ASPECTS OF THE BIOLOGY AND ECOLOGY OF SOME INTERTIDAL HOLOTHURIANS (ECHINODERMATA) ALONG THE EASTERN CAPE COAST OF SOUTH AFRICA

THESIS

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DEDICATION

To the memory of my father

GEORGE WILLIAM FOSTER

who sadly passed away on the

27 June 1993

and who will always be missed.

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ABSTRACT

Four species of dendrochirote holothurians, *Roweia stephensoni* John, *Pseudocnella sykion* Lampert, *Aslia spyridophora* Clark, *Roweia frauenfeldi frauenfeldi* Ludwig, and one aspidochirote holothurian, *Neostichopus grammatus* Clark, are common inhabitants of the intertidal regions of the eastern Cape rocky shores of South Africa. All five species are sympatric for most of their geographical distribution. The intertidal distributions, densities and biomasses of *R. stephensoni*, *P. sykion* and *N. grammatus* were examined at six sites from Port Elizabeth to East London in the eastern Cape. At all sites the three species showed similar overlapping zonations with *R. stephensoni* and *P. sykion* reaching their highest maximum mean densities and biomasses (drained wet weight) in the lower mid-shore region (40/m^2 and 684g/m^2, 18/m^2 and 269g/m^2, respectively at Port Elizabeth), whilst *N. grammatus* in the low-shore region (6/m^2 and 72g/m^2, at Port Elizabeth). The highest densities and biomasses of all three species were recorded on boulder shores that had minimal sand inundation.

Seasonality of reproduction of *R. stephensoni*, *P. sykion* and *N. grammatus* was compared at Port Elizabeth, using gonad index values, gonadal tubule diameters, egg diameters and

spermatozoon content in tubules from January 1992 until August 1993. All three species are dioecious and did not deviate from a 1:1 sex ratio. Sizes at first sexual maturity were 2.5-2.9 cm^3 for *R. stephensoni* and *P. sykion*, and 3.0-3.9cm^3 for *N. grammatus*. The gonads of *R. stephensoni* and *P. sykion* consist of a bunch of unbranched tubules of equal diameter, with males having more tubules of a smaller diameter than females. By contrast, the gonad of *N. grammatus* consists of two tufts of multiple branched tubules. All three species followed annual reproductive cycles, and in *R. stephensoni* and *P. sykion* gametogenesis occurred from March 1992 until August/September 1992, and maturity was maintained until the main spawning event in January 1993. By contrast, in *N. grammatus* gametogenesis occurred from July 1992 until September 1992, and maturity was maintained until the main spawning event in December 1992/January 1993. Unlike *R. stephensoni* and *P. sykion* the reproductive cycle of *N. grammatus* was more pronounced, in that the gonad regressed after spawning.

Aspects of the feeding biology of *R. stephensoni*, *P. sykion*, *A. spyridophora*, *R. f. frauenfeldi* and *N. grammatus* were compared, using the gross buccal tentacle morphology, type and size range of ingested food particles, and gut lengths. Scanning electron microscopy revealed that the buccal tentacles of *R. stephensoni*, *P. sykion* and *A. spyridophora* could be classified as dendritic, branching numerous times with the tips terminating in numerous nodes covered with discs of papillate projections. The buccal tentacle structure of *R. f. frauenfeldi* deviated from the typical dendrochirote dendritic form. The tentacles are stout with few branches which terminate in large bulbous nodes lacking discs of papillate projections. *Neostichopus grammatus* possesses buccal tentacles that could be classified as peltate, terminating in large nodes which also lacked discs of papillate projections. *Roweia stephensoni*, *P. sykion* and *A. spyridophora* are suspension-feeders, ingesting food particles mostly <53µm in size. *Roweia*

f. frauenfeldi was classed as a "heavy" suspension-feeder, ingesting food particles mostly between 250μm-1.18mm, while *N. grammatus* is a deposit-feeder, ingesting benthic sediments mostly between 106-500μm. The gut lengths of the dendrochirote holothurians were found to be significantly longer than that of the aspidochirote, and may be attributed to the nature of the food ingested.

CHAPTER ONE

GENERAL INTRODUCTION

"The echinoderms are the strangest race
That on our World the Lord did place"
Arthur C. Giese

The phylum Echinodermata includes many of the most familiar seashore creatures and is comprised of six classes, viz Asteroidea (starfish), Ophiuroidea (brittle stars), Echinoidea (sea urchins), Crinoidea (feather stars), Holothuroidea (sea cucumbers) (Hyman, 1955), and a recently discovered class the Concentricycloidea (Baker *et al.*, 1986). The class Holothuroidea consists of 6 orders, 25 families and 200 genera encompassing approximately 1250 species. Species in the order Dactylochirotida (≈2% of all holothurians) are rare, and the order Elasipodida (≈10%) are entirely abyssal species. The remaining 4 orders, the Dendrochirotida (≈40%), Aspidochirotida (≈24%), Molpadida (≈6%), and the Apodida (≈18%), are present in many shallow-water marine faunas (Pawson 1982: in Smiley *et al.*, 1991). In general, holothurians are "wormlike" echinoderms with secondary bilateral symmetry imposed over pentaradial symmetry. They usually lie on one side, which may be differentiated, and the

mouth is surrounded by tentacles (Hyman, 1955). Holothurians are distributed widely in all seas and at all depths. In fact, they are one of the few faunal groups that penetrate into the deepest recesses of the ocean (>6000m), where they dominate the invertebrate macrofauna (Billett, 1991). Holothurians are also adapted to live in a wide variety of habitats, with most species being sedentary, although some roam over the substratum (Pawson, 1966).

The southern African subcontinent (south of the Tropic of Capricorn) forms a small landmass, gradually narrowing southwards. It is the meeting place of two of the world's large oceans, viz the Atlantic Ocean to the west and the Indian Ocean to the east, and the coastline (≈4000km) offers numerous marine habitats distributed through tropical, subtropical, warm and cold temperate-waters, which are determined by latitude and prevailing ocean currents (Branch & Branch, 1981). Hence, it is not surprising that approximately 407 species of echinoderms have been recorded from this region, of which ≈47% are endemic (Thandar, 1989a). Holothurians comprise about 108 species of the approximately 407 known southern African echinoderm species. These holothurians can further be divided up into two main faunistic components, that of an Indo-Pacific (≈41%) and endemic (≈41%) origin (Table 1, Thandar, 1989a). Thandar (1989a) further states that as far as intertidal holothurians are concerned there is 100% endemicity of the fauna west of East London (33°01'S/27°55'E) and then northwards into Namibia on the west coast.

Aspects of holothurian biology and ecology have been extensively studied in species from various parts of the world, both shallow-water and deep-sea, ranging from the physiology (e.g. Terwilliger & Read, 1972; Prim *et al.*, 1976; Dimock, 1977; Eylers, 1982; Lawrence & Guille, 1982; Smith, 1983a; Jackson & Fontaine, 1984; Sisak & Sander, 1985), feeding

biology (e.g. Fish, 1967; Yingst, 1976; Fankboner, 1978; Bouland *et al.*, 1982; Hammond, 1982; Roberts & Bryce, 1982; Costelloe & Keegan, 1984; Coulon & Jangoux, 1993), reproductive biology (e.g. Conand, 1982; Costelloe, 1985; Smiley & Cloney, 1985; Smiley, 1988; Sewell & Bergquist, 1990; Billett, 1991; Hamel *et al.*, 1993) to general abundance and distribution (e.g. Bonham & Held, 1963; Lawrence & Guille, 1982b; Muscat, 1982; Pawson *et al.*, 1982; Sloan, 1982; Billett, 1991). Holothurians, especially those that ingest bottom sediments, are regarded as important reworkers of the sediment and fulfil an important role in the ecosystems in which they live (Crozier, 1918; Frankenberg & Smith, 1967; Rhoads & Young, 1971; Hauksson, 1979; Sloan & von Bodungen, 1980; Massin, 1982a). Similarly, filter-feeding holothurians may be important competitors for space with other filter-feeding organisms within their ecosystem (Levinton, 1972).

Although holothurians are reported in numerous ecological surveys of the South African coast and in some instances form a conspicuous component (e.g. Stephenson, 1944; Deichmann, 1948; Day, 1974; Velimirov *et al.*, 1977; Field *et al.*, 1980; Branch & Branch, 1981; McLachlan *et al.*, 1981; Barkai & Branch, 1988; Dower, 1989), their taxonomic status has only recently been resolved (Thandar, 1977, 1985, 1986, 1987a, 1987b, 1988, 1989b, 1989c, 1989d, 1990, 1991; Thandar & Rowe, 1989). In addition, with the exception of a few published papers on some aspects of their physiology (e.g. Hogben & van der Lingen, 1928; van der Lingen & Hogben, 1928; Brown, 1967; Brown & McClurg, 1973), sperm structure (Hodgson & Bernard, 1992) and ecology (Velimirov, 1984; Barkai, 1991), literature on the biology of southern African holothurians is lacking. In fact, in a review on trends in marine littoral research in South Africa during the period 1983-1988, McQuaid (1989) states that 269 papers were published with the major emphasis on taxonomy and ecology (i.e. life-history,

feeding ecology, community structure and biogeography); in addition west coast species and ecosystems have received the most attention. Interestingly, of the 269 publications only 7 concentrated on echinoderms and focused mainly on their taxonomy, while other taxonomic groups such as the Algae (54 papers), Mollusca (84), and the Chordata (57) received the most interest (Table 2, McQuaid, 1989). One may have suspected that such a report might have kindled a greater interest in echinoderm biology. However, since McQuaid's review very little has been published on the biology of South African echinoderms, with only a few articles on echinoid reproduction (Drummond, 1991), gene isolation of *Parechinus angulosus* (Pfeffer & von Holt, 1991), and new geographical records of sea urchins (Marshall *et al.*, 1991) being published. Holothurians, however, have received limited attention, which has mainly concentrated on their taxonomy and geographical range (Thandar, 1989b, 1989c, 1989d, 1990, 1991; Thandar & Rowe, 1989), while Barkai (1991) has investigated aspects of holothurian ecology. Nevertheless, knowledge of holothurian biology and echinoderm biology in general is poor, when compared to that of other taxonomic groups.

Five species of holothurians are particularly common intertidal inhabitants of the eastern Cape rocky shores (region from Cape St Francis (34°12'S/24°52'E) to Kei Mouth (32°41'S/28°23'E); Lubke, 1988a) of South Africa (pers. obs.). The five species represent one aspidochirote of the family Stichopodidae, viz *Neostichopus grammatus* Clark, and four dendrochirotes, of the family Cucumariidae, viz *Roweia stephensoni* John, *Pseudocnella sykion* Lampert, *Aslia spyridophora* Clark, and *Roweia frauenfeldi frauenfeldi* Ludwig (Fig. 1.1) (Thandar, 1985, 1987a, 1987b, 1991). They are all sympatric in their distribution from Cape Agulhas (34°50'S/20°00'E) to East London (Fig. 1.2) (Thandar, 1985, 1987a, 1987b, 1991). *Neostichopus grammatus* can achieve body lengths of up to 120mm, the colour is

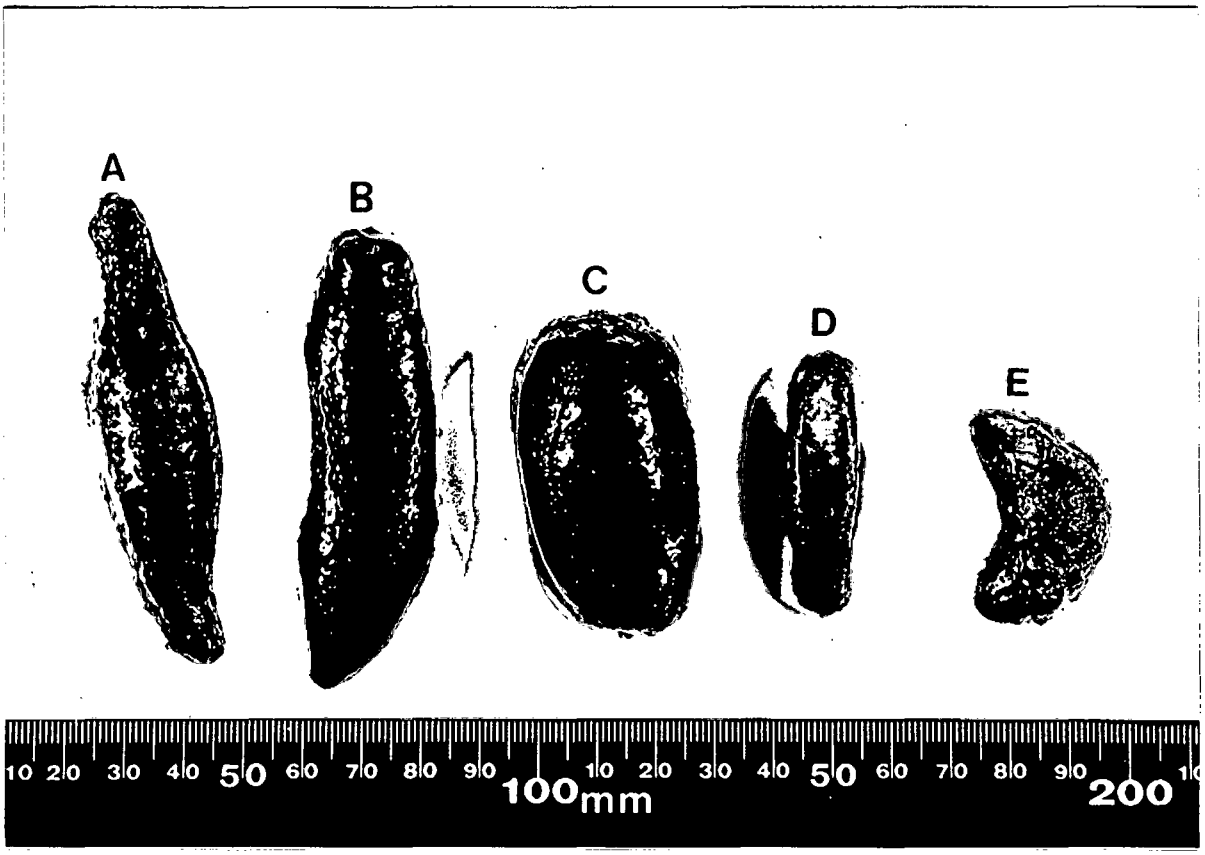


Figure 1.1. Five intertidal holothurian species commonly found on rocky shores along the eastern Cape coast of South Africa. **(A)** *Neostichopus grammatus*; **(B)** *Roweia stephensoni*; **(C)** *Pseudocnella sykion*; **(D)** *Aslia spyridophora*; and **(E)** *Roweia frauenfeldi frauenfeldi*.

usually a shade of red although mottled brown specimens are not uncommon and the body wall is gelatinous in life (Thandar, 1987a); *Roweia stephensoni* reaches a body length of up to 95mm and is uniformly black in colour (Thandar, 1985); *Pseudocnella sykion* is a barrel-shaped species up to 60mm long and is coloured a very dark olive green to black (Thandar, 1987b); *Aslia spyridophora* is a small species (up to 40mm long) and is grey, greyish-brown to maroon in colour (Thandar, 1991); while *Roweia frauenfeldi frauenfeldi* achieves body lengths of up to 90mm and is usually a shade of brown or yellow (Thandar, 1985) (Fig. 1.1). The seemingly high densities that some of these five holothurian species attain on shores within the eastern Cape (pers. obs.) and the important role of holothurians in some ecosystems, prompted an investigation into aspects of the biology and ecology of these five species. The primary aim of this study is to improve our knowledge of the biology of South African holothurians, which in turn is hoped, will lead towards a greater understanding of the role that these invertebrates play in intertidal ecosystems.

The first part of this thesis (Chapter 2) examines the intertidal distribution, density, and biomass of *R. stephensoni*, *P. sykion* and *N. grammatus*. Following on from this, Chapter 3 investigates aspects of the reproductive biology (primarily seasonality of reproduction) of *R. stephensoni*, *P. sykion* and *N. grammatus*, while aspects of the feeding biology of *R. stephensoni*, *P. sykion*, *A. spyridophora*, *R. f. frauenfeldi* and *N. grammatus* are dealt with in Chapter 4. Finally, Chapter 5 presents a general discussion, and suggests avenues for future work.

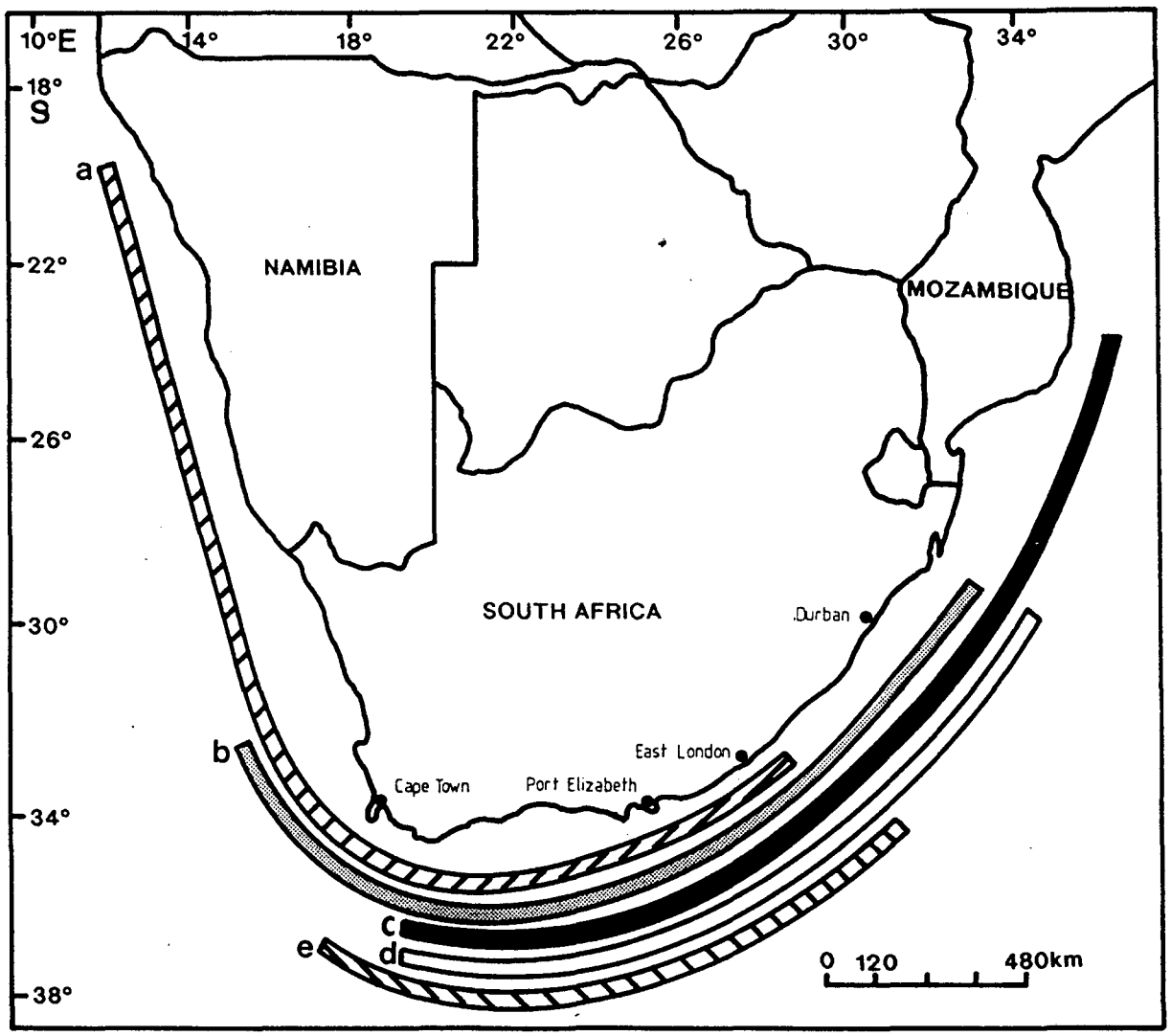


Figure 1.2. The southern African geographical distribution of five intertidal holothurians (after Thandar, 1985, 1987a, 1987b, 1991). **a**: *Roweia frauenfeldi frauenfeldi*; **b**: *Aslia spyridophora*; **c**: *Pseudocnella sykion*; **d**: *Neostichopus grammatus*; and **e**: *Roweia stephensoni*.

CHAPTER TWO

DISTRIBUTION, DENSITY AND BIOMASS OF THREE SYMPATRIC SPECIES OF INTERTIDAL HOLOTHURIANS

2.1 INTRODUCTION

To understand the structure and function of an intertidal ecosystem an adequate knowledge of the community composition, abundance of organisms at each trophic level and the flow of energy to each trophic level is crucial (Odum, 1971). Holothurians are prominent benthic organisms in many marine shallow-water (e.g. Lawrence & Kafri, 1979; Lawrence & Guille, 1982b; Sloan, 1982) and deep-sea ecosystems (review in Billett, 1991), where they are capable of attaining high densities. In addition, they also follow two principal feeding modes, viz suspension-feeding or deposit-feeding (Massin, 1982b), and in some ecosystems suspension-feeding holothurians are reported to constitute an important component of the filter-feeding community (e.g. Velimirov *et al.*, 1977; Field *et al.*, 1980). Levinton (1972), suggests that in such instances there is more likely to be competitive exclusion for feeding space than for food resources with other filter-feeders. By contrast, deposit-feeding holothurians have a large impact on the ecosystem in which they live. For example, Crozier (1918) has estimated that in a small enclosed bay in Bermuda of area 1.7 square miles,

Stichopus species passed between 500 and 1000 tons of substrate through their intestines annually. In the process, they rework the sediment and alter the bottom stability (Massin, 1982a), encourage the return of nutritive elements to the water column (Rhoads & Young, 1971) and increase the amount of sediment-associated bacteria (Frankenberg & Smith, 1967; Hauksson, 1979; Sloan & von Bodungen, 1980). Consequently, due to their densities, and the feeding activity of the deposit-feeders, holothurians must have an important effect on the environment within their ecosystem.

The densities and biomasses of numerous intertidal, subtidal and deep-sea holothurians have been recorded from various parts of the world (e.g. Rutherford, 1973; Nichols, 1975; Lawrence & Kafri, 1979; Sibuet & Lawrence, 1981; Conand, 1982; Engstrom, 1982; Lawrence & Guille, 1982b; Sloan, 1982; Billett, 1991). Holothurians are common marine organisms along the South African coast and have been recorded in numerous intertidal and subtidal surveys (Stephenson, 1944; Deichmann, 1948; Day, 1974; Velimirov *et al.*, 1977; Field *et al.*, 1980; Branch & Branch, 1981; McLachlan *et al.*, 1981; Barkai & Branch, 1988; Dower, 1989), in addition they have been subject to numerous taxonomic studies (Thandar, 1977, 1985, 1986, 1987a, 1987b, 1988, 1989b, 1989c, 1989d, 1990, 1991; Thandar & Rowe, 1989). Although their geographical range is well documented (see works of Thandar, 1977-1991), knowledge of South African holothurian intertidal and subtidal zonation, density and biomass is limited to a few reports for species on the west coast of South Africa (e.g. Velimirov *et al.*, 1977; Field *et al.*, 1980), and is non-existent for species on the eastern Cape coast of South Africa.

In the eastern Cape of South Africa, although the coastline consists of extensive stretches of

sandy beaches, there are numerous types of rocky shores, most of which are mixed shores (neither homogeneous rock or sand) subject to frequent sand scour or inundation (Dower, 1989). Three of the common species of holothurians, *Roweia stephensoni*, *Pseudocnella sykion* and *Neostichopus grammatus*, inhabit these rocky outcrops (Stephenson, 1944; McLachlan *et al.*, 1981; Dower, 1989). *Roweia stephensoni* and *P. sykion* are sedentary species (pers. obs.), whilst *N. grammatus* is highly mobile and roams over the adjacent sediments, consuming it in the process (Chapter 4).

These three species formed the basis of a study on reproductive seasonality (Chapter 3) and therefore it was decided to establish their intertidal distribution, density and biomass prior to the reproductive work. Similarly, holothurians are prominent components of some marine communities in which they play an important role. An assessment of the standing stock of holothurians in any given ecosystem aids in elucidating the impact these invertebrates may have in that ecosystem. Consequently, this study presents data on the intertidal distributions, densities and biomasses of *R. stephensoni*, *P. sykion* and *N. grammatus*, at various sites within the eastern Cape.

2.2 MATERIALS AND METHODS

2.2.1 Species identification

Although the intertidal distributions, densities and biomasses of only three species were investigated in this chapter, the identification procedure used applies to all the species studied (Chapter 4 discusses the feeding biology of five species). Species identification was based on spicule composition, which were extracted by boiling a piece of the body wall in 10% potassium hydroxide solution for several minutes (Mahoney, 1966). The spicule composition

of each species was then compared to that given in the taxonomic keys of Thandar (1985, 1987a, 1987b, 1991). The key to holothurians in Day (1974), using the gross external morphology of the animals, was also used to assist in species identification. Preserved specimens were also sent to Prof. A.S. Thandar (University Durban-Westville, South Africa) for confirmation of species identification.

2.2.2 Study sites

To determine the intertidal distributions, densities and biomasses of *R. stephensoni*, *P. sykion* and *N. grammatus*, six rocky shores were examined from Port Elizabeth to just north of East London (Fig. 2.1). All sites lay within the boundaries which delimit the "eastern Cape region" of South Africa as proposed by Lubke (1988a). In this region two rock types occur: consolidated dune (aeolian calcarenites) and hard quartzitic sandstone (Marker, 1988; Dower, 1989). Dower (1989) recorded that three of the six holothurian species encountered in her study were only found on quartzitic sandstone shores and not on dune rock shores within the eastern Cape. Preliminary inspections of the different shore types confirmed her findings. Consequently, only quartzitic sandstone shores were sampled. The six sites sampled were (from west to east) (Fig. 2.1):- Port Elizabeth (33°58'S/25°38'E), Cannon Rocks (33°44'S/26°35'E), Port Alfred (33°36'S/26°54'E), Kayser's Beach (33°15'S/27°37'E), Gonubie (32°56'S/28°02'E) and Cintsa West (32°50'S/28°09'E) (grid references obtained from Skead, 1973). All sites are exposed to the prevailing westerly swell.

The Port Elizabeth site was characterised by numerous boulders of various sizes (≈10-100cm in diameter), with minimal sand inundation of a coarse grain size, consisting predominantly of numerous small pebbles and shell fragments (>3mm in diameter). Cannon Rocks was

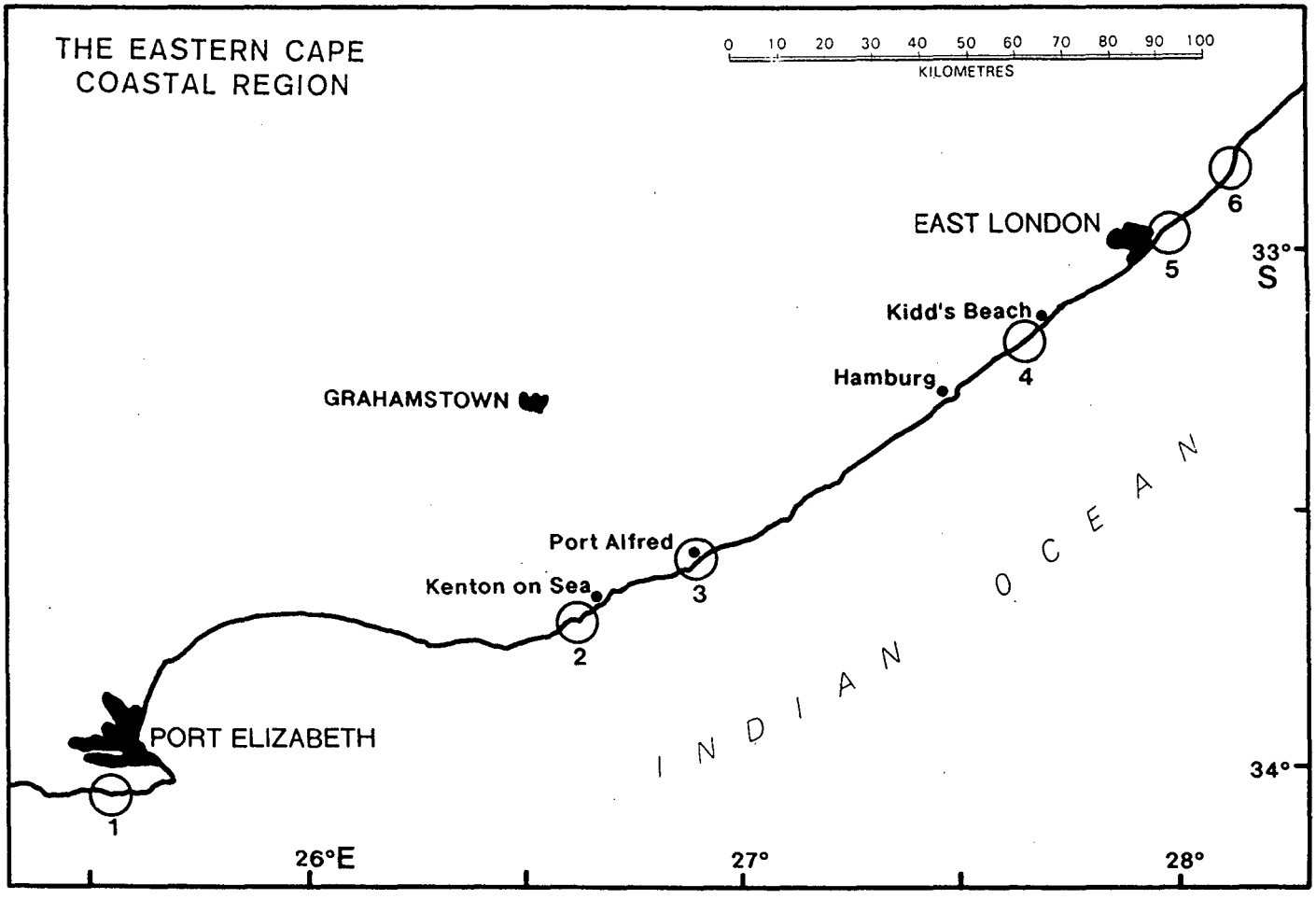


Figure 2.1. The eastern Cape coastal region showing the location of the six study sites: **(1)** Port Elizabeth, **(2)** Cannon Rocks, **(3)** Port Alfred, **(4)** Kayser's Beach, **(5)** Gonubie, and **(6)** Cintsa West.

characterized by low-lying rock outcrops with few boulders, and was heavily inundated with sand of a medium to fine grain size. Port Alfred was characterised by ridges of rocky outcrops with sand-filled gullies (medium to fine grain size) containing some boulders (≈50-80cm in diameter). Kayser's Beach was characterised by a wave cut platform encompassing relatively few crevices, which were free from sand inundation. Gonubie was characterised by numerous boulders of various sizes (≈10-120cm in diameter), and had minimal sand inundation. Lastly, Cintsa West was characterised by ridges of rocky outcrops with gullies filled with moderate amounts of sand (medium to coarse grain size) and some boulders of various sizes (≈20-80cm in diameter).

2.2.3 Sampling procedure

Due to inadequate bench-marks 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 Cape, viz the Littorina, upper Balanoid, lower Balanoid, Cochlear and Infratidal zones (Fig. 21 in Branch & Branch, 1981). The Littorina zone represents the high water spring level, the Balanoid zones the mid-shore, whilst the Cochlear zone represents the low water spring level (Branch & Branch, 1981). The pattern of zonation for the eastern Cape closely resembles that of the southern Cape (Lubke, 1988b).

At each study site three transects, 20 metres apart, were taken from the top of the shore to the low water spring level during spring tides. Along each transect a $1m^2$ quadrat was sampled at 5 or 10 metre intervals, depending on the length of the transects. The three species of

holothurians found within each quadrat were removed and subsequently counted and weighed (drained wet weight). Drained wet weights were used to avoid the influence of water contained within the water-vascular system (Nichols, 1966). The percentage water (drying to constant weight at 60°C) and the energetic content (calculated by bomb-calorimetry) of 100 individuals for each species was determined. This provided a conversion ratio so that the wet weight values of subsequent samples could be easily converted into dry weight and energetic biomass values. Energetic values are the most meaningful units of biomass for ecological research, as it gives an indication of the energy that can be potentially passed through the ecosystem (Odum, 1971).

2.3 RESULTS

2.3.1 Distribution, density and biomass

The intertidal distributions, densities and biomasses (drained wet weight) of *Roweia stephensoni*, *Pseudocnella sykion* and *Neostichopus grammatus*, together with the intertidal profiles are illustrated in Figures 2.2A-C and 2.3A-C. At all sites the three species were always found under boulders or in rock crevices. The holothurians also tended to occur in aggregations under boulders and in crevices causing them to have a patchy distribution, which resulted in large variations in density and biomass within a study site. However, despite this variability a general zonation pattern emerged and where the three species co-occurred, similar overlapping zonation existed.

Tables 2.1 & 2.2 provides data on the average densities and biomasses of *R. stephensoni* and *P. sykion* at the six sites. All species were absent from the high-shore region. *Roweia stephensoni* and *P. sykion* were distributed from the Balanoid zone (mid-shore) to the

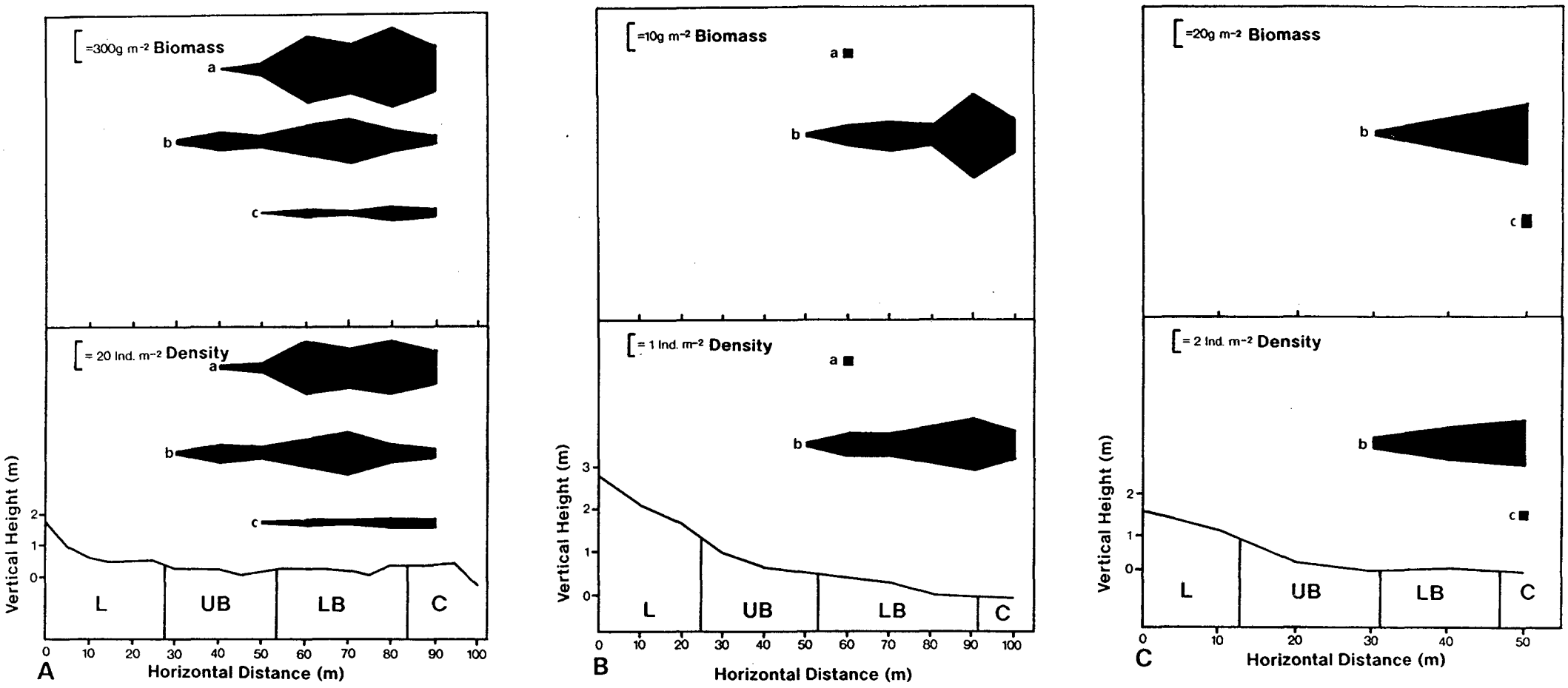


Figure 2.2. Intertidal distributions, densities and drained wet weight biomasses of **a**: *Roweia stephensoni*, **b**: *Pseudocnella sykion* and **c**: *Neostichopus grammatus* at: **(A)** Port Elizabeth, **(B)** Cannon Rocks, and **(C)** Port Alfred.

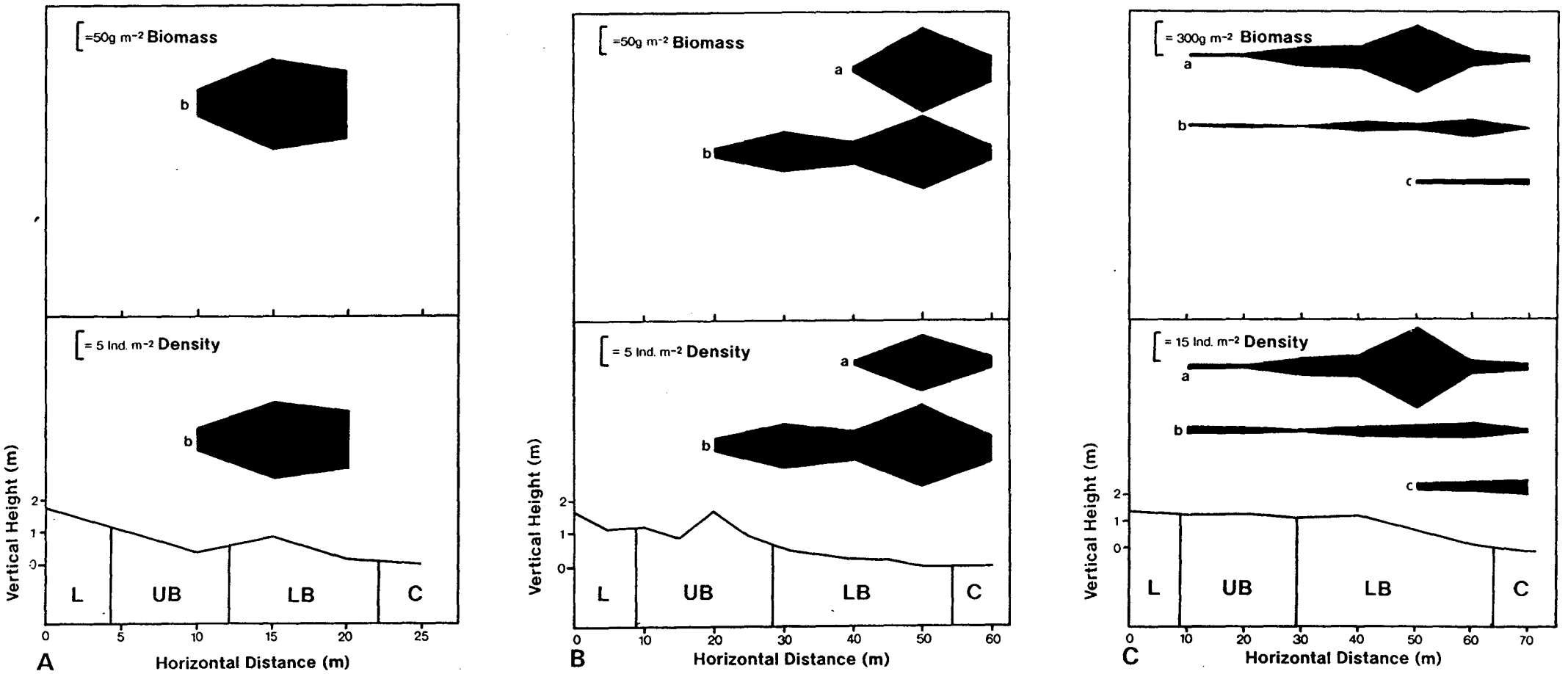


Figure 2.3. Intertidal distributions, densities and drained wet weight biomasses of **a**: *Roweia stephensoni*, **b**: *Pseudocnella sykion* and **c**: *Neostichopus grammatus* at: **(A)** Kayser's Beach, **(B)** Gonubie, and **(C)** Cintsa West.

Cochlear zone (low-shore) (Figs. 2.2A-C & 2.3A-C). At most sites, these two species reached their highest densities and biomasses in the lower Balanoid zone (lower mid-shore) (Tables 2.1 & 2.2). The maximum density recorded for *R. stephensoni* was 132 individuals/m^2 at Port Elizabeth, whilst the maximum recorded for *P. sykion* was 47 individuals/m^2, also at Port Elizabeth. Whereas, *P. sykion* was present at all sites, *R. stephensoni* was only found at Port Elizabeth, Gonubie, Cintsa West, and Port Alfred, and not encountered at the remaining two sites. Although, *R. stephensoni* and *P. sykion* overlap in their distribution on rocky shores, they have different microhabitats. *Roweia stephensoni* was generally found partially buried in the sediments under boulders, and occasionally in crevices. By contrast, *P. sykion* was always found clinging to the underside of boulders but never in the sediments, or they were also found in crevices.

Table 2.1. Average densities and drained wet weight biomasses of *R. stephensoni*, from three transects, in the mid- and low-shore regions at six sites on the eastern Cape rocky shores of South Africa.

	Upper mid-shore		Lower mid-shore		Low-shore	
	Density (m^{-2})	Biomass (g/m^2)	Density (m^{-2})	Biomass (g/m^2)	Density (m^{-2})	Biomass (g/m^2)
Site	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)
Port Elizabeth	3.2 (5.9)	53.4 (107.7)	39.5 (30.1)	683.7 (516.9)	28.4 (31.2)	528.0 (581.9)
Cannon Rocks	0	0	0.1 (0.3)	0.8 (1.6)	0	0
Port Alfred	0	0	0	0	0	0
Kayser's Beach	0	0	0	0	0	0
Gonubie	0	0	3.9 (8.2)	60.5 (129.3)	2.8 (3.0)	57.0 (63.5)
Cintsa West	3.1 (4.6)	55.7 (79.3)	17.5 (21.5)	276.0 (309.9)	4.0 (4.6)	87.7 (100.5)

Table 2.2. Average densities and drained wet weight biomasses of *P. sykion*, from three transects, in the mid- and low-shore regions at six sites on the eastern Cape rocky shores of South Africa.

	Upper mid-shore		Lower mid-shore		Low-shore	
	Density (m^{-2})	Biomass (g/m^2)	Density (m^{-2})	Biomass (g/m^2)	Density (m^{-2})	Biomass (g/m^2)
Site	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)
Port Elizabeth	8.2 (10.7)	116.5 (148.9)	18.0 (14.7)	269.9 (208.5)	6.0 (6.0)	84.2 (84.0)
Cannon Rocks	0.2 (0.3)	0.4 (0.7)	1.7 (1.8)	16.1 (22.2)	1.8 (1.1)	11.7 (2.5)
Port Alfred	0.5 (0.8)	2.6 (4.4)	2.9 (2.0)	25.4 (14.0)	4.2 (4.5)	49.6 (51.8)
Kayser's Beach	2.2 (3.3)	23.4 (39.1)	14.6 (13.1)	169.4 (151.2)	0	0
Gonubie	1.7 (2.7)	12.8 (22.8)	10.3 (10.5)	84.3 (85.3)	3.8 (4.0)	30.3 (32.6)
Cintsa West	1.8 (1.4)	8.6 (6.4)	7.5 (5.5)	76.3 (65.2)	0.9 (1.0)	8.0 (9.2)

Table 2.3 provides data on the average densities and biomasses of *N. grammatus* at the six sites. *Neostichopus grammatus* is distributed from the lower Balanoid zone (lower mid-shore) to the Cochlear zone (low-shore) (Figs. 2.2A-C & 2.3A-C), where it reaches its highest densities and biomasses (Table 2.3). The maximum density recorded for *N. grammatus* was 19 individuals/m^2 at Cintsa West. This species was only found at Port Elizabeth, Cintsa West and Port Alfred, and not encountered at the remaining sites. *Neostichopus grammatus* was generally found on the sediment under boulders, or clinging to the underside of the boulders.

Table 2.3. Average densities and drained wet weight biomasses of *N. grammatus*, from three transects, in the mid- and low-shore regions at six sites on the eastern Cape rocky shores of South Africa.

Site	Upper mid-shore		Lower mid-shore		Low-shore	
	Density (m^{-2})	Biomass (g/m^2)	Density (m^{-2})	Biomass (g/m^2)	Density (m^{-2})	Biomass (g/m^2)
	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)
Port Elizabeth	0.2 (0.4)	2.6 (5.3)	5.7 (5.8)	71.8 (65.2)	5.3 (5.5)	73.7 (77.3)
Cannon Rocks	0	0	0	0	0	0
Port Alfred	0	0	0	0	0.4 (0.5)	13.4 (15.4)
Kayser's Beach	0	0	0	0	0	0
Gonubie	0	0	0	0	0	0
Cintsa West	0	0	2.5 (3.2)	18.9 (21.8)	9.0 (9.2)	49.9 (48.1)

2.3.2 Energetic biomass

Table 2.4 shows the percentage water content and energetic content of the three species. These values were used to calculate the dry weight biomass and hence the energetic biomass from the wet weight biomass values.

Table 2.4. Water and energetic content (dry weight) of three species of eastern Cape intertidal holothurians from South Africa

Species	% Water			Energetic content (kJ/g)		
	n	$\bar{x}$	±SD	n	$\bar{x}$	±SD
R. stephensoni	100	82.3	1.8	100	18.63	1.21
P. sykion	100	71.5	3.2	100	9.55	0.88
N. grammatus	100	89.1	5.1	100	12.11	2.34

Using the energetic values (kJ/g) in Table 2.4, the average energetic biomasses of the three species in the mid- and low-shore regions at all sites were calculated (Table 2.5). The energetic biomass of *R. stephensoni* was greater than the other two species at Port Elizabeth and Cintsa West, but *P. sykion* was the dominant species at Cannon Rocks, Port Alfred, Kayser's Beach and Gonubie. *Neostichopus grammatus* always had the lowest energetic biomass (<100kJ/g) throughout the study sites.

Table 2.5. Average energetic biomasses of *R. stephensoni* (RS), *P. sykion* (PS) and *N. grammatus* (NG), from three transects, in the mid- and low-shore region at six sites on the eastern Cape rocky shores of South Africa.

		Energetic biomass (Kj/m²)		
		Upper mid-shore	Lower mid-shore	Low-shore
Site	Species	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)	$\bar{x}$ (±SD)
Port Elizabeth	RS	176.3 (352.1)	2254.6 (1704.2)	1741.8 (1918.2)
	PS	317.1 (405.3)	734.6 (567.5)	229.2 (228.6)
	NG	3.4 (7.0)	94.8 (86.1)	97.2 (102.1)
Cannon Rocks	RS	0	2.7 (5.3)	0
	PS	1.1 (1.9)	43.8 (60.5)	31.8 (6.8)
	NG	0	0	0
Port Alfred	RS	0	0	0
	PS	7.1 (11.9)	69.1 (38.1)	135.0 (140.9)
	NG	0	0	17.7 (20.3)
Kayser's Beach	RS	0	0	0
	PS	63.7 (106.4)	461.1 (411.5)	0
	NG	0	0	0
Gonubie	RS	0	199.5 (426.4)	187.9 (209.4)
	PS	34.9 (62.1)	229.5 (232.2)	82.5 (88.7)
	NG	0	0	0
Cintsa West	RS	183.7 (261.6)	910.1 (1021.9)	289.1 (331.4)
	PS	23.4 (17.4)	207.7 (177.4)	21.8 (25.0)
	NG	0	24.9 (28.8)	65.8 (63.5)

2.3.3 Biomass indices

The effect of the different biomass indices (e.g. density, wet weight biomass, dry weight biomass and energetic biomass) on the relative importance of each species from each site is given in Figures 2.4, 2.5, 2.6, 2.7 and 2.8. Kayser's Beach was excluded from this comparison as only *P. sykion* was present. The relative proportion that each species contributed to the total biomass of the three species varied according to the way by which biomass was expressed. For example, although *R. stephensoni* always contributed the greatest proportion to the total biomass at Port Elizabeth (Fig. 2.4), when expressed as density *R. stephensoni* comprised 55.6%. Using wet and dry biomass the relative importance of *R. stephensoni* was 60.7% and 51.8%, respectively. The proportional contribution to the energetic biomass was highest at 67.3%. A similar trend can also be seen at Cintsa West. By contrast, *P. sykion* always contributed the greatest proportion to the total biomass of the three species at Cannon Rocks, Port Alfred and Gonubie, while *N. grammatus* always contributed the least at all sites.

2.4 DISCUSSION

The intertidal rocky shore is regarded as one of the most stressful physical environments (Branch & Branch, 1981). This may account for the intertidal distribution of *R. stephensoni*, *P. sykion* and *N. grammatus*. These holothurians were seldom found in the high-shore where aerial exposure is relatively long and the potential for water loss very high. They were, however, most common in the mid- and low-shore regions were aerial exposure is reduced, and desiccation potentially lower. Individuals inhabiting the low shore will only be exposed for a brief period, and this may explain the increased presence of *N. grammatus* as it has a very thin body wall, making it susceptible to rapid desiccation (pers. obs.). Barkai (1991) has indicated that the most important factor influencing the distribution of three South African

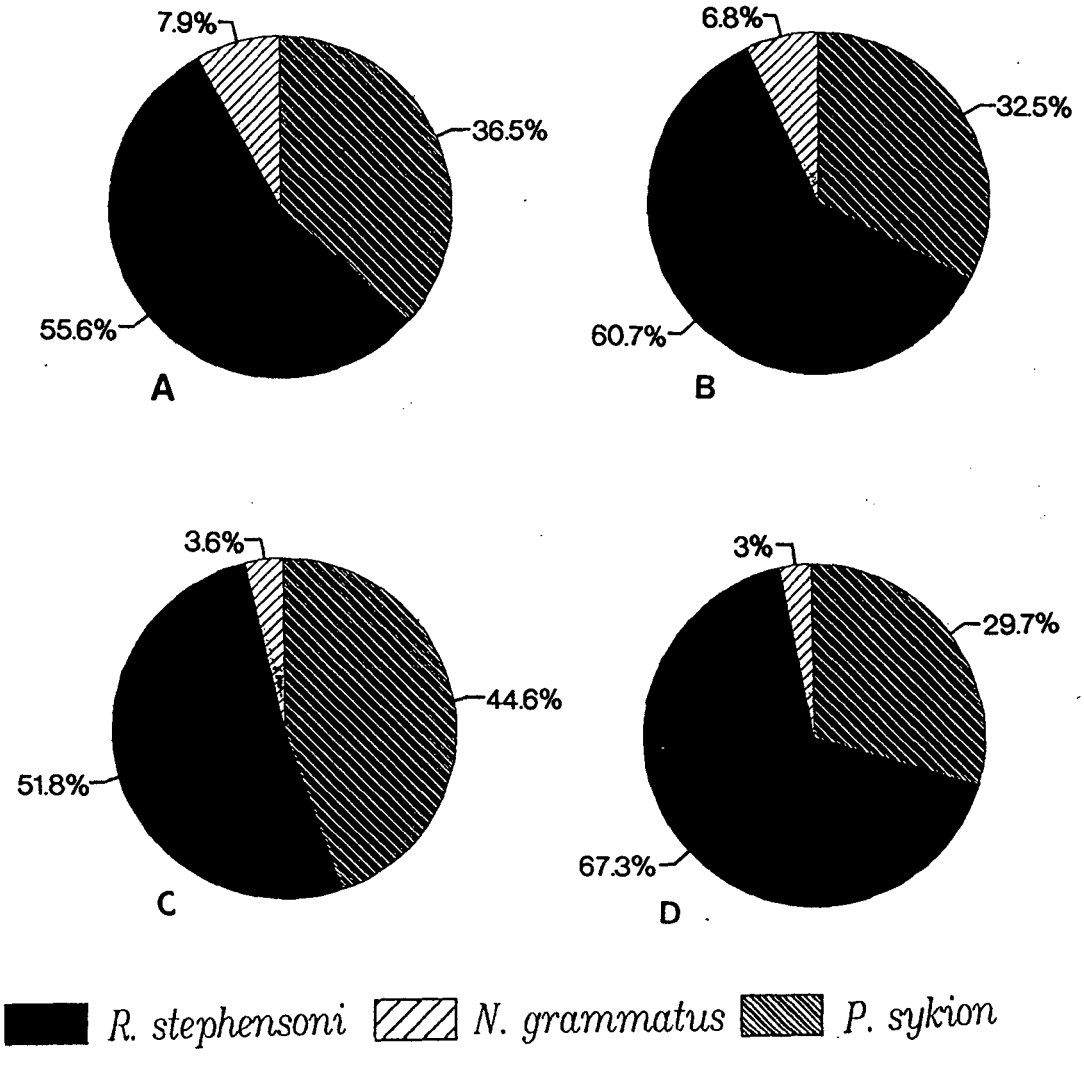


Figure 2.4. Port Elizabeth. Pie charts showing the relative proportion that *Roweia stephensoni*, *Pseudocnella sykion* and *Neostichopus grammatus* contributed to the total shore biomass in terms of: **(A)** density, **(B)** drained wet weight biomass, **(C)** dry weight biomass, and **(D)** energetic biomass.

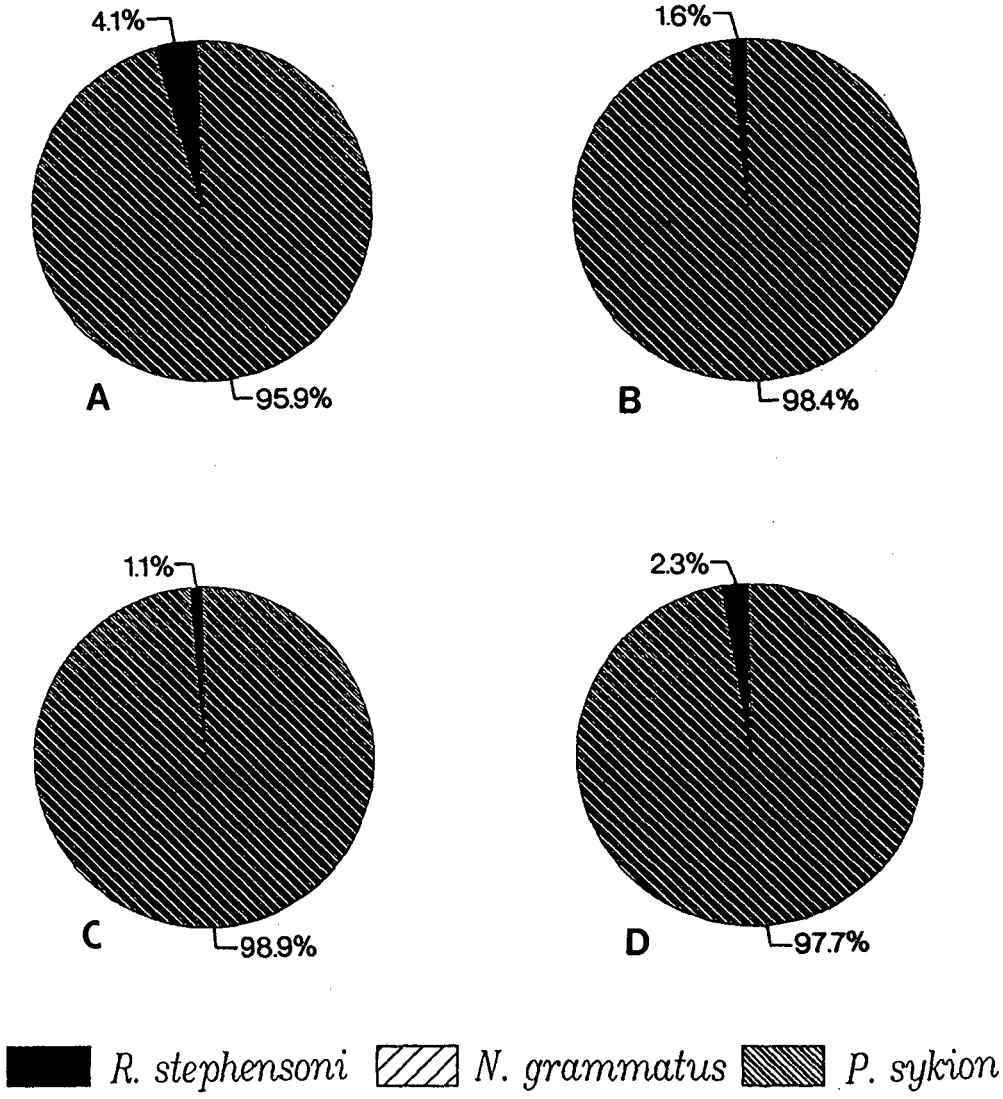


Figure 2.5. Cannon Rocks. Pie charts showing the relative proportion that *Roweia stephensoni*, *Pseudocnella sykion* and *Neostichopus grammatus* contributed to the total shore biomass in terms of: **(A)** density, **(B)** drained wet weight biomass, **(C)** dry weight biomass, and **(D)** energetic biomass.

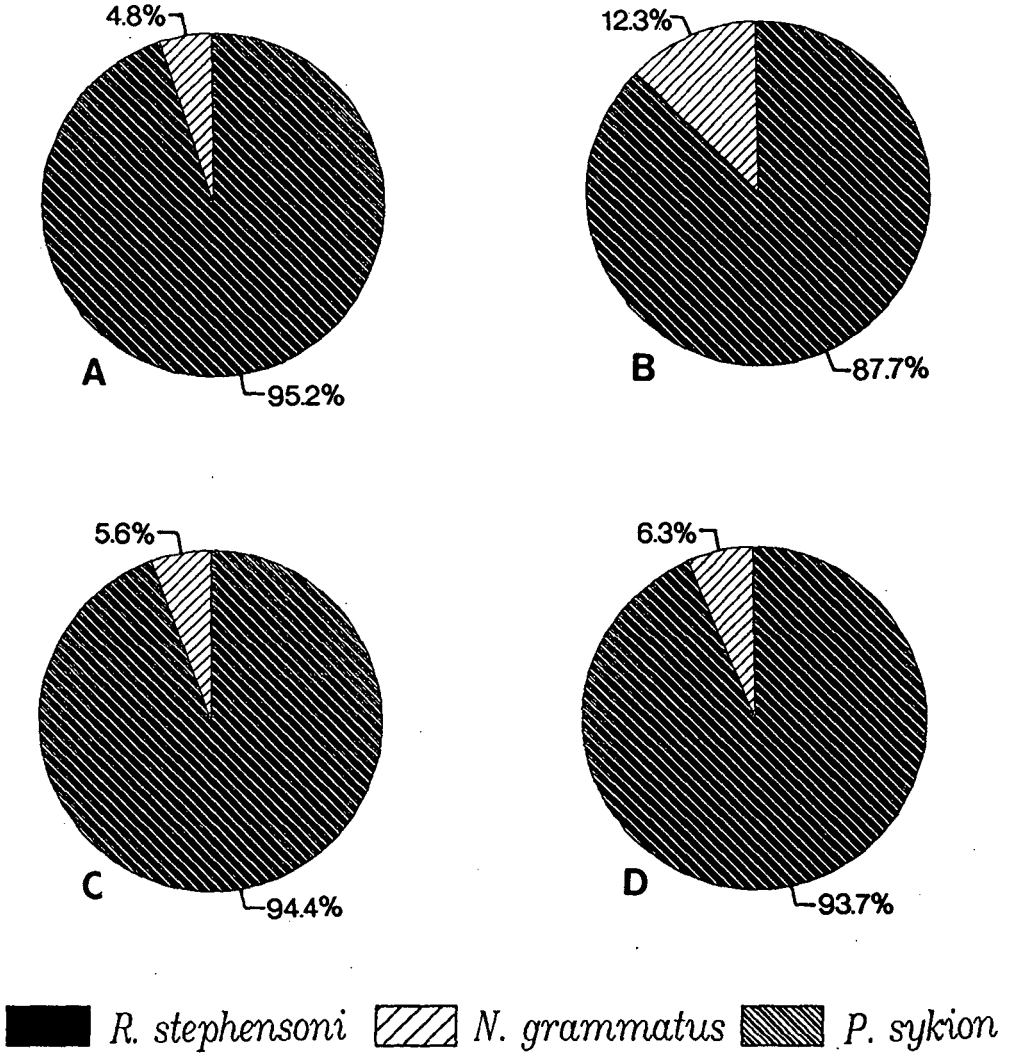


Figure 2.6. Port Alfred. Pie charts showing the relative proportion that *Roweia stephensoni*, *Pseudocnella sykion* and *Neostichopus grammatus* contributed to the total shore biomass in terms of: **(A)** density, **(B)** drained wet weight biomass, **(C)** dry weight biomass, and **(D)** energetic biomass.

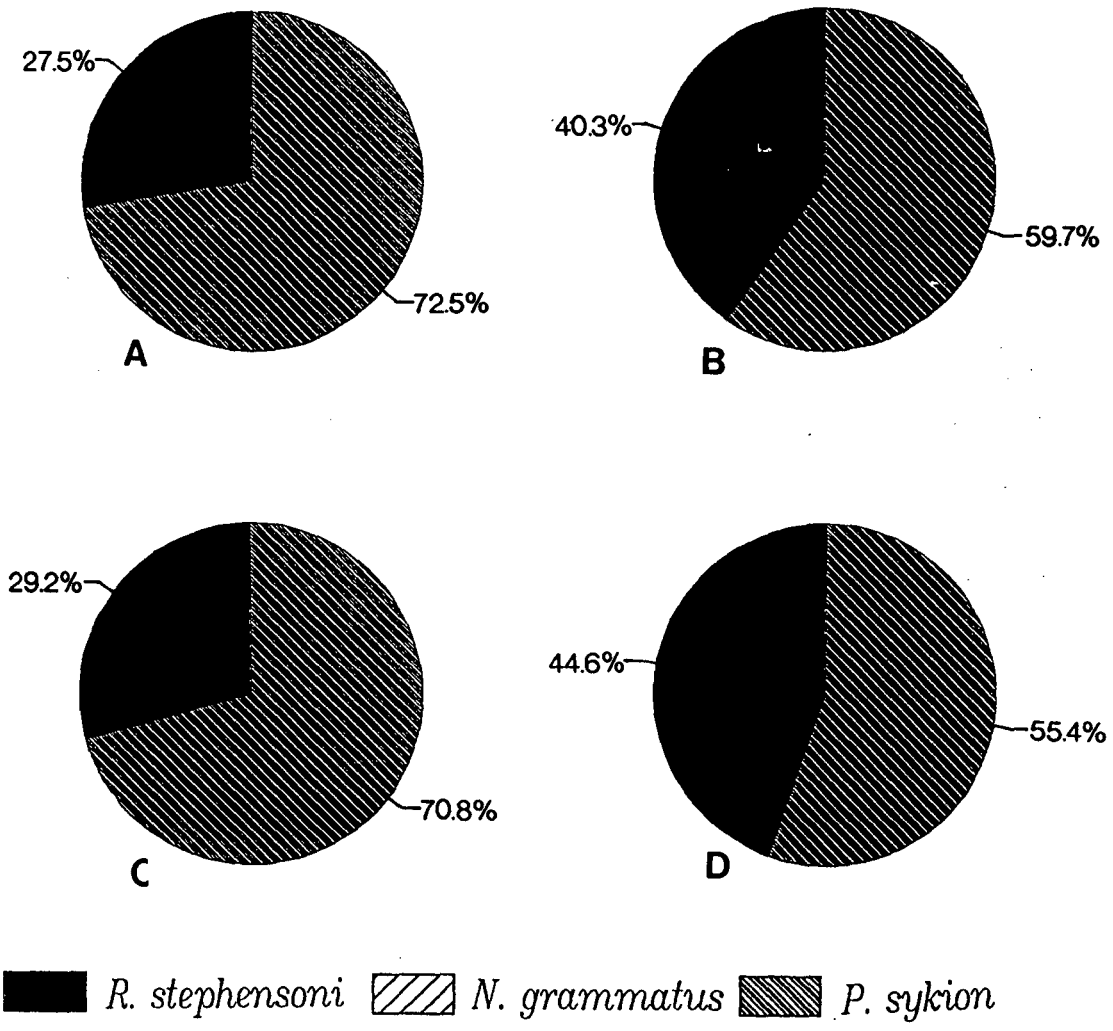


Figure 2.7. Gonubie. Pie charts showing the relative proportion that *Roweia stephensoni*, *Pseudocnella sykion* and *Neostichopus grammatus* contributed to the total shore biomass in terms of: **(A)** density, **(B)** drained wet weight biomass, **(C)** dry weight biomass, and **(D)** energetic biomass.

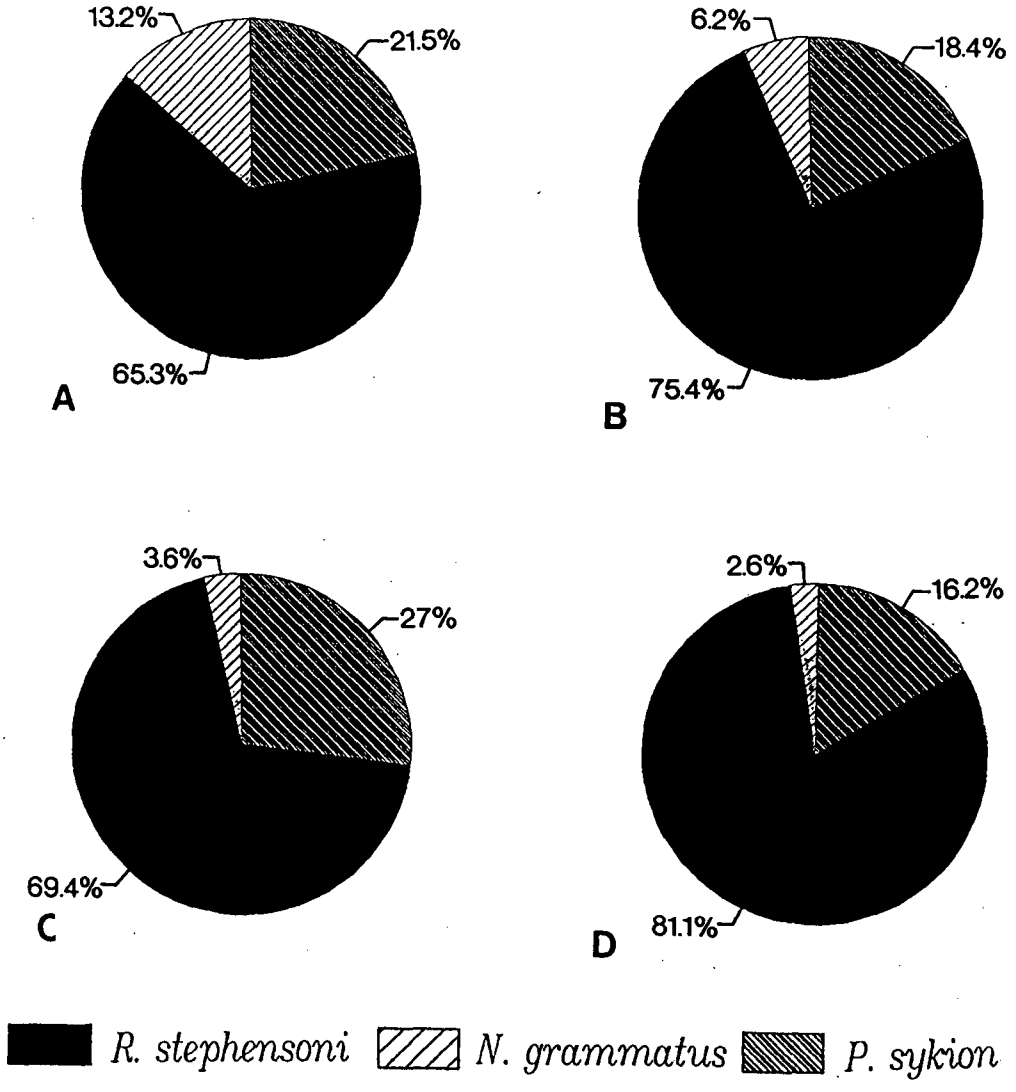


Figure 2.8. Cintsa West. Pie charts showing the relative proportion that *Roweia stephensoni*, *Pseudocnella sykion* and *Neostichopus grammatus* contributed to the total shore biomass in terms of: **(A)** density, **(B)** drained wet weight biomass, **(C)** dry weight biomass, and **(D)** energetic biomass.

sublittoral holothurians was direct water motion. Suspension-feeding holothurians, such as *R. stephensoni* and *P. sykion*, are dependant of natural water flow for efficient feeding (Chapter 4), and consequently this determines their habitat selection (Massin, 1982b). Being situated high up on the shore would not only pose problems of desiccation but also, animals would have less time for feeding.

The observed aggregating behaviour of *R. stephensoni*, *P. sykion* and *N. grammatus* has also been reported for many other echinoderms (Buchanan, 1966; Reese, 1966; Birkeland & Chia, 1971; Velimirov, 1984; Barkai, 1991; Billett, 1991 and references therein; Freire *et al.*, 1992), and the tendency to form aggregations under boulders or in crevices may be adaptive in several respects. Firstly, it may be a strategy to avoid direct sunlight, as many holothurians are regarded to be photonegative (Pawson, 1966). Similarly, Lees & Carter (1972) have indicated that the covering response of the echinoid, *Lytechinus anamesus*, was related to sunlight and water surge. Secondly, it may be a strategy to avoid wave action, as Barkai (1991) has indicated that two South African west coast holothurians, *Thyone aurea* and *Pentacta doliolum*, clumped together in rough waters. The feeding mode of *R. stephensoni* and *P. sykion* (suspension-feeders, see Chapter 4), requires that they inhabit areas of wave action or turbulent water to ensure a constant food supply. Hiding under boulders or in crevices, with only their tentacles exposed, they probably receive some degree of protection. Similarly, *N. grammatus*, although a deposit-feeder (Chapter 4) and less reliant on wave action for a continuous food supply, probably also seeks shelter under boulders from high wave action. Thirdly, it may allow the species to exploit substrates which would be unsuitable for single individuals due to long aerial exposure and desiccation at low tides. Aggregating may result in the retention of water between individuals in the clump, allowing them to

survive long aerial exposure at low tides (Rutherford, 1973). Lastly, it may be a strategy to ensure reproductive success, as Freire *et al.* (1992) have suggested that in species that rely on external fertilization, as in *R. stephensoni*, *P. sykion* and *N. grammatus* (Chapter 3), clumping would be advantageous, allowing for synchronous spawning and the release of male and female gametes in close proximity to one another. Rutherford (1973) has also suggested that the mode of development helps to assure the formation of aggregations. Since most sedentary marine animals, such as *R. stephensoni* and *P. sykion*, settle on substrates which are comparatively limited in area and distribution, it would be "wasteful" if the larvae drifted far from the parent's home. Direct development, as reported for most dendrochirote holothurians (Smiley *et al.*, 1991), not only assures the formation of aggregations but also ensures that the larvae settle on suitable substrates with a minimal loss of young.

The densities and biomasses of *R. stephensoni*, *P. sykion* and *N. grammatus* varied considerably from site to site along the eastern Cape coast, and may be attributed to differences in shore geomorphology between sites. The greatest holothurian richness and densities were recorded at Port Elizabeth and Cintsa West. These sites provide numerous "hiding places" under boulders and ledges, and had minimal to moderate amounts of sand inundation. The lack of holothurian richness and density at the remaining four sites may be attributed to various aspects, from shore geomorphology to human impact. For example, the site at Port Alfred suffered high human impact during the holiday seasons with people constantly turning the intertidal boulders in search of bait. The site at Cannon Rocks can be heavily inundated with sand which will probably result in sand scouring and an unstable substratum, both of which are factors that can reduce species richness and abundance (Coetzee, 1991). On the other hand, the site at Gonubie had practically no sand inundation

which may explain the absence of *N. grammatus*, as it is a deposit-feeder and ingests sand particles in order to obtain food (Chapter 4). Similarly, the site at Kayser's Beach was a wave cut platform offering relatively few "hiding places", and had no sand inundation.

Table 2.6 compares the mean densities and energetic biomasses of *R. stephensoni*, *P. sykion* and *N. grammatus* at Port Elizabeth with that recorded for other species of holothurians. Most abyssal holothurians have extremely low densities and energetic biomasses when compared to shallow-water holothurians (Table 2.6). However, this may be as a result of sampling difficulties (Billett, 1991). As expected, even amongst shallow-water holothurians the densities and energetic biomasses varied considerably from country to country (Table 2.6). However, when compared to other southern African holothurians, the energetic biomasses of *R. stephensoni* (2254 kJ/m^2), *P. sykion* (734 kJ/m^2) and *N. grammatus* (94 kJ/m^2) at Port Elizabeth are similar to values reported for *Pentacta doliolum* (54-4323 kJ/m^2) and *Thyone aurea* (28-2089 kJ/m^2) on the west coast by Velimirov *et al.* (1977). Similarly, the densities of *R. stephensoni* (39.5m^{-2}) and *P. sykion* (18m^{-2}) are similar to that reported for *R. frauenfeldi frauenfeldi* (25-30m^{-2}) on the Cape Peninsula of South Africa by Korrûbel (1991). This study also supports the importance of using energetic values when comparing the biomasses of different species. Wet weight biomass generally over-estimates the importance of animals with a high water content, such as *N. grammatus*, while dry weight biomass generally over-estimates the importance of animals with increased skeletal material, such as *P. sykion.* Similarly, the reduction in the relative importance of *P. sykion*, when expressed in terms of energetic biomass, may be attributed to the large amount of inorganic material in the body wall.

Table 2.6. Mean densities and energetic biomasses of some shallow-water and abyssal holothurians, (*) indicates South African species.

Species	Density (m^{-2})	Biomass (kJ/m^2)	Source
SHALLOW-WATER			
Holothuria glaberina	36	1722	Lawrence & Kafri (1979)
Chiridota rigida	288	129	Lawrence & Kafri (1979)
Afrocucumis africana	546	430	Lawrence & Kafri (1979)
Holothuria difficilis	324	259	Lawrence (1980): in Sibuet & Lawrence (1981)
Holothuria hilla	52	451	
Holothuria leucosiplota	24	815	"
Stichopus chloronotus	15	764	"
Actinopyga mauritana	12	23700	"
Holothuria pervicax	9.3	-	Sloan (1982)
Holothuria impatiens	62.8	-	Sloan (1982)
Stichopus sp.	6.9	-	Sloan (1982)
Stereoderma laevigata	60	480.7	Lawrence & Guille (1982b)
Cladodactyla crocea	100	718	Lawrence & Guille (1982b)
Eumolpadia violacea	1.4	250	Lawrence & Guille (1982b)
Trachythyone elongata	208	585	Lawrence & Guille (1982b)
Aslia lefevrei	70	-	Costelloe & Keegan (1984)
Pentacta doliolum *	-	1585.8	Velimirov *et al.* (1977)
Thyone aurea *	-	924.2	Velimirov *et al.* (1977)
Roweia f. frauenfeldi *	27	-	Korrûbel (1991)
Roweia stephensoni *	39.5	2254.6	Present study
Pseudocnella sykion *	18	734.6	Present study
Neostichopus grammatus *	5.7	94.8	Present study
ABYSSAL			
Benthogone rosea	0.0074	0.41	Sibuet & Lawrence (1981)
Paelopatides gigantea	0.0035	0.34	Sibuet & Lawrence (1981)
Psychropotes longicauda	0.0007	0.27	Sibuet & Lawrence (1981)
Stichopus tremulus	0.0197	-	Billett (1991)
Ypsilothuria talismani	9.5379	-	Billett (1991)
Kolga hyalina	50.170	-	Billett (1991)
Malpadia intermedia	15.2	-	Nichols (1975)

McLachlan *et al.* (1981) have indicated that *Cucumaria frauenfeldi* (= *Roweia frauenfeldi*, Thandar, 1985), was one of the dominant filter-feeders and constituted 6% of the shore biomass at Flat Rocks and Skoenmakerskop, on the Port Elizabeth rocky shore. Skoenmakerskop is only 2-3km from the present study site at Port Elizabeth, and although *R. frauenfeldi* was present it was seldom encountered. This raises two questions: firstly, doubt as to the correct identification of this species by McLachlan *et al.* (1981), because *R. stephensoni* and *P. sykion* were found to be the dominant holothurians in this study and their biomass values indicate that they could make up 6% of shore biomass. Secondly, the holothurian species present on this shore have changed composition in the last 12 years, or holothurian composition changes from site to site along this shore.

In conclusion, in the eastern Cape, holothurians form an important component of intertidal shores which have boulders combined with coarse sediment, and more detailed investigations of other South African shores could reveal a similar situation. Clearly more quantitative surveys on South African holothurian densities and biomasses are required in intertidal, subtidal and deep-sea ecosystems. Future studies may involve monitoring the temporal changes in population structure, and determining the factors that contribute to the variation in densities and biomasses within and between sites.

CHAPTER THREE

ANNUAL REPRODUCTIVE CYCLES OF THREE SYMPATRIC SPECIES OF INTERTIDAL HOLOTHURIANS

3.1 INTRODUCTION

In invertebrates, reproductive modes are diverse and range from primitive to highly complex and in addition, they may either be of a sexual or asexual nature (Giese, 1959). The annual pattern of reproduction in marine invertebrates is very varied and may occur rhythmically or sporadically during part or all of the year (Giese, 1959). It is generally accepted that reproductive patterns are controlled by certain stimuli, which may be of endogenous, or exogenous origin such as temperature, food availability, salinity, and photoperiod (Giese & Pearse, 1974; Grahame & Branch, 1985).

The holothuroid reproductive system is unique amongst invertebrates and differs from all other living echinoderms in that holothurians possess a single gonad (Boolootian, 1966; Tyler & Gage, 1983; Costelloe, 1985; Smiley & Cloney, 1985; Cameron & Fankboner, 1986; Barnes, 1987; Hamel *et al.*, 1993). The gonad is situated in the anterior of the coelom and is comprised of numerous tubules which are united at their bases, and opens to the exterior

via a dorsal pore usually situated amongst the buccal tentacle crown (Hyman, 1955). Holothurians are usually dioecious, and typically only reproduce once a year, releasing their gametes into the sea where they are fertilized (Boolootian, 1966; Smiley *et al.*, 1991). Development of the larvae is usually direct (McEuen & Chia, 1991; Smiley *et al.*, 1991) and in some species larvae may be brooded (Rutherford, 1973; Billett, 1991).

Since the general overview of holothurian reproduction of Boolootian (1966), our understanding of the reproductive biology of holothurians from a wide variety of orders in both shallow-water and deep-sea environments has increased (e.g. Krishnaswamy & Krishnan, 1967; Rutherford, 1973; Green, 1978; Conand, 1981, 1982, 1989; Engstrom, 1982; Tyler & Gage, 1983; Costelloe, 1985; Harriott, 1985; Conand, 1989; Sewell & Bergquist, 1990; Billett, 1991; McEuen & Chia, 1991; Smiley *et al.*, 1991; Hamel *et al.*, 1993). One order in particular, the Aspidochirotida, has received considerable attention mainly because of the commercial importance of these echinoderms (Conand, 1989).

The South African coastline is inhabited by numerous species of holothurians (see Thandar, 1977 - 1991; Thandar & Rowe, 1989 for taxonomic and biogeographical works). Apart from a description of the sperm structure of some South African species (Hodgson & Bernard, 1992), there is no published literature on the reproductive biology of these endemic temperate-water species. Furthermore, there is a general need for reproductive information on holothurians from the southern hemisphere (Smiley *et al.*, 1991). Three species of holothurians, *Roweia stephensoni*, *Pseudocnella sykion* and *Neostichopus grammatus*, are common intertidal inhabitants of the rocky shores on the eastern Cape shoreline of South Africa and occur in relatively high densities (Chapter 2). These three species are also

sympatric in their distribution from Cape Agulhas to East London (Fig. 1.2).

This study investigated the reproductive cycles of *R. stephensoni*, *P. sykion* and *N. grammatus* at a site near Port Elizabeth on the eastern Cape coast of South Africa in relation to environmental conditions for a 20 month period. The aims of the study were:- 1) to determine if the three species followed a cyclical annual reproductive pattern; 2) to describe the stages of gametogenesis; and 3) to assess whether the patterns of reproduction bore any relationship to changes in sea temperature and daylength.

3.2 MATERIALS AND METHODS

The reproductive cycles of *Roweia stephensoni*, *Pseudocnella sykion* and *Neostichopus grammatus* were observed at monthly intervals for twenty months from January 1992 to August 1993. The collection area was restricted to a single site (≈800m^2) close to the Willows (west of Cape Receife) near Port Elizabeth (33°58'S/25°38'E) (Fig. 2.1) from the lower Balanoid to Cochlear zone (Chapter 2, Fig. 2.2A). Thirty specimens of each species were collected monthly at full moon spring low tides. All animals were brought back to the laboratory and subsequently dissected as soon as possible. Although animal length has been used to size many holothurians (e.g. Costelloe, 1985), it was impossible to use this parameter in this study, as the holothurians contracted immediately when touched and a constant length could not be obtained. Consequently, the drained volume of the holothurians was used as a size parameter. Attempts were made to restrict the size range of the holothurians collected thus avoiding any influence of size on the gonadal index (Gonor, 1972). Hence, holothurians collected each month were limited to a ranged from 7-11cm^3 in *R. stephensoni*, 9-14cm^3 in *P. sykion* and 7-11cm^3 in *N. grammatus*.

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

3.2.1 Gonad index and other reproductive parameters

The mean monthly gonad index (GI) values for *R. stephensoni*, *P. sykion* and *N. grammatus* were calculated from 10 males and 10 females of each species, and are expressed as a percentage of gonad weight to entire body weight in the formula:-

GI = (dry gonad weight) ÷ (dry gonad + dry body weight) X 100

(after Gonor, 1972)

In *R. stephensoni* and *P. sykion* dry weight GI values were calculated, whilst for *N. grammatus* wet weight GI values had to be calculated as drying the gonad resulted in amounts too small to be weighed accurately at certain times of the year. All masses were recorded to the nearest 0.01g and dry masses were determined after drying to constant weight at 60°C.

Seasonal changes in the gonadal tubule diameters, oocyte diameters and spermatozoon content of the tubules were also observed for each month from August 1992 to August 1993. For tubule diameters, the gonads of each species were spread out in a shallow dish and the diameter of 20 randomly selected tubules of 10 males and 10 females were determined from a region mid-way along the tubule length, using a Wild M5A stereo dissecting microscope fitted with an eye-piece graticule. For oocyte diameters, oocytes were teased from the tubules of 10 females of each species, and the diameters of the first 50 oocytes encountered were

measured, using a Nikon compound light microscope fitted with a Nikon Filar Micrometre eye-piece. Data from the 10 females were pooled (500 oocytes/sample) and used to determine the mean monthly oocyte diameters. The amount of mature spermatozoa present in the tubules during the same time period was expressed as a percentage of the total tubule cross-sectional area occupied by the mature spermatozoa. The area values were obtained by drawing the necessary tubule details from histological slides (see section 3.2.2 on gametogenic cycle) using a Nikon camera-lucida. Five tubules cut in transverse-section were used from five males of each species. The line drawings were then digitized with the aid of a Summergraphics graphics tablet and SigmaScan (Jandel Scientific) software, and the percentage area the mature spermatozoa occupied was calculated.

3.2.2 Gametogenic cycle

Each month from August 1992 to August 1993, 6-8 randomly selected gonadal tubules were removed from 5 males and 5 females of each species and fixed for at least 24 hours in aqueous Bouin's fixative. Tubules were dehydrated using a graded ethanol series, embedded in Paraplast, and where possible transverse-sections (5-7μm in thickness) were cut on a Leitz rotary microtome. Sections were then stained with haematoxylin and eosin (Humason, 1967). Examination of the sections revealed stages of maturity, oogenesis and spermatogenesis, as well as the cross-sectional area of the tubules occupied by mature spermatozoa. Where necessary, slides were photographed with a Nikon Optiphot compound light microscope.

3.2.3 Sex ratios

Throughout the study period the sex of the individuals of each species collected for GI values was recorded. All species were sexed either macroscopically, or from squash mounts of the

gonad tubules.

3.2.4 Size at first sexual maturity

The size at first sexual maturity was obtained by recording the percentage of individuals of each species with mature gonads in $0.5cm^3$ volume size classes. Ten individuals of *R. stephensoni*, *P. sykion* and *N. grammatus* in each size class were collected during the peak reproductive period and examined macroscopically and histologically to determine sex. An individual was classed as being sexually mature if the tubules of the gonad contained spermatozoa or vitellogenic oocytes. The size at which 50% of the individuals in a given size class were mature, was chosen as an index of first sexual maturity.

3.2.5 Gonadal tubule numbers

In order to determine whether the number of tubules varied between males and females, the gonads of 30 adult males, 30 adult females and ten juveniles of *R. stephensoni* and *P. sykion* from a large size range were examined for each species. The gonads were removed from animals during the peak reproductive period, spread out in a petri-dish and the total number of tubules counted. *Neostichopus grammatus* was excluded from this study as it was determined that as the gonad matures, it branches and ramifies resulting in the formation of more and more tubules. Consequently, the number of tubules varied depending on the stage of development.

3.2.6 Environmental factors

Daylength (at time of animal collection) was calculated from the sunrise and sunset times published in the Daily Dispatch newspaper by the East London airport meterological office.

Average monthly sea temperature readings were obtained from the Port Elizabeth municipality who take daily readings at Humewood beaches. These readings were taken to indicate annual sea temperature fluctuations at the study site, which was only a few kilometres away. The months constituting the seasons are represented in Table 3.1 and are taken from Wood (1993).

Table 3.1. The months constituting the seasons of the northern and southern hemispheres.

	Spring	Summer	Autumn	Winter
N. hemisphere	M A M	J J A	S O N	D J F
S. hemisphere	S O N	D J F	M A M	J J A

3.2.7 Statistical analysis

All statistical procedures were taken from Sokal and Rohlf (1981). Multiple-factor analyses of variance were used to determine whether the gonad index values and the tubule diameters of male and female *R. stephensoni*, *P. sykion* and *N. grammatus* varied significantly over the study period. Multiple regression analyses were performed to determine if the number of tubules differed significantly between males and females in *R. stephensoni* and *P. sykion*. Simple linear regression analyses were performed to determine whether a significant relationship existed between gonad index and sea temperature; and between gonad index and daylength of the three species. Finally, chi-squared tests were performed to determine whether a 1:1 sex ratio existed for the three species.

3.3 RESULTS

3.3.1 General gonad morphology

Roweia stephensoni, *Pseudocnella sykion* and *Neostichopus grammatus* are all dioecious (no hermaphrodites were encountered) and do not exhibit sexual dimorphism. In both *R.*

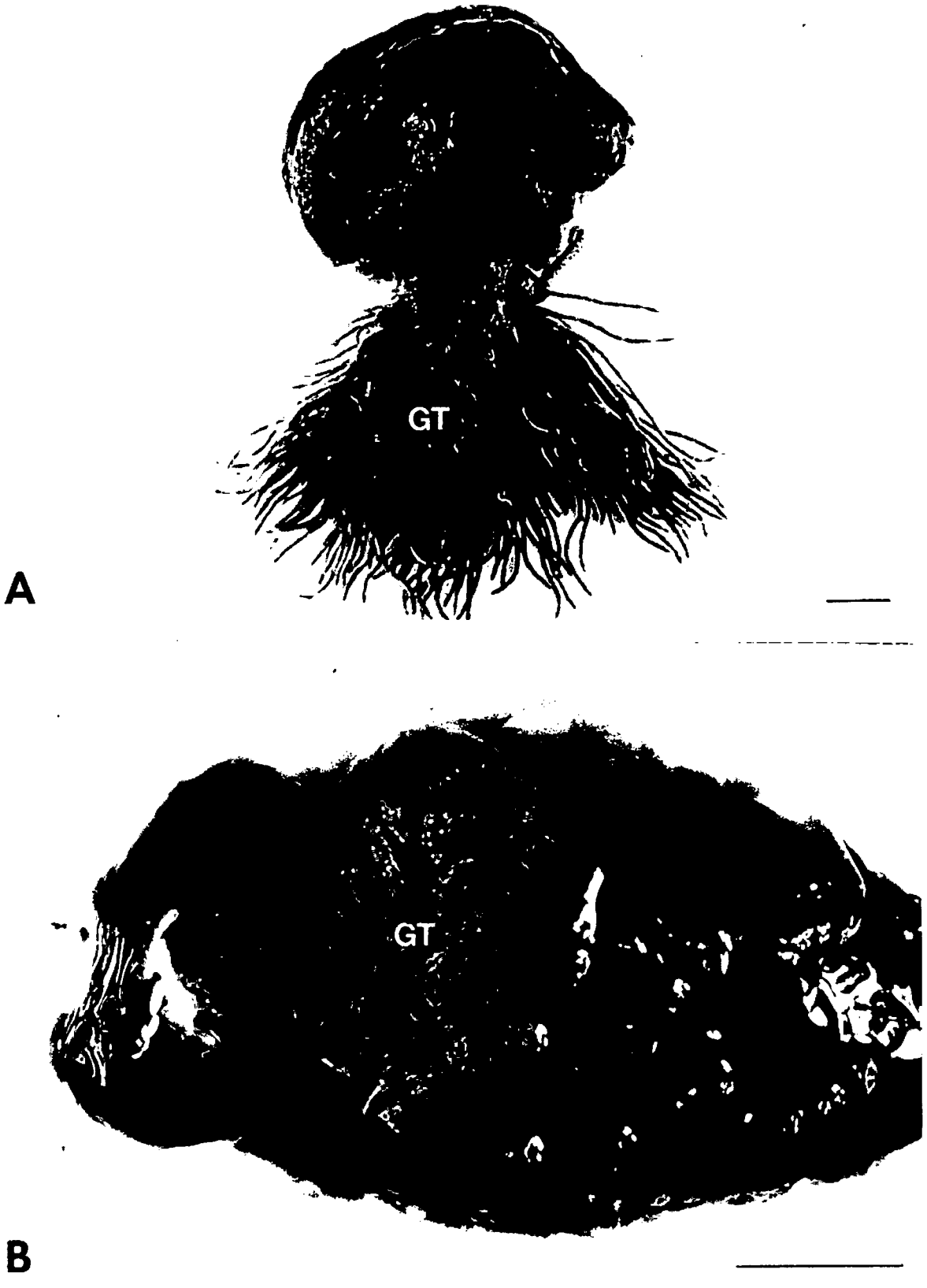
stephensoni and *P. sykion* the gonad consists of a cluster of numerous unbranched tubules located in the anterior of the coelom (Fig. 3.1A). In both of these species the gonad of mature males is yellow in colouration, whilst that of mature females is green. By contrast, in *N. grammatus* the gonad comprises two clusters of multiple branched tubules (Fig. 3.1B). In this species the gonad of mature males is translucent with a yellowish tinge, whilst that of mature females is translucent white in appearance.

3.3.2 Gonad index

In *R. stephensoni* and *P. sykion*, gonads were present throughout the year, and showed a 10 and 4 fold increase in GI values over time, respectively (Figs. 3.2A & 3.3A). There was no significant difference in GI values between males and females for both *R. stephensoni* ($F=2.78$, $P=0.097$) and *P. sykion* ($F=1.13$, $P=0.288$) over the entire study period. Consequently, the GI values for each species represent the pooled GI values of both males and females. In *R. stephensoni*, there was an increase in GI values in March 1992 and in *P. sykion* in April 1992, but for both species maximum gonadal growth only occurred from May 1992 to August 1992, with high GI values from August 1992 to December 1992 (Figs. 3.2A & 3.3A). In both species there was a rapid decrease in GI values in January 1993, suggesting that the gametes had been released. After a brief resting period, of about one month, both species showed GI value increases again to March 1993, with potentially high GI values again from August 1993 (Figs. 3.2A & 3.3A).

By contrast, in *N. grammatus* the gonads underwent reabsorption, either completely or to a few minute threads for part of the year, and showed a 6 fold increase in GI values over time (Fig. 3.4A). Similarly, there was no significant difference in GI values between males and

Figure 3.1. The gross external gonad morphology characteristic of **(A)** *Roweia stephensoni* and *Pseudocnella sykion*, and **(B)** *Neostichopus grammatus*. Scale bars = 1cm. **GT**: gonad tubules.


GT
A
GT
B

females when the gonads were present (F=2.56, P=0.116). Consequently, the GI values for *N. grammatus* represent the pooled GI values of both males and females. *Neostichopus grammatus* showed maximum gonadal growth from June 1992 to October 1992, and maintained high GI values from October 1992 to December 1992 (Fig. 3.4A). Following this, there was a rapid decrease in January 1993, suggesting that spawning had occurred. A long resting period followed from February 1993 to about June/July 1993, during which time the gonads were reabsorbed, before gradually increasing again to potentially high GI values from August 1993 (Fig. 3.4A).

3.3.3 Tubule diameters

Variation in tubule diameter of the three species is illustrated in Figures 3.2B, 3.3B and 3.4B. There was a positive correlation between variations in tubule diameters (i.e. increase and decrease) of the three species with the increases and decreases of their respective GI values. In both *R. stephensoni* (F=277.8, P<0.0001) and *N. grammatus* (F=165.8, P<0.0001) there was a significant difference in the tubule diameters between the sexes. The diameters of the tubules of males were always smaller than those of the females (Figs. 3.2B & 3.4B). By contrast, in *P. sykion* there was no significant difference (F=0.006, P=0.941) in tubule diameters between males and females when the GI values were high (August 1992 to December 1992), but there was a significant difference in tubule diameters between the sexes from January 1993 to August 1993 (F=19.96, P<0.0001) (Fig. 3.3B). In both sexes of all three species, mean maximum tubule diameters were attained from August 1992 to December 1992 in both *R. stephensoni* and *P. sykion* (Figs. 3.2B & 3.3B), whilst from October 1992 to December 1992 in *N. grammatus* (Fig. 3.4B). In all three species there was a rapid decrease in tubule diameters of both sexes in January 1993, suggesting the release of the gametes

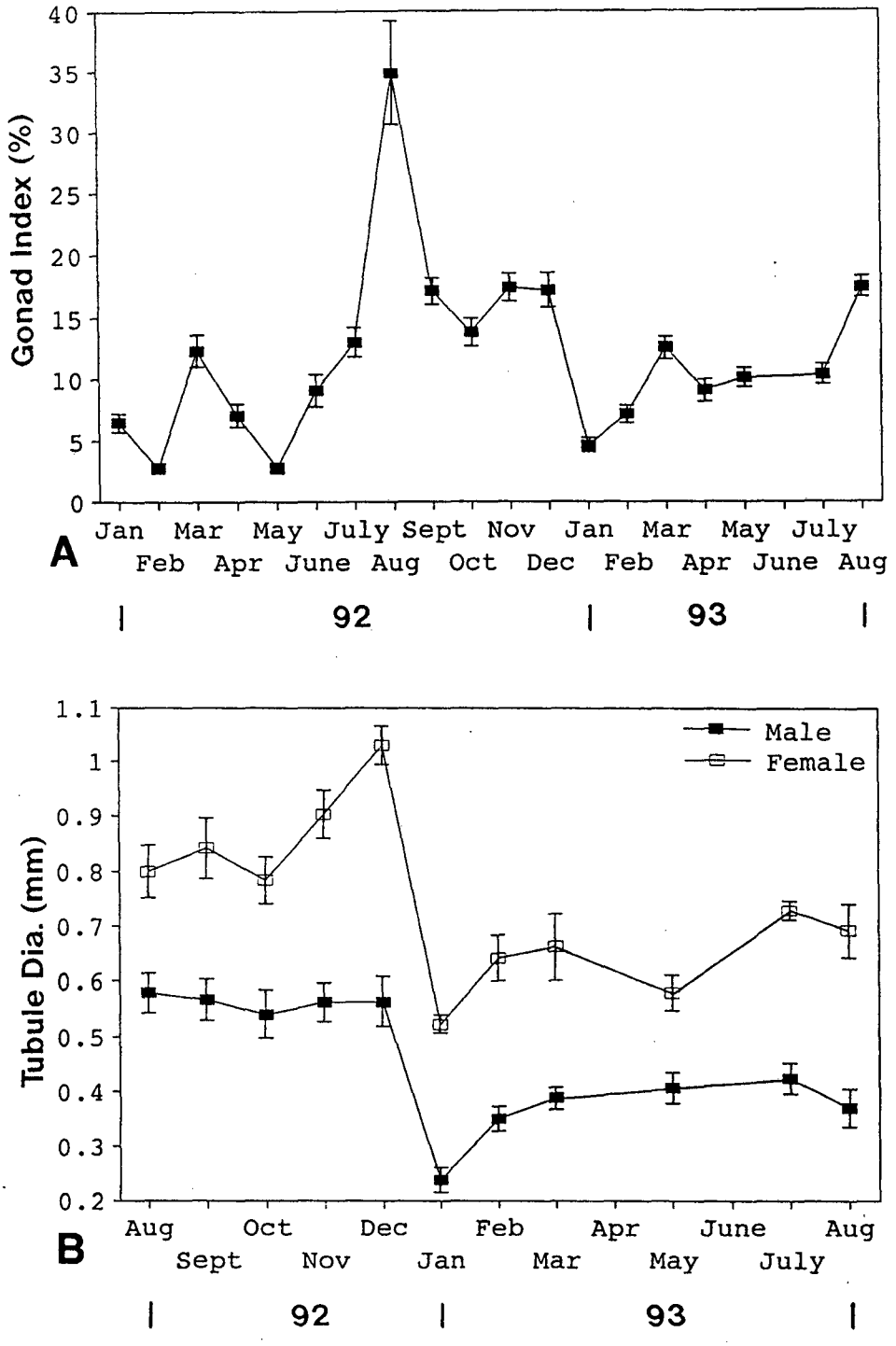


Figure 3.2. *Roweia stephensoni.* **(A)** Seasonal changes in the monthly mean gonad index values (±SE, n=20) from January 1992 to August 1993. **(B)** Seasonal changes in the monthly mean tubule diameters of males and females (±SE, ♂:n=200, ♀:n=200) from August 1992 to August 1993.

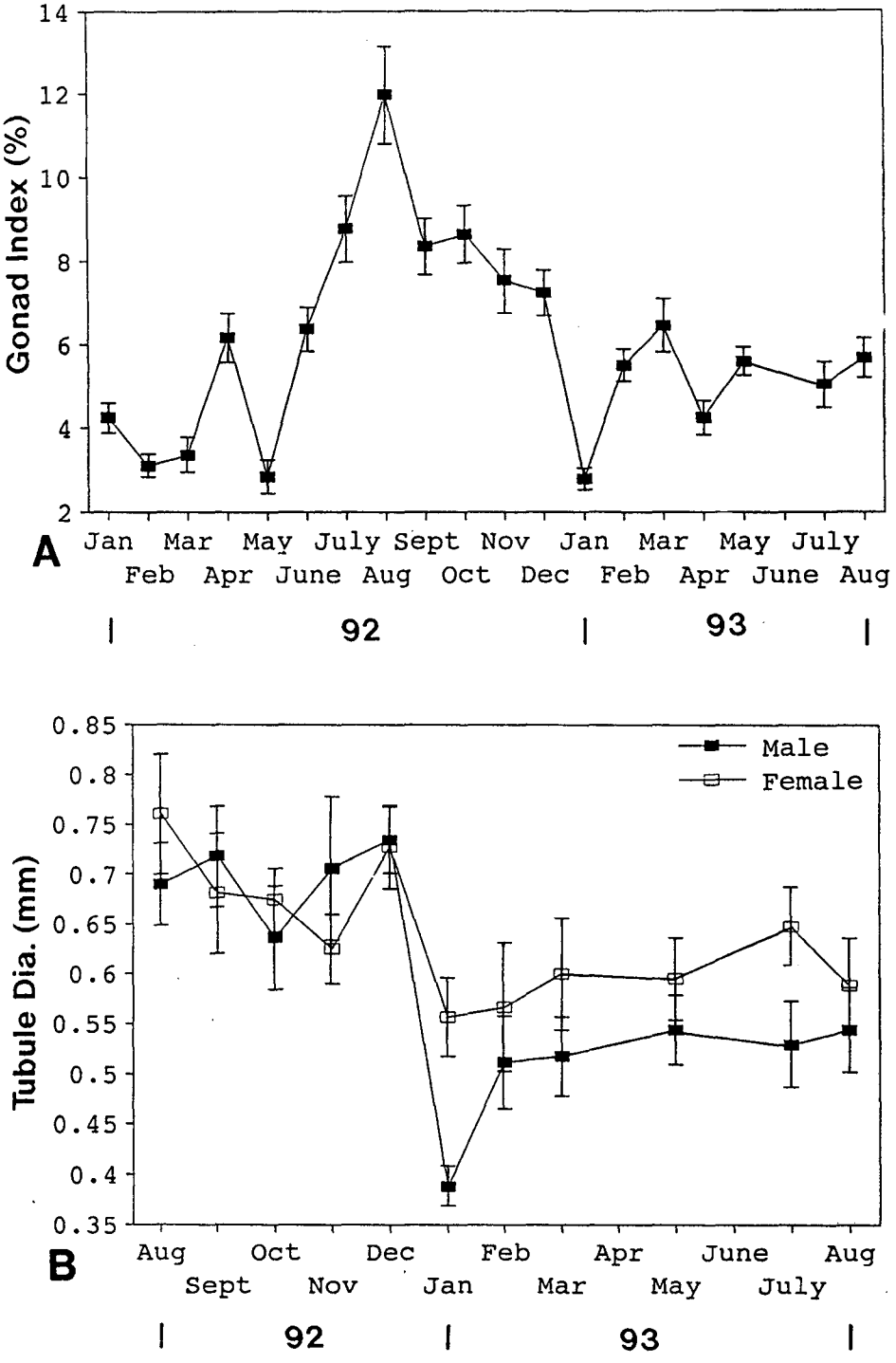


Figure 3.3. *Pseudocnella sykion.* **(A)** Seasonal changes in the monthly mean gonad index values (±SE, n=20) from January 1992 to August 1993. **(B)** Seasonal changes in the monthly mean tubule diameters of males and females (±SE, ♂:n=200, ♀:n=200) from August 1992 to August 1993.

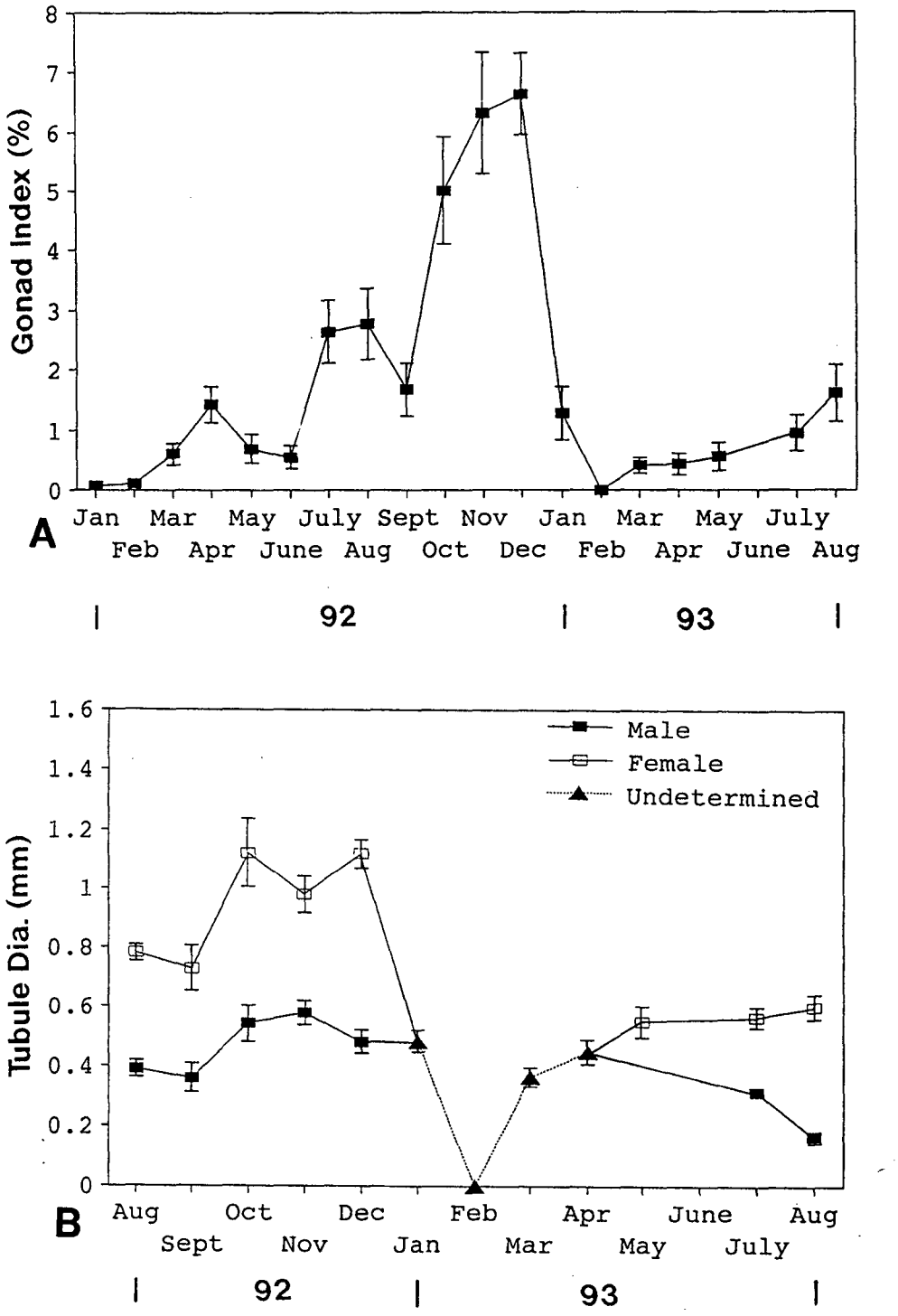


Figure 3.4. *Neostichopus grammatus*. **(A)** Seasonal changes in the monthly mean gonad index values (±SE, n=20) from January 1992 to August 1993. **(B)** Seasonal changes in the monthly mean tubule diameters of males and females (±SE, ♂:n=200, ♀:n=200) from August 1992 to August 1993.

(Figs. 3.2B, 3.3B & 3.4B). After this period, the tubule diameters in both *R. stephensoni* and *P. sykion*, of both sexes, increased slightly in February 1993, whilst in *N. grammatus*, where tubules were present, the diameters remained small (Figs. 3.2B, 3.3B & 3.4B).

3.3.4 Egg diameters and spermatozoon content of tubules

The monthly mean egg diameters and the monthly mean spermatozoon content within the tubules of the three species are illustrated in Figures 3.5A,B, 3.6A,B and 3.7A,B, and correspond positively to the increases and decreases of the specific GI values of each species. In all three species the largest monthly mean egg diameters were encountered from August 1992 to December 1992 in *R. stephensoni* and *P. sykion*, and from October 1992 to December 1992 in *N. grammatus* (Figs. 3.5A, 3.6A & 3.7A), suggesting that the animals were mature. In *R. stephensoni* mature oocytes had a diameter of between 400-450µm, in *P. sykion* 300-350µm, and in *N grammatus* 300-350µm. In all three species the monthly mean egg diameters declined rapidly in January 1993, suggesting that the mature oocytes had been shed (Figs. 3.5A, 3.6A & 3.7A). After this, the monthly mean egg diameters increased slowly to August 1993 in *R. stephensoni* and *P. sykion*, whilst in *N. grammatus* eggs only reappeared in May 1993 and increased to August 1993 (Figs. 3.5A, 3.6A & 3.7A).

The spermatozoon content within the tubules was highest from August 1992 to December 1992 in all three species, suggesting that the animals were mature (Figs. 3.5B, 3.6B & 3.7B). During this period the spermatozoa occupied between 30-70% of the tubule cross-sectional area in *R. stephensoni*, 30-60% in *P. sykion* and 40-90% in *N. grammatus* (Figs. 3.5B, 3.6B & 3.7B). The spermatozoon content within the tubules decreased rapidly in January 1993 in all three species, suggesting that the spermatozoa had been shed (Figs. 3.5B, 3.6B & 3.7B).

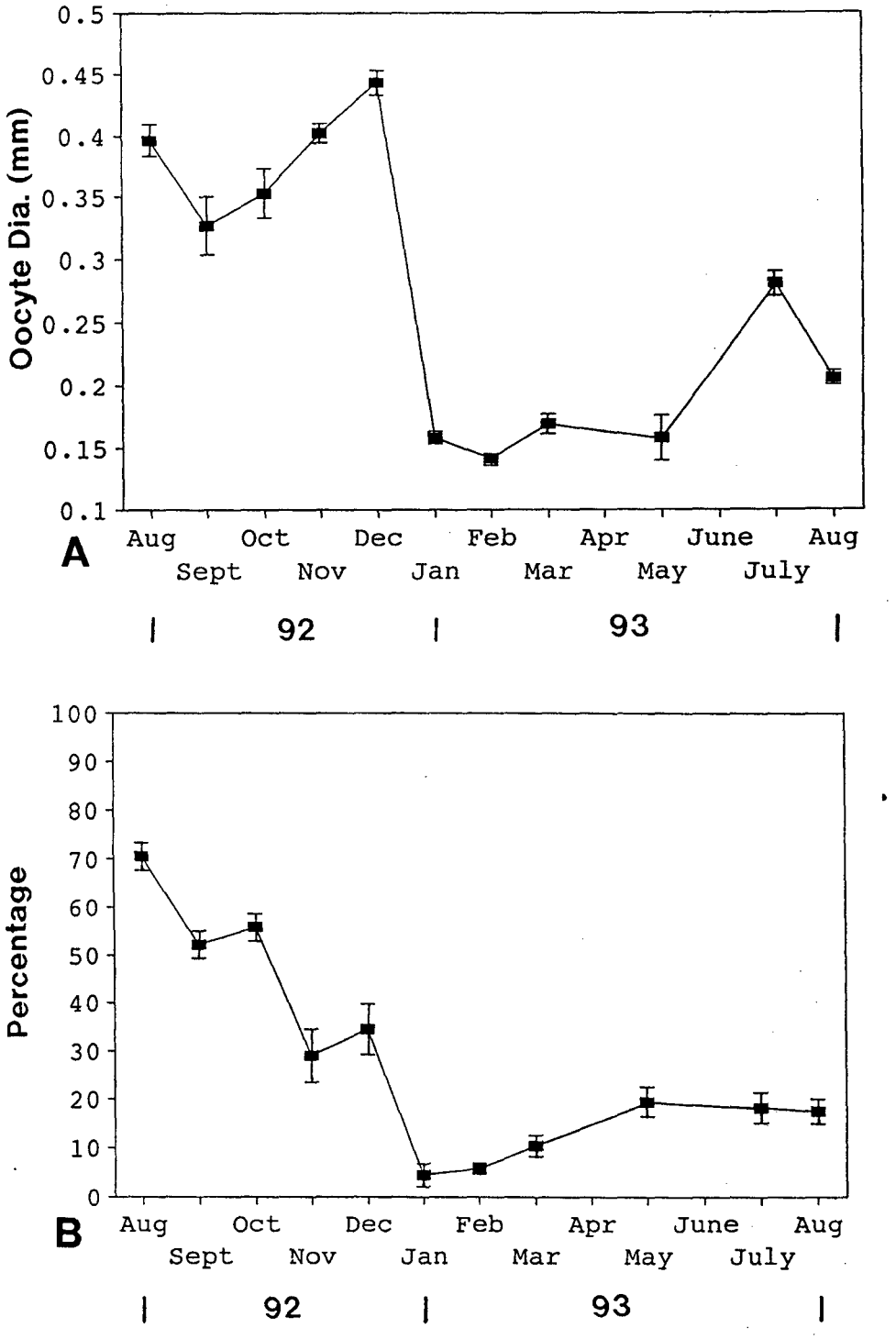


Figure 3.5. *Roweia stephensoni.* **(A)** Seasonal changes in the monthly mean oocyte diameter (±SE, n=500) from August 1992 to August 1993. **(B)** Seasonal changes in the monthly mean proportion of mature spermatozoon (±SE, n=25) component of the tubules from August 1992 to August 1993.

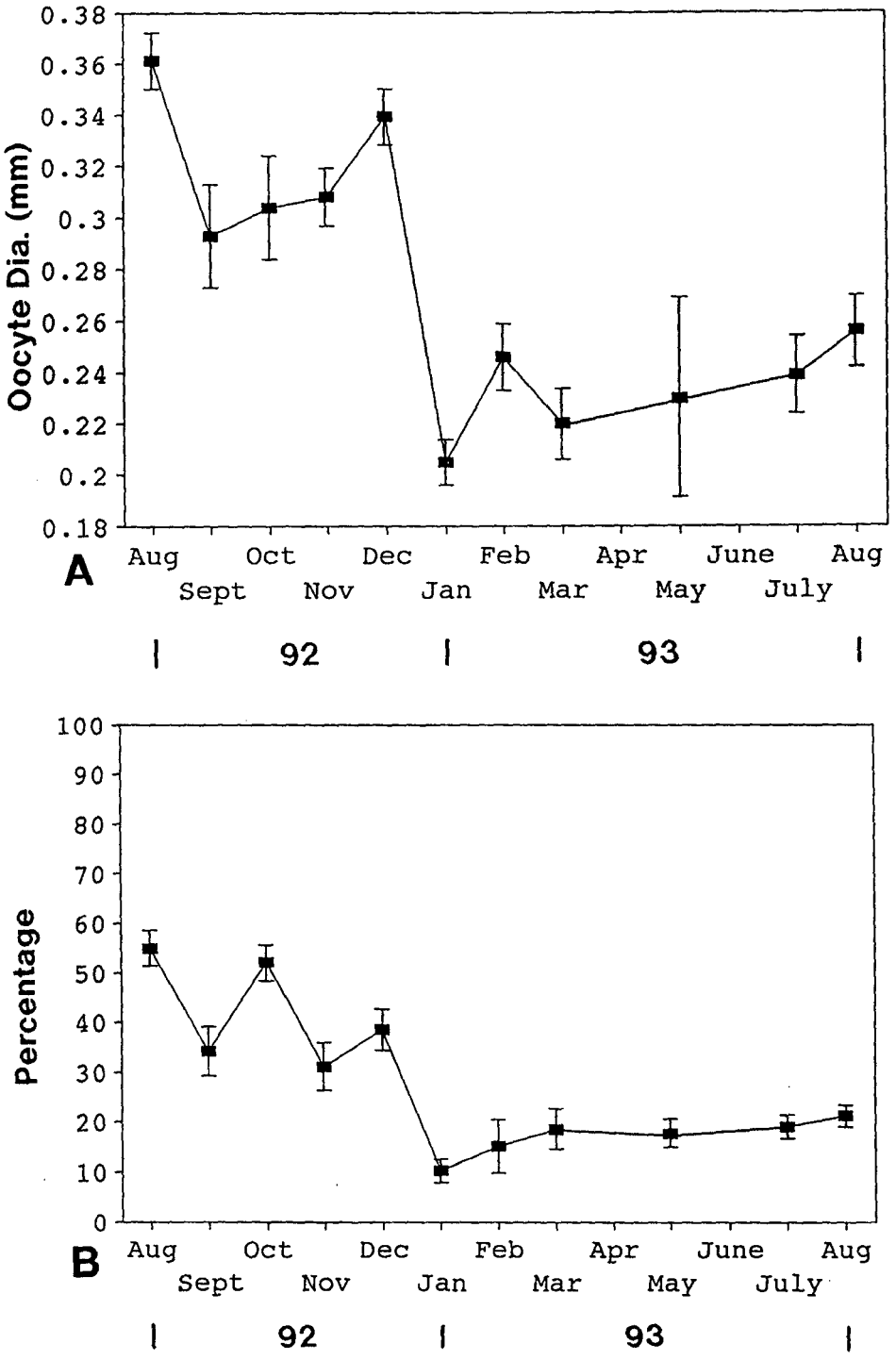


Figure 3.6. *Pseudocnella sykion.* **(A)** Seasonal changes in the monthly mean oocyte diameter (±SE, n=500) from August 1992 to August 1993. **(B)** Seasonal changes in the monthly mean proportion of mature spermatozoon (±SE, n=25) component of the tubules from August 1992 to August 1993.

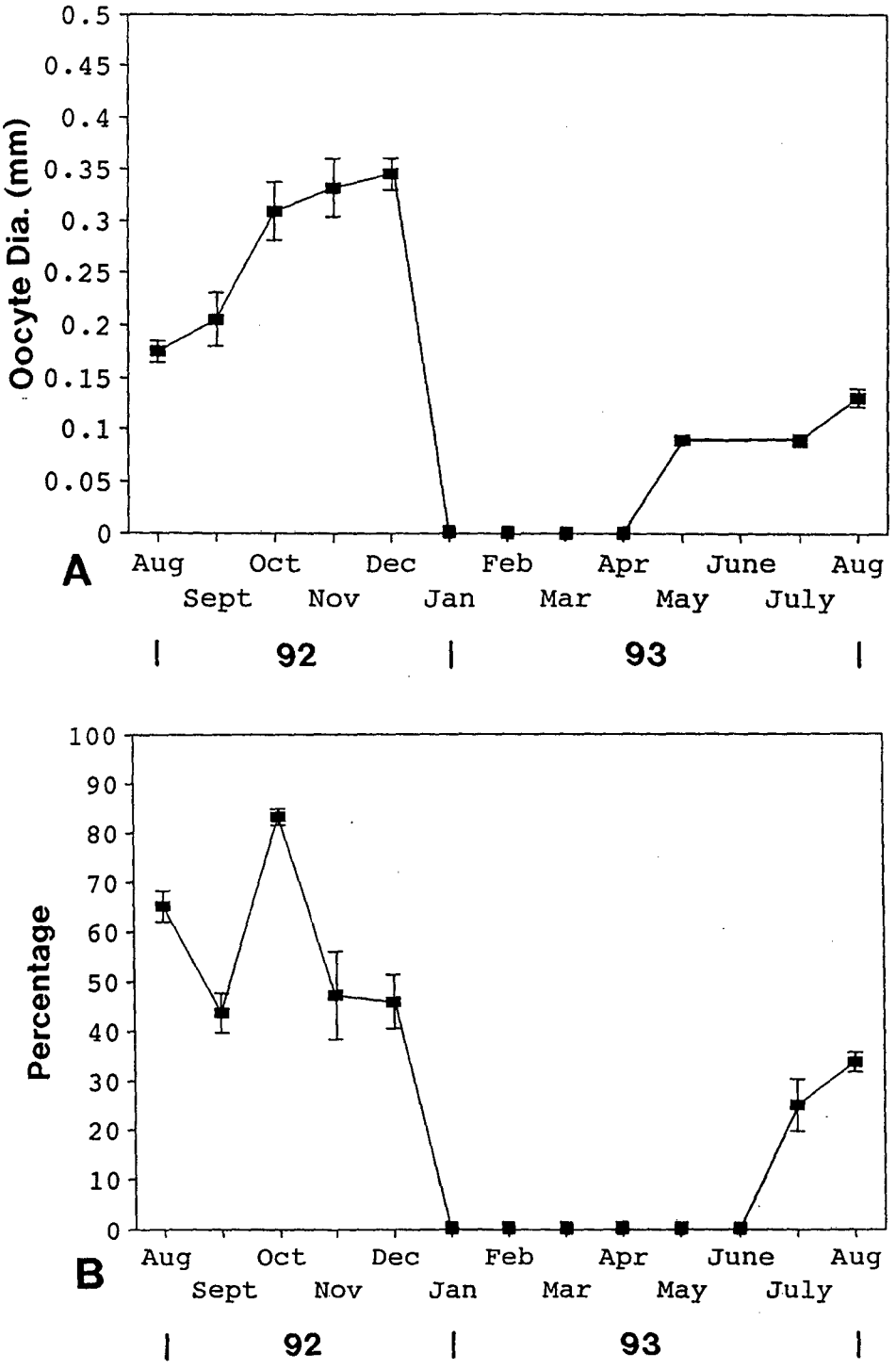


Figure 3.7. *Neostichopus grammatus*. **(A)** Seasonal changes in the monthly mean oocyte diameter (±SE, n=500) from August 1992 to August 1993. **(B)** Seasonal changes in the monthly mean proportion of mature spermatozoon (±SE, n=25) component of the tubules from August 1992 to August 1993.

During this period the spermatozoa occupied less than 10% of the tubule cross-sectional area in *R. stephensoni* and *P. sykion*, whilst in *N. grammatus* no sperm were present (Figs. 3.5B, 3.6B & 3.7B). After this, the spermatozoon content within the tubules increased slowly to August 1993 in *R. stephensoni* and *P. sykion*, whilst in *N. grammatus* spermatozoa only reappeared in July 1993 and increased to August 1993 (Figs. 3.5B, 3.6B & 3.7B).

3.3.5 Sex ratios

Analyses of the ratios of males to females of *R. stephensoni*, *P. sykion* and *N. grammatus* within the population at Port Elizabeth showed similar trends. *Roweia stephensoni* had slightly more females, as of the 344 individuals sampled 163 (47.4%) were male and 181 (52.6%) were female. Similarly, *P. sykion* had slightly more females, as of the 352 individuals sampled 170 (48.3%) were male and 182 (51.7%) were female. Likewise, *N. grammatus* had slightly more females, as of the 163 individuals sampled 78 (47.8%) were male and 85 (52.2%) were female. However, no significant departure from a sex ratio of 1:1 was observed in *R. stephensoni* (df=1, Chi^2=0.941, P=0.331), *P. sykion* (df=1, Chi^2=0.409, P=0.552) and *N. grammatus* (df=1, Chi^2=0.304, P=0.580).

3.3.6 Size at first sexual maturity

In both *R. stephensoni* and *P. sykion*, the onset of first sexual maturity occurred in individuals of about 2.5-2.9cm^3 in size (≈2.4-3.0g drained wet weight), and all individuals below this size were identified as immature. In these two species gonads which lacked colouration were generally immature, although in some instances pigmented gonads were immature due to the absence of mature spermatozoa or vitellogenic oocytes. In *N. grammatus*, the onset of first sexual maturity occurred in individuals of about 3.0-3.9cm^3 in size (≈2.9-4.0g drained wet

wight), and all individuals below this were classed as immature. In *N. grammatus* the presence of the gonad was seldom observed in individuals that were not sexually mature.

3.3.7 Gonad tubule numbers

There was a positive relationship between the number of gonadal tubules and body volume for both males and female of *R. stephensoni* (♂: $F=56.65$, $P<0.0001$, $r^2= 0.67$; ♀: $F=25.56$, $P<0.0001$, $r^2=0.48$), and *P. sykion* (♂: $F=63.06$, $P<0.0001$, $r^2= 0.69$; ♀: $F=13.16$, $P<0.001$, $r^2= 0.32$) (Fig. 3.8A,B). Larger individuals of both sexes tended to have more tubules comprising the gonad than smaller individuals. In addition, the number of tubules constituting the gonad was greater for males than females in *R. stephensoni* ($F=21.46$, $P<0.0001$) and *P. sykion* ($F=46.82$, $P<0.0001$) (Fig. 3.8A,B).

3.3.8 Gametogenesis

Histological examination of the gonads revealed that the gonad wall of both sexes in all three species consisted of an external epithelium, a layer of connective tissue and an inner germinal epithelium (e.g. Figs. 3.9C, 3.10C, & 3.12C). Costelloe (1985) and Hamel *et al.* (1993) have indicated that the gametogenic cycle of holothurians can be divided into five arbitrary stages. Similarly, five stages were easily recognisable in the gametogenic cycle of *R. stephensoni*, *P. sykion* and *N. grammatus*, viz recovery, growth, mature, partly spawned and spent. Since the five stages can be identified in all three species and their development is very similar, they will be dealt with simultaneously, with any specific differences noted.

Oogenesis

1) Recovery stage - Recovery of the female gonad in *R. stephensoni* and *P. sykion* was

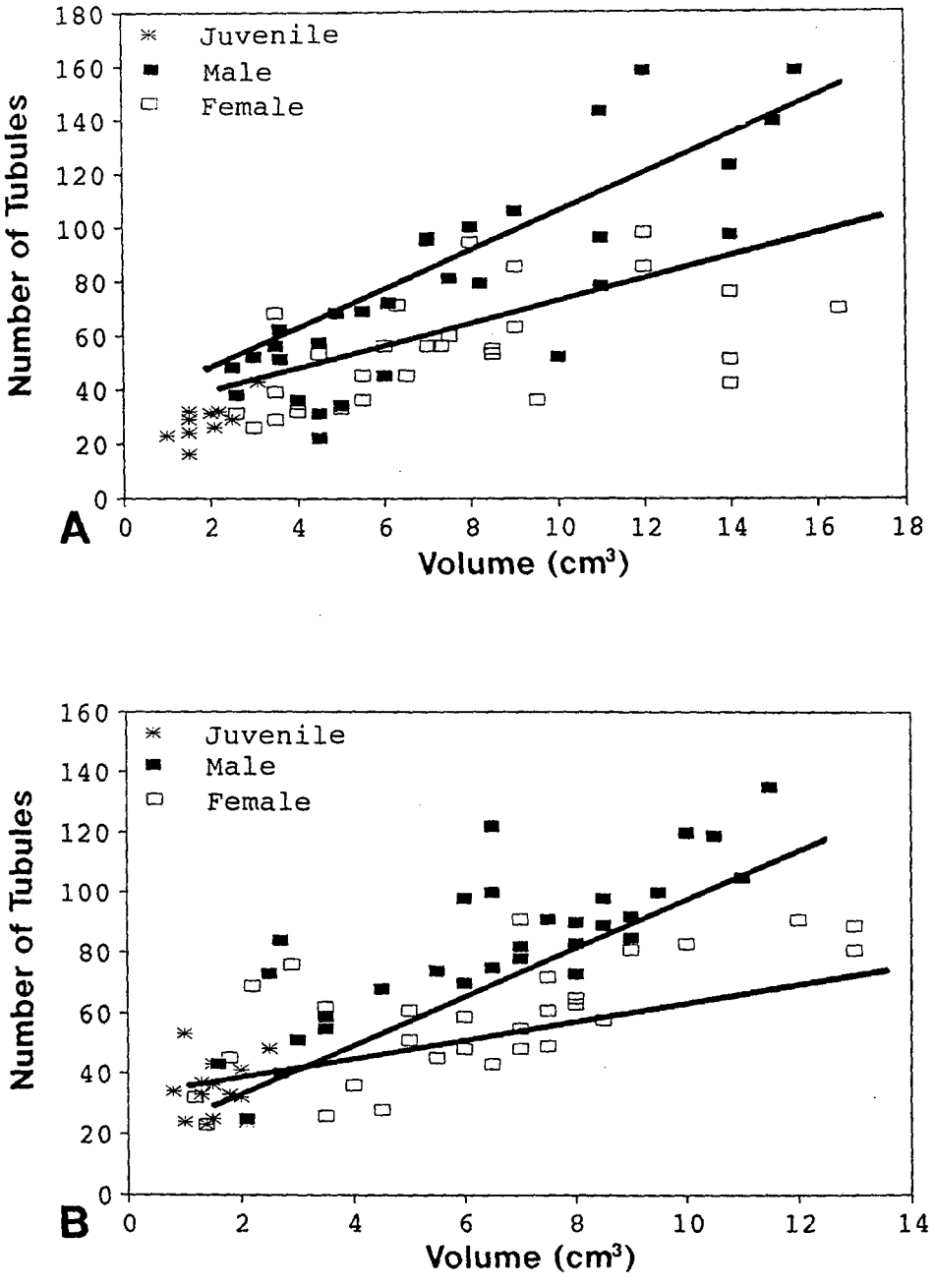


Figure 3.8. The relationship of the number of gonadal tubules present to drained body volume in **(A)** *Roweia stephensoni* for males (n=30, y=7.27x + 35.02, r^2=0.67) and female (n=30, y=4.13x + 32.27, r^2=0.48); and in **(B)** *Pseudocnella sykion* for males (n=30, y=8.01x + 18.27, r^2=0.69) and female (n=30, y=3.03x + 32.02, r^2=0.32).

characterised by a thickening of the external epithelium, and the germinal layer may develop extensions into the lumen (Figs. 3.9A & 3.10A). Oogonia were impossible to identify accurately. By contrast, in *N. grammatus* this stage was characterised by the regression of the gonad.

2) Growth stage - In all three species this stage was characterised by active production of gametes (Figs. 3.9B,C, 3.10B,C & 3.11A,B). Previtellogenic oocytes, ranging in size from 40-180µm in diameter in *R. stephensoni*, 50-185µm in diameter in *P. sykion* and 35-170µm in diameter in *N. grammatus*, were basophilic (Figs. 3.9B,C, 3.10B,C & 3.11A,B), and as vitellogenesis proceeded, form less basophilic vitellogenic oocytes. As the chromatin content of the nucleus undergoes many transformations the chromatin content becomes clearly visible in the nucleoplasm (Figs. 3.9D, 3.10D & 3.11C). The developing vitellogenic oocytes now occupied a larger proportion of the lumen's space and range in size from 200-380µm in diameter in *R. stephensoni*, 190-290µm in diameter in *P. sykion* and 180-290µm in diameter in *N. grammatus*. The oocytes at this stage were attached to the germinal layer by the follicular membrane (Figs. 3.9D, 3.10D & 3.11B).

3) Mature stage - At the mature stage, development of the oogonia becomes less active, but may still persist even though the lumen was full (Figs. 3.9E, 3.11D). The germinal and outer epidermal layer have become reduced (Figs. 3.9E, 3.10E & 3.11D). The oocytes continued to grow until they reached spawning size, 400-450µm in diameter in *R. stephensoni*, 300-350µm in diameter in *P. sykion* and 300-350µm in diameter in *N. grammatus*. By maturity the granular appearance of the nucleoplasm had changed to a homogeneous opaque texture (Figs. 3.9E, 3.10E & 3.11D). In *R. stephensoni* and *P. sykion* 1 to 5 distinct nucleoli were

Figure 3.9. *Roweia stephensoni.* Female gametogenic stages. **(A)** Recovery stage; **(B-D)** growth stage; **(E)** mature stage; **(F)** partly spawned stage; **(G,H)** spent stage. Scale bars = 100μm. **c**: connective tissue; **e**: external epithelium; **f**: follicular membrane; **gc**: gelatinous coat; **ge**: germinal epithelium; **l**: lumen; **m**: mature oocyte; **nu**: nucleolus; **og**: oogonia; **po**: previtellogenic oocyte; **ro**: relict oocyte; **sc**: spherule cell.

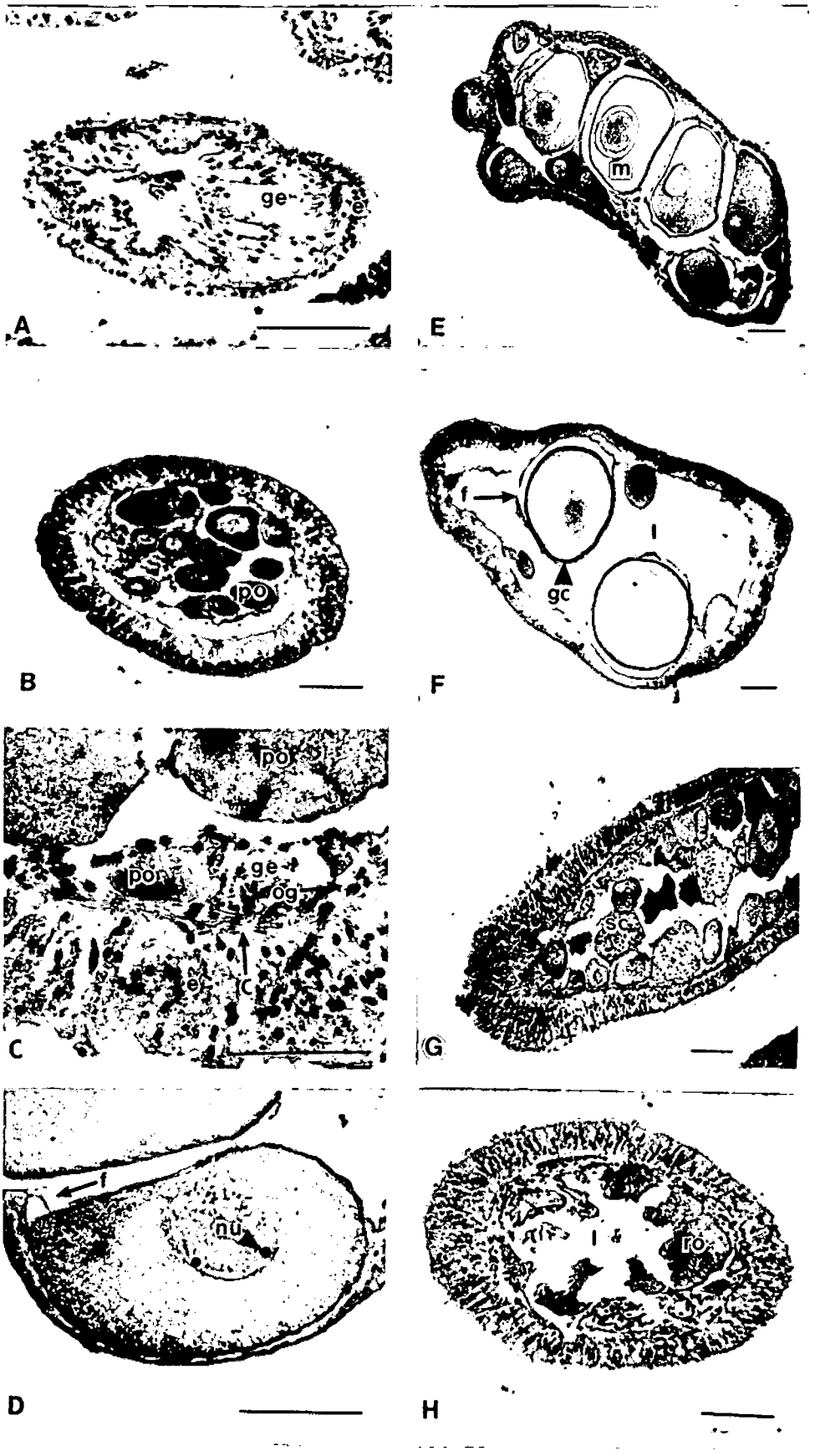

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Figure 3.10. *Pseudocnella sykion.* Female gametogenic stages. **(A)** Recovery stage; **(B-D)** growth stage; **(E)** mature stage; **(F)** partly spawned stage; **(G,H)** spent stage. Scale bars = 100μm. **c**: connective tissue; **e**: external epithelium; **f**: follicular membrane; **gc**: gelatinous coat; **ge**: germinal epithelium; **l**: lumen; **m**: mature oocyte; **nu**: nucleolus; **og**: oogonia; **po**: previtellogenic oocyte; **ro**: relict oocyte; **sc**: spherule cell; **vo**: vitellogenic oocyte.

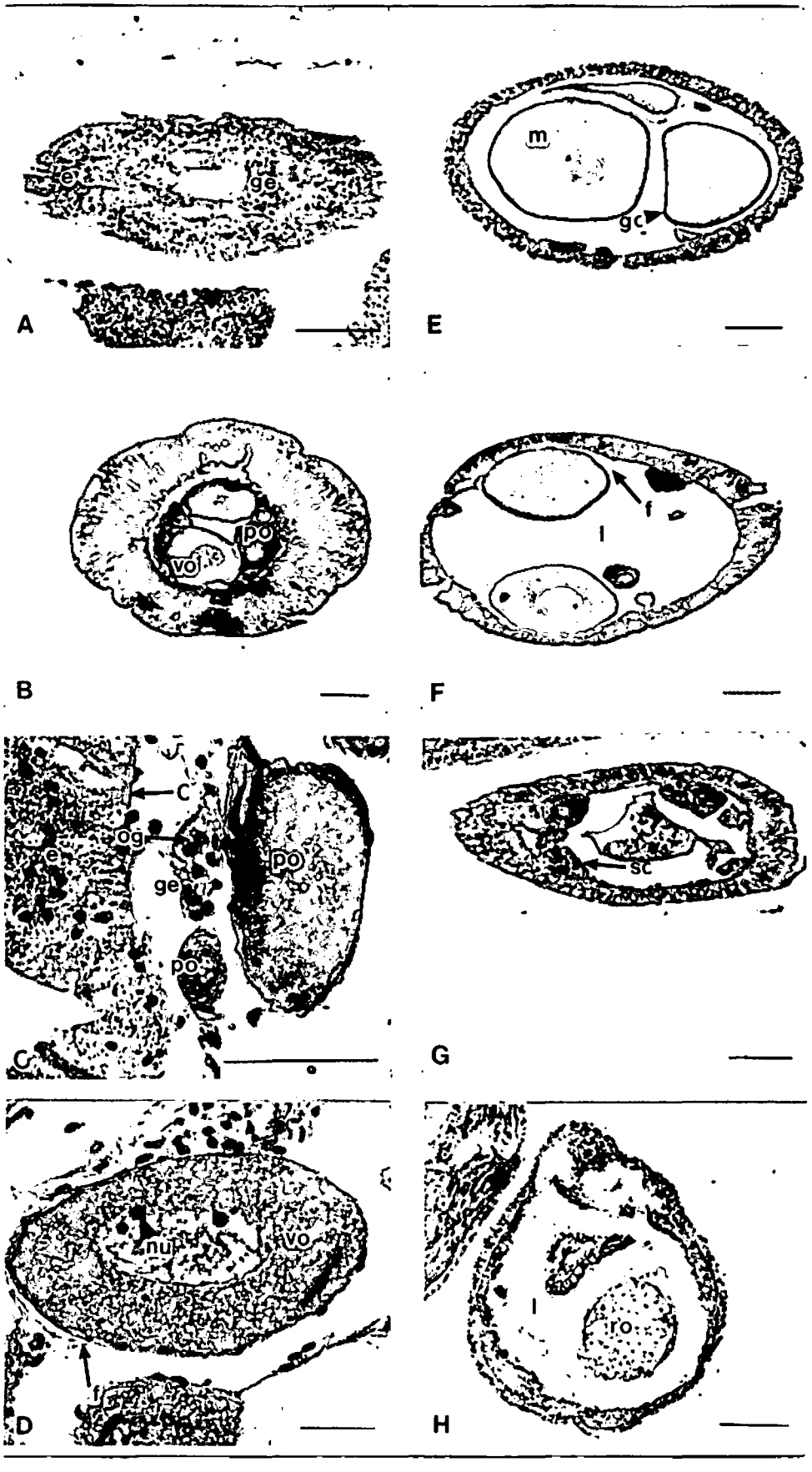
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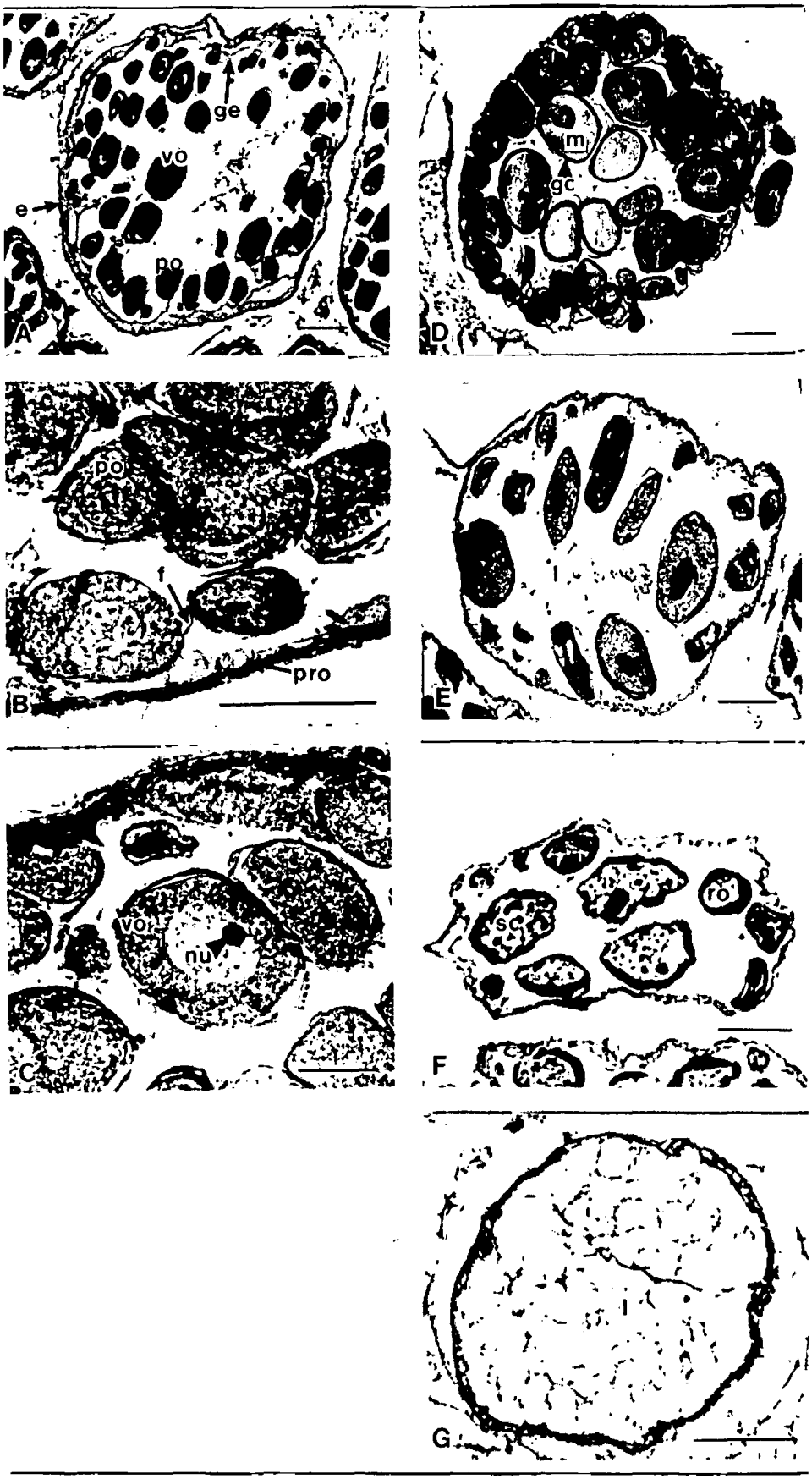
Figure 3.11. *Neostichopus grammatus*. Female gametogenic stages. **(A-C)** Growth stage; **(D)** mature stage; **(E)** partly spawned stage; **(F,G)** spent stage. Scale bars = 100µm. **e**: external epithelium; **f**: follicular membrane; **gc**: gelatinous coat; **ge**: germinal epithelium; **l**: lumen; **m**: mature oocyte; **nu**: nucleolus; **po**: previtellogenic oocyte; **pro**: primary oocyte; **ro**: relict oocyte; **sc**: spherule cell; **vo**: vitellogenic oocyte.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visible attached to the nuclear membrane (Figs. 3.9D,E & 3.10D,E), whilst in *N. grammatus* only one large nucleolus was ever observed (Fig. 3.11C).

4) Partly spawned stage - At this stage most of the mature eggs had been expelled (Figs. 3.9F, 3.10F & 3.11E). At some stage before spawning a thin gelatinous coat developed around the egg (Figs. 3.9F, 3.10E & 3.11D), and the mature ova were freed from the follicular membrane before their release. No further morphological changes were noted in the oocytes before their expulsion into the surrounding water column.

5) Spent - In all three species the entire contents of the gonads were rarely shed during spawning. The remaining large ova were undergoing various stages of resorption, and were phagocytosed forming spherule cells (Figs. 3.9G, 3.10G & 3.11F). After phagocytosis the gonad tubules were devoid of any ova (Figs. 3.9H, 3.10H & 3.11G), and in *N. grammatus* the tubule walls were absorbed as the gonad regressed (Fig. 3.11G).

Spermatogenesis

1) Recovery stage - As in oogenesis, the recovery stage of the male gonad in *R. stephensoni* and *P. sykion* was characterised by a thickening of the outer epithelium, and the germinal layer may develop invaginations into the lumen (Figs. 3.12A & 3.13A). Again, in *N. grammatus* this stage was characterised by the regression of the gonad. Distinct spermatogonia, measuring about 10µm in diameter in *R. stephensoni* and *P. sykion* appeared on the inner germinal layer (Figs. 3.12A & 3.13A).

2) Growth stage - Very active spermatogenesis occurred during this stage. The germinal layer

thickened and developed long extensions into the lumen, increasing the surface area for spermatocyte production (Figs. 3.12B,C, 3.13B,C & 3.14A,B). The primary spermatocytes lined the inner side of the germinal layer, and measured from 5-7μm in diameter in *R. stephensoni* and *P. sykion*, and 6-8μm in diameter in *N. grammatus* (Figs. 3.12C, 3.13C & 3.14B). Early spermatids measuring about 2μm in diameter in *R. stephensoni* and *P. sykion*, and about 3μm in diameter in *N. grammatus* were free from, but in close proximity to the spermatocyte layer (Figs. 3.12C, 3.13C & 3.14B). Some of the spermatids started to give rise to spherical mature spermatozoa, measuring 1.82 ±0.01μm in diameter in *R. stephensoni*, 1.17 ±0.04μm in *P. sykion* and 1.74 ±0.02μm in *N. grammatus* (Hodgson & Bernard, 1992), which began to fill the lumen (Figs. 3.12B, 3.13B & 3.14A).

3) Mature stage - This stage was characterised by a reduction in the thickness of the tubule wall, regression of the germinal layer projections and the mass production of spermatozoa (Figs. 3.12D, 3.13D & 3.14C). Spermatogenesis continued until the lumen was packed with mature active sperm (Figs. 3.12E, 3.13E & 3.14D).

4) Partly spawned stage - At this stage some of the contents of the tubules had been expelled, resulting in empty spaces in the tubule lumen (Figs. 3.12F, 3.13F & 3.14E).

5) Spent - As in the females, the entire contents were rarely shed and relict spermatozoa remained in the lumen, and were probably phagocytosed (Figs. 3.12G, 3.13G & 3.14F). Again in *N. grammatus*, the tubule walls were absorbed as the gonad regresses (Fig. 3.14F).

Figure 3.12. *Roweia stephensoni.* Male gametogenic stages. **(A)** Recovery stage; **(B,C)** growth stage; **(D,E)** mature stage; **(F)** partly spawned stage; **(G)** spent stage. Scale bars = 100µm. **c**: connective tissue; **e**: external epithelium; **ge**: germinal epithelium; **gex**: germinal epithelium extension; **l**: lumen; **pz**: proliferation zone; **rs**: relict spermatozoa; **s**: spermatozoa; **sc**: spermatocytes; **sg**: spermatogonia; **st**: spermatids.

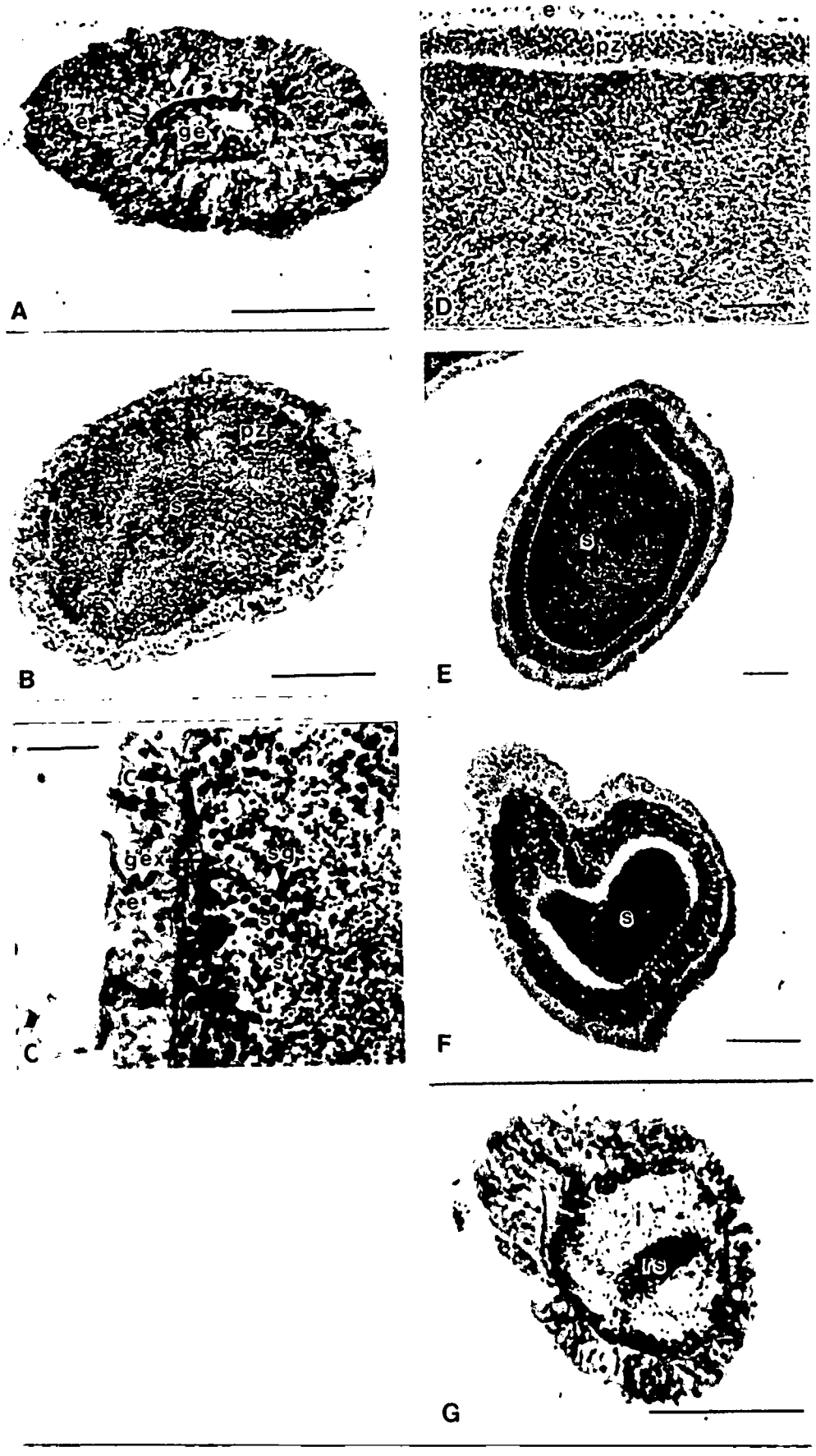
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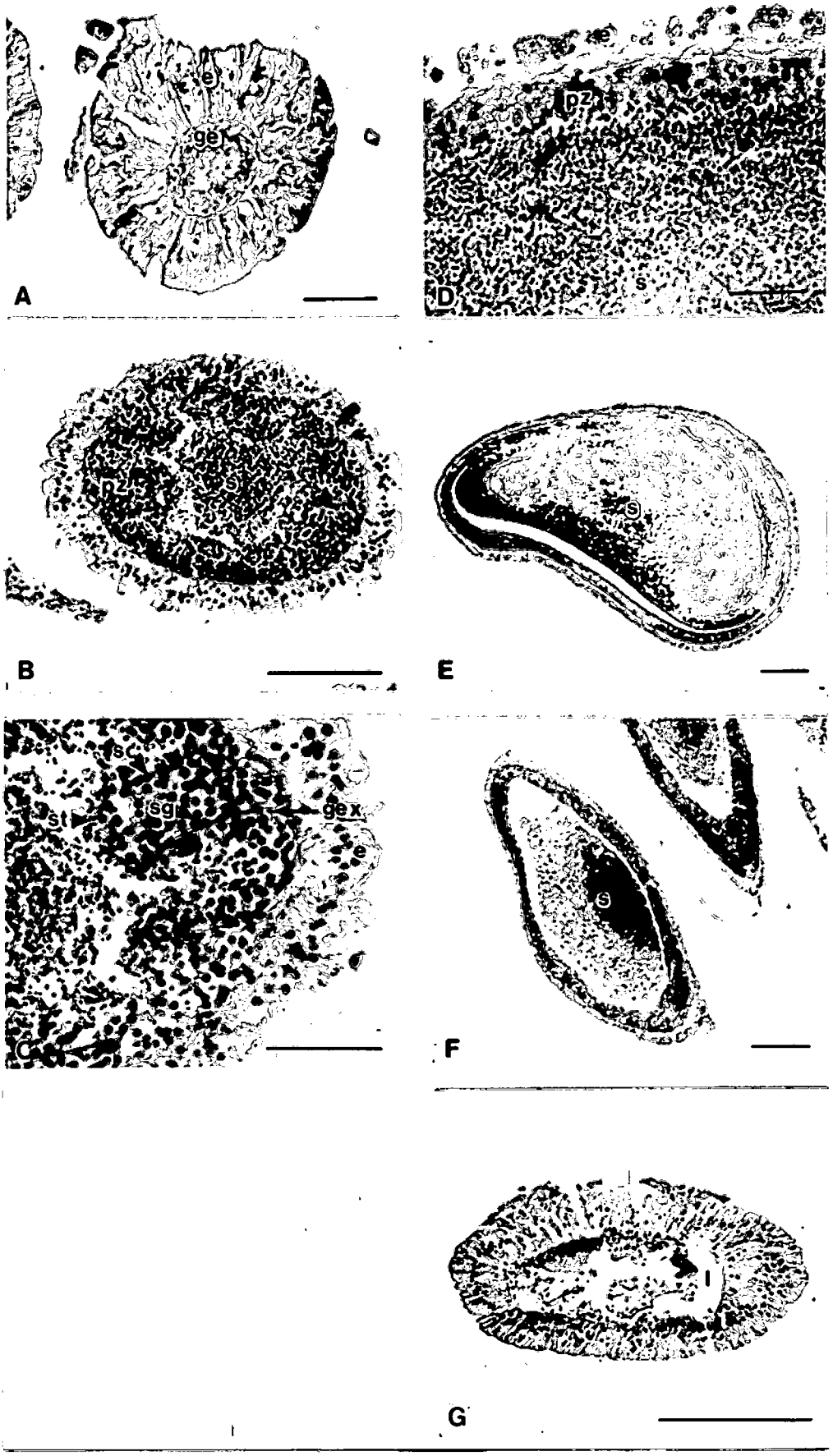
Figure 3.13. *Pseudocnella sykion*. Male gametogenic stages. **(A)** Recovery stage; **(B,C)** growth stage; **(D,E)** mature stage; **(F)** partly spawned stage; **(G)** spent stage. Scale bars = 100µm. **e**: external epithelium; **ge**: germinal epithelium; **gex**: germinal epithelium extension; **l**: lumen; **pz**: proliferation zone; **s**: spermatozoa; **sc**: spermatocytes; **sg**: spermatogonia; **st**: spermatids.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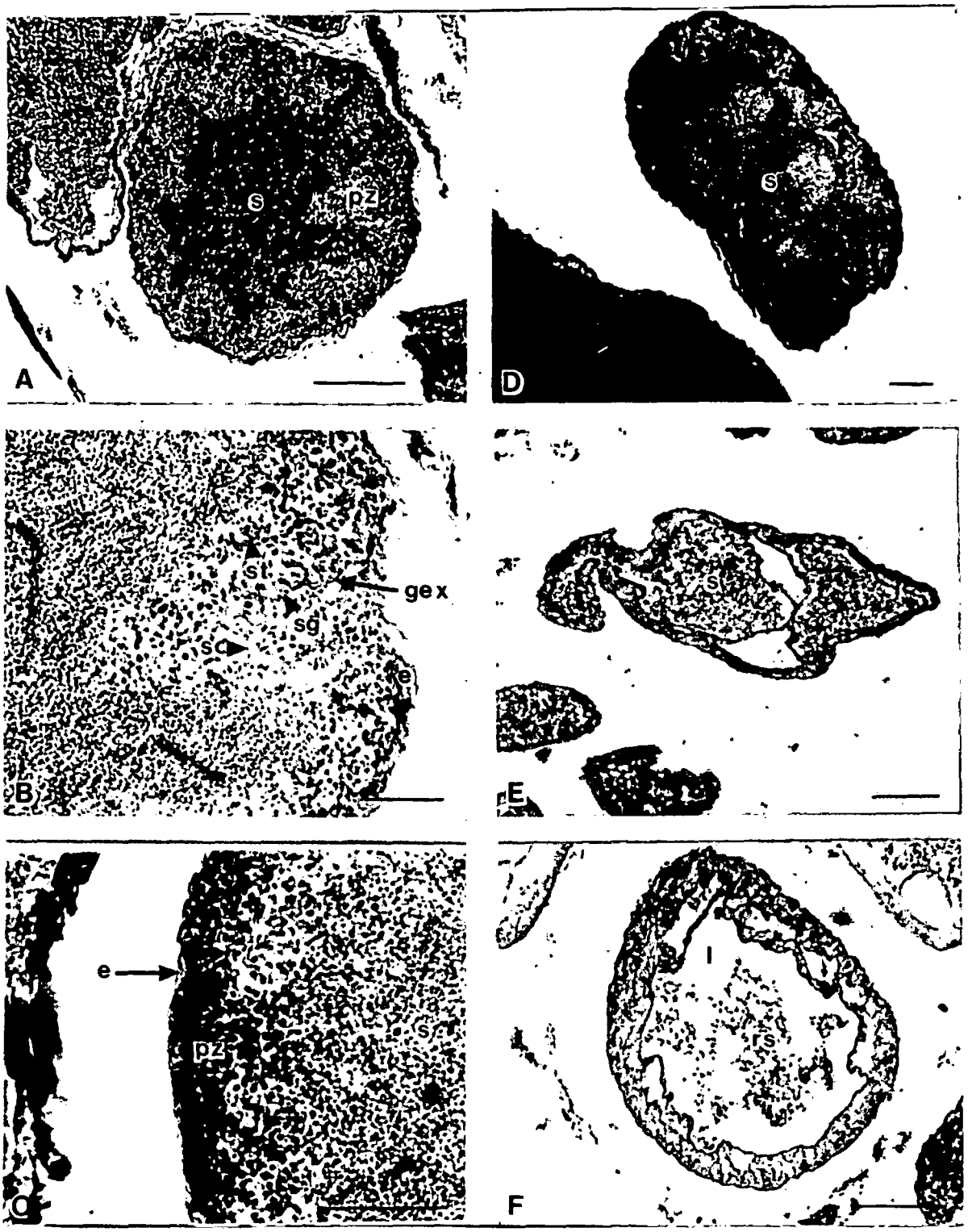


Figure 3.14. *Neostichopus grammatus.* Male gametogenic stages. **(A,B)** Growth stage; **(C,D)** mature stage; **(E)** partly spawned stage; **(F)** spent stage. Scale bars = 100µm. **e**: external epithelium; **gex**: germinal epithelium extension; **l**: lumen; **pz**: proliferation zone; **rs**: relict spermatozoa; **s**: spermatozoa; **sc**: spermatocytes; **sg**: spermatogonia; **st**: spermatids.

3.3.9 Reproductive cycles

The reproductive cycles of *R. stephensoni*, *P. sykion* and *N. grammatus* were best followed from the histological preparations of the tubules, and are summarized in Figures 3.15A,B, 3.16A,B and 3.17A,B. The results of the histological analyses of the reproductive cycles, mirrored that of GI values, gonad tubule and egg diameters, and spermatozoon content. In all three species the reproductive cycles were asynchronous amongst the individuals, but certain trends were apparent. *Roweia stephensoni* and *P. sykion* followed similar reproductive cycles and will be dealt with together. In August 1992 (late winter) both sexes of the two species were mature and their tubules packed with mature gametes (Figs. 3.15A,B & 3.16A,B). A long maturity period was maintained from August 1992 to December 1992 (spring to early summer) during which time partial spawnings occurred in both sexes of the two species. At some stage between December 1992 and January 1993 (mid-summer) the main spawning event occurred, and the majority of individuals of both sexes were spent (Figs. 3.15A,B & 3.16A,B). After a brief recovery period in January/February 1993, gametogenic activity in both sexes had resumed by February 1993 (late summer) and extended until August 1993. However, mature gametes were again present in some of the individuals of both sexes by April/May 1993 (late autumn) (Figs. 3.15A,B & 3.16A,B). It also appeared that there had been a phase shift in the reproductive cycle from 1992 to 1993, as no individuals of both sexes of *R. stephensoni* and *P. sykion* were in the growth phase in August 1992, as they were in August 1993 (Figs. 3.15A,B & 3.16A,B).

Neostichopus grammatus followed a different reproductive cycle. All individuals of both sexes were mature by October 1992 (mid-spring) and a maturity period was maintained from October 1992 to December 1992 (mid-spring to early summer), during which partial

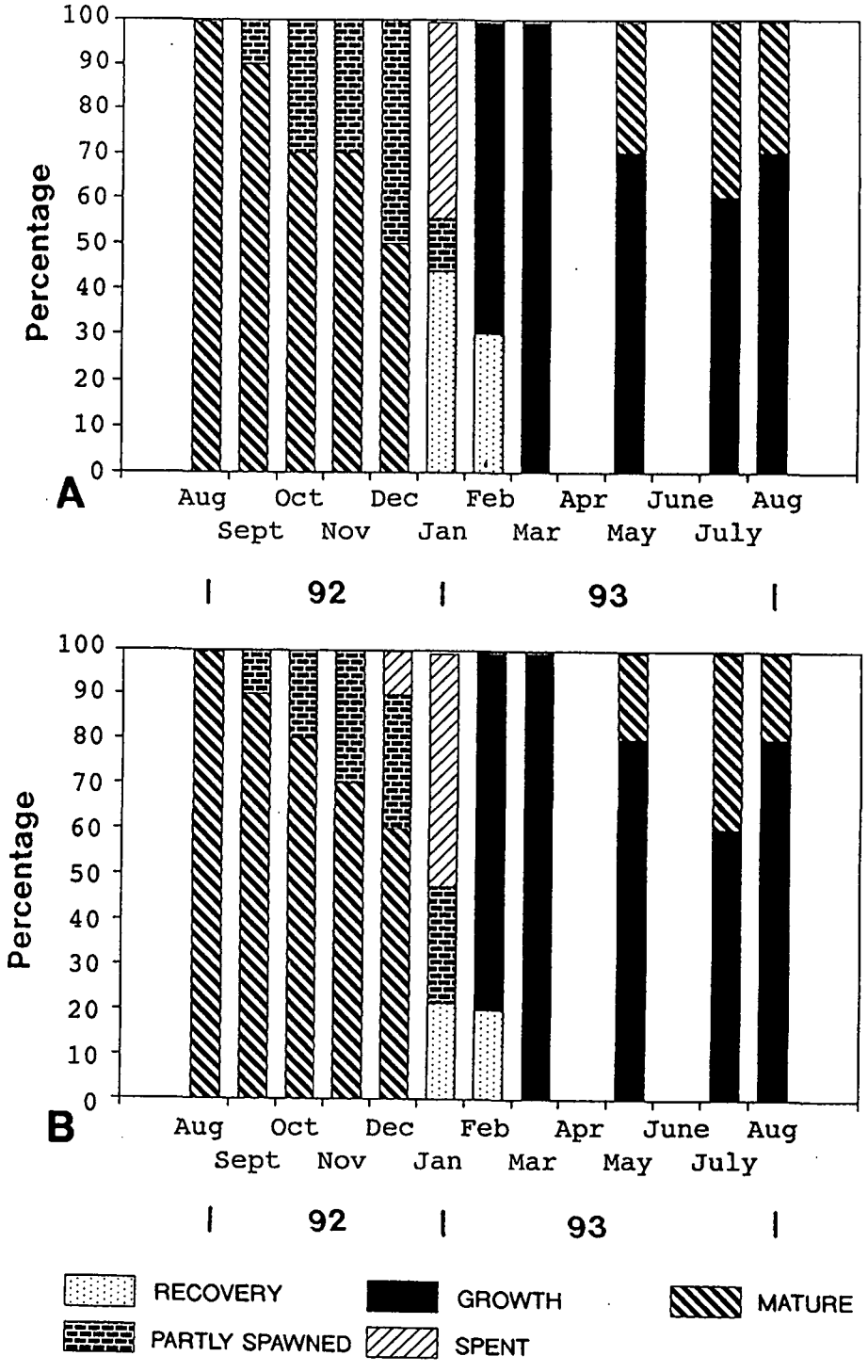


Figure 3.15. *Roweia stephensoni.* Gametogenic cycle. Histograms show relative frequencies of gametogenic stages in **(A)** males and **(B)** females, for the period from August 1992 to August 1993.

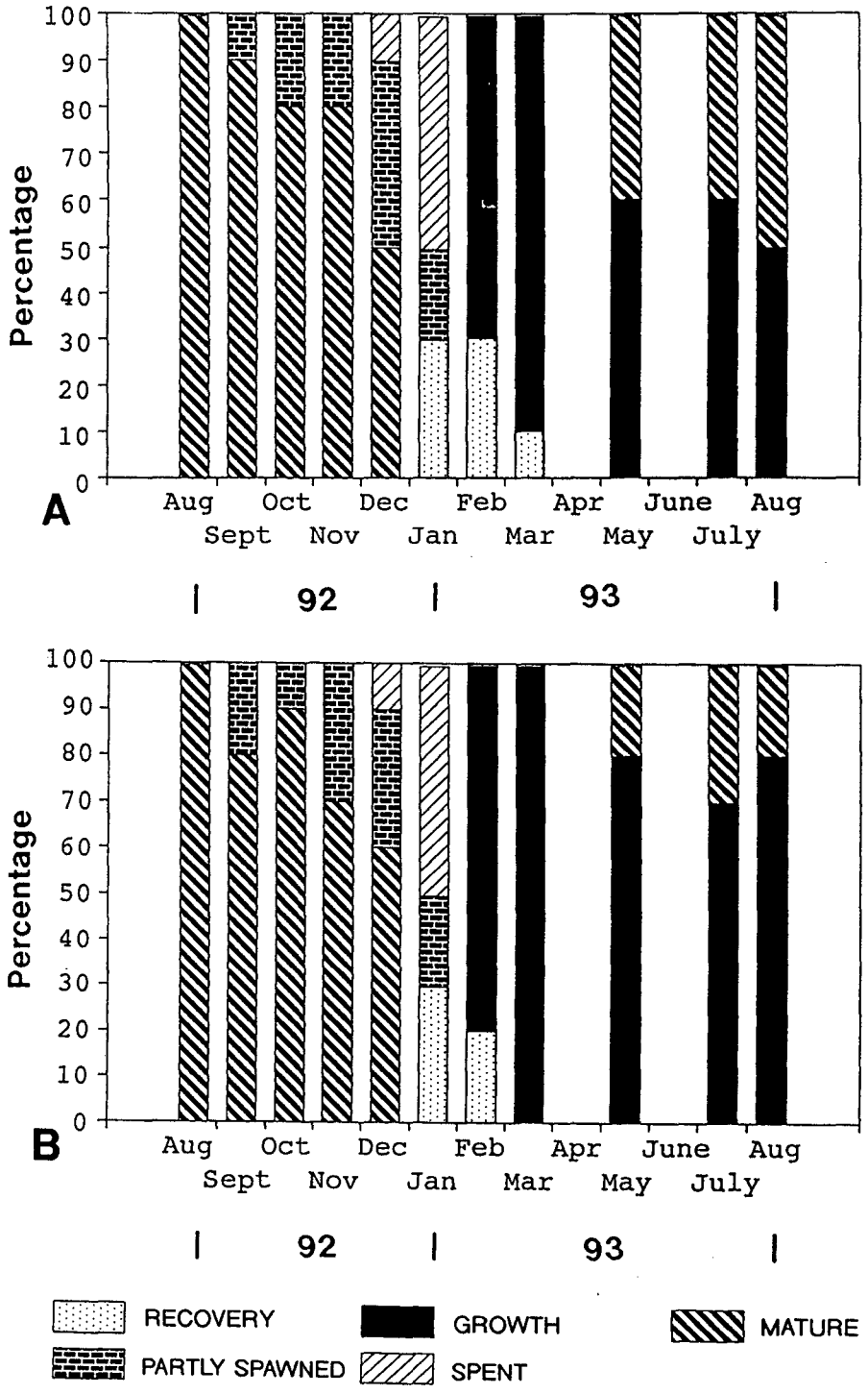


Figure 3.16. *Pseudocnella sykion.* Gametogenic cycle. Histograms show relative frequencies of gametogenic stages in **(A)** males and **(B)** females, for the period from August 1992 to August 1993.

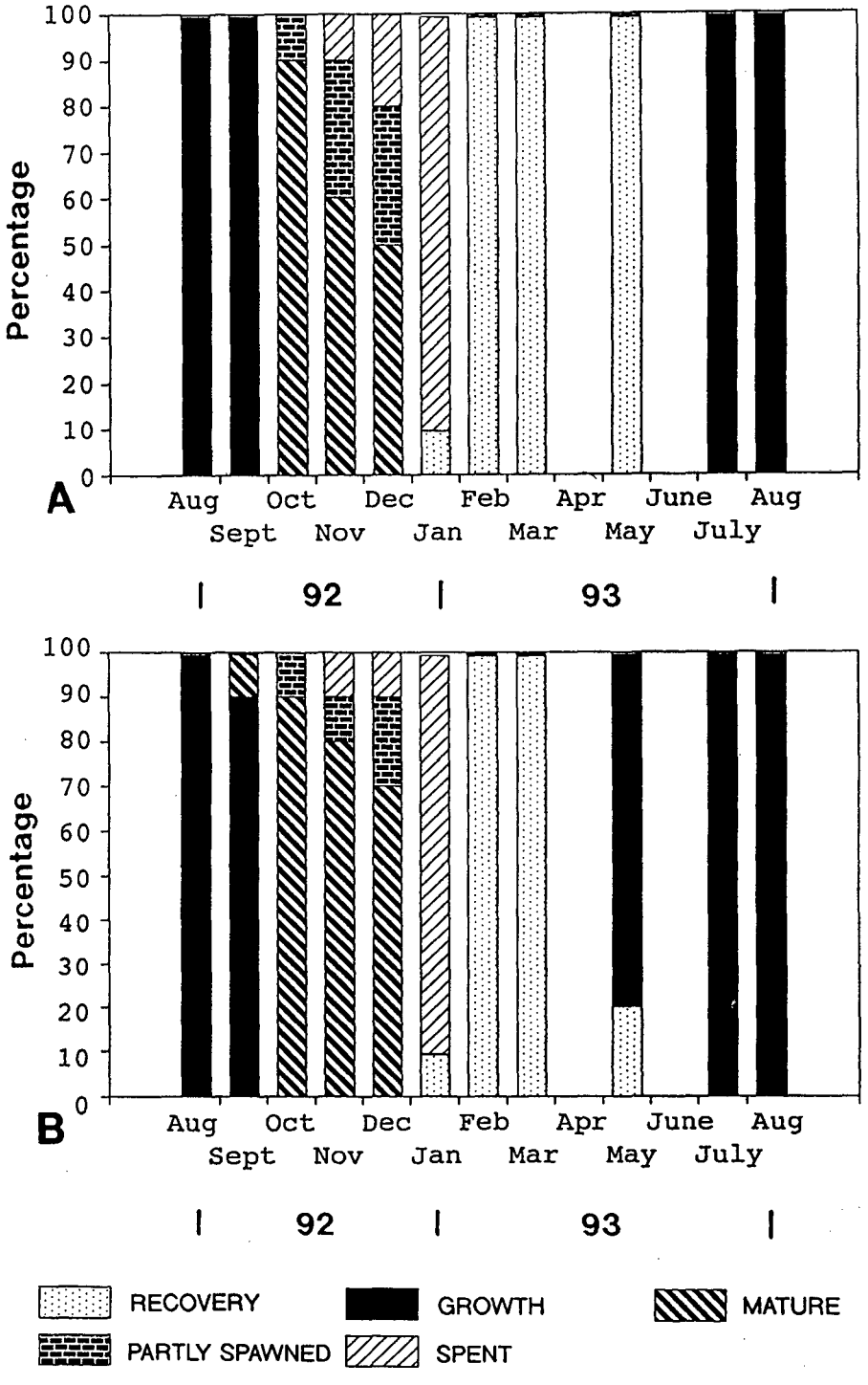


Figure 3.17. *Neostichopus grammatus.* Gametogenic cycle. Histograms show relative frequencies of gametogenic stages in **(A)** males and **(B)** females, for the period from August 1992 to August 1993.

spawnings in both sexes occurred (Fig. 3.17A,B). Again, at some time between December 1992 and January 1993 (mid-summer) the main spawning event occurred in both sexes, and by January 1993 both sexes had begun the long recovery phase (Fig. 3.17A,B), which was characterised by the reabsorption of the gonad either completely or to a few minute threads. The recovery phase of the females was shorter than that of the males, and by May 1993 (late autumn) gametogenesis had begun in the females, while gametogenesis only began in June/July 1993 (mid-winter) in the males (Fig. 3.17A,B). The gametogenic period was also long, as in *R. stephensoni* and *P. sykion.*

3.3.10 Environmental factors

The seasonal variations in sea temperature and daylength during the study period are illustrated in Figure 3.18. Maximum monthly mean sea temperatures of 23.5°C and 23.8°C were recorded in February 1992 and January 1993, respectively. Monthly mean sea temperature minima of 16.8°C and 16.2°C were recorded in September 1992 and August 1993, respectively. Maximum daylengths were recorded in December (857 minutes), while minimum daylengths were recorded in June (597 minutes). The relationship between GI values and sea temperature indicated that there was a significant relationship in *R. stephensoni* ($F=50.45$, $P<0.0001$, $r^2=0.12$) (Fig. 3.19A) and *P. sykion* ($F=34.66$, $P<0.0001$, $r^2=0.08$) (Fig. 3.19B), with maximum GI values being recorded at the lowest temperatures. However, no such relationship existed for *N. grammatus* ($F=3.54$, $P=0.061$, $r^2=0.01$) (Fig. 3.19C). The relationship between GI values and daylength indicated that there was a significant relationship in *N. grammatus* ($F=36.65$, $P<0.0001$, $r^2=0.09$) (Fig. 3.20C), with maximum GI values being recorded during the longest daylengths. By contrast, no such relationship existed for *R. stephensoni* ($F=1.85$, $P=0.173$, $r^2=0.005$) (Fig. 3.20A) and *P. sykion* ($F=0.04$, $P=0.849$,

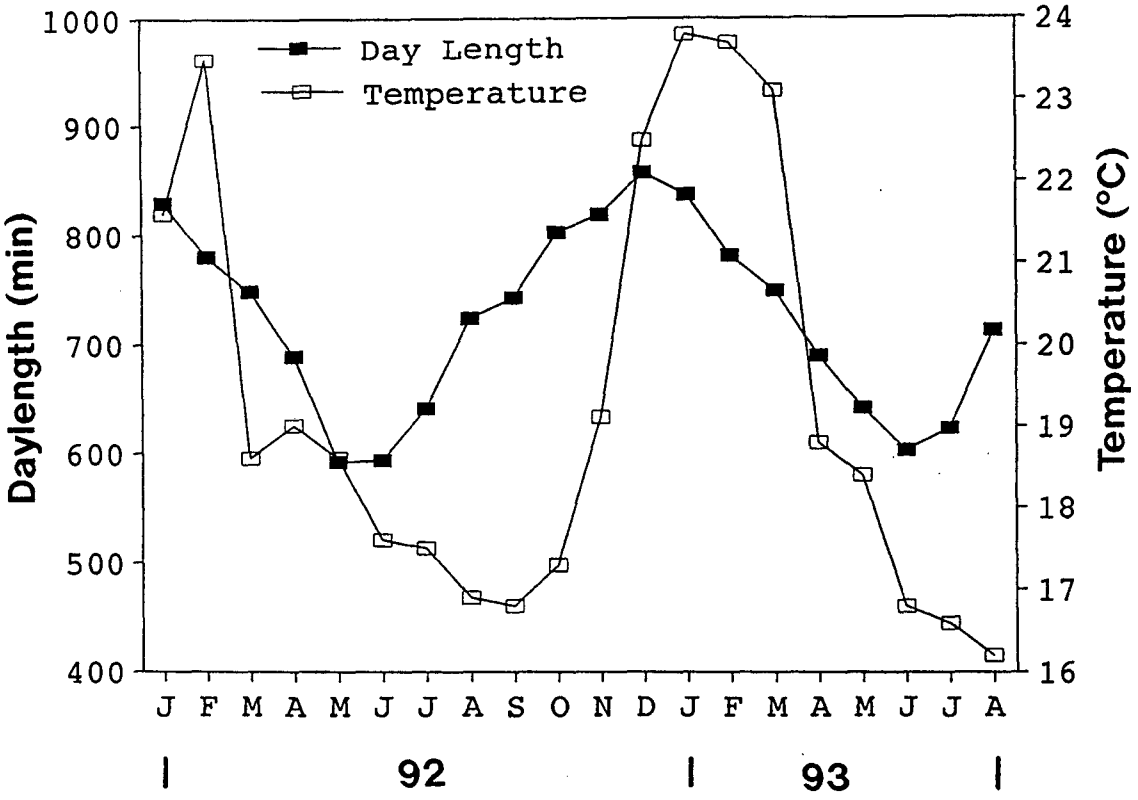


Figure 3.18. Seasonal variations in temperature and daylength during the period January 1992 to August 1993.

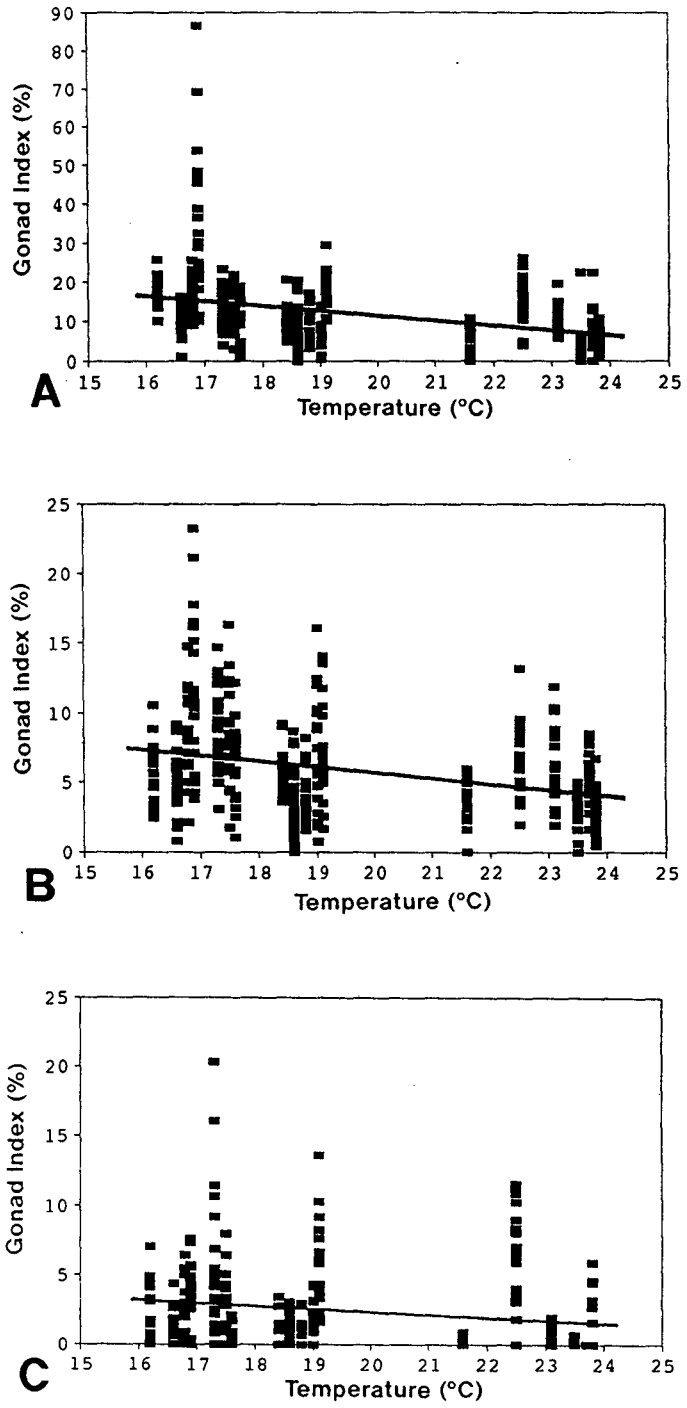


Figure 3.19. The relationship of gonad index values and temperature in **(A)** *Roweia stephensoni* (n=381, y=-1.22x + 35.58, r^2=0.12), **(B)** *Pseudocnella sykion* (n=381, y=-0.40x + 13.72, r^2=0.08) and **(C)** *Neostichopus grammatus* (n=381, y=-0.10x + 3.81, r^2=0.01), from January 1992 to August 1993.

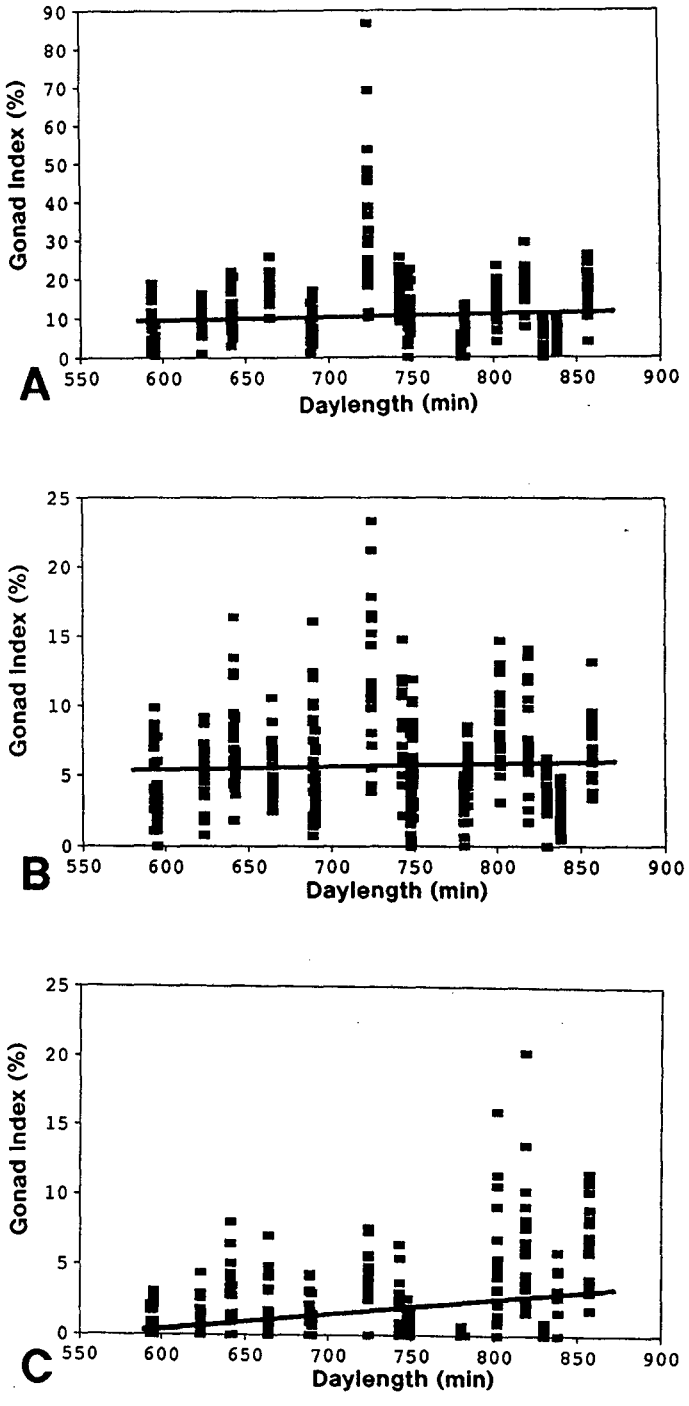


Figure 3.20. The relationship of gonad index values and daylength in **(A)** *Roweia stephensoni* (n=381, y=0.008x + 6.02, r^2=0.005), **(B)** *Pseudocnella sykion* (n=381, y=0.0004x + 5.71, r^2=0.0001) and **(C)** *Neostichopus grammatus* (n=381, y=0.01x + -5.63, r^2=0.09), from January 1992 to August 1993.

r^2=0.0001) (Fig. 3.20B). It appears that in all three species, spawning coincided with increasing sea temperature and daylength, gametogenesis was probably initiated by decreasing or low sea temperature and short daylength, with the gonads reaching maturity during sea temperature minima.

3.4 DISCUSSION

The populations of *Roweia stephensoni*, *Pseudocnella sykion* and *Neostichopus grammatus* studied at Port Elizabeth on the eastern Cape of South Africa exhibited no sexual dimorphism and were all dioecious with a sex ratio of approximately 1:1. Such a sex ratio is characteristic of most holothurians (Conand, 1982; Engstrom, 1982; Mosher, 1982; Costelloe, 1985; Cameron & Fankboner, 1986; Smiley *et al.*, 1991; Hamel *et al.*, 1993). The absence of sexual dimorphism amongst holothurians is the general rule, although hermaphrodism has been reported in some species (Green, 1978; Tyler *et al.*, 1985; Smiley *et al.*, 1991). The gonad of *R. stephensoni* and *P. sykion* consists of one tuft of unbranched tubules, such as those found in other dendrochirote holothurians (Atwood & Chia, 1974; Tyler & Gage, 1983; Costelloe, 1985; Hamel *et al.*, 1993), while that of *N. grammatus* consists of two tufts of branched tubules typical of other aspidochirote holothurians (Atwood, 1974; Smiley & Cloney, 1985; Cameron & Fankboner, 1986; Sewell & Bergquist, 1990). Hamel *et al.* (1993) have reported that the gonad of *Psolus fabricii* (a dendrochirote) is composed of large and small diameter tubules. However, no such distinction in tubule diameter was observed in *R. stephensoni* or *P. sykion*, all the tubules comprising the gonad being of a similar size. The number of gonadal tubules in *R. stephensoni* and *P. sykion* increased with animal size, and males had more tubules than females, as in *Psolus fabricii* (Hamel *et al.*, 1993). By contrast, Costelloe (1985) has indicated that although tubule numbers vary between individuals of *Aslia*

lefevrei, it was not related to the size of an individual. In addition, there was no difference in the gonad size between males and females, as observed for other holothurians (Rutherford, 1973; Costelloe, 1985), although size differs in others (Cameron & Fankboner, 1986; Hamel *et al.*, 1993). The lack of differences in weight (and hence GI values) between males and females in *R. stephensoni* and *P. sykion* may be attributed to males having more tubules of smaller diameter, while females have fewer tubules of greater diameter.

Histological examination of the gonads of the three species revealed that the tubules, like other holothurians (Atwood, 1973, 1974; Costelloe, 1985; Smiley, 1988; Smiley *et al.*, 1991; Hamel *et al.*, 1993), are composed of three tissue layers which are present in both males and females. Similarly, the gametogenic changes recorded in *R. stephensoni*, *P. sykion* and *N. grammatus* are similar to changes in gametogenic condition that are distinctive of most holothurians (Costelloe, 1985; Tyler *et al.*, 1985; Smiley, 1988; Smiley *et al.*, 1991; Hamel, *et al.*, 1993). Oocyte development in all three species followed a typical pattern, with vitellogenesis starting in oocytes of about 200µm diameter in *R. stephensoni*, 190µm diameter in *P. sykion* and 180µm diameter in *N. grammatus*. A mature oocyte of between 400-450µm in diameter in *R. stephensoni*, 300-350µm in *P. sykion* and 300-350µm in *N. grammatus* was then finally produced. Spermatogenesis in holothurians is regarded as very primitive because, amongst other factors, the nucleus of the mature spermatozoa undergoes very little change from that of the original germ cells (Pladellorens & Subirana, 1975), and is typically of the primitive form (Hodgson & Bernard, 1992). During the onset of spermatogenesis the germinal layer becomes convoluted and extends as folds into the lumen thus increasing the surface area for development (Costelloe, 1985; Tyler *et al.*, 1985; Cameron & Fankboner, 1986; Hamel *et al.*, 1993; present study). Spermatogenesis was also clearly stratified in these three species,

as for other holothurians (Costelloe, 1985; Tyler *et al.*, 1985; Hamel *et al.*, 1993), while in others the spermatogonia and spermatids are distributed randomly in the tubule lumen (Atwood, 1973, 1974).

Giese (1959) defines 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. The histological examination of the gonads, in conjunction with the mirrored monthly increases and decreases observed between GI values, tubule diameters, egg diameters and spermatozoon content of the tubules in the three species, indicated that reproduction in *R. stephensoni*, *P. sykion* and *N. grammatus* was seasonal. *Roweia stephensoni* and *P. sykion* exhibited a well defined asynchronous annual cycle of gonadal growth, characterised by a brief resting, long gametogenic and maturity periods. By contrast, although *N. grammatus* also exhibited well defined asynchronous annual cycle, it was characterised by a long resting, long gametogenic and short maturity period. It also appears that there may be temporal variation in the reproductive cycle of *R. stephensoni* and *P. sykion*, as individuals were not in the same reproductive condition in August 1993 as they were in August 1992. Variability in the reproductive cycle is not uncommon in holothurians (Sewell & Bergquist, 1990; Hamel *et al.*, 1993).

Although asexual reproduction, by means of fission, has been recorded in both dendrochirote and aspidochirote holothurians (Bonham & Held, 1963; Harriott, 1985; Smiley *et al.*, 1991), there was no evidence to suggest that it might occur in these three species. During the reproductive cycle, *R. stephensoni* and *N. grammatus* exhibited large increases in GI values (10 and 6-fold, respectively), similar to increases observed in other holothurians

(Krishnaswamy & Krishnan, 1967; Rutherford, 1973; Conand, 1989). *Pseudocnella sykion*, however, showed a comparatively small (4-fold) increase in GI values, which is also not uncommon in some holothurians (Costelloe, 1985; Conand, 1989).

A long asynchronous period of maturity during the spring and most of the summer months was maintained in *R. stephensoni* and *P. sykion*, and to a lesser extent in *N. grammatus*. Similar long maturity periods have also been reported for some holothurians (Sewell & Bergquist, 1990) and other echinoderms (Pearse & Phillips, 1968; Drummond, 1991). The long period of maturity may suggest that the populations are experiencing favourable environmental conditions with adequate nutrition, as numerous studies on echinoids suggest that the quantity and quality of food sources positively influences gonadal growth (Vadas, 1977; Larson *et al.*, 1980; Andrew, 1986). During the period of maturity, partial spawning events occurred in some individuals, but never involved the complete discharge of all gametes. This together with the presence of vitellogenic eggs during the mature stage may indicate that individuals are capable of spawning more than once, as noted for *Holothuria mexicana* (Mosher, 1982). However, it was not until the mid-summer months that the main spawning event occurred and spent individuals of the three species were observed. The main spawning event in these three species occurred at the same time of year as that reported for the east coast South African echinoid *Stomopneustes variolaris* (Drummond, 1991), and a temperate southern hemisphere aspidochirote holothurian, *Stichopus mollis*, from New Zealand (Sewell & Bergquist, 1990). Similarly, most holothurians are noted to spawn during the warmer spring to summer months (Boolootian, 1966; Mosher, 1982; Conand, 1989; Smiley *et al.*, 1991; Hamel *et al.*, 1993), although some do spawn in the cooler winter conditions (Boolootian, 1966; Conand, 1981; Costelloe, 1985).

In all three species, during the spent stage, residual spermatozoa and ova in the tubules are phagocytosed. Similar assimilation processes are reported for holothurians (Krishnan, 1968; Engstrom, 1980; Costelloe, 1985; Smiley & Cloney, 1985; Tyler *et al.*, 1985; Hamel *et al.*, 1993) and other echinoderms (Holland & Giese, 1965; Holland *et al.*, 1975; Byrne, 1990; Drummond, 1991; Laegdsgaard *et al.*, 1991; Vernon *et al.*, 1993). Pearse (1969) has suggested that the phagocytic cells facilitate the transfer of nutrients from the "left-over" gametes, and it has been suggested that the nutrients from phagocytosed "left-over" gametes are accumulated before the next gametogenic cycle begins (Pearse, 1969; Gonor, 1973; Walker, 1982). Costelloe (1985) and Hamel *et al.* (1993) suggest that the thickening of the gonadal tubule walls after spawning, as observed in *R. stephensoni* and *P. sykion*, was due to the accumulation of nutrients derived from the phagocytosis of residual gametes that are stored in the tubule walls. This may then explain the increased GI values in March each year after the brief recovery period observed in *R. stephensoni* and *P. sykion* (Figs. 3.2A & 3.3A), not accounted for in the histological examination of the tubules (Figs. 3.15A,B & 3.16A,B). In addition to gamete reabsorption, gonad tubule reabsorption in *N. grammatus* after spawning was a characteristic feature of this species, a characteristic which is not uncommon to a few species of holothurians (Green, 1978; Tyler & Gage, 1983; Smiley & Cloney, 1985). The nutrients from such reabsorption may be stored in the gut tissues, as a storage function of the gut has been reported in holothurians (Fish, 1967; Krishnan, 1968). However, reabsorption of the gonad tubules did not occur in *R. stephensoni* and *P. sykion*, as in other holothurians (Costelloe, 1985; Hamel *et al.*, 1993). The reabsorption of the tubules, either completely or to a few very short fine threads, raises questions as to where future reproductive cells originate and warrants further investigation.

After the short resting phase in *R. stephensoni* and *P. sykion*, and the longer phase in *N. grammatus*, asynchronous post-spawning growth of the gonads occurred over an extended period, as vitellogenic and spermiogenic activity continued for several months, similar to that of other holothurians (Smiley, 1988; Hamel *et al.*, 1993). The short period from spawning until gonad growth resumed in *R. stephensoni* and *P. sykion* may be attributed to accumulated high stores of reserves during the recovery stage or from the phagocytosis of relict oocytes and spermatozoa.

The seasonality of reproduction observed in *R. stephensoni*, *P. sykion* and *N. grammatus* and in many holothurians (Boolootian, 1966; Costelloe, 1985; Conand, 1989; Smiley *et al.*, 1991; Hamel *et al.*, 1993) and other echinoderms (Byrne, 1990; Drummond, 1991; Pearse & Cameron, 1991; Vernon *et al.*, 1993) raises questions about what environmental factors serve to cue reproduction. It is important that these cues initiate gonadal development at the right time so that spawning occurs at the most favourable time of the year. Annual reproductive cycles and their controlling factors have been investigated in holothurians (Krishnaswamy & Krishnan, 1967; Conand, 1981, 1982). In *R. stephensoni* and *P. sykion* gonadal growth coincided with decreasing sea temperatures, but no such relationship existed with daylength in these two species (Fig. 3.20A,B). Similarly, in echinoids sea temperature has been noted to influenced gonadal growth (Himmelman, 1978; Byrne, 1990). By contrast, gonadal growth in *N. grammatus* was not correlated with sea temperature, but rather with daylength (Fig. 3.20C), as described for some echinoids (Gonor, 1973; Pearse *et al.*, 1986; Bay-Schmith & Pearse, 1987; McClintock & Stephen, 1990; Vernon *et al.*, 1993), and the holothurian, *Psolus fabricii* (Hamel *et al.*, 1993), in that gonadal growth was under photoperiodic control. Lane and Lawrence (1979) have suggested that the environmental factors that prompt spawning

may differ from those that arouse gonadal growth. Although partly spawned individuals were observed as early as September 1992 in *R. stephensoni* and *P. sykion*, and November 1992 in *N. grammatus*, the main spawning event occurred between two successive sampling dates (December 1992 - January 1993) in all three species, indicating that spawning was simultaneous between the species, and that a similar cue initiated this spawning event. Similarly, simultaneous spawning has been reported to occur amongst other species of holothurians (McEuen, 1988), and other echinoderms (Minchin, 1987; Pearse *et al.*, 1988). Temperature is regarded as a spawning signal in many invertebrates (Orton, 1920; Giese, 1959; Giese & Pearse, 1974; Brown, 1984; Grahame & Branch, 1985), and in *R, stephensoni*, *P. sykion* and *N. grammatus* spawning was completed by the longest daylength and before the maximum sea temperature, suggest that rising sea temperature or maximum daylength may serve as a cue for the initiation of spawning. Similarly, the completion of spawning before sea temperature maxima has also been reported in echinoids (Byrne, 1990). However, studies on other holothurians by Costelloe (1985), Cameron and Fankboner (1986) and Hamel *et al.* (1993) suggest that sea temperature does not control spawning, nor did photoperiod in *Psolus fabricii* (Hamel *et al.*, 1993). Other factors such as illumination, pheromones or gametes in the water, and endogenous factors may also play a role in initiating spawning (Himmelman, 1975; Minchen, 1987; McEuen, 1988; Pearse *et al.*, 1988). Similarly, the intensity of illumination has been suggested to influence spawning in some holothurians (Costelloe, 1988; McEuen, 1988; Pearse *et al.*, 1988).

An increase in phytoplankton levels has been shown to induce spawning in some echinoderms (Himmelman, 1975; 1978; Starr *et al.*, 1990, 1992; Hamel *et al.*, 1993), and it has been demonstrated that substances found in diatoms induced spawning in the sea urchin,

Strongylocentrotus droebachiensis (Starr *et al.*, 1992). Spawning during times of phytoplankton blooms would ensure food for developing juveniles (direct developers) and larval (planktotrophic indirect developers) (Smiley *et al.*, 1991). Bearing this in mind, the eastern Cape coast of South Africa is subject to high wind velocities, and during the summer months winds with an easterly component predominate (Taljaard, 1972; Beckley, 1983), which are required to initiate upwelling (Schumann *et al.*, 1982). Such upwelling is capable of reducing sea temperatures by as much as 9°C in a few days (e.g. as during the period 25-28 December 1980; see Beckley, 1983). Such fluctuations in temperature will have a profound effect on the biota living on these shores and may serve as a cue to initiate spawning in *R. stephenson*, *P. sykion* and *N. grammatus*. On the other hand, such upwelling will make nutrients available for phytoplankton, which may result in phytoplankton blooms during this period (Probyn, 1985; Probyn & Lucas, 1987). Such blooms may also be the cues for initiating spawning in these species. However, if food abundance is important for the larvae, the possibility of competition exists. Reese (1968) described the reproductive cycles of three sympatric species of hermit crabs and indicated that the less common species have their reproductive cycles displaced and compressed to avoid larval competition with the most common species. On the eastern Cape shores, *N. grammatus* is the less common species (Chapter 2) and showed a compressed reproductive peak, nonetheless, all three species spawned at a similar time suggesting that food is probably not a limiting factor for the larvae, or that the larvae follow different developmental strategies.

The larval developmental strategies employed by shallow-water invertebrates (Thorson, 1950), holothurians (Rutherford, 1973; Strathmann & Vedder, 1977; Green, 1978; Tyler & Gage, 1983; Billett, 1991; Hamel *et al.*, 1993), and other echinoderms (McClintock & Pearse, 1986;

Bosch & Pearse, 1990) have been related to egg size and fecundity. In echinoderms, eggs with a diameter of about 100µm are usually numerous and will be expected to lead to planktotrophic development. Eggs greater than 1000µm are normally scarce and lead to direct development (Tyler *et al.*, 1982). While eggs of an intermediate size are usually moderately abundant and undergo lecithotrophic development. Based on this assumption, the egg sizes (300-450µm) of *R. stephensoni*, *P. sykion* and *N. grammatus* suggest that development from egg to juvenile is lecithotrophic with a reduced larval stage. However, Bosch (1989) has shown that in some Antarctic seastars the organic content of the egg is not necessarily related to its size, and that eggs of a similar size can develop in different ways. Similarly, this has also been demonstrated in other echinoderms (Strathmann & Vedder, 1977; McClintock & Pearse, 1986; Young *et al.*, 1989). Nevertheless, Smiley *et al.* (1991) have recorded that most holothurian larvae follow a path of direct development (all dendrochirotes), with the exception of indirect development in some members of the orders Aspidochirotida (direct development in deep-water species and indirect development in shallow-water species) and Apodida. Therefore, based on egg size assumptions and the noted presence of direct development in dendrochirotes, *R. stephensoni* and *P. sykion* probably also give rise to direct development larvae. *Neostichopus grammatus* on the other hand may give rise to either direct or indirect development larvae, but until detailed studies are undertaken as to the mode and type of larval development in these three species such statements may be misleading.

In conclusion, it appears as if *R. stephensoni* and *P. sykion* follow a different reproductive pattern to that of *N. grammatus*. *Roweia stephensoni* and *P. sykion* follow a pattern involving early development of gametes after spawning over an extended period, followed by an extended maturity period. By contrast, *N. grammatus* followed a pattern of a long latent phase

after spawning, an extended gametogenic phase, followed by a short maturity period. In addition, the gonad tubules were always retained throughout the year in *R. stephensoni* and *P. sykion*, whilst in *N. grammatus* the gonad tubules regressed after spawning. Larval development may also be different between *R. stephensoni* and *P. sykion*, and *N. grammatus*. The difference in the reproductive patterns observed in these three species may be as a result of their co-existence, or simply a result of phylogenetic differences. Nonetheless, both of these patterns prove to be suitable and ensure the survival of the species. Future work may involve determining the factors that are responsible for the control of spawning and gametogenesis in these three species. In addition, detailed accounts of the larval developmental strategies followed by these three species are still needed.

CHAPTER FOUR

BUCCAL TENTACLE MORPHOLOGY AND FEEDING BIOLOGY OF FIVE SYMPATRIC SPECIES OF INTERTIDAL HOLOTHURIANS

4.1 INTRODUCTION

Holothurians are chiefly deposit- or suspension-feeders, extending their branched buccal tentacles to either sweep them over the adjacent substrate or to hold them out in the sea water (Massin, 1982b). Members of the order Dendrochirotida are generally believed to feed on particles suspended in the water column, using finely branched dendritic tentacles (Fankboner, 1978, 1981; Massin, 1982b; Smith, 1983b; Costelloe & Keegan, 1984). Initially, the capture of suspended material by the tentacles was credited to a coating of mucus applied to them within the pharyngeal cavity (Fish, 1967). It is now widely accepted that food particles are ensnared by means of specialized adhesive areas on the most distal regions of the tentacle branches, with no direct involvement of pharyngeal secretions (Fankboner, 1978, 1981; Smith, 1983b; Costelloe & Keegan, 1984). Similarly, entrapment of food particles between the tentacle branches is regarded as playing a minor role in the feeding process (Fankboner, 1978; Costelloe & Keegan, 1984).

In contrast to suspension-feeding holothurians, members of the order Aspidochirotida are deposit-feeding holothurians which are common in deep-sea (Billett, 1991) and shallow-water communities (Hammond, 1982). In these habitats they are important reworkers of sediments (Bonham & Held, 1963; Bakus, 1973; Hauksson, 1979; Massin, 1982a; Coulon & Jangoux, 1993), as they ingest superficial sediments and feed on non-living detritus and associated micro-organisms (Yingst, 1976; Moriarty, 1982), promote and return nutritive elements to the water column (Rhodes & Young, 1971), and enhance the production of sediment associated bacteria (Amon & Herndl, 1991). These holothurians have peltate oral tentacles (Massin, 1982b), and push sediments into the mouth in a "shovel" or "broom-like" manner (Bouland *et al.*, 1982; Massin, 1982b). Both mechanical (Roberts, 1979; Bouland *et al.*, 1982) and adhesive (Hammond, 1982) feeding models have been ascribed to these tentacles.

The ingestion of a preferred particle sizes from the sediment has been identified in many deposit-feeders, such as polychaetes (Hylleberg, 1975), amphipods (Fenchel *et al.*, 1975), gastropods (Fenchel *et al.*, 1975) and bivalves (Reid & Reid, 1969; Hylleberg & Gallucci, 1975). However, there is disagreement as to whether or not aspidochirotes are selective feeders. Selective feeding has been advocated by Yanamouti (1939), Rhoads and Young (1971), Hauksson (1979), and Roberts (1979), whilst Powell (1977), Sloan & von Bodungen (1980), Hammond (1982), and Yingst (1982) found no evidence for particle size selection.

Despite the ecological importance of holothurians, those in southern Africa have received little attention and our understanding of their feeding biology is very poor. Five species of holothurians, viz *Roweia stephensoni*, *Pseudocnella sykion*, *Aslia spyridophora*, *Roweia frauenfeldi frauenfeldi*, and *Neostichopus grammatus*, are common intertidally on the eastern

Cape coast, some of which can occur in relatively high densities (Chapter 2). They are also sympatric in their distribution from Cape Agulhas to East London (Thandar, 1985, 1987a, 1987b, 1991).

The present study investigated aspects of the feeding biology of these five co-existing holothurians. The aims of the study were:- 1) to compare the structure of the buccal tentacles; 2) to determine the type and size range of food particles ingested by each species; and 3) to determine whether the gut lengths of the five species varied in relation to the food type ingested. Hence, these aims were related to the exploitation of different food resources by the five different species.

4.2 MATERIALS AND METHODS

4.2.1 Specimen collection

Four species of dendrochirotes (*Roweia stephensoni*, *R. f. frauenfeldi*, *Aslia spyridophora*, *Pseudocnella sykion*), and one species of aspidochirote (*Neostichopus grammatus*) were collected from the intertidal region of rocky shores at Port Elizabeth and Port Alfred in the eastern Cape of South Africa. Animals were transported back to the laboratory and either dissected immediately or, when necessary, housed in an aerated aquaria at 20°C.

4.2.2 Buccal tentacle morphology

The buccal tentacles of three individuals of each species were examined by scanning electron microscopy and light microscopy. In order to fix and examine tentacles in an extended state, the usual way of initially preparing tentacles before fixation has been with a chemical anaesthetic, ligaturing and then amputation (Costelloe & Keegan, 1984). A number of

recommended anaesthetics, such as menthol crystals, lithium carbonate, magnesium chloride, magnesium sulphate and carbon dioxide (Mahoney, 1966) were used, but this technique proved unsuccessful as most species did not relax, and the tentacles contracted upon amputation. A cryo-based technique was therefore developed for the preparation of the tentacles, which captured the tentacles in their extended state. The animals were allowed to acclimatize in a small container covered by a minimal amount of sea water. The container was darkened which stimulated the animals to extend their buccal tentacles. On the assumption that the animals were acclimatized with tentacles extended, the lid was removed and the container immediately flooded with an excess of liquid nitrogen. This captured the tentacles in there extended state. The frozen animals were then thawed in a fixative (Bouin's or 2.5% glutaraldehyde in filtered sea water), and allowed to fix for up to 4 hours before the tentacles were amputated. Preparation for scanning electron microscopy following fixation involved dehydration by means of a graded acetone series, which also removed surface mucus, and critical-point drying. Samples were subsequently coated in gold and photographed with a Jeol JSM 840 scanning electron microscope. Light microscopy preparation following fixation involved dehydration by means of a graded ethanol series and embedding in Paraplast. Longitudinal and transverse sections were cut on a Leitz rotary microtome at 5-7μm and stained in haematoxylin and eosin, and Toluine blue for mucous secretions (Humason, 1967).

4.2.3 Gut content and particle size composition

The particle size composition from the gut contents of 100 individuals of each species was pooled and analyzed. The gut contents were removed immediately and placed in 70% alcohol. In addition, the first 1cm layer of sediment was collected from areas where all the species

were found. Holothurians are naturally aquatic feeders, so ingested particles were wet sieved using Endecotts graded sieves. The contents remaining in each sieve were then dried at 60°C to constant weight and expressed as a percentage of the cumulative dry weight. It has been suggested in numerous studies that deposit-feeding holothurians selectively ingest sediments of a particular size (e.g. Hauksson, 1979; Roberts, 1979). Consequently, electivity values were calculated for *N. grammatus* in order to determine if certain sized sediments were selected. Electivity values were computed by the method of Ivlev (1961) as follows:-

$$E = (r_i - p_i) \div (r_i + p_i)$$

where, E is electivity value; r_i, in this case, the percentage of the i^{th} particle size class in the holothurian gut; and p_i, the percentage of the i^{th} particle size class in the sediment. E varies from -1 to +1 and 0 indicates no selection, negative numbers indicate selection against, and positive numbers indicate selection for any particle size class.

In addition, 5 individuals of each species were examined in an attempt to identify gut contents. In *P. sykion* and *N. grammatus* the ingested food particles were sufficiently large enough to be identified under a Wild M5A stereo dissecting microscope. However, due to the fine nature of the ingested food particles in the remaining species, subsamples of the gut contents of each species were analyzed using scanning electron microscopy. The samples were dehydrated through a graded ethanol series, critical-point dried, gold coated and examined in a Jeol JSM 840 scanning electron microscope. After each ethanol series the samples were centrifuged to form a pellet thus ensuring that no particles remained in suspension. All the component food material was identified into one of several large taxonomic groups.

4.2.4 Comparative analysis of gut lengths

Analysis of gut length involved the collection of 100 individuals of each species over a range of sizes. Sizes were based on drained volume (drained in order to eliminate the influence of water in the water-vascular system) in order to achieve a standard animal size for all the species, based on the area the body occupied. Dry weights over emphasised species with increased skeletal material, wet weights over emphasised species with an increased water component of the body tissues, while the length of the animal was impossible to measure as the holothurians contracted when touched. Animals were housed in an aerated aquaria and starved for 2-3 days, by which time the gut contents had been voided, thus allowing for easier handling of the guts. Guts were removed, spread out to their full extent and measured to the nearest millimetre. Statistical analysis comparing the gut lengths of the species involved establishing a standardization value, which was calculated in terms of a gut length:volume ratio for each individual of each species.

4.2.5 Feeding observations

Five individuals of each species were housed in an aerated aquaria to observe tentacle movement during feeding. Similarly, nocturnal field observations were also undertaken at Port Alfred.

4.2.6 Statistical analysis

Simple linear regressions were used to determine if a relationship existed between gut length and body size in each species, and a one-way-analysis of variance (ANOVA) using the standardization values (gut length:volume ratios) coupled with a Sheffe's comparison of range test was performed to determine if the gut lengths differed significantly between the species

(Sokal & Rohlf, 1981).

4.3 RESULTS

4.3.1 Buccal tentacle morphology

On the basis of differing tentacle morphology, two main forms of tentacles are recognized:- 1) a dendritic form, typical of the dendrochirotes and 2) a peltate form, typical of the aspidochirote holothurians.

TRUE DENDRITIC FORM:- possessed by *R. stephensoni*, *P. sykion* and *A. spyridophora*. The tentacle crown of all these species is composed of 10 dendritically branched tentacles of equal length arranged around the mouth in a pentamerous manner. Each tentacle is thickest at its base and branches several times forming secondary, tertiary and sometimes quaternary branches (Figs. 4.1A, 4.2A & 4.3A). The most distal tentacle branches terminate into numerous swellings or nodes, measuring between 80-120μm in diameter in *R. stephensoni* (Fig. 4.1B), 60-100μm in *P. sykion* (Fig. 4.2B) and 100-140μm in *A. spyridophora* (Fig. 4.3B). In *R. stephensoni*, each tentacle node has 4-7 circular areas or discs of papillate projections each containing from 25-39 papillae (Fig. 4.1C). In *P. sykion*, each tentacle node has 5-11 discs of papillate projections each containing from 6-15 papillae (Fig. 4.2C), while in *A. spyridophora*, each tentacle node has 4-12 discs of papillate projections each containing from 5-17 papillae (Fig. 4.3C). The discs vary in diameters from 15-30μm in *R. stephensoni*, 11-15μm in *P. sykion* and 10-14μm in *A. spyridophora* depending on the number of papillae present. In all the species in this group, small stout cilia, measuring about 3μm in length in *R. stephensoni*, and about 2μm in *P. sykion* and *A. spyridophora*, are situated amongst the papillae and often occur in pairs or triplets (Figs. 4.1D, 4.2D & 4.3D).

MODIFIED DENDRITIC FORM:- possessed by *R. f. frauenfeldi*. The tentacle crown consists

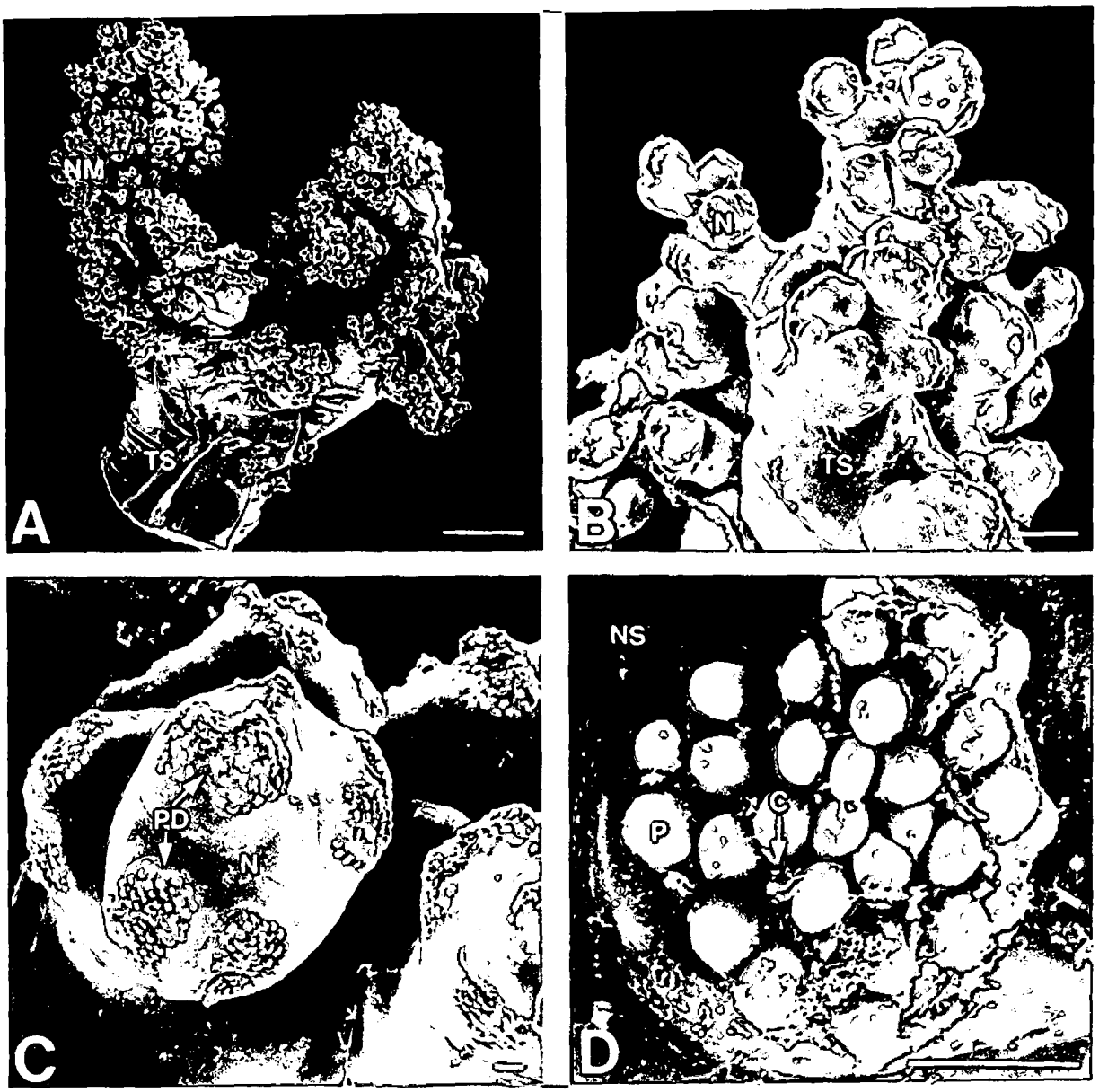


Figure 4.1. *Roweia stephensoni.* **(A)** General view of a whole buccal tentacle showing the shaft and the distal node masses (Scale bar = 1mm); **(B)** distal portion of extended buccal tentacle showing the nodes (Scale bar = 100μm); **(C)** buccal tentacle node covered with discs of papillate projections (Scale bar = 10μm); **(D)** papillate disc on node surface showing numerous papillae interspersed with cilia (Scale bar = 10μm). **C**: cilia; **N**: node; **NM**: node mass; **NS**: node surface; **P**: papillae; **PD**: papillate disc; **TS**: buccal tentacle shaft.

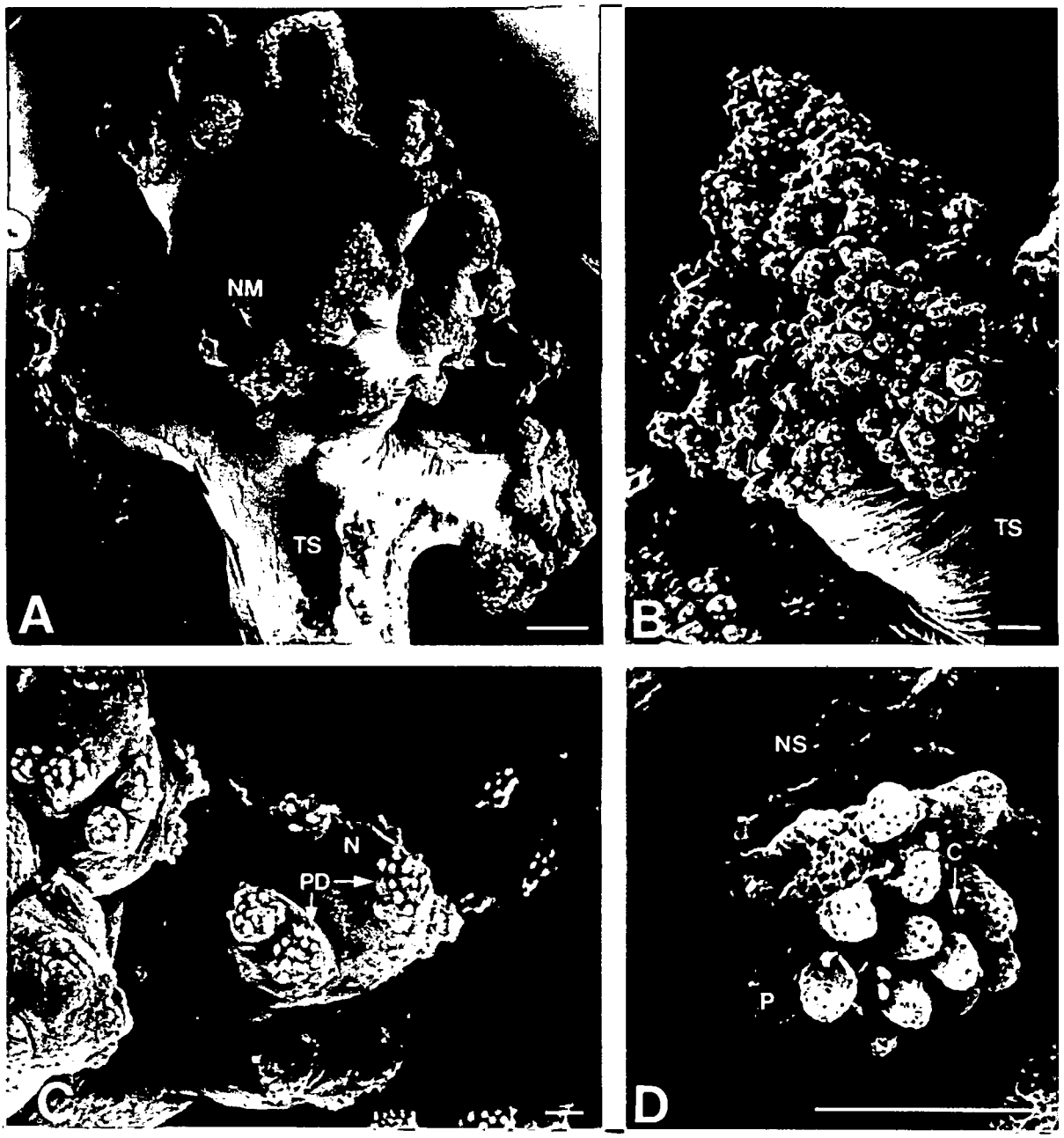


Figure 4.2. *Pseudocnella sykion.* **(A)** General view of a whole buccal tentacle showing the shaft and the distal node masses (Scale bar = 1mm); **(B)** distal portion of extended buccal tentacle showing the nodes (Scale bar = 100μm); **(C)** buccal tentacle node covered with discs of papillate projections (Scale bar = 10μm); **(D)** papillate disc on node surface showing numerous papillae interspersed with cilia (Scale bar = 10μm). **C**: cilia; **N**: node; **NM**: node mass; **NS**: node surface; **P**: papillae; **PD**: papillate disc; **TS**: buccal tentacle shaft.

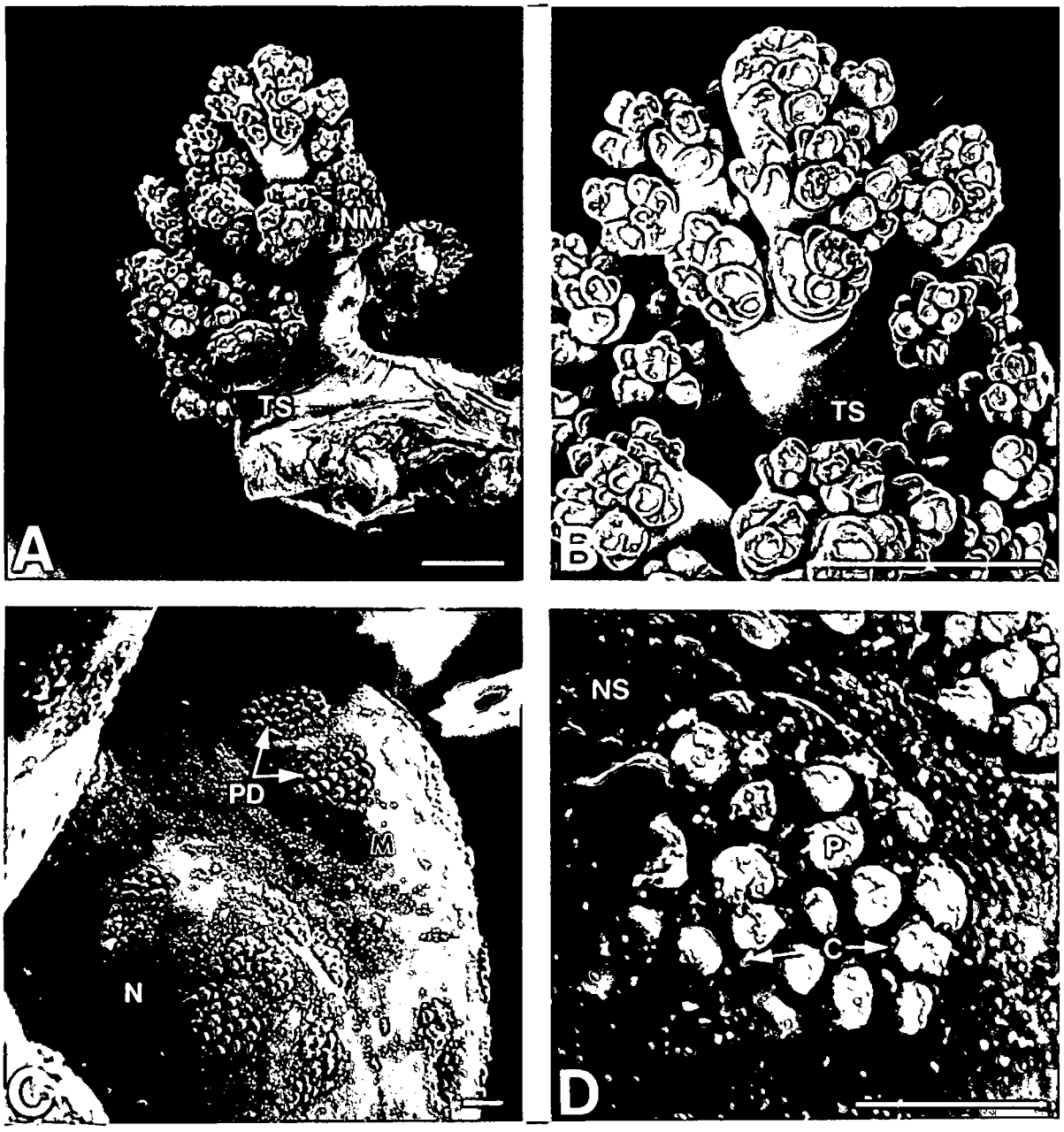


Figure 4.3. *Aslia spyridophora.* **(A)** General view of a whole buccal tentacle showing the shaft and the distal node masses (Scale bar = 1mm); **(B)** distal portion of extended buccal tentacle showing the nodes (Scale bar = 1mm); **(C)** buccal tentacle node covered with discs of papillate projections (Scale bar = 10μm); **(D)** papillate disc on node surface showing numerous papillae interspersed with cilia (Scale bar = 10μm). **C**: cilia; **M**: microvilli; **N**: node; **NM**: node mass; **NS**: node surface; **P**: papillae; **PD**: papillate disc; **TS**: buccal tentacle shaft.

of 10 modified dendritic tentacles of equal length, arranged around the mouth in a pentamerous manner. Each tentacle is thickest at its base and branching is restricted (Fig. 4.4A). Unlike the typical dendritic tentacles characteristic of *R. stephensoni*, *P. sykion* and *A. spyridophora*, in *R. f. frauenfeldi* tentacle branching is reduced and the branches are thick and robust. The relatively few most distal branches form large bulbous nodes, measuring between 230-325µm in diameter (Fig. 4.4B). The tentacle nodes lack discs of papillate projections (Fig. 4.4C), but are covered with numerous microvilli and cilia which often occur in pairs or bunches, measuring about 2µm in length (Figs. 4.4D & 4.4E). The tentacles also have symbiotic ciliates attached to them, with their associated bacteria (Figs. 4.4B & 4.4F).

PELTATE FORM:- possessed by *N. grammatus*. The tentacle crown is composed of 10 peltate branched tentacles of equal length arranged around the mouth. Again each tentacle is thickest at its base and branches several times forming secondary, tertiary and sometimes quaternary branches (Fig. 4.5A). The most distal tentacle branches terminate into numerous nodes, measuring from 80-110µm in diameter (Fig. 4.5B). These form the functional surface of the tentacle when splayed against the substratum during feeding. No discs of papillate projections occur on the tentacle nodes (Fig. 4.5C), but the surface is interspersed with cilia, measuring about 2µm in length (Fig. 4.5D).

The histological structure of the tentacles of all the species studied is similar and comprises of four well defined layers (Fig. 4.6A). On the exterior, a pigmented epithelial layer, underlying this are two layers, one of muscle and the other of connective tissue, and an inner layer of endothelium surrounds the water-vascular system of the tentacle. The tentacle water-vascular system is continuous with the rest of the water vascular system. On the nodes, the epithelial layer consists of elongated epithelial cells (Fig. 4.6B), which have a secretory

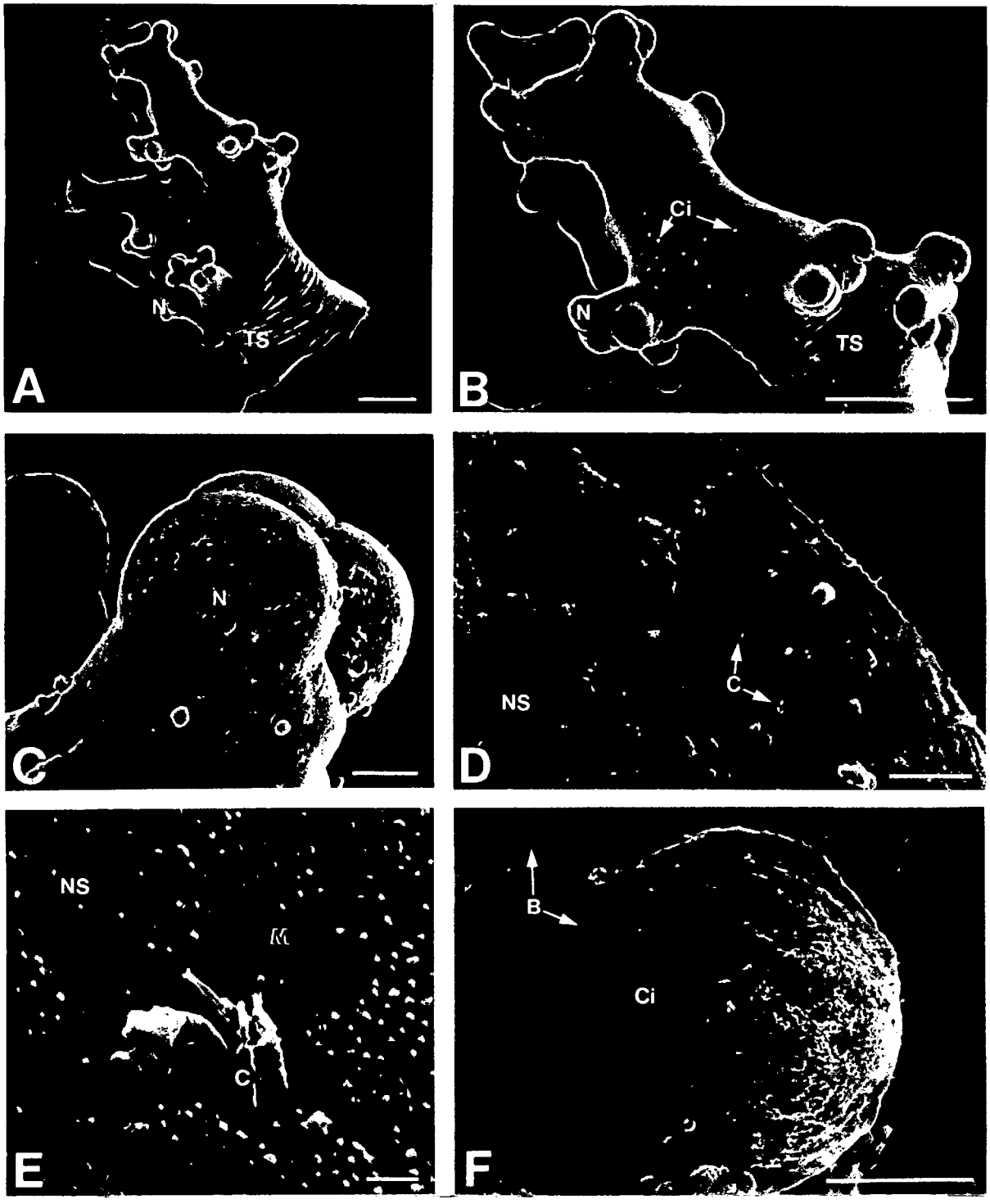


Figure 4.4. *Roweia frauenfeldi frauenfeldi.* **(A)** General view of a whole buccal tentacle showing the shaft and the large distal nodes (Scale bar = 1mm); **(B)** distal portion of extended buccal tentacle showing the nodes and the symbiotic ciliates (Scale bar = 1mm); **(C)** buccal tentacle node lacking discs of papillate projections (Scale bar = 100μm); **(D)** node surface bearing numerous microvilli and cilia (Scale bar = 10μm); **(E)** magnified view of a cilia cluster and the numerous microvilli on the node surface (Scale bar = 1μm); **(F)** symbiotic ciliate with its associated bacteria on buccal tentacle shaft (Scale bar = 10μm). **B**: bacteria; **C**: cilia; **Ci**: ciliate; **M**: microvilli; **N**: node; **NS**: node surface; **TS**: buccal tentacle shaft.

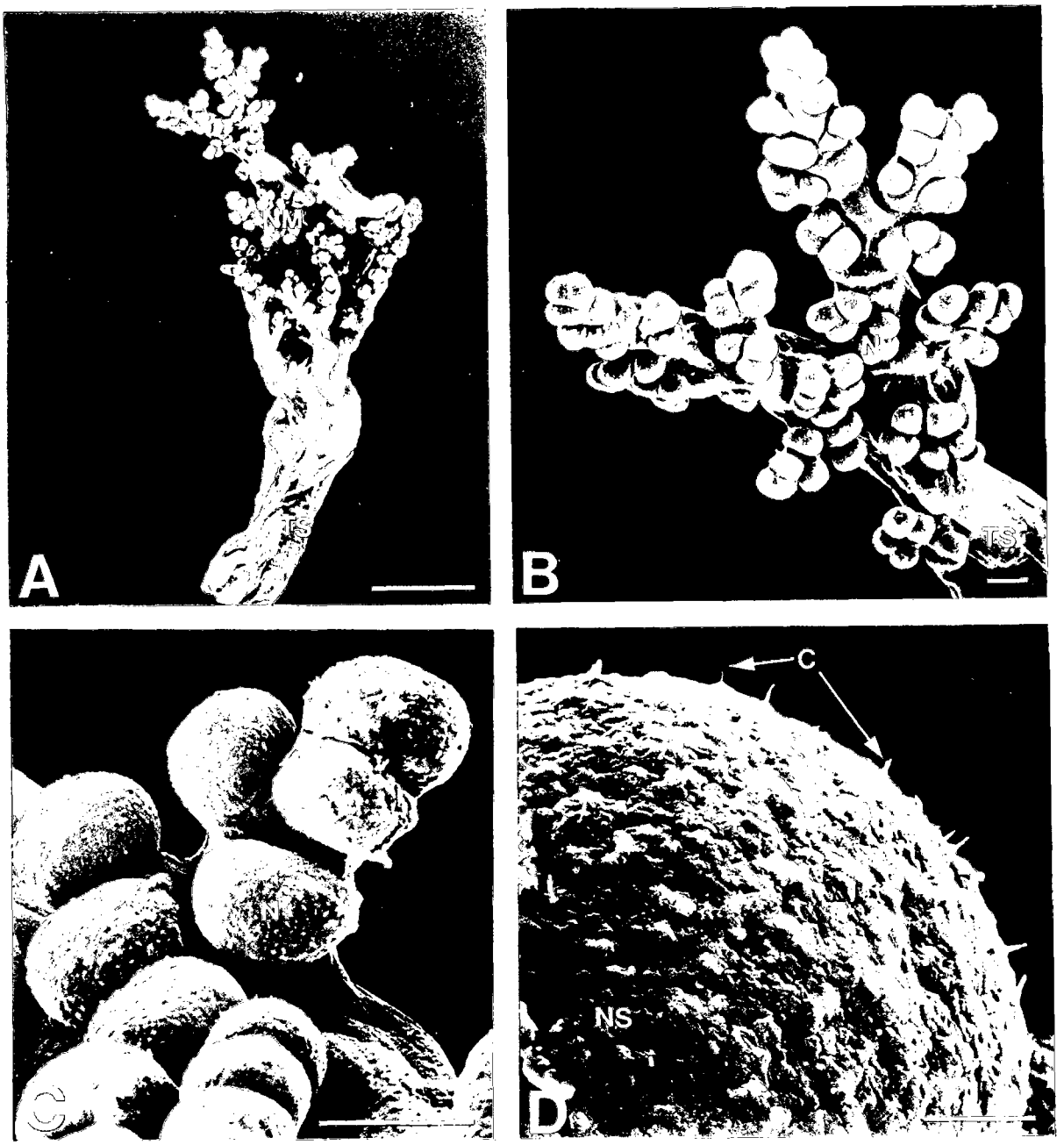
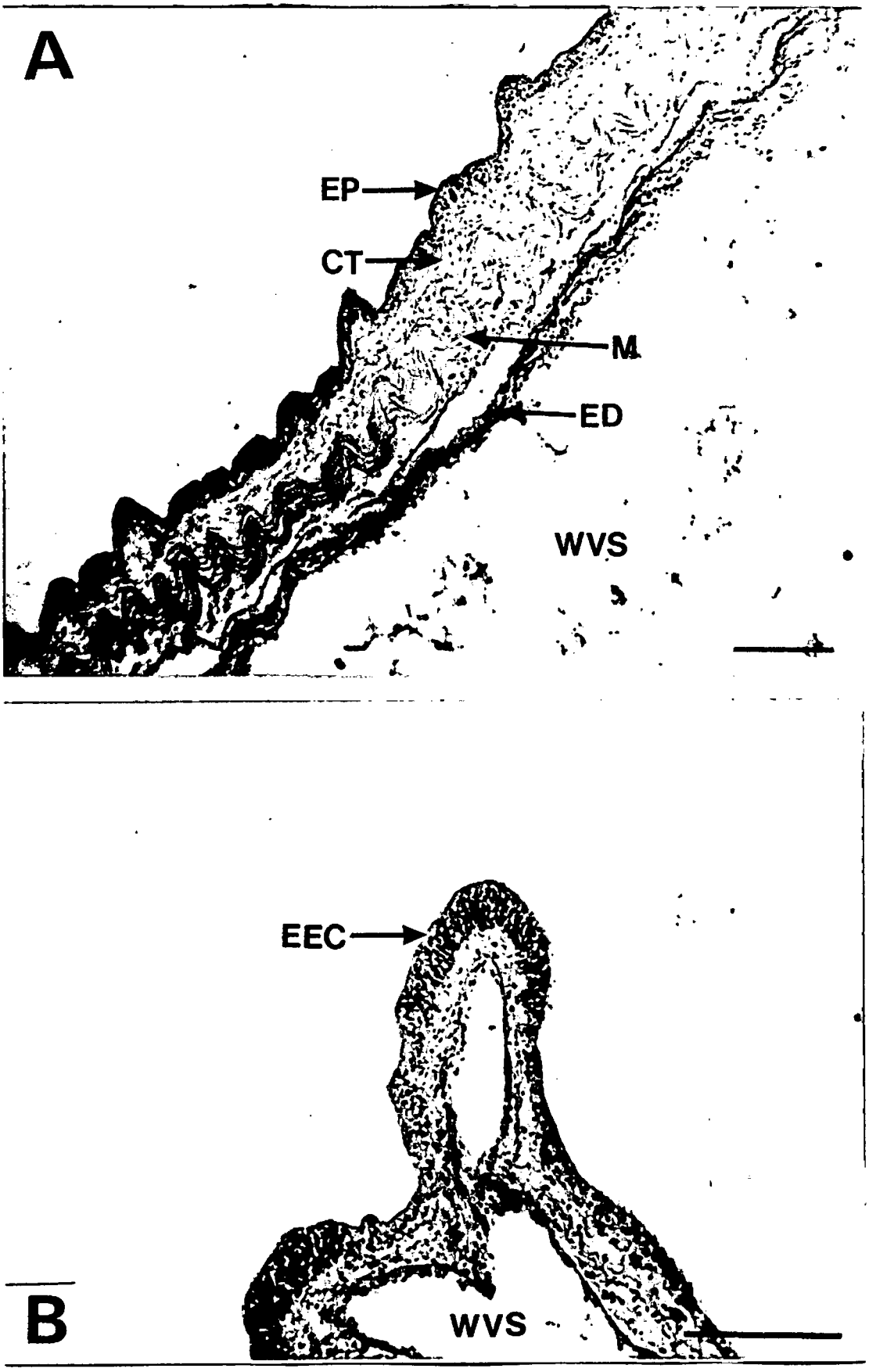


Figure 4.5. *Neostichopus grammatus.* **(A)** General view of a whole buccal tentacle showing the shaft and the distal node masses (Scale bar = 1mm); **(B)** distal portion of extended buccal tentacle showing the nodes (Scale bar = 100μm); **(C)** buccal tentacle nodes lacking discs of papillate projections (Scale bar = 100μm); **(D)** node surface bearing numerous cilia (Scale bar = 10μm). **C**: cilia; **N**: node; **NM**: node mass; **NS**: node surface; **TS**: buccal tentacle shaft.

Figure 4.6. Characteristic tissue layers observed in *Roweia stephensoni*, *Pseudocnella sykion*, *Aslia spyridophora*, *Roweia frauenfeldi frauenfeldi* and *Neostichopus grammatus*. **(A)** Longitudinal section through buccal tentacle shaft; **(B)** longitudinal section through the buccal tentacle node. (Scale bars = 100μm). **CT**: connective tissue; **ED**: endothelium; **EEC**: elongated epithelial cells; **EP**: epithelium; **M**: muscle tissue; **WVS**: water-vascular system.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function.

4.3.2 Gut content and particle size composition

All the species studied were omnivorous and could be divided into one of three groups based on the type and size of the food particles ingested.

SUSPENSION-FEEDERS:- consisted of *R. stephensoni*, *P.sykion* and *A. spyridophora*. Gut contents all comprised largely of unidentifiable organic matter, together with unicellular and filamentous algae, macroalgal fragments, diatoms, dinoflagelates, sponge spicules, fragmented urchin spines, and fragments of arthropod exoskeletons (Fig. 4.7). *Roweia stephensoni* ingested particles ranging from <53µm-1mm, with the greatest component (51%) less than 53µm, another large component (35%) ranged from 106-500µm (Fig. 4.8A). *Pseudocnella sykion* ingested particles ranging from <53-500µm, with the greatest component (51%) less than 53µm, another large component (23%) ranged from 106-250µm (Fig. 4.8A). *Aslia spyridophora* ingested particles ranging from <53-500µm, with the greatest component (72%) less than 53µm, another large component (15%) ranged from 106-250µm (Fig. 4.8A).

"HEAVY" SUSPENSION-FEEDERS:- The gut contents of *R. f. frauenfeldi* consisted of unidentifiable plant matter, gastropod shells, fragments of red, green and brown algae, coralline algae, whole crustacean exoskeletons and appendages, polychaete worm tubes, amphipod exoskeletons, sea urchin spines and sand grains. The ingested particles occupied the entire size range tested, ranging in size from <53µm->3.35mm (Fig. 4.8B). The greatest component (54%) ranged from 250µm-1.18mm.

DEPOSIT-FEEDERS:- The gut contents of *N. grammatus* consisted of a large spectrum of sand grains, unidentifiable plant matter, coralline algae, crustacean appendages, sea urchin spines, calcareous fragments, gastropod and bivalve shells. The ingested particles also

Figure 4.7. Sample of the gut contents of *Roweia stephensoni*, *Pseudocnella sykion* and *Aslia spyridophora*. **(A,B)** food particles commonly found, such as small plant fragments, sponge spicules, filamentous algae, small echinoid spines, diatoms and unidentifiable organic matter (Scale bar = 100μm and 1mm, respectively); **(C,D)** magnified view of diatoms (Scale bars = 10μm); **(E)** magnified view of crustacean fragment (Scale bar = 10μm); **(F)** magnified view of small amphipod exoskeleton (Scale bar = 100μm). **D**: diatom; **E**: echinoid spine; **F**: filamentous algae; **P**: plant fragment; **S**: spicule; **U**: unidentifiable organic matter.

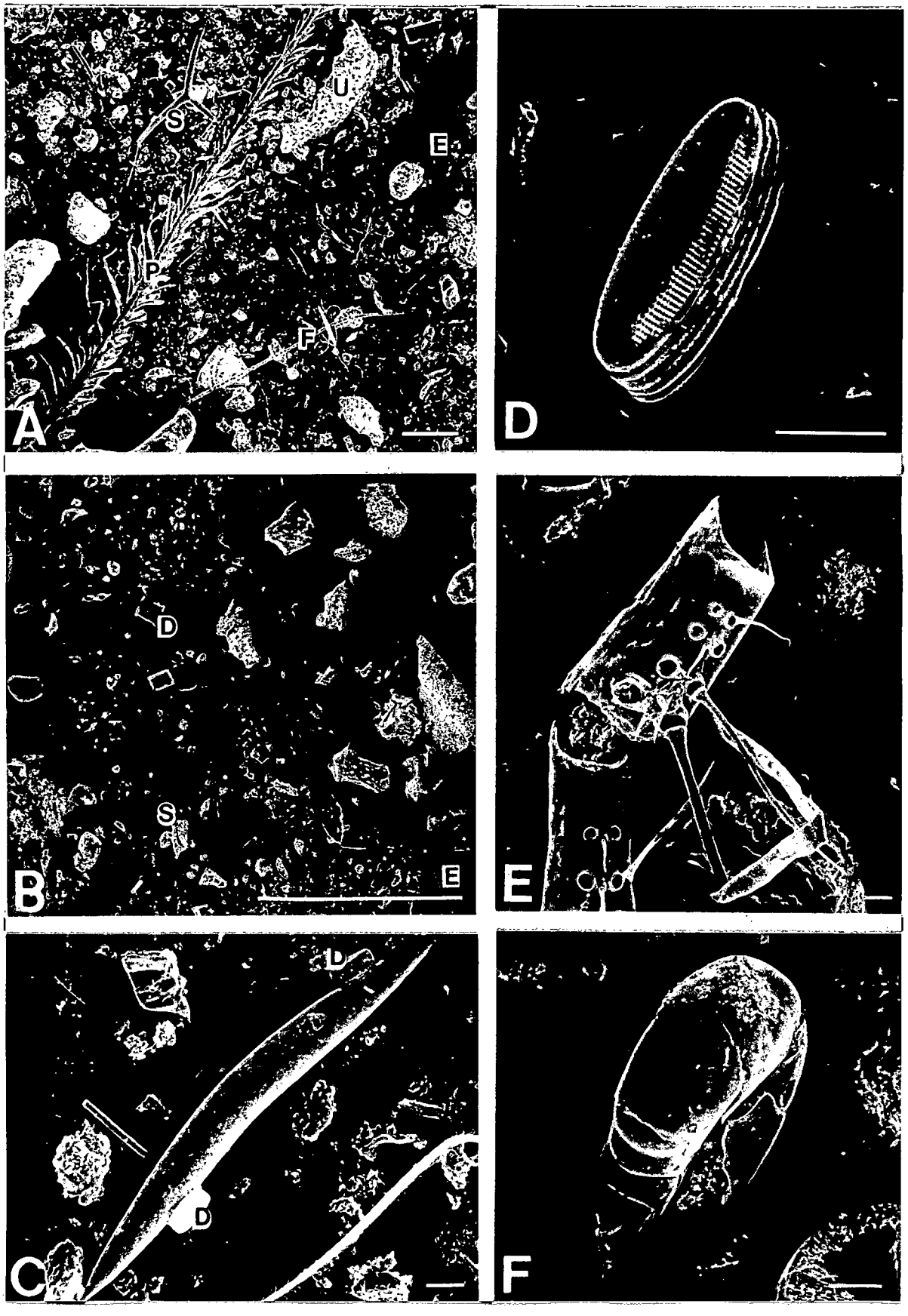
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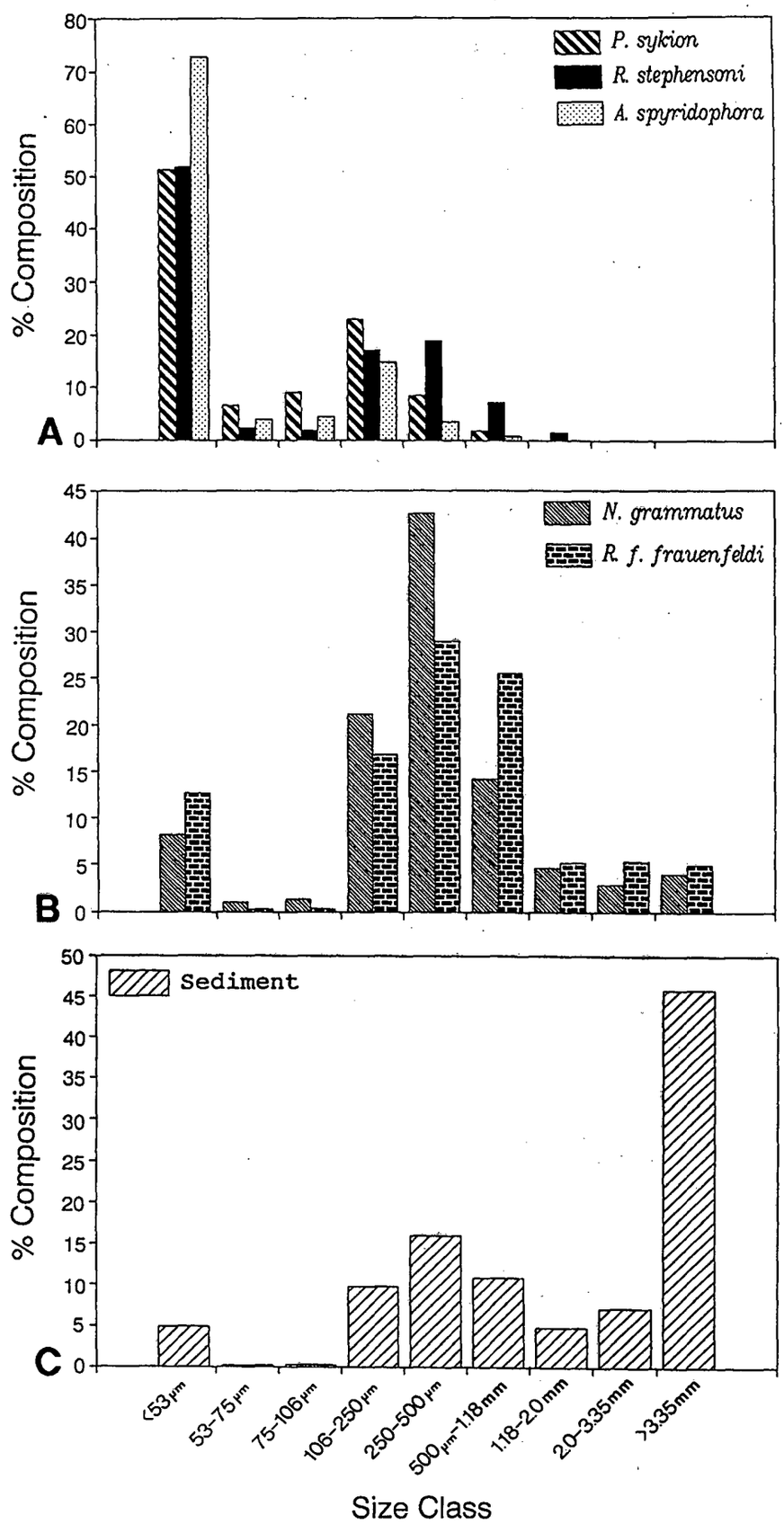
Figure 4.8. The particle size composition of the ingested food, pooled from 100 individuals of each species. **(A)** *Roweia stephensoni*, *Pseudocnella sykion* and *Aslia spyridophora*, **(B)** *Roweia frauenfeldi frauenfeldi* and *Neostichopus grammatus*; and **(C)** particle size composition of the sediments from the region in which the five species were found.



than smaller individuals (Figs. 4.9A,B,C,D,E & F). The gut lengths also varied considerably

occupied the entire range tested, ranging from <53μm->3.35mm (Fig. 4.8B). The greatest component (64%) ranged from 106-500μm. Analysis of the particle size range of the adjacent sediments available for *N. grammatus* to feed upon (Fig. 4.8C) revealed that a large component of sediment (46%) is greater than 3.35mm, and consisted mainly of pebbles and shell fragments. Another large component (35%) ranged from 106μm-1.18mm, and consisted mainly of sand grains and shell fragments. Electivity values comparing gut and adjacent substratum sediments suggested that there was a preference for smaller particles ranging in size form 53-106μm, while there was a negative selectivity for particle greater than 3.35mm (Table 4.1). This interpretation, however, must be viewed with extreme caution, as the substratum layering may be disturbed when taking sediment samples. The actual sediment ingested by the holothurian probably only comes from the upper most surface layer.

Table 4.1. Electivity values for *N. grammatus* calculated from gut and substrate particle size distribution by the method of Ivlev (1961). (•) particles selected for, (*) particles selected against.

	Particle size class								
Species	<53μm	53-75μm	75-106μm	106-250μm	250-500μm	500μm-1.18mm	1.18-2.0mm	2.0-3.35mm	>3.35mm
N. grammatus	0.25	0.67•	0.60•	0.37	0.45	0.14	-0.01	-0.43	-0.84*

4.3.3 Comparative analysis of gut lengths

There was a positive relationships between gut lengths and drained body volume in *R. stephensoni* ($F=95.87$, $P<0.0001$, $r^2=0.49$), *P. sykion* ($F=53.82$, $P<0.0001$, $r^2=0.35$), *A. spyridophora* ($F=103.09$, $P<0.0001$, $r^2=0.51$), *R. f. frauenfeldi* ($F=101.64$, $P<0.0001$, $r^2=0.51$) and *N. grammatus* ($F=78.26$, $P<0.0001$, $r^2=0.44$), with larger individuals having longer guts than smaller individuals (Figs. 4.9A,B,C,D,E & F). The gut lengths also varied considerably

within and between the species, for example an animal of $5cm^3$ in *R. stephensoni* had a gut length of between 20-38cm, *P. sykion* between 15-43cm, *A. spyridophora* between 27-50cm, *R. f. frauenfeldi* between 12-35cm, and *N. grammatus* between 12-28cm. A one-way analysis of variance (ANOVA) using the standardization ratio values (i.e. gut length:volume ratios) indicated that there was a significant difference in the gut length:volume ratios ($F=143.6$; $P<0.001$) between the species. The results from the Scheffe's multiple range test indicated that there was a significant difference between the gut length:volume ratios of all the species, except between *R. stephensoni* and *R. f. frauenfeldi*, and between *R. stephensoni* and *P. sykion* (Table 4.2). Similarly, it indicated that the dendrochirote holothurians had significantly higher gut length:volume ratios than the aspidochirote holothurian.

Table 4.2. Results from a Scheffe's multiple range analysis at 95% confidence level comparing the relationship between gut length:volume ratios of five holothurian species from the eastern Cape coast of South Africa.

Species	Mean gut length: volume ratio (±SD)	Homogeneous groups
N. grammatus	3.71 (±1.62)	X
P. sykion	5.16 (±1.47)	X
R. stephensoni	5.47 (±1.18)	XX
R. f. frauenfeldi	6.38 (±2.62)	X
A. spyridophora	14.94 (±7.53)	X

4.3.4 Feeding behaviour

Table 4.3 lists the major habitat types and feeding techniques of the species studied. The species can be divided into two main groups based on feeding techniques, viz suspension- or deposit-feeders. Amongst the suspension-feeders a distinction must be made between *R.*

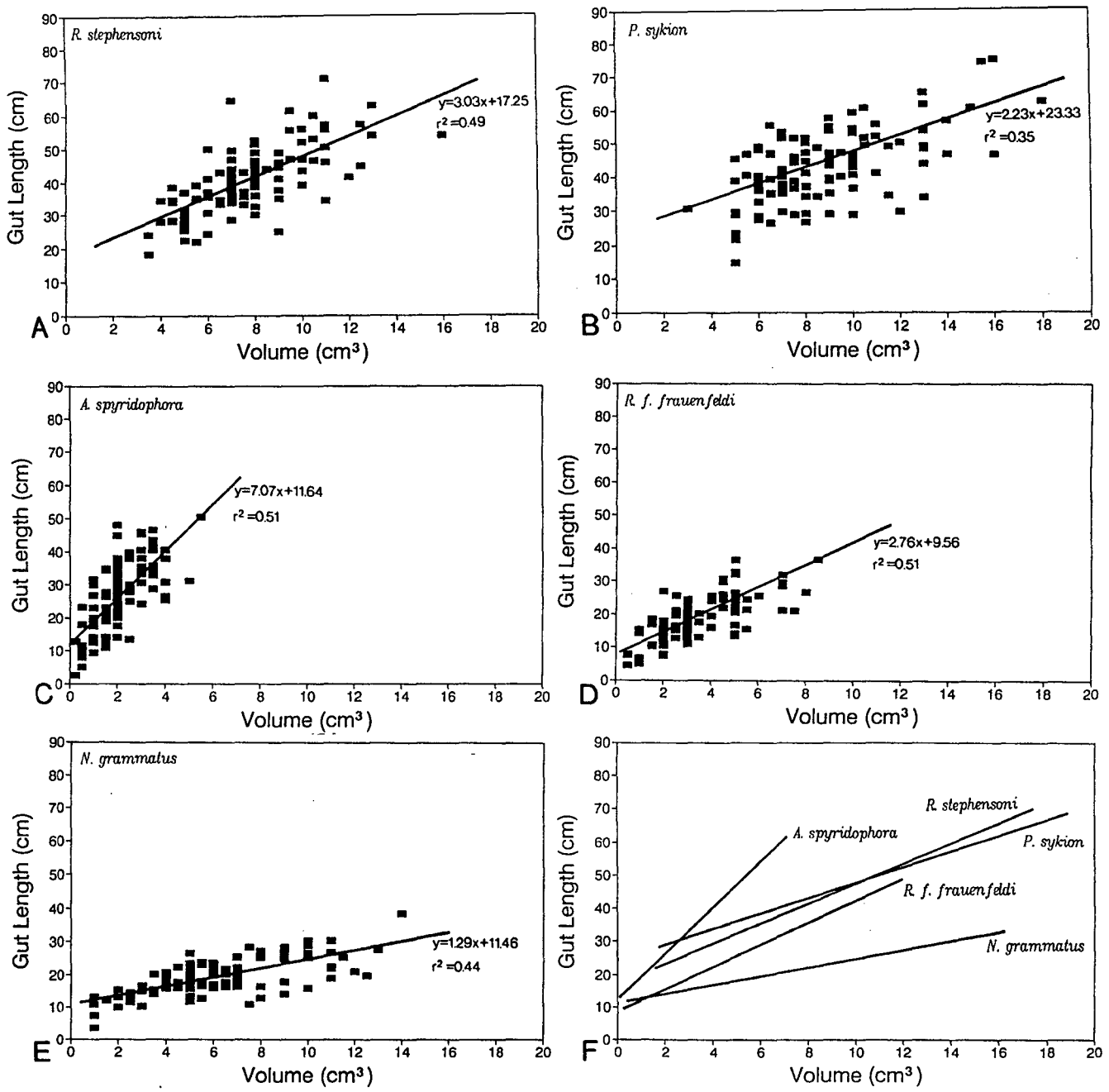


Figure 4.9. Relationship between gut length and drained body volume for **(A)** *Roweia stephensoni* (n=100), **(B)** *Pseudocnella sykion* (n=100), **(C)** *Aslia spyridophora* (n=100), **(D)** *Roweia frauenfeldi frauenfeldi* (n=100), **(E)** *Neostichopus grammatus* (n=100), and **(F)** comparison of all five species.

f. frauenfeldi and the other species. *Roweia f. frauenfeldi* was classified as a "heavy" suspension-feeder as it ensnared large food particles (>250µm) that are suspended in the water column.

Table 4.3. Habitats (after Thandar, 1985, 1987a, 1987b, 1991) and feeding techniques of five holothurian species from the eastern Cape coast of South Africa.

Species	Habitat	Feeding technique
R. stephensoni	Exposed in rock pools and crevices, or under boulders buried in sand.	Suspension-feeder
P. sykion	Common under stones, in pools and in rock crevices. Adults usually exposed.	Suspension-feeder
A. spyridophora	Sand, pebbles broken shells, rock. Usually cryptofaunic or in soft conglomerates of sand, shells and stones.	Suspension-feeder
R. f. frauenfeldi	In crevices or between stone slabs usually embedded in sand.	"Heavy" suspension-feeder
N. grammatus	Common under rocks at spring low tide; found from low tide mark down to about 3m.	Benthic deposit-feeder

SUSPENSION-FEEDERS:- in all of these species the tentacles are held erect in the water column, and were never observed to sweep the substrate for food. Similarly, the animals were not observed to waft their tentacles to and fro in the water column in search of food, but appear to rely upon particles borne on the incoming waves and currents. The tentacles do however, passively sway to and fro with the water currents. By contrast, Korrûbel (1991) observed *R. f. frauenfeldi* to actively sweep its tentacles through the water and even pick up

particulate material from the surrounding sediments, thus making it a suspension- as well as a deposit-feeder. In general, the animals capture suspended food material either by trapping the material amongst the tentacle branches, or by adhesion of particles to the numerous tentacle nodes. Once the suspended particles are ensnared the tentacles arch over and are inserted individually into the pharynx. However, in *R. f. frauenfeldi* two or more tentacles may be inserted depending on the size of the captured particle/s. Once in the pharynx the captured particles are removed from the tentacle before they are withdrawn from the pharynx and extended back into the water column. The tentacles were also observed to intermesh between individuals of the same species that were aggregated. However, in *R. f. frauenfeldi* individuals were never observed in aggregations, thus tentacles never formed intermeshing networks.

DEPOSIT-FEEDERS:- *Neostichopus grammatus* is an active feeder roaming over the substratum in search of food. During feeding the tentacles are spread out over the substratum and deposited particles adhere to the surface of the tentacle as the tentacle is withdrawn from the substratum, the tips of the tentacle branches curl inwards forming a cup which may aid in the mechanical grasping of larger particles. Again the tentacles are arched over into the pharynx where the collected particles are removed, before the tentacle is withdrawn from the pharynx and again splayed against the substratum.

4.4 DISCUSSION

Holothurians feed only by means of their buccal tentacles, with the aspidochirotes representing the highest adaptive radiations in feeding mechanisms with a corresponding high variability in tentacle forms (Roberts, 1979; Hammond, 1982; Roberts & Bryce, 1982). By contrast, the dendrochirotes, which include the most primitive holothuroids (Roberts, 1982),

do not undergo variations in their feeding mechanisms or tentacle forms.

The gross tentacle structure of the suspension-feeding dendrochirotes in this study, with the exception of *R. f. frauenfeldi*, does not differ significantly from that of previously studied dendrochirotes (Fankboner, 1978; Smith, 1983b; Costelloe & Keegan, 1984). However, the tentacle structure of *R. f. frauenfeldi* is sufficiently distinct from the other dendrochirote and aspidochirote tentacles studied to warrant a separate category of tentacle. Fish (1967) suggested that the fore-gut secretions were important in the feeding of *Leptopentacta elongata*, allowing the animal to coat its tentacles thickly with mucus to trap seston. Fankboner's (1981) re-examination of feeding in the forementioned species indicated that the mucus on the tentacles was probably a result of wound trauma. Dissection of the suspension-feeding holothurians in this study whose tentacles were withdrawn into the pharynx, revealed that they were also heavily coated with mucus. Therefore, pharyngeal secretions may play some role in the treatment of food. Adhesion is known to play an important function in echinoderm tube feet (Hermans, 1983). Since holothurian tentacles are modified tube feet (Hyman, 1955), the mucous secretion may play an important role in the capture of food particles. Histological examination indicated that the nodes were the only secretory surfaces on the tentacles. This has also been recorded for *Psolus chitinoides* (Fankboner 1978), *Neopentadactyla mixta* (Smith, 1983b) and *Aslia lefevrei* (Costelloe & Keegan, 1984). Entrapment of seston on the adhesive papillae in *R. stephensoni*, *P. sykion*, *A. spyridophora*, or on the nodes of *R. f. frauenfeldi*, appears to be the principal mode of food capture. While, mechanical entrapment of particles in the inter-papillar spaces of *Psolus chitinoides* has been reported by Fankboner (1978) as playing a minor role in the uptake of suspended food. The extent to which it might operate in *R. stephensoni*, *P. sykion* and *A. spyridophora* could not

be quantified, but it might also be relatively unimportant. However, the large nodes of *R. f. frauenfeldi* appear to have some mechanical role, and act as "hooks" to aid in pushing food into the mouth.

The occurrence of differentiated nodes at the top of each tentacle is presumably of sensory significance. Nodes are clearly distinct from other tentacle areas in having elongated epithelial cells giving rise to papillae containing cilia as in *R. stephensoni*, *P. sykion* and *A. spyridophora*, or simply bearing short cilia or clusters of microvilli as in *R. f. frauenfeldi* and *N. grammatus*. Precisely what stimulates feeding in the species studied remains to be determined. Costelloe and Keegan (1984) have indicated that the tentacles of *Aslia lefevrei* will only be inserted into the mouth if the particles trapped contain nutritive value. Similarly, species of *Holothuria* are able to pick up selectively the organically richest part of the sediment (Sloan & Campbell, 1982). Likewise, Hamel *et al.* (1993) have indicated that *Psolus fabricii* will only feed on four species of diatoms, even though numerous other species are available. Chemosensory capacity of holothurian tentacles is thought to lie within the papillary cells (Fankboner, 1978; Smith, 1983b), and according to Laverack (1974) microvilli are gustatory receptors while cilia are olfactory ones. Mechano-responsiveness to tentacle loading has also been demonstrated in *A. lefevrei*, as there was a difference in the rate at which tentacles were inserted in the mouth in clear and turbid water (Costelloe & Keegan, 1984). Similar observations of mechano-receptiveness of holothuroid cilia have been made by Smith (1983b).

Roweia stephensoni, *P. sykion*, and *A. spyridophora* were most common under boulders in areas where water currents ensure a continuous supply of seston. They feed predominantly

on particles less than 53μm in diameter, which consist of diatoms, unicellular and filamentous algae, and other organic matter suspended in the water column, similar to that of most dendrochirote holothurians (Massin, 1982b; Costelloe & Keegan, 1984; Hamel et al., 1993). The other large component of organic matter (106-250μm) observed in the guts of these three species may be acquired from resuspended organic matter, resuspended from the substrate by wave action. The numerous fine branches of the tentacles are well adapted to capturing these small food particles. When in high density aggregations, neighbouring individuals of *R. stephensoni*, *P.sykion* and *A. spyridophora*, allow their tentacles to intermesh without apparently interfering with the feeding process. This may in fact benefit individuals, as hypothesised by Keegan (1974), by enhancing particle capture within high density aggregations. The baffle effect produced by the expanded tentacle crowns will promote the near-bottom concentration and precipitation of suspended materials. Barkai (1991) has also indicated that water motion is essential for suspension-feeding holothurians in order to supply them with sufficient organic material, therefore the clumping behaviour of holothurians is an adaptation which enhances their ability to survive in regions which are continuously exposed to wave action. The ability of the species to intermesh and disentangle their tentacle without them adhering to each other may also indicate that the adhesive properties of the papillae nodes are rather weak. By contrast, *R. f. frauenfeldi* remains solitary and the tentacles of conspecifics never intermesh. It is seldom seen under boulders and prefers to remain buried in the sediment with only the tentacles protruding into the water currents. It feeds predominantly on particles greater than 106μm in diameter, which consist of macroalgal fragments and dead invertebrates (whole or fragments). Some of these particles are too heavy to remain in suspension and are tossed over the substrate by wave action, and subsequently captured by the tentacles which are robust with few branches. The solitary nature of this

species may indicate that the adhesive property of the node surface is resilient, allowing it to capture and cope with larger particles of food that are showered down on top of the tentacles. Similarly, in *Neopentadactyla mixta*, which avoids physical contact with its conspecifics (Könnecker & Keegan, 1973), papillar adhesion is very resilient (Smith, 1983b).

Deposit-feeding holothurians are regarded as playing an important role in the reworking and processing of surface sediments. It has been estimated that an individual *Stichopus tremulus* is capable on average to process 0.6kg of sediment per annum (Hauksson, 1979), an individual *Holothuria tublosa* 17.5kg dry weight per annum (Coulon & Jangoux, 1993), and a single specimen of *Paracaudina* 56-63kg per annum (Tao, 1930: in Pawson, 1966). On a much larger scale a population of *Holothuria atra* as much as 240000 tons of sand yearly (Bonham & Held, 1963), while Crozier (1918) has estimated that in a small enclosed bay in Bermuda of area 1.7 square miles, *Stichopus* species passed between 500 and 1000 tons of substrate through their intestines annually. Many authors have suggested that deposit-feeding holothurians select grain sizes, and thus feed selectively on the adjacent sediment (Yanamouti, 1939; Rhoads & Young, 1971; Hauksson, 1979; Roberts, 1979). Similarly, sea stars have been shown to be size-selective feeders (Christensen, 1970; Beddingfield & McClintock, 1993), as well as other deposit-feeders (Fenchel *et al.*, 1975; Gladfelter, 1978). However, Hammond (1982), in an extensive review of numerous studies on selective feeding in holothurians, concluded that no studies have unequivably demonstrated that deposit-feeding holothurians are capable of exercising any preference for particular grain sizes. Similarly, *N. grammatus* may not select particular grain sizes when feeding but rather ingests what is available in the adjacent sediment. Although the electivity values indicated that *N. grammatus* selects certain grain sizes, Figures 4.8B and 4.8C indicate that the gut and adjacent sediment conform

closely, indicating that *N. grammatus* probably displays no preference, and consumed all grain sizes in virtually the proportions in which they were available. The discrepancy in gut and sediment particles greater than 3.35mm may be as a result of physical constraints (particle weight, tentacle structure, etc.) experienced by the holothurian in handling particles of that size. The mucous coating which occurs on the tentacles of some aspidochirote holothurians (Hammond, 1982; Roberts & Bryce, 1982), including *N. grammatus*, would cause particles to adhere to the mucous film irrespective of their size, thus supporting none selection.

Studies of detritivores in streams (e.g. Nelson & Scott, 1962), and marine environments (e.g. Newell, 1965) suggest that bacteria provide the main food source for organisms ingesting particulate detrital matter. Epibenthic deposit-feeding holothurians, such as *N. grammatus*, appear to restrict feeding to the topmost few millimetres of the substratum and selectively exploit the layer with the richest bacterial, meiofaunal and microfaunal densities (Yingst, 1976). Moriarty *et al.* (1985) indicated that deposit-feeding holothurians are important bacterial feeders, capable of consuming 10-40% of bacterial carbon produced each day in tropical reefs. A similar situation could exist for *N. grammatus*. Yingst (1976) found that the large energy reserve available to *Parastichopus parvimensis* as plant detritus in shallow inshore waters was not exploited by this species prior to bacterial and fungal decomposition. Bacteria may be important in making dissolved organic matter available to large organisms, by transforming substances into compounds that are easily assimilated (Roberts *et al.*, 1991), and/or create a richer nutrient source (Sibuet *et al.*, 1982). Coprophagy is well known amongst deposit-feeding holothurians (Bakus, 1973; Hauksson, 1979; Sloan & von Bodungen, 1980), with the faeces hosting a rich bacterial fauna. *Neostichopus grammatus* may profitably exploit its own faeces because the casts are recolonized by bacteria and generally have greater

amounts of potential nutrients than surrounding sediments (Johannes & Satomi, 1966; Frankenberg & Smith, 1967; Hauksson, 1979; Sloan & von Bodungen, 1980). In addition, both deep-sea (Roberts *et al.*,1991) and shallow-water holothurians (Holland & Nealson, 1978; McKenzie, 1988) are reported to have integumental subcuticular bacteria over the tentacles that may serve in a nutritive role (Walker & Lesser, 1989) in transferring bacterial metabolites to the holothurian. The gut sediments are also reported to contain higher concentrations of bacteria than the adjacent sediments (Hauksson, 1979), and the digestive tracts of some abyssal holothurians contain bacteria which present little similarity with those separated from the adjacent sediment (Ralijaona & Bainchi, 1982).

It has generally been accepted that bacteria seem to be the main food of deposit-feeding holothuroids (Bakus, 1973; Yingst, 1976; Massin, 1982b; Baird & Thistle, 1986), while diatoms, unicellular algae, suspended organic matter and small invertebrate animals are the main food of suspension-feeding holothurians (Hyman, 1955; Reese, 1966; Fankboner, 1978; Massin, 1982b). The variations in gut length relative to body size between the aspidochirote and dendrochirote holothurians may be of phylogenetic origin, or a functional adaptation to the food type ingested. The short gut length relative to body size in *N. grammatus* may indicate a faster gut passage time than the four species of dendrochirotes studied, due to the ease of digesting bacteria and/or the decomposition of organic matter by bacteria. Hargrave (1970) found that a freshwater deposit-feeding amphipod assimilated different species of bacteria with 60-83% efficiency, while diatoms, green algae and blue-green algae had lower assimilation efficiencies. Similarly, a species of grass shrimp was able to utilize bacteria with 82% efficiency (Adams & Angelovic, 1970), and a gastropod used bacteria with 81-92% efficiency (Callow & Fletcher, 1972: in Yingst, 1976). No cellulase activity has been detected

in many of the deposit-feeding holothurians examined (Yohoe & Yasumasu, 1964; Fish, 1967). Kristensen (1972) also found that 12 species of deposit-feeding invertebrates from Danish waters showed either a very weak ability or none to digest cellulose. The significantly longer gut lengths relative to body sizes in *R. stephensoni*, *R. f. frauenfeldi*, *P. sykion* and in particular *A. spyridophora* may well be due to the high cellulose content of the organic matter ingested, thus requiring longer to digest. Similarly, Hamel *et al.* (1993) have also reported that *Psolus fabricii*, another dendrochirote, has a remarkably long intestine relative to its body size, and suggested that this may be an adaptation to its diet of diatoms which are protected by siliceous frustules. Hargrave (1970), for example, found that *Hyallella azteca*, a deposit-feeding amphipod, utilized the organic matter present in the lake sediments with only 6% efficiency. This low efficiency could be explained by the fact that cellulose and lignin-like substances accounted for over 50% of the organic sediment ingested. Even though the four dendrochirote species ingest similar food types, are all sedentary, and live in a similar region on the shore, *A. spyridophora* has a much longer gut length to body volume than the other three dendrochirotes. Again this may be of phylogenetic origin, or because *A. spyridophora* is much smaller than the other three dendrochirotes, it may have a higher mass specific metabolism and therefore needs to be able to maximise digestion efficiency with a longer gut. This is supported by numerous studies which have indicated that as body size decreases in marine invertebrate, so the mass specific metabolism increases (e.g. Spencer Davies, 1966; Newell, 1979).

The species in this study appear to feed most actively at night, especially *R. f. frauenfeldi* which was never observed feeding during the day. Similar observations have been recorded for other holothurians (Coulon & Jangoux, 1993). It has been shown that predatory fish

influence the activity periods of their bottom-dwelling invertebrate prey (Levinton, 1971; Stein & Magnuson, 1976; Nelson & Vance, 1979). Prey become most inactive when their predators are most active, and at dusk and dawn there can be a significant decline in the foraging activity of predatory fish (McFarland, 1986; Dominguez & Reaka, 1988). Although, holothurians are reported to have very few natural predators (Bakus, 1968; de Vore & Brodie, 1982), *N. grammatus*, *P. sykion* and *R. stephensoni* have been found in the guts of Blacktail (*Diplodus sargus capensis*), Musselcracker (*Sparodon durbanensis*) and Pignose grunter (*Lithognathus lithognathus*) (coastal fishermen, pers. comm.). Buccal tentacles protruding from beneath boulders, crevices, sand or the exposed animal itself may be regarded as tempting "snacks" for some predatory fish. The holothurians may not be killed in such attacks but could suffer severe tentacle damage or loss, which could ultimately lead to starvation. Therefore, emerging at night may be a means of counteracting such attacks.

It has been demonstrated in several studies, that species having very similar ecological requirements are frequently capable of existing together without significant negative effects upon each other (e.g. Harger, 1972; Underwood, 1978; Menge, 1979; Creese & Underwood, 1982). Similarly, it has been suggested that when several species co-occur, each occupies a well-defined ecological niche, and even if trophic requirements are similar, food is different and competition between species minimal. For example, Vasquez *et al.* (1982) studied four co-existing species of sea urchins and concluded that the four species did not overlap in both microhabitat and diet. Those species overlapping in microhabitat did not overlap in diet, and similarly, those overlapping in diet did not overlap in microhabitat. This fits Kohn's (1978) suggestion that particle feeders partition the habitat rather than food resources. Similarly, Sloan & von Bodungen (1980) and Roberts & Bryce (1982) have indicated that partitioning

in some holothurians was predominantly on the basis of habitat preference. Suspension-feeders may collect food particles at different levels in the water column, depending on whether they live in the sediment or are fixed to rock surfaces above the sediment. Levinton (1972), suggested that filter-feeders are more likely to compete for feeding space than for food resources. The feeding techniques observed between some of the five species in this study are sufficiently different to maintain distinct niche separation, and although the dendrochirotes use similar feeding methods they have different microhabitats. *Neostichopus grammatus* appears to be the only intertidal deposit-feeding holothurian on the eastern Cape coast (Thandar, 1987a), and is therefore able to spread into a variety of habitats and exploit a variety of deposits. A similar situation exists for *Isostichopus badionatus* on the Bermudan coast (Sloan & von Bodungen, 1980), and for some predatory gastropod species (Kohn, 1978).

Possible future work on the feeding biology of these holothurians may involve monitoring gut passage time in order to calculate the amount of sediment passed through the intestine of *N. grammatus*. A comparative ultrastructural examination of the gut tissue layers may reveal differences between the suspension- and deposit-feeding species. Similarly, ultrastructural analyses of the node regions of the tentacles may reveal mechano- and chemo-receptive structures. Lastly, regular monitoring of ingested seston may reveal seasonal changes in the diets of the suspension-feeding species.

CHAPTER FIVE

GENERAL DISCUSSION

As with most intertidal organisms the holothurians, *Roweia stephensoni*, *Pseudocnella sykion* and *Neostichopus grammatus* exhibit a distinct pattern of zonation. They are most common in the mid- to low-shore regions where they reach high densities, particularly on shores with minimal sand inundation which also offer suitable microhabitats (Chapter 2). There can be no doubt that the zonation pattern observed on many shores is a result of a gradient of physical stress (Branch & Branch, 1981). However, physical stress may be only one of many factors limiting the distribution of these three species. It is well documented that suspension-feeding holothurians rely on water currents to ensure a constant food supply (e.g. Massin, 1982b), and it has been subsequently shown that water currents play an important role in limiting holothurian distribution (Barkai, 1991). Therefore, the lack of holothurians from high shore regions could be a combination of increased physical stress (e.g. increased risk of desiccation) and reduced feeding time due to long aerial exposure.

This study in conjunction with other ecological surveys on the South African coast, indicates that holothurians can form an important component of the shore biomass. For example,

McLachlan *et al.* (1981) indicated that at some sites along the Port Elizabeth coast holothurians are an important component of the filter-feeding community and constitute 6% shore biomass, similar to that of the mussel, *Perna Perna* (8%). However, at these sites holothurian biomass paled by comparison with the grazer component (82-86%). Similarly, in a recent review by Field and Griffiths (1991) on littoral and sublittoral ecosystems of southern Africa, the importance of holothurians in kelp bed ecosystems on the west coast is again highlighted. Here they state that in such ecosystems, suspension-feeders such as mussels, barnacles and holothurians may constitute 72% of animal biomass. Despite their contribution to biomass in some littoral and sublittoral ecosystems, holothurians remain an overlooked group and aspects of their biology need to be further investigated.

The aggregating behaviour of *R. stephensoni*, *P. sykion* and *N. grammatus*, has been observed in numerous other echinoderm species (Buchanan, 1966; Reese, 1966; Birkeland & Chia, 1971; Velimirov, 1984; Barkai, 1991; Billett, 1991; Freire *et al.*, 1992). Such behaviour may have several adaptive advantages and consequences. Suspension-feeding holothurians, such as *R. stephensoni*, *P. sykion*, and *A. spyridophora*, are thought to increase their ability to capture suspended particles from the baffle effect of their intermeshing tentacles, which causes suspended particulate matter to settle (Keegan, 1974). Similarly, aggregations may arise due to the holothurians seeking shelter in limited habitats, in an attempt to avoid pounding wave action generally associated with intertidal life. This may be particularly true for *N. grammatus* with its soft gelatinous body wall.

Holothurians are broadcast spawners and liberate their gametes into the sea-water where fertilization takes place (Smiley *et al.*, 1991; present study). Due to the aquatic medium

gamete concentrations may be diluted to very low levels making spawning in close proximity to conspecifics crucial for adequate fertilization. Pennington (1986) found that concentrations in excess of 1 million sperm/litre were required to achieve a fertilization success of 50% in echinoids. Therefore, broadcast spawners would be expected to spawn simultaneously and in close proximity to each other. Although, simultaneous spawning was not observed in *R. stephensoni*, *P. sykion* and *N. grammatus*, the main summer spawning event occurred between two successive months in all three species (Chapter 3), indicating that spawning may have been synchronous amongst the species. Simultaneous spawning amongst aggregated individuals, has been observed in many echinoderms (Fishelson, 1968; Hendler & Meyer, 1982; Minchin, 1987; McEuen, 1988; Pearse *et al.*, 1988). Simultaneous spawning in close proximity to members of different species may result in hybridization. In addition, Miller (1985) has indicated that there is very little species specific sperm to egg-water chemotaxis in echinoderms, which only further supports possible hybridization implications. Clearly, this opens up an avenue of future research, which may investigate the possibilities of cross fertilization amongst South African holothurians and other echinoderm species.

The reproductive biology study indicated that the three species studied followed annual cycles of gonadal growth and maturation. It also indicated that the two dendrochirotes, *R. stephensoni* and *P. sykion*, followed a different reproductive strategy to that of the aspidochirote, *N. grammatus* (Chapter 3). The observed differences may be phylogenetic or possible influences of co-existence (Reese, 1968). Similarly, although not investigated, larval development may be different between the dendrochirotes and the aspidochirote. Smiley *et al.* (1991) state that all dendrochirotes give rise to direct development of the larvae, while shallow-water aspidochirote larvae undergo indirect development. Bearing this in mind,

Thandar (1989a) accounts the higher proportion of endemism observed in the South African dendrochirote holothurians, when compared to the aspidochirotes, to the absence of pelagic larvae in the dendrochirotes. Direct development restricts the dispersal ability of a species (Mileikovsky, 1971), but for sedentary species, such as *R. stephensoni* and *P. sykion*, space may be a limiting factor. Having larvae with direct development allows the young to settle in close proximity to the adults on a suitable substrates, hence forming aggregations (Rutherford, 1973). Since, *R. stephensoni* and *P. sykion* are able to attach themselves firmly to the rock surfaces where they are commonly found (pers. obs.), and due to the general lack of natural holothurian predators (Bakus, 1968; de Vore & Brodie, 1982), once such aggregations are established, it is unlikely that other species will be able to displace them. Immediate future work to follow on from this, may investigate the type of larval development of these three species. Furthermore, examination of inter and intragenetic variability within holothurian populations may provide insight into genetic interchange and dispersal capabilities of different holothurian species.

It is often assumed that when niches overlap competitive exclusion is likely to occur (Hutchinson, 1957: in Velimirov, 1984). However, niche overlap does occur without competitive exclusion, as seen for *R. stephensoni*, *P. sykion* and *N. grammatus* (Chapter 2). Possible explanations for this may be due to the different feeding strategies between the aspidochirote and dendrochirote holothurians studied (Chapter 4). *Neostichopus grammatus* ingests bottom sediments, while *R. stephensoni*, *P. sykion*, *A. spyridophora* and *R. f. frauenfeldi* capture suspended particulate matter from the water column. Similarly, even amongst the suspension-feeders, *R. f. frauenfeldi* ingests much larger particle sizes. Likewise, although *R. stephensoni*, *P. sykion* and *A. spyridophora* ingest particles of similar size, their

microhabitat requirements are different. Hence, these species may be able to co-exist because when feeding strategies are similar between species they occupy different microhabitats, and when microhabitats are similar they follow different feeding strategies (Kohn, 1978; Vasquez *et al.*, 1982).

Although it is not known how much sediment is processed by *N. grammatus*, deposit-feeding holothurians do play a major role in reworking the sediment (Crozier, 1918; Bonham & Held, 1963; Rhoads & Young, 1971; Hauksson, 1979; Massin, 1982a; Coulon & Jangoux, 1993). Similarly, it is not know how much organic matter is removed from the water column by the suspension-feeding holothurians. For example, de Villiers (1989) has indicated that a population of macrobenthic filter-feeding bivalve molluscs, *Solen cylindraceus*, in the Kariega estuary (eastern Cape, South Africa) is capable of removing 950kg of suspended particles from the water column each tidal cycle. Similarly, benthic filter-feeders and zooplankton have been recorded to crop large biomasses of phytoplankton (Nichols, 1985). Phytoplankton was a conspicuous component of the ingested food found in the guts of *R. stephensoni*, *P.sykion* and *A. spyridophora* (Chapter 4). As a result of their high densities, these species may be capable of removing substantial amounts of phytoplankton, and thus restricting production rates.

One of the major impacts resulting from high densities of suspension-feeding organisms, is the removal of particles from the water column and the deposition of large amounts of biodeposits on the surface sediments, which are in turn colonized by micro-organisms (de Villiers, 1989). Based on this, a hypothetical trophic pathway can be proposed for the suspension- and deposit-feeding intertidal holothurians found on the eastern Cape coast (Fig.

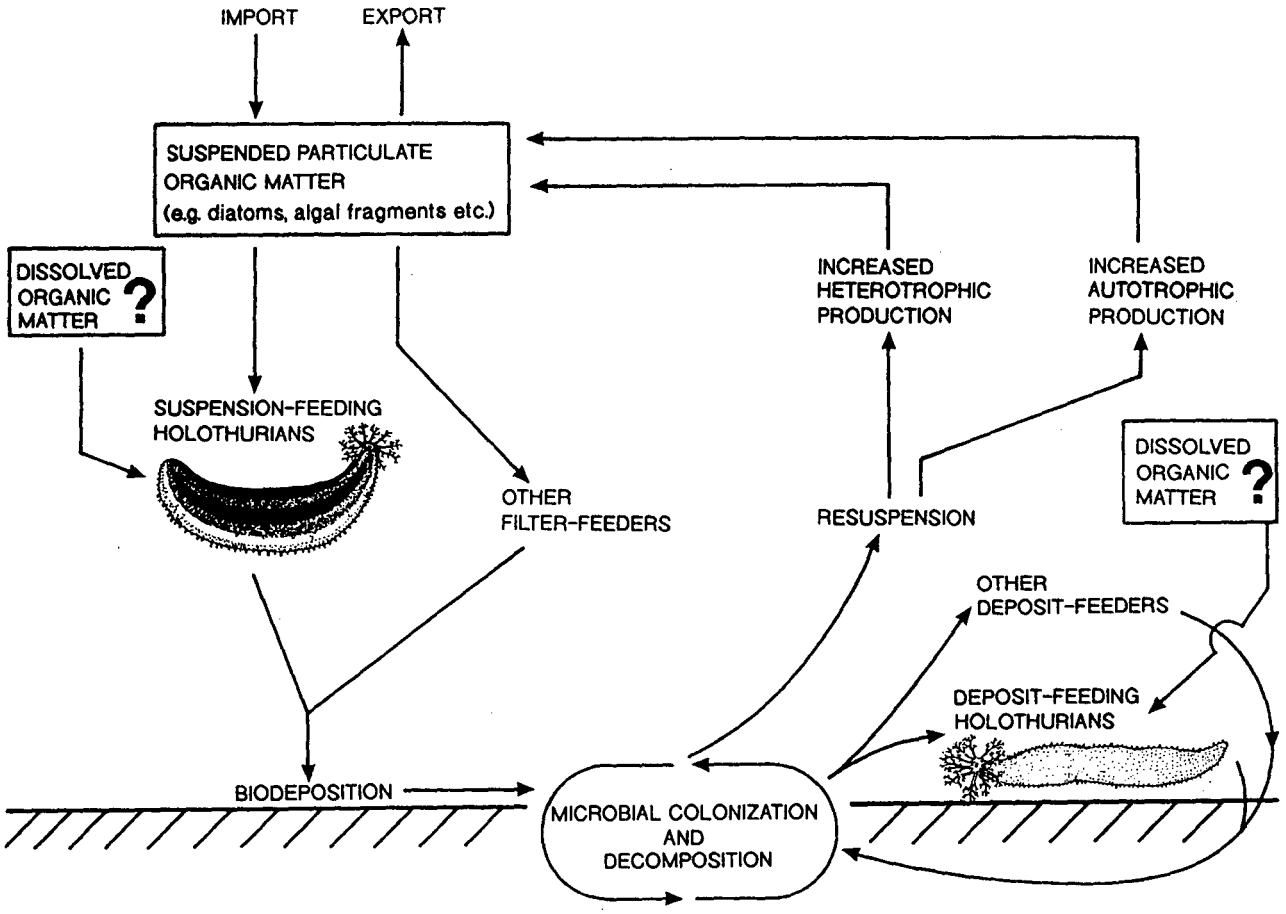


Figure 5.1. Hypothetical model proposed for the role suspension- and deposit-feeding holothurians possibly play in the trophic pathway of intertidal ecosystems. Illustrated is the physical and biological processing of suspended particulate matter and detritus by suspension- and deposit-feeding holothurians; and tidal resuspension of nutrients which are utilized to increase autotrophic and heterotrophic production.

5.1). *Roweia stephensoni*, *P. sykion*, *A. spyridophora* and *R. f. frauenfeldi* will remove particulate organic matter from the water column during their feeding activities, most of which would then be deposited on the intertidal sediment surface as faeces and subsequently colonized by microbial organisms. This in turn provides valuable nutrients for deposit-feeders, such as *N. grammatus*, which rely to a large extent on microbial organisms as a food source (Bakus, 1973; Yingst, 1976; Massin, 1982b; Baird & Thistle, 1986). *Neostichopus grammatus* ingests these nutrient-rich sediments and in turn deposits faeces on the sediment surface which may have an increased bacteria component (Bakus, 1973; Hauksson, 1979; Sloan & von Bodungen, 1980). Wave action probably resuspends these nutrients which are then utilized to enhance heterotrophic and autotrophic production, which in turn are utilized by the suspension-feeding organisms. This model provides a rather crude model of the role of holothurians in intertidal trophic pathways. A more detailed model of energy flow may be proposed if future work concentrates on the amount and the rate at which suspended particulate organic matter and benthic sediments are ingested by suspension- and deposit-feeding holothurians, respectively. In addition, assimilation efficiencies should also be investigated.

Conclusions to be drawn from this study suggest that some holothurians (e.g. *R. stephensoni*, *P. sykion* and *N. grammatus*) which inhabit the eastern Cape coast of South Africa, are able to reach high densities. Such high densities, especially amongst the suspension-feeding holothurians, may result in considerable amounts of particulate organic matter being removed from the water column, which may reduce productivity. Similarly, high densities of the sedentary holothurians may result in competition for space between these holothurians and other sedentary intertidal organisms. Likewise, the ability of deposit-feeding holothurians,

such as *N. grammatus*, to rework benthic sediments may also have a profound effect on the ecosystems in which they live. Finally, although different reproductive strategies are followed between the dendrochirotes (*R. stephensoni* and *P. sykion*) and the aspidochirote (*N. grammatus*), an annual cycle of gonadal growth and maturity with a main spawning event in summer was observed in the three species, which ensures the survival of the species in their harsh intertidal environment.

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ERRATA

1. page 27 line 2: **"dependant of"** should read **"dependent on"**

2. page 30 bottom line: **Malpadia** should read **Molpadia**

3. page 57 bottom line: **regresses** should read **regressed**

4. page 72 line 22: INSERT "some **species** do spawn"

5. page 81 line 14, page 84 line 7: an **aquaria** should read an **aquarium**

6. page 89 line 13: terminate **in** NOT **into**

7. page 107 line 19: **invertebrates**

8. page 113 line 6: delete indefinite article

9. page 114 line 8: **know** should read **known**