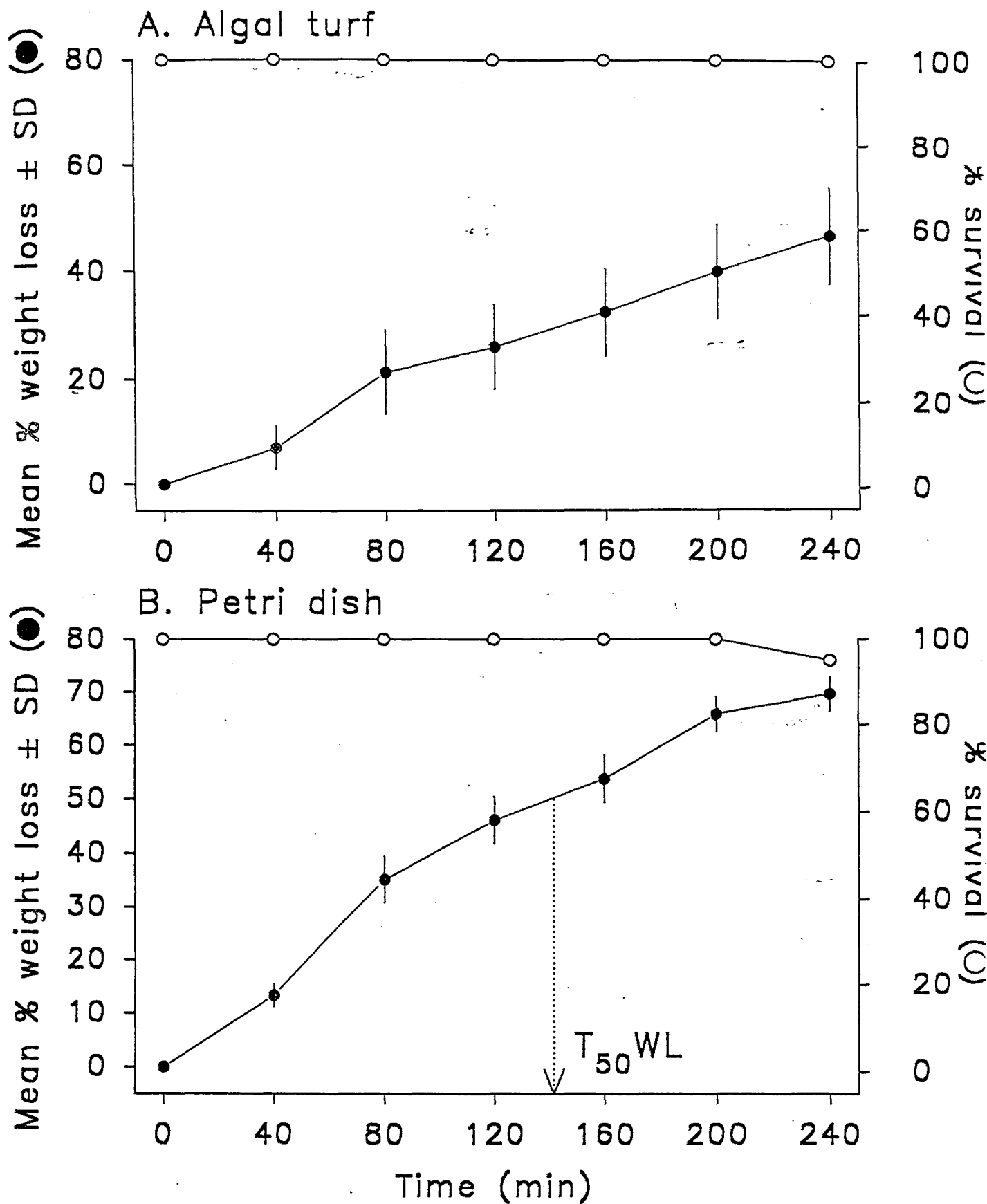


Figure 8. % weight loss (closed circles) and survival (open circles) of *S. serrata* egg masses. A: Petri dish, B: Algal Turf

4.3.4 Field translocation experiments

The results of translocation of egg masses to different microhabitats in the field generally corresponded to the laboratory simulations but, with repeated exposure over a series of low tides, the effects of desiccation were more severe. Egg masses were scored as suffering mortality if they contained any dead embryos. Analysis of survivorship of these egg masses was by 2x2 (*S. capensis*), 2x5 (*S. concinna*) and 2x3 (*S. serrata*) Chi-squared contingency tables. Statistical analysis would have been improved if the lowest values in cells were greater than 5, unfortunately some egg masses failed to adhere for the experimental period. However, for each comparison survival of egg masses was significantly different ($P < 0.001$). For each species, egg mass survival was greater when located in microhabitats experiencing less severe desiccation. Where desiccation was more severe, egg masses often dried out completely with one hundred percent mortality. For example, no mortality was recorded in coralline pools for any species and highest mortality was experienced on bare rock (Table 6). Coiled *S. concinna* egg masses deposited in pools showed improved hatching success than those in concavities, and while coiling of these egg masses did not appear to improve hatching success on the bare rock, it was particularly effective in concavities (Table 2).

Table 2. Embryo mortality in field translocation experiments. N = 20 egg masses in all treatments, where N(total) < 20 egg masses failed to adhere for the duration of the experiment. T = removed from one microhabitat and glued in another microhabitat; S = survival, M = mortality, C = removed from, and glued back into, the same microhabitat.

	<i>S. capensis</i>			<i>S. concinna</i>			<i>S. concinna</i>			<i>S. serrata</i>		
Microhabitat	S		M	Coiled	S	M	Uncoiled	S	M	S		M
Bare rock	T	0	20	T	3	16	T	0	20	T	3	15
Concavities	-	-	-	C	11	4	T	0	17	-	-	-
Algal turf	-	-	-	-	-	-	-	-	-	C	14	0
Pools	T	20	0	T	16	0	-	-	-	T	17	0

Siphonaria serrata egg masses only suffered high mortality on the bare rock (Table 2). In rock concavities there was some mortality of egg masses near the edge of the water, but these were subjected to greater desiccation as the water level was lowered by evaporation during low tide. Unlike *S. capensis*, some *S. serrata* and *S. concinna* egg masses survived on bare rock. However, due to a prolonged developmental period *S. serrata* egg masses would be *in situ* for a further 14-21 days and would probably experience further mortality, especially during the approaching spring tide period when the emersion period is longer.

4.4 DISCUSSION

There are several examples of sympatric species of *Siphonaria* employing different reproductive modes (Boland, 1950; Thorson, 1950; Zischke, 1974; Mapstone, 1978; Chambers & McQuaid, 1994^a). Only one example involves both a planktonic developing and a direct developing species: in the Persian Gulf *S. kurracheensis* occurs on the high shore and *S. sipho* on the low shore (Thorson, 1950). The intertidal distribution of larval developmental type among *Siphonaria* has been attributed to the advantages of pelagic development on the lower shore and direct development on the high shore (Thorson, 1940; Knox, 1955; Morrison, 1963). However, despite a significant correlation between developmental mode and intertidal level for the genus worldwide (Chambers & McQuaid, 1994^a), *Siphonaria capensis* (planktonic development), and *S. serrata* (direct development) do not conform to the model, the latter species co-occurring with another planktonic developer, *S. concinna*. Such a model may be inadequate because it fails to consider the microhabitat differences that exist between life-history stages and egg mass structure, in addition to developmental mode.

Siphonaria capensis generally spends its adult life in crustose algal pools, which are more common on the mid and high shore, where juveniles are also usually found. This is in contrast to some other local shores where it can occur on open rock surfaces. This may be attributable to differences in the rock substratum (Branch & Cherry, 1985). Desiccation experiments on *S. capensis* egg masses in the laboratory, and those placed elsewhere on the shore indicate that spawning in these pools may be necessary for successful hatching. These egg masses have a large surface area to volume ratio

(Figure 3, Chapter 2) and this may aid the diffusion of respiratory gases and reduce developmental period, but the risk of desiccation is correspondingly elevated. A large surface to volume ratio may be advantageous for development in pools due to lower gas diffusion rates in water, but egg masses are particularly vulnerable if deposited elsewhere. Experiments on other planktonic developing *Siphonaria* also indicate higher mortality for egg masses experimentally placed in exposed areas on the high shore (Creese, 1980). These are high shore Australian species which produce pelagic egg masses, and an adaptive response has been postulated as an alternative method of avoiding desiccation during benthic development (Creese, 1980; Quinn, 1988).

On the mid to high shore, *Siphonaria concinna* and *S. serrata* are often found in similar microhabitats, with both direct developing egg masses of *S. serrata* and planktonic developing egg masses of *S. concinna* usually found elsewhere. One interpretation is that spawning rates in some microhabitats are greater than in others, another is that survival of spawn is greater in some microhabitats. However, the most likely interpretation is that both species move to other microhabitats for spawning. Field observations support the latter hypothesis: individuals of *S. concinna* and *S. serrata* are often observed to move, within the same zone, below the adults' normal grazing area for spawning. This behaviour has been reported for other *Siphonaria* (Boland, 1950; Levings & Garrity, 1986). For both *S. concinna* and *S. serrata*, successful hatching depends on a modified egg mass structure, and spawning site. This migratory response could be seen clearly at Cannon Rocks where rings of *S. concinna* egg masses, and occasionally those of *S. serrata*, were often seen around the lower edges of the smaller intertidal boulders when no other microhabitats are available.

For *S. concinna*, the most frequent spawning microhabitats on the mid- to high-shore were rock concavities, crevices and pools. Like many siphonariid limpets, *S. concinna* grazes during low tide on open rock surfaces, crevices and rock concavities before returning to a home scar during high tide (Kilburn & Rippey, 1982; Lasiak & White, 1993). For deposition of egg masses, it moves to other microhabitats where desiccation is likely to be reduced. Experiments indicate that the coiling of these egg masses dramatically reduces mortality resulting from desiccation (Figure 7 and Table 2) by trapping water within the egg masses during emersion. This seems particularly

important for microhabitats such as rock concavities as uncoiled egg masses are unable to survive in what appears to be a common deposition site (Table 2).

Like *S. concinna*, *S. serrata* also grazes on open rock surfaces during low tide. Although the evidence of a home scar is not as clear as that of *S. concinna*, it returns to the same position on the rock where its shell fits the contours of the rock surface. Whilst it also deposits spawn in a variety of microhabitats of reduced desiccation (pools, algal turf and crevices), its egg masses have the most effective structural adaptation against desiccation. These are surrounded by a thick outer layer to which sand and other particles can attach shortly after deposition. A trade-off must then exist between the diffusion of respiratory gases and prevention of water loss by the egg mass. The results of the egg mass desiccation and translocation experiments suggest a higher resistance to desiccation (Table 5), but the longer development period for these egg masses necessitates surviving the spring tide cycle with correspondingly longer periods of emersion. Deposition on algal turf and around the edges of intertidal pools will reduce desiccation and is likely to increase survivorship of egg masses over these longer developmental periods. Whilst these microhabitats may provide the best refuge from desiccation during low tide, it is unclear why algal turf is favoured over other apparently equally benign microhabitats, for example, in pools. It could provide a cryptic habitat against predation of egg masses (reported for other benthic egg masses; Levings & Garrity, 1986) but predation has rarely been observed. The microhabitat differences between *S. serrata* juveniles and egg masses is curious. It may indicate that hatchlings move rapidly away from their hatching site to attach more firmly to the rock substratum, but if this is not the case, it also raises the possibility of a limited capacity for pelagic dispersal of hatchlings, a potential reported for other direct developing gastropods (Martel & Chia, 1991). Larvae emerging from these egg masses, or fragments of an egg mass breaking up during hatching, may be swept to other sites, and potentially to other shores.

For each limpet species the physiological stresses experienced by benthic egg masses in the intertidal appear to be alleviated by a variety of adaptive responses. Semi-lunar periodicity of spawning is well documented for intertidal animals (Underwood, 1979), including siphonariid limpets (Abe, 1940, 1941; Zischke, 1973; Creese, 1980; Hirano & Inaba, 1980). On the South African south coast, neap low tides occur during the

late afternoon and in the early morning when the sun is low, and the timing of deposition corresponds to neap low tides for South African planktonic developers (unpublished data). Daytime spring low tides fall just prior to midday when solar radiation is higher, and synchronising spawning with lunar periodicity to reduce desiccation would be of adaptive value. A similar spawning response when solar incidence is lowest has been reported for two Pacific species of *Siphonaria* (Abe, 1940, 1941; Hirano & Inaba, 1980). Secondly, spawning in specific microhabitats with reduced desiccation during low tide also seems to have adaptive value in enhancing embryonic survival: actively seeking out microhabitats less vulnerable to desiccation for spawning must enhance the reproductive output of these limpets by reducing mortality of egg capsules.

Egg mass structure may be equally important in reducing the physical stresses on embryos as form and size of egg masses will affect both rates of development and resistance to desiccation. An egg mass can be conceptualised as a mass of tissue with no circulatory system, and limits will be imposed on size and shape. Although such structural aspects have received little attention in the literature (but see Strathmann & Chaffee, 1984; Chaffee & Strathmann, 1984), a trade-off must exist between a large surface area for diffusion of respiratory gases and the associated threat of desiccation. Different structures will thus have different tolerances. Such a trade-off appears balanced in favour of diffusion for *Siphonaria capensis* egg masses deposited in intertidal pools where both desiccation and gas diffusion are reduced. For *S. concinna* egg masses this trade-off may be balanced by coiling: a large surface area is exposed during submersion but water retained within the coil reduces desiccation during emersion. Uncoiled, it performs like an *S. capensis* egg mass, although mortality is experienced at a slightly slower rate. This may be attributable to a larger egg capsule size (Chambers & McQuaid, 1994^b) with a lower surface area to volume ratio. Coiled, it performs like an *S. serrata* egg mass, though diffusion of respiratory gases will be enhanced due to a greater surface area. *S. serrata* embryos are larger and have a longer developmental period during which intracapsular metamorphosis occurs. Furthermore, a larger egg capsule size is likely to reduce desiccation of individual capsules (Strathmann & Chaffee, 1984), an important consideration for direct developing species like *Siphonaria serrata* with protracted encapsulation. Evidence

from the genus as a whole indicates that large egg capsule size correlates with direct development (Chambers & McQuaid, 1994^a) and may indirectly influence the structure of an egg mass and rates of development (Strathmann & Chaffee, 1984; Chaffee & Strathmann, 1984) placing further limits on deposition sites. Egg mass structure and available microhabitat may be more important than intertidal height in controlling intertidal distributions of these species, although other factors such as predation and wave exposure may also contribute (Branch & Cherry, 1985).

While the precise adaptive nature of different developmental modes still remains unclear for many species of benthic marine invertebrates, and may be phylogenetically determined (Reid, 1990; Chambers & McQuaid, 1994^a), for *Siphonaria*, both modes of larval development appear equally viable for adults living at the same intertidal level in similar microhabitats. In the absence of vertical migration in order to spawn, reproductively successful populations of these species of *Siphonaria* may be limited to zones where microhabitats suitable egg mass survival are available. A variety of physiological and energetic trade-offs must then exist between suites of co-adapted traits, including egg mass structure, capsule size and fecundity, while several adaptive measures (both behavioural and physiological) contribute to survival and enhanced reproductive output of these limpets.

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CHAPTER 5

REPRODUCTIVE CYCLES AND ENERGETICS OF TWO SYMPATRIC SPECIES OF *SIPHONARIA* (GASTROPODA: PULMONATA) WITH DIFFERENT REPRODUCTIVE MODES.

5.0 ABSTRACT

Siphonaria concinna Sowerby and *S. serrata* (Fischer) co-exist intertidally on many South African rocky shores. They are similar both morphologically and in terms of their intertidal habitat and distribution, but have different reproductive strategies. *S. concinna* has planktotrophic development involving a high fecundity with small eggs. Observations of a field population show a seasonal basis to reproductive activity with the majority of spawning during the summer months. A discrete recruitment event follows after an estimated planktonic period of two months. *S. serrata* has direct development with large eggs and low fecundity. Spawning again has a seasonal basis, although egg masses could be found throughout the year. A single juvenile cohort follows spawning by approximately four months. A comparison of life-history traits between the two species revealed that *S. concinna* reproduced at a smaller size and juveniles grew faster, but suffered higher mortality than *S. serrata* juveniles. Adult growth rates for *S. concinna* were slower but again with higher mortality. Maximum adult size was smaller and life-span was estimated to be less than that of *S. serrata*. By contrast, the life-history of *S. serrata* was characterised by reproduction at a larger size and slower juvenile growth rates but with lower mortality than *S. concinna* juveniles. Adults grew to a larger size, suffered lower mortality and probably live longer. Despite these differences, both species apportion similar amounts of energy into each spawning event and annual energetic investment into spawning also appears equivalent. These life-history attributes correspond with the classic r- and K-selected traits and bet-hedging models, and suggest an evolutionary stable value for reproductive investment.

5.1 INTRODUCTION

The relationship between the diversity of reproductive strategies demonstrated by marine invertebrates and their habitat has become one of the central problems of marine biology. There has been a great deal of evidence to suggest that life-history traits such as egg size and number, time of breeding, age and size at maturity, the

intertidal and geographic distribution of developmental types and a variety of trade-offs between these traits are products of selection, have current utility and can be considered adaptive (see reviews by Jablonski & Lutz, 1983; Grahame and Branch, 1985; Strathmann, 1986). Recent approaches now recognise that adaptation is constrained by phylogeny (Felsenstein, 1985; Coddington, 1988; Wanntorp *et al.*, 1990) and explanations must be congruent with the systematic distribution of the adaptive trait (Baum & Larson, 1991; Stearns, 1992; Reeve & Sherman, 1993). In some groups of marine invertebrates recent phylogenetic studies suggest a systematic basis to reproductive strategy (Reid, 1990; Chambers & McQuaid, 1994^{a,b}). Thus, there are two possible explanations for an observed trait; one of history and relationship examined by phylogenetic studies, and one of process and adaptation examined by ecological investigations. These alternative explanations are tested by considering the evolution of developmental strategy in the siphonariid limpets (Gastropoda: Pulmonata) as part of a general study of the reproductive biology and evolution of *Siphonaria* (Chambers & McQuaid, 1994^{a,b}). In particular, two South African species, *Siphonaria concinna* Sowerby, 1824 and *S. serrata* (Fischer, 1809) are synecological congeners of similar size and behaviour, depositing gelatinous egg masses in the intertidal. They are thought to be in different subgenera (Allanson, 1958, 1959), and have different reproductive strategies; *S. concinna* hatches as a planktonic veliger larva metamorphosing upon settlement, while *S. serrata* hatches as a crawling juvenile following intracapsular metamorphosis (Chambers & McQuaid, 1994^{a,b}). Many existing adaptive models accounting for different reproductive strategies, for example those based on intertidal zonation (Woodward, 1909; Knox, 1955; Mileikovsky, 1975), latitudinal distribution (Thorson, 1950; Spight, 1977; Clark & Goetzfried, 1978; Perron & Kohn, 1985) and body size (Chia, 1974; Giesel, 1976; Underwood, 1979; Gallardo & Perron, 1982), will not apply. This leaves the remaining adaptive explanations, expressed in terms of energetic trade-offs, r-K selection theory and 'bet-hedging' between a variety of reproductive and life-history characteristics (Vance, 1973; Christiansen & Fenchel, 1979; Grahame & Branch, 1985; Stearns, 1976, 1992), as the most plausible.

The definition and measurement of reproductive effort is problematic. Previous studies have used indices which compare the biomass of egg production to the biomass of

parental soma (Menge, 1974; Grahame, 1982; Todd, 1979). Tinkle & Hadley (1975), however, have claimed that the proportion of the animal's total energy budget is the only meaningful measure of reproductive effort. The two South African siphonariids live and graze in similar microhabitats at the same intertidal levels (Chapter 4) and this provides an opportunity to compare the allocation of resources to different reproductive strategies between species which are likely to be influenced by similar selection pressures, and may have similar energy budgets.

In this study a variety of interspecific differences in reproduction between *Siphonaria concinna* and *S. serrata* are quantified. Over a period of nearly two years the timing and energetic input into reproduction of an isolated population of these limpets on a rocky shore on the South African south coast was investigated. Reproductive activity is described in terms of annual spawning and recruitment of offspring, growth and mortality, fecundity and energetic investment into reproduction. As these animals are ecologically proximate, and morphologically and behaviorally similar, the relative energetic investment to reproduction for each species is compared by an estimation of the annual reproductive energy budget. The plausibility of adaptive models based on energetic trade-offs, r-K selection theory and 'bet-hedging' in marine invertebrates are then considered.

5.2 METHODS

5.2.1 Study site

Data were collected on a fortnightly basis on the days following neap tides when spawning is most common for *Siphonaria concinna* (pers. obs.). A study area of approximately 3m² was selected containing a variety of microhabitats occupied by *Siphonaria concinna* and *S. serrata* congeners at Waterloo Bay on the South African south coast. This area corresponded to the most commonly utilised microhabitats of both species identified in Chapter 4. This area was isolated on either side by sand filled gullies and marked off by anti-fouling paint, effectively preventing migration in or out of the area.

5.2.2 Reproductive cycles

Approximately 30 individuals each of *Siphonaria concinna* and *S. serrata* were collected from Waterloo Bay at monthly intervals between August 1991 and

November 1992. Collections were all made at a similar intertidal level over an area of less than 50m². Specimens were maintained in aquaria overnight and then preserved in 70% ethanol before dissection. The blotted wet weights (excluding the shell) of the somatic and gonadal tissue of each individual were measured and the ratio of gonad:somatic weight (g/s ratio) calculated. Parasitism, particularly of gonadal tissue, is not unusual and animals with evidence of parasitism were discarded. A g/s ratio for each month was calculated from the mean of the individual g/s ratios. For both species it was possible to distinguish a 'spent' and an 'active', or 'ripe' gonad from intermediate states, on the basis of size and colour during dissection. Spent gonads appeared small and pale, while ripe gonads were large, occupied a significant part of the body cavity and were either pink or olive green in colour. These distinctions corresponded to gonad size and the percentage of spent and active gonads was calculated for each monthly sample.

5.2.3 Spawning and recruitment

Every neap low tide between January 1991 and March 1993 the study area was searched for egg masses of both species. All *Siphonaria concinna* spawn were counted and removed. Benthic duration of these egg masses is between 5-6 days (Chambers & McQuaid, 1994^b) although evidence of deposition remains for up to 10 days, enabling egg cases that were deposited and hatched between visits to be recorded. Egg masses of *S. serrata* spend a longer period on the shore (approximately 3 weeks: Chambers & McQuaid, 1994^b) and there was little evidence of semi-lunar periodicity of spawning. Individual egg masses were marked with a small notch in the jelly matrix to avoid counting the same egg mass on subsequent visits. Egg masses of *S. serrata* can also be aged as they change colour during development (from a pale clear mass to brown, before they begin to fragment with the onset of hatching) and this assisted identification. Juveniles can be identified to species once larger than 3mm, and total number of recruits in the study area (individuals less than 5mm) were recorded over the same period as spawning.

5.2.4 Population dynamics

Size-frequency data were collected on a regular basis between January 1991 and November 1991 (bi-monthly) and between November 1991 and March 1993 (monthly). The size of all limpets of each species in the study area was determined by measuring shell length *in situ* with vernier callipers to the nearest 0.5mm. Size frequency distributions were constructed for each census date and cohorts were extracted using the software program 'MYX' from Macdonald & Pitcher (1979). The mean size of these cohorts was used to follow growth through time, although only data from clearly distinguishable cohorts were used. Sampling was during neap tides (see above) resulting in wet conditions, and many animals, particularly *Siphonaria serrata*, had thick algal growth on their shells preventing individual marking. This made estimations of growth rates from individuals difficult. Nevertheless, as animals have home scars, or return to the same area of rock, this enables particular individuals to be recognised. Between February 1992 and March 1993 the mean growth rates of 3 size groups (*S. concinna*; 4mm, 10mm, 20mm; *S. serrata*; 4mm, 7mm, 20mm) each initially comprising 10 individuals were monitored for both limpet species.

Where there is prolonged recruitment, juvenile cohorts will be depressed. This can result in underestimations of growth rates, and makes mortality estimates impossible (Creese, 1981). Mortality rates should ideally come from marked individuals, but there were problems with marking (see above). An alternative method uses estimates of mortality determined from cohorts to which recruitment has ceased, by calculating the reduction in density of individual size frequency cohorts through time. This method cannot distinguish between mortality and migration as explanations for change in abundance through time, but this problem was overcome by the isolation of the study site.

5.2.5 Fecundity and Energetics

Animals and freshly deposited egg masses of *Siphonaria concinna* and *S. serrata* were collected from similar microhabitats adjacent to the study area and returned to the laboratory for analysis. Identification of the individual limpet which has spawned a particular egg mass is difficult and only in circumstances where an egg mass could be specifically attributed to a particular individual (for example in an isolated area, or

during deposition) were collections made. Adults were removed from their shells and dried to constant weight to determine the relationship between adult weight and shell length. Each individual egg mass was measured in length and three 1mm sections were cut and pressed between two microscope slides and the number of eggs counted. This enabled estimates of the total number of eggs per egg mass to be calculated. Egg masses were then dried to constant weight, these values were converted to $\log_{10}$ and plotted against shell length for both species (this also enabled the smallest reproductive size to be estimated). Similar plots of fecundity (number of eggs) against shell length were also made. For the determination of the energetic content of egg masses, 10 freshly collected spawn of similar size from 20mm individuals of each species were dried to constant weight for determinations of energy content in a bomb calorimeter (*Calorimeter Systems CP400*).

Finally, an estimation of the annual reproductive energy budget (T) for a standardised 20mm adult individual of each species was made using the equation;

$$T = N.W.E$$

Where N = number of egg masses spawned in a year; W = estimated weight of egg mass produced at 20mm and E = energy content per gram of egg mass. N was estimated from the total number of egg masses spawned in a year and divided by the mean number of adults. This was calculated using running means derived from one-year intervals over the 21 month sampling period to produce an mean annual value for egg masses.adult⁻¹.year⁻¹ between November 1991 and March 1993.

5.3 RESULTS

5.3.1 Reproductive cycles

Spawning can be indicated by a significant decrease in the g/s ratio between consecutive months and a concomitant increase in the number of spent gonads. Both siphonariids show several decreases in the g/s ratio over the study period but these differ in their intensity and duration (Figures 1 & 2). Two major spawning events, indicated by a fall in the g/s ratio, are clearly discernable for *Siphonaria concinna* (Figure 1A). These are associated with an increase in the percentage of spent gonads and a corresponding decrease in the percentage of active gonads, although spent gonads were present during each sampling month (Figure 1B). Beginning at the end

of the winter season, there is a steady build up of the g/s ratio from August to November in 1991 and beginning again earlier in 1992 from July to October. These events appear to have a seasonal basis and the major spawning period follows during the early summer months between November 1991 and February 1992 with up to 40% of the gonads appearing to be spent accompanied by a fall in the g/s ratio. These events began again a month earlier in the following year, during October 1992, although sampling finished before spawning ended. Variation in the g/s ratio between March and June 1992 may be 'noise' in the data (Figures 1A & 1B), however, there were also fluctuations in gonad activity during this period which may be attributable to non-synchronous spawning by some of the population.

The g/s ratio for *Siphonaria serrata* followed a pattern similar to that of *S. concinna* but with more individual investment per spawning, indicated by a higher g/s ratio. The first began during the spring from September 1991 with the least activity from February through to August (Figure 2A). This corresponded to gonad activity, although the condition of the gonads suggest this may event may have begun as early as July (Figure 2B). The g/s ratio began increasing again the following year in the early summer months from August to November 1992 (Figure 2A) but sampling finished before the g/s ratio began to fall again. The g/s ratio was lowest during March-May 1992 (Figure 2A) when the percentage of active gonads was also at its lowest. During these spawning periods spent gonads of *S. serrata* were less common than for *S. concinna*. This may indicate that spawning is more synchronised than *S. concinna*, which showed fluctuations in gonad activity between February and August 1992 (Figure 1B). Over the same period active gonads of *S. serrata* were rare and spent gonads were at their highest levels (Figure 2B). These results suggest either fewer individual spawning events by the *S. serrata* population, or alternatively, greater synchronisation of spawning.

5.3.2 Spawning and recruitment

Seasonality of recruitment and the occurrence of spawn on the shore was clear for *Siphonaria concinna*. Although egg masses could be found throughout the year, they were rarest in the autumn and winter months, from March to August (Figure 3A). Peak spawning corresponded to highest g/s ratios (Figures 1 & 2) for both seasons,

occurring between September 1991 and January 1992 and again the following year during the same months. A smaller peak occurred during June 1992 following a build up from March. Although this was consistent with spent gonads recorded over a similar period (Figure 1B), it may be attributable to spurious data. Peak recruitment of juveniles followed peak spawning in March of 1992, and a minor recruitment peak followed in October 1992. In both cases this lag period between spawning and settlement is approximately 4 months, enabling an estimation of the duration of the planktonic period to be made. Based on juvenile growth rates (see below), the lag between settlement of juveniles and the time when they can actually be observed is estimated at two months, suggesting a planktonic, presumably planktotrophic, period of approximately two months. Recruits could be found throughout the year, but were rare between July 1991 and February 1992, and again rare during August and September of 1992. Sampling ceased at the onset of the next recruitment period, beginning in March 1993, although spawning again appeared on the increase.

Recruitment of *Siphonaria serrata* may also follow a seasonal pattern (Figure 3B). Occurrence of spawn coincided with peaks in spent gonads and high g/s ratios (Figures 2A & B), although peak recruitment of juveniles was later than for *S. concinna*, in June 1992. Throughout the remainder of the sampling period recruitment was very low with less than one juvenile per m² between July 1991 and April 1992. No recruits were found after November 1992. Spawn was most common during the summer months (October 1991-January 1992, and October 1992-January 1993; Figure 3B) but spawning continued during the autumn and winter months and the seasonal peak in spawning indicated by the gonad data (Figure 2B) was less clear (Figure 3B). The relationship between maximum occurrence of spawn and the time juveniles can actually be observed on the shore allows a more accurate estimate of early juvenile period for the direct developing *S. serrata* as recruitment is expected to be primarily local. The lag period is approximately five-six months, from December 1991/January 1992 to July 1992. Smaller recruiting peaks (during February 1992 and October 1992) may correspond to minor peaks in deposition (during September 1991 and April 1992) by a similar time period (Figure 3A & 3B). Allowing three weeks for encapsulation, juveniles must then spend up to four months unobserved on the shore indicating early growth rates for *S. serrata* juveniles of approximately 1mm/month.

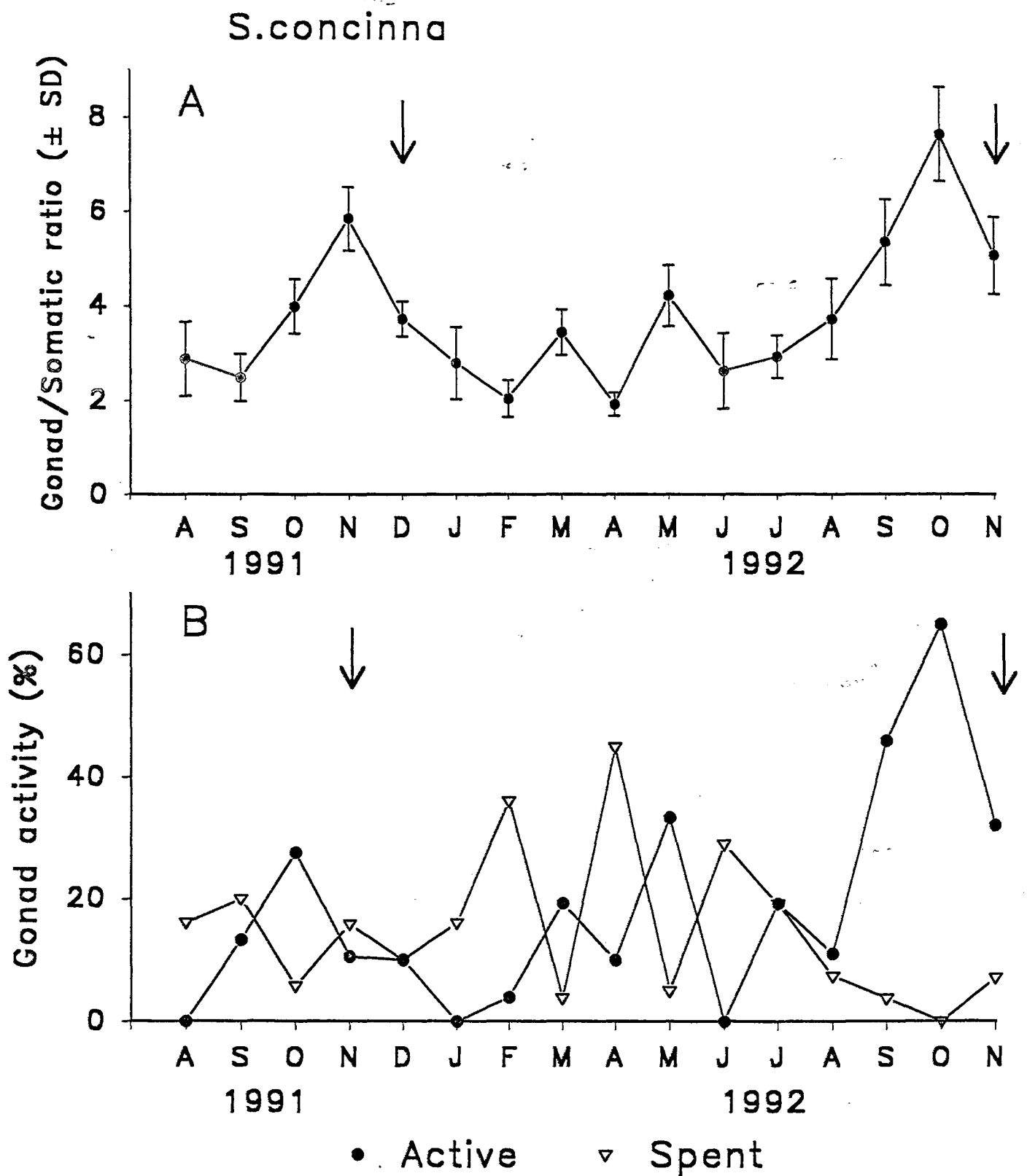


Figure 1. Reproductive cycles of *Siphonaria concinna* (arrows indicate onset of spawning). A: Expressed as mean ($\pm$ SD) gonad/somatic ratio determined from monthly collections. B: Expressed as percentage of spent gonads (triangles) and active gonads (circles) (intermediate gonads not shown).

S. serrata

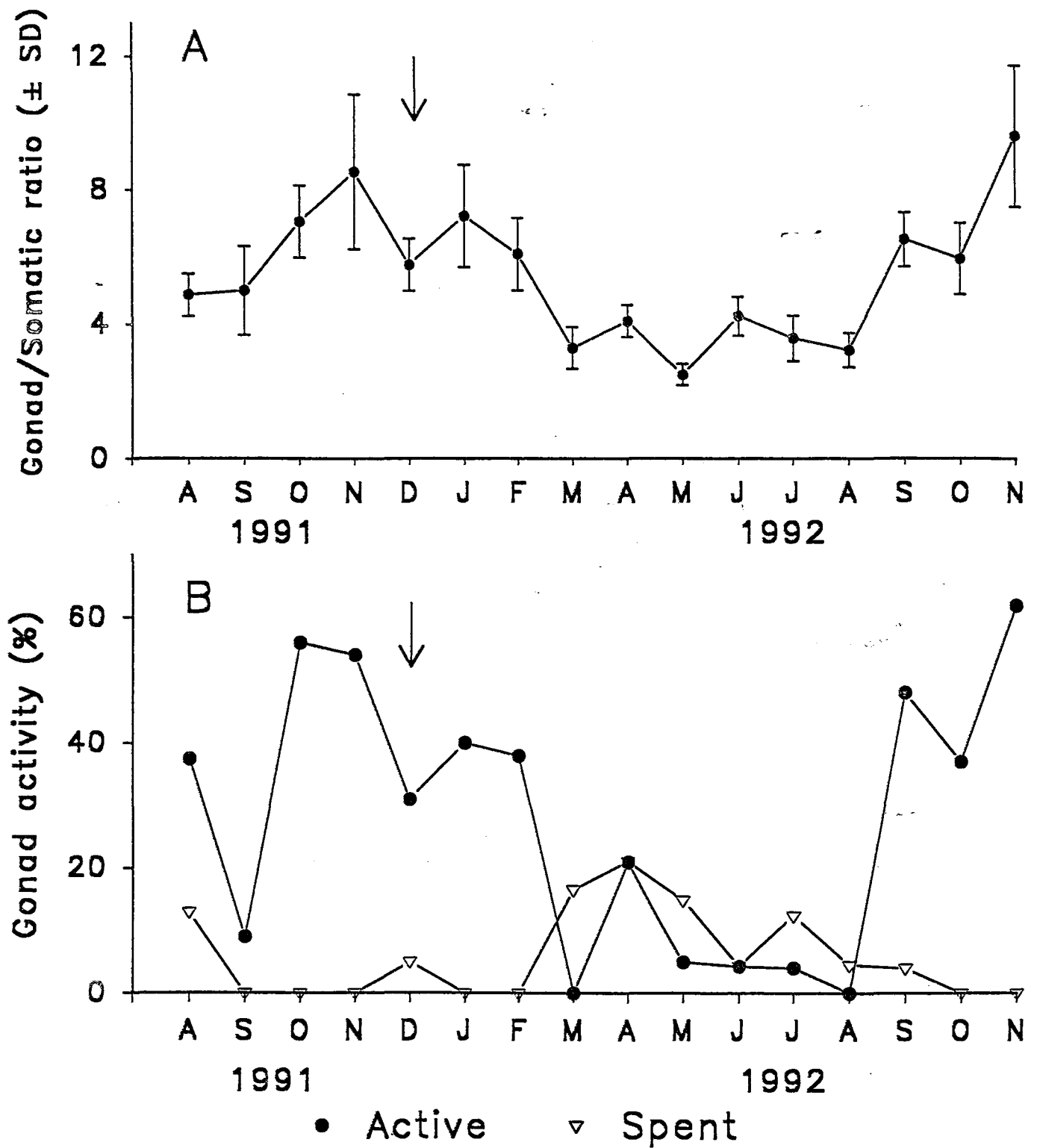


Figure 2. Reproductive cycles of *Siphonaria serrata* (arrows indicate onset of spawning). A: Expressed as mean ($\pm$ SD) gonad/somatic ratio determined from monthly collections. B: Expressed as percentage of spent gonads (triangles) and active gonads (circles) (intermediate gonads not shown).

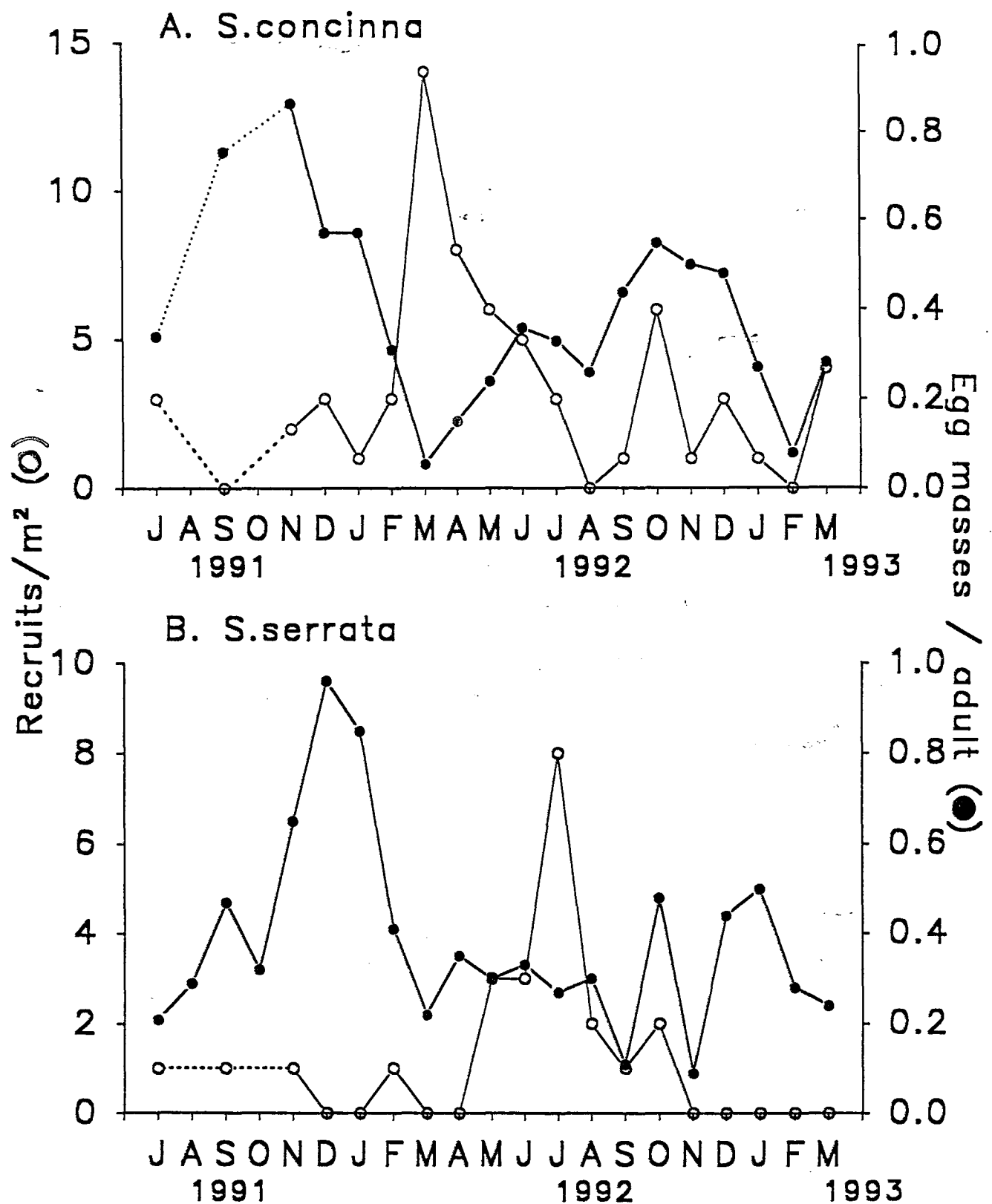


Figure 3. Mean density of recruits (open circles) and egg masses (closed circles) per adult limpet in experimental area at Waterloo Bay. A: *Siphonaria concinna*, B: *S. serrata*.

5.3.3 Population dynamics

Size frequency histograms are shown at bi-monthly intervals between July 1991 and March 1993 (Figures 4 & 5) and from these age cohorts were extracted (Figures 6A & 6B). Identification of discrete adult age cohorts for both species was not possible and cohorts A in Figure 6A and Figure 6B merely represent adult animals of similar adult size (*Siphonaria concinna*, 17-18mm; *S. serrata*, 16-17mm). The growth rate of *S. concinna* adults (Figure 6A) in cohort A was calculated to be 0.33mm.month⁻¹ which was faster than the 0.14mm.month⁻¹ calculated from the marked individuals (Figure 6A, 1). Juvenile cohorts (B,C & D), however, were identifiable, although for *S. concinna* continuous recruitment during the breeding season made distinction difficult. A *S. concinna* juvenile cohort (B) first appeared in November 1991 (Figure 6A) but numbers were insufficient to distinguish it in later months. A second juvenile cohort (C) appearing in February 1992 represented a large number of juveniles and this cohort could be followed until it joined the adult cohort (A) in October of 1992 (Figure. 6A). Juvenile growth rates were estimated to be 1.68mm.month⁻¹ (from groups 2 & 3). A further juvenile cohort (D) appeared in July 1992 but was only discernable again in October 1992. This was smaller than cohort C and continuous recruitment between October 1992 and March 1993 prevented any estimations of growth rates.

Table 1. Growth rates of adults (>16mm, both species) and juveniles (<10mm, *Siphonaria concinna*; <10mm, *S. serrata*) calculated from the increase in mean shell length of cohorts and marked individuals, expressed as mm.month⁻¹

Species	Age class	Time period	Growth rate (SD)
<i>S. concinna</i>	Juvenile cohort (C)	Feb 1992-Aug 1992	1.66(1.14)
"	Marked juveniles (2 & 3; n=8)	Feb 1992-Aug 1992	1.68(0.41)
"	Adult cohort (A)	Nov 1991-Sep 1992	0.33(0.42)
"	Marked adults (1 & 2; n=7)	Feb 1992-Mar 1993	0.14(0.12)
<i>S. serrata</i>	Juvenile cohort (B)	May 1992-Nov 1992	1.75(1.43)
"	Marked juveniles (2 & 3; n=9)	Feb 1992-Nov 1992	1.11(0.3)
"	Adult cohort (A)	Nov 1991-Oct 1992	0.30(0.55)
"	Marked adults (1 & 2; n=8)	Feb 1992-Mar 1993	0.35(0.2)

Far lower levels of recruitment were associated with the *Siphonaria serrata* population, although one juvenile cohort (B) allowed an estimation of juvenile growth rates (Figure 6B). Beginning in March 1992 and growing at $1.75\text{mm}\cdot\text{month}^{-1}$ this cohort joined the adult population 7 months later in December 1992. In this case estimations of marked individual growth rates (2 & 3) were lower ($1.11\text{mm}\cdot\text{month}^{-1}$), but corresponded to estimates of post-hatching growth rates from settlement data at approximately $1\text{mm}\cdot\text{month}^{-1}$. Another juvenile cohort appeared in November 1992, but this could only be differentiated for that month. Adults in cohort A grew at $0.30\text{mm}\cdot\text{month}^{-1}$ which was, unlike juveniles, slower than the marked individuals (1) at $0.35\text{mm}\cdot\text{month}^{-1}$.

Mortality rates were calculated from cohorts to which recruitment had ceased (Table 2). Ignoring the value for cohort D (subject to continuous recruitment), juvenile *Siphonaria concinna* mortality ($13.25\text{ deaths}\cdot 100\text{ individuals}^{-1}\cdot\text{month}^{-1}$) was considerably higher than the rates for adults. These were estimated to be $4.5\text{ deaths}\cdot 100\text{ individuals}^{-1}\cdot\text{month}^{-1}$ for Cohort A and $4.3\text{ deaths}\cdot 100\text{ individuals}^{-1}\cdot\text{month}^{-1}$ for cohorts A + C combined. *S. serrata* juveniles experienced less mortality and calculations from cohort B at $3.6\text{ deaths}\cdot 100\text{ individuals}^{-1}\cdot\text{month}^{-1}$ place juvenile mortality even lower than *S. concinna* adult mortality. Adult *S. serrata* mortality was also correspondingly lower at $1.46\text{ deaths}\cdot 100\text{ individuals}^{-1}\cdot\text{month}^{-1}$ for Cohort A, and $2.0\text{ deaths}\cdot 100\text{ individuals}^{-1}\cdot\text{month}^{-1}$ determined from cohorts A and B combined.

Table 2. Instantaneous rates of mortality calculated from the decrease in density of limpets within particular age cohorts. Rates are expressed as $\text{deaths}\cdot 100\text{ individuals}^{-1}\cdot\text{month}^{-1}$.

* Estimates are likely to be inaccurate due to continuous recruitment.

Species	Cohort designation	Time period	Rate of mortality
<i>S. concinna</i>	A (Adults)	Nov 1991-Feb 1992	4.5
"	C (Juveniles)	Feb 1992-Sep 1992	13.25
"	D (Juveniles)	Oct 1992-Jan 1993	3.33*
"	A+C (Adults)	Oct 1992-Mar 1993	4.3
<i>S. serrata</i>	A (Adults)	Nov 1991-Oct 1992	1.46
"	B (Juveniles)	May 1992-Nov 1992	3.6
"	A+B (Adults)	Dec 1992-Mar 1993	2.0

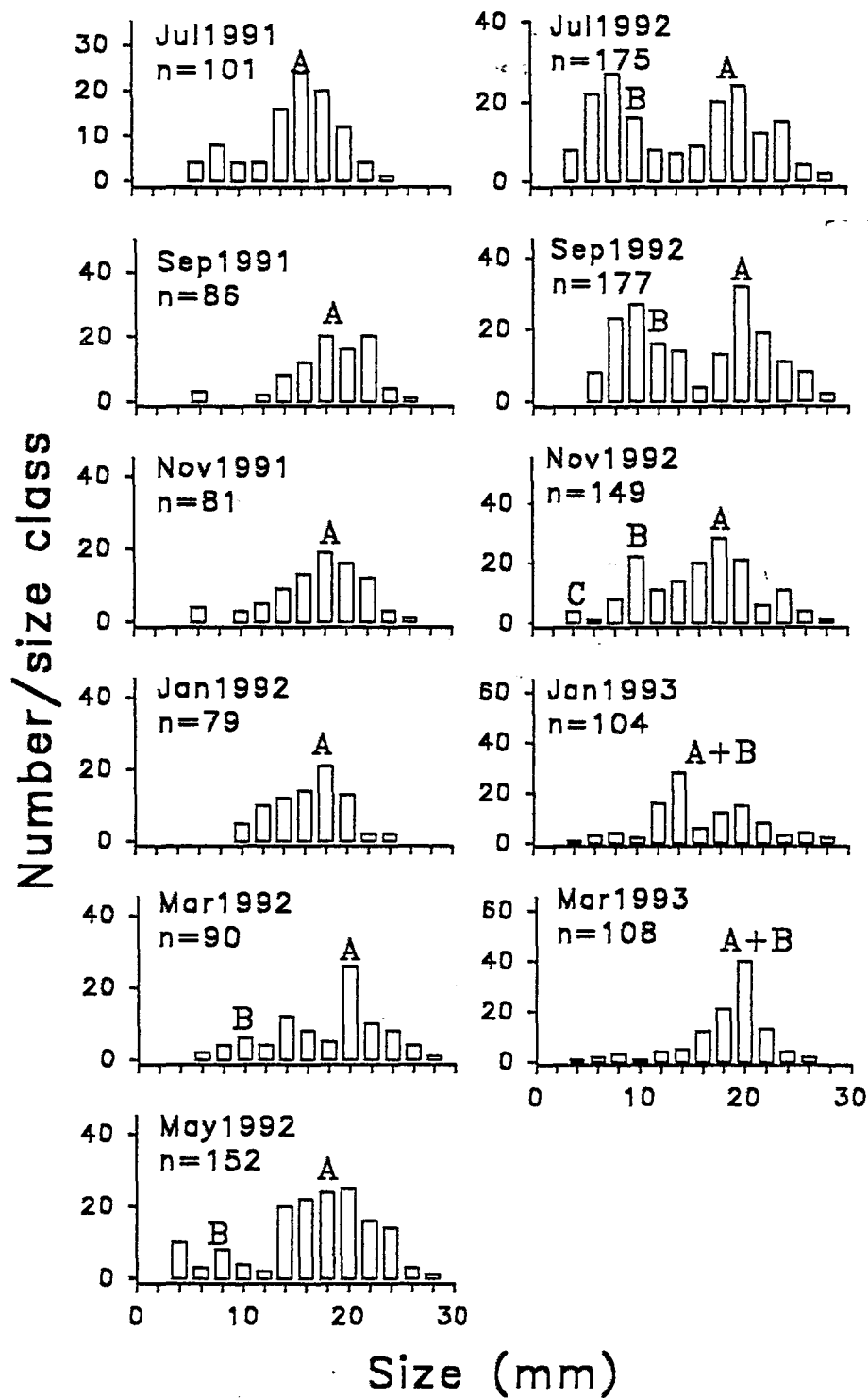


Figure 4. Bi-monthly size frequency histograms for *Siphonaria serrata*.

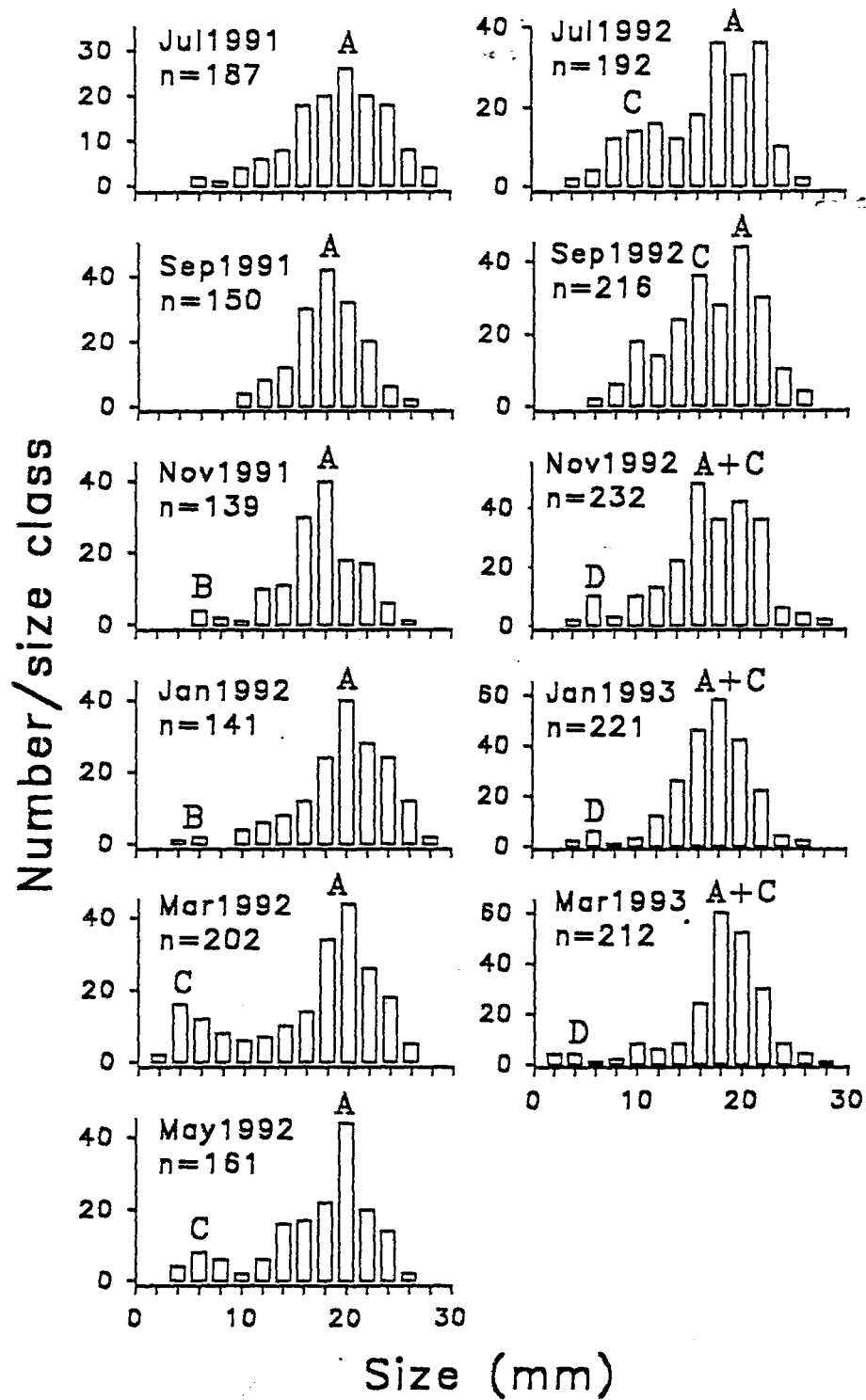


Figure 5. Bi-monthly size frequency histograms for *Siphonaria concinna*.

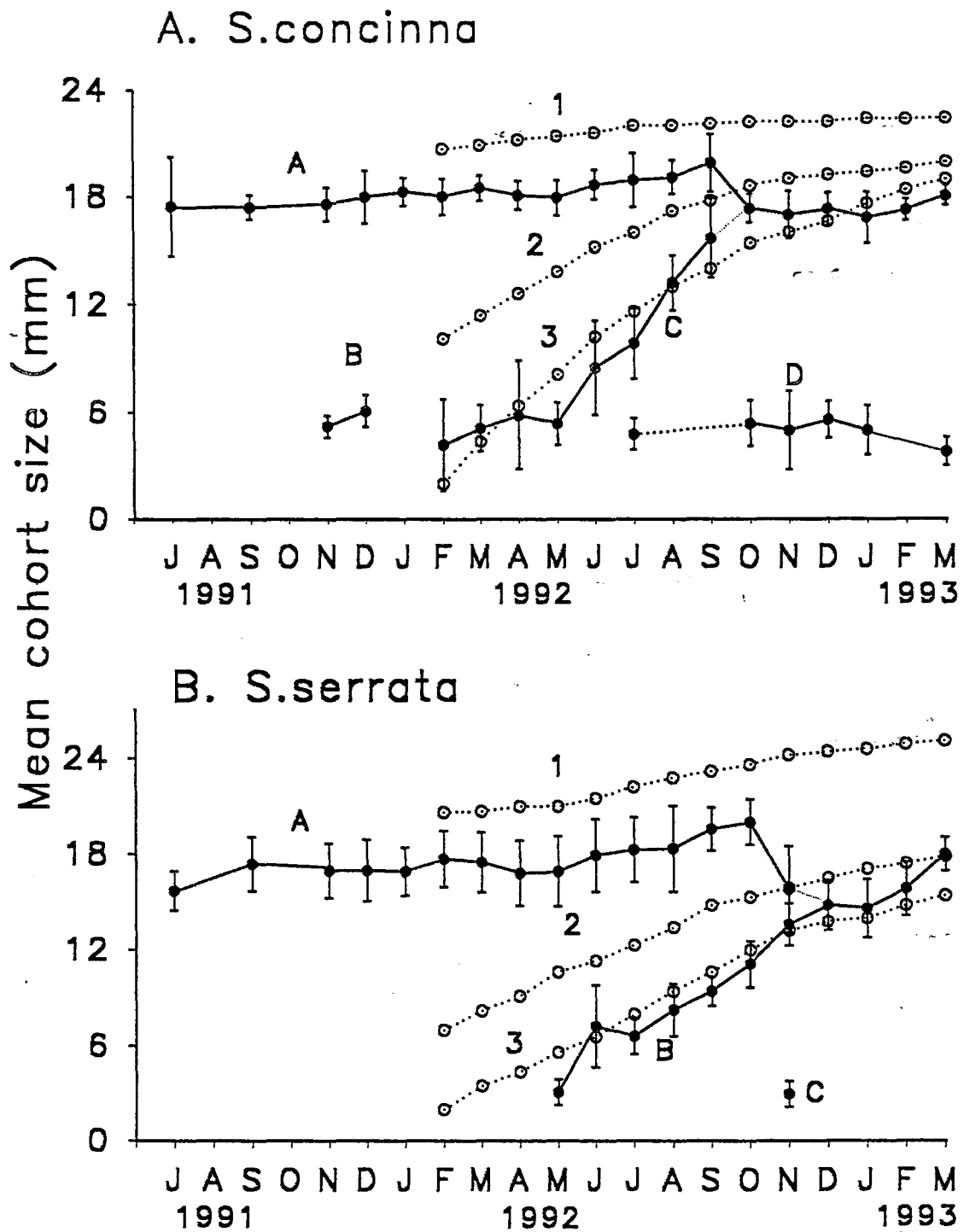


Figure 6. Growth of *Siphonaria concinna* and *S. serrata* determined from the mean size of age cohorts (Figs 6 & 7). A: *S. concinna*; cohorts A-D and groups of marked individuals 1-3. B: *S. serrata*; cohorts A-C and groups of marked individuals 1-3.

5.3.4 Fecundity and energetics

There appeared to be a similar apportionment of energy to shell growth by each species. Shell weight to body weight correlations were made and these were highly significant (*S. serrata*, $r^2 = 64$, $P < 0.001$, $\log_{10} y = \log_{10}(-5.11 + 0.139x)$, $n = 36$; *S. concinna*, $r^2 = 87$, $P < 0.001$, $\log_{10} y = \log_{10}(-6.17 + 0.174x)$, $n = 35$). The shell weight to body weight ratios were not significantly different between the two species (Student's t-test; $P > 0.05$) and adult weight was highly correlated to shell length for both siphonariids (*S. concinna*, $r^2 = 0.87$, $P < 0.001$; *S. serrata*, $r^2 = 0.84$, $P < 0.001$). Consequently, estimations of the relationship between egg mass weight and body size were made using shell length data. The data best fitted a curvilinear relationship for each species and plots were made between $\log_{10}$ egg mass weight and shell length described by $\text{Log}_{10} y = \text{Log}_{10}(a + bx)$, where a and b were the intercepts and gradients respectively. For both species the relationship was highly significant (Figure 7). Similarly, the relationships between shell length and fecundity were also significant (Figure 8). Despite lower r^2 values for fecundity, both correlations enabled estimations to be made with respect to egg mass weight and fecundity for 20mm long individuals of each species (Table 3).

Calorific values of *Siphonaria serrata* and *S. concinna* egg masses yielded different results. Larger capsule size and a thicker jelly matrix for *S. serrata* corresponded to a lower calorific value of spawn (4.68 J.g^{-1}) than for *S. concinna* (6.91 J.g^{-1}) which had smaller more closely packed capsules with less jelly matrix per unit volume. However, the total energetic investment per spawning of a 20mm individual of each species was remarkably similar (Table 3). The estimation of egg masses spawned per year for each species was problematic. Size specific spawning rates are likely for siphonariids (Creese, 1980; Quinn, 1988) and without experimental separation of specific size classes it was impossible to distinguish these rates. Data collected for the g/s ratios indicate that larger individuals were more likely to be reproductively active, but there were insufficient data to quantify this. Despite these problems, some gross estimations were made.

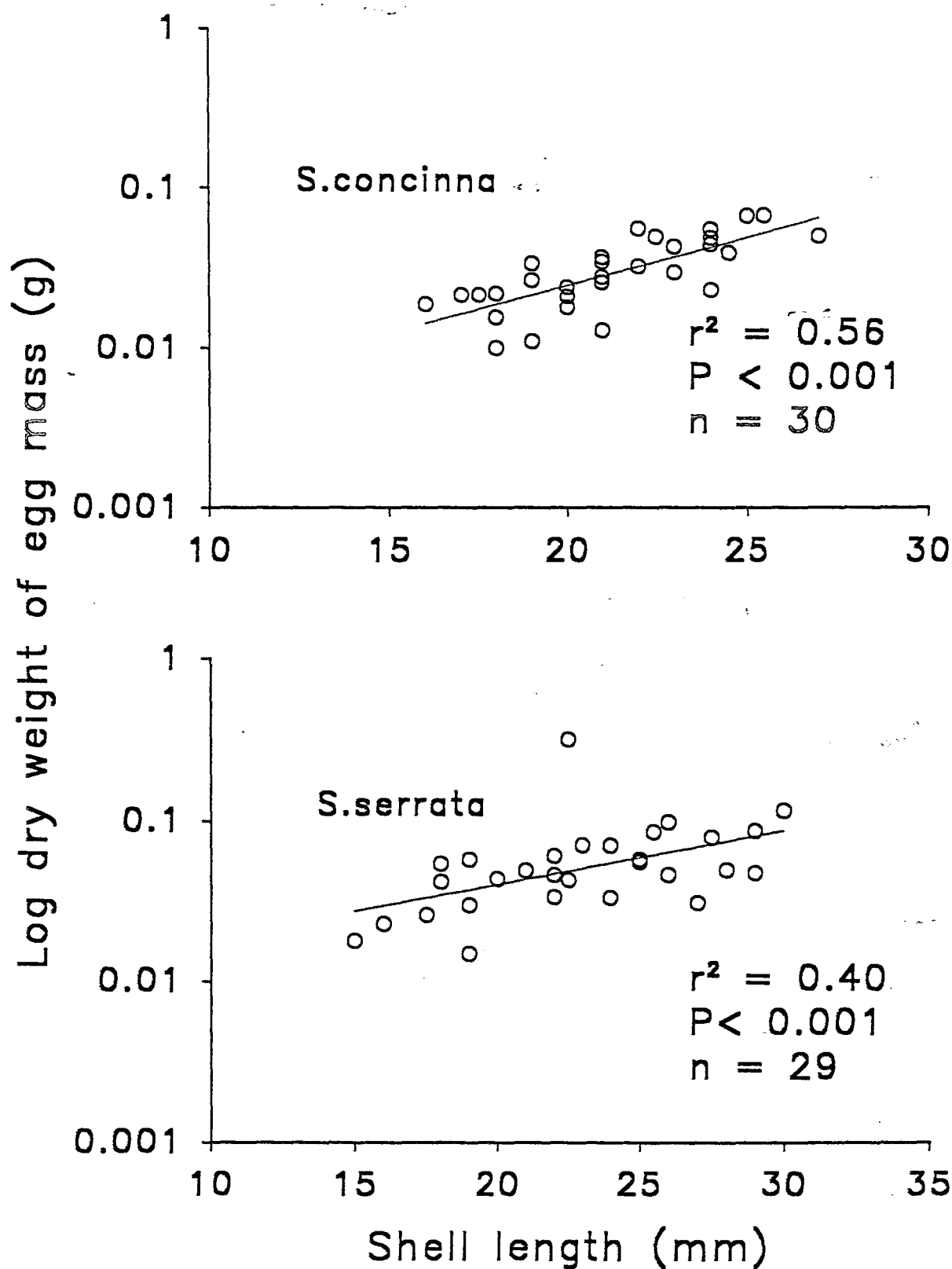


Figure 7. Relationship between $\log_{10}$ dry egg mass weight and shell length for *Siphonaria concinna* ($\text{Log}_n y = \text{Log}_n (-6.49 + 0.1399x)$; $r^2 = 0.56$, $P < 0.001$, $n = 30$) and *S. serrata* ($\text{Log}_n y = \text{Log}_n (-4.88 + 0.0796x)$; $r^2 = 0.40$, $P < 0.001$, $n = 29$).

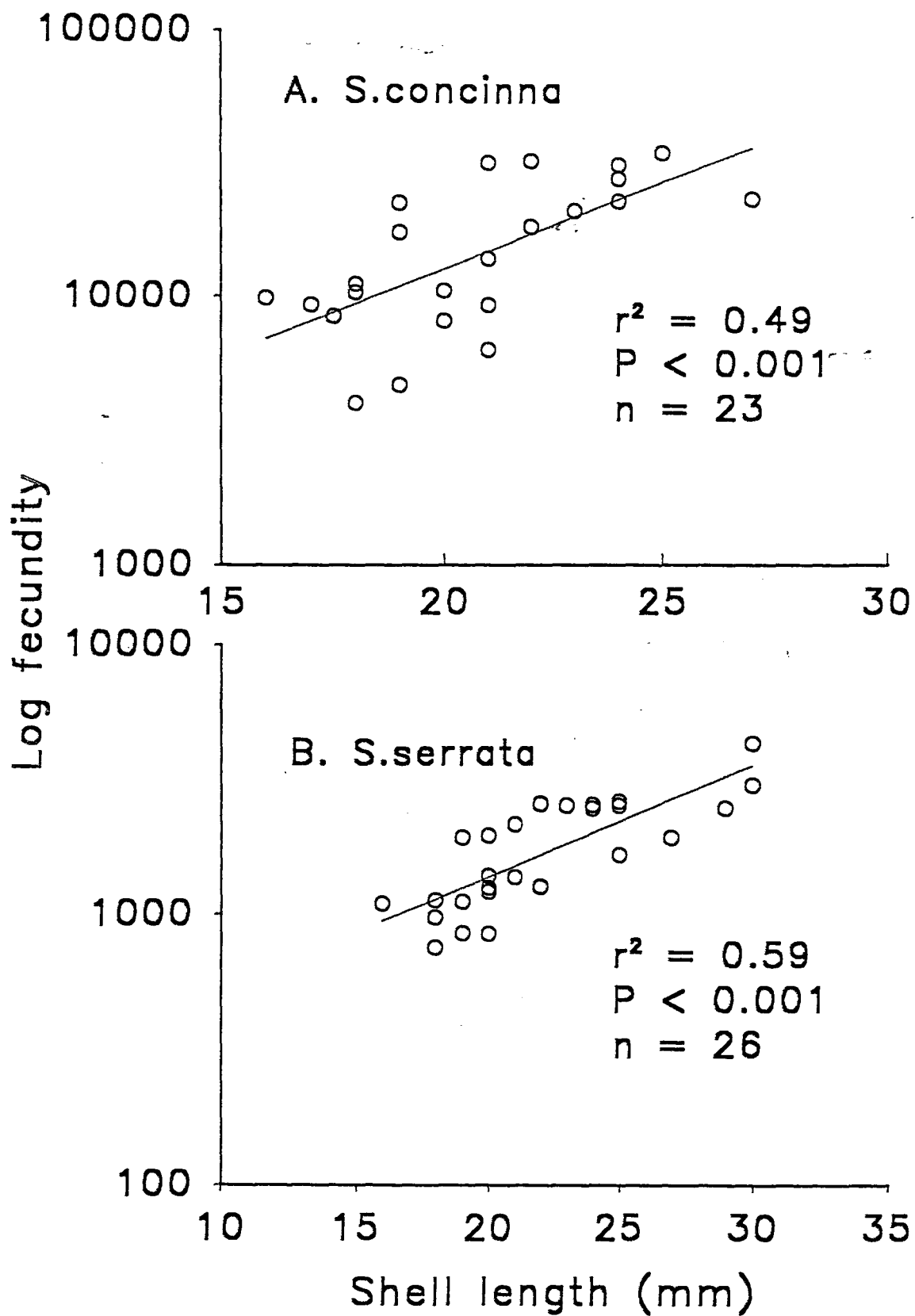


Figure 8. Relationship between $\log_{10}$ fecundity and shell length for *Siphonaria concinna* ($\text{Log}_n y = \text{Log}_n(7.46 + 0.111x)$; $r^2 = 0.49$, $P < 0.001$, $n = 23$) and *S. serrata* ($\text{Log}_n y = \text{Log}_n(5.552 + 0.0844x)$; $r^2 = 0.59$, $P < 0.001$, $n = 26$).

Average adult numbers (calculations repeated for individuals of both species >12mm and >16mm to compensate for potential size-specific spawning differences in spawning rates) were divided by number of egg masses spawned in the study area. This was calculated using means for 6 yearly intervals and yielded similar values for both siphonariids in each size class (Table 3). Consequently, total annual calorific investment (T) in spawning appears equivalent for the two siphonariid species, despite such different modes of reproduction.

Table 3. Reproductive effort of *Siphonaria concinna* and *S. serrata*. Two values of spawn.year⁻¹ (larger size in parentheses) were derived to compensate for potential size-specific spawning rates.

	<i>S. concinna</i>	<i>S. serrata</i>
g.spawn ⁻¹ (W)	0.0249	0.0373
Joules.g ⁻¹ of spawn (E)	6.91	4.68
Joules.spawn ⁻¹	0.1721	0.1746
Spawn.year ⁻¹ (N) >12mm	4.19	4.27
(>16mm)	(6.14)	(5.96)
Joules.year ⁻¹ (T) >12mm	0.721	0.746
(>16mm)	(1.057)	(1.041)

5.4 DISCUSSION

The origin of different reproductive modes may be explained in historical terms of selection, dispersal and the previous habits of ancestors (Jablonski & Lutz, 1983). However, the maintenance of two differing strategies does require explanation in terms of current selection pressures. Due to their different subgeneric status, geographic range and developmental mode (Chambers & McQuaid, 1994^{a,b}) there is no reason to assume that the two siphonariid species considered evolved under the same conditions. *Siphonaria concinna* and *S. serrata* live and graze in the same area in the intertidal and appear to have similar activity rhythms (Allanson, 1958; Chapter 4). Assuming similar grazing, dietary efficiency and investment in shell production, energetic resources available for the two species are likely to be equivalent. The differences in fecundity and egg capsule size indicate that physiological trade-offs exist and each species balances available resources between a variety of reproductive traits.

Both species are very similar in their seasonality of reproduction. During the winter months when epilithic intertidal production is highest (unpublished data), spawning is very low and juveniles are rare on the shore. At the same time the g/s ratio for both species increases and spawning follows between October and March. Peak spawning occurs before the summer months of December and January when physiological stresses such as desiccation are likely to be highest. *S. concinna*'s spawning period is more intense and of a shorter duration, beginning approximately one month earlier than *S. serrata*. There are subsequent synchronous spawning events outside of this main reproductive period by both species; this may result from spurious data, and in the case of *S. concinna* with planktonic development, subsequent recruitment need not necessarily be of local origin. Spawning data for *Siphonaria concinna* shows a greater degree of synchronisation of spawning than *S. serrata* which would have adaptive value for a species releasing planktotrophic larvae. Schumann *et al.* (1982) indicate that the highest periods of inshore pelagic primary production in this area follow upwelling processes during the summer months when spawning by *S. concinna* is highest.

Estimations of growth rates for the two siphonariids, following the establishment of cohorts, also follow different patterns. Most limpet species' growth (both prosobranch and pulmonate) is characterised as indeterminate with highly variable mortality and recruitment (Creese, 1981). Estimates of longevity are best made from marked individuals in this study due to the assumptions of models using age-cohorts. However, the marked adult individuals showed no mortality during the sampling period, preventing more reliable estimates being made. Estimations of growth rates from marked individuals are also likely to be more accurate than those derived from cohort analysis. *S. serrata* adults appear to grow at a faster rate than *S. concinna* adults which appears consistent with the larger adult size attained by *S. serrata* at Waterloo Bay. *S. concinna* individuals of equivalent size (>20mm) may devote more resources to reproduction after this point since they appear to devote less energy to growth. Juvenile growth rates for the two species, however, were different. The appearance of distinct juvenile cohorts follows spawning for both species during the winter months but after different lag periods. *S. concinna* juveniles, after an estimated planktotrophic period of approximately two months, settle, metamorphose, and are

discernable as a juvenile cohort two months later at between 3-5mm long, although juvenile cohorts of *S. concinna* may not necessarily be of local origin. The majority of *S. serrata* juveniles comprising the cohort arriving between March and September 1992 are likely to be from local hatchings, although the potential for occasional pelagic dispersal of direct developing invertebrates (Johannesson, 1988; Martel & Chia, 1991) must be considered.

A variety of life-history characteristics can be summarised for the two siphonariids (Table 4). The most interesting comparison is between the relative energetic investments in reproduction of each species. Despite having large differences in fecundity, capsule size, egg mass structure and developmental strategy, calorific values of each spawn produced by 20mm individuals are remarkably similar (Table 4). If individuals of equivalent size of each species spawn a similar number of egg masses per year, then yearly calorific investment into reproduction by 20mm adults of each species is approximately equivalent. Devoting the same amount of energy to reproduction each year would imply that there may be an optimal allocation of resources between reproduction and other metabolic activities for each species despite differences in their reproductive characteristics. Theoretical predictions and published studies of the relative energetic investments by species into pelagic and non-pelagic development conflict (reviewed by Grahame & Branch, 1985). Work by Grahame (1982) on prosobranchs of the genus *Lacuna* (in which reproductive effort was measured as a portion of the total energy budget) suggests that there may be little difference between species with different reproductive modes. This is consistent with theoretical studies which indicate that there may be an evolutionary stable value for reproductive effort despite differing reproductive modes (Christiansen & Fenchel, 1979; Barnes *et al.*, 1993).

Many species of *Siphonaria* with different reproductive strategies are known to co-exist worldwide (Thorson, 1940; Zischke, 1974; Mapstone, 1978; Creese, 1980, 1981; Chambers & McQuaid, 1994^b). Such situations suggest that more than one life-history pattern can be maintained under the same environmental conditions and involving a variety of trade-offs between reproductive traits (Vance, 1973; Christiansen & Fenchel, 1979). There can be more than one optimal solution to an ecological problem (Parker

& Maynard-Smith, 1990; Stearns, 1992) and there appears to be a clear energetic trade-off between size and number of offspring for these two siphonariids.

Table 4. Life-history characteristics for *Siphonaria concinna* and *S. serrata*. Calculations based on 20mm adults. Values in parentheses derived from calculations based on a larger size at first spawning (see Table 3). * Estimates based on maximum size reached and growth rates.

Trait	<i>S. concinna</i>	<i>S. serrata</i>
Developmental mode	Planktotrophic	Direct
Spawn/year	4.19 (6.14)	4.27 (5.96)
Joules/spawn	0.1721	0.1746
Joules/year	0.721 (1.057)	0.746 (1.041)
Egg capsule size (μ m)	260 x 198	480 x 307
Fecundity	16,000	1350
Potential maximum size	28mm	38mm
Size at first reproduction	12mm	14mm
Seasonality of spawning	Higher	Lower
Seasonality of recruitment	High	High
Adult growth rates	Lower	Higher
Juvenile growth rates	Higher	Lower
Adult mortality rates	Higher	Lower
Juvenile mortality rates	Higher	Lower
Life span*	Shorter	Longer

Long-term demographic studies may reveal the advantages of each developmental mode resulting from their different dispersal capabilities: a temporally stable environment would suit the direct developing *S. serrata* which grows slower but larger, has lower mortality, probably lives longer but is less fecund. This would enable rapid population of local habitats once established. Conversely, temporally unstable habitats would suit *S. concinna* with its faster growth rates, higher mortality, greater fecundity, probably shorter life span and greater dispersal powers enabling colonisation of new environments (or return to old habitats subject to some local catastrophe). On the basis of these differences, *S. concinna* and *S. serrata* appear to sit at different ends on what has been known as the r-K selection continuum. The evidence also supports bet-hedging models based on the relative mortality of different life-history stages

(Stearns, 1976, 1992). While models of r-K selection may have limited application (Hart & Begon, 1982; Boyce, 1984; Barnes *et al*, 1993) most of the life-history traits summarised in Table 4 fit the predictions of both models.

Despite the adaptive attributes of such models, the relationships between various reproductive traits must create barriers to transitional types of development. Simple models of these traits result in adaptive troughs between separate adaptive peaks (Vance, 1973; Caswell, 1981; Grant, 1983; Strathmann, 1986) which selection may only bridge in unusual circumstances. Macroevolutionary processes appear to be responsible for the mode of larval development and egg capsule size (which would involve the disruption of many gene patterns), while selection operating via microevolutionary processes appears to determine a number of quantifiable life-history traits (such as fecundity, seasonality of spawning and recruitment, energetic investment into reproduction and rates of growth and mortality) measured in this study. Explanations for the evolutionary shift between different reproductive modes must derive from the different dispersal capabilities of larval types, or evolutionary constraints resulting from the body size of ancestors (Underwood, 1979), rather than the energetic limitations dictated by particular ecological circumstances.

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CHAPTER 6

DETERMINATION OF GENETIC DIVERSITY OF SOUTHERN AFRICAN INTERTIDAL LIMPETS (GASTROPODA: *SIPHONARIA*) WITH DIFFERENT REPRODUCTIVE MODES USING POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE) OF TOTAL CELLULAR PROTEINS

6.0 ABSTRACT

Using polyacrylamide gel electrophoresis of total soluble proteins, estimates of genetic variability were obtained for seven species of southern African limpets. Greater levels of genetic variability were associated with the planktonic developing species, *Siphonaria concinna*, *S. capensis* and *S. oculus* with clear protein polymorphisms associated with *S. concinna*. The direct developing species, *S. annea*, *S. nigerrima*, *S. serrata* and *S. tenuicostulata* were characterised by lower levels of genetic variability and these were significantly different from the values obtained for planktonic developing species. All species had similar protein banding patterns and on a dendrogram produced from a similarity matrix they nested apart from each other and the three outgroups. The systematic relationships within *Siphonaria* were unclear. Six of the seven species fall into the subgenus *Patellopsis*, but PAGE did not discriminate these from *S. serrata* of the subgenus *Siphonaria*. However, the earlier branching of the planktonic developing species with direct developing species nesting among them on the dendrogram suggests that the planktonic species may be primitive within the *Patellopsis* subgenus.

6.1 INTRODUCTION

Different reproductive modes among benthic marine invertebrates have been thought to lead to different powers of dispersal and consequent gene flow between populations (Jablonski & Lutz, 1983; Grahame & Branch, 1985; Strathmann, 1986). Restricted dispersal should result in low levels of variability due to a small population size and limited genetic exchange between local populations. Conversely, wide dispersal should be characterised by high genetic variability within local populations and homogeneity among neighbouring populations. For marine gastropods the general consensus has been that the production of planktotrophic larvae results in higher dispersal rates and

gene flow than non-planktotrophic larval development (either lecithotrophic or direct) which has restricted dispersal and consequently lower gene flow (Grant & Utter, 1987; Janson, 1987; Behrens Yamada, 1989; Ward, 1990; Hunt, 1993). However, the situation may not be that simple. Wide dispersal of planktonic larvae is dependant upon ocean currents, and recent experimental data have indicated a pelagic dispersal capacity for direct developing larvae, either by floating or rafting events (Highsmith, 1985; Johannesson, 1988; O'Foighil, 1989; Martel & Chia, 1991).

Like many groups of marine gastropods, the siphonariid limpets demonstrate a variety of reproductive modes ranging from planktotrophic larvae to direct development (Berry, 1977; Simpson & Harrington, 1985; Chambers & McQuaid, 1994^{a, b}). The primitive developmental condition in the group is not known but, unlike other marine gastropods which are thought to be primitively planktotrophic (Jablonski & Lutz, 1983; Strathmann, 1986), it may be direct development due their pulmonate ancestry (Chambers & McQuaid, 1994^a). Nine species of *Siphonaria* have been described on South African shores; three planktonic developing species and six direct developing species. Although their taxonomic status has been confused, six species appear to fall into the subgenera *Patellopsis* (Chambers & McQuaid, 1994^b).

Our aim in this paper is primarily to assess levels of genetic variability among South African *Siphonaria* and then to correlate these values to developmental mode. Secondly, to elucidate the systematic relationships among these species to investigate the taxonomic distribution of developmental mode among these *Siphonaria*. Most genetic studies on the dispersal capabilities of different reproductive modes have employed Starch-gel electrophoresis (for example, Grant & Utter, 1987; Janson, 1987; Behrens Yamada, 1989; Ward, 1990; Hunt, 1993) but such methods can be difficult and time consuming. Polyacrylamide Gel Electrophoresis of total soluble proteins (PAGE) has been extensively used in studies of systematics and genetic variation of micro-organisms (Kerstens & De Ley, 1975; Vera Cruz *et al.*, 1984; Jackman, 1985, 1987; Murphy *et al.*, 1990; Qhobela & Claflin, 1992), and more recently has been applied to fish systematics (Nxomani *et al.*, 1994). The technique involves separation of proteins with uniform charge according to their molecular weights, in a gel matrix that acts as a molecular sieve. Proteins react with an anionic detergent, sodium dodecylsulphate (SDS) to form negatively charged complexes. Upon binding to SDS

the proteins denature and stabilize, forming complex rods of a length roughly proportional to the proteins' molecular weights (Smith, 1984). This technique enables the sampling of many loci which improves estimates of genetic variation far more than increasing the number of individuals sampled (Gorman & Renzi, 1979), and can provide similar information to starch-gel electrophoresis from fewer gels.

6.2 METHODS

6.2.1 Sample collection and preparation

Between 25-30 individuals of seven species of *Siphonaria* (subgenera in parentheses) were collected during January and February 1992 from the following South African locations: *Siphonaria (Patellopsis) anneae* Tomlin, 1944, *S. (Patellopsis) nigerrima*, Smith, 1903, *S. (Patellopsis) tenuicostulata* Smith, 1903; Umhlanga Rocks, near Durban (29°25'S, 31°12'E). *S. (Patellopsis) capensis* Quoy & Gaimard, 1833, *S. (Patellopsis) concinna* Sowerby, 1824, *S. (Patellopsis) oculus* Krauss, 1848, *S. (Siphonaria) serrata* (Fischer, 1807); Waterloo Bay, Eastern Cape (33°29'S, 27°9'E). Three further *S. capensis* specimens from the South African west coast (Langebaan Lagoon; 31°15'S, 18°E) were also used. In excess of 900km of coastline separates Waterloo Bay from Langebaan lagoon to the west, and Umhlanga rocks to the east (see Chapter 2, Figure 4). The three pulmonate outgroups, two terrestrial (*Helix sp.*, *Arion sp.*) and one freshwater (*Bunupia sp.*), were collected locally in Grahamstown. For each species, collections were made over an area of 2m². PAGE requires fresh frozen tissue for analysis and two South African species were not investigated. *S. (Sacculosiphonaria) compressa* Allanson, 1959 was too small to provide sufficient proteins, and fresh tissue from *S. (Patellopsis) dayi* Allanson, 1959 was unavailable. Approximately one gram of foot tissue was removed from frozen specimens, rinsed in distilled water, immersed in liquid nitrogen and crushed with a pestle and mortar in 1ml of 0.1M phosphate buffer, pH 7.0. This tissue-buffer emulsion was then decanted into 1.5ml Eppendorf tubes and centrifuged for 10 minutes at 13,000 rpm. The supernatant was then decanted into fresh tubes and stored at 4°C until used for electrophoresis. Protein yields for each sample were determined using the Bradford protein assay method (Hammond & Kruger, 1988).

6.2.2 Electrophoretic analysis of samples

In the presence of the Laemmli buffer system (1970), polyacrylamide slab gel electrophoresis with sodium dodecylsulphate (SDS-PAGE) was performed on the samples. Protein extracts were loaded onto a ten lane gel apparatus (Hoefer Scientific Instruments, model SE 280) with a protein standard (Boeringer Mannheim, (Germany) Combitek Calibration proteins) to enable gel to gel comparisons and determination of molecular weights of the proteins in each sample. Gels were run and stained under the conditions described by Nxomani *et al.* (1994), scanned using the UVP gel documentation system (GDS 2000), and stored as Tag image File Format (TIFF) files for analysis of protein patterns. Numerical analysis of protein patterns was by the fully automated software package GelManager (Version 1.5) by pairwise comparisons of protein absorbance profiles with no manual manipulations of data. Similarity matrices were generated and dendrograms could be produced by use of Pearson's product moment correlation coefficient and the Unweighted Pair Group Method with Averages (UPGMA). Levels of genetic variability can be considered to correspond to similarity values determined from a scale between 0 and +1 produced with the similarity matrices, where 0 is a random relationship and +1 is a perfect relationship. The level at which branches join is equal to the average similarity between the groups of samples joined. A value for overall similarity within a group of samples can be read against a scale produced with the dendrogram, and a mean value can be obtained from a pairwise similarity matrix constructed from distances derived from the dendrogram. Reference markers can be used to correct gel to gel variations, but for both intra- and interspecific comparisons involving many gels, more accurate correction is achieved by designating specific protein bands common to all species as reference points enabling each lane to be standardised, and allowing analysis of high molecular weight proteins near the beginning of the gels.

6.3 RESULTS

Genetic variability estimates derived from the dendrograms were produced by a separate analysis of each species. Two values are given in Table 1. Overall similarity is derived from the initial branching point of the group when compared to a reference marker, and mean similarity represents the average distance between all individuals

calculated from a pairwise distance matrix. For two species, *Siphonaria concinna* and *S. serrata*, dendrograms are shown (Figure 1A & B). Both of these species split into at least three subpopulations, but the similarity within, and between *S. serrata* subpopulations is clearly higher than those of *S. concinna* (samples 14 & 14b were the from the same specimen). The lowest overall similarity and mean similarity values derived from dendrograms produced for each species (i.e. greatest genetic variability within populations) were associated with the planktonic developing *S. capensis*, *S. concinna* and *S. oculus*. For both overall and mean similarity within each species, values were significantly different from the direct developing species (Student's t-test = 6.11, $P < 0.001$ for overall similarity; t-test = 4.89, $P < 0.001$ for mean similarity), which all had similar, but lower levels of variability (Table 1). Of all species, the highest levels of genetic variability were for *Siphonaria concinna* and protein polymorphisms were clear for bands greater than 170 Kilodaltons (Figure 2A). Such variation in this region was, however, not present with the other two planktonic species, *S. oculus* and *S. capensis*. This was despite the fact that the samples of *S. capensis* included three South African west coast specimens from Langebaan Lagoon (Figure 4B, lanes; 7-9) where gene flow to the south coast populations, approximately 1000km away (see Chapter 2, Figure 4), is likely to be restricted. Little significance is attached to the values associated with the outgroups due to low sample numbers. As protein absorbance profiles were generally quite similar for all species including the outgroups (Figures 2A-C), the data gave little insight into the higher systematic relationships amongst the seven South African *Siphonaria*. However, when data on all species were compared in a single dendrogram, individuals of the same direct developing species (usually nine individuals per gel) nested out together and were distinct from each other and the three outgroups (Figure 3). This was not the case for the planktonic developing species. For each of these species, individuals were not grouped in mono-specific clusters, but were mixed with other species. Two of the 3 major subdivisions of the dendrogram also contained a mixture of reproductive types (Figure 3). In cluster 1, *S. capensis* nested with the outgroups with one spurious sample nesting with *S. tenuicostulata*. Within cluster 2 the *S. concinna* population split into two among the Durban direct developers *S. nigerrima*, *S. tenuicostulata* and *S. anneae*. *S. serrata*, a direct developing species of another subgenus, nested separately

with *S. oculus* as cluster 3 (Figure 3). Whilst these exceptions confused the higher systematic relationships of the group, they were consistent with the higher genetic variability associated with the planktonic developers.

Table 1. Summary of genetic similarity values and developmental mode associated with seven species of South African *Siphonaria*.

Species	N	Overall similarity	Mean similarity ($\pm$ SD)	Developmental mode
<i>Siphonaria anneae</i>	26	0.69	0.748 $\pm$ 0.075	Direct
<i>S. capensis</i>	27	0.60	0.672 $\pm$ 0.067	Planktonic
<i>S. concinna</i>	27	0.54	0.646 $\pm$ 0.091	Planktonic
<i>S. oculus</i>	26	0.63	0.689 $\pm$ 0.049	Planktonic
<i>S. nigerrima</i>	25	0.71	0.751 $\pm$ 0.084	Direct
<i>S. serrata</i>	25	0.74	0.806 $\pm$ 0.075	Direct
<i>S. tenuicostulata</i>	26	0.75	0.776 $\pm$ 0.072	Direct
<i>Arion sp.</i>	2	0.96	--	--
<i>Burnupia sp.</i>	2	0.99	--	--
<i>Helix sp.</i>	2	0.98	--	--

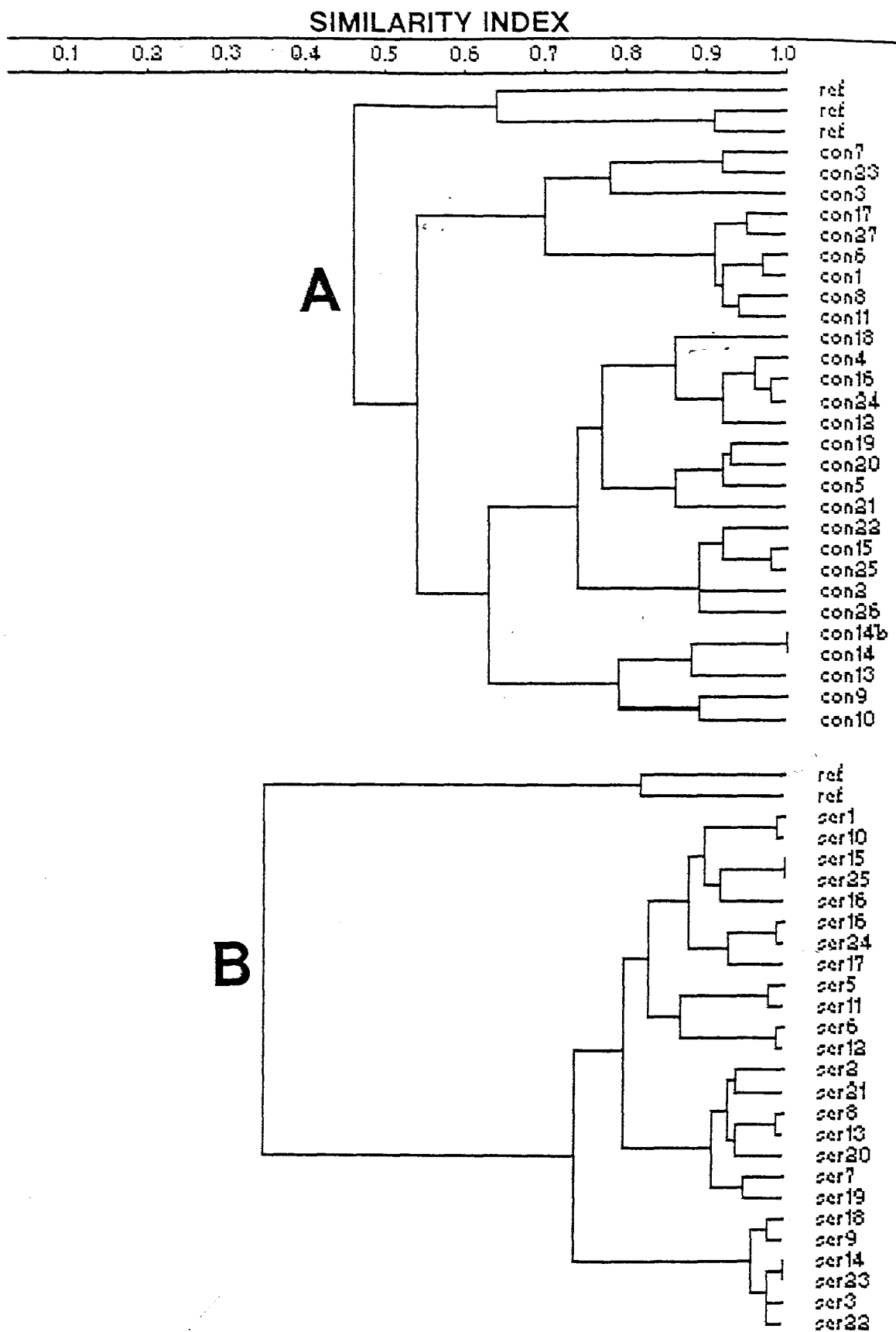


Figure 1. Dendrograms of; A. *Siphonaria concinna*, B. *S. serrata*.

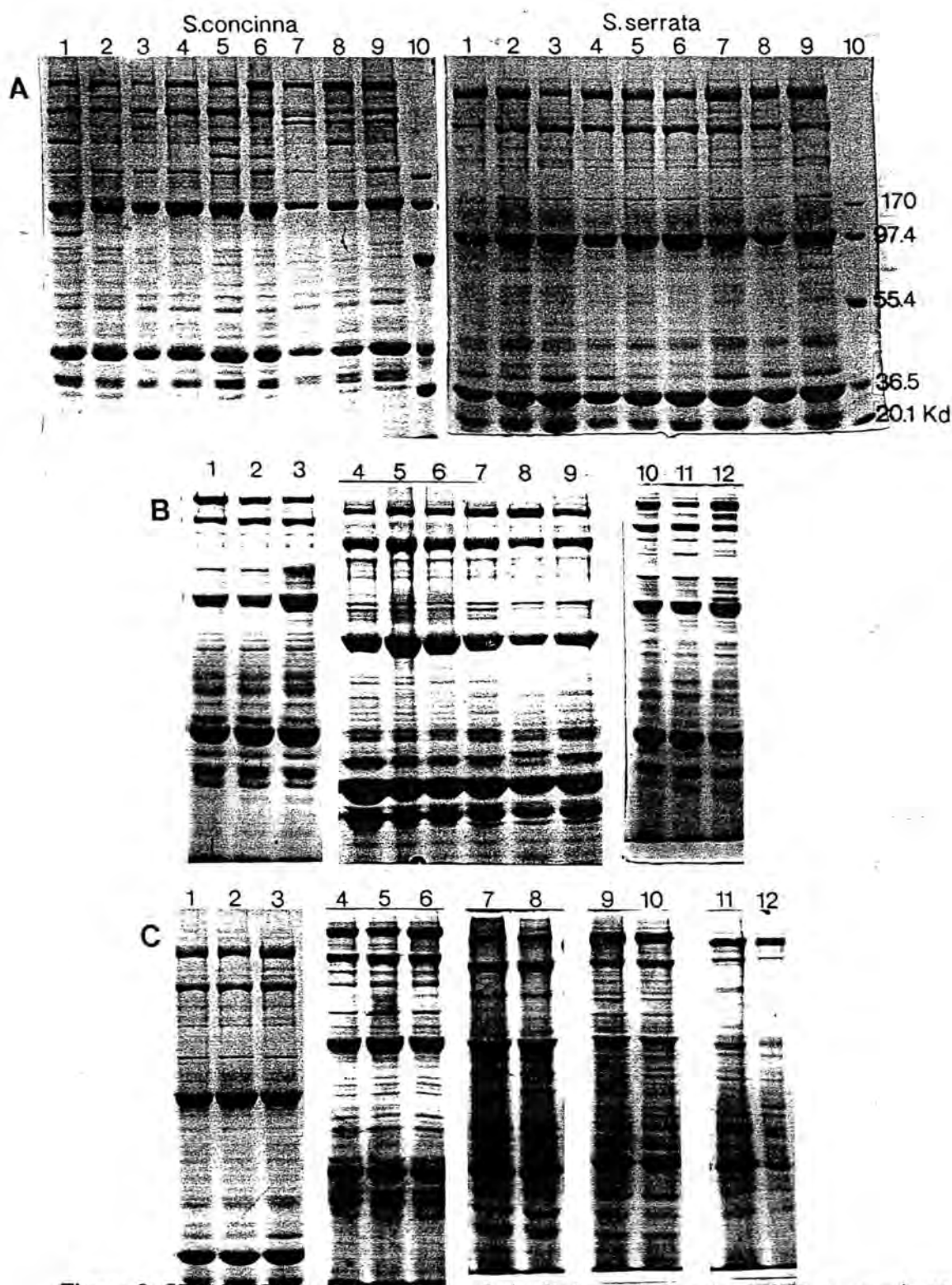


Figure 2. SDS-PAGE protein patterns of; A. *Siphonaria concinna* & *S. serrata* (protein markers in lane 10 in each case). B. *S. anneae* (lanes 1-3); *S. capensis* (lanes 4-6; Waterloo Bay, lanes 7-9; Saldhana Bay.) *S. oculus* (lanes 10-12). C. *S. nigerrima* (lanes 1-3); *S. tenuicostulata* (lanes 4-6); *Arion sp.* (lanes 7 & 8) *Helix sp.* (lanes 9 & 10) and *Burnupia sp.* (lanes 11 & 12). Molecular weights of markers indicated.

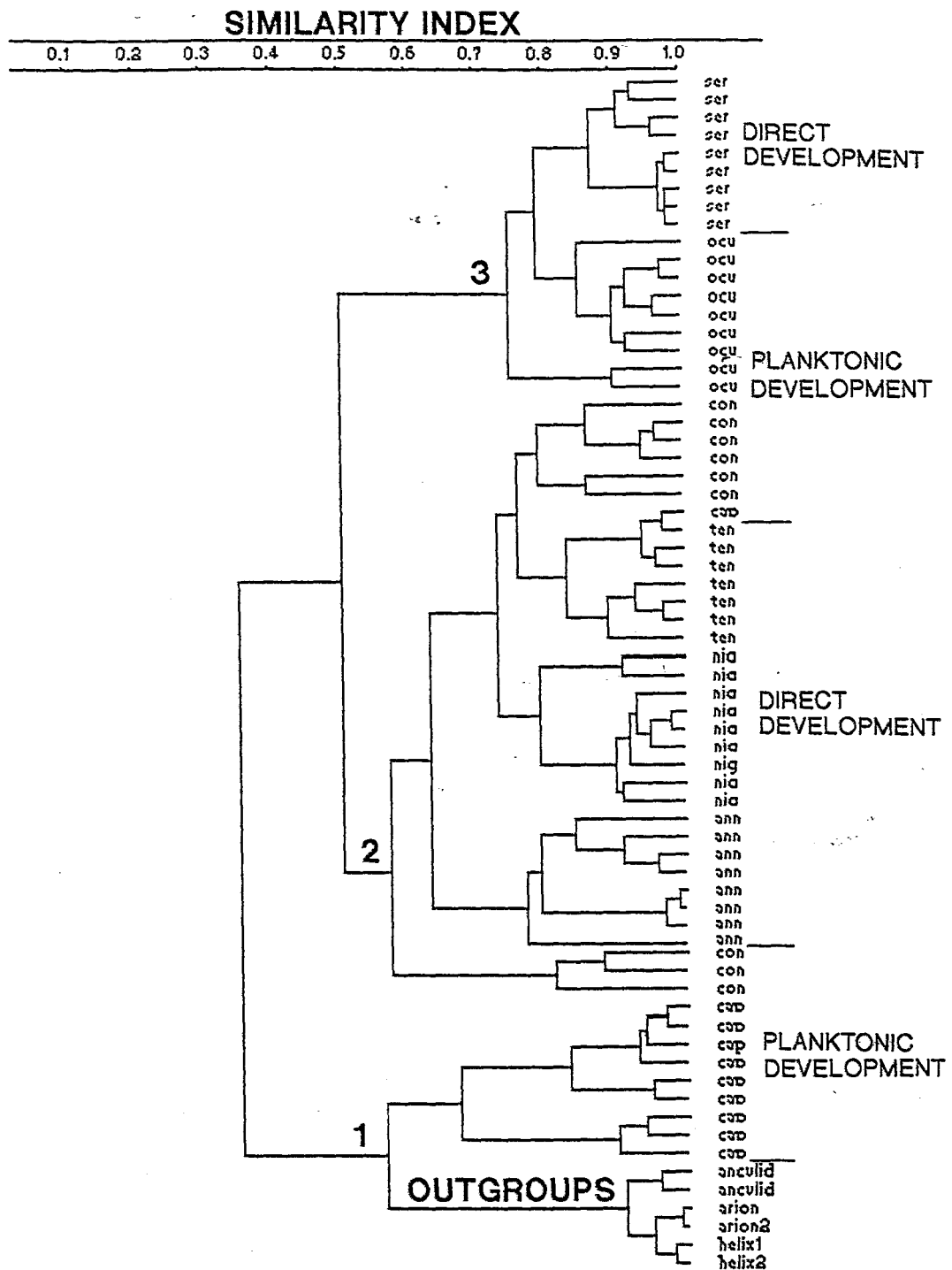


Figure 3. Dendrogram of all *Siphonaria*; *S.serrata* (ser), *S.oculus* (ocu), *S.concinna* (con) *S.tenuicostulata* (ten), *S.nigerrima* (nig), *S.anneae* (ann), *S.capensis* (cap) with the three outgroups; *Helix*, *Arion* and *Burnupia* sp (Ancyliid).

6.4 DISCUSSION

Models correlating greater gene flow, and correspondingly higher levels of genetic variability with a planktonic mode of development (Strathmann, 1986; Grant & Utter, 1987; Janson, 1987; Johannesson et al, 1990; Grant & Lang, 1991; Hunt, 1993) receive some support from this PAGE analysis of South African species. Despite a potential for dispersal associated with direct development (Highsmith, 1985; Johannesson, 1988; O'Foighil, 1989; Martel & Chia, 1991), gene flow is still likely to be less than that of planktonic dispersal, with most recruits to direct developing populations probably originating locally. The lower similarity values associated with *S. concinna* when compared to *S. oculus* and *S. capensis* I attribute to the protein polymorphisms at molecular weights above 170 Kilodaltons. This was despite the fact that three of the specimens of *S. capensis* come from a separate South African west coast population. *S. capensis*, by virtue of an even greater geographic range, may have been expected to demonstrate even greater genetic variability but there were no clear polymorphisms among protein bands of similar molecular weight. The PAGE technique indicates that genetic variation within the genus is small but, with the exception of *S. capensis*, distinct from the three outgroups (Figure 3). *S. serrata*, the only member of the subgenus *Siphonaria*, might have been expected to nest out separately from the remaining species and removal of the *S. capensis* and *S. oculus* from the similarity matrix has this effect. Removal of *S. concinna* from the same matrix implies a close similarity of the three Durban direct developers, suggesting that they may be derived relatively recently from a common ancestor, and would be consistent with a greater potential for speciation within direct developing clades (Jablonski & Lutz, 1983; Strathmann, 1986; but see Hedgecock, 1986).

With the exception of one *S. concinna* group of individuals which branch close to *S. tenuicostulata*, and *S. oculus* nesting with *S. serrata*, perhaps spuriously, all the planktonic species branch earlier than the direct developing species. This suggests that planktonic development may be the ancestral condition and direct development the derived condition, at least within the *Patellopsis* subgenus, if such relationships are indicative of the higher systematic relationships among the subgenus. Further studies, particularly an investigation of the variation at high protein molecular weights, may well provide more information upon which to base systematic information, and further

test estimates of genetic variability. However, it may be possible that only studies at the DNA level would confirm higher evolutionary relationships.

Polyacrylamide gel electrophoresis is a comparable approach to genetic studies to other protein based genetic techniques, such as Starch gel electrophoresis. It is both simpler and quicker to apply, and can potentially yield similar information. The technique can also provide information at three levels; (1) confirmation of species status, (2) determination of levels of genetic variability within species, and (3) the systematic relationships of these species. Whilst the technique failed to determine the systematic relationships of South African *Siphonaria*, and despite the different subgeneric status attributed to *S. serrata*, it indicates that variation within the genus, and between subgenera may be very small.

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CHAPTER 7

USE OF DNA FINGERPRINTING (RAPDS) TO DETERMINE THE SYSTEMATIC RELATIONSHIPS AMONG THE INTERTIDAL LIMPETS, *SIPHONARIA* (PULMONATA: GASTROPODA), WITH IMPLICATIONS FOR THE EVOLUTION OF LARVAL DEVELOPMENT

7.0 ABSTRACT

The systematic relationships among 12 species of *Siphonaria* were assessed using the technique of RAPD-PCR DNA fingerprinting and compared to a related outgroup, a species of *Trimusculus*. Previous systematic relationships proposed by authors on the basis of morphology are confirmed for nine of these species and one subgenus, the *Patellopsis* may be polyphyletic. The status of two species in the subgenus *Sacculosiphonaria* remains unclear. A strong systematic basis to mode of larval development is revealed, and the earlier branching of direct developing species, compared to planktonic developing species, suggests that the primitive developmental condition within *Siphonaria* may be direct development.

7.1 INTRODUCTION

In the field of marine ecology there has been much discussion on the adaptive nature of different modes of reproduction employed by benthic marine invertebrates (see reviews by Jablonski & Lutz, 1983; Grahame & Branch, 1985; Strathmann, 1986). Most studies have focused on correlations between the ecological circumstances under which a trait is presumed to be adaptive. Adaptive explanations for the distribution of developmental types have consistently featured in the published literature (for example, Woodward, 1909; Thorson, 1950; Knox, 1955; Mileikovsky, 1975; Grahame & Branch, 1985; Strathmann, 1986). There are problems with this approach as the argument is linked to the concept of optimal strategies which assume the trait is free to evolve without any phylogenetic constraints (Grahame & Branch, 1985; Reid, 1989; Stearns, 1992). Recent investigations on the systematic distribution of alternative modes of larval development have now begun to consider the importance of

phylogenetic history in determining larval developmental type (Reid, 1990; Chambers & McQuaid, 1994^a).

Unlike the more primitive patellid limpets, with which they often co-exist, the Siphonariidae is a family of limpet-like pulmonates distantly related to the patellids. It has been proposed that the siphonariid limpets are derived from either high shore or terrestrial ancestors (Hubendick, 1947; Boland, 1950; Yonge, 1952; Solem, 1985). However, like many other groups of intertidal gastropods, they demonstrate a variety of developmental types ranging from planktotrophic larval development to the emergence of fully metamorphosed crawling larvae (Thorson, 1950; Knox, 1955; Berry, 1977; Chambers & McQuaid, 1994^a). Two species, *Siphonaria alternata* Say and *S. hispida* Smith, possess a dual development capacity, hatching with both the velar swimming apparatus, and a crawling foot (Marcus & Marcus, 1960; Zischke, 1974). While the evolutionary trend in most groups of intertidal gastropods is from a primitive condition of planktonic development to the derived condition of direct development (Jablonski & Lutz, 1983; Strathmann, 1986) it has been suggested that the siphonariid limpets may represent an interesting evolutionary example of a trend in the other direction (Chambers & McQuaid, 1994^a). The Siphonariidae includes two genera, *Williamia* and *Siphonaria*, the latter being by far the most speciose (Hubendick, 1946, 1947). While both genera are reported to demonstrate planktonic larval development (Marshall, 1981; Chambers & McQuaid, 1994; M. Hadfield, pers. comm.), some primitive *Siphonaria* subgenera appear to be direct developers (Hubendick, 1946, 1947; Boland, 1950; Chambers & McQuaid, 1994^a).

Constructing phylogenies within groups of organisms tells us not only the evolutionary relationships among that group, but can also allow the formulation of evolutionary hypotheses concerning the distribution of shared ecological characteristics and their adaptive significance (Coddington, 1988; Reid, 1989). As yet, no genetic studies on the relationships among the siphonariids have been conducted, and current classification is based on the morphology of the reproductive organs, the respiratory organs and shell characteristics (Hubendick, 1946; Allanson, 1959; Chambers & McQuaid, 1994^b). Despite Hubendick's systematic review of the genus, some relationships still remain unclear (Jenkins, 1981, 1983).

The recent application of molecular biological approaches to classification, using sequence information in particular, has led to new insights into the systematic relationships among organisms. The development of Randomly Amplified Polymorphic (RAPD) DNA markers, generated by the Polymerase Chain Reaction (PCR) enables estimations of genetic variation between organisms without prior knowledge of sequence information (Williams *et al.*, 1990, 1993; Welsh & McLelland, 1990; Hadrys *et al.*, 1992). Short nucleotide sequences, known as primers, determine the number and size of amplified fragments of DNA. Complimentary sequences result in primary sites randomly distributed throughout the genome and polymorphisms in such sites result in differing amplification products, indicated by the presence or absence of fragments. Products are run under an electric field on a gel and the resulting profile is referred to as a 'DNA fingerprint'. This method has been used extensively to detect intra-specific variations (Welsh & McLelland, 1990, 1991; Kambhampati, 1992; Procunier *et al.*, 1993) and more recently, variations between species of aphids (Puterka *et al.*, 1993), parasitic protozoa (Tibayrenc *et al.*, 1993), tilapia fish (Bardakci & Skibinski, 1994) and intertidal gastropods (Crossland, *et al.*, 1993).

In this chapter I use the technique of RAPD-PCR DNA-fingerprinting to investigate the systematic relationships of nine southern African *Siphonaria* and three foreign species. DNA fingerprints generated by these species were compared to another, related southern African pulmonate limpet, *Trimusculus costatus* Krauss, 1848, which also served as an outgroup in the analysis of relationships. Comparisons were made using the fully automated software programme, GelManager (Version 1.5) which constructs dendrograms which can be hypothesised to reflect systematic relationships among groups of organisms. These dendrograms can then be used to formulate hypotheses concerning the evolution of particular characters. The validity of the current system of classification of *Siphonaria* is investigated and the evolution of developmental mode among the genus is considered.

7.2 METHODS

7.2.1 Sample collection

The nine local species belong to three subgenera, given in parentheses. These include *Siphonaria* (*Patellopsis*) *anneae* Tomlin, 1944, *S.*(*Sacculosiphonaria*) *compressa*

Allanson, 1959, *S. (Patellopsis) dayi* Allanson, 1959, *S. (Patellopsis) nigerrima*, Smith, 1903, *S. (Siphonaria) serrata* (Fischer, 1807) and *S. (Patellopsis) tenuicostulata* Smith, 1903 which demonstrate direct development. *S. (Patellopsis) capensis* Quoy & Gaimard, 1833, *S. (Patellopsis) concinna* Sowerby, 1824 and *S. (Patellopsis) oculus* Krauss, 1848 which demonstrate planktonic development. Specimens of these species were collected from locations described in Chapter 2. Two preserved species, *S. (Siphonaria) atra* Quoy & Gaimard, 1833 (direct development) and *S. (Sacculosiphonaria) japonica* Donovan, 1834 (planktonic development) were available from Hong Kong, and preserved specimens of *S. (Kerguelenella) lateralis* Gould, 1846 (direct development) collected from the sub-Antarctic island, South Georgia were also available. *Trimusculus costatus* Krauss, 1848 was collected locally, north of East London (32°45'S, 27°55'E) for use as an outgroup reference. All specimens were preserved in 70% alcohol before DNA extraction.

7.2.2 DNA extraction

Extraction of DNA from muscle tissue essentially followed the method of Gold & Richardson (1991) with some modifications. Parasitism of *Siphonaria* is not uncommon on southern African shores (Hodgson *et al.*, 1993) so, to prevent extraneous DNA contaminating samples, sections of the foot muscle were removed, scraped clean of any tissue from the body cavity and rinsed in sterile, double-distilled water before extraction began. Extraction involved approximately 0.5g of foot muscle cut into small pieces and ground into a fine powder in liquid nitrogen using a pestle and mortar. This powder was re-suspended in 500µl of STE (0.1M NaCl, 50mM-Tris and 1mM EDTA; pH 7.5) in an eppendorf tube. After adding 125µl of 20% SDS (sodium dodecylsulphate), the mixture was vortexed. DNA purification involved successive extractions in an equal volume of 25:24:1 phenol:chloroform:isoamyl alcohol. To the final supernatant 1/10 volume of 2M NaCl and two volumes of ice-cold ethanol were added. Samples were gently mixed and placed overnight at -20°C to precipitate the DNA. Recovery of DNA involved centrifugation at 13,000rpm for 10 minutes at 4°C. The tubes were dried under vacuum for 5 minutes and the pellets were re-suspended in 200µl of TE (10mM Tris-HCl, 1mM Na₂EDTA.H₂O; pH 7.5). A total of five individuals per species were extracted.

7.2.3 DNA amplification

Four decamer primers were used in this investigation (Table 1). Amplification was in a Hybaid Omnigene (UK) thermal cycler. Amplification reactions were performed with 100ng of template DNA, 100pM of the respective primer, 2.5mM of each of the deoxynucleotides (dATP, dTTP, dCTP and dGTP; final concentration of 100mM) (Boeringer Mannheim, Germany), 5µl of 5% acetamide, 24mM MgCl (Biolabs, UK), 5µl Taq polymerase buffer (Promega, USA) and one unit of Taq polymerase (Advanced Biotechnologies, UK). The reactions were carried out in a final volume of 50µl made up with sterile double-distilled water. Each reaction mixture was overlaid with an equal volume of mineral oil to prevent evaporation. One negative control (absence of template DNA) was performed for each set of amplifications.

Amplification cycles were as follows;

1. Initial denaturation @ 94°C for 180 seconds
2. Denaturation @ 94°C for 30 seconds
3. Primer annealing @ 36°C for 30 seconds
4. Primer extension @ 72°C for 60 seconds
5. Final extension @ 72°C for 240 seconds

Steps 2-4 were repeated over 40 cycles.

Table 1. Nucleotide sequences of primers

Primer	Sequence	% GC
2	TGG ATG AAC G	50
6	AAC CGA TGC T	50
10	GTG ATG AAG G	50
11	ACC TGC GTT A	50

7.2.4 Electrophoresis of samples

Approximately 16µl of product were separated electrophoretically in 10% discontinuous gels using the buffer systems of Laemmli (1970) in TBE buffer (0.089M Tris, 0.089M boric acid; pH 8.0). Gels were fixed with 10% ethanol, 0.5% acetic acid

for 10 minutes, stained with 0.1% silver nitrate solution for a further 10 minutes and then rinsed in double-distilled water. Development was in an alkaline solution of 1.5% sodium hydroxide, 0.01% sodium borohydrate and 0.15% formaldehyde. Numerical analysis of gels was by the method of Chapter 6 using the software program GelManager (1.5).

7.3 RESULTS

Each primer produced fingerprints with large numbers of bands (see Figures 1 & 2). While too few bands can give inaccurate estimations of variability, there are problems with too many bands in that difficulties arise in deciding whether or not corresponding bands are identical (Crossland *et al.*, 1993). The GelManager program involves no manual manipulations of data and allows the complex banding patterns obtained in this study to be objectively analyzed (see Nxomani *et al.*, 1994).

Amplifications involving primer 2 were made initially on South African species, with amplifications of further foreign species added to the data matrix later (although the outgroup, *Trimusculus costatus*, was not included in the analysis). As a result, amplification conditions may not be completely standardised for all species using this primer. Gels were run comprising five individuals per gel, totalling 12 gels (one for each species) in the overall analysis for this primer (Figure 3). In this case the first group to nest separately was *Siphonaria (Kerguelenella) lateralis* (branch 1, Figure 3). The remaining species separated into two main groups. The first (branch 2) comprised members of the subgenus *Siphonaria* and *S. (Sacculosiphonaria) japonica*. Members of the subgenus *Patellopsis* and *S. (Sacculosiphonaria) compressa*, clustered together at branch 3 (Figure 3) and constituted all the southern African species, except *S. (Siphonaria) serrata* which nested with the foreign species, *S. (Siphonaria) atra*, *S. (Sacculosiphonaria) japonica* and included one spurious *S. (Patellopsis) nigerrima* individual. Members of the *Patellopsis* clade then separated into two groups. Branch 4 includes the planktonic developing species, *S. concinna*, *S. oculus* and *S. capensis*, and indicated a close similarity between *S. concinna* and *S. oculus*, with *S. capensis* more or less distinct, but also closely related (Figure 3). Branch 5 contained all the southern African direct developers, although *S. compressa* was distinct from these (Figure 3). *S. anneae* and *S. tenuicostulata* nested together and were distinct from *S.*

nigerrima (except the spurious *nig3*) and *S. dayi*, both of which failed to nest as coherent groups (Figure 3). Branch 6 suggested a close similarity between *S. serrata* and *S. atra* (both direct developing species of the subgenus *Siphonaria*), but each species failed to nest as coherent a group. *S. japonica* was distinct from these two species but nested with 3 individuals of *S. atra* and *S. nigerrima* (branch 7, Figure 3). Associations within the primer 2 dendrogram appeared consistent with the subgeneric status assigned to the species, although *S. compressa* and *S. japonica* (subgenus *Sacculosiphonaria*) failed to nest together. Overall, the earlier branching among the dendrogram involved the direct developers, with *S. lateralis* nesting separately first. For primer 6 all amplifications were made over a period of a few weeks with identical reagents. This primer indicated similar associations to those produced by primer 2, but with some exceptions (Figure 4). In this analysis *Trimusculus costatus* was distinct from the remaining species (branch 1, Figure 4), and the next group to nest separately (branch 2) included *S. serrata* and *S. atra*, consistent with the dendrogram generated by primer 2 (Figure 3). Of the remaining species, *S. lateralis* nested as a distinct group but included one spurious *S. serrata* individual, *ser1* & *ser1B* (the same individual amplified twice: Figure 4). This group was distinct from the members of the *Patellopsis* clade, and *S. compressa* and *S. japonica* (subgenus *Sacculosiphonaria*) at branch 3 (Figure 4). *S. japonica* was distinct from all the *Patellopsis* species (branch 4), but *S. compressa* failed to nest as a distinct group and separated among the *Patellopsis* species (branch 5). Subsequent branching of this subgenus was generally consistent with that of primer 2: a separation of planktonic developing species from direct developing species was clear, and close associations between *S. concinna* and *S. oculus*, and *S. annea* and *S. tenuicostulata* were again evident (Figure 4). The remaining two primers tested yielded different, but inconclusive results. Primer 10 after initial amplifications of a few different species indicated very little variation between species (Figure 2) and further amplifications were not made. Primer 11 produced highly variable fingerprints, but differences among species were on the same scale as differences between species and any systematic relationships were unclear (Figure 5). As a consequence, analysis did not involve all individuals of each species since the outgroup, *T. costatus* failed to nest distinctly from the remaining species, although three individuals of this species did nest together (branch 1, Figure 5).

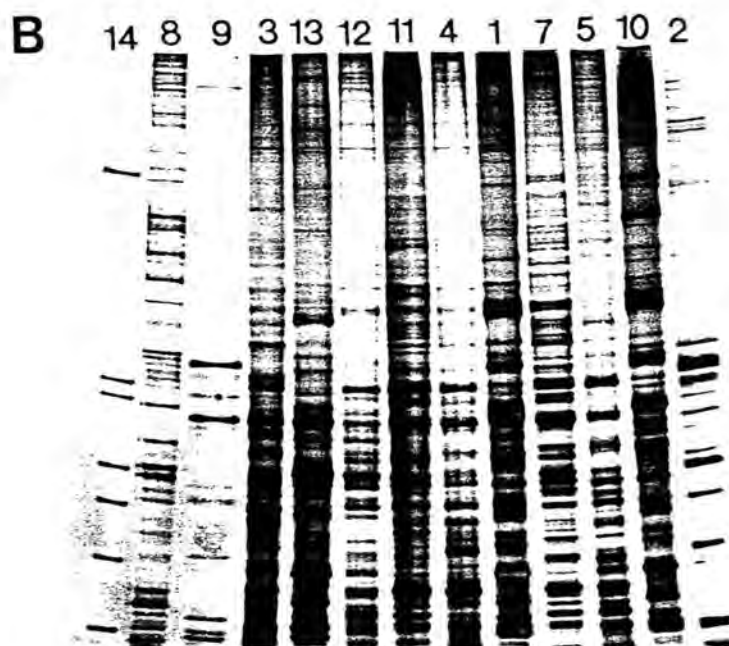


Figure 1. Examples of RAPD patterns from (A) primer 2 and (B) primer 6. (1. *S.anneae*; 2. *S.atra*; 3. *S.capensis*; 4. *S.concinna*; 5. *S.compressa*; 6. *T.costatus*; 7. *S.dayi*; 8. *S.japonica*; 9. *S.lateralis*; 10. *S.nigerrima*; 11. *S.oculus*; 12. *S.serrata*; 13. *S.tenuicostulata*; 14. size marker.

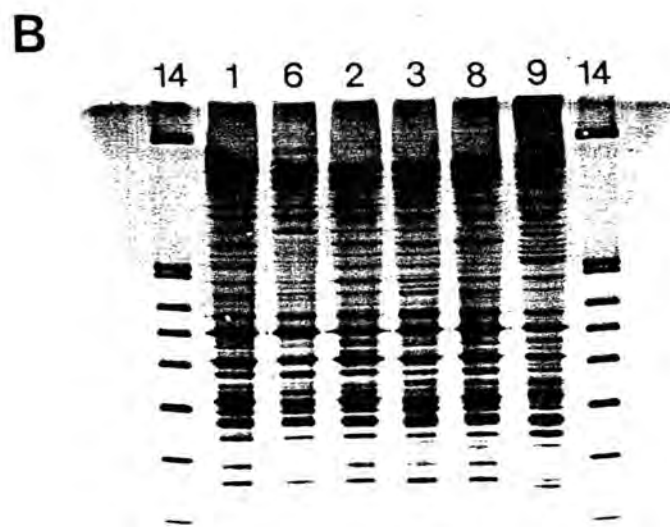
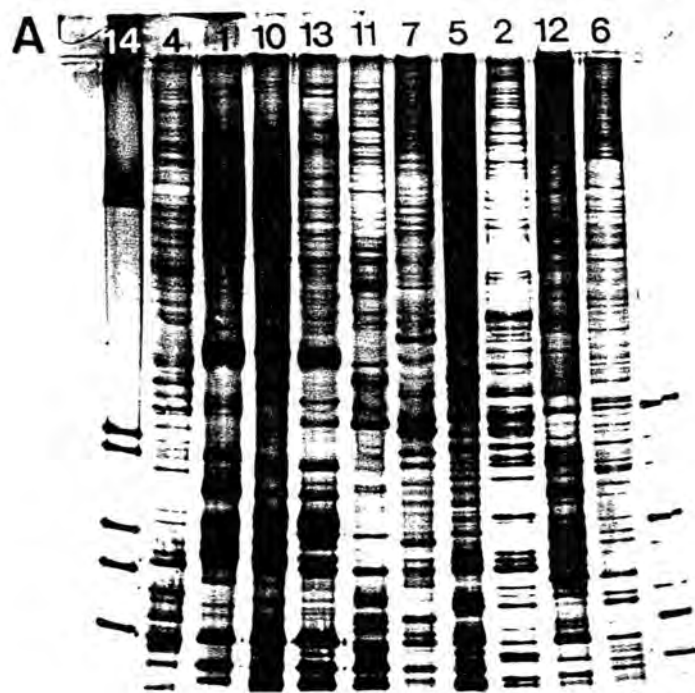


Figure 2. Examples of RAPD patterns from (A) primer 11 and (B) primer 10. Species numbered as in Figure 1.

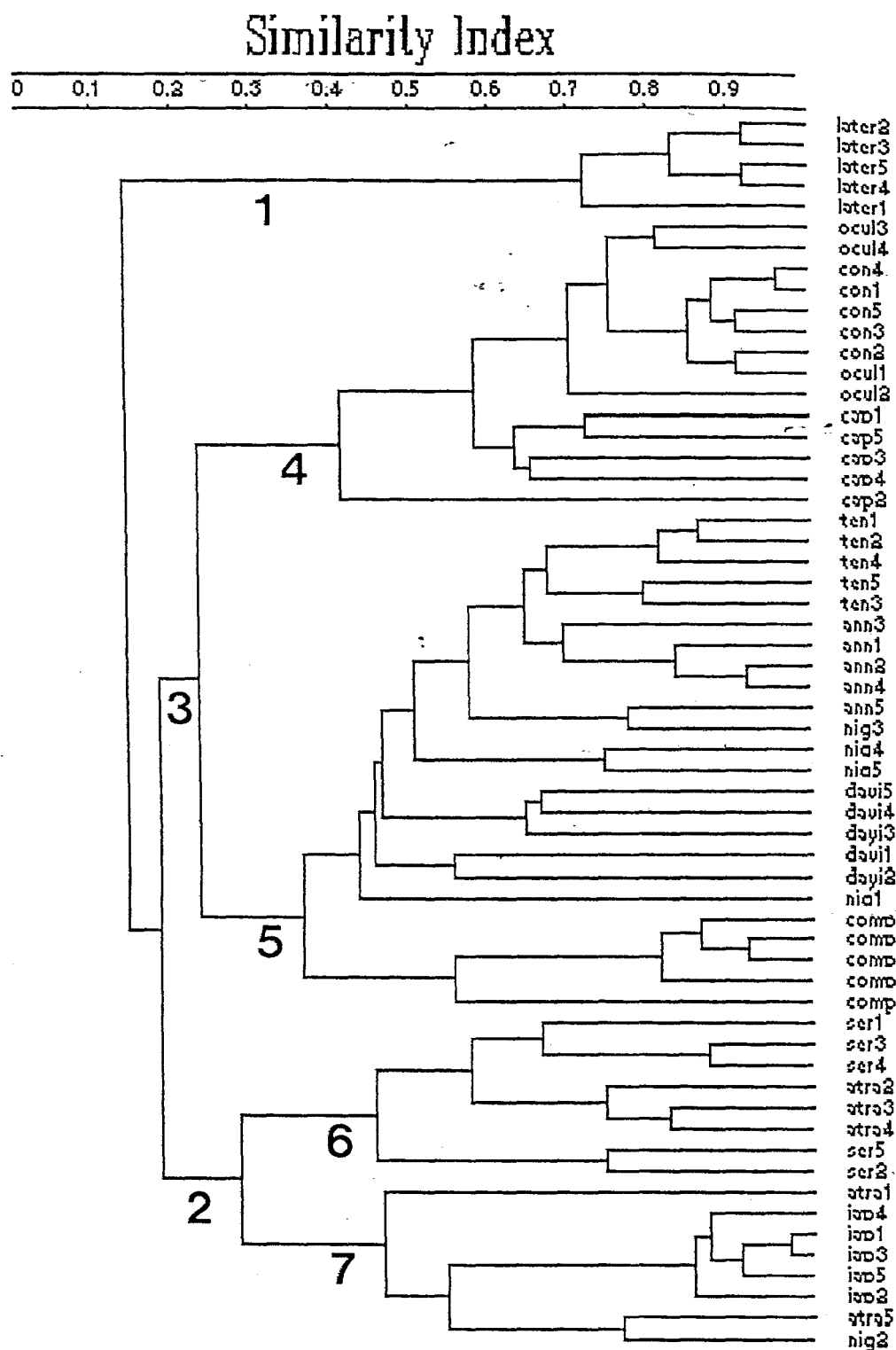


Figure 3. GelManager dendrogram based on primer 2. Abbreviations: ann. *S. annea*; atra. *S. atra*; cap. *S. capensis*; con. *S. concinna*; comp. *S. compressa*; cost. *T. costatus*; dayi. *S. dayi*; jap. *S. japonica*; later. *S. lateralis*; nig. *S. nigerrima*; ocul. *S. oculus*; ser. *S. serrata*; ten. *S. tenuicostulata*. Major clusters: 1. Subgenus *Kerguelenella*, 2. Subgenus *Siphonaria* & *S. (Sacculosiphonaria) japonica*, 3. Subgenus *Patellopsis* & *S. (Sacculosiphonaria) compressa* separating into planktonic (4) and direct (5) developing species.

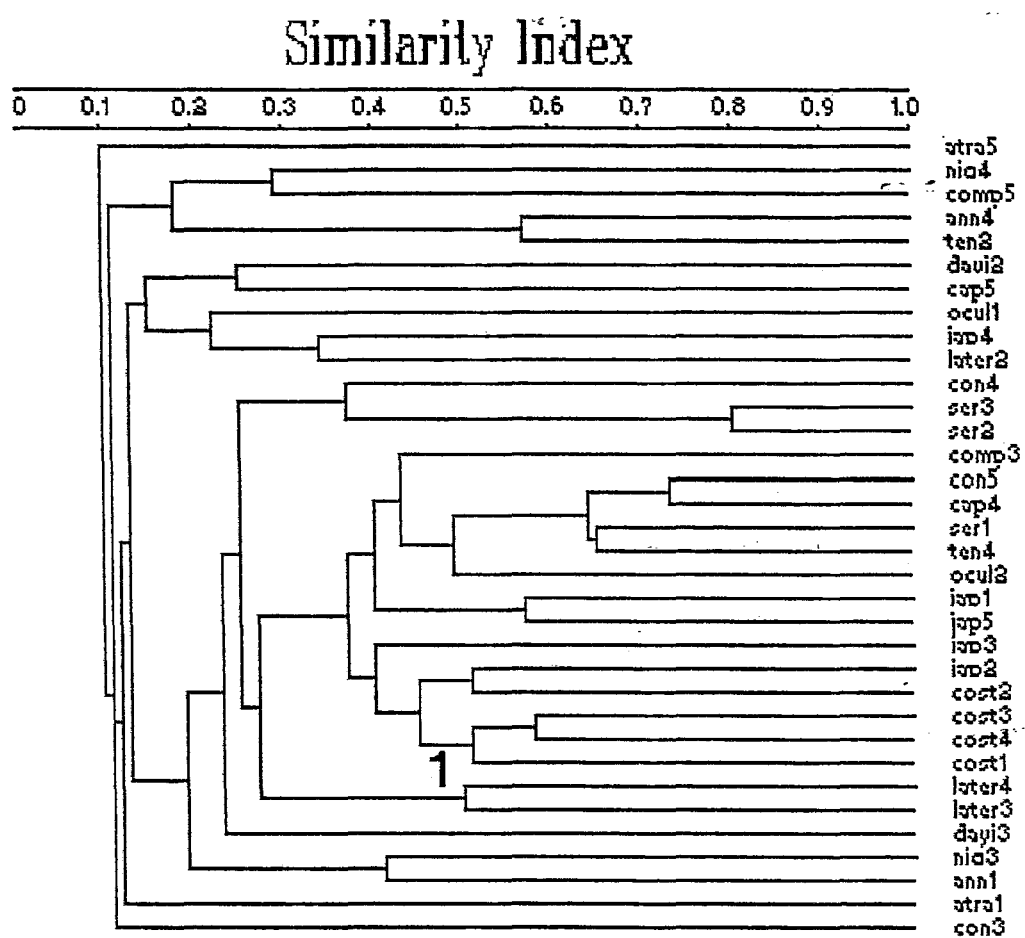


Figure 5. GelManager dendrogram based on primer 11. Abbreviations as in Figure 3.

7.4 DISCUSSION

Although the systematic relationships among *Siphonaria* are unclear, and the subgeneric status ascribed to species by Hubendick (1946) has been questioned (Allanson, 1959; Jenkins, 1981), the results of this study are consistent with the subgeneric status of species described in Chambers & McQuaid (1994^a). Members of the *Patellopsis* clade were distinct from the remaining species and separated into planktonic developing and direct developing groups suggesting that this group may be polyphyletic. This grouping was not apparent from the grouping produced by the PAGE analysis of the previous Chapter, and indicates that the PAGE analysis was insufficient to resolve the higher systematic relationships among the group. The subgeneric association between the direct developing species of the *Siphonaria* clade was also confirmed by this study, but the species of the subgenus *Sacculosiphonaria* failed to constitute a coherent group. Given the ability of RAPD-PCR to discriminate among other species, the taxonomic association between *S. compressa* and *S. japonica* may be incorrect. This is interesting as one (*S. compressa*) is a direct developing species and the other (*S. japonica*) is a planktonic developing species. Although developmental mode may have some ecological basis, its distribution among the *Siphonaria* suggest a systematic basis, as reported for another group of intertidal molluscs, the littorinid periwinkles (Reid, 1989,1990).

A greater potential for speciation by direct developing groups has been recognised due to limited gene flow between populations. Conversely, species with planktonic dispersal are likely to be more widespread and exhibit greater gene flow reducing the probability of both extinction and speciation (Jablonski & Lutz, 1983; Reid, 1989,1990). The close genetic similarities between a number of groups are indications of recent common ancestry. Several direct developing species, particularly those of the subgenus *Patellopsis*, nest very closely together in Figures 3 and 4. Dendrograms of both primer 2 and primer 6 fingerprints indicate a fairly recent common ancestor for *S. tenuicostulata* and *S. anneae*, and possibly for *S. serrata* and *S. atra* as well. In both cases similarities were close enough for these species to nest clearly as coherent groups. This was also true for two similar planktonic species, *S. concinna* and *S. oculus*, which are often sympatric and have very similar egg masses (Allanson, 1959;

Chambers & McQuaid, 1994^a). The employment of further primers may be necessary to discriminate between these species.

Dendrograms of relationships can be considered as hypotheses of phylogenetic relationships among taxa and can also give information concerning the evolution of various characters, and in this case, reproductive mode in particular. Direct developing species nest earlier than planktonic species, generally branching out first, and if the dendrograms are a correct approximation of the phylogenetic relationships within the genus, and we assume direct development to be the plesiomorphic condition, then there is evidence of a shift (in at least some groups) from this primitive condition of direct development to planktonic development. Given the possible high shore, or terrestrial origin of this group of pulmonates (Hubendick, 1947; Boland, 1950; Yonge, 1952; Solem, 1985) we may have an unusual situation where the ancestral condition of the genus is direct development. The evidence to support this is, however, equivocal. The closest related group, *Williamia*, is not reported to show direct development (Marshall, 1981; M. Hadfield, pers. comm.), while another group, once thought to be closely related to *Siphonaria*, the Trimusculidae (Hubendick, 1978). However, this group is now classified into a new order the Eupulmonata, the group now thought to have given rise to the terrestrial pulmonates (see Hazsprunar, 1990; Nordsieck, 1992), and members of this family all appear to be direct developers (Haven, 1973; pers. obs.). Further studies including more species of different subgeneric status would be able to confirm this hypothesis. Whatever the primitive condition within the genus, a shift in mode of larval development may have occurred more than once within the family, given its systematic distribution across several subgenera. Studies on species which appear to have a dual developmental capacity, retaining both the velar apparatus and the crawling foot (see Marcus & Marcus, 1960; Zischke, 1974) may provide clues to the evolutionary processes causing a change in developmental mode, and possibly the direction of that change.

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CHAPTER 8

FINAL DISCUSSION

The concept of adaptation has always been difficult to define (Mayr, 1983; Krimbas, 1984; Reeve & Sherman, 1993). Stearns (1992) defines adaptation in two ways: functionally, it is considered to be a change in phenotype in response to a specific environment; phylogenetically, it requires that we know both the current and ancestral character state and habitat, although this will involve details which may never be available. At the other end of the conceptual spectrum of biological explanation is the concept of constraint; any pattern or state that can be attributed to phylogeny, rather than recent micro-evolutionary processes is a constraint. This can be used as a null hypothesis to test adaptation, and specifies limitations on phenotypic variability caused by either the laws of physics, or the developmental system.

The reproductive strategies of marine invertebrates have been related to various aspects of their ecology. Known as the demographic theory (Reid, 1989), these include the isolation of the habitat, latitude, depth, intertidal height and body size, among others. Different strategies are regarded as optimal solutions resulting from different selective regimes (r-K selection theory), mortality schedules (bet-hedging models) and variations in environmental stability. These models are based on the postulates of resource limitation and optimisation of reproductive strategy by natural selection. However, the different patterns associated with marine reproductive strategies are often extremely complex and it is unlikely that one unifying model will be sufficient to explain these complexities. Further, different dispersal capabilities associated with different developmental modes can result in different rates of extinction and speciation, ultimately affecting patterns of phylogeny (Jablonski & Lutz, 1983; Reid,

1989). Consequently, evolutionary and ecological traits will usually be co-adapted and difficult to separate.

It has been the purpose of this thesis to investigate and try and separate these components among a little studied group of marine invertebrates, the siphonariid limpets. It is for this reason that Chapter 2 re-appraises the range of species on South African shores and characterises for the first time many aspects of their reproductive strategies. The group, and direct developing species in particular, are far more speciose than previously described (Allanson, 1959; Kilburn & Rippey, 1982) with nine species occurring on the South African coast. Among these species there appears to be no clear correlation between developmental mode and habitat or distribution (either intertidal or geographical), but a strong correlation exists between egg capsule size and developmental mode. Worldwide, there is evidence for some demographic models of reproductive strategy among the 26 species reviewed. Planktonic developers are more widespread than direct developing species, and these species often have a greater geographic range. The evidence for other adaptive models, however, is limited. Egg capsule size does seem to be correlated with developmental mode (with only one exception) and intertidal distribution (direct developing species generally occur higher on the shore). There is no clear relationship between body size and developmental mode, although the smallest known species of *Siphonaria* is a direct developer, and the largest a planktonic developer. However, there appears to be some systematic basis to developmental strategy, although the subgenera *Patellopsis* and *Sacculosiphonaria*, including most southern African species, appear exceptional. Two possible explanations exist: either the current subgeneric status of these species is incorrect and re-classification necessary, or, that developmental mode is a more labile trait subject to local selection pressures.

The correlations between egg mass structure and developmental mode among, and between, genera are interesting. Such examples may represent convergent evolution and adaptive explanations can be sought to explain how egg mass structure is controlled by natural selection. In investigating life-history distribution and the structural differences between egg masses, Chapter 3 achieves two things. Firstly, in assessing the intertidal distribution of three sympatric species with different developmental modes and egg mass structure, it is clear that both planktonic (S.

concinna) and direct development (*S. serrata*) are equally viable options under similar selective pressures. Secondly, it reveals an important component of life-history evolution for species depositing benthic egg masses, that of egg mass structure. It appears that while relationships between egg capsule size, developmental mode and egg mass structure may be complex, involving a variety of physiological trade-offs, there is compelling evidence to suggest that the physical structure of egg masses, and their deposition in particular microhabitats represent adaptive responses to local conditions, and mode of larval development. Adults and juveniles of two of these species (*S. concinna* and *S. serrata*) occur in similar microhabitats and are thus likely to be under similar selection pressures. In having different modes of larval development, there appears to be more than one optimal solution in a particular selective regime. However, both species seem to apportion the same amount of energy to reproduction for each spawning episode, and also annually and there seems to be an optimal allocation of resources to reproduction despite such differing life-histories. This suggests that, while selection can act upon some quantitative life-history traits (ie, size and number of offspring) the qualitative differences between different reproductive modes may be phylogenetically constrained.

Genetic investigations using Polyacrylamide gel Electrophoresis (PAGE) confirm the status of species described in Chapter 2 and indicate greater genetic variability associated with planktonic developing species than direct developing species. If this is so, then a greater potential for dispersal must be attributed to a planktonic mode of existence and a greater potential for speciation is likely for direct developing species. These findings are consistent with some of the differences in life-history characteristics of two species, revealed in the previous Chapter. *S. concinna*, a planktonic developer with faster growth rates, higher mortality and greater fecundity would be more likely to survive local extinctions and such characteristics seem suited for temporally unstable habitats. Conversely, the direct developing *S. serrata*, which grows slower but appears to live longer has lower mortality and is less fecund, would be able to colonise local habitats more rapidly, once established. Population bottlenecks and the founder effect would then make speciation episodes more likely. However, it would in turn be more vulnerable to local extinctions.

The relationships revealed by RAPD-PCR DNA fingerprinting support much of the classification systems based on morphology but also have interesting implications with regard to the evolution of larval development. Failure of the two primers used to resolve between some direct developing species, and two related planktonic developing species indicates the close similarity within these groups. This may result from recent speciation events, thought more likely among direct developing species (Jablonski & Lutz, 1983; Reid, 1989, 1990). But the earlier branching of direct developing species in the PAGE study is inconsistent with the results of Chapter 7 which indicate that direct development may be the plesiomorphic condition in, and among, some *Siphonaria* groups. I attach more significance to the higher systematic evidence from the DNA investigations which is controversial as the loss of a veliger stage in larval development is thought to be unlikely, but not impossible (see Strathmann, 1986; Reid, 1989). Furthermore, this process is usually accompanied by the loss of larval organs possessed by planktonic developers, which appear to be retained in direct developing siphonariids. (Ruthensteiner, pers. comm.). However, the ancestral habitats of the group (Boland, 1950; Yonge, 1952; Morton, 1955; Solem, 1985) would make direct development a likely strategy for early, primitive members of this group.

Possible clues to the ancestry of this group lie in their relationships to other primitive pulmonate groups, but these are controversial and remain largely unresolved (see Hubendick, 1978; Solem, 1985; Haszprunar & Huber, 1990; Bieler, 1992; Nordseick, 1992). The recent discovery of their capacity for metabolic rate depression (Marshall & McQuaid, 1991), may suggest a common ancestry with more advanced pulmonates, rather than an independent evolutionary origin of this ability.

One of the pre-requisites for a fully terrestrial existence must be direct developing offspring. The closest related group was thought to be the Trimusculidae, which, along with the Siphonariidae comprised the superfamily, Siphonariacea (Hubendick, 1978). These primitive limpets are known to be direct developers (for example, *Trimusculus conica* (Haven, 1973), *T. costatus* (pers. obs.)), but, on the basis of neural morphology, their relationship with the Siphonariidae is questioned (Haszprunar & Huber, 1990). They have now been classified into a new order, the Eupulmonata, a group thought to have given rise to the terrestrial pulmonates (Bieler, 1992; Nordseick, 1992). Further evidence on the basis of sperm morphology also indicates a more distant

relationship than previously supposed (A. Hodgson, pers. comm.). If this is so, then the group most closely related to *Siphonaria* are the *Williamia* which are reported to have a planktonic larval stage (Marshall, 1981) and planktonic development may indeed be the primitive larval condition among the group.

One must be cautious with this type of speculation. The assumption that if taxa carry primitive (plesiomorphic) characters need not mean that all other characters are necessarily primitive as well. In addition, there are obvious dangers with the phylogenetic approach to the study of adaptive character evolution in that homoplasies and temporal variation in selective regimes can make hypotheses of ancestral character states and selective environments unreliable (Frumhoff & Reeve, 1994).

As far as the life-history characteristics covered in this thesis are concerned, selection seems to operate at two levels: microevolutionary processes appear to shape a variety of life-history traits, whilst macroevolutionary processes operate on qualitative differences such as mode of larval development. Egg mass structure, governed by both local conditions and developmental mode, may fall in between the two. The question then remains: "What drives a change in reproductive mode?" Perhaps such a question can be answered by studies on the species which appear intermediate.

Using the *Siphonaria* as an example, I hope I have gone some way to show that it is a mistake to frame an evolutionary question as a matter of either adaptation or constraint; an evolutionary question has two components, adaptation and lineage-specific effects. Investigations of evolutionary history differ logically from analyses of the maintenance of traits. Phylogenetic history involves investigations of the origin and trajectory of traits over time, whilst the maintenance of traits entails comparisons of the fitness of traits under current selective regimes (O'Hara, 1988; Sherman, 1988; Reeve and Sherman, 1993). A balanced interpretation of an evolutionary pattern must require both approaches.

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