Aspects of the ecology and biology of the isopod, *Exosphaeroma hylocoetes*, (Barnard, 1940) in three temporarily open/closed southern African estuaries.

A thesis submitted in fulfilment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY

of

RHODES UNIVERSITY

by

TONY OSKAR HENNINGER

October 2008

ABSTRACT

Temporarily open/closed estuaries (TOCE's) are the numerically dominant type of estuary accounting for ≈ 70 % of all estuaries along the South African coastline. Despite their numerical dominance, aspects of the biology of organisms within these systems, particularly macrocrustacea remain poorly understood. This study firstly assessed the abundance and biomass of the most common isopod, *Exosphaeroma hylocoetes*, in three Eastern Cape TOCE's, (the West Kleinemonde, East Kleinemonde and Kasouga Estuaries), and their response to mouth breaching events. This study was followed by aspects of the biology of the isopod including their utilisation of submerged macrophytes as a refuge from predation and/or possible food source, as well as the growth rates of the isopods in the laboratory under different environmental conditions.

Mean isopod abundances and biomasses ranged between 0 and 4 791 ind. m^{-2} and 0 and 9.65 mg dwt. m^{-2} in the West Kleinemonde Estuary and between 0 and 108 ind. m^{-2} and 0 and 0.318 mg dwt. m^{-2} in the nearby East Kleinemonde Estuary. In the Kasouga Estuary, the values ranged between 0 and 3 650 ind. m^{-2} and 0 and 5.105 mg dwt. m^{-2}. Temporal and spatial changes in the abundance and biomass of *E. hylocoetes* within the three systems was primarily linked to mouth phase, with populations declining when the mouth was open and to a lesser extent, seasonality. In all three estuaries maximum isopod abundances and biomasses were recorded in their middle and upper reaches, which could be ascribed to the presence of submerged macrophytes particularly *Ruppia maritima*, in two of the estuaries. Males (5.71 ± 0.41 mm) were significantly larger than females (3.99 ± 0.26 mm), but the sex ratios were skewed in favour of females, (a common feature in many isopod populations). Females were found carrying brood throughout the study, releasing offspring directly into the water column, to allow recruitment to the populations. The larger the female, the larger the brood carried (up to a maximum of 72 embryo/mancas).

Results of laboratory experiments indicate that the close association of *Exosphaeroma hylocoetes* with submerged macrophytes is a result of the plant stands providing a refuge against predation by selected ichthyofauna. However stable carbon isotope and fatty acid analyses indicate that *E. hylocoetes* made use of ephiphytic algae and detritus on the stems of *R. maritima*, rather than the submerged macrophyte itself.

There were no significant differences in the growth rates of male and female isopods at combinations of temperature (15 and 25 °C) and salinity (15 and 35 ‰). Laboratory growth studies revealed that males lived longer than females (25.77 ± 3.40 weeks vs 21.52 ± 3.00 weeks), and therefore achieved larger overall size. Females, however, reached sexual maturity (at 2.5 mm) at a faster rate (two to four weeks) than males (5.5 mm after 6.5 to 11 weeks), dependent on temperature.

Exosphaeroma hylocoetes with its association with submerged macrophytes, early maturity, its growth rates and longevity, female-biased sex ratios and year-round breeding, all contribute to its success in temporary open/closed estuaries.

ACKNOWLEDGEMENTS

There are always people to be thanked, as a thesis is not always a solo effort. Firstly, I wish to thank my family: my wife, Catherine, and daughters, Helen and Katharine, for permitting my return to university as a full-time student to complete this thesis at this stage of my life. I have really appreciated your support throughout this time, as it has cost many sacrifices. It has not been an easy time, particularly with our two mothers, Dr Helen Robinson and Gisela Henninger, dying within four months of each other during the second year of this study (2007) and not being here at the end to see this work completed. I am, however, looking forward to crossing the stage with Helen and Katharine at the Graduation Ceremony in April 2009.

My co-supervisors, Professors Alan Hodgson and William Froneman, have always been frank and honest in reviewing my chapters, and for this I am truly grateful. Alan organised my being his teaching assistant before I came back to Rhodes University, allowing us to survive during the three years in Grahamstown. William, who had an office built in his laboratory (as a sanctuary, in which I could work), has always been available with positive suggestions, despite having many other post-graduate students.

The following are to be thanked: Professor Tony Booth (isopod growth rate statistical analyses); Dr Nicole Richoux and Louise Allan (fatty acid analyses); and Dr Doug Downie (*Wolbachia* protocol).

I wish to thank Rhodes University and the National Research Foundation for assistance in financing this research.

TABLE OF CONTENTS

CHAPTER 3: POPULATION DYNAMICS OF THE ESTUARINE ISOPOD, *EXOSPHAEROMA HYLOCOETES*, (BARNARD, 1940)…………………..33

CHAPTER 4: THE ROLE OF MACROPHYTES AS A REFUGE AND/OR FOOD SOURCE FOR THE ESTUARINE ISOPOD, *EXOSPHAEROMA HYLOCOETES* ………..………...69

CHAPTER 5: GROWTH AND LONGEVITY OF *EXOSPHAEROMA HYLOCOETES* UNDER VARYING PHYSICO-CHEMICAL CONDITIONS………………………..…………………………………..92

LIST OF FIGURES

LIST OF TABLES

PREFACE

Data for this study was collected from February 2006 to February 2008.

Chapters 3 and 4 have been accepted for publication:

Henninger, T.O., Froneman P.W. & Hodgson A.N. (2008). The population dynamics of the estuarine isopod *Exosphaeroma hylocoetes*, (Barnard, 1940) within three temporarily open/closed southern African estuaries. *African Zoology* 43: 202 -217

Henninger, Tony O., Froneman, P. William, Richoux, Nicole B. & Hodgson, Alan N. (In press). The role of macrophytes as a refuge and food source for the estuarine isopod *Exosphaeroma hylocoetes* (Barnard, 1940). *Estuarine, Coastal and Shelf Science* (2009), *doi: 10.1016/j. ecss.209.01.017*

Chapter 5 has been submitted for publication:

Henninger, T.O., Froneman P.W., Booth, A.J. & Hodgson A.N. Growth and longevity of *Exosphaeroma hylocoetes* under varying physico-chemical conditions. *Journal of Experimental Marine Biology and Ecology*: JEMBE-D-08-00518

DECLARATION

The following thesis has not been submitted to any other university other than Rhodes University, Grahamstown, South Africa. The work presented here is that of the author, unless stated otherwise.

Chapter 1

General Introduction and Rationale for Study

1.1 General Features and Habitats of Isopods

The objective of this thesis is the autecological study of an isopod inhabiting South African temporary open/closed estuaries. I therefore offer a brief taxonomic summary of the isopods and an overview of South African estuaries before outlining the structure of the thesis.

Isopods are small arthropods that range in size from about 0.5 to 500 mm in length (Brusca and Wilson, 1991). The order Isopoda Latreille, 1817 belongs to the class Malacostraca Latreille, 1802 of the sub-phylum Crustacea Brünnich, 1772 (Martin and Davis, 2001). As a group, the isopods date from the Carboniferous period of the Paleozoic, i.e. about 300 million years ago (Brusca and Wilson, 1991). The thin nature of the isopod exoskeleton, however, tends to make them poor candidates for fossilisation and it is therefore possible that they have a longer ancestry.

The isopod body, which is chitinous, is divided into a head (or cephalon), a thorax (or pereon), and an abdomen (or pleon). The pleon ends in a telson, and as the terminal segments are usually fused, they are jointly known as a pleotelson (Fig. 1.1).

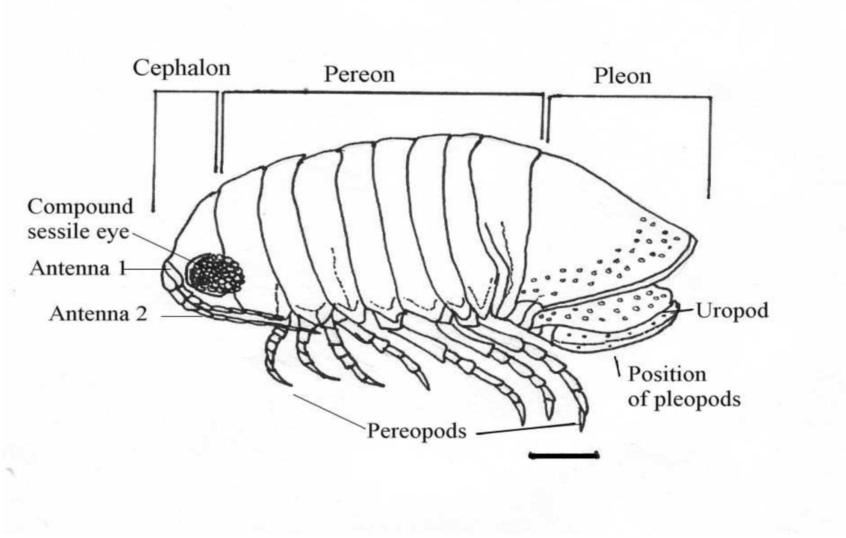

Figure 1.1. Lateral view of the flabelliferan isopod, *Exosphaeroma hylocoetes,* showing different body parts/regions. (Spelling taken from Kensley, 1978). (Scale bar = 1 mm).

Isopods can be found in practically all aquatic environments, including shallow and deep-water marine habitats, estuaries and the intertidal zone, where they are most diverse and abundant; e.g. the sandy beach isopod, *Cirolana harfordi,* was found in densities of up to 12 600 ind. m^{-2} in mussel beds in Monterey Bay, California, (Hewatt, 1937); along the coast of Portugal densities of *Tylos ponticus* and *T. europaeus* were found in average densities of 3 790 ind. m^{-2} in coastal salt marshes, (Dias and Sprung, 2003); and ≈ 50 ind. m^{-2}, (Gonçalves *et al.,* 2005) respectively (Table 1.1). Some have invaded freshwater habitats (possibly from the estuaries), and can even be found in thermal springs and caves (Brusca and Wilson, 1991). The isopods are perhaps the most successful of the terrestrial crustaceans and can even be found in deserts (Warburg, 1968; Shachak *et al.,* 1976; Shackak *et al.,* 1979). It is not surprising that isopods, because of their diverse nature, have a variety of feeding habits, including herbivory, carnivory (including cannibalism), omnivory and detritivory (Johnson, 1976a, b; Perry and Brusca, 1989; Briones-

Table 1.1 Isopod densities in selected studies conducted in the northern- and southern hemispheres.

Location	**Isopod species**	**Density (ind. m^{-2})**	**Source**
A. Northern hemisphere			
Kentucky USA Freshwater stream	*Lirceus fontinalis*	6615	Holomuzki and Short, 1988.
California USA Salt marsh	*Sphaeroma quoyanum*	8000	Tulley *et al*., 2001.
N. Florida USA Marsh	*Cyathura polita*	58 – 75	Kruczynski and Subrahmanyam, 1978.
Portugal Modego Estuary	*Cyathura carinata*	1851 – 5518	Ferreira *et al*., 2004.
Japan Hamanaka Bay	*Tecticeps glaber*	2060	Tooru *et al*., 2004.
B. Southern hemisphere			
Chile North beaches	*Excirolana hirsuticauda*	48 000 – 50 000 *****	Contreras and Jaramillo, 2003.
Chile South beaches	*Excirolana hirsuticauda*	≤ 13 000 *****	Contreras and Jaramillo, 2003.
West Cape, RSA Kelp debris	*Ligia dilatata*	≈ 4300 *	Koop and Field, 1980
West Cape, RSA Bot River (TOCE)	*Exosphaeroma hylocoetes* *Cyanthura carinata*	20 – 1980 80 – 280	Koop *et al*., 1983.
Bot River (TOCE) RSA	*E. hylocoetes* *Cyanthura estuaria*	211 – 738 105 – 486	De Decker and Bally, 1985.
East Cape, RSA Gamtoos Estuary (open)	*Xenanthura sp.* *Cyanthura estuaria* *Cirolana fluviatilis*	17581 278 335	Schlacher and Wooldridge, 1996
	Cirolana fluviatilis	4149 CPUE	Newman *et al*.,2007

* data presented as ind. m^{-1}; TOCE = Temporary Open/Closed Estuary

Fourzán and Lozano-Alverez, 1991; Leonardson, 1992; Shuster and Guthrie, 1999; Barradas-Ortiz *et al.,* 2003; Newman *et al.,* 2007). Some are parasitic on fish (known as "fish lice" and "tongue biters", from the habit of some *Cymothoa* spp. nibbling off fishes' tongues, whilst attached to the floor of the host fish's mouth) as well as on other crustaceans (Kensley, 1978; Briones-Fourzán and Lozano-Alverez, 1991; Wetzer and Brusca, 1997; Thatcher *et al.,* 2003).

1.2 Isopod Taxonomy and Systematics

Published isopod literature is vast and spans roughly 200 years, beginning in Scandinavia when G.O. Sars released his *Crustacea of Norway* series (Sars, 1897 a, b, 1898, 1899 a, b). Much research followed, mainly in the northern hemisphere, carried out by researchers such as Dana (1853a, b, 1854), Lockington (1876, 1877), Benedict (1897, 1898a, b), Boone (1918, 1923) and Wetzer *et al*. (1997). Kussakin (1990) established the existence of two distinct isopod groups: the shallow water and deep sea isopods. Wilson (1998) later demonstrated that most of the deep sea isopods are of the sub-order Asellotta in the northern hemisphere and of the sub-order Flabellifera in the southern hemisphere (Wilson, 1998).

The southern hemisphere supports approximately 1000 species of isopods, and most recent research on them has been carried out mainly in Australia, where studies on the sub-order Phreatoicidea have led to a greater understanding of isopod evolution (Wilson and Ho, 1996; Wilson and Fenwick, 1999; Wilson and Johnson, 1999; Wilson and Keable 1999, 2001, 2002), and to a lesser extent New

Zealand, where numerically, to date, isopod species numbers are low. Bruce (2003) has recently identified new genera and species of sphaeromatid isopods from Australian coastal waters, and has suggested that the described species of *Exosphaeroma* by Barnard (from southern Africa) and Baker (from southern Australia) during the first part of the 20th century be re-examined and re-described.

Taxonomic work on southern African marine isopods began in earnest in the latter half of the 19th century, with various expedition ships visiting the southern African coastline (Kensley, 1978). W. Stimpson collected in False Bay whilst his U.S. North Pacific Exploring Expedition's ship rode at anchor in Simon's Bay in 1853. The *Challenger*, German South Pole and German Deep-Sea Expeditions (Vanhöffen, 1914) also collected material in southern African waters. The Cape Government's research vessel, the *Pieter Faure* did much work (from 1897 to 1907) to lay the foundation of most of the marine taxonomic work in South Africa.

Kensley (1978) reported that the Rev. T.R.R. Stebbing, working from 1900 to 1910, listed 41 species of marine isopods, this total being increased to nearly 200 species by the work of Dr. K.H. Barnard (Barnard 1914, 1940). Barnard continued to collect various isopod species in the 1950s and 1960s. An increase in the number of species collected was aided by the staff of the University of Cape Town's Zoology Department, with the assistance of the *R/V J.D. Gilchrist* and later the *R/V Thomas B. Davie*. Collections were conducted on the Continental Shelf and in estuaries (Kensley, 1978).

The inter-tidal and shallow water marine isopods of South Africa are well documented, particularly in those regions located in the proximity of coastal cities/ towns, e.g. Lüderitz (southern Namibia), and the bays around Cape Town, e.g. Lambert's Bay, Saldanha Bay, Table Bay, False Bay and Still Bay (Kensley, 1978). However, beyond the 200 m water depth relatively few species were known, probably due to a lack of collecting. Work of the S.A. Museum on board the *R/V Meiring Naude* in 1975 and 1976 in the deep water of Natal (southern African east coast) demonstrated a rich and varied fauna, which needed further investigation.

Kensley (1980) recorded seven species (including two new species) from the Vema and Tripp seamounts and the Lüderitz area of the western coastline of southern Africa. These isopods showed strong zoogeographical affinities with the South African isopod fauna (Kensley, 1980). Kensley (1984) followed up on the east coast fauna by recording 51 species (excluding the Anthuridea), as well as identifying a large endemic fauna on the continental shelf and/or slope of the east coast of South Africa.

There are about 10 000 described species of isopods, but the actual number of sub-orders of the Isopoda is presently unresolved, the number ranging from eight (Roman and Dalens, 1999) to ten (Brusca and Wilson, 1991), with Martin and Davis (2001), most recently proposing nine. I shall follow this latest classification in this thesis. As a whole, the isopods appear to be monophyletic (descending from a single ancestor), except for the suborder Flabellifera. The varied and scattered habitats and locations of the families within this suborder would indicate that they are paraphyletic (Martin and Davis, 2001).

The suborder Flabellifera Sars, 1882, is the most species rich suborder (Kensley, 1978; Martin and Davies, 2001) with ≈ 73 species found in southern African waters. Although most are small, the biggest isopod, the 500 mm long *Bathynomus giganteus* that lives in the cold (4 °C) deep waters of the Atlantic and Pacific Oceans (Briones-Fourzán and Lozano-Alverez, 1991) belongs to this group. Flabelliferans are free-living or parasitic on fish and have a body which is normally symmetrical, with lateral uropods. The suborder is subdivided into three groups, (based on the structure of pleopods 4 and 5), the Eubranchiatae, the Platybranchiatae and Hemibranchiatae (Kensley, 1978). The Hemibranchiatae are of relevance here, as *Exosphaeroma hylocoetes*, the focus of this thesis, belongs to this group. The distinguishing feature of this group is the pleated nature of the inner ramus of pleopods 4 and 5, coupled with a membranous outer ramus (Fig. 1.2). The Hemibranchiatae range in size from 5 to 22 mm (Kensley, 1978).

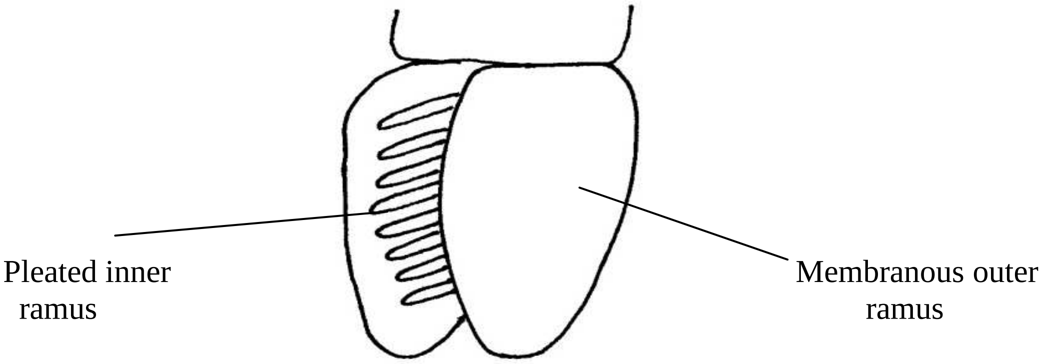

Figure 1.2. Pleopod of the Hemibranchiatae isopods to show the nature of the inner and outer rami. (Adapted from Kensley, 1978).

In southern Africa, there are nine families of Flabellifera (Kensley, 1978), which include the Sphaeromatidae Latreille, 1825 of the group Hemibranchiatae to which *E. hylocoetes* belongs. The Sphaeromatidae are characterised by a body which is usually oval, 3.3 mm to 21 mm in length and often capable of rolling into a ball, known as conglobation (Brusca *et al.*, 2001). The five anterior pleonites are fused and the sixth pleonite is fused with the telson to form a pleotelson. The cephalon is free from pereonite I. The uropodal basis is fused with the endopod, and the exopod can articulate freely (Kensley, 1978). The sphaeromatids are mainly intertidal, but several can be found at greater water depths e.g. 600 m (*Cymodoce* spp.), (Kensley, 1978).

The genus *Exosphaeroma* Stebbing consisits of 11 southern African species that range in size from 4 mm total length (*E. estuarium)* to 15 mm (*E. planum)*. The genus is mostly estuarine and inter-tidal, but some species have been found to a depth of ≈ 100 m (Kensley 1978). *Exosphaeroma hylocoetes* is primarily estuarine.

1.3 Isopod Life Histories

Female isopods use a brood pouch to house the eggs, developing embryos and fully formed offspring, known as manca, before releasing them directly into the water column (Klapow, 1970), i.e. an ovoviviparous system (Fig. 1.3). In many isopods the brood pouch, or marsupium, is external and formed by between three and five pairs of oostegites attached ventrally to the pereonites (Brusca and Wilson, 1991). The external brood pouch may even protrude into the body cavity as found in *Dynamene bidentata* (Harrison, 1984a). However, some species, e.g. certain *Excirolana* species show evidence of internal brood pouches (Klapow, 1970; Fig. 1.3). This feature, i.e. whether the brood pouch is external or internal, has been used as a diagnostic tool to differentiate *Cirolana* from *Excirolana* species (Klapow, 1970). Harrison (1984a, b) has also used this characteristic as a useful generic character in Sphaeromatidae, after investigating 78 genera of this family.

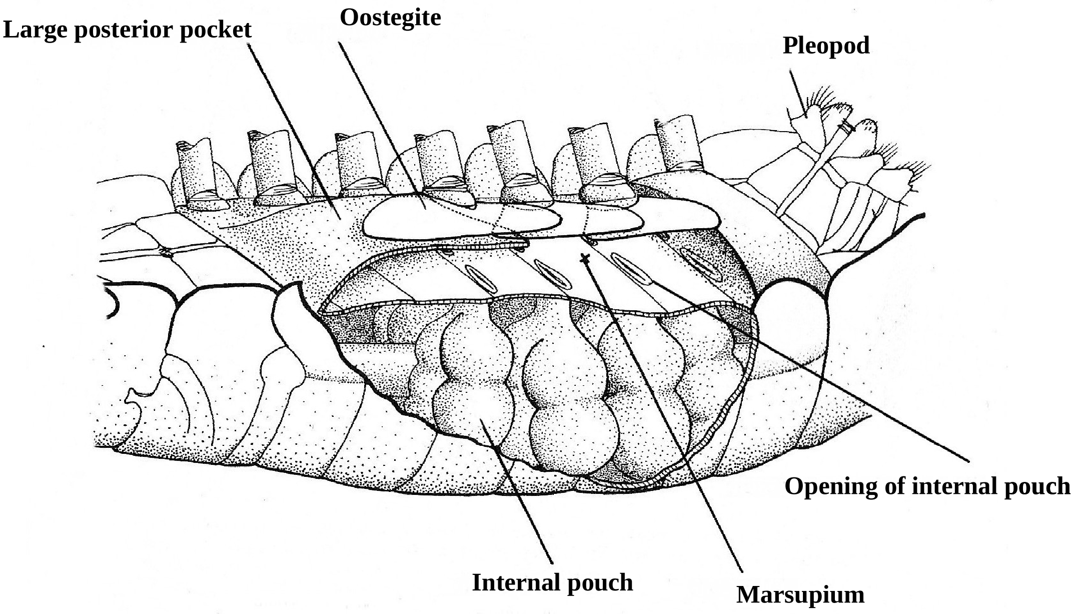

Figure 1.3. Generalised brooding isopod female illustrating possible brood pouch compartments. (Adapted from Harrison, 1984a).

The number of manca released per female is related to female body size (Hatchett, 1947; Jones and Naylor, 1971), and ranges between one and 250 (Ellis, 1961; Carefoot, 1973). Generally, the larger the female, the more manca are released. In boreal species breeding is usually confined to spring and summer, with peaks during summer (Carefoot, 1973). Some species however, e.g. *Jaera albifrons* produce offspring all year round (Jones and Naylor, 1971). Many isopods produce one to two brood per season with females producing brood from between two months and 11 months of age, depending on length of life (Carefoot, 1973). Isopods may live for up to one year in marine and freshwater environments, except *Dynamene bidentata*, an intertidal sphaeromatid, which has males living for two years and females for one year (Carefoot, 1973). Semi-terrestrial (e.g. *Ligia pallasii*) and terrestrial isopods live for between 1.5 and four years, respectively (Carefoot, 1973).

Most marine isopods are sexually dimorphic and have a sex ratio that is biased towards females (Shafir and Field, 1980b). Generally isopod males are larger than females and dominate the older age classes. It is assumed that one male can fertilise several females, resulting in a reduced need for males (Wong and Moore, 1996).

1.4 South African Estuaries

As the focus of this study is an estuarine species of isopod, a brief overview of features and the ecology of South African estuaries, especially temporarily open/closed estuaries is appropriate.

The definition of an estuary varies, depending on whether one is a geologist, oceanographer or biologist (Whitfield, 1992a). Day (1980) defined an estuary as "..a partially enclosed coastal body of water which is either permanently or periodically open to the sea and within which there is a measurable variation of salinity due to the mixture of sea water with fresh water derived from land drainage.” This definition, however, does not adequately describe the variety of estuaries along the South African coastline. Additionally, there is some confusion regarding the terminology. In the past, temporarily closed estuaries have been described as “lagoons” (Begg, 1978; Bird, 1984), “blind estuaries” (Day, 1981a), while Bennett (1989b) referred to them as “seasonally open” and “normally closed”, and Perissinotto *et al.* (2000) as “semi-permanently closed” estuaries. This confusion in terminology led Whitfield (1992a) to refine the terms with regard to estuarine systems in order to create uniformity in understanding and description. Currently, five types of estuaries are recognised along the southern African coastline: river mouths, estuarine bays, estuarine lakes, permanently open estuaries and temporarily open/closed estuaries (Whitfield, 1992a). Salinity has been expressed in terms of the Practical Salinity Scale throughout this thesis and no dimensions or units are thus given.

All South African estuaries share the following common features, (Reddering and Rust, 1990):-

(a) the majority have a small tidal prism $\leq 10^6$ m^3,
(b) they mostly occupy drowned river valleys, while only a few developed on coastal plains,
(c) the tidal inlets are often constricted or blocked by sand bars, and
(d) the flood-tidal plains are usually well developed, while ebb-tidal deltas are poorly developed.

The maximum tidal range of the South African coast is ≈ 2 m between successive high and low spring tides, leading to the estuaries often being described as microtidal systems (Allanson, 1992). The coastal waves are, however, known to control the opening and closing of the temporarily open/closed estuaries (hereafter referred to as TOCEs). Reddering (1988) has found that estuaries situated on open, wave-exposed coastlines tend to close more often than those estuaries on sheltered coasts.

Of the 289 river outlets along the South African coastline only 37 (or 12.8 %) maintain permanent tidal inlets with the sea and can therefore be regarded as permanently open systems (Reddering and Rust, 1990). The overwhelming majority (73 %) of estuaries fall into the TOCE category, making them the dominant type along the South African coastline (Whitfield, 2000; Whitfield and Bate, 2007).

1.4.1 Temporarily open/closed estuaries (TOCEs)

1.4.1.1 Hydrodynamics

TOCEs are characterised by a sandbar at the mouth of the system that separates the estuary from the marine environment. Catchments within these systems are generally small (< 500 km^2), and river flow is frequently very low or absent. They generally have a small tidal prism (< 1 x 10^6 m^3), when the mouth is open, but can appear as river mouths when in flood (Whitfield, 1992a). TOCEs breach when water levels exceed the height of the sandbar (Fig. 1.4); often caused by flood events, resulting in riverine conditions occurring throughout the system (Bennett and Branch, 1990; Wooldridge and McGwynne, 1996; Perissinotto *et al.*, 2000; Walker *et al.*, 2001; Froneman, 2002b; Vorwerk *et al*., 2008).

The mouth phase is strongly linked to the amount of run-off feeding the catchment area. Mesohaline (salinity of 5 to 18) conditions tend to dominate these systems when the mouth is closed, but salinities can reach levels of more than 50 during drought-assisted evaporation (Whitfield and Bruton, 1989). Seepage from the estuary through the sandbar can further increase the salinity of the estuary (Fig. 1.4) during the closed phase (Begg, 1984a; Cooper, 1989; Wooldridge and McGwynne, 1996; Perissinotto *et al.*, 2000). Overtopping of seawater into the estuary during periods of storms leads to a mixing of sea and estuarine waters, particularly in the lower reaches (Fig. 1.4).

Wind drives the mixing of water during the closed phases, while the tides tend to cause mixing during mouth open phases (Harrison *et al*., 2000). Estuarine water temperatures are influenced by the tides during these open phases, but are independent of sea temperatures during the closed phases. There is often seepage of estuarine water through the sandbar into the sea, leading to increased salinity of the estuarine water (Begg, 1984b). Marine and estuarine biota tend to dominate these estuaries, but freshwater organisms can be encountered during oligohaline closed mouth periods (Begg, 1984a).

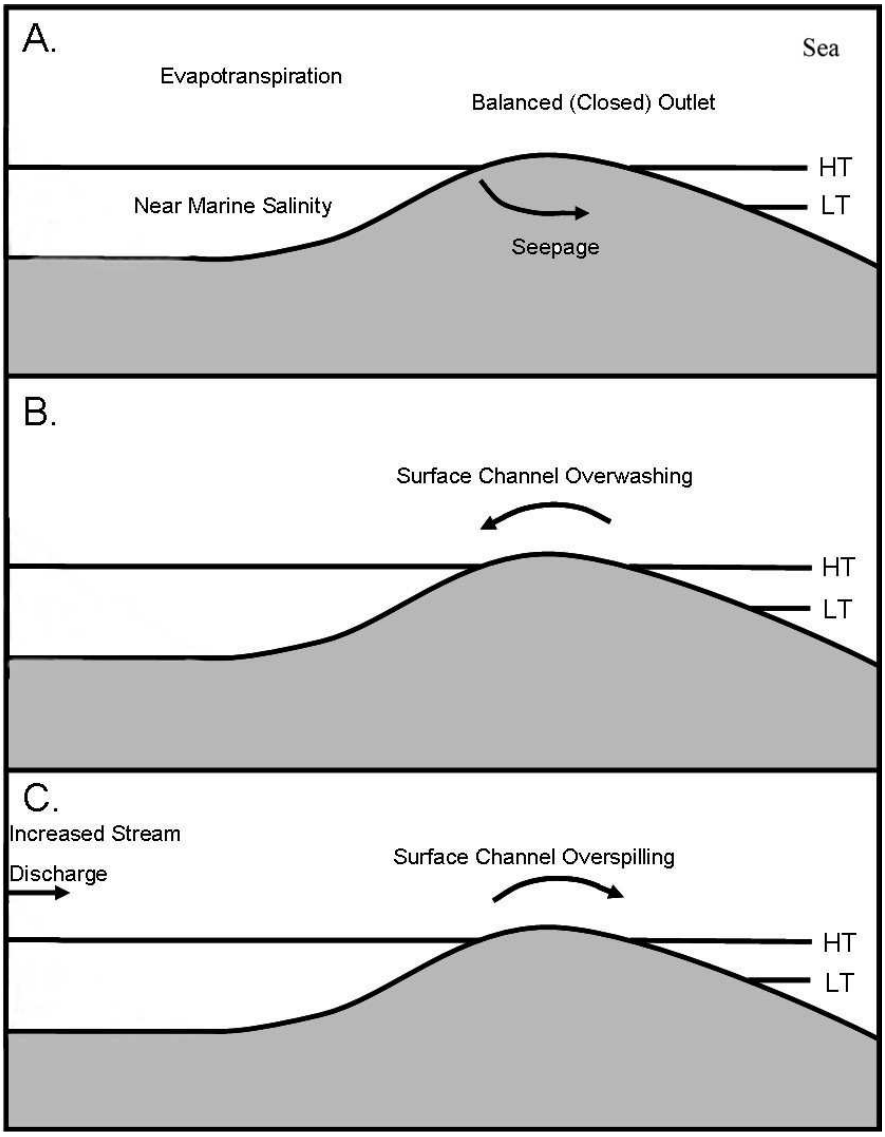

Figure 1.4. Diagram of cross-sections of non-perched and normally closed estuary (TOCE). During balanced conditions (A) stream-flow is balanced by losses through evapo-transpiration and seepage through the berm. During times of high wave energy over-washing (B) brings marine water into the system. Mouth breaching can result following enhanced inputs from over-washing (B) or increased stream-flow (C). Incision depth is low owing to estuarine water levels being so close to the sea level. (Adapted from Harrison *et al*., 2000).

Overtopping and breaching events influence the structure of invertebrate and vertebrate communities of the TOCEs (Vorwerk *et al.*, 2003; Froneman, 2004b, c; Kemp and Froneman, 2004; Perissinotto *et al.*, 2004). TOCEs are also characterised by an absence of horizontal gradients in salinity and therefore the seasonal pattern in biota is linked to the availability of habitat, i.e. submerged macrophytes, rather than a temperature and salinity gradient (Perissinotto *et al*., 2000, 2002; Froneman, 2000, 2002a, 2004b).

During the closed period, once the riverine input reduces the salinity, the physical conditions within the estuary develop into those of a coastal lagoon (Allanson, 1981). Low diversity of species is typical of closed estuaries, resulting from limited recruitment of new species from the sea (Day, 1964). However, repeated and unpredictable mouth breachings interrupt this lagoonal development with catastrophic disturbances producing an unstable system (Fishelson, 1977; Huston, 1979).

The effect of climate change on wave activity is contentious, as it has been postulated that estuaries may become smaller were wave activity to increase, or conversely the tidal scour could increase were tidal activity to become reduced in conjunction with a rise in sea level (Reddering and Rust, 1990). This could lead to presently-classified temporarily open estuaries becoming permanently open, with a stronger marine influence. These systems are therefore extremely dynamic.

South African coastal features have been affected by various changes over time, as the sea level 600 years ago was about 2.5 m above the present mean sea level (Whitfield *et al*., 1983). This has lead to a variety of changes in the river mouth size as the sea level dropped to present level, leading, in turn, to altered water flow patterns, salinity concentrations and sand movements at the mouth.

Geologists regard the lifetime of estuaries as relatively short and describe them as "ephemeral features" of a coastline (Schubel and Hirschberg, 1978). Estuaries have undergone vast transformations in their developments from youthful drowned river valleys proceeding on to mature systems in which sediments fill the valley of the slow moving river (Reddering and Rust, 1990), thus reinforcing the picture of the ongoing dynamic nature of estuaries. The equilibrium of this mature stage can continue to be disrupted by (a) global forces, such as climate changes and associated sea-level fluctuations, and (b) direct human interference, e.g. increased freshwater abstraction as a result of dam building and increased agricultural water usage (Reddering and Rust, 1990). Such human interference generally leads to less water inflow into the river systems leading to sediment deposition within the estuaries (Reddering and Esterhuysen, 1984). Such accumulation of sediment, and reduction of fresh water inflow (from increasing human usage) has led to smaller estuarine channels. These factors, together with increased evaporation during our periodic droughts have a direct effect on the salinity of South African estuaries, e.g. during the drought of 1989 in the Eastern Cape the permanently open Kariega Estuary rose to 42 (Whitfield and Bruton, 1989). Mean salinities of South African estuaries range from 1 to 35 (Whitfield, 1992a).

There is great pressure on the waters of South African estuaries and rivers as there is increasing demand for its usage in the expanding industries, for agriculture and as potable water for the growing population (Reddering and Rust, 1990; Cooper *et al.,* 1999; ETU, 2002). South Africa is regarded as a water-poor region having a mean annual runoff of only ≈ 450 mm yr^{-1}, and experiencing high evaporation rates (DWAF, 2004). The average world runoff is 860 mm yr^{-1}. This has led to the consideration, by water managers, of the construction of more water impoundments along major river systems in South Africa (DWAF, 2004). Before these impoundments can be constructed, however, according to the National Water Act of 1998 (Act 36) the requirements of all river systems must be considered in terms of two Water Reserves, *viz*. a basic human needs reserve and an ecological reserve. This act states that the "ecological reserve" refers to "the water required to protect the aquatic ecosystems of the water reserve", thus encompassing river systems, estuaries and the marine environment. Water running into estuaries prior to the National Water Act of 1998 was considered as "wasted" (Morant and Quinn, 1999). The South African estuaries also have an increasing economic importance with regard to fisheries. Lamberth and Turpie (2003) estimated that estuarine based fisheries produced catches valued at approximately R433 million per year. Estuarine and offshore based penaeid prawn fishery catches have been estimated at R10 million per year (De Villiers *et al*., 1999). Estuaries are, therefore, of great importance to surrounding communities: providing sources of employment, as well as providing a food source by way of recreational fishing (Breen *et al.*, 2004). The trend of building holiday/retirement homes at river mouths and along estuaries has created a social conundrum. Flooding threatens those homes built on the flood plain, which in turn has led to the solution of artificial breaching of the river mouths (Begg, 1984b), altering sediment-moving abilities of the affected rivers and fluctuating salinities during the new open phases, often with devastating affect on the biota of the estuaries.

1.5 Ecology of South African Isopods

A number of studies have focused on the primary production, biology and ecology of invertebrates and vertebrates in TOCEs. Results of these studies indicate that mouth phase plays an important role in structuring the plankton and ichthyofauna within these systems: abundance and biomass of the estuarine fauna decline following a breaching of the estuary mouth, which is ascribed to the estuarine organisms being washed out to sea (Froneman, 2004b). During the open-mouth phase there is recruitment of marine-breeding organisms into the estuary, resulting in dramatic changes within the species diversity and community structure (Bell *et al.,* 2001; Cowley *et al.,* 2001; Vivier and Cyrus, 2001; Froneman, 2004a, b; Kemp and Froneman, 2004). The dominant species within an estuary represent a group of resilient "estuarine" species, able to survive a wide range of changing environmental conditions. The eliminated species possibly represent part of an emerging "lagoonal" fauna having a narrower tolerance of fluctuating conditions (Zajac and Whitlatch, 1982). This finding was based on a study of a small estuary in southern Connecticut, where re-colonisation after a disturbance was dependent on the nature of the changes in the environment, patterns of resource utilisation and the life histories of the colonising species. There has been a strong bias towards the study of the estuarine ichthyofauna, with little work having been carried out on other members of the estuarine food chain. Eastern Cape examples of TOCEs will be discussed in more detail in the next chapter.

Isopods have been recorded in various estuaries and particularly in the intertidal zone along the South African coastline: e.g. MacNae (1957); McLachlan and Grindley (1974); Johnson (1976 a, b); Christie and Moldan (1977); Marsh and Branch (1979); Koop and Field (1981); Shafir and Field (1980a, b); Day (1981b, c); Blaber *et al.* (1983); Dye (1983); Koop *et al.* (1983); Branch and Day (1984); Wallace *et al.* (1984); De Decker and Bally (1985); Byren and Davies (1986); Hodgson (1987); Hanekom *et al.* (1988); Bennett (1989a); Whitfield (1989); De Ruyck *et al.* (1991a, b, 1992); McLachlan and Sieben (1991); Forbes and Cyrus (1992); Schlacher and Wooldridge (1996); Froneman (2000); Wooldridge and Callahan (2000); Bursey and Wooldridge (2003). Results of these studies have demonstrated that isopods are a conspicuous component of estuarine macro-crustacean communities (Simpson *et al.,* 1985; Heck *et al.,* 1995; Hass and Knott, 1998). Densities of up to 17 581 ind. m^{-2} have been recorded in southern African estuaries (Schlacher and Wooldridge, 1996) (Table 1.1). Despite the fact that some species can occur in high densities in estuaries, only a single study has examined the role of these organisms in southern African estuaries. Newman *et al.* (2007) studied the cirolanid isopod, *Cirolana fluviatilis,* in a permanently open estuary where up to 4149 were caught per hour in baited traps. There have been no autecological studies of isopods in TOCEs to date.

The high abundances of estuarine isopods suggest that they play a key ecological role within the nutrient dynamic and energy flow of these systems (Koop and Field, 1980, 1981). Additionally, a number of studies have demonstrated that isopods compose a significant component in the diet of several South African estuarine fish (Blaber 1974, 1976, 1979, 1984; Mason and Marais, 1975; Whitfield and Blaber, 1978; Whitfield, 1980a, b; Coetzee, 1981, 1982a, b; Cyrus and Blaber, 1983; Marais, 1984; Wallace *et al.,* 1984; Coetzee and Pool, 1985; Bennett, 1989a; Bennett and Branch, 1990). Maximum abundances of isopods within estuaries are typically associated with submerged macrophytes, which could either act as an abundant food source, or perhaps provide a refuge for invertebrates against predation (Holomuzki and Short, 1988; Kanouse *et al.*, 2006).

1.6 Structure of Thesis

There have been many studies on estuaries along the southern African coastline, but the vast majority have focused on spatial/temporal patterns in the community structure of fish (e.g. Blaber 1974, 1976, 1984; Mason and Marais 1975; Whitfield and Blaber 1978; Whitfield 1980a, b; Coetzee 1981, 1982a, b; Cyrus and Blaber 1983; Wallace *et al.,* 1984; Bennett 1989b; Bennett and Branch, 1990), and to a lesser extent crustacean zooplankton (Jerling and Wooldridge, 1995; Wooldridge, 1999; Froneman 2002a, b, 2004a, b). The focus of this thesis is a study of the population dynamics (including the reproductive state and sex ratios) of *Exosphaeroma hylocoetes* in three temporarily open/closed estuaries along the southeast coastline of the Eastern Cape Province of South Africa. To date no autecological studies of purely estuarine isopods, (i.e. those within temporarily open/closed temperate estuaries), have been carried in out in estuaries in South Africa.

The study sites are described in Chapter 2, together with major climate details and hydrography affecting the south-east coast of Southern Africa.

The population demographics, i.e. a study of the spatial-temporal patterns (the species composition, abundance, rate of reproduction, biomass, size distribution and sex ratios) of the isopod community within the three estuaries are presented in Chapter 3.

Exosphaeroma hylocoetes was found in greatest profusion amongst submerged marginal vegetation. The role of these submerged macrophytes, particularly *Ruppia maritima,* was therefore investigated to determine its role as either a food source (for the isopods, using stable isotopes and lipids) or as a refuge from fish predation. Results of this study are presented in Chapter 4.

Physico-chemical conditions are known to affect the growth rate of invertebrates. Chapter 5 focuses on the growth of male and female *E. hylocoetes* at two different temperatures and salinity combinations that replicate field conditions.

Finally, Chapter 6 draws together general conclusions of the study on *E. hylocoetes* and suggests future research needed to understand more fully the role of these isopods in temporarily open/closed South African estuaries.

Chapter 2

The Study Sites

2.1 Introduction

The fieldwork for this study was conducted in three temporarily open/closed estuaries (TOCEs), Kasouga, West and East Kleinemonde Estuaries, situated along the south-eastern Eastern Cape Province, between the coastal cities of Port Elizabeth and East London (Fig. 2.1). The three estuaries are approximately 60 km apart and categorised as warm-temperate systems (Harrison, 2004). However, they differ with regard to surface area and length, with the East Kleinemonde Estuary, being the shortest, and the neighbouring West Kleinemonde Estuary the longest (see sections 2.2.1 and 2.2.2). Even though the estuaries are in relatively close proximity to one another they have different characteristics, with different mouth opening phases.

The East and West Kleinemonde Estuaries have been classified as being in good ecological condition, with moderate human impact, while the Kasouga Estuary is regarded as being in excellent condition, indicating minimal anthropogenic impact (Whitfield, 2000). Housing developments are located in the lower reaches of the three systems (Whitfield, 2000), and game, cattle and pineapple farming are the main land use practices in their catchment areas. In order to elucidate the physical and chemical conditions of the estuaries studied, it is necessary to understand some of the climatic and hydrographic influences of the south-eastern South African coastline.

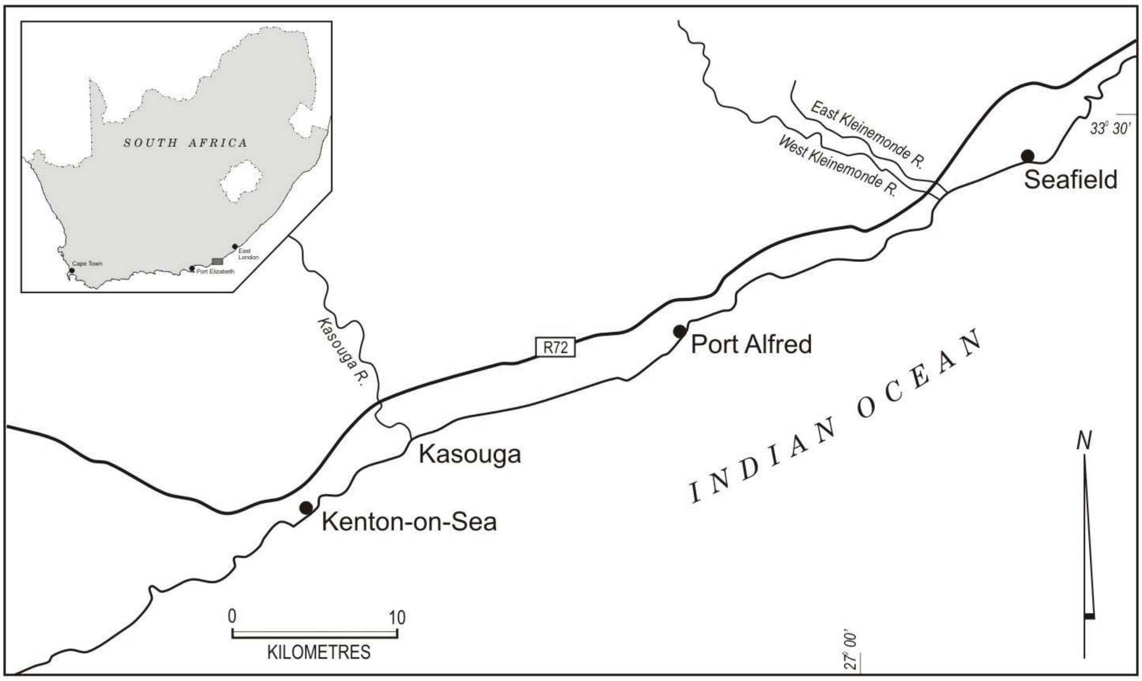

Figure 2.1. Location of three temporarily open/closed estuaries along the south-eastern coast of the Eastern Cape Province of South Africa.

2.1.1 Coastal and climatic influences

The dominant factor influencing the climate of South Africa’s south-east coastline is the warm Agulhas Current (Stone *et al*., 1998). This warm strong surface current contributes to the south-east coastline having a subtropical climate (according to the Koppen system of climate identification). Air temperature ranges from 10 to 22 °C, with an annual rainfall of least 600 mm (Lubke, 1998). The coastal town Port Alfred, for which there is considerable climatic data, is close to the estuaries and gives a good indication of temperature and rainfall for the coastal region, experiencing mean temperatures of between 10 °C (in July, austral winter) and 26 °C (in February, austral summer, Fig. 2.2). Maximum and minimum air temperatures recorded at Port

Alfred between 1996 and 2005 (measured at 08:00, South African Weather Bureau records, *in litt.*) were 38.5 °C (March 2003) and 1.9 °C (July 2002), respectively, although these can be regarded as extreme (Stone *et al*., 1998).

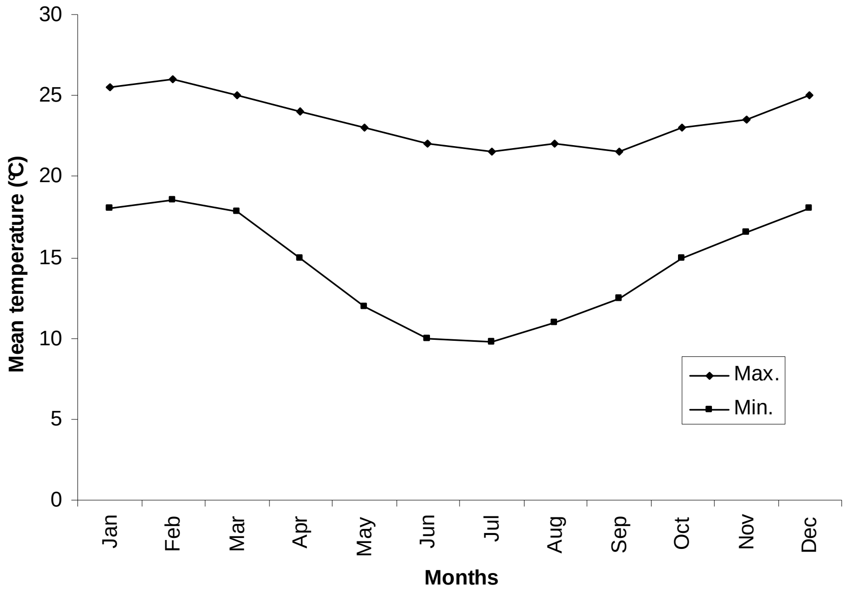

Figure 2.2. Mean maximum and minimum daily air temperature (°C), averaged per month measured at Port Alfred between 1996 and 2005 (South African Weather Bureau records, *in litt*).

Port Alfred recorded a mean annual rainfall, between 1996 and 2005, of 604 mm (Fig. 2.3). A minimum of 396 mm (1999) and a maximum of 731 mm were recorded in 1998 (South African Weather Bureau records, *in litt.*).

The rainfall in the coastal region demonstrates an autumn-spring bimodal pattern (Fig. 2.3) with a maximum rainfall peak occurring during spring (Kopke, 1988). Wet and dry cycles of between 12 and 20 years in duration have been recorded for the south-eastern Cape coastline (Grange *et al*., 2000).

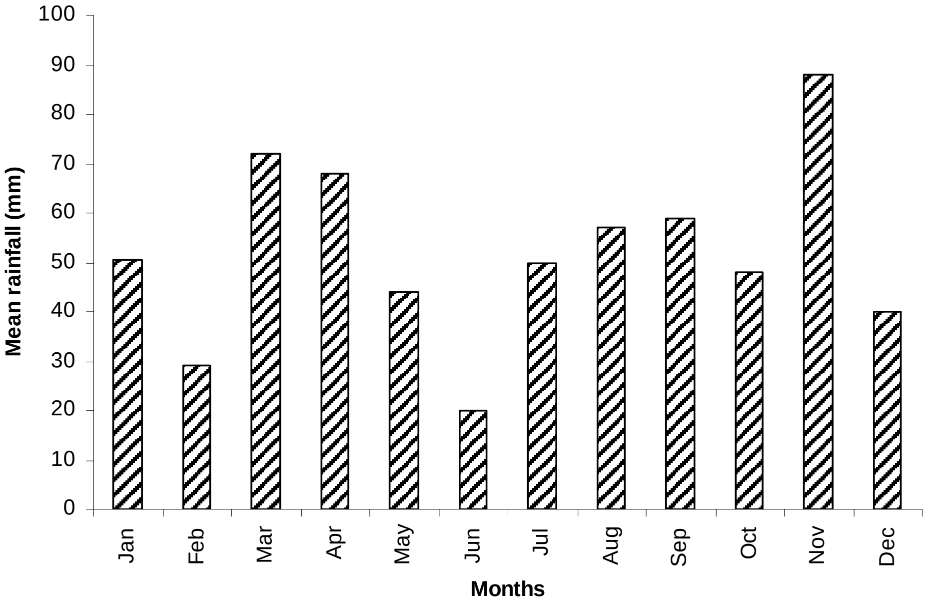

Figure 2.3. Mean monthly rainfall (mm) between 1996 and 2005 recorded at Port Alfred (South African Weather Bureau records, *in litt*).

The coastal area is also influenced by the southern hemisphere sub-tropical high pressure belt and the circum-polar belt of westerly winds (Stone *et al.,* 1998). The high pressure cells move further south in austral summer, to block the westerly winds, and as the high pressure cells move north during austral winter, so the westerly winds can penetrate further northward, blowing onshore. This results in easterly winds occurring in summer, and westerly winds blowing onshore during winter.

The Agulhas Current is narrow (≈ 100 km wide) and averages a flow rate of 1 m sec^{-1} frequently reaching 2 m sec^{-1} at its core, on the surface, and is thus considered fast flowing (Ross, 1988; Lutjeharms, 1998). Its surface temperature varies seasonally between 22 °C and 27 °C (Ross, 1988). The current is closest to the South African coastline at East London (≈ 125 km north east of the East Kleinemonde Estuary), and veers off the coast near Port Elizabeth (≈ 180 km south west of the Kasouga Estuary), after which its flow is less concentrated and slower (Stone, 1988). A counter current then develops offshore, owing to retroflection, and the water circulates in an eastward direction once more. The presence of such retroflective currents from the Agulhas Current can cause rapid changes in sea temperature. In winter a temperature difference of as much as 10 °C can occur between inshore water and the warm Agulhas Current waters further offshore (Stone, 1988).

The Port Alfred upwelling cell, whereby cold deep water from the South Indian Central Water is lifted onto the continental shelf, moves parallel to the Agulhas Current and causes further rapid changes of the sea temperature (Lutjeharms, 1998). The wind affects the surface layers of water, which may be warm, when a thin surface layer of Agulhas Current water covers the cold upwelled water, when blowing from the south-west, and when the wind swings to north-east, the layer of sun-warmed water is displaced to expose the cold upwelled water (Lutjeharms, 1998).

The atmospheric circulation and Agulhas Current, with associated upwellings, and eddies influence the periods of rainfall and storm waves which batter the south-eastern Cape’s coastline, and may result in greater than average rainfalls (> 100 mm) and mouth breaching events (Wooldridge and McGwynne, 1996; Perissinotto *et al.*, 2000; Froneman, 2004a). These breaching events cause the rapid outflow of estuarine water (Fig. 2.4), and the removal of submerged vegetation, both of which affect the populations of estuarine fauna (Vorwerk *et al.*, 2008). The effect of these mouth breaching events will be illustrated and discussed in Chapter 3.

Figure 2.4. Photographs A + B, 15 days apart, illustrating the effect of mouth breaching (C) on water levels in a temporarily open/closed estuary (the Kasouga Estuary), during August 2006.

2.2 The Study Estuaries

2.2.1 Kasouga Estuary

The Kasouga Estuary (33°39’11”S; 26°44’01”E) is a small to medium sized temporarily open/closed system (Fig. 2.5), situated about 15 km west of Kenton-on-Sea, and approximately 20 km west of Port Alfred, along the coastal road (R72). When at its highest level, it is navigable by small boat for ≈ 4 km. It is narrow (30 to 40 m wide), in the upper reaches but can widen to ≈ 200 m in the lower reaches. For most of the year, however, it is generally 100 to 120 m wide in this lower region. The estuary is shallow in the lower and middle reaches never achieving a depth of > 1.8 m at its highest level, and is between 1.5 and 2 m deep (max. depth = 2.1 m) in the main channel in the upper reaches. The estuary is closed for the most part, but will breach following heavy rainfall (> 100 mm in a month), combined with high seas. The sand bar at the mouth is usually very extensive so that overtopping events rarely occur, generally < 4 x per year (Froneman, 2002b, 2004a).

Ruppia maritima (L.) is the dominant submerged macrophyte within the estuary, particularly in the middle and upper reaches, where it conceivably acts as a refuge for a variety of invertebrates and vertebrates (Holomuzki and Short, 1988; Kanouse *et al.*, 2006). The reed, *Phragmites australis* (Cav.) Trin. ex Steud., grows along both banks in the upper reaches, but sparsely in the middle reaches. Preliminary studies indicated that isopods were not found beyond station one, due to the absence of *R. maritima*, and therefore sampling did not occur beyond this point. The catchment area is approximately 45 km^2 in extent, and there is relatively little agricultural development.

The average estuarine water quality index (eWQI) value of the Kasouga Estuary was ≈ 8.4, which indicates good quality (Harrison *et al*., 2000).

The streams and valleys of the catchment are considered undisturbed and pristine (Froneman, 2002b, 2004a). The land within the immediate vicinity of the estuary on the eastern bank is used for the grazing of cattle and holiday housing development of about 100 houses, the Kasouga village. The west bank consists of mainly undeveloped land used primarily for game farming at present, although property developers would like this area for holiday-home development.

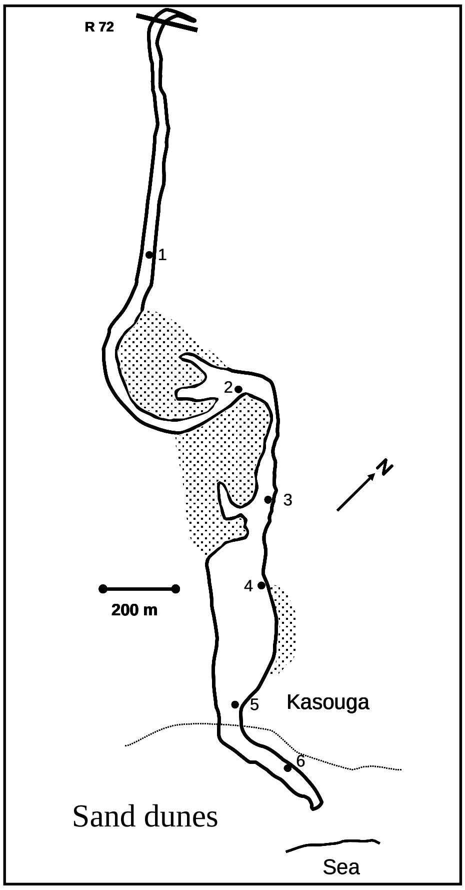

Figure 2.5. Kasouga Estuary with six collection sites indicated. Two sites were located in each of the three reaches of the estuary. The stippled areas represent salt marsh areas adjacent to the estuary.

2.2.2 West and East Kleinemonde Estuaries

The West and East Kleinemonde systems (Fig. 2.6) lie about 20 km east of Port Alfred and occur in narrow valleys. The West Kleinemonde Estuary (33°26’26” S; 27°02’41”E) is the larger of the two and is classified as a large temporarily open/closed system. It is navigable by small motorboat for ≈ 6 km (Bernard and Froneman, 2005) and is relatively wide (up to 200 m) in the lower reaches, but narrower (20 and 30 m) in the upper reaches. This is a shallow system (< 2 m depth at its highest level), particularly in the lower and middle reaches. The mouth is closed for long periods of time, but the sandbar can be breached during periods of heavy rain (> 100 mm in a month). High seas can result in overtopping of seawater resulting in high salinities in the lower reaches, once the mouth has reclosed (Perissinotto, *et al.*, 2000; Cowley and Whitfield, 2001; Harrison, 2004). Over-spilling events comprised of wind-driven estuarine water flowing into the sea were observed during periods when the estuary was at its highest level, (e.g. during March, 2007; Henninger, unpublished data).

The vegetation in the littoral zone of the West Kleinemonde Estuary is dominated by *Ruppia maritima*, in the middle and upper reaches. *Phragmites australis* stands grow along the entire estuary length. The catchment area is long and narrow (about 25 km^2), and most of the land on the west bank is undeveloped cattle grazing area. The narrow range of hills dividing the West and East Kleinemonde has pineapple plantations in the upper reaches of the catchment area, particularly that of the East Kleinemonde. In its lower reaches the estuary is flanked by holiday homes. The average eWQI value for the West Kleinemonde Estuary was ≈ 7.2, indicating moderate quality (Harrison *et al.*, 2000).

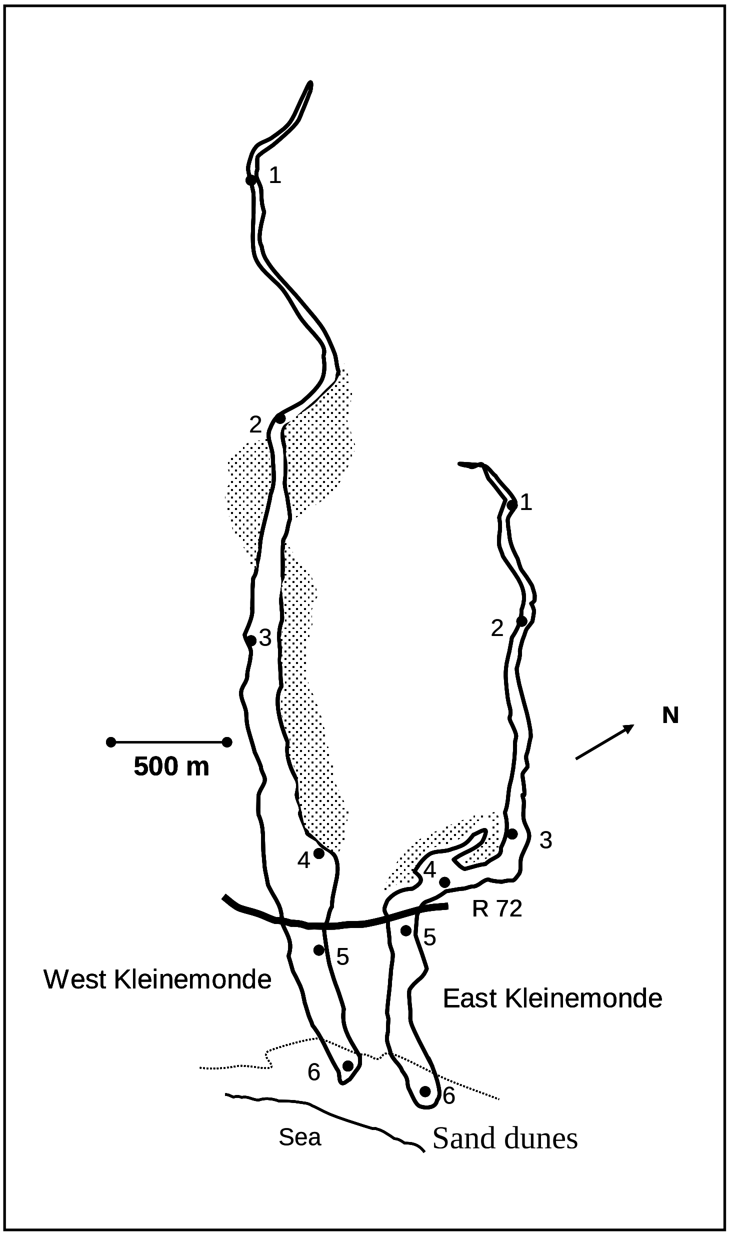

Figure 2.6. West and East Kleinemonde Estuaries showing locations of collection sites. Two sites were located in each of the three reaches (upper, middle and lower) of the estuaries. Stippled areas indicate salt marshes.

The East Kleinemonde Estuary (33°32’18 S; 27° 02’50” E) is classified as a small to medium sized temporarily open/closed estuary and is navigable by canoe for ≈ 2 to 2.5 km (depending on water levels). It is a shallow system with a maximum depth of 1 to 1.5 m in the middle and upper reaches when at its highest level, making it difficult to negotiate by motorboat, particularly during low water spells, when depth may dwindle to 50 cm. The estuary is approximately 100 to 150 m wide in the lower reaches, and about 50 to 100 m wide in the middle and upper reaches. Holiday homes are located on both banks, with the most extensive development (Sea Vale) on the east bank. The catchment area is ≈ 45 km^2 with pineapple plantations dominating the far upper reaches. There is very little agricultural activity in the immediate vicinity of the estuary. The eWQI value was considered moderate at ≈ 7.6 (Harrison *et al*., 2000). Vegetation immediately along the banks is dominated by *Sarcocornia perennis* (Mill.) A.J. Scott*,* *Juncus kraussii* Hochst. and *Sporobolus virginicus* (L.) Kunth., which become inundated when the estuary water levels rise. Small stands of *Phragmites australis* occur in the lower and middle reaches. The mouth is more susceptible to breaching, than either the neighbouring West Kleinemonde, or the Kasouga, with more frequent over-spilling events (Riddin and Adams, 2008; Whitfield *et al*., in press). These events have been recorded over the period 1993 – 1998, and indicate that the mouth was open during every month, except March and July, during this period (Cowley and Whitfield, 2001). In 2006, due to increased river flooding, the estuary was open for 33 % of the days, which is significantly higher than the mean for open mouth conditions (Van Niekerk *et al*., 2008).

Chapter 3.

Population Dynamics of the Estuarine Isopod, *Exosphaeroma hylocoetes*, (Barnard, 1940)

3.1 Introduction

Temporarily open/closed estuaries (TOCEs) are the dominant estuary type (comprising ≈ 73 % of the 289 estuaries) along the South African coastline (Whitfield, 1992a). These estuaries are characteristically shallow (< 2.5 m deep), and generally have low turbidity, except during the open phase (Froneman, 2002a, b). Mouth breaching events following periods of increased rainfall have been shown to have a profound effect on estuarine inhabitants (Vorwerk *et al.*, 2008). These events lead to river conditions dominating these systems (Wooldridge and McGwynne, 1996; Perissinotto *et al.,* 2000; Froneman, 2002a, b) resulting in a decrease in estuarine fauna, possibly due to the removal of submerged vegetation that acts as a refuge area, and/or outflow of biomass-rich estuarine water into the marine environment. Following mouth closure by a sandbar, overtopping events occur, allowing seawater with possible subsequent recruitment of estuarine fauna into the estuary (Wooldridge and McGwynne, 1996; Perissinotto *et al.*, 2000; Froneman 2002a, b; Kemp and Froneman, 2004). Overtopping events occur more frequently than mouth breaching events in Eastern Cape TOCEs (Cowley *et al.*, 2001; Kemp and Froneman, 2004).

Isopods are a conspicuous component of estuarine macro-crustacean communities (Holditch and Harrison, 1983; Simpson *et al.*, 1985; Heck *et al.*, 1995; Hass and Knott, 1998; Ferreira *et al.*, 2004), including those in South Africa (MacNae, 1957; McLachlan and Grindley, 1974; Christie and Moldan, 1977; Hodgson, 1987; Newman *et al.*, 2007). Densities of up to 1 980 individuals m^{-2} have been reported for the temporarily open/closed Bot River (Koop *et al.*, 1983) and 17 581 ind. m^{-2} in the permanently open Gamtoos Estuary along the south-eastern coastline of South Africa (Schlacher and Wooldridge, 1996). Shifts in isopod community structure are correlated to horizontal gradients in salinity and temperature (Wooldridge, 1999; Mouny *et al.*, 2000; Yamada *et al.*, 2007). Maximum abundances of isopods within estuaries are typically associated with submerged macrophytes, which are thought to provide a refuge for invertebrates against predation (Holomuzki and Short, 1988; Kanouse *et al.*, 2006). The high abundances of estuarine isopods suggest that they play a key ecological role within these systems, because isopods are important in the turnover of nutrients (Koop and Field, 1981), and as a food source for several South African estuarine fish (e.g. Blaber 1974, 1976, 1984; Mason and Marais 1975; Whitfield and Blaber 1978; Whitfield 1980a, b; Coetzee 1981, 1982a, b; Cyrus and Blaber 1983; Wallace *et al.* 1984; Bennett 1989a; Bennett and Branch, 1990).

Whilst there has been a great deal of work carried out on the zooplankton of South African estuaries, especially crustaceans (Jerling and Wooldridge, 1995; Wooldridge, 1999; Froneman 2002a, b, 2004b) there has been very little work on epi-benthic Crustacea particularly within TOCEs. The only autecological estuarine isopod study to date is that of Newman *et al.* (2007) on the cirolanid, *Cirolana fluviatilis*, in the permanently open Gamtoos River, within the same geographic region. The isopod *Exosphaeroma hylocoetes* (Barnard, 1940) has been identified as a dominant component of the hyperbenthos within TOCEs. As yet there have been no autecological studies of isopods within these systems. The primary aim of this study is, therefore, to provide the first assessment of population dynamics of this species in three warm temperate TOCEs along the Eastern Cape coastline with emphasis on open and closed phases. The null hypothesis is that mouth openings have no effect on the population dynamics of the isopod *Exosphaeroma hylocoetes*.

3.2 Materials and methods

3.2.1 Study sites

These are described in detail in Chapter 2 (Figs 2.1, 2.5 and 2.6).

3.2.2 Horizontal distribution and habitat of *E. hylocoetes*

To assess the horizontal patterns of the population structure of *E. hylocoetes,* preliminary surveys were carried out in the middle reaches of the Kasouga (four sites) and W. Kleinemonde (six sites) Estuaries. At each site 1 m long sweeps with a hand-held sweep net (200 x 200 mm, 1 mm mesh) were made parallel to the shore every metre along a transect from the bank to the middle of the channel. In addition, a visual estimate of vegetation cover, in a 1 x 1 m quadrat, was made. Three replicates per site at each distance were carried out. As the majority of isopods (up to 95 %) were found within the fringing vegetation (see results section) all subsequent sampling was confined to this habitat.

3.2.3 Abundances and biomasses of *E. hylocoetes*

To assess the spatial and temporal patterns of the population structure of *E. hylocoetes*, monthly surveys were carried out in the three estuaries from February 2006 until August 2007. Six stations were occupied comprising, two stations in each of the upper, middle and lower reaches of each estuary (Fig. 3.1). During each survey isopods were collected using a 200 x 200 mm net with a mesh size of 1 mm. Three replicate 1 m long sweeps per station were collected and bottled for later processing. Additionally, at each station a visual estimation of the vegetation cover was made as described above. Data presented show the mean values for two sites in each reach, with a total of six replicates per reach. At each station water temperature and salinity were measured using a hand-held thermometer and an Atago hand-held refractometer. Measurements were taken in the littoral zone.

In the laboratory the animals were preserved in 4 % formaldehyde for 24 hr then counted and stored in 70 % alcohol. Isopod densities were calculated and expressed as ind. m^{-2}. In addition, each isopod was measured (0.01 mm accuracy) using an ocular micrometer along the mid-dorsal length from the anterior tip of the cephalon, (the point of connection between the cephalon and the flagellae, or antenna one), to the posterior tip of the pleotelson, and then sexed, according to shape and inner ridge of pleotelson (Barnard, 1940). Brood carrying (yellow eggs or mancas) was observed in individuals longer than 2.5 mm, and therefore isopods < 2.5 mm in length were considered as juveniles. The total isopod biomass per station was determined by drying each sample at 60 °C to a point of constant mass and weighing on a Sartorius Electronic Microbalance. Values were expressed as mg dwt. m^{-2}.

Numbers of brood per female were counted and the females' length measured to the nearest 0.001 mm, to determine a relationship between maternal isopod length and brood size. Further, brood was subdivided into that < 1mm, regarded as embryos/eggs and that > 1 mm, which could be recognised as developing and developed mancas, before their release at ≈ 1.5 mm.

3.2.4 Laboratory isopod brood work and sex ratio distortion

In order to determine whether a female biased brood was an artefact of selection pressure in the field, or the result of physical factors, or even an innate factor isopod brood was raised in the laboratory. Isopods were collected in the field and processed in the laboratory. After inspection under a dissecting microscope 30 visibly gravid female *E. hylocoetes* were selected and 15 placed in individual 250 ml plastic containers containing water at a salinity of 15 in a constant environment (C.E.) room at 15 °C. The remaining 15 were placed in a C.E. room set at 25 °C in 250 ml containers in water at a salinity of 15. The females were fed *ad libitum* on commercially available dry fish food. The fish food had the following composition: min 40 % protein; 5 % fat; 5 % fibre and 12 % moisture. The water was changed weekly. The females were observed daily to establish release of brood. Released brood, now mancas, were separated, placed in containers experiencing the same conditions as their mothers. The mancas received the same treatments (with respect to feeding and water changing) as their mothers. The individual mancas were sexed when they reached ≈ 5 mm. This allowed the determination the sex ratios of the brood.

Field studies revealed a sex ratio bias towards females (see results section 3.3.4). Screening for *Wolbachia* as a possible sex ratio distorter was carried out using specific genetic markers in male and female *E. hylocoetes*. DNA was extracted and purified using the spin-column protocol of the *DNeasy Blood and Tissue* handbook 07/2006, (DNeasy 96 – Blood and Tissue Kit, Qiagen, Valencia, CA, USA). Individuals were screened for the presence of *Wolbachia* using a polymerase chain reaction (PCR) to amplify portions of genes used commonly as markers for *Wolbachia* infection: the *Wolbachia* surface protein gene (*wsp*). Amplification of a section of the wsp using the primers Wsp81F and Wsp69R (Zhou *et al.*, 1998) was carried out in a 20 µl PCR reaction. The PCR products were electrophoresed on a 1 % agarose gel using 1 µl loading dye and 5 µl PCR product. Bands were visualised on a UV Transilluminator.

3.2.5 Statistical analyses

One-way ANOVAs were used to determine if there were any significant spatial/temporal patterns in the abundance and biomass of *E. hylocoetes* within the three estuaries. Sources of variation were assessed employing the post hoc Fisher's (LSD) test. T-tests were employed to detect any significant differences in isopod cohort sex ratios at combinations of temperature and/or salinity. The analyses were carried out using *Statistica version 7* (StatSoft) package. Data were not transformed as these met the requirements for ANOVA.

3.3 Results

3.3.1 Physico-chemical results

Rain induced mouth-breaching events occurred on four occasions from August to November 2006 in the W. Kleinemonde. The Kasouga Estuary breached on two occasions in August and October 2006, while in the E. Kleinemonde a total of seven breaching events were recorded, (from June to October and December 2006, as well as during April and June 2007) (Figs 3.1 and 3.2). In all instances, the mouths closed within seven days of breaching.

Water temperatures within all three estuaries showed little variation between reaches and demonstrated a strong seasonal pattern with maximum values (20 to 31 °C) being recorded during January (summer) and minimum values (14 to 16 °C) in winter (Fig. 3.1). Intermediate values were recorded in autumn (16 to 21 °C) and spring (17 to 24 °C). In all three estuaries, inflow of seawater following the overtopping events and freshwater inflow was associated with a decline in water temperatures.

Salinities within all three estuaries were highly variable during the study, ranging from 0 to 33 in the W. Kleinemonde Estuary, 0 to 27 in the E. Kleinemonde and 2 and 32 in the Kasouga Estuary (Fig. 3.2). This variability reflected the influence of both freshwater inflow (due to high winter rainfall within the catchment area) and the inflow of marine waters following breaching events. During the first three months of the survey the mouths were closed and salinity within the three estuaries increased steadily, probably due to a combination of increased evaporation, seepage to the sea via the sandbar and decreased surface run-off, reaching 32 in May 2006 in the Kasouga, 26 in the E. Kleinemonde and 22 in the W. Kleinemonde (Fig. 3.2). Heavy catchment rainfall in June and July 2006, however, diluted salinities to mesohaline and even hyposaline conditions (0 and 2 in August and September 2006) within the three estuaries (Fig. 3.2).

Whilst the mouth of the E. Kleinemonde breached several times during the study starting from the end of July 2006, those of the W. Kleinemonde and Kasouga only started to breach in August 2006 (Figs 3.1 and 3.2). These events resulted in rapid fluctuations in the water levels in the three systems. A key feature was a virtual absence of a salinity gradient in the three systems, where a salinity difference generally did not exceed 4 between the upper and lower reaches. Exceptions occurred between August 2006 and January 2007 in the W. and E. Kleinemonde Estuaries when a gradient was established between the upper and lower reaches. In November 2006 the salinity was 5 in the upper reaches of the W. Kleinemonde Estuary, while it was 31 in the lower reaches. In August 2006, the salinity was 6 in the upper reaches of the E. Kleinemonde Estuary, while at the same time it reached 16 in the lower reaches. After these occasions, i.e. in the following month, the salinity differences were < 4, removing any indication of a salinity gradient.

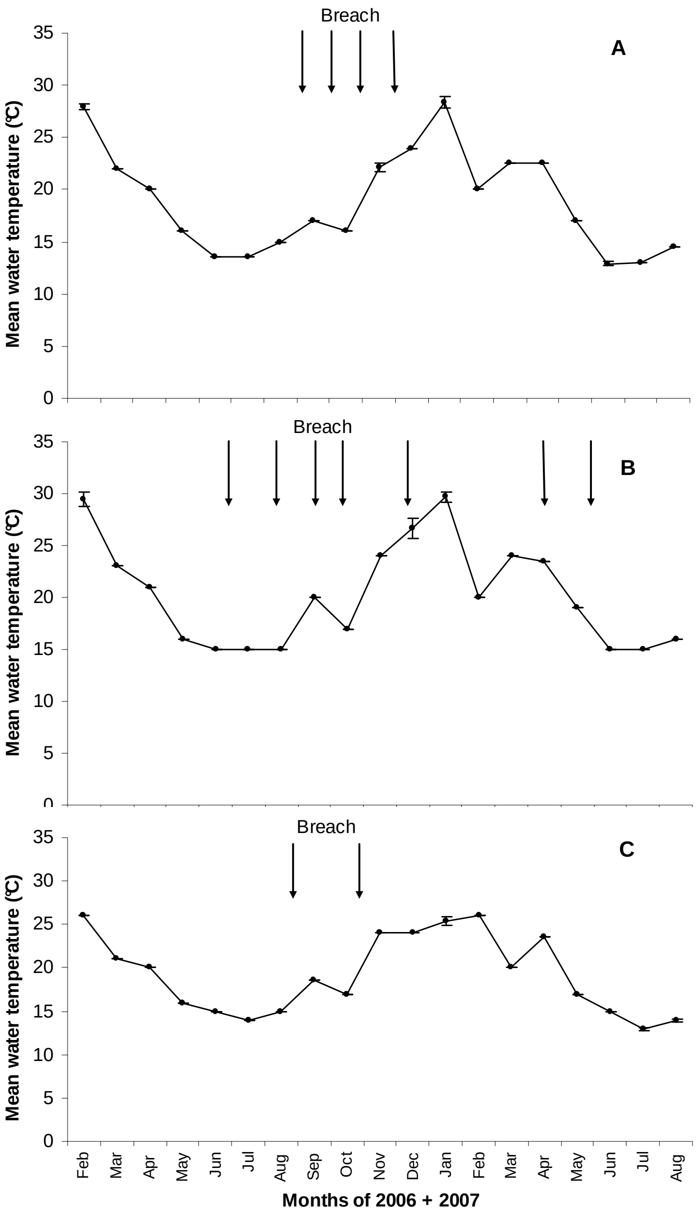

Figure 3.1. Estuarine water temperatures within three Eastern Cape temporarily open/closed estuaries during 2006 and 2007. A = West Kleinemonde; B = East Kleinemonde & C = Kasouga Estuaries. Mouth breaching events are indicated by arrows.

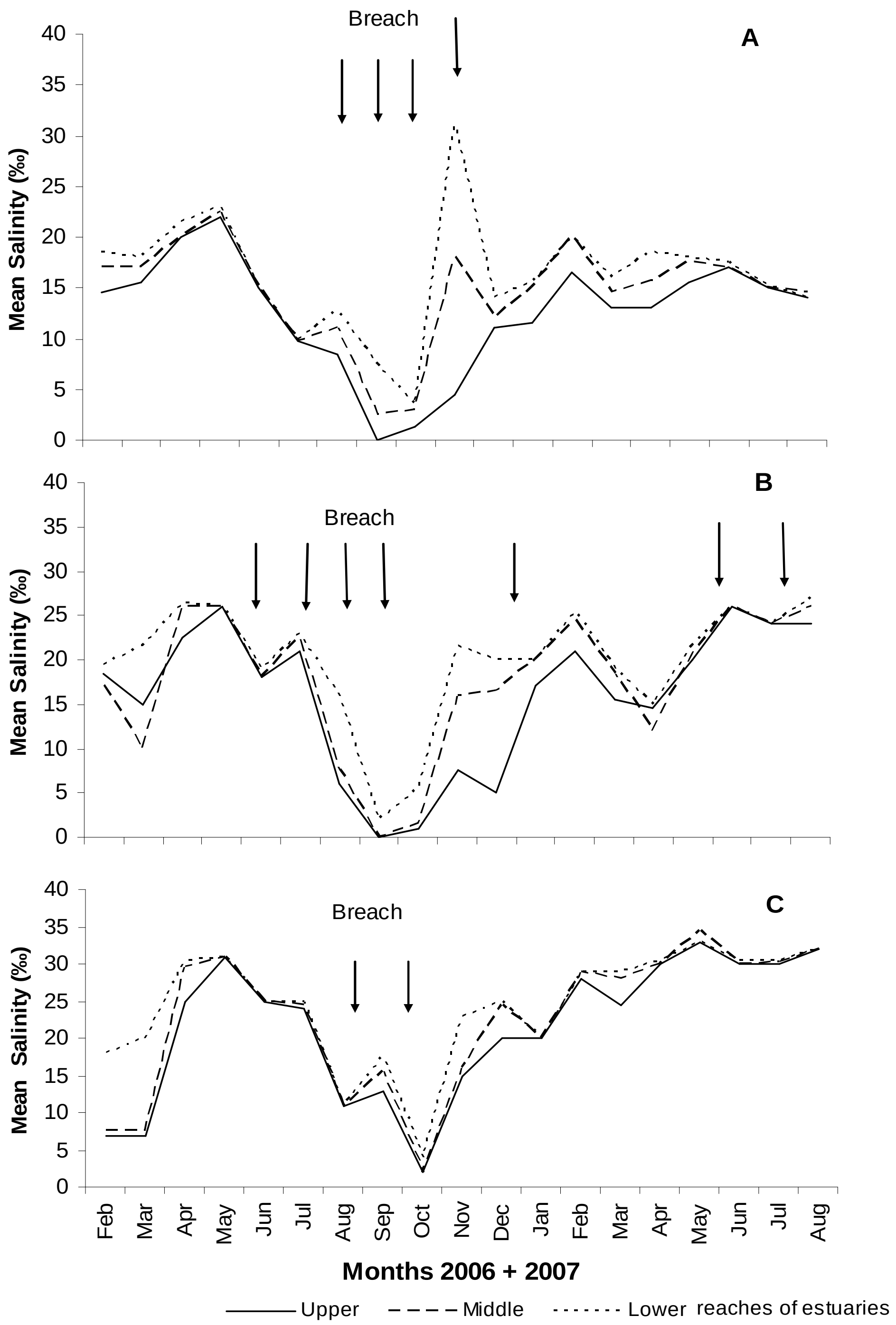

Figure 3.2. Mean salinities of three reaches in three Eastern Cape temporarily open/closed estuaries during 2006 and 2007. A = West Kleinemonde; B = East Kleinemonde & C = Kasouga Estuaries. Mouth breaching events are indicated by arrows. Key refers to the reaches of the estuaries.

3.3.2 Horizontal distribution and habitat of *E. hylocoetes*

Isopod densities were always greatest within the marginal vegetation where percentage vegetation cover was highest (Fig. 3.3). The isopods were virtually absent from the main channels of the estuaries, although a few individuals were found swimming within the water column, over bare mud, within the channel of the Kasouga Estuary (Fig. 3.3).

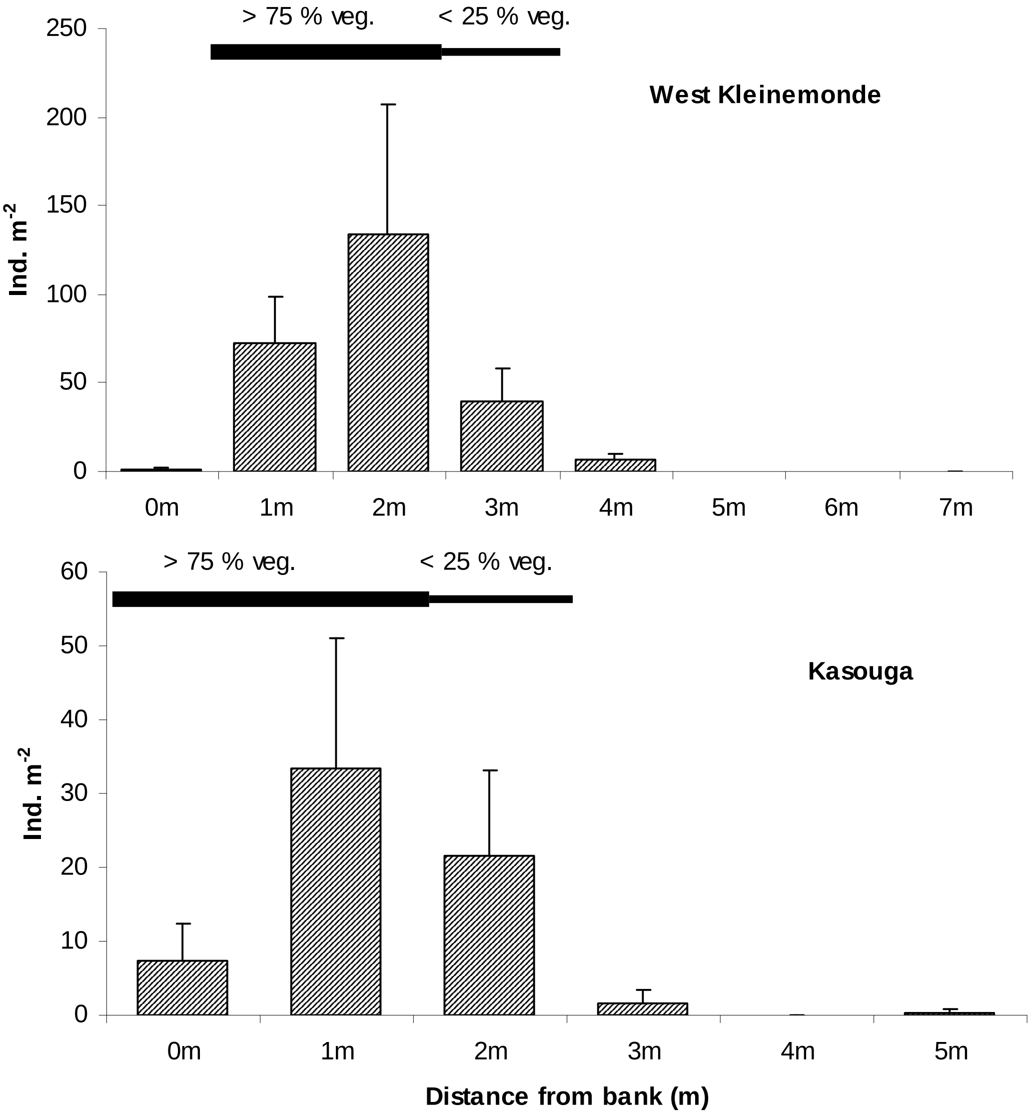

Figure 3.3. Abundance (mean ± S.D.) of *Exosphaeroma hylocoetes* in the West Kleinemonde and Kasouga Estuaries in relation to submerged vegetation cover and distance from the bank.

3.3.3 Vegetation cover

The marginal vegetation cover within the three estuaries was highly variable over both space and time (Fig. 3.4). The upper and middle reaches of the three estuaries always had a higher vegetation cover than the lower reaches. The site located at the mouth of the estuary was characterised by the virtual absence of a vegetation cover. The E. Kleinemonde Estuary had the lowest overall vegetation cover of 29.82 % ± 15.16 over all three reaches during the 19 month study period. The neighbouring W. Kleinemonde Estuary had the highest overall vegetation cover (53.45 % ± 26.82), with submerged vegetation being present in the upper and middle reaches of the estuary throughout the study period (Fig. 3.4). The Kasouga was intermediate between the two having a mean vegetation cover of 32.17 % ± 18.10. Breaching events lead to the complete collapse of the submerged stands of vegetation in the Kasouga and E. Kleinemonde Estuaries (Fig. 3.4) resulting from a rapid decline in the water levels, which either exposed the vegetation (resulting in desiccation within hours) or washed it out of the mouth.

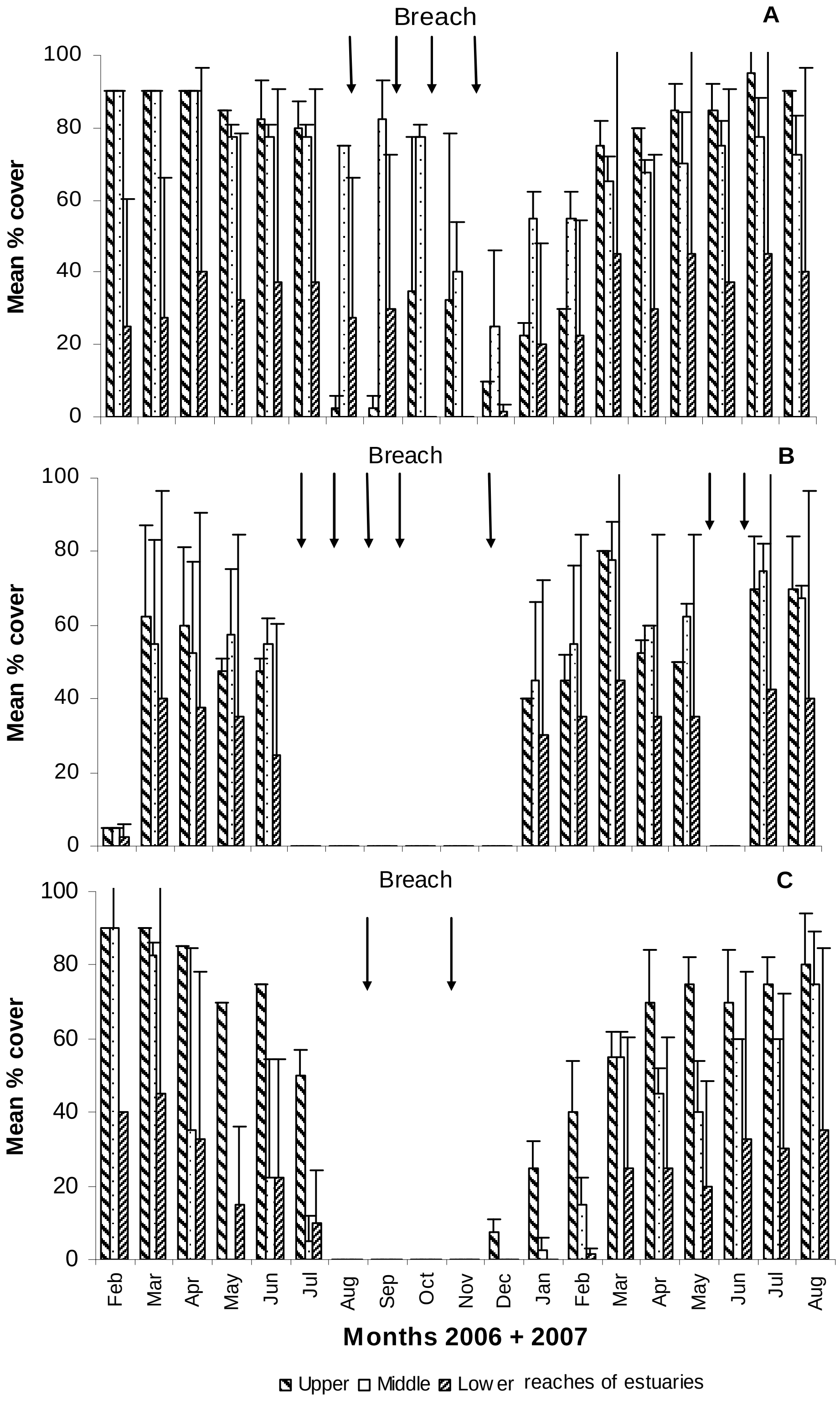

Figure 3.4. Vegetation cover (mean ± S.D) in three regions of three Eastern Cape temporarily open/closed estuaries during 2006 and 2007. A = West Kleinemonde; B = East Kleinemonde & C = Kasouga Estuaries. Mouth breaching events are indicated by arrows. Key refers to the reaches of the estuaries.

3.3.4 Abundances and biomasses of *E. hylocoetes*

Mean abundance and biomass of *E. hylocoetes* within the W. Kleinemonde Estuary ranged between 3 and 4 791 ind. m^{-2} and from < 0.01 to 9.65 mg dwt. m^{-2}, respectively (Figs 3.5 and 3.6). During the first six months of the survey (February 2006 to August 2006), isopod abundance and biomass decreased towards the colder winter months. The four breaching events that occurred between August and November 2006 resulted in a dramatic decline in isopod abundances and biomasses from 564.4 ± 241.9 to 62.5 ± 35.1 and from 1.0 ± 0.5 to 0.15 ± 0.08 mg dwt. m^{-2} (from August to September 2006). Following the closure of the mouth, mean isopod densities and biomasses increased from 3 to 764 ind. m^{-2} and 0 to 1.26 mg dwt. m^{-2}, respectively over a period of seven months (December 2006 to June 2007; Figs 3.5 and 3.6). Estimates of the total isopod abundance and biomass during the open and closed phases were not significantly different from one another ($F_{18, 38} = 1.88$, $p = 0.05$ for abundance and $F_{18, 38} = 1.56$, $p = 0.12$ for biomass, Table 3.1).

Throughout the study the total abundance and biomass of *E. hylocoetes* was significantly higher ($p < 0.05$) within the middle and upper reaches of the W. Kleinemonde Estuary when compared to the lower reaches (Figs 3.5 and 3.6). There was a significant correlation between total abundances of the isopod and percentage vegetation cover ($r^2 = 0.869$; $p < 0.05$; Fig. 3.7). There was no correlation between salinity, temperature and total abundance and biomass of *E. hylocoetes* during the study ($p > 0.05$ in all cases).

The mean isopod abundance and biomass in the East Kleinemonde Estuary prior to the mouth breaching events in June 2006 ranged between 0 and 108.3 ind. m^{-2} and between 0 and 0.32 mg dwt. m^{-2}, respectively (Figs 3.5 and 3.6). After the mouth breached in July, isopods were absent from the estuary. The populations began to recover in February 2007, about one month after mouth closure (in early January). During the subsequent five month period both abundance and biomass increased once more; ranging from 0 to 100 ind. m^{-2} and from 0 to 0.25 mg m^{-2}. There was no significant seasonal pattern in total isopod abundance and biomass, however, the total abundance and biomass of *E. hylocoetes* during the months of February 06; April to June 06; April 07 and July to August 07 were significantly higher than the remaining months (ANOVA p-values ranged between 0.0481 to 0.0177 for abundance and between 0.0482 and 0.0021 with regard to biomass; $F_{18, 38} = 5.22$, $p < 0.001$ for abundance and $F_{18, 37} = 3.50$, $p < 0.001$ for biomass, Table 3.1). There were no significant spatial patterns in isopod abundances and biomass evident during the study ($p > 0.05$ in all cases). Total densities were significantly positively correlated to the percentage vegetation cover ($r^2 = 0.725$; $p < 0.05$; Fig. 3.7).

Mean abundance and biomass of *E. hylocoetes* within the Kasouga Estuary ranged between 0 and 3 650 ind. m^{-2} and from 0 to 5.11 mg dwt. m^{-2}, respectively (Figs 3.5 and 3.6). As for the W. Kleinemonde Estuary, isopod abundance and biomass showed a weak seasonal trend in 2006 decreasing towards the colder winter months during the first five months of the survey (Figs 3.5 and 3.6). Again the mouth breaching events in August and October 2006 coincided with the disappearance of submerged vegetation and isopods within the estuary, with isopod numbers only recovering in April 2007, three months after mouth closure. Between April and August 2007 mean densities and biomasses of the isopods ranged between 0 and 648 ind. m^{-2} and between 0 and 1.52 mg dwt. m^{-2}, respectively (Figs 3.5 and 3.6).

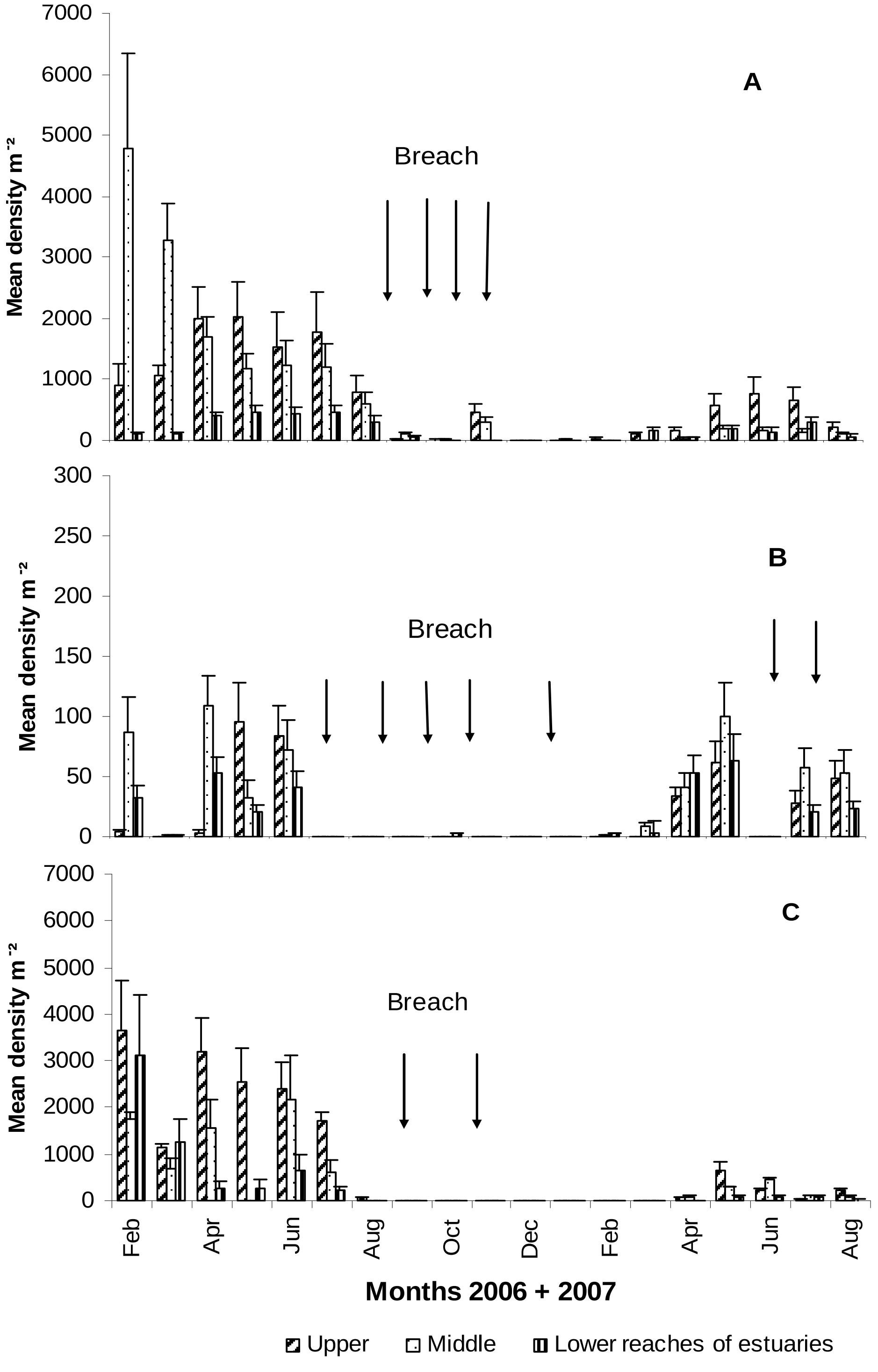

Figure 3.5. Mean density (± S.D.) of *Exosphaeroma hylocoetes* in three reaches of three Eastern Cape temporarily open/closed estuaries. A = West Kleinemonde; B = East Kleinemonde & C = Kasouga Estuaries.
Mouth breaching events are indicated by arrows.

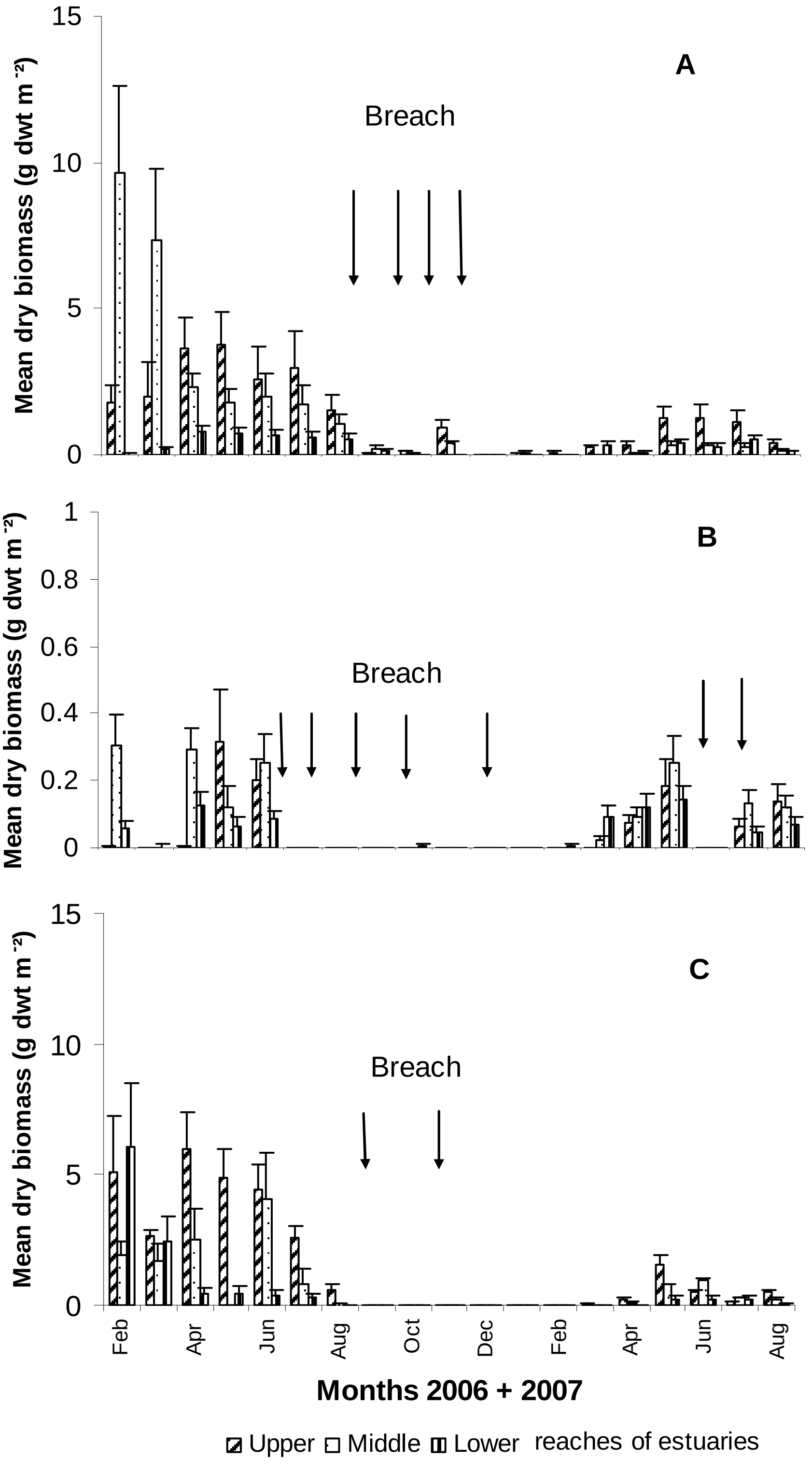

Figure 3.6. Mean biomass (g dwt. m^{-2} ± S.D.) of *Exosphaeroma hylocoetes* in three reaches of three Eastern Cape temporarily open/closed estuaries. A = West Kleinemonde; B = East Kleinemonde & C = Kasouga Estuaries. Mouth breaching events are indicated by arrows.

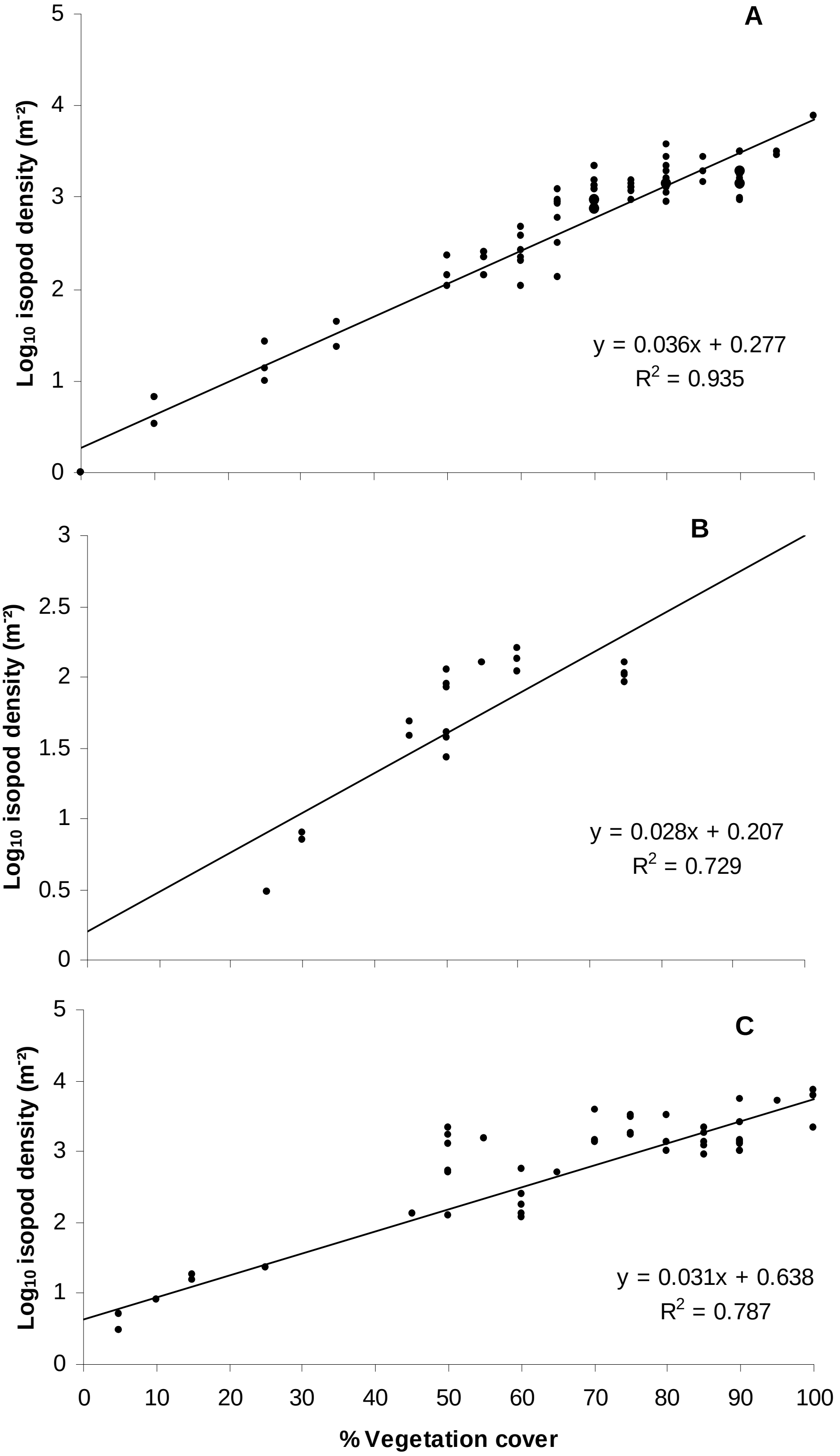

Figure 3.7. Abundance of *Exosphaeroma hylocoetes* related to the percentage vegetation cover in three Eastern Cape temporarily open/closed estuaries. A = West Kleinemonde; B = East Kleinemonde & C = Kasouga Estuaries.

Table 3.1. Results of an ANOVA (*Statistica*, version 7) to determine whether isopod density and biomass demonstrate significant differences between months of the study, site (lower, middle and upper estuary reaches) and mouth state (closed vs. open) in three temporarily open/closed estuaries: (A) West Kleinemonde, (B) East Kleinemonde and (C) Kasouga Estuaries.

Variation	**Sum of squares**	**d.f.**	**Mean squares**	**F-ratio**	**Sig. Level**
A Density					
Months	20290313	18	1127240	1.884	0.050
Site	4184561	2	2092281	2.909	0.063
Mouth state	1643227	1	1643227	2.184	0.145
A Biomass					
Months	70.856	18	3.936	1.558	0.123
Site	14.961	2	7.481	2.660	0.079
Mouth state	5.273	1	5.273	1.795	0.186
B Density					
Months	38822.12	18	2156.78	5.225	0.00001
Site	1844.81	2	922.40	0.946	0.395
Mouth state	1624.98	1	1624.98	1.690	0.199
B Biomass					
Months	0.278	18	0.016	3.498	0.006
Site	0.017	2	0.008	1.040	0.361
Mouth state	0.018	1	0.018	2.300	0.135
C Density					
Months	34783405	18	1932411	5.413	0.00001
Site	2952224	2	1476112	1.756	0.183
Mouth state	1755935	1	1755935	2.073	0.156
C Biomass					
Months	92.606	18	5.145	3.590	0.0005
Site	10.540	2	5.270	2.084	0.134
Mouth state	4.569	1	4.569	1.764	0.190

A significant difference in the overall temporal pattern of isopod abundance and biomasses in the Kasouga Estuary was established, with February 2006 being significantly different to all other months (multiple range tests, p-values ranged between $p < 0.05$ and $p < 0.001$; overall $F_{18, 38} = 5.41$, $p = 0.00001$ for abundance and $F_{18, 38} = 3.59$, $p = 0.00045$ for biomass). The biomass of isopods during February 2006 was similar to those of April 2006 and June 2006, and were significantly different to all other months (p-values between $p < 0.05$ and $p < 0.001$ were recorded). There was no significant difference with regard to site within the estuary and abundance, $F_{2, 54} = 1.76$, $p = 0.18$; or biomass $F_{2, 54} = 2.08$, $p = 0.13$). Isopod abundances and biomasses were typically lowest when the mouth was open, although the values regarding mouth state were highly variable no significant differences were established between the open and closed phase, ($F_{1, 55} = 2.10$, $p = 0.16$ for density and $F_{1, 55} = 1.77$, $p = 0.19$ for biomass, Table 3.1). Once again, a significant positive correlation between total abundances of the isopod and the percentage vegetation cover was noted ($r^2 = 0.79$; $p < 0.05$; Fig. 3.7).

3.3.5 Sexual dimorphism, sex ratios and brooding

Male and female *E. hylocoetes* can be differentiated by studying the pleotelson from the ventral side. Male *E. hylocoetes* have a more pointed, triangular pleotelson, and together with a ridge on the pleotelson when viewed vetrally. Females have a more rounded pleotelson and lack this ridge (Fig. 3.8 A and B). Males longer than 5 mm also develop the characteristic paired pene located anteriorly of the first pleopods (Fig. 3.8 C). Females develop five pairs of vestigial oostegites attached to the ventral side of the pereonites (Chapter 1, Figs 1.1 and 1.3). Brood carrying could be observed in individuals > 2.5 mm. Isopods < 2.5 mm in length were considered as juveniles. Yellow eggs visible through the ventral body wall could identify gravid females. The ventral wall would become distended when mancas were present. Such visually gravid females were then dissected to allow counting and measurement of the brood.

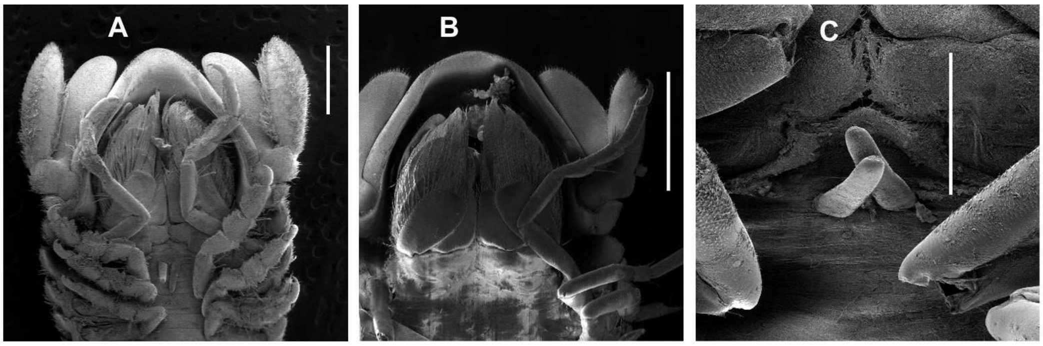

Figure 3.8. Scanning electron micrograph of the ventral view of the pleotelson of *Exosphaeroma hylocoetes* to show differences in the male (A) and female (B) structures. The male pene (C) located in front of the pleopods. Scale bar = 1 mm in A and B; and 0.5 mm in C. Specimens processed using standard techniques and viewed on a Tescan Vega SEM at an accelerating voltage of 5.0 KV

.

Sex ratios within the three estuaries were highly variable. In the W. Kleinemonde and Kasouga Estuaries sex ratios were skewed towards females. These ratios ranged between 1:1 and a maximum of 10.9:1 (during July 2006) in the W. Kleinemonde and between 1:1 and 20.5:1 (during July 2006) in the Kasouga Estuary (Fig. 3.9). The isopods in the E. Kleinemonde Estuary generally demonstrated a 1 : 1 ratio, except during May 2006 when females were outnumbered by males at 0.28 : 1 (Fig. 3.9). The female bias in the W. Kleinemonde Estuary was not only apparent during the longer periods of mouth closure but also during re-colonisations (Fig. 3.9). In the E. Kleinemonde Estuary prior to initial mouth breaching the sex ratio was 1:1 while during the predominantly closed phase in 2007 this became skewed towards females at 1.3:1. The female bias in the Kasouga Estuary lessened during the closed phase of 2007 from 4.6:1 (in the closed phase of 2006) to 1.7: during the closed phase of 2007. However, for nine months the sex ratio was female biased, while only three months saw male dominance (when overall abundances were low) in the Kasouga Estuary (Fig. 3. 9).

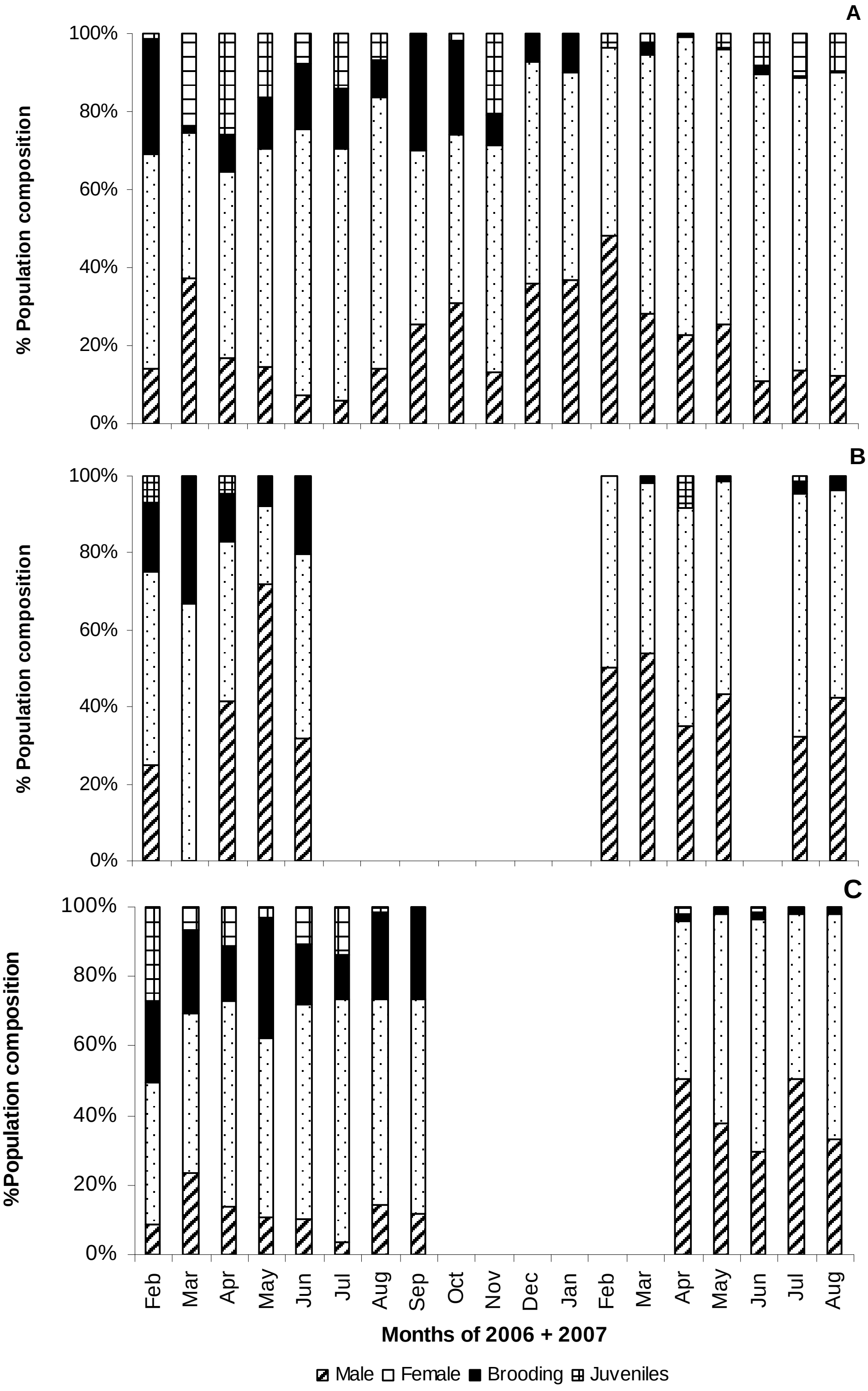

Figure 3.9. Population composition of *Exosphaeroma hylocoetes* in three Eastern Cape temporarily open/closed estuaries. A = West Kleinemonde; B = East Kleinemonde & C = Kasouga Estuaries. Those months with fewer than 10 individuals had data omitted. Brooding refers to gravid females; and juveniles ≤ 2.5 mm.

In the W. Kleinemonde Estuary some females were carrying brood, and juveniles (< 2.5 mm) were present, for most of the year, resulting in no seasonal pattern in juvenile release (Fig. 3.9). During the initial closed phase of the W. Kleinemonde, 29 % of females were found to carry brood, in the mouth-open phase 17 %, but during the closed phase of 2007 this declined to 6.6 %. While brooding females were found in most months when the mouths of the Kasouga and E. Kleinemonde Estuaries were closed, juveniles were scarce in 2007 (Fig. 3.9). In the Kasouga Estuary 42 % of females were found to be carrying brood during the initial mouth-closed phase, but this declined to 3.4 % during the 2007 closed phase. In the E. Kleinemonde Estuary the brood carrying females also showed this decline from 36.8 to 4.8 % in the same two periods (Table 3.1).

Male *E. hylocoetes* (5.71 ± 0.41 mm) were significantly larger (p = 0.003) than females (3.99 ± 0.26 mm) in all three estuaries with males growing to a maximum of 12.0 mm and females 7.5 mm (Fig. 3.10; Table 3.1). There were no significant spatial differences in isopod mean length within each of the estuaries during the 19-month study period, (p = 0.305 in the W. Kleinemonde; p = 0.428 in the E. Kleinemonde and p = 0.059 in the Kasouga Estuary). A one-way ANOVA (Tukey test) between the three estuaries yielded no significant differences in isopod lengths between in the Kasouga and W. Kleinemonde Estuaries (p = 0.102) or between those in the E. Kleinemonde and Kasouga Estuaries (p = 0.550). There was, however, a significant difference in isopod length between the W. Kleinemonde and E. Kleinemonde Estuaries (p = 0.010), with E. Kleinemonde having the longer individuals.

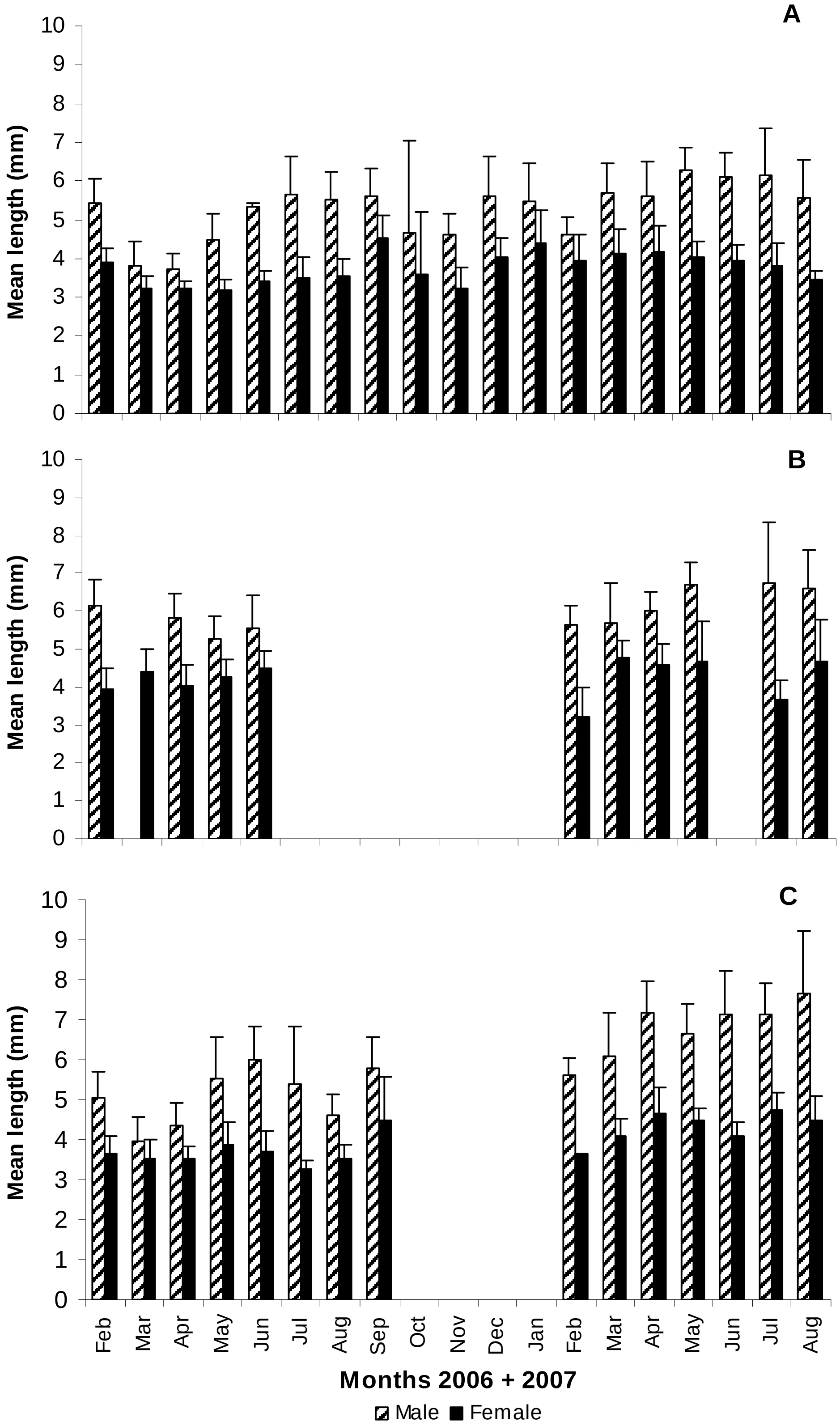

Figure 3.10. Mean lengths (± S.D.) of male and female *Exosphaeroma hylocoetes* in three temporarily open/closed estuaries over the period February 2006 to August 2007. A = West Kleinemonde; B = East Kleinemonde & C = Kasouga Estuaries. Those months with fewer than five individuals had data omitted.

Table 3.2. Mean (± S.D.) and maximum isopod lengths (mm) within all three estuaries from February 2006 to August 2007.

	Mean length (mm)	Maximum length (mm)
Males	5.71 ± 0.41	12.0 (n = 11 608)
Females	3.99 ± 0.26	7.5 (n = 42 277)

Dry mass to length also showed the larger overall male size compared to females, with females carrying brood having a greater mass than males of similar length (Fig. 3.11).

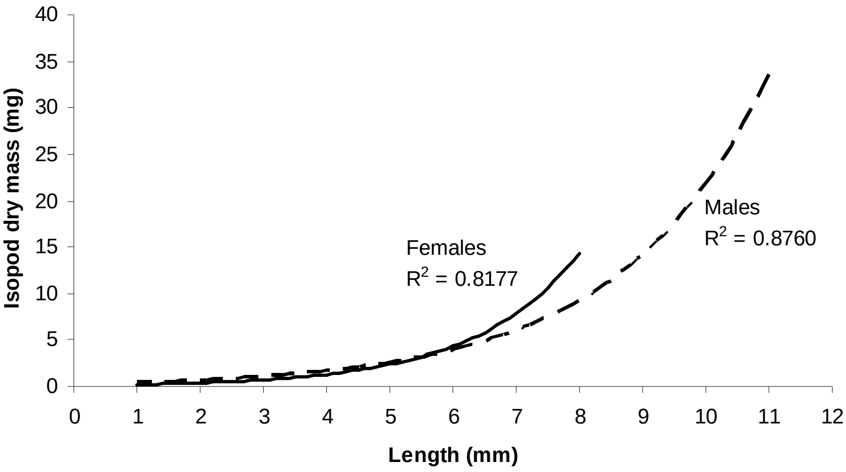

Figure 3.11. Male and female *Exosphaeroma hylocoetes* dry mass to length. Points from scatter diagrams were omitted for clarity.

3.3.6 Female brood size and brood sex ratios

The largest female encountered in the field (7.5 mm) carried the greatest number of brood (72), but these were relatively small at 0.42 mm. The smallest female (3.10 mm) carried only five eggs with a mean size of 0.4 mm. From this it may be concluded that the larger the female body size, the larger the brood size she can carry (Fig. 3.12 A). Eggs/embryos were carried within the female body cavity, rather than within the marsupium created by the oostegites (Fig. 3.13).

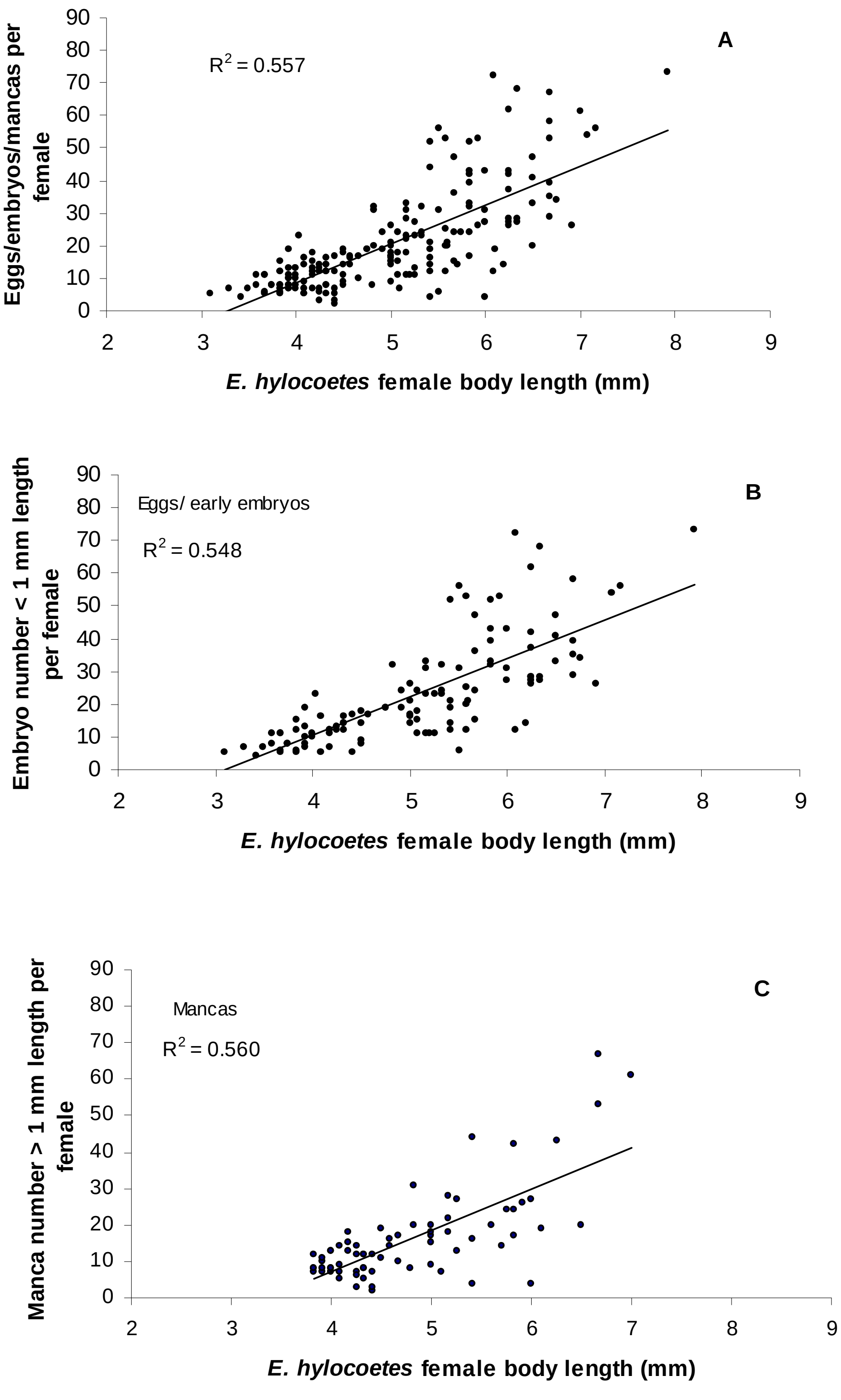

Figure 3.12. Relationship between gravid female *Exosphaeroma hylocoetes* body length and brood size (A); eggs/ early embryos <1 mm diameter (B) and mancas > 1mm (C).

Maximum number of eggs counted were 72, while a minimum of two were found (mean brood size = 20.69 ± 15.23; Fig. 3.12 A). The undeveloped embryos/eggs (< 1mm) had a mean size of 0.54 ± 0.22 mm (ranging in size from 0.17 to 0.92 mm; Fig. 3.12 B). The mancas (> 1mm) ranged in size from one to 1.5 mm (mean length = 1.17 ± 0.14 mm; Fig 3.12 C).

The laboratory-maintained visibly gravid female *E. hylocoetes* ranged in size from 3.10 to 7.92 mm (mean length = 4.99 ± 0.96 mm). The broods of females raised in the laboratory experiments females at two tested temperatures (15 and 25 °C) and one salinity (15) had a significant female bias (p < 0.001in both cases; t = 4.09 at 15 °C and t = 5.67 at 25 °C; Fig. 3.14). At 15 °C the mean number of males per brood was 5.50 ± 3.84, whilst that of females was 17.07 ± 8.48. At 25 °C, the mean number of males per brood was 3.27 ± 2.12 and that of females was 12.12 ± 5.92. There was, however, no significant difference between the sex ratio of males to females at 25 °C and 15 °C (p = 0.134; t = 1.544; df = 28). Males, therefore, made up less than 25 % of the broods at both temperatures (Fig. 3.14). Males were observed to be more cannibalistic than females, even when fed daily. At 15 °C the maximum brood size was 48 with an average brood size of 21.20 ± 12.99. At 25 °C these figures were 28, with an average size of 15.73 ± 7.69. These averages fall within the range found in the previously mentioned field data at 20.69 ± 15.23, but less than the maximum brood (72) found within a female.

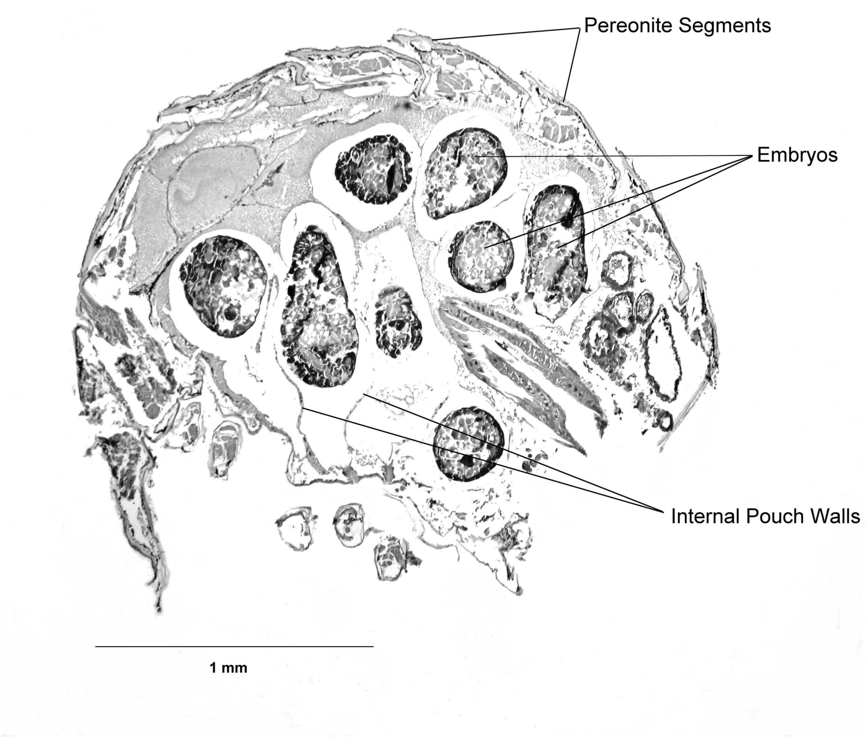

Figure 3.13. Semi-oblique histological section through a gravid female *Exosphaeroma hylocoetes* to reveal location of eggs/embryos within internal pouches in the body cavity. (Compare to Chapter 1, Figure 1.3). Section was stained in haematoxylin and eosin. Tissue was previously fixed in Bouin's fixative and prepared by standard histological methods.

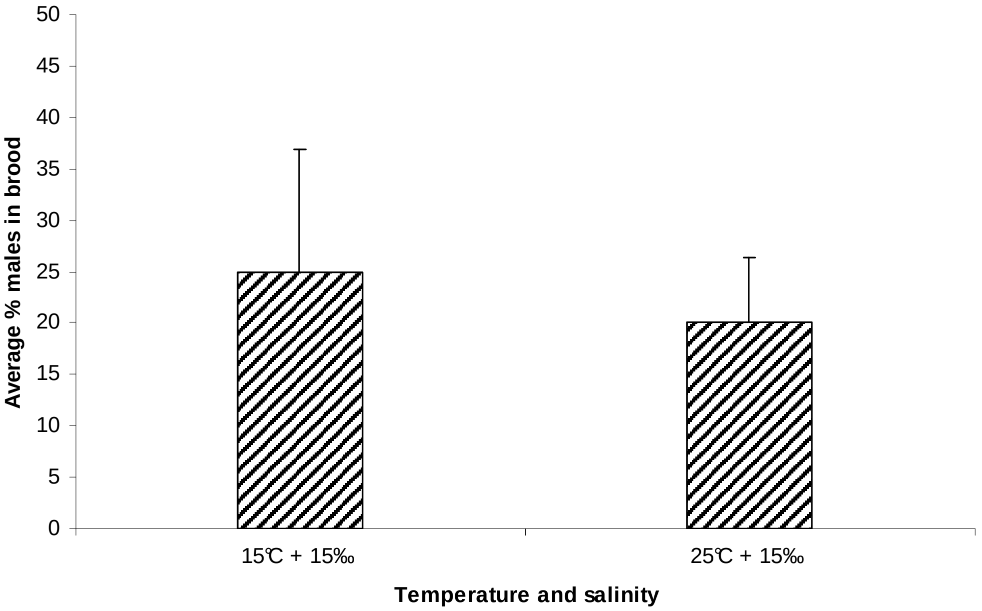

Figure 3.14. Average percentage (+ S.D.) of male *Exosphaeroma hylocoetes* in broods released by females at two temperatures (15 and 25 °C) and at a salinity of 15.

Trials with the *Wolbachia* surface protein gene primer, wsp 81F and wsp 691R, proved negative for *Wolbachia* in *E. hylocoetes* using the polymerase chain reaction (PCR) cycling conditions of 35 cycles (1 min at 94 °C, 1 min at 55 °C, 1 min at 72 °C). Controls for PCR amplification of DNA solutions were carried out using general eukaryotic 285r and 285f primers (Werren *et al.*, 1995). This time PCR cycling conditions were one cycle (1 min at 94 °C, 1 min at 55 °C, 2 min at 72 °C), 35 cycles (15 s at 94 °C, 1 min at 55 °C, 2 min at 72 °C) and one cycle (15 s at 94 °C, 1 min at 55 °C, 7 min at 72 °C; Thermo Hybaid PX2 thermal cycler). A negative result is illustrated in electrophoresis gel in Figure 3.15.

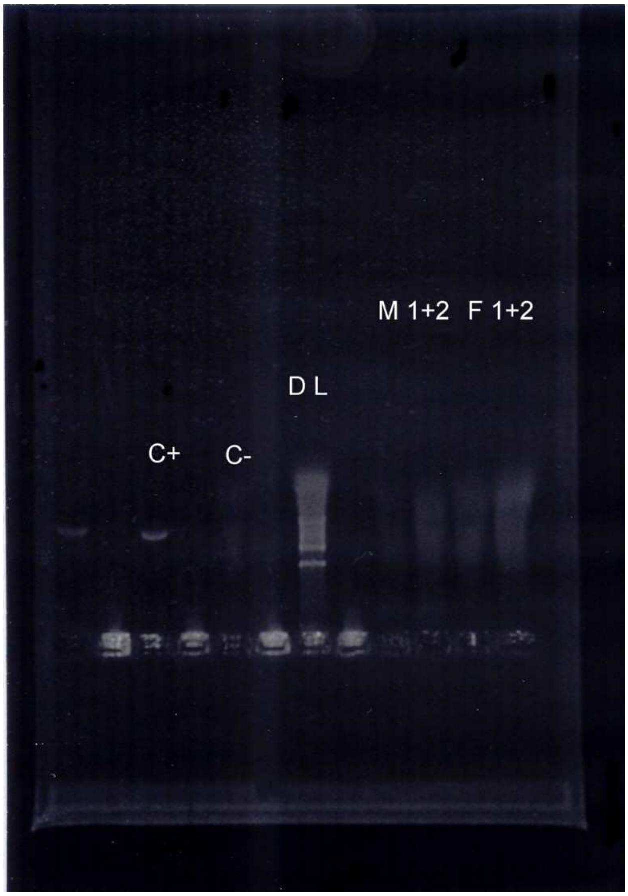

Fig. 3.15. Electrophoresis gel indicating negative *Wolbachia* infection of PCR product of male and female *E. hylocoetes* using *Wolbachia* surface proteins WSP 81 F and WSP 691 R. Key: C+ = positive control; C- = negative control; DL = DNA ladder; M 1+2 = males 1 and 2; F 1+2 = females 1 and 2. Absence of light band, (as in C+), indicates absence of *Wolbachia* infection.

3.4 Discussion

During this study there was no evidence of horizontal temperature gradients in the three estuaries. In addition salinity gradients were only apparent in the two Kleinemonde Estuaries during a few summer months. The absence of any horizontal gradients is a common feature of TOCEs along the southeast coastline of southern Africa (Perissinotto *et al.,* 2000, 2002; Froneman, 2000, 2002a, 2004c), and can be ascribed to a combination of factors including, small river catchment size (here ranging between 23 and 50 km^2) that contributes a limited amount of freshwater run-off entering the systems, shallow water depth (< 1.5 m), and wind-induced horizontal and vertical mixing (Blaber *et al.,* 1983; Perissinotto *et al.,* 2000, 2002, 2004; Froneman, 2002b, 2004c). Mean water temperatures and salinities were similar to those recorded by Harrison (2004) for each of the respective estuaries. The early spring rains coincided with a decrease in salinity, leading to the eventual breaching of the three estuaries. These results concur with those findings of Froneman (2002a, b) for the Kasouga Estuary and with other TOCEs (Perissinotto *et al.,* 2000; Walker *et al.,* 2001; Riddin and Adams, 2008), within the same geographic region.

Water temperature seasonality corresponded to those given for the Eastern Cape coastline (Kopke, 1988). On breaching, cooler marine waters entered the estuaries and lowered the estuarine water temperatures, an effect which was fast negated once the mouths closed and estuarine water temperatures rose once more by 6 °C in the W. Kleinemonde and by 7 °C in the E. Kleinemonde and Kasouga Estuaries between October and November 2006. These changes did not only occur within the lower reaches but along the whole length of the estuary, a result which can be ascribed to the relatively short length and shallowness of the systems (Blaber *et al.*, 1983; Perissinotto *et al.*, 2000, 2002; Froneman, 2002a, b, 2004b, c).

Horizontal gradients in temperature and salinity have been demonstrated to affect the spatial variability in distribution of plankton and nekton in permanently open southern African estuaries (Jerling and Wooldridge, 1995; Wooldridge, 1999; Newman *et al.*, 2007), as well as estuaries in general (Charpentier *et al.*, 2005; Dye and Baros, 2005; Jones and West, 2005; Gladstone *et al*., 2006). Although there was evidence of greater isopod abundance in the middle and upper reaches of the W. Kleinemonde and Kasouga Estuaries during this study, the observed pattern is unlikely to be the result of physico-chemical gradients. Rather, the elevated abundance and biomass values appear to be related to availability of submerged macrophytes. Indeed, the total abundances of isopods within the three estuaries were significantly correlated to percentage vegetation cover ($p < 0.05$ in all cases). Isopods were found in greatest densities when submerged vegetation abundance was > 60 %, and removal of the vegetation during mouth breaching in the E. Kleinemonde and Kasouga Estuaries coincided with the virtual absence of isopods within these systems. Similar conclusions were reached by Kanouse *et al.* (2006) who demonstrated that the biomass of submerged vegetation can determine the nekton distribution in North American marsh ponds. In South Africa, De Decker and Bally (1985), Hodgson (1987), as well as Bernard and Froneman (2005) have all highlighted the importance of submerged vegetation in structuring the estuarine invertebrate communities in both permanently open and temporarily open/closed southern African estuaries. Riddin and Adams (2008) reported similar losses of submerged vegetation in the E. Kleinemonde Estuary, but ascribed this loss to desiccation, within hours, after rapid declines in water levels following mouth breaching. It is now generally accepted that submerged macrophytes provide estuarine organisms with a refuge from predation or alternatively, represent an area of increased food availability (Peterson, 1979; Heck and Thoman, 1981; Orth *et al.*, 1984; Heck *et al.*, 1995; Mattilla *et al.*, 1999; Bernard and Froneman, 2005).

The estimates of abundance and biomass of the isopods reported for the W. Kleinemonde and Kasouga Estuaries during this study are similar to those reported for other South African estuaries, e.g. ≤ 1 980 ind. m^{-2} in the Bot River Estuary (Koop *et al.*, 1983); 17 581 ind. m^{-2} in the Gamtoos Estuary within the same geographic region (Schlacher and Wooldridge, 1996; see also Table 1.1, Chapter 1). On the other hand, the numbers in the E. Kleinemonde are of an order of magnitude less (max. density 108 ind. m^{-2}) than those reported in the literature. These lower estimates can likely be attributed to the increased rate of mouth open events recorded within the system, which were associated with a decline in invertebrate abundances and biomasses (Cowley *et al.*, 2001; Froneman, 2002a). In all three estuaries there was always a dramatic decline in the abundance and biomass of isopods when the estuary mouths breached, resulting in a loss of estuarine water and submerged vegetation. The associated decline in abundance and biomass of isopods is also in agreement with previous studies on other invertebrates (Wooldridge and Callahan, 2000; Froneman, 2002b, 2004b, c; Bernard and Froneman, 2005). Mouth opening/breaching events are, therefore, probably the most significant single ecological event in a TOCE. Although this proves that the status of the mouth had a major impact on isopod densities, the effects of seasonality on the isopod populations cannot be ignored. This study showed evidence of a steady decline in abundance and biomass of isopods within both W. Kleinemonde and Kasouga Estuaries during the first four/five months of the study when the mouth was closed (Figs 3.6 and 3.7).

Although not specifically investigated, the recovery of isopod populations after mouth breaching could occur in any of three ways. Firstly, during overtopping from the marine environment when the mouth is closed: a number of studies have shown that fish (Whitfield, 1992b), ichthyoplankton and macrozooplankton (Kemp and Froneman, 2004; Bernard and Froneman, 2005) enter TOCEs in this way, as do the larvae of the prawn *Palaemon peringueyi* and the mysid *Mesopodopsis wooldridgei*, and so, by deduction, may also *E. hylocoetes*. Secondly, rafting on macrophytes from another estuary when the mouth is open, as has been shown for *Idotea* spp. (Vandendriessche *et al.*, 2006; Teske *et al.*, 2007) could occur. *E. hylocoetes* was observed clinging to *R. maritima* at the mouth of breached rivers (Henninger, pers. obs.). This observation is supported by the findings of Teske *et al.* (2006, 2007) who have shown a strong genetic linkage between all *E. hylocoetes* on the southern coast of South Africa stretching from the Gourits Estuary, east of Cape Agulhas, to the Bulolo Estuary in KwaZulu-Natal (i.e. a homogenous population). Thirdly, refugia within the estuaries prevent the outwash of isopods into the marine environment following breaching events.

Females were observed to be carrying brood throughout the study period, indicating little or no seasonality in the reproduction of *E. hylocoetes*. This is in strong contrast to *Cirolana fluviatilis* in the permanently open Gamtoos Estuary (Eastern Cape), which showed seasonal reproductive patterns (Newman *et al.*, 2007). This absence of distinct seasonal patterns in the reproduction of *E. hylocoetes* could be an important adaptation to the unpredictable nature (i.e. mouth phase/ hydrodynamics) of TOCEs along the eastern coastline of southern Africa.

Most marine isopods show a strong sexual bias towards females (Shafir and Field, 1980b) and sex ratios showing an extreme female bias of up to 14:1 have been reported for various isopods of the genus *Jaera* (Jones and Naylor, 1971; Arrontes, 1992; Pierntney and Carvalho, 1996). It is assumed that one male can fertilise several females (Wong and Moore, 1996), resulting in a reduced need for males. The sex ratios of *E. hylocoetes* were highly female biased in two of the three estuaries. In addition results of this study indicate strong sexual dimorphism, with males being significantly larger than females ($p < 0.05$). Most Sphaeromatidae show a skewness towards larger male size (Kensley, 1978; Brusca and Wilson, 1991; Bruce, 2003). The significance of larger male recorded during this study may be related to mate guarding, as occurs in other species (Jormalainen, 1998).

The larger the female body length the more fecund she can be is in common with other studies of isopods, e.g. *Idotea baltica,* where larger females (20 mm) can produce up to 300 embryos per brood, whereas smaller females (15 mm) produced a maximum of only 140 embryos per brood (Strong and Daborn, 1979). Latitude influences water temperatures and in coastal areas with higher temperature, a higher fecundity and smaller egg size, as in *Betaeus truncates* and *Excirolana hirsuticauda* along the Chilean coast, are usually observed (Lardies and Wehrtmann, 2001; Contreras and Jaramillo, 2003). Female *E. hylocoetes* attain sexual maturity at a relatively small size, ≥ 2.5 mm, deliver offspring (mean number 20.70 ± 15.22) all year round, and can produce as many as 72 offspring at one time. In the Gamtoos Estuary study, *Cirolana fluviatilis* produced up to 47 mancas at once (Newman *et al*., 2007).

Female-biased sex ratios could be the result of biological, e.g. selective predation, or physical factors. The laboratory brooding experiments corroborated the field findings that females outnumbered males even at the early stages of development (Figs 3. 10 and 3.15). The skewed sex ratios, both in the field and in laboratory raised populations could be a result of *Wolbachia* spp. acting as a cytoplasmic sex ratio distorter (Juchault *et al*., 1993; Martin *et al*., 1994; Kageyama *et al*., 1998; Bouchon *et al*., 1998). *Wolbachia* has been shown to affect other Crustacea. *Wolbachia* has been found to be widespread in insects and terrestrial isopods and could have been transferred horizontally from insects to the terrestrial isopods (Werren, 1997). Preliminary results using PCR, however, indicated that *Wolbachia* was not to be responsible for the skewed sex ratios in *E. hylocoetes*, and must be the result of an innate genetic factor, or even another intracellular bacterium belonging to the genera *Anaplasma*, *Cowdria*, *Ehrlichia* or *Rickettsia* (Werren and O'Niell, 1997). *Exosphaeroma hylocoetes* spending all of its time in the estuarine water does not feed in overlapping areas with terrestrial and semi-terrestrial isopods which means that it is unlikely to be exposed to infections from such isopods.

It was often difficult to rear a mating pair together in a container in the laboratory, as inevitably one of the pair would be eaten, despite being fed *ad libitum*. Such cannibalism within mating pairs has been recorded in the parasitic isopod, *Ichthyoxenus fushanensis*, in which resource limitation was suggested as the trigger mechanism for the behaviour (Tsai and Dai, 2003), and in amphipods (Dick *et al*., 1993). Females outnumbered males by ≈ 3:1 in the estuaries, which allowed the isopods to re-colonise an estuary once the mouth has closed. This is in keeping with the results of Ovidiu Vlad (1989) who determined, mathematically, that the best population growth would be expected in female-biased populations. It has been increasingly recognised that the presence and behaviour of males affect the population processes, be it in forcing females into poorer resource habitats, or by exposure by males to greater predation on moving between habitats (Rankin and Kokko, 2007). Females were observed to remain within the *R. maritima* when carrying brood, and they do not feed at this time, possibly to avoid fish predation (see Chapter 4), or to prevent expulsion of embryos/brood from the marsupium (Harrison, 1984a).

The isopods can re-colonise the estuaries within four months after mouth closure. Sex ratios tend to stay female biased even after mouths have breached, again allowing re-colonisation. When all the physical evidence is viewed *en masse*, the TOCEs are revealed as highly dynamic systems which result in the isopods having to develop adaptive strategies to survive, such as those listed here: a strong female bias in sex ratios; year round reproduction; females producing from a relatively small size (≥ 2.5 mm) and producing mancas within the abdominal cavity then releasing these as miniature adults directly into the water column. I believe that is highly likely that *E. hylocoetes* re-colonisation occurs by isopods remaining in refugia, possibly deep submerged algae on the upper layers of mud, and probably also rafting on vegetation, between estuaries (or back into estuary) when the mouths are breached, as reported by Teske *et al.* (2006, 2007).

In conclusion, the results of this study indicate that the horizontal distribution of *E. hylocoetes* is largely linked to habitat (submerged vegetation, especially *Ruppia maritima*), rather than temperature and/or salinity gradients. Shifts in the abundance and biomass of *E. hylocoetes* would seem to be determined by mouth breaching events as these were shown to coincide with dramatic declines in these areas. Mouth breaching events have to been demonstrated to be a major driver in TOCEs (Whitfield *et al.,* in press). The results of this study do therefore not support the proposed null hypothesis and therefore the hypothesis of mouth breaching playing a major role on the population dynamics of *E. hylocoetes* must be accepted. Seasonality was also shown to be influential, although to a lesser extent. *E. hylocoetes* demonstrated strong sexual dimorphism with males significantly larger than females, but sex ratios revealed skewing towards female numerical dominance. Finally, the presence of juveniles throughout the 19 month study indicates that *E. hylocoetes* reproduces throughout the year.

Chapter 4

The Role of Macrophytes as a Refuge and/or Food Source for the Estuarine Isopod, *Exosphaeroma hylocoetes*

4.1. Introduction

It is now well documented that submerged beds of estuarine macrophytes are characterised by elevated diversity, abundance and biomass of both invertebrates and vertebrates (Wallerstein and Brusca, 1982; Wallace *et al.*, 1984; Holomuzki and Short, 1988; Sala and Graham, 2002; Joseph *et al.*, 2006). The elevated abundances and biomasses are thought to reflect the importance of the submerged macrophytes either as a refuge against predation (e.g. Heck and Thoman, 1981; Heck and Wilson, 1987), or as regions of increased food availability (Whitfield, 1980a; Orth *et al.*, 1984; Mattila *et al.*, 1999; Jormalainen *et al.*, 2001; Sala and Graham, 2002; Heck *et al.*, 2003). Similarly, Young *et al*. (1976) found that variations in the intensity of predation account for differences in species abundance in seagrass-colonised compared to exposed areas in North American estuaries. It is also possible that for some species (e.g. polychaetes, gastropods, amphipods and isopods), submerged macrophytes may serve as both a refuge and a source of food. Homziak *et al.* (1982) demonstrated an exponential increase in species abundance and diversity with increased macrophyte cover. Peterson (1979), summarising 11 studies, demonstrated increases in both faunal densities and species richness after the exclusion of predators. Holomuzki and Short (1988), however, point out that for some species predator avoidance by retreating to within the macrophytes could cost the prey species in time and energy thus detracting from their ability to feed and reproduce.

Isopods are a conspicuous component of estuarine macroplankton worldwide (Kensley, 1978; Bruce, 1992a, b; Dias and Sprung, 2003; Gonçalves *et al*., 2005). In southern African estuaries, as in other regions of the world, isopods have been recorded in close association with submerged macrophyte beds (Chapter 3). Among the isopods, *Exosphaeroma hylocoetes* has been demonstrated to be numerically dominant in stands of *Ruppia maritima* in three temporarily open/closed estuaries (TOCEs) along the south-eastern seaboard of southern Africa, where it accounted for up to 95 % of all isopods counted (Chapter 3). Due to their numerical abundance *E. hylocoetes* have been demonstrated to represent an important component in the diets of several fish species, which are highly abundant in estuaries (Blaber, 1984; Coetzee and Pool, 1985; Wallace *et al.*, 1984). For example, the diets of the estuarine fish, *Clinus cottoides* and *Monodactylus falciformis*, include amphipods, copepods, insects, insect larvae and isopods (Whitfield, 1998). Juvenile *Rhabdosargus holubi* (30 mm) are known to feed on copepods, while *Glossogobius callidus* feeds on insect larvae, copepods, ostracods and branchiopods, and both fish species could feed on small isopods (Whitfield, 1993, 1998).

Isopods demonstrate wide-ranging feeding strategies, ranging from carnivory (including cannibalism), to herbivory and detritivory (Johnson, 1976a; Perry and Brusca, 1989; Briones-Fourzán and Lozano-Alverez, 1991; Newman *et al*., 2007). Previous studies employing gut content analysis indicate that the isopod, *E. hylocoetes*, can be regarded as a generalist detritivore (Whitfield, 1989). Unfortunately, due to the advanced state of digestion, the primary source of food for the isopod could not be determined.

Stable isotope ratios, particularly of carbon, ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), have been used since the 1970s to provide information on the flow of energy through aquatic ecosystems (West *et al*., 2006). The ratios of stable carbon isotopes ($C^{13} : C^{12}$) within the body tissues of the producers and the consumers give an indication of food sources (Fry and Sherr, 1984; Peterson *et al*., 1985; Froneman, 2001). The isotope signatures of consumers are closely related to that of their diet, and if food sources are isotopically distinct one can clearly identify a particular food source of a consumer (Peterson *et al*., 1985; France and Peters, 1997). More recently, trophic links within estuarine ecosystems have also been assessed using fatty acid composition (Richoux and Froneman, 2008). Fatty acids, which form structural and functional components of membranes essential for growth and reproduction cannot be synthesised by most organisms and are therefore derived from their diet (Arts *et al*., 2001). The composition of fatty acids within animals can therefore, like stable isotope analysis (Richoux and Froneman, 2007) be employed to examine trophic interactions within aquatic systems.

Although several studies have highlighted the close association between *E. hylocoetes* and beds of submerged macrophytes in southern African estuaries (Chapter 3), it is unclear whether the association reflects the importance of the macrophytes as refugia or as a food source. The null hypothesis would be that the close association between *Exosphaeroma hylocoetes* and submerged macrophytes reflects the availability of both a refuge and increased food availability.

The aims of this study were twofold:

(1) to determine experimentally whether submerged macrophytes serve as a refuge for *E. hylocoetes* against fish predation; and

(2) to establish whether the submerged macrophytes are an important

carbon source for the isopod using a variety of techniques including gut content, stable carbon isotope and fatty acid analyses.

4.2 Materials and Methods

4.2.1. Role of submerged vegetation as a refuge from fish predation.

The experiments were conducted in a constant environment room at Rhodes University during August 2006. The isopods, *Exosphaeroma hylocoetes*, were collected from the Kasouga Estuary (33°39’11”S 26°44’01”E; Chapter 2), using 20 cm^2 hand-nets with a mesh size of ≈ 1 mm. The fish, *Rhabdosargus holubi*, *Glossogobius callidus* and *Monodactylus falciformis,* were netted using a 5 m seine net (mesh size ≈ 1 mm). *Clinus cottoides* were netted in rock pools at the mouth of the estuary.

Experiments were conducted in round polyethylene containers with a diameter of ≈ 50 cm and a height of ≈ 12 cm. Four quadrants were marked in each of the containers which were then filled with estuarine water (32 ‰ at 18 °C). The experimental design included three replicates per treatment. Ten adult isopods were introduced into each container and left overnight to acclimatise. The containers were covered with a plastic lid to prevent evaporation. Densities of isopods in the experimental chambers (50 ind. m^{-2}) were within the range reported for the estuary (Chapter 3).

The study comprised of two experiments:

- assessment of the behaviour of isopods in the presence or absence of vegetation, designated experiment 1.

- assessment of the effectiveness of vegetation as a refuge for isopods from predation by selected fish species in the presence or absence of submerged vegetation (designated experiment 2).

To assess the behaviour of the isopods (experiment 1), two treatments were employed; three containers with no vegetation and three containers with vegetation. A preliminary study indicated that there were no significant differences ($p > 0.05$) in the utilisation of natural and artificial vegetation by the isopods (Froneman and Forbes, unpublished data). Artificial vegetation was created to represent the submerged macrophytes using 10 x 10 cm squares of shade cloth netting with 20 cm strips of shredded plastic threaded into it. The strips had a width of 2 mm, similar to the strands of the submerged macrophyte, *Ruppia maritima*, commonly found in the Kasouga Estuary. The vegetation was threaded to create a density of 760 shoots m^{-2}, which is representative of the category of “high densities of artificial eelgrass” (674 shoot m^{-2}) (Heck and Thoman, 1981). Heck and Thoman (1981) found that low (274 shoots m^{-2}) and intermediate (464 shoots m^{-2}) densities of artificial vegetation did not offer prey organisms significantly more protection than bare areas from predation by killifish on grass shrimp. The vegetation was randomly introduced (by lottery) into one of the four quadrants of three bowls to eliminate site selection by the isopods and to remove edge effects. Ten isopods of similar size (6 to 8 mm body length) were then introduced into each container. After 24 hours the distribution of the isopods in each quadrant of the containers was recorded.

For the predation trials, (experiment 2), the fish were starved for 12 hours prior to the introduction into the experimental containers, to standardise hunger (Gardner, 1981). In each case six replicates of each fish species were used and all experiments were run concurrently. Within each treatment for each fish species, six bowls with no vegetation and six bowls with vegetation in one of the quadrants were employed. The fish varied in length from 68 to 85 mm (mean length ≈ 76 mm SL) for *C. cottoides* and 66 to 72 mm (mean length ≈ 69 mm SL) for *G. callidus*; *R. holubi* varied in length from 75 to 94 mm (mean length ≈ 82 mm SL); while *M. falciformis* ranged in length from 87 to 105 mm (mean length ≈ 94 mm SL). The predatory fish were then placed into the bowls and the live (remaining) isopods were counted at the end of the experiment 24 hours later.

4.2.2 Examination of epibiota on *Ruppia maritima* in the isopod gut contents

Sections of *R. maritima* stems were prepared for scanning electron microscopy (S.E.M) following standard procedures. The sections were fixed in 2.5 % glutaraldehyde in 0.1 M phosphate buffer overnight, followed by dehydration in a series of alcohols to 100 % and critical point drying. The stem sections were then gold coated in a Balzars Sputtering Device. These stem sections would reveal any epiphytes living on the stems which could act as a possible food source. The gut contents of five isopods were carefully dissected, air dried and sputter coated to determine evidence of food sources. The images of these stem sections and gut contents were digitally captured on a Tescan Vega LMU SEM.

4.2.3 Determination of macrophytes as a food source using stable carbon isotope ratios

To determine the primary food source of *E. hylocoetes*, plant matter and isopods were collected from the Kasouga Estuary. The plant matter consisted of *Ruppia maritima*, *Sarcocornia perennis*, *Chenolea diffusa* and *Sporobolus virginicus*. Sediment samples were obtained by removing a core sample of the top 200 mm in 20 mm (diameter) pill vials. The sediments were bulk processed, acidified in 1 M HCl, followed by a thorough rinsing in distilled water. All vegetation and animal samples collected were rinsed in 0.2 µm filtered seawater, after which the plant matter was oven-dried at 60 °C for 24 hours. Isopods were frozen in liquid nitrogen, oven dried at 60 °C for 24 hours, and ground using a pestle and mortar. The isopods were defatted in a solution of chloroform, methanol and water (2:1:0.8; v:v:v) following the method of Bligh and Dyer (1959). Samples were defatted to minimise possible discrepancies arising from variable proportions of fatty tissue within the animal tissue (Froneman, 2002a). Epiphytes growing on the vegetation were gently scraped from the macrophytes using a sharp scalpel blade.

Samples were analysed in the Stable Light Isotope laboratory in the Archaeology Department, University of Cape Town. After sample combustion in an on-line Carlo-Eber preparation unit, $\delta^{13}C$ determination was carried out on a Thermo Finnigan Delta XP Plus mass spectrometer interfaced through a Conflo III device with a Thermo Flash EA 112 elemental analyzer. Results for carbon were reported relative to v-PDB (the fossil Pee Dee Belemnite standard). The raw data were corrected using standards of known isotopic composition (sucrose and lentil) and then normalised against several IAEA reference materials. The analytical precision of the instruments was within ± 0.2 % (1 SD). Results were expressed in parts per thousand (‰) deviating from the standard using the following formula:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000 ‰$$

where X is the element in question and R is the molar ratio of the heavy to light isotope of the sample and standard. Repeated analysis of homogenous material yielded a standard deviation of 0.03 ‰.

4.2.4 Determination of macrophytes as a food source using fatty acid profiles

All biological samples collected were rinsed with GF/F filtered seawater. The isopods were lyophilised for 24 hours and homogenised with a mortar and pestle. The plant samples were cut into short lengths (20 mm) before boiling in distilled water for two minutes in order to deactivate lipolytic enzymes (Budge and Parrish, 1999). Dried and weighed isopod samples (up to 100 mg dry mass), as well as wet plant and sediment samples were placed in 2 ml chloroform under nitrogen in lipid-cleaned vials and stored at -20 °C. Total lipids were extracted and purified using an adapted Folch procedure (Parrish, 1999). Samples were ground, using a metal rod, in 2:1 (v/v) chloroform/methanol, and 0.5 ml of chloroform extracted water added. The bottom organic layer was separated and rinsed using three chloroform washes and all rinses were pooled and concentrated using a gentle flow of nitrogen. A known quantity of internal standard (C19 : 0) was added to each extract to allow quantification of fatty acid peaks. The lipid extracts were suspended in hexane at 80 °C for 1.5 h in the presence of 14 % boron trifloride-methanol to produce fatty acid methyl esters (FAMEs) (adapted from Budge and Parrish, 1998).

Gas chromatographic (GC) analyses of the FAME’s were conducted using a Hewlett Packard 5890A GC equipped with a bonded and cross-linked 78 % cyanopropyl methylpolysiloxane fused silica capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness, Quadrex Corporation) with helium as the carrier gas. Sample aliquots (1 µl) were manually injected at 250 °C with the oven set at 100 °C for 3 mins. The oven temperature was programmed to increase to 150 °C at 5 °C min^{-1}, held for 1 min, then from 150 °C to 220 °C at 3.5 min $^{-1}$. The flame ionisation detector was held at 260 °C. FAMEs were identified by comparing their retention times to those of known standards (37 component FAME standard and marine PUFA no.1, Supelco). Each fatty acid is reported as a proportion of the total identified fatty acids (% TFA). Fatty acids are designated as $x{:}a\omega b$, where x represents the number of carbon atoms in the chain, a refers to the number of double bonds and b is the position of the ultimate double bond from the terminal methyl group. Saturated fatty acids (SFAs) have no double bonds between carbons (e.g. 14:0); monounsaturated fatty acids (MUFAs) contain one double bond (e.g. 16:1ω7), while polyunsaturated fatty acids (PUFAs) have > 1 double bond (e.g. 16: 4ω3). Essential fatty acids, or EFAs, include those PUFAs having > 20C, e.g. 20:4ω6; 20:5ω3 and 22:6ω3. Bacterial fatty acids (BAFAs) are those fatty acids having odd-numbered carbon chains and/or iso- (i-) and anteiso- (ai-) branches, while HPFAs (or higher plant fatty acids) include 18:2 ω6 and 18:3ω3 (Dalsgaard *et al*., 2003).

4.2.5 Data Analyses

The distribution of isopods in the containers without vegetation or fish was analysed using Chi-square (χ^2) tests. To compare the total distribution and abundance patterns of isopods between treatments, one-way ANOVAs were conducted using the computer package *Statistica 7* (StatSoft). Data were not transformed as they met the requirements for ANOVA.

Proportional and quantitative fatty acid data were analysed using Principal Component Analysis (PCA) to demonstrate associations between the potential food sources and the isopods. Proportional data were log-ratio transformed using a modified equation of Seaborn *et al*. (2005): $x_{trans} = ln((x_i + 1)/c_i)$, where x_{trans} is the transformed fatty acid, x_i represents the weight percentage of a given fatty acid and c_i is the weight percent of a reference fatty acid (18:0). The term $x_i + 1$ was used to counteract any zeros within the data set. The groupings in the scores were created using hierarchical cluster analysis. Statistical analyses were completed using PAST 1.42 (Hammer *et al.*, 2001), and variance is reported as one S.D. from the mean.

4.3. Results

4.3.1. Distribution and predation experiments

In the absence of artificial vegetation and predatory fish, there was no significant difference in the number of isopods in the four quadrants of the experimental containers ($\chi^2 = 3.6$; df = 3; $p = 0.31$; Fig. 4.1). No isopod mortality was observed in any of the treatments during the 24 hour period.

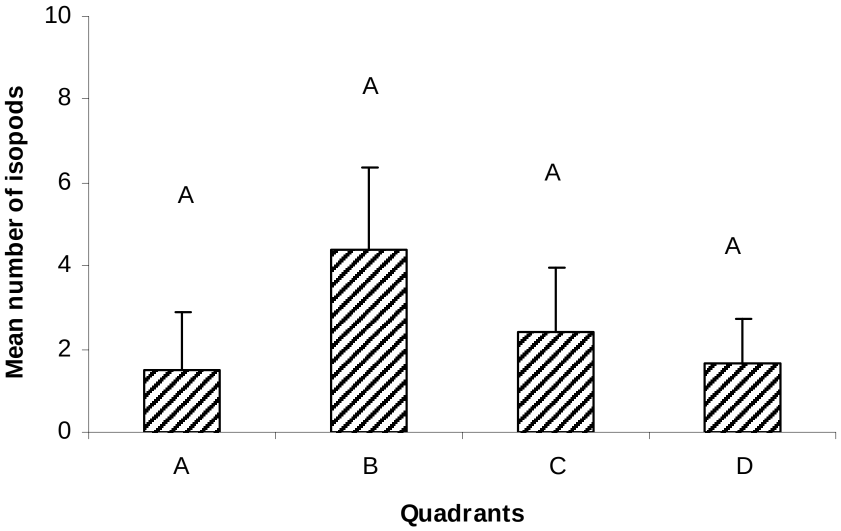

Figure 4.1. Mean number (+ S.D.) of isopods in four quadrants of containers in the absence of both vegetation and fish. The same letter above each bar indicates no significant differences between quadrants.

Isopods aggregated, significantly, on the artificial vegetation in the absence of fish predators (ANOVA; $F_{1,\ 10} = 1320.7$; $p < 0.001$; Fig. 4.2). Mean number of isopods within and away from the artificial vegetation was 9.29 ± 1.10 and 0.24 ± 0.50, respectively.

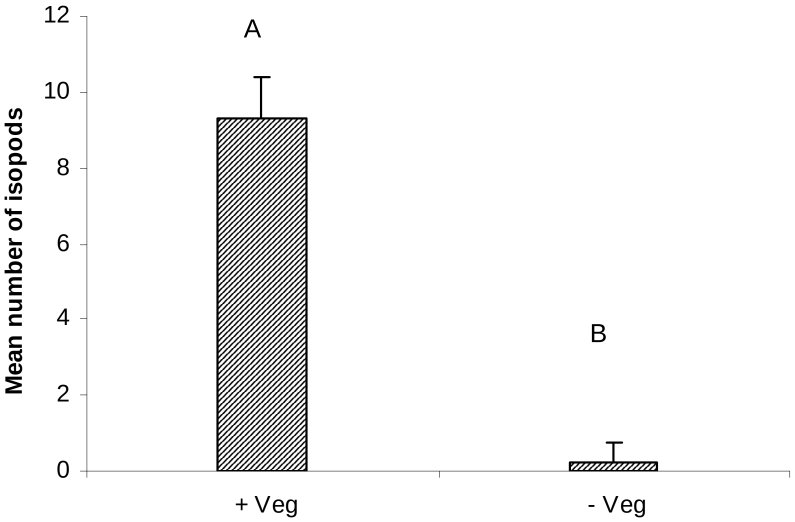

Figure 4.2. Mean number (+ S.D.) of *Exosphaeroma hylocoetes* found in quadrants with and without artificial vegetation after 24 hours. (+ Veg indicates vegetation present; - Veg indicates the absence of vegetation). Different letters indicate a significant difference between treatments.

In all predation experiments, the presence of artificial vegetation resulted in a significant decrease in isopod mortality in the presence of fish over the 24 hour feeding trials (Fig. 4.3). In the absence of vegetation, isopod mortality ranged from 20 to 85 % (average = 47.4 ± 28.4 %) and between 1 to 61 % (22.3 ± 27.0 %) in the presence of vegetation (Fig. 4.3). The extent of predation on the isopods by the different fish species in the different treatments, however, varied considerably. The fish, *C. cottoides* consumed on average 87 ± 5.2 % of the isopods in the absence of vegetation and 63 % ± 5.2 % when vegetation was present (values are significantly different, $p < 0.001$). Similarly, *G. callidus* consumed significantly more isopods (22 ± 10 %) in those treatments where artificial vegetation was absent ($p = 0.025$), than when vegetation was present (8 ± 8 %). The mean isopod mortality in the presence and absence of vegetation in the *R. holubi* treatments was 2 % (± 4) and 40 % (± 13), while in the treatment with *M. falciformis* , the mortality in the absence of vegetation was 52 % (± 17) and 18 % (± 12) in the presence of vegetation (Table 4.1).

Tables 4.1. Results of an ANOVA (*Statistica*, version 7) on the predation experiments with selected estuarine fish species and the mortality rates of *Exosphaeroma hylocoetes* in the presence and absence of vegetation.

Variation	**Mean Squares**	**d.f.**	**Mean squares**	**F-ratio**	**Sig. Level**
Fish predator					
Glossogobius callidus	5.333	1	5.333	6.957	0.0248
Residual	7.667	10	0.767		
Total	13	11			
Rhabdosargus holubi	44.083	1	44.083	49.906	0.000034
Residual	8.333	10	0.883		
Total	52.917	11			
Clinus cottoides	16.333	1	16.333	61.25	0.000014
Residual	2.667	10	0.267		
Total	19.000	11			
Monodactylus falciformis	33.333	1	33.333	15.385	0.0029
Residual	21.667	10	2.167		
Total	55.000	11			

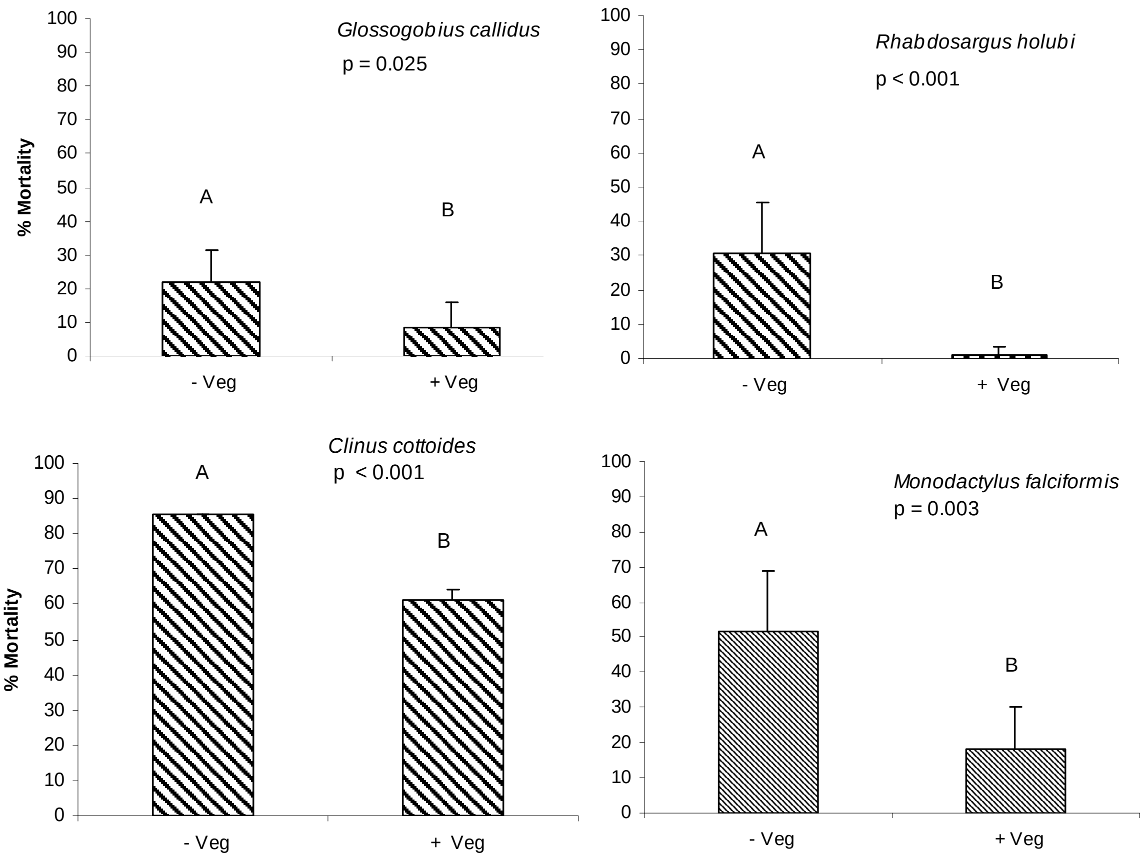

Figure 4.3. The effect of artificial vegetation in providing a refuge for the isopod *Exosphaeroma hylocoetes* against selected estuarine fish. Different letters denote statistical significance; *G. callidus* (p = 0.025), *R. holubi* (p <0.001), *C. cottoides* (p < 0.001) and *M. falciformis* (p = 0.003); n = 6 for each experiment. Key: - Veg = absence of vegetation; + Veg = vegetation present.

4.3.2. Epibiota and gut contents

Diatoms, of the genus *Nitzschia*, bacteria and fungal threads were identified on the stems of *R. maritima* (Fig. 4.4). Analyses of the gut contents of isopods were impossible largely due to the fact that the guts contained a large unidentifiable bolus.

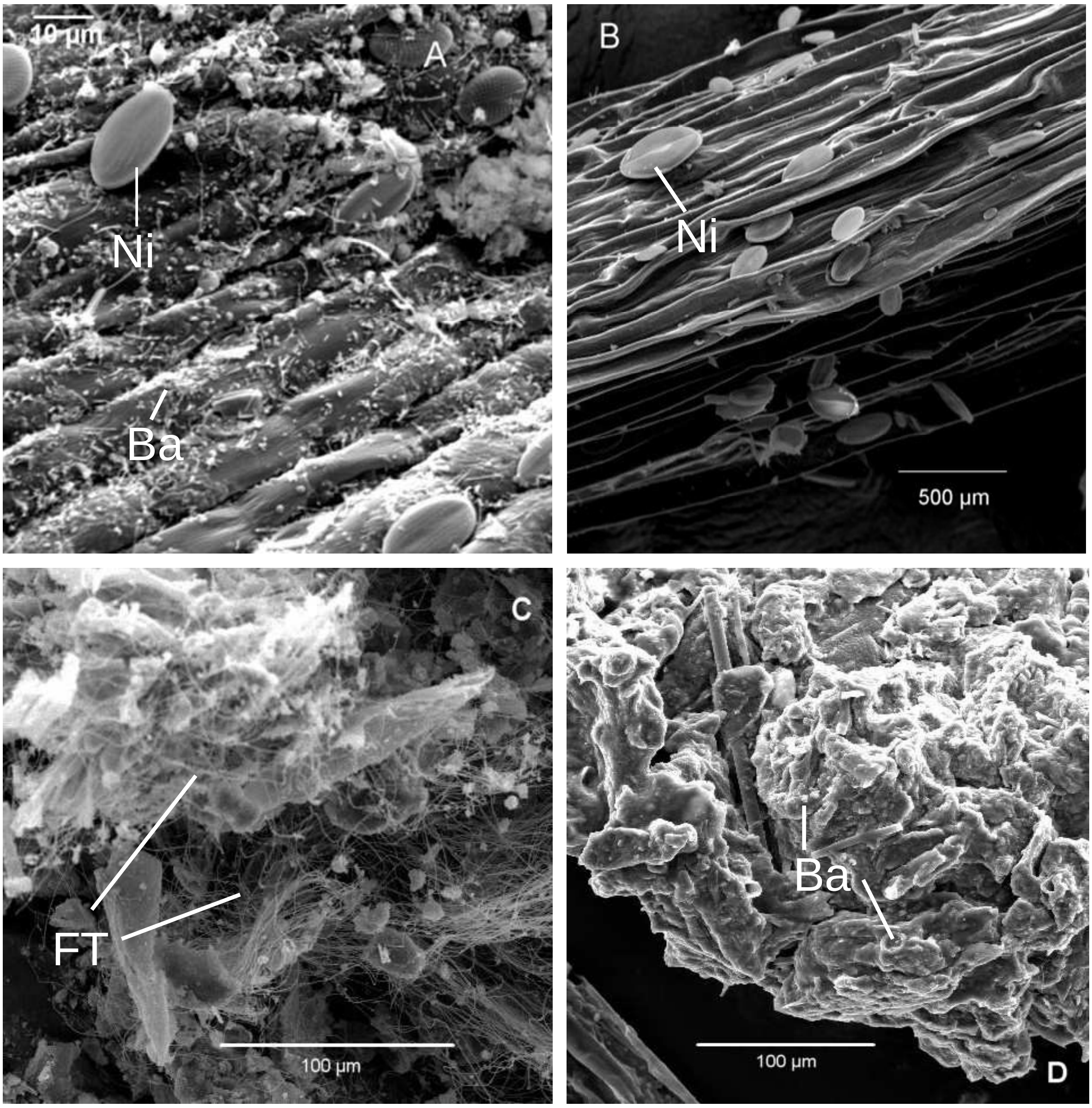

Figure 4.4. A. and B. Diatoms (*Nitzschia* spp.) and bacteria on the stem of *Ruppia maritima*. C. Bacteria and algal/fungal fibres. D. Bacteria. Key: Ni = *Nitzschia* spp.; Ba = bacterium; FT = fungal threads. (Scale bar in A = 10 µm; in B = 500 µm; in C & D = 100 µm). Specimens processed using standard techniques and viewed on a Tescan Vega SEM at an accelerating voltage of 10.0 KV (A + B), 20 KV (C + D).

4.3.3 Stable isotope ratios

The submerged macrophyte, *Ruppia maritima*, had a $\delta^{13}C$ value of –17.02 (± 0.19), while the salt marsh plants, *Sarcocornia perennis* and *Chenolea diffusa*, growing on the fringe of the estuary had more depleted values at –25.19 ± 1.60 and –23.35 ± 0.85, respectively (Fig. 4.5). The $\delta^{13}C$ value of the fringing grass *Sporobolus virginicus* was - 13.61 (± 0.19). The epiphytes on the stems of *R. maritima* had a $\delta^{13}C$ value of - 21.70 (± 1.10), while that of the POM was -20.40 ± 1.05. The mean isotope value of *E. hylocoetes* was -17.41 (± 1.38).

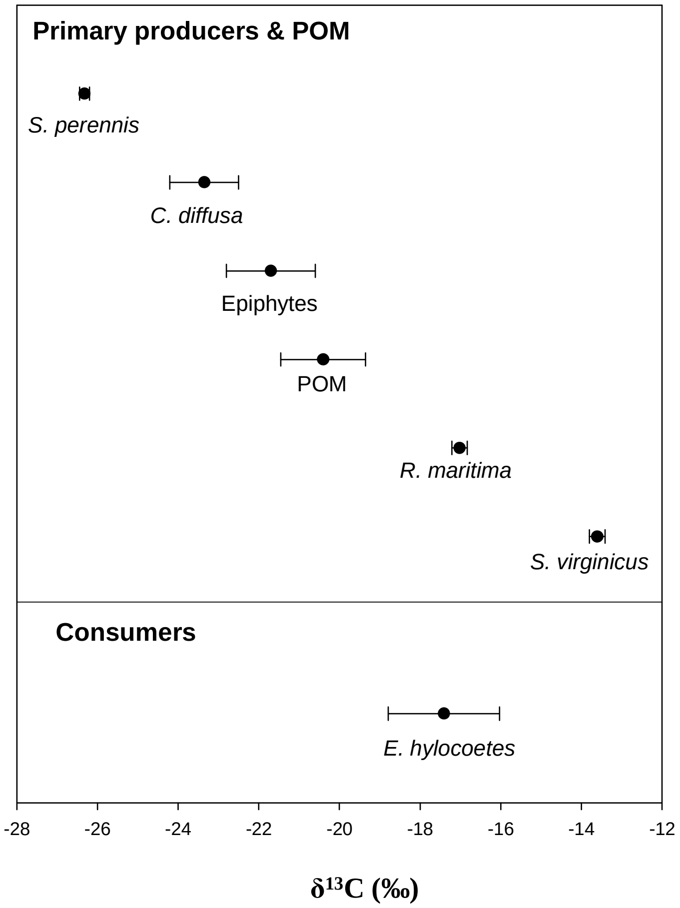

Figure 4.5. Stable carbon isotope $\delta^{13}C$ (‰) signatures with standard errors for the primary producers (*Sarcocornia perennis, Chenolea diffusa, Ruppia maritima* and *Sporobolus virginicus*), POM and the isopod *Exosphaeroma hylocoetes* in the Kasouga Estuary. Error bars are standard deviations.

4.3.4 Fatty acid profiles

Twenty-one fatty acids were detected at concentrations > 1 % of total fatty acids (TFA) in at least one of the potential food sources (Table 4.2). Most plants contained characteristically high proportions of 16:0, 18:2 ω6 and 18:3ω3 fatty acids. Jeffries (1972) showed that in terrestrial plants ≈ 50 to 75 % of their fatty acids are 18 C compounds. Thus the plants examined in this study demonstrate a terrestrial signature. All of the potential food sources contained relatively large proportions (18 to 45 % TFAs) of the SFA 16 : 0, with the alga containing the greatest amount. The SFA 16:0 is the major end product of the most common lipid pathway utilising Type 1 fatty acid synthetase (Dalsgaard *et al*., 2003). The monounsaturated fatty acids (MUFAs) were low in plants, with sediments containing greater proportions (≈ 19 %) of 16:1ω7, similar to that of *E. hylocoetes* (≈ 15 %). Linolenic acid, with 18 carbon atoms and three double bonds (18:3ω3) was the most abundant polyunsaturated fatty acid (PUFA) in marsh plants, e.g. 52 % in *S. maritima* and the submerged macrophyte, *R. maritima* (43.4 %). This fatty acid was found in decreased proportions in the sediments (0.5 %) and the alga (0.1 %). The PUFA 18:3ω3 was found in low levels in the isopod (12 %). The isopods had higher proportions of MUFAs (34 %) and marine (C_{20-22}) PUFAs (≈ 22 %) than the plants.

Bacterial fatty acids (BAFAs) were highest (≈ 19 % TFAs) within the sediments and lowest within the living plants. The sediments contained relatively high levels of 16: 1ω7 and 20:5ω3, which are common diatom markers (Dalsgaard *et al.*, 2003). Sediment PUFA levels were low (≈ 18 %) in comparison to the MUFAs (≈ 27 %) and SFAs (≈ 65 %), consistent with high levels associated with decomposition in sediments (Derieux *et al*., 1998). EFAs were greater in sediments than in other potential food sources owing to elevated (2.4 %) levels of 20:5ω3, however higher plant fatty acids (HPFAs) were very low relative to those found in the plants. The alga, *H. sordida*, contained the highest levels of SFAs (≈ 80 %), but low levels of HPFA (≈ 5 %). The alga contained relatively high levels of 14:0, 16:0, 16:1ω7, 18:1ω9, 18:1ω7 and 18: 2ω6, however, it contained low levels of the EFAs found in the isopod.

The isopod, *E. hylocoetes*, contained a predominance of PUFAs with 20 – 22 carbon atoms, demonstrating a marine pattern (Lovern, 1964), and were particularly rich in 20: ω3 (≈ 17 %), similar to most estuarine zooplankton (Jeffries, 1972; Richoux and Froneman, 2008). The MUFAs were present in comparatively high proportions, particularly 16:1ω7, 18:1ω9 and 18:1ω7. Isopods contained relatively high levels of the diatom markers (16:1ω7 and 20:5ω3) (32 %), and low levels of HPFAs (1.3 %).

The principle components analyses (PCA) indicates a clustering of the sediments around *E. hylocoetes*, whereas the submerged macrophyte and marsh plants had significantly different fatty acid profiles separating them into independent clusters (Fig. 4.6).

Table 4.2. Fatty acid composition (% total identified fatty acids, mean ± S.D.) of *Exosphaeroma hylocoetes*, vegetation and sediments. Only those fatty acids at concentrations > 1 % TFA in at least one of the sample types are included. SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; BAFA: bacterial fatty acid; HPFA: higher plant fatty acid; EFA: essential fatty acid (20:4ω6; 20:5ω3 and 22:6ω3).

Fatty acid	*Exosphaeroma hylocoetes*	Sediments	*Spartina maritima*	*Sporobolus virginicus*	*Ruppia maritima*	*Hincksia sordida*
14:0	3.3 ± 1.0	11.9 ± 0.5	0.5	5.7	1.3	31.0
15:0	0.6 ± 0.2	4.6 ± 1.8	0	1.4	0.3	0
i-15:0	0.5 ± 0.2	5.4 ± 2.3	0	0	0.8	0
ai-15:0	0.2 ± 0.1	4.1 ± 2.0	0	0	0.6	0
16:0	24.1 ± 2.2	32.4 ± 0.8	25.9	34.2	21.1	45.1
ai-16:0	0.6 ± 0.3	1.9 ± 0.9	0	0.9	1.9	0
17:0	1.3 ± 0.3	1.2 ± 1.0	0.4	1.2	1.1	0.7
18:0	4.8 ± 0.9	2.3 ± 0.4	1.9	3.3	2.2	2.0
∑SFA	**36.3 ± 3.9**	**65.2 ± 4.5**	**28.6**	**47.1**	**29.2**	**78.9**
15:1	0.6 ± 0.1	1.1 ± 0.9	0	0	0.2	0
16:1ω7	14.7 ± 3.0	18.7 ± 6.4	2.9	3.7	1.9	4.0
18:1ω9	7.7 ± 1.2	1.6 ± 0.7	1.3	6.1	1.7	4.9
18:1ω7	9.3 ± 0.6	3.4 ± 1.2	0	0	1.1	4.4
20:1ω9	1.5 ± 0.7	0.2 ± 0.2	0	0.3	0	0.1
∑MUFA	**34.1 ± 4.5**	**26.7 ± 3.4**	**4.1**	**11.1**	**4.9**	**13.8**
16:3ω3	1.1 ± 0.8	1.0 ± 1.2	0.6	0	3.5	0.5
16:3ω4	1.6 ± 0.5	0.6 ± 0.9	0	0	0.1	1.0
16:4ω3	0.8 ± 0.4	0.8 ± 1.4	1.1	0	0.5	0
18:2ω6	0.1 ± 0.1	0	13.7	0	18.0	5.1
18:3ω3	1.2 ± 0.2	0.5 ± 0.5	51.5	38.0	43.4	0.1
20:4ω6	3.7 ± 1.3	1.0 ± 0.3	0	0.2	0	0.2
20:5ω3	17.3 ± 1.7	2.4 ± 1.3	0	0.5	0.6	0.1
22:6ω3	1.9 ± 0.4	1.1 ± 0.9	0	0.9	0	0.1
∑PUFA	**29.6 ± 1.2**	**8.1 ± 1.1**	**67.2**	**41.8**	**66.0**	**7.4**
∑BAFA	**4.5 ± 0.6**	**19.3 ± 4.6**	**0.4**	**3.7**	**4.8**	**0.7**
∑HPFA	**1.3 ± 0.2**	**0.5 ± 0.5**	**65.2**	**38.0**	**61.4**	**5.2**
∑EFA	**22.8 ± 1.9**	**4.5 ± 1.4**	**0**	**1.6**	**0.6**	**0.4**

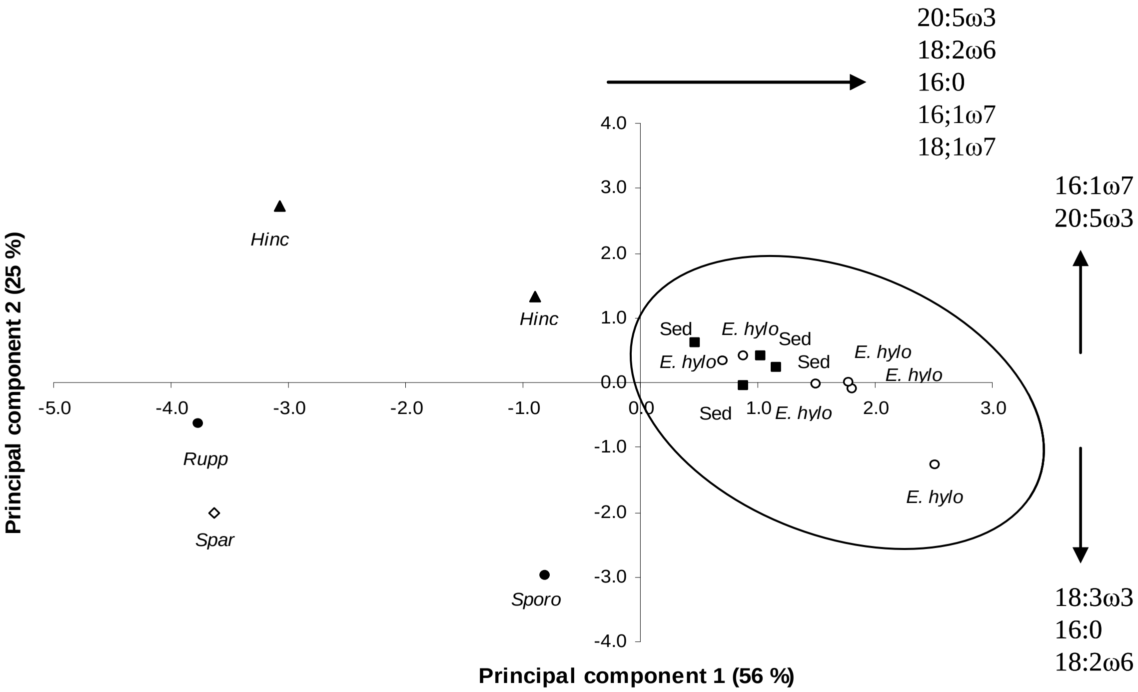

Figure 4.6. PCA ordination of qualitative data (% TFA) on producers (*Rupp* = *Ruppia maritima, Spar* = *Spartina maritima, Sporo* = *Sporobolus virginicus, Hinc* = *Hincksia sordida* and the isopod consumer (*E. hylo* = *Exosphaeroma hylocoetes*) collected in the Kasouga Estuary. Arrows running parallel to each axis indicate the influence of the specified fatty acids having loading values > ± 0.2

4.4 Discussion

Recent studies have demonstrated that maximum abundances of the estuarine isopod, *Exosphaeroma hylocoetes*, within selected southern African temporarily open/closed estuaries is within submerged macrophytes (Chapter 3). This is in agreement with studies conducted on isopods elsewhere in the world. For example, Holomuzki and Short (1988) reported elevated densities of the isopod, *Lirceus fontinalis* in *Cladophora* beds. Similarly, Kanouse *et al.* (2006) demonstrated that nekton (fish and decapod crustaceans) densities were positively correlated to the biomass of submerged aquatic vegetation within brackish marshes of Louisiana, USA. The strong affinity of *E.*

hylocoetes to the beds of artificial submerged macrophytes during the laboratory experiments is thus consistent with the published literature.

The presence of submerged macrophytes resulted in a significant decrease in the predation impact of selected fish species on isopods during this investigation (Fig. 4.3). There was, however, a high variability in isopod mortality dependent on the feeding strategy of the fish. *Clinus cottoides* was the most successful predator of isopods both in the absence and presence of vegetation. The Clinidae are known to be ferocious feeders consuming up to 9 % of their body weight per day, with smaller individuals feeding proportionately more than larger ones (Bennett, 1984). *Clinus cottoides* is typically found within submerged beds of aquatic macrophytes. The limited effectiveness of the submerged macrophytes as a refuge for *E. hylocoetes* against *C. cottoides* predation is therefore, not surprising. The structure of the vegetation, not simply strands as used here, may also play a role in protection from predators (Heck *et al*., 2003). The predator:prey ratio per unit of submerged plant surface area may influence the effectiveness of protection from predation (Mattila *et al*., 2008). There appears to be no significant benefit to individual prey organisms in inhabiting high density submerged aquatic vegetation, unless the predator:prey ratio is lower in the vegetation than elsewhere (Mattila *et al*., 2008). The low predation impact of the goby, *G. callidus*, is likely a result of the small size of individuals employed during the study. On the other hand, the presence of submerged macrophytes did appear to provide the isopod with substantial protection to the remaining fish species. The observed pattern can likely be attributed to the fact that both *R. holubi* and *M. falciformis* are water column or benthic feeders (Whitfield, 1998). The results of predation experiments thus suggest that the submerged beds of macrophytes provide the isopod with an important refuge from predation.

Assuming an enrichment value of 1 ‰ per trophic level (Fry and Sherr, 1984), the results of stable carbon isotope analyses indicated that the salt marsh vegetation (*Sarcocornia perennis*, *Sporobolus virginicus* and *Chenolea diffusa*) as well as the submerged macrophyte, *Ruppia maritima*, could be excluded as important food sources for the isopod (Fig. 4.5). Indeed, the stable isotope analysis failed to identify a single carbon source for the isopod, although the large standard deviation of the isopod $\delta^{13}C$ signature could indicate a diet composed of a combination of sources. The POM and epiphytes on *Ruppia maritima* could be a possible food source, as their $\delta^{13}C$ signatures (-17.02 ± 0.19 ‰) and (-20.40 ± 1.05 ‰) overlap with that of *E. hylocoetes* (-17.41 ± 1.38 ‰). *Ruppia maritima* just as *Zostera marina*, supports a rich epifaunal (Figs 4.4 and 4.6) and infaunal community (including polychaetes, amphipods, gastropods, bivalves and shrimp), which may act as a potentially rich food supply for the isopod (Orth, 1977; Leber, 1985).

Previous research has demonstrated high levels of 18: 2ω6 and 18: 3ω3 in terrestrial plants (Napolitano *et al*., 1997; Budge and Parrish, 1998; Richoux and Froneman, 2008). These fatty acids were present in elevated proportions within all the higher plants in this study (Table 4.1). The low concentration of HPFAs in the isopods indicates that they do not utilize the plants as a significant food source (Table 4.1). Interestingly, the low levels of HPFAs (0.5 %) associated with the sediments indicated that there was very little plant detritus associated with the sediments. The fatty acid profiles of the higher plants were similar to one another, all showing high levels of HPFAs and PUFAs, but low levels of EFAs (Table 4.1). The plant fatty acid profiles are similar to those found in other estuarine studies (Jeffries, 1972; Ames *et al*., 2002, Richoux and Froneman, 2008).

The sediments and the alga, *H. sordida,* contained the greatest proportions of the diatom markers 16:1ω7 and 20:5ω3 (Parrish *et al*., 2000). The presence of these markers in the fatty acid profile of the isopods suggests these two food sources are important in their diet. A second indicator may be employed to identify benthic diatoms viz., ∑c16/∑c18 (Saliot *et al*., 1991; Parrish *et al*., 2000), or the ratio of C16 to C18 fatty acids in the sediments (∑c16/∑c18 = 6.7) and alga (∑c16/∑c18 = 4.1). The isopods' ∑c16/∑c18 ratio was 1.76, further suggesting that diatoms are an important component of their diet. It is worth noting that diatoms of the genus *Nitszchia* were identified in the guts of the isopod during the present study (Fig. 4.4). The PCA analysis results, further, suggests that *E. hylocoetes* feeds mainly on sediments, including the diatoms found within them, as well as epiphytes on the stems of the submerged macrophytes and alga, which all form a group around the isopod. The submerged macrophytes formed a distinct group far removed from the isopod (Fig. 4.6).

The sediments contained higher levels of BAFAs and diatom markers compared to the other potential food sources. The sediments also contained greater proportions of EFAs, reflecting a potential source of EFAs to the isopods. The fatty acid profile of the alga, *H. sordida*, contained high levels of SFAs (≈ 79 %), and low levels of PUFAs, and resembled that of the sediments rather than that of the higher plants. However, *H. sordida* was similar to the higher plants in containing insignificant levels of EFAs. The alga contained elevated levels (31 %) of the fatty acid 14:0, which could be an indication of the decay processes occurring in the alga at that time of year (Chapter 3). The sediments contained relatively high levels (12 %) of this fatty acid and it was found in low levels (3.3 %) in the isopod, indicating its possible incorporation from the alga or sediments. The results suggest that the biota associated with the sediments represent an important food source for the isopod. Alternatively, the isopods may have obtained the EFAs from opportunistically scavenging dead animal material including insects or fish (Richoux and Froneman, 2008). The fatty acid 20:1ω9 has been used as a biomarker to indicate carnivory in marine pelagic food webs and to demonstrate specific predator-prey relationships in calanoid copepods (Saito and Kotani, 2000; Scott *et al.*, 2001). This fatty acid was, however, only present in low quantities in *E. hylocoetes*, indicating that carnivory contributes only a small portion to the total diet of the isopod.

In conclusion, this study has demonstrated that submerged macrophytes provide an important refuge for *E. hylocoetes* against selected fish predators. The effectiveness of the submerged macrophytes as a refugia against fish predators, does however demonstrate a high degree of variability, reflecting the feeding strategies of the different fish species. Results of both stable isotope and fatty acid analyses indicate that submerged macrophytes and salt marsh vegetation can be excluded as important carbon sources for *E. hylocoetes*. Rather, it appears that epiphytic algae growing on the stems of the macrophyte and sediments are the most important food sources for the isopod. The proposed null hypothesis can therefore be accepted as the submerged vegetation plays an important role in being both a refuge from fish predation and an area of increased food availability, despite it not being a direct source of nutrition.

Chapter 5

Growth and Longevity of *Exosphaeroma hylocoetes* under varying Physico-chemical Conditions

5.1 Introduction

Numerous studies have documented the importance of both temperature and salinity in influencing aquatic crustacean metabolic processes, including respiration and growth. For example, increased water temperatures have been shown to increase respiration rates in various species of shrimp (Chen and Nan, 1993; Spanonopoulos-Hernández *et al*., 2005; Allan *et al.,* 2006), and copepods (Isla and Perissinotto, 2004). The response of invertebrates to changes in salinity is, however, more complex largely reflecting their evolutionary origins (Kinne, 1966). For example, Allan *et al.* (2006) demonstrated that the shrimp *Palaemon peringueyi*, which is abundant in South African estuaries (Bernard and Froneman, 2005) did not change its standard metabolic rate significantly between the salinities of 15 and 35 ‰. Juvenile blue swimmer crabs, *Portunus pelagicus,* on the other hand, displayed significantly faster growth and higher survival in response to increasing salinity (Romano and Zeng, 2006). Additional factors that may influence the growth rates of crustaceans include photoperiod (Gambardella *et al*., 1997), food availability (Shuster and Guthrie, 1999) and sex (Newman *et al*., 2007).

Most isopod growth studies have focused on terrestrial isopods using field-determined size frequency histograms, based on cephalothorax width or changes in mass, to establish a growth rate using the von Bertalanffy model (von Bertalanffy, 1938; McQueen and Carnio, 1974; Strong and Daborn, 1979; Caseiro *et al.,* 2000; Li, 2002; Araujo and Bond-Buckup, 2004; Meinhardt et *al.,* 2007). The growth rates of individual isopods (especially marine species) have, however, rarely been studied in the laboratory.

Longevity has been examined mainly in terrestrial isopods, which appear to live longer than marine isopods, varying from 11 to 108 months (Achouri *et al.*, 2003; Warburg, 1993). In terrestrial isopods, females generally achieve a larger overall size, but grow at a slower rate and survive for longer than males (Araujo and Bond-Buckup, 2004; Meinhardt *et al.*, 2007). Females were thus able to produce more offspring, as they had a larger body size permitting greater egg-bearing ability. The only South African isopod growth study, to date, focused on the relationship between body length and the dry mass of fed and unfed *Cirolana fluviatilis* (Newman *et al.*, 2007). The conclusion of that study was that there was no significant difference in the growth rate of males and females.

The estuarine isopod, *Exosphaeroma hylocoetes*, is widely distributed along the south-eastern coastline of southern Africa (Kensley, 1978). These isopods are typically associated with submerged macrophytes in the littoral zones of the middle and upper reaches of estuaries, particularly temporarily open/closed estuaries (Chapter 3). The isopods are primarily detritivores feeding on particulate organic matter (POM) derived from a variety of sources including vegetation, zooplankton, nekton and microalgae attached to the submerged macrophytes (Chapter 4). *Exosphaeroma hylocoetes* demonstrates sexual dimorphism with males being significantly larger than females (Chapter 3). Males attain a maximum length of ≈ 12 mm, while females grow to ≈ 7.5 mm (Barnard, 1940; Chapter 3). In the absence of experimental data, it is not known if the larger size attained by males is the result of elevated growth rates or extended longevity, or a combination of the two. The null hypothesis is that males attain a significantly larger size than females due to a higher growth rate.

The aim of this study, therefore was to determine the growth rates of individual male and female *E. hylocoetes* under a combination of temperatures and salinities which are typically recorded in Eastern Cape temporarily open/closed estuaries (Chapter 3).

5.2. Materials and methods

5.2.1 Collection and laboratory procedures

Exosphaeroma hylocoetes (Barnard, 1940) were collected from the temporarily open/ closed Kasouga Estuary located on the Eastern Cape coastline of southern Africa (see Chapters 2 and 3 for details of site) using a hand-held sweep net. After collection, the isopods were transported back to the laboratory before being maintained in controlled environment (CE) rooms at the following salinity/temperature combinations: 15 /15 °C; 15 /25 °C; 35 /15 °C and 35 /25 °C. All isopods were subjected to a light:dark phase of 12:12 hours. These conditions replicated the natural annual conditions experienced within these TOCEs (Chapter 3). The isopods, in aerated round polyethylene containers (diameter of ≈ 50 cm and a height of ≈ 12 cm), formed the brood stocks. Plastic hair curlers, (in place of vegetation), were placed in the water to act as refugia for the brooding females. The brood stocks were maintained for 18 months to ensure sufficient male and female isopod numbers. Individual juvenile *E. hylocoetes* (n = 30 to 50) within the size range 1.5 to 2.0 mm, from the brood stock, were placed in 250 ml plastic containers of mesohaline (salinity 15) or 100 % seawater (35) and placed in C.E. rooms, (set at either 15 °C or 25 °C). The containers were not aerated as it was considered that the small animals were not under O_2 stress in 250 ml water. Water was changed every seven days and the isopods fed *ad libitum* on commercially available dry fish food. The fish food had the following composition: min 40 % protein; 5 % fat; 5 % fibre and 12 % moisture.

Isopods were measured weekly, until their death, with the aid of a Wild M5A stereomicroscope (Mag. X120) fitted with an ocular micrometer. This entailed measurement, to the nearest 0.01 mm, along the mid-dorsal length from the anterior tip of the cephalon, (in the case of *E. hylocoetes* this was the point of connection between the cephalon and the flagellae, or antenna one), to the posterior tip of the pleotelson (Barnard, 1940). Growth rates were determined from at least 15 males and 15 females in each temperature/salinity combination. These results were used to determine the weekly growth rates, maximum length and longevity of the isopod.

Exosphaeroma hylocoetes can be sexed when ≥ 5 mm, as male *E. hylocoetes* develop two pene situated anteriorly of the pleopods (Barnard, 1940). Males also have a more pronounced triangular pleotelson with a definite inner ridge when view ventrally. This ridge is absent in females and they have a more rounded pleotelson (Barnard, 1940).

5.2.2 Statistical analyses

Initial investigation of the plots of $\frac{dL}{dt}$ against $\bar{L}$ illustrated a quadratic relationship suggesting that growth in *E. hylocoetes* is logistic (Figs 5.1, 5.5, 5.6, 5.7). A quadratic model of the form $\frac{dL}{dt} = k\bar{L}\left(1 - \frac{\bar{L}}{L_\infty}\right) = k\bar{L} - \frac{a\bar{L}^2}{L_\infty}$, where a is the instantaneous growth rate and L_∞ the asymptotic length, was subsequently fitted to each individual isopods' growth data using non-linear least squares regression.

The resultant 133 individual parameter estimates of k and L_∞ were used as input in ANOVAs to determine if salinity, temperature and/or sex had an influence on either growth or asymptotic length rates of male and female *E. hylocoetes*. The data were log (x +1) transformed to ensure that the residuals were normally distributed. The analyses were carried out using S*tatistica 7* (StatSoft).

Growth was further modelled using the logistic growth model of the form

$$\hat{L}_{it} = \frac{L_\infty}{1+e^{-k(t_i - t_0)}}$$

where $\hat{L}_{it}$ is the model predicted length of isopod i at time t_i and t_0 the estimated age at zero length. It was assumed that there was functional relation between each of the parameters and sex, temperature and salinity. Therefore, each of the growth parameters was modelled to include this functional relationship, such that:

$$L_\infty = \beta_0 + \beta_1 \times Sex + \beta_2 \times Temperature + \beta_3 \times Salinity$$

$$k = \beta_4 + \beta_5 \times Sex + \beta_6 \times Temperature + \beta_7 \times Salinity$$

$$t_0 = \beta_8 + \beta_9 \times Sex + \beta_{10} \times Temperature + \beta_{11} \times Salinity\ .$$

Sex was included in the model as a dummy variable with females being assigned a 0 and males a 1.Two other parameters were included in the minimization routine: $\hat{\rho}$, the estimated autocorrelation between successive measurements, and $\hat{\sigma}$, the estimated model standard deviation. The autocorrelation coefficient was required as the data are repeated measures data.

The parameter vector expressed as: $\boldsymbol{\theta} = \{\beta_0, \beta_1, ..., \beta_{11}; \sigma; \rho\}$ contained at most 14 parameters and was estimated by minimizing a negated normal log-likelihood of the form :

$$\ln L = \sum_i \left[\ln \sigma + \frac{1}{2\sigma^2} \sum_j \left(\frac{\varepsilon_{ij} - \rho \varepsilon_{ij-1}}{\sqrt{1-\rho^2}} \right)^2 \right]$$

where $\varepsilon_{ij} = L_{ij} - \hat{L}_{ij}$ is the j th residual for the i th isopod calculated, $\varepsilon_{i0} = 0$.

Likelihood ratio tests were conducted to assess whether individual parameters were statistically significant different. The most parsimonious model was assessed using the AIC (Akaike's Information Criterion) statistic (Akaike, 1974; Hurvich and Tsai, 1989). Parameter variability was calculated using parametric bootstrapping with 250 bootstrap iterations.

A t-test was employed to detect any significant differences in isopod sex longevity at combinations of temperature and/or salinity. The analyses were carried out using S*tatistica 7* (StatSoft) computer package.

5.3 Results

5.3.1 Isopod size and growth rates

Males grew larger (mean body length, 8.08 ± 0.25 mm) in size (body length) than females (6.59 ± 0.25 mm) at all temperature and salinity combinations (Figs 5.2 and 5.3).

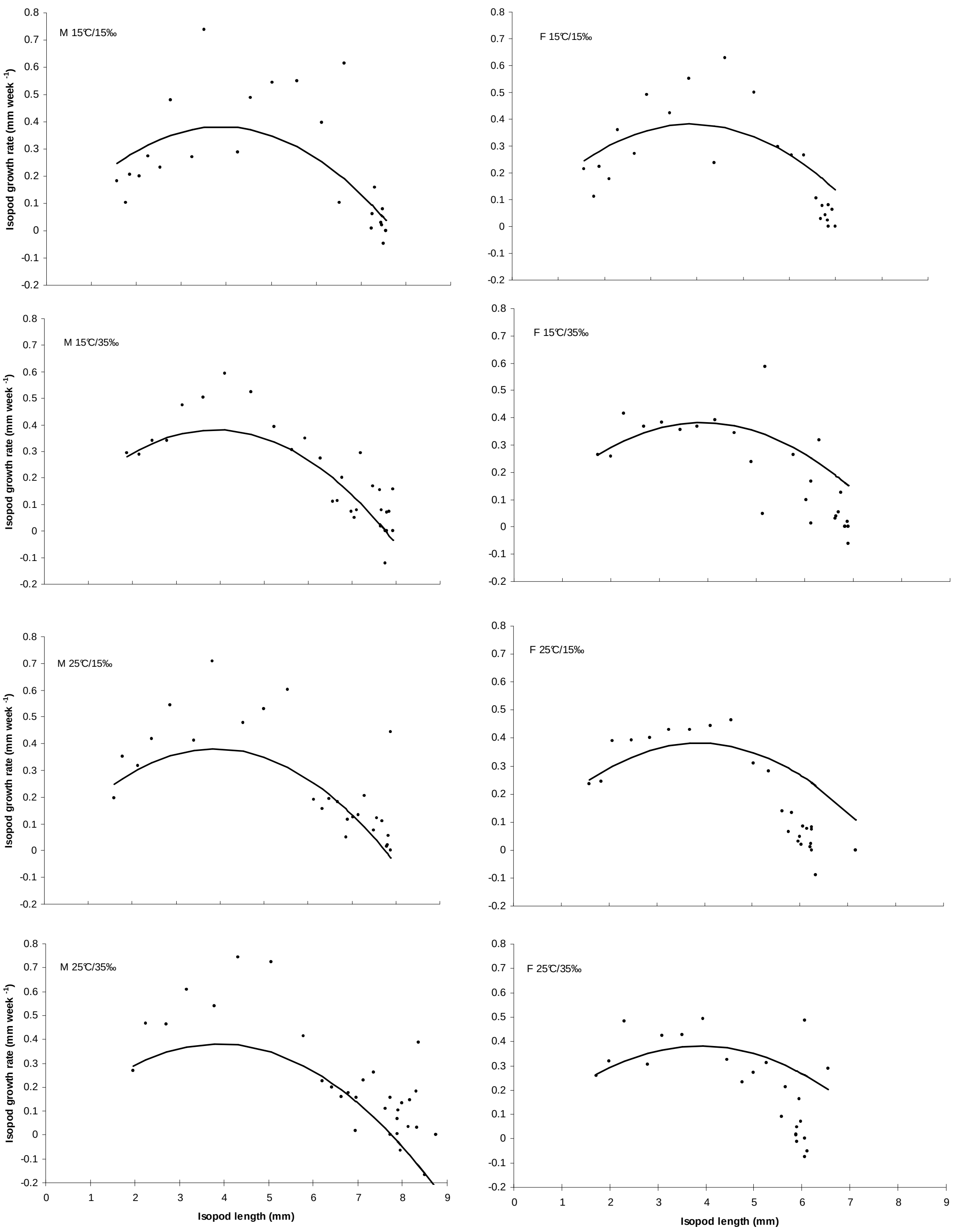

Figure 5.1. Observed mean growth rates (dots) versus predicted growth rates (solid line) of *Exosphaeroma hylocoetes*, at combinations of temperatures (15 and 25 °C) and salinities (15 and 35) based on plots of $\frac{dL}{dt}$ against $\overline{L}$. M = males; F = females. Dots represent observed values; curves represent predicted values

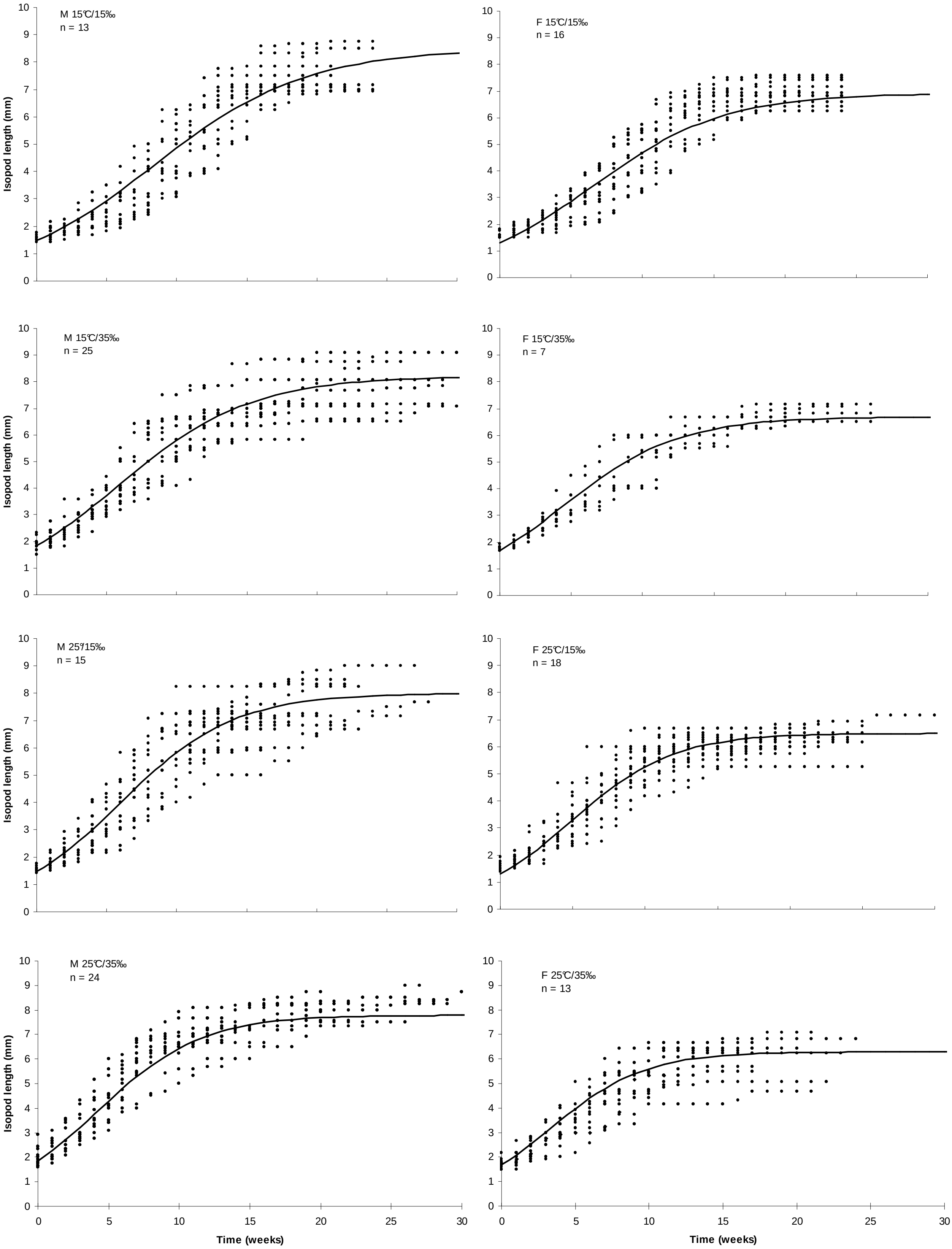

Figure 5.2. Observed male (left) and female (right) growth of *Exosphaeroma hylocoetes* at combinations of two temperatures (15 and 25 °C) and two salinities (15 and 35). The line indicates the predicted growth curve of isopods based on the predicted logistic growth model. M = males; F = females.

Male isopods consistently attained lengths greater than those predicted by the Schnute growth model, while females, with the exception of those incubated at 15 °C and 15, did not attain the predicted lengths (Figs 5.2 and 5.3).

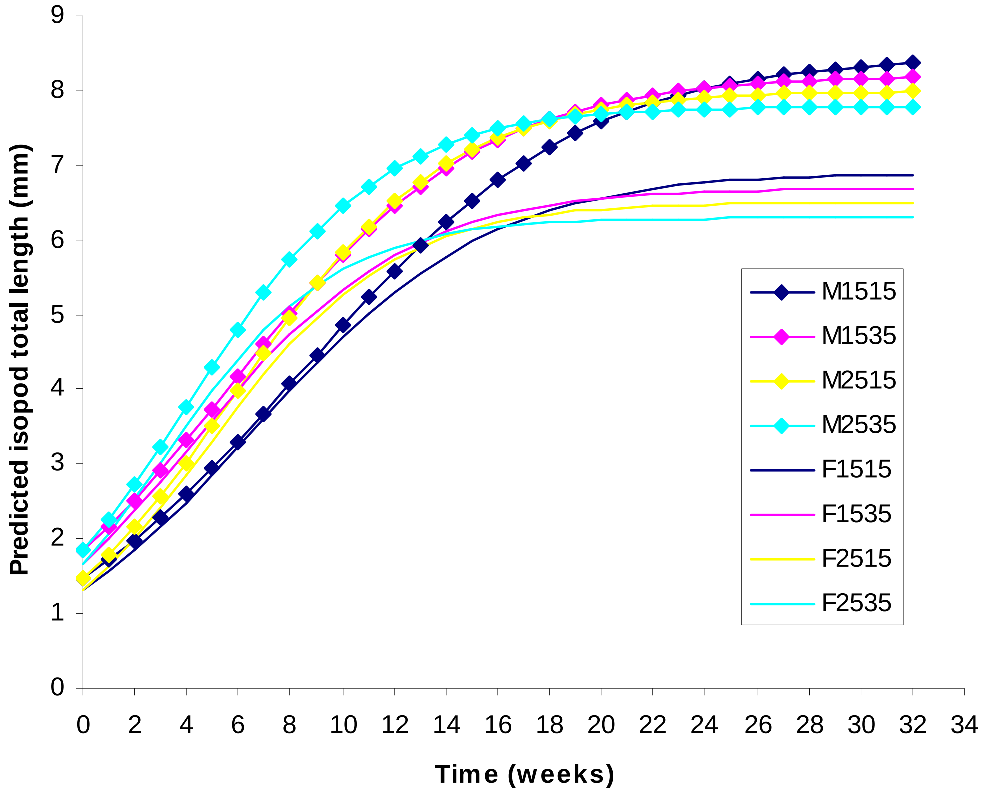

Figure 5.3. Predicted male (M, lines with symbols) and female (F, lines without symbols) isopod lengths in combinations of two temperatures (15 and 25 °C) and two salinities (15 and 35). Lines are the predicted logistic growth models. Output parameters of the model are shown in Table 5.1.

Initial growth occurred at a faster rate at the higher temperature (25 °C) and salinity (35) combination in both males and females, but in both instances these individuals attained the lowest overall length (7.67 mm for males and 6.32 mm for females). They also reached their inflection points, on the curves, at five weeks. After an initial slower growth rate, reaching their inflection points at nine weeks (males) and six weeks (females), the isopods at the lower temperature/salinity combination (15 °C/ 15) attained the longest overall length, 8.03 mm (males) and 6.68 mm (females). Those exposed to the intermediate temperature/salinity combinations demonstrated intermediate growth rates, achieving total lengths midway between the two extreme conditions.

Growth of male and female *E. hylocoetes* was not significantly affected by salinity, based on the LRT and ANOVA analyses ($p > 0.05$), and was therefore removed from the overall model (Fig. 5.3; Table 5.1).

Table 5.1. Logistic growth model maximum likelihood estimates (MLE), their coefficients of variation (CV) and 95% confidence intervals (CI). The model fit is summarised with the negated log-likehood value ($-\ln L$), number of estimated parameters (p) and Akaike Information Criterion (AIC) statistic. The model fitted was $\hat{L}_{it} = \frac{L_\infty}{1+e^{-k(t_i-t_0)}}$ where $L_\infty = \beta_0 + \beta_1 \times Sex + \beta_2 \times Temperature$, $k = \beta_3 + \beta_4 \times Sex + \beta_5 \times Temperature$ and $t_0 = \beta_6 + \beta_7 \times Sex + \beta_8 \times Temperature + \beta_9 \times Salinity$.

	MLE	CV	CI
β_0	7.41	1.76%	(7.17 ; 7.67)
β_1	1.32	3.83%	(1.21 ; 1.41)
β_2	-0.04	15.10%	(-0.05 ; -0.03)
β_3	0.15	9.21%	(0.13 ; 0.18)
β_4	-0.01	44.07%	(-0.03 ; 0.00)
β_5	0.00	14.14%	(0.00 ; 0.01)
β_6	10.64	2.69%	(10.14 ; 11.22)
β_7	0.90	11.79%	(0.70 ; 1.12)
β_8	-0.18	5.98%	(-0.20 ; -0.16)
β_9	-0.08	4.60%	(-0.09 ; -0.08)
ρ	0.60	11.73%	(0.52 ; 0.56)
σ	0.57	6.50%	(-0.46 ; -0.41)
$-\ln L$	-48.41		
p	12		
AIC	-72.81		

Increased temperature, despite depressing the maximum attainable length, allowed the isopods to reach reproductive size sooner, two weeks for females and 6.5 weeks for males (at 25 °C), than females (4 weeks) and males (11 weeks) at 15 °C (Figs 5.3; 5.4; 5.5; 5.6 and 5.7).

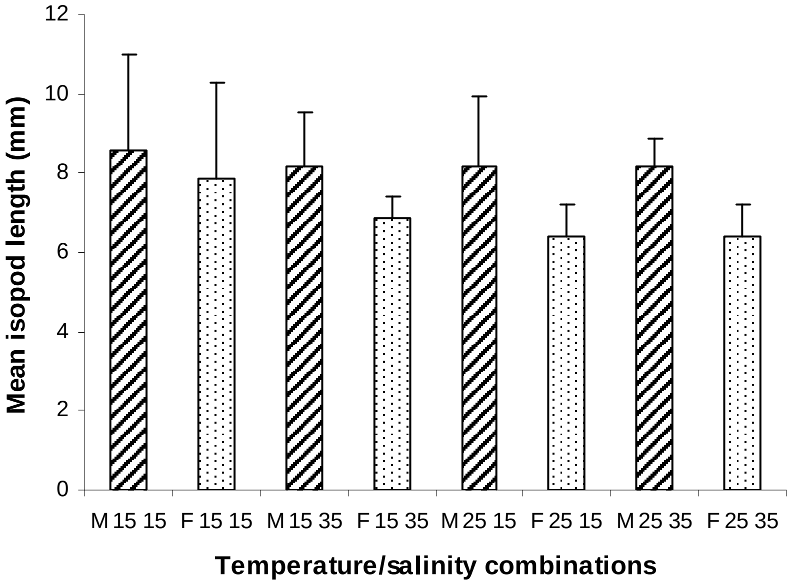

Figure 5.4. Mean lengths (+ S.D.) of male (M) and female (F) *Exosphaeroma hylocoetes* at temperatures of 15 °C and 25 °C and at a salinities of 15 and 35.

There were no significant differences (ANOVA, p = 0.432 to 0.535) in growth rates between males and females under the various treatments (Fig. 5.8).

Overall, ANOVA analyses revealed only significant temperature effects on the growth rates of *E. hylocoetes* ($p < 0.001$; $F_{1,128} = 18.419$; Table 5.2). Salinity was shown to have no significant influence on the sex of the individuals' growth rates ($p = 0.415$; $F_{1,128} = 0.670$; Table 5.2).

Table 5.2Results of an ANOVA (*Statistica*, version 7) for log (x+1) transformed data on factors (salinity, temperature and sex & a combination of salinity and temperature) affecting the growth rates of male and female *Exosphaeroma hylocoetes*.

Variation	**Sum of Squares**	**d.f.**	**Mean squares**	**F-ratio**	**Sig. Level**
Salinity	0.00227	1	0.00227	0.676	0.415
Temperature	0.06247	1	0.06247	18.419	0.000035
Sex	0.00292	1	0.00292	0.862	0.355
Sal:Temp	0.00018	1	0.00018	0.0288	0.865
Residuals	0.4341	128	0.00339		

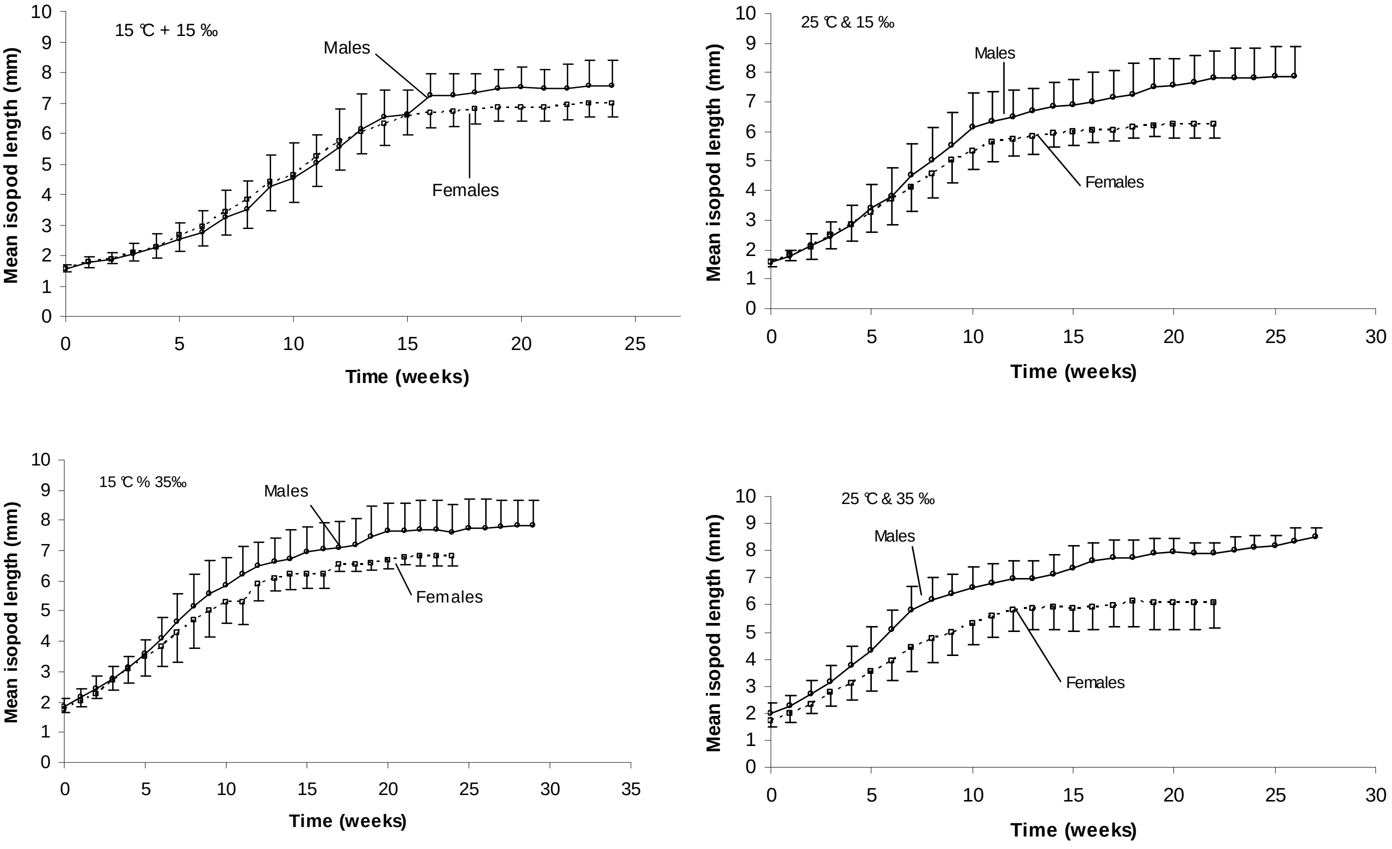

Figure 5.5. Mean male and female isopod length (+ S.D.) achieved over 25 to 30 weeks in individual containers at four combinations of temperature and salinity.

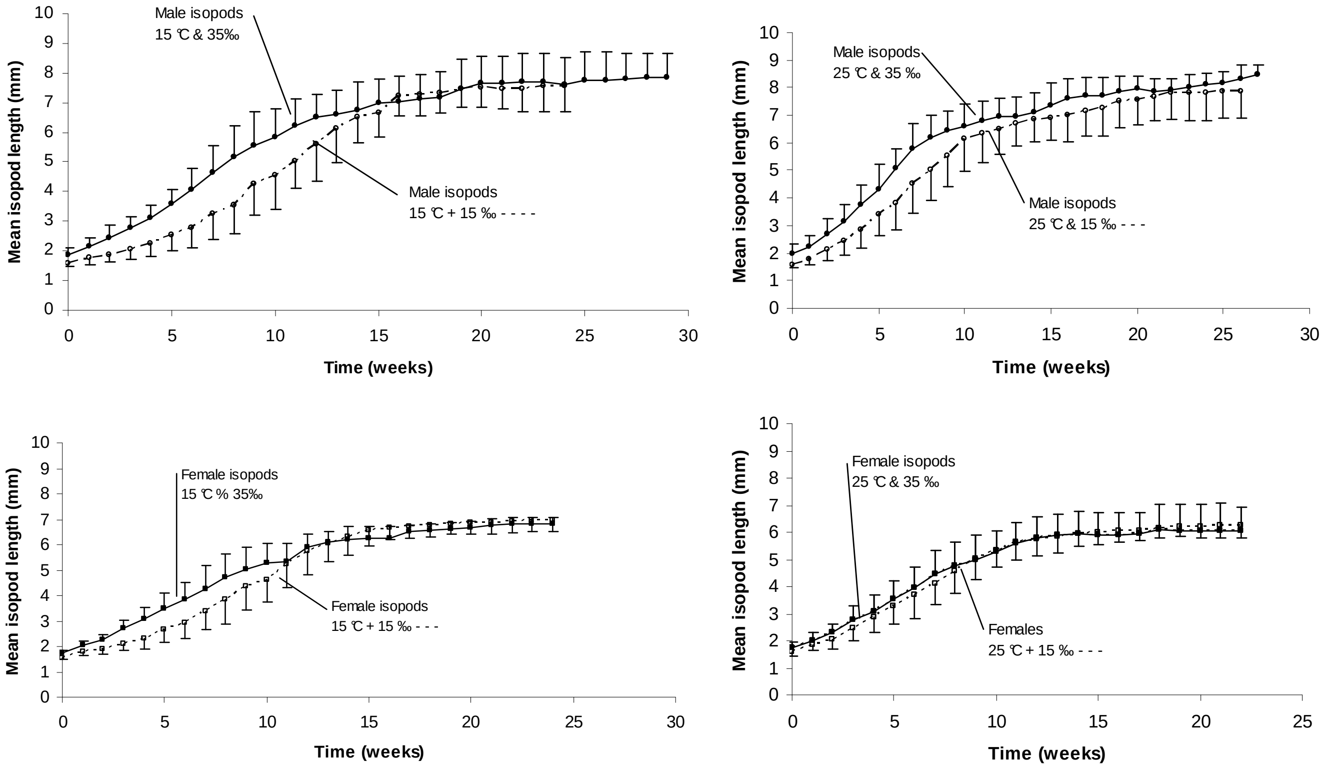

Figure 5.6. Comparisons of male and female *E. hylocoetes* mean length (+ S.D.) achieved at four combinations of temperature and salinity.

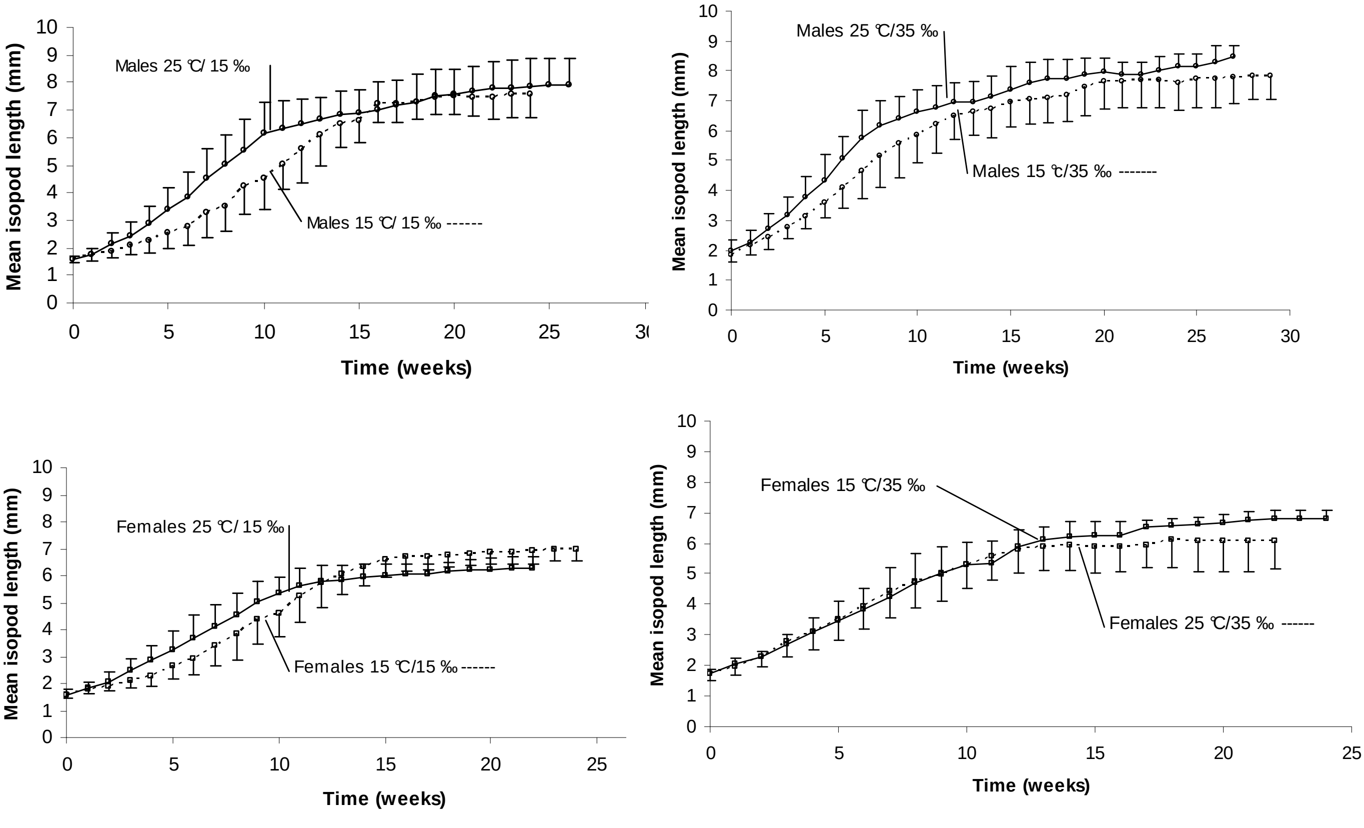

Figure 5.7. Influence of temperature on the growth and mean length (+ S.D.) of male and female *E. hylocoetes* at four combinations of temperature and salinity.

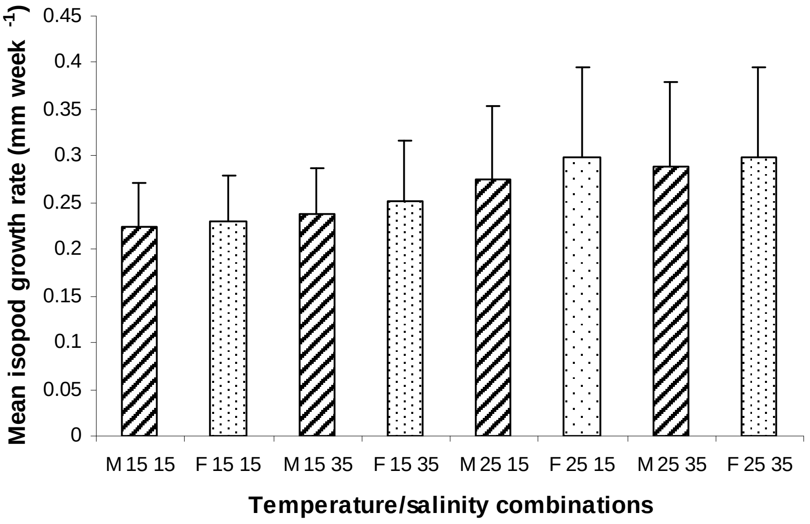

Figure 5.8. Growth rates (+ S.D.) of male (M) and female (F) *Exosphaeroma hylocoetes* at temperatures of 15 °C and 25 °C and at salinities of 15 and 35. Vertical bars indicate 95 % confidence limits.

5.3.2 Survival rates and longevity

Initial trials conducted at 25 ° C and a salinity of 15 indicated that males lived significantly longer than females ($p < 0.001$), with males averaging 25.77 ± 3.40 weeks and females, 21.52 ± 3.00 weeks. Two males lived for 35 weeks, while one female achieved a maximum lifespan of 30 weeks. During this study males reached a maximum length of 11.17 mm, (mean length = 8.00 ± 0.86 mm) and females 7.92 mm (mean length = 6.32 ± 0.59 mm). Longevity contributed to males achieving a significantly larger size than females during the experiments.

At a salinity of 15 and a temperature of 15 °C, male (mean 22.82 ± 1.47 weeks) and female (mean 23.27 ± 1.28 weeks) *E. hylocoetes* showed no significant difference in their life expectancy ($p = 0.415$; $t = 0.829$; $df = 24$), whereas at 25 °C, males (mean 27.73 ± 3.07 weeks) lived significantly longer ($p < 0.001$; $t = 5.615$; $df = 21$) than the females (mean 20.58 ± 3.03 weeks; Fig. 5.3). When comparing males incubated at 15 °C with those incubated at 25 °C, at the same salinity, the males at 25 °C lived significantly longer ($p < 0.001$; $t = 4.784$; $df = 20$) than the males at the lower temperature (Fig. 5.6). Females incubated at 15 °C, however, lived significantly longer than those females at 25 °C at the same salinity ($p = 0.005$; $t = 3.113$; $df = 25$).

At a salinity of 35, males lived significantly longer than females at both 15 °C and 25 °C, ($p <0.001$; $t = 4.738$; $df = 18$ and $p <0.001$; $t = 3.987$; $df = 15$; Fig. 5.3). However, when comparing males at 15 °C (mean 26.42 ± 2.91 weeks) with those at 25 °C (mean 26.67 ± 3.93 weeks) there was no significant difference in life expectancy ($p = 0.880$; $t = 0.153$; $df = 16$). Similarly, females at 15 °C (mean 21.00 ± 1.69 weeks) and 25 °C (19.00 ± 3.72 weeks) did not show any significant difference in their longevity ($p = 0.176$; $t = 1.412$; $df = 17$; Fig. 5.9).

At the temperature of 15 °C, males at a salinity of 35 (mean 26.42 ± 2.91 weeks) did not live significantly longer ($p = 0.880$; $t = 0.153$; $df = 16$) than those males exposed to a salinity of 15 (22.82 ± 1.47 weeks). Similarly, at temperature of 25 °C, males exposed to a salinity of 15 (mean = 27.73 ± 3.10 weeks) did not live significantly longer ($p = 0.546$; $t = 0.618$; $df = 15$) than males exposed to a salinity of 35 (mean 26.67 ± 3.93 weeks; Fig. 5.9).

At a temperature of 15 °C female *E. hylocoetes* at a salinity of 15 (mean 23.26 ± 1.28 weeks) did live significantly longer ($p = 0.002$; $t = 3.621$; $df = 21$) than females exposed to a salinity of 35 (mean 21.00 ± 1.69 weeks). On the other hand, at 25 °C females exposed to a salinity of 15 (mean 20.58 ± 3.03 weeks) did not live significantly longer (p

= 0.273; t = 1.125; df = 21) than females incubated at a salinity of 35 (mean 19.00 ± 3.72 weeks; Fig 5.9).

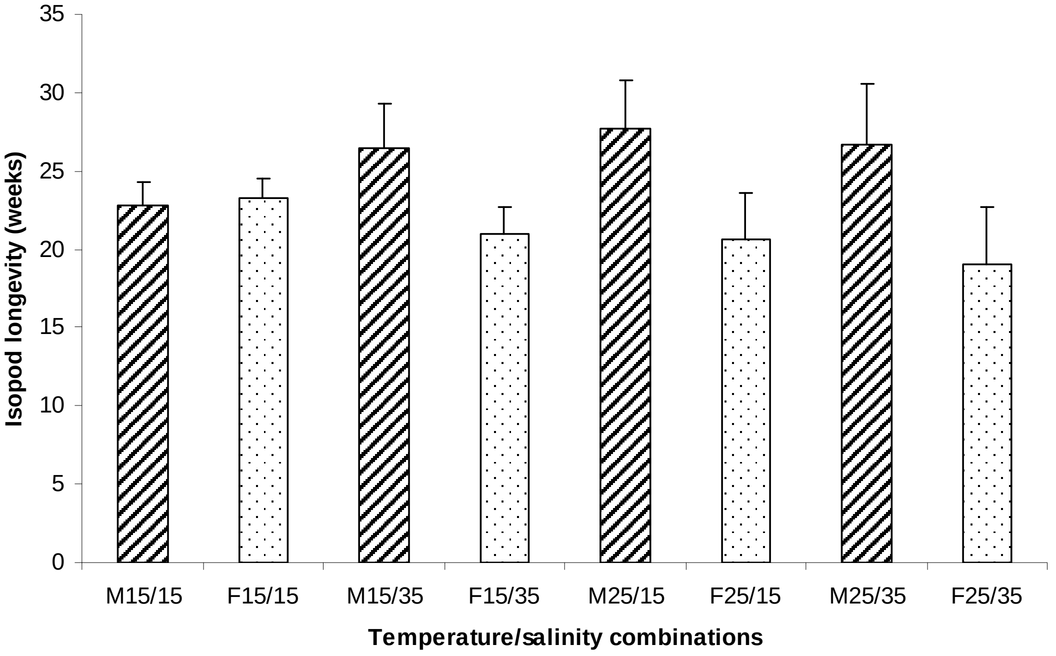

Figure 5.9. Male (M) and female (F) *Exosphaeroma hylocoetes* longevity (mean weeks survived ± S.D.) at temperatures of 15 °C and 25 °C and at salinities of 15 and 35.

5.4 Discussion

Results of the study indicated that male *Exosphaeroma hylocoetes* attained a significantly larger size than the females under all the salinity and temperature combinations (Figs 5.3 and 5.4). The observed pattern is consistent with field studies which have demonstrated that *E. hylocoetes* demonstrates strong sexual dimorphism (Chapter 3). However, the individuals at the lower temperature/salinity combination attained an overall longer size, than those individuals incubated at the higher temperature/salinity. This may support the reproductive drain hypothesis (Koch and

Weiser, 1983; Pauly, 1984; Thorpe, 1987), indicating faster growth at the higher temperature/salinity allowing the isopods to put more effort into reproduction while penalising longer overall growth.

In agreement with several other studies on marine crustaceans (McKenny, 1994; McKenny and Celestial, 1995), both male and female *E. hylocoetes* grew significantly faster at combination of higher temperature and salinity, 25 °C/35, than those individuals incubated at the low temperature/salinity combination (15 °C/ 15). The influence of temperature and salinity on the growth rates of crustaceans is highly variable and appears to be species specific reflecting amongst others ontogeny and specific habitat requirements (Zein-Eldin and Aldrich, 1965; Staples and Heales, 1991; Steele and Steele, 1991; Kondzela and Shirley, 1993; McKenny and Celestial, 1995). For example, the growth of the juvenile amphipod, *Gammarus lawrencianus*, was greatest at intermediate salinities, reflecting the normal conditions in the estuaries where the animals are commonly found (Steele and Steele, 1991). Similarly, the mysid, *Mysidopsis bahia*, demonstrated age-dependent interactions with temperature and salinity. Mysid growth rates, were retarded by both a low temperature-low salinity interaction, and a high temperature-low salinity interaction. Maximum sizes were achieved at high temperature-high salinity combinations (McKenny and Celestial, 1995). Kinne (1964) postulated that changes in osmotic concentration could influence not only metabolic rates, but also metabolic efficiency. Temperatures and salinities beyond those to which the animal is normally acclimatized could induce stress on those animals and alter their respiration and growth rates.

Results of the growth studies indicated that there were no significant differences in the growth rates of male and female *E. hylocoetes* under the range of temperature and salinity combinations ($p > 0.05$; Fig. 5.5). The observed pattern is in contrast to a number of other studies which have demonstrated that growth rates of female Crustacea generally exceed those of the males. For example, females of the kelp forest mysid, *Holmesimysis costata*, grew at a mean rate of 41 µm day^{-1} compared to the 35 µm day^{-1}, for males (Turpen *et al*., 1994). The elevated growth rates observed for the females were related to the increased brood carrying ability of larger sized females. Similar patterns have also been reported for the prawn, *Palaemon xiphias*, and the freshwater shrimp, *Exopalaemon modestus* (Guerao *et al.*, 1994; Oh *et al*., 2002). Berglund (1981) theorizes that a reduced male energy investment in growth could lead to a reduced risk of predation.

Longevity in crustaceans, including shrimps and prawns, have been shown to be strongly influenced by temperature (Guerao *et al.*, 1994; Oh *et al*., 2002). Isopods having a wide latitudinal range show variations in longevity with an increase in longevity generally associated with colder water temperatures (Table 5.2). The results of the longevity experiments obtained here are in the lower range reported for other estuarine and marine isopods of similar size (Table 5.3). For example, *Excirolana braziliensis*, lives for 19 months at a latitude of 20° south, stretching to 31.5 months at 40° south (Cardoso and Defeo, 2004). Similarly, the sponge-dwelling gnathiid isopod, *Elaphognathia cornigera*, lives for more than 36 months (Tanaka, 2003). The reduced life expectancy of *E. hylocoetes* compared to other studies can likely be related to the fact that it occurs in warm temperate waters. Interestingly, terrestrial isopods appear to live longer than their marine counterparts (Table 5.3).

Results of growth trials indicated that longevity of male *E. hylocoetes* was significantly longer than those of the females at the different combinations of temperature and salinity. The extended life expectancy of the males allowed them to attain a significantly larger size than the females ($p < 0.05$). Larger male size in *E. hylocoetes* may be related to mate guarding, as is also found in many other crustaceans, including isopods (Jormalainen, 1998) and crabs (Kobayashi, 2002). Polymorphism, with regard to male body size, has been described in some isopods, in which three distinct male morph types coexist (Shuster, 1987; Shuster and Wade, 1991), and in freshwater prawns (Ra'anan and Sagi, 1985). Larger males, as in *E. hylocoetes*, are thought to have an advantage in male-male conflicts, whereas smaller males could attain maturity faster or engage in alternative mating behaviours, e.g. female mimicry, searching for females located further away from large males, or by sneak mating (Kobayashi, 2002). Smaller female *E. hylocoetes* size may be a result of not feeding during the brooding period when the size of the gut is reduced, as well as greater energy being placed in brooding the offspring (Shuster, 1995).

Table 5.3. Longevity of selected terrestrial and marine isopod species.

Species	**life span (months)**	**Source**
Terrestrial		
Porcellionides pruinosus	11 to 14	Achouri *et al*., 2003
Hemilepistus reaumuri	12	Warburg, 1993
Atlantoscia floridana	♂ = 18; ♀ = 28	Araujo and Bond-Buckup, 2004
Balloniscus glaber	♂ = 28; ♀ = 36	Meinhardt *et al*., 2007
Trichoniscus pygmaeus	24	Sutton *et al*., 1984
Philoscia muscorum	27	Sutton *et al*., 1984
Porcellio scaber	36	Sutton *et al*., 1984
Armadillidium vulgare	42	Sutton *et al*., 1984
Armadillo officinalis	108	Warburg, 1993
Marine		
Dynoides daguilarensis	7 to 12	Li, 2002
Excirolana braziliensis	19 to 31.5	Cardoso and Defeo, 2004
Eurydice pulchra	24	Fish, 1970
Cirolana harfordi	24	Johnson, 1976a
Elaphognathia cornigera	36	Tanaka, 2003
Estuarine		
E. hylocoetes	♂ = 6.5 to 8.5; ♀ = 5.5 to 7.5	Present study

In conclusion, this is the first study to present the findings of a laboratory-based study which examined the growth of individual estuarine isopods (*E. hylocoetes*) under a suite of environmental conditions.

There were a number of important results emanating from the work:

1. There were no significant differences between the growth rates of males and females at all combinations of temperature and salinity. The proposed null hypothesis must therefore be rejected as male and female *E. hylocoetes* grew at the same rate.
2. Males achieved an overall larger size, when compared to females, because they lived longer which allowed them to attain a significantly larger size.
3. Growth in *E. hylocoetes* occurred at a faster rate at the higher temperature (25 °C) than at 15 °C (at a salinity of 15), coinciding with summer conditions commonly found in Eastern Cape estuaries.
4. Female *E. hylocoetes* appeared to attain sexual maturity at a faster rate (smaller overall length) than males.

The above results indicate a true estuarine origin for *E. hylocoetes*. The isopods are well adapted to cope with a wide range of salinities, (here tested from 15 to 35) and temperatures (15 to 25 °C), which enables them to survive in systems with a constantly changing physico-chemical nature such as those occurring in TOCEs of the Eastern Cape.

Chapter 6

General Discussion

Temporarily open/closed estuaries (TOCEs) account for approximately 73 % of all estuaries along the South African coastline (Whitfield, 2000). These estuaries can remain closed for long periods, due to relatively low freshwater inflow, a result of low rainfall in their catchments, high evaporation rates, impoundments and water abstraction (DWAF, 2004). During mouth breaching and overtopping events temporary access to and from the sea is re-established. The increasing need of water for both human and agricultural practices has led to increased levels of freshwater abstraction, and this will probably reduce the incidence of mouth breaching in the future (DWAF, 2004).

Despite infrequent tidal exchanges, TOCEs are characterised by highly variable conditions, i.e. they demonstrate strong temporal variability in physico-chemical parameters. Salinities can vary greatly, rising to 50 during droughts (Whitfield and Bruton, 1989). However, mesohaline conditions, 5 to18, generally dominate when the mouth is closed (Whitfield and Bruton, 1989; Froneman, 2002b). Overtopping of marine water into the system during storms or spring high tides can increase the salinity during the closed phase. These varying physico-chemical conditions affect the fauna living within the estuaries. They need to be tolerant of the changing salinities, often occurring over a short time period, when there is increased freshwater inflow from heavy rainfall after long periods of mouth closure. The overtopping events can have a coinciding secondary biological effect, the recruitment of marine vertebrates and invertebrates into the estuaries (Froneman, 2002c, 2004 b, c; Kemp and Froneman, 2004; Perissinotto *et al.,* 2004). The null hypothesis tested in Chapter 3 concerning mouth opening and the affect on *E. hylocoetes* has not been accepted as these events had a profound effect on their numbers present. These events led to a dramatic loss of the isopod population.

It is now a well documented that permanently open estuaries have a greater species richness than TOCEs (Brown, 1953; Day, 1974; Hodgson, 1987; De Villiers *et al*., 1999). Thus permanently open estuaries support richer macrobenthic communities (often > 60 species), whereas those estuaries experiencing lengthy mouth closures are species poor, often < 35 species (De Villiers *et al*., 1999). When comparing only the warm temperate South African estuaries, an average of 95.1 species can be found in permanently open estuaries compared to only 18.6 in the TOCEs and closed estuaries (De Villiers *et al*., 1999). In the Eastern Cape, the permanently open Kariega River Estuary was found to support 107 species (Hodgson, 1987), whereas the nearby TOCE, the West Kleinemonde Estuary, contained only 34 species (Brown, 1953). Closed Western Australian estuaries also support low species diversities (Platell and Potter, 1996). The greater species richness of permanently open estuaries may be due to greater recruitment from the marine environment, or a more stable marine-influenced environment, and increased habitat availability (Day, 1964; Platell and Potter, 1996).

Many macrobenthic and pelagic species in estuaries have an obligate marine larval phase (Emmerson, 1983; Wooldridge, 1991). Lengthy mouth closure would preclude such a marine larval phase, resulting in a lack of larval recruitment from the marine environment into TOCEs. For example, the sandprawn *Callianassa kraussi* is a brooder with an abbreviated larval period completed within the estuary (Forbes, 1973). It is for this reason that, *C. krausii*, can inhabit TOCEs, whereas the mudprawn, *Upogebia africana* which has an obligate marine larval phase, cannot (Wooldridge, 1999). Endemic macrobenthic estuarine species have been shown to have low species richness, high resilience to environmental changes, and to be structured mainly by sediment characteristics (Teske and Wooldridge, 2001, 2003, 2004). The differences in species richness between open estuaries and TOCEs also applies to the isopods. Day (1974) recorded 19 isopod species within the permanently open tropical Morrumbene Estuary, Mozambique. The local permanently open Kariega Estuary supported eight isopod species (Hodgson, 1987), whereas only four isopod species, *Exosphaeroma hylocoetes, Exosphaeroma estuarium, Excirolana natalensis* and *Pontogeloides latipes* were identified within the three nearby TOCEs investigated in this study. The latter three species occurred in very low densities, (usually < 5 m^{-2}), and the latter two only occurred in the lower reaches, particularly the sand near the mouth, after breaching events (T.O. Henninger, pers. obs.). The elevated species richness in permanently open estuaries is related to the strong horizontal gradients in salinity and the presence of species of both marine and freshwater origins. Additionally, in permanently open estuaries, the mouth status will allow recruitment of isopods throughout the year. The presence of a sandbar at the mouth would restrict the ability of marine isopods to recruit into TOCEs. Thus the isopod fauna in the three TOCEs was dominated by one species only, *E. hylocoetes*. This species is clearly well adapted to TOCE conditions.

Besides differences in life history, feeding strategies and increased habitat availability (e.g. salt marshes) with associated food sources may also dictate success in a TOCE compared to permanently open estuaries. Permanently open estuaries support larger macrophyte beds and salt marshes than TOCEs and therefore, presumably, have higher productivity. However, there has been very little investigation into microbenthic algae and bacterial productivity in estuaries. Deposit/filter feeding may benefit TOCE species, rather than pure filter feeding. For example, *Upogebia africana*, the filter feeding mudprawn is dominant in open estuaries, e.g. the Swartkops River Estuary, where it contributes ≈ 80 %

to the total biomass on the Swartkops intertidal mudbanks (Hanekom *et al*., 1988). The mudprawn is replaced by the deposit/filter feeding sandprawn, *Callianassa kraussi,* in TOCEs (Whitfield, 1989).

In an attempt to identify those features that enable *E. hylocoetes* to be such a successful isopod in TOCEs, the findings of this study are compared to isopod biology in general and to the only other study on a South African estuarine species, *Cirolana fluviatilis* that is an inhabitant of permanently open estuaries.

6.1 Adaptations of *Exosphaeroma hylocoetes* to TOCEs, a Comparison with other Aquatic Isopods.

Success in TOCEs may be established by an organism's abundance in these highly variable environments. Success may therefore, be ascribed to reproductive success (enabling population numbers to recover after mouth breaching events), or an adaptation to habitat, (i.e. tolerance of fluctuating salinities and water temperatures) or a combination of the two factors. Isopods in different estuary types appear to display different characteristics, which may be adaptations to those conditions encountered in either a TOCE or permanently open estuary. Few studies detailing all aspects of isopod life, allowing comparisons, in different estuary types have been carried out to date. The results of this study can be compared with those conducted on *Cirolana fluviatilis* in the permanently open Gamtoos Estuary within the same geographic region (Newman *et al.*, 2007) (Table 6.1). The data on distribution, lengths, brooding period and brood sizes, sex ratios, etc, allow deductions to be made. However, as the data may not be complete in all fields (with respect to longevity, densities), conclusions cannot always be drawn. *Exosphaeroma hylocoetes* is generally smaller and females attain sexual maturity faster than *C. fluviatilis* (Table 6.1). Female *E. hylocoetes* also produce a greater maximum number offspring all year round compared to *C. fluviatilis,* which demonstrate a seasonal breeding pattern. Other life history strategies, e.g. being able to produce large numbers of manca from a smaller female body size together with a female sexual bias probably allows *E. hylocoetes* to re-colonise TOCEs post mouth breaching. By re-examining the results of this study I will attempt to propose some of the underlying reasons for *E. hylocoetes'* success in Eastern Cape TOCEs when compared to other isopods.

6.1.1 Habitat adaptations

Isopod numbers were highest when the estuary mouths were closed and during the summer months (Chapter 3). Isopod numbers declined during winter and they virtually disappeared when the estuary breached. Mouth breaching, therefore, had the most profound effect on isopod abundance and biomass, in similar manner to those of other estuarine macrofauna within South Africa and Australia (De Decker, 1987; Gladstone *et al*., 2006). For a species that is adapted to TOCEs, recovery times, after mouth breaching events were not rapid (≈ 4 months). Because of the infrequent breaching of the mouths of the Kasouga and West Kleinemonde, the populations of *E. hylocoetes* were able to recover. The reduced abundances of *E. hylocoetes* in the East Kleinemonde Estuary must reflect the increased frequency of breaching events and the absence of submerged macrophytes from the system. Recent studies attributed the lower estimates of invertebrate abundances and biomasses to the increased rate of mouth opening events recorded within the system (Cowley *et al.*, 2001; Froneman, 2002a). Riddin and Adams (2008) reported similar losses of submerged vegetation in the E. Kleinemonde Estuary, but ascribed this loss to desiccation, within hours, after a rapid decline in water levels following mouth breaching.

Table 6.1. Comparison of *Exosphaeroma hylocoetes* (TOCE isopod; present study) with *Cirolana fluviatilis* (permanently open estuary isopod; from Newman *et al.*, 2007).

Feature	***E. hylocoetes***	***C. fluviatilis***
Location along S.A. coast:	Table Bay to East London	Breede River to St Lucia
Mean length (mm)		
Males:	5.71 ± 0.41	7.9
Females:	3.99 ± 0.26	7.5
Max. length (mm)		
Males:	12.0	11.3
Females:	7.5	10.2
Length at sexual maturity (mm) Males:	≥ 5	6
Females:	≥ 2.5	5.2
Length: Juveniles:	< 2.5	< 5.2
Mean longevity (weeks) Males:	25.77 ± 3.4	Not presented
Females:	21.52 ± 3.0	
Max. longevity (weeks) Males:	35	
Females:	30	
Gravid period:	All year	Dec. to March (summer)
Mean brood size:	20.7	33.2
Max brood size:	73 (in 7.5 mm ♀)	47 (in 7.9 mm ♀)
Manca size at release:	1.5 mm	2.24 mm
Sex ratio	Female biased 2.9 : 1	Female biased 1.94 : 1
Mean density:	2 849.7 ind. m^{-2}	500 CPUE
Max. density:	7 850 ind. m^{-2}	4 149 CPUE
Location max density:	Middle & upper reaches	Lower & middle reaches
Preferred habitat	Submerged macrophytes	High mud sediments

Another reason for the success of *E. hylocoetes* in TOCEs is its ability to tolerate and breed during long periods of mesohaline conditions (<20). Such conditions can persist for up to 12 months in the West Kleinemonde Estuary (Chapter 3). *Cirolana fluviatilis* was found in greater densities in the lower and middle reaches of the permanently open Gamtoos Estuary, appearing to prefer more stable marine conditions (Newman *et al*., 2007). In contrast, *E. hylocoetes* was found in greatest abundances in the middle and upper reaches of TOCEs. This would indicate a greater tolerance of changes in salinity by *E. hylocoetes* and thus that this species is most probably a truly estuarine species, whereas *C. fluviatilis* is most probably a marine species which has entered open estuaries more recently and is not as well adapted to conditions in TOCEs. Furthermore, the relative absence of *E. hylocoetes* in permanently open estuaries may also indicate their preference for low water flow rates, such as those experienced in TOCEs. Metabolic studies on *E. hylocoetes* could provide useful information on the adaptation of this species to mesohaline conditions.

Densities of the isopod within the three TOCEs were always highest in the middle and upper reaches of the systems (Chapter 3). This distribution pattern appeared to coincide with the highest cover of submerged macrophytes. A similar distribution pattern has been noted in other invertebrates and fish both in southern Africa and elsewhere in the world (Whitfield, 1980b; Heck and Thoman, 1981; Wallerstein and Brusca, 1982; Hanekom and Baird, 1984; Leber, 1985; Emmerson, 1986; Heck and Wilson, 1987; Walsh and Mitchell, 1998; De Villiers *et al*., 1999; Bernard and Froneman, 2005; Joseph *et al.*, 2006).

Exosphaeroma hylocoetes is strongly associated with the submerged macrophytes, (Chapters 3 and 4) and few other isopods were found in this vegetation. The association appears to be linked to fish predator avoidance, as the isopod is able to cling to the slender stems of the *R. maritima* and other submerged macrophytes (T.O. Henninger, pers. obs.) to avoid being detected and eaten. *Ruppia maritima* also supports diatoms on which the isopod feeds (Chapter 4). Submerged macrophytes certainly play an important role in acting as a refuge for invertebrate prey from predatory fish (Chapter 4; Wallerstein and Brusca, 1982; Sala and Graham, 2002; Joseph *et al*., 2006). The laboratory experiments clearly showed that the submerged macrophytes provided a refuge from selected fish predators (Chapter 4). The fish species were differentially successful at finding *E. hylocoetes* within artificial vegetation used to simulate *R. maritima* (Chapter 4). *Clinus cottoides* was the most successful at finding isopods, while *Glossogobius callidus* was the least successful at finding isopods in the artificial vegetation. *Clinus cottoides* was observed pushing its way through the artificial vegetation, while *G. callidus* was not. *Rhabdosargus holubi* were the most commonly encountered fish within the estuary and had intermediate success at finding *E. hylocoetes* within artificial vegetation. *Clinus cottoides* would have limited affect on isopod numbers, as these are mainly intertidal fish, and would only enter estuaries when the mouths are open. Thus the increased submerged vegetation in the TOCEs appears to provide a refuge rather than an increased direct food source as elucidated in Chapter 4, enabling us to reject the proposed null hypothesis.

Several previous studies conducted in other regions of the world have also demonstrated the close association of isopods with submerged macrophytes including *Cladophora* and *Zostera* beds (Hecht, 1973; Robertson, 1980; Hanekom, 1982; Holomuzki and Short, 1988; Joseph *et al*., 2006, Yamada *et al.,* 2007). Interestingly, *E. hylocoetes* has not been observed within *Zostera* beds in the open Kariega Estuary within the same geographic region (P.W. Froneman, pers. comm.; T.O. Henninger, pers. obs.). The findings have indicated that the provision of refuge, rather than a source of food, is the main focus of the macrophytes (Cummins and Klug, 1979). Results of both the stable isotope and fatty acid analyses indicated that the macrophytes could be excluded as an important carbon source for the isopod (Chapter 4). It seems that POM, epiphytic diatoms and the sediments are the primary source of carbon for the isopod.

6.1.2 Reproductive adaptations

Sex ratios of *E. hylocoetes* within the three estuaries were skewed in favour of females. This was also evident in the brood rearing experiments concluded within constant environment rooms (Chapter 3). These skewed sex ratios are in agreement with other studies on isopods (Jones and Naylor, 1971; Arrontes, 1992; Shafir and Field, 1980b; Piertney and Carvalho, 1996) including *Cirolana fluviatilis* that inhabits permanently open South African estuaries (Newman *et al*., 2007). Distorted sex ratios, deviating from an expected 1:1 sex ratio, have been suggested to be the result of bacterial infections by *Wolbachia* spp. in many arthropods, including isopods (Heath and Ratford, 1990; Martin *et al.*, 1994; Werren, 1997; Bouchon *et al*., 1998; Bourtzis and O'Niell, 1998; Kageyama *et al*., 1998; Zimmer, 2001). *Wolbachia* is responsible for feminizing factors, and destroying male brood via cytoplasmic sex factors (CSF) resulting in female-biased progeny in their host (thelygeny). These CSF are only inherited via the egg cytoplasm, changing genetic males into functional females (Martin *et al*., 1994). Terrestrial isopods have been found to test positive for *Wolbachia* infections, and horizontal transfer of bacteria could occur from the terrestrial genera such as *Armadillidium* and *Ligia* to estuarine species which spend part of their life out of the water in the upper intertidal zone (Martin *et al*., 1994; Bouchon *et al*., 1998). *Exosphaeroma hylocoetes* spends its life within the water and does not feed on land, making it unlikely that it would come into contact with terrestrial and semi-terrestrial isopods. Preliminary studies conducted by the author could not detect the molecular markers for *Wolbachia* in *E. hylocoetes* using *DNeasy 96 Blood and Tissue Kit* (Qiagen) to extract DNA by the spin-column method. This was followed by PCR amplification using a *Thermo Hybaid PX2* thermal cycler. It has also been demonstrated that oral uptake of *Wolbachia* is impossible in terrestrial isopods (Juchault *et al*., 1974). However, *Wolbachia* could be transferred in the wild by vectors such as parasites or parasitoides (Martin *et al.*, 1994). The skewed sex ratios in *E. hylocoetes* may be due to inherent genetic factors, or even another intracellular bacterium belonging to the genera *Anaplasma*, *Cowdria*, *Ehrlichia* or *Rickettsia* (Werren and O'Niell, 1997). Whilst the skewed sex ratios in *E. hylocoetes* are not a unique feature, and are possibly due to inherent genetic factors, having a female biased population must facilitate the re-colonisation of TOCEs after mouth breaching events.

In agreement with previous studies on most Sphaeromatidae, *E. hylocoetes* demonstrated strong sexual dimorphism, with males being significantly larger than females (Kensly, 1978; Brusca and Wilson, 1991; Bruce, 2003). Newman *et al.* (2007) in their study of *C. fluviatilis* also found that males were larger (Table 6.1). Larger male size appears to be an advantage when mate guarding (Jormalainen, 1998; Bertin and Cézilly, 2003), a behaviour often observed in laboratory maintained animals (T.O. Henninger, pers. obs.). There were no major differences in the growth rates of male and female *E. hylocoetes*, at most combinations of 15 and 25 °C and salinities of 15 and 35 (Chapter 5), enabling one to reject the proposed null hypothesis. Males (25.77 ± 3.40 weeks) did, however, live longer than females (21.52 ± 3.00 weeks), thus growing for a longer time and attaining an overall larger size reflecting the sexual dimorphism observed in the field (Chapter 3). The increased growth rates recorded at the higher temperatures is consistent with the published literature for prawns and mysids (Staples and Heales, 1991; McKenney and Celestial, 1995). Both male and female *E. hylocoetes* demonstrated a faster growth rate at the higher experimental temperature of 25 °C at a salinity of 15, but these individuals attained the lowest overall length (Chapter 5). The better growth rate under mesohaline conditions would suggest an estuarine origin for *E. hylocoetes* as suggested by Kinne (1964) and Steele and Steele (1991) for other brackish water species. *Exosphaeroma hylocoetes* attained sexual maturity at a smaller size (≥ 2.5 mm) than *C. fluviatilis* (≥ 5.2 mm; Newman *et al.*, 2007; Table 6.1). Isopod life-spans vary, for example, *E. hylocoetes* lives for a shorter period (≈ 6 to 8 months) than most other isopods. *Excirolana braziliensis* lives for between 19 and 31.5 months (Cardoso and Defeo, 2004) and *Elaphognathia cornigera* for ≈ 36 months (Tanaka, 2003). The male intertidal sphaeromatid isopod, *Dynamene bidentata*, lives for 24 months, while females of the species live for only 12 months.

Female *E. hylocoetes* appear to reach sexual maturity at a small size, produce manca all year round and live for a relatively short time (Table 6.1). This is in contrast to *C. fluviatilis*, in the open Gamtoos Estuary, showing a seasonal (summer) gravid period (Newman *et al.*, 2007; Table 6.1). I propose that *E. hylocoetes* females only produce two to a maximum of three broods in their lifetime, despite many other sphaeromatid isopods being semelparous (Harrison, 1984a; Shuster, 1991). This is based on a mean longevity of 22 weeks (5½ months), within which females reach sexual maturity after ≈ 2 weeks and brood for ≈ 2 months; 2 to 3 months in *Excirolana chiltoni* (Klapow, 1972). Isopod females cease feeding during their brooding period (Shuster, 1995), while they remain within the submerged vegetation. The number of offspring produced by a female *E. hylocoetes* in her lifetime would range between an average of 42 and a maximum of 219 (Table 6.1). Female isopods would also need a recovery period, within which they would need to replenish themselves before supporting the next brood.

Although no specific refugia were discovered for *E. hylocoetes*, possible refugia sites could include submerged algae on the upper layer of mud (T.H. Wooldridge, pers. comm.) within the estuaries. It is presumed that some isopods must remain within the estuaries to allow recovery of isopod numbers after mouth closure. Alternatively isopods could raft on submerged macrophytes from breached estuary to neighbouring estuaries (Locke and Corey, 1989; Teske *et al.*, 2006), as commonly occurs in macrobenthic crustaceans (Thiel, 2003). A preliminary of study of 20 net samples, collected during overtopping events from the neighbouring Mpekweni Estuary, revealed four *E. hylocoetes* (accounting for 0.1 % for total invertebrates sampled) being washed into the estuary from the marine environment (Tweddle and Froneman, unpublished data). These data suggest that recruitment of the isopod from other estuaries via the marine environment into an estuary plays only a minor role in the population dynamics of *E. hylocoetes* in TOCEs. The isopod offspring could also recruit directly on the parental raft (Vásquez, 1993; Thiel, 2003). The marine isopod, *Idotea metallica*, demonstrated local reproduction and recruitment on floating algae (Gutow and Franke, 2003), often concluding their complete life cycle on these floating algal rafts (Gutow and Franke, 2003). Teske *et al.*, (2006) reported a southern *E. hylocoetes* mt DNA linage stretching from the Gouritz Estuary (east of Cape Agulhas) to the Bulolo Estuary (near Port St Johns). These southern *E. hylocoetes* do, however, display isolation by distance (Teske *et al.*, 2006), indicating relatively short migrations on floating rafts from estuary to neighbouring estuary.

In conclusion, *E. hylocoetes* with its association with submerged macrophytes, mesohaline adaptations, its growth rates, early maturity, female biased sex ratios and year-round breeding, all contribute to its success within TOCEs.

6.2 Future Research

Exosphaeroma hylocoetes is highly successful in TOCEs and understanding the ecological role of these isopods could assist in the understanding of estuarine invertebrates in general. Future studies to assist the understanding of the role of *E. hylocoetes* could include the following:

- The role of *E. hylocoetes* as a consumer and its role in the recycling of nutrients in estuaries. Also needed is an energy budget for *E. hylocoetes* that could be determined by feeding experiments, determining respiration rates and the energy content of faecal matter. The role of *E. hylocoetes* as contributor to the breakdown of macrophytes could then be evaluated. Production : biomass ratios need investigation.

- The role of *E. hylocoetes* in the energy flow of the estuary, as the isopod forms part of the diet of estuarine fish. Energy budgets, using the results of the above study, as well as those of the other estuarine invertebrates, fish and birds need to be determined. This would entail seasonal gut content investigations and energy budgets of the involved organisms. Studies exist on terrestrial isopods (Lam *et al.*, 1991), intertidal isopods (Johnson, 1976a, b; Koop and Field, 1980, 1981), marine (Lapucki *et al.*, 2005), and freshwater isopods (Adcock, 1982), but little if any on those from TOCEs. In the permanently open Kariega Estuary the carbon flow, using stable isotopes, has been established for many zooplankton and macrofauna (excluding isopods) showing highly complex linkages (Richoux and Froneman, 2007). Nozias *et al*. (2005) looked at a carbon based turnover of meiofauna (particularly nematodes, mites, copepods, ostracods, turbellarians and polychaetes) in a Kwazulu-Natal TOCE, providing estimates of the daily total ingestion rates ranging from 2 to 857 mg C m^{-2}.

- The tolerance of *E. hylocoetes* to changes in salinity, i.e. can the isopod tolerate very low salinities (< 5), as well as high salinities (as salinities in TOCEs can reach 50) and how does it manage this osmotic problem. Their respiration rates in response to exposure to salinity gradients from 0 to 50 at a constant water temperature could be determined. A positive relationship has been established between salinity and macrofaunal species richness, including isopods in seagrass meadows of Japan (Yamada *et al.,* 2007).

- TOCEs mouth breaching frequencies and their influence on the macrofauna, particularly the different species of Isopoda. Several studies have investigated the affect of mouth breaching on estuarine fauna (De Decker, 1987; Whitfield and Bruton, 1989; Froneman, 2000b, c; Gladstone *et al*., 2006). It would be interesting to see the changes in isopod species composition after mouth breaching events. In this study I intermittently encountered *Exosphaeroma estuarium* in low abundances, (≈ 5 m^{-2}), in the closed phases of the TOCEs, but have noticed them in greater abundances in permanently open estuaries. This species could probably have different osmotic constraints limiting its existence in the TOCEs. This is related to the previously mentioned point.

REFERENCES

Achouri, M., Charfi-Cheikhrouha, F. and Marques, J.C. (2003). Biology, population structure, and field-growth rates of *Porcellionides pruinosus* (Brandt, 1833)(Isopoda, Oniscidea) at Garat Nâam (Kasserine, Tunisia). *Crustaceana* **75**: 1241 – 1262.

Adcock, J.A. (1982). Energetics of a population of *Asellus aquaticus* (Crustacea, Isopoda): respiration and energy budgets. *Freshwater Biology* **12**: 257 – 269.

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19**: 716 – 723.

Allan, E. L., Froneman, P.W. and Hodgson, A.N. (2006). Effects of temperature and salinity on the standard metabolic rate (SMR) of the caridean shrimp *Palaemon peringueyi*. *Journal of Experimental Marine Biology and Ecology* **337**: 103 – 108.

Allanson, B.R. (1981). The coastal lakes of South Africa. In: *Estuarine ecology with particular reference to southern Africa*, (ed.) J.H. Day, pp. 331 – 344. A.A. Balkema, Cape Town.

Allanson, B.R. (1992). An assessment of the SANCOR estuaries research programme from 1980 to 1989. *Committee for Marine Science Occasional Report No.1*: 1 – 72.

Ames, A.L., Van Vleet, E.S. and Reynolds, J.E. (2002). Comparison of lipids in selected tissues of the Florida manatee (Order Sirenia) and bottlenose dolphin (Order Cetacea; Suborder Odontoceti). *Comparative Biochemistry and Physiology B* **132**: 625 – 634.

Araujo, P.B. and Bond-Buckup, G. (2004). Growth curve of *Atlantoscia floridana* (van Name) (Crustacea, Isopoda, Philosciidae) from a Brazilian Restinga Forest. *Revista Brasileira de Zoologia* **21**: 1 – 8.

Arrontes, J. (1992). Sex-ratio variation in an intertidal isopod. *Oikos* **63**: 131 – 138.

Arts, M.T., Ackman, R.G. and Holub, B.J. (2001). “Essential fatty acids” in aquatic ecosystems: a crucial link between diet and human health and evolution. *Canadian Journal of Fisheries and Aquatic Sciences* **58**: 122 – 137.

Barnard, K.H. (1914). Contributions to the crustacean fauna of South Africa. 3. Additions to the marine Isopoda, with notes on some previously incompletely known species. *Annals of the South African Museum* **10**: 325 – 442.

Barnard, K.H. (1940). Contributions to the crustacean fauna of South Africa. XII. Further additions to the Tanaidacea, Isopoda, and Amphipoda together with keys for the identification of the hitherto recorded marine and freshwater species. *Annals of the South African Museum* **32**: 381 – 543.

Barradas-Ortiz, C., Briones-Fourzán, P. and Lozano-Álvarez, E. (2003). Seasonal reproduction and feeding ecology of giant isopods *Bathynomus giganteus* from the continental slope of the Yucatán Peninsula. *Deep-Sea Research* I **50**: 495 – 513.

Begg, G.W. (1978). The estuaries of Natal. *Natal Town and Regional Planning Report* **41**: 1 – 657.

Begg, G.W. (1984a). The comparative ecology of Natal’s smaller estuaries. *Natal Town and Regional Planning Report* **62**: 1 – 182.

Begg, G.W. (1984b). The estuaries of Natal (Part 2). *Natal Town and Regional Planning Report* **55**: 1 – 631.

Bell, K.N.I., Cowley, P.D., Whitfield, A.K. (2001). Seasonality in frequency of marine access to an intermittently open estuary: implications for recruitment strategies. *Estuarine, Coastal and Shelf Science* **52**: 327 – 337.

Benedict, J.E. (1897). A revision of the genus *Synidotea*. *Proceedings of the Academy of Natural Science Philadelphia*. **49**: 389 – 404.

Benedict, J.E. (1898a). Two new isopods of the genus *Idotea* from the coast of California. *Proceedings of the Biological Society of Washington* **22**: 53 – 55.

Benedict, J.E. (1898b). The Arcturdae in the U.S. National Museum. *Proceedings of the Biological Society of Washington* **22**: 41 – 51.

Bennett, B.A. (1984). A population energy budget for *Clinus superciliosus* L. with an assessment of the role of resident fish as predators in the intertidal zone. *Marine Biology Letters* **5**: 323 – 334.

Bennett, B.A. (1989a). The diets of fish in three south-western Cape estuarine systems. *South African Journal of Zoology* **24**: 163 – 177.

Bennett, B.A. (1989b). A comparison of the fish communities in nearby permanently open, seasonally open and normally closed estuaries in the south-western Cape, South Africa. *South African Journal of Marine Science* **8**: 43 – 55.

Bennett, B.A. and Branch, G.M. (1990). Relationships between production and consumption of prey species by resident fish in the Bot, a cool temperate South African estuary. *Estuarine, Coastal and Shelf Science* **31**: 139 – 155.

Berglund, A. (1981). Sex dimorphism and skewed sex ratios in the prawn species *Palaemon adspersus* and *P. squilla*. *Oikos* **36**: 158 – 162.

Bernard, A.T.F. and Froneman, P.W. (2005). Population dynamics of the caridean shrimp *Palaemon peringueyi* in a temperate temporarily open/closed southern African estuary. *Estuarine, Coastal and Shelf Science* **63**: 73 – 81.

Bertin, A and Cézilly, F. (2003). Sexual selection, antennae length and the mating advantage of large males in *Asellus aquaticus*. *Journal of Evolutionary Biol*ogy **16**: 491 – 500.

Bird, E.C.F. (1984). Coasts. *An introduction to coastal morphology*. Australian National University, Canberra: 320 pp.

Blaber, S.J.M. (1974).Field studies of the diet of *Rhabdosargus holubi* (Pisces: Teleostei: Sparidae). *Journal of Zoology*, *London* **173**: 407 – 417.

Blaber, S.J.M. (1976). The food and feeding ecology of Mugilidae in the St Lucia Lake system. *Biological Journal of the Linnean Society* **8**: 267 – 277.

Blaber, S.J.M. (1979).The biology of filter feeding teleosts in Lake St Lucia, Zululand. *Journal of Fish Biology* **15**: 37 – 59.

Blaber, S.J.M. (1984). The diet, food selectivity and niche of *Rhabdosargus sabra* (Teleostei: Sparidae) in Natal estuaries. *South African Journal of Zoology* **9**: 31 – 36.

Blaber, S.J.M., Kure, N.F., Jackson, S. and Cyrus, D.P. (1983). The benthos of South Lake, St Lucia following a period of stable salinities. *South African Journal of Zoology* **18**: 311 – 319.

Bligh, E. and Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **37**: 911 – 917.

Boone, P.L. (1918). Descriptions of ten new isopods. *Proceedings of the U.S. National Museum* **54**: 591 – 604.

Boone, P.L. (1923). New marine tanaid and isopod Crustacea from California. *Proceedings of the Biological Society of Washington* **36**: 147 – 156.

Bouchon, D., Rigaud, T. and Juchault, P. (1998). Evidence for widespread *Wolbachia* infection in isopod crustaceans: molecular identification and host feminization. *Proceedings of the Royal Society of London Series B, Biological sciences* **265**: 1081 – 1090.

Bourtzis, K., and O’Niell, S. (1998). *Wolbachia* infections and arthropod reproduction. *BioScience* **48**: 287 – 293.

Branch, G.M. and Day, J.A. (1984). Ecology of southern African estuaries: part XIII. The Palmiet River estuary in the south-western Cape. *South African Journal of Zoology* **19**: 63 – 77.

Breen, C., Adams, J.B., Batchelor, A., Cowley, P.D., Marneweck, G., McGwynne, L., McKenzie, M., Ngulube, P., Paterson, A.W., Sihlophe, N., Taljaard, S., Turpie, J.K., Uys, A., van Niekerk, L., and Wood, A. (2004). Towards the conservation and sustainable use of Eastern Cape Estuaries. TT 237/04. Water Research Commission, Pretoria.

Briones-Fourzán, P. and Lozano-Alvarez, E. (1991). Aspects of the biology of the giant isopod *Bathynomus giganteus* A. Milne Edwards, 1879 (Flabellifera: Cirolanidae), off the Yucatan Peninsula. *Journal of Crustacean Biology* **11**: 375 – 385.

Brown, A.C. (1953). A preliminary investigation of the ecology of the larger Kleinmond River estuary, Bathurst District. MSc thesis, Rhodes University, Grahamstown.

Bruce, N.L. (1992a). A new genus of hemibranchiate sphaeromatid isopod crustacean from tropical Western Australia. *Journal of Natural History* **26**: 1263 – 1272.

Bruce, N.L. (1992b). *Anopsilana barnardi*, a new species of estuarine cirolanid crustacean isopod from tropical eastern Australia. *Memoirs of the Queensland Museum* **32**: 1 – 8.

Bruce, N.L. (2003). New genera and species of sphaeromatid isopod crustaceans from Australian marine coastal waters. *Memoirs of Museum of Victoria* **60**: 309 – 369.

Brusca, R.C. and Wilson, G.D.F. (1991). A phylogenetic analysis of the Isopoda with some classificatory recommendations. In *Proceedings of the 1990 International Crustacean Conference*, ed. P.J.F. Davie and R.H. Quinn. *Memoirs of the Queensland Museum* **31**: 143 – 204.

Brusca, R.C., Coelho, V.R. and Taiti, S. (2001). A guide to the coastal isopods of California. *http://tolweb.org/notes/?note_id=3004* (Accessed 11/04/2006)

Budge, S.M. and Parrish, C.C. (1998). Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, New Foundland. II Fatty Acids. *Organic Geochemistry* **29**: 1547 – 1559.

Budge, S.M. and Parrish, C.C. (1999). Lipid class and fatty acid composition of *Pseudo-nitzschia multiseries* and P*seudo-nitzschia pungens* and effects of lipolytic enzyme deactivation. *Phytochemistry* **52**: 561 – 566.

Bursey, M.L. and Wooldridge, T.H. (2003). Physical factors regulating macrobenthic community structure on a South African estuarine flood-tidal delta. *African Journal of marine Science* **25**: 263 – 274.

Byren, B.A. and Davies, B.R. (1986). The influence of invertebrates on the breakdown of *Potamogeton pectinatus* L. in a coastal marina (Zandvlei, South Africa). *Hydrobiologia* **137**: 141 – 151.

Cardoso, R.S. and Defeo, O. (2004). Biogeographic patterns in life history traits of the Pan-American sandy beach isopod *Excirolana braziliensis*. *Estuarine, Coastal and Shelf Science* **61**: 559 – 568.

Carefoot, T.H. (1973). Studies on the growth, reproduction, and life cycle of the supralittoral isopod *Ligia pallasii*. *Marine Biology* **18**; 302 – 311.

Caseiro, I., Santos, S., Sousa, J.P., Nogueira, A.J.A. and Soares, A.M.V.M. (2000). Optimization of culture conditions of *Porcellio dilatatus* (Crustacea: Isopoda) for laboratory test development. *Ecotoxicology and Environmental Safety* **47**: 285 – 291.

Charpentier, A., Grillas, P., Lescuyerb, F. Coulet, E. and Auby, I. (2005). Spatio-temporal dynamics of a *Zostera noltii* dominated community over a period of fluctuating salinity in a shallow lagoon, southern France. *Estuarine, Coastal and Shelf Science* **48**: 497 – 509.

Chen, J.C. and Nan, F.H. (1993). Changes in oxygen consumption and ammonia-N excretion by *Penaeus chinensis* Obeck at different temperature and salinity levels. *Journal of Crustacean Biology* **13**: 706 – 712.

Christie, N.D. and Moldan, A. (1977). Distribution of benthic macrofauna in Langebaan Lagoon. *Transactions of the Royal Society of South Africa* **42**: 273 – 284.

Coetzee, D.J. (1981). Analysis of the gut contents of the needlefish, *Hyporhampus knysnaensis* (Smith), from Rondevlei, southern Cape. *South African Journal of Zoology* **16**: 14 – 20.

Coetzee, D.J. (1982a). Stomach content analysis of *Gilchristella aestuarius* and *Hepsetia breviceps* from the Swartvlei system and Groenvlei, southern Cape. *South African Journal of Zoology* **17**: 59 – 66.

Coetzee, D.J. (1982b). Stomach content analysis of the leervis, *Lichia amia* (L.) from the Swartvlei system, southern Cape. *South African Journal of Zoology* **17**: 177 – 181.

Coetzee, D.J. and Pool, R.C. (1985). Stomach content analysis of the sea barbel *Galeichthyes feliceps* (Valenciennes in C & V), from the Swartvlei system, southern Cape. *South African Journal of Zoology* **20**: 33 – 37.

Contreras, H. and Jaramillo, E. (2003). Geographical variation in natural history of the sandy beach isopod *Excirolana hirsuticauda* Menzies (Cirolanidae) on the Chilean coast. *Estuarine, Coastal and Shelf Science* **58**: 117 – 126.

Cooper, J.A.G. (1989). Fairweather versus flood sedimentation in Mhlanga Lagoon, Natal: implications for environmental management. *South African Journal of Geology* **92**: 279 – 294.

Cooper, A., Wright, I. and Mason, A.K. (1999). Geomorphology and sedimentology. In: *Estuaries of South Africa*, (eds.) B.R. Allanson and D. Baird, pp. 5 – 25. Cambridge University Press, Cambridge.

Cowley, P.D. and Whitfield, A.K. (2001). *Ichthyofaunal characteristics of a typical temporarily open/closed estuary on the south east coast of South Africa*. Ichthyological Bulletin of the J.L.B Smith Institute of Ichthyology: Report No. 71. J.L.B. Smith Institute of Ichthyology, Grahamstown.

Cowley, P., Whitfield, A.K., Bell, K.N.I. (2001). The surf zone ichthyoplankton adjacent to the mouth of a predominantly closed southern African estuary, with evidence of recruitment during marine overwash events. *Estuarine, Coastal and Shelf Science* **52**: 1 – 10.

Cummins, K.W. and Klug, M. (1979). Feeding ecology of stream invertebrates. *Annual Review of Ecological Systems* **10**: 147 –172.

Cyrus D.P. and Blaber, S.J.M. (1983). The food and feeding ecology of Gerreidae, Bleeker 1859, in the estuaries of Natal. *Journal of Fish Biology* **22**: 373 – 393.

Dalsgaard, J., St. John M., Kattner, G., Müller-Navarra, D. and Hgaen, W. (2003). Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology* **46**: 225 – 340.

Dana, J.D. (1853a). Report on the Crustacea. In: *United States Exploring Expedition under the command of Charles Wilks, U.S.N. 1834 – 1842.* **13**: 690 – 1018.

Dana, J.D. (1853b). *Crusteacea* : 696 – 805. United States Exploring Expeditions during the years 1838, 1839, 1840, 1841, 1842 under the command of Charles Wilkes, U.S.N. C. Sherman, Philadelphia.

Dana, J.D. (1854). Catalogue and descriptions of Crustacea collected in California by Dr. John L. Le Conte. *Proceedings of the Academy of Natural Sciences of Philadelphia* **7**: 175 – 177.

Day. J.H. (1964). *The origin and distribution of estuarine animals in South Africa*. In: *Ecological studies in southern Africa,* (ed.) D.H.S. Davis, pp. 159 – 173. The Hague.

Day, J.H. (1974). The ecology of Morrumbene Estuary Moçambique. *Transactions of the Royal Society of South Africa* **41**: 43 – 97.

Day, J.H. (1980). What is an estuary? *South African Journal of Science* **76**: 198.

Day, J.H. (1981a). Nature, origin and classification of estuaries. In: *Estuarine Ecology with Particular Reference to Southern Africa,* (ed.) J.H. Day, pp.1 – 6. A.A. Balkema, Cape Town.

Day, J.H. (1981b). Summaries of current knowledge of 43 estuaries in Southern Africa. In: *Estuarine Ecology with Particular Reference to Southern Africa,* (ed.) J.H. Day, pp. 251 – 329. A.A. Balkema, Cape Town.

Day, J.H. (1981c). The estuarine fauna. In: *Estuarine Ecology with Particular Reference to Southern Africa* (ed.) J.H. Day, pp. 147 – 178. A.A. Balkema, Cape Town.

De Decker, H.P. (1987). Breaching the mouth of the Bot River Estuary, South Africa: impact on its benthic macrofaunal communities. *Transactions of the Royal Society of South Africa* **46**: 231 – 250.

De Decker, H.P. and Bally, R. (1985). The benthic macrofauna of the Bot River Estuary. With a note on its meiofauna. *Transactions of the Royal Society of South Africa,* **45**: 379 – 396.

De Ruyck, A.M.C., Donn, T.E. JR, and McLachlan, A. (1991a). The activity of three intertidal sand beach isopods (Flabellifera: Cirolanidae). *Journal of Experimental Marine Biology and Ecology* **146**: 163 – 180.

De Ruyck, A.M.C., Donn, T.E. JR, and McLachlan, A. (1991b). Life histories and breeding patterns of three intertidal sand beach isopods. *P.S.Z.N. I: Marine Ecology* **12**: 105 – 121.

De Ruyck, A.M.C., Donn, T.E. JR, and McLachlan, A. (1992). Distribution of three intertidal cirolanid isopods (Flabellifera: Cirolanidae) on a South African sandy beach. *Cahiers Biology de Marine* **33**: 147 – 168.

De Villiers, C.J., Hodgson, A.N., and Forbes, A.T. (1999). *Studies on estuarine macroinvertebrates*. In: *Estuaries of South Africa*, (eds.) B.R. Allanson and D. Baird, pp. 167 – 207. Cambridge University Press, Cambridge.

Dias, N. and Sprung, M. (2003). Population dynamics and production of the isopod, *Tylos ponticus* in a Ria Formosa saltmarsh (south Portugal). *Crustaceana Monographs* **2**: 133 – 149.

Dick, J.T.A., Montgomery, I. and Elwood, R.W. (1993). Replacement of the indigenous amphipod *Gammarus duebeni celticus* by the introduced *G. pulex*: differential cannibalism and mutual predation. *Journal of Animal Ecology* **62**: 79 -88.

DWAF, D.O.W.A.A.F. 2004. *National Water Resource Strategy*. Department of Water Affairs and Forestry, Pretoria. 1 – 150.

Dye, A.H. (1983). Composition and seasonal fluctuations of meiofauna in a Southern African mangrove estuary. *Marine Biology* **73**: 165 – 170.

Dye, A. and Baros, F. (2005). Spatial patterns of macrofaunal assemblages in intermittently closed/open lakes in New South Wales, Australia. *Estuarine, Coastal and Shelf Science* ***64***: 357 – 371.

Ellis, R.J. (1961). A life history study of *Asellus intermedius* Forbes. *Transactions of the American Microscopy Society* **80**: 80 – 102.

Emmerson, W.D. (1983). Tidal exchange of two decapod larvae, *Palaemon pacificus* (Caridea) and *Upogebia Africana* (Thalassinidae) between the Swartkops river estuary and adjacent coastal waters. *South African Journal of Zoology* **18**: 326 – 330.

Emmerson, W.D. (1986). The ecology of *Palaemon pacificus* (Stimpson) associated with *Zostera capensis*, Setchell. *Transactions of the Royal Society of South Africa* **46**: 79 – 97.

ETU, E.a.T.U. (2002). Municipal Service Delivery. http://www.etu.org.za/toolbox/docs/localgov/munservice.html

Ferreira, S.M., Pardal, M.A., Lillebø, A.I., Cardoso, P.G. and Marques, J.C. (2004). Population dynamics of *Cyathura carinata* (Isopoda) in a eutrophic temperate estuary. *Estuarine, Coastal and Shelf Science* **61**; 669 – 677.

Fish, S. (1970). The biology of *Eurydice pulchra* (Crustacea: Isopoda). *Journal of the Marine Biological Association of the United Kingdom* **50**: 753 – 768.

Fishelson, L. (1977). Stability and instability, illustrated by examples from the Red Sea. *Helgolander wissenschaftliche Meeresuntersuchungen* **30**: 18 – 29.

Forbes, A.T. (1973). An unusual abbreviated larval life in the estuarine burrowing prawn *Callianassa kraussi* (Crustacea; Decapoda: Thalassinidea). *Marine Biology* **22**: 361 – 365.

Forbes, A.T. and Cyrus, D.P. (1992). Impact of a major cyclone on a southeast African estuarine lake system. *Netherlands Journal of Sea Research* **30**: 265 – 272.

France, R. and Peters, R. (1997). Ecosystems differences in the trophic enrichment of 13-C in aquatic food webs. *Canadian Journal of Fisheries and Aquatic Science* **54**: 1255 – 1258.

Froneman, P.W. (2000). Feeding studies on selected zooplankton in a temperate estuary, South Africa. *Estuarine, Coastal and Shelf Science* **51**: 543 – 552.

Froneman, P.W. (2001). Stable isotope ($\delta^{13}C$) composition of the food web of the temperate Kariega Estuary (Eastern Cape). *African Journal of Aquatic Science* **26**: 49 – 56.

Froneman, P.W. (2002a). Food web structure in three contrasting estuaries determined using stable isotope ($\delta^{13}C$) analysis. *African Journal of Aquatic Science* **27**: 107 – 115.

Froneman, P.W. (2002b). Seasonal changes in selected physico-chemical and biological variables in the temporarily open/closed Kasouga estuary, Eastern Cape, South Africa. *African Journal of Aquatic Science* **27**: 117 – 123.

Froneman, P.W. (2002c). Response of the plankton to three different hydrological phases of the temporarily open/closed Kasouga estuary, South Africa. *Estuarine, Coastal and Shelf Science* **55**: 535 - 546.

Froneman, P.W. (2004a). Food web dynamics in a temporarily open/closed estuary (South Africa). *Estuarine, Coastal and Shelf Science* **59**: 87 - 95.

Froneman, P.W. (2004b). Zooplankton community structure and biomass in a southern African temporarily open/closed estuary. *Estuarine, Coastal and Shelf Science* **60**: 125 – 132.

Froneman, P.W. (2004c). Seasonal patterns in the zooplankton community structure and biomass in an Eastern Cape temporarily open/closed estuary. *Estuarine, Coastal and Shelf Science* **60**: 693 – 700.

Fry, B. and Sherr, E.B. (1984). $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions in Marine Science* **27**: 13 – 47.

Gambardella, C., Guarino, S.M., Dinardo, C. and de Nicola, M. (1997). Effect of photoperiod on embryogenesis and growth rate in *Idotea baltica* (Isopoda). *Journal of Crustacean Biology* **17**: 412 – 416.

Gardner, M.B. (1981). Effects of turbidity on feeding rates and size selectivity of bluegills. *Transactions of the American Fisheries Society* **110**: 446 – 450.

Gladstone, W., Hacking, N. and Owen, V. (2006). Effects of artificial openings of intermittently opening estuaries on macroinvertebrate assemblages of the entrance barrier. *Estuarine, Coastal and Shelf Science* **67**: 708 – 720.

Gonçalves, S.C., Pardal, M.A., Cardosa, P.G., Ferreira, S.M. and Marques, J.C. (2005). Biology, population dynamics and secondary production of *Tylos europaeus* (Isopoda, Tylidae) on the western coast of Portugal. *Marine Biology* **147**: 631 – 641.

Grange, N., Whitfield, A.K., De Villiers, C.J. and Allanson, B.R. (2000). The response of two South African east coast estuaries to altered river flow regimes. *Aquatic Conservation: Marine and Freshwater Ecosystems* **10**: 155 – 177.

Guerao, G., Pérez-Baquera, J. and Ribera, C. (1994). Growth and reproductive biology of *Palaemon xiphias* Risso, 1816 (Decapoda: Caridea: Palaemonidae). *Journal of Crustacean Biology* **14**: 280 – 288.

Gutow, L. and Franke, H.D. (2001). On the current and possible future status of the neustonic isopod *Idotea metallica* Bosc in the North Sea: a laboratory study. *Journal of Sea Research* **45**: 37 – 44.

Gutow, L. and Franke, H.D. (2003). Metapopulation structure of the marine isopod *Idotea metallica* – a species associated with drifting habitat patches. *Helgoland Marine Research* **56**: 259 – 264.

Hammer, Ø, Harper, D.A.T. and Ryan, P.D. (2001) PAST: palaeontological statistics software package for education and data analysis. Palaeontologia Electronica 4 p 9.
Available at: http://folk.uio.no/ohammer/past/index.html

Hanekom, N. (1982). An ecological study of the Zostera bads in the Kromme Estuary. *University of Prt Elizaberth Report No.,18*, pp. 1 – 163.

Hanekom, N., Baird, D. and Erasmus, T. (1988). A quantitative study to assess standing biomasses of macrobenthos in soft substrata of the Swartkops Estuary, South Africa. *South African Journal of Science* **6**: 163 – 174.

Harrison, K. (1984a). The morphology of the sphaeromtid brood pouch (Crustacea: Isopoda: Sphaeromatidae). *Zoological Journal of the Linnean Society* **82**: 363 – 407.

Harrison, K. (1984b). Some sphaeromatid isopods (Crustacea) from southern and south-western Australia, with the description of two new species. *Records of the Western Australian Museum* **11**: 259 – 286.

Harrison, T.D. (2004). Physico-chemical characteristics of South African estuaries in relation to the zoogeography of the region. *Estuarine, Coastal and Shelf Science* **61**: 73 – 87.

Harrison, T.D., Cooper, J.A.G. and Ramm, A.E.L. (2000). *State of South African Estuaries: Geomorphology, Ichthyofauna, Water Quality and Aesthetics*. State of the Environment Series: Report No. 2. Department of Environmental Affairs and Tourism, Pretoria.

Hass, C.G. and Knott, B. (1998). Sphaeromatid isopods from the Swan River, Western Australia: diversity, distribution, and geographic sources. *Crustaceana* **71**: 36 – 46.

Hatchett, S.P. (1947). Biology of the Isopoda of Michigan. *Ecological Monographs* **17**: 47 – 79.

Heath, D.J. and Ratford, J.R. (1990). The inheritance of sex ratio in the isopod, *Sphaeroma rugicauda*. *Heredity* **64**: 419 – 425.

Heck, K.L. Jr. and Thoman, T. (1981). Experiments on predator-prey interactions in vegetated aquatic habitats. *Journal of Experimental Marine Biology and Ecology* **53**: 125 – 134.

Heck, K.L. Jr. and Wilson, K.A. (1987). Predation rates on decapod crustaceans in latitudinally separate sea grass communities: a study of spatial and temporal variation using tethering techniques. *Journal of Experimental Marine Biology and Ecology* **107**: 87 – 100.

Heck, K.L. Jr, Able, K.W., Roman, C.T., Fahay, M.P., (1995). Composition, abundance, biomass, and production of macrofauna in a New England Estuary: comparisons among eelgrass meadows and other nursery habitats. *Estuaries* **18**: 379 – 389.

Heck, K.L. Jr., Hays, G., and Orth, R.J. (2003). Critical evaluation of the nursery role hypothesis for seagrass meadows. *Marine Ecology Progress Series* **253**: 123 – 136.

Hecht, T. (1973). The ecology of the Kromme River Estuary with special reference to Sesama catenata. Unpublished MSc dissertation, University of Port Elizabeth, pp. 1 – 150.

Hewatt, W.G. (1937). Ecological studies on selected marine intertidal communities of Monterey Bay, California. *American Midland Naturalist* **18**: 161 – 206.

Hodgson, A.N. (1987). Distribution and abundance of the macrobenthic fauna of the Kariega estuary. *South African Journal of Zoology* **22**: 153 – 162.

Holdich, D.M. and Harrison, K. (1983). Sphaeromatid isopods (Crustacea) from brackish waters in Queensland, Australia. *Zoologica Scripta* **12**: 127 – 140.

Holomuzki, J.R. and Short, T. M. (1988). Habitat use and fish avoidance behaviours by the stream-dwelling isopod *Lirceus fontinalis. Oikos* **52**: 79 – 86.

Homziak, J., Fonseca, M.S. and Kenworthy, W.J. (1982). Macrobenthic community structure in a transplanted eelgrass (*Zostera marina*) meadow. *Marine Ecology Progress Series* **9**: 21 – 221.

Hurvich, C.M. and Tsai, C.-L. (1989). Regression and time series model selection in small samples. *Biometrika* **76**: 297 – 307.

Huston, M. (1979). A general hypothesis of species diversity. *American Naturalist* **III**: 81 – 101.

Isla, J.A. and Perissinotto, R. (2004). Effects of temperature, salinity and sex on the basal metabolic rate of the estuarine copepod *Pseudodiamtomus hessei*. *Journal of Plankton Research* **26**: 579 – 583.

Jeffries, H.P. (1972). Fatty-acid ecology of a tidal marsh. *Limnology and Oceanography* **17**: 433 – 440.

Jerling, H.L. and Wooldridge, T.H. (1995). Plankton distribution and abundance in the Sundays River estuary, South Africa, with comments on potential feeding interactions. *South African Journal of Marine Science* **15**: 169 – 184.

Johnson, W.S. (1976a). Biology and population dynamics of the intertidal isopod *Cirolana harfordia*. *Marine Biology* **36**: 343 – 350.

Johnson, W.S. (1976b). Population energetics of the intertidal isopod *Cirolana harfordai*. *Marine Biology* **36**: 351 – 357.

Jones, M.B. and Naylor, E. (1971). Breeding and bionomics of the British members of the *Jaera albifrons* group of species (Isopoda: Asellota). *Journal of Zoology, London* **165**: 183 – 199.

Jones, M.V. and West, R.J. (2005). Spatial and temporal variability of seagrass fishes in intermittently closed and open coastal lakes in southeastern Australia. *Estuarine, Coastal and Shelf Science* **64**: 277 – 288.

Jormalainen, V. (1998). Precopulatory mate guarding in crustaceans: male competitive strategy and intersexual conflict. *The Quarterly Review of Biology* **73**: 275 – 304.

Jormalainen, V., Honkanen, T and Heikkilä, N. (2001). Feeding preference and performance of a marine isopod on seaweed hosts: cost of habitat specialization. *Marine Ecology Progress Series* **220**: 219 – 230.

Joseph, V., Locke, A. and Godin, J-G. J. (2006). Spatial distribution of fishes and decapods in eelgrass (*Zostera marina* L.) and sandy habitats of a New Brunswick estuary, eastern Canada. *Aquatic Ecology* **40**: 111 – 123.

Juchault, P, Legrand, J.J. and Martin, G. (1974). Action interspecifique du facteur feminisant responsible de la thélygénie et de l'intersexualité du Crustacé *Armadillium vulgare* (Isopode Oniscoïde). *Annels de Embryologie et Morphologie* **7**: 265 – 276.

Juchault, P., Rigaud, T. and Mocquard, J.P. (1993). Evolution of sex determination and sex ratio variability in wild populations of *Armasillidium vulgare* (Latr.) (Crustacea, Isopoda): a case of conflict resolution. *Acta Œcologia* **14**: 547 – 562.

Kageyama, D., Hoshikazi, S. and Ishikawa, Y. (1998). Female-biased sex ratio in the asian corn borer, *Ostinia furnacalis*: evidence for the occurrence of feminizing bacteria in an insect. *Heredity* **81**: 311 – 316.

Kanouse, S., La Peyre, M.K. and Nyman, J.A. (2006). Nekton use of *Ruppia maritima* and non-vegetated bottom habitat types within brackish marsh ponds. *Marine Ecology Progress Series* ***327***: 61 – 69.

Kemp, J.O.G. and Froneman, P.W. (2004). Recruitment of ichthyoplankton and macrozooplankton during overtopping events in a temporarily open/closed southern African estuary. *Estuarine, Coastal and Shelf Science* **61**: 529 – 537.

Kensley, B. (1978). *Guide to the Marine Isopods of southern Africa*. Trustees of the South African Museum. Cape Town. 73 pp.

Kensley, B (1980). Decapod and isopod crustaceans from the west coast of southern Africa, including seamounts Vema and Tripp. *Annals of the South African Museum* **83**: 13 – 32.

Kensley, B. (1984). The South African Museum's Meiring Naude cruises. Part 15. Marine Isopoda of the 1977, 1978, 1979 cruises. Annals of the South African Museum 93: 88 - 88

Kinne, O. (1964). The effect of temperature and salinity on marine and brackish water animals. 2. Salinity and temperature-salinity combinations. Oceanography and Marine Biology, Annual Review 2: 281 - 339.

Kinne, O. (1966). Physiological aspects of animal life in estuaries with special reference to salinity. *Netherlands Journal of Sea Research* **3**: 222 – 244.

Klapow, L.A. (1970). Ovoviparity in the genus *Excirolana* (Crustacea : Isopoda). *Journal of Zoology, London* **162**: 359 – 369.

Klapow, L.A. (1972). Fortnightly moulting and reproductive cycles in the sand-beach isopod, *Excirolana chiltoni. Biological Bulletin* **143**: 568 – 591.

Kobayashi, S. (2002). Relative growth pattern of walking legs of the Japanese mitten crab *Eriocheir japonica. Journal of Crustacean Biology* **22**: 601 – 606.

Koch, F. and Weiser, W. (1983). Partitioning of energy in fish: can reduction in swimming activity compensate for the cost of production? *Journal of Experimental Biology* **107**: 141 – 146.

Kondzela, C.M. and Shirley, T.C. (1993). Survival, feeding, and growth of juvenile Dungeness crabs from southeastern Alaska reared at different temperatures. *Journal of Crustacean Biology* **13**: 25 – 33.

Koop, K. and Field, J.G. (1980). The influence of food availability on population dynamics of a supralittoral isopod, *Ligia dilatata* Brandt. *Journal of Experimental Marine Biology and Ecology* **48**: 61 – 72.

Koop, K. and Field, J.G. (1981). Energy transformation by the supralittoral isopod *Ligia dilatata* Brandt. *Journal of Experimental Marine Biology and Ecology* **53**: 221 – 233.

Koop, K., Bally, R. and McQuaid, C.D. (1983). The ecology of South African estuaries. Part XII: The Bot River, a closed estuary in the south-western Cape. *South African Journal of Zoology* **18**: 1 – 10.

Kopke, D. (1988). The climate of the Eastern Cape. In: *Towards an Environmental Plan for the Eastern Cape* (eds.) M.N. Bruton and F.W. Gess, pp 42 – 50. Rhodes University, Grahamstown, South Africa.

Kruczynski, W.L. and Subrahmanyam, C.B. (1978). Distribution and breeding cycle of *Cyathura polita* (Isopoda: Anthuridae) in a *Juncus roemerianus* marsh of northern Florida. *Estuaries* **1**: 93 – 100.

Kussakin, O.G. (1990). Biogeography of isopod crustaceans in the Boreal Pacific. *Bulletin of Marine Science* **46**: 620 – 639.

Lam, P.K.S, Dudgeon, D. and Ma, H.H.T. (1991). Ecological energetics of populations of four sympatric isopods in a Hong Kong forest. *Journal of Tropical Ecology* **7**: 475 – 490.

Lamberth, S.J. and Turpie, J.K. (2003). The role of estuaries in South African fisheries: economic importance and management implications. *African Journal of Marine Science* **25**: 131 – 157.

Lapucki, T., Normant, M., Feike, M., Graf, G. and Szanaiwska, A. (2005). Comparative studies on the metabolic rate of the isopod *Idotea chelipes* (Pallas) inhabiting different regions of the Baltic Sea. *Thermochimica Acta* **435**: 6 – 10.

Lardies, M. and Wehrtmann, I. (2001). Latitudinal variation in the reproductive biology of *Betaeus truncates* (Decapoda: Alpheidae) along the Chilean coast. *Ophelia* **55**: 55 -67.

Leber, K.M. (1985). The influence of predatory decapods, refuge and microhabitat selection on seagrass communities. *Ecology* **66**: 1951 – 1964.

Leonardson, K. (1992). Effects of cannibalism and alternative prey on population dynamics of *Sadura entomon* (Isopoda). *Ecology* **72**: 1273 – 1285.

Li, L. (2002). The taxonomy, biology and behaviour of *Dynoides daguilarensis* (Crustacea : Isopoda) in the Cape D'Aguilar Marine Reserve, Hong Kong. PhD thesis. University of Hong Kong.

Locke, A. and Corey, S. (1989). Amphipods, isopods and surface currents: a case for passive dispersal in the Bay of Fundy, Canada. *Journal of Plankton Research* **11**: 419 – 430.

Lockington, W.N. (1876). Remarks on the Crustacea of the Pacific Coast, with descriptions of some new species. *Proceedings of the California Academy of Science* **7**: 28 – 36.

Lockington, W.N. (1877). Descriptions of seventeen new species of Crustacea. *Proceedings of the California academy of Science* **7**: 41 – 48.

Lovern, J.A. (1964). The lipids of marine organisms. *Oceanography and Marine Biology: an Annual Revue **2***: 169 – 191.

Lubke, R.A (1998). The coastal environment. In: *Field guide to the eastern and southern Cape coasts*, (eds.) R.A. Lubke & I. de Moor, pp. 3 – 9. University of Cape Town Press.

Lutjeharms, J.R.E (1998). Coastal hydrography. In: *Field Guide to the Eastern and Southern Cape Coasts*, (eds.) R.A. Lubke & I. de Moor, pp. 50 – 61. University of Cape Town Press.

MacNae, W. (1957). The ecology of plants and animals in the intertidal regions of the Zwartkops estuary, near Port Elizabeth, South Africa. *Journal of Ecology* **45**: 361 – 387.

Marais, J.F.K. (1984). Feeding ecology of major carnivorous fish from four Eastern Cape estuaries. *South African Journal of Zoology* **19**: 210 -223.

Marsh, B.A. and Branch, G.M. (1979). Circadian and circatidal rhythms of oxygen consumption in the sandy beach isopod *Tylos granulatus* Krauss. *Journal of Experimental Marine Biology and Ecology* **37**: 77 – 89.

Martin, G., Gruppe, S.G., Laulier, M., Bouchon, D., Rigaud, T. and Juchault, P. (1994). Evidence for *Wolbachia* spp. In the estuarine isopod *Sphaeroma rugicauda* (Crustacea): a likely cytoplasmic sex ratio distorter. *Endocytobiosis & Cell Research* **10**: 215 – 225.

Martin, J. W. and Davis, G.E. (2001). An updated classification of the recent Crustacea. Natural History Museum of Los Angeles County Science Series 39, pp. 1 – 71. Los Angeles, CA.

Mason, H. and Marais, J.F.K. (1975). Stomach content analysis of mullet from the Swartkops Estuary. *Zoologica Africana* **10**, 193 – 207.

Mattila, J., Chaplin, G., Eilers, M.R., Heck, K.L. Jr., O'Neal, J.P. and Valentine, J.F. (1999). Spatial and diurnal distribution of invertebrate and fish fauna of a *Zostra maritima* bed and nearby unvegetated sediments in Damariscotta River, Maine (USA). *Journal of Sea Research* **41**: 321 – 332.

Mattila, J., Heck, K.L. Jr., Millstein, E., Miller, E., Gustafsson, C., Williams, S., Byron, D. (2008).Increased habitat structure does not always provide increased refuge from predation. *Marine Ecology progress Series* **361**: 15 – 20.

McKenney, C.L. (1994). Resistance patterns to salinity and temperature in an estuarine mysid (*Mysidopsis bahia*) in relation to its life cycle. *Comparative Biochemistry and Physiology* **109A**: 199 – 208.

McKenney, C.L. and Celestial, D.M. (1995). Interactions among salinity, temperature, and age on growth of the estuarine mysid *Mysidopsis bahia* reared in the laboratory through a complete life cycle. 1. Body mass and age-specific growth rate. *Journal of Crustacean Biology* **15**: 169 – 178.

McLachlan, A. and Grindley, J.R. (1974). Distribution of macrobenthic fauna of soft substrata in Swartkops estuary, with observations on the effects of floods. *Zoologica Africana* **9**: 211 – 233.

McLachlan, A. and Sieben, P.R. (1991). Growth and production of *Tylos capensis* Krauss, 1843 (Isopoda). *Crustaceana* **61**: 43 – 48.

McQueen, D.J. and Carnio, J.S. (1974). A laboratory study of the effects of some climatic factor on the demography of the terrestrial isopod *Porcellio spinicornis* Say. *Canadian Journal of Zoology* **44**: 599 – 611.

Meinhardt, H, Quadros, A.F. and Araujo, P.B. (2007). Growth curve of *Balloniscus glaber* Araujo & Zardo (Crustacea, Isopoda, Oniscidea) from Parque Estadual de Itapuá, Rio Grande do Sul, Brazil. *Revista Brasileira de Zoologia* **24**: 1108 – 1112.

Morant, P.D. and Quinn, N.W. (1999). *Influence of man and management on South African estuaries*. In: Allanson, B.R. and Baird, D. (Eds.). Estuaries of South Africa. Cambridge University Press, Cambridge. 289 – 320.

Mouny, P., Dauvin, J-C. and Zouhiri, S. (2000). Benthic boundary layer fauna from the Seine Estuary (eastern English Channel, France); spatial distribution and seasonal changes. *Journal of the Marine Biological Association of the United Kingdom* **80**; 959 – 968.

Napolitano, G.E., Pollero, R.J., Gayoso, A.M., MacDonald, B.A. and Thompson, R.J. (1997). Fatty acids as trophic markers of phytoplankton blooms in the Bahia Blanca Estuary (Buenos Aires, Argentina) and in Trinity Bay (Newfoundland, Canada). *Biochemical Systematics and Ecology* **25**: 739 – 755.

Newman, B.K., Wooldridge, T.H. and Cockcroft, A.C. (2007). Aspects of the biology and ecology of the estuarine cirolanid isopod, *Cirolana fluviatillis. African Zoology* **42**: 12 – 22.

Nozais, C., Perissinotto, R. and Tita, G. (2005). Seasonal dynamics of meiofauna in a South African temporarily open/closed estuary (Mdloti Estuary, Indian Ocean). *Estuarine, Coastal and Shelf Science* **62**: 325 – 338.

Oh, C-W., Suh, H-L., Park, K-Y, Ma, C-W. and Lim, H-S. (2002). Growth and reproductive biology of the freshwater shrimp *Exopalaemon modestus* (Decapoda: Palaemonidae) in a lake of Korea. *Journal of Crustacean Biology* **22**: 357 – 366.

Orth, R. (1977). The importance of sediment stability in seagrass communities. In: *Ecology of Marine Benthos,* (ed.) B.C. Coull, pp. 281 – 300. University South Carolina Press, Columbia.

Orth, R., Heck, K.L. Jr. and Van Montfrans, J. (1984). Faunal communities in seagrass beds: a review of the influence of plant structure and prey characteristics on predator-prey relationships. *Estuaries* **7**: 339 – 350.

Ovidiu Vlad, M. (1989). The optimal sex ratio for age-structured populations. *Mathematical Biosciences* **83**: 181 – 190.

Parrish, C.C. (1999). Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In: *Lipids in Freshwater Ecosystems*, (eds.) M.T. Arts and B.C. Wainman, pp. 4 – 20. Springer, New York.

Parrish, C.C., Abrajano, T.A., Budge, S.M., Helleur, R.J., Hudson, E.D., Pulchan, K., and Ramos, C. (2000). Lipid and phenolic biomarkers in marine ecosystems: analysis and application. In: *The handbook of environmental chemistry, Vol. 5, Part D* (ed.) P.J. Wangersky, pp. 193 – 223. Springer, Berlin.

Pauly, D. (1984). A mechanism for the juvenile-to-adult transition in fishes. *Journal of Conservation CIEM* **41**: 280 – 284.

Piertney, S.B. and Carvalho, G.R. (1996). Sex ratio variation in the intertidal isopod *Jaera albifrons*. *Journal of the Marine Biological Association of the United Kingdom* **76**: 825 – 828.

Perry, D.M. and Brusca, R.C. (1989). Effects of the root-boring isopod *Sphaeroma peruvianum* on red mangrove forests. *Marine Ecology Progress Series* **57**: 287 – 292.

Perissinotto, R., Walker, D.R., Webb, P., Wooldridge, T.H., and Bally, R. (2000). Relationships between zoo- and phytoplankton in a warm temperate, semi-permanently closed estuary, South Africa. *Estuarine, Coastal and Shelf Science* **51**: 1 – 11.

Perissinotto, R., Nozais, C., Kibirige, I. (2002). Spatio-temporal dynamics of phytoplankton and microphytobenthos in a South African temporarily-open estuary. *Estuarine, Coastal and Shelf Science* **54**: 363 – 374.

Perissinotto, R., Blair, A., Connell, A., Demetriades, N.T., Forbes, A.T., Harrison, T.D., Iyer, K., Joubrt, M., Kibirige, I., Mundree, S., Simpson, H., Stretch, D., Thomas, C., Thwala, X. and Zietsman I. (2004). *Contributions to* *information requirements for the implementation of resource-directed measures for estuaries.* Vol. 2: response of the biological communities to flow variation and mouth state in two Kwazulu-Natal temporarily open/closed estuaries. WRC report No. K5/1247. Water Research Commission, Pretoria, South Africa.

Peterson, C.H. (1979). Predation, competitive exclusion, and diversity in the soft-sediment benthic communities of estuaries and lagoons. In: *Ecological Processes in Coastal and Marine Systems*, (ed.) L.G. Livingstone, pp. 233 - 264. New York. Plenum Press.

Peterson, B.J., Howarth, R.W. and Garritt, R.H. (1985). Multiple stable isotopes used to trace the flow of organic matter through estuarine food webs. *Science* **227**: 1361 – 1363.

Platell, M.E. and Potter, I.C. (1996). Influence of water depth, season, habitat and estuary location on the macrobenthic fauna of a seasonally closed estuary. *Journal of the Marine Biological Association of the UK*, **76**: 1 – 21.

Ra'anan, Z. and Sagi, A. (1985). Alternative mating strategies in male morphotypes of the freshwater prawn *Macrobrachium rosenbergii* (de Haan). *Biological Bulletin* **169**: 592 – 602.

Rankin, D.J. and Kokko, H. (2007). Do males matter? The role of males in population dynamics. *Oikos* **116**: 335 – 348.

Reddering, J.S.V. (1988). Coastal and catchment basin controls on estuary morphology of the south-eastern Cape coast. *South African Journal of Science* **84**: 154 – 157.

Reddering, J.S.V. and Esterhuysen (1984). Research on sedimentation in estuaries. Sedimentation in the Kromme estuary. *Department of Geology, U.P.E., Rosie Report* **6**: 1 – 92.

Reddering, J.S.V. and Rust, I.C. (1990). Historical changes and sedimentary characteristics of Southern African estuaries. *South African Journal of Science* **86**: 425 – 428.

Richoux, N.B. and Froneman, P.W. (2007). Assessment of spatial variation in carbon utilization by benthic and pelagic invertebrates in a temperate South African estuary using stable isotope signatures. *Estuarine, Coastal and Shelf Science* **71**: 545 – 558.

Richoux, N.B. and Froneman, P.W. (2008).Trophic ecology of dominant zooplankton and macrofauna in a temperate, oligotrophic South African estuary: a fatty acid approach. *Marine Ecology Progress Series* **357**: 121 – 137.

Riddin, T. and Adams, J.B. (2008). Influence of mouth status and water level on the macrophytes in a small temporarily open/closed estuary. *Estuarine, Coastal and Shelf Science* **79**: 86 – 92.

Robertson, A.I. (1980). The structure and organisation of an eelgrass fish fauna. *Oecologia* **47**: 76 – 82.

Roman, M.-L. and Dalens, H. (1999). Ordre des Isopodes (Épicarides exclus) (Isopoda Latreille, 1817). In: *Traité de Zoologie. Anatomie, Systématique, Biologie. Tome VII, Fascicule IIIA. Crustacés Péracarides,* (ed.) J. Forest. Memoires de l'Institut Oceanographique Foundation Albert I^{er}, Prince de Monaco, **19**: 177 – 278.

Romano, N. and Zeng, C. (2006). The effects of salinity on the survival, growth and haemolymph osmorality of early juvenile blue swimmer crabs, *Portunus pelagicus*. *Aquaculture* **260**: 51 – 162.

Ross, G.J.B (1988). Coastal hydrography. In: A F*ield Guide to the Eastern Cape Coasts*, (eds.) R.A. Lubke, F.W. Gess, & M.N. Bruton, pp. 31 – 32. Grahamstown Centre of the Wildlife Society of Southern Africa.

Saito, H. and Kotani, Y. (2000). Lipids of four boreal species of calanoid copepods: origin of monoene fats of marine animals at higher trophic levels in the grazing food chain in the subarctic ocean system. *Marine Chemistry* **36**: 233 – 248.

Sala, E. and Graham, M.H. (2002). Community-wide distribution of predator-prey interaction strength in kelp forests. *Proceedings of the National Academy of Science USA*, **99**: 3678 – 3683.

Saliot, A., Laureillard, J., Scribe, P. and Sicre, M.A. (1991). Evolutionary trends in the lipid biomarker approach for investigating the biochemistry of organic matter in the marine environment. *Marine Chemistry* **36**: 233 – 248.

Sars, G.O. (1897a). Isopoda. Part V, VI. Idotheidae, Arcturidae, Asellidae, Ianniridae, Munnidae. *An Account of the Crustacea of Norway with Short Descriptions and Figures of all the Species.* II. Plates XXXIII – LIX. Bergen Museum: Bergen, Norway.

Sars, G.O. (1897b). Isopoda. Part VII, VIII. Desmosomidae, Munnopsidae. *An Account of the Crustacea of Norway with Short Descriptions and Figures of all the Species*. II. Plates L – LXIII. Bergen Museum: Bergen, Norway.

Sars, G.O. (1898). Isopoda. Part IX, X. Munnopsidae (concluded), Ligiidae, Trichoniscidae, Oniscidae. *An Account of the Crustacea of Norway with Short Descriptions and Figures of all the Species*. II. Plates LXIV – LXXXIV. Bergen Museum: Bergen, Norway.

Sars, G.O. (1899a). Isopoda. *An Account of the Crustacea of Norway with Short Descriptions and Figures of all the Species*. II. I -X. Bergen Museum: Bergen, Norway.

Sars, G.O. (1899b). Isopoda. Part XIII, XIV. Cryptoniscidae, Appendix. *An Account of the Crustacea of Norway with Short Descriptions and Figures of all the Species.* II. 233 -270. Plates XCVII – C, Supplement Plates I – IV. Bergen Museum: Bergen, Norway.

Schlacher, T.A. and Wooldridge, T.H. (1996). Axial zonation patterns of subtidal macrozoobenthos in the Gamtoos Estuary, South Africa. *Estuaries* **19**: 680 – 696.

Schnute, J. (1981). A versatile growth model with statistically stable parameters. *Canadian Journal of Fisheries and Aquatic Sciences* **38**: 1128 – 1140.

Schubel, J.R. and Hirschberg, D.J. (1978). Estuarine graveyards, climatic change and the importance of the estuarine environment. In: *Estuarine Interactions*, (ed.) Wiley, M.L., pp. 285 – 303. Academic Press, New York.

Scott, C.L., Falk-Petersen, S., Gulliksen, B., Lønne, O.J. and Sargent, J.R. (2001). Lipid indicators of the diet of the sympagic amphipod *Gammarus wilkitzkii* in the Marginal Ice Zone and in open waters of Svalbard (Arctic). *Polar Biology* **24**: 572 – 576.

Seaborn, G.T. Moore, K.M., Balazs, G.H. (2005). Depot fatty acid composition in immature green turtles (*Chenolia mydas*) residing at two near-shore foraging areas in the Hawaiian Islands. *Comparative Biochemistry and Physiology B* **140**: 183 – 195.

Shachak, M, Chapman, E.A. and Steinberger, Y. (1976). Feeding, energy flow and soil turnover in the desert isopod, *Hemilepstus reaumuri. Oecologia* **24**: 57 – 69.

Shachak. M., Steinberger, Y. and Orr, Y. (1979). Phenology, activity and regulation load in the desert isopod, *Hemilepstus reaumuri. Oecologia* **40**: 133 – 140.

Shafir, A. and Field, J.G. (1980a). Importance of a small carnivorous isopod in energy transfer. *Marine Ecology Progress Series* **3**: 203 – 215.

Shafir, A. and Field, J.G. (1980b). Population dynamics of the isopod *Cirolana imposita* Barnard in a kelpbed. *Crustaceana* **39**: 185 – 196.

Shuster, S.M. (1987). Alternative reproductive behaviours: three discrete male morphs in *Paracerceis sculpta*, an intertidal isopod from the northern Gulf of California. *Journal of Crustacean Biology* **7**: 318 – 327.

Shuster, S.M. (1991). *The ecology of breeding females and the evolution of polygyny in Paracerceis sculpta, a marine isopod crustacean*. In: *Crustacean Sexual Biology,* (eds.) R. Bauer and J. Martin, pp. 91 – 110. Columbia University Press.

Shuster, S.M. (1995). Female reproductive success in artificial sponges in *Paracerceis sculpta* (Holmes) (Crustacea: Isopoda). *Journal of Experimental Marine Biology and Ecology* **19**: 19 – 27.

Shuster, S.M. and Guthrie, E.E. (1999). Effects of temperature and food availability on adult body length in natural and laboratory populations of *Paracerceis sculpta (*Holmes), a Gulf of California isopod. *Journal of Experimental Marine Biology and Ecology* **233**: 269 – 284.

Shuster, S.M. and Wade, M.J. (1991). Equal mating success among male reproductive strategies in a marine isopod. *Nature* **350**: 608 – 610.

Simpson, K.W., Fagnani, J.P., DeNicola, D.M., Bode, R.W., 1985. Widespread distribution of some estuarine crustaceans (*Cyathura polita, Chiridotea almyra, almyracuma proximoculi*) in the limnetic zone of the lower Hudson River, New York. *Estuaries* **8**: 373 – 380.

Spanonopoulos-Hernández, M., Martinez-Palacios, C.A., Vanegas-Pérez, R.C., Rosas, C., Ross, L.G., 2005. The combined effects of salinity and temperature on the oxygen consumption of juvenile shrimps *Litopenaeus stylirostris* (Stimpson, 1874). *Aquaculture* **244**: 341 – 348.

Staples, D.J. and Heales, D.S. (1991). Temperature and salinity optima for growth and survival of juvenile banana prawns *Penaeus merguiensis*. *Journal of Experimental Marine Biology and Ecology* **154**: 251 – 274.

StatSoft, Inc. (2004). STATISTICA (data analysis software system), Version 7. www.statsoft.com

Steele, D.H. and Steele, V.J. (1991). Effects of salinity on the survival, growth rate, and reproductive output of *Gammarus lawrencanus* (Crustacea: Amphipoda). *Marine Ecology Progress Series* **78**: 49 – 56.

Stone, A. W. (1988). Climate and weather. In: A f*ield guide to the Eastern Cape coasts*, (eds.) R.A. Lubke, F.W. Gess, & M.N. Bruton, pp. 19 – 30. Grahamstown Centre of the Wildlife Society of Southern Africa.

Stone, A.W., Weaver, AvB. & West, W.O. (1998) Climate and Weather. In: *Field Guide to the Eastern and Southern Cape Coasts*, (eds.) R.A. Lubke and I. de Moor, pp. 41 – 49. University of Cape Town Press, South Africa.

Strong, K.W. and Daborn, G.R. (1979). Growth and energy utilization of the intertidal isopod *Idotea baltica* (Pallas)(Crustacea: Isopoda). *Journal of experimental marine Biology and Ecology* **41**: 101 – 123.

Sutton, S.L., Hassel, M., Willows, R., Davis, R.C., Grundy, A. and Sunderland, K.D. (1984). Life histories of terrestrial isopods: a study of intra- and interspecific variation. In: *The Biology of Terrestrial Isopods*, (eds.) S.L. Sutton & D.M. Holdich. Symposium of the Zoological Society of London, London **53**: 269 – 294.

Tanaka, K. (2003). Population dynamics of the sponge-dwelling gnathiid isopod *Elaphhognathia cornigera. Journal of the Marine Biology Association of the U.K.* **83**: 95 – 102.

Teske, P.R. and Wooldridge, T.H. (2001). A comparison of the macrobenthic faunas of permanently opn and temporarily open/closed South African estuaries. *Hydrobiologia* **464**: 227 – 243.

Teske, P.R. and Wooldridge, T.H. (2003). What limits the distribution of subtidal macrobenthos in permanently open and temporarily open/closed South African estuaries? Salinity vs. sediment particle size. *Estuarine, Coastal and Shelf Science* **57**: 225 – 238.

Teske, P.R. and Wooldridge, T.H. (2004). Affinities of some common estuarine macroinvertebrates to salinity and sediment type: empirical data from Eastern Cape estuaries, South African. *African Zoology* **39**: 183 – 192.

Teske, P.R., McQuaid, C.D., Froneman, P.W. and Barker, N.P., (2006). Impacts of marine bogeographic boundaries on phylogeographic patterns of three South African estuarine crustaceans. *Marine Ecology Progress Series* **314**: 283 – 293.

Teske, P.R., Papadopoulos, I., Zardi, G.I., McQuaid, C.D., Edkins, M.T., Griffiths, C.L. and Barker, N.P. (2007). Implications of life history for genetic structure and migration rates of southern African coastal invertebrates: planktonic, abbreviated and direct development. *Marine Biology* **152**: 697 – 711.

Thatcher, V.E., de Loyola e Silve, J., Jost, G.F. and Souza-Conceição, J.M. (2003). Comparative morphology of *Cymothoa* spp. (Isopoda, Cymothoidae) from Brazilian fishes, with the description of *Cymothoa catarinensis* sp. nov. and redescriptions of *C. excisa* Perty and *C. oestrum* (Linnaeus). *Revista Brasileira de Zoologia* **20**: 541 – 552.

Thiel, M. (2003). Rafting of benthic macrofauna: important factors determining the temporal succession of the assemblage on detached macroalgae. *Hydrobiologia* **503**: 49 - 57.

Thorpe, J.E. (1987). Smolting versus residency: development conflict in salmonids. *American Fisheries Society Symposium* **1**: 244 – 252.

Tooru, U., Sohei, K. and Hidetsugu, Y. (2004). Spatial distribution and population structure of the benthic sphaeromatid isopod *Tecticeps glaber* in the sandy beach of Hamanaka Bay, northern Japan. *Nippon Suisan Gakkaishi* **70**: 516 – 522.

Tsai, M-L. and Dai, C-F. (2003) Cannibalism within mating pairs of the parasitic isopod, *Ichthyoxenus fushanensis. Journal of Crustacean Biology* **23**: 662 – 668.

Tulley, T.S., Crooks, J.A. and Levin, L.A. (2001). Habitat utilisation and alteration by the invasive burrowing isopod, *Sphaeroma quoyanum*, in Californian salt marshes. *Marine Biology* **138**: 561 – 573.

Turpen, S., Hunt, J.W., Anderson, B.S. and Pearse, J.S. (1994). Population structure, growth, and fecundity of the kelp forest mysid *Holmesimysis costata* in Monterey Bay, California. *Journal of Crustacean Biology* **14**: 657 – 664.

Vandendriessche, S., Vincx, M. and Degraer, S. (2006). Floating seaweed in the neustonic environment: a case study from Belgian coastal waters. *Journal of Sea Research* **55**: 103 – 112.

Vanhöffen, E. (1914). Die Isopoden der Deutschen Südpolar Expidition, 1901 – 1903. *Deutschen Südpolar Expedition* **7**: 447 – 598.

Van Niekerk, L., Bate, G.C. and Whitfield, A.K. (eds.) (2008). An Intermediate Ecological Reserve determination study of the East Kleinemonde Estuary. Water Research Commission, Pretoria.

Vásquez, J.A. (1993). Effects on the animal community of dislodgement of holdfasts of *Macrocystis pyrifera. Pacific Science* **47**: 180 – 184.

Vivier, L. and Cyrus, D.P. (2001). Juvenile fish recruitment through wave overtopping into a closed subtropical estuary. *African Journal of Aquatic Science* **26**: 109 – 113.

Von Bertalanffy, L. (1938). A quantitative theory of organic growth (inquiries on growth laws). *Human Biology* **10**: 181 – 213.

Vorwerk, P.D., Whitfield, A.K., Cowley, P.D. and Paterson, A.W. (2003). The influence of selected environmental variables on fish assemblage structure in a range of south-east African estuaries. *Environmental Biology of Fishes* **66**: 237 – 247.

Vorwerk, P.D., Froneman, P.W., Paterson, A.W. Strydom, N.A. and Whitfield, A.K. (2008). Biological responses to a resumption in river flow in a freshwater-deprived, permanently open southern African estuary. W*ater SA* **34**: 1 – 8.

Walker, D.R., Perissinotto, R. and Bally R.P.A. (2001). Phytoplankton/protozoan dynamics in the Nyara estuary, a small temporarily open system in the Eastern Cape (South Africa). *African Journal of Aquatic Science* **26**: 31 – 38.

Wallace, J.H., Kok, H.M., Beckley, L.E., Bennett, B., Blaber, S.J.M. and Whitfield, A.K. (1984). South African estuaries and their importance to fishes. *South African Journal of Science* **80**: 203 – 207.

Wallerstein, B.R. and Brusca, R.C. (1982). Fish predation: a preliminary study of its role in the zoogeography and evolution of shallow water idoteid isopods (Crustacea: Isopoda: Idoteidae). *Journal of Biogeography* **9**: 135 – 150.

Walsh, C.J. and Mitchell, B.D. (1998). Factors associated with variations in abundance of epifaunal shrimps between and within seagrass meadows. *Marine and Freshwater Research* **49**: 769 – 777.

Warburg, M.R. (1968). Behavioural adaptations of terrestrial isopods. *American Zoologist* **8**: 545 – 559.

Warburg, M.R. (1993). *Evolutionary Biology of Land Isopods*. Springer-Verlag, Berlin, 159pp.

Werren, J.H. (1997). Biology of *Wolbachia*. *Annual Review of Entomology* **42**: 587 – 609.

Werren, J.H. and O'Niell, S.L. (1997). The evolution of heritable symbiots. In: *Influential Passengers: Inherited Micro-organisms and Arthropod Reproduction*, (eds.) S.L. O'Niell, J.H. Werren and A.A. Hoffmann, pp 1 – 41. Oxford University Press, Oxford.

Werren, J.H., Windsor, D. and Guo, L. (1995). Distribution of *Wolbachia* among neotropical arthropods. *Proceedings of the Royal Society of London Series B, Biological Sciences*, **263**: 197 – 204.

West, J.B., Bowen, G.J., Cerling, T.E. and Ehleringer, J.R. (2006). Stable isotopes as one of nature's ecological recorders. *Trends in Ecology and Evolution* **21**: 408 – 414.

Wetzer, R. and Brusca, R.C. (1997). The order Isopoda. Descriptions of the species of the suborders Anthuridea, Epicaridea, Flabillifera, Gnathiidea and Valvalifera. In: *Taxanomic Atlas of the Benthic Fauna of the Santa Maria Basin and Western Santa Barbara Channel, Volume 11, The Crustacea Part 2: Isopoda, Cumacea and Tanaidacea,* (eds.) J.A. Blake and P.H. Scott, pp 9 – 58. Santa Barbara Museum of Natural History, Santa Barbara, California.

Wetzer, R., Brusca, R.C. and Wilson, G.D.F. (1997). The order Isopoda. In: *Taxanomic Atlas of the Benthic Fauna of the Santa Maria Basin and Western Santa Barbara Channel, Volume 11, The Crustacea Part 2,* (ed. J.A. Blake and P.H. Scott), pp 1 – 120. Santa Barbara Museum of Natural History, Santa Barbara, California.

Whitfield, A.K. (1980a). Food chains in Lake St Lucia. In: *Studies on the Ecology of Maputaland,* (eds.) M.N. Bruton and K.H. Cooper, pp. 427 – 431. Rhodes University, Grahamstown.

Whitfield, A.K. (1980b). Distribution of fishes in Mhlanga estuary in relation to food resources. *South African Journal of Zoology* **15**: 159 – 165.

Whitfield, A.K. (1989). The benthic invertebrate community of a southern Cape estuary: structure and possible food resources. *Transactions of the Royal Society of South Africa* **47**: 159 – 179.

Whitfield, A.K. (1992a). A characterization of southern African estuarine systems. *South African Journal of Aquatic Science* **18**: 89 – 103.

Whitfield, A.K. (1992b). Juvenile fish recruitment over an estuarine sand bar. *Ichthos* **36**: 23 - 23.

Whitfield, A.K. (1993). Fish biomass estimates from the littoral zone of an estuarine coastal lake. *Estuaries* **16**: 208 – 298.

Whitfield, A.K. (1998). Biology and ecology of fishes in southern African estuaries. *Ichthyological Monographs of the J.L.B. Smith Institute of Ichthyology*, No. 2, 223 pp.

Whitfield, A.K. (2000). *Available scientific information on individual South African estuarine systems* (217 pp.). Water Commission Report No. 577/3/00, Pretoria.

Whitfield, A.K., Allanson, B.R. and Heinecken, T.J.E. (1983). Report No. 22: Swartvlei (CMS 11). In: *Estuaries of the Cape. Part II: Synopses of Available Information on Individual Systems,* (eds.) A.E.F. Heydorn and J.R. Grindley. *Council for Scientific and Industrial Research Report* **421**: 1 – 62.

Whitfield, A.K. and Blaber, S.J.M. (1978). Resource segregation among iliophagus fish in Lake St Lucia, Zululand. *Environmental biology of Fishes* **3**: 293 – 296.

Whitfield, A.K. and Bruton, M.N. (1989). Some biological implications of reduced freshwater inflow into eastern Cape estuaries: a preliminary assessment. *South African Journal of Science* **85**: 691 – 694.

Whitfield, A.K. and Bate, G.C. (eds) (2007). *A review of information on temporarily open/closed estuaries in the warm and cold temperate biogeographic regions of South Africa, with particular emphasis on the influence of river flow on these systems.* Water Research Commission Report No. 1581/1/07: 214 pp.

Whitfield, A.K., Adams, J.B., Bate, G.C., Bezuidenhout, K., Bornman, T.G., Cowley, P.D., Froneman,P.W., Gama, P.T., James, N.C., Mackenzie, B., Riddin, T., Snow, G.C., Strydom, N.A., Taljaard, S., Terörde, A.L., Theron, A.K., Turpie, J.K., Van Niekerk, L., Vorwerk, P.D. and Wooldridge, T.H. (In press). A multidisciplinary study of a small, temporarily open/closed South African estuary, with particular emphasis on the influence of mouth state on the ecology of the system. *African Journal of Marine Science*.

Wilson, G.D.F. (1998). Historical influences on deep-sea isopod diversity in the Atlantic Ocean. In: *Deep-sea Biodiversity: a Compilation of Recent Advances in Honour of Robert R. Hessler* (ed. C.R. Smith, L.S. Mullineaux and L.A. Levin). *Deep-Sea Research Part II. Tropical Studies in Oceanography* **45**: 279 – 301.

Wilson, G.D.F. and Fenwick, G.D. (1999). Taxonomy and ecology of *Phreatoicus typicus* Chilton (Isopoda, Phreatoicidea). *Journal of the Royal Society of New Zealand* **29**: 41 – 64.

Wilson, G.D.F. and Ho, E.L. (1996). *Crenoicus* Nicholls, 1994, (Crustacea: Isopoda: Phreatoicidea): sytematics and biology of a new species from New South Wales. *Records of the Australian Museum* **48**: 7 – 32.

Wilson, G.D.F. and Johnson, R.T. (1999). Ancient endemism among freshwater isopods (Crustacea, Phreatoicidea). In: *The Other 99%. The Conservation and Biodiversity of Invertebrates* (eds.) W.F. Ponder and D. Lunney, pp. 264 – 268. Transactions of the Royal Zoological Society of New South Wales. Mossman.

Wilson, G.D.F. and Keable, S.J. (1999). A new genus of phreatoicidean isopod (Crustacea) from the North Kimberly Region, Western Australia. *Zoological Journal of the Linnean Society* **126**: 51 - 79.

Wilson, G.D.F. and Keable, S.J. (2001). Systematics of the Phreatoicidea. In: *Isopod Systematics and Evolution* (eds. R.C. Brusca and B. Kensley). Special Publication of the Second Isopod Conference. *Crustacean Issues* **13**: 175 – 194. A.A. Balkema, Rotterdam.

Wilson, G.D.F. and Keable, S.J. (2002). New genera of Phreatoicidea (Isopoda; Crustacea) from Western Australia. *Records of the Australian Museum* **54**: 41 – 70.

Wong, Y.M. and Moore, P.G. (1996). Observations on the activity and life history of the scavenging isopod *Natatolana borealis* Lilljeborg (Isopoda: Cirolanidae) from Loch Fyne, Scotland. *Estuarine, Coastal and Shelf Science* **42**: 247 – 262.

Wooldridge, T.H. (1991). Exchange of two species of decapod larvae across an estuarine mouth inlet and implications of anthropogenic changes in the frequency and duration of mouth closure. *South African Journal of Science* **87**: 519 – 525.

Wooldridge, T.H. (1999). Estuarine zooplankton community structure and dynamics. In: *Estuaries of South Africa,* (eds.) B.R. Allanson, and D. Baird, pp 141 – 166. Cambridge University Press, Cambridge, United Kingdom.

Wooldridge, T.H. and Callahan, R. (2000). The effects of a single freshwater release into the Kromme estuary. 3: Estuarine zooplankton response. *Water SA* **26**: 311 – 318.

Wooldridge, T.H. and McGwynne, L. (1996). *The estuarine environment*. Report No. C31. Institute of Coastal research, Port Elizabeth, South Africa. 91 pp.

Yamada, K., Hori, M., Tanaka, Y., Hasegawa, N.and Kakaoka, M. (2007). Temporal and spatial macrofaunal community changes along a salinity gradient in seagrass meadows of Akkeshi-ko estuary and Akkeshi Bay, northern Japan. *Hydrobiologia* **592**: 345 – 358.

Zajac, R.N. and Whitlatch, R.B. (1982). Responses of estuarine fauna to disturbance. II: Spatial and temporal variations of succession. *Marine Ecology Progress Series* **10**: 15 – 27.

Zein-Eldin, Z.P. and Aldrich, D.V. (1965). Growth and survival of postlarval *Penaeus aztecus* under controlled conditions of temperature and salinity. *Biological Bulletin* **129**: 199 – 216.

Zhou, W., Rousset, F. and O'Niell, S. (1998). Phylogeny and PCR-based classification of *Wolbachia* strains using *wsp* gene sequencing. *Proceedings of the Royal Society of London Series B, Biological Sciences*, **265**: 509 – 515.

Zimmer, C. (2001). *Wolbachia*: a tale of sex and survival. *Science* **292**: 1093 – 1095.