ASPECTS OF THE BIOLOGY OF THE INFAUNAL BIVALVE MOLLUSC *SOLEN CYLINDRACEUS* (HANLEY) IN THE KARIEGA ESTUARY

Dissertation submitted in fulfilment

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by

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FRONTISPIECE: From top left, clockwise: *Solen cylindraceus* (X 2 magnification); Upper reaches of the Kariega estuary; Aerial view of the Kariega estuary during the December 1985 flood.

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1) de Villiers, C.J. & Allanson, B.R. (1988) Efficiency of particle retention in *Solen cylindraceus* (Hanley) (Mollusca : Bivalvia). *Estuarine, Coastal and Shelf Science* 26: 421-428.

2) de Villiers, C.J. & Allanson, B.R. (1989) Osmotic properties of an infaunal estuarine bivalve *Solen cylindraceus* (Hanley). *Journal of Molluscan Studies* 55: 45-51.

3) de Villiers, C.J., Allanson, B.R. & Hodgson, A.N. (1989) The effect of temperature on the filtration rate of *Solen cylindraceus* (Hanley) (Mollusca : Bivalvia). *South African Journal of Zoology* 24: 11-17.

4) de Villiers, C.J., Hodgson, A.N. & Allanson, B.R. (1989) Effect of salinity and temperature on the filtration rate and distribution of *Solen cylindraceus* (Hanley). *Proceedings of the 23rd European Marine Biology Symposium*, Swansea (in press).

Reprints of the above are presented in Appendix I.

ABSTRACT

Solen cylindraceus is an infaunal filter-feeding bivalve inhabiting the intertidal mud banks of many southern African estuaries. It is particularly abundant in the Kariega estuary (33°41'S; 26°42'E) where it reaches densities of 400m^{-2} (192g shell-free dry wt. m^{-2}). The Kariega is a permanently open, marine dominated estuary about 18km in length, and *S. cylindraceus* is most abundant in its middle and upper reaches. Some physical characteristics of the estuary (temperature, salinity, sediment and water turbidity) are described, and the possible role of these factors in determining the density and distribution of *S. cylindraceus* within the Kariega estuary, is discussed.

The structure of the alimentary system, gills and labial palps of *S. cylindraceus* is described, all of which showed no major variation from the "typical" eulamellibranchiate form. *Solen cylindraceus* was found to be a euryhaline osmoconformer with a salinity tolerance range of 15-65‰. When animals were removed from their burrows, osmotic equilibration of the haemolymph was rapid (1-2 hours). By contrast, in animals left undisturbed in their burrows, osmotic equilibration was retarded (72-204 hours). It is suggested that the observed decrease in the rate of change of haemolymph osmolarity for animals in their burrows is linked to the stability of the interstitial salinity. A temperature tolerance range of 5-45°C was determined for *S. cylindraceus* (*in situ*), in which prolonged exposure to 5°C and 40-45°C (12-36 hours respectively) resulted in a decreased burrowing ability, coma and death. Animal burrowing responses were not affected by temperatures in the range 15-35°C.

Field experiments were carried out over several tidal cycles, in which the measurement of crystalline style volume was used as a means of assessing extracellular digestive activity. No major variation in style volume was recorded and it appeared that *S. cylindraceus* did not exhibit any cyclical pattern of style dissolution and regeneration. It is suggested that *S. cylindraceus* feeds continuously from the water column during high tide and possibly within its burrow, at or below the water table, during low tide.

At a suspensoid concentration of 50mg l^{-1}, *S. cylindraceus* was found to filter water almost continuously (90-95% of the time). Time spent filtering dropped to 68% at 100mg l^{-1} and 32% at 500mg l^{-1}. Filtration rates for summer collected animals (25°C) were 22.86 ± 4.36ml $min.^{-1}$, some 3ml $min.^{-1}$ greater than that recorded for winter (16°C) collected animals. Filtration rate may be expressed as a function of shell length by the equations: $y=0.247x^{1.066}$ (winter) and $y=0.758x^{0.826}$ (summer). *Solen cylindraceus* was capable of acclimating its filtration rate to both high and low temperatures under laboratory conditions. Filtration rate exhibited a thermal optimum in the range 15-35°C, declining at higher and lower temperatures. Q_{10} values of filtration decreased rapidly from greater than 4 to less than 2, when the thermal optimum was reached. Maximum rates generally occurred at approximately 5°C above the temperature to which the animal had been acclimated. Optimal filtration rates (19-23ml $min.^{-1}$) were recorded in the salinity range 15-45‰. When subjected to abrupt changes in salinity, filtration rates were immediately depressed. The extent and duration of these decreased filtration rates were dependent upon the magnitude and direction of salinity change, and were always less in animals exposed to hyper- than hyposaline conditions. Animals exposed to increased temperature and simultaneous elevated or unchanged salinity, showed a slight increase in filtration rate followed by rapid acclimation. A decrease in both temperature and salinity resulted in an initial decrease in filtration rate and a longer

acclimation period. The ability of *S. cylindraceus* to acclimate fully within a wide temperature and salinity range, and to filter maximally in hypersaline conditions may, in part, explain its unusually high abundance in the Kariega estuary, despite it being close to the southernmost limit of the animal's geographical distribution. No significant difference in filtration rate was recorded at suspensoid concentrations of 5-100mg l^{-1}. However, at 250 and 500mg l^{-1}, filtration rates decreased significantly, and coincided with increased levels of pseudofaecal production. *Solen cylindraceus* retained particles down to 2.5-3.0µm with great efficiency (ca. 60-90% efficiency). Below this particle size, retention efficiency decreased rapidly and a net production of particles was recorded below 1.5µm. Particle retention was independent of temperature (15 and 25°C) and salinity (15 and 35‰).

Use was made of stable carbon isotope analyses ($^{13}C/^{12}C$ ratios) in an attempt to determine the important food sources of *S. cylindraceus* within the Kariega estuary. The results obtained demonstrated an enrichment in $\delta^{13}C$ values for *S. cylindraceus* from the upper (-27.9‰) to the middle (-25‰) and lower (-21.6‰) reaches of the estuary, with no seasonal variation apparent. The bivalve was substantially more depleted in ^{13}C relative to the dominant aquatic macrophytes *Zostera capensis* (-9.1 to -15.6‰) and *Spartina maritima* (-12.5‰). The use of $\delta^{13}C$ alone, however, to unequivocally "pin point" specific food sources of a filter feeder in a predominantly detritus based food web, is limited. It is suggested that in the Kariega estuary, riparian litter and other terrestrially derived vegetation contribute to the carbon pool. A possible contribution of ^{13}C depleted food sources via chemoautotrophic and/or anaerobic pathways, to the diet of *S. cylindraceus*, is suggested.

CHAPTER ONE

GENERAL INTRODUCTION

Estuaries have a historical and functional importance to man, and many large population centres occur on major estuaries (Kennedy, 1980). They are used as fishing grounds, for commercial aquaculture, waste disposal, a source of industrial cooling water, are often major transportation arteries, and have an important recreational value (Kennedy, 1980; Day, 1981; Kennish, 1986). Thus, an understanding of the ecology of estuaries is important if these systems are to be optimally utilized and successfully managed by man.

Estuarine ecology in southern Africa is largely based upon the work of Professor John Day and colleagues, who examined the distribution and abundance of plants and animals in many southern African estuaries (see Day, 1981 for review of literature). Following on from this descriptive work, estuarine research in South Africa entered an experimental phase, initiated by Professor Brian Allanson and colleagues. The emphasis of this research was not directed at "ecosystems" *per se*, but rather an attempt to assess the importance of various environmental factors to the fauna, so defining their distribution and success. The objective of their work, although strongly reductionist in approach, was based on the rationale, that an understanding of an animal's biology, physiology and ecology would, in turn, lead toward a greater understanding of how estuarine ecosystems function.

Estuarine fauna in general, have a low species diversity, whilst at the same time, these few species may be extremely abundant (McLusky & Elliot, 1980). However, Hodgson (1987) showed that, when compared to other eastern Cape estuaries, the Kariega (33°41'S; 26°42'E) possessed an unusually high species diversity (107 species amongst the macrobenthos alone). Further, the macrobenthos was dominated (both numerically and in terms of biomass) by the two filter feeders, *Solen cylindraceus* (Bivalvia) and *Upogebia africana* (Anomura). Bivalves have been shown to dominate the macrofauna of many estuarine and coastal marine systems and consequently, they play an important role in the trophic structure, energy flow and productivity of such ecosystems (e.g. Keunzler, 1961; Bayne, Thompson & Widdows, 1976; Seed, 1976; Newell, 1979; Wright, Coffin, Ersing & Pearson, 1982; Nichols, 1985; Griffiths & Griffiths, 1987; Hodgson, 1987; Peterson & Black, 1987). Despite the fact that some of these ecosystems may have a high species diversity, the principal unit of study remains the individual organism. Any attempt at understanding the ecology, productivity and energy flow within ecosystems, requires an intimate knowledge of, at least, the dominant species. To this end, bivalves can be excellent animals for such studies (e.g. Bayne, 1976; Newell, 1979; Griffiths & Griffiths, 1987). Research has however, emphasized those species which are not necessarily always the most dominant but, are easily accessible and/or of economic importance. This approach has been largely responsible for infaunal species receiving less attention than their epifaunal counterparts. The peculiarly high abundance of *S. cylindraceus* in the Kariega estuary, prompted an examination of the biology of this infaunal, filter-feeding bivalve, in an attempt to understand not only how physical and chemical environmental variables influence animal function, distribution and success, but also the potential role *S. cylindraceus* may itself play in this estuary.

The research in this dissertation is largely reductionist in approach, and deals with specific aspects of the animal and/or its responses to particular defined conditions, the results of

which it is hoped, will contribute to further our understanding of quantitative estuarine ecology. This thesis is presented as several chapters, each of which comprises an introduction, techniques used and results section, as well as a discussion. Where appropriate, any additional information is given as appendices.

The general anatomy of the digestive tract, as well as the principal feeding organs (gills and labial palps), are described in Chapter 2. The physical characteristics of the estuary, as well as the distribution and abundance of *S. cylindraceus* are discussed in Chapter 3. Following on from this, Chapter 4 examines the tolerance of *S. cylindraceus* to the environmental variables, temperature and salinity, and discusses some advantages of an infaunal existence. Animal activity, in terms of both feeding and digestive rhythms, is investigated in Chapter 5, and related to the animal's infaunal, intertidal existence.

The efficiency of particle retention and the effects of temperature, salinity and combinations of these, as well as suspensoid concentration, on the feeding and filtration rate of *S. cylindraceus* are described in Chapter 6. In Chapter 7, use is made of stable carbon isotope techniques ($\delta^{13}C$) in an attempt to identify the principal carbon sources in the estuary utilized by *S. cylindraceus*. The usefulness of this technique as a means of defining estuarine structure is evaluated. A general discussion is presented in Chapter 8.

CHAPTER TWO

ANIMAL ANATOMY

INTRODUCTION

Members of the molluscan family Solenidae are highly specialized bivalves adapted for vertical burrowing into intertidal or subtidal sand and mud. The animals are characterized by a posteriorly elongated shell and mantle, accompanied by a marked reduction in shell depth, and an umbo that is more or less terminal (Bloomer, 1903; Ghosh, 1920; Owen, 1959). It is this cylindrical-like appearance which has resulted in the common names for these animals of "stickbait", "pencilbait", "finger oysters" or "razor clams".

In some genera e.g. *Pharella* and *Solen*, the mantle is entirely fused along the ventral margin, while in *Siliqua, Phaxus* and *Ensis*, fusion is complete only posteriorly to the fourth pallial aperture (Ghosh, 1920; Owen, 1959). Other features include distinct inhalent and exhalent siphons, which in *Solen* and *Siliqua*, unlike other members of the family, are relatively long and fused (Owen, 1959). *Solen* is apparently the only member of the family capable of complete siphonal autotomy. All members of the family are suspension feeders, and gill form varies from flat and homorhabdic (e.g. *Siliqua* and *Phaxus*), to plicate and heterorhabdic (e.g. *Solen* and *Ensis*) (Atkins, 1936; Owen, 1959; Morton, 1979). In all instances the style sac is completely separate from the midgut and the stomach displays a well-developed posterior sorting area.

Despite their abundance in many regions of the world, documentation of anatomical structure for the genus *Solen* as a whole is somewhat sparse. Bloomer (1901) described the alimentary anatomy of three members of this family; *Solen marginatus, Solen* (=*Ensis*) *ensis* and *Solen* (=*Ensis*) *siliqua*, while Ghosh (1916), described what he considered a dwarfed form of *S. fonesi*, previously described by Bloomer (1906) whose description, according to Ghosh (1916) "gives few details of the internal structure and does little more than compare the foot and mantle etc. with those of *S. vagina* (=*S. marginatus*)". Ghosh (1920) reviewed much of the earlier work (principally Bloomer's) and produced a taxonomic study on the soft parts of some members of the Solenidae upon which he justified a division of the family into three subfamilies. The most recent anatomical description to date of a member of the Solenidae is that of Graham (1931) on *Ensis siliqua* and subsequently his more detailed investigation into the stomach structure of the same in 1949. Other workers, e.g. Nakazima (1958) and Purchon (1960) have concentrated largely on a more detailed description of the stomach structure of *S. gouldi* and *Pharella acuminata* respectively. Owen (1959) gave a basic anatomical description of *Pharella acuminata*, highlighting some of the differences between it and *Ensis siliqua*. In his review on bivalve stomachs, Purchon (1987) summarized the findings of previous workers and presented a diagrammatic representation of the stomach structure of the Solenidae. Purchon (1987) noted that the style sac and midgut of *Solen* are wholly separate but made no mention of any other alimentary structures.

The gills are important components of the feeding and respiratory systems of bivalves. General investigations into gill structure have therefore enjoyed substantial interest over the past few years, with the emphasis falling not only on a physical description of gill and ciliary structure, but more so in relating these findings to gill (and labial palp) function, e.g. particle retention efficiencies and sorting, pumping rates, respiration etc., (e.g. Allen, 1958; Jørgensen, 1960; 1966; 1975a; 1983; Stasek, 1961; Dral, 1967; Moore, 1971; Bernard, 1974; Owen & McCrae,

1976; Palmer & Williams, 1980; Bayne & Newell, 1983; Morton, 1983). Besides the classical work on lamellibranch gill types and ciliary mechanisms by Atkins (1936; 1937a; 1937b) very little has been done on the genus *Solen*.

With the exception of the work of Hodgson (1984) on the autotomy of the siphon, and that of Hodgson, de Villiers & Bernard (1987) dealing with sperm structure, the anatomy of *Solen cylindraceus* is unknown. The objective of this investigation was not an attempt at a detailed description of the fine structure of the gut, but simply to examine the basic form and the associated feeding structures, namely the gills and labial palps. As the major thrust of this work is directed principally at the animal's filtration and ecology, such an anatomical description is thus not out of place.

MATERIALS AND METHODS

A. Animal collection

Animals were collected from the Kariega estuary, and transferred to the laboratory, where they were thoroughly rinsed in filtered (Whatman No. 1) estuarine water (salinity, 35‰) to remove any attached sediment. To prevent gaping, the valves were bound loosely together with cotton thread, and the animals were placed in filtered estuarine water for 12 hours to allow for gut and mantle cavity clearance. Animals were then transferred to a 7% $MgCl_2$ solution (made up in estuarine water) for a period of 12 hours, after which anaesthetized animals were processed further.

B. Light microscopy

Dissected tissue was thoroughly rinsed in 0.45µm (Whatman GFF) filtered estuarine water, to remove as much debris and mucus as possible. Tissue was fixed in an aqueous solution of Bouin's fixative for 72 hours at 10°C. Smaller, whole animals (10-20mm shell length) were

transferred intact, directly to the fixative. Whole animals remained in the fixative for approximately 1 week, the acidity slowly dissolving the $CaCO_3$ of the shell. Once the shell had been fully dissolved, animals were transferred to fresh fixative for 24 hours. Tissues were dehydrated in a graded ethanol series, vacuum infiltrated and embedded in Paraplast. Serial sections (8μm in thickness) were cut, subjected to standard haematoxylin and eosin staining, and mounted. In order to determine crystalline style structure, styles were dissected out of freshly collected animals, placed on glass slides and viewed under a dissecting microscope. Lateral illumination using a cold light source was found to be the most effective means of highlighting the lamellar structure of the style.

C. Scanning electron microscopy

Gills and labial palps dissected from anaesthetized animals, were thoroughly rinsed with cold ($4^{o}C$) 0.45μm filtered estuarine water and transferred to a 2.5% glutaraldehyde solution, (made up in filtered estuarine water), and allowed to fix for 48 hours at $4^{o}C$. Prior to and during fixation, some of the dissected gill lamellae and labial palps were stretched out using insect mounting pins. Fixed tissue was again thoroughly rinsed a number of times with cold 0.1M phosphate buffer (pH 7.2), before being subjected to standard alcohol dehydration and amyl acetate impregnation. Specimens were critical point dried, sputter coated (gold) and viewed on a JEOL JSM - 840 scanning electron microscope.

D. Gut structure

To assist interpretation of the histological serial sections and reconstruction of the alimentary structure, latex moulds were made of the animal's stomach and anterior intestinal regions. Anaesthetized animals were dissected to expose the inhalent chamber and mouth. With the foot deflected to one side, a latex suspension was injected through the mouth into the stomach using a hypodermic syringe fitted with a fine, blunted needle. Injected animals were

left for approximately 20 minutes in the 7% $MgCl_2$ solution, and then transferred to a 50% acetic acid solution and left overnight to facilitate latex setting. Latex moulds were then exposed by digesting away the surrounding tissue with hydrochloric acid.

E. Ciliary currents

Freshly collected *S. cylindraceus* were dissected by slitting the dorsal mantle edge and allowing the valves to gape naturally. Any debris in the mantle cavity was gently flushed out with water from a Pasteur pipette. A suspension of the green algae *Tetraselmis suecica* (Pracinophyceae) and carmine powder was introduced onto the gill area immediately below the siphon. The path of the particles across the gills and labial palps was monitored by the use of a dissecting microscope.

RESULTS

A. General appearance

The external features of *Solen cylindraceus* are shown in Figure 2.1 A and B. It is an elongate animal, measuring some six to seven times its dorso-ventral width. In the Kariega estuary animals seldom attain lengths greater than 90mm. The animal is bilaterally symmetrical and is enclosed ventrally by the concrescence of the mantle edges (Fig. 2.1 A). The periostracum is very often discoloured (dark brown to black), particularly in older specimens. The ligament is situated anteriorly on the dorsal edges of the valves, while the rectal region of the intestine is clearly visible through the dorsal mantle edge (Fig. 2.1 B). The relative position of the anus, which opens into the exhalent chamber, is evident in Figure 2.1 B. The fused, segmented siphons which are readily autotomized, protrude from the posterior end of the animal, and comprise two sections, a ventral inhalent (arrowed, Fig. 2.1 C) and dorsal exhalent.

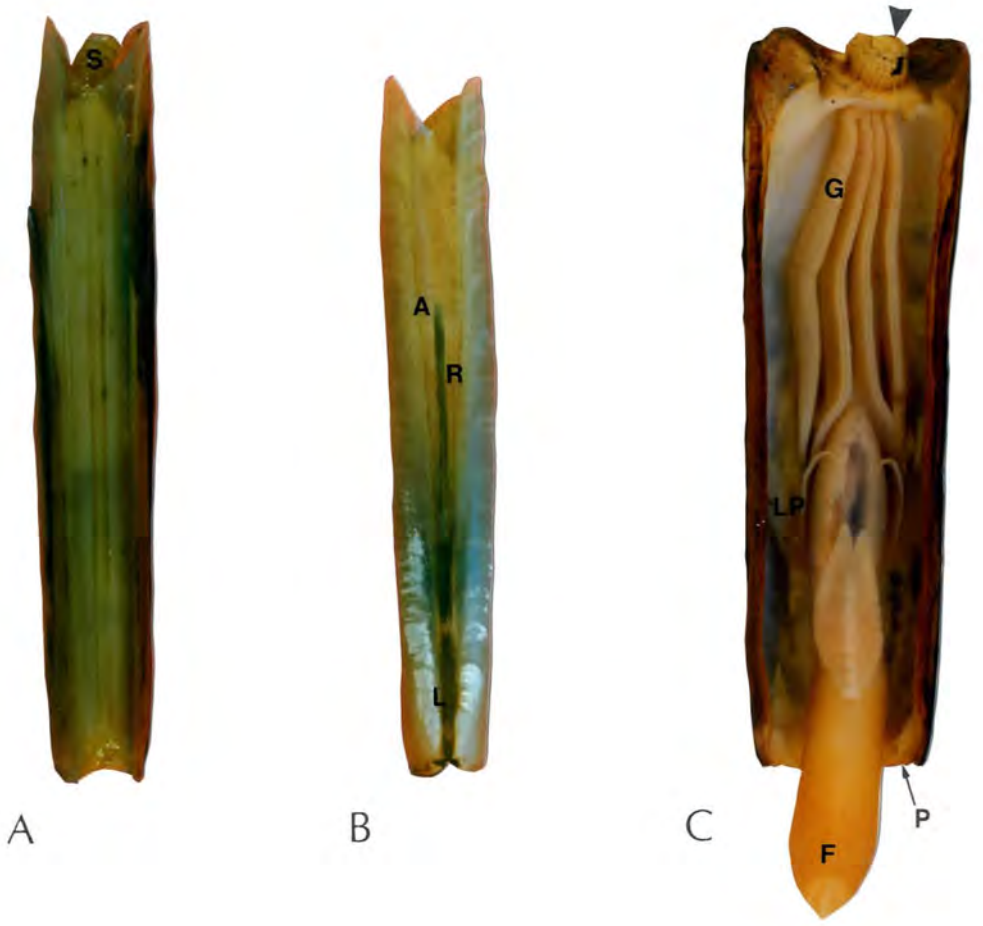


Figure 2.1: *Solen cylindraceus*. **A.** Ventral view. **B.** Dorsal view. **C.** Ventral view, with mantle musculature cut and valves gaping. **Key:** S = siphon (► , inhalent siphon), F = foot, G = gills, L = ligament, LP = labial palps, P = pedal flaps, R = rectal portion of intestine, A = relative position of anus.

Two pairs of gill lamellae originate at the base of the inhalent siphon (Fig. 2.1 C) and extend anteriorly for approximately half the length of the animal. Each pair terminates between the labial palps on either side of the viscero-pedal mass. The mouth, which is at the base of the labial palps, is in a dorsal position relative to the foot, between the "lips" formed by the fusion of the labial palps, and posterior to the anterior adductor muscle. The foot (Fig. 2.1 C) is a large elongated muscular organ which extends anteriorly through the pedal opening, but is capable of being fully retracted. The pedal opening is guarded by two thickened, muscular extensions of the mantle, the pedal valves (Fig. 2.1 C).

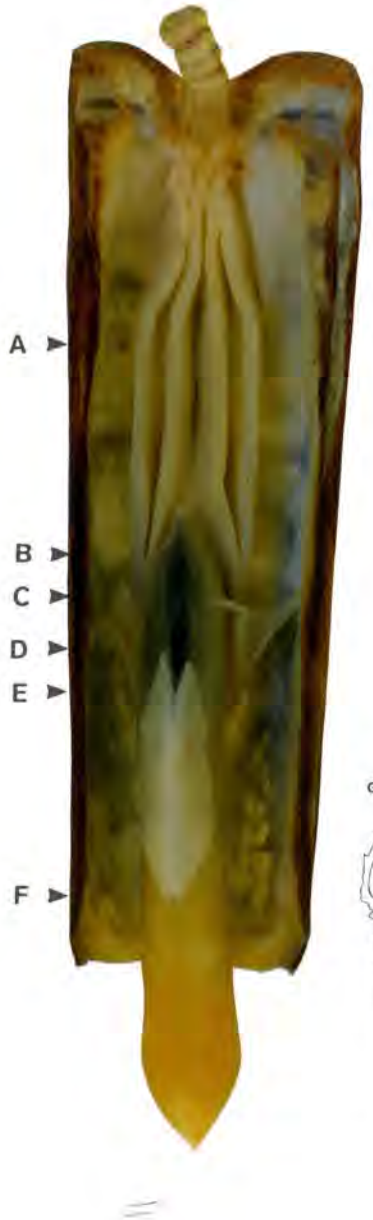
B. Gross anatomy

From serial sections, of which a selected few are illustrated in Figure 2.2, it is clear that the mantle cavity is divided into two chambers by the highly folded gill lamellae - a ventral inhalent and dorsal exhalent (Fig. 2.2 A). The gills extend anteriorly, terminating between the labial palps on either side of the visceral mass (Fig. 2.2 A-E).

i) Alimentary canal

From the mouth, the oesophagus extends posteriorly to the oesophageal portion of the stomach (Figs. 2.3 & 2.4). The oesophagus is highly folded, comprising densely ciliated columnar cells (40-60μm in height), which appear to secrete mucus (Fig. 2.5 A). The stomach is elongate, and lies embedded on the dorsal side of the digestive gland (Figs. 2.2 E & 2.3). Following Bloomer's (1901) nomenclature, the stomach may be divided into three regions: the oesophageal - the anterio-ventral portion where the oesophagus enters; the cardiac - the portion dorsal to and separated from the oesophageal region by a muscular fold; the pyloric - the posterior portion, from which region the midgut and caecum of the crystalline style leave (Fig. 2.3).

Figure 2.2: Selected cross sections from different regions of *Solen cylindraceus*. The approximate position from which each section was taken is indicated on the accompanying plate.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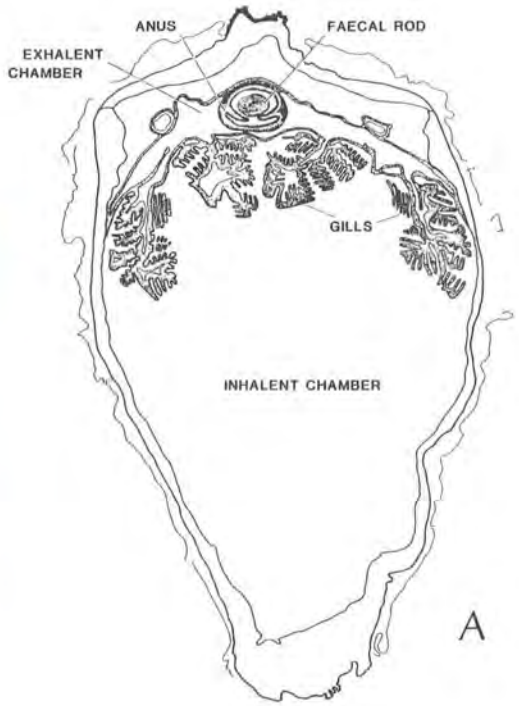
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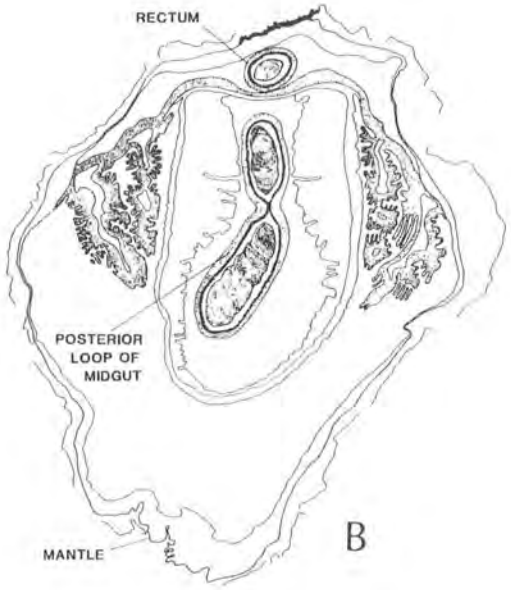
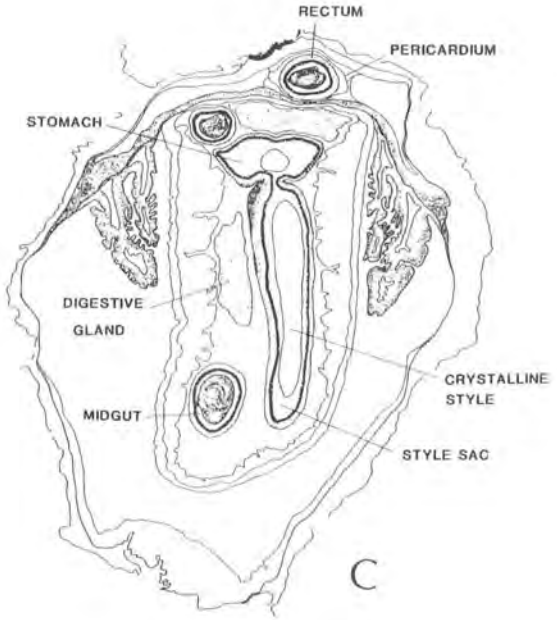
GILLS
INHALENT CHAMBER
A


RECTUM
POSTERIOR LOOP OF MIDGUT
MANTLE
B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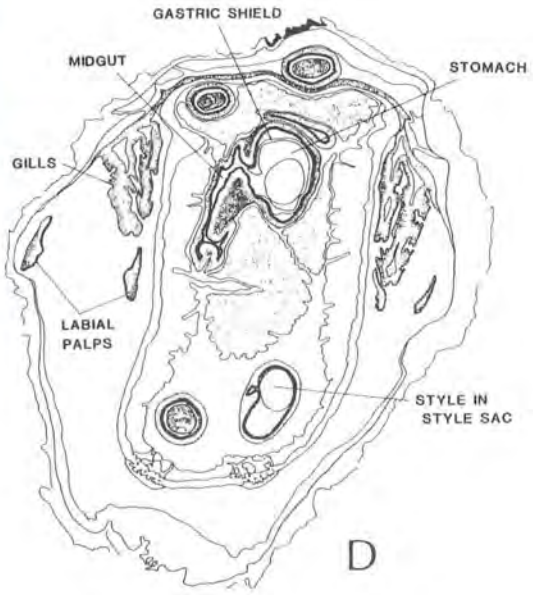
DIGESTIVE GLAND
CRYSTALLINE STYLE
MIDGUT
STYLE SAC
C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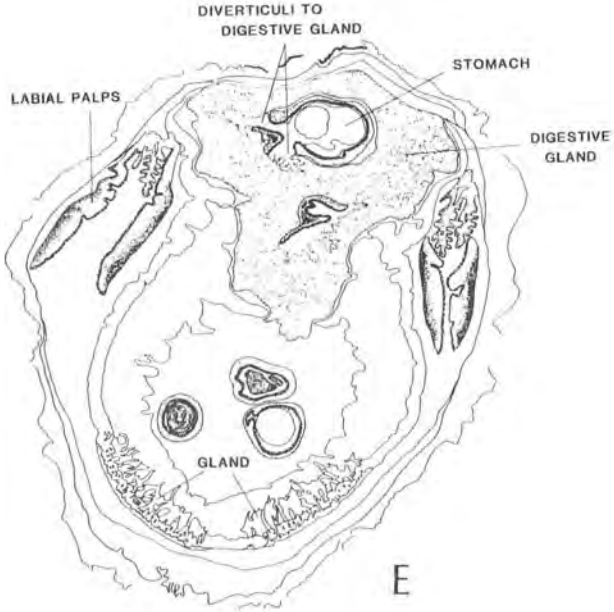
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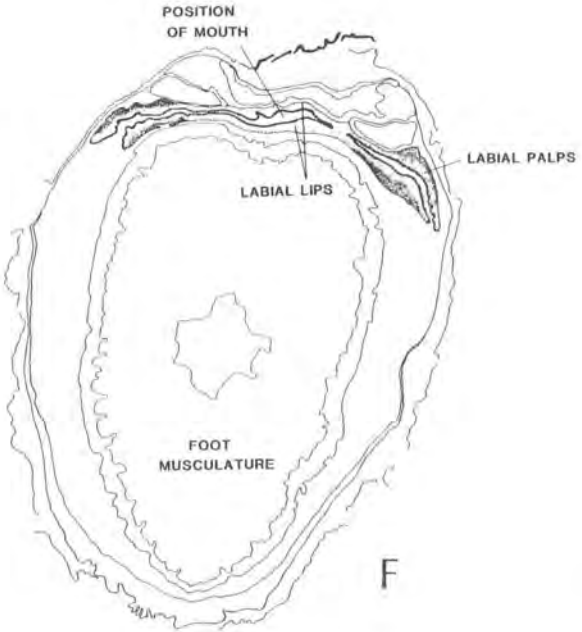

POSITION OF MOUTH
LABIAL PALPS
LABIAL LIPS
FOOT MUSCULATURE
F

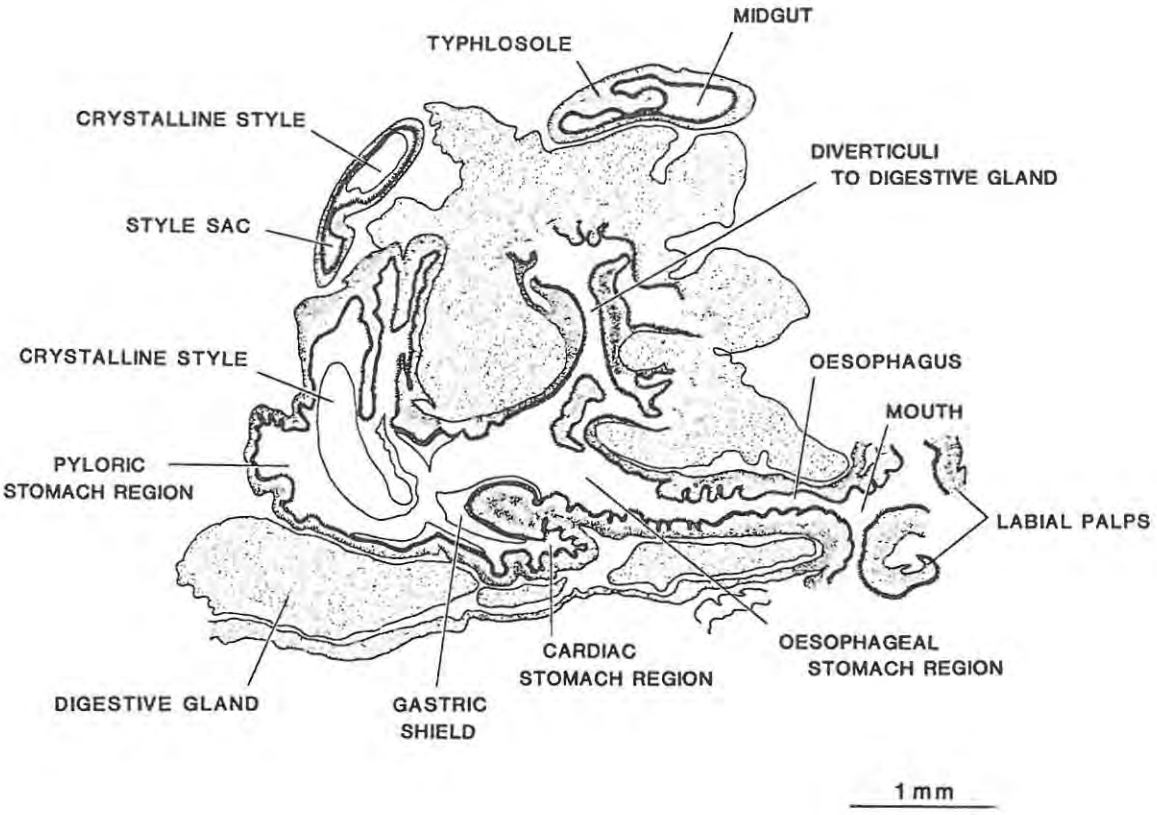


Figure 2.3: Longitudinal section through the stomach region of *Solen cylindraceus*.

Cellular composition of the stomach wall is fairly uniform and consists of densely ciliated columnar cells, some 60µm in height below which there appears to be a thin muscle layer (Fig. 2.5 B & C). The gastric shield appears to be distinct from the gut epithelium, and lies above the cilia (Fig. 2.5 C). Two major diverticuli emerge from the right ventro-lateral surface of the stomach (Figs. 2.2 E, 2.3 & 2.4), into the ventral digestive gland mass. A further opening, serving the dorsal digestive gland (Fig. 2.3) was evident from latex moulds made of the stomach.

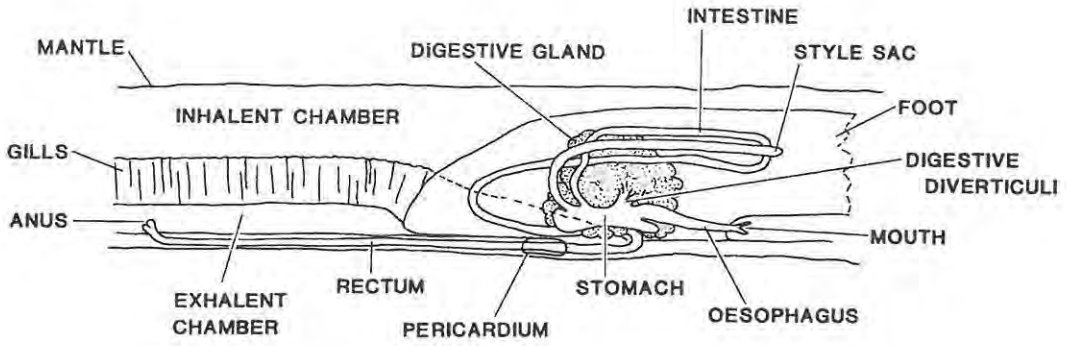


Figure 2.4: Diagrammatic reconstruction of the alimentary system of *Solen cylindraceus*, viewed from the right side.

The caecum of the crystalline style leaves the posterior portion of the stomach ventrally (Figs. 2.2 C, 2.3 & 2.4), curves anteriorly and extends, just below the ventral surface of the viscero-pedal mass, to a position slightly beyond the digestive gland (Fig. 2.4). The midgut leaves the stomach anteriorly next to the style sac (Figs. 2.2 D, 2.3 & 2.4) and follows a course similar to the style sac, to which it is joined by connective tissue. The midgut loops back on itself twice (Fig. 2.4) before leaving the viscero-pedal mass.

The entire epithelium of the midgut and rectum is composed of ciliated cells (ca. 40µm in height), as well as numerous secretory cells (Fig. 2.5 D). The major typhlosole of the midgut (Fig. 2.3) extends approximately one third of the length of the midgut from the stomach. The rectum extends posteriorly along the dorsal mantle edge, passes through the pericardium (Figs. 2.2 C & 2.4) and ends in a bi-lobed anus, which protrudes into the exhalent chamber (Figs. 2.2 A & 2.4). The entire alimentary tract is densely ciliated (Fig. 2.5 A-D).

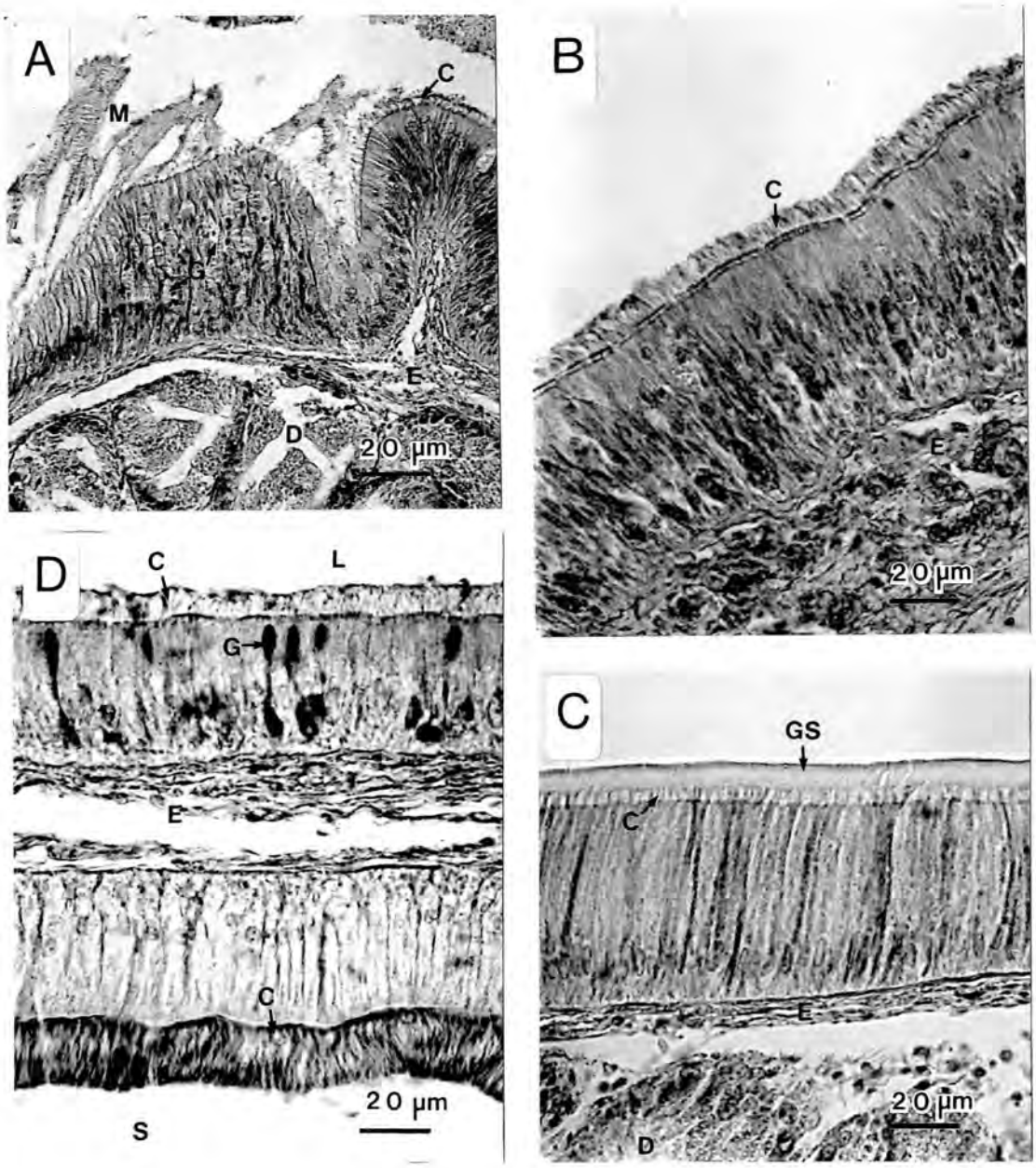


Figure 2.5: Light micrographs of different regions of the gut. **A.** Oesophagus. **B.** Pyloric region of stomach. **C.** Pyloric region of stomach with gastric shield. **D.** Midgut and style sac. **Key:** M = mucus, C = cilia, D = digestive gland, E = muscle and connective tissue, GS = gastric shield, S = caecum of style sac, G = mucus secretory cell, L = caecum of midgut.

The crystalline style of *S. cylindraceus* is a transparent, gelatinous rod-like structure, traversing the entire style sac, ending in the centre of the stomach against the gastric shield (Fig. 2.3). Style length is approximately one third of the animal's shell length, and *in situ*, is retained in a "J" shape (Fig. 2.4), the longer portion present in the style sac. The style is rounded posteriorly, but exhibits an elongated tip on the anterior end, which is very sticky and to which detrital material adheres. The style displays a fine longitudinal lamellar structure which appears to disintegrate and "liquify" in the stomach region.

ii) Gills and labial palps

The gills are long and narrow, with the dorsal edges of the outer demibranch fused with the mantle (Fig. 2.1 C). A similar fusion is present between demibranchs, which ensures complete division of the inhalent and exhalent chambers. Such fusion is, however, not observed in the area around the foot, and the two chambers would then be confluent in this region. The gill is plicate, with 13-16 filaments per plica (Fig. 2.6 A). Ciliation of the gill filaments reflects a typically eulamellibranch structure, comprising lateral, eulatero-frontal and frontal cilia (Atkins, 1936 nomenclature) (Fig. 2.6 A & B). There is, however, some variation in the frontal cilia, showing an apical tract of longer cilia (ca. 20µm in length), bordered on either side by shorter cilia (ca. 6µm long) (Fig. 2.6 B). The eulatero-frontal cilia exhibit a highly organized arrangement (Fig. 2.6 C) comprising a double row of a number of cilia which appear to adhere to one another, forming a cirrus, the apical end of which is branched. The intercirral distance is some 2.5-3.0µm. Ostea (Fig. 2.7 A) occur between filaments and are largely shielded by the lateral and eulatero-frontal cilia. The filaments and plica appear to decrease in height toward the apical edge of the gill, where a well-defined food groove is evident (Fig. 2.7 B).

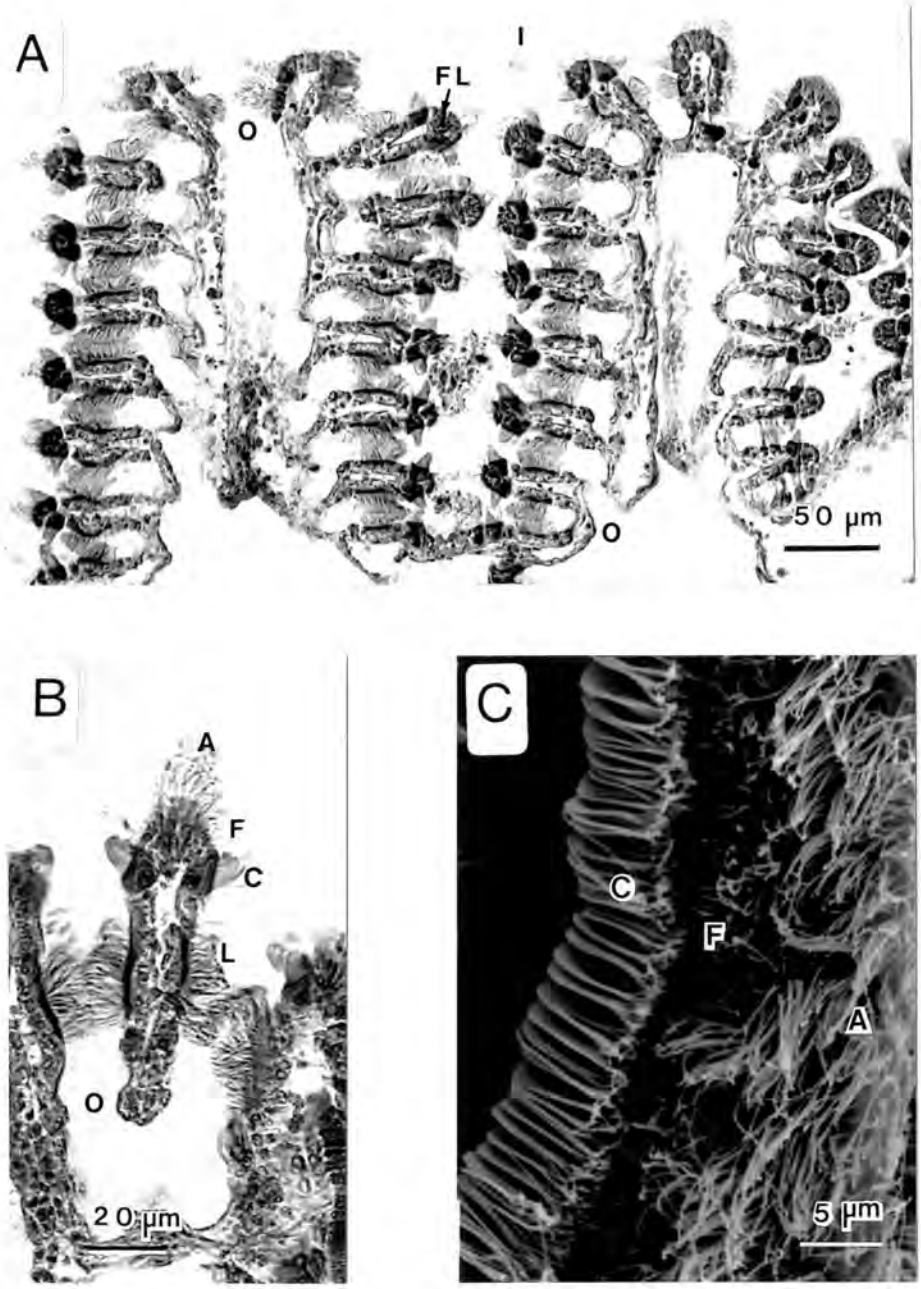


Figure 2.6: Gill structure of *Solen cylindraceus*. **A.** Longitudinal section across a portion of a gill lamella, showing plicate structure and filament arrangement. **B.** Ciliation of individual filament. **C.** S.E.M. showing eulatero-frontal cirrus arrangement. **Key:** O = ostea, C = cirrus, A = longer frontal cilia (apical), F = shorter frontal cilia, L = lateral cilia, I = inhalent chamber, FL = gill filament.

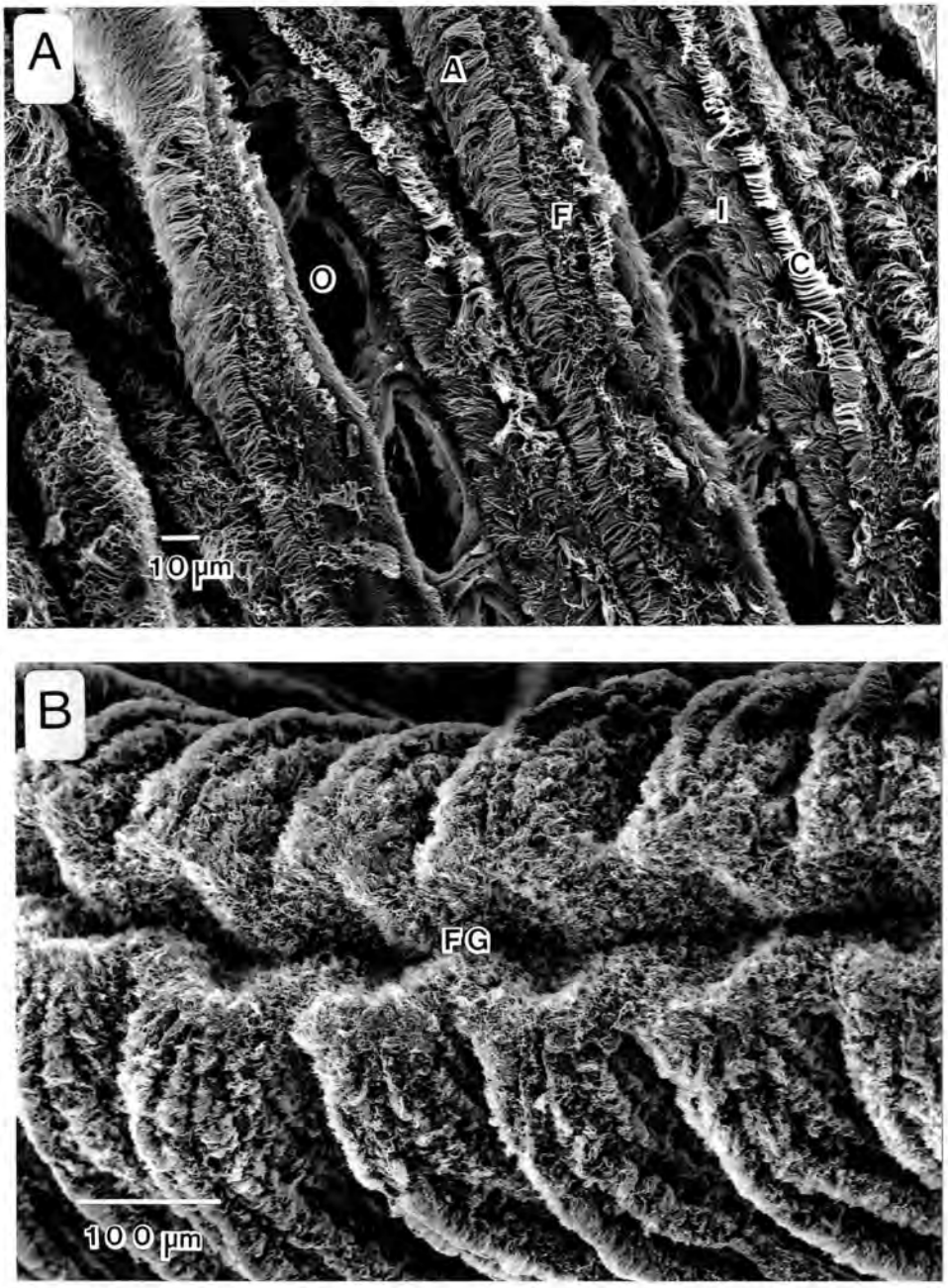


Figure 2.7: S.E.M. of *Solen cylindraceus* gill. **A.** Stretched gill tissue displaying ostea and ciliary arrangement of the filaments. **B.** Apical region of a single gill lamella showing food groove and plicate structure. **Key:** O = ostea, C = cirrus, A = longer frontal cilia (apical), F = shorter frontal cilia, L = lateral cilia, FG = food groove.

Figure 2.8: S.E.M. of labial palp surface of *Solen cylindraceus*. 1 and 2 designate two visibly distinct ciliary tracts.

There are two labial palps, one inner and one outer, on either side of the viscero-pedal mass and mouth. They are roughly triangular in shape, of approximately equal size and are attached dorso-laterally to the viscero-pedal mass. Elongations of the two inner palps fuse anteriorly of the visceral mass and form the ventral lip of the mouth, while similar elongations of the two outer palps fuse to form the dorsal lip. The groove formed between the two palps and the fused extensions is the oral groove, which leads directly to the mouth. The opposed surfaces of the palps are ridged, and densely ciliated. Ridges are set at virtually right angles to the oral groove, and at least two distinct ciliary tracts are evident (Fig. 2.8). The relation between the gills and labial palps is illustrated in Figure 2.9. The inner demibranch extends further into the oral groove than the outer.

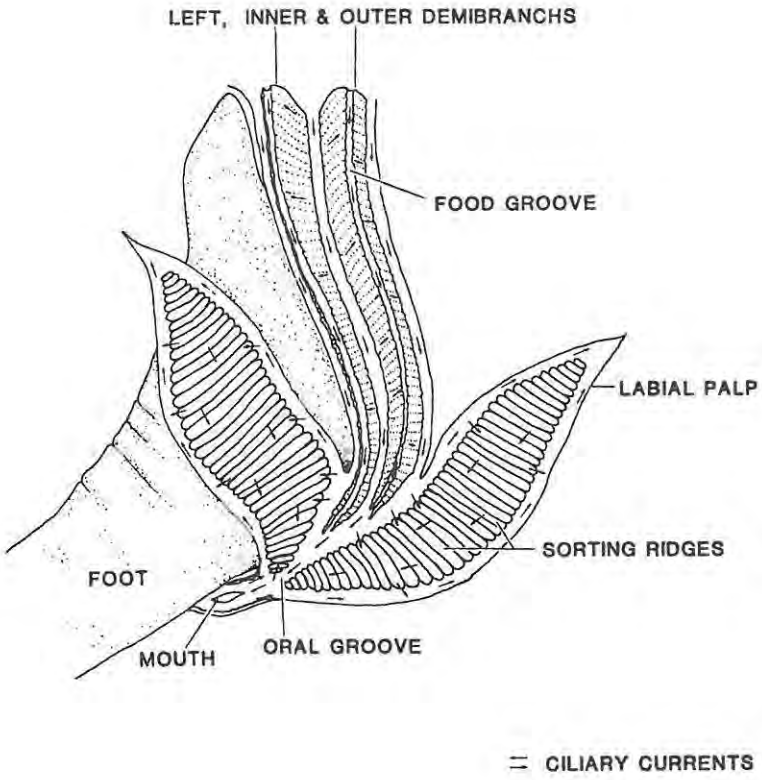


Figure 2.9: Diagrammatic representation of the association between gills and labial palps in *Solen cylindraceus*. Arrows indicate the direction of major ciliary currents.

The ciliary currents of both gills and labial palps are shown in Figure 2.9 and exhibit no major variation from those described for *Solen marginatus* and *Ensis siliqua* (Atkins, 1936). Currents between the bases and along the outer dorsal edges of the demibranchs were all orally directed. A similar orally directed current was observed along the free ventral margin of the demibranchs. Along the plica, only dorso-ventral movement of particles was observed. Between the labial palps, the oral groove extends to the mouth, and the principal rejection currents occur along the lip edges around the mouth, the anterior and posterior margins of the labial palps.

DISCUSSION

The most noticeable anatomical feature of the Solenidae, and one to which (according to Owen, 1959) all other features may be connected, is the marked posterior elongation of the shell. Such elongation and the presence of a large and well-developed foot, have resulted in an animal suitably adapted for vertical burrowing in soft sediments. The siphons, associated feeding organs (gills and labial palps) and alimentary structure are characteristic of a typical suspension feeder as detailed by Jørgensen (1966) and Purchon (1978).

The plicate, heterorhabdic structure of the gills is typical for members of the genus *Solen*, despite having only 13-16 filaments per plica as compared to the 24-26 of *S. marginatus* (Atkins, 1936). Filament ciliary structure is not unlike that described for other eulamellibranchiate bivalves (Atkins, 1936; 1937b) and indicates an organ well-adapted for active water pumping and a filter-feeding existence. The role of the latero-frontal cirri of bivalves (eulatero-frontal in *Solen*, according to Atkins, 1936) as filters which retain suspended particles has been highlighted by a number of workers (e.g. Dral, 1967; Moore, 1971; Owen, 1974a). The cirri in *S. cylindraceus* are branched, and although the extent and periodicity of this branching has not been determined, the intercirral distance (2.5-3.0μm) should nevertheless give an indication of the size of particle which would be retained with great efficiency by the gills. Based on these measurements, *S. cylindraceus* should retain, with virtually 100% efficiency, particles greater than 3.0μm. Assuming a similar branching of the cirri structure as described for *Mytilus edulis* (Owen, 1974a) and *Nucula sulcata* (Owen & McCrae, 1976), *S. cylindraceus* should theoretically be capable of retaining particles down to some 2-2.5μm with great efficiency. The measured particle retention efficiency of *S. cylindraceus* is reported in Chapter 6.

The apparent confluence between exhalent and inhalent chambers in the region of the foot, is described in *S. marginatus* as "a ciliary junction which is easily dissolved" (Atkins, 1936) but whether this is the case in *S. cylindraceus* has not been verified. However, if optimal gill functioning and filtration is to occur, it would not be unlikely that a similar ciliary junction be present in *S. cylindraceus*. The significance of such an easily disruptable junction is unclear, but may assist in preventing unnecessary stretching of the gill lamellae during periods of foot extension. Further, during periods of water ejection from the mantle cavity, either to void faecal and pseudofaecal material or during burrowing, an easily disruptable junction between the major chambers would result in substantially less stress on the gills.

The labial palps are considered the principal sorting structures of bivalves (Purchon, 1978). Purchon (1955) and Allen (1958) have demonstrated the occurrence of several discrete ciliary tracts on the ridged palp surface. The cilia of each tract beat in different orientations, and are responsible for the rigorous sorting process. In the results presented here (Fig. 2.8) only two visually obvious tracts are present. However, much is probably obscured by the contraction of the tissue during fixation, while other smaller tracts may only become apparent in function. Graham (1931) records substantial diversification of ciliary tracts on the labial palps of *Ensis siliqua*. This increased diversification and subsequent more organized sorting mechanism has been suggested to be a consequence of the animal's habitat (Graham, 1931; Purchon, 1978) - *Ensis*, like *Solen*, inhabits intertidal sand and mud banks where a more efficient sorting mechanism would be of advantage.

The alimentary canal presents a number of variations, when compared to that described for *Ensis ensis* (Bloomer, 1901) and *Ensis siliqua* (Bloomer, 1901; Graham, 1931), a result which is not uncommon for species belonging to different genera. However, virtually no differences in the alimentary structure were noted between *S. marginatus* (Bloomer, 1901) and

S. cylindraceus, with the exception that *S. cylindraceus* does not have a convoluted midgut region on the ventral surface of the digestive gland, immediately after leaving the stomach. The stomach is typically elongate with a very large posterior sorting region, just anterior to which the crystalline style enters.

Besides observations as to the gelatinous nature and layered structure of the style, no further properties have been examined. It is "traditionally" assumed that style rotation is continuous, and that rotation against the gastric shield aids the fragmentation and digestion of food particles (Morton, 1952; Bayne, Thompson & Widdows, 1976). Despite the very prominent and extensive ciliation along the entire style sac, I have never observed any rotation of the style in *S. cylindraceus*. Further, it is difficult to imagine rotation of such a fairly turgid gelatine-like rod, which is bent at its anterior end through virtually 300°. More recent work has suggested that the style of bivalves undergoes an intermittent rotation and dissolution, corresponding to tidal influence, feeding and digestive activity (Morton, 1956; Purchon, 1971; Bernard, 1973; Langton & Gabbott, 1974; Langton, 1977). In *S. cylindraceus*, no variation in crystalline style volume with animal filtering activity or tidal inundation has been observed (present study, cf. Chapter 5).

Solen cylindraceus thus exhibits no major variation from the typical eulamellibranch form and is well-adapted to a burrowing and suspension-feeding existence.

CHAPTER THREE

ANIMAL DENSITY AND DISTRIBUTION

INTRODUCTION

Solen cylindraceus is recorded as inhabiting estuaries from Inhambane on the central Moçambique coast to Knysna on the south eastern Cape coast of South Africa (Day, 1981; Kilburn & Rippey, 1982) (Fig. 3.1). The occurrence and abundance of *S. cylindraceus* within the estuaries of its documented range vary (Table 3.1). The bivalve is particularly abundant in the Kariega estuary in which, despite being close to the southernmost limit of the geographical distribution (Fig. 3.1), it reaches densities of 400+ m^{-2} (equivalent biomass of 70g m^{-2} dry wt. (Hodgson, 1987)). Similar densities have been recorded at Gillys point, St Lucia (Table 3.1), but with a maximum biomass of only 14.3g m^{-2} (Blaber, Kure, Jackson & Cyrus, 1983).

The large population of *S. cylindraceus* in St Lucia was attributed to a long period of elevated, stable salinity (Blaber *et al.*, 1983). The success of *S. cylindraceus* in the Kariega may similarly be a result of periods of elevated stable salinities (Hodgson, 1987; de Villiers, Hodgson & Allanson, 1989, in press). The Kariega river drains a catchment which is not only small (686km^2) with a low and variable annual rainfall (500-700mm), but is regulated by three major dams. Consequently, there is no freshwater inflow into the estuary for long periods, resulting in a marine dominated system in which salinities reflect that of the sea (34-36‰) for

its entire length (Allanson & Read, 1987). Hypersaline conditions (40-46‰) are commonly recorded in the upper reaches of the estuary during dry summer months. Such conditions are relieved by infrequent flood events (every 3-5 years, Allanson & Read, 1987), during which waters in the upper reaches may be virtually fresh. A more detailed description of the hydrodynamics and ecology of the estuary is given by Allanson & Read (1987).

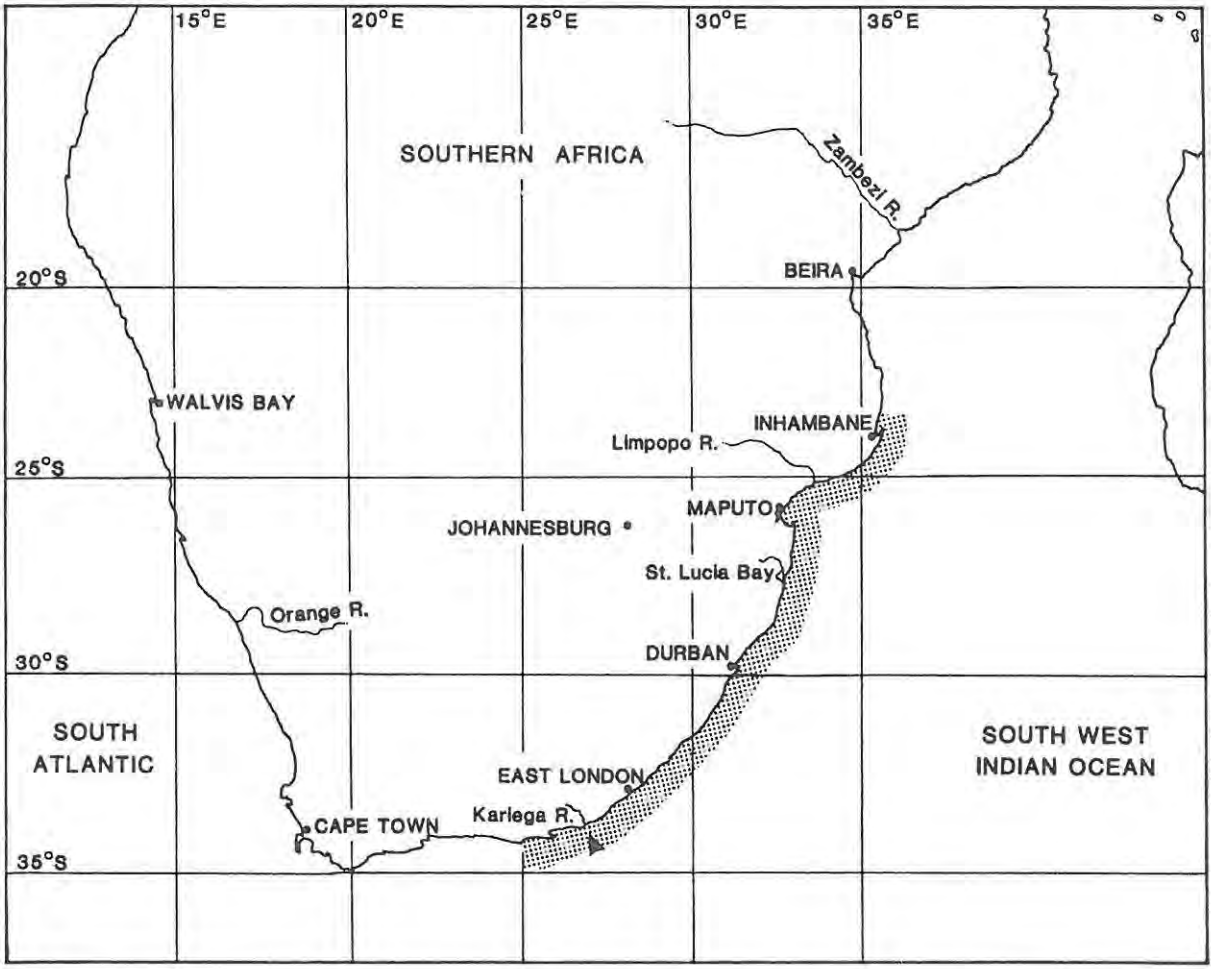


Figure 3.1: Study site (arrowed) and geographical distribution of *Solen cylindraceus*.

Table 3.1: Abundance of *Solen cylindraceus* in some southern African estuaries.

Estuary	*S. cylindraceus* (m^{-2})	Reference
Morrumbene	126+	Day, 1974
St Lucia - Gillys point	498	Blaber *et al.*, 1983
St Lucia - other sites	49-151	Blaber *et al.*, 1983
Mzimkulu	nil	Day, 1981
Mngazana	64	Branch & Grindley, 1979
Great Fish	<1	Taylor (pers. comm.), 1988
Kleinemond*	common	Brown, 1953
Kowie	150	Day, 1981
Kariega	400+	Hodgson, 1987 & present study
Bushmans	10-20	Hodgson, 1987
Swartkops	45-120	McLachlan & Grindley, 1974
		Day, 1981
Kromme	5-15	Baird *et al.*, 1980
Knysna	125	Day, 1967
Seekoei	ß50	Pers. obs., 1989

* Brown records specimens as *Solen capensis*, however, from what is known of the biology of both *S. capensis* and *S. cylindraceus* and the description he gives of the area where animals were found, I feel his initial classification is incorrect and animals were most probably *S. cylindraceus* (see also Scott *et al.*, 1952 and Kilburn & Rippey, 1982). Brown (1953) does not indicate abundance as m^{-2}, but uses the style of Stephenson (1939), common = 32-84 specimens.

Although estuaries are the junction between rivers and the sea, salinity is not the only environmental variable affecting estuarine structure and animal distribution, and consideration must be given to a host of both marine and terrestrial factors. Tidal currents and other oceanic events (e.g. upwelling), the nature of the catchment, precipitation, sediment structure, turbidity, human influences etc., all interact to define estuarine structure, function and, ultimately, faunal richness and success (McLachlan & Erasmus, 1974; McLachlan & Grindley, 1974; Boltt, 1975; Branch & Grindley, 1979; Day, 1981; Blaber *et al.*, 1983; Allanson & Read, 1987; Hodgson, 1987).

Estuaries in the south and eastern region of South Africa tend to be narrow and shallow and consequently, may be subject to rapid changes in physical conditions (Day, 1981). The Kariega is approximately 18km long with an average width of 110m, has a volume of some 3590 X 10^6 m^3, and a tidal prism volume of 2062 X 10^6 m^3 (Allanson & Read, 1987). Water temperatures range seasonally between 7-22^{o}C in winter, and 14-35^{o}C in summer. However, short-term fluctuations of 10-12^{o}C within a single tidal cycle have been recorded (Taylor, 1988), and are largely a consequence of coastal upwelling. Temperatures in the mouth and lower reaches of the estuary tend to reflect that of the sea, whereas in the upper reaches, temperatures are about 5^{o}C higher in summer, with the reverse holding true during winter.

While it is true that the physico-chemical factors in the environment control the nature and distribution of the organisms living in the estuary, it is equally true that biological factors may influence conditions of habitat (Rhoads & Young, 1970; McLachlan & Grindley, 1974; Eagle, 1975). The intertidal mud banks (6-10m wide on average) support three principal macrophyte species: *Zostera capensis* (Setchell), *Spartina maritima* (Curtis) and *Sarcocornia*

perennis (Miller), all present for virtually the entire length of the estuary except in the mouth region (Hodgson, 1987). In the extreme upper reaches, small but dense beds of *Phragmites australis* (Cavanilles) occur. A large amount of terrestrial vegetation lines the edge, and overhangs the intertidal zone of the middle and upper reaches of the estuary. The Kariega is largely a benthic dominated estuarine system with a high species diversity (Allanson & Read, 1987). Hodgson (1987) described the macrobenthic fauna of the Kariega and recorded 107 species. However, his study gives no indication of animal size ranges or any detailed information on intertidal distribution. The aim of this study was not only to expand on Hodgson's data, but also to examine more closely some of the physical parameters e.g. turbidity, sediment nature etc., which may influence the distribution and abundance of *S. cylindraceus* in the Kariega estuary.

MATERIALS AND METHODS

A. Distribution and abundance of *Solen cylindraceus* in the Kariega estuary

A total of 28 stations were sampled along the length of the estuary during October 1986 to January 1987 (Fig. 3.2). Previous work by Hodgson (1987) indicated that four distinct zones are apparent on the intertidal banks. Zone 1, the lowest down the bank, comprises a band of *Zostera capensis*. Zone 2, exposed mud or sand. Zone 3, a belt of *Spartina maritima* and Zone 4 of *Sarcocornia perennis*. Quadrats of $1/8m^2$ were taken within three of these zones, from within the *Spartina* to below the *Zostera*. The number of quadrats taken at each station depended on topography, but attempts were made to sample the following areas:

1. Within the *Spartina*
2. Immediately below the *Spartina*
3. The middle region of the exposed bank
4. ...

4. Immediately above the *Zostera* on the exposed bank
5. *Zostera* fringe (shore side)
6. Within the *Zostera*
7. *Zostera* fringe (river side)
8. Below the *Zostera* (subtidal).

Quadrats were dug to a depth of about 500mm and sieved through a $1mm^2$ mesh sieve. At stations where *S. cylindraceus* were recorded, transects were mapped, using a Leitz Dumpy Level, and the extent of the vegetation cover along the transect line recorded. The total number of animals and their size (mm, shell length) were recorded for each quadrat. Biomass was determined by removing the shell, and drying the specimens at $60^{o}C$ to constant weight (all biomass figures are expressed as shell-free dry weight). Animal and vegetation distribution was then plotted for each station, relative to mean sea level.

B. Physical characteristics of the estuary

Many of the physical characteristics of the estuary have previously been determined, and are more fully described by Allanson & Read (1987) and Taylor (1988). The mud content of the sediments (% subsieves - the percentage of material passing through a sieve mesh of 63µm- (Buller & McManus, 1979; Baird, Hanekom & Grindley, 1986)) was determined at all stations along the estuary. Core samples of 80mm diameter and 300mm deep were taken from the intertidal sand and mud banks (just inshore of the *Zostera* beds). Cores were transported to the laboratory where they were dried on trays at $50^{o}C$ to constant weight. During drying, cores were gently broken up by hand and any animal or plant material removed. Dry weight was recorded, after which samples were washed through a 63µm sieve. The remaining material was again dried and re-weighed. The percentage subsieves was calculated by difference.

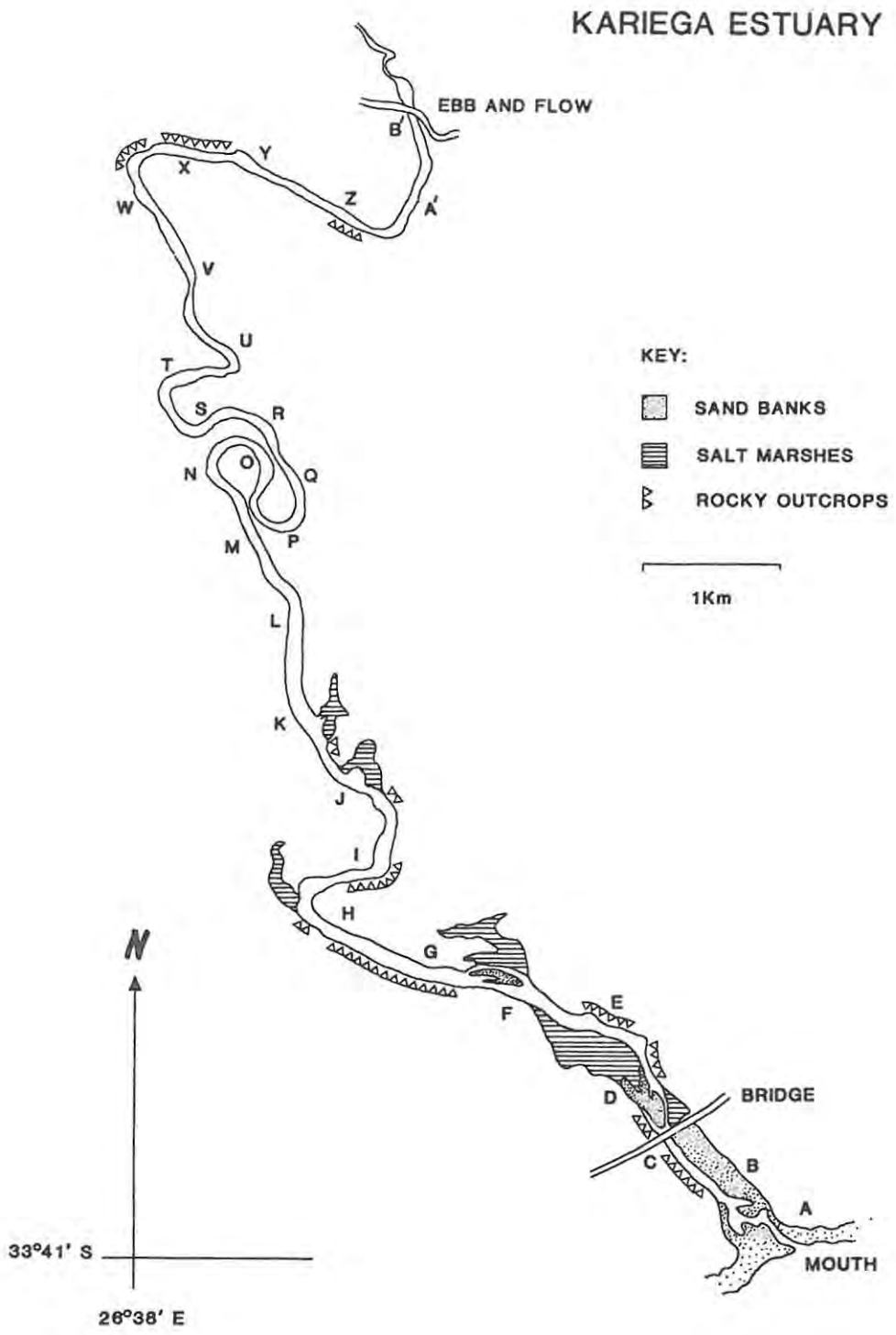


Figure 3.2: Kariega estuary, showing major features and sampling stations.

Turbidity along the length of the estuary was determined at six spring and neap, flood and ebb tides, during September to December 1987. Samples were collected midstream from each station (F-Z, Fig. 3.2) at a depth of 1m, during slack tide. Samples were transported back to the laboratory where turbidity (NTU) was measured, using a HACH model 2100A Turbidimeter. Volumes of 0.5-1.0 l were filtered through pre-ashed, weighed Whatman GFF glass fibre filters (pore size 0.45μm). Filters were dried at 50°C for 72 hours, weighed, and total particulate matter (T.P.M. mg l^{-1}) determined. Total organic content was determined by difference after ashing.

RESULTS

A. Distribution and abundance of *Solen cylindraceus* in the Kariega estuary

No *Solen cylindraceus* were found in the mouth regions (below station G), or at the very head of the estuary (station B^1). The longitudinal distribution of *S. cylindraceus* in the Kariega estuary is illustrated in Figure 3.3.

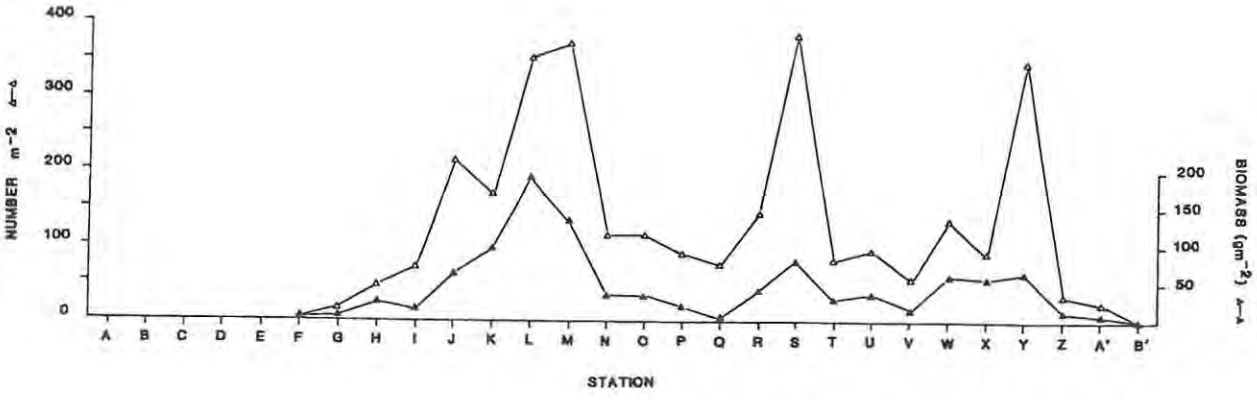


Figure 3.3: Distribution and abundance of *Solen cylindraceus* along the length of the Kariega estuary. Both density (△) and biomass (▲) values are from quadrats taken from the intertidal mud bank, immediately inshore of the *Zostera* fringe.

Three distinct peaks in animal density (>350 m^{-2}) occurred at stations L-M, S and Y. However, only stations L-M demonstrated a corresponding marked increase in biomass (192g m^{-2}). The high numerical, but relatively low biomass values recorded for stations S (77g m^{-2}) and Y (64g m^{-2}), were the result of a larger population of smaller specimens (cf. Fig. 3.4). The transect results of four stations from different regions of the estuary (J,L,S and Y) are shown in Figure 3.4 and serve to illustrate littoral distribution. In all instances the greatest abundance of *S. cylindraceus* was supported by the intertidal mud bank just inshore of, and within the *Zostera* (Fig. 3.4). A similar picture was reflected in terms of biomass distribution (Fig. 3.5). The vertical distribution of *S. cylindraceus* relative to M.S.L. and the *Zostera* zone for all stations measured, is summarized in Figure 3.5. *Solen cylindraceus* appear to have a very narrow range relative to tidal height, and no animals were recorded more than 0.8m above M.S.L., while few were subtidal (Figs. 3.4 & 3.5). The maximum biomass occurred between -0.5 and +0.5m M.S.L., again reflecting a distribution within and immediately inshore of the *Zostera* zone. Such a distribution indicates that the animals will always be submerged during flood tides, but will not always be exposed during ebb tide.

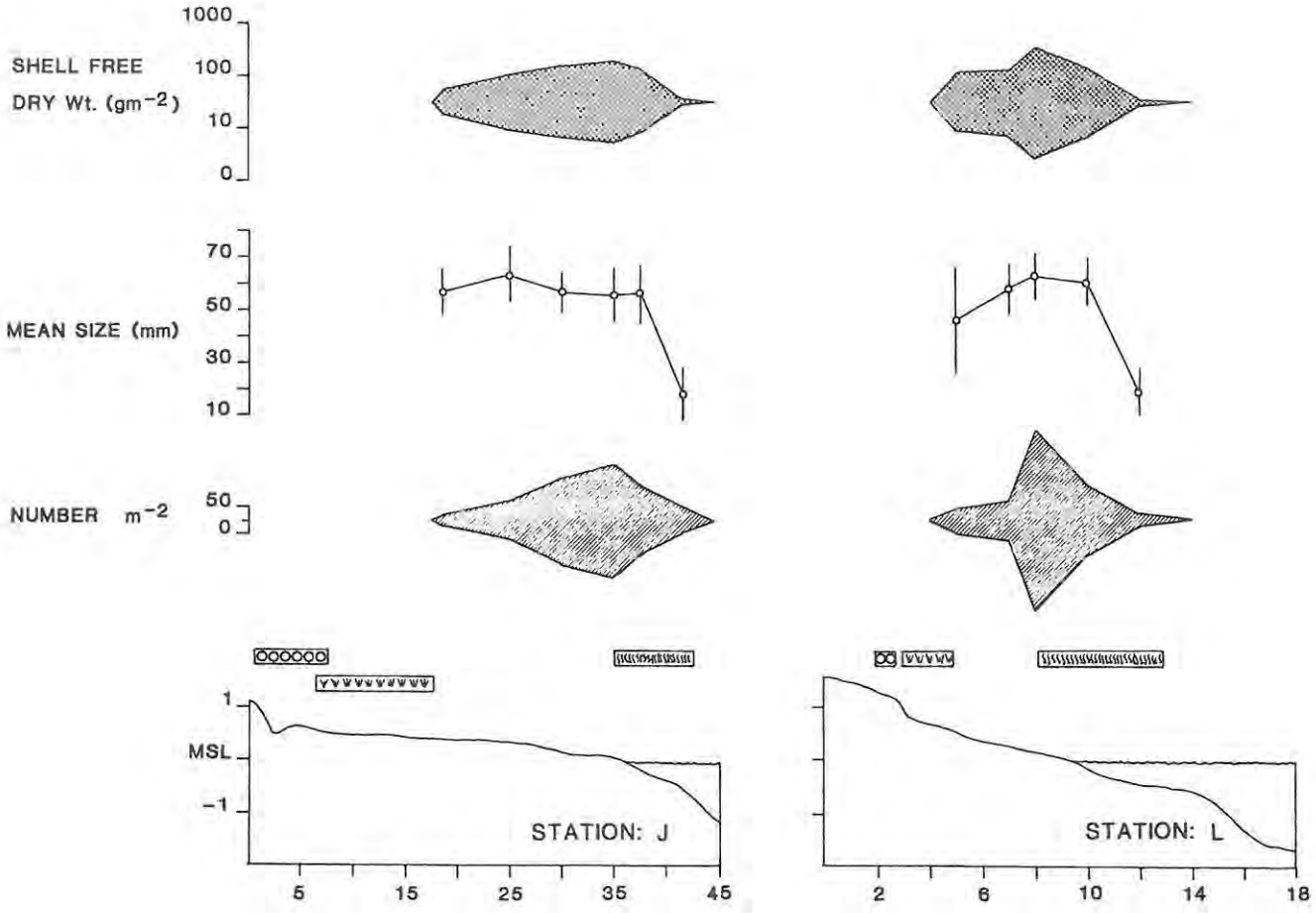
The range of animal sizes collected during sampling was 8-89mm (shell length) and a size frequency histogram using 10mm size class intervals is given in Figure 3.6. The majority of the animals fell within the range 51-70mm. The mean length determined from all the animals collected was 51 ± 16mm (n=1020). In the transect results shown (Fig. 3.4) and at virtually all other stations, the river-side *Zostera* fringe appeared to be inhabited by predominantly smaller size-class animals.

Figure 3.4: Distribution and abundance of *Solen cylindraceus* at four representative stations in the Kariega estuary, relative to mean sea level (M.S.L.), bank topography and principal vegetation types.

Key: = *Zostera capensis*

= *Spartina maritima*

= *Sarcocornia perennis*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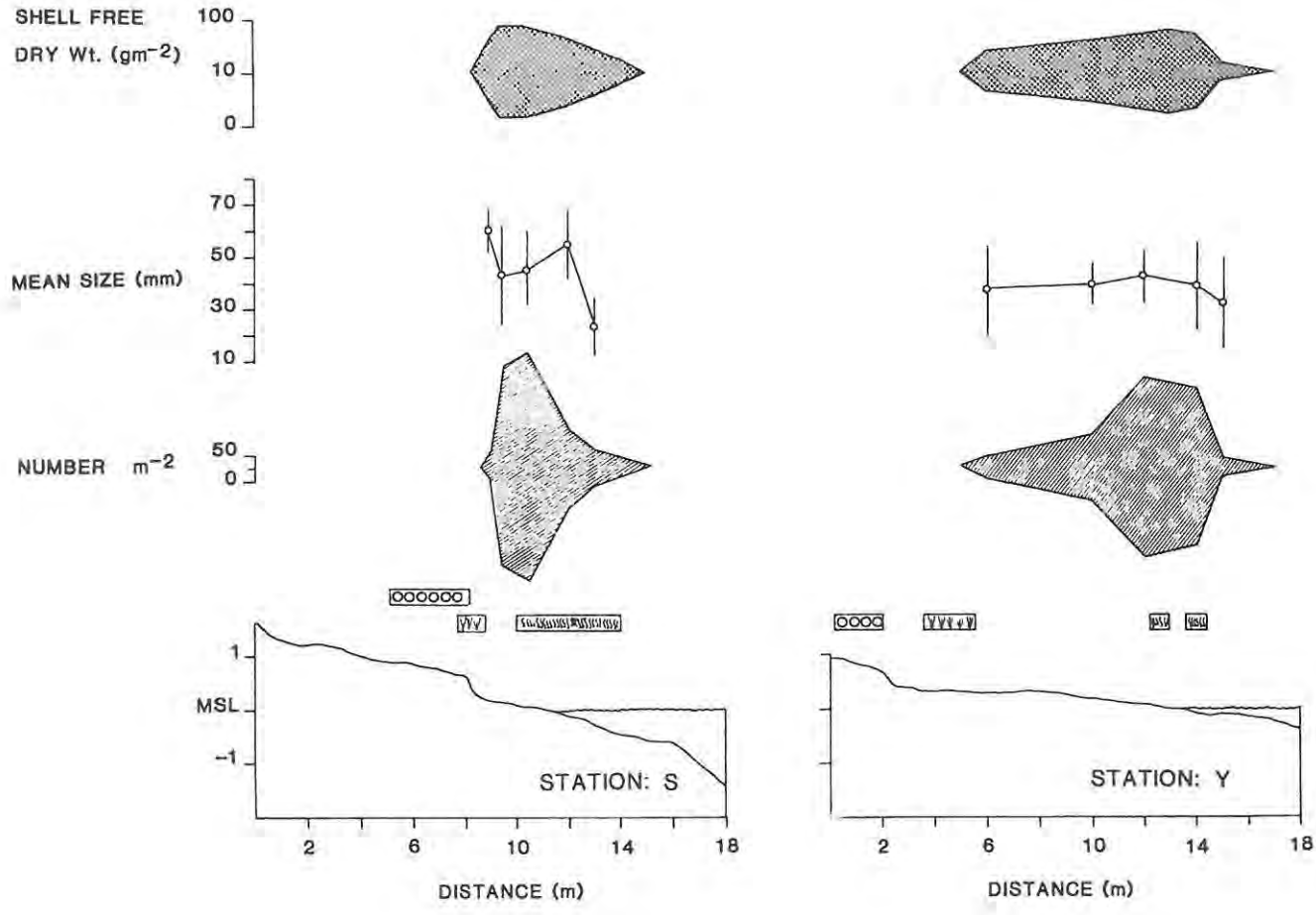
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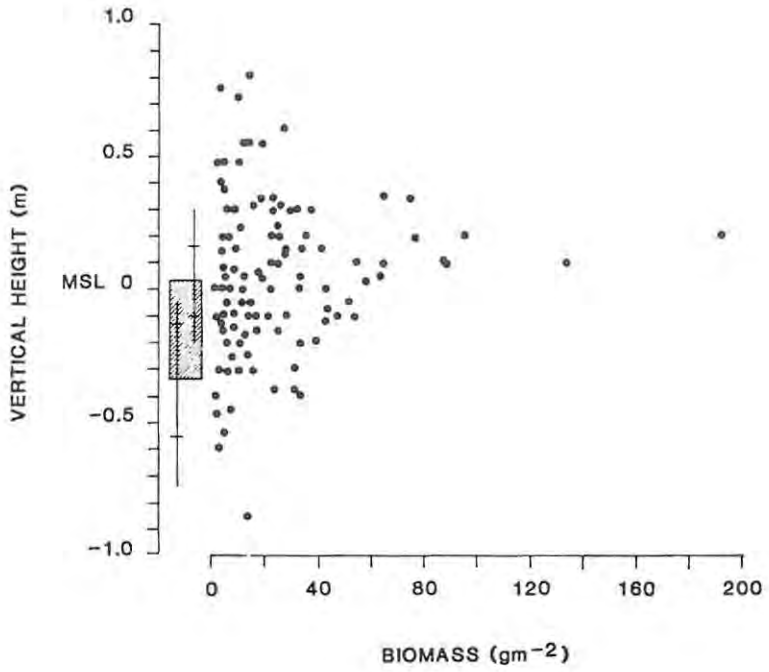


Figure 3.5: Vertical distribution of *Solen cylindraceus* biomass recorded from all stations in the Kariega estuary, relative to mean sea level (M.S.L.) and *Zostera* distribution. The shaded block represents the vertical distance between the mean heights of the upper and lower fringes of the *Zostera* bed, recorded at all stations.

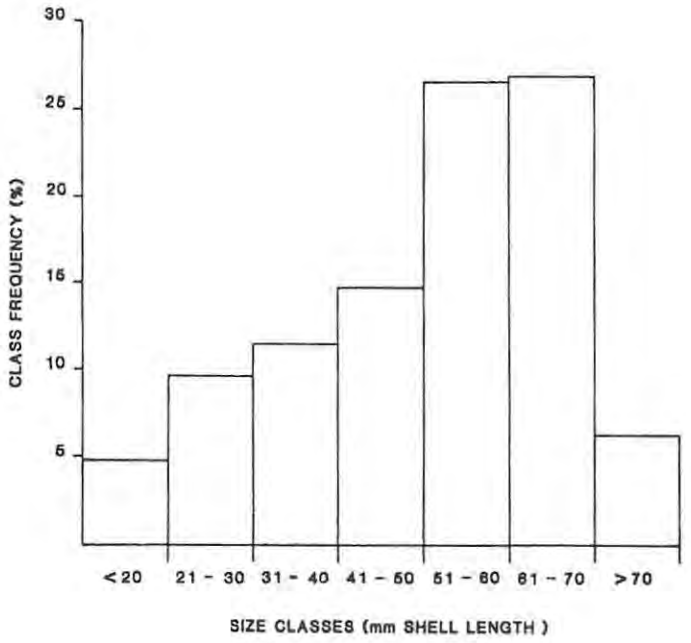


Figure 3.6: Size class frequency histogram for *Solen cylindraceus* in the Kariega estuary at the time of sampling (October 1986 to January 1987).

B. Physical characteristics of the estuary

The percentage mud in the intertidal sediments varied along the length of the estuary. Using this criterion, the Kariega was divided into 4 regions (Fig. 3.7). The mouth area comprised virtually clean sand and contained only approximately 2% subsieves. The lower reaches exhibited a marked increase in sediment mud content (ca. 27%), and consisted of a coarse mixture of sand and finer sediments. Sediments in the middle reaches demonstrated a mud content in the order of some 60%, although two stations (K and N) exhibited substantially lower percentage subsieves. At K there was a large cliff of consolidated sand which had eroded, while at N, the sediment was very stoney. Mud constituted only approximately 32% in the upper reaches of the estuary, and sediments tended to have a coarse texture, similar to that observed in the lower reaches, possibly the result of sand deposition during periodic flood events. Despite the variation in percentage subsieves along the length of the estuary, the intertidal banks were generally firm.

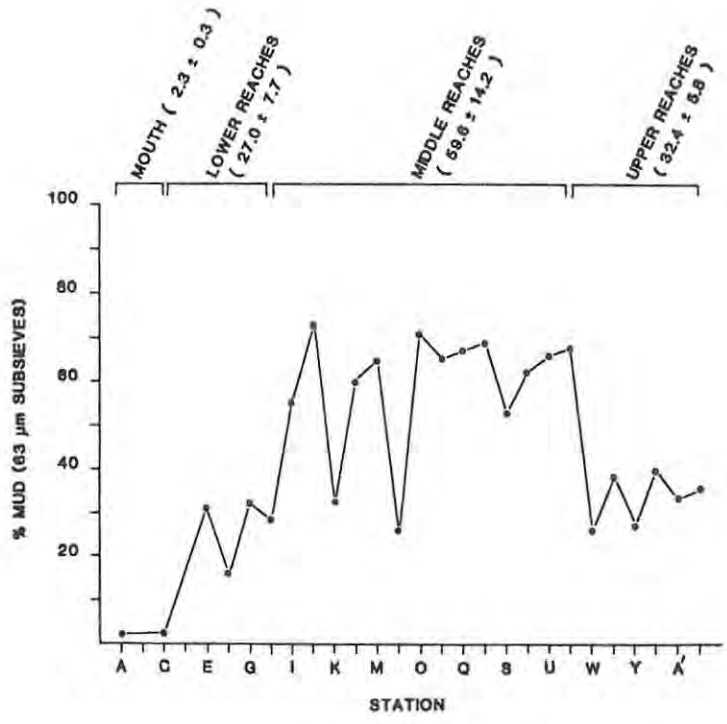


Figure 3.7: Percentage mud (63µm subsieves) in the intertidal sediments along the length of the Kariega estuary. Values in parentheses indicate mean % value ± SD for each region.

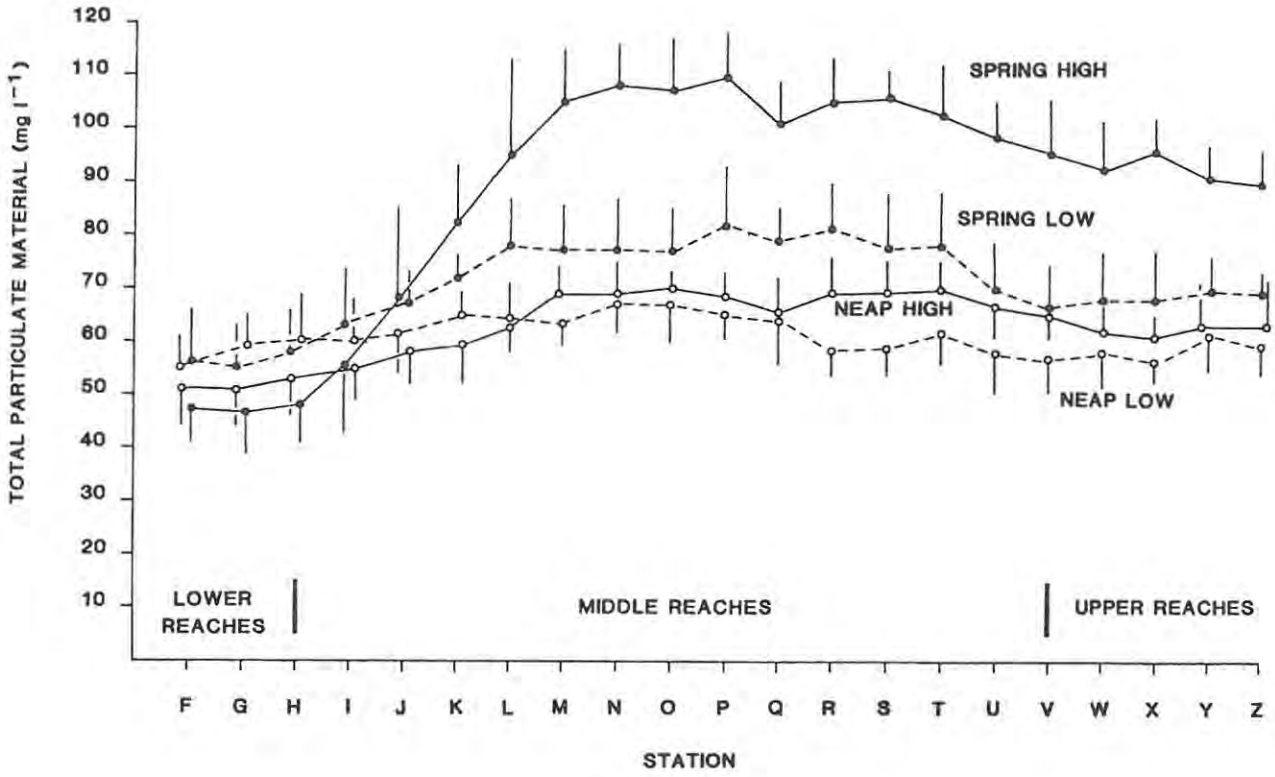


Figure 3.8: Turbidity measurements (as T.P.M.) taken along the length of the Kariega estuary during slack tide. Each data point is a mean + or - SD of 6 tides.

The development of a turbidity maximal zone is demonstrated in Figure 3.8. Turbidity was particularly pronounced during periods of spring flood tides, with T.P.M. values in the range of 80-110mg l^{-1} between stations K and W (organic content, 14-16mg l^{-1}, see Fig. 3.9). Spring ebb, and both neap tides, exhibited lower values in the range 45-80mg l^{-1} and 8-11mg l^{-1} for total and organic particulates respectively. The concentration of suspended particulate material decreased somewhat in the upper reaches, and apparently during slack tide, as levels of particulate material were substantially lower during the following ebb tide. In the lower reaches, the situation was slightly reversed, in that suspended material was

slightly elevated during the ebb tide. There was very little difference in T.P.M. concentration between neap flood and ebb tides. The development of a turbidity maximum zone could again be measured but was not as well-developed as during spring tides. The concentration of suspended material was again slightly higher during the flood tide, in the middle and upper reaches, while slightly elevated loads were recorded in the lower reaches during ebb tides.

DISCUSSION

The distribution of marine and estuarine benthic animals as a direct function of sediment composition has been described by a number of workers (e.g. Beanland, 1940; Rees, 1940; Spooner & Moore, 1940; Holme, 1949; Sanders, 1958; Green, 1968; Bloom, Simon & Hunter, 1972; Boyden & Little, 1973; Newell, 1979). As *S. cylindraceus* appear incapable of burrowing into sandy substrata (pers. obs.) their distribution along the length of the Kariega estuary may be related to sediment nature. A comparison of their distribution to that of sediment percentage subsieves (Fig. 3.9), indicates that the greatest biomass of *S. cylindraceus* coincides with the region of greatest percentage subsieves (approximately stations I-V). McLachlan & Grindley (1974) recorded a similar correlation for *Solen corneus* (=*cylindraceus*), in the Swartkops estuary (approximately 100km west of the Kariega estuary), noting that the animals occurred primarily in the regions where sediments had >20% subsieves, but disappeared where the substrata contained >47.8% subsieves. In the Kariega, the greatest biomass occurred in sediments which had >50% subsieves (stations I-V, Fig. 3.9). This discrepancy may be explained by the possibly firmer intertidal mud banks of the Kariega in which, despite the greater percentage subsieves, the animals can maintain permanently open burrows for their siphons.

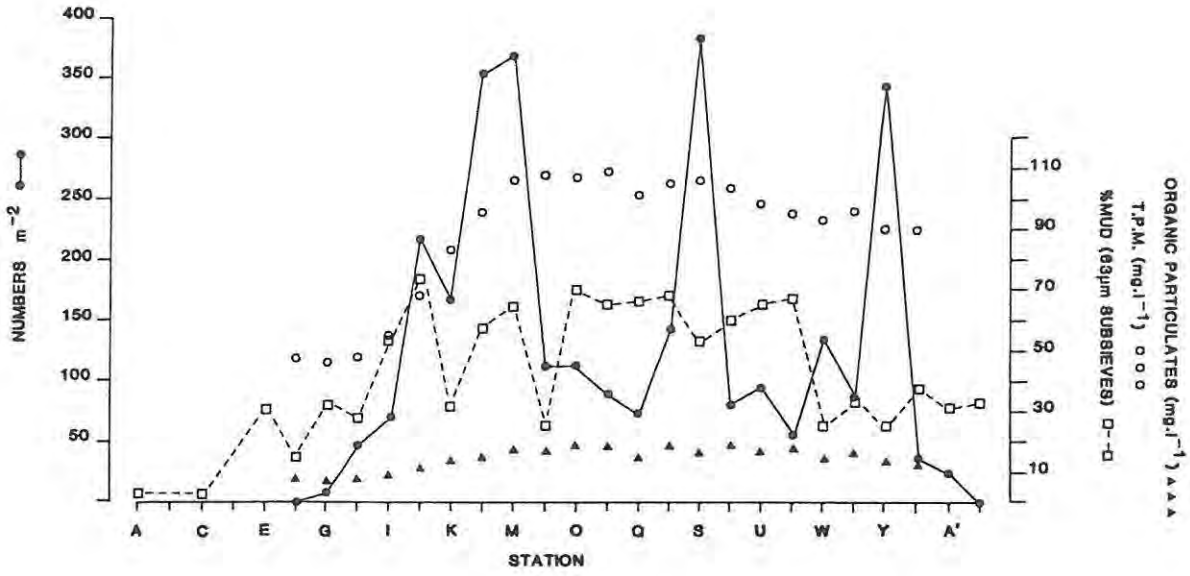


Figure 3.9: Summary diagram comparing the distribution of *Solen cylindraceus* along the length of the Kariega estuary, to the percentage mud of the intertidal substratum, and the concentration of T.P.M. and organic material in the water column.

Despite the suitability (in terms of mud content) of the sediments in the extreme upper reaches of the Kariega (stations A^1 and B^1), the population of *S. cylindraceus* was low. However, during recent sampling (December 1988) a marked increase in the number of *S. cylindraceus* was noted in these head regions. Notwithstanding the estuary being marine dominated and regularly exhibiting hypersaline conditions, populations in the extreme upper regions would bear the brunt of any salinity change. High mortalities have been noted to occur in these regions (Hodgson & de Villiers, pers. obs.), a result of flooding, or even limited freshwater input due to precipitation below the major dams. It is suggested that such localized salinity variations, together with the more "fluid" nature of the mud in these head regions, prevent *S. cylindraceus* from establishing itself for any substantial periods of time. In addition to substratum and salinity, there are undoubtedly other factors which influence the

distribution of *S. cylindraceus*, both directly and indirectly (e.g. temperature, suspensoid concentration etc. (cf. Chapters 5 & 6)). The success of *S. cylindraceus* in the Kariega estuary, may also be linked to food availability. As suspension feeders, the animals would utilize principally the particulate organic material (P.O.M.) in the water column. In the Kariega estuary, the development of a turbidity maximum (a result of tidal shear and wind induced turbulence - Allanson & Read (1987)), is coincident with the distribution of *S. cylindraceus* (Fig. 3.9). Their feeding therefore relies to an extent on water movement. The turbidity maximum values (as T.P.M.; Figs. 3.8 & 3.9) are substantially greater (range: 46.5-110mg l^{-1}) than previously recorded by Allanson & Read (1987), who gave a mean T.P.M. of 23 ± 14mg l^{-1} for the Kariega. It may be that their samples included stations all the way to the mouth, in which region T.P.M. values are generally lower (Taylor, pers. comm.) and may, in part, account for their lower value. Further, they gave no indication as to which tide and at what stage during the tide samples were collected.

The intertidal distribution of *S. cylindraceus* appears closely linked to that of the macrophyte *Zostera capensis* (Figs. 3.4 & 3.5). The potential importance of this association, in terms of the *Zostera* and its associated epiphytic material contributing to the nutritional requirements of the *S. cylindraceus* population are examined in Chapter 7. From Figures 3.4 and 3.10 it is evident that the smaller size groups of *S. cylindraceus* occurred predominantly within or on the river-side fringe of the *Zostera*, while the greater proportion of the population and larger animals occurred either within or immediately inshore of the *Zostera* (Figs. 3.5 & 3.10). Nothing is known of the larval development, spat settlement or adult recruitment of *S. cylindraceus*. The results presented here suggest a possible "migration" of *S. cylindraceus* up the intertidal zone as the animal gets larger. Branch (1975; 1981) described migration in some Patellids, in which small limpets settled lower down on the shore and migrated upward in successive years - a result of increased size and resistance to desiccation and thermal stress.

A similar distribution and migration was recorded for the bivalve *Donax serra* by Ansell & McLachlan (1980), in which adults tended to remain in the intertidal zone while juveniles occurred lower down the shore at the low tide swash zone - a consequence of a lower thermal tolerance. Given the nature of the intertidal muds in the Kariega estuary, it is unlikely that desiccation would be a contributing factor to the observed intertidal distribution of *S. cylindraceus*. The tolerance of smaller *S. cylindraceus* to other physical environmental conditions (temperature and salinity) is unknown. Nevertheless, this observed intertidal distribution may well be a function of burrow depth (cf. Chapter 4; de Villiers & Allanson, 1988). Further, the prolonged periods of submersion and thus available feeding time, and the role of *Zostera* as possible protection against predation by either fish or birds (e.g. Whitfield, 1988; 1989), cannot be excluded in contributing to the success of this species.

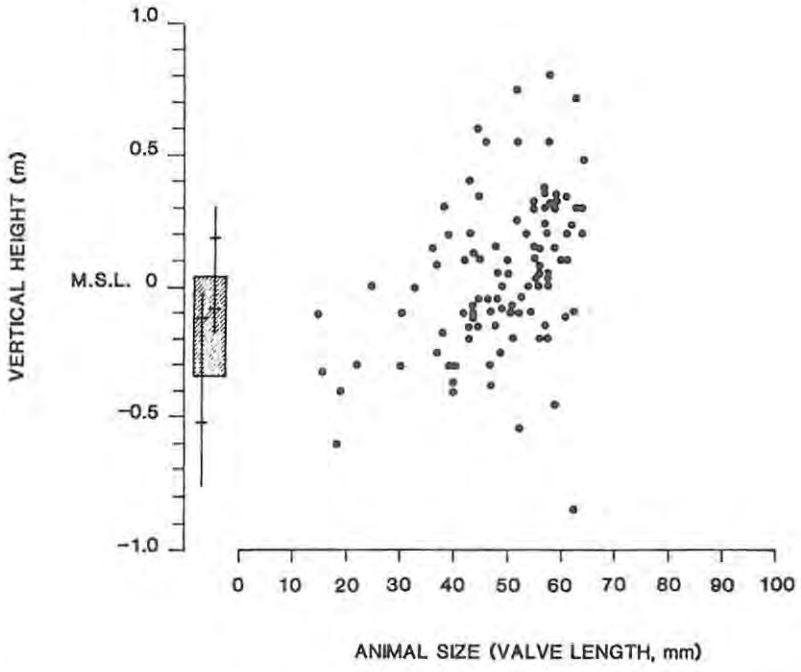


Figure 3.10: Vertical distribution of *Solen cylindraceus* from all stations as a function of size, relative to M.S.L. and *Zostera* distribution. The shaded block represents the vertical distance between the mean heights of the upper and lower fringes of the *Zostera* bed, recorded at all stations.

Hodgson (1987) estimated that 51% of the intertidal invertebrate biomass in the middle reaches of the Kariega were filter feeders, and suggested that such a population must make a considerable impact on the particulate content of the water. However, Hodgson (1987) recorded a maximum biomass of some 70g m^{-2}, while this study, although finding approximately the same number of animals per square metre, records a maximum biomass of 192g m^{-2} in the same region. The potential impact of the *S. cylindraceus* population on the particulate resource in the Kariega, is discussed by de Villiers & Allanson (1988) and in Chapter 6. It is interesting to note that in many estuarine and marine environments, in which the sediments contain a high percentage subsieves, that deposit feeders predominate over filter feeders (e.g. Sanders, 1958; Rhoads & Young, 1970; Boyden & Little, 1973; Branch & Grindley, 1979; Newell, 1979; Kennish, 1986). Rhoads & Young (1970) observed that deposit feeders, by their re-working of the sediments during feeding, reduced the stability of the habitat, resulting in a more "liquid-mud" surface, which may result in the amensalistic (after Rhoads & Young, 1970) exclusion of suspension feeders. In the Kariega estuary, very few deposit feeders occur in any abundance. The most abundant is the bivalve *Macoma litoralis*, which is common intertidally in the lower reaches of the estuary, where the *S. cylindraceus* populations are low (Hodgson, 1987). In the upper and middle reaches however, in the presence of high *S. cylindraceus* populations, it is predominantly sub-tidal. The anomuran prawn, *Upogebia africana*, is another suspensoid feeder (Hill, 1967) and shares many of the intertidal mud banks with *S. cylindraceus* (Hodgson, 1987). Their distribution, although overlapping does not coincide, and *U. africana* inhabits predominantly the lower to lower-middle reaches of the estuary (Hodgson, 1987). McLachlan & Grindley (1974) recorded a similar separation between populations of *U. africana* and *S. cylindraceus*, and suggested that this might be a substratum effect, and indicated that the number of *U. africana* decreased rapidly in substrata containing more than 20% subsieves - separation on this basis is not evident in the Kariega. Further, McLachlan & Grindley (1974) suggested, based on the natural distribution evidence from the

Swartkops estuary, that competition from *U. africana* was probably an important factor limiting the distribution of bivalves. However, nothing is known of the food selectivity spectrum of this species. This study has not considered organism-sediment interactions *per se*, nor the potential amensalistic effect of one trophic type on another (mediated by re-working of the sediment, as proposed by Rhoads & Young (1970) and Bloom *et al.* (1972)). However, such biotic interactions as potentially important factors affecting the density and distribution of *S. cylindraceus* in the Kariega estuary, are not precluded.

In a habitat such as the Kariega estuary, which by impoundment of the catchment has been converted from a normal to a marine dominated and often hypersaline system, the previously existing euryhaline freshwater and many of the true estuarine faunal components (Day, 1981) would have been depleted. It is suggested that as the salinity gradually increased and stabilized, so those "depleted" areas were colonized by the first suitable substratum species to settle - *Solen cylindraceus*. Exhibiting a fairly rapid growth rate and generally large spat falls (Hodgson, pers. comm.), *S. cylindraceus* has remained the dominant filter feeder in these regions. The mud banks of the Kariega are fairly well-consolidated, stable, and no active transport of sediments occur except during severe flood conditions. Such stability is a result not only of a diminished river flow, and the subsequent hydrodynamics of the estuary, but also the growth of substantial intertidal macrophyte beds (*Zostera, Spartina* and *Sarcocornia*). With minimal re-working of the surface sediments by deposit feeders, such firm mud, with its specific dilatency and thixotrophic properties, forms a "classical" habitat for infaunal suspension feeders (e.g. Rees, 1940; Holme, 1949; Bloom *et al.*, 1972; Eagle, 1975; Newell, 1979).

Solen cylindraceus, as an intertidal, infaunal estuarine animal, can avoid external environmental stresses e.g. salinity (see also de Villiers & Allanson, 1989 and Chapter 4), desiccation and temperature extremes, but is controlled by the complex interactions between

the physico-chemical and biological properties of the sediments in which it lives. In the Kariega, where salinities are usually close to that of the sea and stable for long periods, substratum and food availability are probably the two most important factors influencing the distribution of *S. cylindraceus*.

CHAPTER FOUR

TEMPERATURE AND SALINITY TOLERANCE

INTRODUCTION

Temperature and salinity are two important environmental factors which influence the distribution of estuarine and marine fauna (e.g. Day, 1951; Brown, 1953; Smith, 1955; Matthiessen, 1960; Kinne, 1963; 1964; 1971; Sanders, Mangelsdorf & Hampson, 1965; Vernberg & Vernberg, 1972; McLachlan & Erasmus, 1974; McLachlan & Grindley, 1974; Andronikov, 1975; Norse & Estevez, 1977; Newell, 1979; Vernberg, 1981). The lethal limits possessed by a species are rarely a restricting factor within the normal distribution and habitat of that species, and data of this nature are of limited ecological significance (Read & Cumming, 1967). Nevertheless, tolerance extremes remain an important indicator of: 1) the physiological range within which a species may function; 2) definition of a species' distribution limits and, 3) assessing possible effects of variation in physical environmental factors, acting either singly or in concert (e.g. Broekhuysen, 1940; Evans, 1948; Day, 1951; Kinne, 1963; 1964; 1970; Kennedy & Mihursky, 1971; Wolcott, 1973; McLachlan & Erasmus, 1974; Ansell & McLachlan, 1980; Ansell, Barnett, Bodoy & Massé, 1981; Vernberg, 1981).

A) Temperature: The influence of temperature on marine and estuarine organisms has been extensively reviewed by a number of workers, e.g. Kinne, 1963; 1970; Vernberg & Vernberg, 1972; Precht, Christophersen, Hensel & Larcher, 1973; Somero & Hochachka, 1976; Newell, 1979;

Newell & Branch, 1980 and Prosser, 1986. Examination of the thermal limits has suggested that temperature tolerance may exhibit a general correspondence with local environmental conditions. Indeed, the influence of temperature in defining distribution and abundance has been studied for some time (e.g. Broekhuysen, 1940; Evans, 1948; Read & Cumming, 1967; Kennedy & Mihursky, 1971; McLachlan & Erasmus, 1974; Newell, 1976; 1979; Seed, 1976; Ansell & McLachlan, 1980; Ansell, Barnett, Bodoy & Massé, 1980a; 1980b; 1981).

However, the direct lethal effects are not the only manner in which temperature can influence survival and distribution. Sub-lethal effects, which reduce an animal's performance or activity, can prove indirectly lethal, e.g. the effects of moderate temperature changes on growth, feeding, respiration, reproduction and other physiological and behavioural functions may combine to render a population non-viable (e.g. Ali, 1970; Walne, 1972; Ansell & Sivadas, 1973; Bayne, 1973; McLusky, 1973; Feng & Van Winkle, 1975; Bayne, Thompson & Widdows, 1976; Newell & Kofoed, 1977; McLachlan & Young, 1982; Griffiths & Griffiths, 1987; de Villiers, Allanson & Hodgson, 1989). The upper and lower tolerance limits have been examined for *Solen corneus* (=*cylindraceus*), under *in vitro* conditions by McLachlan & Erasmus (1974), and to date comprises virtually the only published knowledge on the thermal tolerances of this species.

B) Salinity: Studies which have examined the effect of salinity on molluscs and their ability to tolerate salinity changes in their natural environment, have demonstrated a correlation between this tolerance and distribution (e.g. Broekhuysen, 1940; Kinne, 1964; 1971; Robertson, 1964; Wilson, 1968; Pierce, 1970; Rename & Schlieper, 1971; Schoffienels & Gilles, 1972; Castagna & Chanley, 1973; Shumway, 1977; Davenport & Fletcher, 1978; Davenport, 1979). Their results indicated that most marine and estuarine bivalves are osmoconformers, only capable of exerting control over the osmolarity of their internal fluids by, principally, behavioural means, such as valve closure. More recently, Costa & Pritchard (1978) and Gainey

(1978; 1987) have indicated the existence of physiological mechanisms, which together with the behavioural responses, would determine the extent of osmotic tolerance and hysteresis.

From available distribution data, *Solen cylindraceus* appears tolerant of a reasonably wide salinity variation. Millard & Broekhuysen (1970) recorded *S. corneus* (=*cylindraceus*) as common in St Lucia (Fig. 3.1), under salinities of 7-19‰. Boltt (1975), also at St Lucia, noted the absence of *S. cylindraceus* in the North Lake and reduced populations in the South Lake, following a 2-3 year period of elevated salinities of 55-60‰ and 45-58‰, respectively. Although the effectiveness ot his use of a Van Veen grab to sample bethos such as *Solen* is questionable, he nevertheless demonstrated a rapid re-colonization by the animal, in both lakes, following a decrease in salinity to approximately 20-40‰. Blaber, Kure, Jackson & Cyrus (1983), recorded high densities of *S. cylindraceus* in St Lucia following a period of reasonably stable salinity (31-45‰). Day (1974) recorded *S. corneus* (=*cylindraceus*) as abundant in regions of the Morrumbene estuary (the northernmost recorded limit of its distribution, Fig. 3.1) in salinities of 11.1-34.4‰, while McLachlan & Erasmus (1974) noted high densities in the Swartkops estuary (close to the animal's southernmost limit) subject to a "normal salinity range" of 15-37‰. McLachlan & Erasmus (1974) have demonstrated that *S. corneus* (=*cylindraceus*) is a euryhaline osmoconformer and that, under *in vitro* conditions, equilibration of their body fluids to the external medium occurred within 24 hours.

As an estuarine species, *S. cylindraceus* encounters periodic fluctuations in both temperature and salinity, the extent of which depends upon the severity of coastal upwelling and episodic flooding by freshwater (cf. Chapter 3). The success of *S. cylindraceus* in the Kariega estuary, despite such events, suggests an ability by the animal to either accommodate, tolerate and/or escape such conditions. This investigation aims to examine the thermal tolerance under *in situ* conditions, and to determine the effect temperature may have on the burrowing ability of

S. cylindraceus. Further, to determine the salinity tolerance and osmotic properties of *S. cylindraceus* under both *in vitro* and *in situ* conditions. The effect of temperature and specific temperature/salinity combinations on the filtration rate of *S. cylindraceus* is examined in Chapter 6.

MATERIALS AND METHODS

A. Tolerance to abrupt temperature changes

Solen cylindraceus of 50-60mm shell length were collected during April 1987, and transferred to mud-filled pots (10-13 animals per pot) at the site of collection. Pots were left in the intertidal zone of the estuary overnight to allow the animals to establish themselves under as near normal conditions as possible. The pots were transferred the following day to laboratory aquaria through which a supply of estuarine water circulated. Temperature and salinity were set to match that measured in the field on the day of collection (field temp: 19°C, laboratory temp: 20°C ± 1°C and salinity of 36‰ ± 2‰). Animals were allowed a 20 day period to acclimate to laboratory conditions of 12:12 h light:dark regime, and a six hourly tidal exposure. Animals were fed a suspension of the green algae *Tetraselmis suecica* every three days, and aquarium water was changed every 7-10 days. The laboratory aquarium arrangement is illustrated in Figure 4.1. The method of determining thermal tolerance was a modification of that used by Ansell & McLachlan (1980) and Ansell *et al.* (1980a; 1980b; 1981).

After acclimation, pots were randomly divided into 9 groups, 8 pots per group. Each group was then transferred to a separate tank, in which the water had been set to one of the following temperatures: 5, 10, 15, 25, 30, 35, 40 or 45°C. One group was maintained at the acclimation temperature (20°C) and served as the control. One pot (10-13 animals) was removed from each of the different temperatures at the following time intervals: 3, 6, 12, 24, 48, 72 and 96 hours, and the number of dead animals counted.

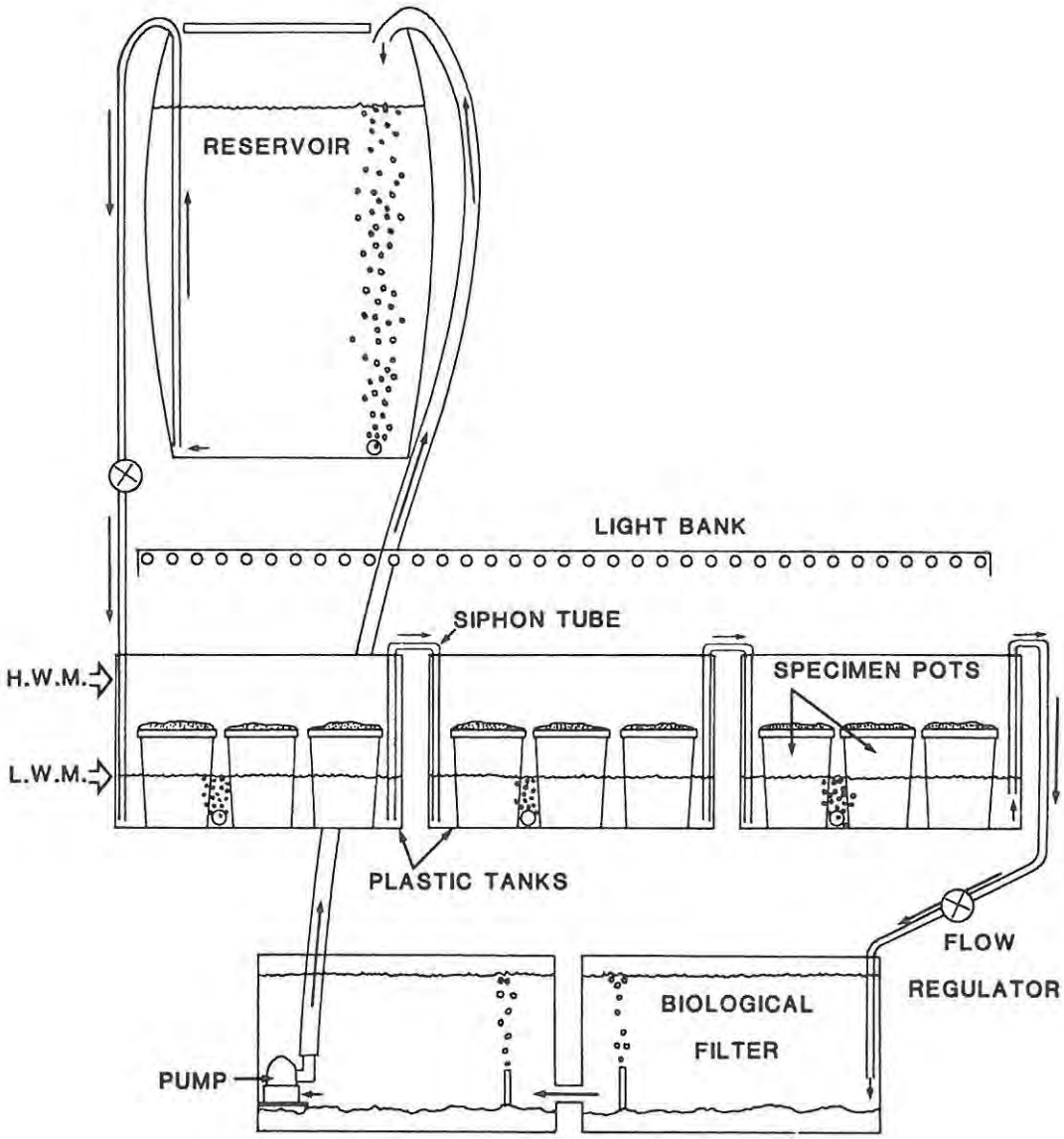


Figure 4.1: Aquarium system used to house *Solen cylindraceus* in the laboratory. Arrows indicate direction of water circulation.

The criteria for death were gaping valves and lack of response on stimulation of the foot, siphons and mantle edge. Pots were then returned to the aquaria and the survivors' ability to burrow back into the mud was determined. Actual burrowing time was not recorded, but simply the ability to re-establish themselves within 1 hour after having been removed. Those animals incapable of burrowing within this time period were removed from the aquaria. No set of pots was counted twice. After the survival and burrowing ability of the animals at both extreme temperatures (5 and 45°C) had been determined, individuals which had not re-established themselves were then transferred to a mud-filled pot held at the control temperature (20°C) and their recovery, in terms of burrowing, assessed. A total of 848 individuals were tested in this investigation.

B. Salinity tolerance

i) Determination of tolerance limits

Animals of 50-60mm valve length were collected as described earlier, and allowed a 20 day acclimation time to laboratory conditions of 35‰, 25°C, 6 hourly tidal exposure and a 14:10 h light:dark regime. After the acclimation period, animals were removed from the pots, rinsed in estuarine water (35‰, 25°C) and the valves tied together with cotton thread to prevent gaping. Ninety six animals were transferred to each of 8 trays of 5 l capacity, containing filtered, aerated water (25°C) of the following salinities: 0, 5, 15, 25, 35, 45, 55 and 65‰. The 35‰ salinity tray constituted the control. From each tray, 12 animals were removed after the following exposure times: 3, 6, 12, 24, 36, 48, 60 and 72 hours, the number of survivors and their response determined, after which they were transferred to a container with water of 35‰ salinity at the same temperature. The number of animals surviving and their response after 4 hours at 35‰ were recorded. Survival was assessed by the response of the animal to stimulation of the foot, mantle edge and siphon.

ii) Osmotic properties under *in vitro* and *in situ* conditions

Solen cylindraceus of 50-60mm valve length, were collected as described earlier, during June/July 1987. Laboratory aquaria were set to field conditions on the day of collection ($19^{o}C \pm 1^{o}C$ and 35‰ ± 2‰). Animals were subjected to the same light:dark and tidal regime and acclimation as previously described. Two experiments were performed, the first a modification of that of McLachlan and Erasmus (1974). Animals were removed from mud pots, thoroughly rinsed and the valves were bound together with cotton thread to prevent gaping. A total of 120 animals were transferred directly from aquaria (salinity 35‰) to each of four trays of 5 l capacity, containing aerated estuarine water at the following salinities: 5‰, 15‰, 25‰ and 45‰. Four animals were removed from each of the experimental trays after the following times:

1. Every 15 min. for the first 2 h
2. Every 30 min. for the next 4 h
3. Every 60 min. for the next 6 h
4. Every 3 h for the next 12 h
5. After 36 h
6. After 48 h

Haemocoelic fluid was extracted from the pedal sinus by hypodermic syringe. Usually 0.5-0.75ml of fluid was obtained in this manner, sufficient for two osmolarity measurements per animal. Osmolarity was determined on volumes of 0.2ml using an Advanced Instruments Inc. Model 3 D digimatic osmometer.

In the second experiment, animals were retained in their burrows and the pots transferred to tanks in which the water had been adjusted to the same experimental salinities as above. Salinities in the experimental tanks were monitored daily, using a Reichert refractometer, and kept close to the desired level (± 1‰) by the addition of distilled water as required. Six animals were removed from the pots at each salinity and the osmolarity of the haemocoelic fluid determined as previously described. Again two readings per animal were possible. Animals were removed every 6 hours for the first 24 hours and every 12 hours thereafter for a total period of 204 hours. In both experiments, the condition of the animals was determined by their response to handling and their reaction to mechanical stimulation of the foot and siphon.

RESULTS

A. Tolerance to abrupt temperature changes

The results are presented in Figure 4.2 A-C. Survival and burrowing ability in the temperature range 15-35^{o}C were virtually identical to the control, and only the control temperature values (20^{o}C) are shown in Figure 4.2 A and B, to avoid cluttering. The full complement of results is tabulated in Appendix II A. Under *in situ* conditions, *S. cylindraceus* exhibited a high survival (range of 91.6-100% after 96 hours) when subjected to temperatures between 5-35^{o}C (Fig. 4.2 A). A slight decrease in the number of survivors was noted at 40^{o}C (72.2-80% after 72-96 hours), while at temperatures of 45^{o}C, mortality increased markedly after 12 hours (Fig. 4.2 A). The time taken for 50% of the animals to die at 45^{o}C was between 36-48 hours, with only a 25 and 27.2% survival recorded after 72 and 96 hours respectively.

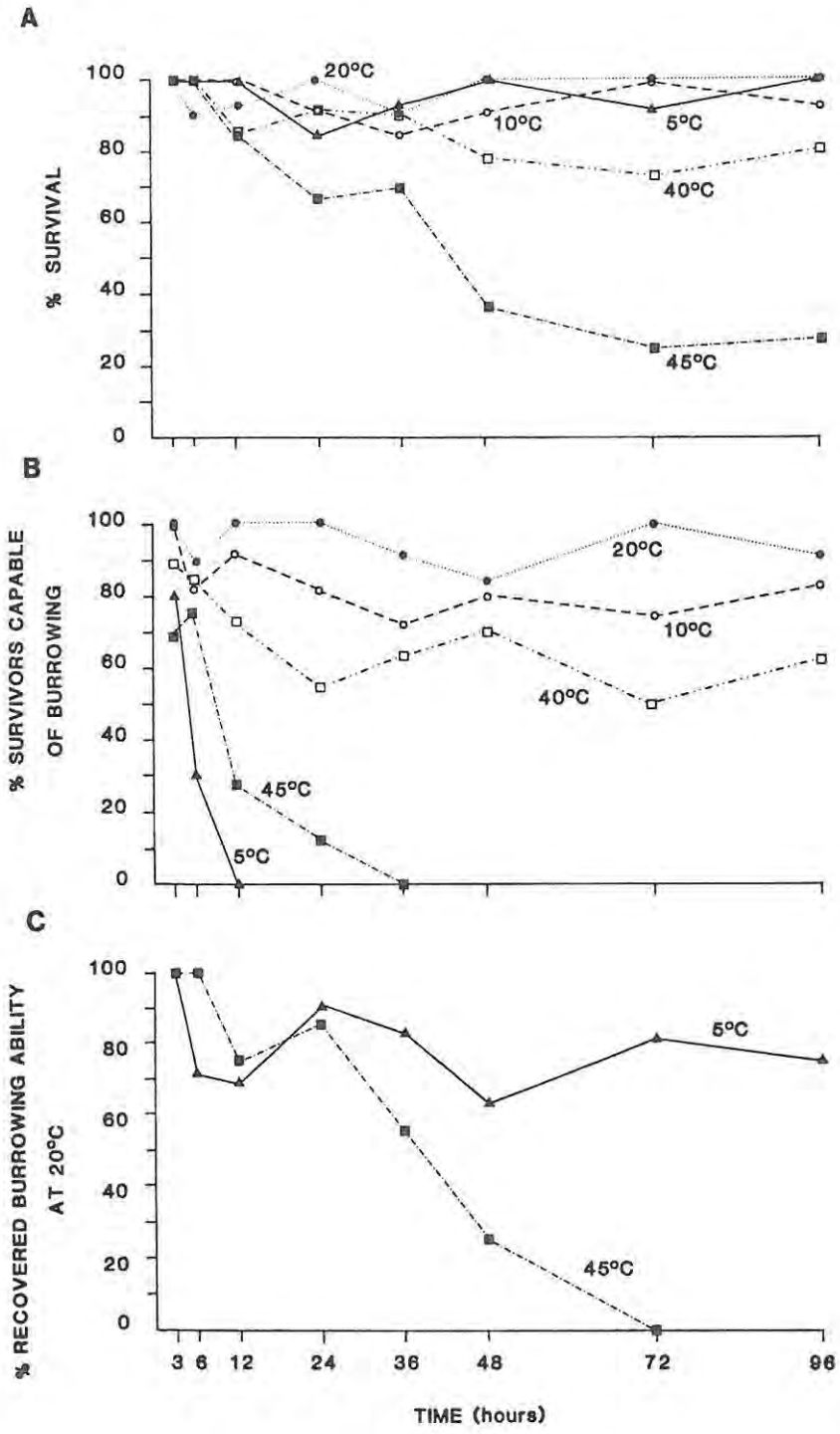


Figure 4.2: Thermal tolerance and burrowing responses of *Solen cylindraceus* under *in situ* conditions. A. Percent survivors at different exposure temperatures (see Appendix II A for full data set). B. Burrowing capability of survivors at different exposure temperatures. C. Recovery of burrowing ability in animals incapable of burrowing at 5 and 45°C, following transfer to 20°C.

Burrowing responses in the 15-35°C temperature range were similar (91.5-94.6% success). A slight decrease in burrowing success was observed at 10 and 40°C, with averaged successes of 78.4 ± 4.7% and 60.1 ± 7.9% respectively after 24 hours (Fig. 4.2 B). Initial, high burrowing success at both 5 and 45°C dropped sharply (Fig. 4.2 B), and no burrowing ability was recorded after 12 and 36 hours respectively.

Figure 4.2 C demonstrates the recovery success of animals initially incapable of burrowing at exposure temperatures of 5 and 45°C, and subsequently returned to 20°C. These data indicate a high recovery (63.6-100%) for animals which were initially exposed to 5°C. Animals initially exposed to 45°C demonstrated a decreased ability to recover normal burrowing activity, following prolonged exposure to such high temperature (100% after 6 hours to 0% after 72 hours).

B. Salinity tolerance

i) Determination of tolerance limits

Results of survival at different salinities are given in Table 4.1. At salinities of 15-65‰, virtually 100% survival was recorded at all stages. Animals exposed to 15‰ appeared to exhibit a sluggish response after 3 and 6 hours only. However, these animals showed signs of complete recovery when transferred back to a salinity of 35‰. On exposure to 5‰ salinity, surviving animals demonstrated an anaesthetized response after 3, 6, 12, 24 and 36 hours. However, on transfer to salinity of 35‰, complete recovery was recorded in all instances, except for the 36 hour group, which, even after the 4 hour period at 35‰ still appeared anaesthetized. Animals, after 3 hours exposure to freshwater, demonstrated virtually no response, and although no deaths were recorded in this period, the animals had not fully recovered after the 4 hour period at 35‰. No response at all was recorded after 6 hours exposure to freshwater and animals were classed as dead.

Table 4.1: Percent survival of *Solen cylindraceus* on re-exposure to 35‰ salinity, following exposure to different salinities for different lengths of time (n = 12 for each time interval).

Exposure time (hours)	Exposure salinity (‰)							
	0	5	15	25	35	45	55	65
3	100	100	100	100	100	100	100	100
6	0	75	100	100	83.3	100	83.3	100
12	0	41.6	100	91.6	100	100	100	100
24	0	16.6	100	100	100	100	100	100
36	0	8.3	83.3	100	100	100	83.3	91.6
48	0	0	100	91.6	100	100	100	100
60	0	0	83.3	100	100	100	100	100
72	0	0	91.6	100	100	100	100	100

These data indicate that *S. cylindraceus* is capable of tolerating salinities in the range 15-65‰, but survives for only short periods at lower salinities. An LT_{50} of approximately 12 hours at 5‰ and between 3-6 hours at 0‰ was recorded, provided that the animals were subjected to an increased salinity within their optimal range following these exposure periods.

ii) Osmotic properties under *in vitro* and *in situ* conditions

The *in vitro* experiment results are given in Figures 4.3 A and 4.4 A. To avoid cluttering, error bars are not shown on the figures, and the full complement of results together with the standard deviations are tabulated in Appendix II B. These data show that the haemocoelic osmolarity of animals removed from their burrows, rapidly conforms to that of the external

medium. The time taken for equilibration depended upon the magnitude of salinity change. Equilibration to 45‰ salinity occurred within 2-2.5 hours, and only 1-2 hours at 25‰. Osmotic equilibration to 15‰ occurred within 4-5 hours, while haemocoelic concentrations of animals exposed to 5‰ stabilized at 195mOsm only after 8-12 hours (Figs. 4.3 A & 4.4 A). Animals held at 5‰ showed an anaesthetized response to handling and stimulation after 2-4 hours. However, after 10-12 hours, no response was observed, even upon insertion of the hypodermic needle, and although the experiment was continued, these animals were considered dead. Animals exposed to 25‰ and 45‰ salinity showed normal responses, while those at 15‰ demonstrated a slightly anaesthetized response after 4-5 hours, which did not persist for the duration of the experiment. No deaths were recorded at the three higher salinities (i.e. 15, 25 and 45‰). It was noted, that transfer of animals from 35‰ to lower salinities, in particular, initiated substantial probing by the foot, accompanied by frequent valve adduction - an activity which would result in substantial irrigation of the mantle cavity.

Results from experiments in which animals were allowed to remain in their burrows and exposed to different salinities are shown in Figures 4.3 B and 4.4 B. The full data set together with the standard deviations is tabulated in Appendix II C. When compared to animals out of their burrows, a marked delay in attaining osmoconformity was observed at all salinity concentrations tested. In animals exposed to 45‰, osmoconformity occurred after 132 hours. For animals exposed to 5, 15 and 25‰, despite substantial decreases in haemocoelic concentrations, osmotic equilibration had not occurred by the end of the experiment (204 hours). Animals exposed to 5‰ exhibited an anaesthetized response only after 144-156 hours, which persisted for the remainder of the experiment. No deaths were recorded at any of the salinities during this experiment. It was noted that animals recovered from pots exposed to 5‰, had all retreated to the base of their burrows, in which position they appeared to remain for the duration of the experiment.

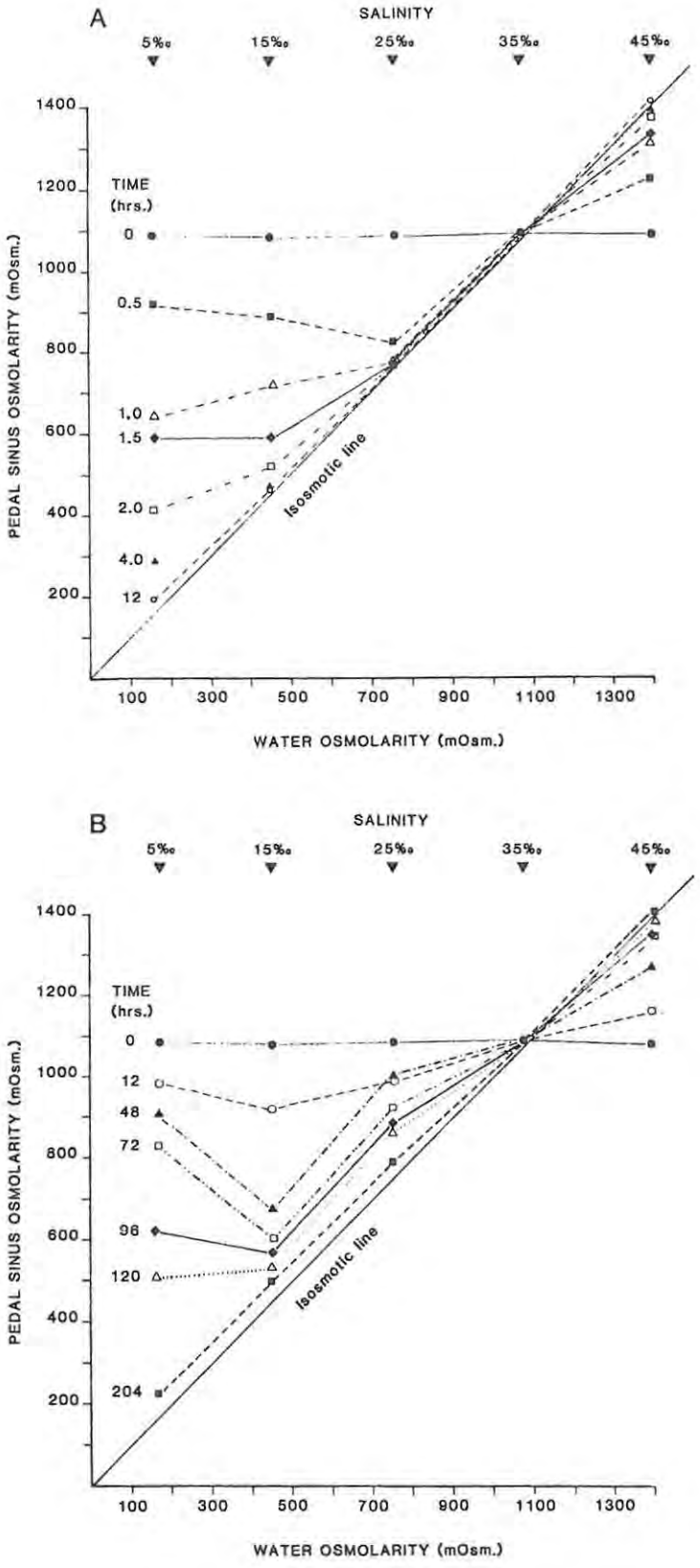


Figure 4.3: Variation in pedal sinus osmolarity of animals acclimated to 35‰ salinity (1074mOsm) and exposed to different salinity concentrations at given time periods, plotted against external water osmolarity. **A.** Animals removed from burrows. **B.** Animals retained in burrows.

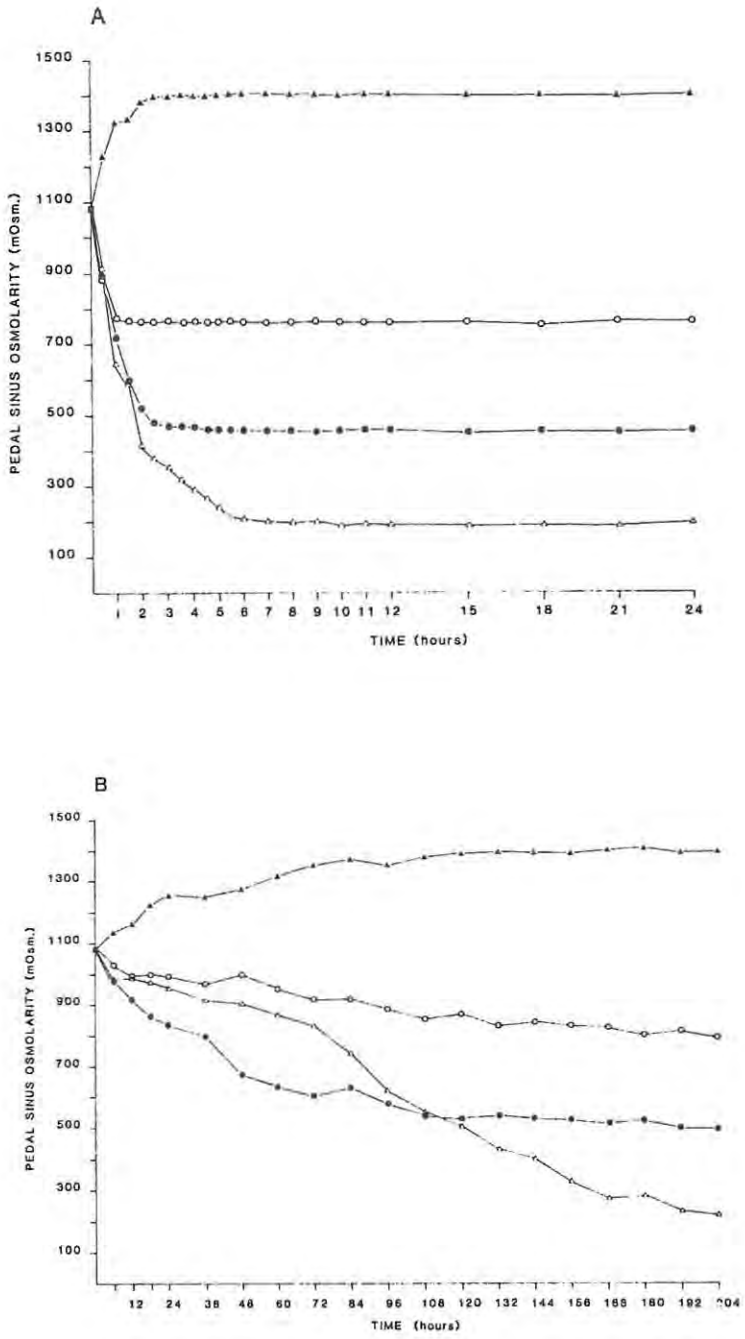


Figure 4.4: Variation in pedal sinus osmolarity with time in animals acclimated to 35‰(1074mOsm) and exposed to: 45‰ (1394mOsm) ▲—▲ ; 25‰ (754mOsm) ○—○ ; 15‰ (451mOsm) ●—● ; 5‰ (167mOsm) △—△ . A. Animals removed from burrows. B. Animals retained in burrows.

DISCUSSION

A. Temperature

The results presented in Figure 4.2 A indicate a temperature tolerance range of 5-45^{o}C for *S. cylindraceus*. In comparing tolerance ranges within species and between populations, it is essential that the assessments are made in a consistent manner (Ansell *et al.*, 1980a). The values found here can, strictly, not be directly compared to the lethal temperature determined for *S. corneus* (=*cylindraceus*), by McLachlan & Erasmus (1974) who used a different experimental procedure. Nevertheless, the upper lethal temperatures recorded are similar, 44.5^{o}C vs 45^{o}C. If Stirling's (1982) indications that the temperature at which an animal loses its ability to burrow into the sediment closely parallels the coma point, is accepted, then exposure to 5^{o}C is virtually the lowest limit *S. cylindraceus* is capable of surviving.

The difference between the mean environmental temperature and the thermal tolerance limit of an animal, is a measure of the limit to which environmental temperature may vary, without adversely affecting the short-term survival of a population (Ansell *et al.*, 1980b). However, these limits may vary, dependent upon the animal's acclimatory history. Seasonal acclimation may then alter the LT_{50} values slightly (values of ca. 0.2^{o}C per ^{o}C difference in acclimation temperature was suggested by Ansell & McLachlan (1980) for *Donax*), within genetically determined limits (Bayne *et al.*, 1976; Newell & Branch, 1980). A number of workers have described a variation in thermal tolerance within and between populations exposed to different thermal regimes (e.g. Henderson, 1929; Broekhuysen, 1940; Evans, 1948; Rao, 1953; Southward, 1958; Hardin, 1968; Vernberg & Vernberg, 1970; 1972; Wolcott, 1973; Crisp, Davenport & Gabbott, 1977; Wilson, 1978; Newell, 1979; Ansell *et al.*, 1980a; 1980b; 1981; Vernberg, 1981; Stirling, 1982). These investigations describe lethal temperature as a function of habitat, in

which adaptation to different latitudinal and microgeographical regimes has taken place. Despite possible acclimatory responses, a water temperature of 45°C is substantially greater than any to which *S. cylindraceus* would be exposed in the natural environment, and it seems unlikely, even in warmer tropical regions, that a higher lethal temperature would be recorded.

In any intertidal zone, diel and tidal periodicity exert a strong measure of control over sediment temperature fluctuations (Johnson, 1965; Harrison, 1985). Numerous studies have demonstrated a marked decrease in sediment temperature fluctuations with depth (e.g. Bruce, 1928; Johnson, 1965; Jansson, 1967; Harris, 1972; Wieser, Schiemer & Gnaiger, 1974; Harrison, 1985). Species living within the top few centimetres of the substratum may thus be exposed to a highly variable thermal regime, while those with a deeper distribution are subjected to more stable temperatures. Interstitial fauna capable of vertical migration within the sediment have an advantage in that they may retreat from the surface as the tide ebbs, so minimizing thermal stress (Newell, 1979). Burrows of adult *S. cylindraceus* are some 200-400mm deep (pers. obs.), and it is suggested that their burrowing ability, together with the thermal stability of the deeper sediments, is the principal mechanism whereby they may reduce thermal stress. Such "vertical migration", although only speculative for *S. cylindraceus*, has nevertheless been recorded in other infaunal groups, e.g. polychaetes (Westheide, 1972), turbellarians (Boaden & Platt, 1971) and sandy shore meiofauna (McLachlan, Erasmus & Furstenberg, 1977). A similar response has been described for *Cardium edule* by Gimazone (1972) in which it is suggested that burrowing in this species is stimulated by increasing temperatures as the tide ebbs, in an attempt to avoid exposure during low tide.

Solen cylindraceus are capable of surviving conditions as low as 5°C, although they do exhibit a decreased burrowing efficiency (Fig. 4.2 B) which is recovered on return to higher temperatures (20°C, Fig. 4.2 C). A similar impairment in burrowing ability on exposure to

reduced temperature has been recorded for *C. edule* (Gimazone, 1972) and for two *Bullia* and *Donax* species (McLachlan & Young, 1982), while Pfitzenmeyer & Drobeck (1967) found a decreased burrowing ability in *Mya arenaria* under conditions of elevated temperature. However, virtually no burrowing impairment was recorded for *S. cylindraceus* on exposure to 10°C (Fig. 4.2 B). Under conditions of coastal upwelling, in which estuarine water temperatures may fall to 8-10°C, it would appear that despite a possible reduction in burrowing efficiency, the population of *S. cylindraceus* would be largely unaffected, provided such conditions do not persist for prolonged periods.

The direct lethal effects of extreme temperatures are not the only manner in which temperature may influence survival and distribution, sub-lethal effects which reduce an animal's performance of any crucial activity may prove indirectly lethal (Kinne, 1963; 1971; Read & Cumming, 1967; Newell, 1979; Ansell & McLachlan, 1980; McLachlan & Young, 1982; Stirling, 1982). The effect of temperature on the burrowing efficiency, feeding, respiration and reproductive potential are all directly implicated in determining the survival and ultimate success of *S. cylindraceus*. The effect of temperature on the filtration rate of *S. cylindraceus* is examined by de Villiers, Allanson & Hodgson (1989); de Villiers, Hodgson & Allanson (1989, in press) and in Chapter 6.

B. Salinity

From previously recorded observations, *S. cylindraceus* is reported as common in salinities ranging from 11,8‰-45‰ (Brown, 1953; Millard & Broekhuysen, 1970; Day, 1974; McLachlan & Erasmus, 1974; Boltt, 1975; Blaber *et al.*, 1983). Results indicated a non-lethal salinity range for *S. cylindraceus* of 15-65‰, compared to the 13-42‰ range determined by McLachlan & Erasmus (1974) for animals from the Swartkops estuary. However, survival at 15‰ was virtually 100%, while exposure to 5‰ resulted in a 50% mortality after approximately 12 hours.

It is suggested that the critical lower tolerance would lie between 5 and 15‰ salinity. Day's (1974) observation of *S. corneus* (=*cylindraceus*) occurring in fairly high numbers at salinities of 11.8‰ in the Morrumbene estuary, must come close to the extreme lower salinity tolerance limit. However, in the Morrumbene estuary there is a marked difference in high and low tide salinity (11.8-33.8‰), as a result, the animals would only be subjected to possible osmotic shock for a brief period during the ebb and flood tides. For most of the flood tide period, the population would be exposed to virtually full strength sea water. The upper tolerance value of 42‰ recorded by McLachlan & Erasmus (1974) is substantially lower than that recorded in the present study (65‰), and may be a result of the *in vitro* technique used by those workers.

Results indicate that under *in vitro* conditions, haemocoelic osmolarity rapidly conformed to that of the external medium (< 6 hours, Fig. 4.4 A). However, osmolarity of the haemolymph was held slightly above that of the external medium over the salinity range tested (Fig. 4.3 A). These findings confirm those of McLachlan & Erasmus (1974) on the same species. Similar weak "control" has been recorded by Pierce (1970) for four species of *Modiolus* and for *Ensis directus* over their non-lethal salinity range, and by Freeman & Rigler (1957) for *Scrobicularia plana*. In experiments in which animals were allowed to remain in their burrows (Figs. 4.3 B & 4.4 B), and were exposed to altered salinity, a marked delay in attaining osmoconformity was observed at all salinities tested.

Many aquatic molluscs have the capability of withstanding large changes in salinity for reasonably long periods of time, by employing a variety of behavioural responses such as valve closure, which is reported for a number of bivalve species e.g. *Glycymeris glycymeris* (Gilles, 1972), *Mytilus edulis* and *S. plana* (Freeman & Rigler, 1957; Hoyaux, Gilles & Jeuniaux, 1976; Shumway, 1977), *Dosina hepatica* (McLachlan & Erasmus, 1974), *Crassostrea gigas* (Shumway, 1977) and *Anadara senilis* (Djangmah, Shumway & Davenport, 1979). The use of the operculum

to tightly seal the shell aperture has been recorded in some gastropod species e.g. *Littorina saxatilis* (Avens & Sleigh, 1965), *Littorina littorea* and *Purpura lappilus* (Hoyaux *et al.*, 1976). Gastropods such as *Siphonaria pectinata* (McAlister & Fisher, 1968) and *Patella vulgata* (Hoyaux *et al.*, 1976) achieve much the same protection by clamping down and adhering firmly to the rock surface. In those bivalve species incapable of complete valve closure e.g. *Mya arenaria*, it is suggested (Shumway, 1977) that the presence of jointed mantle edges and well-developed siphonal sphincter muscles are sufficient to isolate the animal from the external medium, and is comparable to the mechanism of valve closure. *Solen cylindraceus*, like *M. arenaria*, is incapable of complete valve closure and possesses a fused mantle edge and well-developed siphonal musculature (Hodgson, 1984). However, the results presented here (Fig. 4.4 B) suggest, that unlike *M. arenaria* (Shumway, 1977), *S. cylindraceus* effectively utilizes its burrowing ability to avoid short-term effects of any substantial variation in the osmotic concentration of the external medium. When exposed to lower salinities (5 and 10‰), animals retreated to the base of their burrows, where a more osmotically favourable "microhabitat" was likely to be found, resulting in a marked decrease in the rate of osmotic equilibration.

The use of a burrowing habit in this way as a behavioural response in molluscs to osmotic stress, has not been well documented. Shumway (1977) indicated that the burrowing habit of both *S. plana* and *M. arenaria* was not linked to behavioural osmotic control. Djangmah *et al.*, (1979) however, noted that *A. senilis* burrows deeper into the mud substratum to avoid exposure to freshwater, but that this aspect of their behaviour has not been investigated. It is well known that interstitial salinities are reasonably stable and may persist for relatively long periods of time despite variation in the salinity of the overlying water column (Smith, 1956; Capstick, 1957; Sanders, Mangelsdorf & Hampson, 1965; Green, 1968; McLusky, 1968; Kinne, 1971; Chapman, 1981). It is suggested that the observed behavioural osmoregulation in

S. cylindraceus, undisturbed in their burrows, is linked to the stability of the interstitial salinity. Indeed, a number of workers have linked animal distribution to interstitial salinity (e.g. Smith, 1955; Sanders *et al.*, 1965; McLachlan & Erasmus, 1974).

Other methods of osmotic control occur in bivalves e.g. Gainey (1978; 1987) has suggested that osmotic hysteresis in the case of *Polymesoda caroliniana* and *M. edulis*, (in which the valves were wedged open) may be effected by a combination of three possible mechanisms: decreased ciliary activity (*M. edulis*), decreased osmotic permeability, and an increased urine production. While such a hysteretic effect is unknown for *S. cylindraceus*, de Villiers, Hodgson & Allanson (1989, in press) (cf. Chapter 6) have reported a substantial decrease in filtration rate during short-term (*in vitro*) exposure to lowered salinity. This may be explained by Shumway's (1977) proposal of decreased ciliary activity and possible arrest in response to hyposaline stress and reinforced by Matthiessen's (1960) results, who demonstrated that the pumping rate of *M. arenaria* varied directly with salinity (see also Hopkins, 1936; Van Winkle, 1972; Davenport & Fletcher, 1978; Dean & Paparo, 1983; Paparo & Dean, 1984 and Chapter 6). However, under conditions where the animal was removed from its burrow, it seems unlikely that ciliary arrest and the associated decrease in filtration rate would play an important role in maintenance of the animal's osmotic state. On transfer of animals from 35‰ to, in particular, lower salinities, there was initially active probing by the foot accompanied by frequent valve adduction (interpreted as an escape response to unfavourable salinity). Such activity would certainly aid in irrigating the mantle cavity and so increase the rate of osmotic equilibration - as discussed by Davenport (1979), in his work on *M. edulis*. It is responses such as these which contribute to the artificiality of *in vitro* experiments, and indicate the material importance of studies in which environmental disturbance is kept to a minimum.

Retreating down its burrow in response to decreased salinity, effectively isolates *S. cylindraceus* from the external environment. Such a behavioural response may then be equated with valve closure in other molluscs. The physiology of such "isolated" or closed molluscs have attracted much attention, and a variety of workers have indicated physiological responses to cope with such conditions e.g. brachycardia and subsequent decreased respiratory levels (Coleman & Trueman, 1971; Boyden, 1972; Davenport, 1977; Akberali, 1978; Akberali & Trueman, 1985) and anaerobic metabolism (Schlieper, 1957; Crenshaw & Neff, 1969; Moon & Pritchard, 1970; de Zwaan & Wijsman, 1976; Taylor, 1976; de Zwaan, 1977; Akberali, 1978). No such information is at present available for *S. cylindraceus* (or, as far as I am aware for any other member of the genus *Solen*). That *S. cylindraceus* possesses similar physiological adaptations, is a possibility that requires further investigation.

The use of the burrow and its stable interstitial salinities by *S. cylindraceus*, assists the animal in avoiding short-term fluctuations of salinity in the overlying water column, e.g. as described for the Morrumbene estuary (Day, 1974), and would be an important factor dictating the extent of estuarine penetration by this species. In the event of prolonged or permanent changes in salinity, this response would allow the animal a longer acclimatory period in which to come into equilibrium with the external medium. Physiologically, *S. cylindraceus* is thus a euryhaline osmoconformer (McLachlan & Erasmus, 1974), capable of tolerating greater salinity ranges than it generally encounters in its normal distribution. Despite this wide salinity tolerance which, together with its behavioural osmoregulatory responses, allows for the successful occupation of estuarine environments, greatest densities are recorded following periods of stable salinity (Boltt, 1975; Blaber *et al.*, 1983). It is suggested that stable salinities are one of the principal factors contributing to the success and unusually high abundance of this species in the Kariega estuary.

Fauna living near the surface of the sediments, may be limited primarily by their tolerance of physical extremes of temperature and salinity, (Kinne, 1970; Gray, 1974; Newell, 1979). This is perhaps particularly striking in the intertidal zone, in which animal distribution in relation to tidal level has been described by numerous workers (e.g. Davies, 1965; Johnson, 1965; Boaden & Erwin, 1971; Kennedy & Mihursky, 1971; McLachlan & Erasmus, 1974; Newell, 1979; Ansell & McLachlan, 1980; Ansell *et al.*, 1980b). In *S. cylindraceus*, smaller individuals occupy the upper 50mm of the sediment, and occur predominantly lower down in the intertidal zone (Figs. 3.4 & 3.10). As physical conditions near the surface of sediments vary more than at depth, it is possible that juveniles exhibit a wider tolerance range than the adults. However, their lower intertidal distribution, would result not only in decreased periods of exposure, but provide a thermally and osmotically more stable habitat. Variation in tolerance between adult and juvenile has been documented by a number of workers. The relationship is nevertheless not a general one, e.g. both *Donax serra* and *Donax sordidus* juveniles exhibit a lower thermal tolerance than the adults (Ansell & McLachlan, 1980; McLachlan & Young, 1982), while the opposite has been demonstrated by Kennedy & Mihursky (1971) for *Mya arenaria* and *Macoma balthica*, and similarly by Ansell *et al.* (1980b) for *Donax vittatus*, and *Cardium glaucum* (Ansell *et al.*, 1981). Waugh & Garside (1971) and Wilson (1978), however, demonstrated no difference between the adults and juveniles of *Modiolus demissus* and *Tellina tenuis* respectively. Variation in thermal tolerance between adult and juvenile *Donax* species (Ansell & McLachlan, 1980; Ansell *et al.*, 1980b; McLachlan & Young, 1982) has been suggested as a contributing factor which accounts for their varying distribution within the intertidal zone (McLachlan, Woolridge & Van der Horst, 1979). Neither thermal nor salinity tolerances of juvenile *S. cylindraceus* have been examined, and the extent to which the observed distribution of adult and juvenile is a function of physiological tolerance, is uncertain.

The success of *S. cylindraceus* in the Kariega estuary may, in part, be attributed to its wide thermal and salinity tolerance range. It has been established that its burrowing habit, together with the stability of the interstitial environment, allows the animal to effectively avoid any substantial short-term fluctuations of temperature and/or salinity, as may be encountered during either upwelling, flood or run-off from rain water. Further, if such variations in temperature or salinity persist for any length of time, the burrowing habit and interstitial stability would allow a more gradual acclimatory period, in which the animal may accommodate such environmental changes.

CHAPTER FIVE

ANIMAL ACTIVITY

INTRODUCTION

A rhythmic cycle of feeding and digestion in the bivalve *Crassostrea virginica* was first noted by Nelson (1918; 1925). Despite these observations, the general view has been that lamellibranchiate bivalves are, provided conditions are favourable, continuous feeders. More recently, however, studies on intertidal, sub-tidal and freshwater bivalves have indicated rhythmic patterns of feeding and digestion. Such discontinuous feeding and digestive processes have been described for a number of species e.g. *Mytilus edulis* (Rao, 1954; Davids, 1964; Langton, 1975; 1977), *Crassostrea virginica* (Loosanoff & Nomejko, 1946; Brown, 1954; Palmer, 1980), *Cardium edule* (Morton, 1970a), *Crassostrea gigas* (Morton, 1977), *Pecten maximus* (Mathers, 1976), *Anodonta cygnea* (Barnes, 1955; Salanki & Vero, 1969; Morton, 1970b), *Ostrea edulis* (Morton, 1971; Langton & Gabbott, 1974; Mathers, 1974a; 1974b), *Unio pictorum* (Morton, 1970b), *Dreissena polymorpha* (Morton, 1969) and *Lasaea rubra* (Ballantine & Morton, 1956; Morton, 1956).

The question arises whether the observed rhythmicity is endogenous or whether it is co-ordinated with natural feeding cycles imposed by the environment (Owen, 1974b). Such rhythms were originally considered endogenous e.g. Rao (1954) for *Mytilus edulis* and *Mytilus californianus*, and Barnes (1955) for *Anodonta cygnea*. These observations have, however, not

been confirmed by other workers, e.g. Jørgensen (1960) and Davids (1964) in the case of *M. edulis*, or by Salanki & Vero (1969) for *A. cygnea*. Indeed evidence has been presented which suggests that the feeding cycles observed in numerous bivalve species may be modified to accommodate localized variations in the environment (e.g. Mathers, 1976; Morton, 1977; Mathers, Smith & Colins, 1979). Such discontinuous feeding and digestive rhythms have thus been shown to be related to changes in external environmental variables. The significant environmental variable, however, may differ from species to species. The importance of tidal periodicity has been established by Rao, 1954; Ballantine & Morton, 1956; Morton, 1956; McQuiston, 1969; Mathers, 1976; Mathers, Smith & Colins, 1979. Diurnal rhythmicity has been examined by Hiscock, 1950; Salanki, 1966; Morton, 1969; 1970b; 1971; 1978; Morton & McQuiston, 1974, while a combination of both diurnal and tidal factors has been considered by Loosanoff & Nomejko, 1946; Brown, 1954; Morton, 1971; Langton, 1977. Feeding and digestive rhythms may also be influenced by other changes in the physical environment e.g. salinity (Wada, 1969; Morton, 1975) or suspensoid concentration (Loosanoff & Engle, 1947; Davids, 1964; Ali, 1970; Thompson & Bayne, 1972; Foster-Smith, 1975a; 1975b; 1976; Bayne, Thompson & Widdows, 1976; Widdows, Fieth & Worrall, 1979).

Intertidal bivalves are subjected to two major environmental rhythms - tidal and diurnal (Langton, 1977). As arrival of tide and food are two aspects of the same phenomenon, it would appear self-evident that such animals are subjected to an imposed tidal rhythm of feeding and that the tidal, rather than diurnal, cycle would exert the greatest effect (Morton, 1970a; 1977; Langton, 1977). Since tidal variation restricts feeding to particular times, it would not seem unreasonable to assume that the digestive process could follow a similar pattern. It has been suggested that all filter-feeding bivalves may exhibit distinct phases of feeding and digestion, each cycle comprising well-defined periods of feeding, extracellular and intracellular digestion (Morton, 1956; 1973; 1977; McQuiston, 1969). However, there is debate

as to whether the periodicity of feeding and digestion, together with observed structural changes in the gut and digestive glands are synchronized to any particular environmental cycle, or are simply a response to the arrival of food in the stomach (Owen, 1972; 1974b; Langton & Gabbott, 1974; Wilson & La Touche, 1978; Palmer, 1980; Robinson & Langton, 1980).

Digestion in bivalves occurs in two stages:

1. Extracellular digestion in the stomach by the release of enzymes following dissolution of the crystalline style.

2. Intracellular digestion, by the digestive cells of the digestive diverticuli, of principally the products of extracellular digestion.

A comparative measure of these two processes of digestion is to be found in studies of style formation and dissolution and in the cytological structural changes of the digestive tubules, which indicate that extracellular and intracellular digestion often alternate sequentially (Owen, 1972; 1974b; Morton, 1977; 1978). The secretion of the crystalline style in the style sac, and its dissolution in the lumen of the stomach, has been shown to be linked to periods of animal feeding activity, extracellular digestion and accompanying pH changes in the stomach (Kristensen, 1972; Mathers, 1972; 1976; Morton, 1973; 1977; Langton, 1977; Mathers *et al.*, 1979).

Despite considerable interest in the determination of bivalve filtration rates, (for reviews see Jørgensen, 1975b; Winter, 1978; Newell, 1979; Griffiths & Griffiths, 1987; also Chapter 6), only a few investigations take into account animal rhythms of activity and quiescence. Clearly, the demonstration that the processes of feeding and digestion exhibit such a rhythmicity, must have important ecological implications. Notwithstanding a substantial amount of information, consistent with the idea that rhythmicity is not only widespread, but important in co-ordinating the activities of feeding and digestion in bivalves (Winter, 1969; 1973; Widdows,

Fieth & Worrall, 1979; Griffiths, 1980; Palmer, 1980; Hawkins, Bayne & Clarke, 1983), there exists a paucity of data on the feeding behaviour of bivalve molluscs under natural conditions. Further, it has become apparent that the feeding and digestive processes should no longer be viewed in isolation, but rather as co-ordinated aspects of animal function (Purchon, 1971; Hawkins *et al.*, 1983).

As *Solen cylindraceus* is an intertidal animal and as such, has imposed upon it by the tide, a rhythmic periodicity of feeding, the objectives of this study are:

1. To monitor filtration activity, and determine whether during periods of submersion the animal feeds continuously or exhibits any feeding periodicity. In this study, feeding activity is equated to filtration or pumping activity, and is only an indication of whether or not the animal is moving water, as opposed to filtration rate (cf. Chapter 6) which quantifies the volume of water filtered.

2. To examine the effect of different suspensoid concentrations on feeding activity. Under natural conditions *S. cylindraceus* is exposed to varying suspensoid concentrations (50-500mg l^{-1}, cf. Chapter 7) during periods of submersion, dependent on physical environmental factors e.g. state of the tide, wind etc..

3. To use the crystalline style as an "indicator" of extracellular digestion by monitoring style volume and replacement, and to determine if the process of digestion is synchronous with tidal periodicity and feeding activity.

MATERIALS AND METHODS

A. Filtration activity

Solen cylindraceus of 50-60mm shell length were collected from the Kariega estuary during November 1987. Animals were transferred to mud-filled pots at the site of collection and left in the estuary overnight to allow the animals to establish themselves under as normal conditions as possible. Pots were transferred to laboratory aquaria (Fig. 4.1) the following day. Aquarium temperature was set at 25^{o}C and salinity at 36‰ (field conditions: 24^{o}C, 36‰). Animals were allowed a 20 day period to acclimate to laboratory conditions of 14:10 h light:dark regime, and a six hourly tidal exposure. Animals were fed a suspension of the green algae *Tetraselmis suecica* every three days, and aquarium water was changed every 7-10 days.

Natural seston was collected from the estuary, with the use of a bilge pump, during flood tide at a position inshore of the *Zostera* bed directly above the area of greatest density of *S. cylindraceus*. Seston was pumped into a series of large plastic containers through a 64µm sieve. The filtrate was concentrated by continuous centrifugation (15000rpm, flow rate of 200ml min.$^{-1}$) and the resultant "slurry" served as "stock" material from which the different suspensoid concentrations were made up, and to which the animals were exposed. The following suspensoid concentrations were used: 5mg l^{-1} (normal aquarium water, no "slurry" added); 15; 25; 50; 100; 250 and 500mg l^{-1}. Filtration activity at 5mg l^{-1} was determined on animals maintained in the holding aquaria. Activity measurements at the higher concentrations were recorded from single pots, transferred during "low tide" to the experimental aquarium (Fig. 5.1), without being subjected to any change in tidal sequence. The experimental aquarium was filled with water to which an appropriate volume of "slurry" was added to give the desired suspensoid concentration.

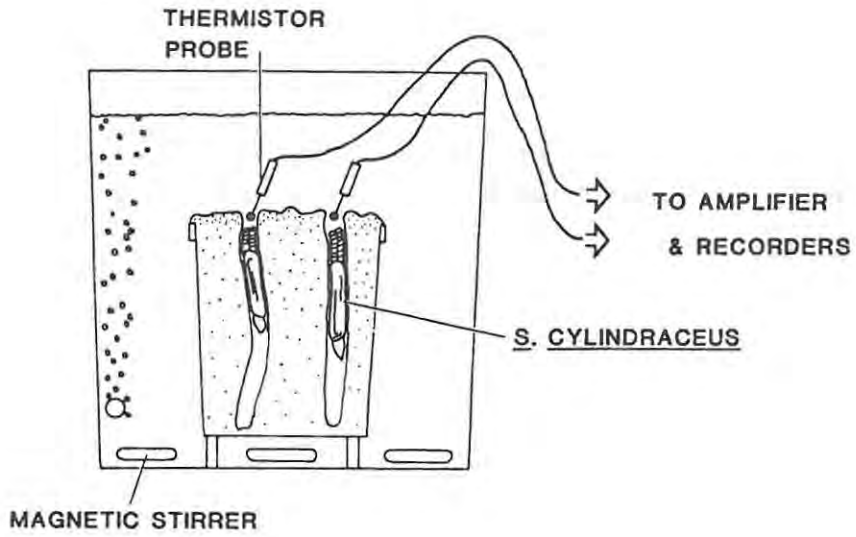


Figure 5.1: Diagram illustrating position of the thermistor probes, for the measurement of animal filtration activity.

Material was maintained in suspension by a series of magnetic stirrers (3) and aeration. The suspensoid concentration of the water was measured at the start, end, and at some stage (ca. after 3 hours) during each experiment. A slight decrease in suspensoid concentration occurred with time, but was felt insufficient to warrant the addition of further "slurry" during the experimental period. Filtration activity was recorded for the full 6 hour submerged period. Available equipment allowed two animals to be recorded simultaneously. A total of 8 animals were recorded in this manner for each suspensoid concentration.

Animal filtration activity was measured by thermistor flow-meters, constructed by Mr. J. Lucas of the Department Physics and Electronics at Rhodes University. A thermistor is a resistor, the resistance of which changes with temperature. As a flow-meter, it is heated slightly above ambient temperature and any flow of water across the probe results in a drop in

temperature of the thermistor bead and subsequent change in resistance, which is detected, amplified and recorded. Thermistor probes were placed at the openings of the *S. cylindraceus* burrows, as shown in Figure 5.1. A miniature bead thermistor (1.5mm diameter) of 2K resistance was used in a self-heating mode, achieved by passing a constant 10mA current through it. The voltage drop across the thermistor at the beginning of the experiment ("background noise", caused by water movement due to aeration etc.) was annulled by an equal voltage (via the zero control, see Fig. 5.2), such that the slight changes (in mA), as a result of the animal pumping, could be detected. Current changes were recorded on a Linear Instrument Corporation, Model 260/MM pen recorder, via a amplifier/buffer (Fig. 5.2). No temperature calibration was incorporated into the circuitry, and the system was operated at a water temperature of 25^{o}C only.

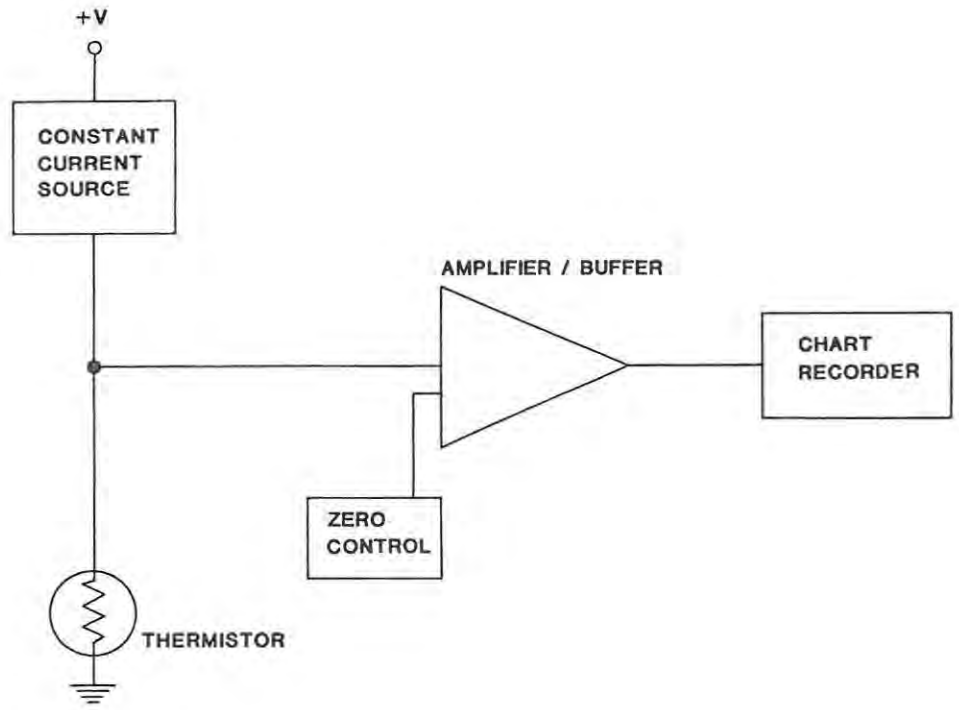


Figure 5.2: Simplified circuit diagram of thermistor flow-meter arrangement.

B. Crystalline style measurements

i) Style volume

In order to determine variation in style volume, two field experiments were carried out. In the first, a range of animal sizes were examined, while in the second, only animals in the size range 50-60mm (shell length) were used. In both instances, animals were collected and transferred to mud-filled pots (ca. 8-10 animals per pot). Pots were then buried, virtually to the rim, approximately 0.5m apart at the same tidal height in the mud bank. This ensured that all animals were subjected to the same tidal periodicity. Pots were left for a 3 week period, to ensure that the animals had fully established themselves and adjusted to the tidal regime.

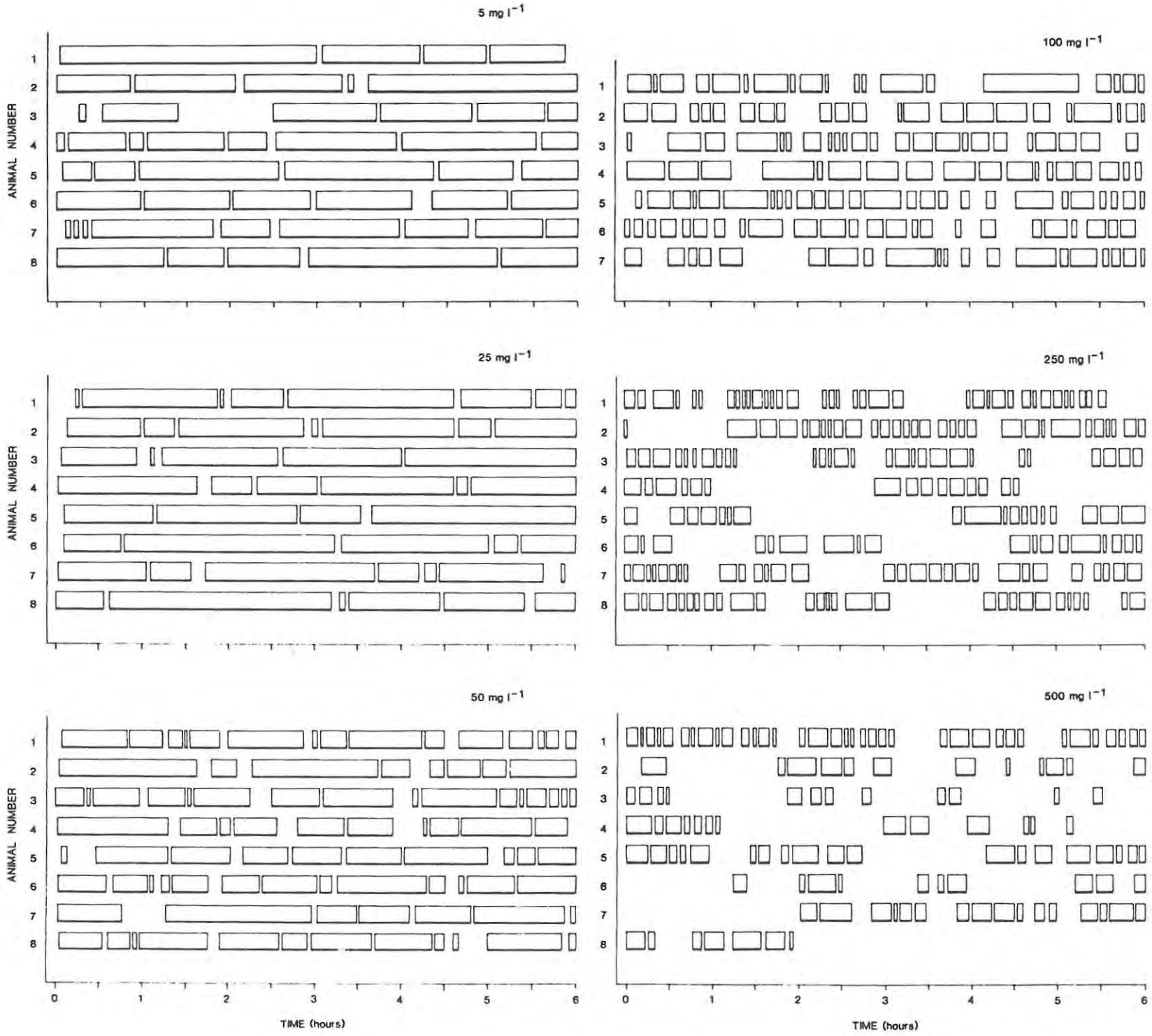
In order to determine possible style dissolution or volume change, single pots were collected at set time intervals, spanning a full flood and ebb tide. Sampling was done hourly in the first experiment, from approximately slack low, through high tide, to the following slack low. A slightly varied sampling strategy was adopted in the second experiment, in which the first specimens were collected during the ebb tide, while animals were still submerged. The second pot was collected after one hour exposure and subsequent pots at 1.5 hour intervals until the next flood tide. Following re-submergence by the flood tide, the next pot was collected after one hour, further specimens were collected every 1.5 hours for the duration of the high tide, with the final sample being taken one hour after re-exposure.

Animals collected at each time interval were removed from their pots and the following data recorded: shell length (mm), style length (mm), style volume (mm^3). Crystalline styles were regarded as cylindrical, and their volumes calculated accordingly. The diameter used in the calculation was a mean of 3 readings taken from different regions of the style (just behind the tip, middle and end) using a dissecting microscope fitted with a graticule.

ii) Style replacement

Solen cylindraceus of 50-60mm shell length were collected and transferred to mud-filled pots on site (ca. 10-12 animals per pot). Pots were then buried alongside one another at the same height in the intertidal zone and left for 20 days, to ensure that animals established themselves under as natural conditions as possible and adjusted to the tidal regime. Pots were then removed and placed in large plastic buckets or baths, containing natural estuarine water in which the vital stain, neutral red (Gurr, BDH Chemicals Ltd.), had been dissolved to give a final concentration of 0.02%. Pilot studies, utilizing a variety of different stains at different concentrations, revealed that neutral red gave the best results, completely staining the crystalline style after 24 hours at the above concentration. Following the 24 hour staining period, pots were replaced in their original positions in the intertidal mud bank. One animal was removed from each pot, the style dissected out, and the extent of staining assessed. This value (expressed as a percentage of total style volume) was regarded as the "time zero" percentage stain. One pot of animals was removed every 2 hours from then on, to a total of 48 hours. The styles were dissected out and the percentage stain remaining, and new style material secreted, determined. A number of additional pots of animals were available to augment those in which the number of *S. cylindraceus* had decreased, a result of movement out of the pot, death or predation. The amount of stain remaining in styles sampled from individual pots, at consecutive time intervals, was averaged and expressed as a percentage of the initial amount of style stained at time zero (96.45%). This change in proportion was regarded as the amount of new style material secreted per sampling interval. Consecutive values were then grouped according to whether they were collected during periods of submergence or exposure, and the sum of the values for each of these groups was taken as the final percentage of new style material secreted per tidal period. Unless otherwise stated, all statistical calculations for validity of differences were made using Students "t" test.

Figure 5.3: Periodicity of filtration activity of *Solen cylindraceus* at different suspensoid concentrations. Bars indicate periods of activity.


5 mg l⁻¹
100 mg l⁻¹
25 mg l⁻¹
250 mg l⁻¹
50 mg l⁻¹
500 mg l⁻¹
ANIMAL NUMBER
1
2
3
4
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6
7
8
0
1
2
3
4
5
6
TIME (hours)

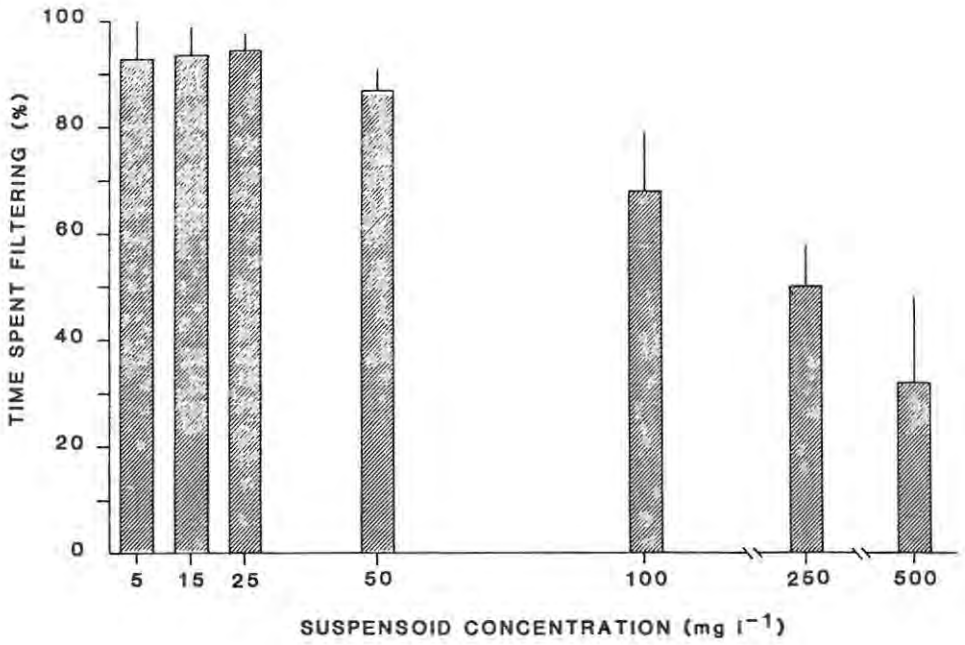


Figure 5.4: Mean total time spent filtering (% + SD) by groups of *Solen cylindraceus* exposed to different suspensoid concentrations (n = 8 in all instances except at 15 and 100mg l^{-1}, where n = 7).

RESULTS

A. Filtration activity

The activity of individual animals at different suspensoid concentrations is shown in Figure 5.3. The mean total time spent filtering by each group of *S. cylindraceus* at each suspensoid concentration is illustrated in Figure 5.4. At suspensoid concentrations of 5-25mg l^{-1}, filtration activity was virtually continuous (90-95% of the time), and was characterized by relatively long ventilatory periods and few, brief, inactive periods. At 50mg l^{-1} the slight decrease ($p < 0.1$) in total time spent filtering (87%, Fig. 5.4) was a consequence of more

frequent periods of inactivity (Fig. 5.3). At the higher suspensoid concentrations, 100, 250 and 500mg l^{-1}, a significant reduction ($p < 0.001$) in the total time spent filtering was observed (68, 50 and 32% respectively, Fig. 5.4). The filtration activity patterns recorded at these elevated concentrations (Fig. 5.3) were all characterized by short ventilatory, and more frequent inactive, periods. Longer periods of quiescence were observed to occur, on exposure to higher suspensoid concentrations (250 and 500mg l^{-1}, Fig. 5.3).

B. Crystalline style measurements

i) Style volume

In order to account for the variation in animal sizes collected in the two experiments, results were normalized by plotting style volume/shell length, for both experiments, against time. These plots are shown in Figure 5.5 A and B. Despite this normalization, values in the first experiment (Fig. 5.5 A), in which a wide range of animal sizes (32-67mm shell length) were analyzed, showed substantial variation (0.19-0.28). No such variation is apparent in the second experiment (0.25-0.29, Fig. 5.5 B) in which only animals in the size range 50-60mm (shell length) were analyzed.

It is suggested that the variation observed in the first experiment is a result of poor experimental design, in using such a wide range of animal sizes. Nevertheless, mean values of style volume/shell length calculated for submerged and exposed conditions in both experiments (0.247 ± 0.074 (submerged) and 0.241 ± 0.058 (exposed) for experiment A, and 0.271 ± 0.038 (exposed) and 0.280 ± 0.042 (submerged) in experiment B), indicated no major variation in style volume. From these data it would seem apparent that the crystalline style of *S. cylindraceus* does not follow any cyclical pattern (tidal) of dissolution and regeneration.

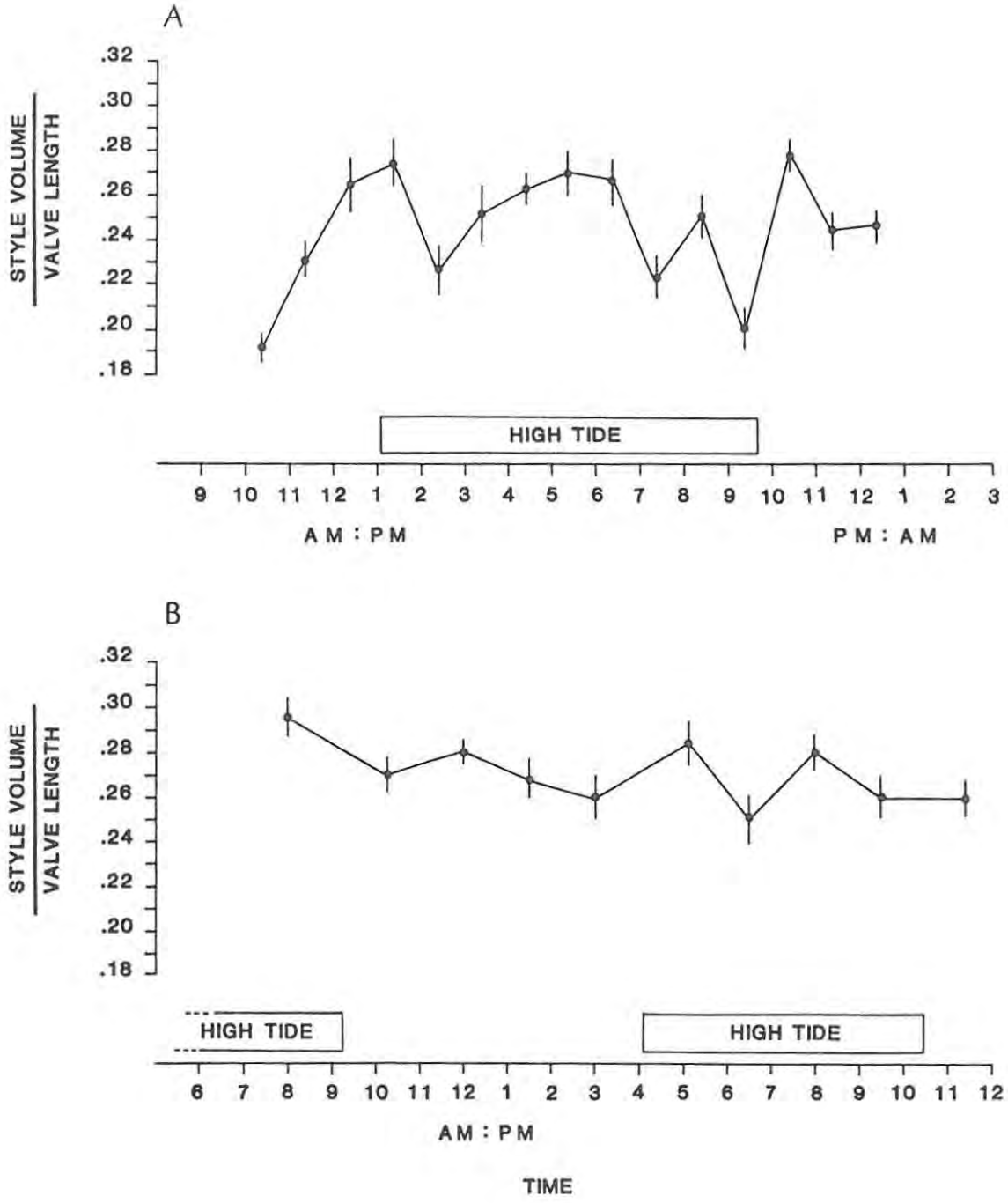


Figure 5.5: Variation in style volume (normalized as; style volume/valve length) with tide. A. Results from animals of different shell lengths (32-67mm) (May, 1985). B. Results of animals of 50-60mm shell length (August, 1985). Each data point is a mean ± SD of all the animals in one pot (n=8-10), collected at that particular time.

ii) Style replacement

The replacement of (stained) style material during flood and ebb tides over a 48 hour period is shown in Figure 5.6. Virtually no stain was visible (only 2.5% by volume) in the crystalline style after this period, and it is suggested that 42-48 hours is the time taken for complete style replacement. The percentage loss of stained style material was greater during the first 3-4 hour period following submergence, than during periods of exposure (Fig. 5.6). Consequently, the relative amount of new style material secreted was greatest during submergence (Fig. 5.7). During periods of submergence, 16, 22, 30 and 10% of the style material was replaced, while only 4, 6, 7, 7 and 2% was recorded during periods of exposure (Fig. 5.7). The low percentage replacement recorded during the last flood tide (10%, Fig. 5.7) is probably an underestimation, the result of the very small amount of stain remaining in some styles while others, by this time, were completely stain-free. From the results presented in Figures 5.6 and 5.7, it appears that there is a cyclical pattern of style replacement, linked to tidal periodicity.

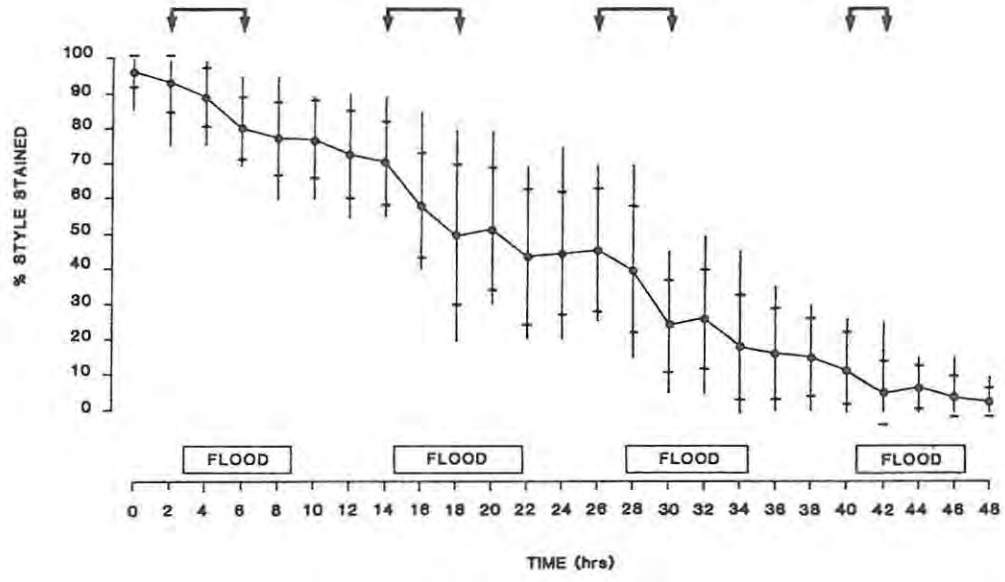


Figure 5.6: The percentage decrease in stained style material during flood and ebb tides, over a 48 hour period. Each data point shows the mean ± SD and range for 10-12 animals. Arrowed areas indicate periods of maximum style utilization.

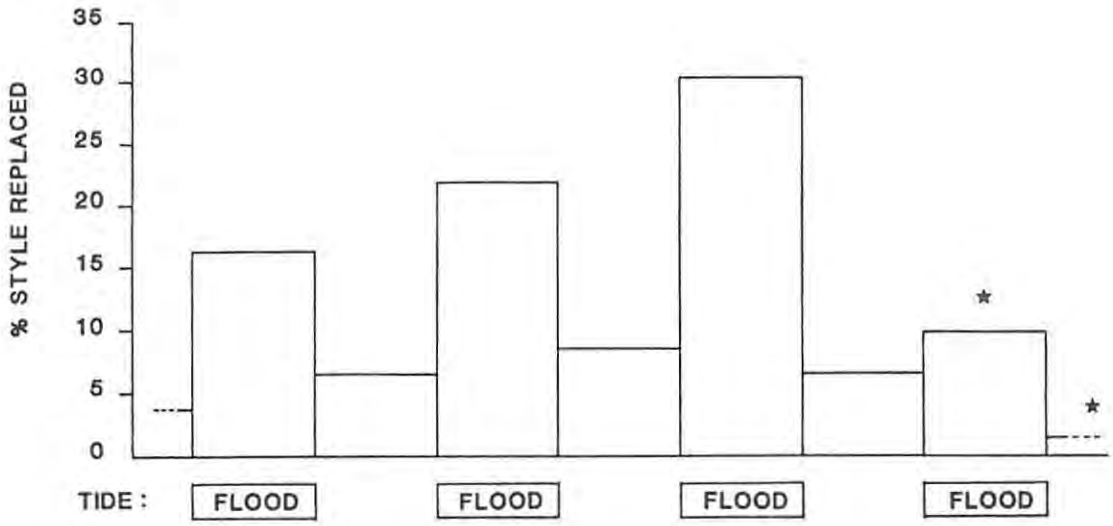


Figure 5.7: Crystalline style replacement during flood and ebb tide periods. ★ = probable underestimated values as many of the styles, by this stage, had very little stain remaining, while others were already completely stain-free.

DISCUSSION

As an intertidal species, it would be expected that *S. cylindraceus* has a tidally mediated rhythmicity of feeding and digestion imposed upon it. Under experimental laboratory conditions, it is evident that during periods of submergence, *S. cylindraceus* has a filtration activity dependent on the concentration of suspended material to which it is exposed. At low suspensoid concentrations, filtration activity is virtually continuous, interrupted by short inactive periods (Figs. 5.3 & 5.4). In the estuary, *S. cylindraceus* is exposed to varying suspensoid concentrations (50-500mg l^{-1}; cf. Chapter 7). However, at elevated concentrations, a marked decrease in filtering time was observed and filtration activity became irregular,

interrupted with more frequent, and often longer periods of quiescence. Such a response is not unique to *S. cylindraceus* and has been reported for a number of bivalve species e.g. *Mytilus edulis* (Davids, 1964; Foster-Smith, 1976), *Mya arenaria, Crassostrea edule, Venerupis pullastra* (Foster-Smith, 1976) and *Ostrea virginica* (Loosanoff & Engle, 1947). These workers recorded not only a decreased pumping time with increasing suspensoid concentrations, but also in many instances a reduced filtration rate, a phenomenon which is consistent with the findings of numerous other authors (e.g. Ali, 1970; Winter, 1970; 1977; Mathers, 1974b; Schulte, 1975; Theissen, 1977; Widdows *et al.*, 1979; Griffiths, 1980; Griffiths & Griffiths, 1987). The effect of suspensoid concentration on the filtration rate of *S. cylindraceus* is examined in Chapter 6.

The precise mechanism regulating filtration activity in *S. cylindraceus*, as well as many other bivalves, is unclear. Foster-Smith (1976) has suggested intermittent closure of the siphons as one possible method. The interrelationship between filtration, suspensoid concentration and pseudofaecal production has been demonstrated by Foster-Smith (1975a) and reviewed by Winter (1977; 1978), in which ingestion rate increased with increasing particle concentration to a certain threshold, above which further material filtered by the gills was rejected as pseudofaeces. Foster-Smith (1975a) has suggested two mechanisms by which bivalves may control ingestion at different suspensoid concentrations: 1) an increased proportion of material rejected as pseudofaeces and; 2) a decreased filtration rate. The irregular filtration activity recorded for *S. cylindraceus* at elevated suspensoid concentrations may, in part, be linked to increased pseudofaecal production (see Fig. 6.5) and more frequent periods of ejection. A number of authors have suggested that feeding and digestion are controlled by patterns of food availability (e.g. Langton & Gabbott, 1974; Owen, 1974b; Wilson & La Touche, 1978; Robinson & Langton, 1980). Palmer (1980) has suggested that this may indeed represent an endogenous mechanism for control of ingestion, in that food is continuously filtered until

such a time that the animal's assimilative capacity is exceeded, upon which filtration ceases or slows. Variation in filtration activity would thus serve to regulate food levels in the stomach and permit relatively consistent intracellular digestion. This hypothesis would fit the observed filtration activity (Fig. 5.3) of *S. cylindraceus*, with longer periods of filtration recorded at low suspensoid concentrations, and shorter food clearance periods in higher concentrations. However, as suspensoid concentrations in the laboratory experiments were maintained at a constant level, if filtration activity was the only regulating factor, a more regular periodicity of pumping and quiescence would be expected. Although it is well known that temporal variations in filtration are components of normal feeding behaviour (Palmer, 1980; Hawkins *et al.*, 1983), the irregular patterns obtained nevertheless suggest the possible interaction of not only increased pseudofaecal production and a decreased filtration rate, as suggested earlier by Foster-Smith (1975a), but also by a reduced time spent filtering.

If Palmer's (1980) hypothesis is accepted for *S. cylindraceus*, the results indicate that while filtration activity varies (possibly serving to regulate gut food levels), extracellular digestion as determined from style volume and replacement rates (Figs. 5.5, 5.6 & 5.7), is continuous. Discontinuous feeding and digestion has been reported for many bivalves, in which cyclical fluctuations in the formation and dissolution of the crystalline style, and the structure of the digestive tubules indicate that feeding, extracellular digestion in the stomach, and intracellular digestion in the digestive diverticuli, often alternate sequentially (e.g. McQuiston, 1969; Morton, 1971; 1977; Owen, 1972; 1974b; Langton & Gabbott, 1974; Mathers *et al.*, 1979; Hawkins *et al.*, 1983). As the digestive glands were not examined in *S. cylindraceus*, no comment can be made with respect to intracellular digestion. The general picture for bivalves exhibiting a tidal feeding and digestive rhythm is that upon exposure, the food source is effectively removed and feeding is halted, after which the style rapidly dissolves. Style reformation occurs just prior to, or at the onset of feeding during the next period of submergence.

However, the crystalline style of *S. cylindraceus* demonstrated no variation in volume during either high or low tide, which would suggest continuous feeding. Given the intertidal distribution of this species, this would seem unlikely. Yonge (1932) and Bernard (1973) have indicated that species with conjoined midgut and style sac have styles which are more short-lived than those in animals with a completely separated style sac. Indeed, most of the work on feeding and digestive rhythms has been performed on species with conjoined style sac and midgut. In the light of this, the effectiveness of using crystalline style volume as a measure of feeding and extracellular digestion in species with separate style caeca, may be questioned.

A consideration of style replacement (Fig. 5.7) indicates a net production of new style material during both flood and ebb tides. These data suggest that the crystalline style is a permanent structure in *S. cylindraceus* and does not appear to undergo rhythmical periods of dissolution. If Langton & Gabbott's (1974) observations that changes in the style may simply reflect variations in available food levels rather than any rhythmic pattern of digestive activity, are applied to *S. cylindraceus*, it would imply continued digestion (and possibly feeding) during not only high, but low tide as well. However, it is evident (Fig. 5.7) that the relative amount of new style material produced during periods of high tide was greater than at low tide. It would appear that in *S. cylindraceus* the process of extracellular digestion is continuous, although exhibiting a marked tidal variation in rate. This is possibly a result of the varying food concentrations in the gut, in turn reflecting food availability. These observations suggest that *S. cylindraceus* may filter in its burrow during periods of low tide. Given the nature of the substratum in which *S. cylindraceus* lives, together with the relatively small tidal variation, it may be argued that the animal in fact experiences no true exposure. The possibility that *S. cylindraceus* may be filtering in its burrow (at or below the water table) during periods of low tide may be reinforced by field observations, in which the animal has been observed to expel water periodically from its burrow. Between 8 and 14 such voidances

have been recorded (pers. obs.) from a single burrow during low tide. Further, at no stage during experimental sampling was the gut found empty, although the volume of food present was substantially less during the latter period of low tide than at high or early low tide.

Morton (1975) has indicated that the intertidal bivalves *Geloina proxima* and *Geloina erosa* may filter interstitial water in their burrows following periods of prolonged exposure. Hughes (1969) reported continuous deposit feeding by *Scrobicularia plana* even during low tide, and further that the animals will feed from the side of their burrows at the level of the water table. The phenomenon of continued filtration and digestion during periods of low tide, amongst infaunal intertidal bivalves, is thus not unique (to *S. cylindraceus*).

Solen cylindraceus thus does not appear to exhibit a totally quiescent phase, characterized by complete or partial style dissolution during periods of "exposure", as described for many epifaunal intertidal bivalves. However, although the processes of feeding and digestion would appear to be continuous, it does exhibit a tidal rhythmicity, with maximal feeding and style replacement occurring at high tide (a possible function of increased food availability). The results of these experiments give a wider implication to studies on the processes of feeding and digestion of particularly infaunal intertidal bivalves, and highlights the need for closer, more detailed investigations.

CHAPTER SIX

PARTICLE RETENTION AND FILTRATION RATE

INTRODUCTION

As a filter feeder, *Solen cylindraceus* utilizes particulate material suspended in the water column brought to it by tidal currents. The amount of food available to a suspension feeding bivalve depends not only upon the concentration of suspended material, the efficiency with which it retains particles and the filtration rate, but also upon the total amount of time the animal spends feeding (filtration activity) (e.g. Vahl, 1972a; Foster-Smith, 1976; Newell, Johnson & Kofoed, 1977; Palmer & Williams, 1980; Wright, Coffin, Ersing & Pearson, 1982; Griffiths & Griffiths, 1987; Peterson & Black, 1987; Matthews, Lucas, Stenton-Dozey & Brown, 1989). The filtration rate and efficiency of particle retention by bivalves have important ecological implications, and the effect that various environmental variables exert on their performance has received a good deal of attention (e.g. Jørgensen, 1960; 1966; 1975b; 1981; Owen, 1966; 1974b; Dral, 1967; Hughes, 1969; 1970; Ali, 1970; Haven & Morales-Alamo, 1970; Vahl, 1972a; 1972b; 1973a; 1973b; Foster-Smith, 1975a; 1975b; 1976; Schulte, 1975; Griffiths & King, 1979; Newell, 1979; Widdows, Fieth & Worrall, 1979; Griffiths, 1980; Newell & Branch, 1980).

In natural marine and estuarine waters, small particles constitute an important fraction of the seston, both in terms of number and volume (Haven & Morales-Alamo, 1970; Vahl, 1972a).

Several investigators have commented on the nutritional importance of the smaller naturally occurring particles to filter feeders. Vahl (1972a) has suggested that the nano- and ultra (sic) plankton may constitute an important fraction of the food ingested by bivalves. The results of Jørgensen (1960; 1975b) as well as Hughes (1969), Vahl (1972a; 1972b), Møhlenberg & Riisgard (1978), Berry & Schleyer (1983), Stuart & Klumpp (1984) and more recently the review by Griffiths & Griffiths (1987), indicated that despite some intra- and inter-specific variability, most bivalves retained up to 100% of particles greater than 4-5μm in diameter, with a marked decrease in retention efficiency as particle size decreases. As many bivalves may thus only retain a portion of the natural suspended material, the concentration of these particles may not always be indicative of available food resources. Documentation of particle utilization is thus essential for any quantitative assessment of food availability to a filter-feeding animal. Further, such differential retention has important implications in the determination of filtration rate, which if determined indirectly, assumes complete retention of the particular particle size range monitored.

Numerous techniques to measure filtration rates of bivalves have been described (e.g. Coughlan, 1969; Winter, 1973; Hildreth & Crisp, 1976; and reviews by Ali, 1970; Winter, 1978). Two approaches have been adopted by researchers:

1. The direct method: which relies upon the physical separation of inhalent and exhalent currents, involving a direct measure of the flow of water from the exhalent siphon (e.g. Davids, 1964).

2. The indirect method: the more favoured approach based upon the rate at which particles of known concentration are cleared from known volumes of suspension over time (e.g. Fox, Sverdrup & Cunningham, 1937; Coughlan, 1969; Bayne, 1971; Widdows & Bayne, 1971; Hildreth & Crisp, 1976).

The determination of filtration rate by the indirect method, may be carried out in either a closed system (of fixed volume) or a flow-through system (of known flow rate), the advantages and drawbacks of each have been dealt with in some detail by Coughlan (1969) and Hildreth & Crisp (1976) respectively. As *S. cylindraceus* possesses fused siphons, the direct approach was not practical, and in the series of experiments presented here, filtration rate was determined indirectly, using both closed and flow-through systems.

Jørgensen (1960) has commented on animal sensitivity, and the problem of animal disturbance when removed from their natural environment and subjected to laboratory experimentation. Further, that the filtration values measured for infaunal bivalves tended to be low, since in most cases measurements were made on animals outside their natural sediment substrata. Attempts to determine filtration rates of *S. cylindraceus, in situ*, with available equipment were, however, unsuccessful, often giving conflicting and even negative filtration rates. Consequently, animals were removed from their burrows and filtration rates determined *in vitro*. An examination of the effect of suspensoid concentration on filtration rate is not new, and has been reported by numerous workers (e.g. Ali, 1970; Thompson & Bayne, 1972; Theissen, 1977; Winter, 1977; Griffiths, 1980; Wilson, 1983; Griffiths & Griffiths, 1987; cf. Chapter 5). Previously (cf. Chapter 5), the effect of suspensoid concentration on filtration activity was examined *in situ*. The employment of similar techniques as described in Chapter 5, allowed the determination of filtration activity of animals under *in vitro* conditions, exposed to the same range of suspensoid concentrations. Such a comparison, I feel, lends credence to the *in vitro* filtration rate determinations.

Newell & Branch (1980) and Prosser (1986) distinguished three time phases in the response of an animal to a change in temperature - which I feel are equally valid for, and may be

extrapolated to, other environmental variables:

1. An immediate (acute or short-term) response.

2. An acclimation (longer or seasonal) response, following on a longer period of exposure to the changed condition.

3. Very long term response to change in environmental conditions over many generations, which may facilitate the appearance of genetic variants, adapted to the new regime.

The general concept of adaptation therefore includes both genetic and non-genetic aspects (Bayne, Thompson & Widdows, 1976; Newell & Branch, 1980; Prosser, 1986). Kinne (1963) distinguished three phases for non-genetic adaptation, i.e. an immediate response to environmental change, the stabilization of this response and finally a new steady state. The genetic aspects which influence factors such as the upper and lower tolerance limits of the species, have been examined for *S. cylindraceus* by McLachlan & Erasmus (1974) and in this study (cf. Chapter 4). To date, their work comprises virtually the only published knowledge on the response of this species to environmental variables. This investigation, by considering filtration rate and filtration activity, aims to examine essentially the "non-genetic" responses of *S. cylindraceus* to the environmental variables of temperature, salinity and suspensoid concentration, to which the animal may normally or episodically be exposed.

MATERIALS AND METHODS

A. Animal collection and maintenance

Animals were collected from the Kariega estuary and transferred to mud-filled pots at the site of collection. Pots were left in the estuary to allow animals to burrow and re-establish themselves under conditions as close to normal as possible. Pots were collected the following day and transferred to laboratory aquaria, through which a supply of estuarine water

circulated (Fig. 4.1). Animals were subjected to a 6 hourly tidal cycle and a 14:10 h and 12:12 h light:dark period for summer and winter collected animals respectively. Temperature and salinity were initially set to match that measured in the estuary on the day of collection. Acclimatory periods and specific rates of temperature and salinity change are described for each experiment. Temperature and salinity were held constant within ± 1°C and ± 2‰ respectively. Aquarium water was changed every 7-10 days, and animals were fed a suspension of the green algae *Tetraselmis suecica* every 3 days.

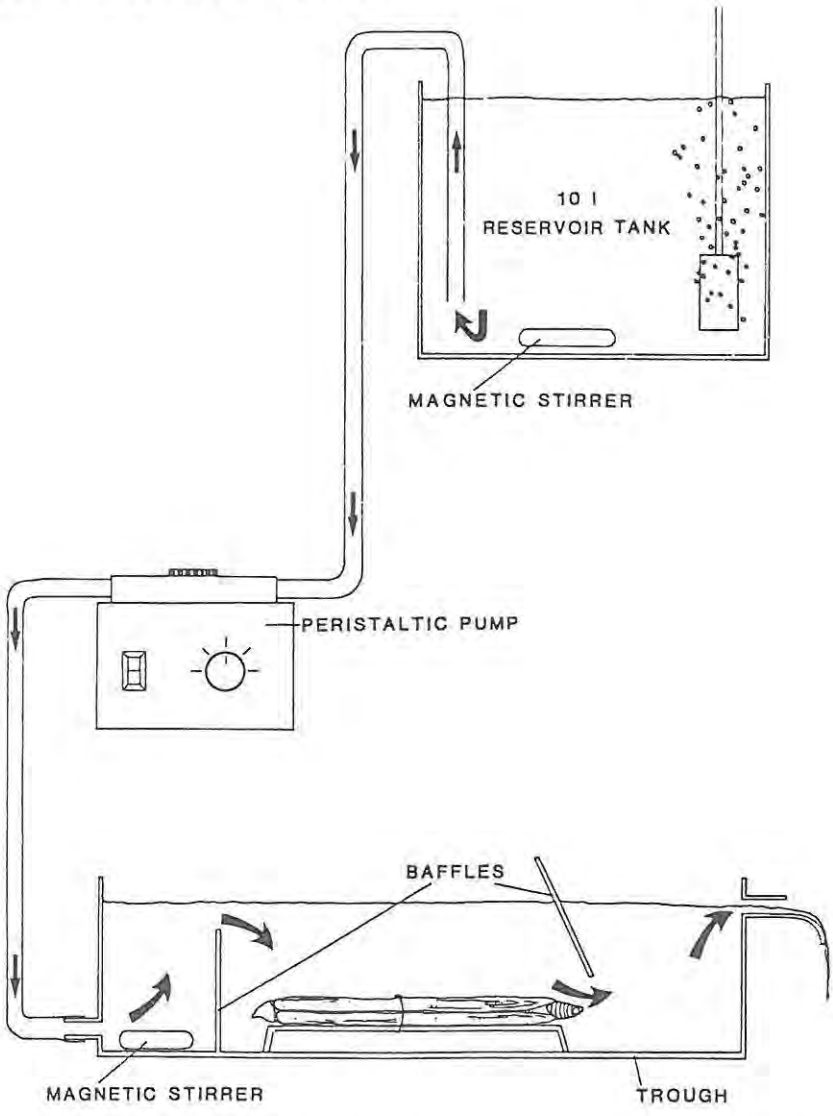


Figure 6.1: Experimental flow-through flume system, used in the determination of retention efficiency and filtration rates of *Solen cylindraceus*. Arrows indicate direction of water flow.

B. Retention efficiency

Animals of 51-60mm valve length were collected during December 1986. Aquarium temperature and salinity were initially set to match that measured in the field on the day of collection (24.2°C, 35‰). Temperature was increased a day later to 25°C. Animals were allowed a 20 day period to acclimate to laboratory conditions. After this period, holding aquaria were divided arbitrarily into 2 groups. In one set, the salinity was maintained at 35‰ while in the second group, salinity was decreased by 2.5‰ day^{-1} to 15‰. Animals in the second group were allowed a 2 day acclimation to 15‰ before being tested. Salinity of the two groups was kept constant (35 and 15‰), while temperature change was effected at a rate of $1°C\ day^{-1}$ to 15°C, with a 3 day acclimation at this temperature prior to testing.

The apparatus used (Fig. 6.1) was a modification of that of Vahl (1972a) and Riisgard (1977). Experimental flumes had internal dimensions of 130mm X 30mm X 35mm and received water via a peristaltic pump from a 10 l storage reservoir, in which suspension of particulate material was effected by aeration and magnetic stirring. An upstream baffle was introduced in the flume, to reduce the circulation effect of the magnetic stirrer required to keep the particles in suspension during flow through the flume. A second downstream adjustable baffle was displaced from the vertical to deflect the exhalent current away from the animal. A flow rate of 50ml $min.^{-1}$ was adopted as standard, which was more than twice the filtration rate calculated for *S. cylindraceus* in preliminary testing and which, in association with the structure of the flume, appeared sufficient to minimize the problem of re-circulation as discussed by Hildreth & Crisp (1976). Natural water from the Kariega estuary of 35‰ and 17‰ (adjusted to 15‰) was used, to which a "spike" of 5 X 10^6 *Tetraselmis suecica* l^{-1} was added.

Animals were taken from pots as required, thoroughly washed to remove mud and tied down onto the specimen stage. Siphons were orientated in the direction of water flow (Fig. 6.1). Animals were allowed a 10-15 minute acclimation period in the flume. During this period, there was extensive foot movement accompanied by some faecal relea e. After about 15 minutes, foot activity decreased and no further faecal release was observed. Resultant debris was removed by Pasteur pipette, after which the flume was flushed with water from the reservoir tank. Particle suspension was allowed to stabilize for a further 10-15 minutes before samples were collected for counting. The control consisted of an identical chamber run simultaneously off the same reservoir tank, the live animal being replaced by plasticine-filled *S. cylindraceus* valves of the same dimensions as the experimental animal.

The number and distribution of particles in the size range 1-10μm was determined using a Model Z_B Coulter Counter fitted with a 50μm aperture. Results were expressed as a mean of three aliquots collected per animal. This procedure was repeated for each of 33, 29, 31 and 34 animals for each of the temperature/salinity groups tested: 25°C, 35‰ ; 25°C, 15‰ ; 15°C, 35‰ ; 15°C, 15‰ respectively. The retention efficiency for the various size fractions was expressed as:

$$1 - \frac{\text{Particle count of test flume}}{\text{Particle count of control flume}}$$

(after Vahl, 1972a).

No pseudofaecal material was produced by the animals during the experiment, and any faecal ropes were removed from the flume.

Filtration rates were determined from counts obtained in the 7-8μm particle size range and calculated according to the expression from Hildreth & Crisp (1976):

$$R_f = \frac{F(C_1-C_2)}{C_1}$$

where R_f = filtration rate (ml min.$^{-1}$), F = water flow rate through the flume (ml min.$^{-1}$), C_1 and C_2 = particle concentration (number ml^{-1}) in the control and test flumes respectively.

C. Effect of suspensoid concentration on filtration rate

Animals of 51-60mm valve length were collected during late summer and transferred to laboratory aquaria, in which temperature and salinity had been set to match those measured in the field (23.8^{o}C, 35‰). Temperature was increased the following day to 25^{o}C, and animals were allowed a 3 week period to acclimate to laboratory conditions.

The apparatus used (Fig. 6.1), had in addition, a thermistor flow-meter (as described in Chapter 5), positioned in front of the exhalent siphon of the experimental animal. This allowed not only for a measure of the time spent filtering, but a more accurate determination of filtration rate, particularly at elevated suspensoid concentrations, by ensuring counts were made only during periods when the animals were active.

Suspended particulate material used, was collected from the estuary at the site of animal collection, during an incoming spring tide as previously described (Chapter 5). Water collected was filtered through a 64μm mesh, and the filtrate was concentrated by continuous centrifugation (15000rpm, flow rate of 200ml min.$^{-1}$). The resultant "slurry" served as a "stock solution" from which experimental concentrations (5, 10, 15, 20, 25, 50, 100, 250 and

500mg l^{-1}) were made. Suspension of material in the reservoir tank was effected by means of a series of magnetic stirrers, mixing propellers and aeration.

Animals were taken from pots as required, transferred to the experimental flume as previously described, and allowed a 15 minute acclimatory period during which time the flume contained aquarium water. On exposure to increased suspensoid concentrations, a 30 minute period was allowed to elapse before measurements were started, to enable the animals to adjust to the increasing concentration gradient and let particle suspension stabilize. Any faecal/pseudofaecal material produced during this time was removed. The control consisted of an identical chamber housing plasticine-filled *S. cylindraceus* valves.

Filtration rates were determined for 8 animals at each suspensoid concentration. Counts were made every 10 minutes (or whenever possible, as dictated by the animal's pumping activity). Generally 4-5 determinations per animal were made during the one hour experimental period. No animal was used more than once. Filtration rates were calculated according to the expression of Hildreth & Crisp (1976) from counts obtained in the 7-8μm particle size range and expressed in ml $min.^{-1}$. During the experimental period, water samples collected periodically from the control flume (every 15-20 minutes) were filtered onto dry, preweighed Whatman GFF glass fibre filters, dried and weighed, so monitoring suspensoid concentration. Any pseudofaecal material produced in the flume during the experimental period was carefully removed by Pasteur pipette, transferred to preweighed glass fibre filters, dried, weighed and expressed as mg h^{-1}.

Total pseudofaecal production (mg h^{-1}), filtration activity (min. h^{-1}) and filtration rates (ml $min.^{-1}$) were all expressed as a mean value obtained from all 8 animals at each suspensoid concentration.

D. Effect of temperature on filtration rate

i) Comparison of summer and winter collected animals

Animals in the size range 38-80mm valve length, were collected during summer (26°C, 36‰) and winter (16.5°C, 35‰). They were transferred to laboratory aquaria, as previously described, in which conditions had been set to match those measured in the field. In the case of the summer collected animals, salinity was adjusted to 35‰ the following day. Animals were allowed a 3 week acclimatory period.

After this period, animals were removed from the pots, thoroughly rinsed to remove any mud, and tied onto a glass platform (50mm X 10mm X 20mm high) with a thin cotton thread. They were then placed in glass jars containing 1 l of 0.45µm filtered estuarine water at the same temperature and salinity. Animals were allowed a one hour period to recover from handling. Any debris produced during this period was removed by siphoning, which resulted in approximately half the volume of water being lost. The remaining volume was made up to 2 l, with freshly filtered estuarine water (35‰ , at the appropriate temperature) containing a suspension of *Tetraselmis suecica*, to give a final concentration of 20 X 10^6 cells l^{-1}. Algal cells were maintained in suspension by a magnetic stirrer. A 10ml aliquot was removed every 30 minutes for three consecutive half-hour periods and the cell count determined immediately. After measurement, the volume of each aliquot was made back up to 10ml (generally only 1-2ml required) using a stock solution of *Tetraselmis* (concentration 20 X 10^6 cells l^{-1}), and returned to the test chamber. This prevented any significant changes in volume or cell concentration as a result of counting.

A control chamber was set up under identical conditions containing plasticine-filled *S. cylindraceus* valves of the same dimensions as the experimental animal. Filtration rate is

expressed as a mean value of the three determinations per animal, and was calculated according to Coughlan (1969):

$$m = \frac{M}{n} \frac{(\log_e C_o - \log_e C_t) - a}{t}$$

where m = filtration rate (ml $min.^{-1}$), M = volume (ml), n = number of animals, t = time (min.), C_o = initial particle concentration, C_t = final particle concentration, a = determined from the control experiment, in which no animals are present, as follows:

$$a = \frac{\log_e C_o' - \log_e C_t'}{t}$$

Only particles in the size range 6-9μm were counted, as this was the modal size range of the *Tetraselmis* culture used (Fig. 6.2). This particle size has been shown to be retained with virtually a 100% efficiency (de Villiers & Allanson, 1988). The use in these experiments of a closed, rather than the flow-through system as previously described, was a consequence of available equipment, which allowed for a greater number of determinations to be run simultaneously.

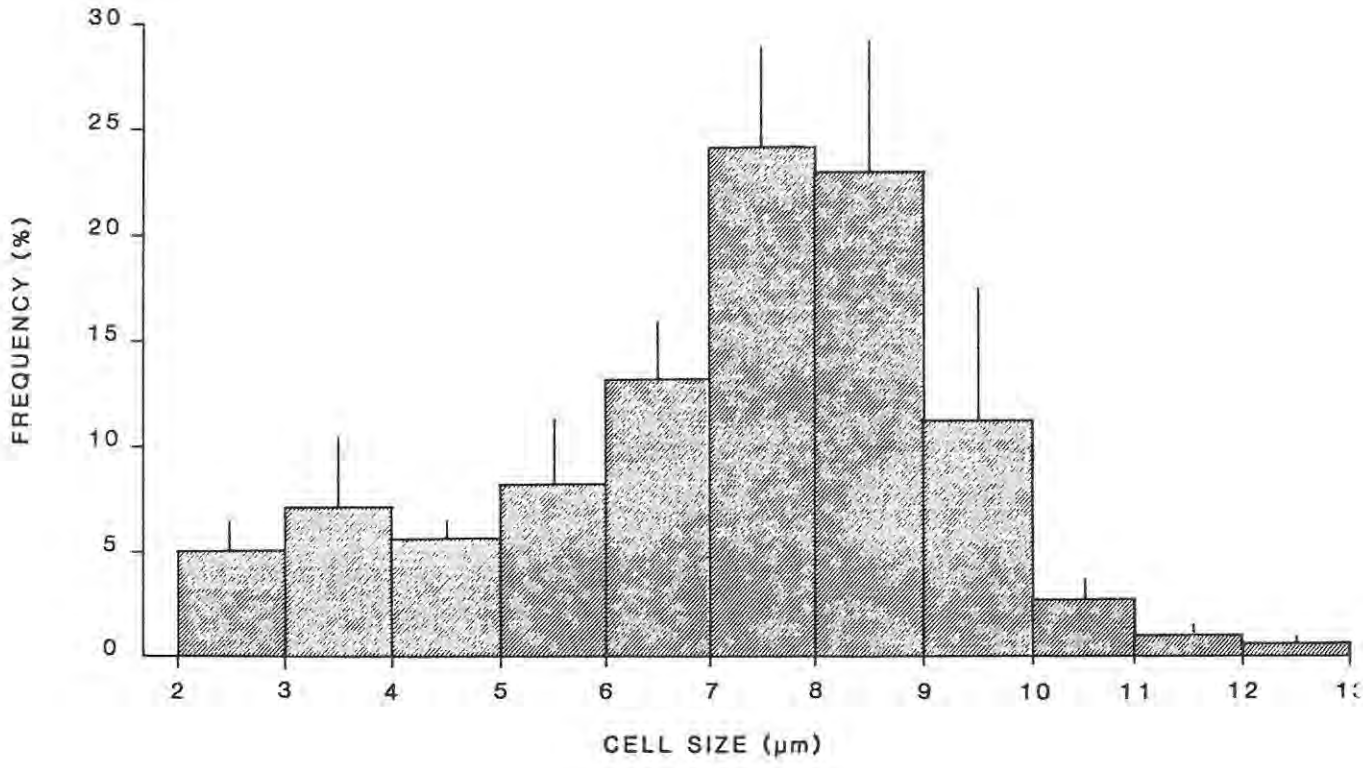


Figure 6.2: Size class frequency histogram for the *Tetraselmis suecica* culture used. Vertical bands indicate + SD.

ii) Filtration rate - temperature response

Only animals in the size range 51-60mm valve length were used in these experiments. Animal collection and transfer to laboratory aquaria were as previously described. Three experiments were performed, to determine:

1. The thermal optimum for filtration.
2. Acute responses of thermally acclimated animals, subjected to abrupt temperature changes.
3. Adjustment of filtration rate following exposure to abrupt temperature change.

The first two are dealt with together:

1 & 2. Six temperature regimes were considered (10, 15, 20, 25, 30 and 35^{o}C), and the experiments were performed in two parts. The first involved animals collected during winter (water temperature, 18^{o}C), and acclimated to temperatures of 10-25^{o}C. In the second, animals were collected in summer (water temperature, 27^{o}C) and acclimated to temperatures of 30 and 35^{o}C. Collection was as previously described. Aquaria temperature and salinity were set to match those recorded in the estuary. Animals were allowed a 3 day acclimation period, after which temperature was changed by 1^{o}C day^{-1}, until the desired experimental temperature had been reached. Animals were then allowed a 30 day acclimatory period at that temperature.

Following the acclimatory period, 10 animals were removed from each of the different experimental temperatures and their filtration rates determined in a closed system, as previously described. The final filtration rates are given as mean values, obtained from three measurements on each animal, for all 10 animals from each acclimation group. An additional 10 animals, from each of the different acclimated groups, were then removed from the pots and transferred to the experimental containers for filtration rate determinations, at each of the exposure temperatures (10, 15, 20, 25, 30, 35 and 40^{o}C). Any specific acclimated group was thus subjected to six exposure temperatures. Results are given as a mean of three determinations per animal for a total of 10 animals. No animal was used more than once.

3. Filtration rate adjustments, following exposure to abrupt changes in temperature, were determined over a 14 day period for animals of 51-60mm valve length. Animals for the winter experiment were collected during winter and early spring (water temperature 16 and 16.5^{o}C), as previously described. Laboratory temperature was set at 15^{o}C and animals were allowed a 3 week acclimatory period. Animals for the summer experiment were collected during late spring and early summer, (water temperature, 20 and 23^{o}C) and transferred to laboratory aquaria in which the temperature matched that during collection. Temperature was

increased from the following day, at a rate of $1^{o}C\ day^{-1}$ to $25^{o}C$, at which the animals were acclimated for 3 weeks. After the acclimation period, aquarium water was replaced with water at the appropriate exposure temperature, which was maintained within $\pm\ 1^{o}C$ for the duration of the experiment. Each group of animals, acclimated to either summer or winter conditions, was thus subjected to six different exposure temperatures. The control in each case, consisted of one group of 10 animals maintained at the original acclimation temperature of 15 or $25^{o}C$. Three animals were removed from each of the different temperature regimes every 12 hours for the first 216 hours (9 days), and thereafter every 24 hours to 336 hours (14 days), for filtration rate determinations, as previously described. Filtration rates are given as a mean of 3 determinations per animal for a total of three animals per time interval.

E. The effect of salinity and specific temperature and salinity combinations on filtration rate

i) Determination of the salinity optimum for filtration

Salinity optima were determined for animals of 50-60mm valve length collected during summer ($25^{o}C$, 35‰) and winter ($15^{o}C$, 35‰). Collection and transfer to the laboratory aquaria were as previously described, and animals were allowed a 7 day period to acclimate to laboratory conditions. Following this period, salinities of individual aquaria were altered at a rate of 2.5‰ d^{-1} until the desired concentrations (5, 10, 15, 25, 35, 45 and 55‰) had been reached. Salinities were held constant (± 2‰) and animals were allowed a 30 day acclimation period.

Filtration rates were determined indirectly in a closed system, as previously described, using a *Tetraselmis* culture. Only particles in the 6-9µm size range were counted. Filtration rate is expressed as a mean value of 3 determinations per animal, for 10 animals at each salinity, except for animals exposed to 5‰ , which were determined after 11 days (n = 3) for summer, and 12 days (n = 4) for winter groups, following a 70% and 60% mortality respectively.

ii) Effect of abrupt salinity change on the filtration rate of animals acclimated at different temperatures

Animals (50-60mm valve length) were collected during summer (27°C, 36‰) and winter (15°C, 35‰) and allowed a 7 day acclimation period to laboratory conditions, as previously described. Summer collected animals were divided into 3 groups. One group was maintained at 25°C, 35‰ , the second was subjected to a temperature increase of 1°C d^{-1} to 35°C with a 7 day acclimation at that temperature. The third group was kept at 25°C but subjected to a salinity decrease of 2.5‰ d^{-1} to 15‰ , and allowed a 14 day acclimation period. A similar rate of change and acclimation time was adopted for the winter collected animals, to yield a further 3 groups, acclimated to conditions of 15°C, 35‰ ; 10°C, 35‰ and 15°C, 15‰. Each acclimation group was divided into 5 batches, four of which were subjected to abrupt salinity changes (to either 5, 15, 25, 35 or 45‰) while the fifth was held constant at the acclimated combination and served as a control.

Animals were removed from the pots, and filtration rates determined indirectly in a closed system, at each of the following times:

1. on immediate exposure
2. two hourly for the first 6 h
3. six hourly to 24 h
4. twelve hourly to 72 h
5. every 24 hours to a total of 216 h.

Filtration rates are expressed as a mean of 3 determinations per animal, for a total of 5 animals at each time interval.

iii) Effect of simultaneous temperature and salinity change on filtration rate

Animals were collected during summer (23°C, 35‰) and winter (16°C, 35‰) and transferred to laboratory aquaria set at 25°C, 35‰ and 15°C, 35‰ respectively, and allowed a 7 day acclimatory period. After this period, 5 animals were removed from each of the temperature/salinity combinations and their filtration rates determined. The mean of these values was regarded as the initial or control rate. Pots containing animals were then transferred to different aquaria in which conditions had been set to: 15°C, 25‰ ; 15°C, 15‰ ; 20°C, 15‰ ; 35°C, 45‰ and 15°C, 35‰ , in the case of the summer acclimated animals, and: 10°C, 25‰ ; 10°C, 15‰ ; 20°C, 15‰ ; 25°C, 45‰ and 25°C, 35‰ , for winter acclimated animals. Filtration rates were determined at the same time intervals as in Section ii, above.

Unless otherwise stated in the text, statistical tests for validity of differences were made using Students "t".

RESULTS

A. Retention efficiency

The retention efficiency and filtration rate results are recorded in Table 6.1 and Figure 6.3. The results show a consistent pattern of particle removal by *S. cylindraceus*. A marked inflection occurred at the 2.5-3μm particle diameter range, below which retention decreased rapidly with virtually zero retention for particles of approximately 1.5μm. In all instances, the concentration of particles measured in the 1-1.5μm size range was greater in the test than in the control flume, indicating a negative retention (Table 6.1 and Fig. 6.3), and a net production of particles in this size range. Retention efficiency data are summarized in Figure 6.4, by setting the retention of the 7-8μm fractions at 100%, and expressing the clearance of the other particle size ranges proportionately. The 7-8μm size range was chosen as it gave

the highest efficiency. However, as the modal size of the *Tetraselmis* culture fell within this size fraction, it may be that this maximum is slightly elevated, if *S. cylindraceus* exhibits any possible selectivity in its feeding (cf. Chapter 7). Particles in the size range 1.5-2μm were retained with a 15-35% efficiency, those in the 2-2.5μm range with a 40-60% efficiency and those of 2.5-3μm with a 70-90% efficiency (Fig. 6.4). Above 3μm, the efficiency did not vary significantly ($p < 0.01$) over the particle size range tested.

The filtration rates determined, varied only minimally for the different salinity and temperature combinations tested (19.4 ± 2.4 to 17.8 ± 2.5 ml $min.^{-1}$; Table 6.1). It would appear that the rates at which both temperature and salinity change were effected, together with the acclimation time used, was sufficient to allow the animal to maintain near "optimal" filtration rates.

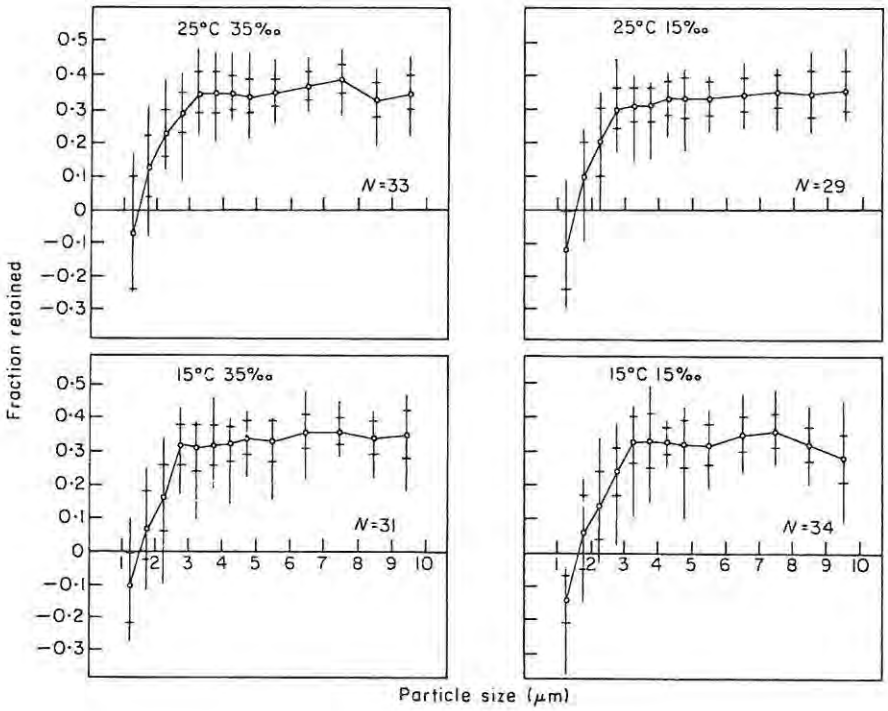


Figure 6.3: Retention efficiency, 1-(test/control), expressed as a function of particle size. O = mean values, ± SD and range. Values plotted midway of each particle size range tested.

Table 6.1: Retention efficiency of *Solen cylindraceus* at different temperature and salinity combinations *.

Particle diameter (µm)	Group 1 25°C, 35‰		Group 2 25°C, 15‰		Group 3 15°C, 35‰		Group 4 15°C, 15‰	
	$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD	$\bar{x}$	±SD
1-1.5	- 0.07	0.12	- 0.12	0.12	- 0.11	0.10	- 0.14	0.07
1.5-2	0.13	0.09	0.10	0.10	0.07	0.11	0.06	0.11
2-2.5	0.23	0.07	0.20	0.10	0.16	0.10	0.14	0.10
2.5-3	0.29	0.06	0.30	0.06	0.32	0.06	0.24	0.07
3-3.5	0.35	0.06	0.31	0.05	0.31	0.07	0.33	0.07
3.5-4	0.35	0.06	0.31	0.05	0.32	0.06	0.33	0.08
4-4.5	0.35	0.05	0.33	0.05	0.32	0.05	0.33	0.04
4.5-5	0.34	0.05	0.33	0.06	0.34	0.05	0.32	0.07
5-6	0.35	0.04	0.33	0.05	0.33	0.06	0.32	0.06
6-7	0.37	0.04	0.34	0.05	0.36	0.05	0.35	0.05
7-8	0.39	0.04	0.35	0.05	0.36	0.05	0.36	0.05
8-9	0.33	0.05	0.34	0.07	0.34	0.05	0.32	0.05
9-10	0.35	0.05	0.35	0.06	0.35	0.07	0.28	0.07
Filtration rate (ml $min.^{-1}$)	19.4	2.4	17.8	2.5	18.2	2.3	18.2	2.6
No. animals	33		29		31		34	

* Values expressed as 1-(number of particles in test flume/number of particles in control flume). Mean ± SD given for each particle size range tested, and for filtration rates. Filtration rates were calculated from the 7-8µm size range.

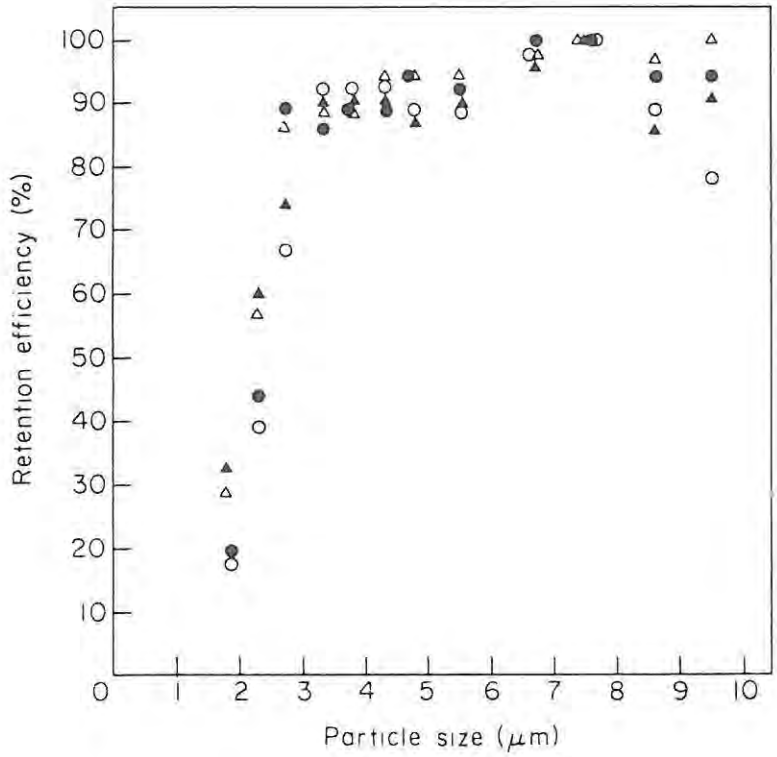


Figure 6.4: Percentage retention of particles by *Solen cylindraceus*. Retention of the 7-8μm size fraction taken to be 100%. Note that the negative retention values for the 1-1.5μm size range are not shown. ▲ 25°C, 35‰ ; △ 25°C, 15‰ ; ● 15°C, 35‰ ; ○ 15°C, 15‰ .

B. Effect of suspensoid concentration on filtration rate

The filtration activity, filtration rate and total pseudofaecal production at different suspensoid concentrations, are shown in Figure 6.5. The results are expressed as a mean value (± SD) for each group of animals (n=8). At suspensoid concentrations of 5-100mg l^{-1}, no significant difference was recorded in either filtration rate (range 18.73 ± 1.90 to 20.78 ± 2.06 ml $min.^{-1}$) or total filtering activity (range 83.3 ± 14 to 96.2 ± 6.22% filtering time). However, a significant decrease in filtration activity was recorded at 250 and 500mg l^{-1} ($p < 0.001$). A similar difference ($p < 0.001$) was recorded in filtration rate at suspensoid concentrations of

250 and 500mg l^{-1}. A significant increase in pseudofaecal production occurred at 50 (10.28 ± 4.37mg h^{-1}) and 100mg l^{-1} (47.16 ± 17.74mg h^{-1}) ($p < 0.001$), after which, however, these values levelled off somewhat, with no significant difference being recorded between 100, 250 and 500mg l^{-1} suspensoid concentrations. From the results obtained above and a knowledge of the suspensoid concentrations to which the animals were subjected, the theoretical ingestion rates were calculated, and are shown in Figure 6.5. Theoretical ingestion rates demonstrated a consistent increase from 5.6mg h^{-1} (at suspensoid concentrations of ca. 5mg l^{-1}) to 44.2mg h^{-1} (at suspensoid concentrations of 50mg l^{-1}) after which it remained fairly constant, through 100 to 500mg l^{-1} suspensions (46.5 and 42.9mg h^{-1} respectively).

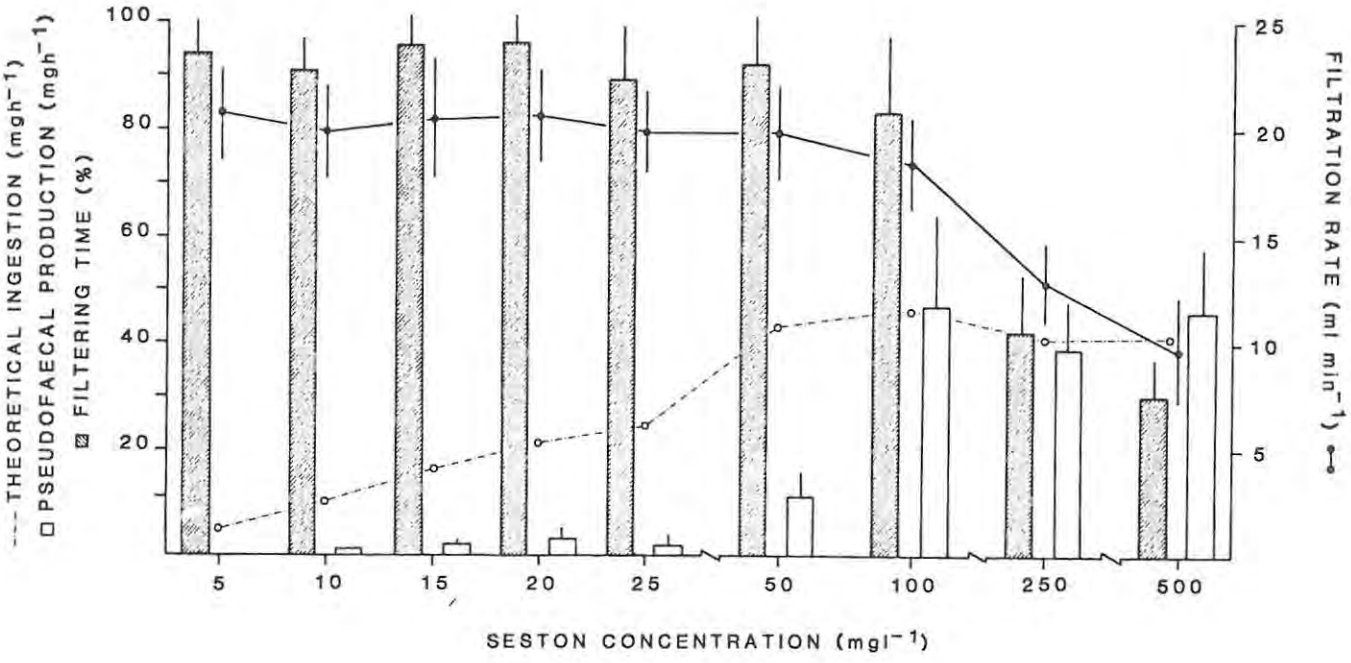


Figure 6.5: The effect of different suspensoid concentrations on filtration rate, filtration time and pseudofaecal production. The theoretical (calculated) ingestion rate is shown (O- -O).

C. Effect of temperature on filtration rate

i) Comparison of summer and winter collected animals

Filtration rates for summer and winter collected animals are shown in Figure 6.6. Fitted power regressions demonstrated almost linear and proportional increases in filtration rate with size. Analysis of variance indicated a significant difference between summer and winter collected groups ($p = 0.000$; F statistic = 31.23). However, a maximum difference of only 3.5ml $min.^{-1}$ was recorded between the regression lines, the biological significance of which, is debatable. The regression equations and values are given in Figure 6.6.

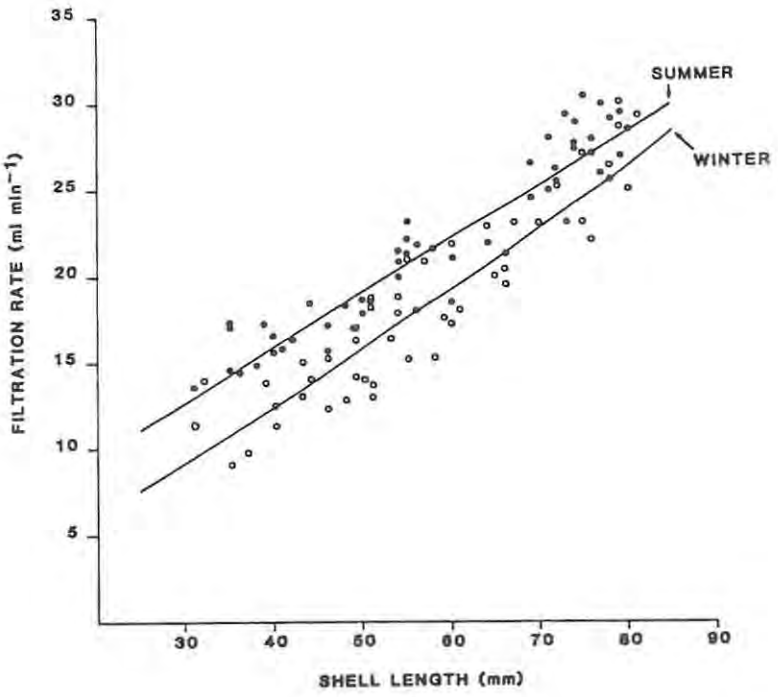


Figure 6.6: Filtration rates of *Solen cylindraceus* as a function of shell length. (●) Animals collected during summer (26°C) ($n = 50$) $y = 0.758x^{0.826}$ ($R^2 = 0.889$). (o) Animals collected during winter (16.5°C) ($n = 49$) $y = 0.247x^{1.066}$ ($R^2 = 0.823$).

ii) Filtration rate-temperature response

The filtration rates of animals acclimated to specific temperatures and exposed to different temperatures, are shown in Figure 6.7 A and B, and Table 6.2. The acclimated filtration rate-temperature curve (Fig. 6.7 A) indicated a thermal optimum for filtration rate over the range 15-35^{o}C, with a maximum of 22.86 ± 4.36 ml min.$^{-1}$ recorded at 25^{o}C. The 15^{o}C and 25^{o}C results compare favourably with the filtration rates measured for summer and winter collected animals of the same size in the previous experiment (Fig. 6.6). The filtration rate values for 40^{o}C, shown in Figure 6.7 A, were obtained by taking a mean value of the last five days data from the 40^{o}C exposure results (Fig. 6.8 B).

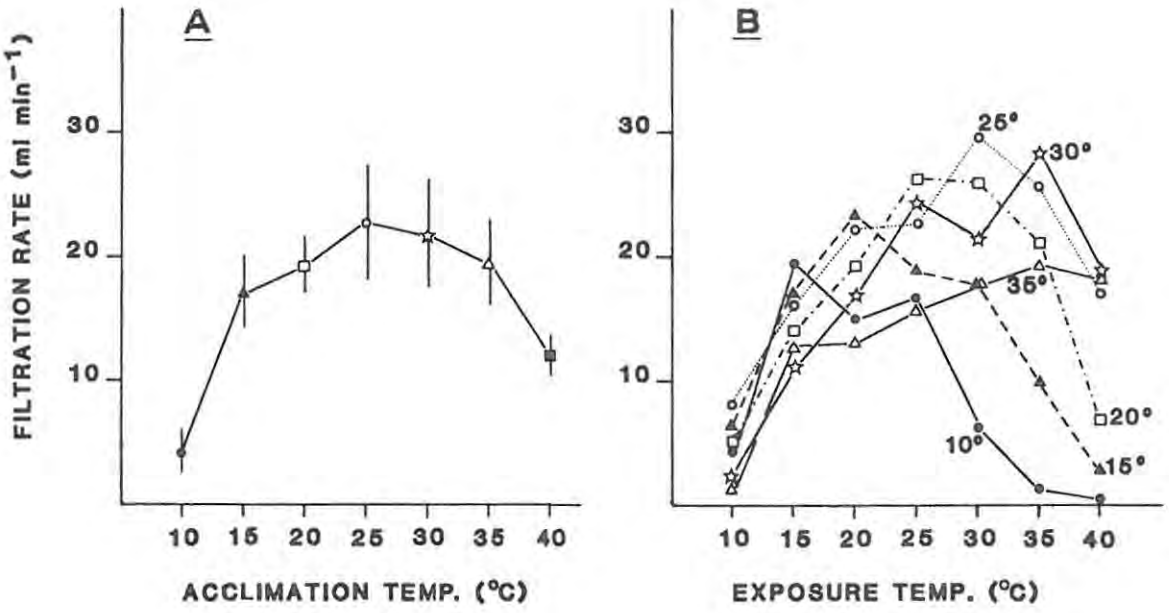


Figure 6.7: A. Acclimated filtration rate of *Solen cylindraceus* (50-60mm shell length). B. The acute filtration rate response of animals acclimated to specific temperatures (indicated on graph) and exposed to different temperatures. To avoid cluttering, standard deviations from the mean are not shown in the figure, see Table 6.2.

Table 6.2: Filtration rates (ml $min.^{-1}$) for groups of *Solen cylindraceus* (50-60mm shell length) acclimated to temperatures of 10-35°C and exposed to temperatures of 10-40°C. Control values for each group are shown in bold. Values for each group are a mean ± SD from 10 animals, three determinations per animal.

Acclimated Temperature	Exposure Temperature 10°C	15°C	20°C	25°C	30°C	35°C	40°C
10°C	**4.20 ± 1.43**	19.54 ± 3.10	14.95 ± 5.60	16.85 ± 4.74	6.10 ± 2.92	1.33 ± 1.35	0.46 ± 0.63
15°C	6.30 ± 2.15	**7.14 ± 2.57**	23.58 ± 3.93	18.88 ± 4.89	17.78 ± 5.01	9.93 ± 3.29	2.69 ± 2.16
20°C	4.99 ± 2.50	13.94 ± 3.17	**19.10 ± 2.06**	26.32 ± 4.50	26.31 ± 3.13	21.02 ± 3.87	6.73 ± 3.10
25°C	8.03 ± 2.56	16.32 ± 3.84	22.48 ± 5.10	**22.86 ± 4.36**	29.88 ± 3.68	25.85 ± 3.64	17.15 ± 4.72
30°C	1.88 ± 1.70	11.04 ± 4.09	16.98 ± 4.45	24.35 ± 5.03	**21.81 ± 4.13**	28.47 ± 3.74	18.25 ± 5.28
35°C	1.28 ± 1.27	12.76 ± 4.00	13.37 ± 2.92	15.85 ± 3.57	17.66 ± 1.64	**19.58 ± 3.61**	18.23 ± 2.17

Maximum filtration rates in most cases were recorded at the exposure temperature immediately above that to which the animals were acclimated (Fig. 6.7 B and Table 6.2). This held for all animals acclimated to temperatures of 10-30°C. However, animals acclimated to 35°C, when exposed to 40°C, exhibited a slight decrease in filtration rate (Fig. 6.7 B). The rate-temperature curve of animals acclimated to 10°C showed a substantial increase in filtration on sudden exposure to temperatures of 15, 20 and 25°C, followed by a rapid decrease as temperature increased further, with virtually no filtering activity recorded at 40°C (Fig. 6.7 B). A similarly shaped curve was described for animals acclimated to 15 and 20°C,

but with slightly elevated filtration rates recorded. Animals acclimated to 25, 30 and 35°C all showed high filtration rates at elevated exposure temperatures. There was a lateral shift of the rate-temperature curve to the right, following acclimation to warmer temperatures (20-35°C). In all instances, temperature had a pronounced effect upon filtration rate below 15°C and in most cases above 30-35°C.

Results for the adjustment of filtration rate by *S. cylindraceus*, acclimated to winter (15°C) and summer (25°C) conditions, and exposed to different temperatures are shown in Figure 6.8 A and B respectively. In both instances, the controls are indicated as a single mean ± SD. Filtration rates of animals acclimated at 15°C, and exposed to 20, 25 and 30°C (Fig. 6.8 A) all increased initially, but decreased and levelled off after approximately 144 hours at somewhat more than the control rate. Initial decreases in filtration rate were recorded at exposure temperatures of 35 and 40°C. At 35°C, filtration rate gradually increased and levelled off at approximately the control value after 180 hours, while those measured at 40°C, stabilized substantially lower. On exposure to 10°C, filtration rate decreased markedly and remained depressed for the duration of the experiment (Fig. 6.8 A). Animals acclimated to 25°C (Fig. 6.8 B) showed an initial increase in filtration rate on exposure to temperatures of 30 and 35°C, which gradually decreased after approximately 156-168 hours to that of the control (ca. 22 ml $min.^{-1}$). No substantial variation from the control value occurred at 20°C. Exposure to 15°C resulted in an initial decreased filtration rate which gradually increased and levelled off at approximately the same level as the 15°C control value in Figure 6.8 A (ca. 17 ml $min.^{-1}$). Exposure to 40°C was characterized by only a slight initial decrease in filtration rate which gradually declined further, eventually stabilizing substantially lower than the control value, but not dissimilar from the 40°C value recorded in Figure 6.8 A. A decrease in temperature to 10°C again resulted in a permanently depressed rate of filtration.

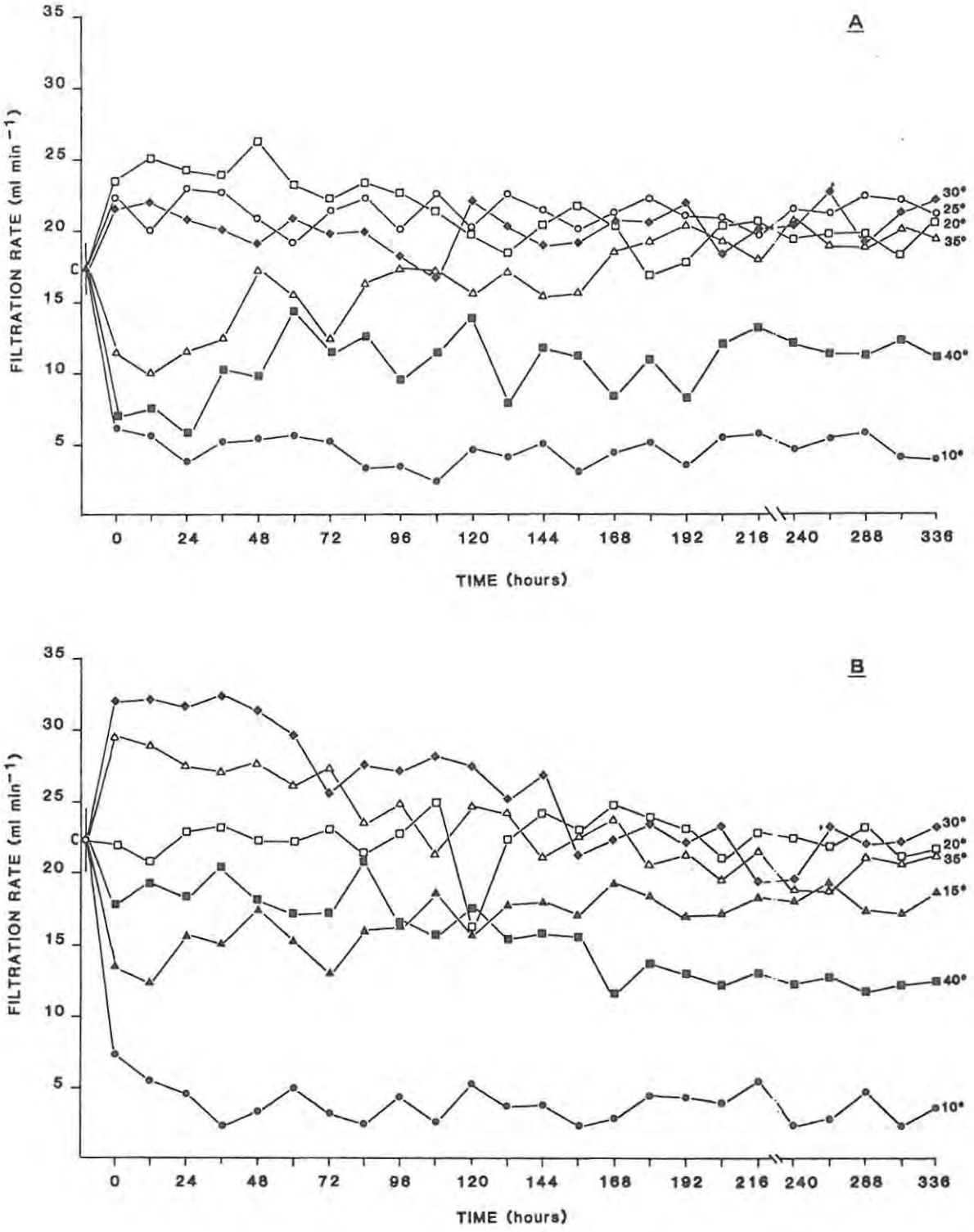


Figure 6.8: Filtration re-acclimation rate of *Solen cylindraceus* acclimated to A. winter (15°C) and B. summer (25°C) conditions, and exposed to different temperatures. C = control value ± SD of animals maintained at the acclimated temperature. Error bars are excluded to avoid cluttering. At no point is the SD greater than ± 2.96.

D. The effect of salinity and specific temperature and salinity combinations on filtration rate

i) Salinity optimum for filtration

Figure 6.9 demonstrates maximum filtration rates of 19-23 ml $min.^{-1}$ in the salinity range of 15-45‰ for both summer and winter acclimated animals. An analysis of variance indicated that salinity significantly influenced filtration rate ($p < 0.01$), but not within the optimal range (15-45‰). At all salinities, season had a significant effect ($p < 0.003$), and winter values were consistently lower than summer rates (range of 1-3 ml $min.^{-1}$), except at 55‰ , where the trend was reversed (Fig. 6.9).

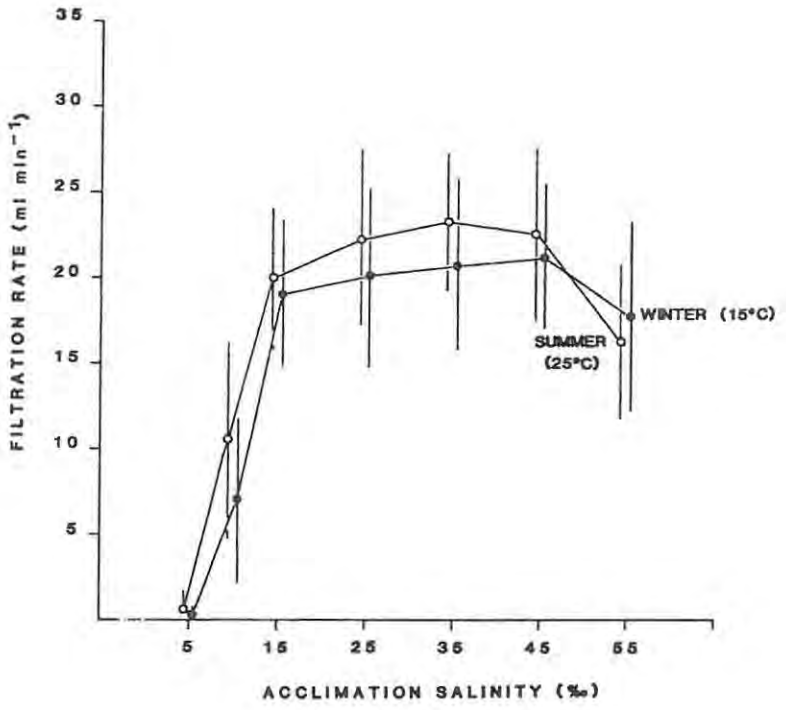


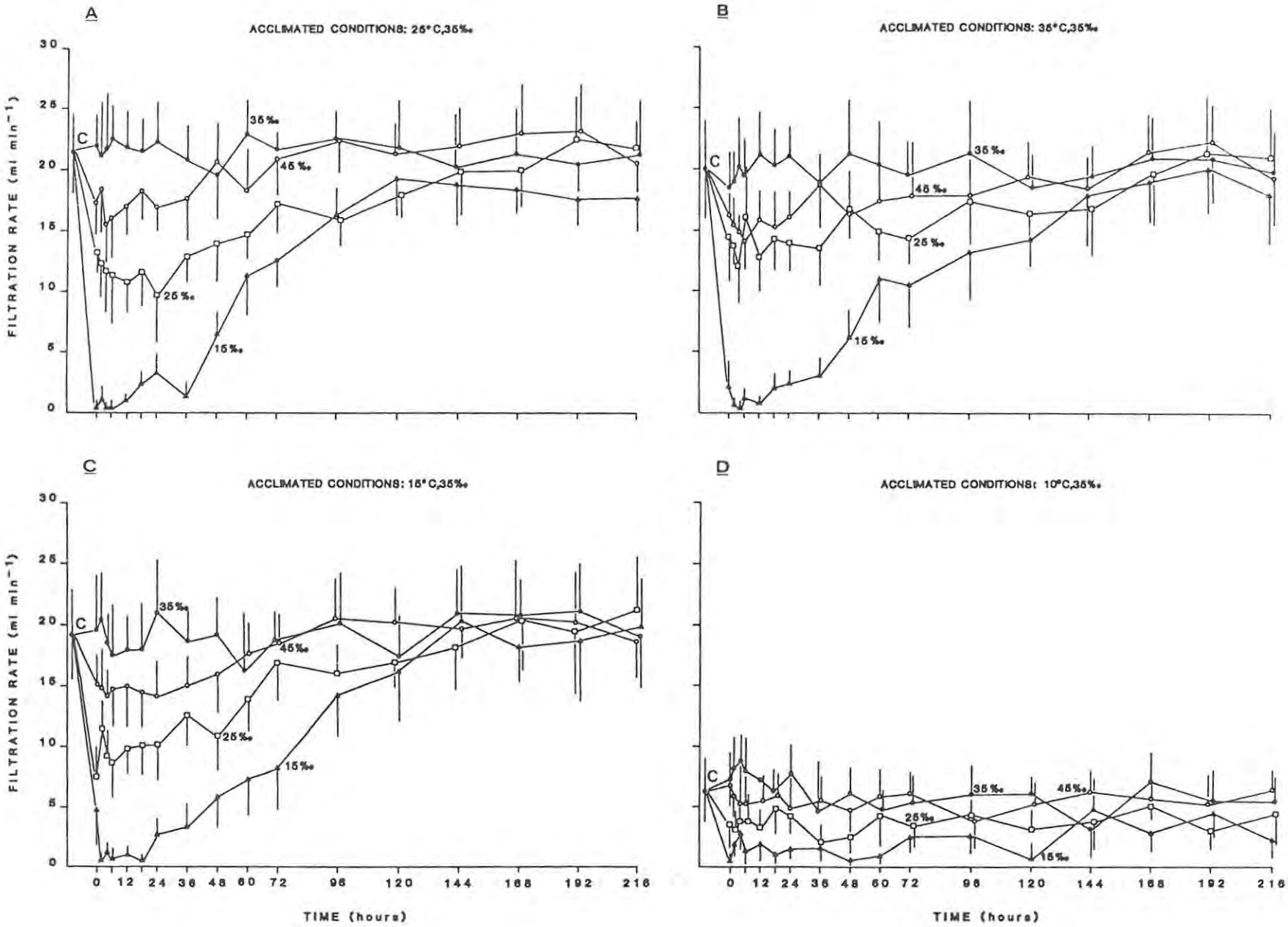
Figure 6.9: Filtration rates of *Solen cylindraceus*, collected during summer and winter, and acclimated to different salinities. Each data point is a mean value of 3 determinations for each of 10 animals, except at 5‰ where only 3 and 4 animals for summer and winter groups respectively were used - a result of high mortality at this salinity.

ii) Effect of abrupt salinity change on the filtration rate of animals acclimated at different temperatures

Animals acclimated to 35‰ , and temperatures of 15, 25 and 35°C all exhibited an initial decrease in filtration rate compared to the control values when exposed to different salinities (Fig. 6.10 A-C). The level to which filtration rate was suppressed, was dependent on the magnitude of salinity change. In all instances, the effect of hypersaline conditions (45‰ , a 10‰ increase) was less marked than hyposaline conditions (25‰ , a 10‰ decrease). However, within 12-24 hours, filtration rates increased to approximately those of the control animals; the increase occurred more rapidly in hypersaline conditions of 45‰ (48-60 hours) than in hyposaline conditions of 25‰ (120-140 hours) and 15‰ (144 hours). Animals held at 10°C (Fig. 6.10 D) all exhibited a consistently depressed rate of filtration (< 10 ml $min.^{-1}$) at all salinities.

Animals acclimated to 15‰ , when exposed to increased or decreased salinities (Fig. 6.11 A & B) showed an initial depression of filtration rate. Animals subjected to 10‰ (a decrease of only 5‰) showed the greatest reduction in filtration rate, which remained depressed at ± 10 ml $min.^{-1}$ for the duration of the experiment. By contrast, the filtration rates of animals exposed to increased salinities returned to approximately the control rates within 60-96 hours. Results for animals exposed to 5‰ are not shown, as their filtration rates were never greater than 1 ml $min.^{-1}$ and furthermore, insufficient animals survived after 96-120 hours to continue the experiment at this salinity.

Figure 6.10: A-D. Filtration rate response (mean, + or - SD) of *Solen cylindraceus*, acclimated to 35‰ salinity and different temperatures, subjected to abrupt salinity changes (indicated on graph). C = control value, overall mean ± SD of animals maintained at 35‰.


A
ACCLIMATED CONDITIONS: 25°C,35‰
B
ACCLIMATED CONDITIONS: 35°C,35‰
C
ACCLIMATED CONDITIONS: 15°C,35‰
D
ACCLIMATED CONDITIONS: 10°C,35‰
FILTRATION RATE (ml min⁻¹)
TIME (hours)
C
35‰
45‰
25‰
15‰
0 12 24 36 48 60 72 96 120 144 168 192 216
0 5 10 15 20 25 30

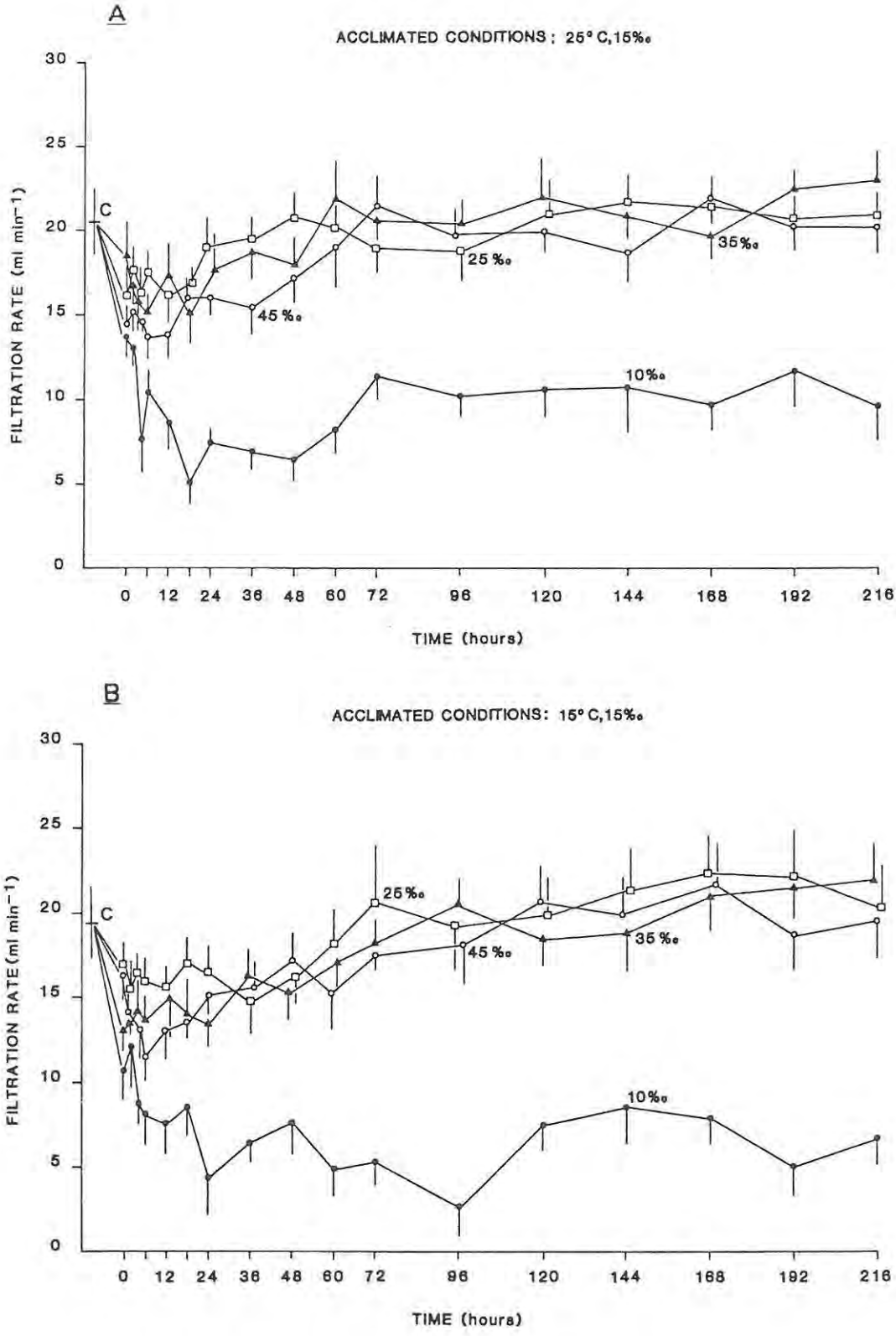


Figure 6.11: A & B. Filtration rate response (mean, + or - SD) of *Solen cylindraceus*, acclimated to 15‰ salinity at 25°C (A) and 15°C (B), to abrupt salinity change.

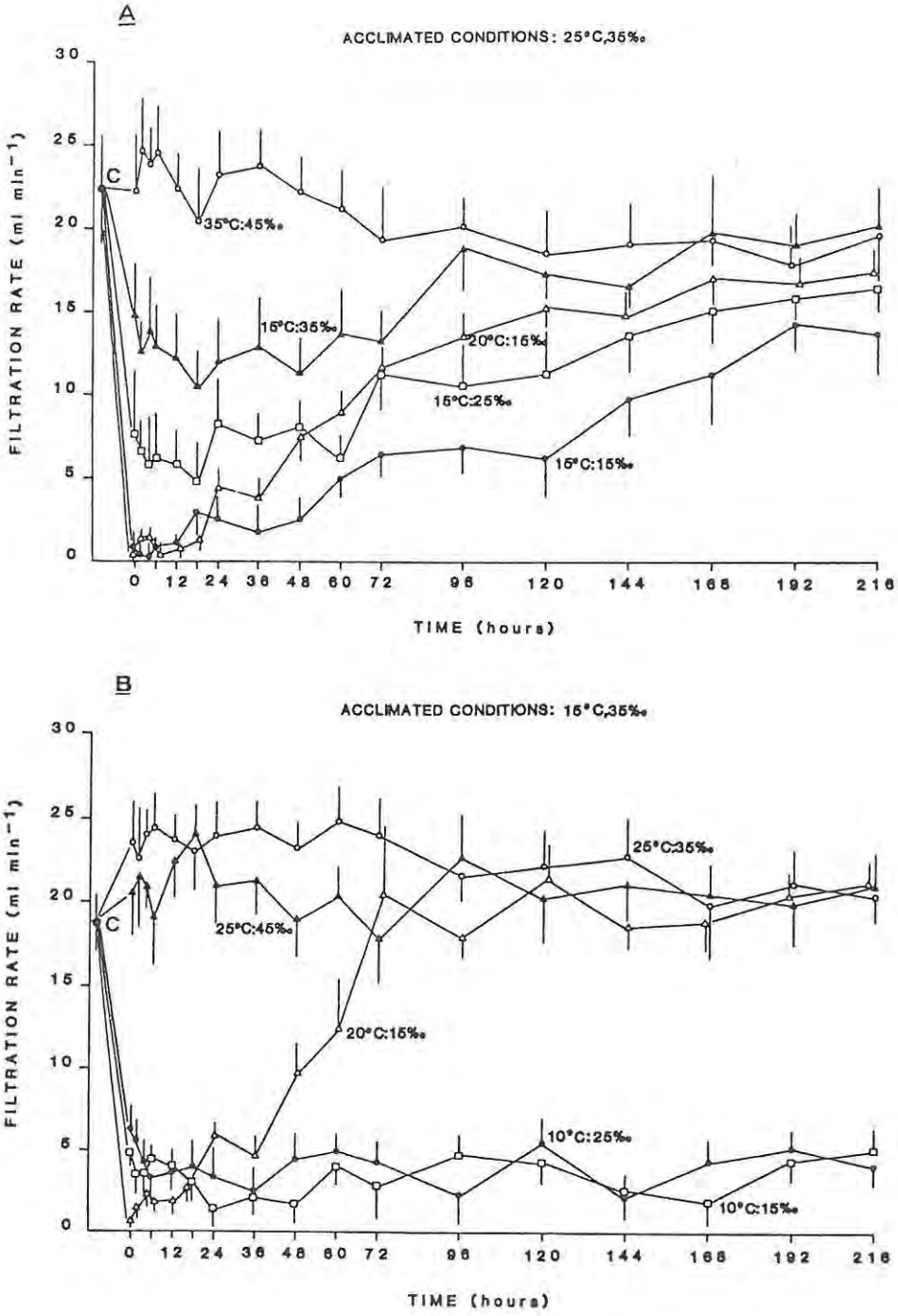


Figure 6.12: A & B. Filtration rate response (mean, + or - SD) of *Solen cylindraceus* acclimated to "standard" summer (A) and winter (B) conditions, to simultaneous, abrupt, temperature and salinity change.

Figure 7.3: Variation in suspensoid concentration (stn. L), measured during neap tides at different positions in the intertidal zone. o—·—o , stn. 1, mid mud bank; ▲—▲ , stn. 2, immediately inshore of the *Zostera*; □·····□ , stn. 3, river side of the *Zostera*; ●—● , stn. 4, centre stream. Wind speeds are indicated as a mean value ± SD for each sampling period.

iii) Effect of simultaneous temperature and salinity change on filtration rate

The filtration rate responses of summer and winter acclimated animals to simultaneous temperature and salinity change are shown in Figure 6.12 A and B. Control values for summer and winter conditions are indicated as a single mean ± SD in the figures with values of 22.39 ± 3.28 and 18.96 ± 1.31 ml $min.^{-1}$ respectively. Exposure to conditions of increased temperature and salinity resulted in an initial increase in filtration rate, which eventually declined (72-96 hours) to approximately that of the control. The response of summer acclimated animals (Fig. 6.12 A) to conditions of lowered temperature coupled with unchanged or decreased salinity, was an immediate and rapid decrease in filtration. Filtration rate remained depressed for 12-24 hours after which it gradually recovered. In most cases however, filtration rates had not fully stabilized by the end of the experiment (216 hours). The response of winter acclimated animals (Fig. 6.12 B) subjected to 10°C and 15 or 25‰ , was a suppressed rate of filtration which persisted for the duration of the experiment. Although exposure to 20°C and 15‰ resulted in an initial depression of filtration, the rate had returned to its original value within 72 hours.

DISCUSSION

Jørgensen (1960) commented on the often conflicting filtration rate results obtained for bivalves, even of the same species, by different workers. Such contradictory estimates of water transport, he attributed principally to different experimental techniques employed and animal sensitivity. Further, Jørgensen (1975b) suggested that many of the data obtained are often of restricted value, due to the uncertainty of laboratory results reflecting unimpeded activity in nature. In the series of experiments presented here, while every attempt was made to maintain conditions as natural as possible, animal disturbance is a real and valid criticism and the results obtained should be viewed with this in mind. However, a clearer understanding of the interactions of environmental variables may be achieved by laboratory

experiments, in which conditions can be precisely controlled. It is possible that the collection, concentration and subsequent resuspension, could have affected the quality of the suspended material used in these experiments, which in turn may have resulted in an altered response by the animal. Nevertheless, the results presented here are, I feel, a reasonable reflection of the natural response of *Solen cylindraceus* to the imposed conditions and, further, that a measure of filtration rate is a useful indication of the animal's potential to accommodate and adapt to fluctuations in environmental conditions.

A. Retention efficiency

Solen cylindraceus exhibited a consistent pattern of particle removal with a marked inflection occurring at approximately the 3μm diameter particle size, below which retention decreased rapidly (Fig. 6.4). However, a negative retention (net production of particles) was recorded in all instances in the 1-1.5μm size range (Fig. 6.3). Vahl (1972a) also recorded a negative retention of smaller particles by *Mytilus edulis*. Møhlenberg and Riisgard (1978) noted occasional negative retention for both *M. edulis* and *Pecten opercularis*, which they regarded as anomalous and due to the disturbance caused by the movement of the animal. In the case of *S. cylindraceus*, the nature and origin of these particles are unknown. They may be due to valve and foot movements, which result in the dislodgement of smaller fragments and possibly bacteria from larger particles. The effect of this increase in the number of smaller particles, would be to decrease the observed fraction of particles which may be retained from the natural seston, with the result that the lower limit of particle retention in *S. cylindraceus* is still uncertain.

Table 6.3 compares the smallest particle sizes retained completely by a number of bivalve species. The size limit for complete retention of particles varies between species, and many workers (e.g. Jørgensen, 1966; Dral, 1967; Haven & Morales-Alamo, 1970; Moore, 1971) have

suggested this to be a function of the gill ciliary component and osteal size. While the effect of osteal size cannot be commented on in this study, (as gill preparations were stretched, cf. Chapter 2, Fig. 2.7 A), the intercirral distance observed for *S. cylindraceus* (2.5-3.0µm, Fig. 2.6 C), is in good agreement with the retention efficiency measured. Similar correlations of particle retention and ciliary structure have been recorded by Jørgensen (1955), Tammes & Dral (1955), Davids (1964), Dral (1967) and Vahl (1972b).

Table 6.3: Comparison of particle size limit for 100% retention in different bivalve species.

Species	Minimum size for complete retention (µm)	Author(s)
Mytilus edulis	4	Vahl (1972a), Jørgensen (1975a)
Ostrea edulis	4	Møhlenberg & Riisgard (1978)
Cardium edule	3	Møhlenberg & Riisgard (1978)
Crassostrea virginica	3	Haven & Morales-Alamo (1970)
Mya arenaria	3-4	Møhlenberg & Riisgard (1978)
Aulacomya ater	4	Stuart & Klumpp (1984)
Perna perna	4	Stuart & Klumpp (1984)
Choromytilus meridionalis	4	Stuart & Klumpp (1984)
Chlamys opercularis	6-7	Møhlenberg & Riisgard (1978)
Chlamys septemradius	6-7	Møhlenberg & Riisgard (1978)
Mactra lilacea	10.9	Matthews, Lucas, Stenton-Dozey & Brown (1989)
Donax serra	4.8	Matthews, Lucas, Stenton-Dozey & Brown (1989)
Solen cylindraceus	3-3.5	Present study

However, retention efficiencies have been shown to vary, and it has been suggested (Davids, 1964; Griffiths & Griffiths, 1987) that the ability to adjust retention efficiency is a consequence of ciliary beat and muscular contraction of the gills, so altering osteal size and interciliary spacing. Recently, Jørgensen (1975a; 1983) emphasized that retention efficiency is not a simple straining process, but depends upon the intergrated activity of all the ciliary systems and the fluid mechanical aspects of the gills.

Solen cylindraceus, in common with other bivalves, effectively retains particles over a wide spectrum of diameters (Table 6.3). A lower limit of 2.5μm (40-60% retention) will allow nanoplankton and bacterial floc assemblages to contribute, along with microplankton, to the diet. Particles of less than 2.5μm diameter are, volumetrically, a significant fraction of the filtrate (Fig. 6.13). If this fraction contains potential food particles, e.g. picoplankton or bacteria, in sufficient quantities, then its relative "unavailability" to *S. cylindraceus* would constitute a potential loss of some significance. However, Allanson & Read (1987) reported bacterial cell concentrations of 9.8 ± 8.82 X 10^5 ml^{-1} (n=260) for the Kariega estuary. At this density, it seems unlikely that bacteria would constitute an important food source to *S. cylindraceus*, as loss of bacterial carbon is estimated from these data to be approximately 1.5 mg C $individual^{-1}$ $tide^{-1}$ (cf. Chapter 8). Further, we are unaware of other picoplankton components in any significant quantity in the Kariega estuary (Allanson & Read, 1987; Grange, pers. comm.).

Solen cylindraceus shares the intertidal mud flats with the anomuran prawn, *Upogebia africana* (cf. Chapter 3), another suspension feeder. Nothing is known of the food selectivity spectrum of this species. Despite the abundance of *S. cylindraceus* in the Kariega estuary, it is estimated that the population only filters some 3-4% of the tidal volume per tide. The

likelihood of any significant resource depletion, as described by Wright, Coffin, Ersing & Pearson (1982) and Peterson & Black (1987) for communities of bivalves with higher filtration rates is unlikely. However, no other suspension or deposit feeder besides *U. africana* has established itself in any great number in the regions of the greatest *S. cylindraceus* density (Hodgson, 1987).

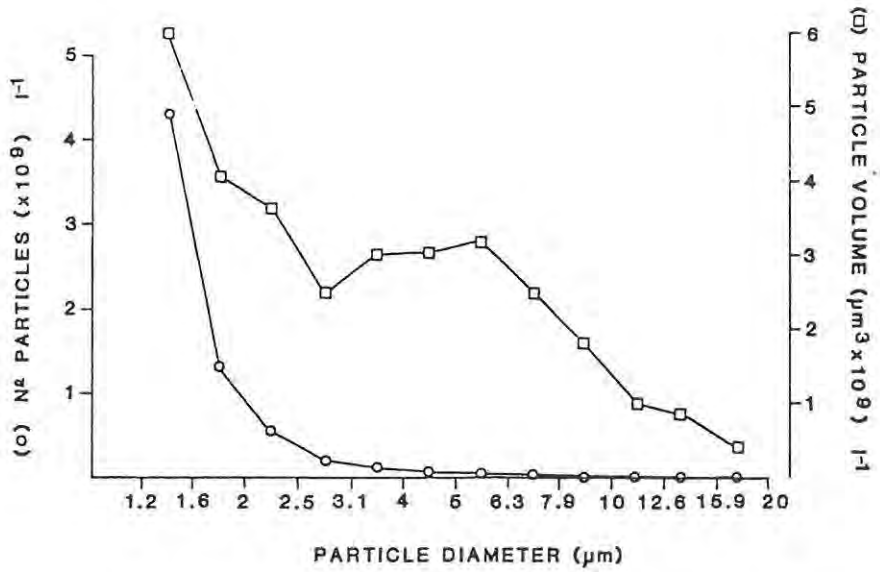


Figure 6.13: Particle size distribution of the Kariega estuary (at the site of animal collection) in terms of both number (o) and volume (□) (after Grange, unpublished results).

B. Effect of suspensoid concentration on filtration rate

There is an abundance of data in the literature dealing with the relationship between the concentration of suspended material, filtration rates and ingestion in bivalve molluscs, with much discrepancy among the results reported (e.g. Theede, 1963; Davids, 1964; Hughes, 1969; Winter, 1969; 1970; 1973; 1977; Ali, 1970; Thompson & Bayne, 1972; 1974; Mathers, 1974b; Foster-Smith, 1975a; 1975b; 1976; Schulte, 1975; Bayne, Thompson & Widdows, 1976; Griffiths & King, 1979; Widdows, Fieth & Worrall, 1979; Griffiths, 1980; Kiørboe, Møhlenberg & Nøhr, 1980;

Winter, Acevedo & Navarro, 1984). However, many of the apparently conflicting results may, in part, be explained by the different techniques, concentration ranges and particle sizes used by the various authors (Griffiths & Griffiths, 1987). Despite some variation, a general picture describing a bivalve's response to increasing particle concentrations emerges, which tends to follow the 3 phases as proposed by Winter (1977):

1. A rapid increase in filtration rate is initiated in response to particle concentrations exceeding some low threshold.

2. A plateau phase extending over optimal feeding levels.

3. A period of decreasing filtration rate as suspensoid concentration increases further, coinciding with increased pseudofaecal production.

Compensation for changes in the concentration of suspended particulates through regulation of filtration rate (*per se*), by bivalves in general, has been questioned (see reviews by Jørgensen, 1975b and Winter, 1978). As optimal ingestion rations may be effected by a variety of other variables besides filtration rate, e.g. filtration activity, pseudofaecal production, as well as behavioural and environmental factors (e.g. Walne, 1972; Foster-Smith, 1976; Mathers, 1976; Epifianio & Ewart, 1977; Winter, 1978; Robinson & Langton, 1980), the importance of not viewing the relationship between suspensoid concentration and filtration rate in isolation, has been stressed (Foster-Smith, 1975a; Palmer, 1980; Griffiths & Griffiths, 1987).

From an examination of filtration rate as a function of suspensoid concentration (Fig. 6.5), it appears that *S. cylindraceus* would follow Winter's (1977) conceptual theme. However, as animals were never held in particle-free water or starved prior to testing (e.g. Davids, 1964; Thompson & Bayne, 1972; Griffiths, 1980), the lower threshold of particle concentration which may initiate an increased filtration rate, has not been determined. *Solen cylindraceus* exhibited optimal filtration rates (the plateau phase - Winter, 1977) over a fairly wide range of particle concentrations (5-100mg l^{-1}). Indeed, within this range, filtration rate appeared

independent of particle concentration, and only decreased significantly once a certain threshold (>100mg l^{-1}) was exceeded. Similar responses in other bivalves have been recorded by the majority of workers, but over a substantially narrower concentration range (e.g. Davids, 1964; Dral, 1967; Winter, 1973; Thompson & Bayne, 1974; Foster-Smith, 1975a; Bayne, Thompson, & Widdows, 1976). As previously discussed (cf. Chapter 5), *S. cylindraceus* exhibited a filtration activity (time spent filtering) which was dependent on the concentration of suspended material to which it was exposed. Indeed, both the *in situ* (Chapter 5) and *in vitro* (this study) filtration activity results are very similar and mutually reinforcing (Figs. 5.3, 5.4 & 6.5). Filtration activity remained relatively consistent (85-95% of the time) up to a particle concentration of approximately 100mg l^{-1}, after which it decreased significantly together with filtration rate, as suspensoid concentrations increased further. A reduction in filtration activity and/or a decrease in filtration rate as a consequence of increased suspensoid concentration has been reported for bivalves by numerous authors (e.g. Loosanoff & Engle, 1947; Ali, 1970; Winter, 1970; 1977; 1978; Mathers, 1974b; Schulte, 1975; Foster-Smith, 1976; Theissen, 1977; Griffiths, 1980; Wilson, 1983; Griffiths & Griffiths, 1987).

The marked decrease in both filtration rate and filtration activity at elevated suspensoid concentrations, was paired with a substantially increased rate of pseudofaecal production. However, it is interesting to note that as suspensoid concentration increased (above ca. 100mg l^{-1}) the amount of pseudofaecal material produced remained relatively constant. This is interpreted as a function of the decrease in both filtration rate and activity - whether this is a physiological response by the animal, or simply a result of an "overloading" of the gill sorting mechanism is unclear (see also Owen, 1974a; Owen & McCrae, 1976). In either instance, the final result is to effectively limit the total volume of material taken in by the animal. It would appear that for *S. cylindraceus* used in these experiments (50-60mm valve

length), a suspensoid concentration of between 50-100mg l^{-1} was probably close to the maximum capable of being sorted by the gills, and consequently the pseudofaecal production threshold would lie within this concentration range.

It is important for filter feeders to maintain a steady and maximum rate of ingestion (Foster-Smith, 1975a). The general picture is that ingestion rates increase with increasing suspensoid concentration until a threshold is reached, above which further material filtered by the gills is carried away and rejected as pseudofaeces (Haven & Morales-Alamo, 1966; Widdows *et al.*, 1979; Griffiths & Griffiths, 1987). *Solen cylindraceus* follows this general trend, reaching a maximum ingestion rate (ca. 40-45 mg h^{-1}) at a suspensoid concentration of approximately 50mg l^{-1}. However, unlike *Cerastoderma edule* or *Venerupis pullastra* (Foster-Smith, 1975a), *S. cylindraceus*, despite a decreased filtration rate and activity, does not appear to demonstrate a decreased ingestion rate with increasing suspensoid concentrations. *Solen cylindraceus* appears to utilize two methods of controlling and ensuring continued optimal ingestion:

1. As suspensoid concentration increases and approaches a certain threshold (ca. 50-100mg l^{-1}), there is a marked increase in the proportion of material ejected as pseudofaeces. During this period and up to this threshold level, filtration rate and activity are relatively unaffected.

2. As suspensoid concentration increases beyond the pseudofaecal production threshold level, the amount of pseudofaeces ejected remains fairly constant, but is accompanied by a reduction in both filtration rate and in the total time spent filtering.

Thus *S. cylindraceus* exhibits a slight variation on Winter's (1977) proposal.

Since ingestion then, is a function of not only suspensoid concentration, but also of the rate of filtration, pseudofaecal production and the total time spent filtering, the present results

indicate an ability by *S. cylindraceus* to effectively compensate for natural fluctuations in suspensoid concentration, which it encounters in the intertidal environment of the Kariega estuary (40-550mg l^{-1}; Figs. 7.2 & 7.3). Such compensation ensures a continual and maximal rate of ingestion even at elevated suspensoid concentrations, so fulfilling the requirements of an "optimal forager" (Lehman, 1976). Nothing, however, is known of the assimilation efficiency or possible selective ingestion by this species.

C. Effect of temperature on filtration rate

Filtration rate has been shown to vary with season in a number of bivalve species. Walne (1972) recorded a definite maximum for *Venerupis decussata* and *Crassostrea gigas* during the summer months, while *Mytilus edulis* and *Ostrea edulis*, although exhibiting an increased rate in summer, did not show as marked a maximum. Worrall, Widdows & Lowe (1983), noted a distinct seasonal variation in filtration rate for *Scrobicularia plana* and further, that filtration rate varied in different *S. plana* populations. Rao (1953) reported a variation in filtration rate in *Mytilus californianus* populations collected at different latitudes, and indicated that these animals were capable of a temperature-compensating response. In populations of *Chlamys opercularis*, a measure of the filtration rate of summer and winter collected animals gave virtually the same values (Vahl, 1972b; McLusky, 1973). Similarly, *S. cylindraceus* showed only a minimal difference in filtration rate between summer and winter collected animals (Fig. 6.6), which is reinforced by filtration rates determined for laboratory animals acclimated to summer and winter conditions (Table 6.1 & 6.2).

According to van't Hoff's Law, filtration rate should double for every 10°C rise in temperature (Schulte, 1975). The temperature coefficients (Q_{10}) for *S. cylindraceus* are compared to a variety of other bivalve species in Table 6.4. An increase in temperature from 10 to 20°C resulted in a rise of approximately 350% (Q_{10} of 4.54) in the filtration rate, while an increase

from 15 to 25°C showed only a 33% rise (Q_{10} of 1.33). A net decrease in the filtration rate of approximately 14% (Q_{10} of 0.85) was recorded when temperature was increased from 25 to 35°C. A temperature increase from 30 to 40°C demonstrated a Q_{10} value of 0.56, corresponding to approximately a 43% decrease in filtration rate. Widdows & Bayne (1971) indicated that Q_{10} values reflect the degree of acclimation, and regarded values approaching unity as indicative of full acclimation. Q_{10} values calculated from acclimated filtration rates (Fig. 6.7 A & Table 6.4) for *S. cylindraceus* in this study, reinforce such a hypothesis (see also Prosser, 1986). A drop in temperature from 15 to 10°C, resulted in approximately a 75% drop in filtration rate, while an increase in temperature from 35 to 40°C showed a decrease of some 37%. The optimal temperature range for filtration is thus fairly clearly defined as 15-35°C, indicative of a eurythermal, sub-tropical species.

The reduced filtration rate at 40°C is interpreted as a response to thermal stress. McLachlan & Erasmus (1974) indicated an upper lethal temperature under *in vitro* conditions of 44.5°C for *S. corneus* (=*cylindraceus*). The thermal tolerance determined for a species may vary, depending on the techniques used, the acclimated state of the animal and the period of exposure (Kinne, 1963; Newell & Branch, 1980). Nevertheless, findings from this work (cf. Chapter 4) indicate an upper lethal limit of 45°C under *in situ* conditions, almost identical to that of McLachlan & Erasmus (1974). Generally, lethal temperatures are determined from short-term exposure experiments, and are of limited ecological significance (Read & Cumming, 1967). Nevertheless, lethal temperatures reflected in a habitat, affect adaptation to different latitudinal and microgeographical regimes (Read, 1967; Kennedy & Mihursky, 1971). The lethal temperature of 45°C and the maximum of 40°C used in these experiments, are both higher than any temperature to which *S. cylindraceus* is likely to be exposed in the natural environment.

Table 6.4: Temperature coefficients (Q_{10}) of acclimated filtration rates for *Solen cylindraceus*, compared to values obtained for other Lamellibranchs.

Species	Temperature (°C)	Filtration rate ($l\ h^{-1}$)	Q_{10}	Author(s)
Arctica islandica	10 20	3.56 4.30	1.23	Winter (1969)
Hiatella arctica	10 20	0.0165 0.0121	0.75	Ali (1970)
Mytilus edulis	10 20	1.100 1.240	1.13	Theede (1963)
	10 20	1.468 1.796	1.22	
	15 25	1.751 1.798	1.001	Schulte (1975)
	20 30	1.796 0.106	0.059	
Solen cylindraceus	10 20	0.252 1.146	4.54	
	15 25	1.028 1.371	1.33	
	20 30	1.146 1.308	1.14	Present study
	25 35	1.371 1.175	0.85	
	30 40*	1.308 0.740	0.56	

* Determined from the last five days values in Figure 6.8.

The rate at which *S. cylindraceus* filters at any particular temperature varies, and is related to its past acclimatory history (Fig. 6.7 B). *Solen cylindraceus* appears to exhibit a lateral shift of the rate-temperature curves to the right, following acclimation to higher temperatures. Similar responses have been reported for other filter-feeding bivalves, *Mytilus edulis* (Schulte, 1975), *Ostrea edulis* (Newell, Johnson & Kofoed, 1977; Buxton, Newell & Field, 1981), as well as for the filter-feeding gastropod, *Crepidula fornicata* (Newell & Kofoed, 1977). The initial response of animals, and the time taken to acclimate to different exposure temperatures varied (Fig. 6.8 A & B), depending upon the conditions to which the animals were acclimated, but generally followed the three phases for non-genetic adaptation as described by Kinne (1963) i.e. an immediate response to environmental change, the stabilization of this response, and a new steady state. In the experiment presented here, animals which were previously acclimated to 15°C and 25°C, subsequently re-acclimated to exposure temperatures in the range 15-35°C, within 120-168 hours (5-7 days). However, animals exposed to extreme temperatures of 10°C, exhibited no acclimatory response, while those at 40°C displayed a gradual, limited acclimation. As an intertidal animal, it is unlikely that *S. cylindraceus* will be exposed to only one single temperature, but will respond rather to short-term fluctuations within the tidal period, reflecting the difference between estuarine and sea water temperatures. To this end, the acute responses will probably reflect more accurately the filtering activity of the population.

It is suggested that for *S. cylindraceus* there is a temperature range within which complete acclimation (Precht type 2) occurs, and beyond which only a partial (Precht type 3 for 40°C) or no (Precht type 4 for 10°C) acclimatory response is recorded (see Newell & Branch, 1980). *Solen cylindraceus* exhibits an effective seasonal compensation in filtration rate and appears to acclimate fairly rapidly over its thermal optimal range (15-35°C), provided exposure conditions persist for several days. Such "plasticity" would allow for optimal filtration throughout the

year. However, *S. cylindraceus* appears incapable of adjusting its filtration rate sufficiently rapidly so as to fully accommodate large short-term temperature fluctuations (within a single tidal cycle) which, under conditions of severe upwelling, may vary by as much as 10 to 12^{o}C (Taylor, 1988).

D. The effect of salinity and specific temperature and salinity combinations on filtration rate

A salinity tolerance range of 13-42‰ has been reported for *S. corneus* (=*cylindraceus*) by McLachlan & Erasmus (1974) under *in vitro* conditions. However, the recorded distribution of *S. cylindraceus* in estuaries with salinities varying from 7-55‰ (Millard & Broekhuysen, 1970; Day, 1974; Boltt, 1975; Blaber, Kure, Jackson & Cyrus, 1983), suggests a substantially wider tolerance, and the values of 15-65‰ recorded under *in situ* conditions (cf. Chapter 4), seem more realistic. The present work demonstrates a salinity optimum for filtration, in the range 15-45‰ , with only partial acclimation occurring at 10‰ (Fig. 6.11) and 55‰ (Fig. 6.9), while no acclimatory response is recorded at salinities of 5‰. This would suggest a non-lethal salinity range of approximately 10-65‰. Pierce (1970), using ventilation rates as a measure of establishing salinity limits for a number of species of *Modiolus*, recorded individual ranges varying between 3-48‰ for four different species. Similarly, Hopkins (1936) recorded normal pumping rates at 25-39‰ for *Ostrea* (=*Crassostrea*) *gigas*, and noted only partial acclimation to salinities of 20‰ , and no effective pumping below 13‰. Anderson & Prosser (1953) recorded *Venus* (=*Mercenaria*) *mercenaria* as ceasing to pump below 50% sea water, while *Modiolus demussus* stopped pumping only below 35% sea water. Matthiessen (1960) and Akberali (1978) indicated a variation in filtration rate on exposure to different salinities in *Mya arenaria* and *Scrobicularia plana* respectively. Numerous investigations considered the effect of salinity, temperature and combinations of these, on ctenidial ciliary activity of bivalves, and indicated an acclimatory response by the gill tissue to both salinity and temperature change (e.g. Van Winkle, 1972; Dean & Paparo, 1983; Paparo & Dean, 1984).

The pattern of acclimation by *S. cylindraceus* to different salinities was reasonably consistent (Figs. 6.10 A-C & 6.11). The rate of acclimation to salinity change appeared independent of temperature within the thermal optimal range (15-35^{o}C) (Fig. 6.10 A-C). However, at 10^{o}C (Fig. 6.10 D) irrespective of salinity, *S. cylindraceus* exhibited a permanently depressed rate of filtration. Season and acclimation temperature have been recorded as having a marked effect on the salinity tolerance in a number of species as well as on gill ciliary action (e.g. Schlieper, 1955; Vernberg, Schlieper & Schneider, 1963; Kinne, 1964; 1971; Castagna & Chanley, 1973). Despite filtration rate being a function of gill ciliary action (Jørgensen, 1981), no marked effect in relation to salinity change at the different acclimation temperatures (within the thermal optimal range) was noted for *S. cylindraceus*. The responses observed, generally followed the three phases for non-genetic adaptation as described by Kinne (1963). Responses of this nature were also reflected in much of the work done on the effect of salinity on gill ciliary activity (e.g. Vernberg *et al.*, 1963; Van Winkle, 1972; Castagna & Chanley, 1973; Davenport & Fletcher, 1978; Dean & Paparo, 1983; Paparo & Dean, 1984). The general picture is one in which rapid change in salinity results in an inhibition of filtration activity, followed by no, partial or complete recovery. In all instances (Figs. 6.10 & 6.11), the initial inhibition and subsequent rate of recovery of filtration in *S. cylindraceus*, appeared to be dictated not only by the magnitude, but also direction of salinity change. Conditions of increased salinity induced a lesser initial inhibition and subsequent shorter acclimatory time than a decrease in salinity of the same magnitude. Similar observations of a greater tolerance by some bivalves to hypersaline than hyposaline conditions, have been made by a number of workers e.g. Dean & Paparo (1983) and Paparo & Dean (1984) for *Crassostrea virginica*, Schlieper, Flugel & Theede (1967) for *Modiolus auriculatus*, Van Winkle (1972) for *C. virginica, Mercenaria mercenaria, Modiolus demissus* and *Mytilus edulis*, and Shumway (1977) who noted that *Mya arenaria* conformed to higher salinities more rapidly than to lower salinities.

Despite a more rapid acclimation to higher salinities, the initial response is also influenced by the past acclimatory history of the animal. Figure 6.10 A-C shows a substantial initial inhibition of filtration in animals acclimated to 35‰ and exposed to 15‰ , despite the latter falling within the salinity optimal range. Animals acclimated to 15‰ and exposed to 10‰ (Fig. 6.11 A & B) exhibited a less marked initial response and a gradual decline to the new steady state. Van Winkle (1972) noted a similar response, in which animals acclimated to low salinities demonstrated a less inhibited ciliary activity when exposed to lower salinities.

Animals acclimated to 25°C, 35‰ and exposed to conditions of 15°C, 35‰ ; 15°C, 25‰ and 15°C, 15‰ (Fig. 6.12 A) demonstrated the effect of a decreased salinity and temperature on filtration rate. Exposure to 15°C, 35‰ reflected a response to temperature change only, and resulted in an initial decrease of some 45% in filtration rate. A comparison of this curve to the 15°C, 25‰ and 15°C, 15‰ exposure curves, showed a greater initial inhibition of filtration in the latter two (ca. 75 and 95% respectively), coinciding with the magnitude of salinity change. A comparison of the 15°C, 15‰ exposure curve (Fig. 6.12 A) to that in Figure 6.10 C, demonstrated that animals subjected to simultaneous change in temperature and salinity, required a substantially longer acclimatory period. However, the 20°C, 15‰ (Fig. 6.12 A) and 15°C, 15‰ (Fig. 6.10 C) curves are virtually identical. Although the substantial initial depression in filtration rate is considered primarily due to a decrease in salinity, it is suggested that the more rapid acclimation at 20°C is a consequence of a reduced thermal shock. Davenport & Fletcher (1978) recorded that for *Mytilus edulis*, salinity alone was capable of depressing frontal gill ciliary activity by some 39.5% on exposure to a salinity of 20‰. Further, they noted that this depression was reversible under conditions in which salinity was increased back to normal. The slight increases in filtration rates recorded under exposure conditions of increased temperature and salinity (Fig. 6.12 A & B) are interpreted as

due principally to temperature, as an increased salinity on its own induced an initial decrease in filtration rate (Figs. 6.10 & 6.11), while temperature increases were shown to increase the filtration rate in *S. cylindraceus* (cf. Section C) and other bivalves (e.g. Jørgensen, 1975b; Newell, 1979; Griffiths & Griffiths, 1987). A consideration of the 20°C, 15‰ exposure curves from both summer and winter acclimated conditions (Fig. 6.12 A & B) indicated an initial inhibition of filtration, of the same magnitude in both instances. However, the acclimation rate varied, and appeared to be a function of the exposure temperature and past acclimatory history of the animal. The more rapid acclimation was recorded under conditions in which the exposure temperature was greater than the original acclimatory temperature. Exposure to a temperature of 10°C, irrespective of the exposure salinity (Figs. 6.10 D & 6.12 B), produced consistently depressed rates of filtration, suggesting an overriding influence of temperature outside the thermal optima.

There appears therefore to be a threshold for both temperature and salinity, beyond which the animal cannot, or may only partially acclimate. Within these optimal ranges however, *S. cylindraceus* is capable of complete acclimation, the rate of which would be a function of the specific exposure conditions and past acclimatory history of the animal. Further, by varying filtration rate, filtration activity and pseudofaecal production, and so ensuring a maximal rate of ingestion, *S. cylindraceus* appears capable of optimally utilizing the suspended material over the naturally occurring concentration ranges in the intertidal region of the Kariega estuary.

CHAPTER SEVEN

POTENTIAL FOOD SOURCES

INTRODUCTION

As an intertidal suspension feeding animal, *Solen cylindraceus* will utilize particulate material brought to it by water currents. The variation in suspensoid concentration along the length of the Kariega estuary, and the development of a turbidity maximal zone, as described in Chapter 3 (Figs. 3.8 & 3.9), although coincident with the distribution of *S. cylindraceus*, was considered to be of only secondary advantage (to the animal), a result of substratum choice. Further, the development and measurement of suspensoid concentration in the centre of the estuary has limited application to an infaunal intertidal species. In this study, the variation in suspensoid concentration across the intertidal zone is measured, in an attempt to determine the range of suspensoid concentrations to which the *S. cylindraceus* population would naturally be exposed. The nature and origin of this suspended material has important implications, nutritionally and ecologically. The Kariega contains fairly large beds of *Zostera capensis* (ca. 20ha, with a mean biomass for the entire estuary of 190g m^{-2} dry wt., Hodgson, pers. comm.) on which the amount of epiphytic material is highly variable (station H, 1.66g; stn. L, 6.07g; stn. Z, 0.94g epiphytic material g^{-1} *Zostera* (dry wt.), de Villiers unpublished results). The intertidal distribution of *Zostera* together with its associated epiphytes (Fig. 3.4), suggests the potential importance of these materials as a food source to the *S. cylindraceus* community. A measure of the naturally occurring $^{13}C/^{12}C$ isotope ratios is used in an attempt to assess

the contributions of *Zostera* and its associated epiphytic material, and to determine the principal sources of carbon, utilized by the *S. cylindraceus* population in the Kariega estuary.

The identification of food sources by gut analysis of filter feeders provides, at best, only a very rough indication of the nature of the material ingested, with minimal information as regards the original carbon source. Stable carbon isotope analyses, if interpreted with caution, provide a useful tool in assisting in the identification of original sources of carbon, and their relative importance to the diets of the fauna in estuarine ecosystems (e.g. Haines, 1976a; 1976b; De Niro & Epstein, 1978; Thayer, Parker, LaCroix & Fry, 1978; Fry & Parker, 1979; Haines & Montague, 1979; McConnaughey & McRoy, 1979a; 1979b; Incze, Mayer, Sherr & Macko, 1982; Stephenson & Lyon, 1982; Fry, 1984; Fry & Sherr, 1984; Knox, 1986b; Whitfield, 1989).

Inorganic carbon exists in two naturally occurring, isotopically stable forms, ^{13}C (1.1%) and ^{12}C (98.9%) (Thayer *et al.*, 1978). The use of ^{13}C as a natural tracer in the identification of carbon flow from plants to animals in a variety of ecosystems, is based on the fact that different groups of plants, dependent on their photosynthetic pathway, synthesize organic carbons with distinct $^{13}C/^{12}C$ ($\delta^{13}C$) ratios (Smith & Epstein, 1971; Benedict, 1978). Plants possessing the C_4 photosynthetic pathway have $\delta^{13}C$ values ranging from -9 to -19‰ , while those with a C_3 pathway have values of -24 to -34‰ (Thayer *et al.*, 1978). Results are normally negative, as samples are more depleted in ^{13}C than the international standard against which they are measured (Chicago Pee Dee belemnite) (Craig, 1957). The isotope ratios for plants are thus determined by the particular photosynthetic fixation of CO_2, while that of animals is a function of diet (Haines, 1976b; De Niro & Epstein, 1978; Thayer *et al.*, 1978; Rau & Hedges, 1979; Teeri & Schoeller, 1979; Rau & Anderson, 1981; Fry, 1984).

Although this technique is conceptually simple (you are what you eat) and despite the fact that plant material $\delta^{13}C$ values remain unchanged once the plant dies and forms part of the detritus pool (Smith & Epstein, 1970; Haines, 1977; Stephenson & Lyon, 1982; Fry, 1984; Fry & Sherr, 1984; Knox, 1986b), isotopic fractionation and variations in $^{13}C/^{12}C$ ratios during metabolism, together with isotopic mixing in the natural environment, all limit its precision (De Niro & Epstein, 1978; McConnaughey & McRoy, 1979a; 1979b; Tieszen, Boutton, Tesdahl & Slade, 1983; Fry & Sherr, 1984; Knox, 1986b). However, mixing of carbon sources aside, such fractionation and variation in isotopic ratios are small (Teeri & Schoeller, 1979; Stephenson & Lyon, 1982; Fry, Anderson, Entzeroth, Byrd & Parker, 1984; Fry & Sherr, 1984, Knox, 1986b). The $\delta^{13}C$ values of marine animals in most instances closely resemble dietary values, with relatively small variations (1-2‰ $\delta^{13}C$) among individuals of the same species fed identical diets (Fry & Arnold, 1982; Fry & Sherr, 1984). In many instances, slightly more positive $\delta^{13}C$ values (^{13}C enrichment) have been recorded at higher than at lower trophic levels (ca. 1-2‰) (De Niro & Epstein, 1978; Fry, Joern & Parker, 1978; Fry & Parker, 1979; McConnaughey & McRoy, 1979a; Fry, Scalan & Parker, 1983; Rau, Mearns, Young, Olson, Schafer & Kaplan, 1983; Rodelli, Gearing, Gearing, Marshall & Sasekumar, 1984; see also Table 3 in Fry & Sherr, 1984). However, documentation of such systematic enrichment is not universal (e.g. Stephenson, Tan & Mann, 1986), and no consistent enrichment fractionation value has emerged (e.g. Petelle, Haines & Haines, 1979; Teeri & Schoeller, 1979; Fry & Arnold, 1982; Fry & Sherr, 1984).

Although many of the primary sources of carbon in the environment may have distinctly different isotopic values, a measure of the particulate material available to a suspension feeder such as *S. cylindraceus* would, due to fractionation and mixing of these sources, essentially give a diluted or averaged $\delta^{13}C$ signal. Intermediate values obtained for such consumers may, in part, be explained by various isotope mixing models (Rau & Anderson, 1981; Fry, 1984; Rodelli *et al.*, 1984; Stephenson, Tan & Mann, 1986; see also Figure 1 in Fry & Sherr, 1984),

and are dependent on a knowledge of the relative contribution of organic material from the primary carbon sources (Fry & Sherr, 1984). Unfortunately, quantification of carbon from various sources into the particulate detrital pool in the natural environment, is difficult to document.

The objectives of this study were to use $\delta^{13}C$ measurements in an attempt to identify important carbon sources in the estuary, the results of which are interpreted as indicative of general, rather than specific, sources of fixed carbon. Further, to evaluate the potential use of stable isotope analyses as an accessory tool in defining estuarine structure and function.

MATERIALS AND METHODS

A. Determination of the variation in suspensoid concentration across an intertidal region of the estuary

In order to sample the water column at different positions across the intertidal zone, a temporary jetty was erected at station L (cf. Fig. 3.2) - which is approximately halfway along the length of the estuary, and at which one of the greatest densities ($>350m^{-2}$) of *S. cylindraceus* was recorded. The jetty extended from the shore, above the high water spring tide level, to beyond the outer fringe of the *Zostera* (Fig. 7.1). To enable the mud banks and vegetation to stabilize and recover from the trampling which resulted during the building process, a two month period was allowed to elapse prior to any sampling being undertaken.

Water samples were collected from 3 positions in the intertidal region by means of bilge pumps, placed on small platforms positioned on the mud surface:

1. In the middle of the mud bank
2. Immediately inshore of the *Zostera*
3. Immediately outside the *Zostera* (see Fig. 7.1).

A further water sample was collected from the centre of the estuary at a depth of 1m. Water samples of approximately 2 l were collected in this way from each position every 30 minutes, over a full tidal cycle (12 hour period), for a total of 6 spring and 6 neap tides. Samples were transported back to the laboratory where they were filtered onto pre-ashed and weighed Whatman GFF glass fibre filters. Filters were dried at 50°C to constant weight, and the total suspended material determined and expressed as mg l^{-1}. To determine tidal movement and periods of slack water, current speeds were monitored, using a Savonius rotor, immediately after collection of the water samples. Wind speed was recorded with a Wilh. Lambrecht model 1443 Vane anemometer, every 30 minutes.

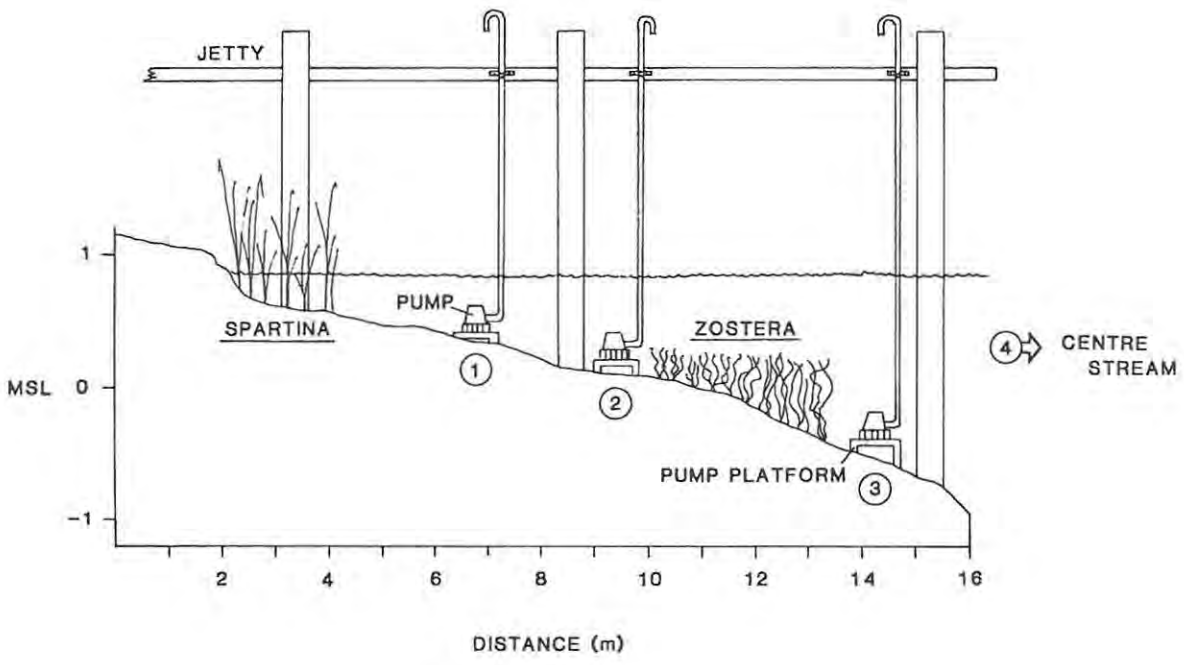


Figure 7.1: Diagram showing the positions from which water samples were collected in the intertidal zone.

B. Determination of stable carbon isotope ratios

The initial pilot study for $\delta^{13}C$ analysis was carried out during autumn (April, 1988), and sampling was restricted to only one station in the estuary (stn. L), with additional water samples collected at the mouth. A second set of samples was collected during winter (June, 1988), from the same stations and above the ebb-and-flow. A more extensive set of samples was collected during summer (December, 1988) from three stations (H, L and Z) as well as from the mouth and at the ebb-and-flow (cf. Fig. 3.2). During collection, attempts were made to identify and sample as many potentially important sources of carbon as possible. Further, in addition to *S. cylindraceus*, the prawns *Upogebia africana* and *Callianassa kraussi*, representing the other dominant infaunal component, were also collected. A full list of all samples collected is shown in Appendix III.

i) Sample collection and preparation

a) Animals

Animals were collected from $0.25m^2$ quadrats. *Solen cylindraceus* (50-60 mm shell length) were collected at stations H, L and Z (n=22-102, see Appendix III), *Upogebia africana* at stations H and L (n=62 and 48) and *Callianassa kraussi* at station Z (n=4). Animals were thoroughly rinsed and kept in clean estuarine water overnight, to allow gut clearance. Flesh from *S. cylindraceus* was dissected out of the shell, homogenized and freeze-dried. Entire prawns were similarly homogenized and freeze-dried.

b) Vegetation and epiphytes

Spartina maritima and *Zostera capensis* were cropped just above the sediment surface, by hand, from $0.25m^2$ quadrats at stations H, L and Z. *Spartina* material was rinsed in clean estuarine water and oven dried at $50^{o}C$ to constant weight. Samples of *Zostera* were carefully

transferred to large plastic basins of clean (Whatman GFF filtered) estuarine water, and epiphytic material was carefully removed. The *Zostera* was then rinsed a second time in filtered estuarine water and oven dried (50°C) to constant weight. The epiphytic material removed from the *Zostera* was concentrated by centrifugation (10000rpm for 120 min.) and freeze-dried. Samples of two other common estuarine flora, *Sarcocornia perennis* and *Chenolea diffusa* were harvested from $0.25m^2$ quadrats from station L only, thoroughly rinsed and oven dried (50°C) to constant weight. Dried plant material was broken up and powdered using a Waring blender. Debris originating from the overhanging terrestrial vegetation was collected by hand from the intertidal mud surface at stations H, L and Z. Tidal wrack was collected from the highwater spring tide level at stations where it was present (H and Z) and comprised a mixture of both terrestrial and estuarine vegetation. Samples of flood wrack were removed from the scrub and trees lining the stream above the ebb-and-flow. All material was rinsed, oven dried and powdered as previously described.

c) Sediment samples

Samples were collected at stations H, L and Z and at the mouth. All large pieces of debris e.g. leaf litter and twigs etc. were removed from the surface prior to collection. In the pilot study, only samples from station L were taken and comprised two core samples (8cm diameter X 2cm depth) from the intertidal and subtidal region. These were washed through a $2mm^2$ sieve to remove any large stones, shell fragments etc., oven dried at 50°C, and ground using a mortar and pestle. Scrapings of the top 1-3mm of the mud surface were taken from the high, mid and low intertidal region at station L, by means of a clean glass slide and similarly dried and powdered as described above. Only surface scrapings from the mid intertidal region were taken at stations H and Z.

d) Suspended particulate material

Water samples were collected by bilge pump into large plastic containers, and transported to the laboratory where the suspended material was concentrated by continuous centrifugation (14000rpm, flow rate of 200ml $min.^{-1}$) and freeze-dried. All water samples were collected during flood tide. During the pilot study, water samples were collected only at station L (from both the central stream and intertidal region), as well as the mouth and upstream of the ebb-and-flow (riverine material). Subsequent samples were taken from the intertidal region only (immediately inshore of the *Zostera*, above the region of greatest density of *S. cylindraceus* (cf. Fig. 3.4)) at stations H, L and Z, and from the centre stream at the mouth. The water sample collected at station L was fractionated, by sieving, into the following size ranges: >20µm; 10-20µm; <10 µm.

ii) $^{13}C/^{12}C$ analysis

All $^{13}C/^{12}C$ analyses were performed by Mr. A.S. Talma and Mrs. G. von la Chevallerie at the Division of Earth, Marine and Atmospheric Science & Technology of the C.S.I.R. (Pretoria). All samples were pre-treated with dilute HCl, washed, dried and combusted in a closed system with ultra high pressure O_2 (as described by Schiegl & Vogel, 1970). Resultant CO_2 was frozen out and isotope ratios measured on a VG SIRA 24 mass spectrometer against an internal standard calibrated to the International Chicago Pee Dee belemnite standard. By convention (Craig, 1957), the $^{13}C/^{12}C$ results are reported as $\delta^{13}C$ in ‰ , where

$$\delta^{13}C = (^{13}C/^{12}C_{sample}) / (^{13}C/^{12}C_{PDB}) - 1 \times 10^3 \ (‰)$$

The analytical precision of these measurements, based on replicate analyses is $< \pm 0.2‰$ (A.S. Talma, pers. comm.).

RESULTS

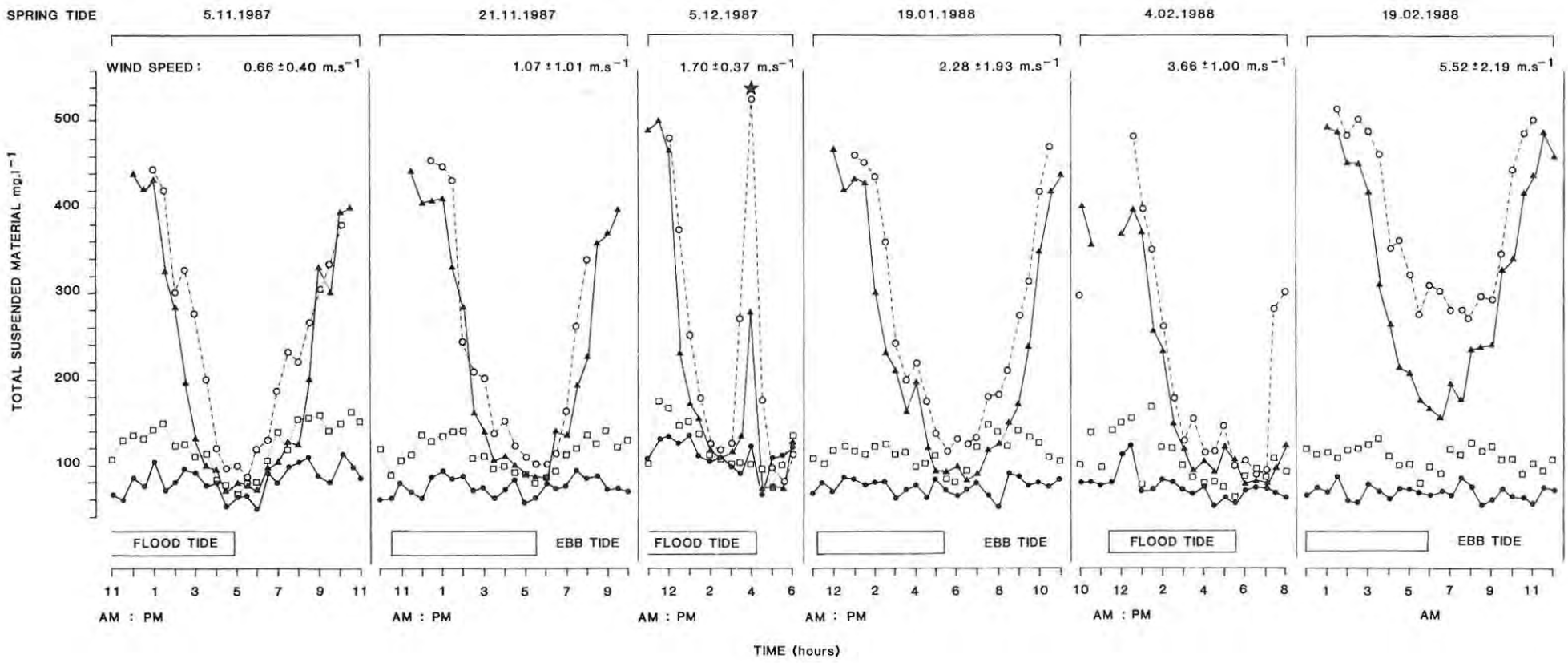
A. Variation in intertidal suspensoid concentration

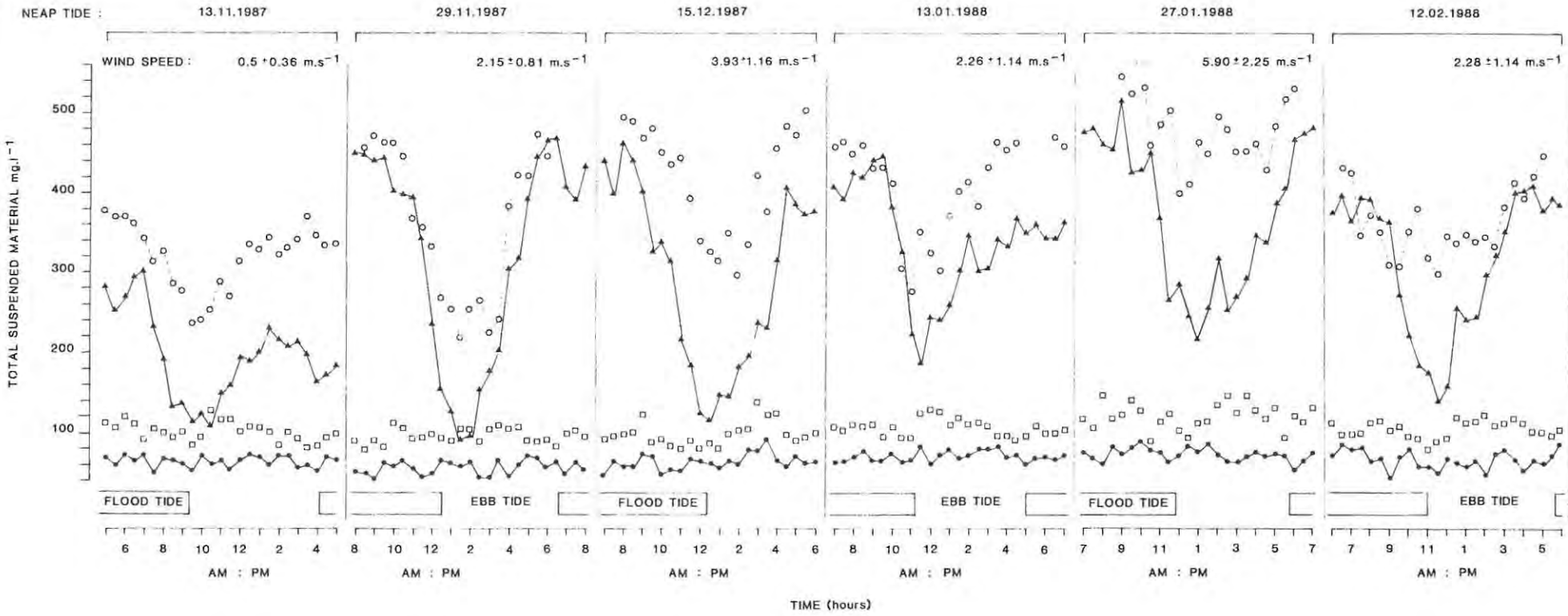
The variation in suspensoid concentration (mg l^{-1}) measured at different stations across the intertidal zone at both spring and neap tides, is shown in Figures 7.2 and 7.3 respectively.

At stations 1 and 2 (on the shore side of the *Zostera*, Fig. 7.1), a marked increase in suspensoid concentration (to 400-500mg l^{-1}) was recorded during the incoming tide. As the period of slack water approached, large amounts of the suspended material sedimented, which resulted in approximately a 4-fold decrease in the total suspensoid concentration (to ca. 80-100mg l^{-1}, measured during slack tide). During the ebb tide, as the water level receded, a noticeable increase in suspensoid concentration (to ca. 300-500mg l^{-1}) was observed. Such tidally mediated variations in suspensoid concentration within the intertidal zone are evident and recurrent during both spring and neap tides.

The extent to which sedimentation occurs, and the relative suspensoid concentration measured during periods of slack water, appear to be influenced not only by tidal current and depth, but also prevailing wind conditions. The effect of wind induced turbulence was particularly noticeable in the shallower regions of the intertidal zone (station 1, Fig. 7.1), in most of the neap, and last 2 of the spring tide surveys, during which on-shore winds, with mean speeds of greater than approximately 2.2m s^{-1} were recorded. The effect of recreational activity (two passes by a motorboat with ski) on the resuspension of intertidal sediments is demonstrated by the large peak recorded during the period of slack tide on the 15.12.1987 survey (Fig. 7.2).

Figure 7.2: Variation in suspensoid concentration (stn. L), measured during spring tides at different positions in the intertidal zone. o---o, stn. 1, mid mud bank; ▲—▲, stn. 2, immediately inshore of the *Zostera*; □····□, stn. 3, river side of the *Zostera*; ●——●, stn. 4, centre stream. Wind speeds are indicated as a mean value ± SD for each sampling period. ★ = Effect of motorboat and ski.


SPRING TIDE
5.11.1987
21.11.1987
5.12.1987
19.01.1988
4.02.1988
19.02.1988
WIND SPEED :
0.66 ±0.40 m.s⁻¹
1.07 ±1.01 m.s⁻¹
1.70 ±0.37 m.s⁻¹
2.28 ±1.93 m.s⁻¹
3.66 ±1.00 m.s⁻¹
5.52 ±2.19 m.s⁻¹
TOTAL SUSPENDED MATERIAL mg.l⁻¹
500
400
300
200
100
FLOOD TIDE
EBB TIDE
FLOOD TIDE
EBB TIDE
FLOOD TIDE
EBB TIDE
11 1 3 5 7 9 11
11 1 3 5 7 9
12 2 4 6
12 2 4 6 8 10
10 12 2 4 6 8
1 3 5 7 9 11
AM : PM
AM : PM
AM : PM
AM : PM
AM : PM
AM
TIME (hours)


NEAP TIDE :
13.11.1987
29.11.1987
15.12.1987
13.01.1988
27.01.1988
12.02.1988
WIND SPEED :
0.5 ± 0.36 m.s⁻¹
2.15 ± 0.81 m.s⁻¹
3.93 ± 1.16 m.s⁻¹
2.26 ± 1.14 m.s⁻¹
5.90 ± 2.25 m.s⁻¹
2.28 ± 1.14 m.s⁻¹
TOTAL SUSPENDED MATERIAL mg.l⁻¹
500
400
300
200
100
FLOOD TIDE
EBB TIDE
FLOOD TIDE
EBB TIDE
FLOOD TIDE
EBB TIDE
6 8 10 12 2 4
8 10 12 2 4 6 8
8 10 12 2 4 6
8 10 12 2 4 6
7 9 11 1 3 5 7
7 9 11 1 3 5
AM : PM
AM : PM
AM : PM
AM : PM
AM : PM
AM : PM
TIME (hours)

The concentration of suspended material measured at station 3 (river side of the *Zostera*, Fig. 7.1), exhibits a tidally mediated pattern which is more clearly defined during conditions of spring than neap tide, with values of approximately 100-180mg l^{-1} (flood and ebb periods) and 60-100mg l^{-1} (slack tide) recorded. Values during neap tides were relatively constant at approximately 80-120mg l^{-1}. Samples collected from the centre of the stream (station 4, Fig. 7.1) showed no apparent tidal variation in suspensoid concentration, although concentrations measured during spring tide were generally greater than those during neap tide (overall mean values of 80.27 and 65.94mg l^{-1} for spring and neaps respectively; see also Fig. 3.8). From the results presented here (under the conditions measured), wind did not appear to influence the concentration of suspended materials at stations 3 or 4.

B. $^{13}C/^{12}C$ analyses

The isotope values for all samples collected are shown in Figure 7.4 and Appendix III. *Solen cylindraceus* exhibited a marked enrichment in $\delta^{13}C$ values from the upper (stn. Z, -27.9‰), to the middle (stn. L, -25.3 to -24‰) and the lower reaches (stn. H, -21.6‰) of the estuary. Stable isotope values for *S. cylindraceus* from station L, included both summer and winter collected specimens and no seasonal variation was apparent. The prawn, *Upogebia africana* demonstrated a simila- enrichment from station L (-23.2‰) downstream to station H (-18.4‰). No *U. africana* occurred at station Z, and were replaced by *Callianassa kraussi* which had a value of -26.8‰. Suspended particulate material collected from the mouth during early winter gave values of -15.9 and -19.7‰ , while a value of -18.5‰ was recorded for summer collected material. $\delta^{13}C$ values of the total suspended material from stations H (-21.0‰) and L (-20.7 and -20.4‰) were more enriched than those values recorded at station Z (-23.4‰) and the ebb-and-flow (-23.8‰). Suspended particulates collected at station L, which were size fractionated, demonstrated enriched values of the >20µm (-20.8‰) and <10µm (-21.4‰) fractions relative to the 10-20µm fraction (-24.6‰).

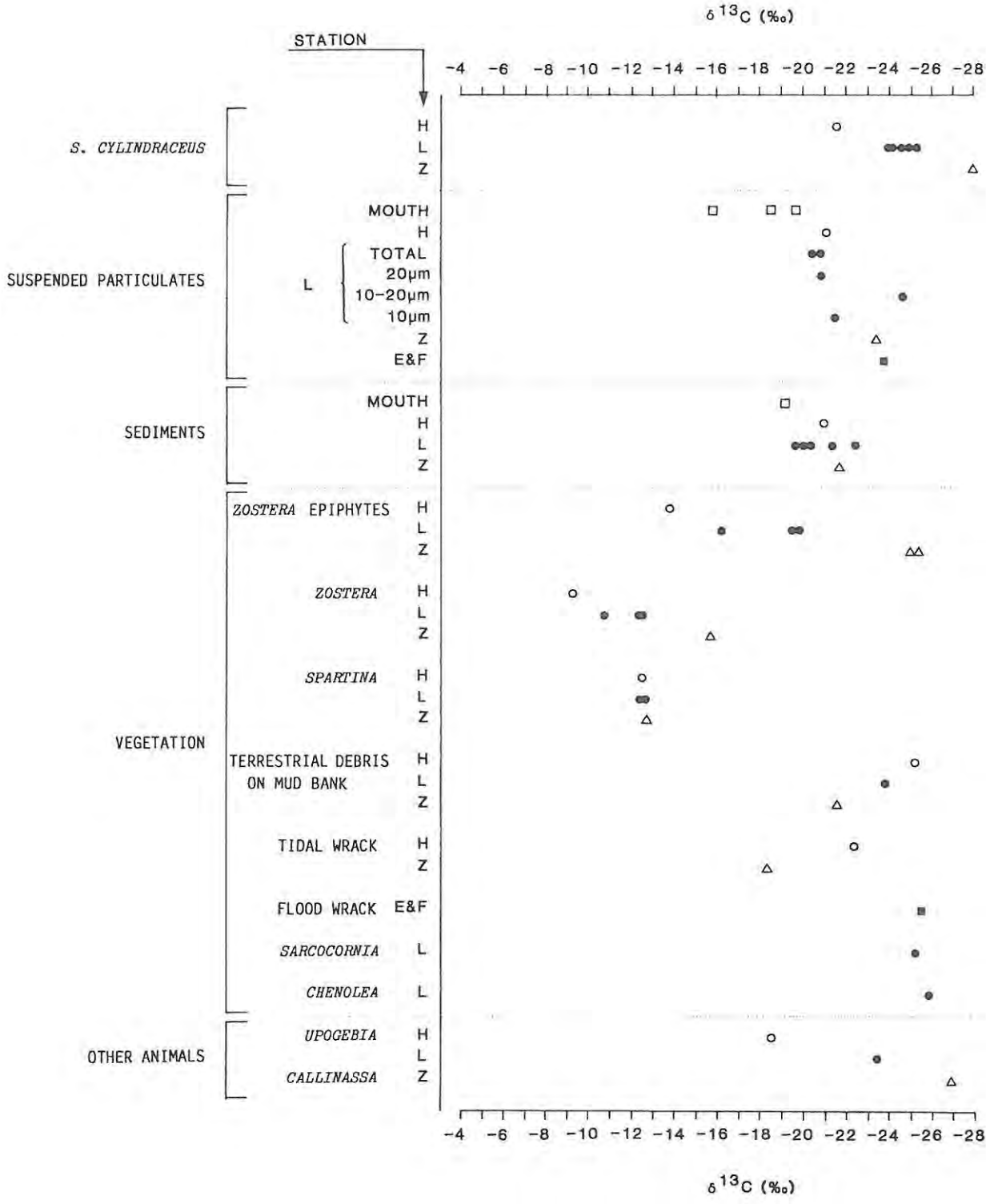


Figure 7.4: Stable carbon isotope composition ($\delta^{13}C$, ‰) of animals and potential food sources sampled in different regions of the Kariega estuary. Mouth (□), station H (o), L (●), Z (△) and ebb-and-flow (E & F).

Subtidal and intertidal sediment samples collected at station L showed a remarkable consistency (-20.3‰ in both instances), while surface sediment scrapings had values of -19.6 to -22.4‰ Sediment surface values at the mouth were the least negative (-19.3‰), while at stations H and Z values of -20.9 and -21.6‰ respectively, were recorded. Epiphytic material from *Zostera* had a similar enrichment in $\delta^{13}C$, from the upper (-25.3 and -25.1‰) to the lower reaches (-13.8‰) of the estuary. A comparison of epiphyte values collected at station L during winter (-19.5 and -19.8‰) and summer (-16.1‰) indicates possible seasonal variation in $\delta^{13}C$. Similarly, *Zostera* had a marked $\delta^{13}C$ enrichment from the upper to mouth regions (-15.6 to -9.1‰). It is suggested that this observed enrichment may, (at least in part) be attributed to insufficient washing of the *Zostera*, and subsequent contamination of the signal from remaining associated epiphytic material. Stable isotope ratios of the macrophyte *Spartina maritima* showed minimal variation along the length of the estuary (-12.5 to -12.6‰). The other common intertidal plants *Sarcocornia perennis* and *Chenolea diffusa*, which are inundated only during very high spring tides, had values of -25.2 and -25.8‰ respectively, very close to that of the terrestrial flood wrack (-25.3‰) collected at the ebb-and-flow. Terrestrial debris collected off the intertidal mud banks exhibited an enriched $\delta^{13}C$ value from the lower to upper reaches of the estuary (-25.1 to -21.5‰), the reverse of that which has previously been described for the suspended particulates, sediments and epiphytic material measured. A similar trend was observed for the tidal wrack collected, with values of -22.2‰ at station H and -18.1‰ at station Z. The less negative value obtained at station Z is attributed to the large amount of *Zostera* which formed the bulk of the tidal wrack, while very little was present in the sample collected at station H.

DISCUSSION

The distribution of *Solen cylindraceus* both intertidally and along the length of the estuary, is coincident with the regions of greatest suspensoid concentration in the water column. Such a distribution would be of advantage to these suspension feeders as, within the turbidity range measured (ca. 60-550mg l^{-1}), ingestion by the animals would be maximal (cf. Fig. 6.5). In the Kariega, *S. cylindraceus* is most abundant just inshore of and within the *Zostera* beds (Hodgson, 1987; present study, cf. Fig. 3.4). It is therefore possible that *Zostera* may be a potentially important food source for *S. cylindraceus*. Indeed, seagrass beds have been shown to be productive systems inhabited and utilized by many invertebrate and vertebrate species (e.g. Odum, 1971; Marsh, 1973; Thayer, Adams & LaCroix, 1975; Adams, 1976a; 1976b; Penhale, 1977; Fry & Parker, 1979; McConnaughey & McRoy, 1979b; Stoner, 1980; Fry, Scalan & Parker, 1983; Whitfield, 1989). Seagrass is considered to be an important carbon source in many aquatic ecosystems, and is made available to the faunal community through detrital food chains (Harrison & Mann, 1975; Fenchel, 1977; Fry & Parker, 1979; McConnaughey & McRoy, 1979b; Fry, Scalan & Parker, 1983; Watson, Robertson & Littlejohn, 1984). Although little is known of the processes of this transfer (Young & Young, 1978), recent work by Benner, Peele & Hodson (1986) and Whitfield (1989) has demonstrated the importance of both bacteria and macroinvertebrates respectively, in this process. Further, Fenchel (1977) has suggested that seagrass detritus together with associated microorganisms, may constitute important sources of food for many invertebrate species.

Results from this study demonstrate lighter (more negative) $\delta^{13}C$ values for *S. cylindraceus* than that measured for either *Zostera* or the equally abundant macrophyte, *Spartina*. Unless significant depletion in the $\delta^{13}C$ values occurs during degradation and decomposition of both these aquatic macrophytes - which in the light of previous work seems unlikely (Smith & Epstein, 1970; Haines, 1977; Thayer *et al.*, 1978; Haines & Montague, 1979; Ivlev, Kaloshin,

Radyukin, Sholin & Pozdnyakova, 1982; Schwinghamer, Tan & Gordon, 1983; Fry & Sherr, 1984), neither appears to be an important food source for *S. cylindraceus*. However, Peterson, Howarth, Lipshultz & Ashendorf (1980) have indicated that S-oxidizing bacteria, often present in intertidal muds, and responsible for anaerobic decomposition of material, have $\delta^{13}C$ values substantially more negative than their substrates, and may themselves be important sources of carbon. They calculated that seston containing a mixture of 30% such bacterial carbon (-35‰) and 70% *Spartina* detritus (-12‰) would give an averaged $\delta^{13}C$ value of approximately -19‰. Such a value is not dissimilar from those recorded for total suspended particulates in the Kariega. However, given the fairly firm and stable nature of the intertidal muds of the Kariega estuary (cf. Chapter 3), the contribution of $\delta^{13}C$ depleted carbon from such a pathway could be considered minimal.

As a filter feeder, *S. cylindraceus* would primarily derive its carbon from the particulate matter in the water column. Given its intertidal distribution, such suspended particulates would comprise not only phytoplankton and detritus normally in suspension, but also resuspended material (cf. Figs. 7.2 & 7.3) originating from both the intertidal mud surface and epiphytic material dislodged from the *Zostera*, during flood and ebb tides. Such resuspended materials have been shown to be important additional sources of food for both benthic and pelagic filter feeding communities (Roman & Tenore, 1978; Baillie & Welsh, 1980; de Jonge & van den Bergs, 1987). $\delta^{13}C$ values of *S. cylindraceus* showed a marked enrichment from the upper (-27.9‰) to the middle (mean = -24.5‰) and lower (-21.6‰) reaches of the estuary. In view of the high degree of analytic precision of this technique, these variations represent significant between-site differences. As such differences are considered too great to be a result of normal animal metabolism (De Niro & Epstein, 1978; Fry & Arnold, 1982; Stephenson & Lyon, 1982; Fry *et al.*, 1984; Fry & Sherr, 1984), they must be attributed to the ingestion of food of different isotopic composition.

Similar trends of $\delta^{13}C$ enrichment were evident in both the total suspended particulates and epiphytic material analyzed at each station. Surface sediments, however, were surprisingly uniform and exhibited only a very slight enrichment towards the mouth. $\delta^{13}C$ values for the size fractionated suspended particulates at station L (middle reaches), demonstrated a significantly lighter value (-24.6‰) in the 10-20μm size range relative to the other fractions, and were virtually identical to the mean value for *S. cylindraceus* (-24.5‰) at that station. Such a correlation might suggest a possible selective feeding action by the animal (certainly the 10-20μm size range would be retained with optimal efficiency - cf. Chapter 6). A similar selective feeding activity may be suggested for the sediment processing detritivore (Branch & Branch, 1981) *Callianassa kraussi*, which is substantially lighter in $\delta^{13}C$ than the sediments it processes.

Epiphytic material has been indicated as an important nutritional source and may form the primary basis of food webs in many estuaries and seagrass meadows (Fry, 1984; Kitting, 1984; Kitting, Fry & Morgan, 1984; Morgan & Kitting, 1984; Knox, 1986a). However, epiphytes, total suspended particulates and surface sediments in the Kariega, all demonstrated enriched $\delta^{13}C$ values relative to *S. cylindraceus* at each particular sampling site. This suggests, particularly in the middle and upper reaches of the estuary, a substantial contribution to the carbon pool from a significantly lighter food source. Three possible sources of $\delta^{13}C$ depleted carbon have been identified:

1. Salt marsh vegetation, principally *Sarcocornia perennis* and *Chenolea diffusa*.

2. Fresh water derived inputs, mostly terrestrial detritus and vegetation deposited following periodic flood events.

3. Riparian litter, mainly natural terrestrial vegetation overhanging the intertidal zone - dominated by four species; *Sideroxylon inerme, Rhus glauca, Schotia afra* and *Cussonia spicata*.

Sarcocornia and *Chenolea*, which exhibited depleted $\delta^{13}C$ values, are fairly abundant in the Kariega estuary (Hodgson, 1987; Taylor, 1988). Their contribution to the particulate organic carbon (P.O.C.) available to *S. cylindraceus* would, however, be limited by the fact that they are only infrequently inundated, during conditions of unusually high spring tides. Further, Taylor (1988) has indicated only minimal P.O.C. export from the salt marshes on the Kariega.

Flood wrack and suspended particulates collected above the ebb-and-flow, were both $\delta^{13}C$ depleted. Although the Kariega is a marine dominated system, with major freshwater inflow occurring only during flood events, it is suggested that the amount of carbon imported during such events (made available by deposition and flocculation), constitutes a substantial input into particularly the upper and middle reaches of the estuary. Indeed, Allanson & Read (1987) have demonstrated substantial increases in P.O.C. following flood events in another eastern Cape estuary, the Keiskamma. Because of the hydrodynamics of the Kariega (Allanson & Read, 1987), such flood-borne material would remain in the upper reaches of the estuary for prolonged periods and so allow for incorporation into the food web.

Despite the catchment of the Kariega having been disturbed due to dam construction, the estuary itself is undisturbed. Farming activities have not encroached the banks, and recreational activity is restricted largely to the lower reaches. The banks of the middle and upper reaches are lined with dense, natural vegetation, which overhangs much of the intertidal regions. Many of the lower branches of these trees and bushes are nearly always submerged at high tide (Hodgson, 1987; pers. obs.). The intertidal banks are consequently heavily littered with debris originating from this riparian vegetation, as well as other wind-borne material (pers. obs.). Litterfall has been shown to constitute a significant and often major source of organic material in many aquatic ecosystems (e.g. Cummins, Petersen, Howard, Wuycheck & Holt, 1973; Fisher & Likens, 1973; Gasith & Hasler, 1976). As for the flood-borne material,

this riparian and wind-borne litter would largely remain in the upper reaches of the estuary, to be incorporated into the detrital food web.

An unknown factor in this study is that of the contribution of phytoplankton. It was not, unfortunately, possible to determine the $\delta^{13}C$ value for the phytoplankton component of the Kariega estuary. However, its contribution would be reflected in the "averaged" seston values, and perhaps the closest this study comes to determining phytoplankton $\delta^{13}C$ values is from the 10-20µm seston fractionation result (-24.6‰). Phytoplankton values available from the literature, range from -18 to -24‰ for the temperate marine species (Haines & Montague, 1979; Fry & Sherr, 1984), and from -24 to -30‰ for river/estuarine species (McConnaughey & McRoy, 1979b; Fry & Sherr, 1984). Further, estimations of phytoplankton productivity (988kg tidal cycle^{-1} overall estuary^{-1}; Allanson & Read, 1987), indicate a contribution some three times greater than that of *Zostera*, to the carbon pool of the Kariega estuary. If *S. cylindraceus*, as previously suggested, possesses the potential for selective feeding, phytoplankton may well constitute a carbon source of some magnitude. However, Hughes & Sherr (1983) have indicated that phytoplankton carbon is a more important food source for subtidal than intertidal animals. This aspect clearly requires closer investigation.

Allanson & Read (1987) have indicated that marine imports account for 77% of the P.O.C. in the Kariega estuary. While such a value is realistic for the mouth and lower reaches, the present study does not support the view of such a dominant marine influence, but suggests rather a significant contribution of terrestrial carbon into the middle and upper reaches of this "marine dominated" estuary. Despite its distribution and abundance in the Kariega, the productivity of *Zostera* (340kg tidal cycle^{-1} overall estuary^{-1}; Allanson & Read, 1987) remains unexpressed in this study.

Solen cylindraceus would appear to be an indiscriminate suspension feeder, utilizing particulate material of both marine and terrestrial origin - the relative amounts of which are determined by local hydrology and its position in the estuary. Similar influences of terrestrial material contributing to faunal diets in some estuarine and marine food webs, have been reported by a number of workers (e.g. Odum & Heald, 1972; Haines, 1977; Hackney & Haines, 1980; Incze *et al.*, 1982; Stephenson & Lyon, 1982; Fry, 1984; Rodelli *et al.*, 1984). A knowledge of the contribution of organic materials of marine and terrestrial origin, to estuarine ecosystems and food webs, is of importance in understanding the functioning of such systems. To this end, $\delta^{13}C$ analyses are an essential accessory tool, not only because they can delineate carbon flow and indicate important materials contributing to secondary production, but also that animal $\delta^{13}C$ values represent a time-integrated average of food assimilated. Admittedly such measurements, while useful, may be equally frustrating. However, the use of multiple tracer techniques (^{13}C; ^{15}N; ^{34}S; D) together with traditional approaches would allow for a greater resolution and understanding of estuarine structure and function. Such an understanding forms the basis from which intelligent decisions and wise management policy on the conservation and future use of such systems, may be made.

CHAPTER EIGHT

GENERAL DISCUSSION

In general, the fauna of estuaries is characterized by having relatively few species, whilst at the same time these few may be extremely abundant (McLusky & Elliott, 1980). In comparison to other eastern Cape estuaries, the Kariega exhibits an unusually high species diversity, within which *Solen cylindraceus* is particularly abundant (Hodgson, 1987; present study). The abundance of estuarine animals leads to the recognition of estuaries as productive habitats, in which high productivity is based upon the ability of the estuary to retain detrital material from both autochthonous and allochthonous sources (McLusky, 1971; Correll, 1978; McLusky & Elliott, 1980; Whitfield, 1983). The Kariega is a marine dominated estuary, a result of upstream impoundment, with very little or no freshwater entering the system except during episodic flood events (every 3 to 5 years). A consequence of such a reduction in freshwater input is a decreased flushing time (Allanson & Read, 1987). Any autochthonous production and allochthonous material delivered into the estuary is thus largely retained within the system (particularly in the upper and middle reaches), and incorporated into the food web. Despite very sporadic freshwater inflow, the Kariega maintains a permanently open mouth to the sea, due to the presence of a rocky prominentry. The resultant hydrodynamic structure of this predominantly homogeneous (in terms of salinity) estuary produces a "plug" of water which, under tidal influence, moves up and down the estuary, having limited exchange with the marine environment. Indeed, Taylor (1987 and pers. comm.) has indicated minimal export of

organic carbon from the estuary to the sea under such conditions (dependent, however, upon mesoscale atmospheric/oceanic events). Such a hydrodynamic structure would assist in the retention of detrital material within the system for prolonged periods.

Limited freshwater input, and the fact that the estuary receives no anthropogenic wastes, has resulted in a fairly clear, quiet-water, macrophyte dominated system (Allanson & Read, 1987), in which the eelgrass *Zostera capensis* and chordgrass *Spartina maritima*, occur along virtually the entire length of the estuary (Hodgson, 1987). Seagrasses are considered to be an important carbon source in many aquatic ecosystems (e.g. Fenchel, 1977; Fry & Parker, 1979; McConnaughey & McRoy, 1979b; Allanson & Read, 1987). However, only a small amount of this plant material is consumed directly by herbivores, and the majority contributes to the detrital pool (Marsh, 1973; Harrison & Mann, 1975; Morgan & Kitting, 1984; Kennish, 1986; Whitfield, 1989). In an estuarine ecosystem, two types of food chain exist:

1. grazing - in which the live green plants and phytoplankton form the basis
2. detrital - with detritus and its associated microorganisms forming the basis (Odum, 1980).

The latter predominates as the principal channel of energy flow in estuaries (Odum, 1980; Watson, Robertson & Littlejohn, 1984; Whitfield, 1989).

It has been demonstrated (Peterson & Howarth, 1987) that, due to its refractory nature, much of the macrophyte derived detritus requires extensive processing by the microbial community before being available to the macrofauna. Detritus and its associated microorganisms have been identified as the single most important food source for resident invertebrates in some systems (Newell, 1965; Harrison & Mann, 1975; Fenchel, 1977; Tenore, 1977; Whitfield, 1989). In the Kariega, which may to an extent function as a detrital trap, material may be reprocessed several times over. *Solen cylindraceus* will ingest plant fragments and detrital

particles together with the associated microorganisms, and deposit faeces and pseudofaeces (biodeposits) on the sediment surface. These deposits will in turn be recolonized, further broken down by physical action, bacteria, fungi, protozoa etc., and eventually reingested. Such recycling and coprophagous feeding habits are a normal consequence of a detrital feeding existence, and have been described for a variety of estuarine fauna (e.g. Johannes & Satomi, 1966; Frankenberg, Coles & Johannes, 1967; Whitfield, 1989).

The nutritional quality of the detritus will vary according to the animal's digestive capability, the origin, age and decompositional state of the plant material, and the extent of colonization by microorganisms (Zimmerman, Gibson & Harrington, 1979). It is generally assumed that detrital consumers receive most of their nutritional requirements from the attached microorganisms, with only a small proportion being directly derived from the decomposing plant material (Fenchel, 1977; Newell, 1979; Kennish, 1986; Knox, 1986a). Wetzel (1976), using ^{14}C labelled substrate, showed that *Nassarius obsoletus* retained primarily microbial and benthic derived carbon, while Fenchel (1972) and Stuart, Field & Newell (1982) have demonstrated a high removal and absorption of bacteria from detrital material in *Macoma balthica* and *Aulocomya ater* respectively. Tunnicliffe & Risk (1977) have shown that densities of *M. balthica* are positively correlated with sediment bacterial densities, and further, that they feed on these bacteria. Nevertheless, there does remain some uncertainty concerning the proportion of the macrodetritivore diet attributable to the microorganisms associated with detritus, and the detrital material itself (Tunnicliffe & Risk, 1977; Newell, 1979; Knox, 1986b; Levinton & Bianchi, 1980). There is evidence which indicates that in a number of systems, bacterial standing stock alone is not sufficient to supply the necessary food requirements, and suggestions are that benthic microalgae contribute a substantial proportion to the diet of a number of macrodetritivores (e.g. Wetzel, 1976; Jensen & Siegismund, 1980; Levinton & Bianchi, 1980; Bianchi & Levinton, 1984).

In the Kariega estuary, Allanson & Read (1987) recorded a bacterial population of $0.98 \pm 0.86 \times 10^6$ ml^{-1}. At this density it is unlikely that bacteria would constitute an important food source for *S. cylindraceus* (de Villiers & Allanson, 1988). However, their values were for bacteria in suspension within the central stream water column of the estuary, and no information is available on the microbial densities in suspension in the intertidal zone or within the intertidal sediments. The resuspension of surface sediments by tidal currents and wind induced turbulence results in the development of a turbidity maximum region in the central stream and intertidal zone of the estuary (Allanson & Read, 1987; present study, Figs. 3.8, 7.2 & 7.3). Such resuspension of surface materials has been shown to result in significant increases not only in primary production, but also microbial heterotrophy associated with detrital particles (Tenore, 1977; Odum, 1980). This tidally mediated resuspension would result in a recycling of sedimented carbon and so augment the food available to the intertidal filter-feeding *S. cylindraceus* community. Such tidal resuspension of the intertidal sediments and subsequent stimulation of both autotrophic and heterotrophic production is illustrated in Figure 8.1.

One of the major impacts resulting from high densities of suspension feeders, 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 recolonized by microorganisms and resuspended (Fig. 8.1). Yingst & Rhoads (1980) have indicated significantly elevated rates of bacterial growth and biomass in sediments which are highly bioturbated, and suggest a positive feedback mechanism between bioturbating species and decomposers (see also Gerlach, 1978; Newell, 1979; Knox, 1986b). As a filter feeder, the role of *S. cylindraceus* as a bioturbator is, in the strict sense of the term, limited. However, it is estimated that the population of *S. cylindraceus* in the Kariega estuary removes some 950kg (dry wt.) of suspended material per tide, most of which would be redeposited on the intertidal mud surface as faeces and pseudofaeces. Further,

unlike the compact faecal rods produced by other filter feeders (Rhoads, 1974), those of *S. cylindraceus* tend to be loose and rapidly disintegrate upon resuspension by tidal or wind induced turbulence (pers. obs.). In the light of these factors, it is suggested that in the Kariega estuary, biodeposition by *S. cylindraceus* may make a substantial contribution to the detrital pathway. Further, that a similar "positive feedback mechanism" for the enhancement of microbial production as described by Yingst and Rhoads (1980) for deposit feeders, may be equally valid for this infaunal filter-feeding community. Such a feedback mechanism through biodeposition, detrital recolonization, tidal resuspension and subsequently enhanced heterotrophic and autotrophic production (Fig. 8.1), would result in a very efficient mechanism with considerable nutrient conservation potential within the Kariega estuary. The importance of bacteria as a food source for the filter-feeding *S. cylindraceus* population in the Kariega has yet to be fully assessed. At least two lines of research should be considered in future:

1. Bacterial number, productivity, biomass and correlations to both the organic content and faunal component of the sediments.

2. Assimilation by the various animal species of the different food components ingested.

The specific carbon sources contributing to the diet of macrobenthic organisms inhabiting estuaries are generally poorly defined (Wetzel, 1976; Newell, 1979; Knox, 1986b). In the Kariega estuary a number of potential carbon sources may contribute either directly or indirectly to the food requirement of *S. cylindraceus*:

1. Allochthonous contributions include riverine and terrestrial material deposited following a flood event, as well as riparian litter and marine imports.

2. Autochthonous production of aquatic macrophytes (e.g. *Zostera, Spartina,* algae), autotrophic and heterotrophic microorganisms (e.g. benthic microalgae, bacteria, diatoms etc.) and phytoplankton (Fig. 8.2).

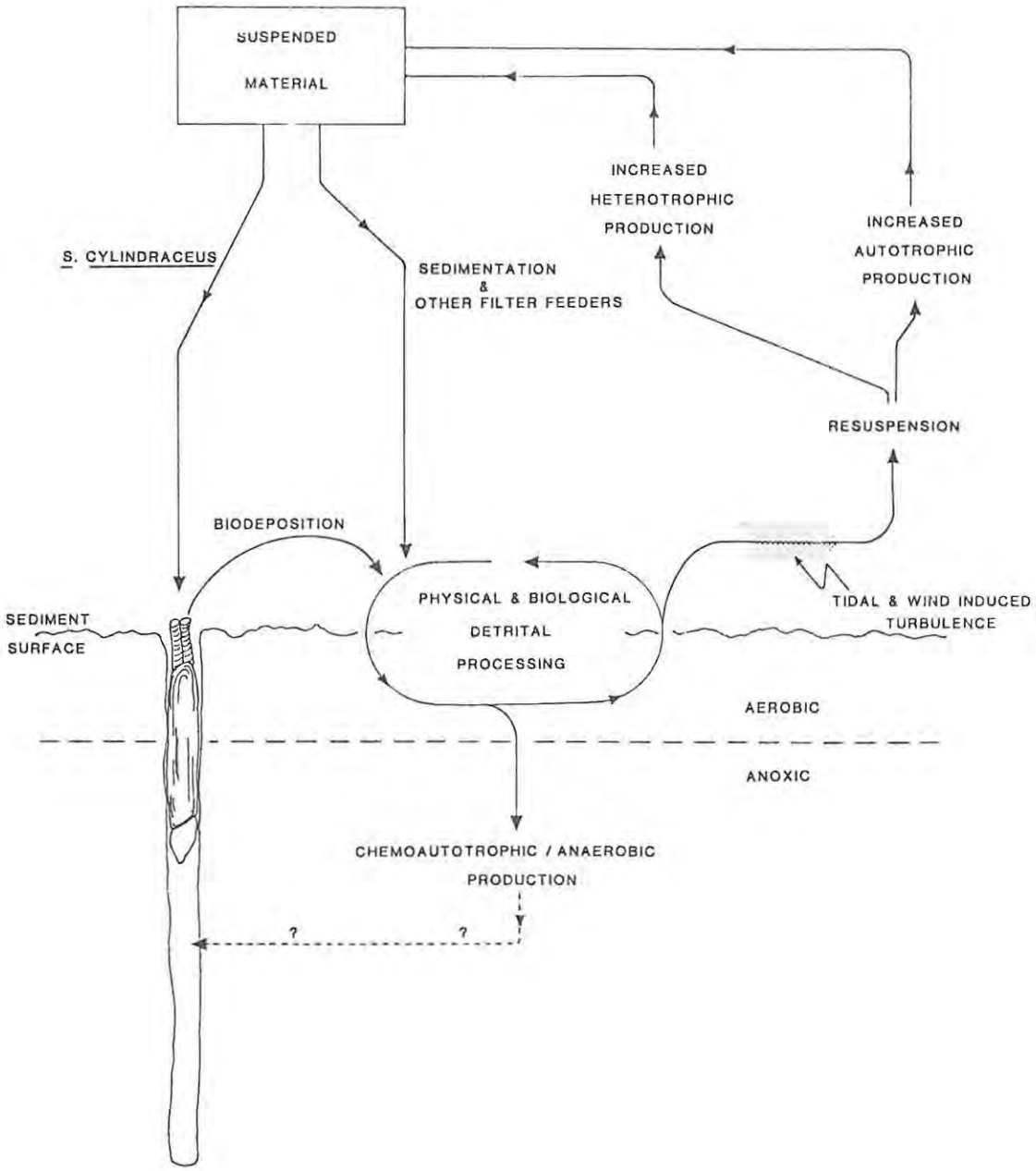


Figure 8.1: Hypothetical model proposed for the intertidal zone of the middle and upper reaches in the Kariega estuary. Illustrated is the physical and biological processing of detritus, and the tidally mediated resuspension and subsequent augmentation of this food resource by related increased autotrophic and heterotrophic production, and by biodeposition. A possible pathway for the contribution from chemoautotrophic and/or anaerobic production in the sediments, to the nutritional requirements of *Solen cylindraceus*, is also shown.

Based on observations of gut content, *S. cylindraceus* may be loosely defined as a detrital feeder. Such a classification is, however, indicative of a feeding strategy and does not quantify specific carbon and other food sources. Such quantification is difficult, primarily because ingestion is not equivalent to assimilation and because detritus is a "mixed-bag" category, derived from many sources with a variety of associated microorganisms. The use of $\delta^{13}C$ may assist in the identification of particular carbon sources and in the interpretation of food web structure. Used on its own, however, this technique is not sufficient to unambiguously indicate carbon sources in the natural habitat (e.g. due to isotope mixing), and additional information is thus required (see Fry & Sherr, 1984 for review; also Chapter 7). Nevertheless, it is of use in demonstrating major influences and general trends which in themselves supply valuable information as to the overall structure and functioning of estuarine ecosystems.

Solen cylindraceus exhibits a marked enrichment in $\delta^{13}C$ values from the upper (-27.9‰) to the lower reaches of the estuary (-21.6‰), with an intermediate value of -24.5‰ recorded from the middle region (cf. Fig. 7.4). This difference is a consequence of diet and possibly reflects a greater terrestrial contribution to the particulate pool in the upper reaches, while a greater marine influence is indicated in the lower reaches. It has previously been suggested (Chapter 7) that terrestrial material contributes substantially to the detrital pool, and its retention in the estuary is largely a result of the particular hydrodynamics of the system. This allochthonous material would become available to the *S. cylindraceus* community primarily via the detrital pathway, although direct assimilation of fine fragments may occur (Fig. 8.2).

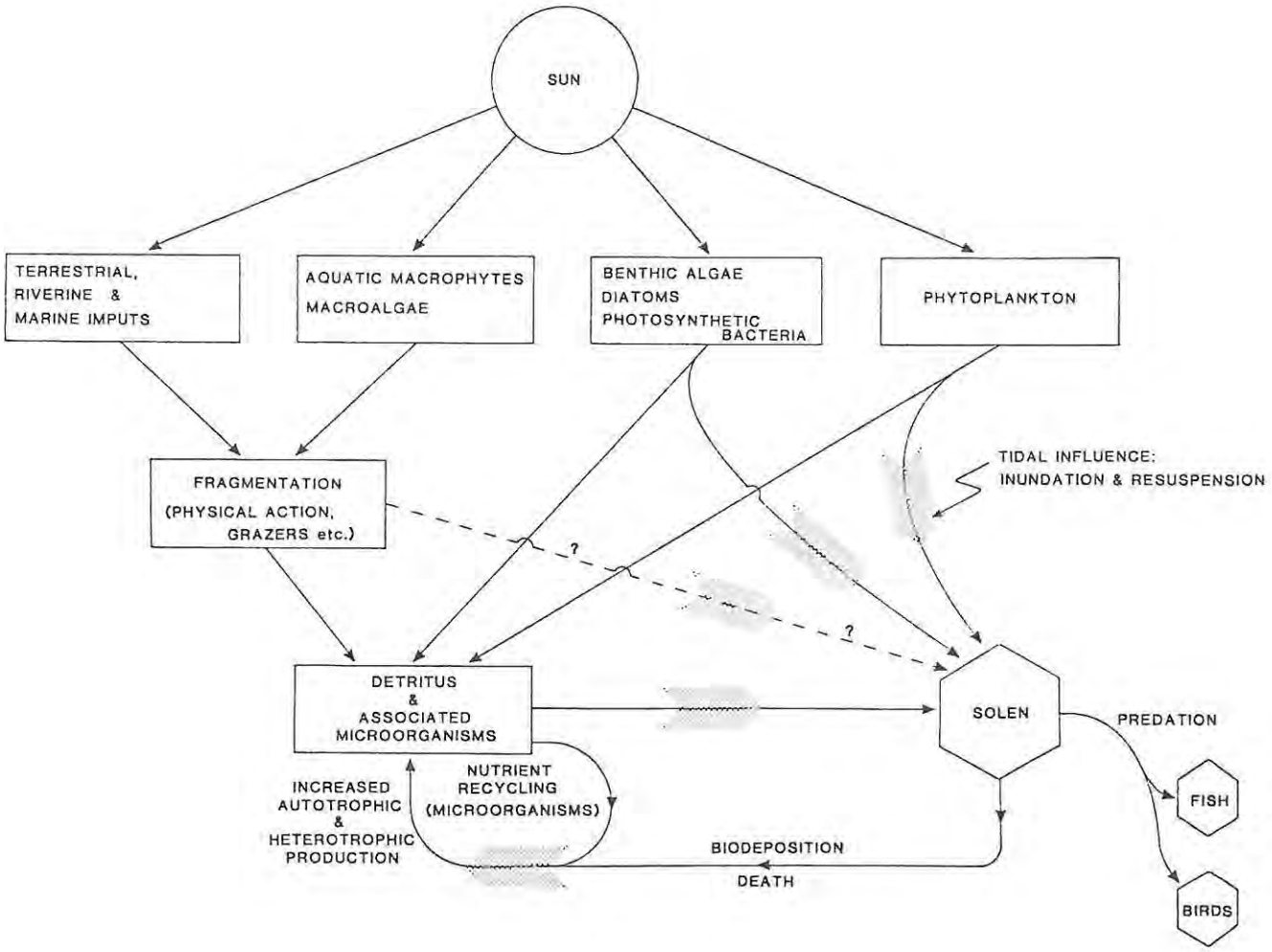


Figure 8.2: Flow diagram illustrating the possible food sources and pathways for *Solen cylindraceus* in the Kariega estuary. It is suggested that the detrital/microbial pathway, via both terrestrial and aquatic macrophytes, predominates.

Given the distribution and abundance of *Zostera* and *Spartina* within the estuary, and the hydrodynamics of the system, both these macrophytes must be potentially important food sources to *S. cylindraceus* (Fig. 8.2). Both *Zostera* and *Spartina* have $\delta^{13}C$ values of approximately -12‰ , a value which is not directly expressed in the *S. cylindraceus* community. The $\delta^{13}C$ value of *S. cylindraceus* represents a time integrated average of all assimilated food, and the values recorded for *Zostera* and *Spartina* may thus be "diluted" by the more negative values from the terrestrial contribution (cf. Fig. 7.4). However, with the exception of station

H (cf. Fig. 7.4), the $\delta^{13}C$ values for *S. cylindraceus* are more negative than that recorded for even the terrestrial debris. Further, although marine animals closely resemble their $\delta^{13}C$ dietary values in most instances, an enrichment of about 1-2‰ is recorded for each increasing trophic level (De Niro & Epstein, 1978; Fry & Parker, 1979; Fry & Arnold, 1982; Fry & Sherr, 1984). If, therefore, *Zostera* and *Spartina* comprise even a small percentage of the diet of *S. cylindraceus*, $\delta^{13}C$ values recorded for these animals, particularly in the upper and middle regions of the estuary, suggest a contribution from a much more negative carbon source.

Peterson, Howarth, Lipshultz & Ashendorf (1980) have indicated that S-oxidizing bacteria, present in many intertidal muds, are responsible for anaerobic decomposition and have $\delta^{13}C$ values very much more negative than their substrates (e.g. $\delta^{13}C$ of -35‰ for bacteria utilizing *Spartina* (-12‰) as a substrate). These bacteria may themselves be an important source of carbon. Previously (cf. Chapter 7) it was considered that given the firm nature of the intertidal substratum, any substantial contribution from such an anaerobic pathway was unlikely. However, assuming $\delta^{13}C$ values of -35‰ (after Peterson *et al.*, 1980), then if such ^{13}C depleted material contributed even a small proportion to the diet, the $^{13}C/^{12}C$ ratio of the animal would be "significantly" affected.

Based on the crystalline style data presented (Fig. 5.7), in which a net production of new style material was recorded during both flood and ebb tides, it was suggested that *S. cylindraceus* continues to feed in its burrow at, or below, the water table during periods of tidal "exposure". Such continuous feeding below the mud surface, during periods of low tide, would allow anaerobic bacteria themselves and/or their metabolites to contribute to the animal's diet. Assuming a value of -35‰ for this anaerobic bacterial carbon, and that *S. cylindraceus* exhibits no food selection capabilities, it is estimated that a contribution of some 15% to the diet in the upper and middle reaches of the estuary would be required to

account for the $\delta^{13}C$ values of *S. cylindraceus* at each of these locations. In addition, the sandier texture and subsequent greater water flow through the sediments in the lower reaches of the estuary would suggest predominantly aerobic decomposition pathways. In the finer-grained sediments of the upper and middle reaches, however, an anaerobic region occurs some 3-5cm below the sediment surface into which the *S. cylindraceus* burrow penetrates some 30-40cm. This may allow for leaching or diffusion of anaerobic bacteria and their metabolites into the burrow in which, it is thought, the animal would still be feeding. The possibility of such anaerobic/chemoautotrophic contributions to the nutritional requirements of *S. cylindraceus* is illustrated in Figure 8.1.

Although the Kariega is a macrophyte dominated system in which a detrital food web predominates, phytoplankton production still contributes a large proportion of the available carbon. Indeed, Allanson & Read (1987) have established a net contribution by phytoplankton of 988kg C tidal cycle^{-1} overall estuary^{-1}, some three times as much as that recorded for *Zostera*. The phytoplankton contribution, either directly or indirectly (Fig. 8.2), to the food requirement of *S. cylindraceus*, may be substantial. However, a measure of phytoplankton concentration (as chlorophyll-a) in the Kariega gives a value of 4.5 ± 3.5µg l^{-1} (Allanson & Read, 1987). This value falls into the oligotrophic and lower levels of the mesotrophic categories of Sakomoto (1966), and suggests that phytoplankton are unlikely to be an important food resource to the benthic filter feeders. Further, Lucas (1986) and Allanson & Read (1987) have indicated that in marine dominated systems, despite a greater light penetration of the water column, phytoplankton production is generally low due to nutrient limitation, a consequence of a reduced or no freshwater inflow. Similarly, Qasim & Sankaranarayanan (1972) have found that terrestrial and aquatic macrophytes contributed the greater proportion of the P.O.C. in a tropical estuary, while the phytoplankton contribution was less than 1%.

Benthic filter feeders and zooplankton have been recorded to crop large biomasses of phytoplankton and thus restrict production rates (e.g. Wolff, Vegter, Mulder & Meijs, 1976; Nichols, 1985). Although the Kariega is dominated by the macrobenthic filter feeders, *S. cylindraceus* and *Upogebia africana* (notwithstanding that little is known of the feeding and particle selectivity of *U. africana*), it nevertheless seems unlikely that these populations, given their intertidal distribution, would remove large proportions of phytoplankton. The impact of the zooplankton community is unknown, but is presently receiving attention. Indications are, however, that these may crop substantial amounts of phytoplankton (Grange, pers. comm.) and so limit direct availability to the benthic filter-feeding community.

Documentation of energy transfer through communities has resulted in the development of various conceptual and simulation models, which have greatly enhanced our understanding of the processes involved (see Knox, 1986a & 1986b for review). What has become abundantly clear is that the traditional "trophic level" concept has little relevance in estuarine food webs. Macrobenthic animals (filter and deposit feeders) feed on organic particles, bacteria, benthic microalgae, diatoms, micro- and meiofauna, as well as eggs and larvae of both their own and other species. The availability of such a mixed diet is aided by the turbatory effects of both the fauna themselves e.g. biodeposition and the reworking of the surface sediment layers (e.g. Yingst & Rhoads, 1980; Knox, 1986b), as well as physical processes e.g. tidal and wind induced turbulence, with subsequent sediment resuspension (Tenore, 1977; Odum, 1980). Basic to our understanding of the complex food web structure of estuaries, is thus not only the question of the role of detritus and its associated microbial community, but that of the estuarine sediments as sites of accumulation, production, consumption and remineralization of organic material.

The estuarine ecosystem consists of biotic communities and an abiotic environment which are interactive. Such interaction between an organism and the environment dictates its distribution and success. However, the pertinent causal factors and relationships are not always immediately apparent. This study suggests that the success of *S. cylindraceus* in the Kariega is a consequence not only of its broad tolerance limits (cf. Chapter 4), its adaptive capabilities and behavioural responses (cf. Chapters 5 and 6), but also the particular physical characteristics of the estuary. These characteristics, the prolonged periods of stable salinity, temperature variation within the animal's optimal range, the nature of the intertidal sediments, detrital entrapment and estuarine retention time, together with the development of a tidally mediated turbidity maximum, are all a result of the hydrodynamics of the system. This, in turn, is mediated by minimal freshwater inflow into the estuary for long periods of time and a mouth which is permanently open to the sea.

FUTURE RESEARCH DIRECTIONS

1. Determination of the assimilation efficiency and development of an energy budget for *S. cylindraceus*.

2. A more detailed examination of estuarine sediment processes and animal/sediment interactions.

3. Possible resource partitioning between filter-feeding communities within the estuary.

4. Reproduction, larval development and recruitment of *S. cylindraceus*.

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APPENDIX I

PUBLICATIONS

Estuarine, Coastal and Shelf Science (1988) **26**, 421–428

Efficiency of Particle Retention in *Solen cylindraceus* (Hanley) (Mollusca: Bivalvia)

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Solen cylindraceus retains particles down to 2·5–3 μm with great efficiency. Below this particle size, retention efficiency decreases rapidly and a net production of particles is recorded below 1·5 μm. Particle retention is independent of temperature (15 °C and 25 °C) and salinity (15‰ and 35‰). Filtration rate is shown to vary minimally at the different temperature and salinity combinations tested: 19·4 ml min^{-1} recorded at 25 °C and 35‰, and 18·2 ml min^{-1} at 15 °C and 15‰. Despite being a dominant member of the estuarine macrobenthos, it is estimated that the *Solen* population filters only 3–4% of the tidal volume per tide and is likely to play a minor role in any resource depletion.

Introduction

Solen cyclindraceus comprises a substantial portion of the infaunal animal biomass in the marine dominated Kariega estuary on the east coast of South Africa (Hodgson, 1987). As filter feeders these animals utilize detritus and plankton brought to them by tidal currents. The amount of food available to a suspension feeding bivalve depends upon the concentration of the suspended particles, the volume of water transported through the gill, and the efficiency with which particles are retained (Palmer & Williams, 1980). The retention efficiency of various particle sizes and the filtration rate are therefore important ecological parameters and have received considerable attention in recent years. In natural marine and estuarine waters, small particles constitute an important fraction of the seston, both in terms of number and volume (Haven & Morales-Alamo, 1970; Vahl, 1972*a*; Grange, unpubl. results) (Figure 4). Several investigators have commented on the nutritional importance of the smaller naturally occurring particles to filter feeders. Vahl (1972*a*) has suggested that nano and ultra plankton may constitute an important fraction of the food ingested by bivalves. The results of Jørgensen (1960, 1975) as well as Hughes (1969), Vahl (1972*a*, *b*), Møhlenberg and Riisgard (1978), Berry and Schleyer (1983), and Stuart and Klumpp (1984) indicate that, despite some intra- and inter-specific variation, most bivalves retain up to 100% of the particles greater than 4–5 μm in diameter, with a decrease in retention efficiency as particle size decreases. As many bivalves may retain only a portion of the natural suspended particles, the concentration of these particles may not

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always be indicative of available food resources. Documentation of particle-retention efficiency is thus essential to any quantitative assessment of food availability to a filter feeding animal.

The objectives of this study were to determine the relative particle retention efficiency for *S. cylindraceus* of naturally occurring seston found in the Kariega estuary, at different temperature and salinity regimes.

Materials and methods

Animal collection and acclimation

Animals of 51–60 mm shell length (mean size range measured in estuary) were collected from the Kariega River estuary on the East Cape coast (33°41'S, 26°42'E) during December 1986, and transferred to 12 pots filled with sediment from the site of collection. The pots were left in the estuary to allow the animals to burrow and re-establish themselves under conditions as close to normal as possible. Pots were collected the following day and transferred to aquaria through which a supply of estuarine water circulated. The temperature was initially set to that measured in the field on the day of the collection (24·2 °C) and was increased a day later to 25 °C. Animals were allowed a 20-day period to acclimate to laboratory conditions of a 14:10 h light:dark regime and a six-hour tidal exposure. Aquarium water was changed every 7–10 days and animals were fed a suspension of the green algae *Tetraselmis suecica* (Prasinophyceae) every three days. Holding tanks were divided arbitrarily into two groups: in one set the salinity was maintained at 35‰; in the second group salinity was decreased by 2·5‰ day^{-1}, until a concentration of 15‰ was reached. Animals in the second group of tanks were allowed a two-day acclimation to 15‰ salinity before being tested. Temperature changes were effected at a rate of 1 °C day^{-1} to 15 °C with a three day acclimation at that temperature prior to testing. Temperature and salinity were kept constant at $\pm 0{\cdot}5$ °C and $\pm 1{\cdot}0$‰, respectively.

Retention efficiency

The design of the apparatus used was modified from Vahl (1972*a*) and Riisgard (1977) (Figure 1). The experimental flumes had internal dimensions of 130 mm × 30 mm × 35 mm, and received water via a peristaltic pump from a storage reservoir in which suspension of particulate material was effected by aeration and magnetic stirring. An upstream baffle was introduced in the flume to reduce the circulation effect of the magnetic stirrer which was required to keep the particles in suspension during flow through the flume. A second, and downstream adjustable baffle was displaced from the vertical to deflect the exhalent current away from the animal. A discharge rate of 50 ml min^{-1} was adopted as standard, which is more than twice the filtration rate previously calculated for *S. cylindraceus* (unpubl. data) and which, in association with the structure of the flume, appeared sufficient to minimize the problem of re-circulation as discussed by Hildreth and Crisp (1976). Water from the Kariega estuary of 35‰ and 17‰, adjusted to 15‰, was used to which was added a 'spike' of 5×10^6 *Tetraselmis seucica* l^{-1}.

Animals were taken from pots as required, thoroughly washed to remove mud and tied down onto the specimen stage. Siphons were orientated in the direction of water flow (Figure 1). Animals were allowed a 10–15 min acclimation period in the test chamber. During this period there was extensive foot movement and some faecal release. After about 15 min, foot activity decreased and no further faecal release was observed. The

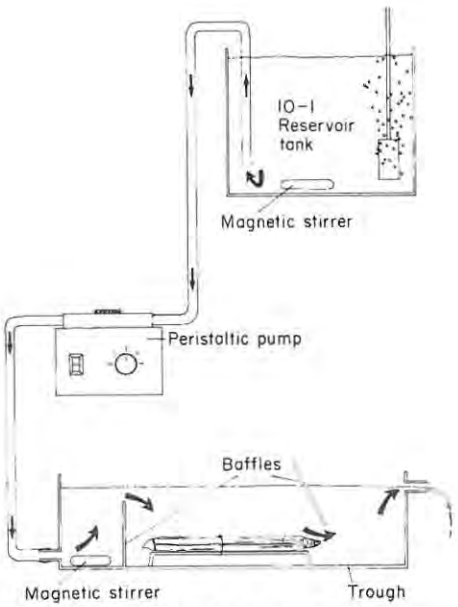

Figure 1. Experimental set up. Arrows indicate direction of water flow.

resultant debris was removed and the flume flushed with clean water from the reservoir tank.

The particle suspension was allowed to stabilize for a further 10–15 min before samples were collected for counting. The control consisted of an identical chamber run simultaneously in which the live animal was replaced by plasticene filled *Solen* valves. The number and distribution of particles in the size range 1–10 μm was measured in three aliquots collected from the test and control flumes, using a Model Z_B Coulter Counter fitted with a 50-μm aperture. This procedure was replicated for each of 29, 31, 33, and 34 animals within the groups assigned to the four temperature and salinity combinations (See Table 1). The retention efficiency for the various size fractions is expressed (Vahl, 1972*a*) as:

$$1 - \frac{\text{particle count of test flume}}{\text{particle count of control flume}}.$$

No pseudofaecal material was produced by the animals during the experiment. Faecal ropes were removed from the flume using a Pasteur pipette.

Filtration rate

Filtration rate was determined using counts obtained for the 7–8 μm particle-size range and calculated according to the expression of Hildreth and Crisp (1976):

$$R_f = \frac{F(C_1 - C_2)}{C_1}$$

where R_f is the filtration rate (ml min^{-1}), F is the water flow rate through the vessel (ml min^{-1}), and C_1 and C_2 are the particle concentrations (No. particles ml^{-1}) in the control flume and test flume, respectively.

TABLE 1. Retention efficiency of *S. cylindraceus* at different temperature and salinity combinations[a]

Particle diameter (μm)	Group 1 25 °C, 35‰		Group 2 25 °C, 15‰		Group 3 15 °C, 35‰		Group 4 15 °C, 15‰	
	$\bar{x}$	±Sx	$\bar{x}$	±Sx	$\bar{x}$	±Sx	$\bar{x}$	±Sx
1–1·5	−0·07	0·12	−0·12	0·12	−0·11	0·10	−0·14	0·07
1·5–2	0·13	0·09	0·10	0·10	0·07	0·11	0·06	0·11
2–2·5	0·23	0·07	0·20	0·10	0·16	0·10	0·14	0·10
2·5–3	0·29	0·06	0·30	0·06	0·32	0·06	0·24	0·07
3–3·5	0·35	0·06	0·31	0·05	0·31	0·07	0·33	0·07
3·5–4	0·35	0·06	0·31	0·05	0·32	0·06	0·33	0·08
4–4·5	0·35	0·05	0·33	0·05	0·32	0·05	0·33	0·04
4·5–5	0·34	0·05	0·33	0·06	0·34	0·05	0·32	0·07
5–6	0·35	0·04	0·33	0·05	0·33	0·06	0·32	0·06
6–7	0·37	0·04	0·34	0·05	0·36	0·05	0·35	0·05
7–8	0·39	0·04	0·35	0·05	0·36	0·05	0·36	0·05
8–9	0·33	0·05	0·34	0·07	0·34	0·05	0·32	0·05
9–10	0·35	0·05	0·35	0·06	0·35	0·07	0·28	0·07
Filtration rate (ml min^{-1})	19·4	2·4	17·8	2·5	18·2	2·3	18·2	2·6
No. animals	33		29		31		34	

[a]Values expressed as 1-(number of particles from test flume/number of particles from control flume). Mean ± SD given for each particle size range tested and for filtration rates. Filtration rates were calculated from the 7–8 μm size range.

Results and discussion

Jørgensen (1960) comments on the often conflicting results obtained by different workers on the same species. He attributes this principally to the use of different experimental techniques as well as to animal sensitivity, i.e. changes in their environment may cause them to react by altering water transport rates and even the mode of functioning of the feeding organs. In the series of experiments presented here, animal disturbance is a real and valid criticism and the results obtained should be viewed with this in mind.

Retention-efficiency and filtration-rates results are recorded in Figure 2 and Table 1. The results show a consistent pattern of particle removal by *S. cylindraceus*. A marked inflection occurs at the 2·5–3·5 μm particle diameter range, below which retention decreases rapidly with apparently zero retention for a particle size of ca. 1·5 μm. These data are summarized in Figure 3 by setting the retention of the 7–8 μm fractions at 100% and expressing the clearance of the other particle-size ranges proportionately. The 7–8 μm size range was chosen as it gave the highest efficiency but, as the modal size of the *Tetraselmis suecica* culture fell within this size fraction, it may be that this maximum efficiency is slightly biased due to possible selectivity by the animal. Particles in the 1·5–2 μm range are retained with a 15–35% efficiency, those in the 2–2·5 μm range with a 40–60% efficiency, and those in the 2·5–3 μm range with a 70–90% efficiency. Above 3 μm the efficiency did not vary significantly over the particle size range tested. However, in all cases, the concentration of particles measured in the 1–1·5 μm range was greater in the test than in the control flume, indicating a negative retention and net production of particles in

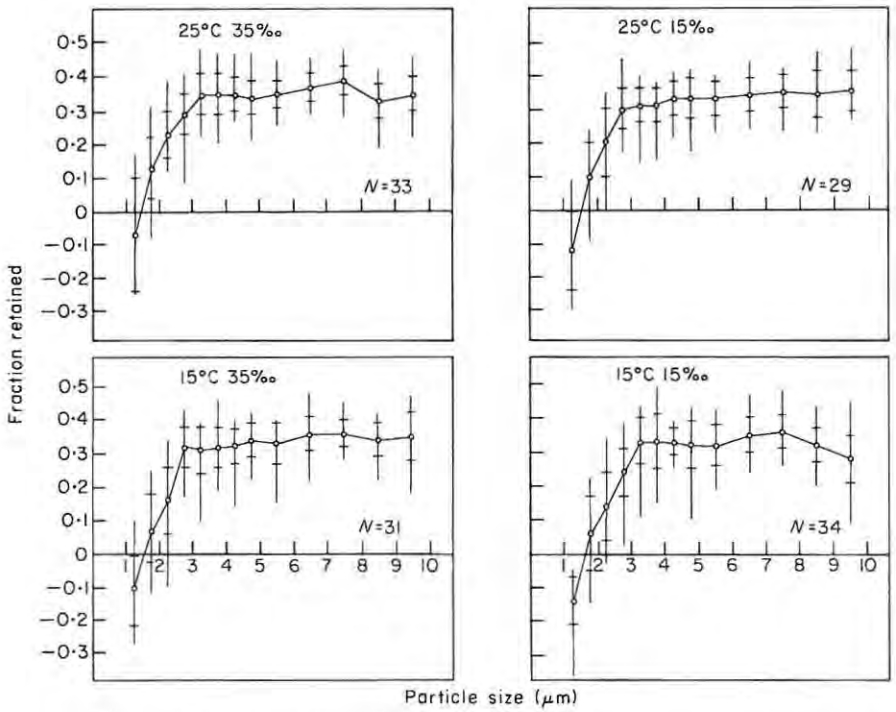

Figure 2. Retention efficiency [1-(test/control)] expressed as a function of particle size. ○, mean values. SD, range. Values plotted midway of each particle size range tested. *n*, Number of individuals tested at each temperature and salinity combination.

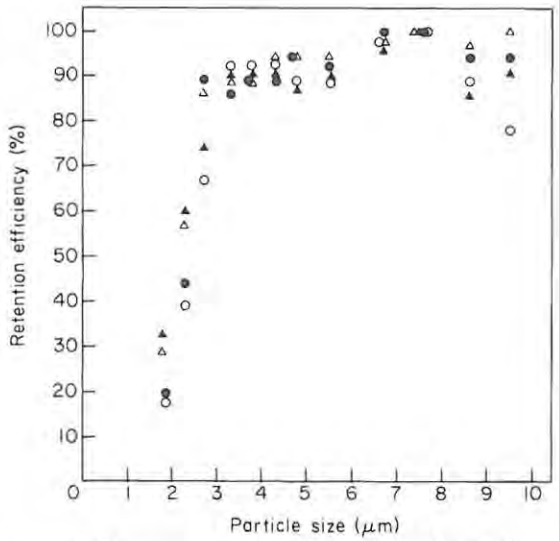

Figure 3. Percentage retention of particles by *S. cylindraceus*. Retention of 7–8 μm size fraction taken to be 100%. Note that negative retention values for 1–1·5 μm size range are not shown. ▲, 35‰, 25 °C; △, 15‰, 25 °C; ●, 35‰, 15 °C; ○, 15‰, 15 °C.

TABLE 2. Comparison of particle size limit for 100% retention in different bivalve species

Species	Minimum size for complete retention (μm)	Reference(s)
Mytilus edulis	4	Vahl (1972*a*), Jørgensen (1975)
Ostrea edulis	4	Møhlenberg & Riisgard (1978)
Cardium edule	3	Møhlenberg & Riisgard (1978)
Crassostrea virginica	3	Haven & Morales-Alamo (1970)
Mya arenaria	3–4	Møhlenberg & Riisgard (1978)
Aulacomya ater	4	Stuart & Klumpp (1984)
Perma perma	4	Stuart & Klumpp (1984)
Choromytilus meridionalis	4	Stuart & Klumpp (1984)
Chlamys opercularis	6–7	Møhlenberg & Riisgard (1978)
Chlamys septemradius	6–7	Møhlenberg & Riisgard (1978)
Solen cylindraceus	3–3·5	Present study

that size range. Vahl (1972*a*) also records a negative retention of the smaller particles by *Mytilus edulis*. Møhlenberg and Riisgard (1978) noted occasional negative retention for both *M. edulis* and *Pecten opercularis*, which they regard as anomalous and to be due to the disturbance caused by movement of the animal. In the case of *S. cylindraceus*, the origin and nature of these particles are unknown. They may be due to valve and foot movements which result in the dislodging of smaller fragments and possibly bacteria from larger particles. The effect of this increase in the number of smaller particles would be to decrease the observed fraction of particles which may be retained from the natural seston, with the result that the lower limit of particle retention in *S. cylindraceus* is still uncertain.

Table 2 gives a comparison of the smallest particle sizes retained completely by a number of bivalve species. The size limit for complete retention of particles varies between species, and many workers (e.g. Jørgensen, 1966; Haven & Morales-Alamo, 1970; Moore, 1971) have suggested this to be a function of the gill ciliary component. However, as emphasised by Jørgensen (1975, 1983), this retention efficiency is not a simple straining process, but depends upon the integrated activity of all the ciliary systems and fluid mechanical aspects of the gill.

Filtration rates for *S. cylindraceus* have been shown to vary with short-term change in temperature and salinity (de Villiers, unpubl results), a phenomenon not uncommon to bivalves (Theede, 1963; Ali, 1970; Widdows & Bayne, 1971; Bøhle, 1972; Walne, 1972; Ansell & Sivadas, 1973, Newell *et al.* 1977; Widdows, 1978; Buxton *et al.* 1981). However, the filtration rates calculated here vary only minimally for the different temperature and salinity combinations tested. *Solen cylindraceus* has a wide osmotic tolerance (13–43‰; McLachlan & Erasmus, 1974), and it would appear that the rate of both salinity and temperature change effected in the laboratory was sufficiently gradual to enable the animal to maintain near 'optimum' filtration rates. As the estuary salinity varied only infrequently during summer, filtration rates in this species were maintained near to optimum for long periods.

In common with other bivalves, *S. cylindraceus* effectively retains particles over a wide spectrum of diameters (Table 1). A lower limit of 2·5 μm (40–60% retention) will allow nanoplankton and bacterial floc assemblages to contribute, along with microplankton, to the diet. Particles of less than 2·5 μm diameter are a volumetrically significant fraction of

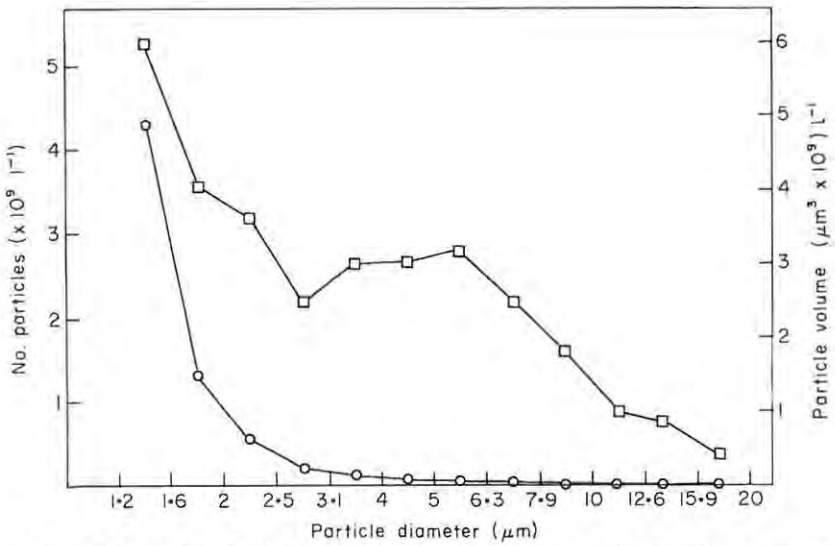

Figure 4. Particle-size distribution of the Kariega estuary (at the site of animal collection) in terms of both number (○) and volume (□) (after Grange, unpubl. results).

the filtrate (Figure 4). If this fraction contains potential food particles, for example picoplankton or bacteria, in sufficient quantity then its ' unavailability ' to *Solen* could constitute a potential loss of some significance. However, Allanson and Read (1987) report bacterial cell concentrations of $9{\cdot}8 \pm 8{\cdot}82 \times 10^5$ ml^{-1}, $n = 260$ for the Kariega estuary. At this density it is unlikely that bacteria would constitute an important food source to *Solen*. The loss of bacterial carbon is estimated from our data to be 1·5 mg C individual^{-1} tide^{-1}. We are also unaware of other picoplankton components in any significant quantity.

Solen cylindraceus shares the intertidal mudflats with the anomuran prawn *Upogebia africana*, another suspensoid feeder. Nothing is known of the food selectivity spectrum of this species. However, in view of the small fraction of the tidal volume which is filtered by the *Solen* population [an estimate of 3–4% per tidal cycle is available from de Villiers (unpubl. results)], the likelihood of significant resource depletion as described by Wright *et al.* (1982) and Peterson and Black (1987) for communities of bivalves with higher filtration rates is unlikely.

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OSMOTIC PROPERTIES OF AN INFAUNAL ESTUARINE BIVALVE *SOLEN CYLINDRACEUS* (HANLEY)

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ABSTRACT

Solen cylindraceus is an infaunal euryhaline osmoconformer with a wide salinity tolerance range, 13-45‰. Under conditions in which the animal is removed from its burrow, osmotic equilibration is rapid (1-12 h). When the animal remains undisturbed in its burrow, equilibration is retarded (72-204 h). It is suggested that the observed decrease in the rate of change of haemolymph osmolarity in animals in their burrows is linked to the behavioural response of the animal and to the stability of the interstitial salinity. Doubt is also cast upon the usefulness of *in vitro* experimentation in osmotic studies of estuarine intertidal burrowing animals.

INTRODUCTION

Solen cylindraceus (Hanley) is a dominant member of the intertidal estaurine infauna of the marine dominated Kariega estuary on the south eastern coast of southern Africa (Hodgson, 1987). The influence of the sea is felt along most of the length of the estuary, so that salinities normally remain close to that of sea water (34-36‰). During periods of drought a negative salinity gradient is set up with salinities of up to 43‰ in the upper reaches. Brackish water, salinities 0-5‰ are recorded in the same region during periods of flood.

The ability of molluscs to tolerate changes in the salinity of their natural environment has been studied by numerous authors (Robertson, 1964; Schoffienels & Gilles, 1972; Pierce, 1970; Shumway, 1977 & Davenport, 1979). Their results indicate that most marine and estuarine bivalves are osmoconformers, which can only exert control over the osmolarity of their internal fluids by behavioural means such as valve closure (Shumway, 1977; Davenport & Fletcher, 1978). More recently, Costa and Pritchard (1978) & Gainey (1978 & 1987) have indicated the existence of physiological mechanisms which, together with the behavioural responses of the animal would determine the extent of osmotic tolerance and hysteresis. McLachlan and Erasmus (1974) have demonstrated that both *Solen capensis* and *Solen corneus* (= *cylindraceus*) are euryhaline osmoconformers and that equilibration of their body fluids to the osmotic concentrations of the external media occurred within 24 h under conditions in which the animals were removed from their burrows. The success of *Solen cylindraceus* in the middle to upper reaches of the Kariega estuary, despite periodic flooding, prompted a closer examination of the change in haemolymph osmolarity when the animal remains undisturbed in its burrow during periods of variation in the overlying salinity. This investigation deals primarily with a comparison of the osmotic properties of *Solen cylindraceus* under: (i) *in vitro* conditions; (ii) *in situ* conditions.

MATERIALS AND METHODS

Solen of 50-60 mm valve lengths were collected from the Kariega estuary during June/July 1987. Animals were transferred to mud-filled pots at the site of collection and the pots left in the estuary to allow the animals to burrow and establish themselves under as near normal conditions as possible. Pots were transferred the following day to laboratory aquaria, through which a supply of estuarine water circulated. Aquarium water was changed every 7-10 days. Temperature and salinity were set to match that measured in the field on the day of collection, (18.9°C ± 1°C and 35‰ ± 2‰). The animals were allowed a twenty day period to acclimate to laboratory conditions of 12:12 h light:dark regime, and a six hourly tidal exposure. Animals were fed a suspension of the green algae *Tetraselmis suecica* every three days. Two experiments

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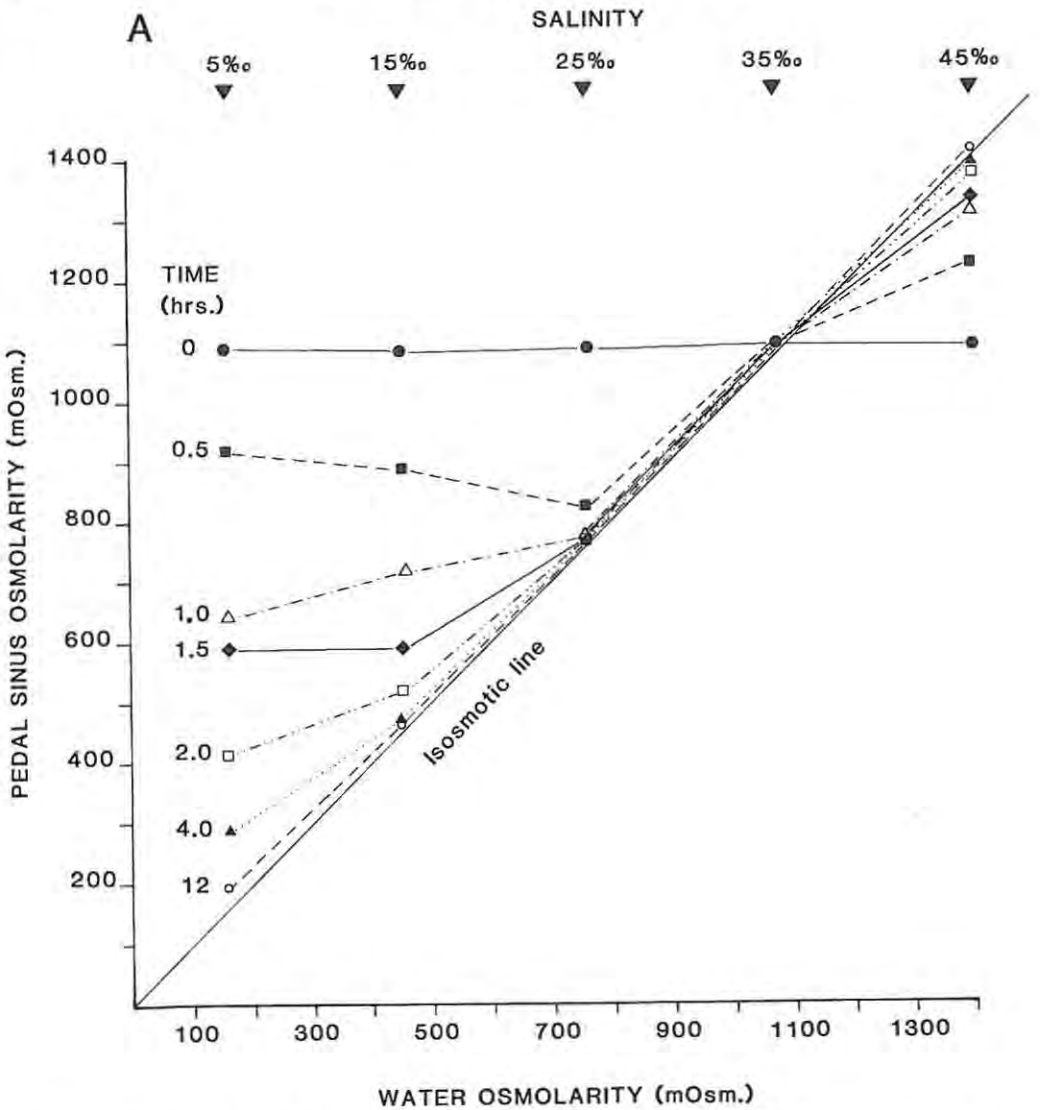

Fig. 1. Variation in pedal sinus osmolarity of animals acclimatized to 35‰ salinity (1074 mOsm), and exposed to different salinity concentrations, at given time periods, plotted against external water osmolarity. A = Animals removed from burrows, B = Animals retained in burrows.

were performed, the first a modification of that of McLachlan and Erasmus (1974). Animals were removed from the mud pots, thoroughly rinsed and valves were bound together with cotton thread to prevent gaping. A total of 120 animals were transferred directly from aquaria (salinity 35‰) to each of four trays of 5 l capacity containing aerated sea water at the following salinities: 5‰, 15‰, 25‰ and 45‰. Four animals were removed from each of the experimental trays after the following times:

(i) Every 15 min for the first 2 h
(ii) Every 30 min for the next 4 h
(iii) Every 60 min for the next 6 h
(iv) Every 3 h for the next 12 h
(v) After 36 h
(vi) After 48 h.

Haemocoelic fluid was extracted from the pedal sinus by hypodermic syringe. Usually 0.5–0.75 ml of fluid was obtained in this manner, sufficient for two osmolarity measurements per animal. Osmolarity was

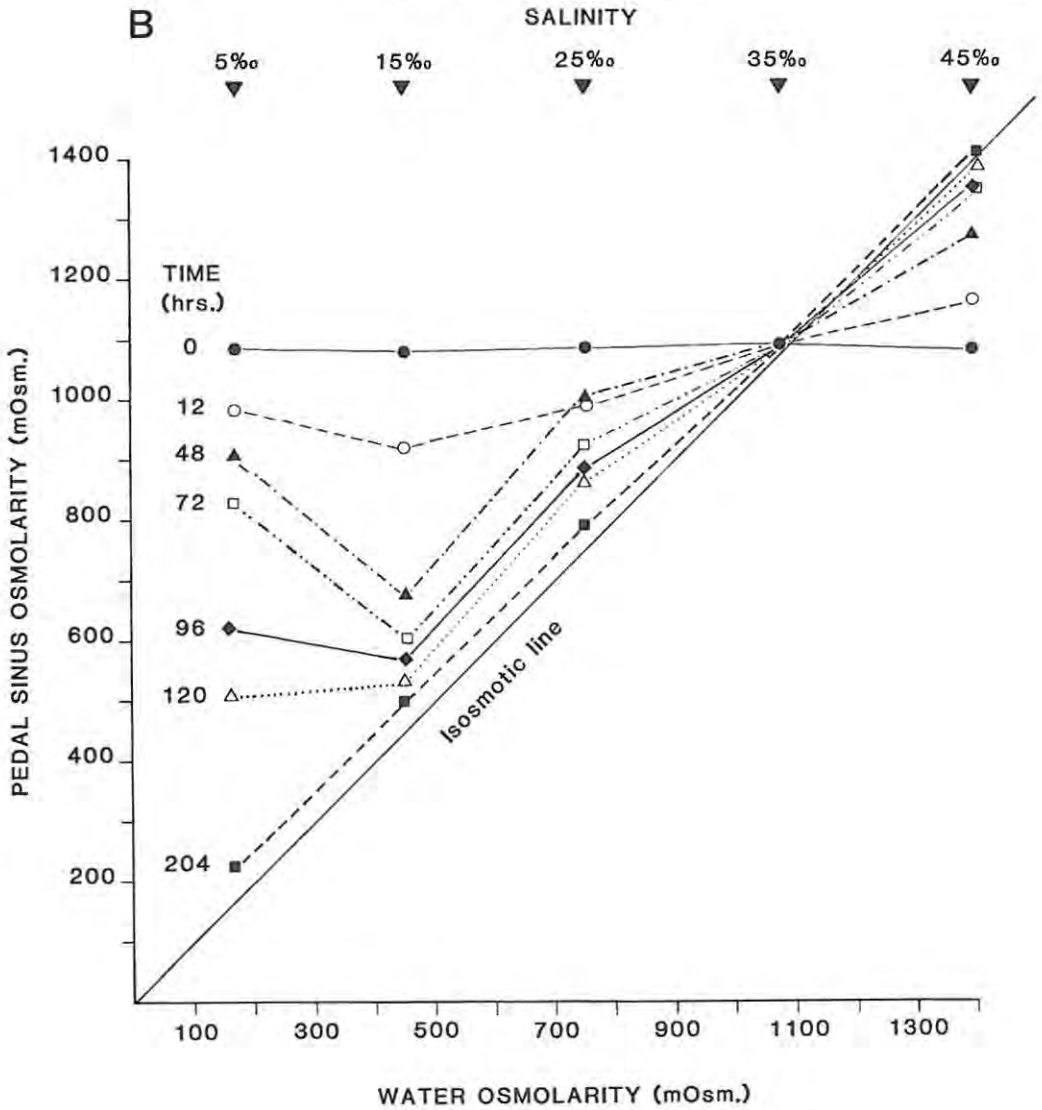

Fig. 1. Cont.

determined on volumes of 0.2 ml using an Advanced Instruments Inc. Model 3D digimatic osmometer.

In the second experiment, animals were retained in their burrows and the pots transferred to tanks in which the water had been adjusted to the same experimental salinities (as in the first experiment). Salinities in the experimental tanks was monitored daily using a Reichert refractometer and kept close to the desired level (± 1‰) by the addition of distilled water as required. Six animals were removed from the pots at each salinity and the osmolarity of the haemocoelic fluid determined as previously described. Again, two readings per animal were possible. Animals were removed every 6 h for the first 24 h and every 12 h thereafter for a total period of 204 h. In both experiments, the condition of the animals was determined by their response to handling and their reaction to mechanical stimulation of the siphon or foot. The mean values for certain groups of animals (as shown in Fig. 1A & B), together with the stadard deviations are given in Table 1.

RESULTS AND DISCUSSION

The results of the *in vitro* experiments are given in Figs. 1A, 2A and Table 1A. These data show

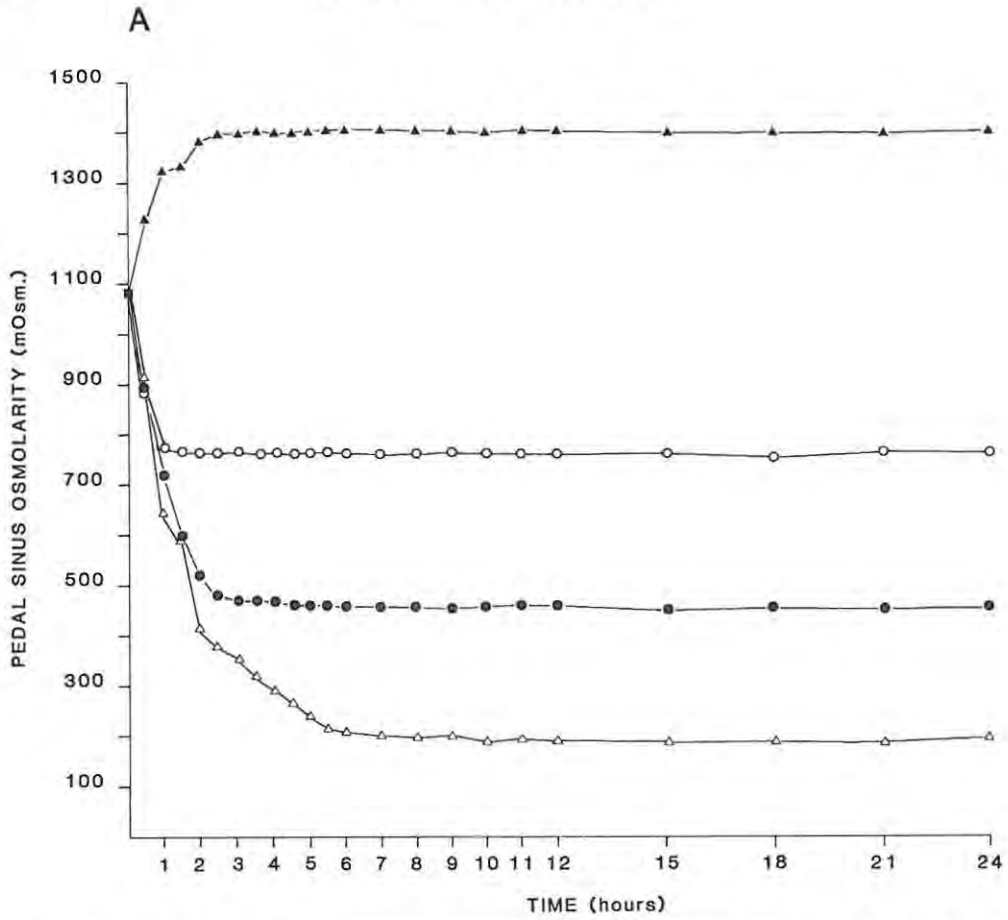

Fig. 2. Variation in pedal sinus osmolarity with time in animals acclimatized to 35‰ (1074 mOsm) and exposed to; 45‰ (1394 mOsm) ▲——▲: 25‰ (754 mOsm) ○——○; 15‰ (451 mOsm) ●——●; 5‰ (167 mOsm) △——△ A = Animals removed from burrows, B = Animals retained in burrows.

that the haemocoelic osmolarity of the animals rapidly conforms to that of the external medium, and confirms the findings of McLachlan and Erasmus (1974) on the same species (*Solen corneus* (= *cylindraceus*)). The osmolarity of the haemolymph is held slightly above that of the external medium over the salinity range tested. Similar weak control has been recorded by Pierce (1970) for four species of *Modiolus* over their non-lethal salinity range, and by Freeman & Rigler (1957) for *Scrobicularia plana*. Animals held at 5‰ exhibited an anaesthetized response after 2-4 h. Virtually no foot or valve response was obtained after 6-8 h. After 10-12 h there was no response even after inserting a hypodermic needle and although the experiment was continued, the animals were deemed dead!

Results of experiments in which animals were allowed to remain in their burrows and exposed to altered salinities are recorded in Figs. 1B, 2B and Table 1B. A marked delay in attaining osmoconformity is observed at all salinity levels tested. In animals exposed to 45‰, osmoconformity occurred after 132 h. For animals exposed to 5, 15 and 25‰, despite substantial decrease in haemocoelic concentrations, osmotic equilibration had not occurred by the end of the experiment (204 h). Animals exposed to 5‰, exhibited an anaesthetized response only after 144-156 h, which persisted for the remainder of the experiment. No deaths were recorded in any of the salinities used in the second experiment. The striking decrease in the rate of equilibration recorded at 5‰ (Fig. 1B and 2B)

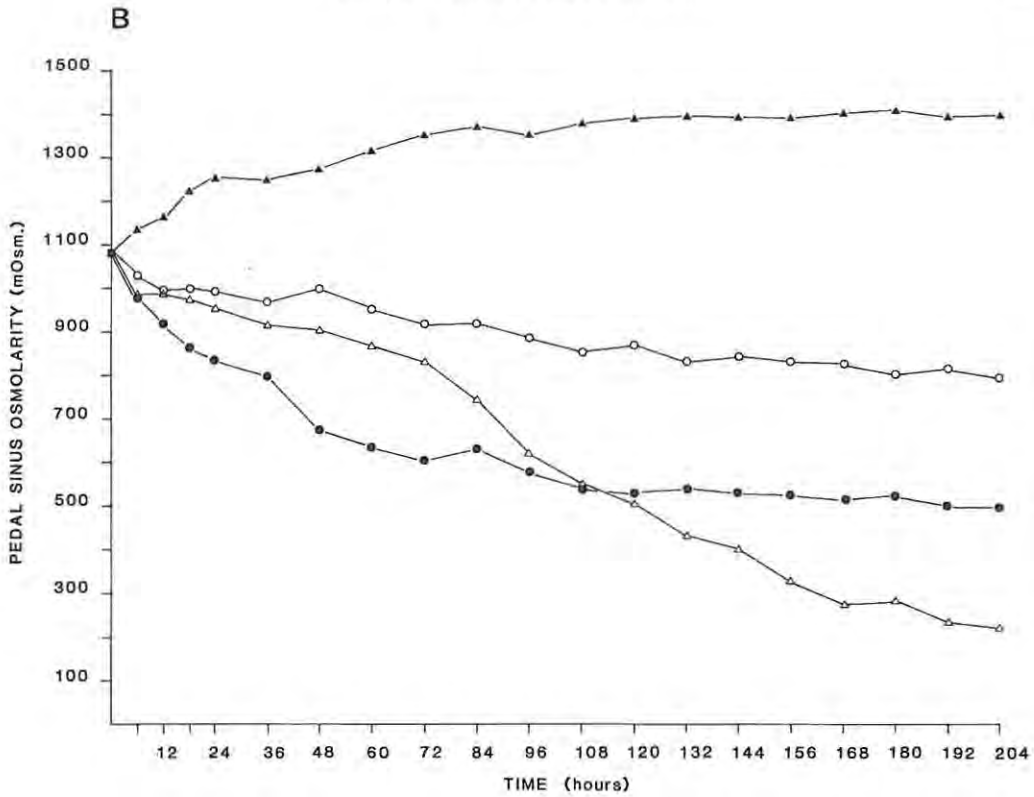

Fig. 2. Cont.

is considered to be due to a response by the animal to substantial and unfavourable salinity change. It was noted that animals, when removed from pots exposed to 5‰, had retreated to the base of their burrows, where a more osmotically favourable 'microhabitat' is likely to be found, and where they remained for the duration of the experiment.

Many aquatic molluscs have the capability of withstanding large changes in salinity for reasonably long periods of time by employing a variety of behavioural responses such as valve closure, which is reported for a number of bivalve species, e.g. *Glycymeris glycymeris* (Gilles, 1972), *Mytilus edulis* and *Scrobicularia plana* (Hoyaux, Gilles & Jenniaux, 1976; Shumway, 1977). *Crassostrea gigas* (Shumway, 1977) and *Anadara senilis* (Djangmah, Shumway & Davenport, 1979). The use of the operculum to tightly seal the shell aperture has been recorded in some gastropod species, e.g. *Littorina saxatilis* (Avens & Sleigh, 1965), *Littorina littorea* and *Purpura lapillus* (Hoyaux *et al.*, 1976). Gastropods such as *Siphonaria pectinata* (McAlister & Fisher, 1968) and *Patella vulgata* (Hoyaux *et al.*, 1976) achieve much the same protection by clamping down and adhering firmly to the rock. In those bivalve species incapable of complete valve closure, e.g. *Mya arenaria*, it is suggested (Shumway, 1977), that the presence of joined mantle edges and siphonal sphincter muscles, are sufficient to isolate the animal from the external medium and is comparable to the mechanism of valve closure. *Solen cylindraceus*, like *Mya arenaria* is incapable of complete valve closure and possesses a similarly fused mantle edge. However, the results presented here indicate that unlike *Mya arenaria* (Shumway, 1977), *Solen cylindraceus* effectively utilizes its burrowing ability to buffer short term effects of any substantial variation in osmotic concentration of the external medium. The use of a burrowing habit in this way as a behavioural response in molluscs to osmotic stress has not been well documented. Shumway (1977) has indicated that the burrowing habit of both *Scrobicularia plana* and *Mya arenaria* is not linked to behavioural

Table 1. Variation in haemolymph osmotic pressure in animals, A: removed from burrows, and B: retained in their burrows, acclimatized to 35‰ (1074 mOsm) and exposed to different salinities. Haemocoelic concentrations expressed as a mean value ± STD deviation.

Time (h)	Exposure Salinity 5‰ (167 mOsm)	15‰ (451 mOsm)	25‰ (754 mOsm)	45‰ (1394 mOsm)
A: Animals Removed from Burrows (n = 8, see text)				
0	1089 ± 3.67	1088 ± 1.77	1084 ± 2.03	1087 ± 4.17
0.5	912 ± 4.91	891 ± 10.93	882 ± 3.66	1225 ± 3.52
1.0	643 ± 5.47	720 ± 3.88	776 ± 4.83	1322 ± 4.43
1.5	590 ± 4.56	594 ± 3.77	768 ± 6.54	1330 ± 3.87
2.0	415 ± 3.38	519 ± 2.13	765 ± 4.77	1378 ± 3.73
4.0	290 ± 3.02	470 ± 1.88	762 ± 8.67	1399 ± 1.72
12.0	193 ± 3.64†	460 ± 1.30	762 ± 5.44	1403 ± 1.98
B: Animals Retained in Burrows (n = 12, see text)				
0	1084 ± 4.05	1080 ± 1.98	1086 ± 3.74	1085 ± 4.13
12	986 ± 12.58	919 ± 14.06	992 ± 11.24	1163 ± 22.50
48	903 ± 15.42	674 ± 12.48	999 ± 17.25	1271 ± 16.61
72	830 ± 20.70	605 ± 15.08	922 ± 14.12	1350 ± 12.56
96	621 ± 13.83	576 ± 9.12	866 ± 10.06	1350 ± 9.95
120	507 ± 13.75	529 ± 13.60	868 ± 26.06	1387 ± 8.60
204	224 ± 11.06	499 ± 12.75	793 ± 23.73	1395 ± 7.72

† Animals classified dead.

osmotic control. Djangmah *et al.* (1979) note that *Anadara senilis* burrows deeper into the mud substrate to avoid exposure to fresh water, but that this aspect of their behaviour has not been investigated.

It is known that interstitial salinities are reasonably stable and persist for relatively long periods of time despite salinity variations in the overlying water column (Sanders, Mangelsdorf & Hampson 1965; Green, 1968; McLusky, 1968; Kinne, 1971). It is suggested that the observed behavioural osmoregulation in *Solen cylindraceus* which are undisturbed in their burrows, is linked to the stability of the interstitial salinity.

Other methods of osmotic control occur in bivalves. Gainey (1987) has suggested that osmotic hysteresis in the case of *Mytilus edulis* (in which the valves were wedged open), may be effected by a combination of 3 mechanisms: (i) decreased ciliary activity, (ii) decreased osmotic permeability, (iii) an increase in urine production. While such a hysteretic effect is unknown for *Solen*, de Villiers (in press) has reported a substantial decrease in filtration rate during short term (*in vitro*) exposure to lowered salinity. This may be explained by Shumway's (1977) proposal of decreased ciliary activity and possible arrest in response to hyposmotic stress, and reinforced by Mattissen's (1960) results who demonstrated that the pumping rate of *Mya arenaria* varies directly with salinity.

However, under conditions where the animal is removed from its burrow, its seems unlikely that ciliary arrest and the associated decrease in filtration rate would play an important role in the maintenance of the animal's osmotic state. On transfer of animals from 35‰ to, in particular, lower salinities, there is, initially, active probing by the foot accompanied by frequent valve adduction. This activity would certainly aid in irrigating the mantle cavity and so possibly increase the rate of osmotic equilibration, as discussed by Davenport (1979) in his work on *Mytilus*. Such responses contribute to the artificiality of *in vitro* experiments and indicate the material importance of studies in which environmental disturbance is kept to a minimum.

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Effect of salinity and temperature on the filtration rate and distribution of *Solen cylindraceus* (Hanley)

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Abstract

Filtration rates of the infaunal estuarine bivalve *Solen cylindraceus* were greatest (19- 23 ml·min^{-1}) within the salinity and temperature range 15-45‰ and 15-35 °C. Below and above these ranges, filtration rates decreased markedly. When subjected to abrupt changes in salinity (final salinity range of 5 to 55 ‰), filtration rates were immediately depressed. The extent of this initial depression was always less in animals exposed to hyper- than hyposaline conditions, with a coincident shorter recovery time of filtration rate, to approximately pre-experimental levels. Animals exposed to conditions of 10 °C irrespective of salinity, and 10‰ irrespective of temperature all exhibited a permanently depressed rate of filtration. Animals exposed to increased temperature and simultaneous elevated or unchanged salinity, show a slight increase in their filtration rate, followed by a rapid acclimation. A decrease in both temperature and salinity resulted in an initial decrease in filtration and subsequent longer acclimation period. It is suggested that the ability of *S. cylindraceus* to acclimate fully within a wide temperature and salinity range, and to filter maximally in hypersaline conditions may explain its unusually high biomass in the Kariega estuary, despite it being close to the southernmost limit of the animals geographical distribution. This estuary is marine dominated and is characterized by elevated, stable salinities (35-45 ‰) in the middle and upper reaches, which is where *S. cylindraceus* is found.

Keywords: estuarine bivalve, filtration rate, salinity, temperature.

Introduction

Estuaries in the south and eastern regions of South Africa tend to be narrow and shallow, and consequently may be subject to rapid changes in physical conditions (Day 1981). One such estuary, the Kariega (33°41'S; 26°41'E), owing to a small and highly regulated catchment, is a marine dominated system in which hypersaline conditions (45-46‰) are commonly recorded in the upper reaches (Allanson & Read 1987). Such conditions are relieved by infrequent flooding (approximately once every 5 years) which results in salinities of < 5.0‰ at the head and middle regions of the estuary. Water temperatures fluctuate seasonally between 7-22 °C in winter and 14-35 °C in summer. However, shorter-term temperature fluctuations of 10-12 °C, within a single tidal cycle, have been recorded occasionally (Taylor 1988). The extent of such episodic physical variations, to which the benthic infauna may be subjected, depends upon the magnitude of flooding by fresh water, and severity of coastal upwelling.

Successful occupation of estuarine habitats requires an ability to respond (behaviourally and/or physiologically) to both long- and short-term changes in the environment. One of the dominant macrobenthic organisms of the Kariega estuary, is the intertidal bivalve mollusc, *Solen cylindraceus* (Hanley, 1843). Although it inhabits estuaries from central Mocambique to the south coast of South Africa (Day 1981, Kilburn & Rippey 1982), it is particularly abundant in the Kariega where, despite being close to the southernmost limit of its geographical distribution (Figure 1), reaches densities of 400·m^{-2} and a biomass of 70 g·m^{-2} dry wt (Hodgson 1987). Similar densities have also been recorded at St Lucia, but with a maximum biomass of only 14.3 g·m^{-2} dry wt (Blaber *et al.* 1983). Despite its abundance in some estuaries, very few studies on the biology of *S. cylindraceus* have been published. McLachlan & Erasmus (1974) have recorded the salinity tolerance and upper lethal temperature (*in vitro*) for *S. corneus* (= *cylindraceus*) as 13-42‰ and 44.5 °C, while Blaber *et al.* (1983) have recorded the presence of *S. cylindraceus* in estuaries with salinities ranging from 7 to an excess of 55‰. More recent studies on *S. cylindraceus* (de Villiers & Allanson 1988, de Villiers, Allanson & Hodgson in press) have indicated that the bivalve exhibits a seasonal compensation in filtration rate, and acclimates fairly rapidly over its thermal optimal range (15-35 °C), provided such conditions persist for a few days. *Solen* is, however, incapable of adjusting its filtration rate rapidly enough to acclimate to short-term temperature fluctuations, i.e. within a single tidal cycle (de Villiers *et al.* in press).

The aims of this study were to determine the effect of salinity, and the interaction of temperature and salinity, on filtration rate. Filtration rates were used as indices of the animals potential to adapt to environmental fluctuations in temperature and salinity. These results were then used to assess the relative importance of

the two environmental factors in determining the distribution of *Solen cylindraceus* and its unusually high abundance in the Kariega estuary.

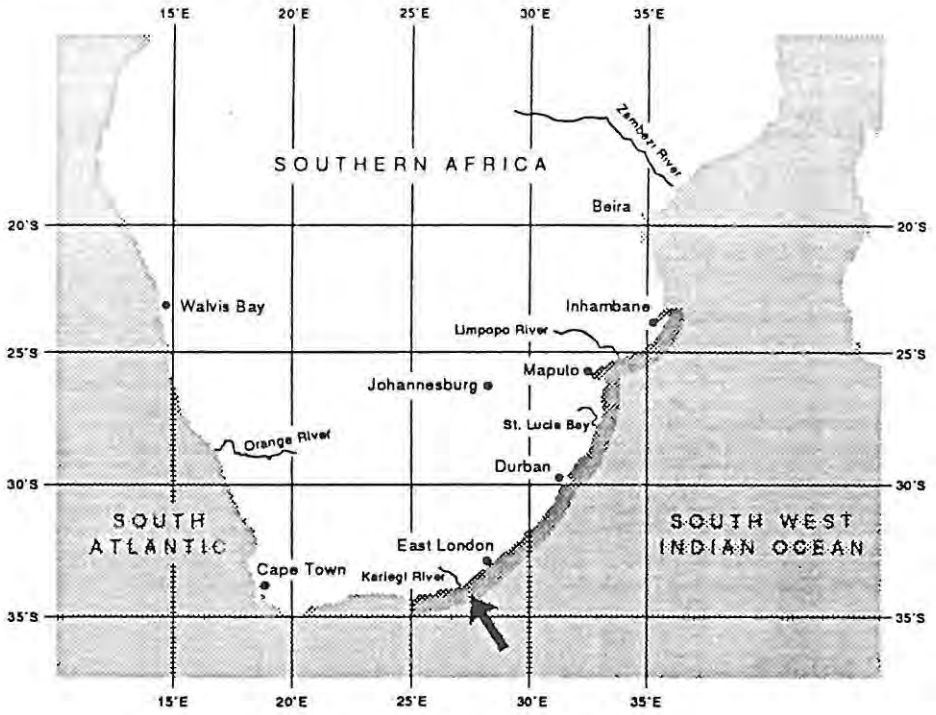

Figure 1.
Study area (arrowed) and distribution of *S. cylindraceus*.

Materials and methods

A. *Determination of the salinity optimum*

Salinity optima were determined for summer and winter collected animals of 50-60 mm shell length (modal size for *Solen cylindraceus* in the Kariega estuary). Animals were placed in mud-filled pots at the site of collection, left for 24 hours and then transferred to laboratory aquaria, conditions in which matched those measured in the estuary, 25 °C & 35 ‰ (summer) and 15 °C & 35 ‰ (winter) (± 1 °C and ± 2 ‰). These mid-season values were regarded as being 'standard' summer and winter conditions, respectively. Animals were allowed 7 days to acclimate to laboratory conditions of a 6 hourly tidal cycle and 14L:10D hr and 12L:12D hr photoperiod for summer and winter collected specimens, respectively. After this period, salinities of individual aquaria were altered at a rate of 2.5 ‰·d^{-1} until the desired concentrations; 5, 10, 15, 25, 35, 45 and 55 ‰ had been reached. Salinities were held constant (± 2 ‰) and animals were allowed a 30 day acclimation period.

Filtration rates were determined indirectly in a closed system as described by de Villiers *et al.* (in press), using a Coulter Counter and a culture of the green algae *Tetraselmis suecica*. Only particles in the size range 6-9 µm (modal size for culture) were counted. This particle size has been shown to be retained with 100% efficiency (de Villiers & Allanson 1988). The filtration rate is expressed as a mean value of 3 determinations per animal, for 10 animals at each salinity, except for animals exposed to 5 ‰, which were determined after 11 days (n = 3) for summer, and 12 days (n = 4) for winter groups, following a 70 and 60% mortality, respectively. Filtration rates were calculated according to Coughlan (1969). Previous work using a flow-through system (de Villiers & Allanson 1988) with filtration rates calculated according to Hildreth & Crisp (1976) gave comparable results. A control chamber was set up under identical conditions, and contained plasticine filled *Solen* valves of the same dimensions as the experimental animal.

B. *Effect of abrupt salinity change on the filtration rate of animals acclimated at different temperatures*

Animals were collected during summer (27 °C & 36 ‰) and winter (15 °C & 35 ‰) and allowed a 7 day acclimation period to laboratory conditions as previously described. Summer-collected animals were divided into 3 groups. One group was maintained at 25 °C & 35 ‰, the second was subject to a temperature increase of

1 °C·d^{-1} to 35 °C with a 7 day acclimation time at that temperature. The third group was kept at 25 °C but subjected to a salinity decrease of 25 ‰·d^{-1} to 15 ‰, and allowed a 14 day acclimation period. A similar rate of change and acclimation time was adopted for the winter-collected animals, to yield a further 3 groups, acclimated to conditions of 15 °C & 35 ‰; 10 °C & 35 ‰ and 15 °C & 15 ‰. Each acclimation group was divided into 5 batches, four of which were subject to abrupt salinity changes (to either 5, 15, 25, 35 or 45 ‰) while the fifth was held constant at the acclimated combination and served as a control. Animals were removed from the pots and filtration rates determined (as described) at each of the following times:

1) on immediate exposure, 2) two-hourly for the first 6 hours, 3) six hourly to 24 hours, 4) twelve hourly to 72 hours, 5) every 24 hours to a total of 216 hours. Filtration rates are expressed as a mean of 3 determinations per animal, for a total of 5 animals at each time interval.

C. *Effect of simultaneous temperature and salinity changes, on filtration rate*

Animals were collected during summer (23 °C & 35 ‰) and winter (16 °C & 35 ‰), transferred to aquaria and allowed a 7 day acclimation period to 'standard' conditions as previously described. After this period, 5 animals were removed from each of the temperature:salinity combinations and their filtration rates determined. The mean of these was regarded as the initial or control rate. Pots containing animals were then transferred to different aquaria in which conditions had been set to 15 °C & 25 ‰; 15 °C & 15 ‰; 20 °C & 15 ‰; 3 °C & 45 ‰ and 15 °C & 35 ‰ in the case of the summer-acclimated animals, and 10 °C & 25 ‰; 10 °C & 15 ‰; 20 °C & 15 ‰; 25 °C & 45 ‰ and 25 °C & 35 ‰ for winter-acclimated animals. Filtration rates were determined at the same time periods as described in Section B.

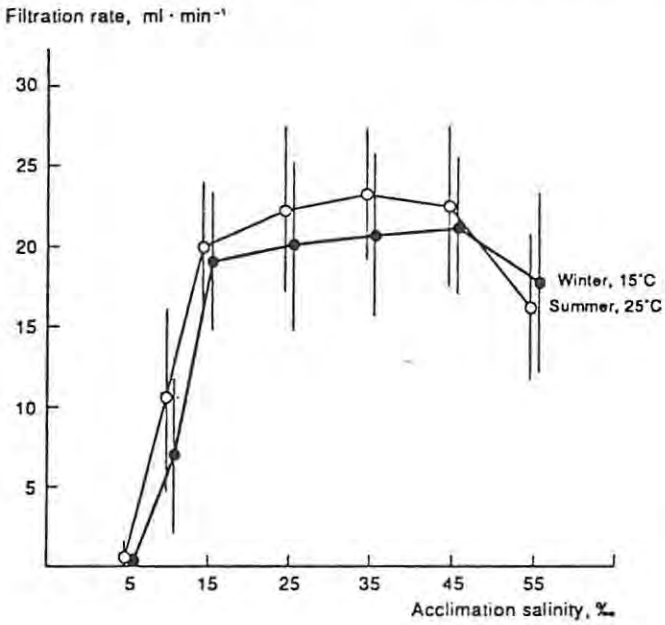

Figure 2.
Filtration rates ($x \pm SD$) for *Solen* at different acclimation salinities.

Results

A. *Salinity optimum*

Figure 2 indicates maximum filtration rates of 19-23 ml·min^{-1} in the range 15-45 for both summer- and winter-acclimated animals. Analysis of variance indicates that salinity significantly influenced filtration rate ($p < 0.01$), but not within the optimal range (15-45 ‰). At all salinities, season did have a significant effect, and winter values were consistently lower than summer rates (range of 1-3 ml·min^{-1}), except at 55 ‰, where the trend was reversed.

B. *Effect of abrupt salinity change on filtration rate at different acclimation temperatures and salinities (Figures 3 & 4)*

Animals acclimated to 35 ‰ and temperatures of 15, 25 and 35 °C, all exhibited an initial decrease in filtration rate (Figure 3A-C), when exposed to different salinities. The level to which filtration rate was suppressed, was correlated with the magnitude of salinity change. In all instances, the effect of hypersaline conditions (45 ‰, a 10 ‰ increase) was less marked than hypersaline conditions (25 ‰, a 10 ‰ de-