GROWTH, REPRODUCTION AND FEEDING BIOLOGY OF *TURBO SARMATICUS* (MOLLUSCA: VETIGASTROPODA) ALONG THE COAST OF THE EASTERN CAPE PROVINCE OF SOUTH AFRICA

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Turbo sarmaticus **Linnaeus 1758**

CONTENTS

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ABSTRACT

Investigations were carried out on aspects of the biology of the vetigastropod *Turbo sarmaticus*. Studies included: 1) the distribution and standing stock of this animal at four sites along the coast of the Eastern Cape Province of South Africa; 2) the growth rate of animals on a wave-cut platform; 3) the reproductive cycle of an intertidal population; and 4) aspects of the feeding biology examining the ability of this mollusc to consume and digest six macroalgae, the influence of algal diet on growth rate and reproductive fitness and the polysaccharolytic activity of the digestive enzymes.

On eastern Cape shores, *T. sarmaticus* had a size related distribution, with smaller animals being found towards the upper mid-shore and larger animals being found in a downshore direction. The mean shore densities of *T. sarmaticus* at three sites where exploitation of animals was minimal, were very similar (1.2 - 1.7 individuals/m^2). The largest animals (up to 110 mm shell length) were found on an offshore island. This may have been a result of animals not being exploited, as well as a possible increase in primary productivity and food availability. The lowest density (0.2 individuals/m^2) and animal size (< 70 mm shell length) was recorded at a site (Kelly's beach - Port Alfred) where exploitation was more intense. It is probable that intense over-exploitation was threatening the populations at this site.

The growth rate of *T. sarmaticus* was determined by means of the von Bertalanffy growth model and expressed by the equation $L_t = 81.07(1-e^{-0.544(t)})$. The initial growth rate of *T. sarmaticus* (up to ≈ 80 mm shell length) was similar on shores with different geomorphologies (*i.e.* boulder shores and wave-cut platforms). Growth rates of individuals were variable, which means that individuals within a population reached exploitable size (3 - 6 years old) and sexual maturity (1½ - 2 years old) at different ages.

Seasonality of reproduction of *T. sarmaticus* was determined using gonad index, egg diameters and spermatozoa content within the gonad. *Turbo sarmaticus* was dioecious and had a sex ratio in favour of males (1.2:1). Animals attained sexual maturity at a size of about 52.5 mm shell length. There was little variation in the reproductive cycle over time with gametogenesis occurring from March/April until August/September, whilst maturity (Gonad Index = 15%) was maintained until the spawning event from December to March. After spawning the gonad regressed.

Field and laboratory observations of the feeding biology of *T. sarmaticus* confirmed that this mollusc was a generalist grazer capable of consuming and digesting algae from the Rhodophyta, Chlorophyta and Phaeophyta. The consumption rates (juveniles: 1.45 - 9.50% body weight/day, adults: 1.06 - 6.08%) and digestibility (9 - 75% apparent dry matter) of six macroalgae was found to vary. For most algae, juvenile *T. sarmaticus* had higher consumption rates (1.6 - 2.8 times higher) and digestibility values (12 - 24% higher) than adults. It is suggested that consumption rates were dependent on the digestibility of the algae. In addition, it is suggested that the consumption rates of the different algae were not related to the nutritional content, but rather the energetic content of the algae. In both juvenile and adult animals, temperature had a positive influence on consumption rates, resulting in an increase at higher temperatures. However, in both juvenile and adult *T. sarmaticus*, algal digestibility was not affected by temperature. Finally, it was proposed that *Ulva rigida*, *Codium*

extricatum, *Ecklonia radiata* and *Gelidium pristoides* would provide the best nutritional value for growth and reproductive fitness in *T. sarmaticus*, whilst *Iyengaria stellata* and *Corallina* spp. would provide the poorest.

Experiments on the effects of four algal diets on the biology of *T. sarmaticus* showed that the best growth rate (up to 13.8 mm shell length increase per annum), reproductive fitness (Gonad Index up to 33%) and energy levels (up to 4.76% glycogen in the foot) were achieved when *T. sarmaticus* was fed *G. pristoides*, *U. rigida* or a mixed diet. *Turbo sarmaticus* fed *Corallina* spp. showed reduced growth (2.4 mm shell length increase), reproductive fitness (Gonad Index up to 4.4%) and energy levels (up to 3.42% glycogen in the foot).

A study of the polysaccharolytic enzyme activity of *T. sarmaticus* indicated that this mollusc possesses enzymes that can, at least partially, digest most of the storage and structural polysaccharides found in the Chlorophyta, Rhodophyta and Phaeophyta. This further supported the findings that *T. sarmaticus* was a generalist grazer. Two levels of activity were detected: 1) high levels of enzyme activity (up to 328.2 μg/mg/ml/hr) occurred on the storage polysaccharides that occur in the Rhodophyta and Chlorophyta, and 2) lower levels of activity were detected on the storage polysaccharides (up to 44.8 μg/mg/ml/hr) of the Phaeophyta and on all the structural polysaccharides tested (<45.5 μg/mg/ml/hr). It was suggested that *T. sarmaticus* did not rely heavily on structural carbohydrates as a source of carbon.

Finally, the results of this study were discussed in relation to the future management of *T. sarmaticus* stocks, the possible role of this macroalgal grazer in the intertidal zone and the effects of over-exploitation of this animal. The potential aquaculture of this mollusc was also addressed briefly.

CHAPTER ONE

GENERAL INTRODUCTION

The phylum Mollusca is one of approximately twenty major divisions of the animal kingdom (Cox 1962). More than 80% of molluscs are gastropods, and few gastropod superfamilies rival the marine Trochacea (Prosobranchia: Vetigastropoda) in numbers of genera, species and individuals (Hickman & McLean 1990). The Trochacea comprises the families Turbinidae, Skeneidae and Trochidae (Hickman & McLean 1990). The Turbinidae are characterized by the ability to add calcium carbonate to their opercula. They have a worldwide distribution, being found at all latitudes and depths, ranging from intertidal to subtidal and with a few in the bathyal zone (Joll 1980, Worthington & Fairweather 1989, Hickman & McLean 1990). Turbinids are primarily restricted to hard substrates, with a strong affinity for calcium carbonate (Hickman & McLean 1990). They attain their greatest diversity in warm tropical and subtropical waters (Hickman & McLean 1990).

Four species of *Turbo* (Turbinidae) are commonly found on the rocky shores of South Africa (Stephenson 1944, Day 1974, Velimirov *et al.* 1977, Field *et al.* 1980, McQuaid 1980, McLachlan *et al.* 1981, Dower 1989, Lasiak 1991, Branch *et al.* 1994). These four endemic species are: *Turbo sarmaticus* Linnaeus, 1758, *Turbo coronatus* Gmelin, 1791, *Turbo cidaris cidaris* Gmelin, 1791 and *Turbo cidaris natalensis* Krauss, 1848. *Turbo sarmaticus* is the largest of the four, reaching a shell length in excess of 100 mm (Day 1974, Kilburn & Rippey 1982).

Turbo sarmaticus is a herbivorous gastropod with a geographical distribution ranging from False Bay to Natal (Figure 1.1) where it occurs in the lower littoral and shallow sublittoral regions (to a depth of about 8 metres) of rocky shores (Kensley 1973, Kilburn & Rippey 1982, Branch *et al.* 1994). Although recorded in Table Bay (Kensley 1973) and in Natal (Kensley 1973, Richards 1981), it is not always found at these localities (Jackson 1976, Lambert 1976). Therefore, these localities are often excluded from the distribution range of this species (Barnard 1963, Day 1974, McLachlan & Lombard 1981).

Turbo sarmaticus has been utilised as food by the human inhabitants of the coastal regions of the southern Cape for as long as the archaeological record exists (more than 70000 years) (Voigt 1973, Lasiak 1991). It is still considered a delicacy by those who are able to collect these animals (Wilkinson 1981). Unlike the abalone, *Haliotis midae* (Hecht & Britz 1990), *T. sarmaticus* is not at present exploited commercially, despite the fact that large populations may develop in the lower littoral, particularly in the Eastern Cape Province

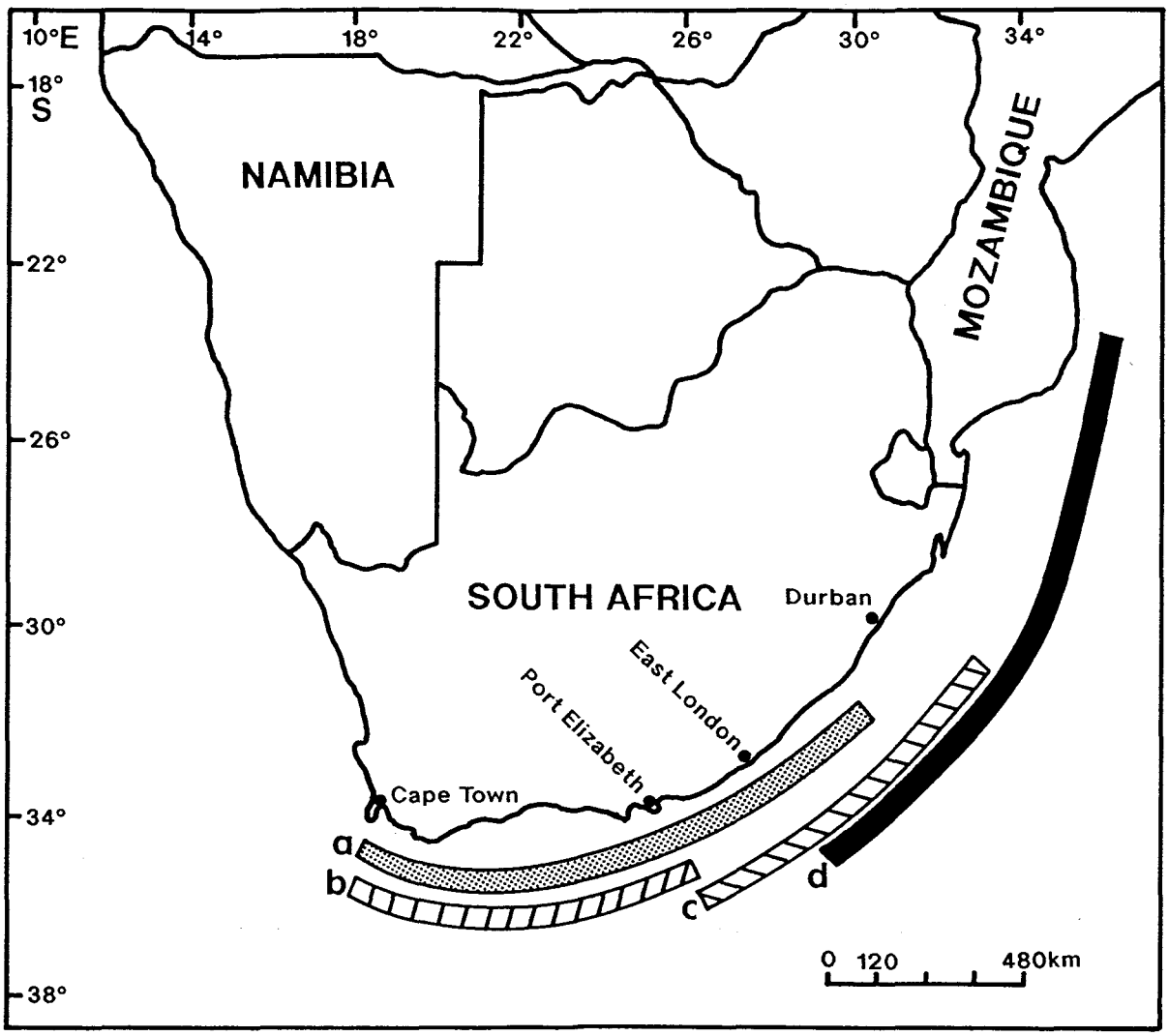

Figure 1.1. The southern African geographical distribution of four turbinids (after Kensley 1973, Kilburn & Rippey 1982, Branch *et al.* 1994). **a**: *Turbo sarmaticus*, **b**: *Turbo cidaris cidaris*, **c**: *Turbo cidaris natalensis* and **d**: *Turbo coronatus*.

(McLachlan *et al.* 1981, Yssel 1989, Bruton *et al.* 1991, *pers. obs.*). It is, however, becoming increasingly difficult to find large specimens in the intertidal zone (except in marine reserves - Yssel 1989), despite regulations limiting its exploitation. Legislation for the protection of this mollusc has existed since 1973. Regulations declared in terms of the Sea Fisheries Act (1988) limit both the number of animals (5/person/day) and minimum size which may be collected. Animals removed must not be able to be passed through a ring 63.5 mm in diameter (≈ 73.7 mm shell length - Bruton *et al.* 1991).

The region of the rocky shore inhabited by *T. sarmaticus* is a variable and dynamic environment characterized by great species diversity, marked zonation and niche differentiation (Stephenson & Stephenson 1949, Lewis 1964, Newell 1979). The physiological ecology and population dynamics of the coexisting littoral and shallow sublittoral assemblage of species has received considerable attention in recent years, especially the more prominent mollusc species, *viz.*: *Haliotis midae* (*e.g.* Newman 1966, 1967, 1968, 1969, Wood & Buxton 1996a, 1996b), *Haliotis spadicea* (Muller 1984), *Patella* spp. (Branch 1971, 1974a, 1974b, 1975a, 1975b, 1975c, 1975d, 1978, 1979, 1986), *Choromytilus meridionalis* (Griffiths 1977), *Pyura stolonifera* (Van Driel 1977, Berry 1982), *Perna perna* (Berry 1978), *Aulacomya ater* (Griffiths & King 1979a, 1979b, Stuart 1982), *Littorina africana knysnaensis* (McQuaid 1981a, 1981b) and *Oxystele* spp. (McQuaid 1983, Van Rooyen 1985).

Although the four species of *Turbo* are common to the littoral and sublittoral regions of rocky shores of the southern and eastern African coasts (Figure 1.1), little is known about their biology. To date, studies have included the reproductive cycle of *T. coronatus* (Lasiak 1986) and a comparative investigation of sperm morphology of three species (Hodgson & Foster 1992). Studies on *T. sarmaticus* have included limited investigations of its energetics, population dynamics and biochemical composition (Lombard 1977, McLachan & Lombard 1980, 1981, Yssel & Robinson 1988, Yssel 1989, Bruton *et al.* 1991).

Turbo sarmaticus is one of the larger and more prominent macroalgal grazers inhabiting rocky shores of the south coast of South Africa where it can comprise 22 - 36% of shore biomass (McLachlan *et al.* 1981). This species, therefore, forms an important component of rocky shore ecosystems. Although thought to be a generalist macroalgal grazer, nothing has been published on its feeding biology. As *T. sarmaticus* is exploited as a food source (which is possibly leading to declining stocks), has great potential for aquaculture and

has possible potential as a small scale fishery (*pers. comm.* - A. Eesterhuizen, Skerpioen Kraal Farm, Western Cape), a more detailed investigation into aspects of the biology and ecology of this species was initiated. Management of an exploited species must be based on a sound knowledge of an animal's biology. Microphagous feeding has been well documented within the vetigastropods (Steneck & Watling 1982, Hawkins & Hartnoll 1983) but, with the exception of *Haliotis* spp. (macroalgal grazers), little is known about macroalgal grazing within this group. Therefore, the overall objective of this study was to improve our knowledge of the growth, reproduction and feeding biology of *T. sarmaticus*. At the same time, this data will assist in the future management of this species and improve our knowledge of macroalgal/grazer interactions within the vetigastropods.

The first part of this thesis briefly examines the intertidal population structure and standing stocks of *T. sarmaticus* at four sites along the coast of the Eastern Cape Province (Chapter 2). Following this, Chapter 3 compares the growth rate of *T. sarmaticus* on an aeolian sandstone wave-cut platform to that previously determined for animals inhabiting boulder shores. Aspects of the reproductive biology (primarily seasonality) of *T. sarmaticus* are examined in Chapter 4. Having established the above fundamental data, the remainder of the thesis focuses on the biology of feeding. Chapter 5 presents the results of a study on the consumption rate and digestibility of six intertidal macroalgae by *T. sarmaticus*, whilst experiments on the effects of diet on growth rate and reproductive fitness are given in Chapter 6. Chapter 7 documents the results of the polysaccharolytic activity of the digestive enzymes of *T. sarmaticus*. Finally, Chapter 8 presents a general discussion in which avenues for future research are also proposed.

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CHAPTER TWO

POPULATION STRUCTURE AND STANDING STOCK OF *TURBO SARMATICUS* AT FOUR SITES ALONG THE COAST OF THE EASTERN CAPE PROVINCE

INTRODUCTION

When a large, unbiased sample is taken from an invertebrate population, lengths of individuals may be measured and graphed as a length-frequency plot (King 1995). From such plots it is possible to establish the health of a population of animals in relation to other populations subject to exploitation mortality (Schiel & Breen 1991, King 1995). As a population becomes more heavily exploited, larger animals are removed and, as long as recruitment is not affected, smaller ones are continually added to the exploitable part of the population. This results in a decrease in the mean length of the population (Pitcher & Hart 1982, King 1995).

Exploitation mortality, however, is not the only cause for differences in the size-frequency distributions of animal populations. Differential growth rates of populations at different geographical regions can occur. This may be due to differences in habitat and food quality, quantity and availability between geographical regions (Breen 1980, Tegner 1989). Size distributions could also reflect differential recruitment patterns. Poor recruitment for a few years, as a result of unsuccessful spawning or larval mortality, could result in the absence of smaller animals. This, in turn, could be misinterpreted as being caused by over-exploitation (King 1995). Finally, natural mortality resulting from adverse conditions, lack of food, competition, high levels of predation (King 1995) or red tides (*e.g.* Horstman *et al.* 1991) could be higher in some regions, thereby causing discrepancies in size classes between sites. Differences in size-frequency distributions of populations can, therefore, be explained by any one or all of the above factors. Only experimentation (Schiel & Breen 1991) or extensive sampling giving a long-term series of data can determine the cause of any observed differences in size distribution (*e.g.* Hayashi 1980).

Recruitment variability is a major problem when trying to understand the dynamics of exploited mollusc populations (Shepherd & Godoy 1989). Sustained productivity in wild populations is largely dependent on the natural recruitment of juveniles (Prince *et al.* 1987, Brown 1991) and incidences of recruitment over-exploitation have resulted in the subsequent collapse of mollusc fisheries (*e.g.* Breen & Adkins 1980, Sluczanowski 1984, Breen 1986). In addition, extensive or restricted larval dispersal will determine whether or not adult stocks are dependent on larvae from inter- or intra-population recruitment.

South Africa has a long, wave-exposed coastline which extends for some 2570 km from the Namibian border (28°S/16°E) in the west to Mozambique (26°S/32°E) in the east. The coast of the Eastern Cape Province of South Africa, although consisting of extensive stretches of sandy beaches, has numerous types of rocky shores, most of which are mixed shores (neither homogeneous rock nor sand) subject to frequent sand scour or inundation (Marker 1988, Dower 1989). Quantitative studies on species abundance on rocky shores within the Eastern Cape Province are limited to studies by McLachlan *et al.* (1981), Dower (1989), Van Erkom Schurink & Griffiths (1990), Bruton *et al.* (1991), Wood (1993), Foster (1994) and Gray (1996).

Turbo sarmaticus is one of the larger species of intertidal gastropods found on eastern and southern Cape rocky shores (Lombard 1977, McLachlan *et al.* 1981, Yssel 1989, Bruton *et al.* 1991). Examination of shell middens from the Transkei coast (Eastern Cape Province) has indicated that *T. sarmaticus* was, and still is, a preferred food item of impoverished communities (Lasiak 1991). Extensive shellfish gathering is known to result in changes in the intertidal community structure and function (Hockey & Bosman 1986). Where *T. sarmaticus* populations have been over-exploited, either naturally or experimentally, recovery of these populations has taken up to four years (*e.g.* Tsitsikamma Coastal National Park - Yssel & Robinson 1988, Yssel 1989) or has not occurred (*e.g.* Transkei coast - Dye 1992).

Within the Eastern Cape Province, standing stocks of *T. sarmaticus* have been determined on shores between Port Elizabeth and Tsitsikamma (McLachlan & Lombard 1981, McLachlan *et al.* 1981, Yssel 1989, Bruton *et al.* 1991). The extent of exploitation of *T. sarmaticus* along this coast, and the effect of this on its population structure, has not been examined. A further assessment of the standing stocks of *T. sarmaticus* could therefore be valuable in determining the status of this large invertebrate on local shores. Such information is vital to the management of this shellfish resource.

The aim of this chapter was to document and compare the density, biomass and size-frequency of *T. sarmaticus* at more sites in the Eastern Cape Province (sites between Port Elizabeth and Port Alfred). The results obtained would then be related to variation in exploitation pressures on these shores. In addition, this study aimed to identify a site which would sustain long-term (24 months) sampling of animals for a study on the reproductive seasonality of *T. sarmaticus*.

MATERIALS AND METHODS

Study sites

In the Eastern Cape Province rocky shores consist of one of two types: consolidated dunes (aeolian calcarenites) which form wave cut platforms, and hard quartzitic sandstone which forms boulder shores, reefs or boulder-reef shores (Marker 1988, Dower 1989). Four sites were selected within a 120 km stretch of shoreline, *viz.*: Chelsea Point and Cape Recife (both at Port Elizabeth), Bird Island (Algoa Bay) and Kelly's Beach (Port Alfred) (Figure 2.1). These sites were selected as they all had very similar plant and animal community assemblages. They were all boulder-reef shores consisting of quartzitic reefs extending perpendicularly from the shore out to sea, with numerous boulders between the reef ridges (Figure 2.2). With the exception of Bird Island, which had a steep shore gradient, all sites had shores with shallow gradients (Figure 2.3). All sites were exposed to the prevailing westerly swell.

Exploitation pressures were very different at the four sites. Bird Island is a marine reserve and the collection of marine organisms is prohibited. This, together with its inaccessibility, means that exploitation of *Turbo sarmaticus* is non-existent. Cape Recife is a terrestrial reserve and entry into it can only be obtained by means of a permit. Access to the shoreline is therefore limited which restricts the collection of *T. sarmaticus*. Although Chelsea Point is a popular recreational area, bait collecting is rare (*pers. obs.*), thus exploitation of *T. sarmaticus* is probably very low. Finally, Kelly's Beach is subject to high exploitation pressure by impoverished people who search for food and bait daily, and in the process remove many undersized *T. sarmaticus* (*pers. obs.*, *pers. comm.* - Dept. Sea Fisheries, Port Alfred).

Sampling procedure

One of the main concerns of any sampling procedure is whether or not the sample is representative of the population (Cochran 1963). No attempt was made to sample the shallow sublittoral region as heavy wave action at all sites makes this region inaccessible in all, but

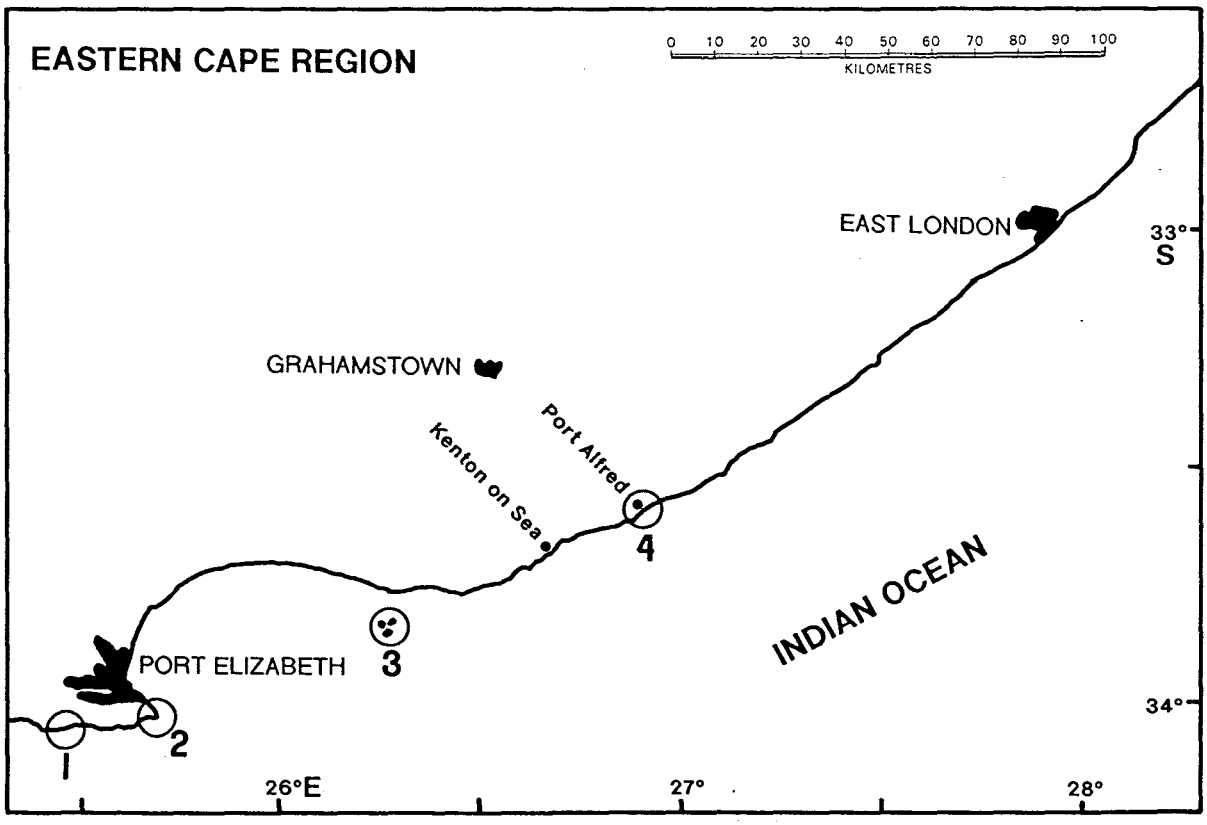


Figure 2.1. The Eastern Cape Province coastal region showing the location of the four study sites: **(1)** Chelsea Point, **(2)** Cape Recife, **(3)** Bird Island and **(4)** Kelly's Beach.

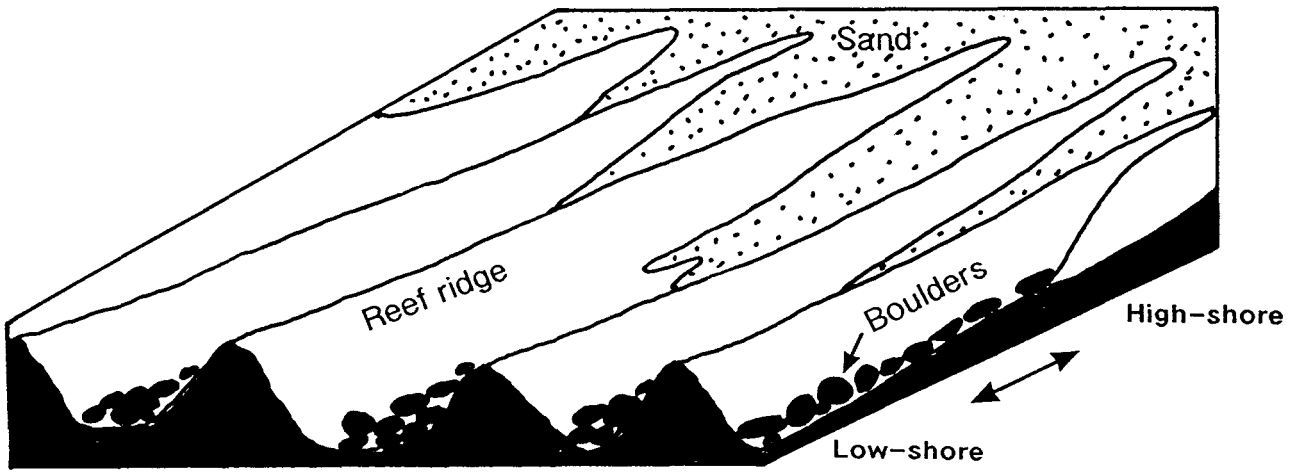


Figure 2.2. Diagrammatic representation of a quartzitic sandstone boulder-reef shore. Reef ridges extend perpendicularly out to sea and numerous boulders lie between these ridges.

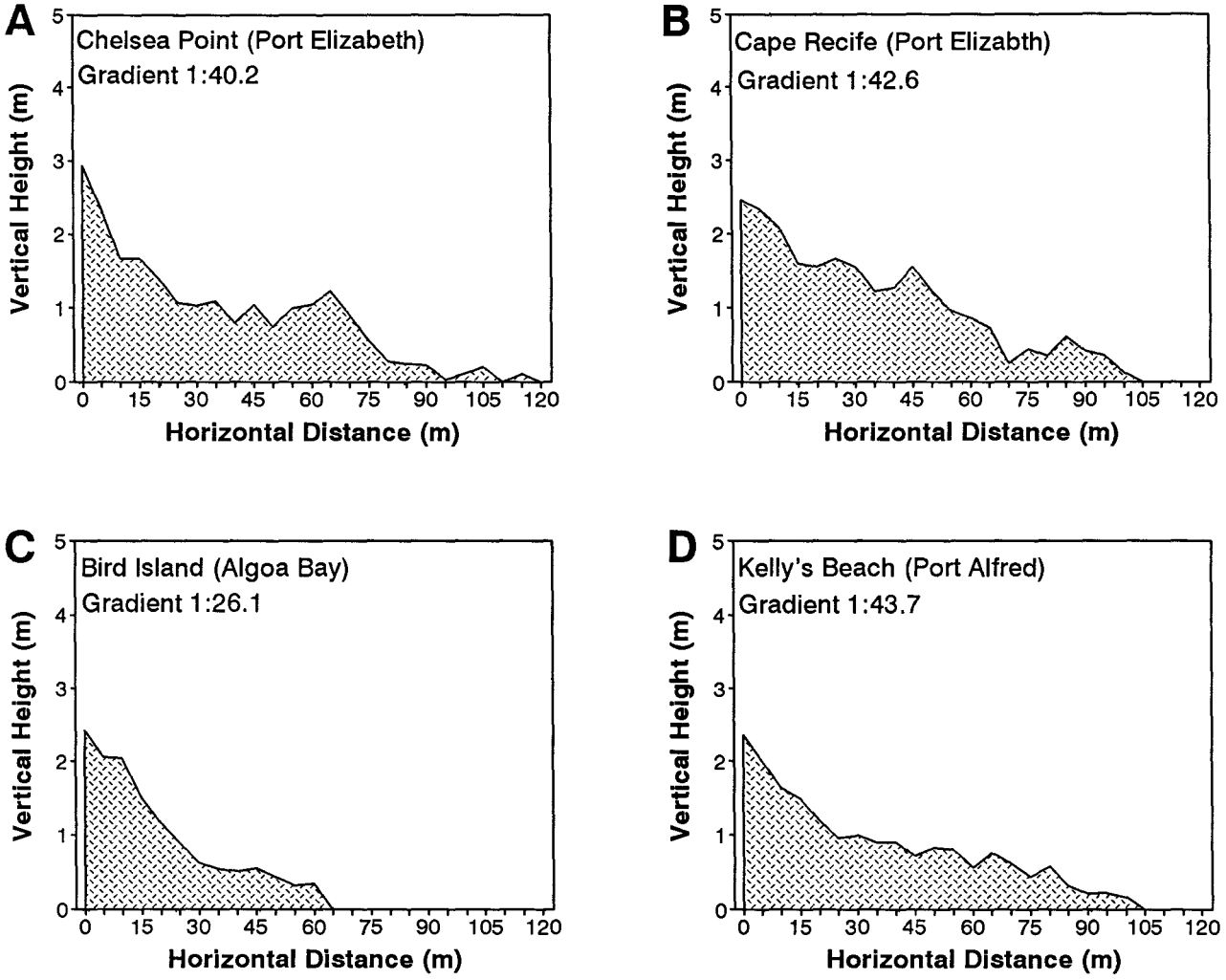


Figure 2.3. Intertidal profiles of the four study sites from the coast of the Eastern Cape Province. Profiles were determined using a staff and dumpy-level.

the calmest weather conditions. Therefore, the population of *T. sarmaticus* for this study, and subsequent chapters, is defined as those individuals found in the rocky littoral region of each study site. Generally, this is usually the only part of the population that is easily available for exploitation by man.

Due to inadequate benchmarks and the complications of extrapolating the mean low water spring levels from empirical values of tide levels published by the South African Naval Hydrographer, characteristic indicator species were used to determine the high-, mid- and low-shore regions. Five distinct zones are recognized on rocky shores of the southern and eastern Cape: Littorina, upper Balanoid, lower Balanoid, Cochlear and Infratidal zone (Branch & Branch 1981, Lubke 1988). The Littorina zone (high-shore) represents the high water spring level (HWS) and is characterized by the presence of the snail *Littorina knysnaensis* (Branch & Branch 1981). The Balanoid zones represent the mid-shore from the high water neap to the low water neap tidal levels. The upper Balanoid zone indicates the upper mid-shore and is characterized by the presence of the animals *Chthamalus dentatus*, *Tetraclita serrata* and *Helcion pectunculus*, and the seaweeds *Ulva* spp. and *Iyengaria stellata*. The lower Balanoid zone indicates the lower mid-shore and is characterized by the presence of the animals *Octomeris angulosa*, *Balanus* spp., *Patella longicosta* and *Oxystele sinensis*, and the seaweeds *Gelidium pristoides* and *Codium lucasii capensis* (Branch & Branch 1981, Seagrief 1988). The Cochlear zone represents the low-shore from the low water neap to the low water spring tidal level (LWS) and is characterized by the presence of the animals *Patella cochlear* and *Pyura stolonifera*, and the seaweeds *Caulerpa filiformus* and *Plocamium corallorhiza* (Branch & Branch 1981, Seagrief 1988).

At each study site, a transect area ten metres wide was taken from the HWS to the LWS at the lowest spring tides and within this area twenty, one square metre quadrats were sampled from each zone (n = 4), except the Infratidal zone which is mostly inaccessible due to high wave action. All the *T. sarmaticus* found within each quadrat were removed, counted and the shell lengths measured to the nearest 0.1 mm (Figure 2.4). After this, they were returned to their position on the shore. Estimates of biomass were calculated from the measurements of shell length using the equations (after Bruton *et al.* 1991):

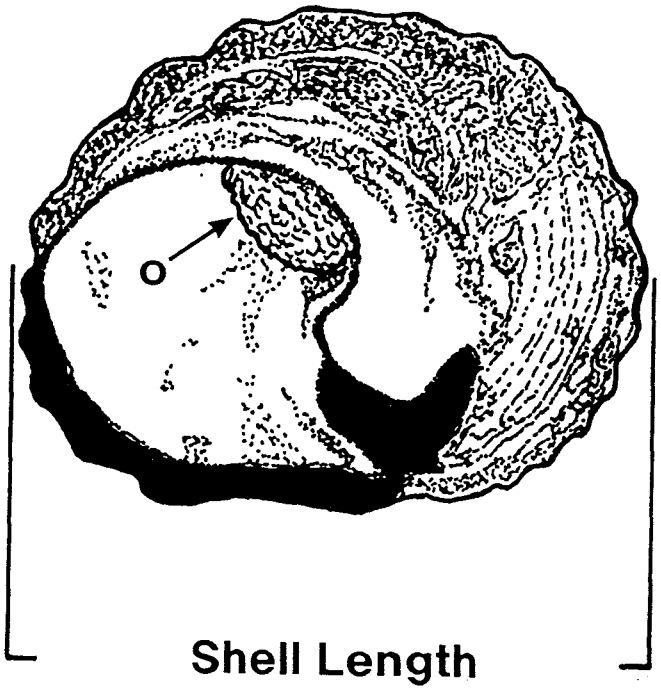


Figure 2.4. Diagram of the shell of an adult *Turbo sarmaticus* indicating the dimension measured as shell length (mm). **O**: operculum. Scale = life size.

$$1) \quad Dry\ mass = 2.47 \times 10^{-5} (L^{2.93325})$$

$$2) \quad Ash\text{-}free\ dry\ mass = 0.027 + 0.8224\ dry\ mass$$

$$3) \quad Total\ wet\ mass = 14.768 * ash\text{-}free\ dry\ mass + 3.196$$

Equation 1 was used to determine the dry biomass (flesh only in grams), whilst equation 3 was used to determine the total wet biomass (shell, operculum and flesh in grams). Although these equations were derived for *T. sarmaticus* from the Cape St. Francis region (≈ 80 km west of Port Elizabeth), they were regarded as representative for the *T. sarmaticus* populations at the present study sites. In this way the unnecessary destruction of animals was avoided. All study sites were sampled between September and December 1995.

Statistical analysis

Due to sample counts within each quadrat being less than twenty and the implication of zero values, all data were transformed using the low counts in quadrats transformation ($\sqrt{x+1}$) to comply with the requirements of ANOVA tests (Fry 1993). Multi-factor ANOVA and One-way ANOVA tests, coupled with Scheffe's multiple range tests, were used to determine if density and biomass differed significantly between the sites and within the zones at each site (Fry 1993). Chi-squared contingency table tests were used to determine if size-frequency distributions differed significantly between sites and zones (Fry 1993). All statistical calculations were done using a StatGraphics V7.0 computer package (Statistical Graphics Corporation, USA).

RESULTS

Density

At all study sites, the *Turbo sarmaticus* sampled were probably not completely representative of the total population for the following reasons: 1) smaller animals (< 10 mm

shell length) may have been overlooked and therefore were not fully represented in the data, and 2) the sublittoral population was not sampled. Nevertheless, as the sampling method was standard it was possible to compare the sites.

During daytime, *T. sarmaticus* at all the sites was found aggregated under boulders or in rock crevices. No animals were found in the high-shore region and animals were distributed from the upper mid-shore to the low-shore.

The mean densities of *T. sarmaticus* in each zone at the four sites are given in Table 2.1. There was no significant difference in the density of animals (1.28 to 1.73/m²) at Chelsea Point, Cape Recife and Bird Island. However, the density at Kelly's Beach (0.26/m²) was significantly lower (Tables 2.2 & 2.3). The maximum density recorded for *T. sarmaticus* during this study was 17/m² at Chelsea Point.

Table 2.1. Shore and zonal densities (mean ± S.E./m²) of *Turbo sarmaticus* at four sites along the Eastern Cape Province.

Site	Shore average (No./m²)	High-shore (No./m²)	Upper mid-shore (No./m²)	Lower mid-shore (No./m²)	Low-shore (No./m²)
Chelsea Point	1.73 ± 0.40	0	0.05 ± 0.04	2.90 ± 0.93	2.30 ± 0.67
Cape Recife	1.28 ± 0.21	0	0.55 ± 0.24	1.60 ± 0.44	1.70 ± 0.36
Bird Island	1.50 ± 0.32	0	0.80 ± 0.38	2.85 ± 0.78	0.85 ± 0.28
Kelly's Beach	0.26 ± 0.08	0	0	0.45 ± 0.18	0.35 ± 0.18

There was a significant difference in the density of animals between the different zones (Table 2.2) and these zonal differences were affected by the site (Table 2.2). With the exception of Bird Island, all sites had their highest *T. sarmaticus* densities in the lower mid-shore and low-shore zones (Table 2.4). At Bird Island only the lower mid-shore had a significantly higher density of animals (Table 2.4).

Table 2.2. Results of a Multi-factor ANOVA to determine whether the density of *Turbo sarmaticus* differed between sites and zones at four shores along the coast of the Eastern Cape Province.

Source of variation	Sum of squares	D.F.	Mean square	F-ratio	Sig. level
Main effects					
A:Zone	8.09	2	4.04	14.33	$p < 0.001$
B:Site	5.63	3	1.87	6.65	$p < 0.001$
Interactions					
AB	3.79	6	0.63	2.23	$p = 0.040$
RESIDUAL	64.36	228	0.28		
TOTAL	81.88	239			

Note - comparisons of density were carried out only for zones where animals were found (n = 3).

Table 2.3. Results from a Scheffe's multiple range test to determine where differences in the shore density of *Turbo sarmaticus* occurred between four sites along the coast of the Eastern Cape Province. (X's in same column indicate no significant differences).

Site	Homogeneous groups	
Kelly's Beach	X	
Cape Recife		X
Bird Island		X
Chelsea Point		X

Table 2.4. Results from a One-way ANOVA and Scheffe's multiple range test to determine whether the density of *Turbo sarmaticus* differed between the zones of a site, and where the differences occurred between the zones at four sites along the coast of the Eastern Cape Province. (X's in same column indicate no significant differences).

Zone	One-way ANOVA Sig. level	Homogeneous groups	
Chelsea Point	p = 0.002		
Cape Recife	p = 0.045		
Upper mid-shore		X	
Lower mid-shore			X
Low-shore			X
Bird Island	p = 0.014		
Upper mid-shore		X	
Lower mid-shore			X
Low-shore		X	
Port Alfred	p = 0.036		
Upper mid-shore		X	
Lower mid-shore			X
Low-shore			X

Note - comparisons of density were carried out only for zones where animals were found (n = 3).

Biomass

The mean dry biomass of *T. sarmaticus* at the four sites and their respective zones are given in Table 2.5. There was no significant difference between the mean shore biomass (mean dry weight: 4.42 to 9.41 g/m²) at Chelsea Point, Cape Recife and Algoa Bay (Tables 2.6 & 2.7). However, the biomass of animals at Kelly's Beach (mean dry weight: 0.41 g/m²) was significantly lower that at the other sites (Tables 2.6 & 2.7). The maximum dry biomass recorded for *T. sarmaticus* during this study was 78.62 g/m² at Bird Island. With the exception of Bird Island, all sites showed a downshore increase in biomass (Table 2.5).

Table 2.5. Shore and zonal dry weight biomass (mean ± S.E. g/m²) of *Turbo sarmaticus* at four sites along the Eastern Cape Province, South Africa.

Site	Shore average	High-shore	Upper mid-shore	Lower mid-shore	Low-shore
Chelsea Point	5.55 ± 1.52	0	0.02 ± 0.01	6.51 ± 2.05	11.84 ± 3.93
Cape Recife	4.42 ± 1.12	0	0.45 ± 0.21	3.52 ± 1.04	9.29 ± 2.93
Bird Island	9.41 ± 2.19	0	3.48 ± 2.14	16.99 ± 5.23	7.77 ± 2.79
Kelly's Beach	0.41 ± 0.15	0	0	0.60 ± 0.31	0.65 ± 0.31

There was a significant difference in biomass between the different zones, but these zonal differences were not affected by the site (Table 2.6). At all the sites, the lower mid-shore and low-shore had a significantly higher biomass of animals than the upper mid-shore (Tables 2.5, 2.6 & 2.8).

Table 2.6. Results of a Multi-factor ANOVA to determine whether the dry biomass (g/m²) of *Turbo sarmaticus* differed between sites and zones at four shores along the coast of the Eastern Cape Province.

Source of variation	Sum of squares	D.F.	Mean square	F-ratio	Sig. level
Main effects					
A:Zone	40.72	2	20.36	17.51	$p < 0.001$
B:Site	29.38	3	9.79	8.42	$p < 0.001$
Interactions					
AB	13.54	6	2.25	1.94	$p = 0.075$
RESIDUAL	265.06	228	1.16		
TOTAL	348.71	239			

Note - comparisons of density were carried out only for zones where animals were found (n = 3).

Table 2.7. Results from a Scheffe's multiple range test to determine where differences in the shore biomass of *Turbo sarmaticus* occurred between four sites along the coast of the Eastern Cape Province. (X's in same column indicate no significant difference).

Site	Homogeneous groups	
Kelly's Beach	X	
Cape Recife		X
Chelsea Point		X
Bird Island		X

Table 2.8. Results from a Scheffe's multiple range test to determine where differences in the zonal biomass of *Turbo sarmaticus* occurred at four sites along the coast of the Eastern Cape Province. (X's in same column indicate no significant difference).

Site	Homogeneous groups	
Upper mid-shore	X	
Lower mid-shore		X
Low-shore		X

Population structure

The size-frequency analyses of *T. sarmaticus* at each site are presented in Figures 2.5A to 2.8A. There was a significant difference ($Chi^2 = 80.52$, d.f. = 33, $p < 0.001$) in the size range of animals between the sites (Figures 2.5A - 2.8A, Table 2.9). The largest animals were found at Bird Island (110 - 119 mm shell lengths) and Cape Recife (100 - 109 mm). The largest size class recorded at Kelly's Beach, however, was only 60 - 69 mm shell length (Figure 2.8A, Table 2.9).

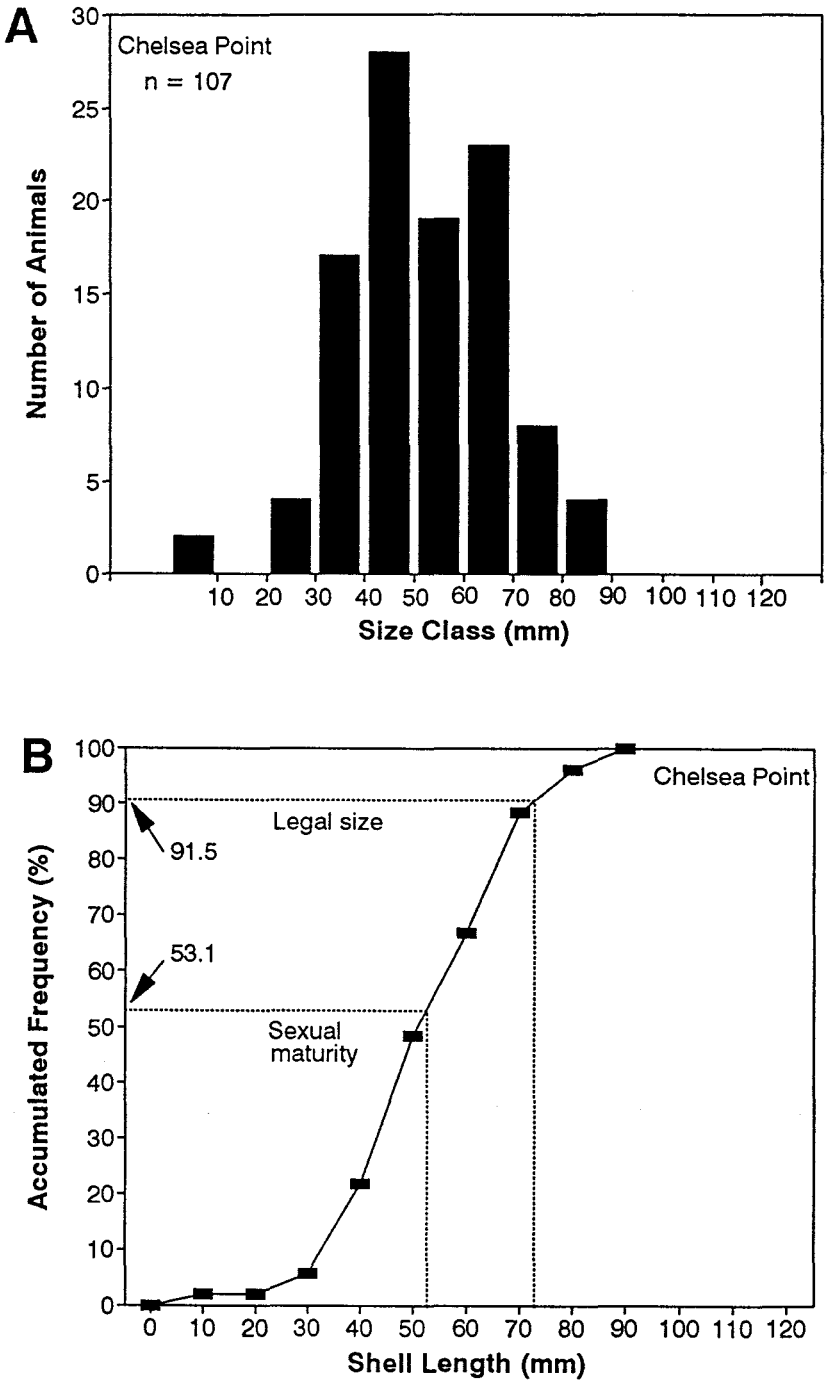


Figure 2.5. Chelsea point (Port Elizabeth). **(A)** Size-frequency distribution (shell length in mm) of *Turbo sarmaticus* (n = 107) on the shore. **(B)** Accumulated frequency plot for *T. sarmaticus* indicating the percentage of the population that are juveniles (< 52.5 mm shell length) and under the present exploitable legal size (≈ 73.7 mm shell length).

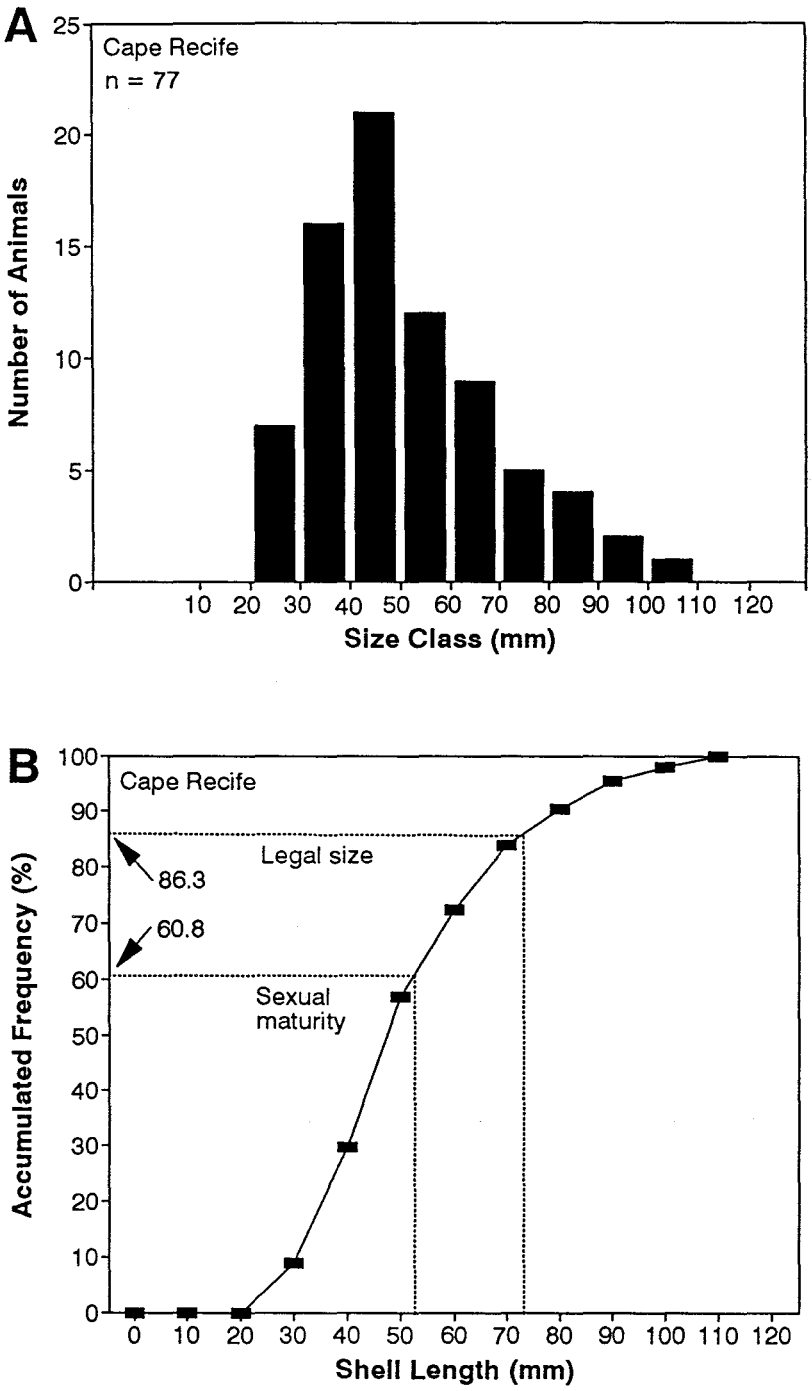


Figure 2.6. Cape Recife (Port Elizabeth). **(A)** Size-frequency distribution (shell length in mm) of *Turbo sarmaticus* (n = 77) on the shore. **(B)** Accumulated frequency plot for *T. sarmaticus* indicating the percentage of the population that are juveniles (< 52.5 mm shell length) and under the present exploitable legal size (≈ 73.7 mm shell length).

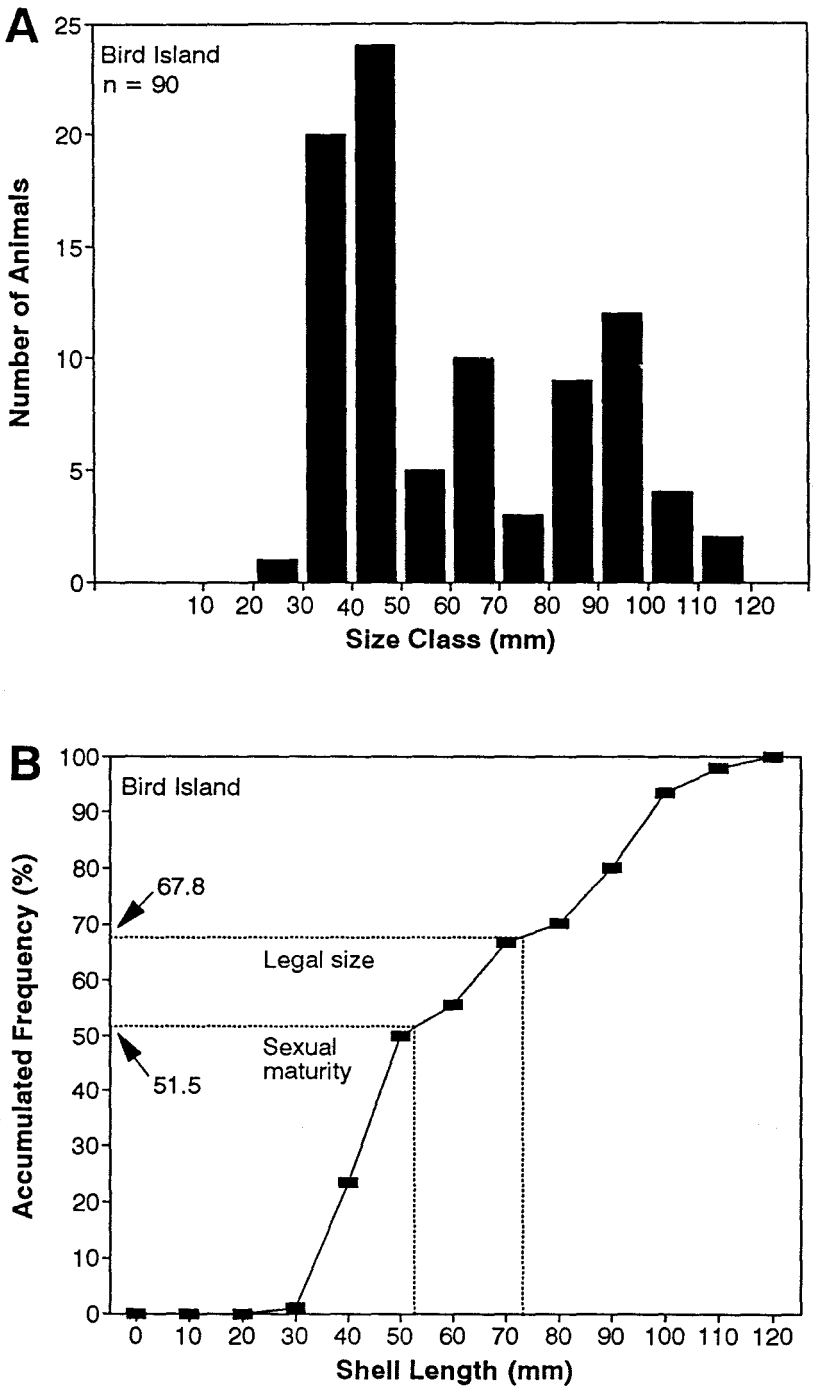


Figure 2.7. Bird Island. **(A)** Size-frequency distribution (shell length in mm) of *Turbo sarmaticus* (n = 90) on the shore. **(B)** Accumulated frequency plot for *T. sarmaticus* indicating the percentage of the population that are juveniles (< 52.5 mm shell length) and under the present exploitable legal size (≈ 73.7 mm shell length).

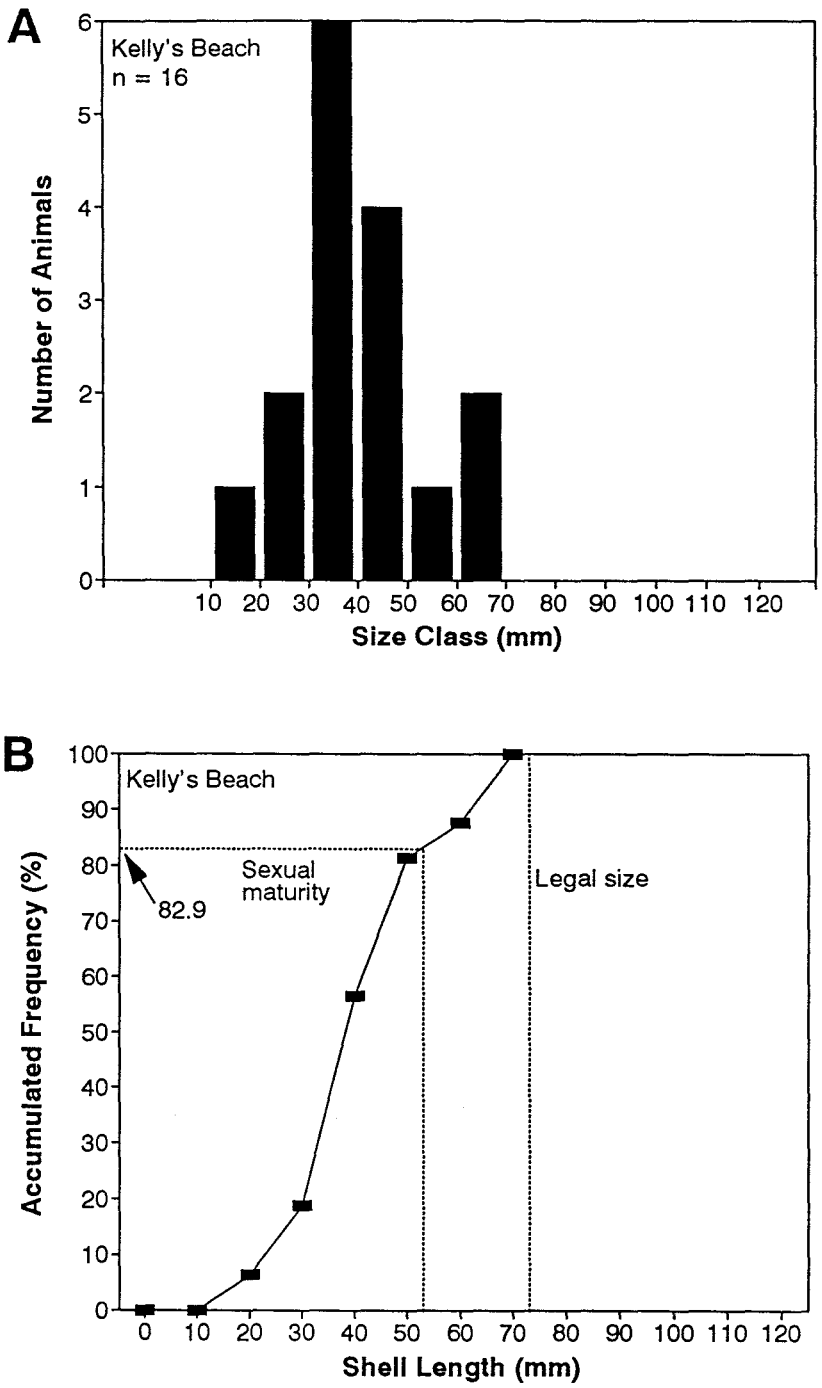


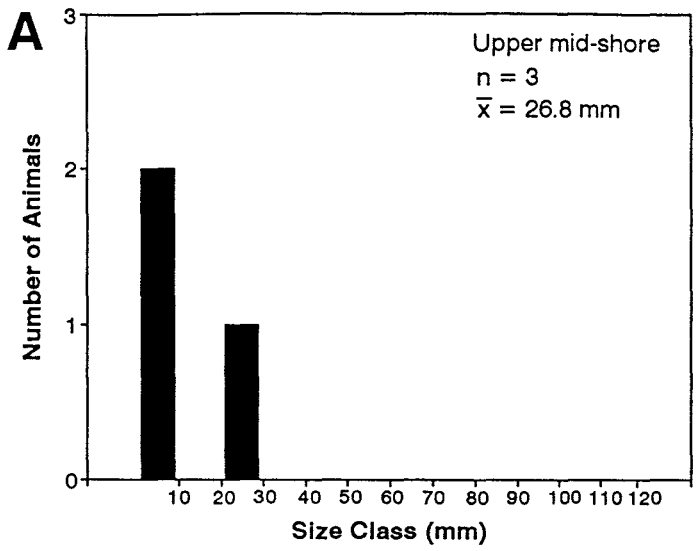
Figure 2.8. Kelly's Beach (Port Alfred). **(A)** Size-frequency distribution (shell length in mm) of *Turbo sarmaticus* (n = 16) on the shore. **(B)** Accumulated frequency plot for *T. sarmaticus* indicating the percentage of the population that are juveniles (< 52.5 mm shell length) and under the present exploitable legal size (≈ 73.7 mm shell length).

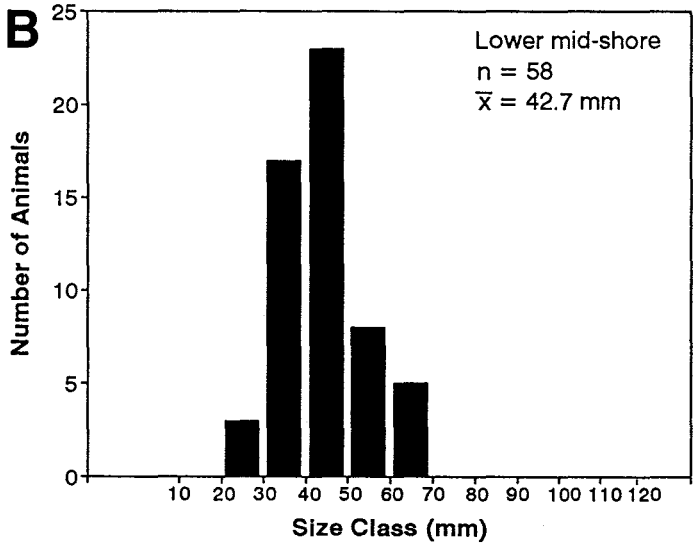
Table 2.9. Comparative data on the population structure of *Turbo sarmaticus* at four sites along the coast of the Eastern Cape Province.

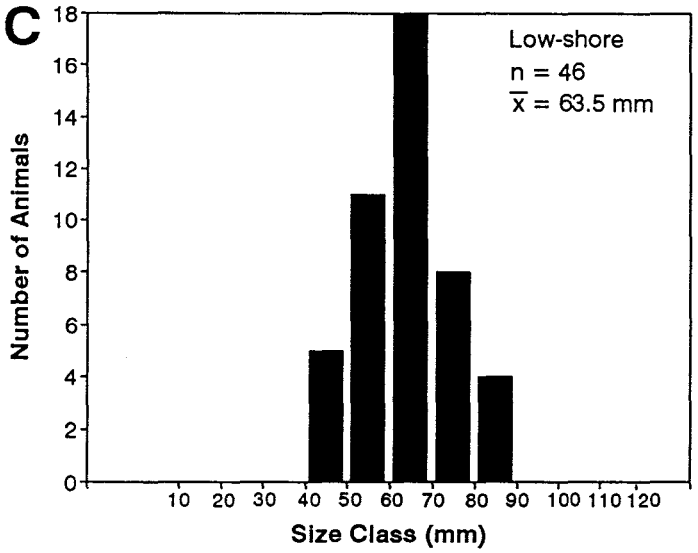
Site	Mean size (mm)	Modal size class (mm)	Smallest size class (mm)	Largest size class (mm)
Chelsea Point	44.3	40 - 49	< 10	80 - 89
Cape Recife	46.9	40 - 49	20 - 29	100 - 109
Bird Island	61.6	40 - 49	20 - 29	110 - 119
Kelly's Beach	40.0	30 - 39	10 - 19	60 - 69

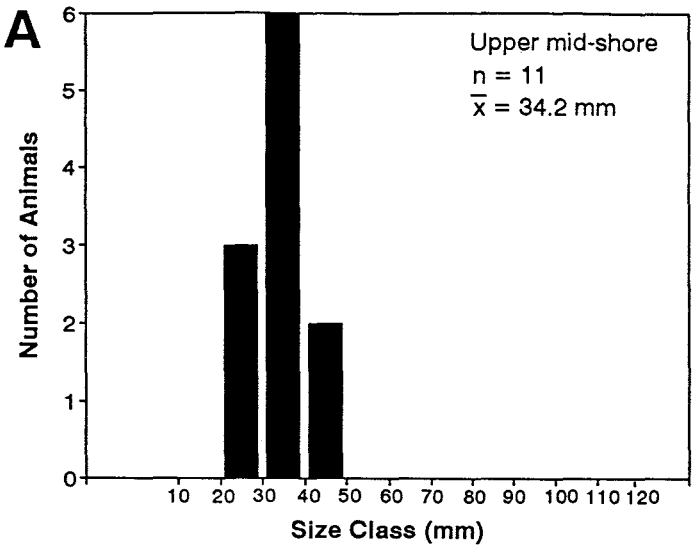
Accumulated frequency plots derived from the size-frequency data (Figures 2.5B to 2.8B) enabled: 1) the percentage of the population that were juveniles (*i.e.* below 52.5 mm shell length - Chapter 3), and 2) the percentage of the population below the exploitable legal size limit (≈ 73.7 mm shell length) to be calculated for each site. At Kelly's Beach, 82.9% of the animals were juveniles (Figure 2.8B), whilst at Bird Island only 51.5% were of this category (Figure 2.7B). Bird Island had the most animals of exploitable size (≈ 32.2%) (Figure 2.7B), whilst Kelly's Beach had no legal sized animals in the population (Figure 2.8B).

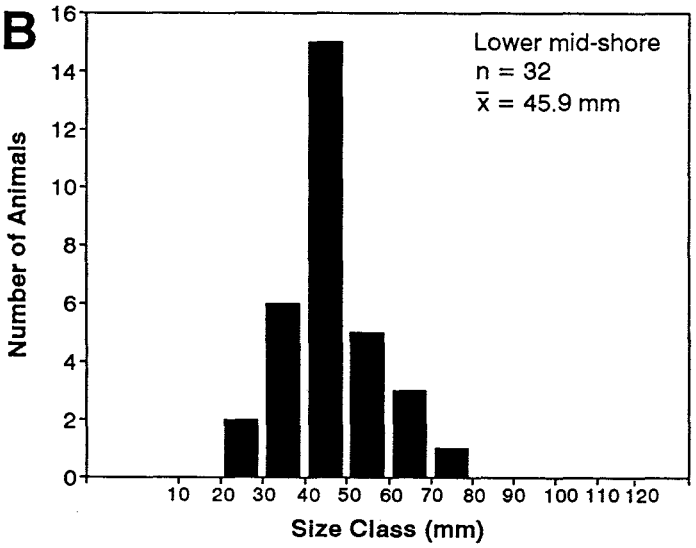
Size-frequency distributions of animals in each zone at each site are presented in Figures 2.9 to 2.12. At Chelsea Point and Cape Recife animal size increased significantly from the upper mid-shore to the low-shore (Figures 2.9 & 2.10, Table 2.10). At Chelsea Point the mean size of the animals increased from 26.8 mm shell length (upper mid-shore) to 63.5 mm shell length (low-shore), while the mean size of the animals at Cape Recife increased from 34.2 mm shell length (upper mid-shore) to 60.7 mm shell length (low-shore) (Figures 2.9 & 2.10). Although there was a downshore increase in the mean size of the animals at Bird Island (52.8 to 72.0 mm shell length) and Kelly's Beach (36.3 to 43.7 mm shell lengths), the size range of animals between the zones were not significantly different (Figures 2.11 & 2.12, Table 2.10).

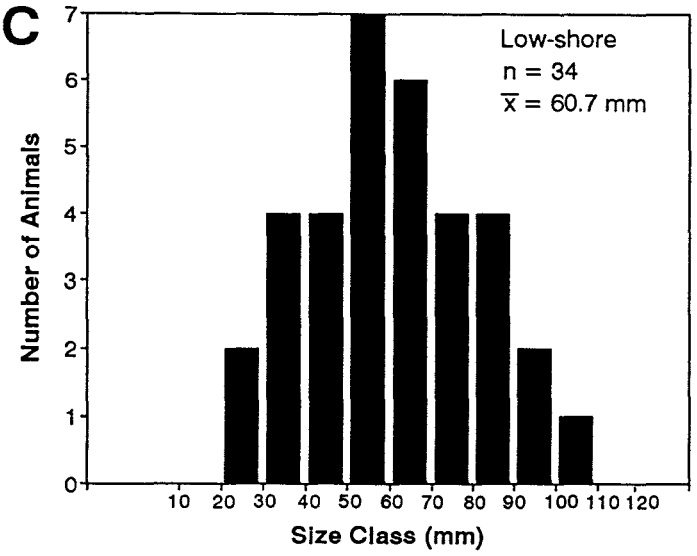

A
Upper mid-shore
n = 3
x̄ = 26.8 mm
Number of Animals
0
1
2
3
10 20 30 40 50 60 70 80 90 100 110 120
Size Class (mm)

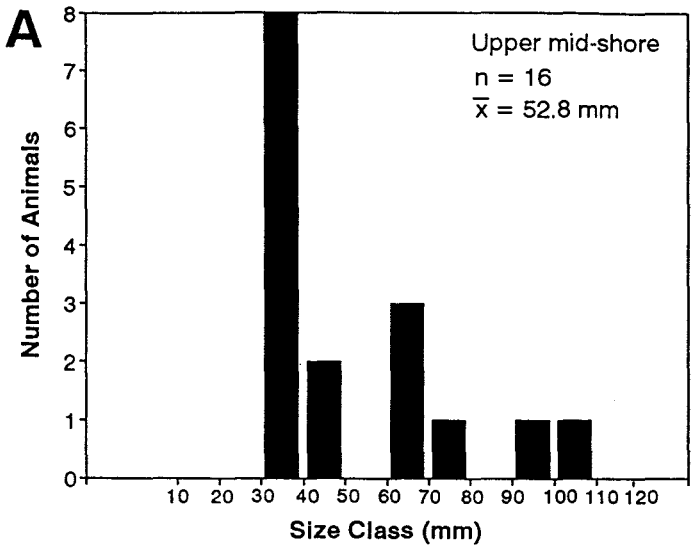

B
Lower mid-shore
n = 58
x̄ = 42.7 mm
Number of Animals
0
5
10
15
20
25
10 20 30 40 50 60 70 80 90 100 110 120
Size Class (mm)

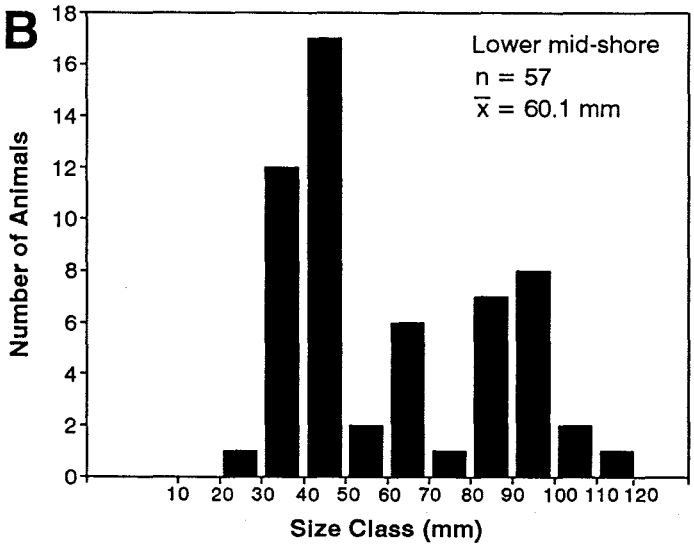

C
Low-shore
n = 46
x̄ = 63.5 mm
Number of Animals
0
2
4
6
8
10
12
14
16
18
10 20 30 40 50 60 70 80 90 100 110 120
Size Class (mm)

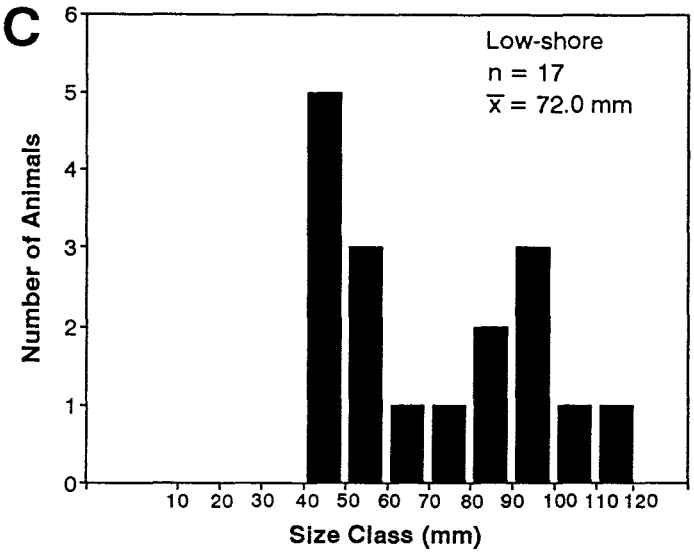

A
Upper mid-shore
n = 11
x̄ = 34.2 mm
Number of Animals
0 1 2 3 4 5 6
10 20 30 40 50 60 70 80 90 100 110 120
Size Class (mm)

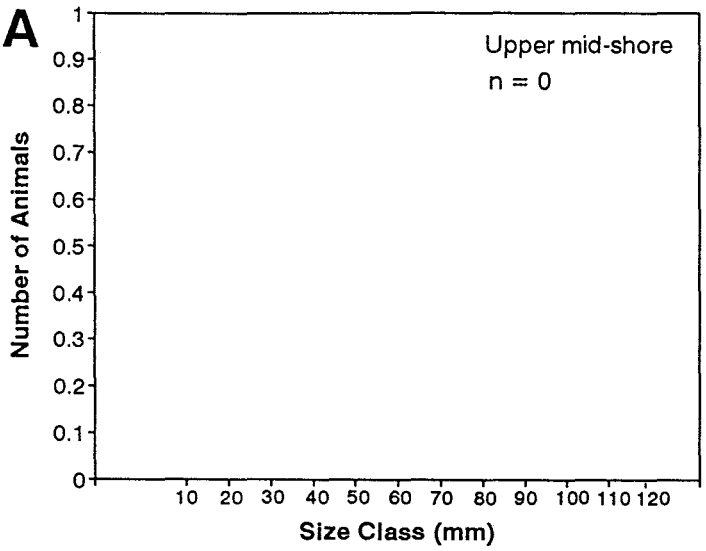

B
Lower mid-shore
n = 32
x̄ = 45.9 mm
Number of Animals
0 2 4 6 8 10 12 14 16
10 20 30 40 50 60 70 80 90 100 110 120
Size Class (mm)

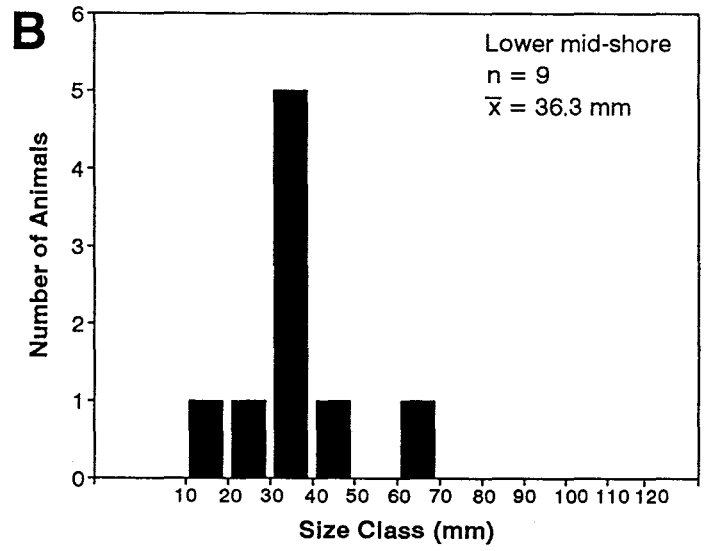

C
Low-shore
n = 34
x̄ = 60.7 mm
Number of Animals
0 1 2 3 4 5 6 7
10 20 30 40 50 60 70 80 90 100 110 120
Size Class (mm)


A
Upper mid-shore
n = 16
x̄ = 52.8 mm
Number of Animals
0 1 2 3 4 5 6 7 8
10 20 30 40 50 60 70 80 90 100 110 120
Size Class (mm)


B
Lower mid-shore
n = 57
x̄ = 60.1 mm
Number of Animals
0 2 4 6 8 10 12 14 16 18
10 20 30 40 50 60 70 80 90 100 110 120
Size Class (mm)


C
Low-shore
n = 17
x̄ = 72.0 mm
Number of Animals
0 1 2 3 4 5 6
10 20 30 40 50 60 70 80 90 100 110 120
Size Class (mm)


A
Upper mid-shore
n = 0
Number of Animals
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
10 20 30 40 50 60 70 80 90 100 110 120
Size Class (mm)


B
Lower mid-shore
n = 9
x̄ = 36.3 mm
Number of Animals
0
1
2
3
4
5
6
10 20 30 40 50 60 70 80 90 100 110 120
Size Class (mm)

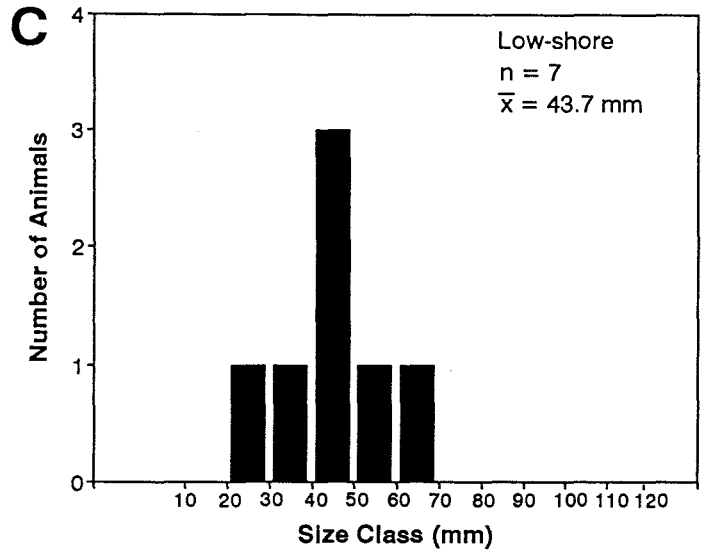

C
Low-shore
n = 7
x̄ = 43.7 mm
Number of Animals
0
1
2
3
4
10 20 30 40 50 60 70 80 90 100 110 120
Size Class (mm)

Table 2.10. Summary statistics from a Chi² contingency table analyses to determine whether the size-frequency distributions of *Turbo sarmaticus* differed between zones at four sites along the coast of the Eastern Cape Province. (* indicates a significant difference).

Sites	Chi²	D.F.	Sig. level
Chelsea Point	50.90	6	$p < 0.001$ *
Cape Recife	16.85	8	$p = 0.031$ *
Bird Island	24.20	18	$p = 0.148$
Kelly's Beach	5.50	5	$p = 0.357$

Standing stock

The standing stocks for each site were extrapolated from the density and biomass data. Standing stock was expressed in terms of animal numbers and total wet weight biomass (flesh and shell/kg) (Materials and Methods - Equation 3) per kilometre of coast line for each shore region. All rocky shores were considered to be suitable habitats for *T. sarmaticus*. While estimates of stocks obtained in this way were only first approximations, they nevertheless provided a useful index of relative abundance for this animal.

The Chelsea Point region had the greatest number of animals (10700/km), whilst the Kelly's Beach region had the fewest (1600/km) (Table 2.11). Bird Island, with its larger animals, had the greatest total wet weight biomass (709.1 kg/km), whilst Kelly's Beach had the lowest biomass (36.5 kg/km) (Table 2.11). Bird Island, although having fewer animals per kilometre shore line than Chelsea Point, had the most legal sized animals (2898/km) (Table 2.11). However, the Chelsea Point region had the greatest number of sexually mature individuals per kilometre shore line (5018/km), whilst the Kelly's Beach region had the lowest, having only 273 animals per kilometre (Table 2.11).

Table 2.11. Estimates of intertidal standing stocks of *Turbo sarmaticus* per kilometre of shore line for four coastal regions of the Eastern Cape Province.

Shore region	Numbers (per km)	Total wet biomass (kg/km)
Chelsea Point	10700	449.3
legal size	*909*	
sexually mature	*5018*	
Cape Recife	7700	349.9
legal size	*1055*	
sexually mature	*3018*	
Bird Island	9000	709.1
legal size	*2898*	
sexually mature	*4365*	
Kelly's Beach	1600	36.5
legal size	*0*	
sexually mature	*273*	

DISCUSSION

The intertidal rocky shore is one of the most physically stressful environments (Branch & Branch 1981) and availability of food and space are major limiting factors for organisms in these ecosystems (Lewis 1964, Newell 1979). Within the intertidal region, *Turbo sarmaticus* is limited to, but not exclusive to, the mid- to low-littoral areas. Similarly, *Turbo undulatum* is also reported to be most abundant low on the shore and in lower mid-shore rock pools (Worthington & Fairweather 1989).

The mean shore densities found for *T. sarmaticus* in this study, with the exception of Kelly's Beach, were similar to those reported for other sites along the coast of the Eastern Cape Province (Table 2.12). In addition, these densities were similar to those of other equal-sized *Turbo* species (0.5 - 5/m^2) from various regions of the world (*e.g.* New Zealand - Davidson & Chadderton 1994, Indonesia - Ompi 1994, Cook Islands - Preston *et al.* 1995).

Table 2.12. Shore density (mean ± S.E.) of *Turbo sarmaticus* at various sites along the coast of the Eastern Cape Province.

Shore region	Site sampled	Sampling period	Density (No./m²)	Reference
Tsitsikamma National Park	Swartrif	1972 - 1987	1.25 ± 0.45	Yssel 1989
Cape St Francis	Various sites in St. Francis Bay	Apr - June 1990	1.33 ± 0.38	Bruton *et al.* 1991
Port Elizabeth	Flat Rocks	Mar 1975 - June 1976	0.68	McLachlan & Lombard 1981
	Skoenmakerskop	Feb 1975 - July 1976	1.27	McLachlan & Lombard 1981
	Chelsea Point	Sept 1995	1.73 ± 0.40	This study
	Cape Recife	Dec 1995	1.28 ± 0.21	This study
Algoa Bay	Bird Island	Oct 1995	1.50 ± 0.32	This study
Port Alfred	Kelly's Beach	Nov 1995	0.26 ± 0.08	This study

It is interesting to note that the densities of *T. sarmaticus* at two exploited sites (*e.g.* Chelsea Point and Cape St. Francis) were similar to that at the reserve sites (Bird Island, Tsitsikamma National Park, Skoenmakerskop and Cape Recife) (Table 2.12). However, within the reserves the animals were usually larger (> 90 mm shell length), such animals constituting about 3.8% (Cape Recife) to 22.2% (Bird Island) of the population. Other exploited sites (*e.g.* Kelly's Beach and Flat Rocks) (McLachlan & Lombard 1981) had much lower densities of *T. sarmaticus*. In addition, Kelly's Beach had smaller animals and no legal sized specimens were found in the transect.

Many factors may influence the density, biomass and population structure of intertidal animals. Even sites which are in close proximity and experience similar physical conditions can show radical differences in species abundance (*e.g.* Barkai & Branch 1988). The degree of wave exposure (Stephenson & Stephenson 1972, Underwood 1981, McQuaid & Branch 1984, 1985, Takada & Kikuchi 1990, Bruton *et al.* 1991, Takada 1993, Ompi 1994), substratum type (McQuaid & Branch 1985), primary productivity (Bosman & Hockey 1988a, 1988b, Dye & White 1991, Potter & Schleyer 1991, Lasiak & White 1993, Bustamante *et al.* 1995) and temperature (Branch 1984, Brink 1987) are all known to affect species abundance.

It is suggested, however, that the density, biomass and population structure of *T. sarmaticus* at the different shores in this study were unlikely to be influenced by wave activity, substratum type and temperature, as all the sites had similar physical conditions.

Differences in primary productivity between sites may have contributed to the observed differences in density, biomass and size-frequency of *T. sarmaticus*. Primary productivity is known to vary around the South African coast (Bustamante *et al.* 1995) and may even vary at different tidal levels on a shore (Bosman & Hockey 1988b, Dye & White 1991). The accumulation of guano from birds that roost and nest on Bird Island may have a fertilizing effect in the intertidal zone which may increase algal productivity, as observed for other islands (Bosman & Hockey 1986, 1988b, Branch *et al.* 1987). Increased algal productivity on such nutrient-rich islands has been shown to increase the size of grazers (Bosman & Hockey 1988b). It is therefore possible that the larger sized *T. sarmaticus* found on Bird Island was linked to increased food availability.

Unlike most sites examined in this study, *T. sarmaticus* at Kelly's Beach was subject to intense exploitation and disturbance (turning of boulders in search of bait - *pers. obs.*, Dept. Sea Fisheries - Port Alfred). Human exploitation was probably the main cause for the lower density, biomass and smaller sized animals at this site. High human exploitations of other *T. sarmaticus* populations (*e.g.* Flat Rocks - McLachlan & Lombard 1981), as well as other molluscs (Branch 1975, Van Erkom Schurink & Griffiths 1990, Keough *et al.* 1993, Addessi 1994), has been shown to reduce the standing stocks and maximum size of the animals. Even if animals are not collected and removed from the intertidal, high-intensity human trampling (particularly during holiday seasons) of the rocky shore organisms is capable of directly or indirectly having a negative influence on intertidal populations (Povey & Keough 1991). Chelsea Point, and to some extent Cape Recife, is subject to low intensity exploitation which may explain why these sites had large animals (> 80 mm shell length) (Rondo 1995). Although human exploitation is thought to be the main explanation for the observed differences in size-frequencies of *T. sarmaticus* at some sites along the eastern Cape coast, other factors may also play a role. For example, erratic distribution and settlement patterns are known to influence size composition and modify populations (Lasiak & Dye 1989).

The density and size structure of *T. sarmaticus* subjected to low intensity exploitation (*e.g.* Chelsea Point) was similar to that found on unexploited shores (see Table 2.12). The

resilience to low levels of exploitation may have been a result of *T. sarmaticus* extending its distribution into the subtidal. Similarly, other exploited turbinids (Keough *et al.* 1993) and gastropods (Poiner & Catterall 1988) obtain some degree of resilience by replenishing their exploited intertidal populations from subtidal populations. It is not known to what degree *T. sarmaticus* benefits from replenishing intertidal populations from subtidal stocks and this warrants further investigation.

Bruton *et al.* (1991), using yield-per-recruit models for *T. sarmaticus* populations in the Cape St. Francis area, indicated that the present exploitable legal size (≈ 73.7 mm shell length) should not be decreased as this would result in a considerable drop in a potential yield of *T. sarmaticus*. Very often, the legal size limits are not adhered to and numerous undersized individuals are removed (Kelly's Beach being particularly prone to such exploitation). This excessive removal of undersized animals may have resulted in the lower density, biomass and smaller sized *T. sarmaticus* at Kelly's Beach. The Kelly's Beach site, at the time of sampling, yielded no legal sized animals and only offered a standing stock of approximately 1600 per km of coast. This was considerably lower than reserve sites (*e.g.* Bird Island) and low intensity exploited sites (*e.g.* Chelsea Point), which yielded standing stocks of 9000 and 10700 animals per km of coast, respectively, and in addition, a relatively large proportion of the animals in these sites were over the exploitable legal size.

Intraspecific zonation patterns are often exhibited by herbivorous gastropods (*e.g.* Bertness 1977, McQuaid 1981, Cushman 1989, Takada 1996). Vermeij (1972) has proposed two main types of size-specific gradients. Firstly (type 1), shell size tends to increase in an upshore direction in species that are characteristic of the high-shore, and secondly (type 2), shell size increases in a downshore direction in species typical of the lower tidal areas. In the type 1 gradient, mortality is caused by physical factors such as high temperature or desiccation. In the type 2 gradient, mortality is generally caused by predation or other biotic interactions. This study, in conjunction with observations made by other workers (Lombard 1977, McLachlan & Lombard 1981, Kilburn & Rippey 1982, Yssel 1989, Bruton *et al.* 1991) has shown that *T. sarmaticus* has a "type 2" size gradient, with animals being most common on the low-shore and shell size increasing downshore. Similar size increases downshore have been reported for other turbinids (Yukihira *et al.* 1995).

Many factors have been reported to cause and maintain size gradients in populations

on shores (Walsby 1977, McQuaid 1981, McCormack 1982, Chen & Richardson 1987, Peckol *et al.* 1989). These range from predation (Markowitz 1980) and post-larval mortality (Vermeij 1972) to active migration in response to various cues (Bertness 1977, Gendron 1977, Doering & Phillips 1983). Several of these factors may control the size structure gradient of *T. sarmaticus*. On exposed shores, snails are often displaced by wave action (Smith & Newell 1955, Walsby 1977). The intense wave action experienced at the study sites may have maintained the vertical size gradient, as smaller individuals may have been dislodged from the lower littoral regions. Hence, *T. sarmaticus* may have been forced to live in the mid-littoral regions until larger and able to withstand greater wave action. Avoidance of possible predation on the low-shore by crabs and octopuses was given as the cause of vertical distribution patterns in whelks (Phillips 1969, Feare 1970). Both of these predators are relatively abundant in the low-shore regions of the Eastern Cape Province (*pers. comm.* - A.N. Hodgson, Rhodes University). Therefore, larvae and smaller individuals are likely to prosper better in the mid-shore region because of less predation. Larger individuals with robust shells and thick opercula, however, may be more protected against predation. Yssel (1989) demonstrated that when a large portion of an existing population of *T. sarmaticus* was removed (as in over-exploitation), the size structure gradient collapsed. This suggests that intraspecific competition may exist between small and large *T. sarmaticus* for food or shelter, rather than interspecific competition or avoidance of predators.

Branch (1984) has identified temperature as a factor controlling the distribution of populations of many organisms on rocky shores. Behaviourial and physiological adaptations of snails to desiccation are important for their survival (Garrity 1984, Marchetti & Geller 1987). Lombard (1977) has shown that large *T. sarmaticus* are more suspectable to rapid temperature changes than small individuals. This may explain why few large animals were found in the upper shore zone. On rocky shores, if refuges such as holes and crevices are in short supply, high temperature may cause large animals to migrate down the shore, while juveniles and small animals escape into refuges (Emson & Faller-Fritsch 1976, Raffaelli & Hughes 1978). Even though the underside of boulders in the upper shore region may offer plenty of refuges, the large size attained by adult *T. sarmaticus* may prevent the utilization of these refuges, thus necessitating the downward migration of animals.

In some intertidal gastropods, habitat selection plays a major role in maintaining their

pattern of vertical distribution (review in Underwood 1979, Byers & Mitton 1981, Janson 1983, Woodbury 1986, Byers 1989). In some cases, preferences in habitat selection change during the snail's life-history as often, tidal level preferences differ between juveniles and adults (McQuaid 1981, 1982). In addition, Mitchell (1980) has pointed out that the interspecific vertical distribution of six trochids (*Diloma* spp.) was not directly related to resistance to high temperatures and desiccation, but to the conditions likely to be experienced during low tides within the microhabitat occupied by each species. Similarly, the different low tide microhabitat requirements of small and large *T. sarmaticus* may have resulted in the establishment of the vertical size gradient observed in this study.

Differences in the algal dietary requirements of intertidal herbivorous gastropods have been known to affect their vertical distribution (*e.g.* Branch 1975, Takada 1996). Thus, any differences in the dietary requirements of small and large *T. sarmaticus*, may have influenced their distribution within the intertidal. For example, very small *T. sarmaticus* (< 10 mm shell length) have been observed to settle in the upper littoral zone on *Ulva* spp. at Kenton-on-Sea (*pers. obs.*). The migratory ability of *T. sarmaticus* may have been important in maintaining the size gradient, as this species is extremely mobile (Lombard 1977, Yssel 1989, *pers. obs.*). Various studies have attempted to determine the cues used by species that actively migrate to maintain their size distributions. A wide variety of possible cues have been determined from light and gravity (Bertness 1977, Doering & Phillips 1983) to wave action (Gendron 1977). Since larger *T. sarmaticus* seemingly prefer areas of high wave action (Bruton *et al.* 1991), the low-shore and adjacent subtidal region with their higher wave action may have attracted the adult animals, thus establishing the observed size gradient.

In conclusion, *T. sarmaticus* is a large and abundant grazer on intertidal shores of the Eastern Cape Province. Due to its abundance on some shores, it may play a pivotal role in the structuring of some communities. The density, biomass and size-frequency of *T. sarmaticus* within reserves and exploited sites (*e.g.* Chelsea Point) were found to be very similar. The larger animals found at Bird Island may have been a result of no exploitation, as well as a suggested increase in primary productivity and food availability. It is probable that the intense over-exploitation of animals and disturbance of the shore at Kelly's Beach (Port Alfred) contributed to the lower density and biomass, and smaller size of *T. sarmaticus*. In addition, it is suggested that over-exploitation of animals at Kelly's Beach is threatening

the *T. sarmaticus* population. Preventing the removal of *T. sarmaticus* from this site may allow stocks to recover, although this may take at least four years (Yssel 1989). The standing stocks of *T. sarmaticus* at Chelsea Point (Port Elizabeth) were found to be relatively high, therefore, it was concluded that this site could sustain monthly sampling for a long-term reproductive study. Finally, *T. sarmaticus* exhibited a distinct size gradient, with larger animals being found in a downshore direction. Although the causal factors of this size gradient were not investigated, a number of possible reasons have been discussed. This vertical size gradient of *T. sarmaticus* has important consequences for its management as a large portion of the reproductively active population can only be collected from the intertidal during spring low tides or specialized equipment is required to collect them from the subtidal region. Future studies on *T. sarmaticus* may involve monitoring the temporal changes in population structure of animals at exploited and unexploited sites. This data may assist in determining the effect of exploitation on *T. sarmaticus* populations. In addition, exploitation experiments will determine the time required for *T. sarmaticus* populations to recover. Finally, determining the factors that contributed to the variation in density and biomass between sites, and those that resulted in the establishment of vertical size gradients in *T. sarmaticus*, need to be investigated.

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CHAPTER THREE

GROWTH RATE OF *TURBO SARMATICUS* FROM A WAVE-CUT PLATFORM

INTRODUCTION

The population structure and productivity of any species can only be determined when data on growth rate, population age structure, maximum age and mortality are available (Underwood 1979). Furthermore, the quantification of growth is pivotal in the expression of population dynamics (Beverton & Holt 1959). For species that are exploited, such as the vetigastropod, *Turbo sarmaticus*, information on growth rate and the factors that affect growth will be important for future management of stocks. Research on abalones (Vetigastropoda) has indicated that growth rate data are necessary for calculating yield-per-recruit and egg-per-recruit models. These models can determine the time lag between spawning and reproductive recruitment (Day & Fleming 1992). This time lag is significant in population dynamics as it sets the period of pre-reproductive mortality and the proportion of juveniles surviving to reproductive recruitment. Any density-dependent growth will also alter the stock-recruitment relationship. In addition, mathematical models used in the formulation of management strategies rely heavily on the accurate determination of age and growth data to: 1) provide a basic framework for the assessment of the status of exploited stocks (Ricker 1975); 2) propose regulatory measures (Butterworth *et al.* 1989) and 3) ensure sustainable utilization of stocks (Poore 1972).

Variation in the growth rate of a species over time, between sites or between individuals at a site has important consequences for stock-recruitment relationships (Day & Leorke 1986). If variation between sites is significant, then management models will have to be determined for each site. Similar concerns apply to variation over time as growth may be measured in an unusual year (Sainsbury 1980). Many marine gastropods are known to show seasonal variation in growth rate (Horikoshi 1967, Todd 1979, McQuaid 1981, Ekaratne & Crisp 1984, Burgett *et al.* 1987, Miyamoto *et al.* 1995). Growth rate appears to be determined by external factors such as temperature (Ekaratne & Crisp 1984), desiccation (Roberts & Hughes 1980) and food supply (Horikoshi 1967, McQuaid 1981, Cubit 1984). Some of these factors have been investigated experimentally (see review by Hahn 1989), but often, numerous factors act simultaneously to affect growth (Underwood 1984).

Growth in molluscs is usually measured in terms of increases in shell size and the process of shell formation has been discussed by Wilbur (1964). A number of methods have

been used to determine growth in molluscs such as internal banding patterns of the shell, cohort analysis and tagging studies (Crisp 1989, Day & Fleming 1992, Shepherd & Breen 1992). A reliable method for determining growth of individuals is to tag measured animals and to remeasure them after a set time interval (Forster 1967, Newman 1968, Branch 1974, Day & Fleming 1992).

Growth rates have been studied in turbinids from various regions of the world (*e.g.* Yoshiya *et al.* 1986, You *et al.* 1987, Guanes Mercado & Torres Moye 1991, Yukihira *et al.* 1995), but little attention has been given to the growth rate of South African turbinids. The growth rate of *T. sarmaticus* has been determined at a few sites along the South African coast (Lombard 1977, McLachlan & Lombard 1981, Yssel 1989). The growth models derived from these studies, however, were based on poor recapture of tagged animals and were restricted to one shore type (quartzitic boulder shores) even though *T. sarmaticus* is known to inhabit shores of other geomorphologies (*e.g.* wave-cut platforms and reefs - *pers. obs.*).

The aims of this study were to produce a growth model derived from a higher count of returned animals and to establish whether the growth rate of *T. sarmaticus* resident on a wave-cut platform was different to that of animals on boulder shores (Lombard 1977, McLachlan & Lombard 1981, Yssel 1989). Wave-cut platforms are regarded as having a higher algal richness and biomass than boulder shores (McQuaid & Branch 1984, McQuaid *et al.* 1985). This increased food availability may positively influence the growth rate of *T. sarmaticus*, thereby influencing the age at which animals reach sexual maturity and exploitable legal size.

MATERIALS AND METHODS

Study site

Lombard (1977) and Yssel (1989), in their growth rate studies, found that many of the marked *Turbo sarmaticus* moved away from the initial study site, thereby reducing recapture. In an attempt to restrict long-shore migration of marked animals, a wave-cut platform at Kenton-on-Sea which was bounded on both sides by sand-filled gullies was selected (Figure 3.1). As *T. sarmaticus* is sand-intolerant (Yssel 1989, *pers. obs.*), the gullies should have

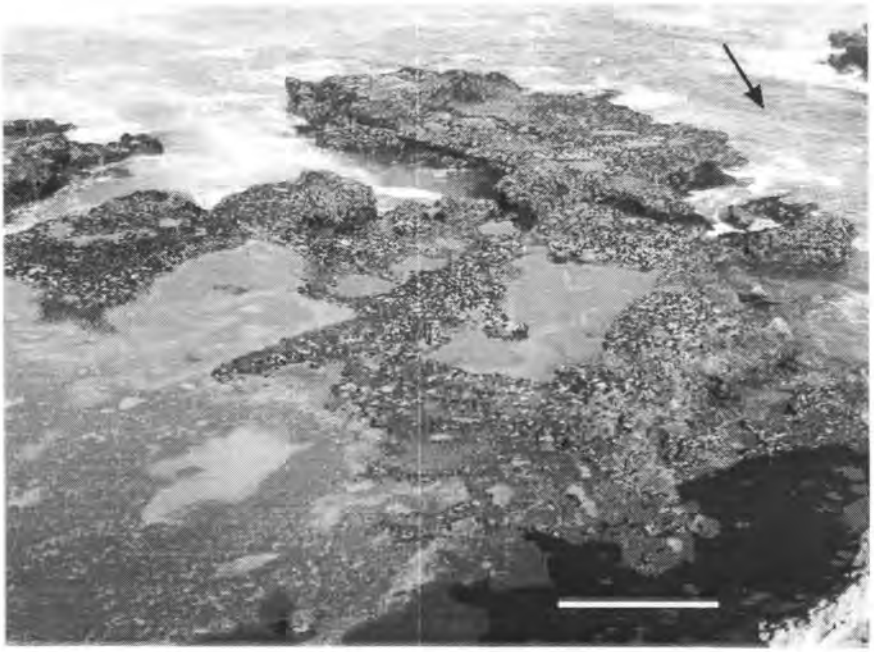

Figure 3.1. Photograph of the wave-cut platform (spring low tide) at Kenton-on-Sea where the growth study of *Turbo sarmaticus* was undertaken. The arrow indicates one of the deep sand-filled gullies isolating long-shore migration of *T. sarmaticus*. Scale bar = 5 m.

limited the movement of *T. sarmaticus* away from the platform.

Prior to tagging, the size-frequency of *T. sarmaticus* at Kenton-on-Sea was determined in order to ensure that the animals collected for the growth rate study were representative of the entire size range present. During an evening spring low tide (January 1995) the shell lengths of all the animals found on the platform were measured. These data were then used to plot the size-frequency distribution of the population at this site.

Growth rate

Two methods were used to determine the growth rate of *Turbo sarmaticus*. The first involved measuring the shell lengths of labelled individuals at regular intervals, and the second used internal shell micro-growth banding.

A) Tagging experiment

A total of 562 *T. sarmaticus* ranging from 19 - 92 mm shell length were collected during an evening spring low tide in 1995. The animals were immediately transported back to the laboratory where numbered tags made from "Dymo" were attached to the outer lip of the shells. Two holes ($\approx$ 1 mm ϕ) were drilled through the shells using a dentist's drill, and the tags were then fastened to the shells with nylon fishing line (Figure 3.2). Preliminary studies showed that the holes in the shells were repaired within 2 - 3 weeks and it was therefore assumed that the labelling technique did not interfere with the animal's normal growth and behaviour. Following labelling, shell lengths were measured (0.1 mm) and the animals were then returned to the study site on the 1st February 1995.

The number of animals in each size class, given as a percentage of the total number of *T. sarmaticus* labelled (n = 562), at Kenton-on-Sea is presented in Table 3.1. These animals represented the entire size range present at the Kenton-on-Sea study site (Figure 3.3).

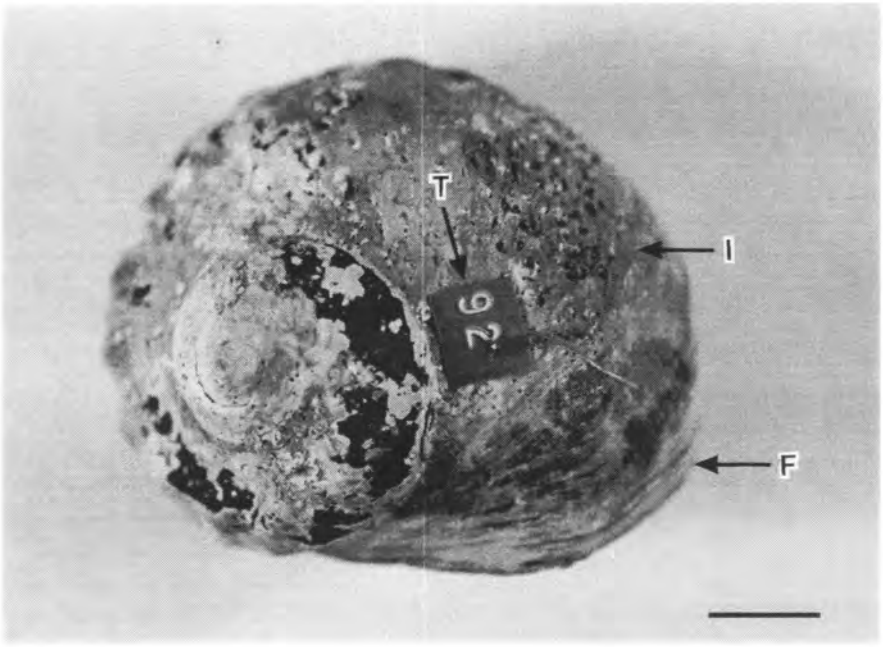

Figure 3.2. An example of *Turbo sarmaticus* tagged with a "Dymo" number which is attached to the shell by means of nylon fishing line. **T**: "Dymo" tag, **I**: initial shell length, **F**: final shell length after a year of growth. Scale bar = 1 cm.

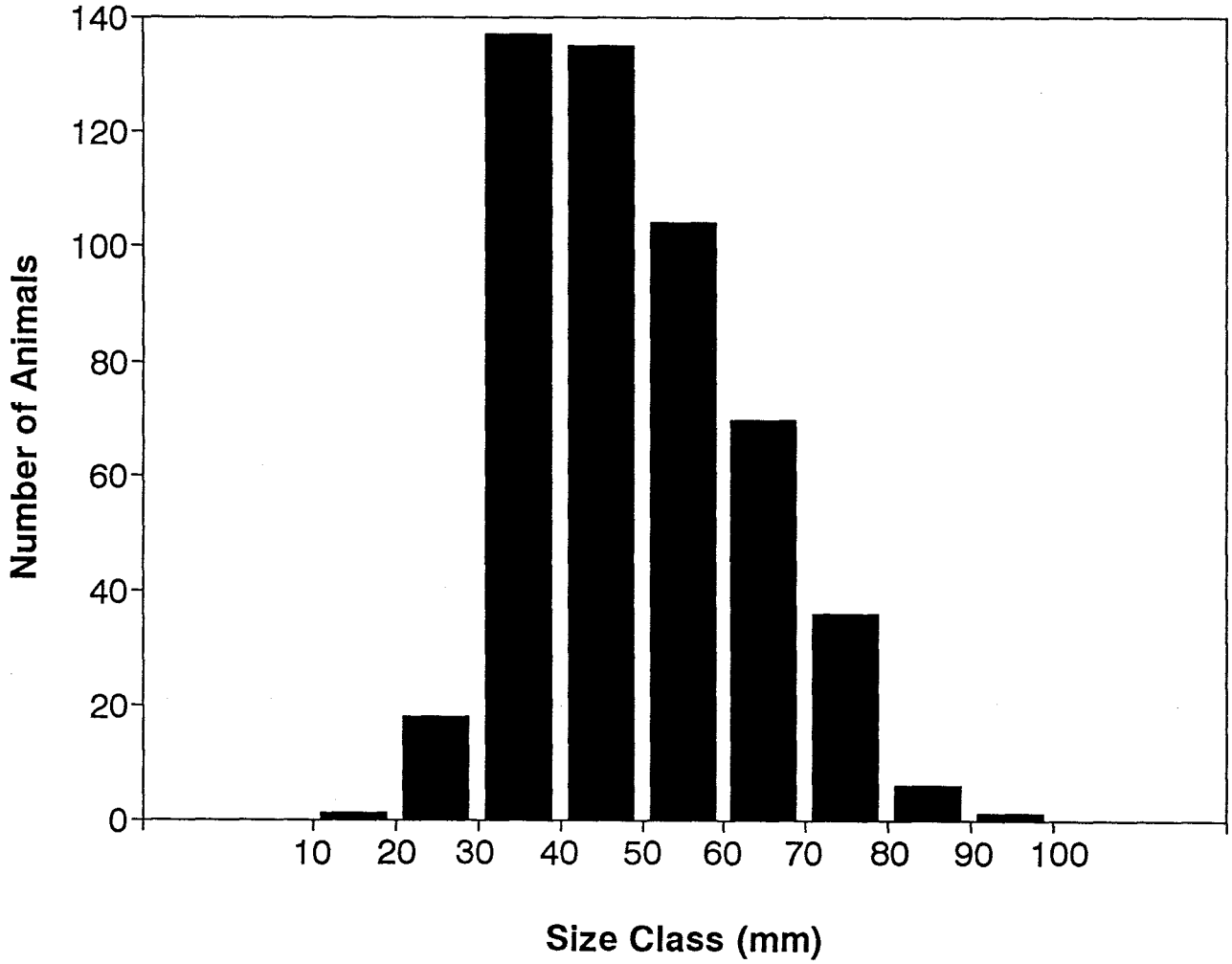

Figure 3.3. Size-frequency distribution (shell length in mm) of *Turbo sarmaticus* (n = 508) present on the wave-cut platform at Kenton-on-Sea (January 1995).

Table 3.1. The number of animals in each size class as a percentage of the total number of *Turbo sarmaticus* labelled (n = 562) at Kenton-on-Sea.

Size class (mm shell length)	Actual numbers	Percentage
10 - 19.9	1	0.2
20 - 29.9	18	3.2
30 - 39.9	153	27.1
40 - 49.9	151	26.7
50 - 59.9	115	20.5
60 - 69.9	77	13.7
70 - 79.9	40	7.1
80 - 89.9	6	1.1
90 - 99.9	1	0.4

Monthly searches were done during evening spring low tides until 1st February 1996 (12 months) and the size (shell length) of the tagged individuals found were measured.

To calculate the growth of *T. sarmaticus*, a Ford-Walford plot was constructed to estimate the constants L_∞ and K of the von Bertalanffy equation. These values were then used in the von Bertalanffy growth equation (Beverton & Holt 1959) to produce a growth curve:

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

where, $\mathbf{L_t}$ = length at age t; $\mathbf{L_\infty}$ = theoretical maximum length; **e** = base of Napierian logarithm (2.71828); **K** = constant reflecting rate of change of length increments ($K = -\text{Log}_e k$); **t** = time; $\mathbf{t_0}$ = time at which growth commences from L = 0 (in this instance taken to be 0).

B) <u>Growth banding</u>

Attempts were made to examine micro-growth bands in the calcareous operculum and internal spiral columella of the shell. Five opercula and five internal spiral columellae were

embedded in polyester casting resin (Metaserv s.w. resin 137/12742). The interior surfaces of the opercula (side attached to foot - Figure 3.4) were sanded down until smooth using a series of graded sandpapers (100 - 600 grade waterpaper). Following this, the smooth surfaces were polished using a metal polish (Brasso). The spiral columellae were cut longitudinally and transversely using a diamond saw, sanded smooth and then polished as above. The polished surfaces were then etched in 0.01 M HCl for two minutes. Acetate peels (Agar Scientific Ltd. # G255), soaked in acetone until almost molten (about 35 seconds), were placed on the etched surfaces and allowed to dry (about five minutes). After drying, the acetate peels were placed between two slides and viewed under an Olympus BX40 phase-contrast light microscope to observe any banding. In addition to acetate peel observations, the etched opercula and spiral columellae were gold coated and viewed in a Jeol JSM 840 scanning electron microscope to observe growth banding.

Some evidence of banding was seen in both the opercula and spiral columellae, but it was impossible to determine whether the banding was due to growth rings or structural anomalies in the shell. Due to this uncertainty, growth banding was discarded as a method for determining growth rate in *T. sarmaticus*.

RESULTS

The size-frequency of the *Turbo sarmaticus* population at Kenton-on-Sea is shown in Figure 3.3. Very few small (< 30 mm shell length) animals were found with most ranging from 30 - 70 mm shell length. The largest animals found at this site were about 90 mm in length but they were very scarce (Figure 3.3).

Of the 562 tagged *T. sarmaticus*, 54.6% were not recovered. The monthly recovery rate was low, ranging from 4 - 14% (Figure 3.5), and at the end of 12 months, only 70 animals could be located (12.4% return). The recovery rate of these 70 animals during the year was very erratic (8 were found 5 times, 15 four times, 16 three times, 22 twice, and 9 only once) (see Appendix III.I) thus making it impossible to determine if there were any seasonal differences in growth rate.

Animals as large as 90 mm shell length were labelled at the start of the tagging

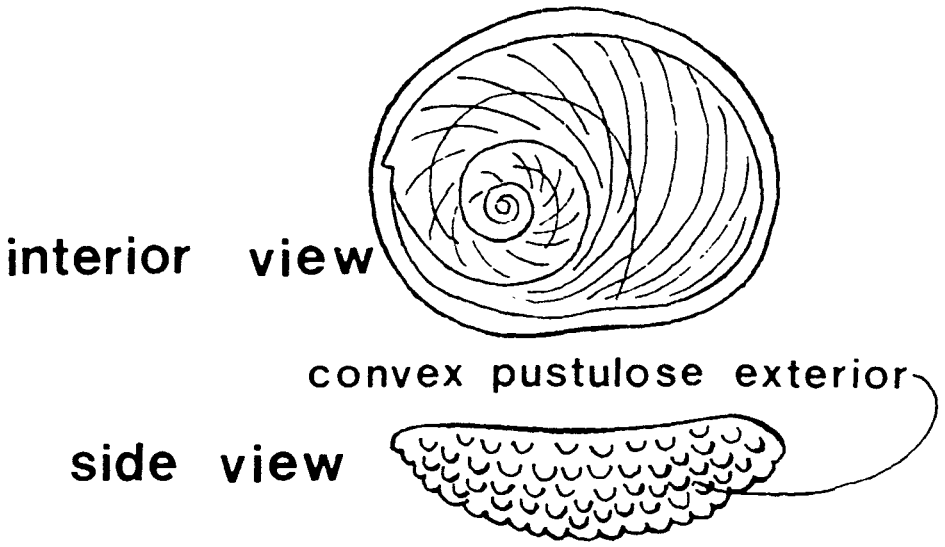

Figure 3.4. Diagrammatic representation of the operculum of *Turbo sarmaticus* indicating the surface examined for growth lines.

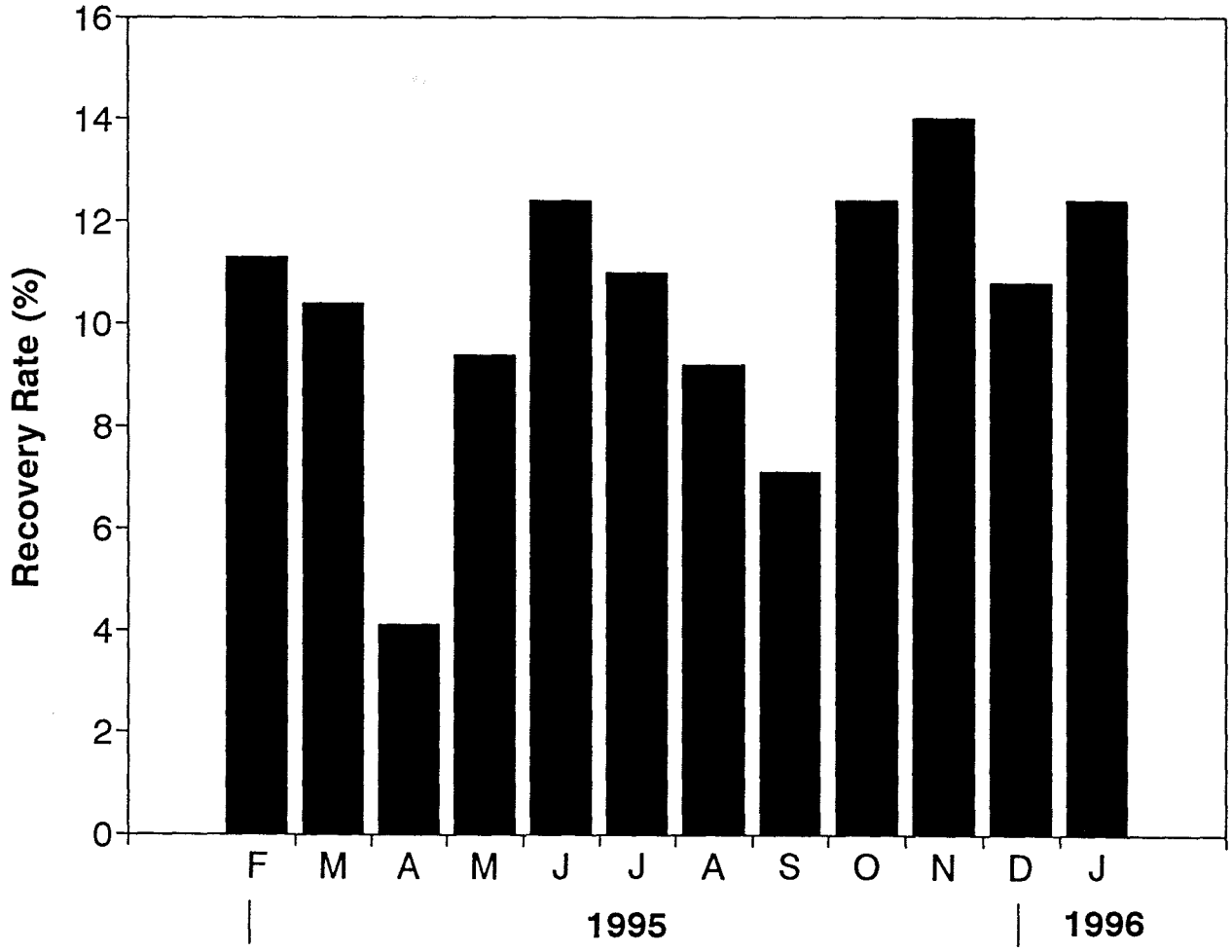

Figure 3.5. Monthly recovery rates (% of total animals) of tagged individuals used for calculating the growth rate of *Turbo sarmaticus* on the wave-cut platform at Kenton-on-Sea.

experiment. However, the largest animal recaptured at Kenton-on-Sea at the end of the study was only 76.2 mm shell length, which is not representative of the upper size class found at this site (Figure 3.3). As a result of not recapturing animals greater than 77 mm shell length, the von Bertalanffy growth equation is limited and can only represent the growth rates of animals up to about 80 mm shell length. Despite this limitation, the von Bertalanffy growth equation provided useful information about the initial growth rate of *T. sarmaticus* up until sexual maturity (≈ 52.5 mm shell length) and exploitable legal size (≈ 73.7 mm shell length) at the Kenton-on-Sea site.

The fastest growth occurred in smaller individuals which had the greatest growth increments from their initial shell lengths. For example, an animal of 30 mm shell length increased by approximately 25 mm in a year, whereas an animal of 70 mm shell length only increased by approximately 5 mm (Figure 3.6). A Ford-Walford plot of the data from Kenton-on-Sea gave an estimate of 0.544 for K and 81.07 mm for L_∞ (Figure 3.7A). A growth curve based on the von Bertalanffy growth equation showed the size/age relationship of *T. sarmaticus*, $L_t = 81.07\ (1\text{-}e^{-0.544(t)})$, and indicated that the growth up to the size of 65 mm shell length (first 3 years) was rapid, after which it slowed down (Figure 3.7B). The growth curve further indicated that at Kenton-on-Sea the mean age of exploitable legal sized animals was 4.4 years (range: 2.9 - 5.8) and the mean age of sexually mature animals was 1.9 years (range: 1.6 - 2.2) (Figure 3.7B).

DISCUSSION

Internal shell growth lines can be a very valuable in determining age and growth in many marine molluscs (Crisp & Richardson 1975, Lutz 1976, Hilbish 1986, Richardson 1987, Crisp 1989, Nakaoka & Matsui 1994, Crisp *et al.* 1990, Gray 1996). However, no internal micro-growth banding was readily distinguishable in either the spiral columella of the shell or the operculum of *Turbo sarmaticus*. Similarly, Lombard (1977), McLachlan & Lombard (1981) and Yssel (1989) also found internal growth lines in the shell and operculum to be an unreliable method for determining age in *T. sarmaticus*.

Growth curves should express the pattern of change in growth of a population with time (Welch 1970). The growth of *T. sarmaticus* of less than 80 mm shell length was

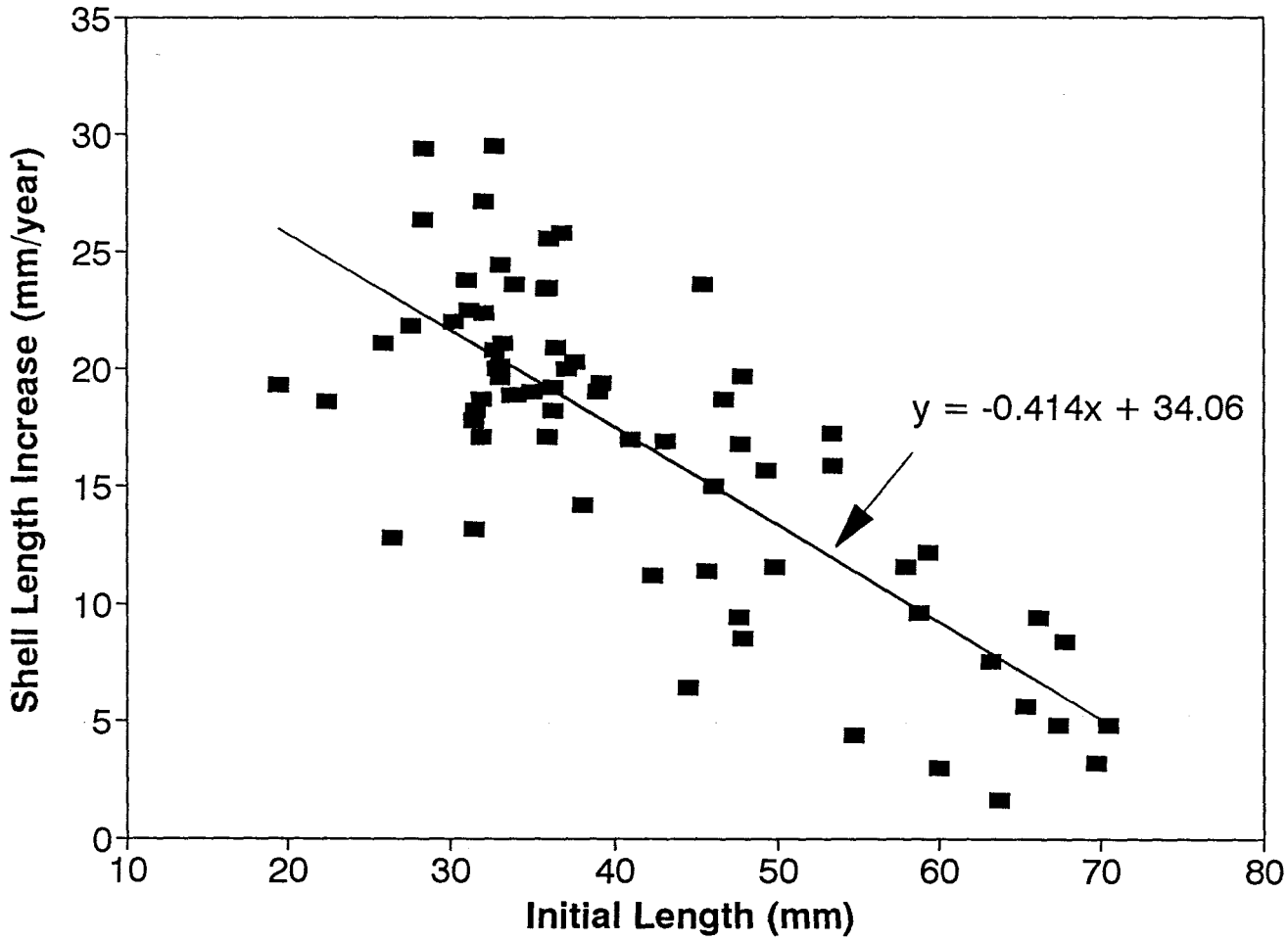

Figure 3.6. Relationship between shell length increases (mm/year) and initial shell lengths of tagged *Turbo sarmaticus* (n = 70) at Kenton-on-Sea from February 1995 to February 1996.

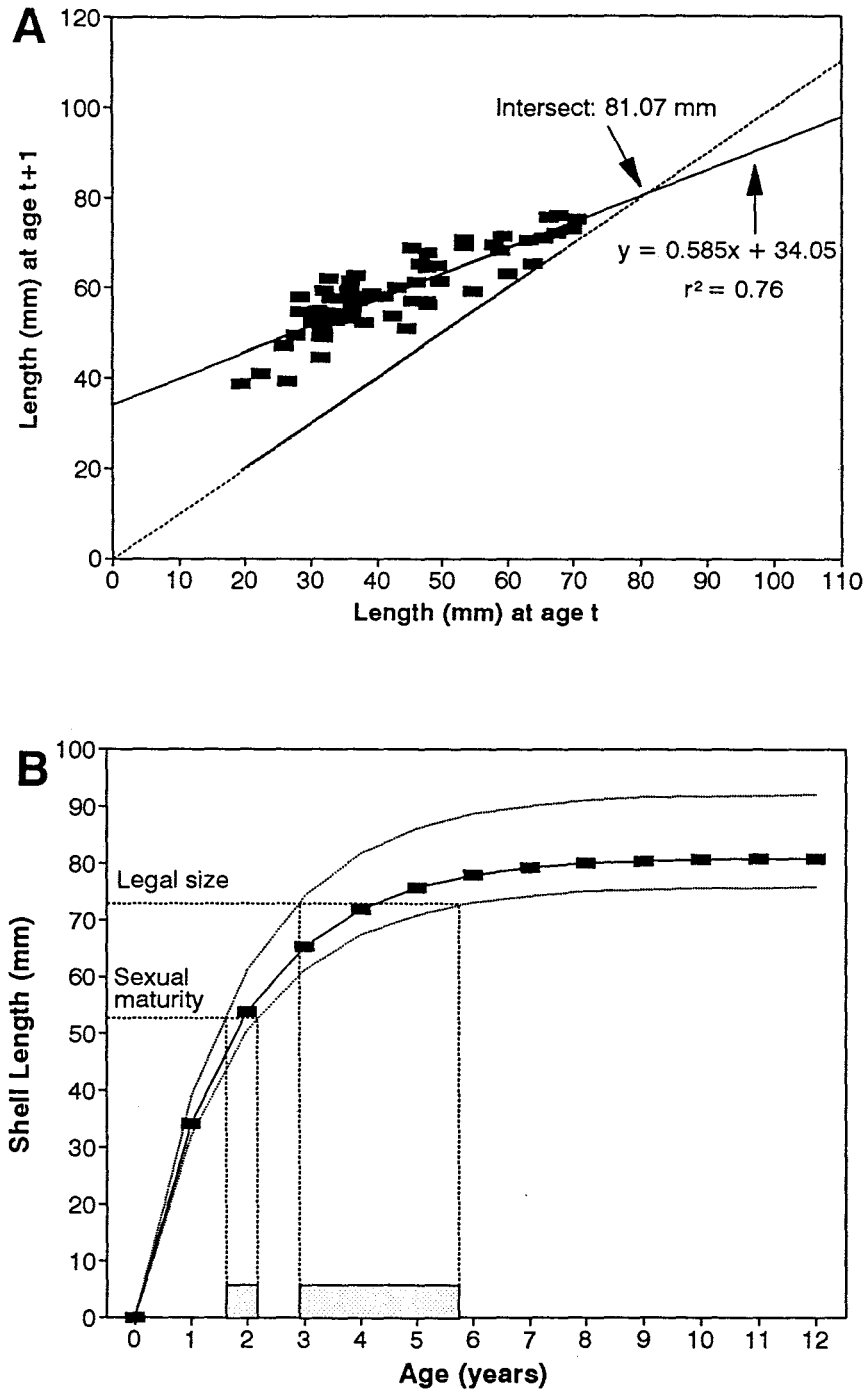

Figure 3.7. (A) Ford-Walford plot for *Turbo sarmaticus* (n = 70) at Kenton-on-Sea obtained by plotting initial shell length against shell length after one year. **(B)** Von Bertalanffy growth curve for *T. sarmaticus* at Kenton-on-Sea calculated from the K and L_∞ values obtained from a Ford-Walford plot.

described accurately by the von Bertalanffy growth model. Comparison of the growth curve of *T. sarmaticus* from the wave-cut platform at Kenton-on-Sea with those for animals from boulder shores in the eastern Cape, indicates that growth rate (up to exploitable legal size ≈ 73.7 mm shell length) is similar at all sites (Figure 3.8). Animals at Kenton-on-Sea attained sexual maturity at an age (≈ 1.9 years) similar to those from Tsitsikamma National Park (Sandbaai: ≈ 2.0 years, Bester's Hole: ≈ 2.2 years) and Port Elizabeth (Flat Rocks: ≈ 2.4 years, Skoenmakerskop: ≈ 1.6 years) (Lombard 1977, McLachlan & Lombard 1981, Yssel 1989).

The levelling off of the growth curve (at about 80 mm shell length) for *T. sarmaticus* at Kenton-on-Sea was sooner than for animals from the other sites (Figure 3.8). This may be explained by the low L_∞ (81.07 mm) calculated for *T. sarmaticus* due to no animals greater than 77 mm shell length being recaptured. The fact that larger animals (> 90 mm shell length) were found at Kenton-on-Sea (Figure 3.3) means that the L_∞ calculated was inaccurate. If the 90 mm size class animals released had been recaptured, then the L_∞ would have been greater. The growth curve may then have been similar to that calculated for *T. sarmaticus* at Port Elizabeth (Figure 3.8) (Lombard 1977, McLachlan & Lombard 1981). *Turbo sarmaticus* achieves sizes up to 115 mm shell length at other sites (*e.g.* Bird Island) within the same geographical region (*i.e.* temperate South-East region - Chapter 2). Therefore, the growth curves calculated for animals at Tsitsikamma National Park are probably the most accurate predictions of growth rate for this mollusc. The absence of larger animals at Kenton-on-Sea may have been due to a number of factors. Larger animals may have lived subtidally (only the intertidal population was sampled), growth rates may have been slower and/or there may have been an increased mortality of larger individuals (natural or due to exploitation by man) (Tarr 1995).

The high variability in growth rate between individuals at Kenton-on-Sea produced the wide scatter of growth increments observed in Figure 3.6. For example, animals of 30 mm initial shell length increased by as little as 14 mm or as much as 30 mm shell length per year. Similar variability in growth rate between individuals of the same species has been reported for other molluscs (*e.g.* Poore 1972, Branch 1974, Kojima *et al.* 1977, Nash 1992), including *T. sarmaticus* from other sites along the coast of the Eastern Cape Province (Lombard 1977, McLachlan & Lombard 1981, Yssel 1989).

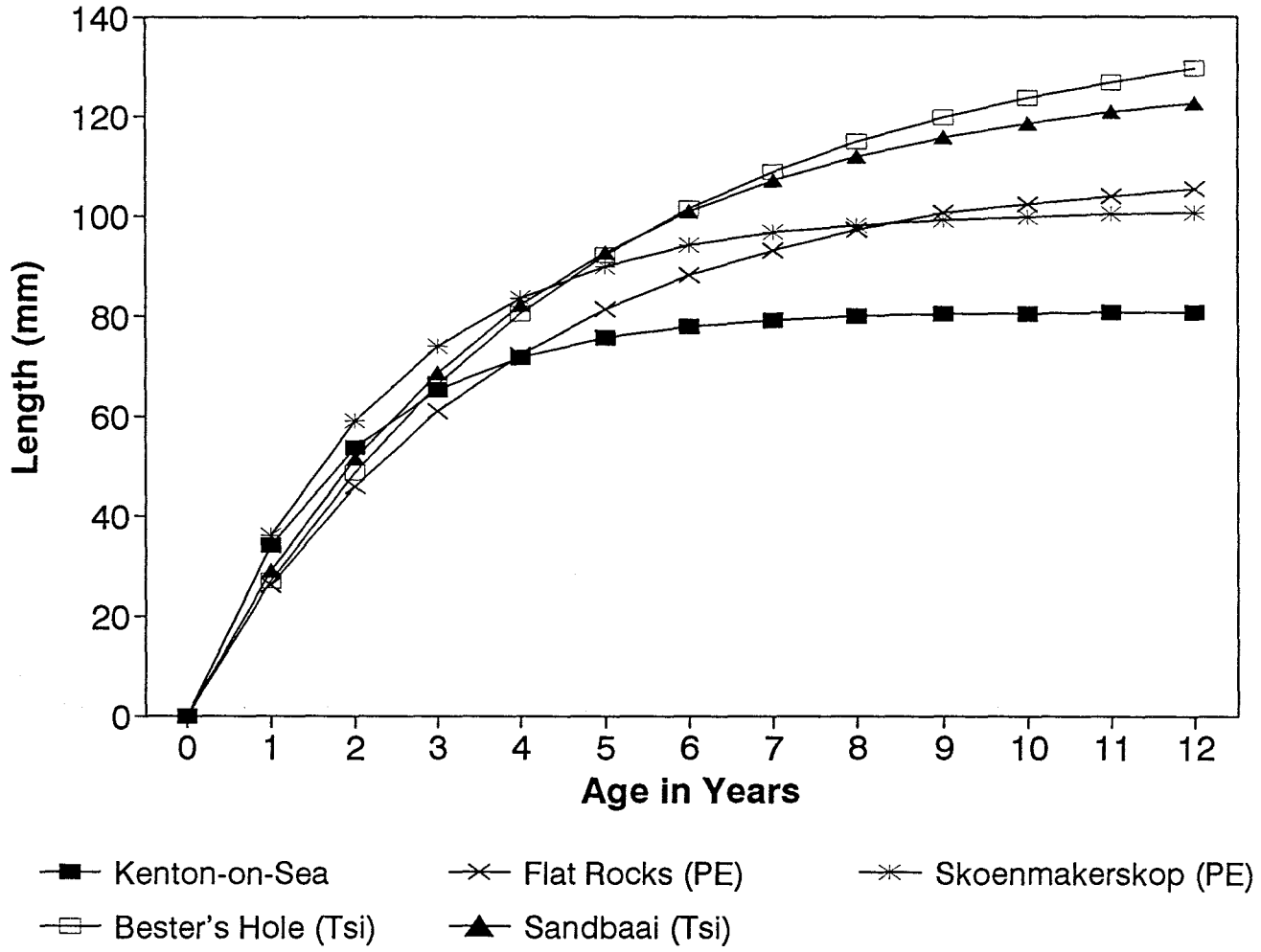

Figure 3.8. Comparison of von Bertalanffy growth curves for populations of *Turbo sarmaticus* previously studied at Port Elizabeth (Lombard 1977, McLachlan & Lombard 1981) and Tsitsikamma Coastal National Park (Yssel 1989) with that at Kenton-on-Sea (this study). PE = Port Elizabeth, Tsi = Tsitsikamma Coastal National Park.

Sources of intra- and inter-specific variation in growth rate of molluscan populations are many and varied. Exposure to wave action (Breen & Adkins 1979, Hayashi 1980, Brown & Quinn 1988), local temperature regimes (Newman 1968, Quayle 1971, Leighton 1974), different habitats (Fournier & Breen 1983, Sloan & Breen 1988, Arnold *et al.* 1991), genetic variation (Hara 1990) and differences in animal density (Breen 1980, Calvier 1982) can all influence growth rates. In addition, locality is important as it incorporates the effects of food supply (Breen 1980, Shepherd & Hearn 1983). One common explanation for differences in growth rate in molluscs is food supply, with algal abundance and productivity being of major importance (Leighton & Boolootian 1963, Hirose 1974, McQuaid 1983, Shepherd & Hearn 1983, Underwood 1984, Riisgard 1991, McGrath 1992, Takada 1995). However, Newman (1968) has stated that while aspects such as dietary differences may play a role, temperature difference is probably the primary factor causing the discrepancies in growth rates between different regions. The similarity in initial growth rates of *T. sarmaticus* at different sites within the Eastern Cape Province (Figure 3.8) may have been due to the animals experiencing similar sea surface temperature as result of the warm Agulhas current (Branch & Branch 1981). The increased algal richness and biomass often associated with wave-cut platforms (McQuaid & Branch 1984, McQuaid *et al.* 1985) did not have a positive influence on the growth rate of *T. sarmaticus* at Kenton-on-Sea. It is possible, however, that the platform at Kenton-on-Sea did not have enhanced algal quality and/or quantity, thereby resulting in the similar initial growth rate of *T. sarmaticus* at all sites (Figure 3.8). The reasons for the individual variation in growth rates of *T. sarmaticus* at Kenton-on-Sea are not known. The steep profile of the lower-balanoid and cochlear zones on the wave-cut platform meant that these zones were compressed (*pers. obs.*) and therefore, similar wave action, temperature and food availability would have been experienced by all individuals. The variation in growth rates amongst individuals may therefore have been attributed to parasitic infestation (Huxham *et al.* 1993, Mouritsen & Jensen 1994) as some *T. sarmaticus* were found to be infested at Kenton-on-Sea (*pers. obs.*). In addition, genetic variability (Hara 1990) amongst the individuals may have contributed to the variations in growth rate.

In most molluscs, growth rates reach their maximum in early life and then decrease progressively with age (Branch 1974, Wood 1993, McNamara & Johnson 1995, Shepherd *et al.* 1995, Gray 1996). This was found to be the case for *T. sarmaticus* which had an initial

increase in shell length of about 23 mm per year, declining in larger animals (> 65 mm shell length) to about 5 mm per year. A number of factors are responsible for decreased rates of shell deposition with age. Decreases in molluscan carbonic anhydrase activity (Kawai 1955) and metabolism (Davies 1966) with age are known to decrease growth rates. Another factor influencing growth rate in molluscs is gamete production as energy intake is partitioned between growth and gamete development (Forster 1967, Griffiths & King 1979, Shepherd & Hearn 1983). Due to the complexity of shell deposition, however, it is difficult to ascribe a single cause and effect relationship (Branch 1974).

It was not possible to determine whether there were any seasonal differences in growth rate of *T. sarmaticus* in this study as the number of animals recaptured with regularity during the year was very small (see Appendix III.I). Nevertheless, other turbinids (Yukihira *et al.* 1995), as with *T. sarmaticus* from the same geographical region (Eastern Cape Province) of the South African coast (Lombard 1977, McLachlan & Lombard 1981, Yssel 1989), have been reported to exhibit seasonal differences in growth rate. It is therefore likely that this is true for the Kenton-on-Sea population. These data from previous studies on *T. sarmaticus*, however, must be viewed with caution as they were determined from low recapture rates. Seasonal changes in growth rates of molluscs are often reported and attributed to seasonal variation in temperature, food availability, reproductive cycle or a combination of these factors (Cox 1962, Sakai 1962, Newman 1968, Blackmore 1969, Dame 1972, Hirose 1974, Arnold *et al.* 1991, Yukihira *et al.* 1995).

The low recovery rate of *T. sarmaticus* (Figure 3.5) highlights two important features of this animal. Firstly, it is cryptic and difficult to find. Being cryptic will offer this species some degree of protection from exploitation by man which may in part, explain the poor recovery rate. Secondly, the poor recovery rate may also have been a result of the high mobility of individuals that constantly moved in and out of the study site. Lombard (1977) found a similar situation with tagged *T. sarmaticus* at Port Elizabeth, while Robinson (1974) reported that *T. sarmaticus* has been observed to move as far as 30 metres in less than a month. Being mobile may offer *T. sarmaticus* populations some degree of resilience against exploitation as the larger, reproductively active animals may move into the deeper, less accessible subtidal waters.

In conclusion, the results of this study, when combined with those of previous work

(Lombard 1977, McLachlan & Lombard 1981, Yssel 1989), have shown that the initial growth rate of *T. sarmaticus* was similar on shores with different geomorphologies. However, due to larger animals not being recaptured, the growth curve for *T. sarmaticus* at Kenton-on-Sea does not accurately represent the animal's growth above 80 mm shell length. The growth curve was limited by the low L_∞ which was dependent on the size of the animals recaptured. This raises doubts as to the usefulness of determining growth rates by means of the von Bertalanffy equation as L_∞ affects the equation. Growth rates of individuals were variable, which means that individuals in the population were reaching exploitable size at different ages. Consequently, exploitable size was reached at an age of approximately three to six years, whilst sexual maturity was reached after approximately one-and-a-half to two years. These data indicate that for *T. sarmaticus* at Kenton-on-Sea the present exploitable size ($\approx$ 73.7 mm shell length) is set at a number of years (1 - 4) after sexual maturity. This level may afford a degree of protection to the spawning stock by enabling animals to reproduce before becoming available for exploitation. Future research may predict the growth rate of *T. sarmaticus* from other wave-cut platforms where large animals (> 100 mm shell length) reside. In addition, the factors influencing individual growth rate warrant further investigation.

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Appendix III.I. The monthly recovery and size at recovery of labelled *Turbo sarmaticus* (n = 70) from February 1995 to January 1996 at Kenton-on-Sea.

	Date labelled (1995)	Date of recovery										
Animal number	Feb 95	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan 96
	Initial shell length (mm)	Shell length at recovery (mm)										
g8	28.3			34.6								54.7
g10	47.6								52.3			57.0
g20	45.6											57.0
g26	32.0						45.6		51.4	52.6		54.4
g27	42.3		44.4									53.5
g30	32.7							50.6				53.5
g36	36.8		40.0				52.4					62.6
g47	39						48.4	50.8				58.0
g48	47.7			53.0					61.4	63.4		64.5
g57	47.9						45.0		49.2			56.4
g61	35.9		37.9					50.6		55.3		59.4
g66	27.6		30.2	34.6					44.0	47.1		49.4
g73	45.4											69.0
g82	38.0											52.2
g89	43.1								55.8	59.0		60.0
g95	37.6			43.0								57.9
r21	53.4									67.7		70.6
b4	33.0		34.7									53.1
b5	31.4											44.6
b25	47.9									64.6		67.6
b52	36.4			41.1								57.3
b73	46.7		48.7	50.5					59.9	62.0		65.4
b81	60.0											63.0
gc91	34.0											52.9
gc37	33.7											52.6
gc83	36.2			40.6			45.9	48.8		53.8		55.4
gc68	28.4							40.0				57.8
gc33	35.9									50.8		53.0
gc58	31.0											54.8
gc57	32.8			38.4			42.9					52.8
gc28	30.2			35.2			41.0		46.4	51.4		52.2

Appendix III.I. Continued.

	Date labelled (1995)	Date of recovery										
Animal number	Feb 95	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan 96
	Initial shell length (mm)	Length at recovery (mm)										
gc61	35.8			39.6					50.4	54.0		59.2
gc41	33.9											57.5
rc62	31.9		35.5	38.8	40.0			44.9				49.0
rc53	36.2			42.0	44.3							54.4
rc25	32.0			36.5	40.6							59.2
rc56	35.0				41.6				48.8			54.0
rc51	33.2		35.0	38.5	41.4							54.3
rc4	36.0		39.0	42.5	44.5							61.6
rc88	31.9		35.5	38.8	39.8							50.6
rc74	69.8						71.6		72.0			73.0
rc44	70.5						72.9					75.3
rc43	54.7		55.3				57.4			58.0		59.1
rc94	49.8			54.1			57.4		58.5			61.4
rc35	58.0						64.9			69.0		69.6
rc75	67.8						72.0					76.2
bck	33.0		35.7					45.4		50.4		52.6
bce	63.8							65.3	65.5	65.6		65.8
ba6	25.9							37.2		42.0		47.0
bb8	53.4			56.6				62.2	62.8			69.3
bb0	58.8							64.0				68.4
bc8	22.4							32.0	33.5			41.0
bd1	33.0		35.7					48.4				57.5
bd2	32.7							53.6	55.6			61.9
bd5	46.1			52.1				56.6		59.1		61.1
bd0	19.4							28.8				38.7
be9	59.3							65.6				71.5
bf3	44.5						47.2	48.1				50.9
bf6	31.4		33.8					45.7	47.2			49.2
bf8	37.0							54.6				57.0
bg0	63.2							68.5				70.7
bh5	31.5							42.0				49.7

Appendix III.I. Continued.

	Date labelled (1995)	Date of recovery										
Animal number	Feb 95	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan 96
	Initial shell length (mm)	Length at recovery (mm)										
bh7	66.2							71.7				75.6
bj0	26.4							31.5				39.2
bl0	49.3							59.4				65.0
bm2	39.2			45.0				51.0	52.4	54.8		58.6
bm3	65.4							68.1	68.4	69.6		71.0
bm4	67.4							70.2				72.2
bm6	31.1			36.7				44.5	46.5	49.5		53.6
bn7	41.0		43.4	46.0			50.0	53.6				58.0

CHAPTER FOUR

THE ANNUAL REPRODUCTIVE CYCLE OF *TURBO SARMATICUS*

INTRODUCTION

The reproductive biology of prosobranch gastropods is more varied than in any other group of molluscs (Fretter 1984). This is related to their wide range of structure and habitat. The majority of prosobranchs are gonochoristic (Fretter 1984) and exhibit two grades of organization: diotocardian, and the more advanced monotocardian. Primitive taxa such as the Turbinidae have a diotocardian organization, with each sex having a single gonad (where the gonad and digestive gland occupy the visceral coil) which opens by the functional right kidney and fertilization is external (Fretter & Graham 1994).

Annual patterns of reproduction in marine invertebrates are varied and may occur rhythmically or sporadically during part or all of the year (Giese 1959, Giese & Kanatani 1987). It is generally accepted that reproductive patterns are controlled by stimuli which may be endogenous or exogenous in origin (Giese & Pearse 1974, Grahame & Branch 1985, Giese & Kanatani 1987). One of the simplest reproductive strategies found in marine invertebrates is broadcast fertilization during which the gametes are released into the environment at spawning and fertilization is external. Most shallow water marine invertebrates (including broadcast fertilizers) are seasonal in their reproduction (Giese 1959, Kinne 1963, Clark 1965, Webber 1977, Fretter 1984, Fretter & Graham 1994).

A variety of methods can be used to determine the reproductive cycle and spawning season in marine invertebrates (Giese 1959, Boolootian 1966, Giese & Pearse 1974, Perez Ruzafa & Marcos Diego 1985). Ideally, histological studies in conjunction with gonad indices are recommended. Both of these, together with other parameters, were examined in this study.

Within trochacean gastropods, reproduction and development has been most extensively studied in Trochidae, whilst little is known about the Turbinidae (see Hickman 1992 for review). There are some accounts, however, of the seasonality of reproduction in turbinids from various geographical locations (*e.g.* Underwood 1974, Joll 1980, Yamamoto 1985, Belmar Perez *et al.* 1991, Ohashi 1991, Hickman 1992, Yukihira *et al.* 1995) including South Africa. Of the four South African species of *Turbo*, studies on reproductive seasonality have been limited to *Turbo sarmaticus* (Lombard 1977, McLachlan & Lombard 1980) and *Turbo coronatus* (Lasiak 1986a). Although Lombard (1977) and McLachlan & Lombard (1980) examined seasonality of reproduction in *T. sarmaticus*, these studies were short-term

(12 months) and did not determine the seasonal changes in the sperm content, oocyte diameter within the gonad, or sex ratio of the animals.

The aims of this study were to provide a more detailed account of seasonality of gametogenesis, including sperm and egg production, and to examine the reproductive cycle over a longer time period (24 months). In addition, by re-examining a population of animals at Port Elizabeth, the results could be compared with those obtained 20 years previously (Lombard 1977 - later published in McLachlan & Lombard 1980), thereby revealing any changes in the reproductive cycle over time. Estimates of standing stock (Chapter 2) of *T. sarmaticus* established that the shore at Port Elizabeth would be a suitable study site. The population of adult animals at this site was estimated to be large enough to sustain monthly sampling for the entire reproductive study (24 months).

MATERIALS AND METHODS

Animal collection

The reproductive cycle of *Turbo sarmaticus* was determined over 24 months from July 1994 to June 1996. The collection of animals was restricted to a single site (≈ 800 m²) at Chelsea Point (west of Cape Recife), Port Elizabeth (33°58'S/25°38'E). To ensure that 10 males and 10 females were obtained per month, thirty-five specimens (75 - 85 mm shell lengths) were sampled during the lowest spring tide. The animals were then taken to the laboratory and immediately dissected. The number of animals collected each month never exceeded thirty-five thus ensuring that the population was not over-exploited.

Sex ratio

It was possible to sex *T. sarmaticus* throughout the year macroscopically or from squash mounts of the gonad. Results from all the sacrificed animals during the course of the study were pooled (n = 404) to determine the sex ratio.

Size at sexual maturity

In order to determine the size at which *T. sarmaticus* reaches sexual maturity, animals were collected (25 - 90 mm shell lengths) when the gonad index was greatest (November). Animals were divided into 5 mm size classes (shell length). This size class division was chosen as it represented only a small difference in size between the animals. Ten individuals in each size class were examined macroscopically and histologically for mature gonads. Animals were classed as mature if the acini of the gonad contained spermatozoa or vitellogenic oocytes. The size at which 50% of the individuals in a given size class had mature gonads was chosen to represent the onset of sexual maturity. This percentage evaluation technique is a standard method used in fisheries management for the determination of sexual maturity in molluscs (Pitcher & Hart 1982, King 1995).

Reproductive cycle

A) Gonad index

The mean monthly gonad index (GI) for *T. sarmaticus* was calculated for 10 males and 10 females using the formula of Gonor (1972):

$$GI = \frac{\text{Dry Gonad Weight}}{\text{Dry Gonad Weight + Dry Body Weight}} \times 100$$

Animals were fixed for at least 72 hours in 4% formal saline to separate the gonad from the digestive gland. After fixation, the gonad was peeled away from the digestive gland, allowing body and gonad to be weighed separately. All dry masses (drying to constant weight at 60°C) were recorded to the nearest 0.01 g.

B) Gametogenic cycle

Each month, from July 1994 to June 1995, a small portion of the formalin fixed gonad

was removed (from the same region each time) from five males and five females. The portions were dehydrated using a graded ethanol series, embedded in Paraplast and transverse sections (6 μm in thickness) were cut on a Leitz rotary microtome. The sections were stained with haematoxylin and eosin (Humason 1967) and examined. The gametogenic condition of each animal was then assessed and scored as one of the following stages: 1) Early gametogenesis - characterised by an abundance of previtellogenic oocytes and spermatocytes, 2) Late gametogenesis - characterised by numerous developing vitellogenic oocytes and spermatids/spermatozoa, 3) Mature - characterised by an abundance of mature oocytes (with jelly coats) and active sperm, and 4) Spawned/spent - characterised by an absence of mature oocytes or sperm with any remaining gametes undergoing resorption. Where necessary, slides were photographed with a Nikon Optiphot compound light microscope.

In order to quantify seasonal changes in egg size within the gonad, oocyte diameters were measured each month from July 1994 to June 1995. The diameters of oocytes from five females were measured from histological slides. The diameters of the first fifty oocytes, observed in mid-longitudinal section, were measured (including egg jelly coats) using a Nikon compound light microscope fitted with a Nikon Filar Micrometre eyepiece. Data from the five females were pooled (250 oocytes/sample) and used to determine the monthly size range of oocytes.

In order to quantify seasonal changes in the sperm content within the gonad, the abundance of spermatozoa in the acini of the gonad was determined each month from July 1994 to June 1995. The abundance of mature spermatozoa was expressed as a percentage of the total gonad cross-sectional area they occupied. The area values were obtained by viewing histological slides (n = 5) under an Olympus BX40 microscope and capturing the image with a Panasonic WV-CM 1000 video monitor. The images were then archived using a Jeol Winstor image archiver and analysis system. SigmaScan/Image software (Jandel Scientific) was then used to calculate the percentage area occupied by the mature spermatozoa.

Environmental factors

The mean monthly daylength was calculated from sunrise and sunset tables published by the South African Royal Naval Hydrographer. Average, maximum and minimum monthly

sea temperatures were obtained from the Port Elizabeth municipality who took daily readings at Humewood beach. These readings were taken to indicate annual sea surface temperature fluctuations at the study site which was only a few kilometres away.

Statistical analysis

All statistical procedures were taken from Fry (1993) and computed with a StatGraphics V7.0 statistical computer program (Statistical Graphics Corporation, USA). All data were tested for homogeneity of variance and where necessary percentage data were transformed using the arcsine transformation ($\sin^{-1}\sqrt{x}$) to comply with the requirements of ANOVA tests. Multiple-factor ANOVA and two sample t-tests were used to determine whether the GI of males and females varied significantly over the study period. Chi-squared contingency table tests were used to determine if oocyte size class distributions differed significantly during the study period. Finally, a chi-squared test was performed to determine whether a 1:1 sex ratio existed for *T. sarmaticus*.

RESULTS

Sex ratio

Turbo sarmaticus was found to be dioecious (no hermaphrodites were encountered) and did not exhibit external sexual dimorphism (*i.e.* no difference between the shell sizes of males and females or in their external morphology) (Figure 4.1). The gonads of mature males, however, were creamy-yellow in colouration, whilst those of mature females were olive green. After spawning, the male gonads appeared brownish-cream, whilst in females they were brownish-green in colour. This made it possible to determine the sex of individuals throughout the year.

Of the 404 animals sampled at Port Elizabeth, 223 (55.2%) were male and 181 (44.8%) were female, giving a sex ratio of 1.2:1 in favour of males. This ratio departed significantly from a 1:1 sex ratio (Table 4.1).

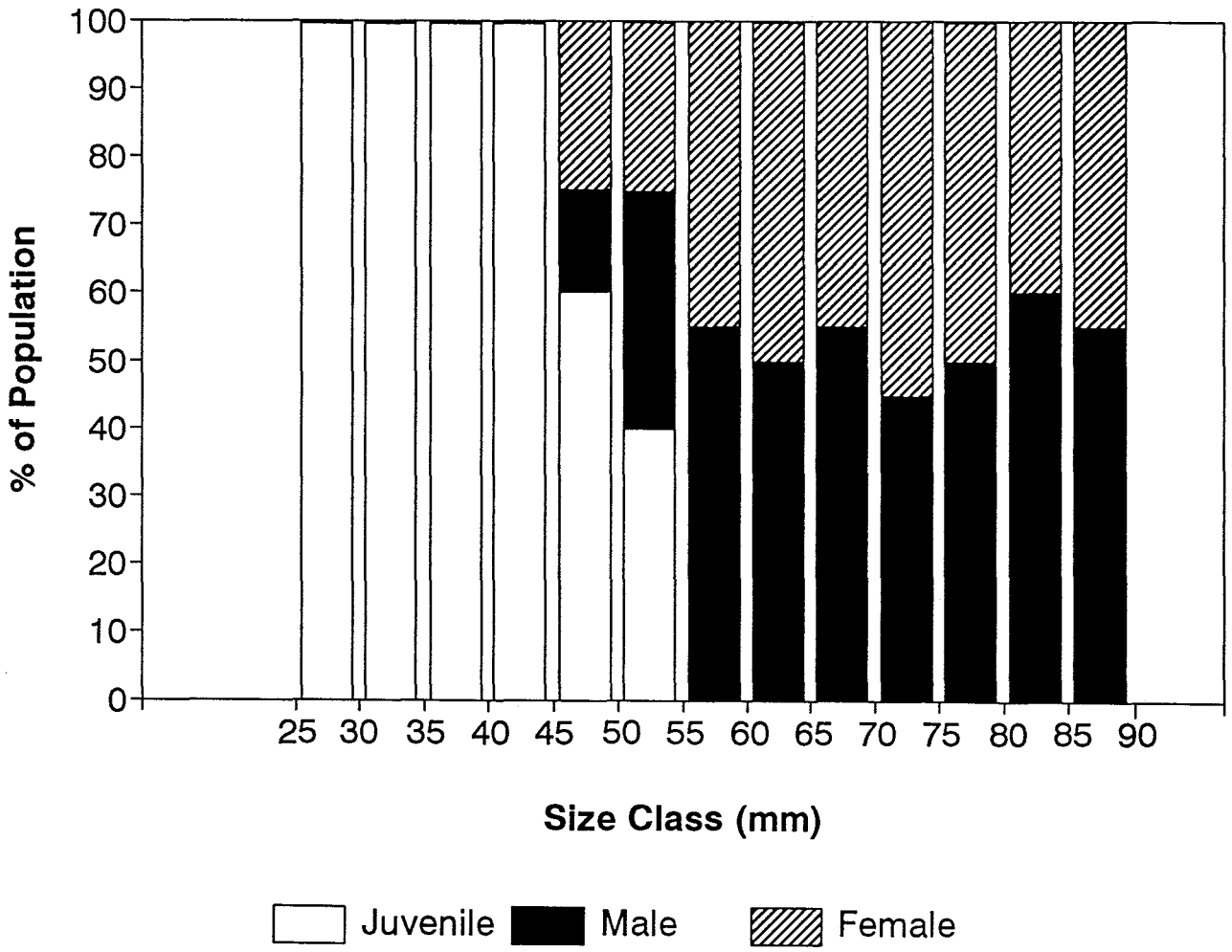


Figure 4.1. Distribution of juvenile, male and female individuals (n = 20) of *Turbo sarmaticus* within different size classes.

Table 4.1. Results of a Chi-square goodness-of-fit test to determine whether the sex ratio of male to female *Turbo sarmaticus* from a population at Port Elizabeth deviated from a 1:1 ratio.

Observed frequency	Expected frequency	Chi-square
♂ 223	202	2.18
♀ 181	202	2.18
Chi-square = 4.36 with 1 d.f. Sig. level: P = 0.036		

Size at sexual maturity

Sexual maturity was found to develop in individuals with a shell length of about 50 - 55 mm. All animals below this size range were immature (Table 4.2) and had either no gonad or the gonad was visible as a small streaky white layer containing very few developing gametes.

Table 4.2. The percentage of *Turbo sarmaticus* in each size class which were sexually mature. All animals were collected in November 1996, the time when the GI was greatest.

Shell length (mm)	Percentage
25 - 29.9	0
30 - 34.9	0
35 - 39.9	0
40 - 44.9	0
45 - 49.9	40
50 - 54.9	60
55 - 59.9	100
60 - 89.9	100

Reproductive cycle

Although the gonad of *T. sarmaticus* was present throughout the year, there was annual

variation in gonad size and gametogenic condition (Figures 4.2 & 4.3). During the reproductive cycle (1995), the GI of males and females varied significantly with an 8-fold increase and decrease in GI (Table 4.3, Figure 4.2). In addition, during the pre- and post-spawning period of the reproductive cycle, males had a significantly higher GI than the females (Table 4.3, Figure 4.2).

Table 4.3. Results of a Multi-factor ANOVA to determine whether the GI of *Turbo sarmaticus* differed between sexes of the animals and between months of the year at Port Elizabeth.

Source of variation	Sum of squares	D.F.	Mean square	F-ratio	Sig. level
Main effects					
A:Sex	0.30	1	0.30	50.34	$p < 0.001$
B:Month	2.11	23	0.09	15.28	$p < 0.001$
Interactions					
AB	0.32	23	0.01	2.34	$p < 0.001$
RESIDUAL	2.60	432	0.01		
TOTAL	5.34	479			

Although the 1995 - 1996 cycle was more defined than the 1994 - 1995 cycle (Figure 4.2), similar trends in GI can be observed. As the stages in gametogenic development of *T. sarmaticus* in this study were no different to those described previously for *T. sarmaticus* (Lombard 1977) and other gastropods (*e.g.* Orton *et al.* 1956, Bedford 1965), only a brief description follows.

Gonad indices were usually lowest (2 - 4%) from March to May (late summer to autumn) (Figure 4.2). At this time, the gonads of both sexes were showing signs of early spermatogenesis and oogenesis, and in some instances, stages of late gametogenesis were visible (Figure 4.3). The ovary mainly contained small pre- and early vitellegenic oocytes (< 120 μm diameter) which were not surrounded by a thick jelly coat (Figures 4.4 & 4.5A). A few larger oocytes (> 200 μm diameter), however, were present in some individuals in April 1995 (Figure 4.4J). In males, the spermatozoan content within the gonad acini was between 8 - 15% (Figures 4.6 & 4.7A). The GI started to increase in May/June, and the gonads of both

sexes were showing signs of late gametogenesis (Figures 4.2, 4.3, 4.5B & 4.7B). During this time, there was a significant increase in the frequency of larger vitellogenic oocytes and in sperm abundance within the gonad acini (Figures 4.4 & 4.6, Tables 4.4 & 4.5). The highest GI (♂: up to 14%, ♀: up to 12%, Figure 4.2) was recorded from August/September to December (spring to mid-summer). During this time both sexes had mature gonads (Figure 4.3). The females were mostly packed with large mature oocytes (280 - 360 μm in diameter) with thick jelly coats (51 ± 9.8 μm, n = 50) (Figures 4.4 & 4.5C). The males were packed with mature spermatozoa (measuring 6.6 ± 0.05 μm in head length, n = 50) which occupied 50 - 60% of the gonad acini (Figures 4.6 & 4.7C). Unlike 1995, there was a decrease in GI in October/November 1994 in both sexes (Figure 4.2). In females, the decrease in GI in November 1994 coincided with no large vitellogenic oocytes being found in the ovary, possibly a result of spawning (Figures 4.3 & 4.4E). Although the GI of the males decreased in October 1994, they were still mature (Figures 4.2 & 4.3). This was not repeated in 1995. During summer there was a decrease in GI until March/April (Figure 4.2) and during this time many individuals of both sexes were spent (Figures 4.3, 4.5D & 4.7D). During the spent stage any remaining oocytes and sperm were broken down and reabsorbed (Figure 4.5D).

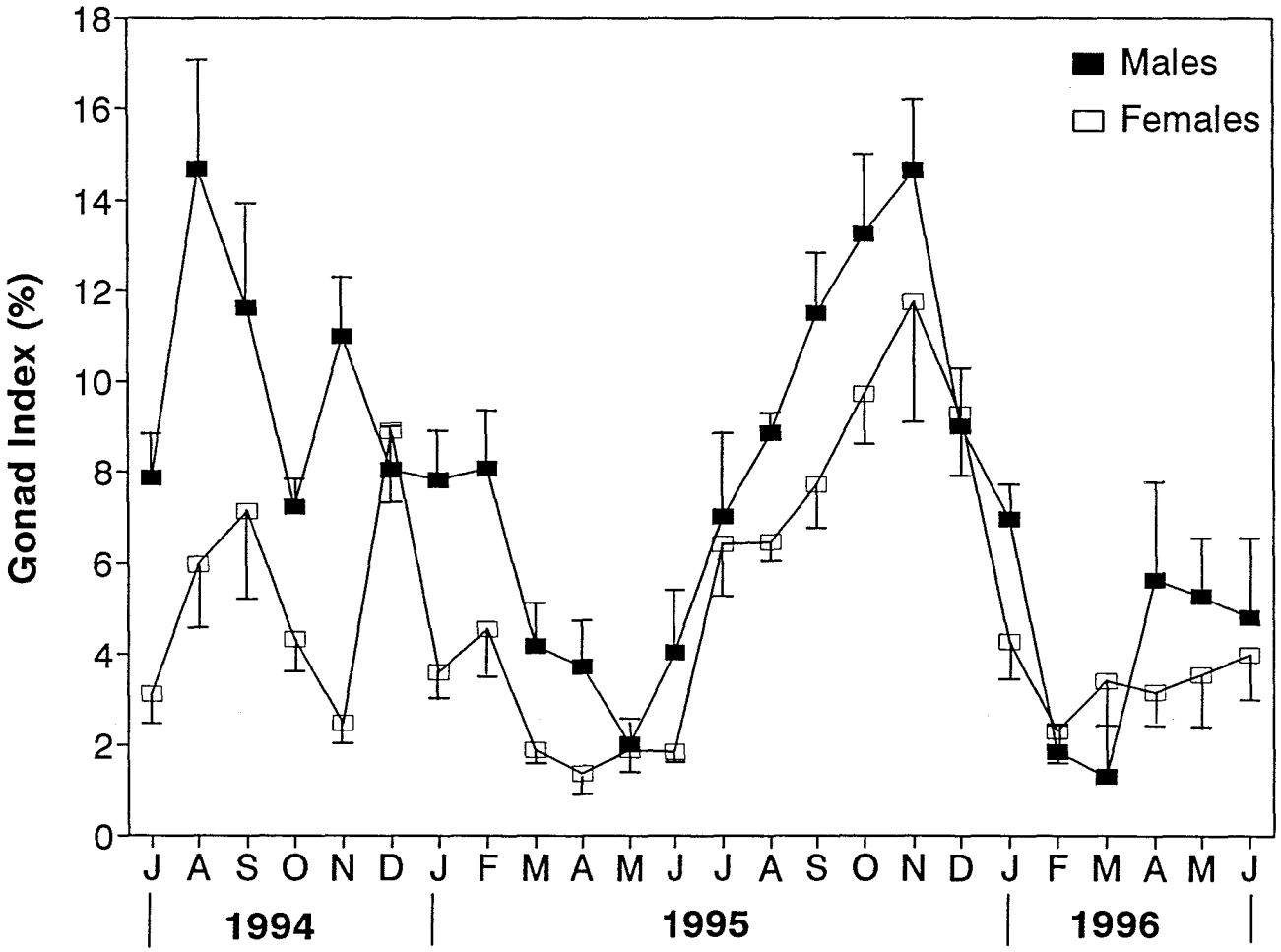


Figure 4.2. Seasonal changes in the monthly gonad index values (mean ± S.E., ♂ n = 10, ♀ n = 10) of *Turbo sarmaticus* from July 1994 to June 1996.

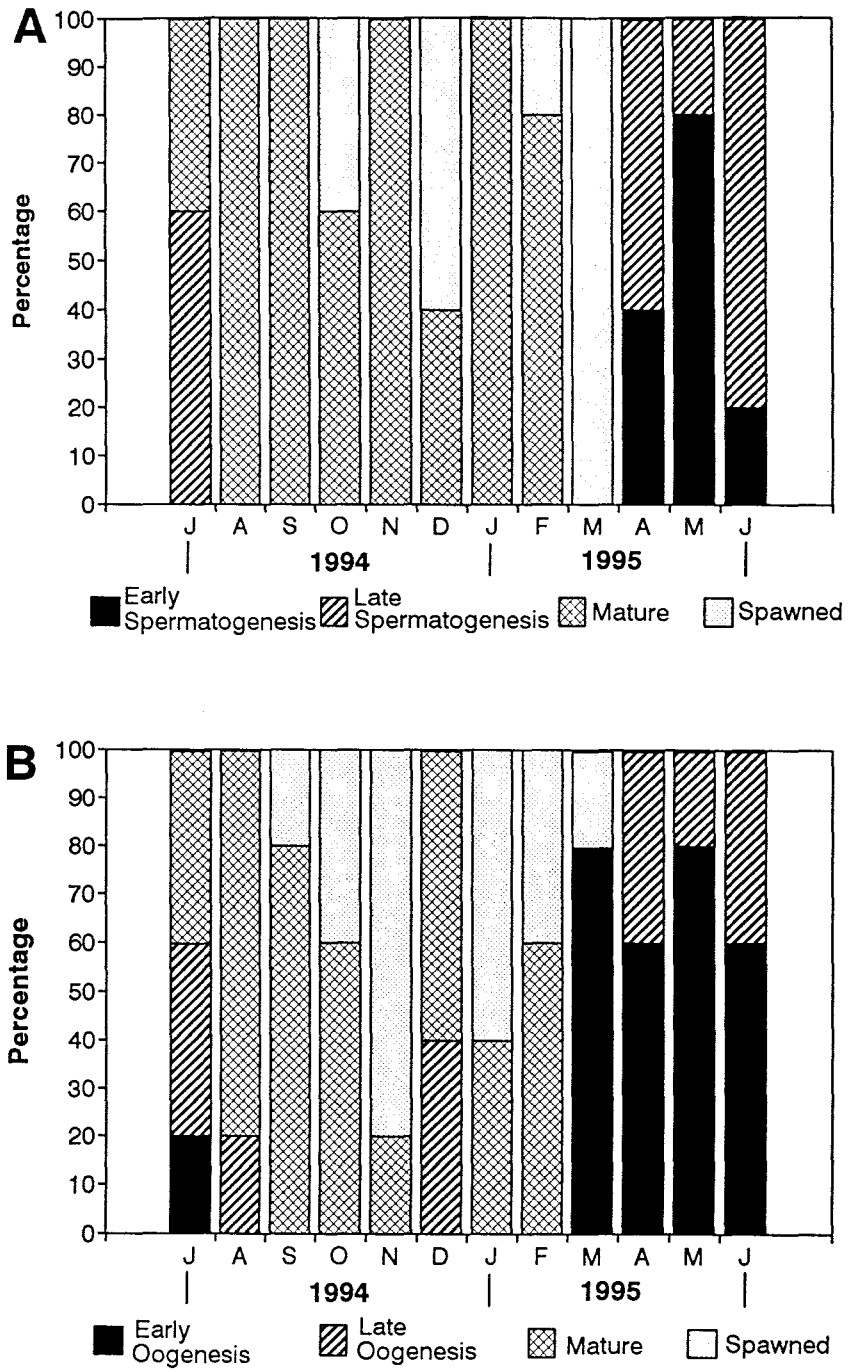
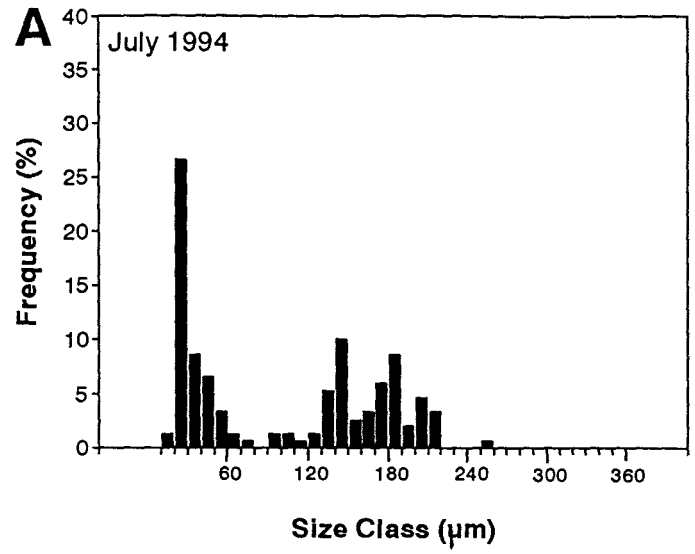


Figure 4.3. Gametogenic cycles of *Turbo sarmaticus*. Histograms show relative frequencies of gametogenic stages in **(A)** males (n = 5) and **(B)** females (n = 5) for the period July 1994 to June 1995.

Figure 4.4. Seasonal changes in the size-frequency distribution of oocytes (n = 250) of *Turbo sarmaticus* on a monthly basis from July 1994 to June 1995.


A
July 1994
Frequency (%)
0
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20
25
30
35
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60
120
180
240
300
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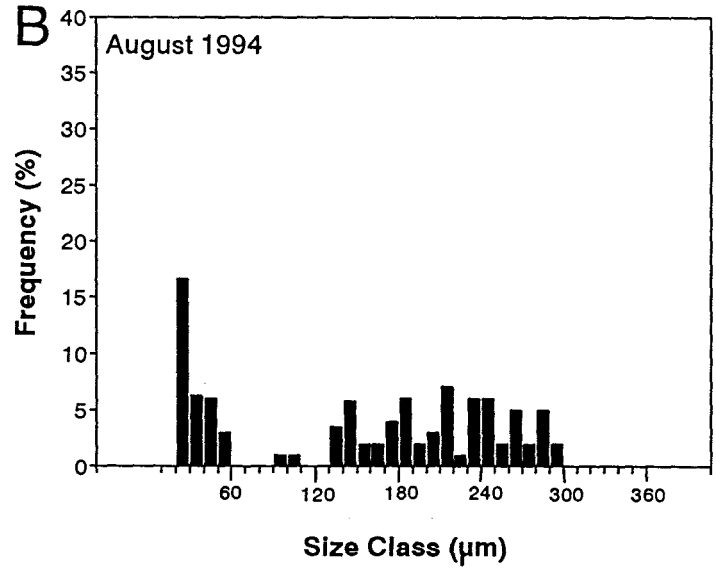
Size Class (µm)


B
August 1994
Frequency (%)
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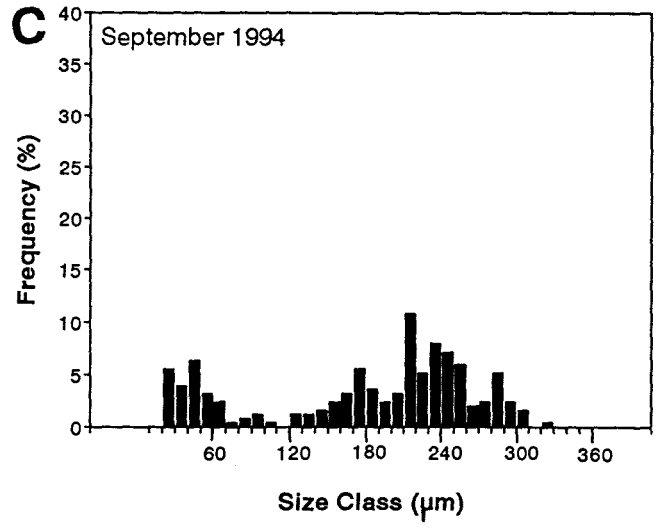
Size Class (µm)


C
September 1994
Frequency (%)
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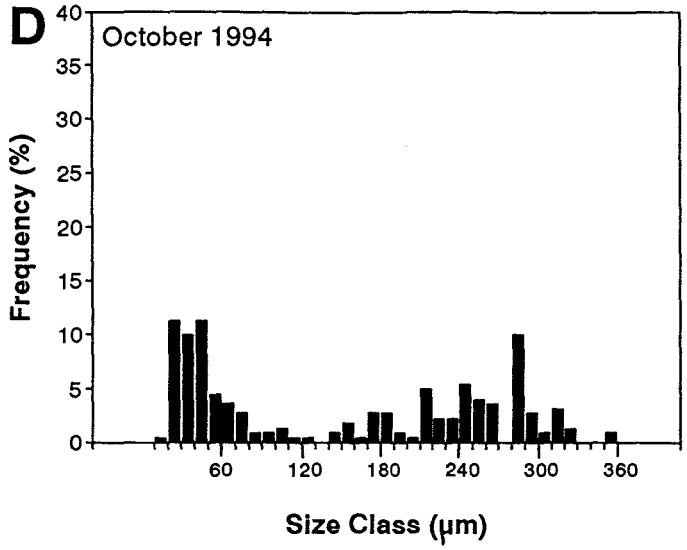
Size Class (µm)

Figure 4.4. Continued. ▸


D
October 1994
Frequency (%)
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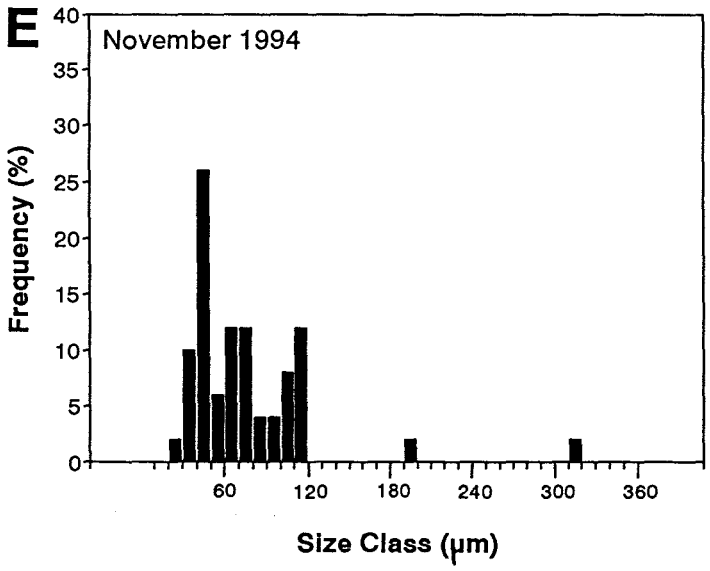
Size Class (µm)


E
November 1994
Frequency (%)
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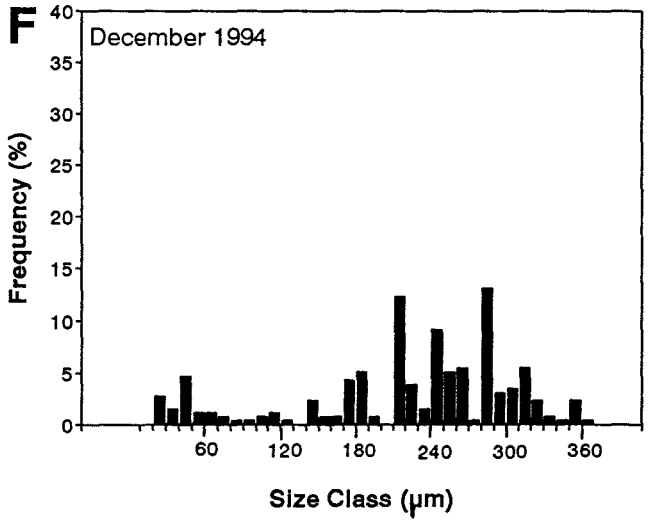
Size Class (µm)


F
December 1994
Frequency (%)
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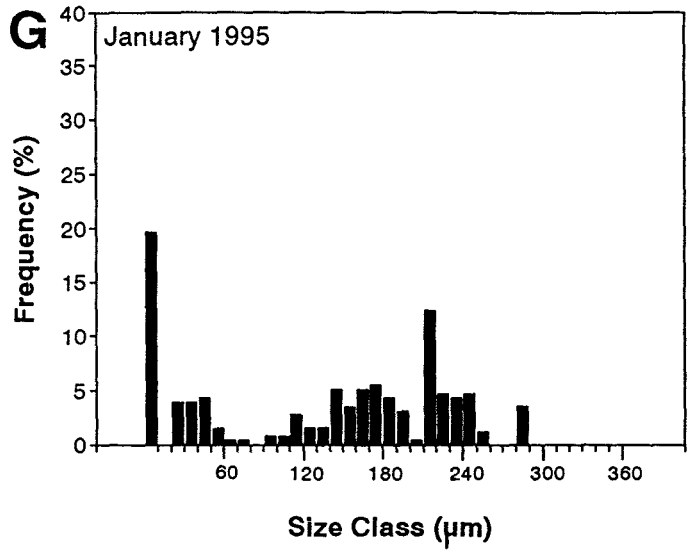
Size Class (µm)

Figure 4.4. Continued. ▸


G
January 1995
Frequency (%)
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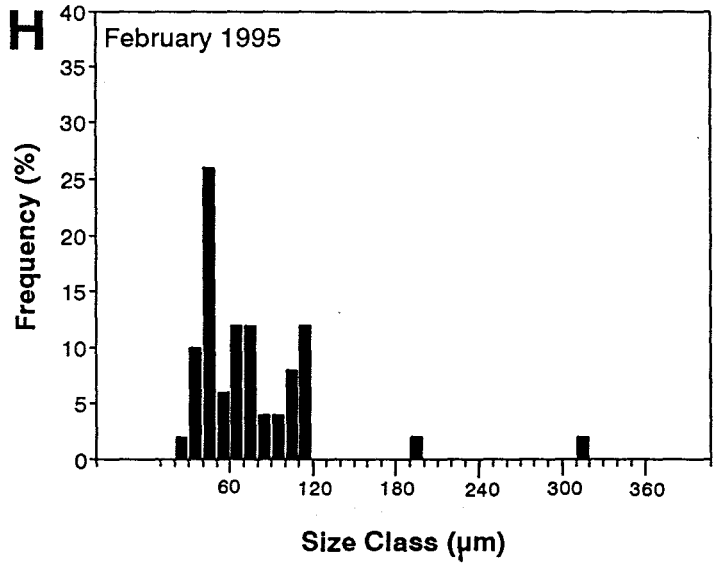
Size Class (µm)


H
February 1995
Frequency (%)
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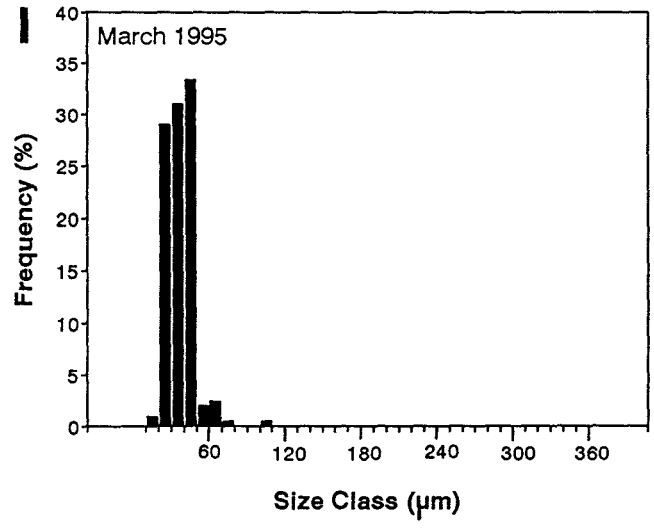
Size Class (µm)


I
March 1995
Frequency (%)
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5
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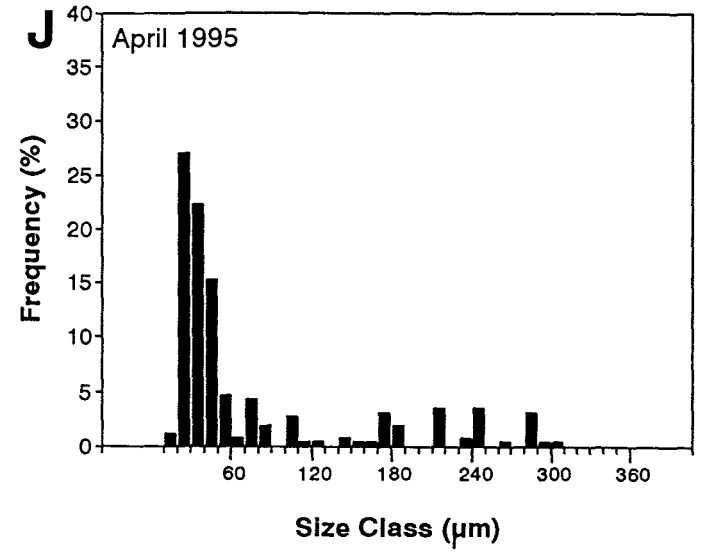
Size Class (µm)

Figure 4.4. Continued. ▸


J
April 1995
Frequency (%)
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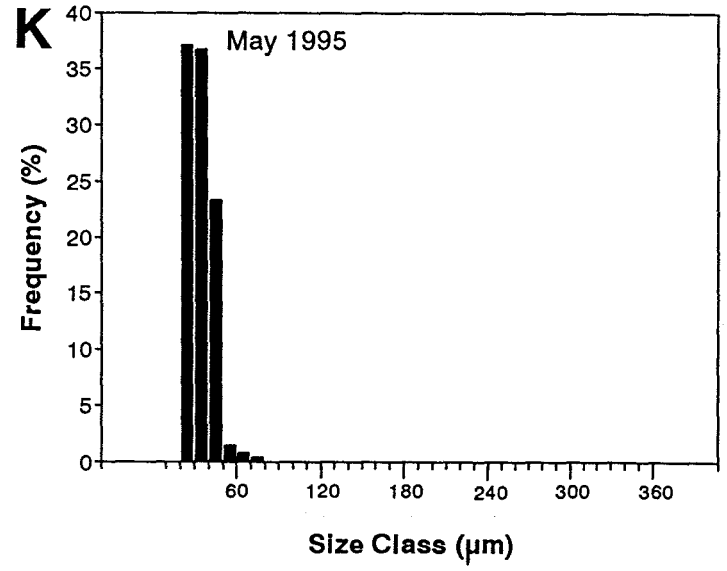
Size Class (µm)


K
May 1995
Frequency (%)
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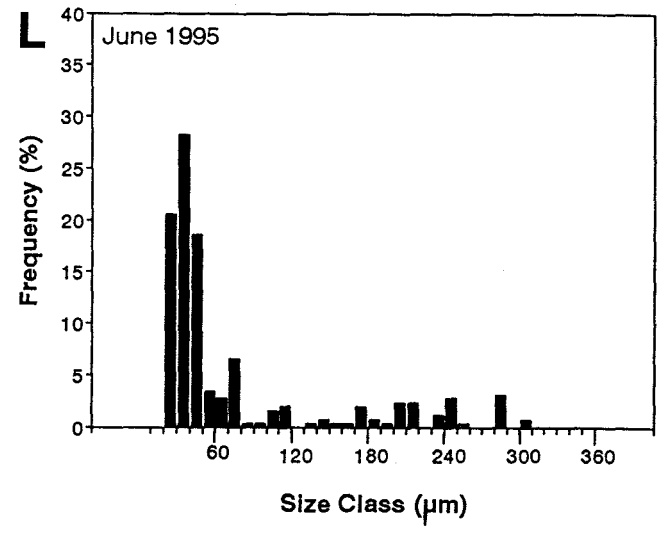
Size Class (µm)


L
June 1995
Frequency (%)
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15
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Size Class (µm)

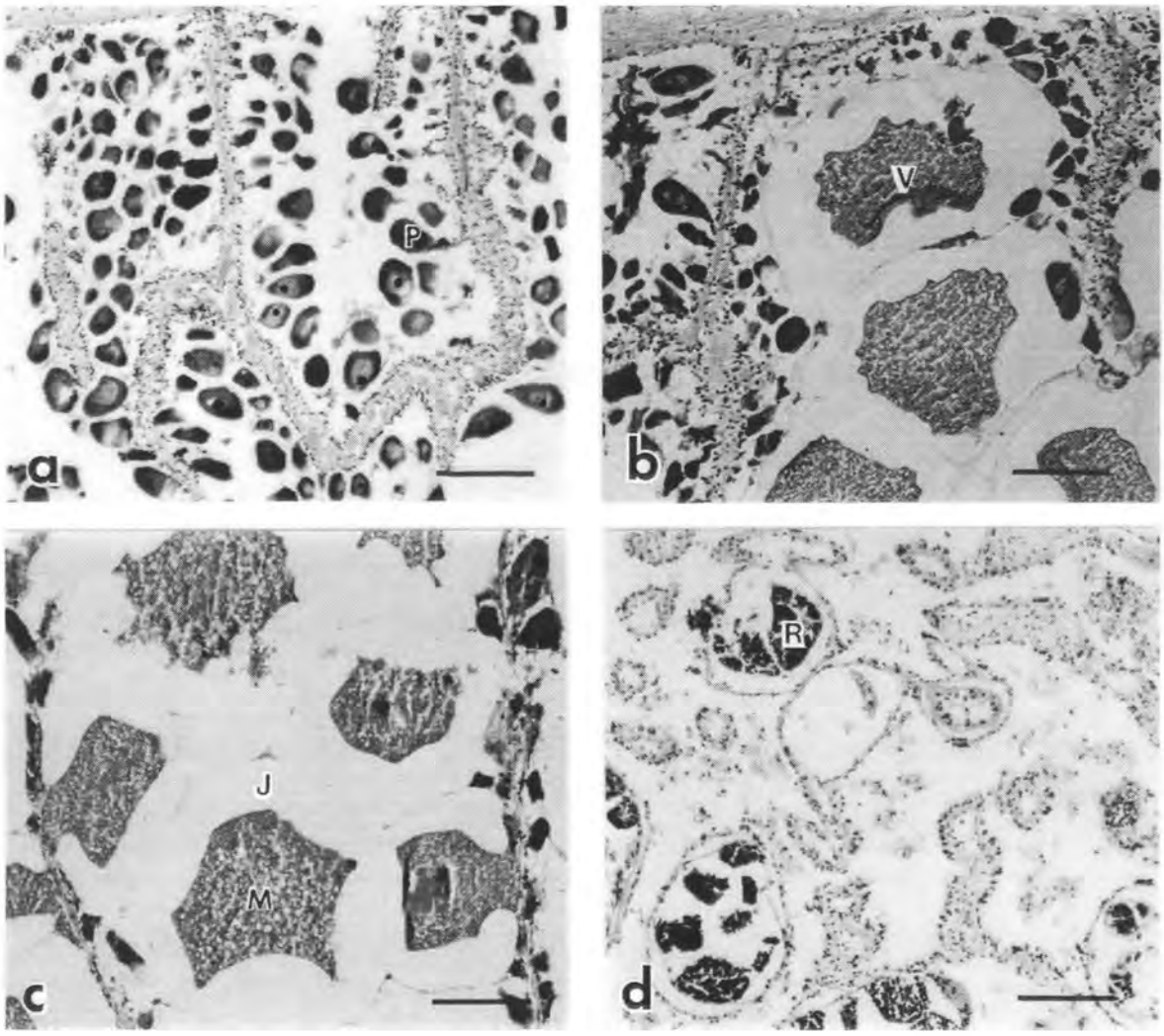

Figure 4.5. Gametogenic stages for female *Turbo sarmaticus*. **(A)** Early gametogenesis. **(B)** Late gametogenesis. **(C)** Mature. **(D)** Spawned/Spent. Scale bars = 100 μm. **J**: jelly coat, **M**: mature oocyte, **P**: previtellogenic oocyte, **R**: relict oocyte, **V**: vitellogenic oocyte.

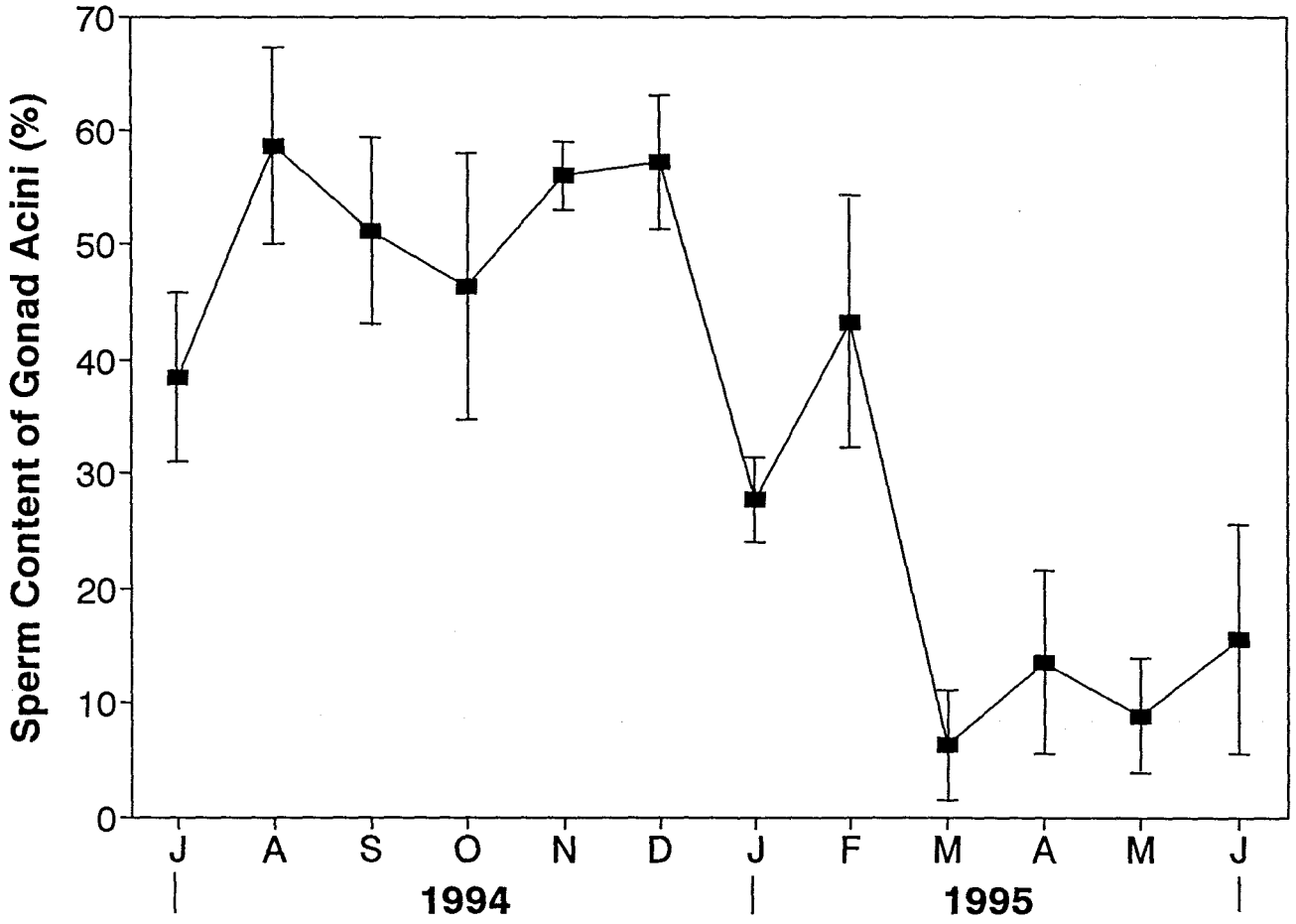

Figure 4.6. Seasonal changes in the monthly abundance of mature spermatozoa (mean ± S.E., n = 5) in the acini of the gonad of *Turbo sarmaticus* from July 1994 to June 1995.

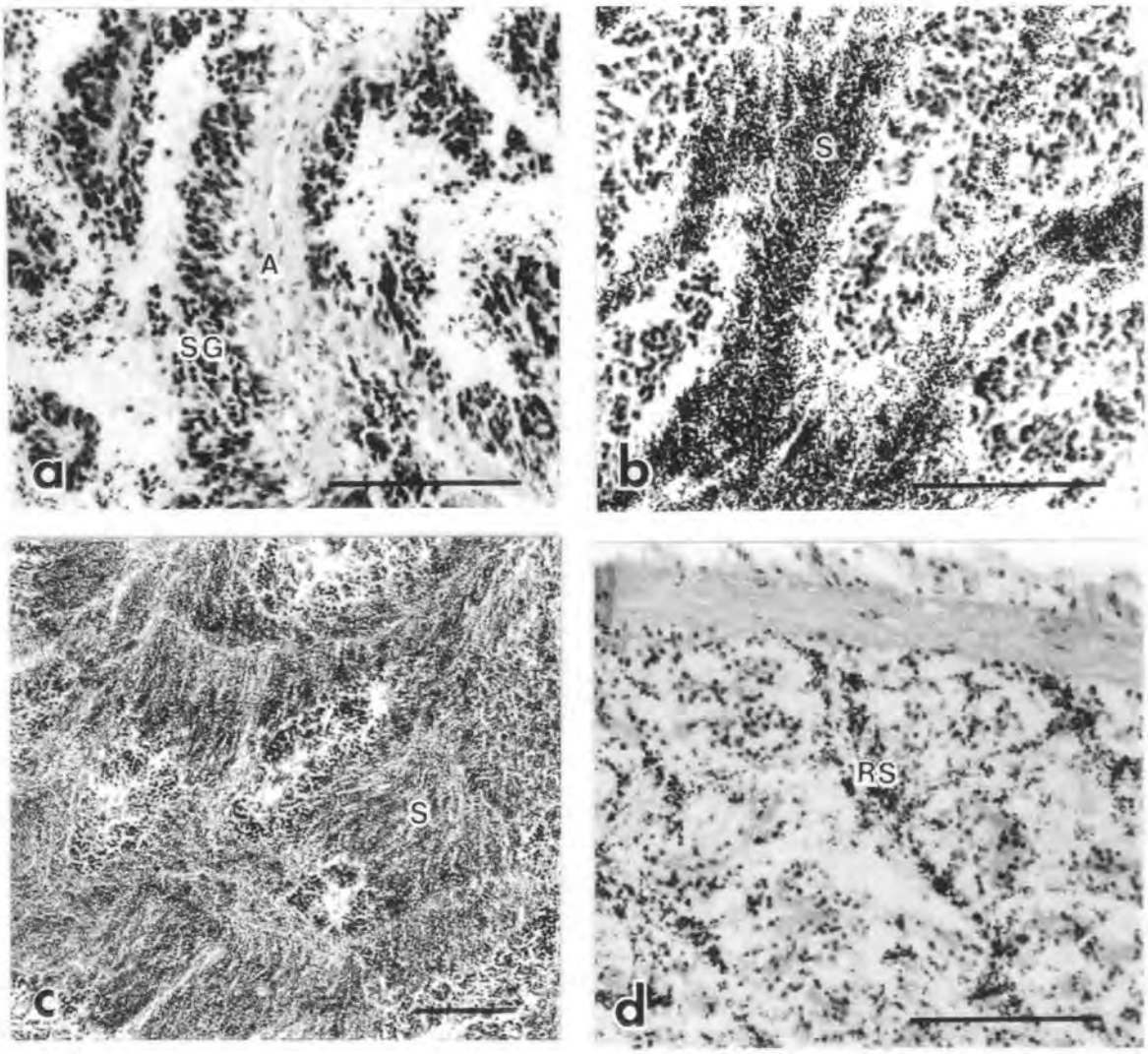

Figure 4.7. Gametogenic stages for male *Turbo sarmaticus*. **(A)** Early gametogenesis. **(B)** Late gametogenesis. **(C)** Mature. **(D)** Spawned/Spent. Scale bars = 100 μm. **A**: developing acini layer, **S**: spermatozoa, **SG**: spermatogonia, **RS**: relict spermatozoa.

Table 4.4. Results from a Chi-Square contingency table analysis to determine whether oocyte size differed during the reproductive cycle of *Turbo sarmaticus*.

Chi-square	D.F.	Sig. level	
2665.17	324	$p < 0.001$	
Statistic	Symmetric	With rows dependent	With columns dependent
Lambda	0.140	0.070	0.207
Uncertainty Coeff.	0.190	0.169	0.217
Somer's D	-0.313	-0.316	-0.310

Table 4.5. Results from a One-way ANOVA to determine whether the spermatozoan content differed within the gonad of *Turbo sarmaticus* during the reproductive cycle.

Source of variation	Sum of squares	D.F.	Mean square	F-ratio	Sig. level
Between groups	4.19	11	0.38	8.47	$p < 0.001$
Within groups	2.16	48	0.04		
TOTAL	6.35	59			

Environmental factors

The seasonal variations in sea surface temperature and daylength during the study period are illustrated in Figure 4.8. Maximum monthly mean sea temperatures were recorded in the summer months of February 1995 (21.3°C) and January 1996 (22.5°C), whilst monthly mean sea temperature minima were recorded in the winter months of July 1994 (15.5°C), July 1995 (14.9°C) and June 1996 (15.3°C) (Figure 4.8A). The greatest sea temperature variability occurred in summer, where the sea temperature ranged from 15°C to 24°C (*e.g.* December 1994), whilst in winter the sea temperature only ranged from 14°C to 16°C (*e.g.* July 1995) (Figure 4.8A). Maximum mean daylength was recorded in the summer month of January (14 hrs 23 min), whilst minimum mean daylength was recorded in the winter month of July (9 hrs 55 min) (Figure 4.8B). Higher gonad indices were recorded during warmer sea temperatures and longer daylengths, while lower gonad indices were recorded during cooler sea temperatures and shorter daylengths.

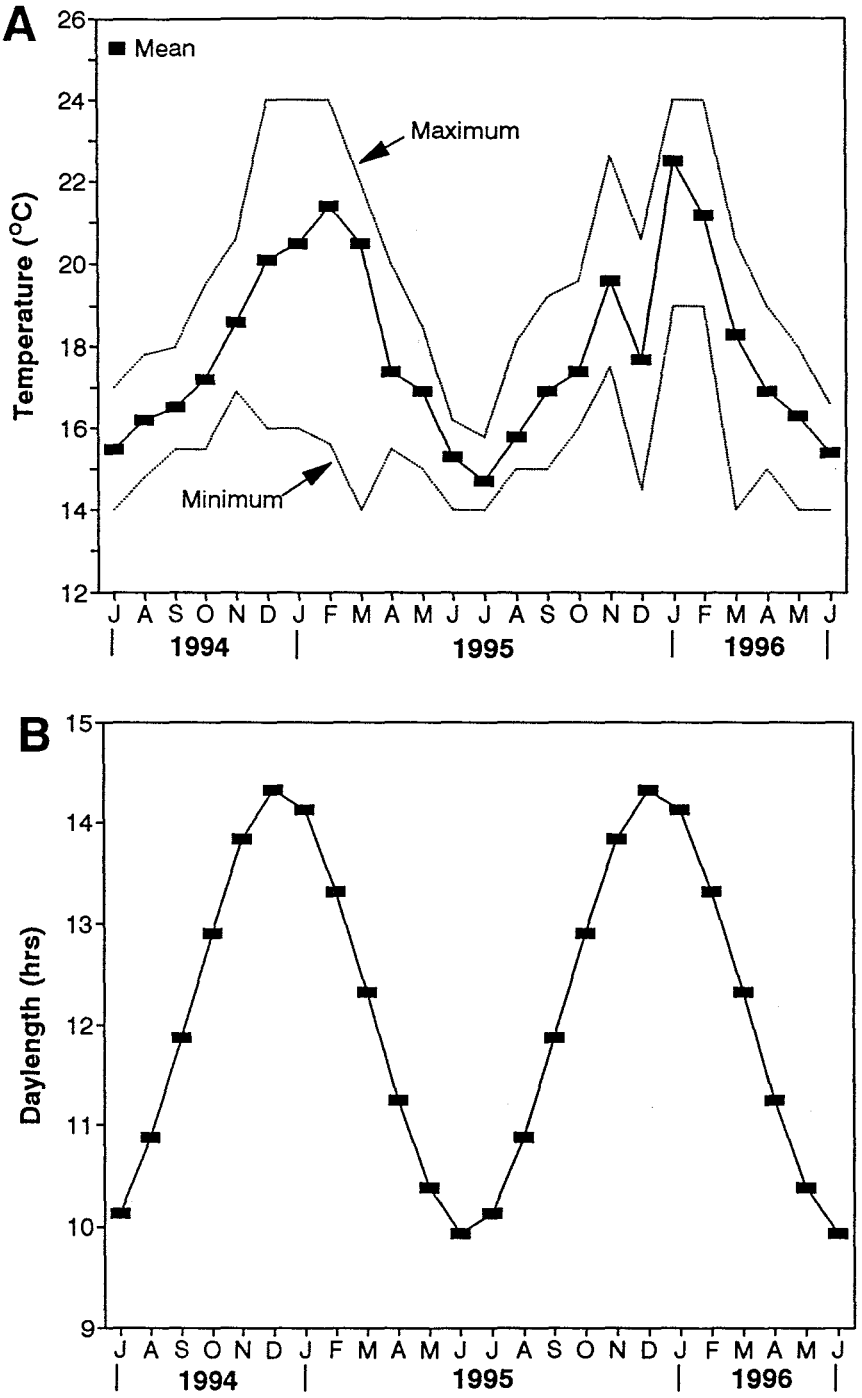

Figure 4.8. Seasonal variation in **(A)** temperature and **(B)** daylength during the period July 1994 to June 1996.

DISCUSSION

The population of *Turbo sarmaticus* studied at Port Elizabeth in the Eastern Cape Province of South Africa was dioecious, but individuals exhibited no external sexual dimorphism. Similarly, in most prosobranchs where the reproductive system is very simple (*i.e.* no copulatory organs), there is no sexual dimorphism apart from the structure and colour of the gonad (Webber 1977, Runham 1992). Sexual dimorphism, however, has been observed in the turbinid, *Turbo marmoratus*, which showed differences in the size and shape of the papillae of the right kidney opening between the sexes (Kikutani *et al.* 1994).

In many dioecious molluscs, females tend to be more numerous than males (Fretter & Graham 1964, Fretter 1984). In this study on *T. sarmaticus*, however, a sex ratio of 1.2:1 in favour of males was observed. Similarly, a sex ratio in favour of males has been observed for some haliotids (Sinclair 1963, Poore 1973, Pearse 1978, Wells & Keesing 1989), most South African *Patella* spp. (Branch 1974, Gray 1996) and several chitons (Stephenson 1934, Glynn 1970, Pearse 1978). Glynn (1970) has suggested that an abundance of males may be common in intertidal species which broadcast their gametes. The abundance of males could counter sperm dilution caused by the high turbulence often found in the intertidal zone. Sex ratios, however, are affected by a number of factors including: biased sampling, sex changes, differential growth or extreme fishing mortality where in size classes above the minimum exploitable legal size, one sex predominates (Giorgi & DeMartini 1977, Shepherd & Hearn 1983). *Turbo sarmaticus* did not change sex or exhibit differential growth between the sexes (Figure 4.1). In addition, the population structure and density of animals at Port Elizabeth were similar to those of an unexploited population (Chapter 2). Therefore, the observed sex ratio of *T. sarmaticus* at Port Elizabeth is unlikely to be caused by the above factors. Nevertheless, the data observed must be regarded with caution until further shores can be sampled in the Port Elizabeth region.

No animals smaller than 50 mm shell length were sexually mature, thus all animals below this size were regarded as juveniles. The onset of sexual maturity in the population of *T. sarmaticus* in this study (50 - 55 mm shell lengths) was similar to that previously recorded at Port Elizabeth (53 - 57 mm) (Lombard 1977) and occurred when animals were about two years old (Chapter 3). The size at sexual maturity is important for management of exploited

stocks as the harvestable size of an animal is set at a size which will protect some of the spawning stock (Muller 1984). At the present exploitable size (≈ 73.7 mm shell length) *T. sarmaticus* would be about four years old (Chapter 3) which means that individuals would potentially have two breeding seasons before becoming available for exploitation. This period may therefore offer the spawning stock some degree of protection.

Histological sections revealed that the mature oocytes of *T. sarmaticus* are enclosed in a thick jelly coat (Figure 4.5C) which is regarded as a unique and typical feature of trochacean gastropods (Hickman 1992). Simpson (1977) considered such a covering to be indicative of broadcast spawning and external fertilization which takes place in most turbinids (Hickman 1992). Some turbinids, however, produce gelatinous benthic egg masses (Eisawy & Sorial 1974, Mooers 1981, Bandel 1982) while others are brooders (Burn 1976).

Giese (1959) defined the reproductive cycle as the events from the time of activation, through growth, gametogenesis, spawning, recession of gonadal activity and the length of the resting period between cycles. The histological examination of the gonads, along with the mirrored monthly increases and decreases observed between GI, as well as oocyte diameters and spermatozoan content of the gonad acini, indicated that reproduction in *T. sarmaticus* was seasonal with a well-defined annual cycle of gonadal growth. A comparison of the results of this study with those of Lombard (1977) revealed that there was little variation in the reproductive pattern over time. *Turbo sarmaticus*, like most other South African marine invertebrates (Table 4.6), spawned during summer. Boolootian *et al.* (1962, see summary) have also indicated that approximately 85% of marine molluscs are summer spawners. The histology of the gonad of *T. sarmaticus* indicated that, like eastern Cape populations of *Haliotis midae* (Wood & Buxton 1996), a portion of the population (< 30%) spawned prior to the main spawning period (November to March). During the main spawning event of *T. sarmaticus*, all the mature eggs and spermatozoa were released. Similarly, complete spawning has been observed in other turbinids (Joll 1980), although instances of incomplete spawning have also been reported (Underwood 1974, Lasiak 1986a).

Table 4.6. Summary of the spawning periods of some South African marine invertebrates.

Species	Geographical location	Spawning period	Reference
Turbinids:			
Turbo coronatus	Former Transkei	Dec - Feb	Lasiak 1986a
T. sarmaticus	Port Elizabeth	Nov - Mar	This study
Trochids:			
Oxystele tabularis	Former Transkei	Continuous	Lasiak 1987a
O. variegata	Former Transkei	Continuous	Lasiak 1987a
Monodonta australis	Former Transkei	Feb - June	Lasiak 1987a
Haliotids:			
Haliotis midae	West coast	Oct - Dec Mar - May	Newman 1967
	Eastern Cape	April - June	Wood & Buxton 1996
H. spadicea	Port Elizabeth	Oct - Jan	Muller 1984
Limpets:			
Patella concolor	Former Transkei	Sept - Nov Feb - Mar	Lasiak 1987b
P. aphanes	Natal	Jan - Feb Apr - June	Robson 1986
P. argenvillei	Kommetjie (W. Cape)	May - June	Branch 1974
P. barbara	Kommetjie (W. Cape)	May - June	Branch 1974
P. granatina	Kommetjie (W. Cape)	May - June	Branch 1974
P. oculus	Kalk Bay (False Bay)	Sept	Branch 1974
P. longicosta	Kalk Bay (False Bay)	Oct - Nov	Branch 1974
P. granularis	Kommetjie/Kalk Bay	May - June	Branch 1974
P. cochlear	Kommetjie/Kalk Bay	May - June	Branch 1974
Helcion pectunculus	Bloubergstrand	Apr - May Nov - Dec	Gray 1996
	Port Elizabeth	Apr - May Nov - Dec	Gray 1996
Cellana capensis	Former Transkei	Sept - Oct Feb - Apr	Lasiak 1987b
Siphonaria concinna	Waterloo Bay	Nov - Dec	Chambers 1994
Littorinids:			
Littorina kraussi	Former Transkei	Dec - Mar	Lasiak 1987c

Table 4.6. Continued.

Species	Geographical location	Spawning period	Reference
Nodolittorina natalensis	Natal	Dec - Feb	Potter 1984
L. africana africana	Natal	Continuous	Potter 1984
Bivalves:			
Choromytilus meridionalis	False Bay	Aug - Feb	Griffiths 1977
	Table Bay	Aug - Sept Dec - Mar	Griffiths 1977
Aulacomya ater	False Bay	Aug - Mar	Griffiths 1977
	Table Bay	Dec - Mar June - Aug	Griffiths 1977
Perna Perna	Former Transkei	Feb - Sept	Lasiak 1986b
	Natal	May - August Sept - Oct	Berry 1978
Crassostrea cucullata	Former Transkei	Feb - March	Lasiak 1986b
Echinoids:			
Stomopneustes variolaris	East Coast	Dec - Feb	Drummond 1991
Echinometra mathaei	Ramsgate	Dec - April	Drummond 1995
Diadema savignyi	Isipingo	Dec - April	Drummond 1995
Holothurians:			
Roweia stephensoni	Port Elizabeth	Dec - Jan	Foster & Hodgson 1995
Pseudocnella sykion	Port Elizabeth	Dec - Jan	Foster & Hodgson 1995
Neostichopus grammatus	Port Elizabeth	Dec - Jan	Foster & Hodgson 1995

Any spermatozoa and eggs remaining after spawning were degenerated and reabsorbed during the short spent phase. This process also occurs in other prosobranchs and allows all valuable components of superfluous gametes to be re-used for metabolic purposes (De Jong-Brink *et al.* 1983). Gametogenesis commenced about two to three weeks after spawning in *T. sarmaticus*. Similarly, a rapid initiation of gametogenesis has been recorded in haliotids (Webber & Giese 1969). *Turbo sarmaticus*, as with other turbinids (Underwood 1974, Joll 1980, Lasiak 1986a, Belmar Perez *et al.* 1991, Yukihira *et al.* 1995) had a long period of sexual maturity (about 6 months). By contrast, shorter periods (about 2 months) have been

recorded for other turbinids (Grange 1976, Yamamoto 1985). The long period of maturity may suggest that the *T. sarmaticus* population was experiencing favourable environmental conditions with adequate nutrition (Vadas 1977, Larson *et al.* 1980, Andrew 1986). In addition, Giese (1959) has indicated that an extended breeding season can mean either that individuals of a population are producing several broods a year or that they are breeding asynchronously. *Turbo sarmaticus* had only one main spawning period a year. However, it is possible that asynchronous breeding may have occurred between individuals of the population. This, however, warrants further investigation using a larger sample size of animals.

The timing of reproductive cycles may have evolved to maximise reproductive success (Pianka 1976). The reproductive seasonality of *T. sarmaticus* and other prosobranchs (Fretter 1984) raises questions about which environmental factors serve to cue reproduction. It is important that these cues initiate gonad development at the right time so that spawning occurs at the most favourable time of the year. In broadcast spawners, synchronization of gametogenesis within the population and the simultaneous release of gametes by males and females enhances the likelihood of successful fertilization. Hence, many broadcast spawners maximize their chance of reproductive success by having restricted, synchronized breeding periods (Giese 1959). Despite the advantages supposedly gained by synchronization of reproductive activity, several protracted breeders have been shown to exhibit asynchronous spawning (Simpson 1977, Joska & Branch 1983, Lasiak 1990). The adoption of this strategy may increase the spread of potential settlement periods (Newell *et al.* 1982).

The exogenous and, in particularly, the endogenous factors which regulate reproductive activity in prosobranchs are poorly understood (Webber 1977, Fretter 1984). However, a variety of factors are known to trigger spawning in gastropod molluscs including temperature, mechanical stimuli and hormones (Orton 1920, Linke 1933, Gabe 1951, 1953, Loosanoff & Davis 1952, Hancock 1960, Kessel 1964, Berry & Chew 1973, Alifierakis & Berry 1980, Fretter 1984, Geraerts *et al.* 1991, Moran 1997). In addition, Rawlings (1994) has demonstrated that the presence of predators can stop spawning in the rocky shore gastropod, *Nucella emarginata*, thereby illustrating that chemical cues released by potential predators can also have a profound effect on gastropod reproduction. Prosobranchs do not lend themselves to the necessary experimental work to determine endogenous factors (Fretter 1984). Most

attention has therefore been directed at determining the key environmental factors that synchronize the reproductive cycle of marine gastropods to prevailing environmental conditions. Exogenous factors are especially applicable to temperate regions where there are distinct annual cycles of light intensity, temperature, nutrients and winds which drive cycles of productivity (Wilk *et al.* 1990). Several studies have indicated correlations between environmental parameters and reproductive cycles (*e.g.* Webber & Giese 1969, Sutherland 1970, Pearse 1978). Underwood (1974) suggested that only the first phase of the reproductive cycle (gametogenesis) is likely to depend on environmental triggers, while vitellogenesis is dependent on nutrient availability and spawning on the completion of vitellogenesis.

The reproductive cycle of *T. sarmaticus* may be related to general trends in sea surface temperature, as temperature is regarded as a spawning signal in many invertebrates (Orton 1920, Giese 1959, Fretter & Graham 1964, Newman 1967, Smith & Carefoot 1967, Giese & Pearse 1974, Kikuchi & Uki 1974, Hayashi 1980, Brown 1984, Grahame & Branch 1985). Grahame & Branch (1985) have suggested that if temperature varies seasonally, it is almost inevitable that breeding can be associated with some change in temperature, and other cues may be overlooked. The gametogenic progression from March/April to July/August in *T. sarmaticus* coincided with increasing sea temperature and daylength (Figures 4.3 & 4.8). Likewise, gametogenic progression in *Turbo coronatus* has also been related to sea temperature along the east coast of South Africa (former Transkei coast) (Lasiak 1986a). On the other hand, gonad growth and gamete production in some species of haliotids were controlled by factors other than temperature, such as photoperiod (Webber & Giese 1969) and seasonal changes in food supply (Boolootian *et al.* 1962, Sutherland 1970).

The peak spawning activity of *T. sarmaticus* occurred when sea temperature and daylength were greatest. During this period sea temperature also varied the most (Figure 4.8A). Studies on turbinids have shown that spawning can be induced by temperature changes (Ai 1965) and increasing temperature has been found to enhance spawning in other gastropods (*e.g.* Underwood 1972, Clare 1990). In addition, spawning can be initiated by photoperiod (Webber & Giese 1969) as pairing, copulation and egg-laying in the queen conch, *Strombus gigas*, were all positively related to photoperiod (Stoner *et al.* 1992). In some tropical littorinid gastropods spawning was related to lunar phases (Berry 1986), although Grange (1976) found no relationship between spawning and time of day (night or day), state of the

tides (position in the tidal cycle) or time of the month (position in the lunar cycle) in New Zealand trochids and turbinids. Rough water has also been found to initiate spawning in some trochids, turbinids (Grange 1976, Yukihira *et al.* 1995) and other marine molluscs (Branch 1974, Catalan & Yamamoto 1993). Since adult animals of *T. sarmaticus* inhabited the low-shore, an area characterized by continuous high wave action (Chapter 2) and preferred shores with high wave exposure (Bruton *et al.* 1991), spawning may have been influenced by this parameter. Gray (1996) has shown that strong westerly winds (onshore causing storm conditions at Port Elizabeth) often blow during the months when spawning occurs in many Eastern Cape Province intertidal species including *T. sarmaticus*. Branch (1974) has indicated that spawning during storms has the advantage of reducing desiccation of newly settled limpets, and accompanying onshore winds may prevent larvae from being carried out to sea, thus ensuring that the larvae remain onshore.

Although strong westerly winds predominate during the summer months, the coast of the Eastern Cape Province is frequently subjected to strong easterly winds which may persist for several days (Taljaard 1972, Beckley 1983). These easterly winds have been related to rapid decreases in sea temperature which are associated with upwelling events (Schumann *et al.* 1982, Goschen & Schumann 1995). Recent evidence, however, suggests that these upwelling events are not caused by the wind direction but rather occur at random, due to an unusual feature of the Agulhas current as it passes along the coast of the Eastern Cape Province (*pers. comm.* - J.R.E. Lutjeharms, University of Cape Town). Such upwelling events are capable of reducing summer sea temperatures by as much as 9°C in a few days (Beckley 1983, Goschen & Schumann 1995). These rapid fluctuations in summer sea temperature could have a profound effect on the intertidal biota and may serve as a cue to initiate spawning in *T. sarmaticus*, as it does in other turbinids (Ai 1965). Upwellings may also create nutrient rich water which often initiates phytoplankton blooms (Probyn 1985, Probyn & Lucas 1987, *pers. comm.* - C.D. McQuaid, Rhodes University). Although trochacean larvae are non-planktotrophs (Hickman 1992), molluscan veliger larvae can absorb dissolved organic matter from the surrounding water (Welborn & Manahan 1990). Therefore spawning, coinciding with the upwelling of organic nutrients may be advantageous to development. However, Bonardelli *et al.* (1996) have shown that spawning in the giant scallop, *Placopecten magellanicus*, was not related to the abundance of phytoplankton or particulate organic carbon and nitrogen in

the water, but consistently occurred during abrupt summer temperature changes.

In conclusion, the *T. sarmaticus* population at Port Elizabeth exhibited no external sexual dimorphism and the sex ratio (1.2:1) was in favour of males. Although there was some variation in GI between 1995 and 1996, the overall trend of the reproductive cycle was similar. The reproductive cycle of *T. sarmaticus* involved the development of the gametes soon after spawning, whilst the maturity phase of the gonad was protracted (about 6 months) and spawning occurred from November to February/March. It is suggested that spawning could have been triggered by rapid changes in summer sea temperatures caused by upwelling events. In addition, these upwelling events may have produced nutrient-rich waters which could have been utilized by the larvae. In order to validate these suggestions, laboratory experiments need to be undertaken to determine if rapid changes in water temperature will trigger spawning in *T. sarmaticus*. Further experiments should investigate the ability of *T. sarmaticus* larvae to absorb nutrients from surrounding water. The onshore winds (westerlies) which predominate during the summer months probably ensured that larvae were not transported far from the shore. It is critical that future research investigates larval development. These data will provide useful information about larval settlement time and time in the water column, which will give an indication of the dispersal ability of *T. sarmaticus* larvae.

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CHAPTER FIVE

CONSUMPTION RATES AND DIGESTIBILITY OF SIX INTERTIDAL MACROALGAE BY *TURBO SARMATICUS*

INTRODUCTION

The transfer of energy and nutrients from primary producers to grazers is important in all ecosystems, and inshore benthic ecosystems in which algae are the primary producers are no exception (Hawkins & Hartnoll 1983). Optimization theory predicts that the processes of foraging, feeding and digestion maximize energy intake (Schoener 1971, Pyke *et al.* 1977, Hughes 1980). Hence, a basic tenet of nutritional theory is that animals, with few exceptions, eat to satisfy their energetic requirements (Smith 1989). Most dietary studies on animals have focused on foraging and feeding (*e.g.* Charnov 1976, Hughes 1980) whilst, despite their importance (Sibly 1981), digestive processes have been generally neglected (Penry & Jumars 1987). Digestion and assimilation are vital to the transfer of energy from one trophic level to the next.

The bulk of algal biomass, excluding water, consists of structural and storage carbohydrates (Percival 1979, Painter 1983) and thus a macroalgal grazer such as *Turbo sarmaticus* will derive most of its energy from carbohydrates. The amount of algae consumed and the efficiency of digestion will influence the amount of energy gained from such food. As the metabolic rate of poikilotherms, and hence their energetic requirements, is primarily determined by temperature and body size (Fry 1971, Uki 1981, Hahn 1989, Smith 1989), the amount of algae consumed will be controlled by these parameters (Uki 1981, Uki *et al.* 1986, Peck 1989, Lyon 1995).

Studies on animal nutrition have used the terms assimilation efficiency and absorption efficiency (*e.g.* Callow & Fletcher 1972, Calow 1975, Bowen 1981, Bordner *et al.* 1983, Buxton & Field 1983, Barkai & Griffiths 1987) to describe the ability of an organism to utilise a food resource. Since values of assimilation and absorption efficiencies are calculated in a similar way (*i.e.* difference between the amount ingested and that egested), this thesis will make use of the term assimilation efficiency to avoid confusion of these terms. In addition, it is possible to compare assimilation efficiency and values of digestibility (*pers. comm.* - P.J. Britz, Rhodes University) therefore such comparisons are made in this study.

Feeding rates and assimilation efficiencies of marine invertebrate herbivores vary considerably often depending on the nature of the food (Carefoot 1967a, 1967b, Paine & Vadas 1969, Lawrence 1975, Bordner & Conklin 1981, Hawkins 1981, Hawkins & Hartnoll

1983, Peduzzi 1987, Brown *et al.* 1990, Rogers *et al.* 1995, Semura 1995). Factors such as plant toughness (Padilla 1985, Pennings & Paul 1992, McShane *et al.* 1994), food quality (Bedford & Moore 1985), palatability (Imrie *et al.* 1990), feeding repellents (Hay & Fenical 1988, Harada 1992) and feeding stimulants (Harada & Kawasaki 1982, Sakata *et al.* 1988, Sakata & Ina 1989, Imrie *et al.* 1990) will all influence the utilization of a food source.

One way of measuring nutrient availability is to determine the digestibility of a food. The digestibility coefficient is defined as the fraction of the food which is absorbed, expressed as a percentage of the food ingested, on a dry matter basis. Digestibility trials have been used to assess the potential nutritive value of food for fish (Austreng 1978, De la Noue & Choubert 1985, 1986, Kirchgessner *et al.* 1986, Epko & Bender 1989, Spyridakis *et al.* 1989, Pongmaneerat & Watanabe 1991), crustaceans (Bordner & Conklin 1981, Bordner *et al.* 1983, Brown *et al.* 1986, 1989, 1990) and marine molluscs (Carefoot 1970, Barkai & Griffiths 1987, Dixon 1992, Wee *et al.* 1992, Britz 1995). These studies have established a standard method for determining the digestibility of a wide range of foods.

Calculation of digestibility coefficients makes allowances for incomplete digestion which represents the greatest loss between the quantity of the nutrient present in the diet and the amount finally utilized by the animal (Crampton & Harris 1969, Jobling 1983, McDonald *et al.* 1984). There are two general methods of measuring digestive efficiency (De la Noue & Choubert 1986). The "direct method" (Post *et al.* 1965, Ogino *et al.* 1973, McDonald *et al.* 1984) requires the collection of the total faecal matter produced after consumption of a known amount of food, and the "indirect method" omits the quantitative collection of faeces by measuring the concentration of an inert indicator in the food and faeces (Maynard & Loosli 1969, Choubert *et al.* 1979, McDonald *et al.* 1984, Hardy 1989). Complete collection of faecal matter is tedious and impossible in many circumstances. Mason (1970) found that in *Helix aspersa* the calculated assimilation efficiency was the same whether determined by the crude gravimetric method (direct) or ash ratio method (indirect). By contrast, Grahame (1973) found that for *Littorina littorea*, the crude gravimetric method gave considerably lower assimilation efficiency values (57%) than the ash ratio method (89%). Since the indirect method of determining digestibility coefficients is the most accurate and frequently used, it was used in this study.

Two types of indicators are commonly used in the indirect method: 1) those added to

a diet such as chromic oxide (Cr_2O_3), and 2) naturally occurring indicators such as ash. Research in ruminants (Van Keulen & Young 1977), pigs (McCarthy *et al.* 1974), poultry (Vogtmann *et al.* 1975) and fish (Atkinson *et al.* 1984) has validated the use of acid-insoluble-ash (AIA) (mainly silica) as a naturally occurring marker for digestibility trials. Many digestibility studies support the use of AIA as a natural marker as it gives very similar results to those obtained from artificial markers like chromic oxide (*e.g.* Atkinson *et al.* 1984, Maguire *et al.* 1993).

Despite the usefulness of determining digestibility coefficients they have several limitations (De Silva 1989, Smith 1989, Maguire *et al.* 1993). The most important limitation is that the digestibility coefficient relies on the assumption that all faecal matter represents the proportion of the diet that is not digested and absorbed. This is, however, not entirely correct as a portion of the faecal matter is composed of waste metabolites from the body itself in the form of unabsorbed digestive enzymes, epithelial cells abraded from the intestinal wall and mucus (Morrison 1954, Crampton & Harris 1969, McDonald *et al.* 1984). These metabolic secretions result in the underestimation of the digestibility coefficient. The coefficient must therefore be referred to as the apparent dry matter digestibility coefficient (hereafter abbreviated as ADMDC) which distinguishes it from the true digestibility coefficient which is difficult to determine accurately (Maynard & Loosli 1969, Calow & Fletcher 1972, McDonald *et al.* 1984, Leavitt 1985).

Data on consumption rates and assimilation efficiency of South African marine molluscs are limited to studies on *Bullia digitalis* (Stenton-Dozey & Brown 1988), *Patella cochlear* (Branch 1981) and *Haliotis midae* (Barkai & Griffiths 1987, 1988, Dixon 1992, Britz 1995). *Turbo sarmaticus* is regarded as a generalist grazer, feeding on a variety of macroalgae. However, the amount of algae consumed, their relative nutritional importance and ease of digestion are not known. A knowledge of consumption rates and digestibility of food sources is essential for studies on energetics and for the evaluation of the dietary value of food. In addition, digestibility studies of naturally ingested food by populations are proving useful to evaluate and understand the success or failure of species (*e.g.* fish populations - Bowen 1981, De Silva 1985).

The aim of this study was to establish whether *T. sarmaticus* could consume various intertidal macroalgae, hence justifying its classification as a generalist grazer. This study also

aimed to compare the consumption rate and ADMDC of various algae by *T. sarmaticus* in order to evaluate the dietary value of these algae. In addition, the effect of temperature on consumption rate and apparent dry matter digestibility of three macroalgae by *T. sarmaticus* was also investigated.

MATERIALS AND METHODS

Preliminary feeding observations

In order to ascertain which species of algae were consumed by *Turbo sarmaticus*, nocturnal feeding observations of field animals and analysis of gut contents were undertaken. The results revealed that *Gelidium pristoides*, *Ulva rigida* and *Corallina* spp. were the most commonly consumed algae. Three other algal species, *Codium extricatum*, *Iyengaria stellata* and *Ecklonia radiata*, although not distinguishable in gut contents or observed to be eaten, were often abundant in habitats occupied by *T. sarmaticus*. The ability of *T. sarmaticus* to consume and digest these algae was therefore also examined.

Energy content and nutritional composition of algae

In order to determine some aspects of the food value of the six algae, the energy content and nutritional composition (protein, carbohydrate, lipid content) was examined in algae collected from Kenton-on-Sea.

The energy content of the algae fed to *T. sarmaticus* was determined on a monthly basis from September 1995 to September 1996 in order to determine any seasonal variability. Samples of each alga were returned to the laboratory, immediately cleaned, shaken to remove excess water and dried to constant weight at 60°C. Each dried sample was finely ground and homogenized using an electric mill (sieve size < 0.1 mm). Their energetic contents were then determined using an MC-1000 modular ballistic bomb calorimeter calibrated with benzoic acid. Each month, three determinations of energy content were obtained for each alga. A mean was then calculated and taken to represent the energetic content.

Examination of the nutritional composition of the six algae involved a collection once

in summer (December) and once in winter (July) in order to determine any seasonal variability. The samples collected were cleaned, rinsed and dried to a constant weight at 60°C. The dried samples were then finely ground and homogenized using an electric fine mill (sieve size < 0.1 mm), and their chemical composition analysed. Three determinations of each nutritional constituent were obtained for each alga. Means were then calculated and taken to represent the different nutritional constituents.

Ash content was measured by combusting approximately 1 g of a sample in a muffle furnace at 500°C for 4 hrs (Dawes 1981).

Protein content was estimated by dye binding (Bradford 1976). Samples of dried material (50 mg) were digested in 5 ml of 1 N NaOH for 18 hrs with occasional shaking. The reagent, Coomassie Brilliant Blue G-250 (Sigma) was added (1 ml) to three aliquots of each sample and mixed immediately with a vortex mixer. Absorbance of the protein-dye complex was then measured at 595 nm and compared to a bovine serum-albumin standard curve (Kochert 1978).

Soluble carbohydrates (potentially available) were extracted from samples of approximately 5 mg by the phenol-sulphuric acid method (Dubois *et al.* 1956). The weighed samples were heated with 10 ml of 5% trichloroacetic acid for 3 hrs with occasional shaking. After centrifugation, three 0.2 ml aliquots of each sub-sample were treated with phenol (5%) and concentrated H_2SO_4 . Absorbencies were measured at 490 nm and compared to a glycogen standard curve (Dawes 1981).

The total lipid content of the samples was determined gravimetrically. Samples of ≥ 100 mg dry material were used and lipids extracted at 60°C with a 2:1 (v/v) chloroform-methanol mixture for 15 minutes. The resulting solution was mixed, filtered through Whatman # 541 filter-paper and washed with water (Dawes 1981). The solvent was separated out by centrifugation and then evaporated in a pre-weighed round bottom flask by rotary evaporation. Total lipid content was then determined by comparing pre- and post-evaporation flask weights.

Study animals

All *T. sarmaticus* (juveniles: 40 - 45 mm shell lengths, adults: 70 - 75 mm shell lengths) were collected from the same rocky shore at Kenton-on-Sea, Eastern Cape Province,

and taken to the laboratory. New animals were collected for each experiment to avoid pseudoreplication and to ensure that animals were not affected by long-term laboratory exposure. For each experiment, ten juveniles and ten adults were placed in twenty aerated aquaria (25 x 15 x 20 cm, one animal per aquarium) and allowed to acclimatize for seven days prior to experimentation. During this period, animals were starved for five days to void gut contents and then pre-fed for two days on an experimental diet. All aquaria were housed in a constant environmental room (12 hrs light:12 hrs dark regime) at either 15°C, 20°C or 25°C depending on the experimental conditions. In addition, all animals were kept constantly submerged and were provided with shelters (small plastic flower pots) to hide from the light. The water in each aquarium was replaced daily with fresh filtered sea water (35 ‰).

Consumption rates of algae

The following algae were selected on the basis of their intertidal abundance and feeding observations of *T. sarmaticus*: Chlorophyta - *U. rigida*, *C. extricatum*; Phaeophyta - *I. stellata*, *E. radiata*; Rhodophyta - *G. pristoides*, *Corallina* spp. Each animal was provided with an excess of fresh algae daily which it could feed on *ad lib*. The amount offered was blotted damp-dry and weighed to the nearest 0.001 g. The following day, uneaten food was removed, blotted damp-dry and reweighed. This procedure was followed for five consecutive days for each alga and at each temperature. A mean was calculated for the five days from each juveniles and each adult and used to determine the daily food consumption rates and specific consumption rates of juveniles and adults (n = 10/algae). Daily food consumption rate (dry weight) was calculated using the formula (after Britz 1995):

$$C_g = F - R$$

where, $\mathbf{C_g}$ = consumption (g); **F** = initial food weight; **R** = weight of food remaining after feeding. Daily food consumption (C_g) was then standardized for body weight and expressed as a daily specific consumption rate (% of animal dry body weight) using the formula (after Britz 1995):

$$C_{\%b.wt} = \frac{C_g}{W} * 100$$

where, $C_{\%b.wt}$ = animal food consumption (% of body weight); C_g = daily food consumption; W = animal weight (g). Repeated determinations of wet and dry weights (n = 5/alga) gave a percentage dry weight conversion value for each alga (Table 5.1). The dry weight was determined by drying wet algae to constant weight at 60°C.

Table 5.1. Percentage dry weight determined from wet weight of six intertidal algae from the Eastern Cape Province.

Species	% (dry)
Ulva rigida	21.9
Codium extricatum	7.9
Ecklonia radiata	17.9
Iyengaria stellata	12.8
Gelidium pristoides	29.8
Corallina spp.	58.4

To determine the effect of temperature on consumption rates, feeding experiments were carried out at 15°C, 20°C and 25°C. These temperatures represent the minimum, mean and maximum sea surface temperatures, respectively, for the Eastern Cape Province. At 15°C and 25°C only, the consumption rates of *U. rigida*, *G. pristoides* and *Corallina* spp. were determined as these algae were the main components of the diet of *T. sarmaticus*.

Digestibility of algae

Digestibility experiments were run in conjunction with the consumption rate experiments. The effects of temperature on the ADMDC were tested at the same temperatures and with the same algae as for the consumption rate experiments (see above). The indicator

method, using acid-insoluble-ash (AIA) as an internal marker, was used to calculate the ADMDC for the algae.

Faeces produced during the consumption of the algae were collected for five days. Faeces were collected twice daily (09h00 and 16h00) to prevent re-ingestion and to minimize the effects of decomposition. Every attempt was made to ensure that only intact faeces were collected. Faeces were pipetted into beakers and then poured onto Whatman hardened ashless filter-paper discs in a funnel attached to a vacuum pump. All filter-paper discs were dried and pre-weighed (0.0001 g). The filter-paper discs and faeces were then dried to constant weight at 60°C for three days and then weighed (0.0001 g). Faecal mass was then determined by subtraction. Due to the small quantities of faeces collected daily from each animal, the faeces were pooled for the 5-day period for each experimental animal and stored in a desiccator until subsequent analysis. The ADMDC using AIA content of the algae and the associated faeces was determined using the method outlined by Atkinson *et al.* (1984). Samples of algae and corresponding faeces were ashed for 16 hrs at 600°C to ensure complete combustion of the organic material in the samples. The resulting ash was boiled in 75 ml of HCl (2 M) for 3 minutes, filtered through ashless filter-paper, and the residue washed with hot water. Ashing the filter-paper and residue again for 16 hrs at 600°C and reweighing gave the AIA. The ADMDC was calculated using the formula (after McDonald *et al.* 1984):

$$ADMDC = \frac{\%\ faecal\ indicator - \%\ diet\ indicator}{\%\ faecal\ indicator} * 100$$

Statistical analysis

All statistical analysis was calculated with a StatGraphics V7.0 statistical computer program (Statistical Graphics Corporation, USA). All data were tested for homogeneity of varience and where necessary percentage data were transformed using the arcsine transformation ($\sin^{-1}\sqrt{x}$) to comply with the requirements of ANOVA tests (Fry 1993). One-way ANOVA, Scheffe's multiple range tests and 2-sample t-tests (Fry 1993) were used to test for differences in consumption rates and digestibility between the different algal diets for both

adult and juvenile animals.

RESULTS

Energy content and nutritional composition of algae

The energetic content of the different algal species ranged from 2.5 to 16.5 kJ/g (Figure 5.1), being highest for *Gelidium pristoides* and lowest for the heavily calcified *Corallina* spp. (Figure 5.1C). There was little monthly variation in the energetic content of the phaeophytes and rhodophytes, however, monthly differences were found in the chlorophytes. The energetic content of *Ulva rigida* was highly variable with low values being recorded in November, December, April and July. *Codium extricatum* had a seasonal trend in energetic content, being low (≈ 5 kJ/g) during the autumn/winter months and high (≈ 10 kJ/g) during the spring/summer period (Figure 5.1A).

The nutritional composition of the six algae varied between species but there was little seasonal variation within a species (Table 5.2). With the exception of *Ecklonia radiata* (≈ 28%) and *G. pristoides* (≈ 15%), all the algae had an ash content greater than 50%, with the highest content being found in *Corallina* spp. (≈ 80%). The highest carbohydrate content was found in *G. pristoides* (≈ 40%) and the Chlorophyta (*C. extricatum*: ≈ 20%, *U. rigida*: ≈ 17%), whilst the lowest carbohydrate content was found in *Corallina* spp. (≈ 4%) and *Iyengaria stellata* (≈ 2%) (Table 5.2). All the algae had a protein content between 5 - 10% with *E. radiata* and *G. pristoides* having the highest values (≈ 10%) (Table 5.2). All the algae were low in lipid content (≤ 1.2 %) (Table 5.2).

Figure 5.1. Monthly energetic content of some Eastern Cape Province intertidal macroalgae belonging to: **(A)** Chlorophyta, **(B)** Phaeophyta and **(C)** Rhodophyta.

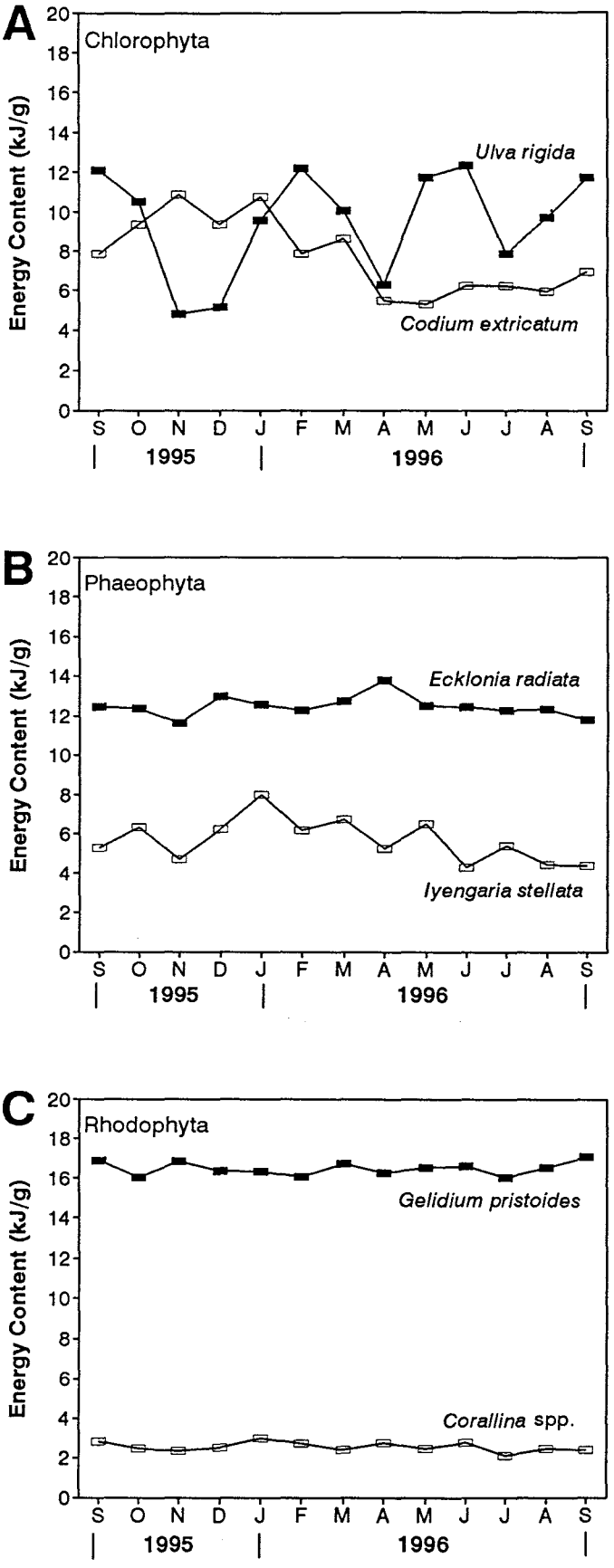

A
Chlorophyta
Energy Content (kJ/g)
Ulva rigida
Codium extricatum
B
Phaeophyta
Energy Content (kJ/g)
Ecklonia radiata
Iyengaria stellata
C
Rhodophyta
Energy Content (kJ/g)
Gelidium pristoides
Corallina spp.
S O N D J F M A M J J A S
1995
1996

Table 5.2. Nutritional composition of six intertidal macroalgae from the coast of the Eastern Cape Province.

	Algae	Ash content (% dry)	Soluble carbohydrates (% dry)	Protein (% dry)	Total lipids (% dry)
	SUMMER (December)				
Chlorophyta:	*Ulva rigida*	52	18.1	6.4	0.3
	Codium extricatum	64	20.9	9.9	0.9
Phaeophyta:	*Ecklonia radiata*	32	13.3	10.1	1.1
	Iyengaria stellata	64	2.5	5.8	0.6
Rhodophyta:	*Gelidium pristoides*	14	43.1	11.8	0.9
	Corallina spp.	77	4.7	6.4	0.7
	WINTER (July)				
Chlorophyta:	*Ulva rigida*	47	17.3	5.9	0.6
	Codium extricatum	66	21.2	7.3	1.1
Phaeophyta:	*Ecklonia radiata*	24	15.3	10.2	0.9
	Iyengaria stellata	68	2.3	4.6	0.8
Rhodophyta:	*Gelidium pristoides*	16	41.4	10.8	1.2
	Corallina spp.	80	4.2	6.1	1.1

Consumption rates of algae

A) Consumption rates of six species of algae at 20°C

Turbo sarmaticus readily consumed all the algae tested. At 20°C, the daily specific consumption rates of juvenile animals ranged from 1.45% b.wt./day (*E. radiata)* to 9.50% b.wt./day (*Corallina* spp.) (Figure 5.2, Table 5.3). The daily specific consumption rates for adult animals ranged from 1.06% b.wt./day (*G. pristoides*) to 6.08% b.wt./day (*Corallina* spp.) (Figure 5.2, Table 5.3). In juvenile animals, the consumption rate of *Corallina* spp., *U. rigida* and *C. extricatum* was significantly higher than the other diets (Tables 5.4 & 5.5). Significantly more *I. stellata* was consumed than *G. pristoides*, whilst *E. radiata* was the least consumed alga (Figure 5.2, Tables 5.4 & 5.5). In adult animals, the consumption rate of

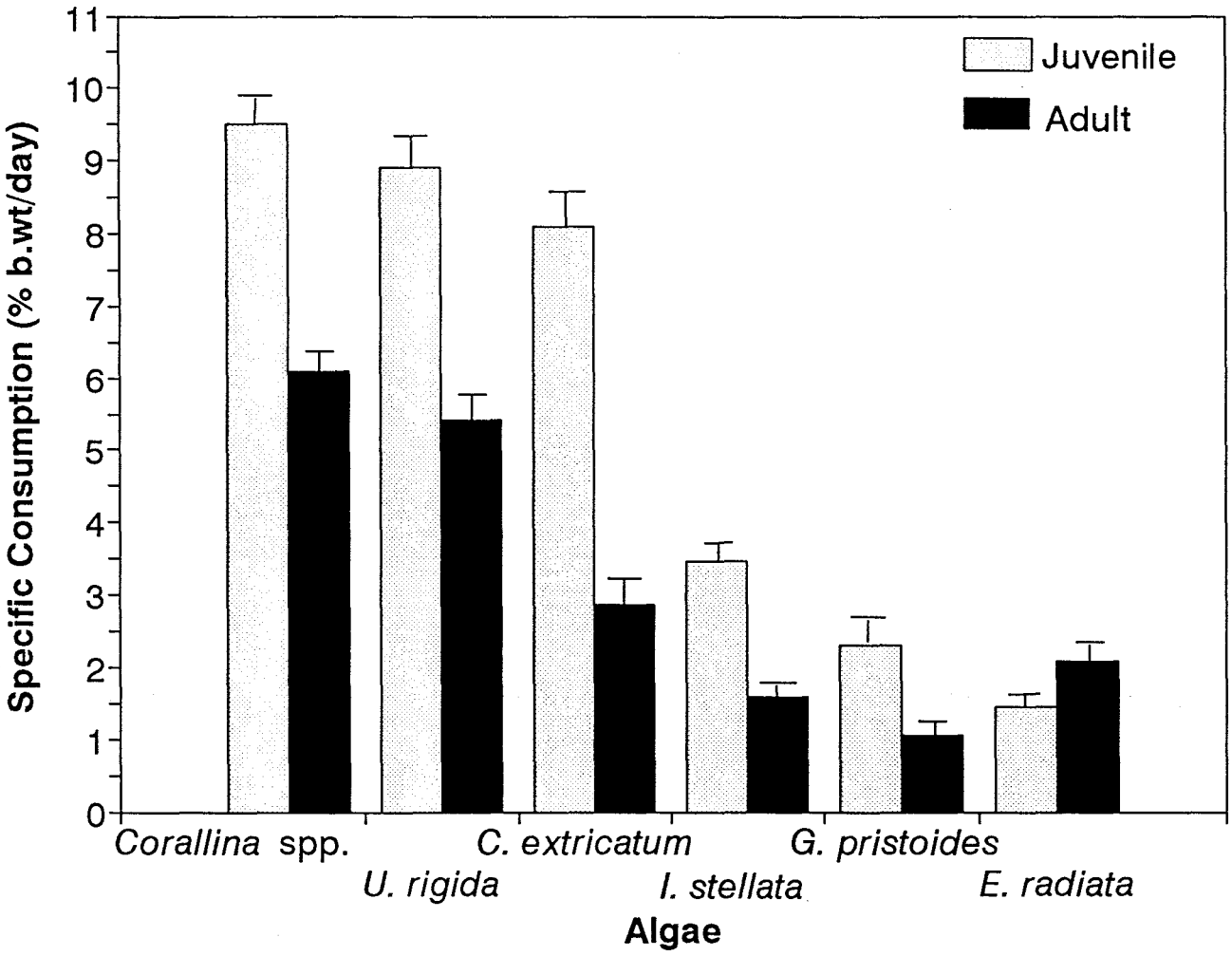


Figure 5.2. Daily specific consumption rates (mean ± S.E) (% dry body weight) of *Corallina* spp., *Ulva rigida*, *Codium extricatum*, *Iyengaria stellata*, *Gelidium pristoides* and *Ecklonia radiata* at 20°C by juvenile (n = 10) and adult (n = 10) *Turbo sarmaticus*.

Corallina spp. and *U. rigida* was significantly higher than other diets (Figure 5.2, Tables 5.4 & 5.5). Significantly more *C. extricatum* wasconsumed than *E. radiata*, *I. stellata* and *G. pristoides* which were consumed the least (Figure 5.2, Table 5.5).

Table 5.3. Daily specific consumption rates (mean ± S.E.) (% dry body weight) at 15°C, 20°C and 25°C for juvenile and adult *Turbo sarmaticus* fed various algal diets.

	Mean daily specific consumption rates (% dry body weight)					
	Juvenile			Adult		
Algae	15°C	20°C	25°C	15°C	20°C	25°C
Ulva rigida	2.85±0.69	8.91±0.45	7.33±1.09	3.68±0.47	5.42±0.35	5.82±0.49
Gelidium pristoides	1.14±0.21	2.30±0.41	2.32±0.58	1.26±0.19	1.06±0.20	2.09±0.45
Corallina spp.	1.60±0.28	9.50±0.33	9.39±1.41	2.96±0.32	6.08±0.34	5.99±0.66
Codium extricatum	-	8.09±0.43	-	-	2.86±0.34	-
Iyengaria stellata	-	3.46±0.24	-	-	1.59±0.11	-
Ecklonia radiata	-	1.45±0.21	-	-	2.09±0.33	-

Table 5.4. Results of a One-way ANOVA to determine whether the daily specific consumption rates of juvenile and adult *Turbo sarmaticus* differed when fed six algal diets at 20°C.

Source of variation	Sum of squares	D.F.	Mean squares	F-ratio	Sig. level
Juveniles					
Between groups	0.369	5	0.073	84.02	$p < 0.001$
Within groups	0.047	54	0.001		
TOTAL	0.416	59			
Adults					
Between groups	0.183	5	0.036	37.21	$p < 0.001$
Within groups	0.053	54	0.001		
TOTAL	0.236	59			

Table 5.5. Results from a Scheffe's multiple range test to determine where differences in the daily specific consumption rates of juvenile and adult *Turbo sarmaticus* occurred when fed six algal diets at 20°C. (X's in same column indicate no significant differences).

Juveniles		Adults	
Algae	Homogeneous groups	Algae	Homogeneous groups
Ecklonia radiata	X	*Gelidium pristoides*	X
Gelidium pristoides	X	*Iyengaria stellata*	X X
Iyengaria stellata	X	*Ecklonia radiata*	X
Codium extricatum	X	*Codium extricatum*	X
Ulva rigida	X	*Ulva rigida*	X
Corallina spp.	X	*Corallina* spp.	X

Juvenile animals had specific consumption rates significantly higher than those of the adults for all the algal diets, except *E. radiata* in which no significant difference was observed (Table 5.6). Specific consumption rates of juveniles were 1.6 (*U. rigida*) to 2.8 (*C. extricatum*) times higher than those of the adults (Figure 5.2, Table 5.3).

Table 5.6. Results from a 2-sample t-test to determine whether the daily specific consumption rates differed between juvenile and adult *Turbo sarmaticus* when fed on six algal diets at 20°C. (* Indicates a significant difference).

Algae	t-value	Sig. level
Ulva rigida	6.068	$p < 0.001$ *
Iyengaria stellata	6.862	$p < 0.001$ *
Codium extricatum	9.364	$p < 0.001$ *
Corallina spp.	7.100	$p < 0.001$ *
Gelidium pristoides	2.685	$p = 0.015$ *
Ecklonia radiata	-1.210	$p = 0.241$

B) <u>Consumption rates of three species of algae at 15°C, 20°C and 25°C</u>

With the exception of *G. pristoides*, both juvenile and adult animals fed on *Corallina* spp. and *U. rigida* at 15°C had significantly lower daily specific consumption rates than when

fed at higher temperatures (20°C and 25°C) (Figure 5.3, Tables 5.3, 5.7 & 5.8). However, there was no significant difference in the daily specific consumption rates of *U. rigida* and *Corallina* spp. at 20°C and 25°C (Figure 5.3, Tables 5.3, 5.7 & 5.8).

Table 5.7. Results of a One-way ANOVA to determine whether the daily specific consumption rates of juvenile and adult *Turbo sarmaticus* differed with temperature (15°C, 20°C and 25°C) when fed on *Corallina* spp., *Ulva rigida* and *Gelidium pristoides*.

	Source of variation	Sum of squares	D.F.	Mean square	F-ratio	Sig. level
JUVENILE	*Corallina* spp.					
	Between groups	0.210	2	0.105	17.34	$p < 0.001$
	Within groups	0.163	27	0.006		
	TOTAL	0.373	29			
	Ulva rigida					
	Between groups	0.116	2	0.058	17.44	$p < 0.001$
	Within groups	0.090	27	0.003		
	TOTAL	0.206	29			
	Gelidium pristoides					
	Between groups	0.010	2	0.005	2.18	$p = 0.132$
	Within groups	0.066	27	0.002		
	TOTAL	0.077	29			
ADULTS	*Corallina* spp.					
	Between groups	0.028	2	0.014	5.29	$p = 0.011$
	Within groups	0.071	27	0.002		
	TOTAL	0.100	29			
	Ulva rigida					
	Between groups	0.016	2	0.008	6.77	$p = 0.004$
	Within groups	0.032	27	0.001		
	TOTAL	0.048	29			
	Gelidium pristoides					
	Between groups	0.010	2	0.005	2.73	$p = 0.083$
	Within groups	0.050	27	0.001		
	TOTAL	0.060	29			

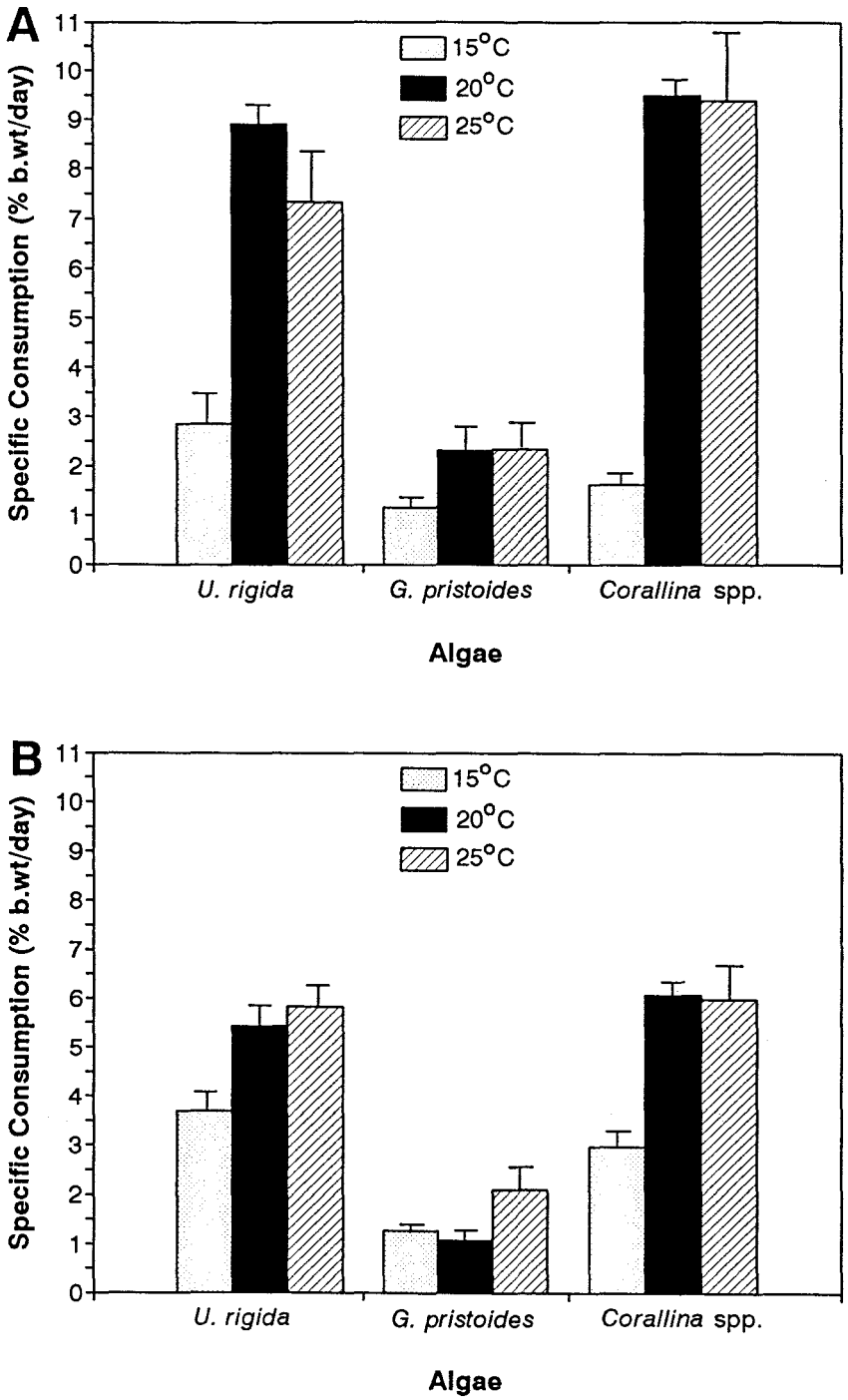


Figure 5.3. Daily specific consumption rates (mean ± S.E) (% dry body weight) of *Ulva rigida*, *Gelidium pristoides* and *Corallina* spp. at 15°C, 20°C and 25°C by **(A)** juvenile (n = 10) and **(B)** adult (n = 10) *Turbo sarmaticus*.

Table 5.8. Results from a Scheffe's multiple range test to determine where differences in daily specific consumption rates for juvenile and adult *Turbo sarmaticus* occurred when fed three algal diets at 15°C, 20°C and 25°C. (X's in the same column indicate no significant difference).

	Juvenile		Adult	
Temperature	*Corallina* spp. and *Ulva rigida*	*Gelidium pristoides*	*Corallina* spp. and *Ulva rigida*	*Gelidium pristoides*
15°C	X	X	X	X
20°C	X	X	X	X
25°C	X	X	X	X

Digestibility of algae

A) <u>Digestibility of six species of algae at 20°C</u>

At 20°C the ADMDC of the various algae for juvenile and adult animals ranged from as little as 9% (*Corallina* spp.) to as great as 75% (*G. pristoides*) (Figure 5.4, Table 5.9). In both juveniles and adults, there was a significant difference in the ADMDC among all the algal diets, with *G. pristoides* having the highest and *Corallina* spp. having the lowest (Figure 5.4, Tables 5.10 & 5.11).

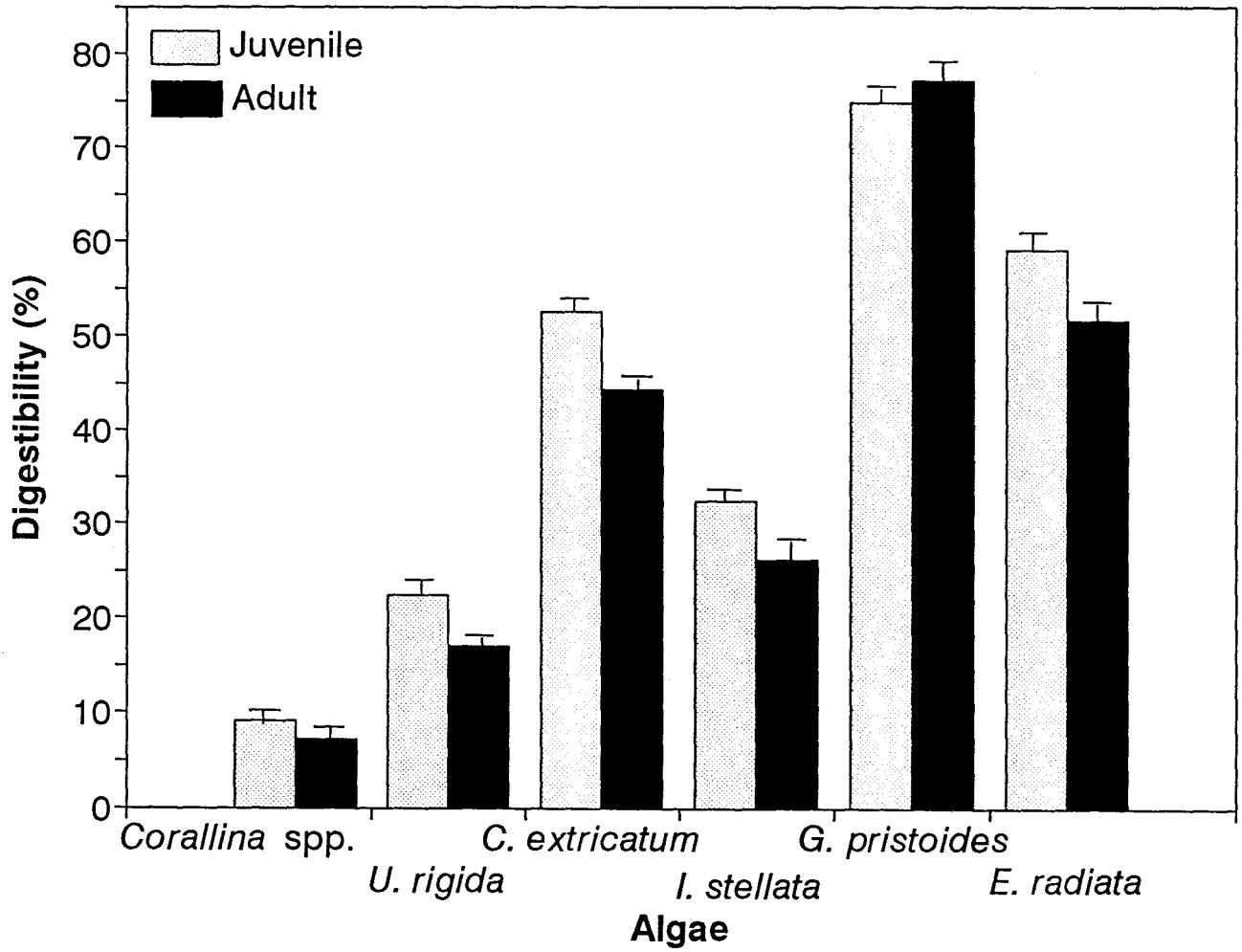


Figure 5.4. Apparent dry matter digestibility coefficient (mean ± S.E) (%) of *Corallina* spp., *Ulva rigida*, *Codium extricatum*, *Iyengaria stellata*, *Gelidium pristoides* and *Ecklonia radiata* at 20°C by juvenile (n = 10) and adult (n = 10) *Turbo sarmaticus*.

Table 5.9. Apparent dry matter digestibility coefficient (mean ± S.E.) of six algae consumed by juvenile and adult *Turbo sarmaticus* at 15°C, 20°C and 25°C.

	Apparent Dry Matter (%)					
	Juvenile			Adult		
Algae	15°C	20°C	25°C	15°C	20°C	25°C
Ulva rigida	22.6±4.8	22.4±5.1	26.0±3.9	16.9±2.9	17.1±3.7	18.5±3.5
Gelidium pristoides	73.3±6.4	74.8±6.4	79.4±6.8	76.5±5.9	77.1±6.3	81.4±6.6
Corallina spp.	8.7±1.1	9.1±1.4	10.0±1.5	7.4±0.9	7.3±1.2	8.0±1.2
Codium extricatum	-	52.6±4.5	-	-	44.4±5.5	-
Iyengaria stellata	-	32.4±4.4	-	-	26.2±3.2	-
Ecklonia radiata	-	59.1±6.1	-	-	51.6±5.4	-

Table 5.10. Results of a One-way ANOVA to determine whether the apparent dry matter digestibility coefficients differed for six algae consumed by juvenile and adult *Turbo sarmaticus*.

Source of variation	Sum of squares	D.F.	Mean squares	F-ratio	Sig. level
Juveniles					
Between groups	3.735	5	0.747	246.11	$p < 0.001$
Within groups	0.163	54	0.003		
TOTAL	3.899	59			
Adults					
Between groups	4.159	5	0.831	299.84	$p < 0.001$
Within groups	0.149	54	0.002		
TOTAL	4.309	59			

Table 5.11. Results of a Scheffe's multiple range test to determine where differences in apparent dry matter digestibility coefficients occurred for six algae consumed by juvenile and adult *Turbo sarmaticus*. (X's in same column indicate no significant differences).

	Juvenile	Adult
Algae	Homogeneous groups	Homogeneous groups
Corallina spp.	X	X
Ulva rigida	X	X
Iyengaria stellata	X	X
Codium extricatum	X	X
Ecklonia radiata	X	X
Gelidium pristoides	X	X

With the exception of *G. pristoides*, juvenile animals had significantly higher ADMDC values than the adults for all the algae (Figure 5.4, Tables 5.9 & 5.12). The ADMDC values for juveniles were 12% (*C. extricatum*) to 24% (*U. rigida*) higher than those for the adults (Figure 5.4, Table 5.9).

Table 5.12. Results of a 2-sample t-test to determine whether apparent dry matter digestibility coefficients differed between six algae consumed by juvenile and adult *Turbo sarmaticus*. (* Indicates a significant difference)

Diet	t-value	Sig. level
Ulva rigida	2.660	p = 0.015 *
Iyengaria stellata	3.554	p = 0.002 *
Codium extricatum	3.626	p = 0.001 *
Corallina spp.	3.120	p = 0.005 *
Gelidium pristoides	-0.782	p = 0.444
Ecklonia radiata	2.895	p = 0.009 *

Finally, in both juveniles and adults, temperature did not affect the ADMDC of *Corallina* spp., *U. rigida* and *G. pristoides* (Figure 5.5, Tables 5.9 & 5.13).

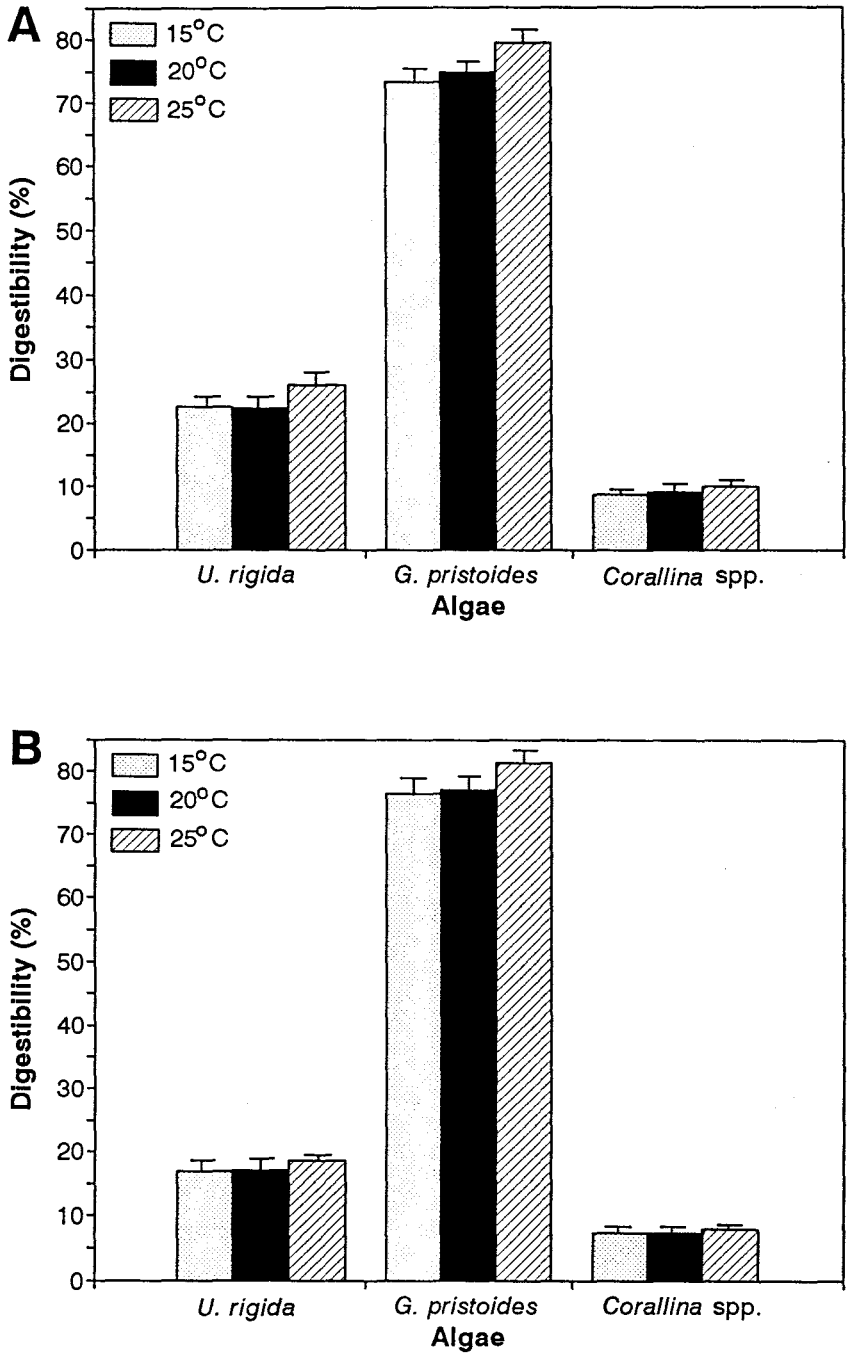


Figure 5.5. Apparent dry matter digestibility coefficient (mean ± S.E) (%) of *Ulva rigida*, *Gelidium pristoides* and *Corallina* spp. at 15°C, 20°C and 25°C by **(A)** juvenile (n = 10) and **(B)** adult (n = 10) *Turbo sarmaticus*.

Table 5.13. Results of a One-way ANOVA to determine whether apparent dry matter digestibility coefficients differed for three algae consumed by juvenile and adult *Turbo sarmaticus* at 15°C, 20°C and 25°C.

	Source of variation	Sum of squares	D.F.	Mean square	F-ratio	Sig. level
JUVENILE	*Corallina* spp.					
	Between groups	0.002	2	0.001	2.50	p = 0.100
	Within groups	0.013	27	0.001		
	TOTAL	0.016	29			
	Ulva rigida					
	Between groups	0.011	2	0.005	1.90	p = 0.168
	Within groups	0.082	27	0.003		
	TOTAL	0.093	29			
	Gelidium pristoides					
	Between groups	0.029	2	0.014	2.49	p = 0.101
	Within groups	0.161	27	0.005		
	TOTAL	0.191	29			
ADULTS	*Corallina* spp.					
	Between groups	0.001	2	0.001	1.07	p = 0.355
	Within groups	0.012	27	0.001		
	TOTAL	0.013	29			
	Ulva rigida					
	Between groups	0.002	2	0.001	0.68	p = 0.511
	Within groups	0.052	27	0.001		
	TOTAL	0.054	29			
	Gelidium pristoides					
	Between groups	0.024	2	0.012	1.82	p = 0.180
	Within groups	0.181	27	0.006		
	TOTAL	0.206	29			

DISCUSSION

Macrophagous herbivory is the dominant type of feeding in *Turbo sarmaticus*. This seems to contradict the general trend observed for other vetigastropods and patellogastropods which are dominated by microphagous herbivory (Steneck & Watling 1982). *Turbo sarmaticus* is probably an opportunistic macroalgal feeder, and although it has been observed to consume only *Gelidium pristoides*, *Ulva rigida*, and *Corallina* spp. in the field, the results from this study have shown that it is able to can consume and digest a greater variety of algae. Similar findings have also been reported for some fissurellids (Franz 1990).

Depending on the alga and the age of *T. sarmaticus*, animals ingested between 1.06% and 9.50% of their dry body weight per day. These values are similar to those reported for other gastropods (Grahame 1973, Clarke 1989, Dixon 1992, Britz 1995). The ingestion rate of *T. sarmaticus* decreased with increasing body size. Similar decreases in ingestion rates with body size have been observed in other gastropods (Bayne & Scullard 1978, Wickens & Griffiths 1985, Stenton-Dozey & Brown 1988), sea urchins (Buxton & Field 1983) and mangrove crabs (Emmerson & McGwynne 1992). It is likely that the higher specific consumption rate in juvenile *T. sarmaticus* and lower rate in adults was due to differences in metabolic rates (Branch 1981, Peck *et al.* 1987).

The assimilation efficiency of gastropods can be highly variable, being directly influenced by the quality rather than the quantity of food, as well as the degree of parasitization (Carefoot 1967a, Paine & Vadas 1969, Calow 1975, Lawrence 1975, Hawkins 1981, Hawkins & Hartnoll 1983). The ADMDC of *T. sarmaticus* varied considerably and was dependent on the diet as well as the animal's body size, with smaller individuals having greater digestibility efficiencies. These findings were in contrast to results from sea urchins (Fuji 1962, 1967, Buxton & Field 1983), mangrove crabs (Emmerson & McGwynne 1992) and other gastropods (Bayne & Newell 1983, Barkai & Griffiths 1987) where digestibility was not dependent on body size. The reason for these contrasting results is unknown and further investigations are needed.

Most of the algae consumed by *T. sarmaticus* had digestibility values ranging from 40 - 75%, which are similar to those reported for algae consumed by other molluscs (Carefoot 1967a, Paine 1971, Kofoed 1975, Streit 1975, Conover 1978, Jensen & Siegismund 1980,

Peduzzi 1987, Loo 1992, Wee *et al.* 1992). In addition, these digestibility values are comparable with those for food consumed by carnivorous gastropods (52 - 95%) (Bayne & Newell 1983, Stenton-Dozey & Brown 1988). The higher digestibility values for food consumed by carnivorous molluscs can be attributed to animal tissue usually being more digestible than plant tissue (Hughes 1986). Barkai & Griffiths (1987) have indicated an assimilation efficiency of 37% for *Ecklonia maxima* consumed by the South African abalone, *Haliotis midae*, whilst Dixon (1992) reported digestibility values of 29.9% and 70.7% for *Plocamium corallorhiza* and *Gelidium amanzii*, respectively, consumed by the same animal. Some very high assimilation efficiencies have been reported for algae consumed by *Patella cochlear* (93%) and *Acanthopleura granulata* (96%) (Branch 1981, Mook 1986). In addition, very high assimilation efficiencies have been reported for bacteria (81 - 98%) consumed by fiddler crabs and deposit-feeding gastropods (Dye & Lasiak 1987).

Temperature and salinity can have a marked effect on consumption in gastropods (Stickle *et al.* 1985, Dixon 1992, Britz 1995). When allowed constant access to *U. rigida* and *Corallina* spp., the amount of algae consumed by juvenile *T. sarmaticus* was 3 - 5 times greater at 20°C and 25°C than at 15°C. Adult animals fed on the same algae had a consumption rate 1.5 - 2 times greater at 20°C and 25°C, than at 15°C. Similarly, when allowed constant access to an artificial aquaculture diet, the food intake of *H. midae* doubled from 15°C to 22°C (Dixon 1992). Key factors that may have influenced the ADMDC values at different temperatures are ingestion rate, digestive enzyme activity and the transit time of the ingested material through the gut (Prosser & Brown 1961, Condrey *et al.* 1972). Gut transit time, which is an inverse function of both temperature (Horn & Gibson 1990) and ingestion rate (Elliot & Persson 1978, Fange & Grove 1979, Jobling 1980, Fauconneau *et al.* 1983), would have tended to decrease with increasing temperature. Despite the increase in algal consumption by *T. sarmaticus* from 15°C to 20°C, and a probable concomitant reduction in transit time, the digestibility values did not differ significantly between temperatures. This may have been due to an increased secretion of enzymes (Luquet 1979, Hepher 1988) and/or a decrease in the reaction time of the enzymes with the substrates (Hepher 1988). This would have resulted in similar digestibility values despite the ingested food possibly being passed through the gut at a faster rate, which would have reduced contact time with enzymes. In direct contrast to *T. sarmaticus*, however, data obtained for *H. midae* indicated higher

digestibility values when temperature was increased from 15°C to 18°C (Dixon 1992).

The importance of environmental effects on metabolism and physiology have been demonstrated in aquaculture management (Brett 1979). In contrast to homeothermic animals (Lovell 1989), temperature influences basal and active metabolic rates in poikilotherms (Bullock 1955). Poikilotherms exposed to higher temperatures have the choice of three strategies in order to maintain an energetic intake that exceeds basal requirements. They can either increase their rate of ingestion or increase their digestive efficiency of food, or a combination of both (Newell & Branch 1980). The increased consumption rate associated with temperature may be regarded as an attempt by *T. sarmaticus* to increase energetic intake to meet the rise in metabolic costs. A positive relationship between temperature and food intake has also been observed in other gastropods (Edwards & Huebner 1977, Lee *et al.* 1988, Gao *et al.* 1990), crustaceans (Serfling & Ford 1975, Bordner & Conklin 1981) and fish (Luquet 1979, Horn & Gibson 1990, Wax & Pote 1990). *Turbo sarmaticus*, therefore, does not increase its digestive efficiency at higher temperatures, but rather increases its ingestion rate in order to compensate for increased energetic demands.

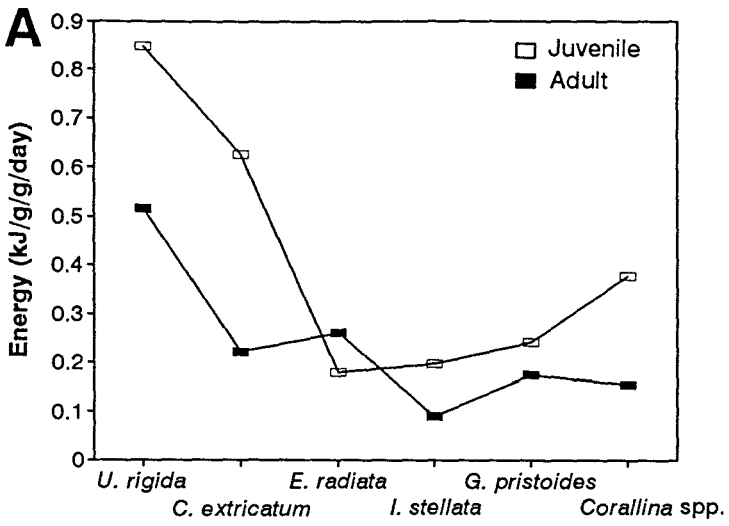
A significant feature of the energy input of an organism is not the amount consumed but the absorbed fraction. A complex of different factors (*e.g.* palatability, availability, energy content, ease of ingestion, assimilation efficiency) are responsible for the amount of energy available to a grazer (Hawkins & Hartnoll 1983). In addition, algal quality may affect digestion rates and apparent digestibility, both of which will affect the amount of nutrients available for absorption (Hughes 1986). There is some evidence in the literature to suggest that when offered a choice of food, animals select those which provide maximum energy (Carefoot 1967a, Paine & Vadas 1969, Hawkins & Hartnoll 1983). By contrast, Fleming (1995) has indicated that *Haliotis rubra* selects algal species on the basis of their digestible nitrogen content, and that the energetic content of the algae does not correlate with the algae selected. It is possible that when an animal consumes food of poor nutritional content (low energy and/or protein levels), more is consumed to meet the animal's nutritional needs (*i.e.* a nutrient threshold). Increased consumption of a poor quality food would therefore, result in a similar energetic and nutrient intake to a decreased consumption of a good quality food source. To some extent this theory holds true for *T. sarmaticus*, as the different consumption rates of certain algae (*Corallina* spp., *G. pristoides*, *I. stellata* and *E. radiata*) resulted in

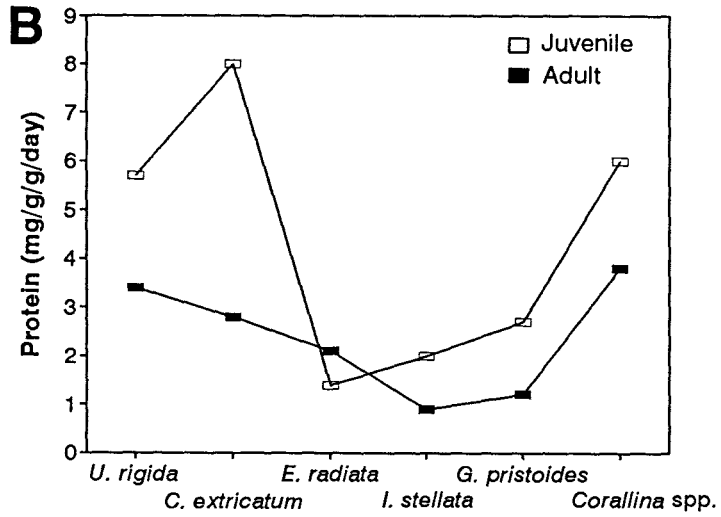
similar potential energy intakes for these algae (Figure 5.6A). The potential nutrient (protein and carbohydrate) intake, however, was variable, regardless of the consumption rates of the different algae (Figure 5.6B & C). Further investigations are needed to determine the digestibility of these nutrients (protein and carbohydrates) in order to establish their potential utilization. A general pattern emerged in that the potential energy and nutrient intake was greater for green algae (*U. rigida* and *C. extricatum*) than the red and brown algae (Figure 5.6). This may be attributed to their relatively high energy and nutrient contents (Table 5.2), and their high consumption rates (Figure 5.2).

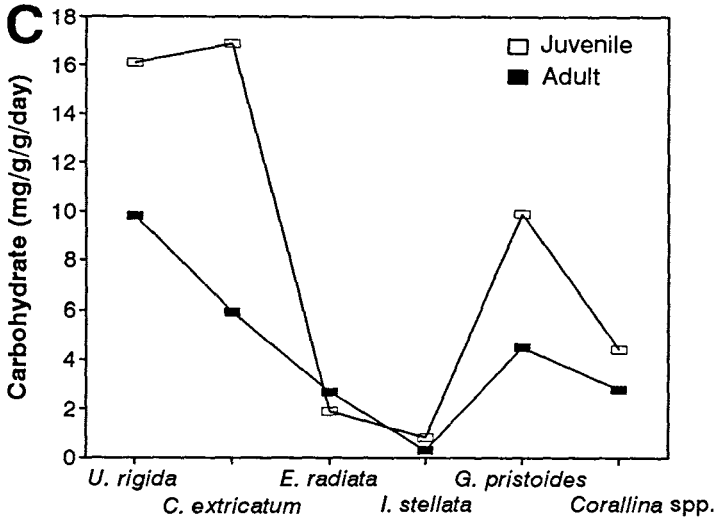
The higher specific consumption rates of *T. sarmaticus* when feeding on *Ulva rigida*, *Corallina* spp., and *Codium extricatum* may have indicated a preference for these algae. However, food preference studies need to be carried out before such a conclusion can be drawn. It is suggested that the higher specific consumption rates may have been a result of compensation by *T. sarmaticus* for the lower digestibility of these algae. This is supported by the relationship which existed between specific consumption rate and apparent dry matter digestibility for *T. sarmaticus* (Figure 5.7). A similar relationship between food quality and ingestion rates was observed in two freshwater gastropods where increased intake compensated for a poor quality diet (Calow 1975). In addition, McShane *et al.* (1994) have indicated that ingestion rates in *H. rubra* were varied and depended on plant toughness rather than on chemical composition. Conversely, it has been shown in sea urchins, that they can improve their digestive efficiency, rather than increase their ingestion rate when a food intake is of poor quality (Lawrence *et al.* 1989).

The energy value of the organic component of an organism's food supply varies widely (Finlay & Uhlig 1981). Furthermore, high energy components of food may not be available for utilization because their complex chemical structures may be difficult to break down. *Turbo sarmaticus* had high digestibility values for *G. pristoides* ($\approx$ 75%), *E. radiata* ($\approx$ 55%) and *C. extricatum* ($\approx$ 50%) which suggested that it had developed the ability to utilize such high energy components in these algae. This ability has been demonstrated in other animal groups (Condrey *et al.* 1972). *Turbo sarmaticus*, however, has temporal limitations on its food supply because of its nocturnal grazing behaviour (*pers. obs.* and therefore the utilization of such high energy components in the diet may have been an adaptation to more efficiently utilize a limited feeding window.

Figure 5.6. Daily nutritional intake of **(A)** energy (kJ/gram of dry algae/gram of dry animal weight/day), **(B)** protein (mg/g/g/day), and **(C)** carbohydrate (mg/g/g/day) calculated from the differing daily specific consumption rates of six intertidal macroalgae ingested by *Turbo sarmaticus*.


A
Energy (kJ/g/g/day)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Juvenile
Adult
U. rigida
E. radiata
G. pristoides
C. extricatum
I. stellata
Corallina spp.


B
Protein (mg/g/g/day)
9
8
7
6
5
4
3
2
1
0
Juvenile
Adult
U. rigida
E. radiata
G. pristoides
C. extricatum
I. stellata
Corallina spp.


C
Carbohydrate (mg/g/g/day)
18
16
14
12
10
8
6
4
2
0
Juvenile
Adult
U. rigida
E. radiata
G. pristoides
C. extricatum
I. stellata
Corallina spp.

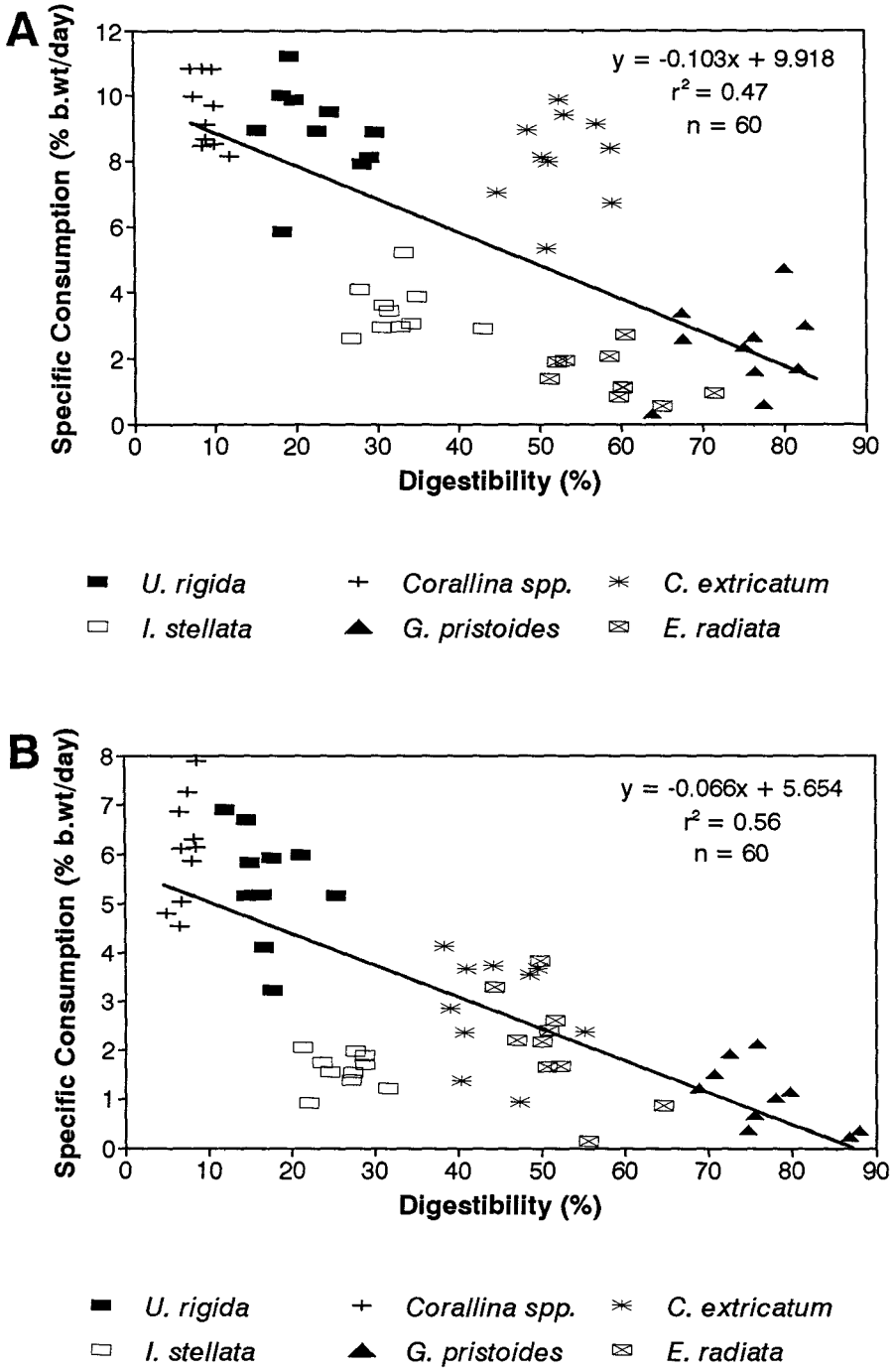


Figure 5.7. Relationship between apparent dry matter digestibility (%) and daily specific consumption rate (% b.wt) for **(A)** juvenile and **(B)** adult *Turbo sarmaticus* fed on six algal species.

Boolootian & Lasker (1964) have suggested that marine herbivores eat algae which are compatible with the enzymes and other equipment of digestion and which support good growth. These authors found that the sea urchin, *Strongylocentrotus purpuratus*, had higher digestibility values for the algae it consumed in its natural habitat, than for other algal diets. By contrast, *T. sarmaticus* did not follow this pattern, as algae consumed in the field did not always have the highest digestibility values. For example, *Corallina* spp. although extensively consumed, was poorly digested.

Differences in chemical and energy contents of algae may indicate differences in their value as food for herbivores. A food of high value may be defined as one that is readily digested and which provides a relatively large proportion of required energy and nutrients to the grazer per weight of food. Paine & Vadas (1969), in an extensive survey of temperate zone marine algae, found green algae to be highest in energy and lowest in ash of the three algal divisions Rhodophyta, Chlorophyta and Phaeophyta. Similarly, Linn Montgomery & Gerking (1980) found the Chlorophyta superior to Phaeophyta, which in turn were superior to Rhodophyta as potential foods. By contrast, Kaehler & Kennish (1996) examined numerous algal species from Hong Kong and found the Phaeophyta superior, whilst the Chlorophyta were of intermediate or inferior quality with respect to most nutrient components. Although only a few representatives of each algal division were analysed in this study, on the basis of their energetic and nutritional composition, *G. pristoides*, *E. radiata* and *U. rigida* were the superior algae. However, with the exception of *U. rigida*, these algae were not always the most consumed.

Total lipid was generally low in the three algal divisions. Lipid levels in algae, examined in this and other studies (Russell-Wells 1932, Black & Cornhill 1951, Paine & Vadas 1969, Idler & Wiseman 1970, Jensen 1972, Dawes *et al.* 1974, Hayashi *et al.* 1974, Kaehler & Kennish 1996), were found to be low, seldom constituting more than 5% of dry weight. In general, algae are also low in protein, a component critical to growth (Prosser 1973, Cowey 1975). The values for protein content on a dry weight basis in this study (6 - 11%) were consistent with the values obtained for other algal species (Jensen 1972, Dawes *et al.* 1974, Kaehler & Kennish 1996).

The major problem in algal digestion resides with the hydrolysis of polysaccharides which are a greater energy reserve than proteins or lipids (Linn Montgomery & Gerking

1980). Soluble carbohydrate values in this study were comparable to those obtained for other algal species (*e.g.* Paine & Vadas 1969, Kaehler & Kennish 1996). Alpha-linked polysaccharides, such as starch, are more susceptible to amylases than beta-linked polymers like cellulose and its derivatives. Chapter seven discusses polysaccharide digestion in detail and has indicated that amylase activity in the digestive regions of *T. sarmaticus* is much higher than the cellulases. Such differences in activity will have an effect on the potential food value of the algal divisions. Brown algae are characterized by storage compounds and extracellular substances that are beta-linked polymers (Percival & McDowell 1967, Craigie 1974, Mackie & Preston 1974). In contrast, green algae store starch, an alpha-linked polymer, but the cell walls are high in resistant beta-linked polymers (Craigie 1974, Mackie & Preston 1974). Red algae store starch, an alpha-linked polymer, and their extracellular compounds are dominated by alpha- and beta-linked polysaccharides (Percival & McDowell 1967). On the basis of this, red algae are more susceptible to digestion than the green and brown algae. This trend was observed for *T. sarmaticus* where the highest digestibility value was obtained for a red alga.

The articulated Corallinacaea, as observed in this study, are characterised by extremely high ash content and subsequently low energy and nutrient value (Kaehler & Kennish 1996). High ash levels in these species result from the calcium carbonate, which in addition to limiting the abundance of nutrients, is also thought to interfere with the digestive capabilities of herbivores (Horn 1989, Hay *et al.* 1994). The Corallinaceae are therefore of extremely poor quality as a potential food source, and the exclusion of such algae from an animal's diet may even have a selective advantage. However, this is not the case in *T. sarmaticus*, some other turbinids, gastropod molluscs (Galli & Giese 1959, Leighton & Boolootian 1963, Tsuda & Randall 1971, Clarke 1988, Bode 1989, Worthington & Fairweather 1989, Aguilar Rosas *et al.* 1990, Franz 1990, Wood & Buxton 1996) and some crabs (Kennish *et al.* 1996) which readily consume coralline algae. Similarly, the lower apparent digestibility values for *T. sarmaticus* fed on *U. rigida* and *I. stellata* may indicate that there are more structural carbohydrates, calcareous material and refractory components which offer more resistance to digestion in these algal species.

In conclusion, *T. sarmaticus* is a generalist grazer capable of consuming and digesting algae from the Rhodophyta, Chlorophyta and Phaeophyta. There was little variation in the

seasonal energetic and nutritional content of six intertidal macroalgae from the Eastern Cape Province, indicating that they would have had a similar nutritional value for *T. sarmaticus* all year round. The consumption rates and digestibility of the different algae, however, varied considerably. Most of the algae consumed and digested by juvenile *T. sarmaticus* had significantly higher specific daily consumption rates and ADMDC than adult animals. It is suggested that consumption rates were dependent on the ADMDC of the algae. In addition, it is further suggested that the consumption rates of the different algae were not related to the nutritional composition of the algae, but more likely the energetic content. This would have ensured that the energy intake met a minimum threshold level. Finally, in both juvenile and adult animals, temperature had a positive influence on consumption rates, resulting in increased rates at higher temperatures. However, in both juvenile and adult *T. sarmaticus*, algal ADMDC was not affected by temperature.

Based on the energetic content, nutritional composition, consumption rate and digestibility of the six algae, a summary table indicates the potential nutritional value of the six algae for *T. sarmaticus* (Table 5.14). It is suggested that *U. rigida*, *C. extricatum*, *E. radiata* and *G. pristoides* have the best nutritional value for growth and reproductive fitness in *T. sarmaticus*, whilst *I. stellata* and *Corallina* spp. have the poorest nutritional value. The influence of three of these algae on the growth rate and reproductive fitness of *T. sarmaticus* are examined in Chapter 6.

Table 5.14. Summary of the potential nutritional value of six macroalgae from the Eastern Cape Province for *Turbo sarmaticus*.

	Chlorophyta		Phaeophyta		Rhodophyta	
	Ulva rigida	*Codium extricatum*	*Ecklonia radiata*	*Iyengaria stellata*	*Gelidium pristoides*	*Corallina spp.*
Organic content (%)	Medium	Medium	High	Medium	High	Low
Energy (kJ/g)	Medium	Medium	High	Medium	High	Low
Protein (%)	Medium	Medium	High	Low	High	Medium
Carbohydrate (%)	Medium	Medium	Medium	Low	High	Low
Digestibility (%)	Medium	Medium	Medium	Medium	High	Low
Consumption rate (% b.wt/day)	High	High	Low	Medium	Medium	High

Index of evaluation

Organic content: High ≥ 80%, Medium 30 - 60%, Low < 30%
Energy: High ≥ 10 kJ/g, Medium 3 - 9 kJ/g, Low < 3kJ/g.
Protein: High ≥ 10%, Medium 6 - 9%, Low < 6%.
Carbohydrate: High ≥ 40%, Medium 10 - 39%, Low < 10%.
Digestibility: High ≥ 70%, Medium 10 - 69%, Low < 10%.
Consumption: High ≥ 8%, Medium 2 - 7%, Low < 2%.

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CHAPTER SIX

THE INFLUENCE OF DIET ON THE GROWTH RATE, REPRODUCTIVE FITNESS AND OTHER ASPECTS OF THE BIOLOGY OF *TURBO SARMATICUS*

INTRODUCTION

The relationship between algae and herbivores is complex and has stimulated much research (see review Hawkins & Hartnoll 1983), yet little is known about the nutritional needs of algal-eating invertebrates. The prime directive of all organisms is to obtain food for survival, growth and reproduction (White 1993). The food type chosen is often selected to satisfy physiological needs and contributes most to the growth and eventual reproduction of the organism (Nicotri 1980, Watanabe 1984). It is thought that in the mutual evolution between algae and herbivores, an alga which gives good growth would be the one most preferred, yet this is often not the case (Leighton & Boolootian 1963, Clarke 1988). An alga which is common in the animal's habitat may be ignored for other less abundant algae (Hawkins & Hartnoll 1983, Mazzella *et al.* 1992).

Feeding behaviour is regarded to represent long-term (evolutionary) adaptations to optimize growth, reproduction and ultimately the production of offspring (Schoener 1971, Pyke *et al.* 1977). The fitness of an organism can be defined as the proportionate contribution of individuals to future generations (Begon *et al.* 1986). This is determined by how efficiently an organism obtains, digests and assimilates nutrients from organic matter, and how it uses these nutrients for maintenance, growth, storage and ultimately reproduction (Avery *et al.* 1993).

Several factors influence the fitness a consumer derives from exploiting a given food resource (Sih 1987). The most obvious is the nutritional value of the food resource relative to the needs of the consumer. Most studies equate the nutritional value of a food resource to its energetic content but nutrient composition may also be important (Lowe & Lawrence 1976). Another factor affecting fitness is the time required for handling food. Some plants have evolved structural and chemical defences which increase the handling costs for the consumer (Hay & Fenical 1988, Padilla 1989). Finally, fitness may be affected by the time and costs involved in searching for food. Here, critical factors are food abundance and distribution (patchiness), and in the case of animal prey, prey behaviour (Lima & Dill 1990).

Although the plant material utilized by herbivores may have a more predictable mass and availability than animal foods, it is nutritionally inferior (Boney 1965, Mattson 1980, White 1985, Wolcott & O'Connor 1992). Marine herbivores are thought to be nutritionally

limited by their food sources and consequently, they select for nutritionally rich algae (Himmelman & Nedelec 1990, Horn *et al.* 1990, Duffy & Paul 1992). Plants have a low nitrogen content which can prove to be an important limiting nutrient for herbivores, since energy in the form of carbon (carbohydrates) is more readily available in plants (Boyd & Goodyear 1971, Mattson 1980, White 1993). Therefore, a herbivore will maximise its growth and reproductive fitness on nitrogen-rich foods. In fish, for example, body condition and size of gonads have been shown to be directly related to abundance of high-quality food (*e.g.* Montgomery *et al.* 1989, Caceres *et al.* 1994).

Declining abalone fisheries worldwide have accelerated the development of abalone aquaculture (Ino 1952, Tong 1983, 1987, Illingworth 1986, Moss 1986), with much interest being placed on research of suitable algae diets and the development of artificial diets (*e.g.* Uki *et al.* 1985, Hahn 1989, Britz *et al.* 1994). This has provided a good grounding from which the influences of diet on growth and reproductive fitness in other marine molluscs can be evaluated. A number of studies have determined the dietary value of macroalgae for growth in abalone (Sakai 1962, Kikuchi *et al.* 1967, Koike *et al.* 1979, Uki 1981, Hayashi 1982, Uki *et al.* 1986, Sato & Notoya 1988, Hahn 1989, Peck 1989). Based on this research, it is now known that various parameters affect growth rates in marine molluscs. Low temperature reduces growth by affecting feeding rates, feeding duration (Uki 1981) and food absorption efficiency (Peck 1989). Similarly, photoperiod affects feeding rates and absorption efficiencies (Ebert & Houk 1984) and therefore growth. In addition, reduced salinity and low oxygen concentration also reduce growth (Chen 1984). Most studies have focused on, and have shown the importance, diet in determining growth rates (Aranda *et al.* 1989, Hahn 1989, Laing 1990, Wikfors *et al.* 1991, Day & Fleming 1992, Hanisak 1992, Pennings *et al.* 1993, Viana *et al.* 1993). Growth rates of animals fed on various diets can be related to the growth-promoting ability of the food such as nutrient composition, edibility (structure, texture), digestion, absorption by the animal and availability in the habitat (*e.g.* Leighton & Boolootian 1963, Carefoot 1967a, 1967b, Lawrence 1975).

Numerous species of algae are available for consumption by *Turbo sarmaticus* on eastern Cape rocky shores (Seagrief 1988, Branch *et al.* 1994). *Turbo sarmaticus* is a generalist macroalgal grazer, however, three species of algae form the bulk of its diet, *viz.*: *Corallina* spp., *Gelidium pristoides* and *Ulva rigida* (Chapter 5). Although the potential

dietary value of these algae has been determined (Chapter 5), their effect on the growth rate and reproductive fitness of *T. sarmaticus* has not been investigated. Over-exploitation of shellfish (*e.g.* limpets and mussels) along the former Transkei coast of the Eastern Cape Province has caused changes in the macroalgal community structure (Dye 1992). For example, where mussel beds have been removed, coralline algae have rapidly and extensively taken their place. Such changes in the intertidal community could influence growth rate and reproductive fitness of macroalgal grazers such as *T. sarmaticus*. This, in turn, could affect the ability of *T. sarmaticus* populations to endure exploitation by man. The aim of this study was to determine the dietary value of three macroalgal species (*Corallina* spp., *G. pristoides* and *U. rigida*) commonly consumed by *T. sarmaticus*. These algae were evaluated by relating the growth, reproductive fitness and energy levels (in the form of glycogen) attained by *T. sarmaticus* when fed these algae alone or in a mixture.

MATERIALS AND METHODS

Experimental system

All experiments were carried out at a small research laboratory in Port Alfred (33°36'S/26°54'E). The laboratory uses sea water pumped from the mouth of the Kowie River. The experimental aquaria and system parameters are summarised in Table 6.1. Two groups of experimental animals were used in this series of experiments. The first was a juvenile group in which the effects of diet on growth rate, reproductive fitness, shell strength and thickness, and body energy (glycogen) levels were determined. The second was an adult group in which the effect of diet on reproductive fitness and body energy (glycogen) levels were determined. Despite the fact that the juveniles attained sexual maturity by the end of the growth rate experiment, they are still referred to as the juvenile animals in this study in order to distinguish them from the adult experimental group. All experimental animals were labelled with "Dymo" tags (see method in Chapter 3) in order to identify individuals.

Table 6.1. System parameters of the experimental aquaria at the Port Alfred laboratory.

Parameter	Experimental regime
Temperature	Ambient sea surface temperature.
Salinity	35 ± 1 ‰
Water supply	Partial recirculation through a biological filter with 25% replacement per day from Kowie River.
Flow rate	6 changes/experimental container/hour.
System volume	10 000 litres.
Experimental containers	Plastic, 110 cm X 70 cm X 30 cm, ≈ 230 litres. Situated outdoors.
Light regime	Ambient sunlight and daylength (but shaded from direct sunlight).
Animal shelter	Shelters were made from plastic piping (40 cm long X 11 cm diameter) cut in half longitudinally. Shelters were provided to give the animals a daytime hiding place.

Influence of diet on growth rate

Similar sized juvenile (≈ 42 mm shell length, n = 80) *Turbo sarmaticus* were collected from the intertidal zone at Kenton-on-Sea. Juvenile animals were chosen as they grow more rapidly than older sexually mature animals (Chapter 3). Therefore, the effect of diet on growth increments would be more apparent. Twenty animals were placed in each of four experimental containers and fed one of four diets. There was no significant difference in the mean shell length and total mean wet weight (shell and flesh) of the animals in each container at the start of experimentation (Table 6.2).

Table 6.2. Results of a One-way ANOVA to determine whether the mean shell length and total mean wet weight of juvenile *Turbo sarmaticus* differed between containers at the start of experimentation.

Source of variation	Sum of squares	D.F.	Mean square	F-ratio	Sig. level
Shell length					
Between groups	0.001	3	0.001	0.376	p = 0.770
Within groups	0.125	76	0.002		
TOTAL	0.127	79			
Wet weight					
Between groups	0.013	3	0.004	0.330	p = 0.803
Within groups	1.028	76	0.013		
TOTAL	1.042	79			

The experiment was conducted from 1st April 1995 - 1st April 1996. Animals were fed on algal diets that consisted of either *Corallina* spp., *Gelidium pristoides*, *Ulva rigida* or an equal volume mixture of all three algae. The algae were obtained from the same source (Kenton-on-Sea) every month to minimize possible ecotypic variations in energetic and nutrient content. Algae were provided in excess, animals were allowed to feed *ad lib*, and uneaten algae were replaced each week with fresh algae. All experimental tanks were cleaned regularly (once a week) to remove the growth of filamentous algae which may have been consumed by the animals, thereby influencing the results.

Monthly measurements of shell lengths (0.1 mm) and total wet weights (0.01 g) of all *T. sarmaticus* were taken. Before measurements were taken, excess water was drained from the animals by suspending them in a nylon net bag for five minutes. After this they were blotted dry. Growth rates of individuals (shell length increases per month) fed different algal diets were calculated from the formula:

$$SL = \frac{L_f - L_i}{t}$$

where, **SL** = growth rate (shell length increase in mm/month); $\mathbf{L_f}$ = final mean shell length;

L_i = initial mean shell length; **t** = time. Similarly, growth rates of individuals (total wet weight gained per month) fed different algal diets were calculated from the formula:

$$TWW = \frac{W_f - W_i}{t}$$

where, **TWW** = growth rate (total wet weight gained in grams/month); W_f = final total wet weight (g); W_i = initial mean total wet weight (g); **t** = time. These data were compared with the growth rate (Chapter 3) of similar sized field animals (controls).

Influence of diet on reproductive fitness

By the end of the growth rate experiment, the juvenile *T. sarmaticus* had grown to a size at which they would have reached sexual maturity (≈ 52 mm shell length - Chapter 4). To compare the effect of diet on gonad development, animals were removed (n = 20/diet) from their shells and their GI calculated (see Chapter 4 for method). These data were compared with the GI of field animals (controls).

To examine the effect of diet on reproductive development of larger adult *T. sarmaticus* (≈ 80 mm shell length), animals (n = 80) were collected from Kenton-on-Sea in May 1996 (when the GI of field animals were at their lowest - Chapter 4). Ten animals were placed in each of eight aquaria and fed on one of four algal diets (*Corallina* spp., *G. pristoides*, *U. rigida* or an equal volume mixture of these algae; n = 20/diet) for six months. In November 1996 (when the GI of animals in the field were at their highest - Chapter 4), the animals were removed from their shells and their GI calculated. In addition, to compare GI between diets, these data were compared with the GI of similar sized field animals (controls).

Influence of diet on shell strength and thickness

At the end of the juvenile growth rate experiment, the shell strength and thickness of the juvenile *T. sarmaticus* fed on the four algal diets (*Corallina* spp., *G. pristoides*, *U. rigida*

and an equal mixture of these algae; n = 20/diet) were measured. Shell strength was determined on a small section of shell (10 x 10 mm) taken from about 10 mm from the growing edge (Figure 6.1). The pieces of shell were placed in an Instron 4301 (load, extension and strain machine) and the load required (in Newtons) to break the shell by compression with a 3 mm diameter steel rod was taken as a parameter of strength. All shell pieces were tested wet as drying can reduce shell strength (Currey 1979).

Shell thickness was determined from the same shell pieces (n = 10/diet). Shell pieces were dried (at 60°C), gold coated and viewed in a Jeol JSM 840 scanning electron microscope. Five measurements were taken along the length of the shell piece for each layer (prismatic and nacreous) (Figure 6.2). The data from the 10 shells for each diet were pooled (n = 50/layer) and used to determine mean shell layer thicknesses for animals fed on each diet. The shell strength and thickness data were compared with that of similar sized field animals (controls).

Ninety percent of the calcified layers (prismatic and nacreous) of molluscan shells are composed of calcium carbonate (Watabe 1988). In order to see if the amount of calcium present in the algal diets influenced the shell strength and thickness of the layers, the calcium content of the three algae was determined. Each alga (*U. rigida*, *G. pristoides* and *Corallina* spp.) was washed in freshwater (to remove sea salts and marine invertebrates) and dried to constant weight at 60°C. The algae were then finely ground and homogenized using an electric mill (sieve size < 0.1 mm). Following this, the organic matter was removed by ashing 1 g of dry algae to constant weight at 600°C. The resultant ash from each alga was then treated with an excess of concentrated HCl, liberating the calcium from calcium carbonate. The solutions (repeated in triplicate for each alga) were then centrifuged (3000 rpm) for 5 minutes and the supernatants were removed. The supernatants were then diluted so that their calcium contents fell within the limits of the spectroscope (calcium range). Then the calcium contents were determined using a GBC 909 atomic absorption spectroscope fitted with a calcium hollow cathode lamp (lamp current: 10.0 mA, flame type: nitrous oxide-acetylene). The concentration (in ppm., later calculated to percentage) of calcium in each solution was calculated by the spectroscope, which had been pre-calibrated with known amounts of calcium solution. A mean was calculated from the triplicate determinants of calcium content obtained for each alga. This mean value was taken to represent the calcium content.

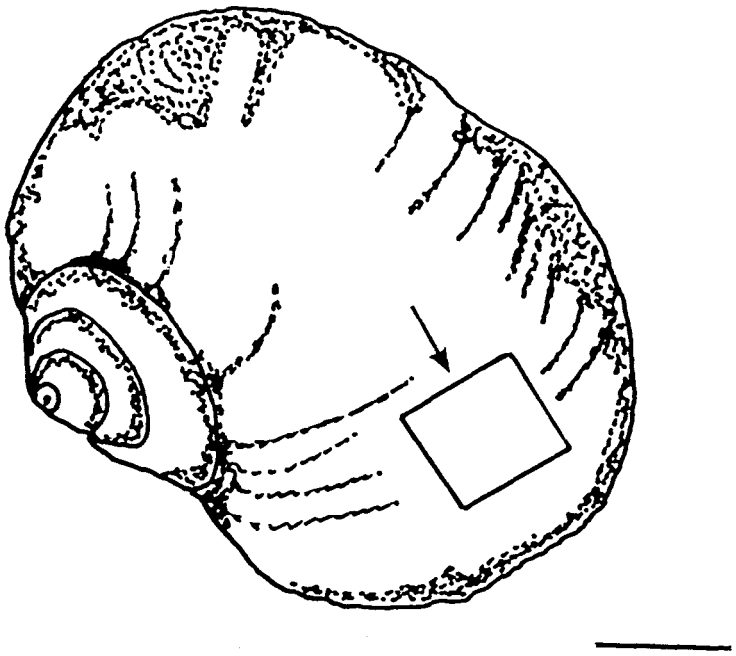

Figure 6.1. Diagrammatic representation of the area of shell removed for determining shell strength and shell layer thickness of *Turbo sarmaticus* (n = 10) fed on four algal diets. The arrow points to the piece of shell removed. Scale bar = 1 cm.

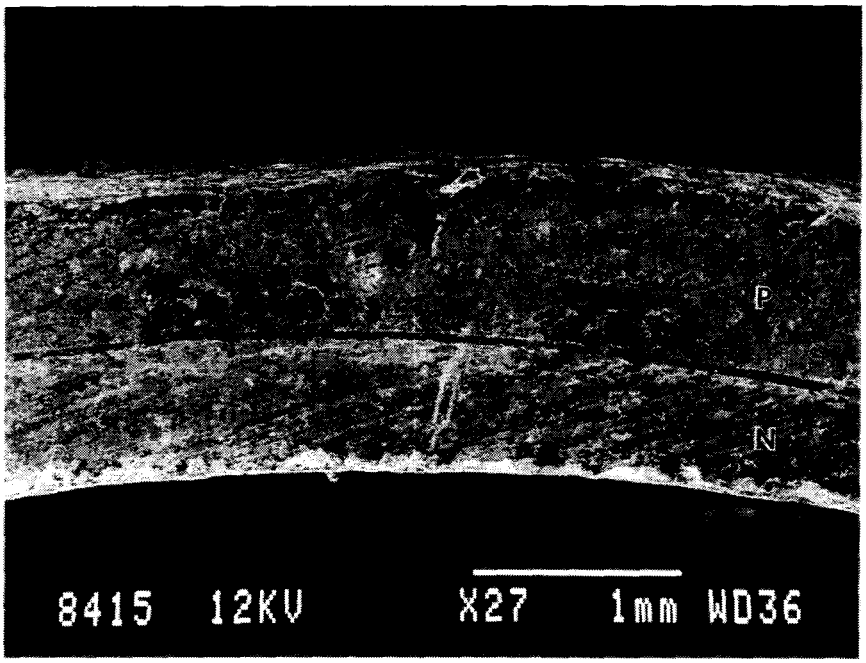

Figure 6.2. Scanning electron micrograph of a transverse section of shell used to measure and compare the thicknesses of the prismatic and nacreous layers of *Turbo sarmaticus* (n = 10) fed on four algal diets. **P**: prismatic layer, **N**: nacreous layer. Scale bar = 1 mm.

Influence of diet on tissue glycogen levels

Glycogen storage levels in the foot tissues of five juvenile and five adult *T. sarmaticus* fed on the different algal diets were compared in order to determine energy levels at the end of the growth and reproductive fitness experiments. In addition, the glycogen levels in the foot of five similar sized adult field animals (controls) were determined at the beginning and end of the adult reproductive fitness experiments. Foot tissue was used as this is a well-documented site for glycogen storage in marine molluscs (Webber 1970).

Glycogen levels were determined with minor modifications to the method described by Oser (1965). Tissue from each foot (n = 5) was homogenized in ice-cold distilled water. The homogenate was then freeze-dried and the resultant powdered tissue stored at 5°C. Glycogen was extracted by boiling 100 mg of powdered tissue in 3 ml of 30% KOH for 30 minutes. The glycogen was precipitated by adding 0.5 ml of saturated Na_2SO_4 and 3.5 ml of 95% ethanol and heating again until the mixture boiled. The mixture was then cooled and centrifuged at 3000 rpm for 5 minutes at 5°C, afterwhich the supernatant was discarded. The sediment was dissolved in 2 ml water and reprecipitated with 2.5 ml of 95% ethanol. The resulting sediment was hydrolysed (yielding glucose from glycogen) for one hour in 2 ml of 0.5 M HCl in a boiling water bath. The hydrolysate was cooled, neutralized with 0.5 M NaOH and made up to a volume of 100 ml. Glucose levels were then determined with Anthrone reagent (0.2 g of anthrone in 100 ml of 95% H_2SO_4) by adding 10 ml of reagent to: 1) 5 ml of each neutralised hydrolysate and 2) 5 ml of distilled water which served as a blank. The test tubes were covered with glass marbles and then heated for 10 minutes in boiling water, cooled immediately and the absorbancies read in a Shimadzu UV-1201 spectrophotometer at 620 nm. Glucose levels were then determined from a standard curve of known concentrations of glucose (Figure 6.3). In order to calculate the glycogen content of the tissue, 1 g of glycogen yielded 1.11 g of glucose on hydrolysis.

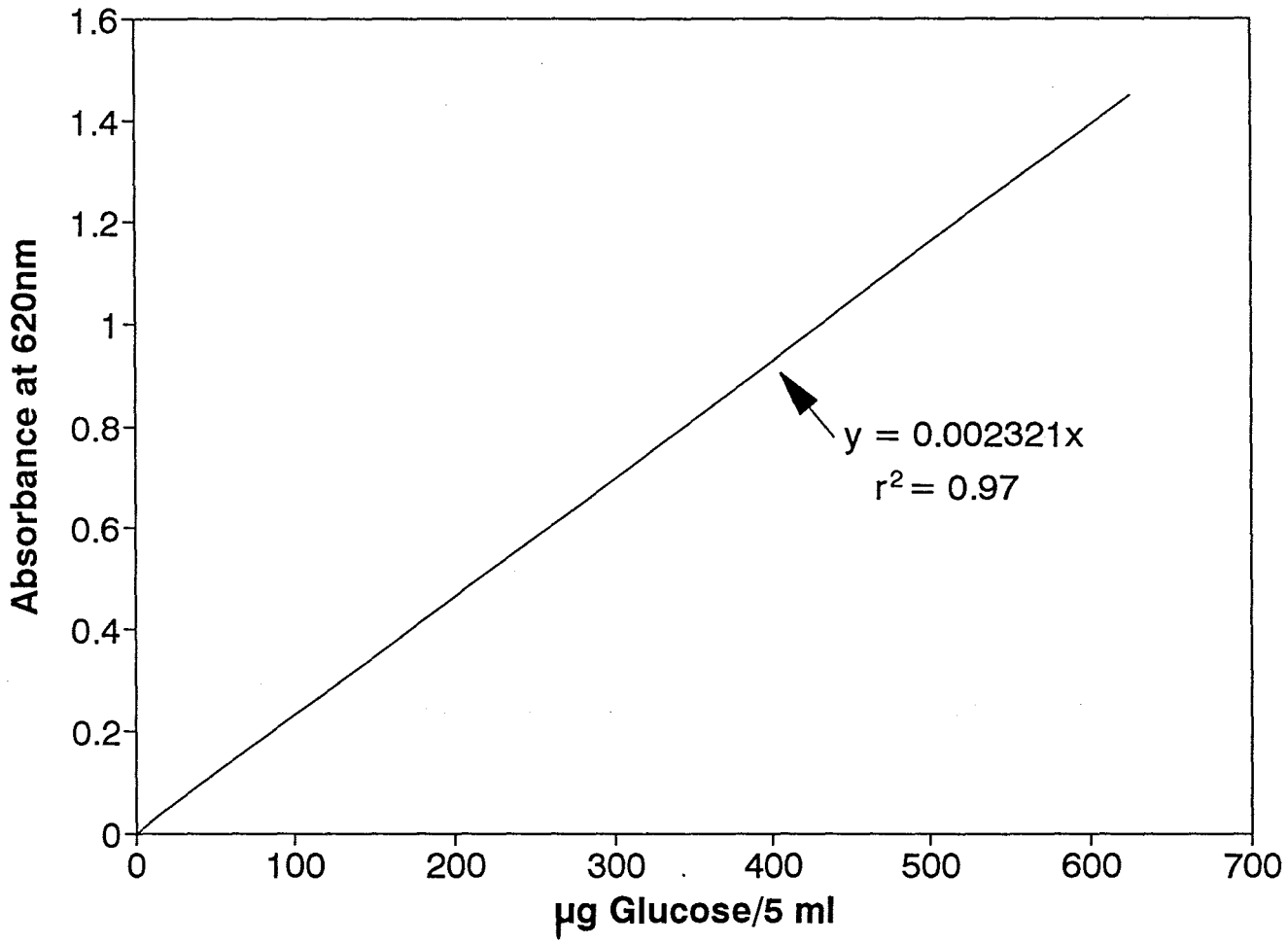

Figure 6.3. Glucose standard curve.

Statistical analysis

All data were tested for homogeneity of variance and where necessary transformed using the log transformation for population data (ln[x+1]) and the arcsin transformation for percentages ($\sin^{-1}\sqrt{x}$) to comply with the requirements of ANOVA (Fry 1993). Paired t-tests were performed to determine if mean animal size (shell length and total wet weight) increased when fed on various algal diets. One-way ANOVA coupled with Scheffe's multiple range tests were used to determine if shell strength and thickness, gonad development (GI) and glycogen levels were influenced by diet for the respective experiments. All statistical analyses were calculated using a StatGraphics V7.0 computer package (Statistical Graphics Corporation, USA).

RESULTS

Influence of diet on growth rate

After an initial period of no growth (2 months for *Corallina* spp. and 1 month for other diets), all juvenile *Turbo sarmaticus* fed on the different macroalgal diets increased in size (shell length) (Figures 6.4 & 6.5, Table 6.3) and total wet weight (Figure 6.6, Table 6.3) over the 12-month experimental period. The increases in shell length and total wet weight differed significantly between some algal diets (Figure 6.7, Table 6.4).

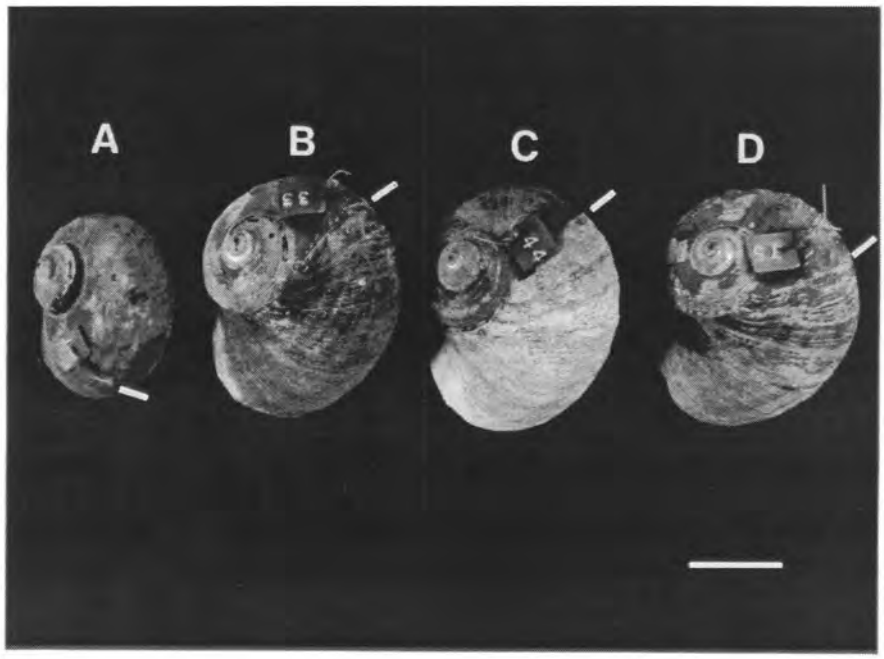

Figure 6.4. Examples of shells of juvenile *Turbo sarmaticus* fed four algal diets over 12 months. **(A)** *Corallina* spp., **(B)** *Gelidium pristoides*, **(C)** *Ulva rigida* and **(D)** Mixed diet. Lines indicate initial shell lengths. Scale bar = 2 cm.

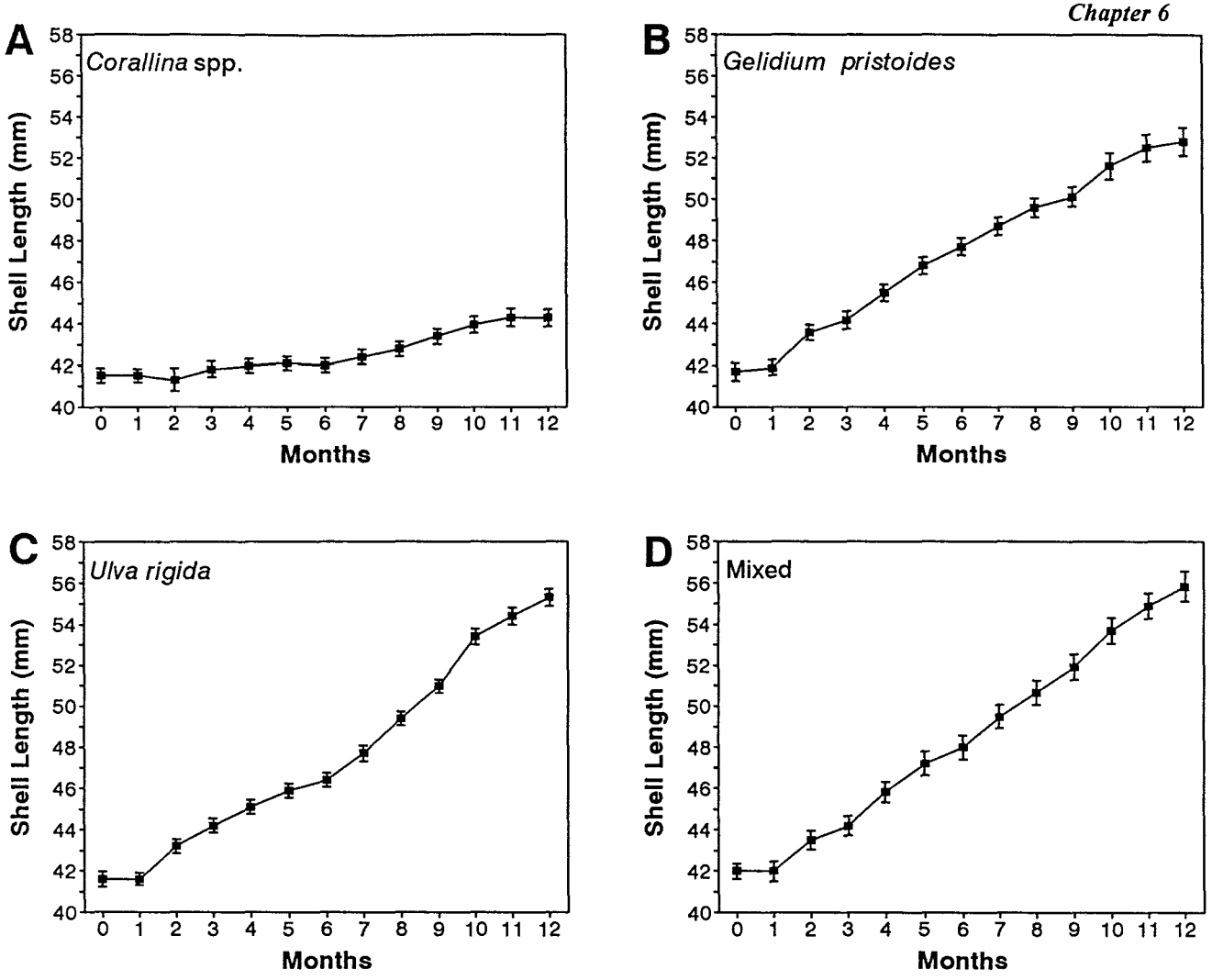

Figure 6.5. Growth rates (shell length: mean ± S.E.) of juvenile *Turbo sarmaticus* (n = 20) fed four algal diets over 12 months. **(A)** *Corallina* spp., **(B)** *Gelidium pristoides*, **(C)** *Ulva rigida* and **(D)** Mixed diet.

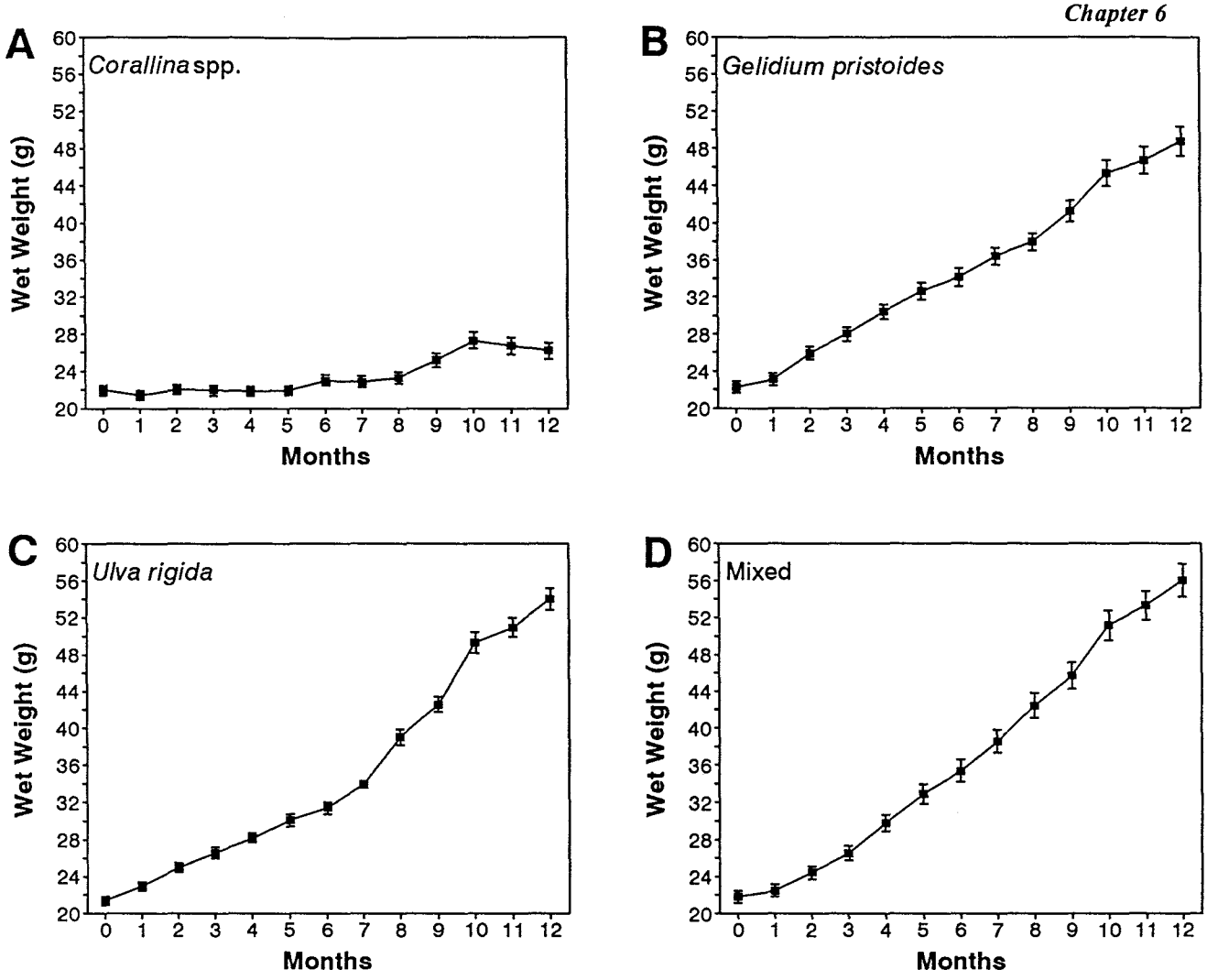

Figure 6.6. Growth rates (total wet weight: mean ± S.E.) of juvenile *Turbo sarmaticus* (n = 10) fed four algal diets over 12 months. **(A)** *Corallina* spp., **(B)** *Gelidium pristoides*, **(C)** *Ulva rigida* and **(D)** Mixed diet.

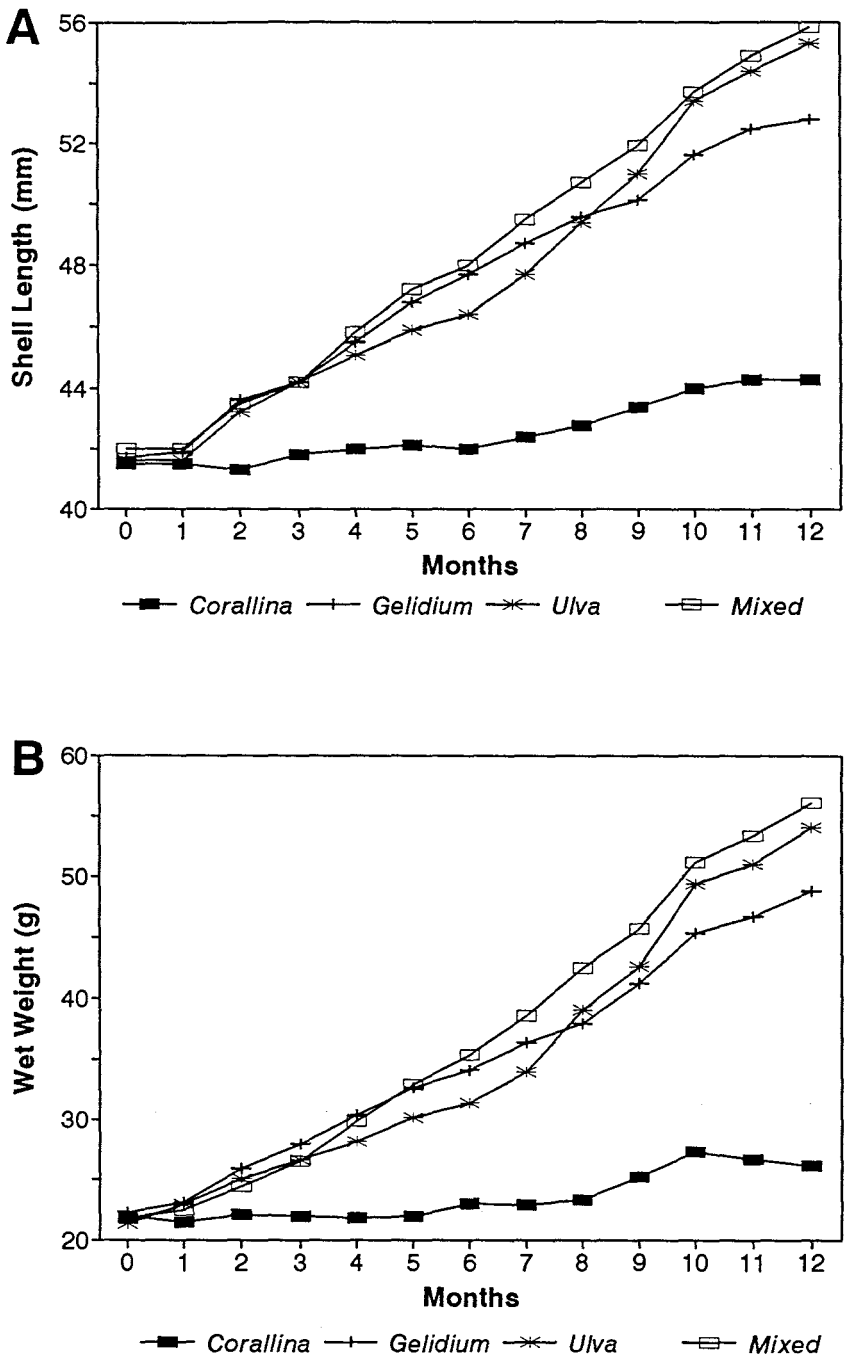

Figure 6.7. Comparison of growth rates of juvenile *Turbo sarmaticus* (n = 20) fed on four algal diets over 12 months. **(A)** shell length increase and **(B)** total wet weight gained. Standard error bars have been omitted for clarity.

Table 6.3. Results of a paired t-test to determine whether the mean shell length and total wet weight of juvenile *Turbo sarmaticus* increased when fed on four different diets over a 12-month period.

	Algal diet	t-statistic	Sig. level
Shell length	*Corallina* spp.	5.69	$p < 0.001$
	Gelidium pristoides	13.23	$p < 0.001$
	Ulva rigida	29.87	$p < 0.001$
	Mixed diet	18.04	$p < 0.001$
Wet weight	*Corallina* spp.	5.77	$p < 0.001$
	Gelidium pristoides	15.84	$p < 0.001$
	Ulva rigida	31.71	$p < 0.001$
	Mixed diet	21.65	$p < 0.001$

Table 6.4. Results of a One-way ANOVA to determine whether the growth rate (shell length and total wet weight) differed between juvenile *Turbo sarmaticus* fed on four different diets over a 12-month period.

Source of variation	Sum of squares	D.F.	Mean square	F-ratio	Sig. level
Shell Length					
Between groups	0.711	3	0.237	100.69	$p < 0.001$
Within groups	0.178	76	0.002		
TOTAL	0.890	79			
Wet Weight					
Between groups	7.257	3	2.419	128.56	$p < 0.001$
Within groups	1.429	76	0.018		
TOTAL	8.687	79			

Growth was most rapid in animals fed on the mixed diet (mean shell length and wet weight increase of 13.8 mm and 34.26 g, respectively) and *U. rigida* (mean shell length and wet weight increase of 13.7 mm and 32.68 g, respectively). Animals fed on these diets grew significantly faster than those fed on *G. pristoides* and *Corallina* spp. (Figure 6.7, Table 6.5). Animals fed *G. pristoides* had a mean shell length and wet weight increase of 11.1 mm and 26.53 g, respectively (Figure 6.7, Table 6.5). Growth of animals fed *Corallina* spp. was significantly reduced with a mean shell length and wet weight increase of only 2.4 mm and 4.23 g, respectively (Figure 6.7, Table 6.5). The average monthly shell length increases and total wet weight gains for animals fed the mixed diet were ≈ 1.15 mm/month and ≈ 2.85g/month, respectively and for *U. rigida*, ≈ 1.14 mm/month and ≈ 2.72 g/month, respectively. Animals fed *G. pristoides* showed growth gains in shell length and wet weight of ≈ 0.93 mm/month and ≈ 2.21 g/month, respectively. The growth rate of animals fed on *Corallina* spp. was ≈ 0.20 mm/month and ≈ 0.35 g/month, respectively. Similar sized field animals had a shell length increase of ≈ 1.48 mm/month (calculated from von Bertalanffy growth model - Chapter 3).

Table 6.5. Results from a Scheffe's multiple range test to determine where differences in the growth rate (shell length and wet weight) of juvenile *Turbo sarmaticus* occurred when fed on four algal diets over 12 months. (X's in same column indicate no significant differences).

Algal diet	Homogeneous groups	
	Shell length	Wet weight
Corallina spp.	X	X
Gelidium pristoides	X	X
Ulva rigida	X	X
Mixed diet	X	X

Influence of diet on reproductive fitness

The gonad indices of the juvenile and adult animals fed on different algal diets are summarised in Table 6.6. Only 30% of the juveniles fed on *Corallina* spp. developed gonads. The mean GI for both juveniles and adults varied significantly with diet (Table 6.7). With the exception of the animals fed on *Corallina* spp., all the animals (juvenile and adult) fed on the various diets had well-developed gonads with a GI of 29 - 33% in juveniles and 24 - 27% in adults (Table 6.6). The animals fed only *Corallina* spp. (juveniles: < 1%, adults: ≈ 4%) had significantly smaller gonads (Tables 6.6 & 6.8).

Table 6.6. The gonad index (mean ± S.E., n = 20) of *Turbo sarmaticus* fed on various algal diets and that of similar sized field animals. Juveniles and adults were fed diets for 12 months and 6 months, respectively.

Algal diet	Juvenile	Adult
Corallina spp.	0.88 ± 0.35	4.40 ± 1.11
Gelidium pristoides	29.75 ± 1.41	24.02 ± 1.64
Ulva rigida	31.77 ± 1.09	23.97 ± 1.56
Mixed diet*	33.24 ± 0.76	27.13 ± 1.27
Field animals[1] (n = 20)		2.37 ± 2.89
Field animals[2] (n = 20)		13.49 ± 1.68

* Mixed diet of equal volumes of *Corallina* spp., *G. pristoides* and *U. rigida*.
1 GI of field animals during April.
2 GI of field animals at the termination of experimentation.

Table 6.7. Results of a One-way ANOVA to determine whether the GI of juvenile and adult *Turbo sarmaticus* differed when fed on four algal diets over 12 months.

Source of variation	Sum of squares	D.F.	Mean square	F-ratio	Sig. level
Juvenile					
Between groups	3.279	3	1.093	342.85	$p < 0.001$
Within groups	0.178	56	0.003		
TOTAL	3.458	59			
Adult					
Between groups	1.230	3	0.410	72.86	$p < 0.001$
Within groups	0.315	56	0.005		
TOTAL	1.545	59			

Table 6.8. Results of a Scheffe's multiple range test to determine where differences in GI of juvenile and adult *Turbo sarmaticus* occurred when fed on four algal diets over 12 months. (X's in same column indicate no significant differences).

Algal diet	Homogeneous groups	
	Juvenile	Adult
Corallina spp.	X	X
Gelidium pristoides	X	X
Ulva rigida	X	X
Mixed diet	X	X

Influence of diet on shell strength and thickness

The shell strengths (in Newtons) of the juvenile animals fed on the various diets and those of similar sized animals in the field are summarised in Table 6.9. The force required to break the shell pieces ranged from 113.4 N (mixed diet) to 143.7 N (*U. rigida*).

Table 6.9. Shell strengths (mean ± S.E., n = 10) of juvenile *Turbo sarmaticus* fed various algal diets and that of similar sized field animals.

Algal diet	Shell Strength (Newtons)
Corallina spp.	134.4 ± 10.7
Gelidium pristoides	120.1 ± 6.3
Ulva rigida	143.7 ± 5.7
Mixed diet	113.4 ± 6.4
Field animals[1]	112.5 ± 8.0
Field animals[2]	126.5 ± 5.6

1 Similar sized field animals to that of the experimental animals fed on *Corallina* spp.
2 Similar sized field animals to that of the experimental animals fed on *U. rigida*, *G. pristoides* and the mixed diet.

Animals fed on *Corallina* spp. did not have significantly stronger shells than those of similar sized field animals (Table 6.10). However, there was a significant difference in the shell strengths of the animals fed on the other diets (Table 6.10). Animals fed on *U. rigida* had significantly stronger shells than those fed on *G. pristoides* and the mixed diet (Table 6.11). However, the shell strengths of animals reared on *U. rigida* were not significantly stronger than those of similar sized field animals (Table 6.11).

Table 6.10. Results of a One-way ANOVA to determine whether the shell strength differed for field animals and juvenile *Turbo sarmaticus* fed *Corallina* spp., *Gelidium pristoides*, *Ulva rigida* and a mixed diet.

Source of variation	Sum of squares	D.F.	Mean square	F-ratio	Sig. level
Corallina spp. and field animals					
Between groups	0.273	1	0.273	2.14	p = 0.151
Within groups	4.841	38	0.127		
TOTAL	5.115	39			
Gelidium pristoides, *Ulva rigida*, mixed diet and field animals					
Between groups	0.714	3	0.238	4.72	p = 0.004
Within groups	3.783	75	0.050		
TOTAL	4.498	78			

Table 6.11. Results of a Scheffe's Multiple range test to determine where differences occurred in mean shell strength for juvenile *Turbo sarmaticus* fed on three algal diets and for similar sized field animals. (X's in same column indicate no significant differences).

Algal diet	Homogeneous groups	
Gelidium pristoides	X	
Mixed diet	X	
Field animals	X	X
Ulva rigida		X

The thicknesses (μm) of the prismatic and nacreous shell layers of the juvenile animals fed on the various diets and those of similar sized field animals in the field are summarised in Table 6.12.

Table 6.12. Shell layer thicknesses (mean ± S.E., n = 10) of juvenile *Turbo sarmaticus* fed various algal diets and those of similar sized field animals.

Algal diet	Shell layer thickness (μm)	
	Prismatic	Nacreous
Corallina spp.	566 ± 24	590 ± 20
Gelidium pristoides	656 ± 16	515 ± 20
Ulva rigida	598 ± 16	613 ± 11
Mixed diet	586 ± 13	515 ± 14
Field animals[1]	408 ± 15	574 ± 17
Field animals[2]	498 ± 27	539 ± 21

1 Similar sized field animals to those of the experimental animals fed *Corallina* spp.
2 Similar sized field animals to those of the experimental animals fed *U. rigida*, *G. pristoides* and the mixed diet.

The animals fed *Corallina* spp. had significantly thicker prismatic layers than similar sized field animals (Table 6.13). However, there was no significant difference between the thickness of their nacreous layers (Table 6.14). Animals reared on *U. rigida*, *G. pristoides* and the mixed diet had significantly thicker prismatic layers than similar sized field animals (Tables 6.13 & 6.15). In addition, the prismatic layer of the animals fed *G. pristoides* and *U. rigida*

was significantly thicker than that of the animals fed the mixed diet (Table 6.15). The nacreous layer of *T. sarmaticus* fed *U. rigida* was significantly thicker than that of the animals reared on *G. pristoides* and the mixed diet, and that of similar sized field animals (Tables 6.14 & 6.15).

Table 6.13. Results of a One-way ANOVA to determine whether the prismatic shell layer thickness differed for juvenile *Turbo sarmaticus* fed four algal diets and for similar sized field animals.

Source of variation	Sum of squares	D.F.	Mean square	F-ratio	Sig. level
Corallina spp. and field animals					
Between groups	2.376	1	2.376	24.41	$p < 0.001$
Within groups	9.539	98	0.097		
TOTAL	11.916	99			
Gelidium pristoides, *Ulva rigida*, mixed diet and field animals					
Between groups	2.953	3	0.984	15.06	$p < 0.001$
Within groups	12.808	196	0.065		
TOTAL	15.761	199			

Table 6.14. Results of a One-way ANOVA to determine whether the nacreous shell layer thickness differed for juvenile *Turbo sarmaticus* fed four algal diets and for similar sized field animals.

Source of variation	Sum of squares	D.F.	Mean square	F-ratio	Sig. level
Corallina spp. and field animals					
Between groups	0.009	1	0.009	0.17	$p = 0.680$
Within groups	5.200	98	0.530		
TOTAL	5.209	99			
Gelidium pristoides, *Ulva rigida*, mixed diet and field animals					
Between groups	1.284	3	0.428	8.13	$p < 0.001$
Within groups	10.312	196	0.052		
TOTAL	11.596	199			

Table 6.15. Results of a Scheffe's multiple range test to determine where differences occurred in mean shell layer thickness for juvenile *Turbo sarmaticus* fed four algal diets and for similar sized field animals. (X's in same column indicate no significant differences).

Algal diet	Homogeneous groups	
	Prismatic	Nacreous
Field animals	X	X
Gelidium pristoides	X	X
Ulva rigida	X X	X
Mixed diet	X	X

The calcium content of *Corallina* spp. was twice that of *U. rigida* and ten times that of *G. pristoides* (Table 6.16). The amount of calcium appeared to be related to the ash content of these algal species (Table 6.16).

Table 6.16. Percentage ash and calcium present in three intertidal macroalgal species from the Eastern Cape Province.

Algae	Ash (%)	Calcium (%)
Corallina spp.	77	26.8
Gelidium pristoides	14	2.1
Ulva rigida	52	11.0

Influence of diet on tissue glycogen levels

The levels of glycogen in the foot of the juvenile animals fed on the various diets are summarised in Table 6.17.

Table 6.17. The foot glycogen levels (mean ± S.E., n = 5) of juvenile *Turbo sarmaticus* fed on four algal diets over 12 months.

Algal diet	Glycogen levels (mg/100 mg tissue)
Corallina spp.	3.42 ± 0.15
Gelidium pristoides	3.66 ± 0.16
Ulva rigida	4.09 ± 0.26
Mixed diet	4.76 ± 0.06

Juvenile animals fed on the mixed diet had glycogen levels which were significantly higher than those of the animals fed the other diets. Animals fed on *Corallina* spp. had the lowest glycogen levels (Tables 6.18 & 6.19).

Table 6.18. Results of a One-way ANOVA to determine whether the foot glycogen levels of juvenile *Turbo sarmaticus* differed when fed four algal diets.

Source of variation	Sum of squares	D.F.	Mean square	F-ratio	Sig. level
Between groups	0.204	3	0.068	9.67	p = 0.001
Within groups	0.112	16	0.007		
TOTAL	0.317	19			

Table 6.19. Results of a Scheffe's multiple range test to determine where differences in foot glycogen levels of juvenile *Turbo sarmaticus* occurred when fed four algal diets. (X's in same column indicate no significant differences).

Algal diet	Homogeneous groups		
Corallina spp.	X		
Gelidium pristoides	X	X	
Ulva rigida		X	
Mixed diet			X

The levels of glycogen in the foot of the adult animals fed on the various diets as well as those of field animals (controls) are summarised in Table 6.20.

Table 6.20. The foot glycogen levels (mean ± S.E., n = 5) of adult *Turbo sarmaticus* fed on four algal diets over six months. Values for similar sized field animals at the beginning and end of experimentation are also given.

Algal diet	Glycogen levels (mg/100 mg tissue)
Corallina spp.	0.58 ± 0.03
Gelidium pristoides	1.65 ± 0.06
Ulva rigida	1.35 ± 0.04
Mixed diet	1.87 ± 0.04
Field animals[1]	7.22 ± 0.35
Field animals[2]	2.52 ± 0.12

1 Similar sized field animals sampled at the beginning of experimentation (April).
2 Similar sized field animals sampled at the end of experimentation (November).

Adult field animals at the start of experimentation had significantly higher glycogen levels (≈ 7%) than at the end (≈ 2.5%) (Tables 6.20, 6.21 & 6.22). Field animals at the end of experimentation had significantly higher glycogen levels than animals fed the experimental diets (Tables 6.21 & 6.22). With the exception of animals fed *G. pristoides* and the mixed diet, there were significant differences between the glycogen levels of the adult animals fed on the different algal diets (Tables 6.21 & 6.22). Animals fed on *Corallina* spp. had the lowest glycogen levels (≈ 0.5%) while animals fed on the mixed diet had the highest (≈ 1.8%) (Tables 6.20 & 6.22).

Table 6.21. Results of a One-way ANOVA to determine whether the foot glycogen levels differed for adult *Turbo sarmaticus* fed four algal diets and for similar sized field animals.

Source of variation	Sum of squares	D.F.	Mean square	F-ratio	Sig. level
Between groups	7.597	5	1.519	359.36	$p < 0.001$
Within groups	0.101	24	0.004		
TOTAL	7.698	29			

Table 6.22. Results of a Scheffe's Multiple range test to determine where differences occurred in foot glycogen levels for adult *Turbo sarmaticus* fed four algal diets and for similar sized field animals. (X's in same column indicate no significant differences).

Algal diet	Homogeneous groups			
Corallina spp.	X			
Ulva rigida		X		
Gelidium pristoides			X	
Mixed diet			X	
Field animals[2]				X
Field animals[1]				X

1 Similar sized field animals sampled at the beginning of experimentation (April).
2 Similar sized field animals sampled at the end of experimentation (November).

DISCUSSION

The nutritional quality of marine macroalgae is generally poor (Boney 1965, Mattson 1980, White 1985, Kennish 1996) and therefore limits herbivore fitness. Due to their comparatively high C:N ratio, most plants are especially low in nitrogen and protein (Mattson 1980, Horn & Neighbors 1984, Begon *et al.* 1986). It is advantageous for any species to eat food which gives the best growth and the maximum possible production of offspring. Accordingly, many grazers are thought to feed selectively on food which maximises the intake of important nutrients (Mattson 1980, White 1985, Horn *et al.* 1986, Duffy & Paul 1992, McShane *et al.* 1994).

The present study represents a first step towards defining the nutritional requirements of *Turbo sarmaticus* for growth and reproduction. The growth rates of juvenile animals that were fed a mixed diet (equal volume *Corallina* spp., *Gelidium pristoides* and *Ulva rigida*) were slightly greater than those fed certain monospecific diets (*U. rigida*). Furthermore, animals fed the mixed diet and *U. rigida* grew more rapidly than those fed *G. pristoides* and *Corallina* spp.. Such differences in growth rate have been observed in other molluscs (Ino 1952, 1958, Swan 1961, Leighton & Boolootian 1963, Carefoot 1967a, Hanisak 1992) as a result of the considerable variability in nutritional values of algae consumed. These results agree, to some extent, with those of previous studies which have shown that the best growth

in marine herbivores is attained when they are fed a mixed diet (Wood 1968, Walne 1974, Koike *et al.* 1979, Gorfine & King 1991, Morrison & Whittington 1991, Mercer *et al.* 1993, Stuart & Brown 1994).

Although *Corallina* spp., *G. pristoides* and *U. rigida* are the main algae consumed by *T. sarmaticus* on Eastern Cape Province rocky shores (Chapter 5), other algae are probably also consumed. It remains to be determined whether such algae provide nutrients deficient in the above three algae. Selecting a mixed diet can be a way for an organism to ensure that its nutritional needs are met (Mercer *et al.* 1993). Consuming a mixed diet also reduces searching time and optimises the inclusion of trace nutrients in the diet (Westoby 1974). Many of the algae on rocky shores contain physical and chemical defences (Duffy & Hay 1990) which can deter feeding and affect dietary preference (Van Alstyne & Paul 1990, Irelan & Horn 1991, Meyers & Paul 1992). Ingesting a mixed diet reduces interference from these compounds (Kitting 1980, Mattson 1980). In addition, Lamare (1990, cited in Stuart & Brown 1994) showed that the food conversion efficiency by *Haliotis iris* fed a mixed diet of three algae was greater than when the algae were consumed alone.

Growth rates of herbivorous molluscs are affected by a number of factors which include the palatability, cell wall toughness, digestibility and nutritional value of their algal diets (Kitting 1980, Bertness *et al.* 1983, Wolcott & Wolcott 1984, 1987, Norton & Manley 1990, O'Connor 1992, White 1993). Even if a diet meets the nutritional requirements of an animal, the digestion and conversion of nutrients to biomass is often limited (Morse 1984). It is suggested that the differences in growth rates observed for juvenile *T. sarmaticus* in this study were mainly due to differences in the nutritional composition of the algae and their digestibility. The greater overall energetic and nutritional values of *G. pristoides* (energy = 16.5 kJ/g, protein ≈ 12%, carbohydrate ≈ 42% - Chapter 5) and *U. rigida* (energy = 9.5 kJ/g, protein ≈ 6.5%, carbohydrate ≈ 18% - Chapter 5), combined with the greater digestibility (at 20°C) of these algae (*G. pristoides* ≈ 75%, *U. rigida* ≈ 20% - Chapter 5) when compared to *Corallina* spp. (energy = 2.5 kJ/g, protein ≈ 6.4%, carbohydrate ≈ 5%, digestibility ≈ 9% - Chapter 5), resulted in the faster growth and weight gains by *T. sarmaticus*.

In order to establish the true value of an alga for growth of a herbivore, experimental trials must be long-term (see review Day & Fleming 1992). Growth may be influenced by factors other than food during short-term trials and may not reflect the long-term effects of

a single-species diet (Day & Fleming 1992). Both the previous diet of the test animals and the disturbance suffered by the animals when the trials are set up may influence growth in short-term trials (Day & Fleming 1992). *Turbo sarmaticus* showed an "acclimatisation period" with no growth for the first to second month of experimentation (Figure 6.7) which may be attributed to disturbance (*e.g.* collection, labelling, change of environment, *etc.*). Similarly, studies on growth rates of other laboratory reared marine molluscs have shown an initial period of no or slow growth (Darling 1965, Koike *et al.* 1979, Katoh 1989, Day & Fleming 1992).

Another factor that requires testing by long trial periods are the long-term effects of a single-species diet on growth. Trials in which *Haliotis rubra* were fed on single-species diets of dried algae showed that animals ceased to grow after a period of 50 - 200 days (Day & Fleming 1992). This suggests that these animals were not receiving all the nutrients necessary for growth. Duncan & Klekowski (1975) noted that "essential" substances may become limiting where animals are provided with only one type of food in long-term experiments. No such reduction in the growth rate of *T. sarmaticus* was observed after a year of feeding animals single-species diets. However, there was a slight decrease in body weight towards the end of the experimental period in the group fed on *Corallina* spp. Leighton & Boolootian (1963) also reported weight loss in *Haliotis cracherodii* which was fed on coralline algae alone, even though such algae are a common component of the animal's natural diet.

As some single-species algal diets do not promote growth in some abalone species, the suitability of experiments for testing diet quality and the use of single-species diets in aquaculture must be questioned. Although an alga may not support sustained growth when given alone, it may be of great value in supplying essential nutrients as part of a mixed diet. Koike *et al.* (1979) have reported that offering mixed diets to *Haliotis tuberculata* did not improve growth rates, but they may have been valuable in sustaining growth over time. Duncan & Klekowski (1975) stated that it is rare for a species to feed on the same food for its whole life which suggests that most species can only obtain the full range of required nutrients from a mixed diet. These results suggest that a good quality diet in the field is dependent on the availability of a regular supply of a mixture of algae.

Gamete production is a protein and energy demanding process, often dependent on the levels of previously stored body reserves (Fishelson *et al.* 1985, Montgomery *et al.* 1989,

Prop & Deerenberg 1991, Marken Lichtenbelt 1993, Kennish 1997). Kennish 1996, 1997 highlighted the importance of protein levels to the fitness of a herbivorous crab, and concluded that reproduction and, more importantly, storage of nutrients could not occur if the protein levels of the diet were too low, even if energy levels were high. It is important that animals allocate a certain amount of the energy assimilated from food to reproduction despite the competing demands of growth and maintenance. In order to maximise fitness, a herbivore should, according to the optimal foraging theory, maximise the energy in its diet by selecting the most energy-rich foods (Pyke *et al.* 1977, Hughes 1980, Pyke 1984).

Many studies have shown that gonad size, and hence reproductive fitness, of a marine herbivore is dramatically influenced by diet. When animals are placed under nutritive stress (*e.g.* starvation), reproductive output decreases (Chester 1995). By contrast, reproductive output usually increases when an abundance of good quality food is available for consumption (Carefoot 1967a, MacDonald & Bayne 1993, Caceres *et al.* 1994, Lemire & Himmelman 1996) or when food resources are supplemented with protein (Kennish 1996). Similarly, the reproductive fitness of *T. sarmaticus* was affected by the different diets. When fed a mixed diet, *G. pristoides* or *U. rigida*, reproductive fitness increased in comparison to field animals. This implies that the field animals did not reach their potential level of reproductive fitness. The lower reproductive fitness of field animals may have been due to their natural diet being in short supply. However, the time available in the field for feeding in any 24-hour period is limited as *T. sarmaticus* does not feed when exposed at low tides (*pers. obs.*). In this study, all experimental animals had food in excess and their feeding activities were not interrupted by tidal fluctuations. Thus, their energetic and protein intake may have been elevated by an unlimited food supply and uninterrupted grazing periods.

As nutrient gains in a diet decrease, the fitness of the herbivore will decrease, firstly by affecting reproductive performance, and secondly by affecting growth (Lemire & Himmelman 1996). When nutrient levels are below that required for maintenance, survivorship will be affected (Rushton & Hassall 1983, Kennish 1996). Juvenile *T. sarmaticus* fed on the poorer quality *Corallina* spp. had a decreased growth rate and small gonads. Therefore, this diet was sufficient to maintain the animals, but insufficient to promote adequate growth and reproductive fitness. Similarly, when nutrients in the diet of crabs were limited, the gonads did not produce gametes (Sastry 1983, Kennish 1996). These findings

have important ecological implications for *T. sarmaticus*. In South Africa, the practice of removing shellfish (*e.g.* limpets and mussels) to supplement the diets of impoverished communities is increasing. Where over-exploitation of shellfish has occurred (*e.g.* Transkei coast of the Eastern Cape Province), a rapid and extensive establishment of coralline algae has resulted (Dye 1992). Since the type of algal communities present on a shore often affect the diet of herbivores (Cubit 1984, Williams 1993, Ito *et al.* 1996, Kennish *et al.* 1996), the extensive presence of a poor quality food source such as *Corallina* spp., could result in the reduced growth rate and reproductive fitness of *T. sarmaticus*. This, in turn, may result in the depletion of *T. sarmaticus* populations on such rocky shores.

When marine invertebrates are placed under nutritive stress, the most quickly affected tissue or organ stores are lipid and glycogen (Von Brand *et al.* 1957, Emerson & Duerr 1967, Gabbot & Bayne 1973, Pieters *et al.* 1980, Hayashi 1983, Whyte *et al.* 1990, Young *et al.* 1991, Ram & Young 1992, Carefoot *et al.* 1993). In addition, the reproductive cycle of many marine invertebrates is energetically demanding and relies on the utilisation of accumulated stored glycogen reserves which are converted into the lipid reserves of the developing eggs (Soniat & Ray 1985). The utilization of these reserves results in their depletion by the end of the spawning period of the reproductive cycle (Webber 1970, Bayne *et al.* 1982, Fernandez Castro & Vido de Mattio 1987, Rao *et al.* 1988, Sreedhar & Radhakrishnan 1995, Kennish 1997). Similarly, there was a decrease in glycogen levels from about 7% to about 2.5% in adult field *T. sarmaticus*. However, despite unlimited access to food, laboratory reared adult animals fed *G. pristoides*, *U. rigida* and the mixed diet showed an even greater decrease in glycogen levels to about 1.5% when compared to field animals. Animals fed *Corallina* spp. showed the greatest decrease in glycogen levels to about 0.5% when compared to field animals. This suggests that glycogen utilization in *T. sarmaticus* may be under the influence of an endogenous reproductive cycle. In addition, juvenile *T. sarmaticus* fed on *Corallina* spp. also had the lowest glycogen levels. These low glycogen levels were accompanied by decreased reproductive fitness in both adult and juvenile *T. sarmaticus* and resulted in decreased growth rates in juvenile animals. This may indicate that all available energy reserves were channelled into the maintenance of the animals and no energy was available for storage.

Although calcium is particularly important for shell building in molluscs, it is also

required for other functions such as blood clotting, muscle function and nerve transmission (Lovell 1989). Shell building or repair is an energetically costly process (Geller 1990, Palmer 1992), requiring adequate nutrition from the organism's food. *Turbo sarmaticus*, as with other turbinids (Currey & Taylor 1974) build thick shells probably because they live in areas of intense wave activity. *Turbo sarmaticus* and other gastropods (Clarke 1988) ingest *Corallina* spp. algae which have no benefit for growth or reproductive fitness. It is possible, however, that *T. sarmaticus* benefits from the high calcium levels provided by *Corallina* spp. for shell construction and strength. The shell strengths of animals fed on *Corallina* spp. alone were no different from those of field animals, indicating no benefit in that regard. The thinner prismatic layer of the field animals, when compared to those of the laboratory reared animals, were probably a result of greater shell wear in the field animals. Similarly, Coote *et al.* (1996) have shown that the shell weight:area ratio of the abalone, *Haliotis laevigata* was unaffected by the dietary calcium levels. It was impossible to compare the shell strength of animals reared on *Corallina* spp. alone to those deprived this alga (differences in size - see results section). However, animals fed *U. rigida* (which has a relatively high calcium content) had stronger and thicker shells than those fed *G. pristoides*. This indicates that the calcium content of the diet may have been important for shell development. Further research is still needed as these results are inconclusive.

Coote *et al.* (1996) have shown that dietary calcium levels are unrelated to the growth rate of the abalone, *Haliotis laevigata*. This, however, is not surprising as many aquatic molluscs absorb most of their calcium directly from the surrounding water (Thomas & Lough 1974). Similarly, *T. sarmaticus* may absorb most of its calcium requirements from the water, thus making dietary calcium less important as calcium carbonate in the diet is not easily utilised (Coote *et al.* 1996). Phosphorus has been shown to be an important element capable of increasing growth rates (Coote *et al.* 1996). Hatcher (1994) has indicated that an evaluation of potential food would be better accomplished by measurement of the carbon:phosphorus or nitrogen:phosphorus ratios rather than the commonly used carbon:nitrogen ratio. Future research, therefore, should evaluate the influence of dietary phosphorus on the growth rate of *T. sarmaticus*.

In conclusion, this study has illustrated the necessity of measuring both growth rate and reproductive output when examining the effects of diet on a herbivore's ecology. Quality

(energetic and nutritional composition - Chapter 5) of diet directly influences the level of fitness of *T. sarmaticus* by affecting growth, reproductive output and energy reserves. Therefore, as predicted in Chapter 5, the best growth rate, reproductive fitness and energy levels were achieved when *T. sarmaticus* was fed a good quality monospecific diet (*G. pristoides* and *U. rigida*) or a mixed diet. *Turbo sarmaticus* fed a poor quality diet (*Corallina* spp.) showed decreased growth, reproductive fitness and energy levels. Although *T. sarmaticus* was able to sustain itself on a poor quality diet, it could not reproduce without consuming other algae. The benefits of calcium in the diet for shell strength and thickness in *T. sarmaticus* still needs to be investigated fully as the data obtained in this study was inconclusive. This study only examined the influence of intertidal algae on the growth and reproductive fitness of *T. sarmaticus*. However, *T. sarmaticus* extends its distribution into the subtidal region where it would encounter a different suite of macroalgae. The extent to which this algal flora would affect growth and reproduction needs to be investigated. Offshore populations would only be able to benefit from the intertidal macroalgae if pieces became detached and were carried to these populations by currents.

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CHAPTER SEVEN

POLYSACCHAROLYTIC ACTIVITY OF THE DIGESTIVE ENZYMES OF *TURBO SARMATICUS*

INTRODUCTION

The bulk of algal biomass, excluding water, consists of structural and storage carbohydrates (polysaccharides). Marine molluscs that consume algae therefore derive most of their energy from carbohydrates. Of the approximately 250000 known species of marine algae, only the polysaccharides of 190 have been studied (Painter 1983). These few studies have established that marine algae produce polysaccharides of almost unparalleled diversity and complexity (Painter 1983). Painter (1983) has suggested that the evolution of algal polysaccharides may have occurred in association with the morphological evolution of algae. It is therefore possible to loosely group polysaccharide types with the major classes of algae. For example, alginates and laminarin occur exclusively in the Phaeophycae (Percival 1979).

A generalist marine herbivore requires mechanisms to release and digest the variety of structural algal polysaccharides present in algae. Differential ability to digest different algal polysaccharides could have profound ecological implications (Lubchenco & Gaines 1981). Even if the structural material is not utilized as a food source itself, it has to be degraded to allow access to the cell contents. There are three possible mechanisms that a herbivore can employ to degrade the structural polysaccharides of algae: 1) mechanical, using mouth parts to break up macroalgae for ingestion; 2) biochemical, using an enzyme system to break down ingested material extracellularly; and 3) microbial, by enteric bacteria which break down ingested structural material. These mechanisms need not be mutually exclusive and any combination of them may suffice.

The first step in the digestive process in most herbivorous gastropods is the use of the radula to mechanically break down algal material by rasping and scraping (Salvini-Plawen 1988, Fretter & Graham 1994). The first glands associated with the buccal cavity are the salivary glands. Very few prosobranchs secrete digestive enzymes in the saliva; it usually contains only mucus and occasionally protein (Fretter & Graham 1994, Voltzow 1994). The main organs of digestive enzyme production are the oesophageal glands and the digestive glands which, in herbivores, secrete digestive fluids capable of attacking carbohydrates (Salvini-Plawen 1988, Fretter & Graham 1994). The oesophageal glands, of which the mid-oesophageal is the most conspicuous (often known as the crop), consist of pouch-like extensions formed by the expanded lateral walls of the oesophagus (Fretter & Graham 1994,

Voltzow 1994). The digestive gland is a large asymmetrical organ that generally forms two lobes each connected to the stomach by a duct (Fretter & Graham 1994, Voltzow 1994). Food is led into the stomach, mixed with digestive enzymes from the oesophageal glands and enzymes secreted by the digestive gland are then added (Fretter & Graham 1994). The digested food is then passed into the ducts and tubules of the digestive gland for absorption, after which the undigested material is excreted (Fretter & Graham 1994).

Numerous studies have been conducted on the polysaccharidases of freshwater and marine invertebrate herbivores. Some studies have investigated a number of polysaccharidases in several invertebrate groups/phyla (Mansour-Bek 1954, Huang & Giese 1958, Stone & Morton 1958, Sumner 1969, Kristensen 1972, Wojtowicz 1972, Elyakova *et al.* 1974, 1981, Moldotsov *et al.* 1974, Piavaux 1977, Yamaguchi *et al.* 1989), while others have investigated specific polysaccharidases (Nair 1955, Eppley & Lasker 1959, Yokoe & Yasumasu 1964, Okada *et al.* 1966, Thanassi & Nakada 1967, Koopmans 1970, Sova *et al.* 1970, Crosby & Reid 1971, Favorov & Vaskovsky 1971, Elyakova 1972, Moldotsov & Vafina 1972, Piavaux 1977, Gianfreda *et al.* 1979). Studies have mainly concentrated on three taxa: Crustacea (*e.g.* Telford 1970, Friesen *et al.* 1985, Stuart *et al.* 1985, McConville *et al.* 1986, Musgrove 1988, Fang & Lee 1992), Echinodermata (Hultin & Wanntrop 1966, Elyakova *et al.* 1981, see Lawrence 1982 for review, Sweijd 1990) and Mollusca (*e.g.* Alexander *et al.* 1979, Gianfreda *et al.* 1979, Kesler 1983, Hughes 1986, Mayasich & Smucker 1986, Suzuki *et al.* 1986, Barlocher *et al.* 1989).

The comparative studies by Kristensen (1972), Elyakova *et al.* (1981), and to a lesser extent Huang & Giese (1958), have shown that molluscs possess an extensive suite of carbohydrases and that these enzymes are far stronger than the enzymes produced by crustaceans, echinoderms and annelids. Furthermore, Van Weel (1961) has stated that "there seems to be no other group in the animal kingdom with such an array of digestive enzymes, particularly carbohydrases". Collectively, molluscs have been shown to digest cellobiose, amylose, glycogen, laminarin, carboxymethyl cellulose, cellulose, chitin, xylan (Huang & Giese 1958, Kristensen 1972), agar, alginic acid (Favarov & Vaskovsky 1971, Gomez-Pinchetti & Garcia-Reina 1993), inulin (Van Weel 1961), carrageenin (Horiuchi & Lane 1966) and fucoidan (Huang & Giese 1958, Alexander *et al.* 1979).

In a review, Van Weel (1961) has stated that food specialization does not seem to

exclude certain enzymes in molluscs. In addition, Kesler (1983) has shown that in certain freshwater snails diet did not significantly affect cellulase activity. However, correlations have been observed between the enzymes produced/enzyme activity and diet in molluscs (Ladd Prosser & Van Weel 1958, Seiderer *et al.* 1982, Morton 1983, Fang & Lee 1992). It may therefore be possible to make tentative predictions about the diet of a herbivorous organism based on the relative importance of the enzymes which are active on the plant polysaccharides.

There are three basic methods used to assay polysaccharidases. The suitability of each assay depends largely on the properties of the polysaccharide concerned: 1) viscosimetric assays (*e.g.* Epply & Lasker 1959, Hultin & Wanntrop 1966); 2) artificial chromogenic substrates which assay specific enzymes (*e.g.* Klinger 1984); and the most common assay 3) polysaccharide end-product analysis which measures change in the levels of reducing sugars (*e.g.* Claereboudt & Jangoux 1985, Yamaguchi *et al.* 1989). The Nelson-Somogyi method for the determination of reducing sugars (Nelson 1944, Somogyi 1952) is a standard biochemical assay. When polysaccharides are hydrolysed, reducing sugars are released, and the subsequent increase in reducing sugars represents enzyme activity. A calibration curve for the different reducing sugars must be determined (Fielding *et al.* 1986) over a range of concentrations, and the concentration of reducing sugar in the assay sample is then calculated from this curve. A shortcoming of this assay is that it detects the presence of all reducing sugars (di-, tri-, and oligomers) and not only monomers (Mayasich & Smucker 1986). This can result in an over-estimation of the importance of the enzyme and subsequent substrate that are digested as the substrate can only be utilised in metabolic pathways once it has been reduced to its monomeric form (Mayasich & Smucker 1986, Boetius & Felbeck 1995). In spite of this, it has been found to be the best technique for determining enzyme activity (Fielding *et al.* 1986).

Unlike other turbinids worldwide (Muramatsu *et al.* 1977, 1993, Muramatsu & Egawa 1980, Shun *et al.* 1984), no digestive enzyme studies have been attempted on South African species. *Turbo sarmaticus* consumes a wide range of algae from thin walled Chlorophyta (*e.g. Ulva rigida*) to calcarious Rhodophyta (*e.g. Corallina* spp.) (Chapter 5). The purpose of this study was therefore to investigate the digestive capabilities of *T. sarmaticus*. Nine naturally occurring and one artificial polysaccharide were incubated with enzyme extracts obtained from

the mid-oesophageal gland and digestive gland of *T. sarmaticus*. This was done in order to determine the range and extent of polysaccharide activity and thereby provided an indication of the marine macroalgal groups that could be utilized. Since the radula assists in the mechanical breakdown of algae, it was also decided to identify the radula structure and formula in order to determine the grazing ability of *T. sarmaticus* (Steneck & Watling 1982).

MATERIALS AND METHODS

Radula structure

The radulae of five adult (> 80 mm shell length) *Turbo sarmaticus* were removed and placed in a 10% solution of sodium hypochlorite for 30 minutes. During this time they were periodically cleaned with an ultrasonic cleaner. The radulae were cut into smaller strips, dehydrated in a graded alcohol series (70%, 85%, 100% for 1 hour in each) and then allowed to dry on slides. The radulae were then mounted on scanning electron microscopy (SEM) stubs, gold coated and viewed in a Jeol JSM 840 scanning electron microscope.

Polysaccharide assays

A) Animal collection

In order to determine the enzyme activity in both juvenile (< 50 mm shell length) and adult (> 80 mm shell length) *T. sarmaticus*, animals were collected at a spring low tide at Kenton-on-Sea in August 1996. They were immediately transported to the Grahamstown laboratory (60 km away) and processed on ice.

B) pH of the digestive organs

The pH of the mid-oesophageal and digestive gland homogenates were determined from freshly dissected animals (n = 5 pooled) with a Hanna Instruments 8520 pH meter (Table 7.1). Sodium phosphate buffer (0.1 M) at the required pH was used in all enzyme

extractions and enzyme assay procedures. This was done in order to ensure that each *in vitro* enzyme activity assay took place at a similar pH to that of its specific digestive organ, thus eliminating the effects of pH changes on enzyme activity.

Table 7.1. pH of different digestive organs from adult and juvenile *Turbo sarmaticus*.

	pH	
Digestive organ	Adult	Juvenile
Mid-oesophageal gland	6.4	6.2
Digestive gland	6.2	6.3

C) Enzyme extraction

To extract digestive enzymes, the mid-oesophageal and digestive glands of ten adult and ten juvenile animals were removed and immediately washed in sodium phosphate buffer (0.1 M). For each digestive organ, the tissues were pooled and homogenized in chilled buffer using an electric homogeniser to give a standard 10% weight/volume homogenate. The homogenate was centrifuged at 12000 rpm for 2 hrs at 4°C. The supernatant was then drawn off and centrifuged at 30000 rpm for 2 hrs at 4°C. These centrifugations were done using a Du Pont Sorvall RC-5 superspeed refrigerated centrifuge. The final supernatant was then stored in aliquots of 7 ml at -15°C for no longer than one month. Preliminary tests showed that the activity of the extract was unaffected during this period.

D) Substrate polysaccharides

Substrates were selected in order to represent the polysaccharides present in as wide a range of algae as possible (Table 7.2). At least one structural and one storage polysaccharide from each of the major algal classes (Chlorophyta, Phaeophyta and Rhodophyta) were included. All substrates used were of natural origin, except for carboxymethyl cellulose (CM-cellulose).

Table 7.2. Algal polysaccharide substrates present in the Chlorophyta, Phaeophyta and Rhodophyta.

Polysaccharide	Function	Monomer	Algal class	Reference
Amylose	Storage	Glucose	Rho/Chl	1,2,3
Glycogen	Storage	Glucose	Rho	1,3
Laminarin	Storage/ Structural	Glucose	Pha	1,2,3,4
Inulin	Storage	Fructose	Chl	1,3,4
CM-cellulose	Structural	Glucose*	Rho/Chl	4,5
Fucoidan	Structural	Fucose*	Pha	2,5
Carrageenan	Structural	Galactose*	Rho/Chl	2,3,5
Xylan	Structural	Xylose	Chl/Rho	2,3,4,5
Mannan	Structural	Mannose	Chl	2,4,5
Alginic acid	Structural	Glucose*	Pha	2,3,4,5

* The monomers of these polysaccharides are derivatives of the indicated sugar, but are unavailable and thus the indicated monomer was used to generate the standard curve.

Rho - Rhodophyta, Chl - Chlorophyta, Pha - Phaeophyta

1 - Manners & Sturgeon 1982
2 - McCandless 1982
3 - Painter 1983
4 - Percival 1979
5 - Percival & McDowell 1982

E) Monomer standard curves and protein assays

Standard curves were generated using monomers which most closely matched the monomer units of the polysaccharide substrate in question. A concentration series (0 - 0.2 mg sugar/ml) was made for each monomer (Table 7.2) and assayed (see assay procedure below). Absorbance values (510 nm) at the different concentrations for each monomer were measured using a Shimadzu UV-1201 spectrophotometer. Linear regressions were plotted to obtain the relationship between absorbance and monomer concentration (Figure 7.1). Only data which produced an r-squared value greater than 0.9 were accepted for the standard curve. Since this method only gave an indirect measure of enzyme activity after hydrolysis (based on monomer concentration), and since only suitable substitutes were used in place of the polysaccharide

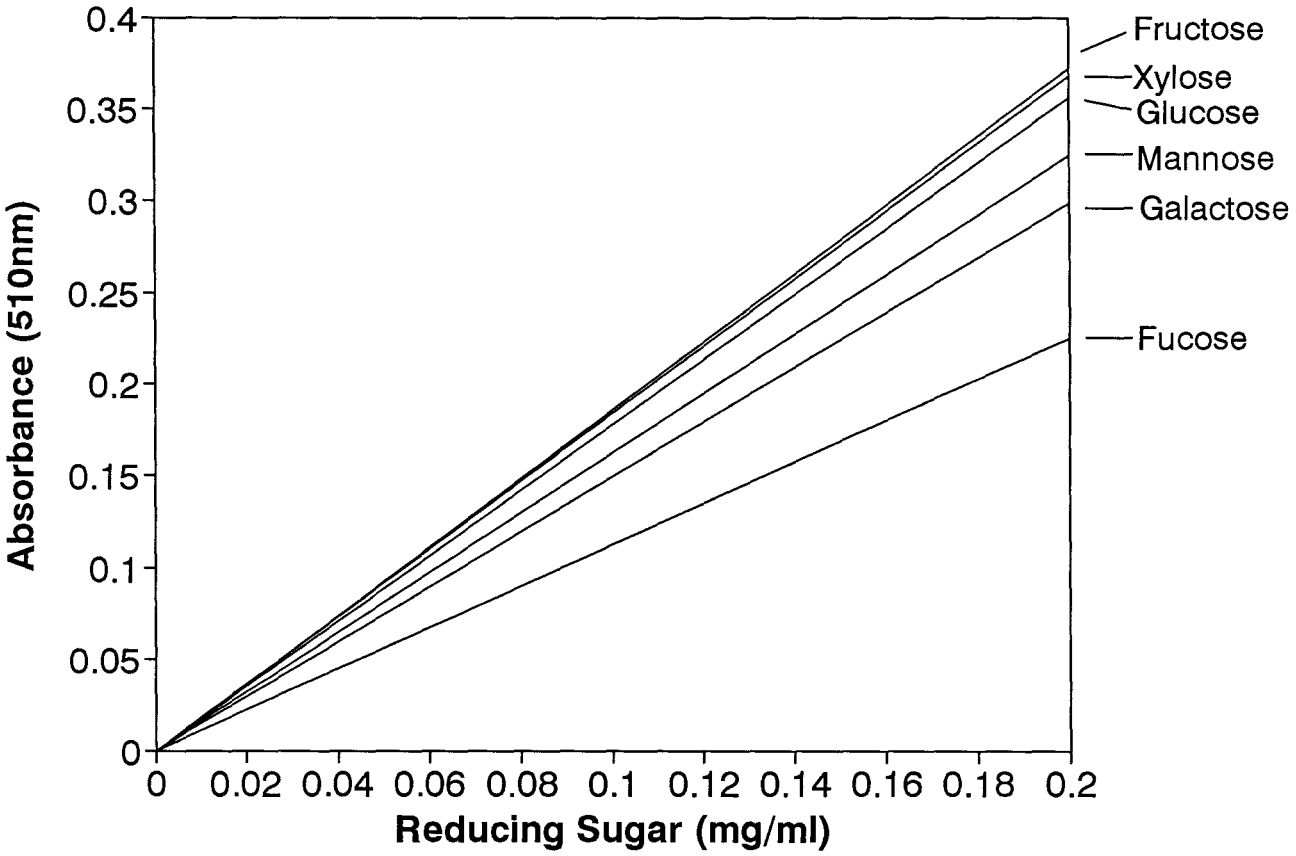


Figure 7.1. Standard curves obtained for different reducing sugars (monomers) at the same concentrations.

monomers (it is often impossible to obtain the monomers of these polysaccharides), the results may not have always accurately reflected true enzyme activity.

The protein content present in each enzyme extract (supernatant) was determined by the Folin-Lowry method described by Plummer (1978) using Bovine Serum Albumin (BSA) as a standard (Table 7.3).

Table 7.3. Amount of protein (mg/ml) in the enzyme extract (supernatant) of different digestive organs of adult and juvenile *Turbo sarmaticus*.

Digestive organ	Adult (mg/ml)	Juvenile (mg/ml)
Mid-oesophageal gland	1.95	1.85
Digestive gland	5.57	5.42

F) Assay procedure

Reducing sugar levels were determined by the Nelson-Somogyi method described by Plummer (1978). The enzyme mixture (1 ml) consisted of 0.2 ml supernatant, 0.5 ml substrate and 0.3 ml buffer. A 2% stock solution of each substrate was made up, giving a 1% final concentration in the enzyme mixture. Since this method measures total reducing sugar content, the intrinsic reducing sugar levels in both the enzyme extracts (supernatant) and substrate solutions must be accounted for in experimental design. Two controls were prepared in the same way as above. One contained boiled enzyme extract (denatured) to determine intrinsic reducing sugar levels in the substrate, while the other contained no substrate to determine intrinsic reducing sugar levels in the enzyme extract. All assays were performed for a 60-minute period at 20°C and repeated in triplicate. After the incubation period, protein was removed from the test solutions using barium hydroxide (0.3 mol/l) and $ZnSO_4$ (50 g/l), and the solutions were centrifuged for 2 minutes in a bench-top microfuge. The resulting supernatants were further processed using alkaline copper tartrate solution (4 g $CuSO_4$, 24 g Na_2CO_3, 16 g Na K tartrate, and 180 g $NaSO_4$ in water per litre) and arsenomolybdate reagent (available commercially). The resulting solutions were diluted to a convenient volume and the

reducing sugar levels were determined by reading the absorbancies in a Shimadzu UV-1201 spectrophotometer at 510 nm. This wavelength was chosen to minimise the effects of variation in reagent blank and reoxidation of cuprous oxide.

G) Data analysis

A mean was calculated from triplicate determinations (to compensate for experimental error) of the polysacharolytic activity on each polysaccharide for each digestive organ homogenate. Since the enzyme extracts were prepared by homogenizing ten mid-oesophageal glands and ten digestive glands, an n-value of only one was obtained in each case. For this reason it was impossible to perform statistical comparisons of polysaccharolytic activity levels on each polysaccharide substrate. In addition, it was impossible to increase the n-values due to the time constraints of enzyme extract preparation and the variability of enzyme activity on a temporal basis (enzyme activity can vary on a daily basis) (Niederholzer & Hofer 1979, Stuart *et al.* 1985, Bayne 1993, Flari & Lazaridou-Dimitriadou 1996) which may have complicated data interpretation.

RESULTS

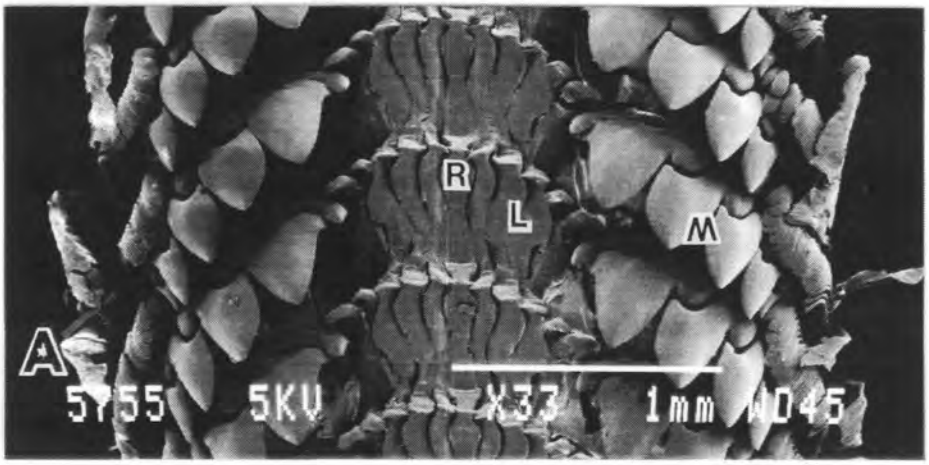
Radula structure

Turbo sarmaticus was found to have a rhipidoglossan type radula (Figure 7.2A) with the formula:

$$\infty + 1 + 4 + R + 4 + 1 + \infty$$

The rachidian tooth (R) was small and not cusped (Figure 7.2B). On either side of it were five lateral teeth with the outermost dominating (D). Beyond these were numerous marginals which were divided into a vast array of needle-like outermost marginals and enlarged cusped innermost marginals (Figure 7.2C). The second innermost marginal was found to be the largest tooth.

Figure 7.2. The rhipidoglossan radula of *Turbo sarmaticus*. **(A)** Whole radula (Scale bar = 1 mm), **(B)** Rachidian and lateral teeth (Scale bar = 100 μm) and **(C)** Numerous marginal teeth (Scale bar = 100μm). **D**: dominant lateral, **IM**: inner marginal teeth, **L**: lateral teeth, **M**: marginal teeth, **OM**: outer marginal teeth, **R**: rachidian tooth.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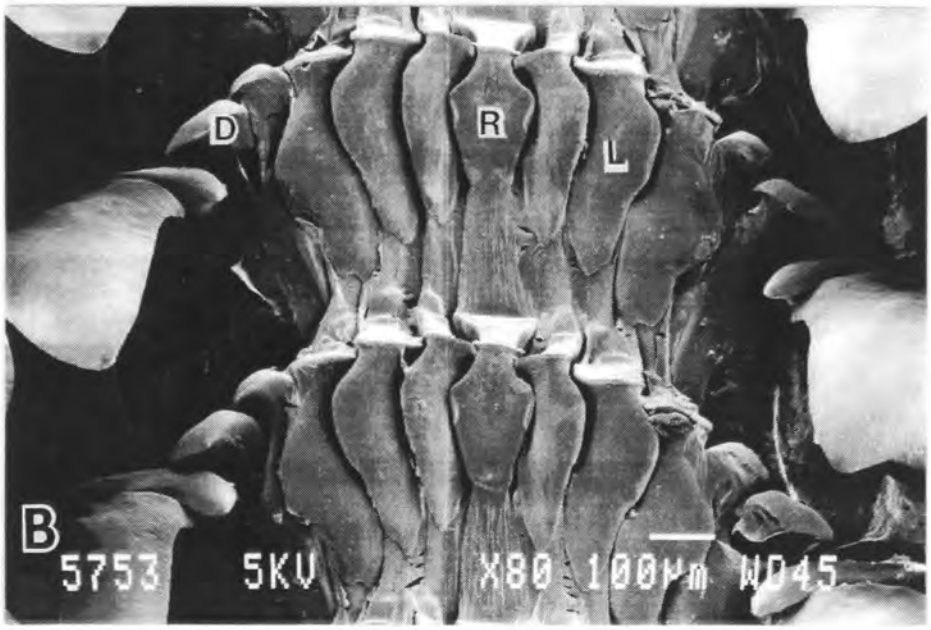
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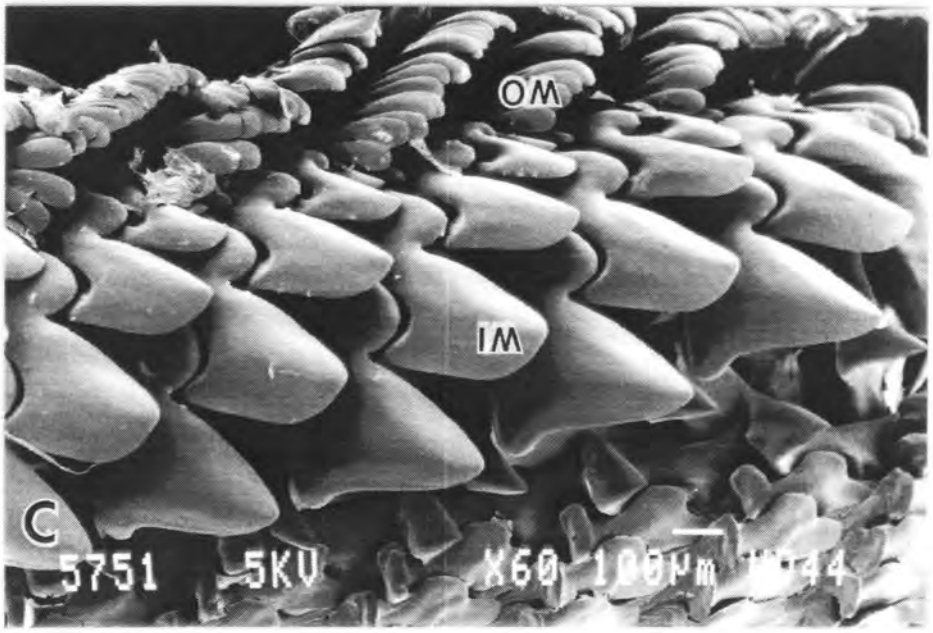
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Polysaccharide assays

A) Oesophageal gland

Reducing sugar assays on the oesophageal gland of adult and juvenile *T. sarmaticus* demonstrated the presence of enzymes which hydrolysed amylose, glycogen, laminarin, CM-cellulose, xylan and alginic acid (Figure 7.3A). However, no enzyme activity was observed for inulin, fucoidan, carrageenan and mannan (Figure 7.3A). The polysaccharolytic activity on the various polysaccharides was different in both adult and juvenile animals (Figure 7.3A). With the exceptions of xylan and alginic acid, enzyme activity on the other polysaccharides was much higher (*e.g.* up to 4 times - glycogen) in juveniles than in adults (Figure 7.3A).

In adult animals, the highest levels of enzyme activity occurred for the structural polysaccharides alginic acid (112.8 μg/mg/ml/hr) and xylan (95.6 μg/mg/ml/hr), and the storage polysaccharide glycogen (75.1 μg/mg/ml/hr) (Figure 7.3A). The storage polysaccharide amylose (41.0 μg/mg/ml/hr) showed the next highest activity, whilst the lowest levels of activity occurred for the structural polysaccharide CM-cellulose (17.0 μg/mg/ml/hr) and the storage/structural polysaccharide laminarin (15.3 μg/mg/ml/hr) (Figure 7.3A).

In juvenile animals, the highest level of enzyme activity occurred for the storage polysaccharide glycogen (328.2 μg/mg/ml/hr) followed by the storage polysaccharide amylose (137.0 μg/mg/ml/hr) and the structural polysaccharide alginic acid (122.6 μg/mg/ml/hr). The structural polysaccharides xylan (100.9 μg/mg/ml/hr) and CM-cellulose (93.7 μg/mg/ml/hr) showed the next highest activity, whilst the lowest levels of activity occurred for the storage/structural polysaccharide laminarin (43.2 μg/mg/ml/hr) (Figure 7.3A).

B) Digestive gland

Reducing sugar assays on the digestive gland of adult and juvenile *T. sarmaticus* demonstrated the presence of enzymes which hydrolysed amylose, glycogen, laminarin, CM-cellulose, fucoidan, carrageenan, xylan and alginic acid (Figure 7.3B). However, no enzyme activity was observed for inulin and mannan (Figure 7.3B). Polysaccharolytic activity was

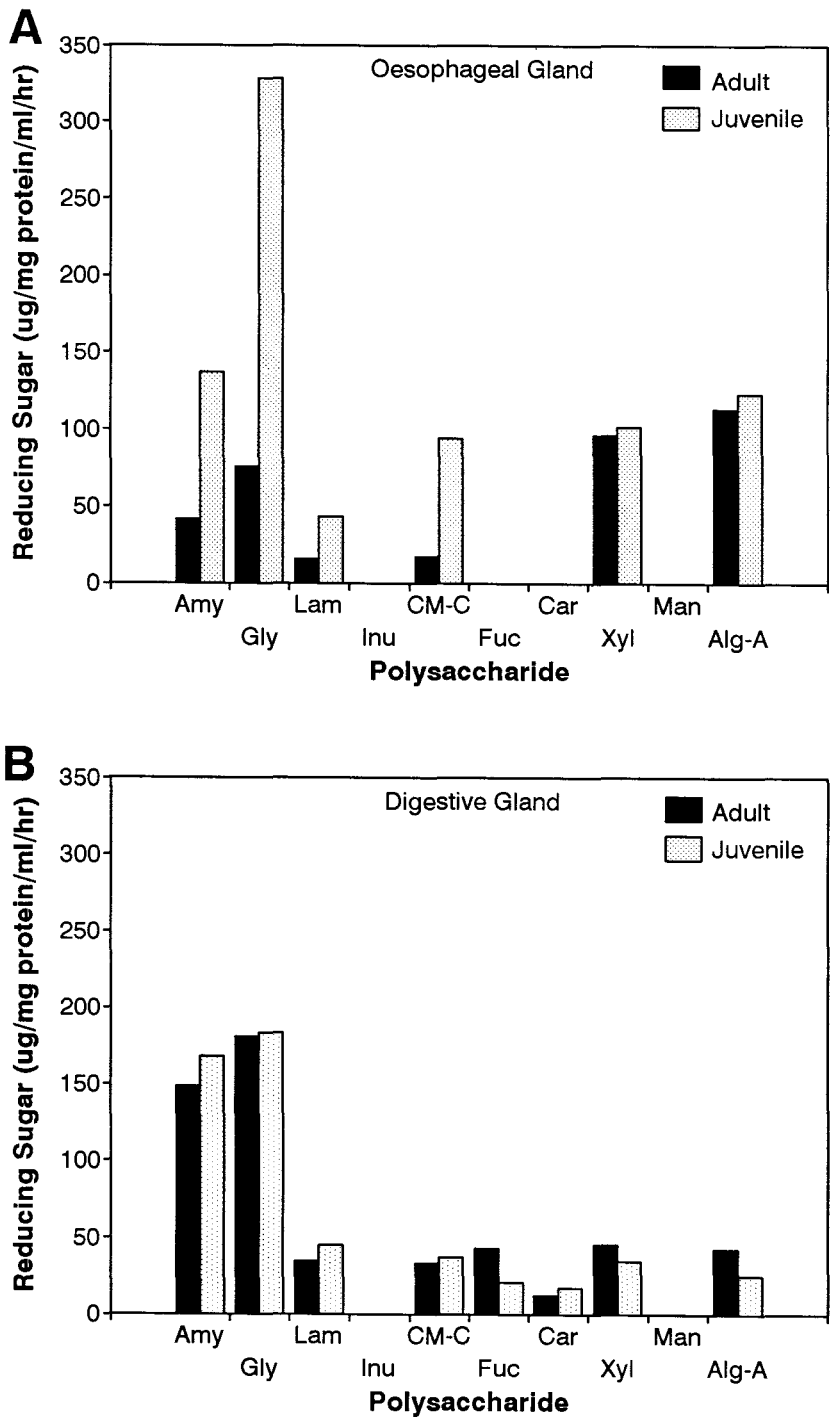

Figure 7.3. Reducing sugar levels (μg/mg protein/ml/hr) indicating enzyme activities of juvenile and adult *Turbo sarmaticus* on algal polysaccharides for the **(A)** Oesophageal gland and **(B)** Digestive gland. Amy = Amylose, Gly = Glycogen, Lam = Laminarin, Inu = Inulin, CM-C = CM-cellulose, Fuc = Fucoidan, Car = Carrageenan, Xyl = Xylan, Man = Mannan, Alg-A = Alginic acid.

different for the various polysaccharides in both adult and juvenile animals (Figure 7.3B). With the exception of fucoidan (adults double that of juveniles), enzyme activity was similar between juvenile and adult animals (Figure 7.3B).

In adult animals, the highest levels of enzyme activity occurred for the storage polysaccharides glycogen (180.8 μg/mg/ml/hr) and amylose (147.9 μg/mg/ml/hr) (Figure 7.3B), followed by the structural polysaccharides xylan (45.5 μg/mg/ml/hr), fucoidan (43.1 μg/mg/ml/hr) and alginic acid (42.5 μg/mg/ml/hr) (Figure 7.3B). The storage/structural polysaccharide laminarin (34.1 μg/mg/ml/hr) and the structural polysaccharide CM-cellulose (32.9 μg/mg/ml/hr) showed the next highest activity, whilst the lowest levels of activity occurred for the structural polysaccharide carrageenan (11.9 μg/mg/ml/hr) (Figure 7.3B).

In juvenile animals, the highest levels of enzyme activity occurred for the storage polysaccharide glycogen (183.2 μg/mg/ml/hr) and amylose (167.8 μg/mg/ml/hr). The storage/structural polysaccharide laminarin (44.8 μg/mg/ml/hr) and the structural polysaccharides CM-cellulose (36.8 μg/mg/ml/hr) and xylan (34.4 μg/mg/ml/hr) showed the next highest activity, whilst the lowest levels of activity occurred for the structural polysaccharides fucoidan (20.8 μg/mg/ml/hr), alginic acid (25.1 μg/mg/ml/hr) and carrageenan (17.1 μg/mg/ml/hr) (Figure 7.3B).

Comparison of the enzyme activity of adult and juvenile animals indicated that in adults the greatest digestive enzyme activity for amylose, glycogen, laminarin and CM-cellulose occurred in the digestive gland. The greatest activity for xylan and alginic acid occurred in the mid-oesophageal gland (Figure 7.3). In juvenile animals the greatest digestive enzyme activity for glycogen, CM-cellulose, xylan and alginic acid occurred in the mid-oesophageal gland (Figure 7.3). The greatest activity for amylose occurred in the digestive gland (Figure 7.3).

DISCUSSION

The radula of *Turbo sarmaticus* was found to be of the rhipidoglossate type which is common to most herbivorous and scavenging gastropod molluscs (Salvini-Plawen 1988, Hickman & McLean 1990, Nazneen *et al.* 1993, Fretter & Graham 1994), except true limpets (Patellogastropoda) (Steneck & Watling 1982). It is the most complex prosobranch radula,

having numerous teeth per row, and a myriad of muscles in the buccal mass (Graham 1973, Fretter & Graham 1994).

Examination of the diets of many rhipidoglossan grazers has revealed that, with the exception of a few feeding on macrophytes (*e.g. Haliotis* spp.), there is a predominance for feeding on microalgae and delicate filaments (Steneck & Watling 1982). *Turbo sarmaticus* is capable of macrophyte grazing, and with the use of its radula, it can rasp off fragments from various macroalgae with different morphologies (thin walled *Ulva* to calcarious *Corallina* spp.). Therefore, digestion is, in part, facilitated by the radula, which breaks food into small particles. In this way, a larger surface area is exposed to enzyme activity and soluble nutrients are released.

The methods used to study the polysaccharidases of marine invertebrates are very inconsistent. This makes it difficult to compare enzyme activity between species as often the units of activity vary. However, qualitative information relating to the presence or absence of an enzyme, and a descriptive reference of their activity levels can be deduced.

There is considerable evidence to suggest that marine herbivores and micro-organisms are able to digest the complex and wide range of structural, matrix and storage carbohydrates of seaweeds (Galli & Giese 1959, Lewis 1964, Horiuchi & Lane 1966, Nisizawa *et al.* 1968, Kristensen 1972, Wojtowicz 1972, Alexander *et al.* 1979, Benitez & Macaranas 1979, Torzilli & Andrykovitch 1980, Elyakova *et al.* 1981, Stark & Walker 1983, Liu *et al.* 1984, Morrice *et al.* 1984, Onishi *et al.* 1985, Preston *et al.* 1985, Suzuki *et al.* 1986, Boyen *et al.* 1990). Some of these studies have shown that CM-cellulose is the most widely digested structural carbohydrate, while digestion of other structural carbohydrates is less common. *Turbo sarmaticus* was found to have a similar enzyme suite to that of other turbinids (Table 7.4). Most of the digested sugars were polymers of glucose, indicating that the polysaccharidases were mainly glucanases.

Table 7.4. Polysaccharides digested and enzyme activity detected in *Turbo* species.

Species	Polysaccharides digested	Reference
Turbo sarmaticus	Amylose Glycogen Lamanarin CM-cellulose Fucoidan Carrageenan Xylan Alginic acid	This study
Turbo cornutus	Agar Alginate Carrageenan CM-cellulose Porphylan β-1,4-Mannan β-1,3-Xylan β-1,4-Xylan Casein	Yamaguchi *et al.* 1989
Turbinidae spp., *Turbo* spp., *Turbo argyrostomus*	Amilopectin Laminarin Lychenin Pachyman Yeast glucan CM-cellulose	Elyakova *et al.* 1981
	Enzymes	
Turbo rugosum	Laminarinase	Piavaux 1977
Turbo cornutus	Cellulase	Yokoe & Yasumasu 1964
	Alginate lyase	Muramatsu *et al.* 1977
Turbo spp.	Cellulase β-galactosidase Xylanase Amylase Alginase Protease	Liu *et al.* 1984

Based on the enzymes produced by the mid-oesophageal and digestive glands it is possible to suggest the role and importance each gland would have in the digestive process for *T. sarmaticus*. The radula would be responsible for breaking up macroalgae into smaller ingestible pieces. In so doing, it would assist in the digestive process by creating a larger food surface area on which the enzymes could act. Once the food is passed into the oesophagus,

it would encounter enzymes from the mid-oesophageal glands. These enzymes would initiate the first phase of the digestive process. Enzymatic activity would occur on the structural polysaccharides of the green, red (xylan, CM-cellulose) and brown algae (alginic acid) (Figure 7.3A), thus making the storage polysaccharides available for digestion. High enzymatic activity would occur on the storage polysaccharides of the red and green algae (amylose and glycogen), and to a lesser extent on the storage polysaccharides of the brown algae (laminarin) (Figure 7.3A). The digestive process would continue further in the stomach where the digestive enzymes would be released from the digestive gland. Digestion of the structural polysaccharides xylan and alginic acid would continue, but to a lesser extent than in the region of the mid-oesophageal glands (Figure 7.3). Further enzymatic activity would occur resulting in the additional digestion of structural polysaccharides of the green, red (carrageenan) and brown algae (fucoidan) (Figure 7.3B). This would further assist in the release of storage polysaccharides from the green, red (amylose and glycogen) and brown algae (laminairin). Enzymatic activity would still continue on these storage polysaccharides by enzymes released from the digestive gland (Figure 7.3B).

Polysaccharides digested by Turbo sarmaticus and other invertebrates

Alginic acid is a mannuronic glucuronic acid polymer and is the principle structural polysaccharide of the Phaeophycae (Percival & McDowell 1982). The digestion of alginic acid has previously been demonstrated in other molluscs (Horiuchi & Lane 1966, Favorov & Vaskovsky 1971, Kristensen 1972, Liu *et al.* 1984, Gomez-Pinchetti & Garcia-Reina 1993, Erasmus 1996), echinoderms (Eppley & Lasker 1959, Favorov & Vaskovsky 1971) and crustaceans (Kristensen 1972).

Carrageenans are a wide range of galactans occurring in the Rhodophycae and Chlorophycae (Percival & McDowell 1982). Little evidence exists for the digestion of carrageenan by marine invertebrates. However, digestion has been demonstrated in the gastropods, *Strombus gigas* (Horiuchi & Lane 1966), *Haliotis midae* (Erasmus 1996) and weak activity has been found in the asteroid, *Asteria rubens* (Kristensen 1972).

The hydrolysis of xylan is slightly more widespread than carrageenin and activity has been demonstrated in molluscs, crustaceans, annelids and echinoderms (Kristensen 1972,

Alexander *et al.* 1979, Liu *et al.* 1984, Sweijd 1990).

Fucoidan is a term used to describe a range of sulphated fucose polymers which are important structural polysaccharides in the Phaeophycae (McCandless 1982). Studies have shown that activity on fucoidan is found mainly in grazing gastropods (Huang & Giese 1958, Galli & Giese 1959, Alexander *et al.* 1979).

Inulin is another fucan found in marine algae. It is a simple deoxy-fucose polymer found in the Chlorophycae (Painter 1983). No inulin activity was observed in *T. sarmaticus*. Although Sweijd (1990) found slight inulinase activity in the sea urchin, *Parechinus angulosis*, and Van Weel (1961) made a brief reference to inulinase activity in molluscs, no further evidence for the digestion of inulin has been reported in marine herbivores.

The occurrence of an α- and β-mannosidase was reported by Moldotsov *et al.* (1974) for a variety of echinoids. However, Sweijd (1990) found no activity on mannan in the South African urchin, *Parechinus angulosus*. Similarly, no activity was found in *T. sarmaticus*. The only other evidence for the digestion of mannan has been found in the freshwater crayfish, *Paranephrops zealandicus* (Musgrove 1988).

The digestion of cellulose and cellulose derivatives (such as CM-cellulose and cellobiose) has been demonstrated in a wide range of marine invertebrates (Crosby & Reid 1971, Elyakova 1972, Gianfreda *et al.* 1979). The complete digestion of cellulose requires three enzymes: C_1 cellulase, C_x cellulase and cellobiase, all of which are reported to be commonly produced by the digestive gland of several invertebrates (Nair 1955, Horiuchi & Lane 1966, Kristensen 1972, Mathers 1973, Alexander *et al.* 1979, Stark & Walker 1983, Teo & Sabapathy 1990, Gomez-Pinchetti & Garcia-Reina 1993). There is, however, still uncertainty as to whether eukaryotes produce true cellulase and none of the studies have indicated whether the cellulase was of microbial origin. Comparative studies have shown that these enzymes are limited in their distribution and where they occur, their activity is relatively low (Hultin & Wanntrop 1966, Elyakova 1972, Elyakova *et al.* 1981, Klinger 1984). Similar activity on CM-cellulose was found in *T. sarmaticus*.

Amylase activity has been observed in molluscs as early as the beginning of this century (Bailey & Worboys 1960) and is always common to marine invertebrates (Huang & Giese 1958, Kristensen 1972, Elyakova *et al.* 1981). Both juvenile and adult *T. sarmaticus* had the ability to hydrolyse amylose and glycogen (glycogen is used as an analog of the

storage polysaccharides of the Rhodophyta - Painter 1983) to a greater extent than any of the other carbohydrates. Similarly, many studies have shown that α-glucosidase activity (using amylose and glycogen as substrates) in invertebrates is generally higher than the activity of other enzymes (*e.g.* Elyakova *et al.* 1981, Klinger 1984).

Laminarinases hydrolyse the β-(1,3)-linked glucan laminarin which is the storage glucan in the Phaeophycae (Painter 1983). Laminarinase activity has been found in coelenterates, annelids, crustaceans, molluscs and echinoderms (*e.g.* Sova *et al.* 1970, Kristensen 1972, Elyakova *et al.* 1981). Sova *et al.* (1970) and Elyakova *et al.* (1981) have found that laminarinase activity was highest in molluscs, especially bivalves. In the present study, laminarin was digested to some extent by *T. sarmaticus*.

Enzyme activity and its importance for Turbo sarmaticus

A distinction must be drawn between the levels of polysaccharidase activity. Most research has shown that polysaccharides are broken down either completely, resulting in high activity levels, or partially, resulting in low, often barely detectable, levels of enzyme activity. It is suggested here that high levels of activity represented the utilization of that polysaccharide by *T. sarmaticus* as a carbon source. However, low levels of activity require further consideration. Molecular weight is one of the factors affecting the gelling properties and viscosity of agars, carrageenans, alginates, fucans and other structural polysaccharides of marine algal (Whistler & BeMiller 1973, Kloareg & Quatrano 1988). Thus, even cleavage of these long-chained polysaccharides at one point can significantly reduce their gelling properties and consequently their efficiency in the cell wall. If cleavage were to occur at a number of points along the polysaccharide chain, the increase in the level of reducing sugars released may be very small. However, the secondary and tertiary structures of these molecules may change significantly. Whether this increase is detectable, will depend on the degree of hydrolysis. Thus, low levels of enzyme activity could be interpreted as representing cleavage of long chain polysaccharides to lower molecular weight polymers. The implication for *T. sarmaticus* is that this degree of activity would be enough to cause disruption of the cell wall, although not enough to allow utilization of the cell wall as a source of carbon.

Many studies suggest that there is not always a strong relationship between the

presence of an enzyme and the animal's diet (Van Weel 1961, Alexander *et al.* 1979, Gianfreda *et al.* 1979, Boetius & Felbeck 1995). However, dietary preferences have been reported to influence the enzyme activity of a number of marine invertebrates and a number of theories have been put forward to explain these differences in enzyme activity. Kristensen (1972) has shown that even if the enzymes do not differ between taxonomic groups, the relative importance of the different enzymes may be linked to diet. Harris *et al.* (1986) found a difference in the enzyme activity of adult *Calanus helgolandicus* fed diets with different proportions of starch. They suggested that there may be a compensatory mechanism between digestive enzymes and the substrate ingested. In another study, Knauer *et al.* (1996) found that juvenile *Haliotis midae* fed on diatoms and artificial food exhibited different lipase and amylase activities. A study on two species of mussels showed that, although the two species had a similar array of enzymes, the specific activities were different (Seiderer *et al.* 1982) due to different metabolic requirements of the two species. Finally, the digestive enzymes of the shrimp *Penaeous monodon* changed between nauplius and adult and this was attributed to a change in diet (Fang & Lee 1992). However, these results must be treated with caution as none of the studies proved unequivocally that the enzymes were endogenous. More recently, Erasmus (1996) has shown that endogenous polysaccharidase activity is related to diet in *H. midae*. Therefore, it may be possible to deduce, from the relative importance of the different enzymes, which algae are important in the diet of *T. sarmaticus*.

Activity on inulin and mannan has not been widely tested and where tested for, it has been usually absent (Mathers 1973, Stark & Walker 1983, Payne & Thorpe 1993, Simon 1997, this study). This suggests that these carbohydrates are not widely used by either grazers or filter feeders. In addition, many studies have indicated that activity on alginic acid, carrageenin, fucoidan and xylan is greater in algal grazing molluscs than filter feeding molluscs (Galli & Giese 1959, Horiuchi & Lane 1966, Favarov & Vaskovsky 1971, Kristensen 1972, Seiderer *et al.* 1982, Liu *et al.* 1984, Erasmus 1996, Simon 1997). This implies that there is a strong relationship between diet (algal as apposed to seston) and the ability to digest structural carbohydrates.

In the present study, a number of structural carbohydrates (laminarin, CM-cellulose, fucoidan, carrageenan, xylan and alginic acid) were digested by *T. sarmaticus*, but the digestive activity on these was low. This suggests that structural carbohydrate digestion by *T.*

sarmaticus acts primarily as a medium to cause a partial hydrolysis of cell walls, thus releasing the digestible storage carbohydrates (amylose and glycogen) (Kristensen 1972). Therefore, digestion of these structural carbohydrates only acts as a secondary source of food. In addition, it has been reported that cellulase may aid in the digestion of other nutrients and not just cellulose (Newell & Langdon 1986). Although structural carbohydrate digestion by *T. sarmaticus* was low, the possible role of gut bacteria in the digestive process cannot be ruled out. Many studies have shown that gut bacteria are capable of breaking down structural carbohydrates, thus making the products of digestion available to the animal (Huang & Giese 1958, Crosby & Reid 1971, Fong & Mann 1980, Sweijd 1990, Kushmaro 1995). This aspect of digestion has not been examined in this study and warrants further investigation.

Turbo sarmaticus exhibited digestive activity on structural and storage polysaccharides present in red, green and brown algae which implies that *T. sarmaticus* can utilize all these algal types. Inspection of the gut contents and nocturnal feeding observations supported this as *T. sarmaticus* mainly ingested red and green algae as part of its natural diet (Chapter 5). A possible reason for the scarcity of brown algae in the diet may have been as a result of this algae being the least abundant along the coast of the Eastern Cape Province (Seagrief 1988).

Invertebrates are capable of changing their enzymes in response to their diet (Stuart *et al.* 1985, Harris *et al.* 1986, Erasmus 1996). Furthermore, a change in digestive enzyme activity in response to changes in food availability may occur in as short a time period as a few days (Bayne 1993). Therefore, the enzyme activity detected in this study may have reflected algal availability rather than the endogenous enzyme activity of *T. sarmaticus*.

In conclusion, this study has shown that *T. sarmaticus* possessed enzymes that could digest most of the structural polysaccharides present in the algae tested. Two levels of activity were detected. High levels of enzyme activity occurred on the storage polysaccharides of Rhodophycae and Chlorophycae. These algae were also consumed and, in most instances, digested the most (Chapter 5), which allowed *T. sarmaticus* to obtain a high energy intake, particularly from the Chlorophycae (Chapter 5, Figure 5.6). Lower levels of activity were detected on the storage polysaccharides of the Phaeophycae and on all the structural polysaccharides tested. These results indicate that *T. sarmaticus* is capable of digesting the cell walls of red, green and brown algae. This supports that *T. sarmaticus* is a generalist grazer. Furthermore, it is suggested that *T. sarmaticus* does not rely heavily on structural

carbohydrates as a source of carbon.

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CHAPTER EIGHT

GENERAL DISCUSSION

The expanding human population increases the pressure on the environment. There is therefore an urgent need for the sustainable management of exploited species such as *Turbo sarmaticus*. The main objective for the management of any exploited marine species should be to maximize its yield, by maintaining a particular stock level to act as a buffer against poor recruitment years and by maintaining a minimum spawning stock (Pitcher & Hart 1982, King 1995). The results of the present study will therefore be used to discuss the future management of *T. sarmaticus*, the possible role of this macroalgal grazer in the intertidal zone and the effects of over-exploitation of this mollusc. Finally, the possible aquaculture potential of *T. sarmaticus* will be addressed briefly.

Although a wide range of intertidal species are exploited from regions of the South African coast, shellfish gatherers have been reported to be selective, both in terms of species and size classes removed (Bigalke 1973, Siegfried *et al.* 1985, Hockey & Bosman 1986). The marked increase in the intensity of shellfish gathering in recent years has resulted in the depletion of certain shellfish stocks (Van Erkom Schurink & Griffiths 1990) and in some instances has changed the structure of intertidal communities (Hockey & Bosman 1986).

Assessments of the impact of human exploitation on rocky intertidal biota have been based on comparisons of community structure and/or population size structure of key species within protected and non-protected areas (Moreno *et al.* 1984, Castilla & Duran 1985, Siegfried *et al.* 1985, Hockey & Bosman 1986, Ortega 1987). This thesis has used similar comparisons of *T. sarmaticus* populations between exploited and unexploited areas (Chapter 2). The underlying assumption is that significant differences between sites result solely from the presence or absence of human exploitation. However, the size structure of organisms is known to be affected by predators, physiological tolerances and interspecific competition (Seed 1976). In addition, erratic distribution patterns have been attributed to irregular cycles of settlement, competition, predation, denudation and resettlement (Lewis 1964). These factors are difficult to determine and therefore their impact on the different study sites were not known. Thus, attributing differences between protected and unprotected areas solely to human interference is questionable. Since every attempt, however, was made during the present study to ensure that exploited and unexploited sites had similar biotic and abiotic factors, it is believed that differences were mainly due to human exploitation pressures. Similarly, other studies along the South African coastline have shown the adverse effects of exploitation (Van

Erkom Schurink & Griffiths 1990, Lasiak 1991, Dye 1992), as where high exploitation of shellfish has occurred these populations have become depleted. In this study, the site that was subjected to intense exploitation (Kelly's Beach - Port Alfred) had fewer animals than the unexploited sites (Bird Island - Algoa Bay) or the sites subjected to low intensity exploitation (Chelsea Point - Port Elizabeth). Furthermore, the intensely exploited site had no legal sized animals (≈ 73.7 mm shell length), while the less exploited sites had larger populations and many legal sized animals (Chapter 2).

Turbo sarmaticus is highly susceptible to over-exploitation along the coast of the Eastern Cape Province for a number of reasons:

1) Although *T. sarmaticus* is capable of considerable movement in its rocky shore habitat (*pers. obs.*), it is probably restricted in its long-shore movement by the patchiness of the rocky shores along this coastal region (Lubke 1988, Dower 1989). These isolated populations are likely to be particularly sensitive to extreme exploitation.

2) *Turbo sarmaticus* has a preference for shallow water and, although they may be found down to a depth of 8 metres (Kensley 1973, Kilburn & Rippey 1982, Branch *et al.* 1994), they are usually restricted to the rocky intertidal and shallow subtidal reefs (*pers. obs.*, *pers. comm.* - local divers).

3) Legal sized animals occupy exposed portions of the intertidal, and despite being cryptic and hiding in crevices they are easily recognised by experienced shellfish gatherers (*pers. comm.*). This aspect, together with their restricted and shallow distribution, makes them easily accessible to collectors.

4) *Turbo sarmaticus* exhibits a vertical size gradient with smaller immature animals upshore and larger adults downshore (Chapter 2). Most of the immature individuals (< 52.5 mm shell length) are exposed in the intertidal at low tides. Many animals are therefore removed before they can reach sexual maturity, thus potentially reducing the numbers of reproductively active animals.

5) Their protracted spawning period (≈ 4 months - Chapter 4) together with possibly having pelagic larval stages (as characterised by most trochaceans - Hickman 1992) in the unstable rocky intertidal surf zone may contribute to unpredictable recruitment.

At present there is a restriction imposed on the collection of *T. sarmaticus*. The minimum legal size is set at a level above its size at sexual maturity (≈ 73.7 mm shell length)

and the catch limit is set at five animals/person/day. Based on results from this study, the present legal size is set at a safe level, allowing animals to have reached sexual maturity for at least two years, thus allowing for two breeding seasons (Chapters 3 & 4). However, the relevance of size at sexual maturity depends on its relationship with the size preferred by shellfish gatherers. If the size at sexual maturity is less than the rejection size, then exploited populations should always include some reproducing individuals. If, however, the size at maturity is greater than the rejection size there is a serious risk that removal of large numbers of reproductively active individuals will drastically reduce the recruitment potential of the population. Examination of the size composition of exploited species relative to their size at maturity has indicated that along the former Transkei coast (Eastern Cape Province) of South Africa approximately 40% of the *T. sarmaticus* collected were immature (Lasiak 1991). If uncontrolled, this may result in a serious depletion of the reproductive stock which, in turn, will result in the demise of these *T. sarmaticus* populations.

Turbo sarmaticus spawns during a period (November - February) which coincides with the peak holiday season (Christmas vacation) (Chapter 4). During this vacation, intertidal rocky shores experience increased exploitation pressure (*pers. obs.*) with numerous undersized *T. sarmaticus* being removed (*pers. comm.* - Dept. Sea Fisheries). In addition, these rocky shores are subjected to increased disturbance (*i.e.* turning of boulders) as holiday makers search for bait organisms other than *T. sarmaticus*. A closed season during the spawning season of *T. sarmaticus* may be considered an additional management option. However, studies must first be undertaken to determine whether *T. sarmaticus* is more vulnerable during the spawning period. If this is the case, then the closed season should incorporate the spawning period. However, if this is not the case, then the effectiveness of a closed season during the breeding season must be questioned and other options such as closed areas on a rotational basis amongst populations could be considered.

The concept of closed areas which are free from exploitation has been recognized as a viable management option for the *Haliotis midae* fishery (Tarr 1992). The protection afforded the spawning stock in closed areas would benefit the immediate and nearby populations, but depleted stocks further along the coast would only benefit if larval dispersal and/or migration were extensive (Tegner & Butler 1985). Migratory tendencies have been reported for abalone (Koike *et al.* 1972) which moved from adjacent areas to repopulate

exploited areas. It is suggested that this would have limited relevance in the Eastern Cape Province as rocky shores are typically small, discontinuously distributed and separated by vast expanses of sand (Lubke 1988, Dower 1989) which would probably preclude the movement of *T. sarmaticus*. Stocks are probably maintained by either direct input from the resident spawning stock or by larval drift from nearby populations. However, the extent to which the larvae of *T. sarmaticus* are capable of dispersing is yet to be determined.

The recruitment of pelagic larvae from less accessible unexploited populations may contribute to the replenishment of stocks in exploited areas (King 1995). Along the coast of the Eastern Cape Province, the larvae from *T. sarmaticus* might come from inaccessible subtidal populations, from stocks protected within nature reserves or from stocks associated with inaccessible areas where exploitation is limited. Since most trochacean gastropods have a relatively short larval life-span of less than 10 days (Grange 1976, Hickman 1992), Lasiak (1991) has suggested that their dispersal range will probably be limited. In contrast, Moran (1997) has stated that a short larval life-span does not necessarily mean that dispersal will be limited as the speed of oceanic currents can result in larvae with even a short life-span being carried many kilometres by these currents. The coastal hydrography of the Eastern Cape Province is dominated by the Agulhas current which flows close to the shore between Durban (29°52'S/31°00'E) and Port Elizabeth (33°58'S/25°38'E), and thereafter diverges from the coast (Ross 1988). It is likely that the larvae of *T. sarmaticus* remain close inshore by virtue of localised currents. However, if offshore winds were to persist for several days larvae may be carried further offshore, possibly resulting in the larvae of *T. sarmaticus* populations north of Port Elizabeth becoming entrained in the Agulhas current. This current has an average velocity of about 1 m/sec (Ross 1988). Therefore, *T. sarmaticus* larvae may be able to disperse $\approx$ 86.4 km/day. Thus, the role of inshore currents in the dispersal ability of *T. sarmaticus* needs to be investigated. In addition, determining the genetic variability between and amongst populations of *T. sarmaticus* at various locations along the South African coast may give a further indication of the dispersal ability of this animal. Populations showing genetic homogeneity may indicate that the dispersal ability of such populations is limited (Grant & Lang 1991, Hunt 1993) and excessive exploitation pressure will have adverse effects.

The presence of extensive subtidal populations outside the reach of shellfish gatherers

may buffer intertidal populations of *T. sarmaticus*. Catterall & Poiner (1987) have suggested that species with adjacent subtidal populations may contribute to localized reproduction within exploited areas if they have sexually mature mobile benthic stages capable of migrating up into the intertidal area. If the intertidal areas are not searched too frequently by shellfish gatherers, such upshore migration could buffer the effects of local exploitation. Since most of the reproductively active individuals of *T. sarmaticus* occur in the lower intertidal or subtidally, the contribution made by upwardly mobile individuals may act as a buffer against low exploitation. This may explain why low intensity exploited sites (*e.g.* Chelsea Point) had similar densities and population structures to those of unexploited sites (*e.g.* Bird Island) (Chapter 2). It is suggested that when exploitation pressures are high, this buffering effect is lost and the *T. sarmaticus* population begins to decline, as at Kelly's Beach (Chapter 2). If populations of *T. sarmaticus* are not managed properly, the continued degradation of these populations, as at Kelly's Beach, may result in such coastlines becoming unsuitable for future exploitation. This may have significant implications for the ecology of such shores as the role of *T. sarmaticus* in such ecosystems will be reduced or eliminated.

Hawkins & Hartnoll (1983), in a review on algal grazing by marine invertebrates, have indicated that there are four basic feeding patterns in molluscs (modified after Branch 1981): 1) generalists, feeding mainly on microalgae, 2) species feeding on macroalgae, 3) territorial species, closely linked to a particular food plant and 4) epiphytic stenotopic species which feed on their host plant. This study has shown that *T. sarmaticus* is a generalist macroalgal grazer capable of consuming and digesting macroalgae from the Chlorophyta, Rhodophyta and Phaeophyta (Chapters 5 & 7).

Most chitons (Boyle 1977, Newell 1979, Steneck & Watling 1982), prosobranch limpets and snails (particularly rhipidoglossan species) are generalist grazers feeding on any microflora or detritus available on rock surfaces (Boyle 1977, Newell 1979, Underwood 1979, Branch 1981, Steneck & Watling 1982). Other molluscs, particularly mesogastropods, feed on large erect algae as well as microflora or encrusting forms, and do exhibit choice in diet. Various studies of food preference have been made with winkles and topshells (*e.g.* Lubchenco 1978, Underwood 1979, Mooers 1981) and generally green algae are preferred to red or brown algae. Although food preference studies were not undertaken for *T. sarmaticus*, this mollusc consumed green and red algae to a greater extent than the brown algae (Chapter

5). In addition, these two algal types were also more readily digested than the brown algae (Chapter 5) as a result of the greater enzyme activity of *T. sarmaticus* on the storage and structural polysaccharides of these algae (Chapter 7). It is therefore likely that, although *T. sarmaticus* can consume and digest algae from the three algal divisions, it may show a preference for green and red algae when presented with a choice.

Kitting (1980) stated that the maintenance of a particular mixed diet will probably be found primarily among species rarely exhibiting major influences on their food resources, or among free-roaming animals moving readily to new patches of food if any desirable food becomes rare. In addition, generalist grazers living in environments with diverse food assemblages may be allowed to consume preferable and more beneficial, well-mixed diets than they would consume in less diverse food assemblages. Similarly, *T. sarmaticus* achieved its best growth and reproductive fitness when fed a mixed diet or good quality monospecific diets (*Ulva rigida* and *Gelidium pristoides*) (Chapter 6). *Turbo sarmaticus* fed on one of its natural diets alone (*Corallina* spp.) which had a poor energetic and nutritional content (Chapter 5) showed slow growth and its reproductive output decreased dramatically (Chapter 6). The benefit of eating *Corallina* spp. for the dietary requirements of *T. sarmaticus* is still unknown and warrants further investigation.

It has been suggested that the form of the radulae and feeding movements are adapted to the types of food eaten (Hawkins & Hartnoll 1983). Prosobranch radulae can be divided into various types but only the rhipidoglossan, taenioglossan and docoglossan types are used in grazing (Hawkins & Hartnoll 1983). Rhipidoglossan molluscs (most vetigastropods) are regarded as feeding microphagously and this has been attributed to their weak jaws which are unable to remove chunks of material from the substratum (Hawkins & Hartnoll 1983). However, in *T. sarmaticus* this is not the case, as these molluscs use their rhipidoglossan type radulae (Chapter 7) to feed on macroalgae. In addition, *T. sarmaticus* used this radula type to feed on macroalgae ranging from thin walled chlorophytes to calcareous rhodophytes (Chapter 5). Therefore, this study supports the warnings of Raffaelli (1985) that predicting diet from radula morphology alone should be viewed with caution. It is more likely that algal availability as well as feeding capability will determine diets (Hawkins *et al.* 1989).

Hawkins & Hartnoll (1983) have stated that most of the macroalgal production on rocky shores is not used by herbivores but passed to detritivores as the majority of grazers

feed on microalgal films on rock surfaces. Any energy transfer to detritivores from macroalgae is therefore likely to occur only from the natural decay of these algae. It is generally assumed that detrital consumers receive most of their nutritional requirements from the attached microorganisms, with only a small proportion being directly derived from the decomposing plant material (Fenchel 1977, Newell 1979, Kennish 1986, Knox 1986). Therefore, macroalgal grazers, such as *T. sarmaticus*, play an important role in the transfer of energy from macroalgae to the detritivore trophic level.

Intertidal and subtidal marine ecosystems rely primarily on herbivores, detritivores and filter-feeders for the distribution of energy through the system (Barnes & Hughes 1982). Three invertebrate macroalgal grazers are prominent on rocky shores in South Africa, *viz.*: *Haliotis midae* (vetigastropod), *Parechinus angulosus* (echinoderm) and *T. sarmaticus* (Day 1974). Of these, *T. sarmaticus* makes an important contribution to shore biomass (22 - 36%) along the Port Elizabeth coast (McLachlan *et al.* 1981). Since the densities of *T. sarmaticus* at study sites examined in this thesis were similar to those previously reported for Port Elizabeth (Chapter 2), it is likely that this mollusc also makes an important contribution to shore biomass at all the studied sites along the coast of the Eastern Cape Province. Since macroalgal grazers (*e.g.* sea urchins) can have profound influences on the structure of benthic communities (see Lawrence & Sammarco 1982 for reviews), other large macroalgal grazers such as *T. sarmaticus* may also play an important role.

Although *T. sarmaticus* is a generalist macroalgal grazer, three species of algae form the bulk of its diet, *viz.*: *Ulva rigida*, *Gelidium pristoides* and *Corallina* spp. (Chapter 5). In Chapter 2, the standing stock (individuals/km shoreline) of *T. sarmaticus* at exploited and unexploited sites was determined. In addition, the amount of these three algae consumed was determined in Chapter 5. Therefore, it is possible to calculate (although only a rough estimate) the amount of algae consumed by *T. sarmaticus* at these sites per annum. The daily amounts of algae consumed (dry weight) by juvenile and adult animals were averaged to produce an estimate of algae consumed by *T. sarmaticus*. These amounts were then compared to the standing stocks of animals at a low intensity exploited site (Chelsea Point - Port Elizabeth) and a highly exploited site (Kelly's Beach - Port Alfred). Based on these calculations, the *T. sarmaticus* population at Chelsea Point could potentially consume (dry weight at 20°C) approximately 954.4 kg/km shoreline/year of *U. rigida*, 199.2 kg/km shoreline/year of *G.*

pristoides and 960.7 kg/km shoreline/year of *Corallina* spp.. In contrast, at Kelly's Beach where the standing stock of *T. sarmaticus* was much lower as a result of exploitation, only 142.1 kg/km shoreline/year of *U. rigida*, 29.7 kg/km shoreline/year of *G. pristoides* and 143.6 kg/km shoreline/year of *Corallina* spp. were consumed.

Grazers of macroalgae along the South African coast have been found to ingest between 8% (≈ 3260 kJ/m²/year) (*e.g. Haliotis midae* - Barkai & Griffiths 1988) and 14% (7985.8 kJ/m²/year) (*e.g. Parechinus angulosus* - Buxton & Field 1983) of the estimated annual production of macroalgae in their respective habitats. Based on the density of *T. sarmaticus* at Chelsea Point and Kelly's Beach, the daily consumption rates and energetic content of *U. rigida*, *G. pristoides* and *Corallina* spp. (Table 8.1), the annual population energetic intake was calculated for *T. sarmaticus* populations at these two sites.

Table 8.1. The density of *Turbo sarmaticus*, daily consumption rate and energetic content of three algal species used to calculate the annual energetic intake of *Turbo sarmaticus* populations at two sites.

Site	Density (No./m²)
Chelsea Point	1.73
Kelly's Beach	0.26
Algal species	**Consumption rate (g/day dry)**
Ulva rigida	0.244
Gelidium pristoides	0.051
Corallina spp.	0.246
Algal species	**Energetic content (kJ/g dry)**
Ulva rigida	9.53
Gelidium pristoides	16.47
Corallina spp.	2.55

The annual population ingestion at Chelsea Point was 1458.1 kJ/m²/year for *U. rigida*, 525.1 kJ/m²/year for *G. pristoides* and 396.0 kJ/m²/year for *Corallina* spp., whilst at Kelly's Beach it was 220.5 kJ/m²/year for *U. rigida*, 78.8 kJ/m²/year for *G. pristoides* and 59.5 kJ/m²/year

for *Corallina* spp.. However, the annual production of macroalgae along the coast of the Eastern Cape Province has not been determined, thus making it is impossible to predict what percentage *T. sarmaticus* consumed. Nevertheless, *T. sarmaticus* probably plays an important role in the processing of macroalgae and assists in the transfer of energy from primary producers to some predators and the detritivore trophic level. Since exploitation lowers the standing stocks of *T. sarmaticus*, the amount of energy that can potentially be transferred to the next trophic levels will be considerably reduced.

Mussels are one of the preferred shellfish collected on shores along the South African coast (Lasiak & Dye 1989). They have been extensively removed along the former Transkei coast and in their place coralline algae have rapidly and extensively become established (Dye 1992). A herbivore's diet is often dictated by the algal community on shores (Cubit 1984, Williams 1993, Ito *et al.* 1996, Kennish *et al.* 1996). Therefore, the abundances of a poor quality food source such as coralline algae which is consumed by *T. sarmaticus* will ultimately result in the reduced growth rate and reproductive fitness of this mollusc (Chapter 6). In addition, the fact that *T. sarmaticus* is at its geographical extreme and may experience recruitment failure, along with the removal of many immature animals are factors that will further compound to the demise of *T. sarmaticus* stocks along the former Transkei coast. Therefore, excessive exploitation of *T. sarmaticus* would reduce the role of this mollusc in the transfer of energy to the particulate feeder trophic level (*e.g.* detritivores).

Despite South Africa's relatively large size and long coast line (≈ 3000 km), a number of geographical and climatic features impose restrictions to the development of mariculture (Hecht & Britz 1990). These include an exposed, high energy coastline and differing water temperatures caused by the cold Atlantic waters in the west and the warmer Indian Ocean in the east. Nevertheless, there are a number of successful types of aquaculture which include oysters, mussels and more recently, abalone (Hecht & Britz 1990). Although *T. sarmaticus* is collected as a food source, it is as yet not exploited commercially in South Africa. In addition, Hecht & Britz (1990), in a review on aquaculture in South Africa, did not include *T. sarmaticus* as a prospective species for potential aquaculture. Turbinids are exploited commercially from other regions of the world and in some instances, certain stocks are becoming depleted (Alagarswami 1987, Chantrapornsyl 1995, Yamaguchi 1995). *Turbo sarmaticus* can achieve densities similar to those of other exploited turbinid populations

worldwide (see Chapter 2). In addition, results from this thesis have indicated that *T. sarmaticus* is a hardy mollusc capable of surviving aquaculture conditions. When fed on certain algal diets (*Ulva rigida* and a mixture of *U. rigida*, *G. pristoides* and *Corallina* spp. - Chapter 6), growth was still good (although about 20% slower than in the field), while reproductive output was dramatically enhanced (≈ 100%). If *T. sarmaticus* was to be harvested at a "cocktail" size (55 - 60 mm shell lengths), it is predicted that this size would be achieved after 2 - 3 years of growth (Chapter 3). It is therefore suggested that this mollusc should be considered as a future aquaculture species. However, much research is still needed to determine whether growth rates can be enhanced in aquaculture species with the use of artificial diets (Britz 1995). In addition, research needs to determine successful techniques to ensure artificial spawning and to ensure that larval development until settlement is successful.

In summary, *T. sarmaticus* plays an important role in the ecosystems in which it lives by making energy from macroalgae available for particulate feeders. This mollusc is an important source of protein for certain impoverished communities, and it is therefore vital that this mollusc does not become over-exploited. Over-exploitation will result in the demise of stocks thereby reducing the role this mollusc plays in its ecosystem, resulting in the lowering of yields and a loss of this mollusc as a protein source. It is suggested that a closed season (from November to February) be implemented at areas experiencing stock depletions to assist in the conservation of this species. Finally, because turbinids are sought after molluscs for exploitation worldwide, *T. sarmaticus* may be considered as a future aquaculture species. In addition, South Africa has a further three species of turbinids and at least one trochid (*Oxystele sinensis*) that need investigating for their aquaculture potential.

Avenues of future research

1) A detailed examination of the larval life-history and settlement time of *T. sarmaticus* will assist in recruitment predictions.

2) An investigation into the genetic variation of *T. sarmaticus* populations along the South African coast will assist in the determination of larval dispersal.

3) The determination of an energy budget for *T. sarmaticus* will provide comparable data to that of other gastropods. This would be of interest not only because of the potential economic importance of *T. sarmaticus*, but also because of the large size and long life cycle of this species relative to those of other gastropods.

4) Comprehensive investigations into the effects of exploitation of *T. sarmaticus* standing stocks would predict the consequences of over-exploitation for this mollusc and its role in the ecosystem.

5) A detailed assessment of the aquaculture potential of *T. sarmaticus* would be important as the introduction of this mollusc as a viable aquaculture species may contribute to economic growth and employment opportunities in South Africa.

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