POPULATION STRUCTURE, PHYSIOLOGY AND FEEDING ECOLOGY OF THE MYSID *Mesopodopsis wooldridgei* (WHITTMANN) IN A LARGE PERMANENTLY OPEN ESTUARY

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ABSTRACT

This project had three main aims. The first aim was to investigate the metabolic physiology of the mysid *Mesopodopsis wooldridgei*. Secondly, the study examined the contribution of the mysid to the zooplankton community. Finally the study attempted to clarify the primary sources of carbon utilised by the mysid using stable carbon isotope analysis. The study was conducted in the freshwater dominated permanently open Great Fish Estuary (33°30'S; 27°08'E) located along the south-eastern coastline of southern Africa.

Mass specific oxygen consumption rates of *Mesopodopsis wooldridgei* ranged between 0.11 and 8.38 µl O_2 mg ww^{-1} h^{-1} and increased with an increase in water temperature. There were no significant differences in the respiration rates between the male and female mysids ($P > 0.05$). At a constant temperature (20°C), the oxygen consumption rate of *M. wooldridgei* decreased with an increase in salinity. Salinity tolerance of the mysid was strongly modified by temperature. At 5‰ and 30°C, mysids exhibited mass mortality. The Q_{10} value for *M. wooldridgei* at 15, 25 and 35‰ was estimated at 2.34, 1.44 and 2.14, respectively. Results of the study suggest that *M. wooldridgei* is well adapted to surviving in environments characterised by variations in temperature and salinity.

Within the Great Fish Estuary, total chl. *a* concentration ranged between 2.68 µg L^{-1} and 31.12 µg L^{-1} and was always dominated by large phytoplankton cells (>5 µm). Average zooplankton abundance ranged between 62 and 28 917 ind. m^{-3} and biomass between 10 and 203 mg Dwt m^{-3}. The zooplankton community was numerically dominated by the calanoid copepod *Pseudodiaptomus hessei*, which comprised up to 100% (range between 12 and 100%) of the total zooplankton counted. Total zooplankton biomass during the day was dominated by copepods and by mysids during the nighttime. Among the mysids, *M.*

wooldridgei was the most numerically abundant mysid and comprised <10% of the total zooplankton abundance. Numerical analysis found no seasonal pattern in the total zooplankton abundance (including mysids). The lack of any seasonality could be attributed to the continuous freshwater inflow into the estuary due to the inter-basin transfer of water from the Gariep Dam to the Fish River system. A distinct spatial pattern in the zooplankton community structure was evident with the upper stations almost entirely dominated by the copepod *P. hessei*, while at stations occupied in the lower reaches of the estuary, the zooplankton community comprised a mixture of freshwater, estuarine and marine breeding zooplankton species. The mysids also demonstrated a distinct spatial pattern in their distribution. Mysids were generally absent from the upper reaches, while in the middle reaches *Rhopalophthalmus terranatalis* and *M. wooldridgei* were numerically dominant. *Gastrosaccus brevifissura* dominated in the lower regions of the estuary.

Stable isotope analysis ($\delta^{13}C$) indicated that the dominant source of carbon utilised by the numerically dominant copepods and juvenile *M. wooldridgei* within the estuary was derived from the extensive phytoplankton stocks within the system. In contrast, sub-adult and adult mysids (*R. terranatalis*, *M. wooldridgei* and *G. brevifissura*) appeared to consume a combination of phytoplankton and copepods. The contribution of the various sources of carbon to the total carbon intake of the mysid remains unknown.

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DECLARATION

The work described in this thesis was carried out in the Department of Zoology and Entomology at Rhodes University under the supervision of Dr. William Froneman. These studies represent original work by the author and have not been submitted in any form to another university. Where use was made of the work of others, it has been duly acknowledged in the text.

CHAPTER 1 - GENERAL INTRODUCTION

Mysids are a ubiquitous component of the plankton in a variety of aquatic environments including in freshwater, estuarine and marine systems (Viherluoto, 2001). These organisms have been demonstrated to have a wide variety of feeding habits (Jerling and Wooldridge, 1995a), ranging from a herbivorous to carnivorous feeding mode (Fulton, 1982a). As a consequence, mysids are important consumers of both primary and secondary production within aquatic systems (Fulton, 1982b; Grange, 1992). Because mysids also represent an important food source for a number of predators including fish, birds and carnivorous invertebrates (Mauchline, 1980), they can be considered as a key component of aquatic foodwebs, particularly in estuaries.

The distribution of mysids from a variety of aquatic systems has been shown to be largely influenced by temperature and salinity or a combination thereof (Baldó *et al*, 2001; Roast *et al*, 1999). For example, the distribution of *Schistomysis spiritus* in the Bristol Channel was found to result from combined effect of both temperature and salinity (Williams and Collins, 1984). A similar pattern was demonstrated for the mysid, *Rhopalophthalmus mediterraneus* in an estuary in southwest Spain (Baldó *et al*, 2001). Biological factors, which affect the distribution of mysids within aquatic systems include feeding, reproduction and predation (Froneman, 2001a; Jerling and Wooldridge, 1995a; Roast *et al*, 2000; Webb *et al*, 1987; Wooldridge and Webb, 1988). The availability of appropriate food sources can likewise affect mysid distribution within estuaries.

Previous studies on feeding ecology of mysids has shown ontogenetic development strongly impacts the mysid feeding strategies (Siegfried and Kopache, 1980; Toda and Wada, 1990; Viherluoto *et al*, 2000). While adult mysids appear largely to be carnivorous, juveniles appear to feed mainly on phytoplankton. Immature mysids feed on a combination of both phytoplankton and heterotrophic carbon sources and can therefore be regarded as omnivorous (Siegfried and Kopache, 1980; Toda and Wada, 1990; Viherluoto *et al*, 2000; Froneman, 2001a).

1.1 SOUTH AFRICAN ESTUARIES

The South African coastline can broadly by divided into three distinct zones, the cool temperate along the west-coast, the warm south temperate along the east coast and the subtropical zone along the north coast (Figure 1.1). Within these three zones are 258 functional estuaries. The most widely accepted definition of South Africa's estuaries is that of Day (1980), which defines 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 freshwater derived from land drainage."

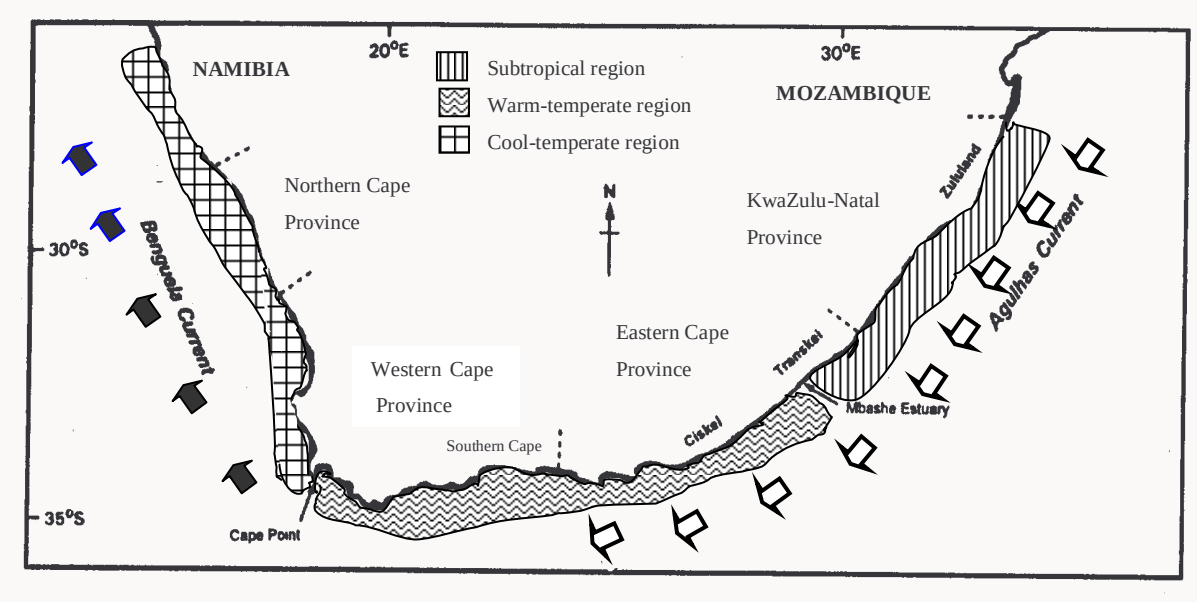

Figure 1.1: Map of southern Africa showing the three major biogeographic regions (adapted from Whitfield, 2000).

Day's definition adequately describes the variety of functional estuaries recorded along the South African coastline. Whitfield (1992) classified the estuaries within each region according to physico-chemical variables and mouth state. Based on these variables, 5 types of estuaries can be identified: permanently open estuaries, temporarily open/closed estuaries, river mouths, estuarine lakes and estuarine bays (Table 1.1). Among the five categories, the permanently open and temporarily open/closed estuaries account for approximately 98% of all estuaries along the southern African coastline (Whitfield, 1992).

Table 1.1: Physical characteristics of the 5 main types of South African estuaries (adapted from Whitfield, 1992).

System	Tidal prism	Mixing process	Average salinity
Estuarine bay	>10 x 10^6 m^3	Tidal	20-35‰
Permanently open estuary	1-10 x 10^6 m^3	Tidal/riverine	10->35‰
River mouth	<1-10 x 10^6 m^3	Riverine	<10‰
Estuarine lake	<0.1 x 10^6 m^3	Wind	1->35‰
Temporarily open estuary	Absent	Wind	1->35‰

Permanently open estuaries are generally characterized by the presence of horizontal and vertical salinity gradients, moderate tidal prism and catchment areas ranging between 500 and 10 000 km^2 (Whitfield, 1992). Salinities within the estuary may vary between 5 - 30‰ but hypersaline conditions are possible under conditions where freshwater impoundments or abstraction dramatically reduces the freshwater inflow into the system (eg: Kariega Estuary along the Eastern Cape coast) (Whitfield, 1992). The water temperature regime within the estuary is determined by the prevailing influence of the sea or the magnitude of freshwater inflow and at times a mixture of the two sources (Whitfield, 1992).

Permanently open estuaries have been the focus of the bulk of the research despite the fact that they comprise < 30% of the estuaries along the South African coastline (Perissinotto *et al*, 2000). The bias towards the larger systems can be ascribed to the fact that these estuaries often support large commercial fisheries (vertebrate and invertebrate) and that they represent focal points for coastal development (Morant and Quinn, 1999).

1.2 ZOOPLANKTON COMMUNITY

The zooplankton species composition, biomass and distribution in permanently open southern Africa estuaries have been investigated on a number of occasions (Wooldridge, 1999 and references therein). Results of these studies indicate that the zooplankton community is numerically dominated by copepods of the genera *Pseudodiaptomus, Acartia, Halicyclops* and *Oithona*, which may at times contribute up to 95% of the total zooplankton. The contribution of the remaining groups including mysids, isopods, cumaceans and decapods to

total zooplankton abundance is generally less than 20%. However in terms of biomass, mysids contribute a significantly greater portion of the zooplankton community (Froneman, 2001a; Grindley, 1981). For example, in the Great Fish Estuary, mysids contributed up to 70% of the total zooplankton biomass (Grange, 1992). Shifts in the zooplankton community structure, biomass and distribution in permanently open estuaries has been linked to amongst other, freshwater inflow, temperature, food availability (Froneman, 2001a; Fry and Sherr, 1984; Grange *et al*, 2000; Paterson and Whitfield, 1997; Roast *et al*, 1999) and biological interactions including inter- and intra-specific competition (Wooldridge and Bailey, 1982; Wooldridge and Webb, 1988) and predation by both vertebrates and invertebrates.

Estimates of the zooplankton abundance and biomass in permanently open southern African estuaries are highly variable reflecting the differences in the physico-chemical variables between the different systems (Table 1.2). In general, the highest zooplankton abundance and biomass values are recorded in those systems characterised by sustained freshwater inflow (Table 1.2). In these systems, zooplankton abundance and biomass may exceed 10^5 ind. m^{-3} and 10^3 mg Dwt m^{-3}, respectively. Conversely, in freshwater deprived systems, average zooplankton biomass is generally less than 50 mg Dwt m^{-3} (Grange *et al*, 2000). The reduced zooplankton biomass found in permanently open freshwater deprived estuaries is thought to result from reduced food availability (chl. *a*) as freshwater flow represents the primary source of nutrients necessary to sustain the growth of phytoplankton (Froneman, 2000a).

Table 1.2: Summary of the available abundance and biomass values for the total zooplankton community in selected permanently open estuaries along the Eastern Cape coastline.

Estuary	Rate of freshwater input	Abundance (ind. m^{-3})	Biomass (mg m^{-3})	Reference
Kromme	Deprived	4919.7 (avg)	160.7 (avg)	Scharler *et al* (1998)
Swartkops	Intermediate	7530 (avg)	<10-100 (range)	Grindley (1981)
Sundays	Continuous	14805.9 (avg)	<10-1450 (range)	Wooldridge and Bailey (1982)
Kariega	Deprived	126-16 468 (range)	20-110 (range)	Froneman (2001a); Grange (1992)
Great Fish	Dominated	N/A	256-11 000 (range)	Grange (1992)

The zooplankton distribution within permanently open estuaries largely reflects the physiological adaptations of individual zooplankton species. For example, peaks in the abundances of *Acatia longipatella* and *A. natalensis* occur in different zones along the estuarine salinity gradient (Wooldridge and Melville-Smith, 1979). In addition to influencing the total zooplankton abundance and biomass in estuaries, freshwater inflow also plays an important role in determining the taxonomic diversity in estuaries. Generally, zooplankton taxonomic diversity is lowest in those permanently open estuaries characterised by reduced freshwater inflow (Froneman, 2001a). The observed pattern be related to the low contribution of freshwater zooplankton to the total zooplankton diversity and the virtual absence of true estuarine fauna within these systems (Grange *et al*, 2000). Further, axial gradients in salinity generated by freshwater inflow into estuaries influences the magnitude of recruitment of marine breeding species into estuaries.

1.2.1 Mysids

Although, mysids often contribute substantially to the total zooplankton biomass, few studies have specifically focused on the ecological role of these organisms in southern African estuaries (Wooldridge, 1999). There are five species of mysid commonly found in the waters of southern African estuaries, *Mesopodopsis wooldridgei*, *M. africanca*, *Gastrosaccus brevifissura*, *G. gordonae* and *Rhopalophthalmus terranatalis* (Wooldridge, 1999). Among

the mysids, *M. wooldridgei* demonstrates the widest distribution (Wooldridge, 1983; Wooldridge, 1999; Wooldridge and Bailey, 1982) and is thus the optimal choice for a study animal. Below is a summary of the main findings of studies, which have investigated the ecological role of *M. wooldridgei* in permanently open southern African estuaries (Table 1.3).

1.2.2 Spatial and temporal patterns in distribution of *M. wooldridgei*

M. wooldridgei is numerically abundant in estuaries although they can also be found in high numbers offshore (Wooldridge, 1983; Wooldridge, 1999). Estimates of the abundance and biomass of *M. wooldridgei* in permanently open estuaries are highly variable reflecting different physico-chemical variables within these systems (Wooldridge and Bailey, 1982). In general, highest abundances and biomass values of *M. wooldridgei* are attained in those estuaries characterised by sustained freshwater inflow (Table 1.3). In previous studies, *M. wooldridgei* was most abundant in the middle reaches of the estuary with maximum values attained during spring and summer months (Grange, 1992; Wooldridge and Bailey, 1982). On the basis of its osmoregulatory capacity, Webb *et al* (1997) suggested that the distribution of *M. wooldridgei* is determined by biological rather than physiological factors. *M. wooldridgei* exhibits diel vertical migration patterns which is largely thought to serve a predatory avoidance strategy (Froneman, 2001a; Wooldridge, 1999). Alternatively, it is also thought that *M. wooldridgei* undertakes diel vertical migration to maintain its position within the water column (Wooldridge and Erasmus, 1980). Within the middle and lower reaches of estuaries, distribution of the mysid is thought to reflect the biological interactions between various species of mysid. *Rhopalophthalmus terranatalis* has been noted to predate on the juvenile forms of *M. wooldridgei* (Wooldridge and Webb, 1988). *M. wooldridgei* is thus generally found to be spatially segregated from *R. terranatalis* (Wooldridge and Bailey, 1982). Female *M. wooldridgei* have different feeding requirements, activity levels and distribution patterns that of males in order to redirect their energy towards reproduction (Wooldridge, 1983). As a consequence, females tend to be spatially segregated from the remaining life history stages of *M. wooldridgei* (Wooldridge, 1983).

Table 1.3: Summary of the available abundance and biomass values for *M. wooldridgei* in selected permanently open estuaries along the Eastern Cape coastline.

Estuary	Rate of freshwater input	Abundance (ind. m^{-3})	Biomass (mg m^{-3})	Reference
Kromme	Deprived	83.0 (avg)	29.7 (avg)	Scharler *et al* (1998)
Swartkops	Intermediate	97.1 (avg)	38 (avg)	Scharler *et al* (1998)
Sundays	Continuous	69.9-155.5 (range)	119.4-132.2 (range)	Scharler *et al* (1998)
Kariega	Deprived	<10-100 (range)	N/A	Froneman (2000a)
Great Fish	Dominated	N/A	<10-10 100 (range)	Grange (1992)

1.2.3 Reproduction

There have been very few studies conducted on the life histories of mysids (Gorokhova and Hansson, 2000) in southern African estuaries, with only one laboratory study conducted by Wooldridge (1986) on *R. teranatalis*. In that study *R. terranatalis* reproduced for 8 months of the year with brood size depending on the size of the individual mysid and time of the year. There is presently no information available on the reproductive periodicity of *M. wooldridgei* in southern African permanently open estuaries. A study conducted in the Sundays Estuary and in Algoa Bay demonstrated that juvenile *M.slabberi* (now *M wooldridgei*) were present in samples collected throughout the year (Wooldridge, 1983). These data tentatively suggest that *M. wooldridgei* breeds continuously throughout the year. Finally, a previous study on *M. slabberi* in the United Kingdom indicated that optimal survival rates of newly released young occurred in waters where salinity values exceeded 7‰ (Greenwood *et al*, 1989).

1.2.4 Feeding

Based on their feeding structures, *M. wooldridgei* can be considered as a filter feeder (Webb *et al*, 1987; Wooldridge and Webb, 1988). Studies conducted in the freshwater dominated Sundays River Estuary indicate that *M. wooldridgei* feeds extensively on phytoplankton and

when available, protozooplankton (Webb *et al*, 1987). In contrast, in the freshwater deprived Kariega Estuary, juvenile *M. wooldridgei* can largely be regarded as herbivorous and the adults carnivorous, feeding mainly on protozooplankton and copepods (Jerling and Wooldridge, 1995a; Froneman, 2001a). Immatures consume a combination of phytoplankton and protozooplankton. Differences in feeding activities of the mysids between the two estuaries, appears to be related to the size structure of the phytoplankton. In the Kariega Estuary, the phytoplankton community is largely comprised of picophytoplankton (<2 μm), which is too small to be efficiently fed on by *M. wooldridgei* (Froneman, 2001a). As a consequence, mysids feed on alternative carbon sources (Froneman, 2001a). In contrast in the Sundays Estuary, the phytoplankton are directly available for utilisation by *M. wooldridgei*.

Although the mysid *M. wooldridgei* has been demonstrated to make a substantial contribution to the total zooplankton biomass in a variety of permanently open estuaries, few studies have examined the spatial and temporal patterns in their distribution or indeed their feeding ecology in these systems. Furthermore, no information is available on the factors that determine the distribution of the mysid in permanently open estuaries.

1.3 AIMS

The aims of this study are as follows:

- To investigate the physiology of *Mesopodopsis wooldridgei* in response to a range of salinities and temperatures and a combination thereof.
- To determine the spatial and temporal patterns in the distribution and abundance of *M. wooldridgei* in a permanently open freshwater dominated estuary.
- To investigate the feeding ecology of *M. wooldridgei* using stable isotope analysis.

To address these aims, investigations were conducted in the permanently open, freshwater dominated Great Fish Estuary located on the Eastern Cape Coastline of southern Africa.

1.4 STUDY SITE

The permanently open Great Fish Estuary (33°30'S, 27°08'E) is ≈ 20 km long (Day, 1981) and has a catchment area of approximately 29 937 km^2. The upper reaches are freshwater dominated, the lower marine water and the middle reaches a mixture of the two (Grange *et al*,

2000). Due to the Gariep interbasin transfer, the estuary is now regarded as a freshwater dominated system (O'Keeffe and De Moor, 1988), with a clear salt wedge evident throughout the year (Grange *et al*, 2000).

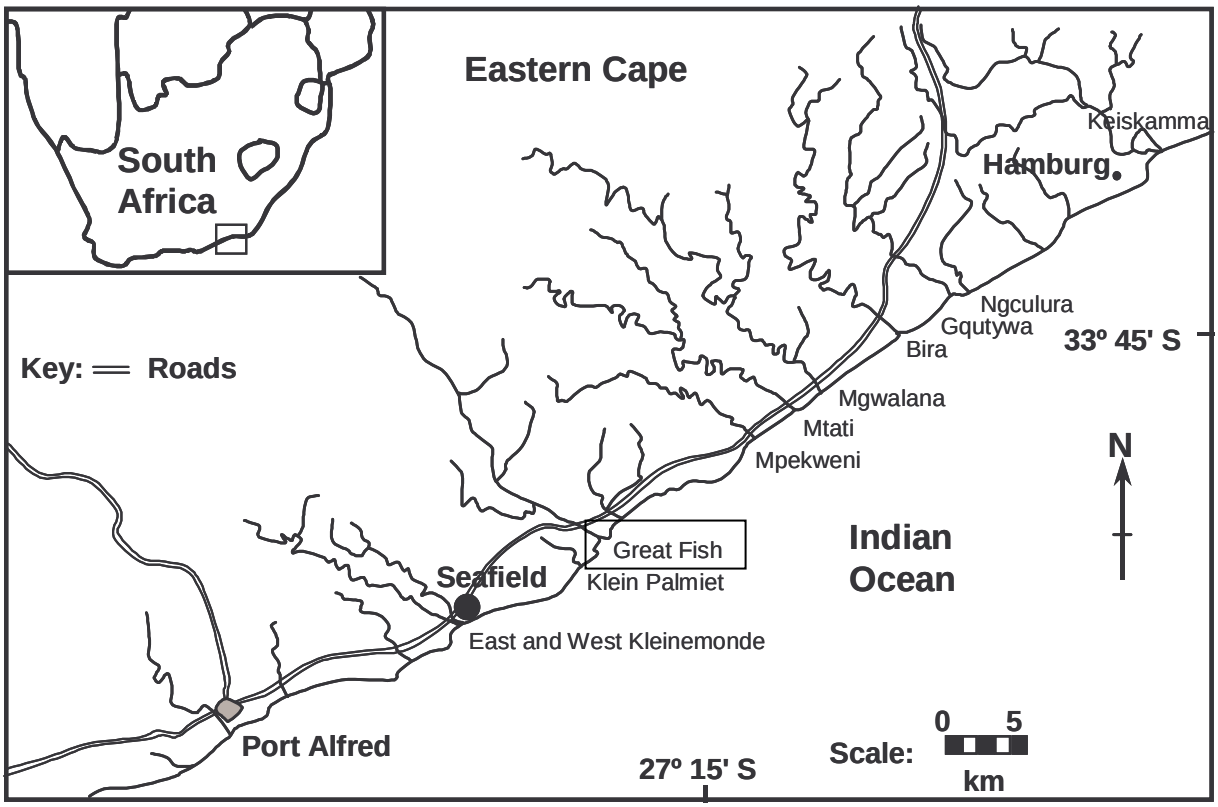

Figure 1.2: Map of the Eastern Cape coastline showing the location of the Great Fish Estuary (adapted from Walton, 1984).

The average depth of the estuary is 2.24m (SE±0.52) with an annual mean river discharge of 224 x 10^6 m^3 (Grange *et al*, 2000) (Fig. 1.2). The estuary is classified as a partially-stratified system due to the tidal prism volume of 1.624x106 m^3 and a flow ratio of 0.17 (Grange, 1992).

Physico-chemical and selected biological variables, including size fractioned chlorophyll *a* (chl. *a*) and zooplankton samples, were investigated at eight stations (Figure 1.2) along the length of the estuary over the period of June 2003 to May 2004 (Figures 1.3-1.7).

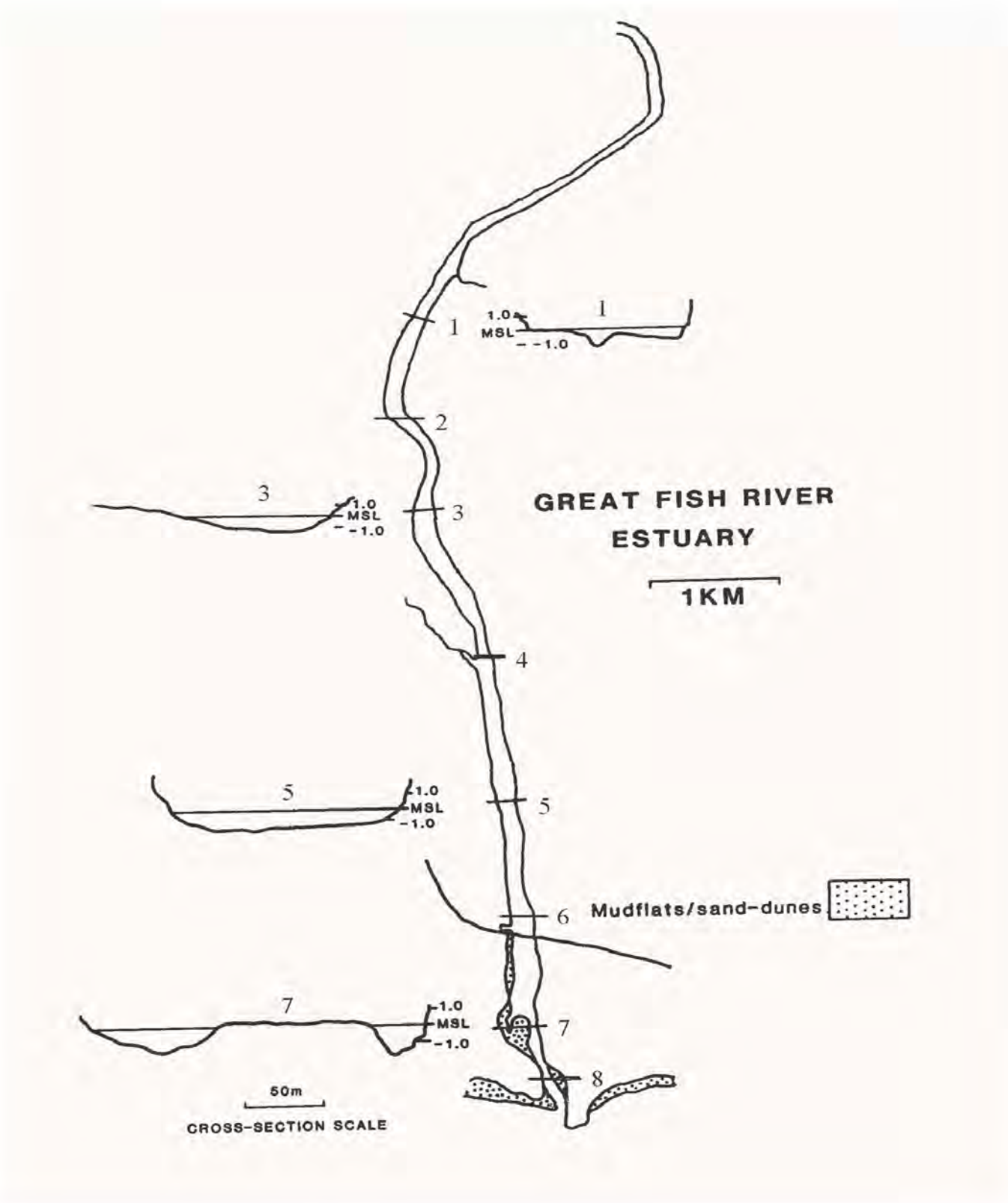

Figure 1.3: Map of the Great Fish Estuary showing the 8 stations where zooplankton sampling was undertaken. Cross-sectional dimensions of odd numbered stations are indicated (adapted from Grange, 1992).

Figure 1.4: Station 1 at the Great Fish Estuary showing the first river outlet. This station was freshwater throughout the 12-month survey.

Figure 1.5: Station 4 at the Great Fish Estuary showing the second river outlet. This station was normally a mixture of the fresh and seawater.

Figure 1.6: Station 6 at the Great Fish Estuary showing the bridge. This station was normally a mix of fresh and seawater.

Figure 1.7: Station 8 at the Great Fish Estuary showing the rocks leading to the sandy mouth region. This station was normally marine dominated.

CHAPTER 2 - RESPIRATORY RESPONSES OF THE MYSID, *Mesopodopsis wooldridgei*, TO CHANGES IN SALINITY AND TEMPERATURE

2. 1 INTRODUCTION

A recent study suggested that the distribution of the estuarine mysid *Gastrosaccus brevifissura*, was determined by its inability to withstand low salinities (Marshall *et al*, 2003). At present there are no data available on the physiological responses of *Mesopodopsis wooldridgei* to changes two abiotic factors, namely temperature and salinity. The main aim of this study is to investigate the metabolic physiology of *M. wooldridgei* over a range of temperatures and salinities and a combination thereof. The temperatures and salinities selected were within the range typically recorded in estuarine environments along the southern African coastline. These data will provide information on the factors determining the distribution of *M. wooldridgei* in southern African estuaries, which differs from a previous study that found biological factors being the main determinant of mysid distribution (Wooldridge and Webb, 1988).

2.2 METHODS

Mysids were collected from the middle reaches of the Great Fish Estuary in February and March 2003. Net tows were conducted during the day from the shore using a modified WP-2 net (nominal mouth size 0.25 m^2; mesh size, 100 μm). The animals collected were immediately transferred to a well-aerated 50L polyethelene container and transported back to the laboratory. Mysids collected were kept in well- aerated fish tanks filled with unfiltered seawater in a constant environment room (CE) at 20°C with light dark phase of 12:12. Mysids were provided with standard fish food daily. The experimental procedures employed during this study are similar to that presented in Marshall *et al* (2003) and Roast *et al* (1999) and are described below. Only females without developing embryos and those mysids that were actively swimming were employed in the physiology experiments.

2.2.1 Temperature

In order to test the effects of temperature on the rates of oxygen uptake of adult *M. wooldridgei*, male and female mysids were exposed to 4 different temperatures (15, 20, 25

and 30°C) in sterilized seawater (35 ppt) (micro-waved) that has been passed (vacuum <5cm Hg) through a 0.2 µm filter. Mean size (total length) of males and females were estimated at 7.813 mm (± 0.372 mm) and 8.119 mm (± 0.639 mm), respectively. Mysids were acclimated at experimental temperature for 24 hours prior to respiration rates being measured. The temperature range selected represents the mean seasonal temperatures in the Great Fish Estuary (Grange *et al*, 2000) and were maintained within ± 0.2°C using a water bath. A single mysid was sealed into an 11ml glass vial and the oxygen consumption was determined after 2-4 hours of exposure in the dark (minimise activity; Marshall *et al*, 2003) (n = 5-8 for each temperature). The values were then compared to the average oxygen concentration of the control vials without mysids. The difference in the oxygen concentration between the control and treatment vials was assumed to correspond to the oxygen consumed by the mysid (Marshall *et al*, 2003). Two control vials were employed for every 8 treatments.

Oxygen concentrations were determined using the Winkler titration method according to the method described in Strickland and Parsons (1968). On completion of the incubations, the water was removed using a 10ml syringe. Manganous sulphate reagent was immediately added followed by an alkaline iodide solution and the contents of the bottle were then mixed. Once the precipitate formed and had fallen approximately one third of the way down the bottle, sulphuric acid was added and the contents mixed once again until all the precipitate had dissolved. After acidification, the solution was then transferred into a new flask and titrated using a thiosulphate solution. The titration was completed once a very pale straw colour remained. From the measurements obtained during the titration the oxygen consumed could be calculated. The mysids were sacrificed for subsequent length measurements (from between the eyes to the tip of the telson; Marshall *et al*, 2003) using a Nikon dissecting microscope fitted with an eye piece micrometer and operated at 60X magnification. Measurements were made to the nearest 0.1mm. Thereafter, the mysid wet weights were determined using a Sartorious microbalance after removing excess water with blotting paper. Mass specific oxygen consumption rates of the mysids for the various temperatures were then expressed as µl oxygen consumed per mg wet weight per hour (µl O_2 mg ww^{-1} h^{-1}) (Roast *et al*, 1999).

2.2.3 Salinity

In order to test for the effect of salinity on the metabolic physiology of *M. wooldridgei*, mysids were exposed to 4 different salinity (5, 15, 25 and 35‰) concentrations at a constant temperature (20 ± 0.2°C). Salinities were prepared by diluting filtered (0.2 µm) micro-waved seawater with double distilled de-ionised water. Oxygen concentration after 2 - 4 hours of exposure (in the dark) was then measured in the treatments and compared to the average oxygen concentration of the control vials employing the method described above.

2.2.4 Temperature and Salinity

To assess the combined effects of salinity and temperature on the metabolic physiology of *M. wooldridgei*, mysids were exposed to a range of temperature (15, 20, 25 and 30°C) and salinity (5, 15, 25 and 35‰) combinations. Experimental procedure employed during these experiments is described above.

The Q_{10} values of the mysids during the various experiments were determined employing the equation: $Q_{10} = (V_2/V_1)^{10/T2-T1}$ (Garnacho *et al*, 2001). This is the coefficient for specific physiological rates (V_2 and V_1) at their respective temperatures (T_1 and T_2) (Garnacho *et al*, 2001).

Statistical analysis

Differences in the mass specific oxygen consumption rates of the mysids in the various treatments (temperature, salinity and the combined data) were tested using ANOVA (Statistica, 1995).

2.3 RESULTS

2.3.1 Temperature

There was no significant difference in the respiration rates of adult males and females (those with empty brood pouches) ($P > 0.05$). As a consequence, values for males and females have been pooled. The mass specific oxygen consumption rates of the mysid during the incubations ranged between 0.111 and 8.383 µl O_2 mg ww^{-1} h^{-1} (Figure 2.1A) and increased with an increase in water temperature (Figure 2.1A). Indeed, significant differences were found in the respiration rates of the mysids incubated at the four temperatures ($p < 0.05$)

(Table 2.1). The Q_{10} value for the combined data set was estimated at 2.138 over the temperature range of 20 – 30°C.

Table 2.1: Statistical comparison of respiration rates of *M. wooldridgei* over a temperature range of 15 to 30°C (where S is significant and N is not significant).

Temperatures	P value	F	F crit	
15/30°C	<0.01	53.200	5.318	S
15/25°C	<0.01	23.305	4.747	S
15/20°C	0.044	4.782	4.494	S
20/30°C	0.017	7.368	4.600	S
20/25°C	0.759	0.097	4.414	N
25/30°C	<0.01	22.597	4.965	S

2.3.2 Salinity

Oxygen consumption rates of the mysid at the various salinities ranged between 0.762 – 5.264 µl O_2 mg ww^{-1} h^{-1} (Fig. 2.1B) and decreased with increasing salinity. There was only one significant difference in the mass specific respiration rates of the mysids in the various treatments (P> 0.05). Mysids respired significantly more at 5‰ when compared to 35‰ at 20°C (P< 0.05).

2.3.3 Temperature and salinity

Mass specific respiration rates of *M. wooldridgei* at the various salinities increased with an increase in temperature (Figure 2.2). With two exceptions, 15°C/35‰ and the 30°C/5‰ treatments, differences in the mass specific respiration rates of the mysid in the various treatments were not significantly different (Table 2.2). In the 30°C/5‰ treatment, mysids exhibited mass mortality. The Q_{10} values (temperature range of 20-30°C) of the mysids in the 15, 25 and 35‰ treatments were estimated at 2.339, 1.441 and 2.138, respectively.

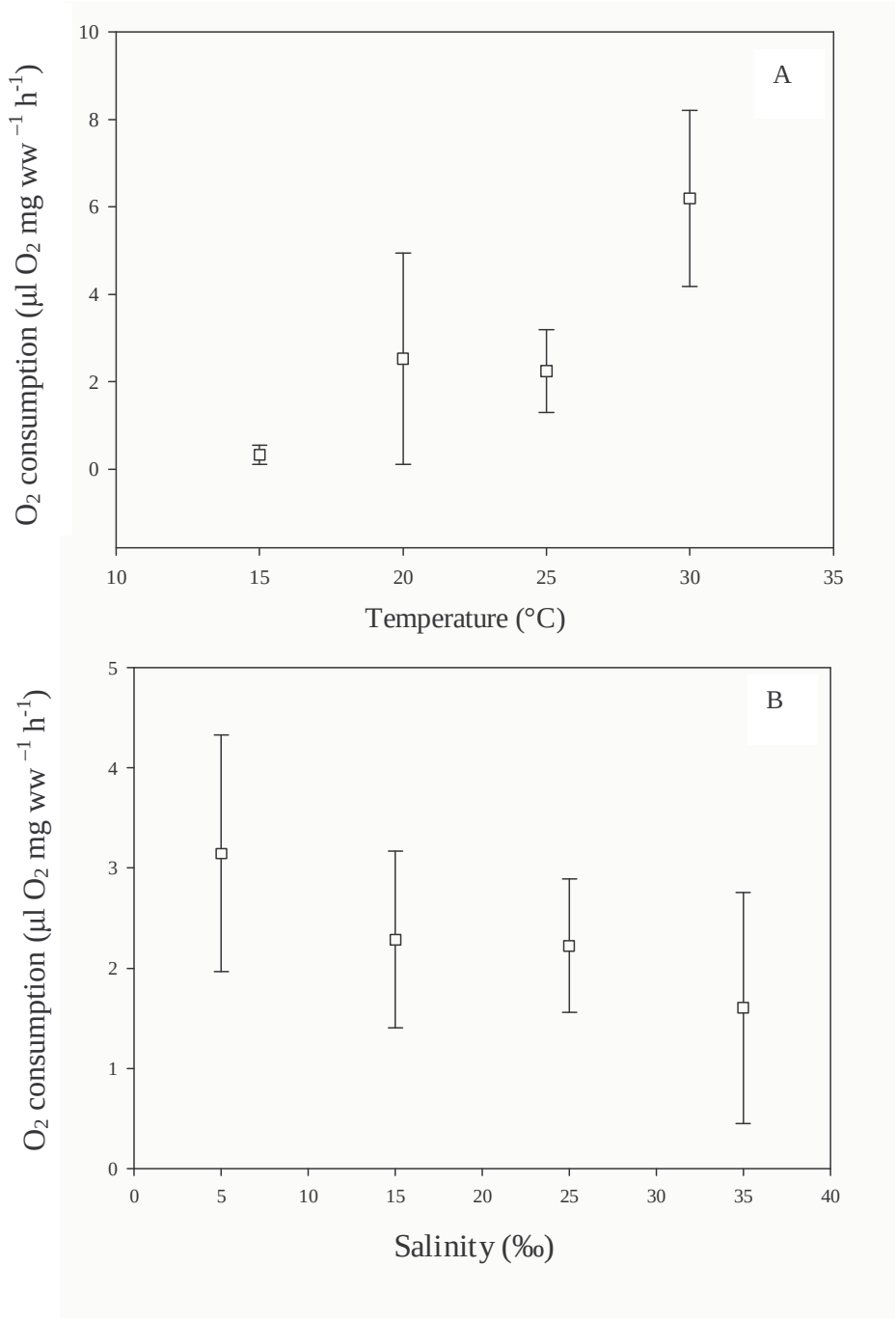

Figure 2.1: A and B. Mass specific respiration rates of *M. wooldridgei* for a range of temperatures (A) and salinities (B). Error bars are standard deviation. n= 5-8 for each treatment.

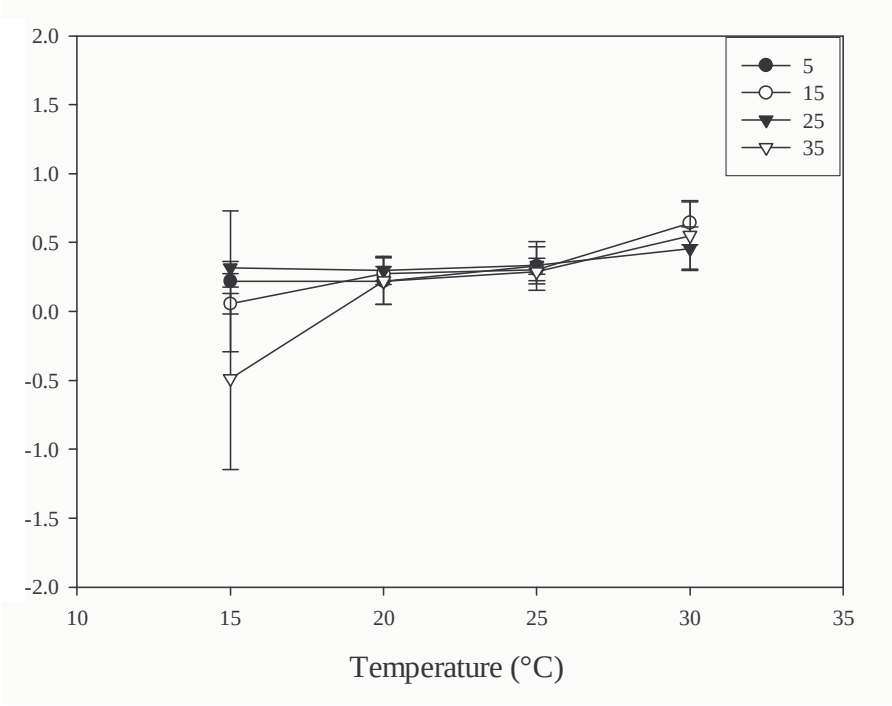

Figure 2.2: Mass specific respiration rates of the mysid, *M. wooldridgei*, for a range of temperatures (°C) and salinities (‰). Error bars are standard deviation. n= 5-8 for each treatment.

Table 2.2: Statistical analysis of the mass specific respiration rates of *M. wooldridgei* over a range of temperatures and salinities (S is significant, N is not significant).

15°C	P value	F	F crit	
35/5‰	<0.01	70.790	5.117	S
35/15‰	0.0456	5.212	4.965	S
35/25‰	<0.01	21.932	5.318	S
25°C	P value	F	F crit	
35/5‰	0.818	0.055	4.747	N
35/15‰	0.627	0.248	5.667	N
35/25‰	0.884	0.022	4.965	N

2.4 DISCUSSION

The increase in mass specific respiration rate of *M. wooldridgei* with an increase in temperature reported here is in agreement with a number of previous studies investigating the metabolic physiology of mysids (Marshall *et al*, 2003; Roast *et al*, 1999). It is generally accepted that extreme deviations from a Q_{10} value of 2 can be interpreted as a stress response (Marshall *et al*, 2003). The comparatively low Q_{10} value (2.138) of *M. wooldridgei* obtained during this study suggests that the mysid is unlikely to risk thermal related extinction in permanently open southern African estuaries were summer temperatures rarely exceed 25°C (Grange *et al*, 2000). It is worth noting that the Q_{10} value of *M. wooldridgei* during this investigation was similar to that of estuarine mysids from other geographic regions. For example, the Q_{10} value of *Neomysis integer* was estimated at 2.52 for females and 2.48 for males (Roast *et al*, 1999). Similarly, the Q_{10} value of the southern African mysid, *Gastrosaccus brevifissura*, was estimated at 2.147 (Marshall *et al*, 2003). Although gender differences in the oxygen consumption rates of mysids has previously been documented (Roast *et al*, 1999) no significant differences were not found in the mass specific respiration rates between male and female *M. wooldridgei* during this study ($P > 0.05$). The importance of sex in accounting for some of the variance in the data cannot, however, be discounted.

Metabolic responses of mysids to changes in salinity are highly variable, largely reflecting the evolutionary origin of the different species. For example, Marshall *et al* (2003) showed that the respiration rates of the estuarine mysid, *G. brevifissura*, did not change in response to variations in salinity. On the other hand, an increase in salinity led to a significant reduction in the rate of oxygen consumption for the mysid, *N. interger* (Roast *et al*, 1999). During the present study, mass specific oxygen consumption rates of *M. wooldridgei* at a constant temperature, decreased with an increase in salinity (Figure 2.1B). The response of *M. wooldridgei* to variations in salinity is therefore, equivalent to Kinne's (1971) second category which states that the rate of oxygen consumption of the organism increases in sub- and supranormal salinities.

In agreement with recent studies (McKenney, 1994; Roast *et al*, 1999), the salinity tolerance of *M. wooldridgei* was strongly modified by temperature (Figure 2.2). At 5‰ and 30°C only, *M. wooldridgei* exhibited mass mortality during the experiments. Mortality did not occur in any other combination of temperature or salinity. The apparent inability of *M. wooldridgei* to

persist in waters of low salinity and high temperature may be fundamental in accounting for the virtual absence of this species in temporarily open/closed estuaries where warm, mesohaline (5-17‰, temperatures may attain 28°C) conditions typically prevail (Froneman, 2002c). At moderate temperatures and salinities, there were no significant differences in the mass specific respiration rate of the mysid ($P > 0.05$). The Q_{10} values of *M. wooldridgei* at 15, 25 and 35‰ Q_{10} were estimated at 2.34, 1.44 and 2.14, respectively. The lack of any significant physiological response over a wide range of physico-chemical variables suggests that *M. wooldridgei* is well adapted to estuarine environments where variations in temperature and salinity prevail. The observed pattern reported here is in agreement with field studies, which have shown that *M. wooldridgei* is common in the lower and middle reaches of permanently open estuaries, particularly the Great Fish Estuary (Grange *et al*, 2000; Wooldridge and Bailey, 1982). The results presented here support the findings of Webb *et al*(1997), which suggest that biological factors play an important role in determining the distribution of *M. wooldridgei* in the middle and lower reaches of estuaries.

The distribution of invertebrates within estuaries reflects the complex interaction between physicochemical and biological variables (Wooldridge, 1999). Results of this investigation indicate that, *Mesopodopsis wooldridgei*, is physiologically well adapted to surviving in estuarine systems where the environmental variables vary over short time periods. Future studies should assess the importance of biological variables, including food availability and predation in determining the distribution of *M. wooldridgei* in the southern African estuaries. Finally the inability of *M. wooldridgei* to survive in freshwater may limit the upper distribution of the mysid in permanently open estuaries.

CHAPTER 3 - ZOOPLANKTON DISTRIBUTION IN THE GREAT FISH ESTUARY WITH SPECIAL EMPHASIS ON THE MYSID, *Mesopodopsis wooldridgei*

3.1 INTRODUCTION

The impact of varying freshwater flow on the biology of estuaries has been the focus of a great deal of research throughout the world (Kaartvedt and Aksnes, 1992; Kimmerer, 2002; Mallin *et al*, 1993; Smatacek, 1986) and indeed, in South Africa (Allanson and Read, 1995; Froneman, 2002b; Grange *et al*, 2000; Grange and Allanson, 1995; Jerling, 1998; O'Keeffe and De Moor, 1988; Wooldridge and Callahan, 2000).

A previous study conducted in the freshwater dominated Great Fish Estuary showed that the zooplankton community in the upper reaches of the system was numerically and by biomass dominated by the copepod, *Pseudodiaptomus hessei*, while in the lower and middle reaches of the estuary, the mysid *Mesopodopsis wooldridgei* dominated total zooplankton biomass (Grange *et al*, 2000; Grange and Allanson, 1995). The observed pattern was in agreement with previous studies conducted in the permanently open Sundays Estuary within the same geographic region (Wooldridge and Bailey, 1982). The changes in the zooplankton community structure observed in the Great Fish Estuary was ascribed to freshwater inflow into the system (O'Keeffe and DeMoor, 1988). To the authors' knowledge, there have been no subsequent studies on the zooplankton community structure within the Great Fish Estuary. The aim of the current study is to examine the composition and distribution of the zooplankton community along the length of the Great Fish Estuary, with specific emphasis on the mysid, *M. wooldridgei*.

3.2 MATERIALS AND METHODS

Physico-chemical and selected biological variables, including size fractioned chlorophyll *a* (chl. *a*) and zooplankton samples, were investigated at eight stations (Figure 1.1) along the length of the estuary over the period of June 2003 to May 2004.

Mean monthly freshwater discharge rates during each monthly sampling trip were obtained from the Department of Water Affairs and Forestry (DWAF).

3.2.2 Physico-chemical variables

Water samples (600ml) were collected from the surface (0.5 m) and at a depth of ≈1.5 meter using a 5L Niskin bottle. Temperatures at two depths were determined on-site using a YSI temperature probe, and the salinity with a hand-held refractometer. Seston concentrations at each depth were determined by gently filtering 300mL water sample through pre-weighed glass fibre filters (GF/F), which was oven dried at 60°C for 24 hours. The resulting difference between final and initial weights corresponded to seston concentration and was expressed as $\mu g\ L^{-1}$.

3.2.3 Biological variables

Chlorophyll *a* analysis

Chlorophyll *a* (Chl. *a*) concentrations were determined, at the surface and at 1.5 meter depth, from a 250ml water sample, collected using the Niskin bottle. The sample was serially filtered (vacuum <5 cm Hg) through a 5.0 Nucleopore filter and a GF/F filter. Filters were then extracted in 90% acetone for 24 hours in the dark at -20°C. Chl. *a* concentrations were then determined fluometrically using a Turner Designs 10AU fluorometer, according to the method of Holm-Hansen and Riemann (1978). Results were expressed as $\mu g\ L^{-1}$.

3.2.4 Zooplankton

During each monthly sampling trip, plankton tows were conducted at each station at low tide in triplicate during the daytime and nighttime to account for the zooplankton diel vertical migrations. Daytime sampling normally began before 12 pm and nighttime sampling after sunset. A modified WP-2 net (nominal mouth size of 0.25 m^2; mesh size 100 μm) fitted with a General Oceanics flow meter to determine the amount of water filtered during each tow and was used to collect zooplankton. The net was towed next to the boat so that the entire apparatus was submerged just below the surface (approximately 5-10 cm). Volumes filtered during the tows ranged between 1.07 and 7.87 m^3. Samples collected were immediately preserved in 4% buffered (hexamine) formalin for later analysis in the laboratory.

3.2.5 Zooplankton community analysis

The total zooplankton abundance and biomass (excluding mysids) were determined from sub-samples (1/2 or 1/4) obtained using a Folsom plankton splitter. Zooplankton biomass was determined by passing the sub-sample through a GF/F pre-weighed filter and then oven dried at 60°C for 24 hours. The biomass was determined by subtracting the final from the initial weight and was expressed as mg Dwt m^{-3}.

All mysids were removed from the plankton sample and sub-sampled (1/2 or 1/4) when an excess of 250 mysids were present in the sample. The mysids *Rhopalophthalmus terranatalis, Mesopodopsis wooldridgei* and *Gastrosaccus brevifissura*, were identified, sexed and measured. Seven different size classes of *M. wooldridgei* were identified according to the method of Wooldridge and Bailey (1982) and Froneman (2001a).

The size classes for *M. wooldridgei* were as follows:

1) Juveniles (<4mm)
2) Immatures (5-7 mm)
3) Adult male (>7mm) – 4th pleopod
4) Adult female (>7 mm)- empty brood pouch
5) Adult female (>7 mm)- brood pouch with eggs
6) Adult female (>7 mm)- brood pouch with developing/eyeless young
7) Adult female (>7 mm)- brood pouch with eyed young

In order to estimate the biomass of the mysids, the length weight (dry mass) relationships of Wooldridge and Bailey (1982) for three mysid species were employed.

M. wooldridgei: $\log_{10}$ Mass (mg) = 2.87 $\log_{10}$ L (mm) – 2.7846
R. terranatalis: $\log_{10}$ Mass (mg) = 2.81 $\log_{10}$ L (mm) – 2.6975
G. brevifissura: $\log_{10}$ Mass (mg) = 3.15 $\log_{10}$ L (mm) – 2.8258

3.2.6 Numerical analyses

The spatial and temporal patterns in the distribution of the zooplankton and mysids in the Great Fish Estuary were analysed employing a non-parametric multivariate

analysis from the PRIMER (version 5.2.4 for Windows) software package. Preliminary results employing the entire data set did not reveal the presence of any significant spatial or temporal patterns in the distribution of the total zooplankton or the mysids. The absence of any pattern could likely be attributed to the extreme variability in the zooplankton abundances (data not shown). As consequence, the zooplankton abundance data were combined into stations for spatial comparison and into months for temporal comparison. Only nighttime samples were employed in order to analyse the entire zooplankton community.

A similarity matrix of the data was produced by log transforming (log (x+1)) the zooplankton abundance data. A cluster analysis of the data was then performed with a complete hierarchical sorting strategy. Dendrograms were generated to determine the spatial and temporal relationships. To determine sources of significance between the groupings identified with the cluster analyses, the ANOSIM routine package was employed. The SIMPER analysis was then run to identify the main species, which contributed to the similarity in each of the groupings identified with the hierarchical cluster analysis. Zooplankton diversity indexes within each grouping were calculated using the Margalefs' equation:

$$d = (S-1)/\text{Log}\,(N)$$

where: d is the Margalef diversity index, S is the number of species and N is the total number of individuals

3.2.7 Statistical analysis

A Shapiro-Wilk test was performed on all the data using STATISTICA 6.0 to determine normality. Where appropriate a Mann-Whitney test was used to determine significant differences between nonparametric data. When the data was not normal, it was transformed using log (x+1) (Hampton, 1994). The Neuman-Keuls multiple range test was then performed after ANOVA to determine where any differences lay.

3.3 RESULTS

3.3.1 Physico-chemical variables

Water temperatures displayed a distinct temporal pattern, with the lowest values recorded in the winter (12.8°C) (Figure 3.1) and highest during summer (27°C) (Figure 3.2). Intermediate temperatures were recorded in spring and autumn (ranging between 17°C to 27°C).

There were distinct differences in water temperature between the surface and bottom water, with a maximum of 6.7°C variation. A clear salinity gradient was evident along the estuary during most months, with the upper three stations generally characterized by freshwater (salinities <5‰) while the lower three stations were typically marine dominated (salinities between 30 and 35‰). Stations within the middle reaches of the estuary were normally composed of a mixture of marine and freshwater. Salinities within this region typically ranged between 10 and 25‰.

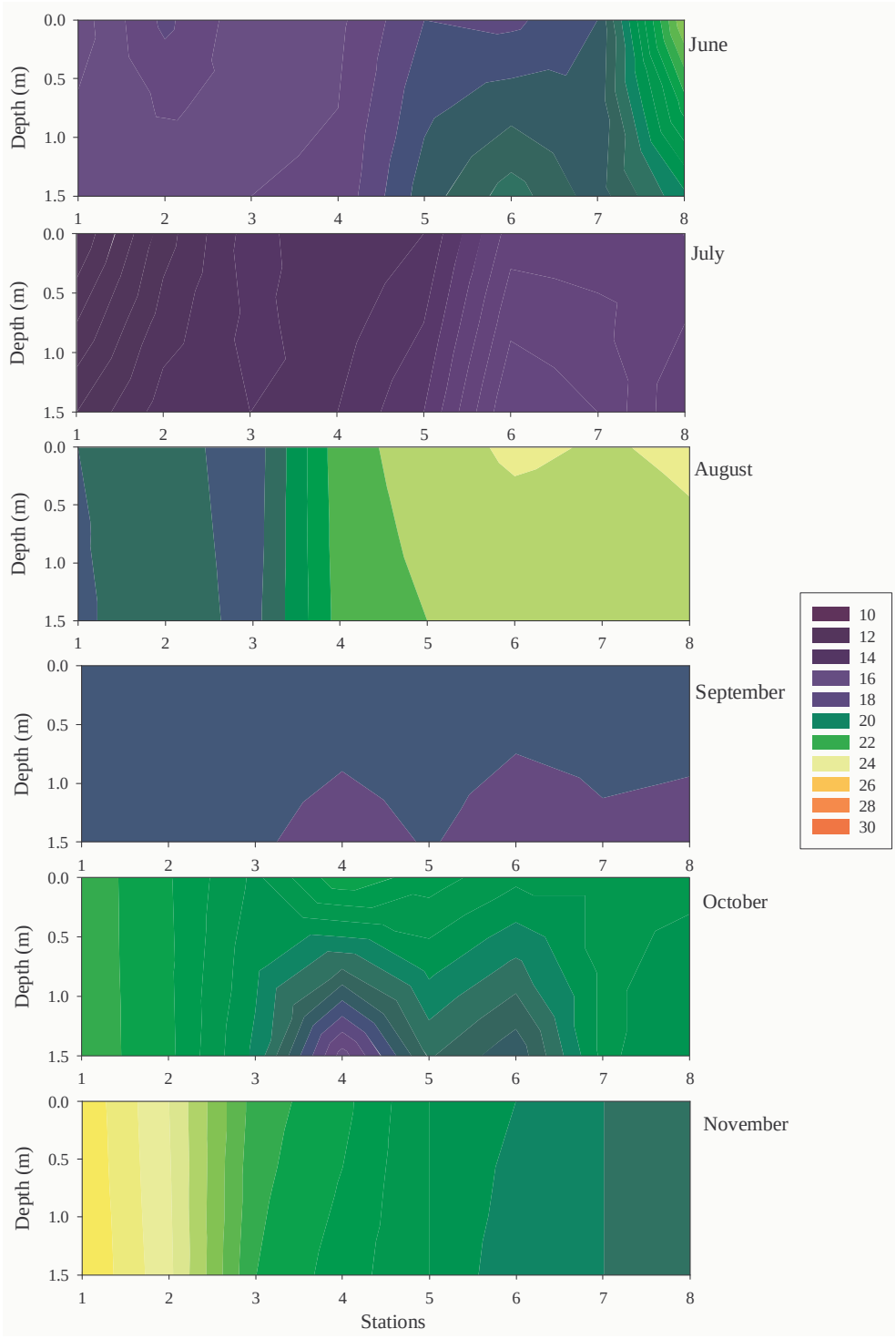

Figure 3.1: Water temperature profiles (°C) for the winter and spring 2003 surveys in the Great Fish Estuary.

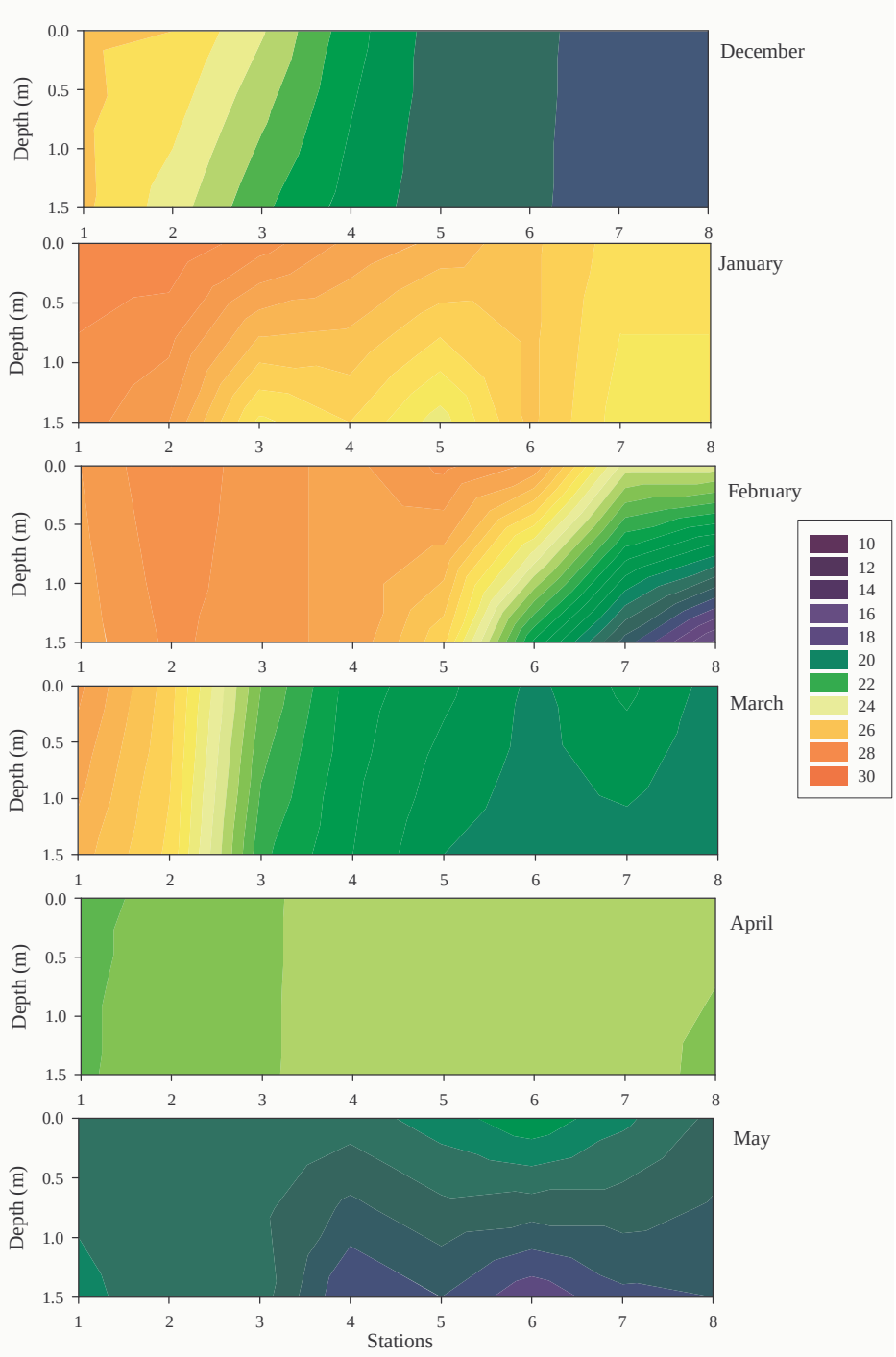

Figure 3.2: Water temperature profiles (°C) for the summer and fall 2003/2004 surveys in the Great Fish Estuary.

A distinct salt wedge was observed during all sampling trips (Figure 3.3) Exceptions were recorded in February and April 2004 where freshwater was observed along the entire length of the estuary (Figure 3.4*)*. The absence of any gradient in salinity

during February and April 2004 could be attributed to the marked increase in the freshwater discharge into the system (Table 3.1).

Table 3.1: Mean monthly discharge into the Great Fish River at the Matomela's weir located upstream of the Great Fish River Estuary. Data was obtained from Department of Water Affairs and Forestry.

Month	Mean discharge (m^3s^{-1})
June	10.04
July	9.427
August	5.974
September	5.215
October	4.841
November	4.840
December	6.937
January	8.188
February	11.989
March	7.02
April	15.67

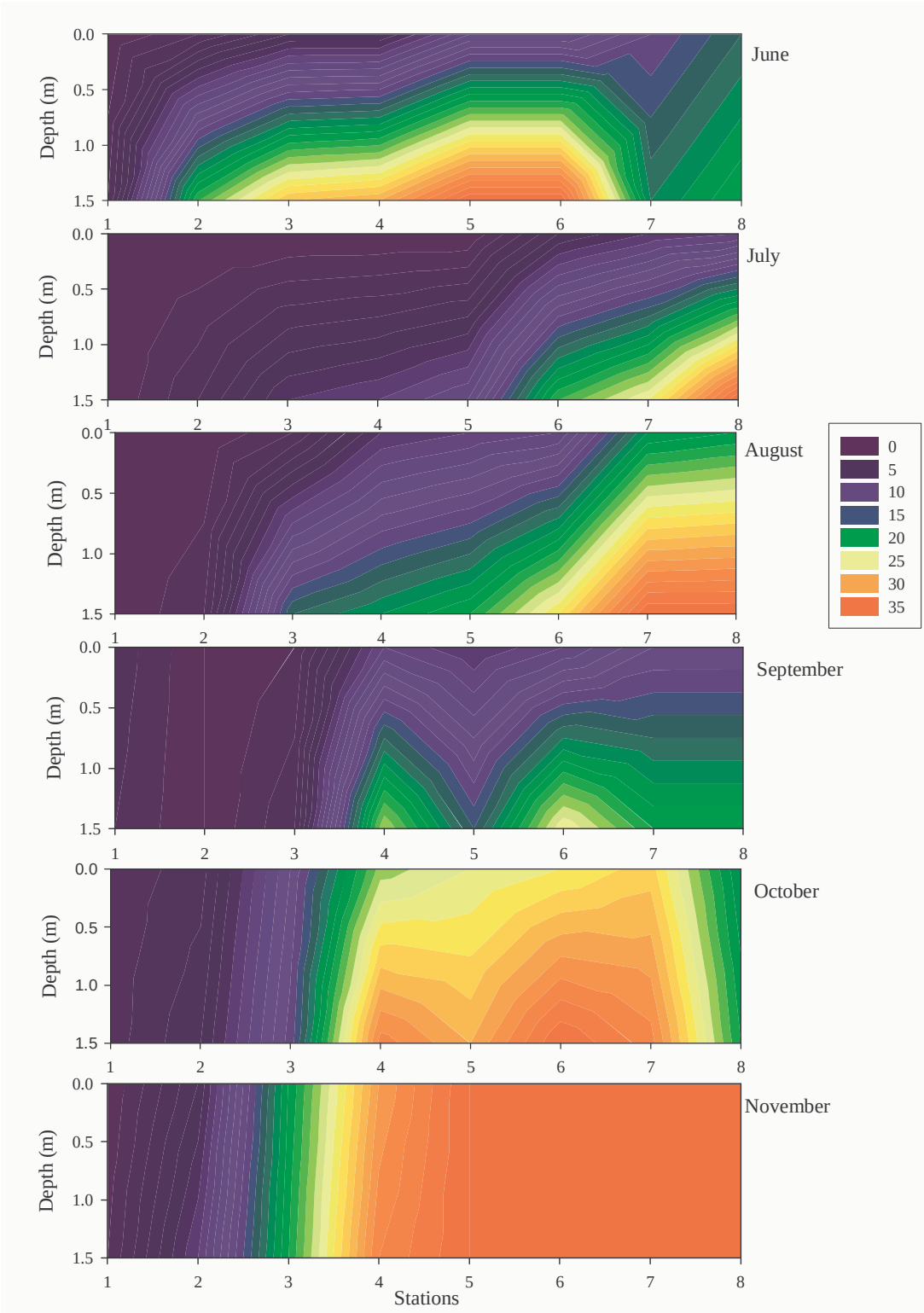

Figure 3.3: Depth profile of salinity (‰) for the winter and spring 2003 surveys in the Great Fish Estuary.

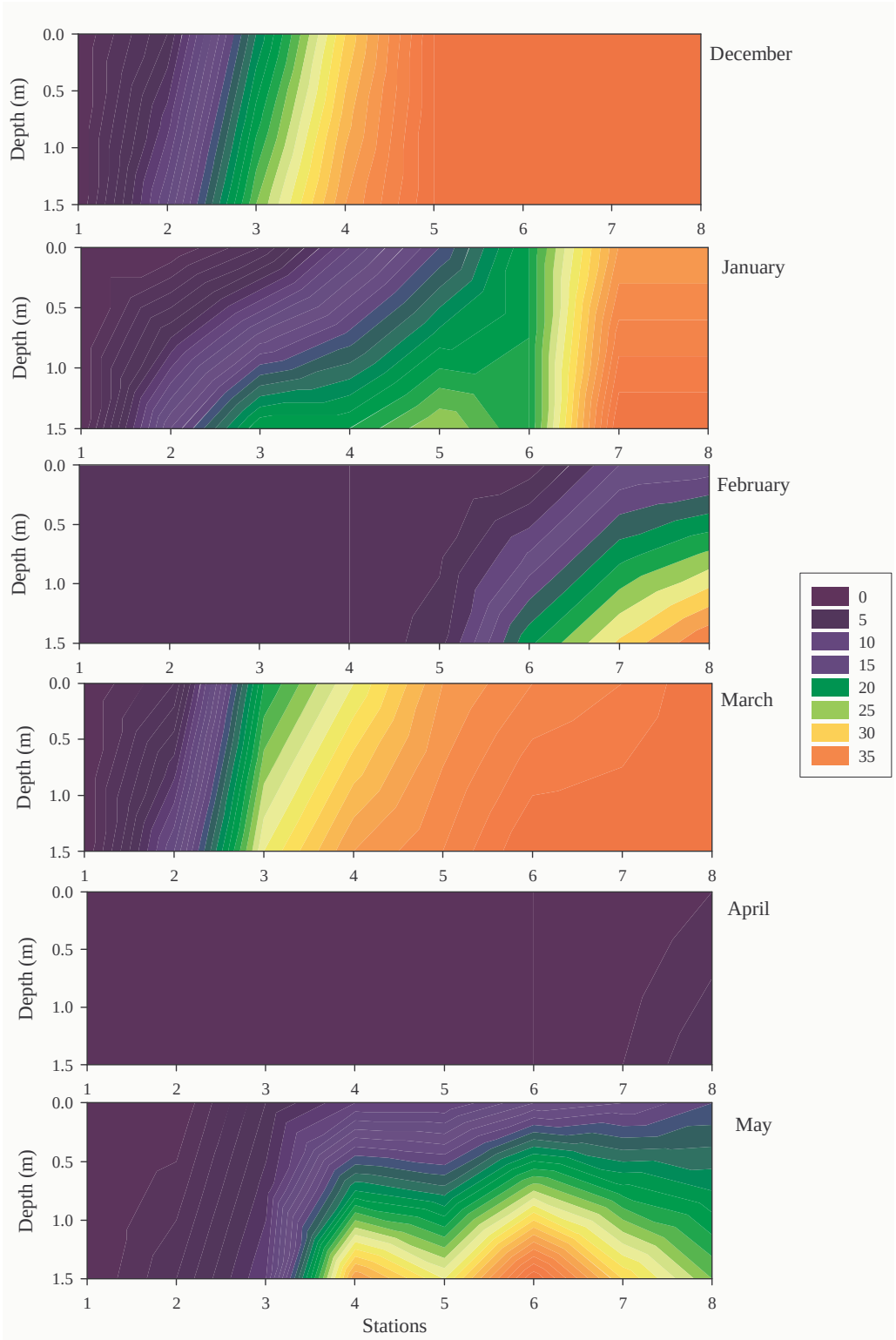

Figure 3.4: Depth profile of salinity (‰) for the summer and autumn 2003/2004 surveys in the Great Fish Estuary.

3.3.2 Seston

Seston values during the study ranged between 58.03 µg L^{-1} and 528.75 µg L^{-1}. There were no significant differences in seston concentrations between surface and subsurface depths or indeed between the stations during each month (P>0.05 for all cases). As a consequence, the mean seston values for surface and subsurface depth (approximately 1.5m) for each month is presented below (Figure 3.5). Generally, the highest seston values corresponded to these months (February and April 2004) when an increase in river discharge was observed.

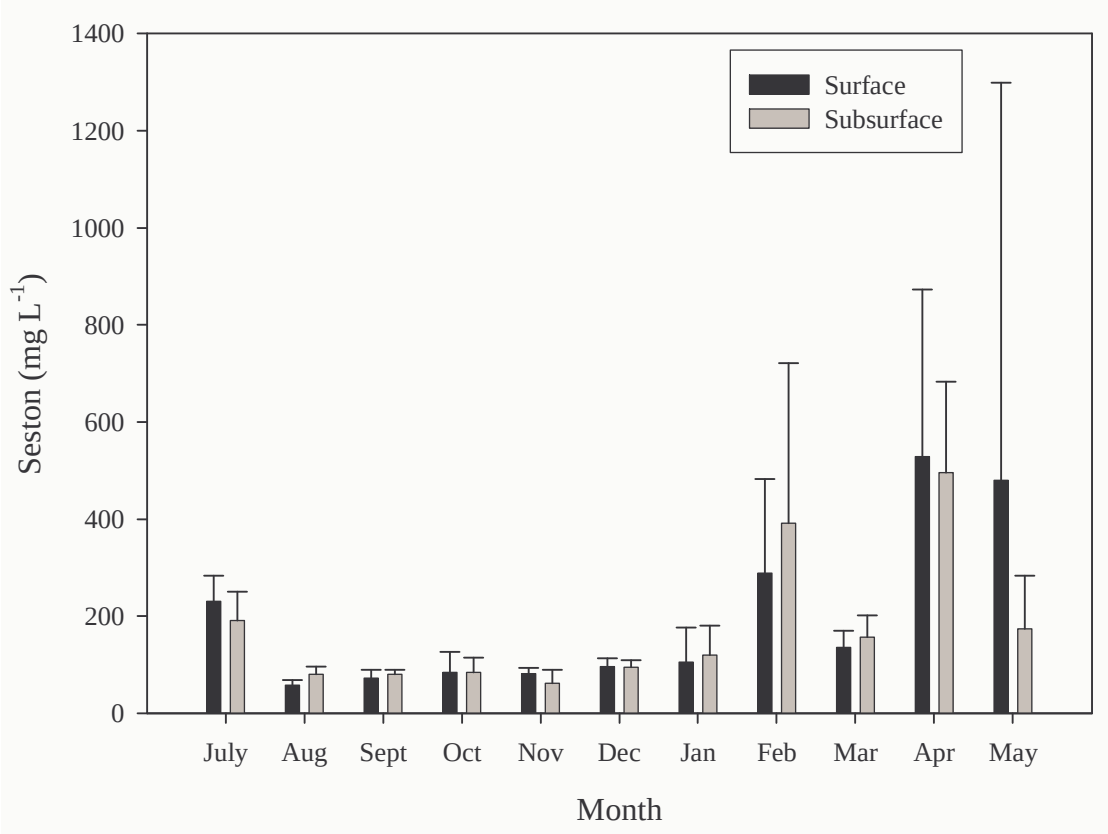

Figure 3.5: Average monthly seston concentrations (mg L^{-1}) from the Great Fish Estuary from surface and depth (~1.5 m) samples.

3.3.3 Chlorophyll *a* concentrations

Total chl. *a* concentrations between surface waters and depth were not significantly different from one another (P>0.05) and ranged between 0.085 and 300.85 µg L^{-1}. There was no horizontal pattern in total chl. *a* concentration evident during the study. Throughout the investigation, total chl. *a* concentration was dominated by >5 µm size class, which contributed between 76 and 95% of the total pigment. Concentrations of

the >5 μm chl. *a* fraction ranged between 1.20 and 222.19 μg L^{-1} and between 0.32 and 14.30 μg L^{-1} for the <5 μm fraction. Total chl. *a* concentrations did not increase during periods of increased freshwater flow. The highest chl. *a* concentrations were recorded during the winter months (May through August- Figures 3.6 and 3.7).

3.3.4 Zooplankton

There were no significant temporal patterns in total zooplankton abundance and biomass during the study (P>0.05). Total zooplankton abundance and biomass values during nighttime were significantly higher than daytime values (P<0.01). Among the zooplankton, the various developmental stages of copepods (mainly *P. hessei* and *A. longipatella*) numerically dominated the zooplankton counts. The numerical dominance of the copepods was particularly evident at stations occupied during the day and in the upper reaches of the estuary during the nighttime sampling. Among the copepods, *P. hessei* was identified as the single most numerically abundant species with daytime densities ranging between 6.81 and 13 703.80 ind. m^{-3} or between 23 and 100% of the total zooplankton abundance. Nighttime densities of *P. hessei* ranged between 16.36 and 73 778.4 ind. m^{-3}. These values corresponded to between <1 and 100% of total nighttime zooplankton abundance. Also well represented among the copepods were species belonging to the genera *Acartia*, *Oithona* and *Halicyclops*. Combined, these copepods generally contributed <5% of the numerical abundance at all stations. With the exception of mysids, the contribution of the remaining groups including amphipods, isopods, decopods and cumaceans was equivalent to <1% of the total zooplankton abundance at all stations. Total zooplankton biomass ranged between 9.96 to 146.14 mg Dwt m^{-3} during the daytime (Figure 3.8) and between 1.29 and 203.01 mg Dwt m^{-3} at nighttime (Figure 3.9).

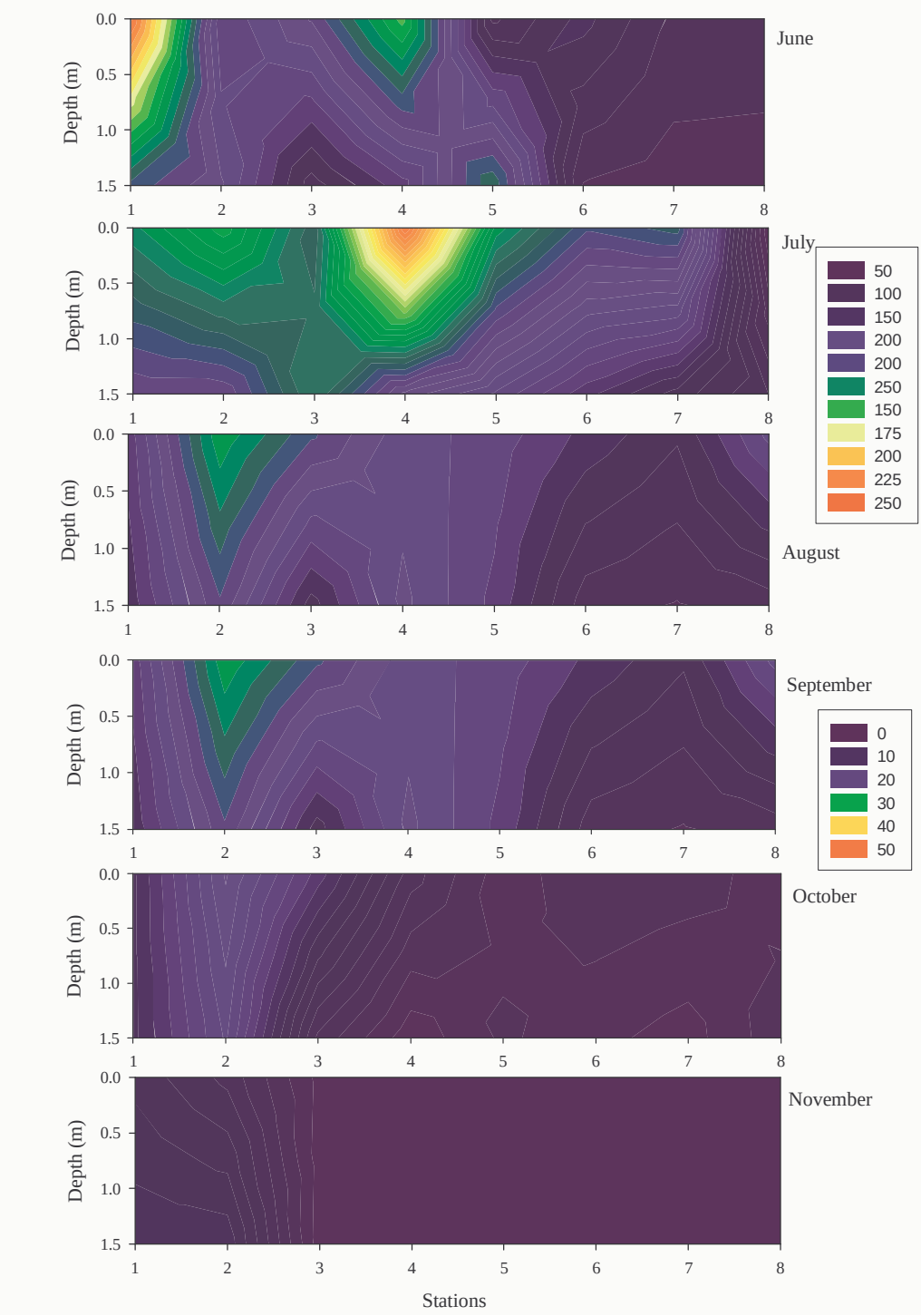

Figure 3.6: Depth profile of total chl. *a* concentration (µg L^{-1}) from the Great Fish Estuary during winter and spring 2003/2004. Note the separate legend for the month of July.

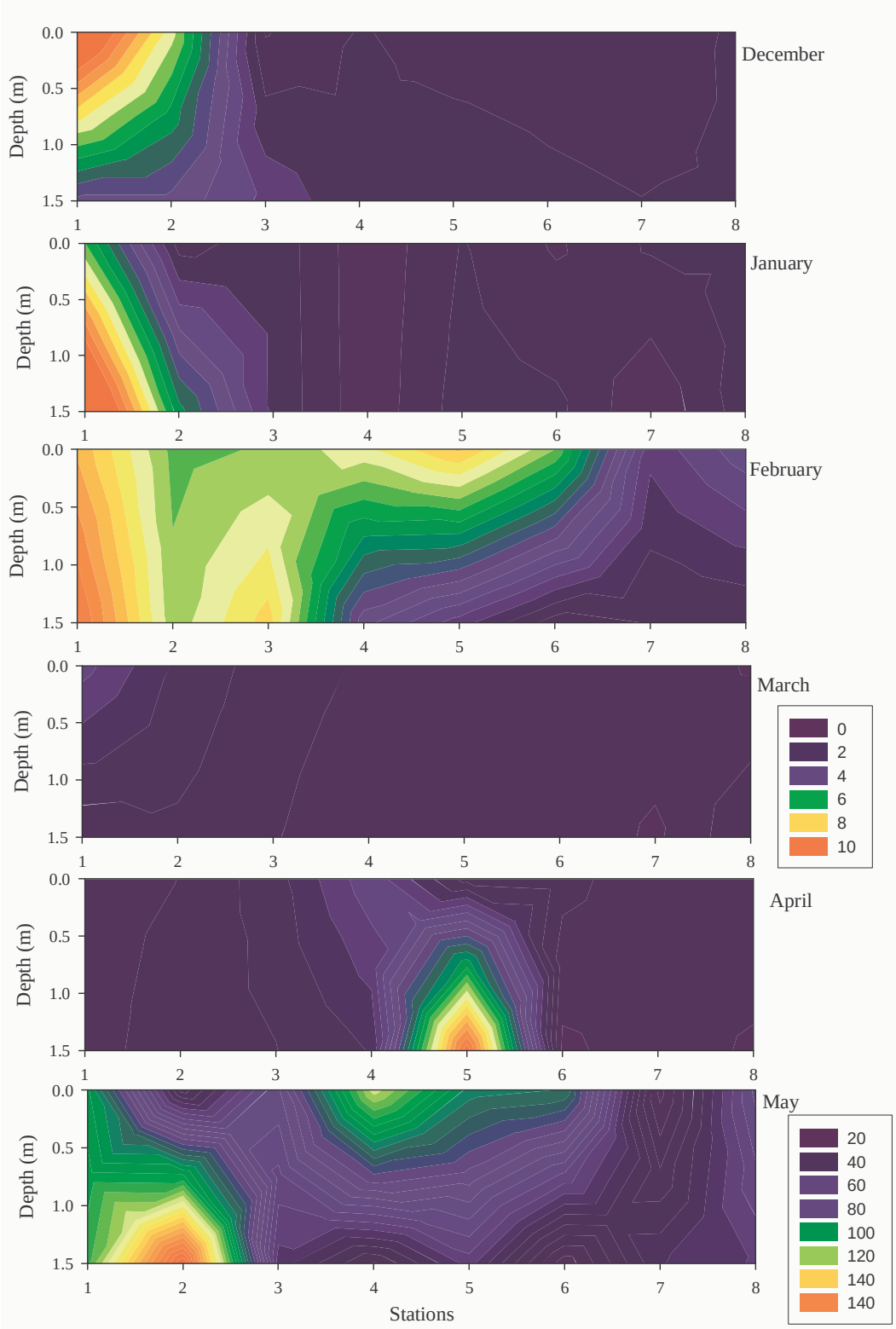

Figure 3.7: Depth profile for total chl. *a* concentration (µg L^{-1}) from the Great Fish Estuary during summer and autumn 2004. Note the separate legend for May only.

3.3.5 Mysids

Averaging the plankton tows is a more reliable method to determine a realistic biomass of mysids, as all these species are known to swarm (Connell, 1974; Tattersall, 1952; Wooldridge, 1983). Mysid abundances during the daytime ranged from 0.6 to 172.13 ind. m^{-3} (dominated almost entirely by *M. wooldridgei*) and between 0.14 to 108.19 ind.m^{-3}during the nighttime. These values correspond to <1% of the total zooplankton abundance during the daytime and between 1 and 9% (average 3%) of the nighttime zooplankton abundance. Abundance and biomass values of mysids and the remaining zooplankton are displayed in figures 3.10 A and B and 3.11 A and B, respectively.

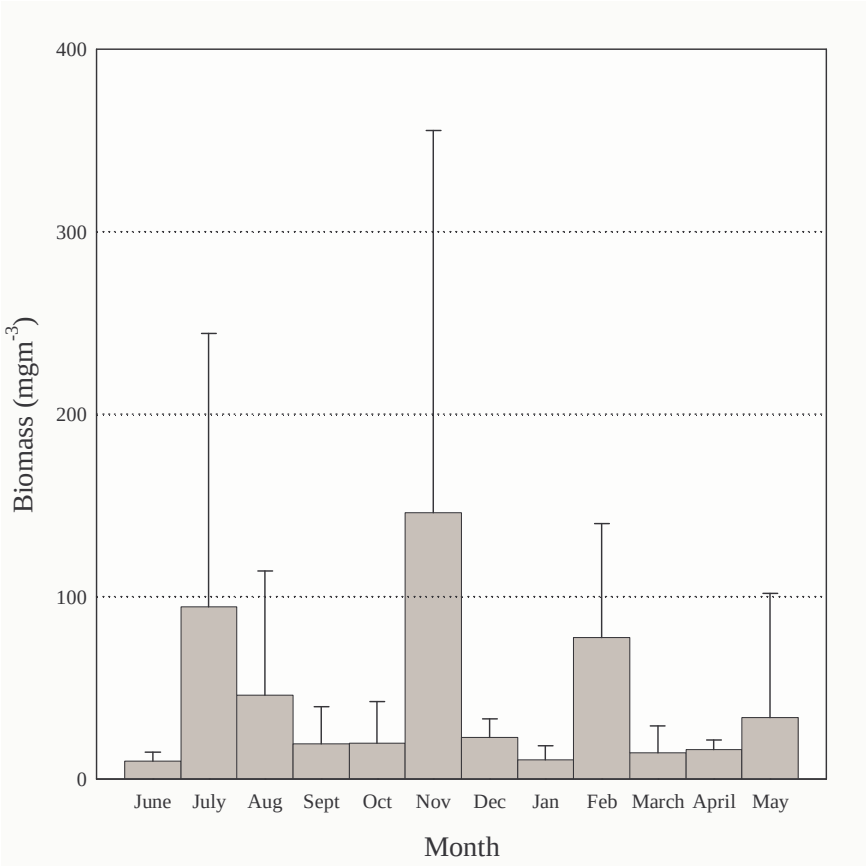

Figure 3.8: Total zooplankton biomass (mg Dwt m^{-3}) during the daytime sampling over the 12-month study period within the Great Fish Estuary. Values for the 8 sampling stations have been pooled for each month. Error bars are standard deviation. n=24 for each month.

Total mysid abundance and biomass during nighttime samples were significantly higher than daytime values ($P<0.05$). Total mysid biomass for the morning ranged between 0.095 and 15.81 mg Dwt m^{-3} and between 0.31 and 190.66 mg Dwt m^{-3}

during nighttime. Mysids contributed between <1% and 48% (average 14%) of the morning zooplankton biomass and between <1 and 95% (average 44%) of the total zooplankton biomass during the nighttime (Figure 3.11 A and B).

Mysids were absent from the morning samples in April and the nighttime samples of February 2004. A distinct spatial pattern in mysid biomass was evident with middle reaches (Stations 3-5) of the estuary having significantly higher mysid biomass ($P<0.01$, $F= 6.04$) and abundance ($P<0.01$, $F= 4.94$) values than those recorded at stations occupied in the upper (Stations 1-2) and lower (Stations 6-8) reaches of the estuary.

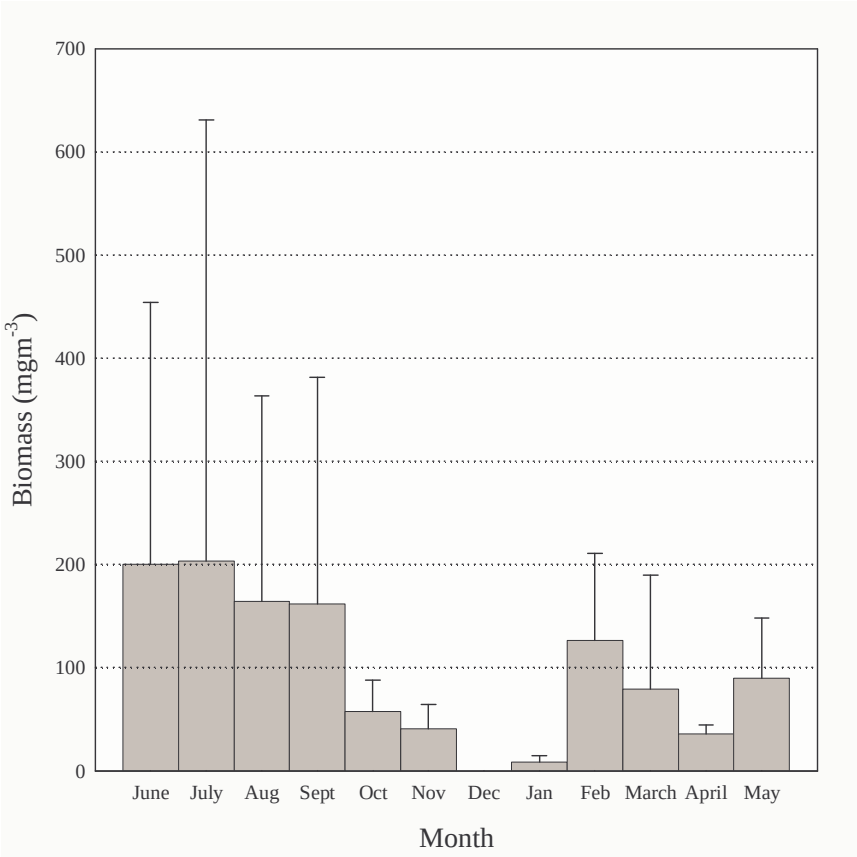

Figure 3.9: The total zooplankton biomass (mg Dwt m^{-3}) during nighttime sampling within the Great Fish Estuary. Values for the 8 sampling stations have been pooled for each month. Error bars are standard deviation. n=24 for each month.

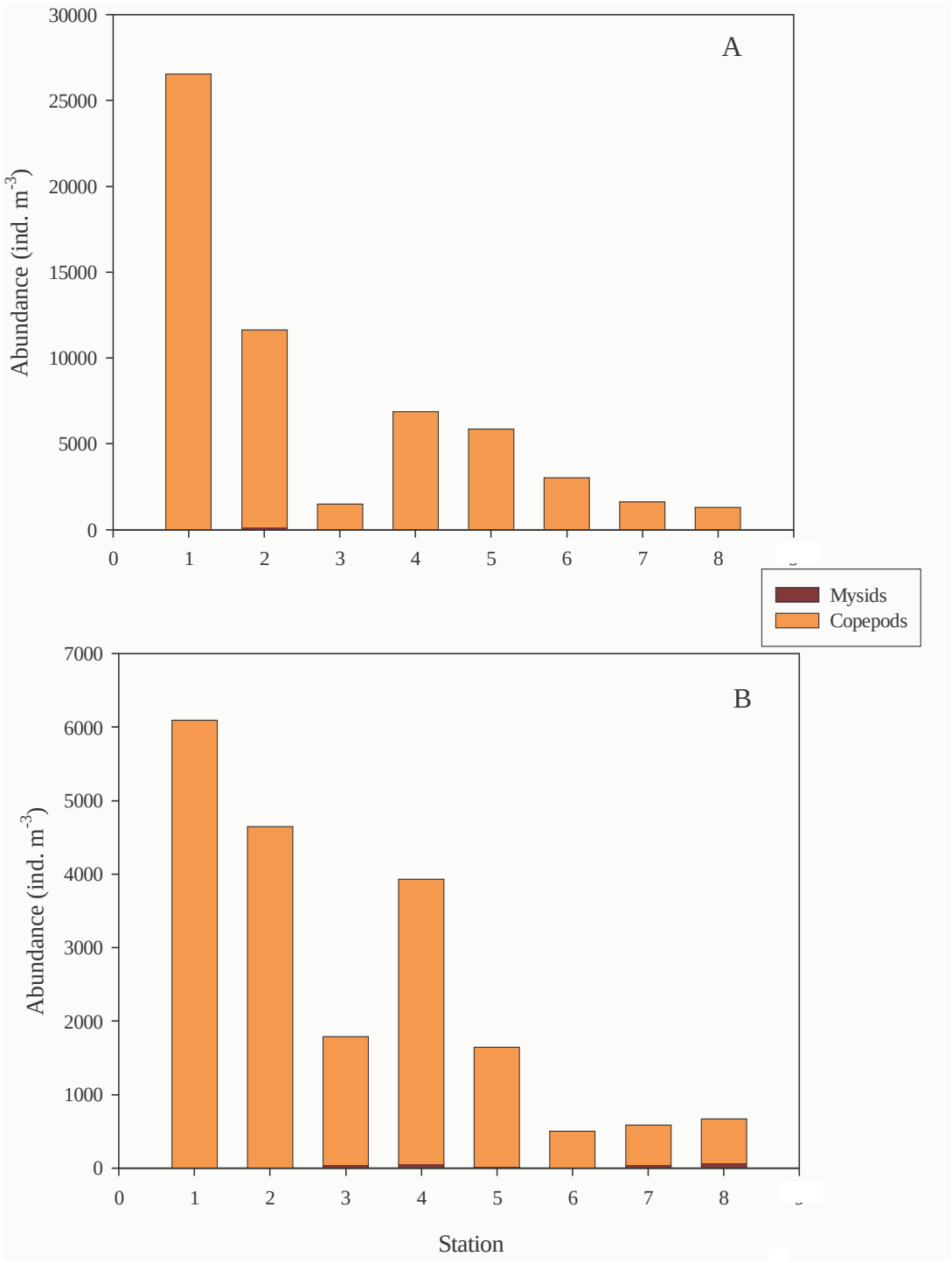

Figure 3.10: Spatial abundance (ind. m^{-3}) of mysids and copepods at the 8 sampling stations at the Great Fish Estuary during the daytime (A) and nighttime (B). Values have been pooled for each station over the 12-month sampling period.

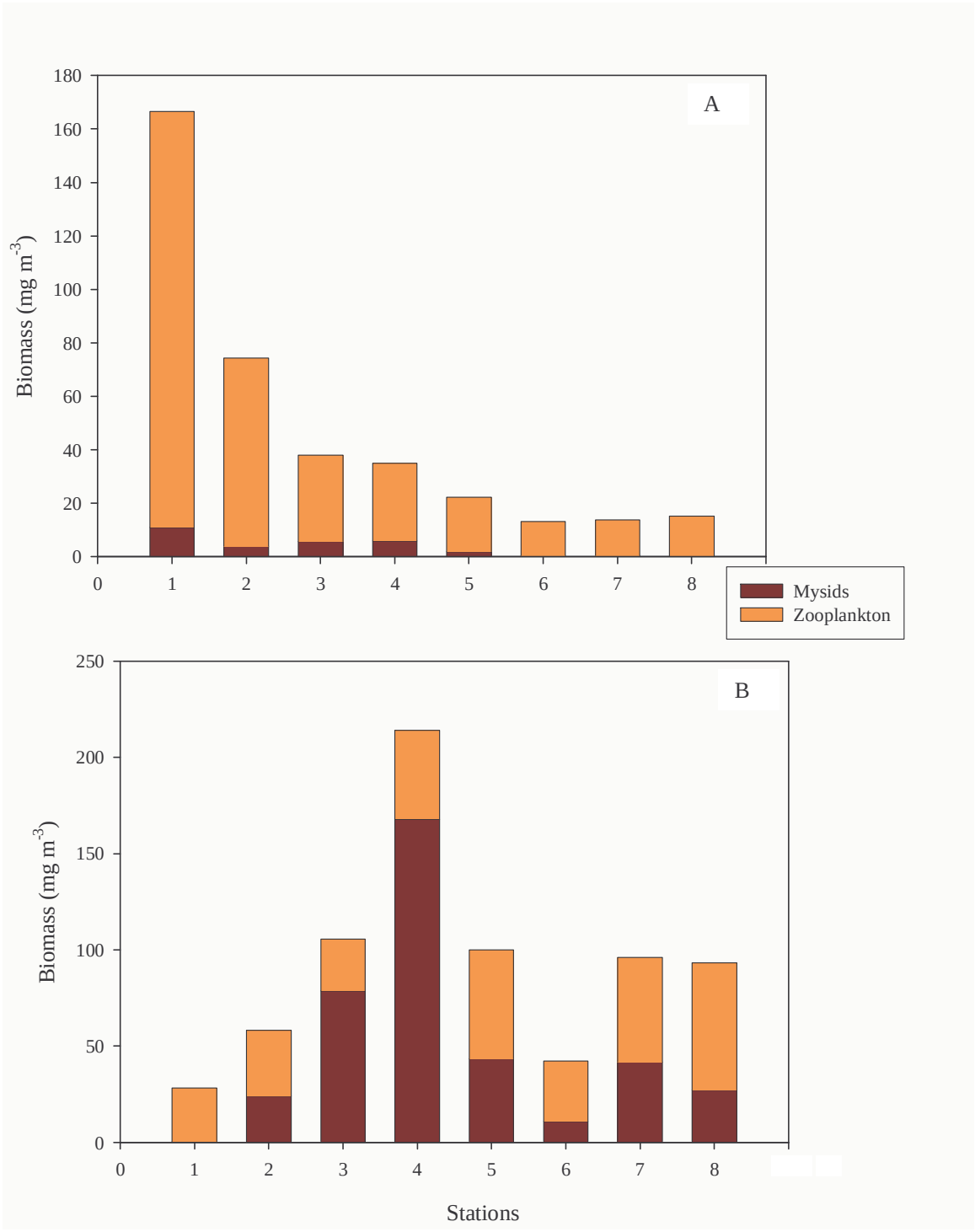

Figure 3.11: Spatial biomass (mg m^{-3}) of mysids and the remaining zooplankton at the 8 stations at the Great Fish Estuary during the daytime (A) and nighttime (B). Values have been pooled for each station over the 12-month sampling period.

3.3.6 *Mesopodopsis wooldridgei*

Population structure and distribution of *Mesopodopsis wooldridgei*

Abundance values of *M. wooldridgei* during the daytime ranged between 0.6 and 172.13 ind. m^{-3} or between <1% to 18% (average 2%) of the total zooplankton abundance. During nighttime abundance values ranged between 0.55 and 84.50 ind. m^{-3}. These values corresponded to between <1% and 74% (average 7%) of the total zooplankton abundance. Total abundances of *M. wooldridgei* in the middle reaches (Stations 3-6) of the estuary were found to be significantly higher when compared to station 1 ($F= 4.94$, $P<0.05$). There was no significant difference between the daytime and nighttime abundance values of *M. wooldridgei* ($P>0.05$). The virtual absence of *M. wooldridgei* during the months of February and April 2004 corresponded to peaks in freshwater inflow into the estuary.

The abundances of the various life stages (juvenile, immature, male and female) of *M. wooldridgei* over the 12-month study are presented in Figures 3.12 and 3.13. Results of the investigation indicated that there were no significant spatial differences in the distribution patterns of the various developmental stages of *M. wooldridgei* ($P> 0.05$ in all cases). The abundance of adults during June showed significant difference compared to all other months. When pooled, the data for all months and stations showed significantly more adults are present than juvenile and immature size classes ($P<0.05$). Female *G. psammodytes* with brood pouches have been demonstrated to be spatially separated from other reproductive or developmental stages (Wooldridge, 1983). During the current investigation no such pattern was observed as females were consistently recorded in samples comprising male and the earlier developmental stages of *M. wooldridgei* (Figure 3.14 A and B). There were no significant correlations found between *M. wooldridgei* and the physico-chemical and biological variables measured during the study ($P>0.05$ in all cases).

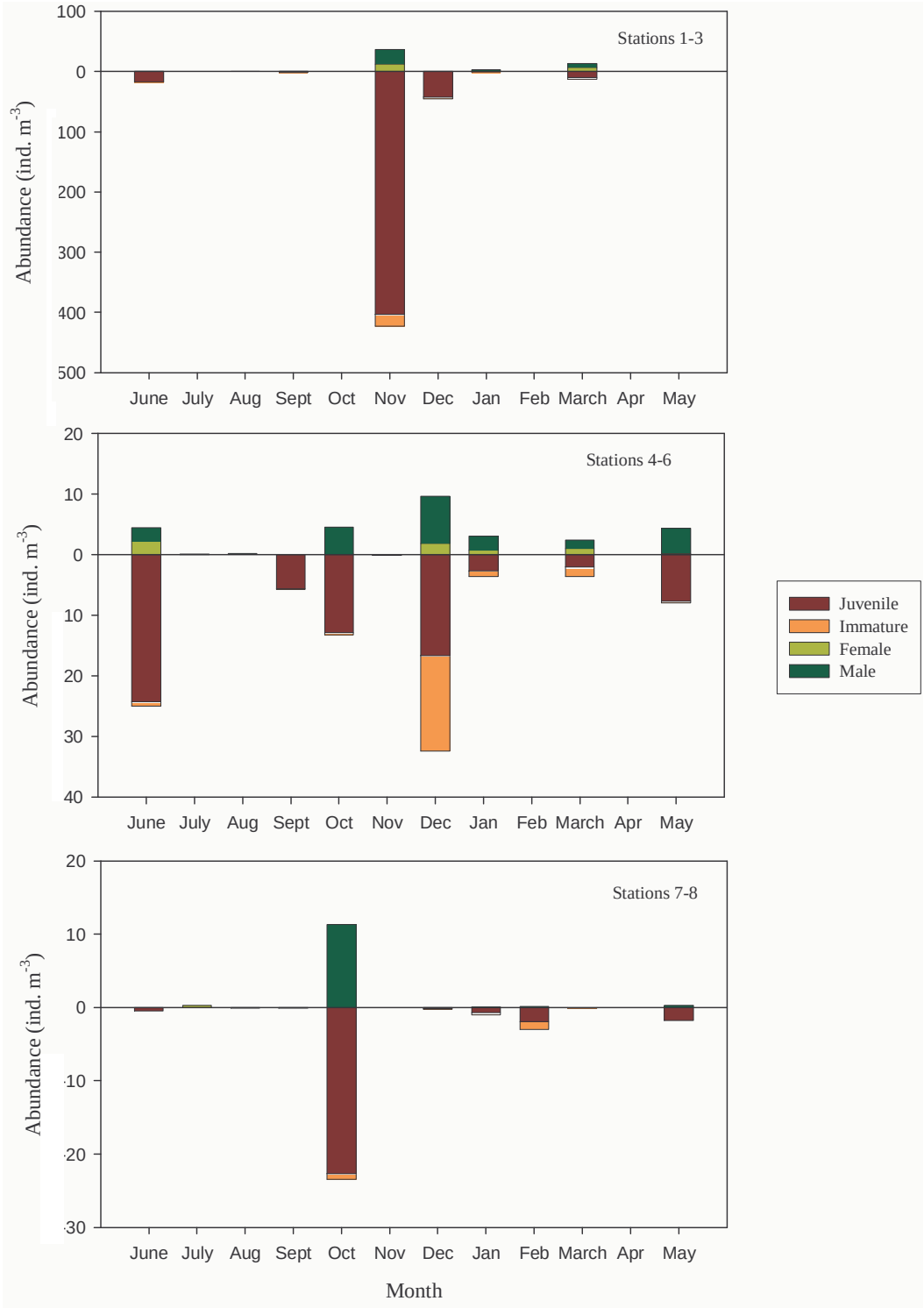

Figure 3.12: Spatial and temporal abundances (ind. m^{-3}) of the various developmental stages of *M. wooldridgei* in the upper (St. 1-3), middle (St. 4-6) and lower (St. 7-8) reaches of the Great Fish Estuary during the daytime. Note the different magnitudes of the y-axis. The adults are shown above the line while the immatures and juveniles are below the line.

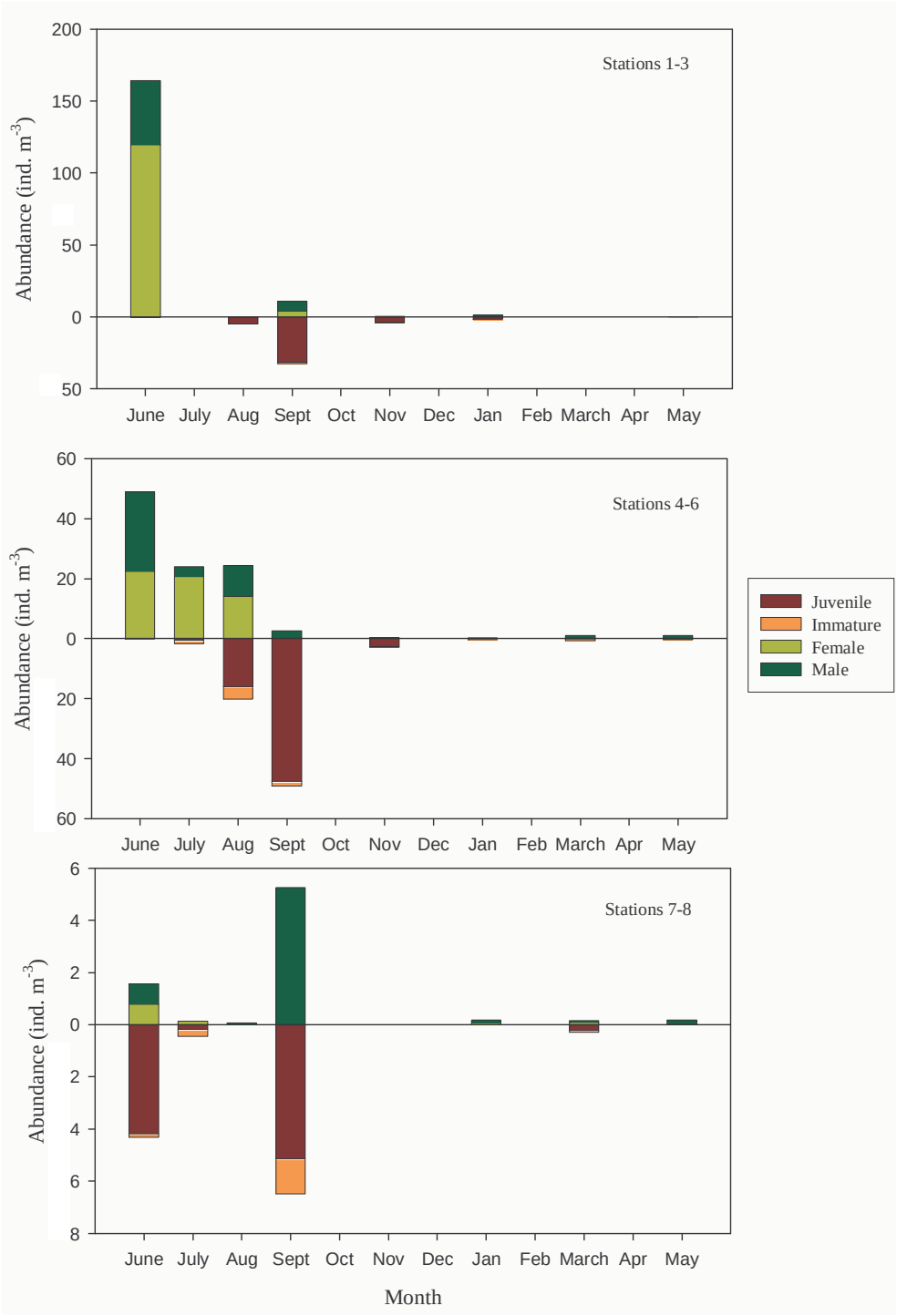

Figure 3.13: Spatial and temporal abundances (ind. m^{-3}) of the various developmental stages of *M. wooldridgei* in the upper (St. 1-3), middle (St. 4-6) and lower (St. 7-8) reaches of the Great Fish Estuary during the nighttime. Note the different magnitudes of the y-axis. The adults are shown above the line while the immatures and juveniles are below the line. Note, due to logistical constraints no data were collected during the nighttime October and December sampling trips.

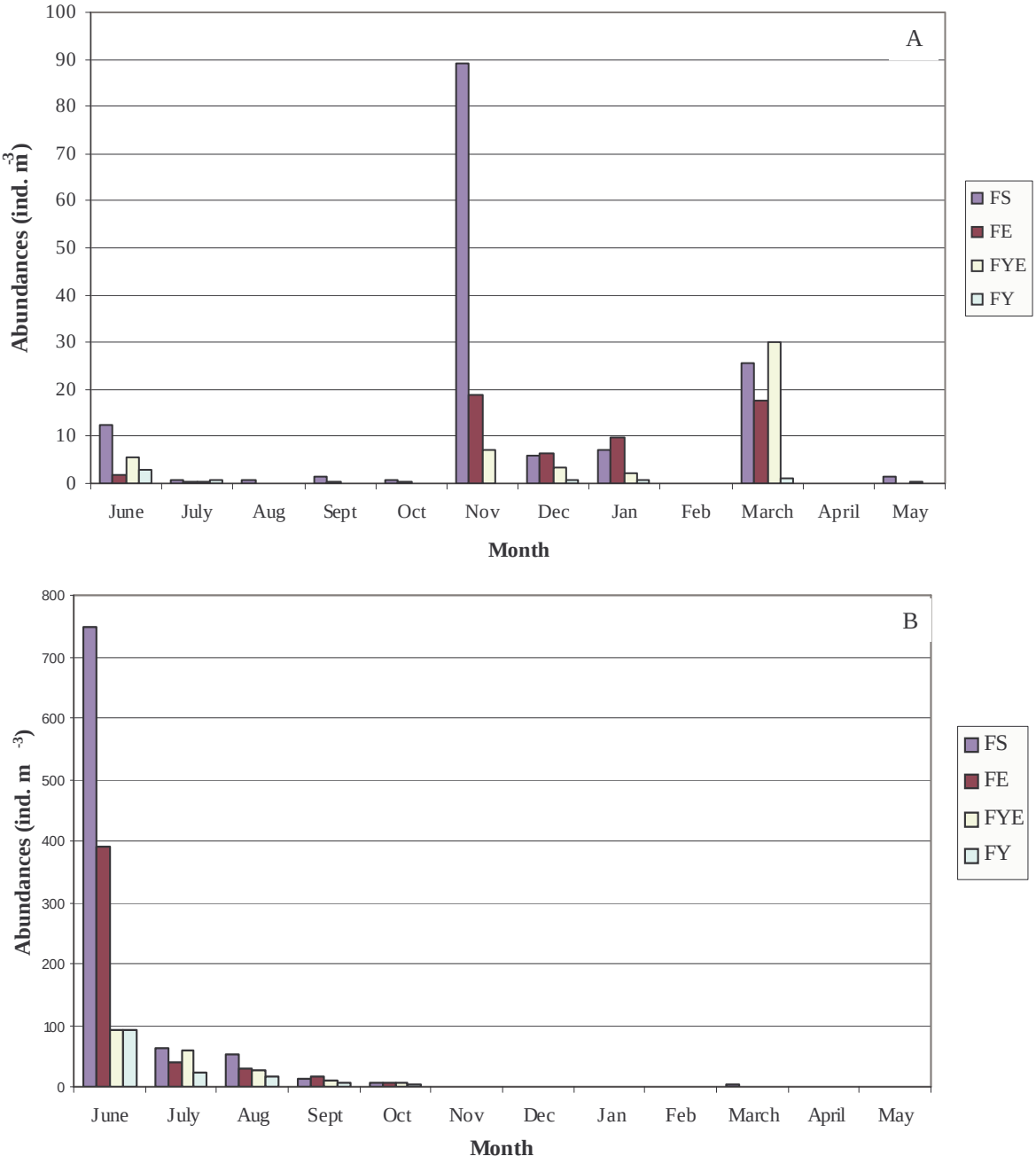

Figure 3.14: A and B: Temporal distribution of the female reproductive stages of *M. wooldridgei* during the day (A) and night (B) over the 12-month sampling period in the Great Fish Estuary. FS- empty brood sac, FE- brood sac containing eggs, FYE- brood sac containing young with eye spots, FY- brood sac containing young without eye spots. Note the different y-axis values. Note, due to logistical constraints no data were collected during the nighttime October and December sampling trips.

The average biomass of *M. wooldridgei* in the morning and at night, ranged between 0.12 and 15.81 mg Dwt m^{-3} and between 0.42 and 160.44 mg Dwt m^{-3}, respectively (Figures 3.15 A and B). These values comprised between <1 and 48% of the total

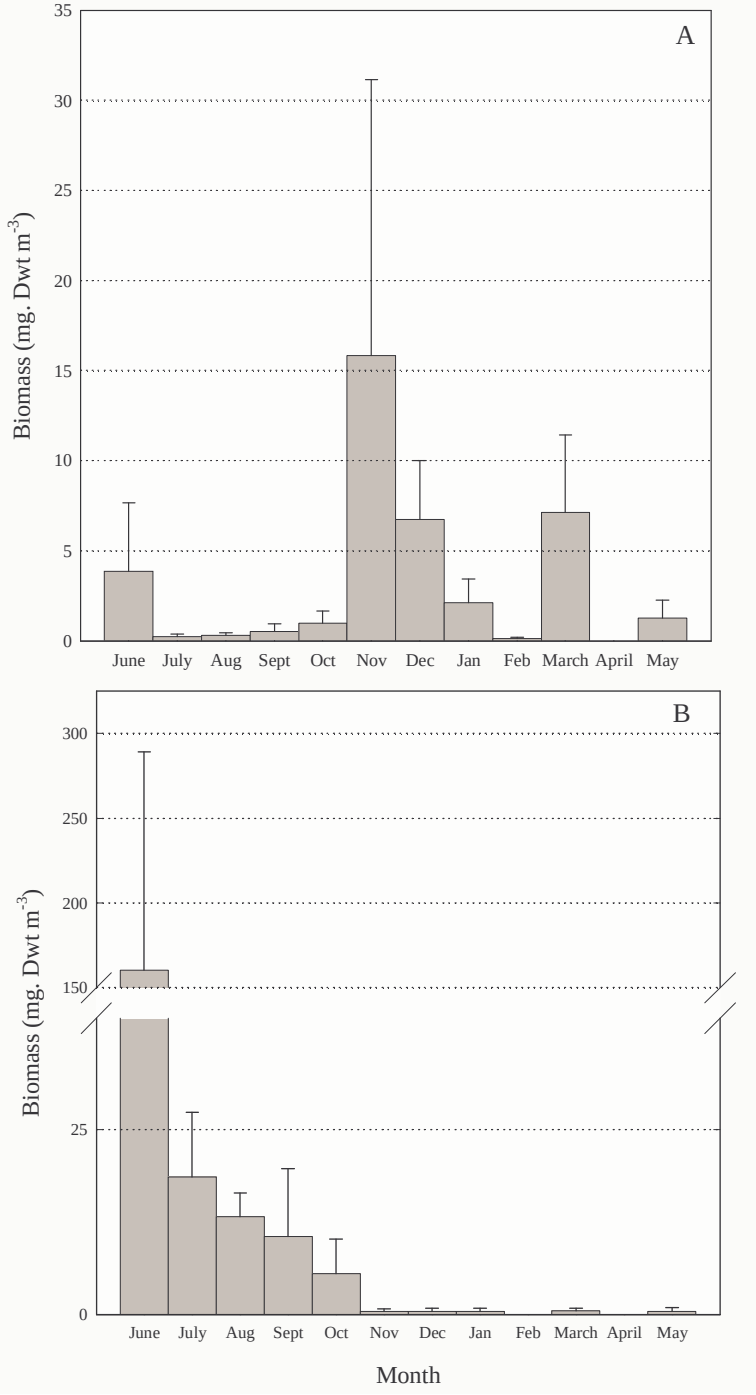

Figure 3.15 A and B: Average biomass (mg m^{-3}) of the mysid, *M. wooldridgei*, during the daytime (A) and nighttime (B) sampling in the Great Fish Estuary. Values for the 8 sampling stations have been pooled for each month. Error bars are standard deviation. Note the different magnitudes of the y-axis.

zooplankton biomass during daytime and between < 1 and 80% during the nighttime. The biomass values of *M. wooldridgei* in the middle reaches of the estuary were significantly higher than those values obtained in the upper (stations 1 and 2) and lower (stations 6-8) regions of the estuary ($F= 7.80$, $P<0.01$). Nighttime biomass values were significantly higher than the daytime values ($P<0.05$).

3.3.7 Numerical analyses

Total zooplankton abundance

Temporal patterns

Unfortunately due to technical problems (boat engine failure), no nighttime samples were collected for the months of October and December. The numerical analyses of the mean monthly zooplankton abundance data indicated the presence of three significantly different groupings, designated Groups 1 to 3 (ANOSIM routines; $P < 0.05$) (Table 3.2). Group 1 was identified as an outlier and comprised the survey conducted in June. Group 2 comprised the surveys conducted in February and April (Figure 3.16). The remainder of the surveys comprised Group 3 (Figure 3.16). SIMPER analysis showed that the differences between the various groupings identified with the numerical analyses could be ascribed to changes in the relative abundances of the most abundance species rather than the presence or absence of individual species. The 5 most numerically abundant species accounting for up to 75% of the similarity within each grouping identified with the numerical analyses is presented in Table 3.1. Margalef diversity index values for Group 2 ranged between 2.20 and 2.80 and between 2.27 and 3.18 in Group 3. The diversity index value for Group 1 was estimated at 3.22.

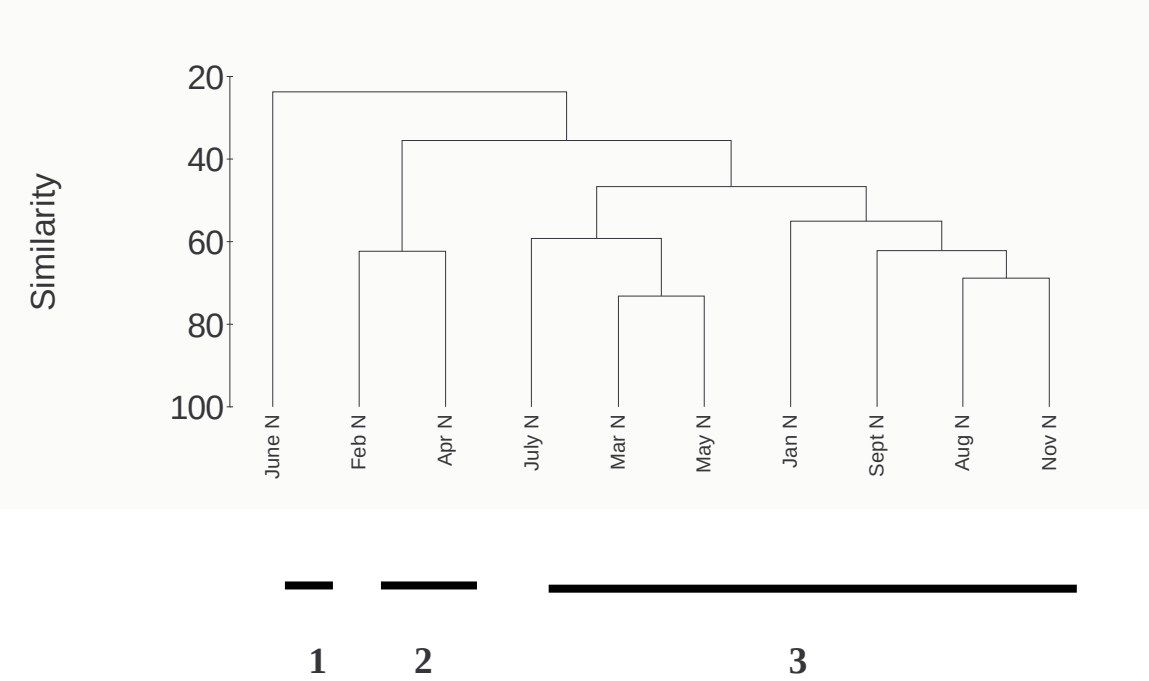

Figure 3.16. Dendrogram showing the classification of the mean monthly zooplankton abundance data collected in the Great Fish Estuary over the period June 2002 to April 2003. n = 8 for each month. Data for October and December 2003 are omitted as samples were not collected at night due to logistical constraints.

Table 3.2. Mean monthly average abundances of the seven most numerous zooplankton species within each grouping identified with the hierarchical cluster analysis. Values in brackets are ind. m^{-3}.

Group 1	**Group 2**	**Group 3**
Pseudodiaptomus hessei (2.3); *Mesopodopsis wooldridgei* (84.50); *Gastrosaccus brevifissura* (1.60); *Oithona nana* (8.5); *Halicyclops* spp. (1.3)	*Pseudodiaptomus hessei* (2877); *Grandidierella lignorum* (244.1); *Halicyclops* spp. (34.4); *Oithona nana* (42.1); *Calanus* spp. (34.4)	*P. hessei* (1611.6); *M. wooldridgei* (10.6); *O. nana* (284.4); *G. brevifissura* (23.3); *Halicyclops* spp. (111.3); Calanus spp. (146.4)

Spatial patterns

Results of the numerical analyses conducted to assess the spatial trends in the distribution of the total zooplankton abundances in the Great Fish estuary is presented in Figure 3.17. Hierarchical cluster analyses identified two significantly different groupings of stations (ANOSIM routine; $P < 0.05$). Group 1 comprised stations 5 to 8 and Group 2, stations 1 to 4 (Figure 3.17). SIMPER analyses indicated that the differences between the two groupings could be attributed to differences in the total zooplankton abundance and the taxonomic diversity within the different reaches of the estuary. The 8 most abundant species accounting for up to 90% of the similarity within the two groupings identified with the numerical analyses is presented in Table 3.3.

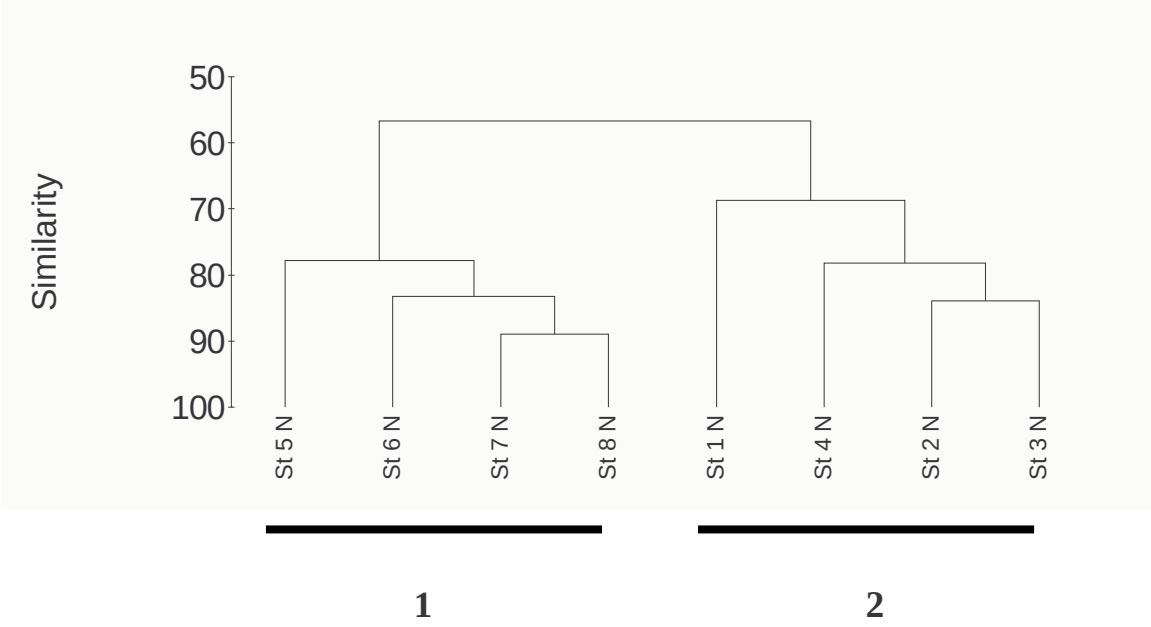

Figure 3.17: Dendrogram showing the classification of the mean monthly abundances of total zooplankton collected at each station collected from the Great Fish Estuary over the period June 2003 to May 2004.

Stations in Group 1, were characterised by moderate zooplankton abundances (1356 to 2817 ind m^{-3}) and high species diversity (Margaleff diversity index values ranged between 4.28 and 5.26). The high species diversity at the Group 1 stations could be attributed to the presence of both estuarine (e.g. *Pseudodiaptomus hessei*) and marine breeding species including the caridian shrimp *Palaemon peringyui* and the mysid, *Gastrosaccus brevifissura*. In Group 2, abundances of zooplankton at the various stations were higher ranging between 4997 and 7332 ind. m^{-3}. The zooplankton

community in Group 2 was almost entirely dominated by the copepod, *Pseudodiaptomus hessei*, which comprised on average 79% (range 68-83%) of the total zooplankton abundance. The Margalef diversity index values for stations within Group 1 ranged between 1.89 and 3.62.

Table 3.3: Mean average abundances of the 8 most numerically abundant species at each group of sampling station accounting for up to 85% of the similarity within each grouping identified with the hierarchical cluster analysis. Values in brackets are mean abundances (ind. m^{-3}).

Group 1	**Group 2**
Pseudodiaptomus hessei (670.5); *Oithona nana* (101.0), *O. brevicornis* (182.6); *Acartia longipatella* (258.2) *Limacina* spp. (44. 7); *Halicyclops* spp. (139.8); *Palaemon peringyui* (larvae) (79.3); *Grandidierella lignorum* (74.1); *Gastrosaccus brevifissura* (32.6)	*P. hessei* (4850.1); *O. nana* (93); *A. longipatella* (42.5) *P. peringyui* (larvae) (3.11); *Calanus* spp. (22.8); insecta (19.2); *G. lignorum* (2.4)

Mysids

Temporal patterns

The numerical analyses did not identify any significant seasonal patterns in the mysid abundances during the study. Three significant different groupings (designated Groups 1 to 3) were identified with the hierarchical cluster analyses. ANOSIM routine demonstrated that the groupings were significantly different from one another ($P < 0.05$). Differences between the Group 2 and 3 could largely be attributed to shifts in the relative contributions of the three mysid species to the total mysid abundances (Figure 3.18).

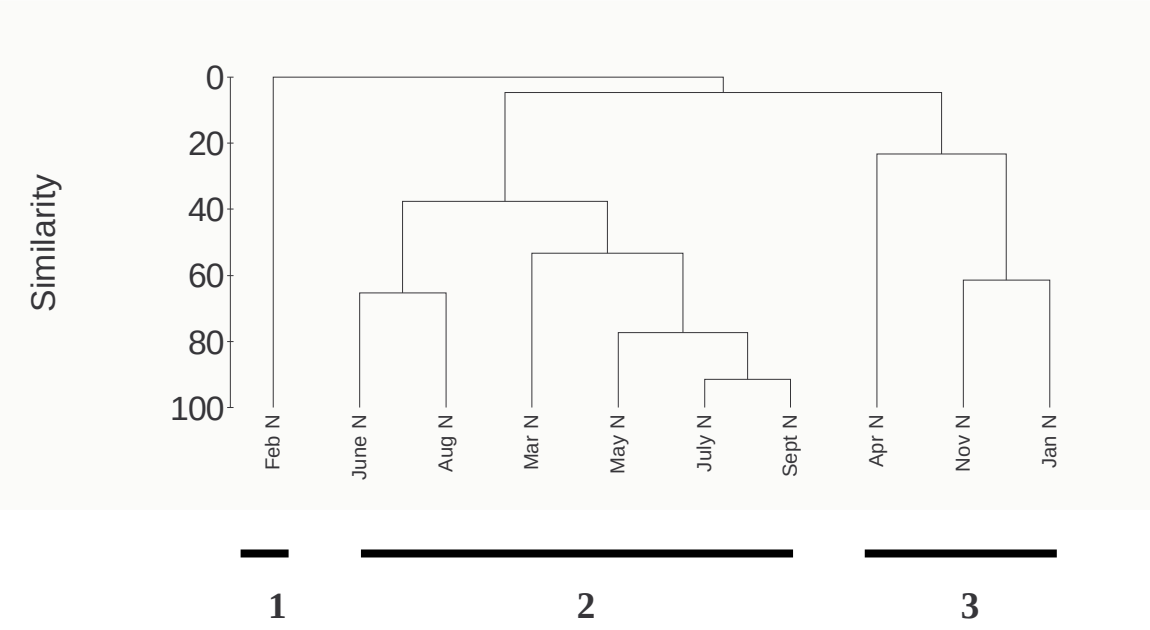

Figure 3.18: Dendrogram showing the classification of the mean monthly mysid abundance data collected in the Great Fish Estuary over the period June 2003 to May 2004.

In contrast Group 1, was characterised by the absence of any mysids during the sampling survey. Group 2 was characterised by elevated abundances of the all three mysid species. Finally in Group 3, *R. terranatalis* was absent from the samples and the abundances of *M. wooldridgei* and *G. brevifissura* were < 2 ind. m^{-3}. The mean abundances of the mysids within the three groupings identified by the numerical analyses are presented in Table 3.4.

Table 3.4: Mean average mysid abundances (ind. m^{-3}) in the three groupings identified with the numerical analyses. Analyses were undertaken using the PRIMER statistical computer package (Clarke and Warwick 1994).

Mysid species	Group 1	Group 2	Group 3
Mesopodopsis wooldridgei	0.00	25.45	1.40
Rhopalophthalmus terranatalis	0.00	13.44	0.00
Gastrosaccus brevifissura	0.00	27.45	0.22

Spatial distribution of mysids

Results of the numerical analyses conducted to assess the spatial distribution patterns of the mysids in the Great Fish Estuary is presented in Figure 3.19. Hierarchical cluster analyses identified three groupings of stations. Group 1, comprised station 1, Group 2, stations 2 to 5 and finally Group 3, stations 6 to 8. ANOSIM routine indicated that the groupings were significantly different from one another ($P < 0.05$). SIMPER analyses indicated that the differences between the three groupings could be ascribed to shifts in the relative contribution of the three mysids species to the total mysid abundance. An exception was presented in Group 1 where mysids were absent or present in extremely low concentrations (< 0.5 ind m^{-3}) (Table 3.5). Group 2, was numerically dominated by *M. wooldridgei* and *R. terranatalis* and was characterised by the virtual absence of *G. brevifissura*. Finally, Group 3 was numerically dominated by *G. brevifissura*. Mean average abundances of *R. terranatalis* and *M. wooldridgei* in Group 3 were always < 4 ind. m^{-3}.

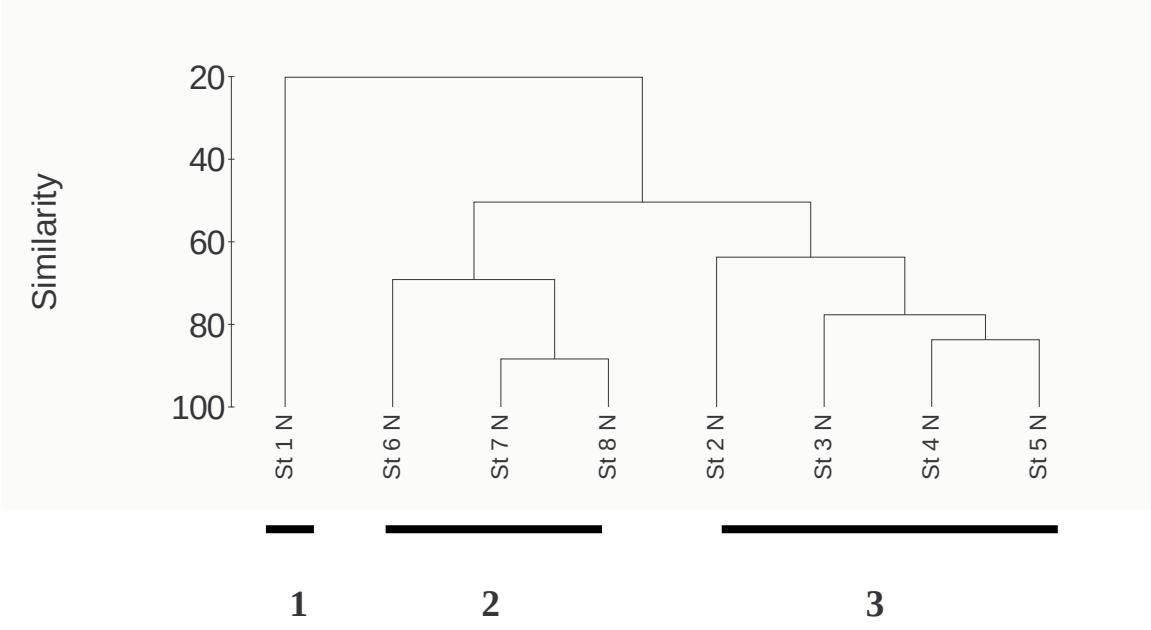

Figure 3.19: Dendrogram showing the classification of the mean mysid abundance data at each station occupied in the Great Fish Estuary over the period June 2003 to May 2004.

Table 3.5: Mean average abundances (ind. m^{-3}) of the three mysid species in the groupings identified with the hierarchical cluster analysis. Group 1 comprised station 1, Group 2, Stations 6 to 8 and Group 3, stations 2-5. Analyses were conducted using the SIMPER statistical package of the PRIMER computer package.

Mysid species	Group 1	Group 2	Group 3
Mesopodopsis wooldridgei	0.43	3.44	28.65
Rhopalophthalmus terranatalis	0.00	2.69	14.23
Gastrosaccus brevifissura	0.00	43.06	0.00

3.4 DISCUSSION

Temporal changes in total chlorophyll *a* concentrations and zooplankton abundance and biomass in permanently open southern African estuaries has been linked to seasonal patterns in water temperature and rainfall (Allanson and Read, 1995; Baldó *et al*, 2001; Froneman, 2004; Jerling, 1998; Wooldridge, 1977; Wooldridge, 1986; Wooldridge, 1999; Wooldridge and Bailey, 1982). The results of the numerical analyses conducted on both the log-transformed total zooplankton abundance and mysid abundance data did not reveal the presence of any seasonality (Figures 3.16 and 3.18). The absence of any seasonal pattern can probably be ascribed to the sustained freshwater inflow into the estuary due to the inter-basin transfer of water from the Gariep dam to the Fish River system (Grange *et al*, 2000). On the other hand, alterations in the magnitude of the freshwater inflow into the estuary did contribute to the aseasonal patterns in zooplankton community structure. During February and April 2004, the increased outflow of freshwater into the marine environment coincided with a significant decrease in total zooplankton abundances (including mysids) in the estuary (designated Group 2 in the results of the numerical analysis, Figure 3.16). The decrease in the zooplankton abundance and biomass values could likely be attributed to the transport of the zooplankton out of the estuary into the marine environment. SIMPER analysis indicated that the difference between the communities of Group 2 and 3 could largely be attributed to the relative abundance of species. The numerical analysis of the temporal distribution of mysids differs from the zooplankton community in that the effect of the freshwater pulse is only evident in February (Group 1) and not April.

Although sustained freshwater inflow into the estuary masked the seasonal patterns in zooplankton community composition and abundance, the strong axial gradient in salinity contributed to a distinct spatial pattern in the zooplankton community structure and biomass in the Great Fish Estuary (Figure 3.17). Results of the numerical analysis conducted on the total zooplankton abundance data indicated that the estuary could largely be divided into two discreet zones, designated Group 1 and 2. Group 1 in the lower reaches of the estuary was composed of a diverse zooplankton community with both estuarine and marine breeding species (including copepods, mysids and amphipods) present. In contrast, Group 2 comprised those stations occupied in the upper reaches of the estuary, which were almost entirely numerically dominated by the calanoid copepod, *P. hessei*, which contributed up to 90% of the total zooplankton abundance. *P. hessei* is considered to be a pioneer species (Wooldridge and Bailey, 1982) able to withstand large fluctuations in salinity and temperature (Grindley, 1972; Wooldridge and Melville-Smith, 1979). Peaks in the abundance of *P. hessei* corresponded to freshwater pulses (Wooldridge and Bailey, 1982). Estimates of zooplankton abundance (62 and 28 917 ind. m^{-3}) and biomass (10 to 203 mg Dwt m^{-3}) during this study are in the range reported for other permanently open estuaries with sustained freshwater inflow (See Table 1.2) (Wooldridge and Bailey, 1982, Wooldridge, 1999). In contrast, in permanently open freshwater deprived estuaries total zooplankton abundance and biomass values are substantially lower (Grange *et al*, 2000). The elevated zooplankton abundance and biomass values recorded in permanently open estuaries with sustained freshwater inflow appears to be linked to increased food availability (chl. *a*) within these systems (Allanson and Read, 1995).

In agreement with total zooplankton abundance data, the distinct axial gradient in salinity within the Great Fish Estuary also contributed to discreet spatial patterns in the mysids distribution. Numerical analysis indicated the presence of three distinct groupings (Figure 3.16). Group 1 comprised station 1, which was always freshwater dominated and was characterised by the virtual absence of mysids. Group 2 comprised those stations occupied in the lower reaches of the estuary and was numerically dominated by the marine breeding mysid, *G. brevifissura*. Finally, within Group 3, the mysids *M. wooldridgei* and *R. terranatalis* numerically dominated. Physiological constraints have been shown to restrict the distribution of mysids within

estuaries particularly within the upper reaches (Baldó *et al*, 2001; Wooldridge and Bailey, 1982). Results of the physiology experiments conducted during this study showed that the respiration rates of *M. wooldridgei* are optimal at moderate (20°C) temperatures and salinities (15‰ and 25‰). Conversely, the mysids exhibited mass mortality in freshwater (see Chapter 2). During the present study, maximum biomass of *M. wooldridgei* was recorded within the middle reaches of the estuary where moderate temperature and salinities prevail. On the other hand *M. wooldridgei* was largely absent from the upper reaches of the estuary where freshwater dominated. These data suggest that the upper distribution of *M. wooldridgei* within the Great Fish Estuary is determined by physico-chemical variables. Within the middle and lower reaches of the estuary, the distribution of *M. wooldridgei* appears to reflect biological interactions.

Freshwater inflow into estuaries has been shown through nutrient loading to promote primary production in estuarine systems, particularly larger diatoms (Mallin *et al*, 1993). The trapping of riverine phytoplankton as well as allochthonous imports further enhance the phytoplankton biomass within these systems (Lucas, 1986). There were no apparent temporal patterns in total chl. *a* concentration. The lack of seasonality in total chl. *a* concentrations could largely be ascribed to sustained freshwater inflow into the system. Total chl. *a* concentration ranged between 2.68 μg L^{-1} and 31.12 μg L^{-1} and was always dominated by large phytoplankton cells (>5 μm). The predominance of large cells can be attributed to the increased availability of macronutrients (primary source in freshwater), which promotes growth of the larger cells (Allanson and Read, 1995). The values reported here are substantially higher than those recorded in the freshwater deprived Kariega and Kasouga estuaries located within in the same geographic region (Froneman, 2001a; Grange *et al*, 2000). In the Kariega and Kasouga estuaries picophytoplankton (<2 μm) dominated the total chl. *a* concentrations (Froneman, 2002b; Grange *et al*, 2000). The present chl. *a* concentrations also exceed the mean for the Sundays Estuary which reached 25 μg L^{-1} in the upper section of the system (Hilmer and Bate, 1990).

Abundance values of *M. wooldridgei* during the daytime ranged between 0.06 and 172.13 ind.m^{-3}, and between 0.55 and 84.50 ind.m^{-3} during nighttime. The average biomass for *M. wooldridgei* in the morning and at night, ranged between 0.12 and

15.81 mg Dwt m^{-3} and between 0.42 and 160.44 mg Dwt m^{-3}, respectively. The contribution of mysids to total zooplankton abundance was relatively low, with an average of 2% during the daytime and 10% during the nighttime. On the other hand, the mysids contributed a much greater proportion of the total zooplankton biomass, with an average of (range: <1 and 48%) 14% during the day and 44% (range: <1 and 95%) during the nighttime. Among the mysids, *M. wooldridgei* consistently contributed most to the total mysid biomass. Indeed, *M. wooldridgei* contributed on average 12% of the daytime and 13% of the nighttime total zooplankton biomass. The biomass values presented here are comparable to the lower range found in other studies conducted in the same geographic region. For example, values ranging between <10 and 1450 mg m^{-3} were obtained in the Sundays Estuary, where mysids comprised between 70 and 90% of the total zooplankton biomass (Wooldridge and Bailey, 1982). Avoidance of surface water by mysids in water with strong salinity stratification could lead to an underestimation of abundance, as only the upper water column was sampled (Figure 3.3 and 3.4).

The significantly higher biomass values for *M. wooldridgei* obtained during the nighttime can be attributed to the distinct vertical migration patterns demonstrated by this species (Froneman, 2001a; Wooldridge, 1983). The vertical migration is generally thought to have evolved as a predator avoidance strategy (Gliwicz, 1986). Wooldridge and Erasmus (1980) suggested that diel vertical migration pattern demonstrated by *M. wooldridgei* may serve as a mechanism for maintaining its position within the water column. *M. wooldridgei* has been shown to be spatially segregated from *R. terranatalis* in the Sundays Estuary with *G. brevifissura* restricted to stations near the mouth region. This pattern was thought to reflect the impact of adult *R. terranatalis* preferentially feeding on juvenile *M. wooldridgei* (Wooldridge and Webb, 1988) coupled with the inability of *G. brevifissura* to withstand low salinities (Marshall *et al*, 2003). During the present study, *G. brevifissura* was largely limited to the mouth region where the salinities were near that of seawater. On the other hand, *M. wooldridgei* and *R. terranatalis* did not appear to demonstrate any clear spatial segregation.

Wooldridge (1983) showed that reproductively active *M. slabberi* (now *M. wooldridgei*) were spatially separated from the various developmental stages of the

mysid. During the present study no such pattern was observed (Figures 3.12 and 3.13). In agreement with previous studies, female *M. wooldridgei* with brood pouches were recorded throughout the study period suggesting continual breeding throughout the year (Wooldridge, 1983). This is further supported by the presence of newly released juveniles during most months of the survey (Figures 3.12 and 3.13). This is in contrast to *R. terranatalis* which breeds for only 8 months of the year and has an overwintering brood (Wooldridge, 1986). The apparent lack of an overwintering brood in *M. wooldridgei* could be attributed to the high food availability, in the form of chl. *a*, within the Great Fish Estuary. Sustained freshwater inflow into the Great Fish Estuary resulted in the absence of seasonal patterns in the biology. The strong axial gradient caused by the interaction of marine and freshwater also helps to determine the distribution of this mysid.

In conclusion, results of the present study indicate that the sustained freshwater inflow into the Great Fish Estuary coincided with elevated chl. *a* concentrations and contributed to a distinct horizontal pattern in the zooplankton community structure and composition. Seasonality in zooplankton abundance and biomass was masked by the sustained freshwater inflow into the estuary. The zooplankton total abundances were numerically dominated by copepods although mysids at times dominated total zooplankton biomass.

CHAPTER 4 - FOOD WEB STRUCTURE USING CARBON ISOTOPE ANALYSIS ($\delta^{13}C$) IN A LARGE PERMANENTLY OPEN ESTUARY

4.1 INTRODUCTION

Estuarine food webs present a particular challenge to researchers due to their complexity. The complexity can be related to the variety of potential sources of food available to heterotrophic organisms within systems including terrestrial, marine and freshwater (Haines and Montague, 1979). Feeding studies on zooplankton in southern African estuaries have traditionally employed *in vitro* incubations or the gut florescence approach (Froneman, 2000a; Froneman, 2001a; Froneman, 2001b; Jerling and Wooldridge, 1995a; Webb *et al*, 1987; Wooldridge and Webb, 1988). These techniques are limited as they do not consider the wide variety of potential food sources available to the zooplankton. Furthermore these techniques do not take into account the important contribution of detritus (Rau *et al*, 1983) or indeed the assimilation of the various carbon sources (Créach *et al*, 1997).

Several studies have employed isotopes to assess carbon flow and trophic links in aquatic ecosystems (del Giorgio and France, 1996; Fry and Sherr, 1984; Haines and Montague, 1979). Carbon isotope analysis can be a useful tool in determining the dominant food sources especially when they are isotopically distinct as ^{13}C is thought to have an enrichment factor of 1‰ per trophic level (Fry and Sherr, 1984). Stable isotope analyses has been employed on a number of occasions to determine the main sources of carbon utilised by zooplankton and fish in permanently open estuaries along the south-eastern coastline of southern Africa (Froneman, 2000b; Froneman, 2002a; Jerling and Wooldridge, 1995c; Mbande *et al*; Paterson and Whitfield, 1997). Results of studies conducted in freshwater deprived estuaries have highlighted the important contributions of salt marsh vegetation and submerged macrophytes to total carbon flow (Froneman, 2002a; Paterson and Whitfield, 1997; Froneman, 2001c). These results were in contrast to studies conducted in estuaries characterised by freshwater inflow, where the main carbon utilised by the zooplankton was derived from phytoplankton, freshwater in origin (Froneman, 2002a; Jerling and Wooldridge, 1995c). The low contribution of phytoplankton to total carbon flow in the Kariega

Estuary could largely be attributed to the size structure of the phytoplankton, which are too small to be directly utilised by the zooplankton within the system (Froneman, 2001c; Froneman, 2002a).

The main aim of this study is to determine the primary food sources consumed by the zooplankton community with special emphasis on the three main mysid species, *Rhopalophthalmus terranatalis*, *Mesopodopsis wooldridgei* and *Gastrosaccus brevifissura* in the freshwater dominated, Great Fish Estuary.

4.2 MATERIALS AND METHODS

Plant, zooplankton and particulate organic matter (POM) samples for isotope analysis were collected in July (winter) and November(summer) 2003 from the middle reaches of the Great Fish Estuary (Table 4.1).

4.2.1 Sample collection

Vegetation was sampled from the riparian zone, salt marsh and littoral zone. All vegetation sampled was rinsed in seawater, which had been filtered through a 0.2 μm Nucleopore filter and then oven-dried at 60° C for 24 hours. Benthic filamentous diatom samples were collected using a 5cm corer. On collection the top 2mm of the sediment was removed and frozen for later analysis. In the laboratory the sample was defrosted and the filamentous algal mat separated from the detritus by washing in filtered seawater. The sample was dehydrated as above.

Particulate organic <20 μm samples were obtained by filtering a 5l water sample which had been pre-screened through a 20 μm filter through pre-combusted GF/F filter (vacuum <5cm Hg). This was followed by the manual removal of all visible zooplankton and other contaminants using a Heerenburg dissecting microscope operated at 100x magnification. For the determination of the >20 μm POM fraction, a 10l water sample was gently filtered through a 20 μm Nitex mesh. Material retained on the mesh was collected and placed in a petri-dish. All zooplankton was then removed from the filtrate, using a dissecting microscope. Samples were then oven dried at 60°C for 24 hours before isotope analysis was conducted.

Zooplankton samples were collected from the channel at night using a modified WP-2 net (mouth area 0.25m^2, mesh size 100 μm). In total, three tows were conducted. Samples were only collected within the middle reaches of the estuary as a previous study showed that there were no significant spatial differences in the isotopic signatures if the zooplankton in the Great Fish Estuary (Froneman, 2002a). Immediately after collection, samples were gently passed through a 20 μm mesh and the zooplankton retained were frozen with liquid nitrogen and transported to the laboratory for analysis. In the laboratory, zooplankton was separated into the following groups: copepods, mysids and amphipods. Three size classes of mysid were considered: adults, immatures and juveniles, according to Froneman (2001a). A detailed list of zooplankton species analysed during the study is presented in Table 4.1.

4.2.2 Chlorophyll *a* concentrations

Size fractioned chl. *a* was determined using two classes, those cells <20 μm and those >20 μm. A 250ml sample was initially filtered through as 20 Nucleopore filter (vacuum <5cm Hg) then through a glass fibre filter. Filters were then extracted in 90% acetone for 24 hours in the dark at -20°C. Chl. *a* was then determined fluometrically as described previously (see Chapter 3).

Table 4.1: The zooplankton and vegetation used for isotope analysis collected from the Great Fish Estuary during winter and summer. A: adults, I: immature, J:juvenile.

Species	Estuarine Habitat
Vegetation	
Acacia karoo	Riparian
Chrysanthemoides monilefera	Riparian
Euphorbia triangularis	Riparian
Phragmites australis	Riparian
Chenola diffusa	Salt marsh
Sarcorcornia perennis	Salt marsh
Juncus krausii	Salt marsh
POM	Channel
Zooplankton	
Amphipoda	
Grandidierella lignorum	Channel
Copepoda	
Acartia longipatella	Channel
Pseudodiaptomus hessei	Channel
Halicyclops spp.	Channel
Mysidae	
Mesopodopsis wooldridgei (A,I, J)	Channel
Rhopalophthalmus terranatalis (A, I)	Channel
Gastrosaccus brevifissura	Channel

4.2.3 Sample preparation

Zooplankton samples were defatted in a solution of chloroform, methanol and water (2:1:0.8) adapted from the method of Bligh and Dyer (1959). This procedure was carried out to minimize discrepancies resulting from differences in the proportion of fatty tissue between different organisms.

4.2.4 Isotope analysis

$\delta^{13}C$ determination was carried out using a Finnigan-MAT 252 stable light isotope mass spectrometer after sample combustion in and on-line Carlo-ebre preparation unit. Merk gelatine was used as an internal standard, calibrated against IAEA reference materials. Results are expressed in the standard delta notation as:

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

Where X = element in question and R = ratio of the heavy over the light isotope. Repeated analysis of homogenous material yielded a standard deviation of 0.04‰.

4.2.5 Data analysis

Newman-Keuls multiple range test were performed after a one-way ANOVA analysis to determine whether there were any significant difference in the isotopic signatures of the dominant producers and consumers in the Great Fish Estuary. This analysis was conducted using the statistical computer package STATISTICA version 6.0.

4.3 RESULTS

There was no significant difference between summer and winter isotope values of the primary producers and zooplankton ($P>0.05$). As a consequence, values for the two seasons have been pooled.

4.3.1 Chlorophyll *a* concentrations

The mean total chlorophyll *a* concentration for cells <20 μm was 11.34 μg L^{-1} and 18.38 μg L^{-1} for cells >20 μm.

4.3.2 Vegetation and particulate organic matter

In total, 7 species of plants were collected, including 3 terrestrial, one aquatic and 3 salt marsh vegetation types (see Table 4.1). Particulate organic matter (POM) of two

size fractions were considered, <20 μm and >20 μm. The isotopic values for the POM of <20 and >20 μm in size were found to be -27.9 (±1.56)‰ and -26.5 (±1.34)‰, respectively. Of the primary producers considered, the salt marsh plant, *Chenola diffusa*, was found to have the most depleted isotopic value, calculated at -25.8 (±0.8)‰. From the results of the statistical analysis, all plants were found to have similar $\delta^{13}C$ signatures (Figure 4.1) with only *Acacia karoo* (-22.87±0.07)‰ being significantly different from *Chenola diffusa* (-25.8±0.8‰) ($P<0.05$). The remaining terrestrial plants, *Chrysanthemum moilifera* and *Euphorbia triangularis* had values in the mid-range -23.7 (±0.31)‰ and -24.7 (±0.09)‰. The isotopic values for salt marsh plants, *Sarcorcornia perennis* and *Juncus krausii* were of -24.4 (±0.96)‰ and -22.9 (±0.12)‰, respectively. The only aquatic plant sampled, *Phragmites australis*, had an isotopic value of -23.9 (±0.2)‰.

4.3.3 Zooplankton

In total seven species of zooplankton were samples, with the mysids, *R. terranatalis* and *M. wooldridgei* represented by 2 and 3 size classes, respectively. Collectively the zooplankton analysed during this study comprised >95% of the total zooplankton abundance in the Great Fish Estuary (see previous chapter). The zooplankton sampled could be separated into 2 significantly different groups (Figure 4.1). The first group was composed of larger individuals including the amphipod *Grandidierella lignorum* (-24.6±1.69‰), adult *M. wooldridgei* (-24.2±0.88‰), and adult (23.9±1.24‰) and juvenile (24.1±0.59‰) *R. terranatalis*. The second grouping comprised the remaining zooplankton species including the copepods, *P. hessei* (-26.5±0.91‰), *A. longipatella* (-26.3±0.87‰), *Halicyclops* spp. (-25.9±1.22‰), and juvenile (-25.8±1.27‰) and larval (-26.2±0.63‰) mysids, *M. wooldridgei* and *G. brevifissura* (-25.7±0.39‰).

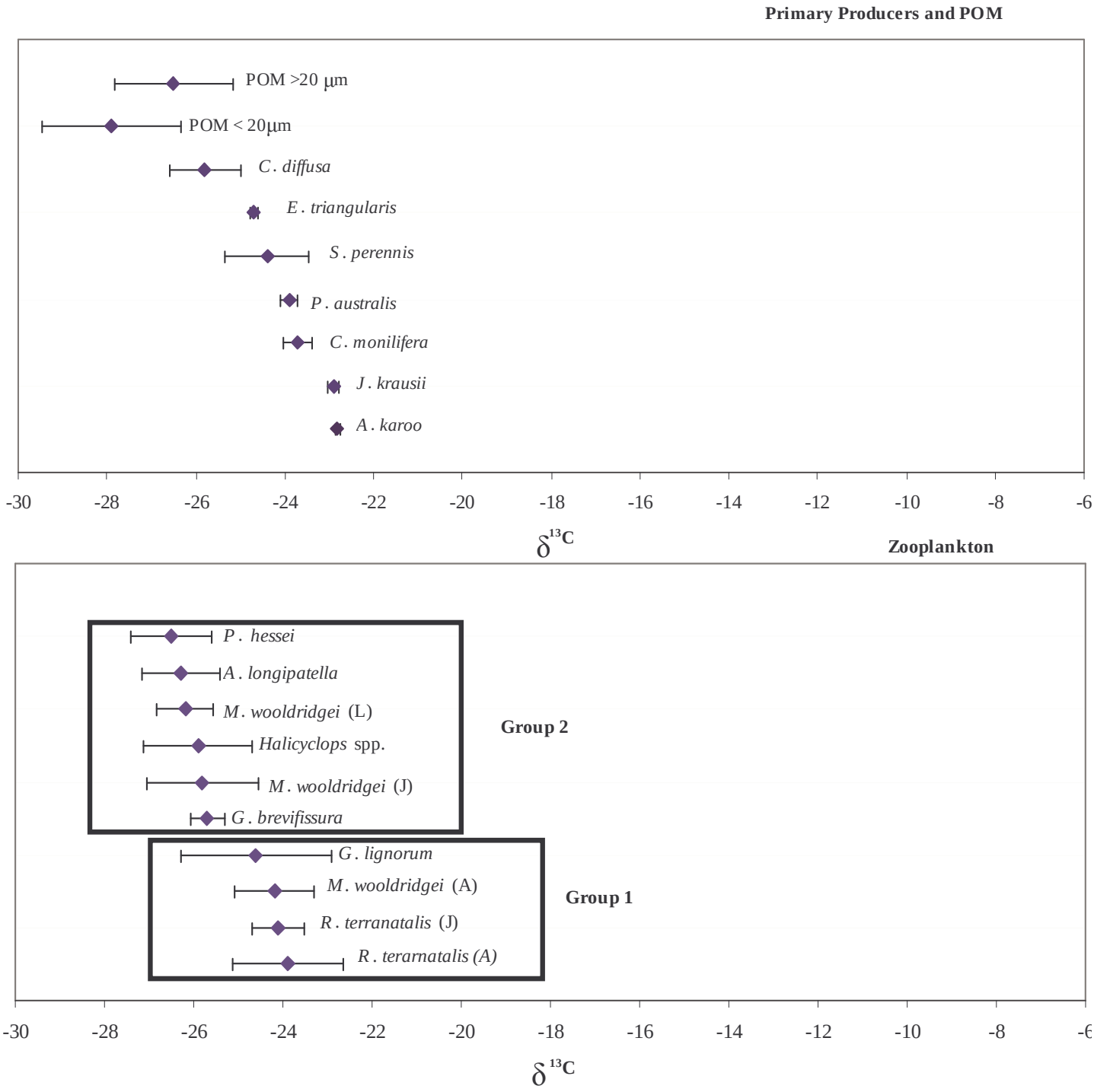

Figure 4.1: $\delta^{13}C$ (‰) values for primary producers and zooplankton collected from the Great Fish Estuary during summer and winter 2004. A: adults, I: immature, J: juveniles. Error bars are standard deviation, with n=6 for each value.

4.4 DISCUSSION

Seasonality in isotopic signatures of primary producers and zooplankton has been linked to amongst others, temperature, water flow and changes in food availability (Créach *et al*, 1997). Results of the present study indicated that there are no clear seasonal trends in total chl *a* concentrations or zooplankton biomass in the Great Fish Estuary (see Chapter 3). The lack of seasonality in the isotopic signatures of the primary producers and zooplankton is thus not surprising.

It is often not possible to determine the exact source of detritus within the water column when an intermediate value between plankton and other plant material is found (Peterson and Fry, 1987). This is reflected in the composition of the POM as there are several potential sources of carbon in the Great Fish Estuary including salt marsh and terrestrial vegetation and marine and freshwater phytoplankton. Previous studies conducted in permanently open and temporarily open/closed estuaries (TOCE) within the same geographic region have shown that salt marsh derived carbon makes a substantial contribution to the total carbon flow within these systems (Froneman, 2000b; Froneman, 2001c; Froneman, 2002a). This type of vegetation was once thought to make up the majority of organic seston in estuaries (Haines, 1977). However, in the current study, the isotopic signatures of the salt marsh vegetation (*S. perennis*, *J. krausii* and *C. diffusa*) are too enriched (-22.9 to -25.8‰) to have made significant contribution to the POM, whose isotopic values are highly depleted. In fact, a similar argument can be made for the terrestrial vegetation. It is worth noting that isotopic values of freshwater phytoplankton are typically thought to range between -25‰ to -30‰ (Fry and Sherr, 1984; Jerling and Wooldridge, 1995c). These values fall well within the range of the POM (-22.9 to -25.8‰) of the Great Fish Estuary. These facts suggest that POM is largely comprised of freshwater phytoplankton.

Two significantly different groupings of zooplankton were identified during the study. The first grouping of zooplankton comprised mainly copepods with $\delta^{13}C$ values ranging between -26.3 to -26.5‰. Assuming 1‰ enrichment per trophic level it is evident that copepods are consuming mainly phytoplankton. This result is in agreement with previous grazing studies conducted in the estuary which showed that

carbon derived from the consumption of phytoplankton was sufficient to meet the basic metabolic requirements of the dominant copepods in the Great Fish Estuary (Froneman, 2002a). The second group comprised the larger zooplankton, including adult and juvenile *R. terranatalis*, adult *M. wooldridgei* and the amphipod, *G. lignorum*. Upon closer inspection, it is apparent that *G. lignorum* should be considered separately from the mysids as it is a benthic burrower (Read and Whitfield, 1989) and thus would likely utilize a different carbon source than the mysids. Zooplankton have been shown to forage across isotopically distinct groups (France and Peters, 1997) and this is reflected in the carbon values of the mysids which were 2 to 3‰ more enriched than the POM. This suggests that the adult mysids are consuming a different carbon source than that of the copepods. The isotopic signatures for *M. wooldridgei* indicated that this mysid feeds mainly on phytoplankton as a juvenile and on copepods when an adult (Figure 4.1). *R. terranatalis* had the most enriched value (-23.0±1.24‰) of all the zooplankton. According to Wooldridge and Webb (1988), *R. terranatalis* can be considered an omnivore. The results of this study suggest that this mysid can largely be considered a carnivore as the isotopic value is too depleted to reflect a substantial contribution of phytoplankton or detritus derived from terrestrial or salt marsh vegetation.

Results of previous isotope studies conducted in permanently open estuaries within the same geographic region have demonstrated that carbon derived from both the terrestrial environment (including salt marsh) and channel (phytoplankton) contribute to carbon flow within the system (Froneman, 2002b; Mbande *et al*, 2004; Paterson and Whitfield, 1997). In contrast, results of this investigation indicate that the zooplankton within the Great Fish Estuary are largely sustained by the substantial phytoplankton stocks within the estuary, with terrestrial vegetation making a minor contribution. Differences in results of the various studies can largely be attributed to freshwater inflow. The primary source of macronutrients necessary to sustain the growth of phytoplankton is derived from inflow of freshwater (Allanson and Read, 1995). With the exception of the Great Fish Estuary, the permanently open estuaries examined previously can largely be regarded as freshwater deprived systems. As a consequence, phytoplankton stocks within these systems are low (generally <10 μg L^{-1}) and are generally dominated by phytoplankton cells too small to be grazed efficiently by the dominant zooplankton within these systems (Froneman,

2000a; Froneman, 2001b). On the other hand, the Great Fish Estuary is a freshwater dominated system characterized by extensive phytoplankton stocks (up to 200 μg L^{-1}), which are readily available for consumption by the dominant zooplankton within the system. The high contribution of the phytoplankton derived carbon to total carbon intake of the numerically dominant zooplankton in the estuary is thus not surprising.

CHAPTER 5 - FINAL DISCUSSION

The main objectives of this investigation were three-fold. The first aim was to investigate the physiology of the mysid, *Mesopodopsis wooldridgei* to variations in temperature and salinity and a combination thereof. Secondly, the study assessed the spatial and temporal distribution of the zooplankton, with particular emphasis on mysids, within a large permanently open estuary. Finally, the study investigated the primary carbon sources utilised by *M. wooldridgei* using stable carbon isotope analysis. The results of the various investigations are summarised below.

5.1 PHYSIOLOGY

Several factors have been shown to impact on the metabolic activities of invertebrates, with the most prevalent being temperature (eg: Newell and Branch, 1980; Weisse and Rudstam, 1989; Winkler and Greve, 2002) and salinity (eg: Marshall *et al*, 2003; Verslycke and Janssen, 2002; Webb *et al*, 1997). In a previous study, Webb *et al* (1997) found the body fluids of *Mesopodopsis wooldridgei* and *Rhopalophthalmus terranatalis* ranged from hyperosmotic to hypotonic depending on the medium in which they were submerged. *Gastrosaccus brevifissura* was found to maintain its body fluid at a higher concentration then all the media, including seawater (Webb *et al*, 1997). The moderate Q_{10} value of 2.14 observed for *M. wooldridgei* during this study, suggests that the mysid is unlikely to experience thermal extinction in warm temperate estuaries. The highest respiration rates were obtained when a combination of salinity and temperature were altered, and mass mortality was experienced at high temperatures (30°C) and low salinities (5‰). These data suggest that the upper distribution pattern of *M. wooldridgei* is likely to be determined by physiological constraints. The moderate Q_{10} values (1.44 – 2.34) obtained over a range of salinities (15 – 35‰) between 20 – 30°C, suggests that *M. wooldridgei* is well adapted to living in an environment characterised by variability in temperature and salinity. It is worth noting that maximum abundances of *M. wooldridgei* were recorded in the middle reaches of the Great Fish Estuary during the course of the study (Figure 3.11 A and B). Within this region of the estuary, water temperatures rarely exceeded 20°C and salinities were generally in the range of 10 to 25‰. The maximum abundances of *M. wooldridgei*, thus coincided with optimum physiological conditions for the mysid. It

is likely that within the middle reaches of the estuary, biological interactions including food availability and predation contribute to the spatial distribution of *M. wooldridgei*.

5.2 ZOOPLANKTON DISTRIBUTION

Under natural conditions, permanently open estuaries are described as having perennial riverine flow, but are acknowledged as being variable systems, reliant on changes in river flow, mouth condition and tidal range (Whitfield, 1992). The variability in the freshwater inflow into the estuary contributes to the temporal changes in the biology within these systems (Wooldridge, 1999). Due to the inter-basin transfer of water from the Orange River system to the Great Fish River system, much of the natural variability in flow rates of the Great Fish River has been lost (O'Keeffe and De Moor, 1988). The absence in the variability in the flow rates of freshwater into the Great Fish Estuary contributed to the lack of seasonality in the total chlorophyll *a* concentration (Figures 3.6 and 3.7) and zooplankton abundance (Figures 3.8 and 3.9) observed during the present study. Indeed, numerical analyses conducted on the log-transformed total zooplankton abundance data and the mysid abundance data did not reveal the presence of any seasonality in the zooplankton community (Figure 3.16). It should be noted, however, that dramatic increases in the magnitude of freshwater inflow into the Great Fish Estuary (February and April 2004) coincided with aseasonal changes in the zooplankton abundances within the estuary (designated Group 2 in the numerical analysis; see Figure 3.16). During the periods of increased freshwater inflow into the estuary, the total zooplankton abundances (including the mysids) were among the lowest recorded during the entire study. The low abundances of the zooplankton during these periods can likely be ascribed to the transport of the zooplankton out of the estuary into the marine environment.

In agreement with previous studies conducted along the Eastern Cape coastline (Jerling and Wooldridge, 1995b; Wooldridge and Bailey, 1982), the sustained inflow of freshwater into the Great Fish Estuary contributed to the spatial pattern in the distribution of zooplankton within the estuary (Grange *et al*, 2000). In the upper reaches, the zooplankton community was almost entirely numerically dominated by the calanoid copepod, *Pseudodiaptomus hessei* and was characterised by the virtual absence of marine species. The absence of marine species in the upper reaches of the

estuary was evident from the low Margalef diversity index values obtained within the region. In the lower reaches of the estuary, zooplankton community was more diverse reflecting an increased contribution of marine breeding decapods, amphipods and mysids (Table 3.3 and Figure 3.17).

Freshwater inflow has been shown to significantly impact the community composition of the zooplankton within estuaries (Allanson and Read, 1995). For example, in the marine dominated Kariega Estuary within the same geographic region, the zooplankton community is numerically dominated by the copepods *Pseudodiaptomus hessei* and *Acartia longipatella*. This is in contrast to the freshwater dominated Great Fish Estuary, where zooplankton community is numerically dominated by the mysid shrimp, *M. wooldridgei* and the copepod, *P. hessei* (Grange *et al*, 2000). The average monthly total zooplankton abundances were highly variable and ranged between 16 and 13 700 ind. m^{-3}. The various developmental stages of the calanoid copepod, *P. hessei* numerically dominated these samples, comprising at times up to 100% (range 23 to 100%) of the total zooplankton abundance. The predominance of *P. hessei* within the estuary is not surprising as a number of studies have demonstrated that the copepod is able to withstand large variations in temperature and salinity and can be regarded as a pioneer species in estuaries (Wooldridge and Melville-Smith, 1979). The total zooplankton biomass ranged between 5 to 194 mg Dwt m^{-3}, and was dominated by mysids comprising on average 32% (range between <1 and 98%) of the total biomass. Among the mysids, *M. wooldridgei* accounted for on average for 96% of the total mysid biomass (up to 160 mg Dwt m^{-3}). These values are comparable to previous studies within large permanently open estuaries with sustained freshwater flow. For example, in the Sundays Estuary the biomass values of *M. wooldridgei* ranged between 224 and 1117 mg m^{-3} (Wooldridge and Bailey, 1982). Similarly, in a previous study in the Great Fish Estuary total biomass values of *M. wooldridgei* ranged between 256 and 194 mg m^{-3} (Grange *et al*, 2000).

The numerical analysis of the spatial distribution of the log-transformed mysid abundance data showed that the estuary could be divided into three distinct regions. Station 1 (freshwater dominated) demonstrated very low abundances of *M. wooldridgei* with *R. terranatalis* and *G. brevifissura* absent. This is in agreement with a previous study in the Sundays Estuary, which demonstrated that mysids were absent

from the riverine section of the estuary (Jerling and Wooldridge, 1995b). *R. terranatalis* and *M. wooldridgei* occupied the same region of the estuary, with maximum abundance recorded in the middle reaches of the estuary. This result is in contrast to Wooldridge and Bailey (1982), which demonstrated separate areas of maximum abundance for *R. terranatalis* and *M. wooldridgei*. The spatial separation between the two mysids is thought to reflect the predatory-prey relationship between *R. terranatalis* and *M. wooldridgei*. Indeed, in laboratory experiments adult *R. terranatalis* have been shown to prey on *M. wooldridgei*, with the juveniles being most susceptible (Wooldridge and Webb, 1988). Finally, in the lower reaches of the estuary *G. brevifissura* predominated. A recent study by Marshall *et al* (2003) found that changing temperature and salinity impacted significantly on the oxygen consumption of the mysid *G. brevifissura*. Although the mysid was seen to regulate its metabolism without incurring stress within most naturally occurring salinities (15-35‰), it was sensitive to large changes in temperature. *G. brevifissura* was found in greatest abundances in the lower reaches of this system, where the salinity was normally between 20 and 35‰. The distribution of *G. brevifissura* thus reflects its physiological requirements.

5.3 STABLE ISOTOPE ANALYSIS

Results from the stable isotope analysis indicated that the majority of the zooplankton (mainly copepods) within the Great Fish Estuary are sustained by the extensive phytoplankton stocks that prevail within the system (Figure 4.1). This result is in agreement with a previous study conducted in the Sundays River Estuary characterised by sustained freshwater inflow (Jerling and Wooldridge, 1995c). Seasonality in the diets of zooplankton within estuaries has been linked to amongst others temperature and changes in freshwater inflow (Froneman, 2002a). The absence of any seasonal trends in the diets of the numerically dominant zooplankton within the Great Fish Estuary Fish estuary, can largely be attributed to the continuous input of freshwater into the system due to the inter-basin transfer of water from the Gariep dam to the Great Fish River (Grange *et al*, 2000). Indeed, it is worth noting that total chl. *a* concentrations did not demonstrate and seasonal trends. Results obtained during the present study are in contrast to findings conducted in the freshwater deprived permanently open Kariega Estuary within the same geographic region

(Froneman, 2001a; Froneman, 2002a). In the Kariega Estuary, the zooplankton were largely sustained by carbon derived from a variety of sources including the salt marsh vegetation, submerged macrophytes and phytoplankton (Froneman, 2001a; Froneman, 2002a). Differences in the results obtained between this and previous studies appear to be linked to the magnitude of freshwater inflow into the estuary. The total chl. *a* concentration in the freshwater deprived Kariega Estuary was found to be dominated small picophytoplankton (<2 μm) which are considered too small for direct utilization by the dominant zooplankton in the system (Froneman, 2001). As a consequence, the numerically dominant zooplankton within the estuary consumed alternate carbon sources (Froneman, 2001a; Froneman, 2004). During the present study, total chl. *a* concentration were always dominated by cells >5 μm , which are readily consumed by the dominant zooplankton in the estuary (Froneman, 2001a; Grange, 1992). The high contribution of phytoplankton derived carbon to total daily carbon intake by the numerically dominant zooplankton (copepods) is thus not surprising.

Based on their mandible edge indices, *M. wooldridgei* can be considered as herbivorous and *R. terranatalis* carnivorous (Jerling and Wooldridge, 1994; Wooldridge and Webb, 1988). Results of the stable isotope analysis study suggest that adult *M. wooldridgei* can largely be regarded as omnivorous feeding on both phytoplankton and copepods (Figure 4.1). This result is in contrast to a similar study conducted within the Kariega Estuary, where adult *M. wooldridgei* was carnivorous (Froneman, 2002a). Selective feeding by *M. slabberi* (now *M. wooldridgei*) has largely been shown to be determined by food particle size and ease of capturability (Siegfried and Kopache, 1980; Webb *et al*, 1987). As mentioned previously, in the Kariega Estuary, the bulk of the phytoplankton are <2 μm and thus largely not available for direct utilisation by *M. wooldridgei*. On the other hand, in the Great Fish Estuary, the phytoplankton are largely and directly available to the mysid. The apparent alteration in feeding regime between the various systems appears to be related to the availability of large sized phytoplankton within the water column, which dominates the chl. *a* of the Great Fish Estuary. It is worth noting that diet of *M. wooldridgei* appears to change with ontogenic development from a herbivorous feeding mode to a more omnivorous feeding mode.

5.4 FUTURE RESEARCH

The present study has generated new information on a number of aspects on the biology of the mysid, *Mesopodopsis wooldridgei*, in a large permanently open estuary. The investigation has also highlighted three aspects of the ecology of *M. wooldridgei* that require further investigation in the future.

1- Carbon isotope analysis has been successfully employed to investigate the food webs of a number of South African estuaries (Froneman, 2001c; Froneman, 2002a; Jerling and Wooldridge, 1995c; Mbande *et al*; Paterson and Whitfield, 1997). Results of these studies have highlighted the important contribution of carbon derived from phytoplankton and salt marsh vegetation to total daily carbon intake of the numerically dominant zooplankton in permanently open estuaries (Froneman, 2001a). The lack of a definitive carbon source for the larger mysids during this study, however, highlights the limitations of stable isotope analysis. Further isotope studies could provide important information on the contribution of the different carbon sources to the mysids within the Great Fish Estuary. Nitrogen analysis can be excluded, as this isotope is extremely variable and should not be used in detritus based food web studies (Benner *et al*, 1991). Sulphur isotope studies (Fry and Sherr, 1984) combined with carbon analysis may be a more effective approach in determining the carbon sources utilised by the mysids within this estuary. Alternatively, further information on the trophic links between the mysids and the plankton could be assessed employing lipid biomarkers (Stübing and Hagen, 2003). The approach would integrate the trophic information over a longer time period. While lipid biomarkers are unable to identify species specific interactions, the technique would provide valuable trophic information on the level of large taxonomic groups (Stübing and Hagen, 2003).

2- During the current study increases in freshwater inflow into the Great Fish Estuary coincided with significant decreases in zooplankton abundance and biomass. Mass mortality of zooplankton associated with rapid changes in salinity has been previously documented (Kaartvedt and Aksnes, 1992; Wooldridge and Bailey, 1982). It is not known whether the zooplankton within the Great Fish Estuary were simply moved offshore or whether mortality was experienced due to drastic alterations in salinity.

At present there is little information known about the movement of mysids in and out of estuaries. Future research should investigate the link between the nearshore marine environment and estuaries.

3- There is evidence in literature to suggest that *M. wooldridgei* is spatially segregated from *R. terranatalis* (Wooldridge and Bailey, 1982; Wooldridge and Webb, 1988). The observed pattern is through to reflect the predatory impact of *R. terranatalis* on early developmental stages of *M. wooldridgei* (Wooldridge and Bailey, 1982; Wooldridge and Webb, 1988). However, results of the present study did not identify any spatial segregation between the two mysids species. The absence of any spatial pattern in distribution of these two mysid species may be due to an artefact of sampling approach or technique employed during the present study. In order to assess the spatial patterns of the two mysid species more accurately, a higher intensity survey is required. Further, the vertical distribution of the two mysid needs to be investigated to determine if any differences in vertical patterns of the two species exists.

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