STRUCTURE AND FUNCTIONING OF FISH ASSEMBLAGES IN TWO SOUTH AFRICAN ESTUARIES, WITH EMPHASIS ON THE PRESENCE AND ABSENCE OF AQUATIC MACROPHYTE BEDS.

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ABSTRACT

Temporarily open/closed estuaries (TOCEs) are the dominant estuary type in South Africa. These systems are often characterized by extensive beds of submerged macrophytes, which form important foraging and shelter habitats for fishes, especially for estuary-dependent fish species such as the Cape stumpnose *Rhabdosargus holubi* and Cape moony *Monodactylus falciformis* that are commonly associated with them. A loss of submerged macrophytes from an estuary has been shown to affect the fish community as well as reducing overall system productivity.

The TOC East Kleinemonde Estuary, situated in the warm-temperate biogeographic region of South Africa has been subject to an ongoing long-term fish monitoring project since 1995. During the period 1995 to 2002, this estuary contained large beds of the submerged macrophytes *Ruppia cirrhosa* and *Potamogeton pectinatus*. However, subsequent to a major flood event in 2003 these macrophytes have been largely absent from this system.

The effect of the loss of submerged macrophytes on the East Kleinemonde fish assemblage was investigated through an analysis of seine and gill net catch data. Seine net catches for a 12 year period, encompassing six years of macrophyte presence and six years of macrophyte senescence, revealed changes in the relative abundance of certain fish species. Vegetation-associated species such as *R. holubi* and *M. falciformis* decreased in abundance whereas sediment-associated species, especially members of the family Mugilidae, increased in abundance following loss of the macrophytes in this estuary. The critically endangered pipefish *Syngnathus watermeyeri* was only recorded in catches during years in which macrophyte beds were present.

In addition to the analysis of catch data, the importance of macrophytes as a primary energy source for selected estuarine fishes was explored through the analysis of carbon and nitrogen stable isotopes. Prior to conducting these analyses, common methodological practices to address the presence of carbonates and lipids within isotope samples were evaluated. A subset of samples were either acid washed to remove carbonates, or lipids were removed according to the method of Bligh and Dyer (1959) as both of these compounds have been shown to affect stable carbon and nitrogen isotope ratios. The suitability of the lipid normalization models of Fry (2002) and Post *et al*. (2007) for samples of estuarine fish muscle were also tested. Based on this evaluation both models are suitable for use with estuarine fish muscle tissue, however since neither carbonate nor lipid content of any of the samples used in this study was high all samples were left untreated in the following analysis.

Carbon isotope ratios from a wide range of fish species collected from the East Kleinemonde Estuary during the macrophyte-senescent phase were compared with individuals of the same species from the neighbouring West Kleinemonde Estuary (where extensive beds of *R. cirrhosa* and *P. pectinatus* were present) and revealed the influence of submerged macrophyte material in the diet of fishes in the latter system. However, it was apparent that these plants are not directly consumed but rather contribute to the detrital pool that forms a food source for most invertebrate and some fish species. The most significant source of carbon for East Kleinemonde fishes during the macrophyte senescent phase appeared to have a more depleted origin; probably from benthic or pelagic microalgae.

In conclusion, while the importance of macrophyte beds as shelter and foraging habitats for estuarine fishes are well documented, their role in terms of the structuring and functioning of fish assemblages in TOCEs remains somewhat uncertain. The findings of this study were possibly masked by the resilience of vegetation-associated species to the loss of this habitat, as well as by life history characteristics of species such as *R. holubi* that allow their numerical dominance despite habitat change. Nonetheless, macrophyte senescence in the East Kleinemonde Estuary resulted in the loss of at least one species and the reduced abundance of vegetation-associated species, probably reflective of reduced food resources and/or increased vulnerability to predation. As a result, beds of submerged macrophytes are an important habitat within TOCEs.

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DECLARATION

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

CHAPTER ONE: GENERAL INTRODUCTION

1. SOUTH AFRICAN ESTUARIES

The term ‘estuary’ has been variously defined to describe the seaward portion of a river system where freshwater becomes mixed with seawater (*inter alia* Pritchard, 1967; Day, 1981). Whitfield (1992) classified southern African estuaries based on their physiographical, hydrological and salinity characteristics into five basic classes, namely estuarine bays, river mouths, estuarine lakes, permanently open systems and temporarily open/closed estuaries. Using a much broader classification, estuaries may simply be categorized on their hydrodynamics as either being permanently open to the sea (permanently open estuaries, POEs) or having periods of mouth closure (temporarily open/closed estuaries, TOCEs). Unlike northern hemisphere systems which are typically large and deep, permanently open, with well-defined horizontal salinity gradients, the majority of South African estuaries are small, intermittently open systems. In fact, of the approximately 250 functional estuaries along the South African coastline more than 70% are TOCEs (Perissinotto *et al*., 2000; Cowley *et al.*, 2001).

In these TOCEs, marine sediment deposited mainly by onshore and longshore drift in the mouth region builds up until the system becomes blocked off from the sea by a sand berm. Mouth state is determined by a number of forces, these being separated into scouring forces that remove sand from the mouth, and forces that lead to sand deposition. Scouring forces are river inflow, related to catchment size and runoff, and tidal flow, while wave energy and factors related to the sediment load in waves such as grain size and beach slope are forces that lead to mouth closure (van Niekerk, 2007). All South African estuaries have these forces working on them continually. The mouth state that results is dependent on the relative strength of the mouth-opening versus mouth-closing forces, and may be influenced by factors that enhance or inhibit their action, such as the presence of a rocky promontory at the mouth that dissipates wave action and encourages sediment scour.

Along the south-eastern seaboard of South Africa, rainfall patterns are erratic and evaporation rates high. The coastline is typically high energy with wave action being described as heavy and continuous (McLachlan *et al*., 1981), and this, combined with the low freshwater runoff, results in many estuaries within this region being predominantly closed. In addition, freshwater abstraction for agricultural and/or human development purposes may also influence the mouth opening regime of TOCEs. The consequences of reduced freshwater inputs include (i) a reduction of the scouring effects when the mouth does open, resulting in open mouth conditions persisting for much shorter periods, (ii) the possible creation of hypersaline conditions once the mouth closes, and (iii) reduced recruitment opportunities for certain estuarine-dependent marine organisms (Whitfield & Bruton, 1989; Whitfield, 1996; Perissinotto *et al*., 2000; Bell *et al*., 2001; James *et al*., 2007; van Niekerk, 2007; Whitfield *et al.* 2008). Other common threats to the functioning of TOCEs include development of the river banks and floodplain which results in increased sediment and pollutants being washed into the estuary as well as the mouth being artificially breached as these developments become threatened by rising water levels.

A closed mouth state affects both biotic and abiotic aspects of an estuary. Salinities in a TOCE are a result of the interaction between freshwater input by river flow and runoff, evaporative effects and seawater intrusion. During the open mouth phase river inflow is high and salinity patterns mimic those of POEs, being most saline near the mouth and progressively fresher towards the head of the estuary. However, during the closed mouth phase some systems may become hypersaline under low river flow conditions, or mesohaline to oligohaline under high flow conditions (Harrison 2004).

Salinity is an important determinant of plant and animal community composition in estuaries (Harrison & Whitfield, 2006; Riddin & Adams, 2008a). Salinity regimes within a TOCE, affected by prolonged periods of mouth closure may cause significant physiological stress to the biota of a TOCE; salinities exceeding 20 ppt have been linked to senescence of certain submerged macrophytes, e.g. *Potamogeton pectinatus* (Riddin &

Adams, 2008a), and Froneman and Henninger (2009) suggested that salinities of > 35 ppt in the Kasouga Estuary contributed to the reduced abundance of the estuarine isopod *Exosphaeroma hylocoetes*.

In contrast to stress caused by hypersaline conditions, increased freshwater input which leads to low salinities may be equally devastating to certain fish species. Blaber and Whitfield (1976) reported the death of at least 100 000 fish of 11 species in the Lake St Lucia system, attributed to osmoregulatory failure caused by a combination of very low salinities (< 3 ppt) and low temperatures. Similarly, heavy rainfall during the summer of 1980-1981 increased freshwater input into the Bot Estuary and reduced salinities in the system to 2-3 ppt, resulting in the estimated mass mortality of over 7 000 fish belonging to nine species (Bennett, 1985).

2. FISH COMMUNITIES IN TOCES

The ichthyofauna associated with estuaries may be divided into two major groups based on their patterns of estuary utilization; these being estuarine resident species and marine or freshwater species that occupy estuaries for more limited periods. Fish communities found in South African estuaries thus vary in time and space.

A number of biotic and abiotic factors have been identified as key in structuring estuarine ichthyofaunal communities. These factors include salinity, temperature, turbidity, estuary size and type (Whitfield, 1996; Harrison & Whitfield, 2006). Apart from the health of a system and its ability to offer appropriate food and shelter, the most important factor affecting the community composition of TOCEs is mouth state (Whitfield, 1996; James *et al*., 2008b). Since many TOCEs are predominantly closed, fish recruitment is determined by the frequency, timing and duration of open mouth events (Bell *et al*., 2001; Kemp & Froneman, 2004; James *et al*., 2008b).

Whilst an open mouth may represent the easiest opportunity for organisms to recruit into estuaries, it has been shown that marine overwash during rough sea conditions greatly serves to increase the accessibility of temporarily open estuaries to the biota that use them (Cowley *et al*., 2001; Vivier & Cyrus, 2001; Kemp & Froneman, 2004). This is particularly important for TOCEs in the Eastern Cape Province of South Africa where mouth opening events are sporadic and short-lived due to erratic rainfall patterns and limited freshwater inputs. Bell *et al*., (2001) showed that overwash events not only increased the number of recruitment opportunities, but being fairly common events they also reduced the interval between consecutive access opportunities; a factor important for species with breeding seasons of limited duration.

Not all fish species are able to take advantage of overwash events to recruit, but for those that can these events are very important and contribute greatly to the success of these species in utilizing estuarine habitats. Dominance by the sparid *Rhabdosargus holubi* in warm-temperate TOCEs, and the mugilid *Liza richardsonii* in cool-temperate TOCEs have been attributed to their prolonged breeding seasons and serial spawning behaviour, together with an ability to utilize overwash events to recruit into estuaries (Cowley & Whitfield, 2001b).

TOCEs have slightly different fish assemblages to POEs. In general, fish species richness is lower in small TOCEs than large TOCEs, but highest in POEs (Vorwerk *et al*., 2003). This pattern is directly related to recruitment opportunities as more marine taxa are often absent or present in lower numbers in small TOCEs or those that are predominantly closed (James *et al*., 2007). In such systems, the ichthyofauna is dominated by estuarine-spawning species such as *Gilchristella aestuaria* or *Glossogobius callidus*. Larger TOCEs on the other hand generally have higher habitat diversity and more frequent mouth opening, which contributes to the increased species richness (Whitfield, 1996).

Where regular access to the sea occurs, juveniles of marine taxa are common in TOCEs, and may dominate the ichthyofauna. These systems, especially the vegetated littoral habitat within them, thus function as important nursery areas for marine fish species

(James *et al.*, 2007). While marine species may dominate by taxa and biomass, estuarine residents such as *Atherina breviceps* and *Gilchristella aestuaria* are well adapted to estuarine conditions and may be numerically dominant (Potter *et al.*, 1990).

3. ECOLOGICAL ROLE OF SUBMERGED MACROPHYTES

Beds of submerged macrophytes are important habitats for estuarine fauna, as they provide shelter and a food-rich habitat due to their structure, productivity and associated epibiota (Whitfield, 1984; Henninger *et al.*, 2009). For example, maximum abundances of the estuarine isopod *Exosphaeroma hylocoetes* have been recorded in macrophyte beds in a number of South African TOCEs (Froneman & Henninger, 2009; Henninger *et al.*, 2009). Similarly, highest abundances of the caridean shrimp *Palaemon peringueyi* in the Kasouga Estuary were recorded in the macrophyte-dominated upper reaches (Froneman & Henninger, 2009).

Juvenile fishes in particular have been found to make use of the habitat offered by submerged macrophytes, with the result that submerged plant beds characteristically have higher abundances and biomass of fishes associated with them compared to surrounding habitat types. Branch and Grindley (1979) found a higher biomass of juvenile fishes in macrophyte habitats, including salt marshes dominated by *Sarcocornia* spp. and seagrass beds dominated by *Zostera capensis*, than in other habitat and substrate types. Beckley (1983) reported elevated fish densities in seagrass beds (*Z. capensis*), as well as a higher species abundance in the beds than in the channel of the Swartkops Estuary. Hanekom and Baird (1984) found macrophytes to be especially important to juvenile fishes in estuaries where turbidity was low and water movement high as they provided shelter from visual predators. The importance of macrophyte beds to the estuarine fauna may also differ between estuarine types, with more than double the species richness associated with *Z. capensis* beds in the permanently open Knysna Estuary when compared to the same habitat in the intermittently open Swartvlei Estuary (Whitfield *et al.*, 1989).

Although juveniles of many fishes utilize macrophyte beds, this habitat is of particular importance to certain species. Hanekom and Baird (1984) found *Rhabdosargus holubi* and *Monodactylus falciformis* in significantly higher numbers in *Zostera* beds than in non-*Zostera* areas of the Kromme Estuary. These two fish species were suggested to be the most common consumers of invertebrate species associated with submerged macrophyte beds (Froneman & Henninger, 2009). Senescence of submerged macrophytes may affect estuarine fish community structure, species abundance and biomass. Loss of this habitat from an estuary has been shown to result in a shift to numerical dominance of sediment-associated species from those associated with vegetation in the Swartvlei estuarine lake (Whitfield, 1986) and a decreased condition of the vegetation-associated *R. holubi* and *M. falciformis* in the same system (Whitfield, 1984) as well as the disappearance of vegetation-associated species e.g. *Diplodus sargus capensis* from the Swartkops Estuary (Beckley, 1983) and *Syngnathus watermeyeri* from the East Kleinemonde Estuary (Whitfield, Cowley & James *unpublished data*).

As macrophyte beds can become extensive, estuaries that have macrophytes present in them will be more productive than those without (Wortmann *et al*., 1998). This is directly through macrophytic growth, but also through the increase in area for epiphytic attachment on the stems and leaves of the submerged plants (Dame, 2008). Taylor (1983) estimated a loss in primary production of 60% associated with a loss of the macrophyte *Potamogeton pectinatus* and charophyte beds from the Swartvlei estuarine lake.

The importance of a range of estuarine macrophytes as a food source to estuarine consumers has been found to vary. The emergent macrophyte *Spartina alterniflora* and its associated microalgae have been shown to be important food sources in North Carolina marshes (Currin *et al*., 1995), as were mangroves and seagrasses in Australia (Abrantes & Sheaves, 2009). Mangroves were also found to be an important food source to estuarine fauna in a New Zealand estuary, but were not exclusively consumed by any animal studied (Alfaro *et al*., 2006). The eelgrass *Zostera marina* was found to be of limited dietary importance to some invertebrates in a Korean estuary, while benthic and pelagic microalgae were the more important food sources to estuarine consumers (Choy *et al*., 2008). Similarly, carbon from mangrove plants was found to be of limited importance to detritivorous fish in a subtropical lagoon in Taiwan (Lin *et al*., 2007). Smit *et al*. (2006) found minimal contribution by the seagrass *Posidonia sinuosa* to animal biomass in a Western Australian estuary, although it was a major component of the seston and sedimentary organic matter.

The Cape stumpnose *R. holubi* grazes aquatic macrophytic material but due to a lack of cellulase only assimilates the epiphytic component (Blaber, 1974a). In general, macrophytes are not directly grazed by the aquatic fauna (Mann, 1988) but do form detritus that is an important food source for a number of estuarine species (Carr & Adams, 1973; Branch & Grindley, 1979; Hanekom & Baird, 1984; Vizzini *et al*., 2002). Moreover, aquatic macrophyte beds are collecting sites for detritus (Carr & Adams, 1973) as they decelerate the flow of water and allow material to be deposited and retained within the submerged plant habitat. It has been suggested that detritus provides the major energy flow pathway in estuaries (Odum, 1971; Mann, 1988; Bennett, 1989a; Whitfield & Lubke, 1998), and so contribution to this pool by macrophytes is likely to be important within TOCEs.

4. ESTUARINE FOOD WEBS AND THE USE OF STABLE ISOTOPES

TOCE food webs

Food webs vary in complexity, primarily determined by the number of trophic levels represented within a system, with terrestrial food webs typically having three levels and aquatic systems four (Preisser, 2008). Trophic efficiency, which is the fraction of the total carbon input to a trophic level that is transmitted to the next one (Baird & Ulanowicz, 1993), generally decreases with increasing trophic level and this is the main reason why the number and biomass of top predators that a food web can support is small (Preisser, 2008).

Trophic compartments within a range of South African estuaries cover primary producers, invertebrate consumers, fishes and birds (Baird *et al.*, 1991; Baird & Ulanowicz, 1993; Baird, 1999). The same compartment may have a different trophic function between systems depending on the representative species (Baird, 1999). Birds, for example, may function as herbivores (e.g. coots), carnivores feeding on invertebrates (e.g. sandpiper) or piscivores (e.g. heron or cormorant).

Studies on southern African estuaries reveal that although detritivores dominate the fauna, the suite of feeding groups of fishes within these systems covers the whole range of trophic guilds, from herbivorous and detritivorous feeders (e.g members of the family Mugilidae, van der Elst, 1993) to carnivores (e.g. *Lithognathus lithognathus*, Bennett, 1979; Bennett, 1993) and top piscivorous predators (e.g. *Lichia amia* and *Elops machnata*, Whitfield & Blaber, 1978; Bennett, 1979; Marais, 1984). A typical South African estuarine food web is presented in Figure 1.1.

Primary producers in an estuary include macrophytes, phytoplankton and the phytobenthos. Although macrophyte production is generally an order of magnitude higher per unit area than that of phytoplankton, phytoplankton usually contributes more than macrophytes to overall system primary production (Adams *et al.*, 1999). Phytobenthic production is, however, estimated at being a far more important contributor to total system production than phytoplankton, with Baird and Ulanowicz (1993) reporting a ratio of pelagic phytoplankton production to benthic production of 1 : > 5 for the Kromme and Swartkops estuaries.

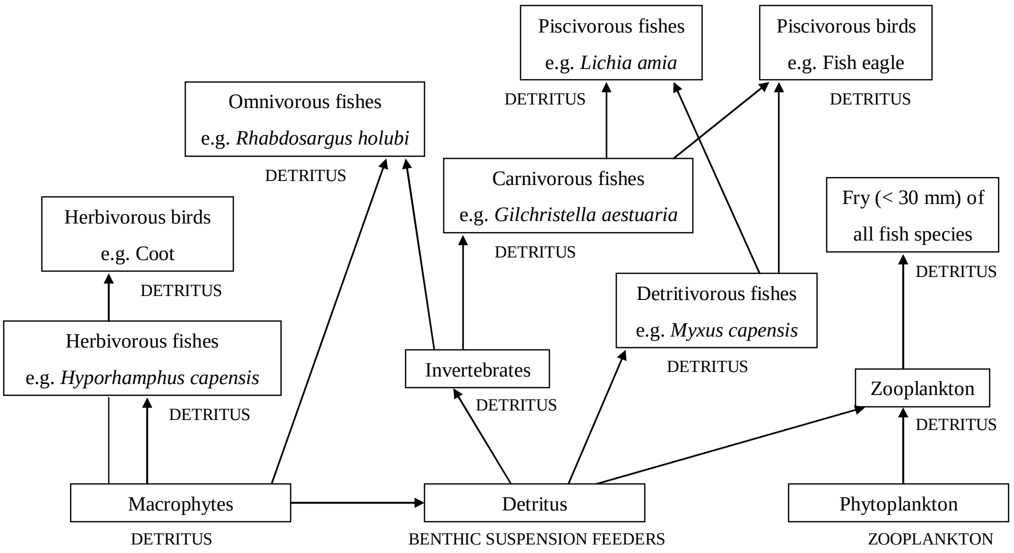


Figure 1.1 Typical food web of an aquatic macrophyte-dominated South African estuary (adapted from Bennett, 1989a, for three Cape estuaries). Blocks represent compartments in the food web (producers or consumers). The text below each block represents the most common sink for the energy produced by each compartment (after Baird, 1999)

The flexible nature of feeding habits by estuarine fish predators results in the diet of individuals reflecting relative abundance of prey items (Bennett, 1979; Blaber, 1979; Marais, 1984). Whitfield and Blaber (1978) list abundance, type and size of prey as being the most important factors in selection of prey items. In a similar manner, the relative contribution of plant material to the diet of omnivores such as *R. holubi* tends to reflect the availability of submerged aquatic macrophytes and abundances of invertebrate prey. Plant material is harder to digest and less nutritious than invertebrates and an increase in dietary contribution by plants may indicate a decreased abundance of alternative prey (Bennett, 1989a). Conversely, following the loss of aquatic macrophytes the diet of such consumers becomes exclusively carnivorous.

In terms of vertebrate consumers, Eastern Cape estuaries typically have few dominant pathways of energy flow (Marais, 1984). Certain components of the estuarine food web are common to most energy pathways. For example, the prawns *Upogebia africana* and *Callianassa kraussi* are prey items common to a wide variety of large zoobenthic fish predators; *U. africana* in particular being favoured by *Pomadasys commersonnii* but is also consumed by typically piscivorous fish species (Marais, 1984). Similarly, the estuarine round-herring *Gilchristella aestuaria* is a favoured prey item for a wide range of piscivorous predators (Coetzee, 1982a; Talbot & Baird, 1985) as are species of the family Mugilidae (Marais, 1984).

Detritus is the most important source of energy in an estuarine foodweb (Day, 1951; Whitfield, 1980; Marais, 1984; Mann, 1988; Bennett, 1989a). Detritivores are usually very abundant in South African estuaries (Whitfield, 1980; Harrison & Whitfield, 2006). However, Cowley & Whitfield (2002) found that the omnivorous *R. holubi* accounted for the bulk of fish production (> 74%) in the East Kleinemonde Estuary, while iliophagous detritivores accounted for only 7% of total fish production. In this system, species such as *R. holubi* play an important role in energy flow within the estuary, as well as between the estuary and the nearshore marine environment (Cowley & Whitfield, 2002). The importance of detritivory as a fish feeding mechanism thus varies between systems, depending on the degree to which primary production is utilized directly or indirectly by primary and secondary fish consumers (Baird, 1999).

Stable isotopes as a method for studying estuarine food webs

Elements exist in nature as a number of isotopes of varying atomic mass. The ratio of heavy to light isotopes of an element changes in predictable ways and it is the changes in these ratios that have allowed scientists to use stable isotopes in a wide variety of applications, including trophic studies. Unlike other techniques for examining diet, stable isotope analysis is time-integrated and provides not only source-sink information on energy flow in a system but also an indication of the pathways along which energy is transferred between consumers through a system. Depending on the question, it is often used in combination with or in place of standard techniques such as gut content analysis which provide a 'snapshot' of a potentially varied diet (Pinnegar & Polunin, 1999).

Variation in isotopic value is the result of a number of processes occurring on various scales, from the global cycling of N and C to fractionation during biochemical reactions within the tissues of a consumer (Eggers & Jones, 2000). Groups of primary producers have carbon isotopic values that are due not only to the value of atmospheric C available to the plants but also influenced by fractionation that occurs due to their photosynthetic pathway. Discrimination by the carboxylating enzyme responsible for CO_2 fixation in photosynthesis contributes to the variability of isotope values amongst C_3 and C_4 primary producers (Michener & Schell, 1994).

The $\delta^{13}C$ and $\delta^{15}N$ values of a consumer are enriched relative to the food source by a predictable value, and this allows one to identify which food sources have been assimilated by an individual based on its isotopic values relative to that of the food items. In the case of ^{13}C, the accepted average enrichment per trophic level is 0 to 1 ‰ (DeNiro & Epstein, 1978; Peterson & Fry, 1987; Michener & Schell, 1994), balanced by the $\delta^{13}C$ depletion of respired CO_2 and excretory products (DeNiro & Epstein, 1978).

Due to the preferential excretion of ^{14}N (DeNiro & Epstein, 1980; Peterson & Fry, 1987) and fractionation during assimilation (Post, 2002), a consumer will typically be enriched in ^{15}N by about 3 to 5 ‰ relative to its diet. Values of $\delta^{15}N$ are commonly used to indicate trophic level of a consumer (Vander Zanden & Rasmussen, 1999; Jacob *et al.*, 2005).

Early work has shown estuarine food webs to be largely based on detritus (Day, 1951; Odum, 1971; Whitfield, 1980; Marais, 1984; Mann, 1988; Bennett, 1989a), derived from submerged vegetation within the estuary or from adjacent saltmarshes, wetlands or from the marine or freshwater environment. Because of the small particle size and decayed nature of detritus it is difficult to identify its origin. Stable isotope analysis therefore represents an ideal mechanism to trace the relative importance of different primary producers in an estuarine system. Furthermore, such studies on South African estuaries are scarce and information provided by such an investigation is therefore valuable to understand the trophic functioning of these ecosystems.

5. AIMS AND OBJECTIVES

An episodic flood event in May 2003 along the south-eastern coastal region of the Eastern Cape Province of South Africa caused the mouths of all TOCEs in the region to breach and scour to a level seldom recorded during previous opening events. The impact of the flood was particularly severe on the East Kleinemonde Estuary and resulted in significant scouring of sediments from the mouth and lower regions of the estuary. Apart from the removal of part of the aquatic macrophyte sedimentary seed bank, the low water levels persisted for an extended period thus leading to desiccation and senescence of the intertidal plant beds. The effects of the 2003 flood on the neighbouring West Kleinemonde Estuary were, however, less severe due to a rocky sill in the mouth region, and the submerged macrophytes remained relatively unaffected. The distinct differences between the two estuaries, particularly in terms of macrophyte abundance, over the past six years provided an opportunity to investigate differences in the trophic structure and role of macrophytes in these two TOC estuarine food webs and fish assemblages.

The primary aims of this project were to investigate the role of aquatic macrophytes in terms of (i) structuring TOCE fish assemblages in a TOCE and (ii) their dietary importance for selected fish species in two TOCEs. Long-term catch records were analysed and compared, and a stable isotope comparison of food webs between a macrophyte-dominated estuary and one in which these plants are absent conducted to test the following hypotheses:

1. Submerged macrophytes are an important factor structuring TOCE fish assemblages within TOCEs (Chapter 3).

2. Aquatic macrophytes are an important energy source for fishes in TOCEs (Chapter 5).
3. The relative importance of food web components is different between estuaries in which macrophytes are either abundant or absent (Chapter 5).

Following an introduction to the relevant topics and study site in Chapters 1 and 2, this thesis will take the following layout:

Chapter 3 will address the role of submerged macrophyte beds in structuring fish assemblages within TOCEs. Potential change in catch records from the East Kleinemonde Estuary will be examined over a period of more than a decade, and encompassing a phase within which macrophytes were dominant and one following their senescence.

Chapter 4 will address common methodological inconsistencies in the preparation of samples for analysis of carbon and nitrogen isotope ratios, while Chapter 5 will make use of the analysis of stable isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) in order to assess the importance of submerged macrophytes as an energy source for fishes within TOCEs. Isotopic ratios of individuals from the same fish species will be compared between the East Kleinemonde Estuary, from which macrophyte beds are absent, and the West Kleinemonde Estuary, which has extensive beds of submerged macrophytes.

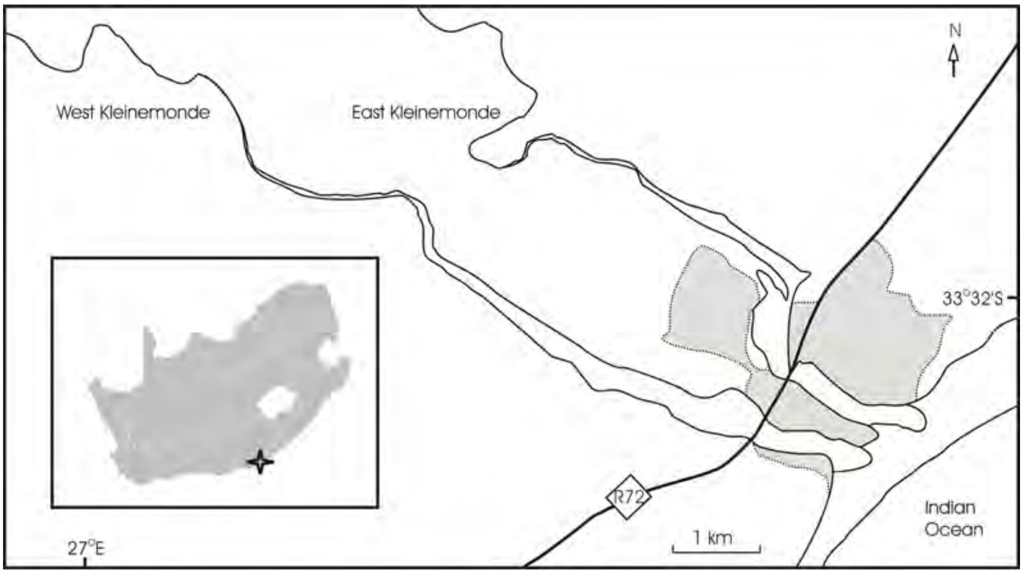
In Chapter 6 the findings of this study will be summarized and further discussed, and conclusions drawn as to the importance of submerged macrophytes to fishes within TOCEs, and hence their role in such systems. Results from this study will assist in making informed decisions around the long-term maintenance of these plant populations that will ultimately affect the management of TOCEs within this region.

CHAPTER TWO: STUDY SITE

1. INTRODUCTION

The approximately 300 estuaries found along the South African coastline can be divided into different bioregions based on water temperature, rainfall and river flow of the area within which they are found (Day, 1981; Harrison 2004). The East Kleinemonde (33°32'S; 27°03'E) and West Kleinemonde (33°33'S; 27°02'E) estuaries, situated in the Eastern Cape Province on the southeast coast of South Africa are classified as warm-temperate systems.

Figure 2.1. Map showing the location of the East Kleinemonde and West Kleinemonde estuaries. The settlement of Seafield is indicated by light grey shading



Temporarily open/closed estuaries (TOCEs) in the Eastern Cape typically have low turbidities and high salinities due to the low rainfall and run-off, high evaporation rates and a relatively high seawater input when compared to KwaZulu-Natal TOCEs (Harrison, 2004). Salinity in a system during the closed phase is however variable, and is dependent on the degree of seawater intrusion during the previous open or tidal phase as well as the prevalence of overwash events (Whitfield *et al.*, 2008). Systems are generally shallow, and while waters rapidly heat up in summer, mouth opening and an influx of seawater may reduce water temperature in the estuary very quickly, especially along the south-east coast of South Africa where sea temperatures peak at 19°C (Harrison, 2004).

Characteristics of the East Kleinemonde catchment and estuary are summarized in Table 2.1. The estuary is a small, shallow system which has a maximum surface area and volume during the closed phase (35.7 ha and 664 000 m^3) and a minimum during its open phase (11.6 ha and 16 000 m^3). Due to a small catchment size, low mean annual runoff (MAR) and limited tidal prism this system is predominantly closed, with the duration of open mouth events being short-lived.

Berm dimensions are related to wave conditions, sediment characteristics and aeolian transport (Theron & Bornman, 2008). Historical data show the berm width to be in the region of 200 m, although observations made during 2006 provide a range of 70 m to 120 m, with an estimated height of 1.2 to 2.9 m above MSL depending on the tide (Theron & Bornman, 2008).

Land-use practices in the catchment area of the East Kleinemonde River include limited pineapple and more extensive cattle farming. Apart from this, the area is relatively undisturbed (van Niekerk *et al.*, 2008). The main direct anthropogenic impact on the morphology of the East Kleinemonde Estuary can be attributed to the R72 bridge and embankments constructed in the 1960s, which fixed the deep channel against the southwest bank and widened the downstream northeastern sandbank (Theron & Bornman, 2008). The increased downstream sedimentation that resulted from the construction of the northeastern approach to the bridge has allowed reed beds of *Phragmites australis* to develop in this area of the estuary. There is also potential threat of microbial contamination of the estuary from septic tank overflows from the Seafield settlements (Cowley & Whitfield, 2001b), as well as increased freshwater and fertilizer runoff into the estuary which has contributed to a change from beds of submerged *Ruppia cirrhosa* to reeds (Riddin & Adams, 2008b).

The TOC West Kleinemonde Estuary is larger than the East Kleinemonde Estuary (surface area *c*. 80 ha, Riddin & Adams, 2009). The mouth area below the R72 bridge is the widest region of the estuary, with an average width of 200 m. The estuary becomes increasingly narrow as one moves into the upper reaches, with steep banks, a channel deepening to about 3 m and width decreasing to about 20 – 30 m (Blaber, 1973). An artificial weir 5 km upstream of the mouth used to separate estuarine and riverine waters but has since been destroyed, thus restoring the natural extent of the estuary (10 km).

Anthropogenic impacts on the West Kleinemonde include accelerated sedimentation attributed in the lower reaches to the construction of the R72 bridge, allowing for the development of sand banks (Badenhorst, 1988). Development on the western bank has fixed the highest permissible water level at MSL + 2.3m (Badenhorst, 1988). Most of the area on the eastern bank above the road bridge is undeveloped, and used for cattle grazing (Henninger *et al*., 2008).

Table 2.1. Characteristics of the East and West Kleinemonde catchments and estuaries, under a closed mouth state. Values for open conditions are included in parentheses where available. (Blaber, 1974b; Badenhorst, 1988; Bernard & Froneman, 2005; Henninger *et al*., 2008; Hughes, 2008; Riddin & Adams, 2008b; Whitfield *et al*., 2008)

	East Kleinemonde	West Kleinemonde
Catchment size (km²)	46.3	93.7
Mean annual runoff (m³)	2×10^6	4.04×10^6
Estuarine surface area (ha)	35.7 (11.6)	80
Estuary length (km)	3.7	10
Mean depth (m) of estuary	2 (1)	3
Maximum width (m) of estuary	210 (120)	200

Both of the Kleinemonde estuaries are located in the Ndlambe Municipality and there are three settlements at the mouth of these systems. Presently, 300 plots have been developed and are supplied with freshwater by the Wellington Dam, which is situated on a tributary of the East Kleinemonde River and collects water from 9% of the catchment area (Hughes, 2008). Daily water utilisation varies with season, with an average of 275 m^3 consumed per day during the peak holiday months of December, January and April, and approximately 140 m^3 per day for the rest of the year (Hughes, 2008). Present rates of consumption and the storage capabilities of Wellington Dam impact minimally on natural river flow (Hughes, 2008).

2. CLIMATIC CONDITIONS

The Eastern Cape has a climate that is characterized by a high degree of inter-annual variability (Lubke, 1998). The coastal zone is regarded as subtropical with maximum and minimum temperatures of 22.2 and 10 °C, and minimum rainfall of 60 mm for all months (Lubke, 1998). Inland temperature extremes characteristic of both summer and winter are moderated in the coastal zone by the cooling and warming effects of the sea (Stone *et al.*, 1998).

The section of Eastern Cape coastline within which the Kleinemonde estuaries are found is a transition zone between winter rainfall (Southern Cape up to Port Elizabeth) and summer rainfall (towards and above Kei Mouth) (Lubke, 1998). Rainfall in this region occurs throughout the year, with a larger peak in spring and autumn (Figure 2.2) (Lubke, 1998; Harrison, 2004), influenced by the south-easterly winds which occur predominantly during these two seasons (Stone *et al.*, 1998). Prevailing winter winds along the Eastern Cape coastline are southerly or south-westerly, and north-easterly in the summer months (Lubke, 1998).

Rainfall records (Table 2.2) indicate a high degree of spatial variability over a relatively small area within the Eastern Cape coastal region. This pattern of variability is likely due to the topography of the region (Stone *et al*., 1998). Rainfall can also be variable between years, with 2008 being an example of a drier year (total rainfall of 550 mm compared to the annual average of 893 mm in 2006/7 for Kleinemonde, Table 2.2).

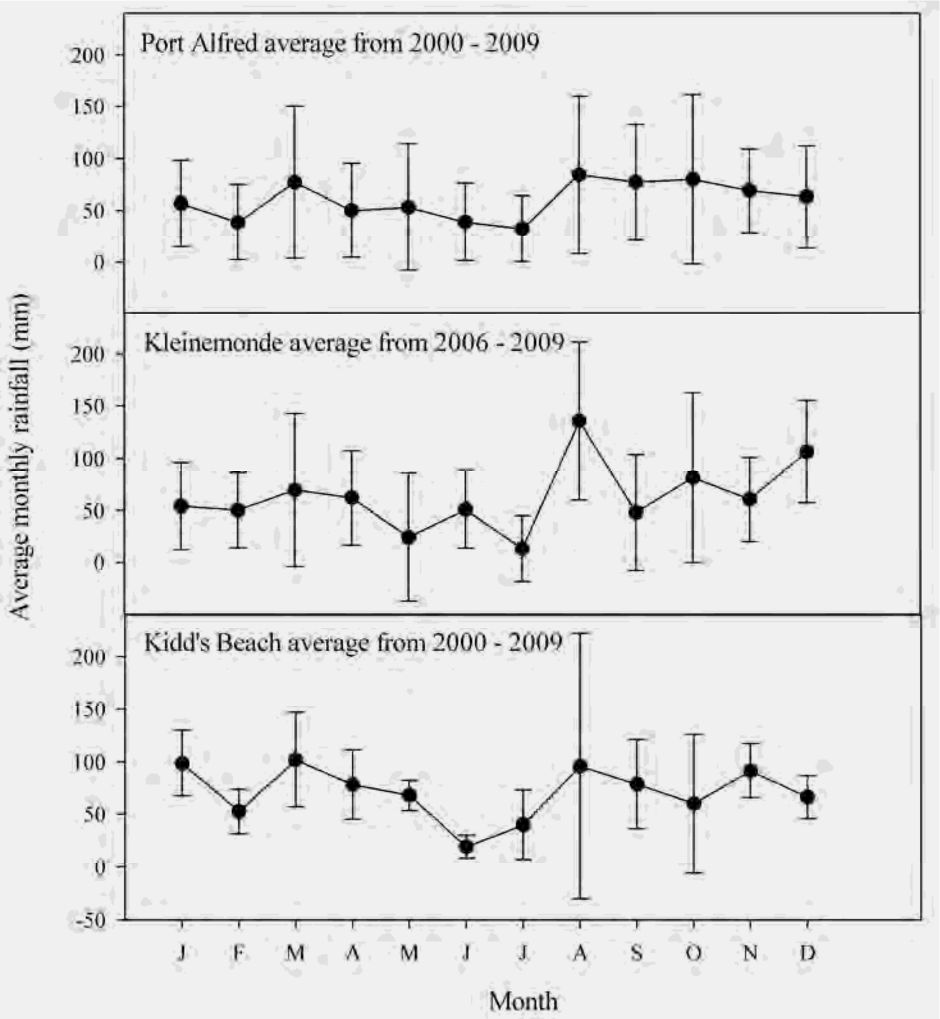


Figure 2.2. Average monthly rainfall (mm) values for three coastal localities, Eastern Cape Province. Error bars indicate standard deviation. Data for Port Alfred and Kidd's Beach courtesy of the South African Weather Service (SAWS) and that for Kleinemonde from P. Cowley

Table 2.2. Total annual rainfall (mm) as recorded at four coastal localities in the Eastern Cape Province. All data courtesy of the South African Weather Service (SAWS) except that for Kleinemonde from P. Cowley

Location	2000	2001	2002	2003	2004	2005	2006	2007	2008	Annual avg. ± SD
Alexandria	925	887	873	758	691	675	705	725	450	743 ± 143.9
Port Alfred	368	683	634	735	706	1288	806	686	468	708 ± 256.5
Kleinemonde							956	831	550	779 ± 207.7
Kidds Beach	915	832	1050	573	927	1126	1047	747	548	863 ± 207

3. THE COASTAL ENVIRONMENT

In the 300 km stretch of coastline between Port Elizabeth and East London there are a total of 38 estuaries, of which 80% are classified as temporarily open/closed systems according to Whitfield (1992) (Cowley & Whitfield, 2001b).

The coastline from the north-east of Cape Padrone is fairly straight, with a narrow (approx. 30 km wide) continental shelf (Lutjeharms, 1998). The section between Kenton-on-Sea and Fish River Mouth is largely sandy beaches, with rocky shores and wave-cut platforms being common features (Lubke, 1998). Within this stretch a total of six estuaries are present – half of these being permanently open systems (such as the Kowie at Port Alfred) and the rest closed (Lubke, 1998). Salt marsh habitats are often present in estuaries in this area, depending on physical conditions within each system (Lubke, 1998).

Based on rainfall patterns, dune thicket is the dominant vegetation type in this area with grasslands also being common. Land use practices include cattle and pineapple farming. Settlements are mostly resorts, concentrated around the estuary mouths (Lubke, 1998), and these have only really developed since the stretch of coastline between Woody Cape and the mouth of the Great Fish Estuary became accessible with the opening of the R72 coastal road.

Coastal hydrography

The warm (surface temperature of 28 and 20 °C in summer and winter respectively, Lutjeharms, 1998) Agulhas Current flows in a predominantly southwesterly direction along the edge of the continental shelf off the Eastern Cape coast. The temperature of the sea along this particular stretch of coast (approximately 19 to less than 17 °C in summer and winter respectively) is, however, directly influenced by the direction of the prevailing wind (Lubke, 1998). Water movement close inshore is largely wind-driven, with a persistent onshore leading to a rise in sea-level (Lutjeharms, 1998).

In the vicinity of Port Alfred, the change from narrow to broader continental shelf sets up an upwelling cell that may or may not be a permanent feature (Lutjeharms, 1998). A persistent north-easterly wind forces a surface layer of warm water (up to 50 m depth) offshore and allows cold upwelled water to move onshore, with south-westerly winds causing the situation to work in reverse (Lutjeharms, 1998). The effects of the upwelling cell, most dramatic of which is a sudden change in temperature, affects the coastal environment in the vicinity of Port Alfred and even as far away as Algoa Bay (Lutjeharms, 1998).

Spilling waves, which gradually dissipate energy, are most common along the shore in the region of the Kleinemonde estuary mouths (Theron & Bornman, 2008). During high tides however, relatively high wave energy conditions occur near the mouths as the surf zone narrows to include the steeper, more reflective area of the beach profile and deeper water over the outer sandbar allows more wave energy to pass inshore. Dominant beach conditions in this area are dissipative to intermediate, being affected by the nature of the surf-zone, the breaker type and beach slope.

Sediment transport

Ample sediment is provided to the mouth region of the East and West Kleinemonde estuaries by the aeolian-headland-bypass system at Black Rock Point from the southwest, with the wide sand beach and dunes also providing sediment from the northeast. The major aeolian sediment input is from the east-northeast (Theron & Bornman, 2008). Net sediment transport is longshore. Turbulent waves in the surf zone entrain high sediment loads and deposit them near the mouths where there is a low-energy environment. Ample sediment is therefore available in the mouth regions and its transport into the systems is determined by the net transport capacity of the ebb and flood tidal flows near the mouths (Theron & Bornman, 2008).

4. THE ESTUARINE ENVIRONMENT

4.1 The East Kleinemonde Estuary

Both abiotic and biotic characteristics of the East Kleinemonde Estuary have been extensively studied (Bell *et al*., 2001; Cowley & Whitfield, 2001a, 2001b, 2002; Teske & Wooldridge, 2001, 2003; Strydom *et al*., 2003; Henninger *et al*., 2008; Riddin & Adams, 2008a, 2009; van Niekerk *et al*., 2008). This estuary is also subject to an ongoing monitoring project that began in 1994 when data were first collected on the mouth state and fish community of this system (James *et al*., 2008a, 2008b), and in 2005/2006 it was the subject of a multidisciplinary research program where emphasis was placed on the effect of mouth phase on the ecology of the system (Whitfield *et al.*, 2008).

Salinity regimes within the East Kleinemonde depend on mouth state and rainfall, and range from oligohaline (< 5 ppt) to mesohaline (5 – 18 ppt) conditions (Cowley & Whitfield, 2001b). Salinity measurements made during 2006 indicate an almost homogenous water body when the estuary is closed, with some stratification occurring at the head when freshwater enters the system (van Niekerk *et al*., 2008). Strong vertical salinity gradients were present during periods when the estuary was open (Taljaard *et al.*, 2008). Saltwater intrusion, the degree of which is determined by the depth of the mouth and volume of river inflow, causes salinities in the lower reaches to attain > 30 ppt (Taljaard *et al.*, 2008). After the mouth closes, overwash events and evaporation may increase salinities in the estuary (Cowley & Whitfield, 2001b).

Water temperature shows a strong seasonal pattern, with warmer waters of 20-25 °C being characteristic of the summer months and winter having colder temperatures of 13-20 °C (Harrison, 2004; Taljaard *et al.*, 2008). Some vertical stratification does occur, with bottom temperatures being lower than surface, particularly in the summer months (Cowley & Whitfeld, 2001b). Turbidity of the water column increases during open phases, related to an increase in freshwater inflow (Taljaard *et al.*, 2008). Measurement of physico-chemical features during a closed period in spring/summer by Harrison (2004) indicated that average levels of dissolved oxygen (6.37 mg l^{-1}) and turbidity (3.7 NTU) are within the range expected for warm-temperate estuaries, and were higher than those of the West Kleinemonde for the same sampling period (4.92 mg l^{-1} and 1.1 NTU respectively).

Mouth dynamics

According to Whitfield *et al.* (2008) the East Kleinemonde has five recognisable hydrodynamic states (Figure 2.3). The closed phase is the dominant phase for this estuary (Table 2.3 and 2.4), and is characterized by the presence of a well-developed sandbar (berm) at the mouth that separates the estuary from the sea. Some seepage may occur through the sandbar to the sea when river flow is minimal and the estuary water level is high (< 0.3 x 10^6 m^3, Whitfield *et al.*, 2008). The outflow phase occurs when the berm is either completely or partially breached by river flood water leaving the estuary. However mouth breaching may also be facilitated by marine overwash that flattens the berm and raises the estuary water level such that outflow occurs. Normally mouth breaching is associated with periods of river flooding when the estuary volume is high (1.5 – 2.5 m MSL) and is very short in duration (< 2 days). Following breaching and the completion of the outflow phase, the estuary may enter a tidal regime, with duration of this phase being dependent on the maintenance of an open mouth (Whitfield *et al.*, 2008). As the berm rebuilds only water from the peak high tide enters the estuary, with the ebb tidal flow being prevented from leaving the estuary by the developing sand bar. When water from a spring high tide is prevented from entering the estuary by the height of the berm, the estuary is in a closed phase once more. Overwash events may occur during high marine seas when the estuary mouth is closed. These marine events are usually associated with a high tide (small events, < 3 h) and/or stormy seas with large waves (large events, > 3 h) (Whitfield *et al.*, 2008).

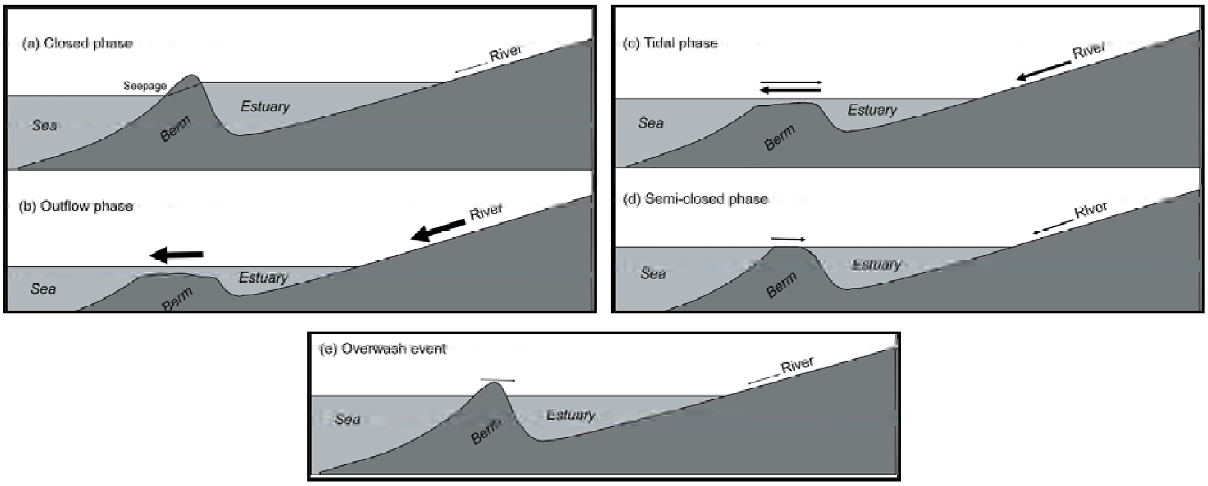


Figure 2.3. Diagrammatic representation of the major hydrodynamic phases and events in the East Kleinemonde Estuary (from Whitfield *et al.*, 2008)

An extensive, ongoing dataset exists on mouth condition of the East Kleinemonde Estuary. Information is presented from 1994 to June of 2008 (Tables 2.3 and 2.4). From 1994, the mouth of the East Kleinemonde Estuary was closed on average 74% of the year. Open mouth conditions occured approximately 31 days a year (8.6%), while on 94 days a year (26%) the estuary was accessible for organisms to recruit from the marine environment. Overwash events had an average occurrence almost twice that of open mouth events (a combined total of 62 days a year for small and large overwash compared to 31 days a year for open mouth events, Table 2.3), highlighting the importance of such events as recruitment opportunities for estuarine associated biota.

Mouth opening is sporadic, and linked to rainfall events and increased freshwater inflow. The duration of mouth closure is variable. The mouth may remain closed for long periods during drought years (2008 – Table 2.3) but in wet years frequent opening may occur (2006 – Table 2.3). The mouth of the East is more susceptible to opening than that of the West (Henninger *et al.*, 2008). Between 2004 and 2008, the mouth of the East Kleinemonde Estuary was open on 238 days while that of the West Kleinemonde on only 162 days (Tables 2.3 and 2.6).

Table 2.3. State of the East Kleinemonde Estuary mouth (in days) recorded between 1994 and June of 2009. Average values exclude 2009. (Small OW = overwash events < 3hr in duration, Large OW = overwash events > 3hr in duration, EAO = estuarine access opportunities) (Source: P. Cowley *unpublished data*)

Year	Closed	Small OW	Large OW	Open	Link	EAO
1994	270	78	5	12	0	95
1995	154	188	15	8	0	211
1996	319	38	6	3	0	47
1997	296	53	5	11	0	69
1998	354	7	3	1	0	11
1999	365	0	0	0	0	0
2000	308	25	4	29	0	58
2001	259	44	1	55	6	106
2002	279	22	11	48	5	86
2003	229	53	19	64	0	136
2004	325	27	5	9	0	41
2005	179	62	34	90	0	186
2006	152	85	12	112	4	213
2007	229	83	21	26	6	136
2008	344	16	5	1	0	22
2009 June	170	8	3	0	0	11
Average ± SD	271 ± 70	52 ± 46	10 ± 9	31 ± 35	1 ± 2	94 ± 70

Table 2.4. State of the East Kleinemonde Estuary mouth (percentage) recorded between 1994 and June of 2009. Average values exclude 2009. (Small OW = overwash events < 3hr in duration, Large OW = overwash events > 3hr in duration, EAO = estuarine access opportunities) (Source: P. Cowley *unpublished data*)

Year	Closed	Small OW	Large OW	Open	Link	EAO
1994	74	21.4	1.4	3.3	0	26
1995	42.2	51.5	4.1	2.2	0	57.8
1996	87.2	10.4	1.6	0.8	0	12.8
1997	81.1	14.5	1.4	3	0	18.9
1998	97	1.9	0.8	0.3	0	3
1999	100	0	0	0	0	0
2000	84.2	6.8	1.1	7.9	0	15.8
2001	71	12.1	0.3	15.1	1.6	29
2002	76.4	6	3	13.2	1.4	23.6
2003	62.7	14.5	5.2	17.5	0	37.3
2004	88.8	7.4	1.4	2.5	0	11.2
2005	49	17	9.3	24.7	0	51
2006	41.6	23.3	3.3	30.7	1.1	58.4
2007	62.7	22.7	5.8	7.1	1.6	37.3
2008	94	4.4	1.4	0.3	0	6
2009 June	93.9	4.4	1.7	0	0	6.1
Average ± SD	74.1 ± 9.1	14.3 ± 12.7	2.7 ± 2.5	8.6 ± 9.6	0.4 ± 0.7	25.9 ± 19.1

The relatively low berm height of the East Kleinemonde is controlled by wind erosion and/or regular marine overwash (van Niekerk *et al.*, 2008), thus overwash events are a frequent occurrence for this system. Data from a water level recorder at the East Kleinemonde have shown that what may appear as a sustained open mouth event is actually a series of rapid breaching and closing events (van Niekerk *et al.*, 2008).

Closing forces dominate in small TOCEs because river inflow and tidal exchange are insufficient to maintain an open mouth (van Niekerk *et al.*, 2008). High wave energy and a large availability of coastal sediments also promote estuary mouth closure. In the East

Kleinemonde, timing of opening is unpredictable (Riddin & Adams, 2008b). An unusually long open mouth event at the end of 2004 lasted for 37 days, although the majority of recorded breaching events showed the mouth closing within a few tidal cycles (van Niekerk *et al*., 2008). Open mouth conditions that last for longer than a day (e.g. 23 – 26 June 2006 or 3 – 16 August 2006) are due to an elevated average river flow of > 0.04 $m^3 s^{-1}$ that is maintained for longer than a day (van Niekerk *et al*., 2008). The increased volume of water flowing out of the mouth prevents mouth closure. Sediment scour occurs mainly during the flood outflow phase but not during the subsequent base flow period that is characteristic of the tidal phase.

Macrophyte dynamics

Unfortunately, annual records of the percentage vegetation cover within the East Kleinemonde Estuary are unavailable. The data presented below are therefore gleaned from the existing literature or follow verbal communication with workers who have been involved with this system. The range of habitat types present in the East Kleinemonde Estuary, and the area covered by each in 2006 is shown in Table 2.5. When conditions are ideal for macrophyte growth (stable water level, little water movement and low turbidities) this habitat can be extensive. The vast area of exposed sand and mudbanks may be colonized by intertidal salt marsh when water levels are low or by aquatic macrophyte beds when the estuary is closed and water levels rise (Riddin & Adams, 2008b). *Phragmites australis* is restricted to areas where freshwater is available and it is likely that the increased output by sewers (septic tanks), stormwater drains or runoff from the road has allowed this reed to spread in recent years.

Table 2.5. Habitat types and area cover in the East Kleinemonde Estuary (after Riddin & Adams, 2008b)

	Area covered (ha)
Intertidal saltmarsh	2.45
Sarcocornia perennis dominant	
Supratidal saltmarsh	2.77
Sporobolus virginicus	
Sarcocornia pillansii	
Limited *Limonium scabrum*	
Saltmarsh mosaic	1.78
Juncus species	
Sarcocornia species	
Triglochin striata	
Samolus porosus	
Sporobolus virginicus	
Bassia diffusa	
Other sedges	
Salicornia species	1.02
Wetland grass	0.57
Paspalum species	
Juncus kraussi, *acutus*	1.81
Reeds and sedges	1.01
Phragmites australis	
Exposed mudbanks	9.83
Estuarine water area (open, spring low)	12.69
Sandbank (open, spring low)	1.78
Submerged macrophytes (ideal conditions)	14.5

Figure 2.4 shows the actual distribution of different macrophyte types in the East Kleinemonde, and is based on data compiled from a survey conducted in 2006.

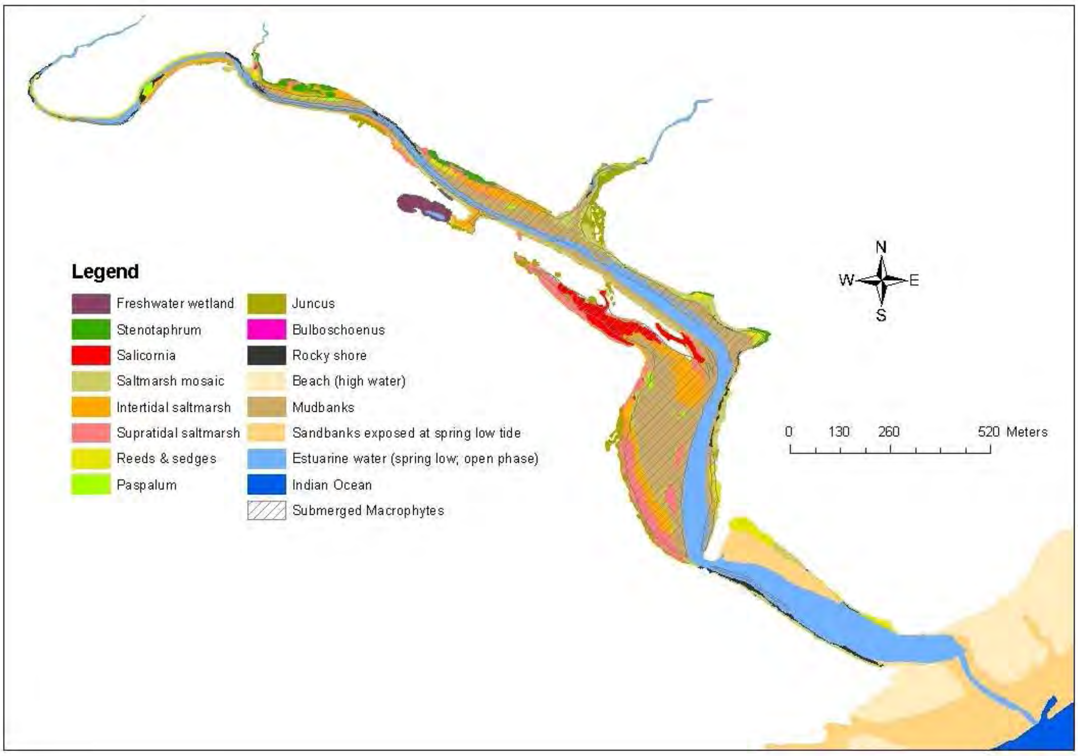


Figure 2.4. Vegetation map for the East Kleinemonde Estuary, showing areas covered by each macrophyte type in 2006. (Source: Riddin, T. and Adams, J.B.)

Submerged macrophyte dynamics reflect the changing physical conditions within an estuary, most notably those of salinity and water level, linked to mouth state. *R. cirrhosa* was the dominant species present in the East Kleinemonde during the 1990s and early 2000s, with the presence of extensive beds being attributed to the prolonged periods of mouth closure in most years (Table 2.3). Higher freshwater inflow relative to seawater inputs from 1999 to 2001 reduced salinity within the estuary and allowed *P. pectinatus* to expand into the middle and upper reaches, such that by 2001 it had become the dominant macrophyte in the East Kleinemonde Estuary (Riddin & Adams, 2008b).

A flood event in May 2003 along the south-eastern coastal region of the Eastern Cape Province of South Africa caused the mouths of all TOCEs in the region to breach. The impact of the flood resulted in a major mouth breaching event with large amounts of sediments being removed from both systems. Removal of the subtidal *P. pectinatus* beds during the flood and subsequent salinities > 15 ppt prevented the recovery of this species in the East Kleinemonde when the mouth closed. The major flood would have also depleted surface sediment seed banks of *R. cirrhosa* and this may have hampered its re-establishment during the subsequent closed phases when salinities were more suitable for this species. As a result of the environmental conditions impacting negatively on both aquatic macrophyte species, only a limited area of the East Kleinemonde Estuary was covered by submerged plant beds in 2006 (Adams & Riddin, 2007).

By the beginning of this study in January 2008, *P. pectinatus* was not present and only small beds of *R. cirrhosa* were found. By winter of 2008 *R. cirrhosa* had spread throughout the extent of the East Kleinemonde according to its salinity tolerance (0 – 45 ppt, Riddin & Adams, 2008b) with *P. pectinatus* being confined to the upper reaches of the estuary. In 2009 increased salinities caused *P. pectinatus* to be absent from the East Kleinemonde Estuary although *R. cirrhosa*, with its higher salinity tolerance, persisted throughout this period and could be found throughout the length of the estuary.

4.2 The West Kleinemonde Estuary

The West Kleinemonde is also predominantly closed, with the mouth opening following heavy rains or major overwash events. Overwashing is a frequent occurrence due to the non-perched mouth, and because of this a marked salinity gradient is present, with the lower reaches being characterized by salinities of 25 – 30 ppt and the upper reaches often having substantially fresher waters (5-10 ppt) (Bernard & Froneman, 2005). During drought conditions however, reduced freshwater inflow results in salinity throughout the estuary being far more homogenous. Temperatures show seasonal variation, with surface summer temperatures commonly ranging between 22 - 29 °C, and winter 12 - 15 °C.

Physico-chemical measurements made during a closed phase in a spring period by Harrison (2004) indicate that the West Kleinemonde has a relatively warm water temperature during this season (average 23.2 °C). Salinity was in the region of 19 ppt, dissolved oxygen an average of 5 mg l^{-1} and turbidity low (1.1 NTU) at the time of the study, these factors being influenced by mouth state among other features. Dissolved oxygen is a function of water temperature, and the values recorded for the West Kleinemonde were in the range expected for warm-temperate estuaries (5 – 8 mg l^{-1}, Harrison, 2004).

Mouth dynamics

Unlike the East Kleinemonde Estuary, long-term data on the estuary's fish community is unavailable for the West Kleinemonde Estuary. Daily mouth condition has however been recorded for the West Kleinemonde Estuary since 2004. This information is presented in Tables 2.6 and 2.7. The mouth of the West Kleinemonde is predominantly closed. Over the last five years, this mouth phase has taken up the majority of each year (295 days a year on average, or 80.6%). Open mouth events occur about as frequently as overwash events, with overwash (both small and large) taking place on approximately 35 days a year and open mouth conditions an average of 32 days a year. There is a high degree of variability between years however. In 2007 overwashing occurred on 42 days in the West Kleinemonde, with open mouth days being zero for the West Kleinemonde during this same year.

In terms of estuarine access (recruitment) opportunities, the East Kleinemonde seems to be slightly more accessible to recruiting fauna than the West (EAOs representing roughly 25.9% of a year, or an average of 94 days compared to 19.4% or 71 days per year for the West; Tables 2.3 and 2.4, and 2.6 and 2.7 respectively). The number of opportunities for recruitment, related to rainfall, may be highly variable between years however, with recruitment being facilitated by the high number of access opportunities in 2005 (41.9%) compared to only 3.3% in 2008 in the West Kleinemonde Estuary (Table 2.7).

Table 2.6. State of the West Kleinemonde Estuary mouth (in days) recorded between 2004 and June of 2009. Average values exclude 2009. (Small OW = overwash events < 3hr in duration, Large OW = overwash events > 3hr in duration, EAO = estuarine access opportunities) (Source: P. Cowley *unpublished data*)

Year	Closed	Small OW	Large OW	Open	Link	EAO
2004	347	10	2	7	0	19
2005	212	36	20	96	1	153
2006	245	28	25	59	8	120
2007	315	30	12	0	8	50
2008	354	9	3	0	0	12
2009 June	176	2	3	0	0	5
Average ± SD	295 ± 63	23 ± 12	12 ± 10	32 ± 43	3 ± 4	71 ± 63

Table 2.7. State of the West Kleinemonde Estuary mouth (percentage) recorded between 2004 and June of 2009. Average values exclude 2009. (Small OW = overwash events < 3hr in duration, Large OW = overwash events > 3hr in duration, EAO = estuarine access opportunities) (Source: P. Cowley)

Year	Closed	Small OW	Large OW	Open	Link	EAO
2004	94.8	2.7	0.5	1.9	0	5.2
2005	58.1	9.9	5.5	26.3	0.3	41.9
2006	67.1	7.7	6.8	16.2	2.2	32.9
2007	86.3	8.2	3.3	0	2.2	13.7
2008	96.7	2.5	0.8	0	0	3.3
2009 June	97.2	1.1	1.7	0	0	2.8
Average ± SD	80.6 ± 17.2	6.2 ± 3.4	3.4 ± 2.8	8.9 ± 11.9	0.9 ± 1.2	19.4 ± 17.2

Macrophyte dynamics

The presence of a species of submerged macrophyte within a temporarily open/closed estuary is variable in both space and time, dependent on physical conditions within the system. Unfortunately, in the absence of annual records the changes in percentage vegetation cover within the West Kleinemonde Estuary described below are pieced together from verbal communications from workers involved with this system or where briefly described in the literature. Figure 2.5 shows the distribution of different macrophyte types in the West Kleinemonde during 2006.

The same flood event that removed the submerged macrophyte beds from the East Kleinemonde in May 2003 had a less pronounced effect on the vegetation within the West Kleinemonde Estuary. Although the mouth breached and sediment was removed from the system, the macrophyte beds did not show the same extent of destruction than in the East Kleinemonde.

Between 2003 and 2004, Bernard and Froneman (2005) recorded submerged vegetation cover being less than 10% of total area in the upper reaches, while the middle reaches had extensive cover (> 70%). Vegetation cover in the lower reaches was patchy, and covered less than 20% of the total area. An increase in cover was observed for all regions of the estuary during the study but no indication of the plant species present was made. In 2006 these plant beds were almost purely *Ruppia cirrhosa* due to the preceding prolonged period of mouth closure (2 years) and salinities > 15 ppt (Riddin & Adams, 2008b). Maximum potential biomass for this species in the West Kleinemonde in August 2006 was estimated at 2883 g dry mass m^{-2}, while the corresponding value for the East Kleinemonde was 706 g dry mass m^{-2} (Riddin & Adams, 2008b).

By the beginning of this study in January 2008, *P. pectinatus* had become dominant in the upper reaches of the West Kleinemonde with both *P. pectinatus* and *R. cirrhosa* present in the middle reaches. This was due to a prolonged closed phase that allowed freshening of the West Kleinemonde Estuary, facilitating the expansion of *P. pectinatus* further down into the estuary from its head. By winter of 2008 *R. cirrhosa* was found from the lower into the middle reaches of the West Kleinemonde, with *P. pectinatus* confined to the upper reaches. In 2009 salinities exceeded 20 ppt throughout the length of the West Kleinemonde, resulting in the absence of *P. pectinatus* but the persistence of *R. cirrhosa* in all regions of the estuary throughout this period.

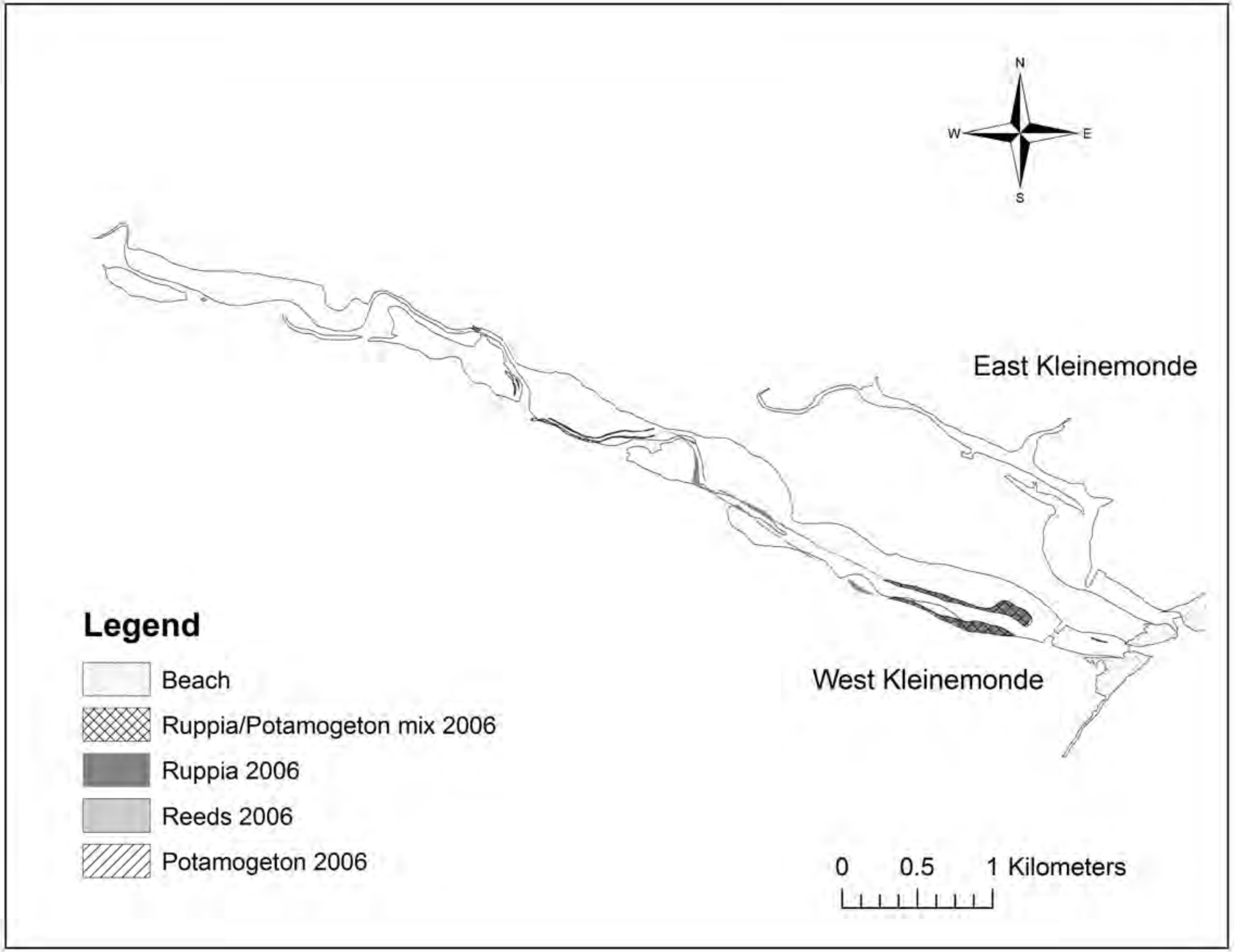


Figure 2.5. Submerged macrophyte map for the West Kleinemonde Estuary. Data for 2006 (Source: Riddin, T. and Adams, J.B.)

CHAPTER THREE: FACTORS INFLUENCING THE FISH ASSEMBLAGES OF THE EAST AND WEST KLEINEMONDE ESTUARIES, WITH EMPHASIS ON SUBMERGED MACROPHYTES

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1. INTRODUCTION

Habitat variability is a factor contributing to an increase in species diversity within estuarine systems (Whitfield, 1996), although it is often overlooked. Beds of submerged macrophytes are important habitats, especially for juvenile fishes, and contribute significantly to the goods and services that estuarine systems offer. They are food-rich due to the associated epiphytes (Hanekom & Baird, 1984) and epifauna (Davies, 1982; Henninger *et al*., 2009) and also offer shelter from predation for both invertebrates (Emmerson, 1986; Henninger *et al*., 2009) and fishes (Adams *et al*., 2004). As a result, the nursery function of an estuary is closely related to aquatic plant communities (Wallace & van der Elst, 1975; Beckley, 1983). Macrophytes further contribute to the detrital pool within an estuary, and act as collecting beds for this important food source by slowing the flow of water (Carr & Adams, 1973). Added to this, they may well be an important component of the suite of organic olfactory cues that assist the larvae of certain marine fishes to locate and recruit into estuaries (Whitfield, 1994; James *et al*., 2005).

The loss of beds of submerged macrophytes from estuarine systems has been linked to a decrease in primary productivity (Taylor, 1983), as well as a decline in fish species richness (Beckley, 1983; Cowley & Whitfield, 2001b) and a change in the relative abundance of species (Whitfield, 1984, 1986). In the same way, highest abundance and biomass of invertebrates have been recorded from beds of submerged macrophytes (Bernard & Froneman, 2005; Froneman, 2006; Froneman & Henninger, 2009), and a loss of this habitat has been linked to a decline in invertebrate biomass within estuarine systems (Kikuchi, 1974; Orth, 1975; Davies, 1982).

Variability in estuarine systems

Estuarine ecosystems are dynamic, being susceptible to wide fluctuations in physical conditions (Day *et al.*, 1989; Harrison & Whitfield, 2006). Such changes vary in both time and space. Short-term fluctuations include extremes of salinity, linked primarily to mouth phase and freshwater inflow that may cause significant physiological stress to the biota and, in documented cases, have led to reduced reproductive success (Froneman & Vumazonke, 2007; Froneman & Henninger, 2009) and even death of estuarine fauna and flora (Blaber & Whitfield, 1976; Bennett, 1985; Riddin & Adams, 2008a). Such short term variation is relatively easy to document and has therefore been the subject of a number of studies (e.g. Bennett, 1985, 1994).

Longer-term changes that may take place over a period of years, however, are harder to measure, but important in their effects on estuarine functioning. Such long-term variation may be due to a number of short- or long-term influences including random climatic anomalies such as severe droughts or storms, climatic trends such as El Niño events, or human-induced factors such as the effects of dredging or pollution (Nichols, 1985). The identification of the persisting effects thereof are only possible if estuarine communities are monitored over correspondingly long time periods (Flint, 1985). An increase in sea surface temperature for example has been linked to the appearance and continued presence of certain tropical fish species in the East Kleinemonde Estuary since 1995 (James *et al.*, 2008a), a pattern that is only detectable by the existence of a long-term dataset. Flint (1985), using an 11 year dataset, identified the importance in episodic flooding events to productivity in the Corpus Christi Bay, Texas, and Desmond *et al.* (2002) were able to identify physical predictors of estuarine invertebrate and fish communities with the help of an 11 year dataset.

Background and aims of this study

A severe flood in 2003 was responsible for a number of changes in the estuarine environment of the East Kleinemonde; immediate effects including a prolonged open phase and tidal conditions with an increase in salinity, with more sustained effects including a lower closed phase water level due to the lower berm height. Linked to these physico-chemical changes was the desiccation and die-back of large beds of the submerged macrophytes *Ruppia cirrhosa* and *Potamogeton pectinatus*, leading to a significant loss of estuarine plant habitat that has not fully recovered six years after the event. In contrast, the neighbouring West Kleinemonde Estuary has extensive beds of submerged macrophytes, the presence of which has been stable over a long time period.

The primary aim of this study was to assess changes in the fish assemblage of the East Kleinemonde Estuary following the loss of the macrophyte beds from the system. Seine net catch data were compared between the periods 1998 – 2003 (macrophyte-dominated phase) and 2004 – 2009 (macrophyte-senescent phase). In addition, the fish composition and abundance data were compared between the East and West Kleinemonde estuaries for the period 2008 - 2009. It was hypothesized that species richness, as well as the abundance of macrophyte-associated species such as the Cape stumpnose *Rhabdosargus holubi* and Cape moony *Monodactylus falciformis*, will be lower in the macrophyte-senescent phase of the East Kleinemonde Estuary when compared to the period during which submerged macrophyte beds were present or in the macrophyte-dominated West Kleinemonde Estuary.

2. METHODS

2.1 Field sampling

As part of an ongoing long-term monitoring project, the ichthyofaunal community of the East Kleinemonde Estuary has been sampled biannually in summer (January or February) and winter (June or July) since December 1994 (James *et al*., 2008a, 2008b). The estuary was divided longitudinally into lower, middle and upper reaches, with fixed sampling sites positioned within each reach (Figure 3.1) and effort was therefore distributed along the estuary length. A range of gear types were used to ensure that every group of the fish assemblage would be adequately represented (Rozas & Minello, 1997). This same protocol was followed in the West Kleinemonde Estuary during 2008 and 2009.

Marine-spawning fish species and those that spawn within the estuarine environment were targeted separately using seine nets of different mesh sizes. A small mesh seine (30 m x 2 m) with a 5 mm bar mesh was used to sample the estuarine-spawning fishes and a large mesh seine net (50 m x 2 m) with a 15 mm bar mesh for the marine spawners (Cowley & Whitfield, 2001b). Six monofilament gill nets (10 m x 2 m) with sections of 45 mm, 75 mm and 100 mm stretch meshes were set overnight along the length of each estuary in order to capture the larger species that actively avoid a seine net, with effort divided equally amongst the reaches of each system.

The seine nets were laid in a semi-circle from the shore using a motorized boat and hauled ashore by three to four people. From each seine haul, individuals to be used for the isotope study were removed from the catch and placed on ice to be processed later. In order to compliment the existing long-term database on the East Kleinemonde Estuary, catch data from all gear types were recorded. Fish were identified to the species level and standard length (SL) was recorded for each fish to the nearest mm. Where catches were large, a sub-sample of approximately one hundred individuals of each species was measured, but all individuals were counted. The gill net and large mesh seine net catches were processed in the field, while small mesh seine catches were retained and processed in the laboratory.

Over the period 2008 – 2009, the number of hauls per sampling trip completed in the East Kleinemonde Estuary ranged between six and nine for the small mesh seine net and seven and nine for the large mesh seine, with at least two hauls per reach of the estuary. In the West Kleinemonde Estuary, between six and 12 hauls were made per sampling trip using the small mesh seine net and between six and nine for the large mesh seine net, again with a minimum of two hauls per region of the estuary.

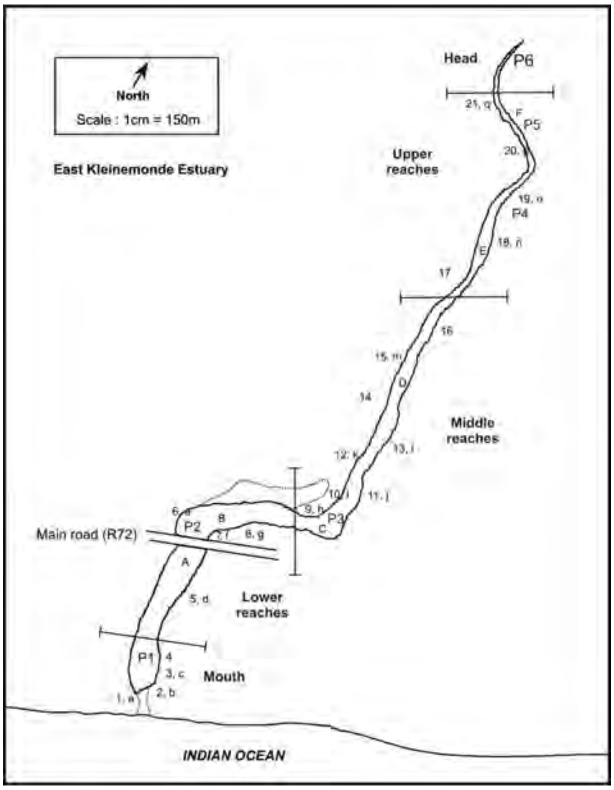

Figure 3.1 Map of the East Kleinemonde Estuary showing the sampling sites for the long-term monitoring project; a – q small mesh seine sites, 1 – 21 large mesh seine sites, A – F gillnet sites, P1 – P6 physico-chemical sites (from James *et al*., 2008b)

2.2 Environmental variables

Although it is possible to analyse an almost endless range of physical variables, this study focused on those that could be related to potential changes in the composition of the ichthyofaunal community in the Kleinemonde estuaries over the period 1998 to 2009. These factors must therefore show inter-annual rather than intra-annual variability, and reliable data should exist for them over the period in question. Variables chosen according to these criteria were estuary mouth state, in particular the occurrence of a spring mouth opening each year which has been shown to be a strong determinant of fish assemblage composition (James *et al.*, 2008b), as well as salinity, annual rainfall and the presence or absence of beds of submerged macrophytes.

Data on daily mouth state from the East Kleinemonde Estuary exist for 1993 – 2009 as part of the long-term monitoring project, and have been recorded for the West Kleinemonde Estuary from 2004. Whitfield *et al*. (2008) identified five different mouth conditions; these defined as follows (i) open, (ii) closed, (iii) outflow, (iv) tidal and (v) semi-closed. For use in this thesis the categories defined by Whitfield *et al*. (2008) have been further simplified such that the categories of estuary mouth condition are (i) open, when the sand berm is breached due to increased river flow, (ii) link, where water trickles out of the estuary, (iii) overwash of marine water into the estuary at high tide or during stormy sea conditions, including both small (< 3h in duration) and large (> 3h in duration) events and (iv) closed conditions during which time the sand berm separates the estuary from the sea. As fishes caught in the large mesh seine net would have recruited into the estuary the previous year, the dataset analysed for mouth condition corresponded to a year earlier than that of the fishes, namely 1997 – 2008 for the East Kleinemonde Estuary and 2007 – 2008 for the West Kleinemonde Estuary. Daily rainfall data were obtained from the South African Weather Service's Port Alfred station and summed for the year. Salinity records for summer and winter of each year, with a few exceptions, were available as part of the long-term monitoring project for both estuaries. Seasonal values are averages from a number of sites over the length of each estuary (Figure 3.1).

2.3 Data analysis

Species abundances are presented as catch per unit effort (CPUE) for the large mesh seine net. Classification and ordination of small and large mesh seine net data between 1993 and 1997 from the East Kleinemonde Estuary of regional (lower, middle and upper reaches) fish assemblage data showed that with the exception of catches associated with the sandy mouth region, assemblages were similar throughout the system (Cowley & Whitfield, 2001b). Similarly, CPUE data as previously analysed by James *et al.* (2008b) for the period summer 1995 to winter 2005 indicated no major seasonal change in individual fish species CPUE. Regional, as well as seasonal data have therefore been grouped in this study to form annual values.

Available data were divided into different groupings of years according to the presence or absence of submerged macrophyte beds within a system, in an attempt to elucidate the influence that availability of this habitat was having on the fish assemblage. Groupings allowed for comparison of potential change in fish community within an estuary following loss of this habitat, as well as between estuaries with differing patterns of macrophyte abundance. Thus, the comparisons included: (i) 1998 – 2003 (macrophyte-dominated phase) versus 2004 – 2009 (macrophyte-senescent phase) within the East Kleinemonde Estuary, and (ii) 2008 – 2009 in both the West (macrophyte-dominated) and East (macrophyte-senescent) Kleinemonde estuaries.

Changes in fish assemblages and how these relate to the selected physical variables were analysed using the PRIMER package (V. 5.2.4., PRIMER-E, 2001). Only data from the large mesh seine net were used in this analysis as it was the only gear type where the CPUE was high enough for use in PRIMER. The Bray-Curtis similarity measure was used to produce an association matrix, during which process the data were fourth root transformed. This transformation downplays the contribution by dominant species and allows the intermediate and rarer species to contribute to the analysis (Clarke & Warwick, 1994). Data were not standardized as CPUE is already a measure of relative abundance and so further treatment was not necessary. Data were then ordinated using the non-metric multidimensional scaling (nMDS) technique in the PRIMER package. Analysis of similarities (ANOSIM) indicated whether the groupings identified by ordination were significant, and similarity percentages (SIMPER) then identified species most responsible for the Bray-Curtis dissimilarity between groups (Clarke & Warwick, 1994). Finally, maximum Spearman's rank correlation (ρ_w) between the observed data assemblages and physical variables measured was calculated using the BIO-ENV procedure, results indicating how well a single or combination of environmental variables match the community structure (Clarke & Warwick, 1994). Various phases of mouth status were entered as a percentage of the year that the mouth displayed each category, with the occurrence of a spring opening entered as having taken place (1) or not (0). Similarly macrophyte beds were analysed as present (1) or absent (0) from the system.

Ecological functioning within a system may be independent to change in species composition but rather linked to functional groupings (Bremner *et al*., 2003; Hewitt *et al*., 2008). To gain a better understanding of the effects following the loss of submerged macrophytes from the East Kleinemonde Estuary, components of the fish assemblage were divided into different functional groups and variation in annual CPUE assessed; this providing an additional level of analysis of change (e.g. Mistri *et al*., 2000; Bremner *et al*., 2003; Dumay *et al*., 2004). The groupings included firstly those species caught in higher abundances in beds of submerged macrophytes (vegetation-associated species) and those more typically associated with a bare substratum, and secondly trophic guilds within the fish species comprising piscivores, zoobenthivores, zooplanktivores, omnivores and detritivores. These divisions were an attempt to assess where in the estuarine fish assemblage the influence, if any, of the presence of the submerged macrophyte beds was strongest.

The significance of change in CPUE between the fish populations was statistically tested using the non-parametric Wilcoxon matched pairs test for dependent samples where the change was within one system and the Mann-Whitney U test where variation was between estuaries. Both tests were performed using the STATISTICA Software package (version 8.0) at the $P < 0.05$ level of significance.

3. RESULTS

3.1 Physical environment

Total annual rainfall in most years between 1998 and 2008 at Port Alfred in the Eastern Cape was between 600 – 800 mm (Figure 3.2). Exceptions to these are 1999, 2000 and

2008 which were exceptionally dry years (total annual rainfall 382.8, 367.6 and 468.1 mm respectively), and 2005 which was exceptionally wet (1287.8 mm). Although at the time of this study rainfall data were not complete for the whole of 2009, not much rain had fallen in the first half of the year with a January - June total of 173.3 mm compared to 350.8 mm during the same period in 2000 (driest year) and 538.3 mm in 2005 (wettest year).

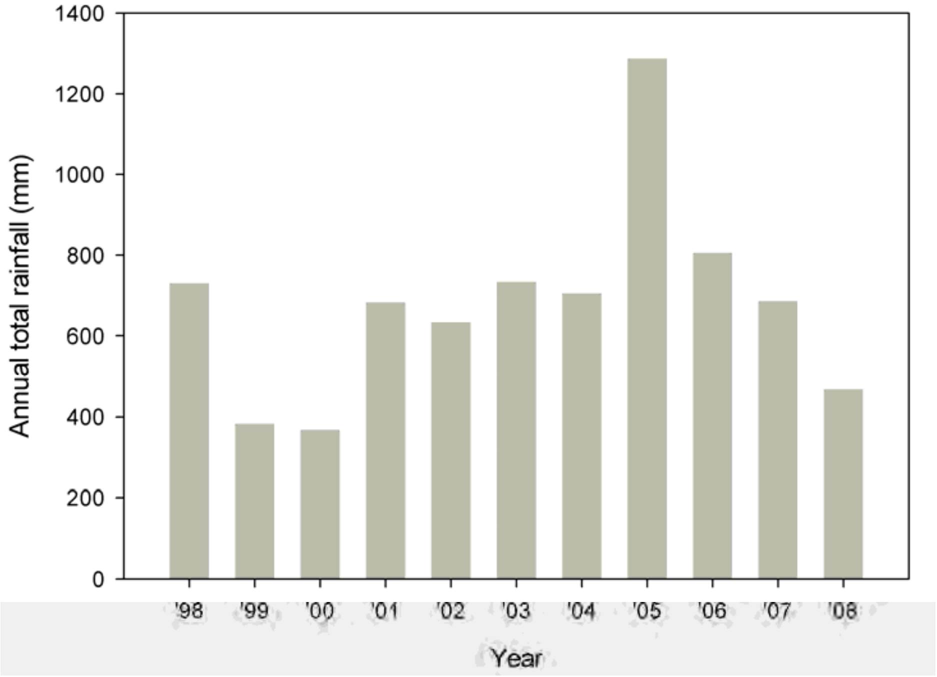

Figure 3.2 Total annual rainfall (mm) over the period 1998 – 2008, as recorded at Port Alfred (Source: South African Weather Service)

Mouth phase is presented for the period 1997 – 2008 in the figure below. Between 1997 and 2008 the mouth of the estuary was predominantly closed, ranging from 100% of the days in 1999 to a minimum of 42% of the days in 2006. However, all years except for 2005 and 2006 showed a closed state for more than 60% of the days. Accordingly, 2005 and 2006 provided the most recruitment opportunities for fishes, predominantly in the form of overwash events during 2005 and open mouth events in 2006 (26% and 31% of each year respectively). Excluding 1999, the percentage of days in which overwash occurred ranged between 3% (1998) and 29% (2007), with the range for an open mouth state being between 0% (1998) and 31% (2006).

In terms of the type of recruitment opportunity, marine overwash events were the most common, occurring on a higher percentage of the days in all years except 2001, 2002 and 2006. In some cases (e.g. 2007) the difference is quite marked (overwash = 28%, open mouth = 7%), while other years show similar periods of both events (e.g. 2005 overwash = 26%, open mouth = 25%). The estuary mouth breached during spring in 2000, 2001, 2002, 2003, 2005 and 2006, and overwash conditions during spring occurred in all years except 1999.

Between 2007 and 2008 the West Kleinemonde Estuary was mainly closed off from the sea. Closed mouth conditions made up 86.3% of the days in 2007 and 96.7% in 2008. The mouth did not open at all in either year, with overwash events offering the only opportunity for recruitment of marine-spawning species. Overwash events occurred on 11.5% of the days in 2007 and 3.2% in 2008; spring overwash events accounting for 0.3% and 1.4% of these totals for 2007 and 2008 respectively.

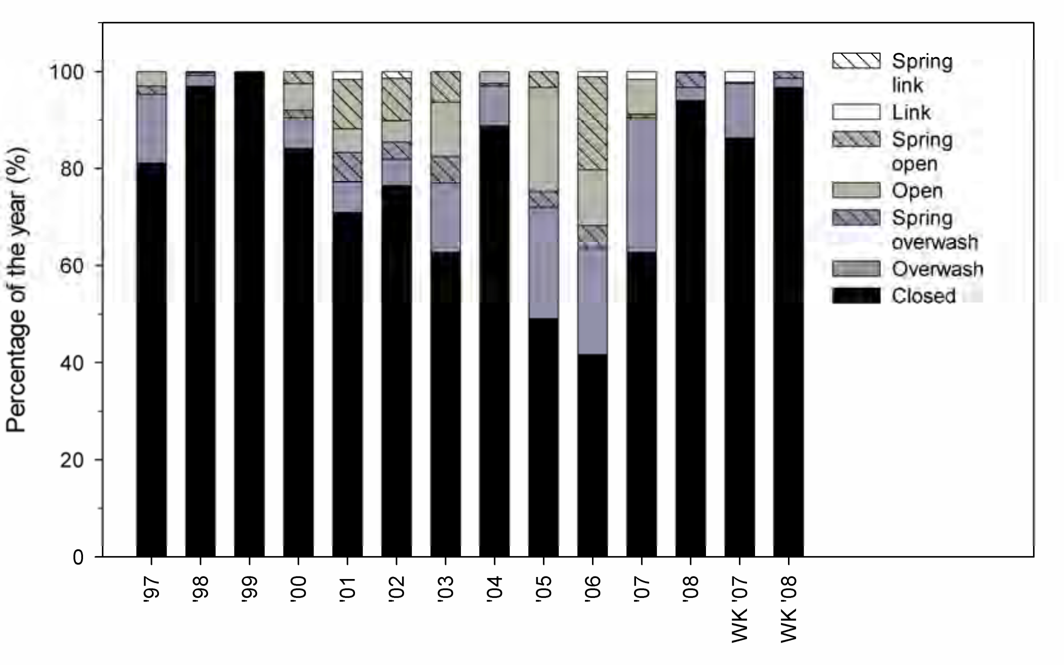

Figure 3.3 Annual mouth state of the East Kleinemonde Estuary over the period 1997 – 2008, and the West Kleinemonde Estuary over the period 2007 – 2008

As a general pattern winter salinity values seem to be higher than those of summer in the East Kleinemonde Estuary, possibly linked to reduced freshwater input during this season (Figure 3.4). Although in some years (2004 and 2009) salinity increased to euryhaline values, average salinity seems to fluctuate between meso- (5 – 17.9 ppt) and polyhaline (18 – 29.9 ppt) conditions.

Salinity values in the West Kleinemonde Estuary were similar to those in the East Kleinemonde, falling within the polyhaline range for 2008 and 2009 (Figure 3.4). Although rainfall during 2008 and 2009 was low, the East and West Kleinemonde estuaries experienced different salinity regimes for these years. In both years, the average salinity of the East Kleinemonde Estuary was higher than that of the West Kleinemonde, which has the larger catchment and therefore a greater freshwater inflow potential.

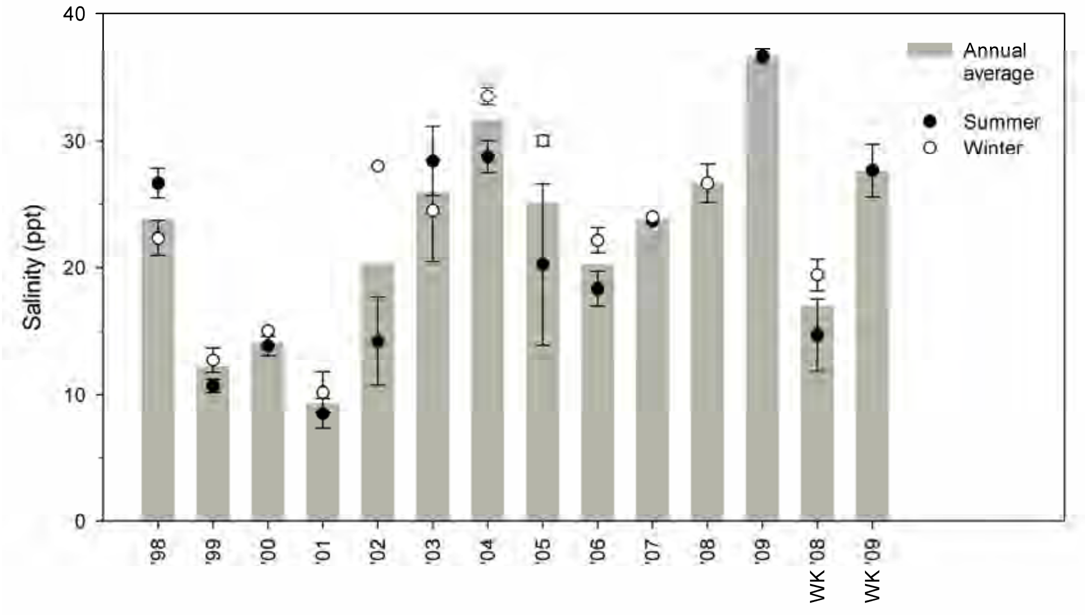

Figure 3.4 Summer, winter, and annual average salinity (ppt) readings from the East Kleinemonde Estuary, over the period 1998 to 2009, and from the West Kleinemonde Estuary for 2008 and 2009. Seasonal values are averages over at least three sites with error bars representing standard deviation. Where data are missing for seasons, annual average reflects that of a single season

3.2 Fish assemblage structure

Total catch within the East and West Kleinemonde estuaries was dominated by marine-spawning fishes (Category II) (Table 3.1). Over the sampling period, a total of 40 species from 18 families were recorded in the various gear types. The East Kleinemonde Estuary's catches included 37 of these species from 18 families, while 28 species from 14 families were recorded in the West Kleinemonde Estuary (Table 3.1). Those species being recorded only from the East Kleinemonde Estuary were *Caranx sexfasciatus*, *Glossogobius giuris*, *Pomadasys olivaceum*, *Pomatomus saltatrix*, *Syngnathus acus*, *Terapon jarbua*, *Hyporhamphus capensis*, *Valamugil buchanani*, *Valamugil cunnesius*, *Valamugil robustus*, *Pseudorhombus arsius* and *Rhabdosargus sarba*; the first six of which, together with *V. buchanani* were unique to the macrophyte-senescent phase between 2004 and 2009. Despite the much shorter sampling period, catch records from the West Kleinemonde Estuary revealed three unique species, namely *Trachurus trachurus*, *Diplodus cervinus hottentotus* and *Syngnathus temmincki*.

A similar number of species were recorded from both estuaries during the macrophyte-dominated sampling phases. A total of 26 species from 15 families were recorded from the East Kleinemonde Estuary (1998 – 2003) and 28 species representing 14 families from the West Kleinemonde Estuary (2008 – 2009). However, total effort was not comparable as a total of 208 large mesh and 159 small mesh seine net hauls were performed in the East Kleinemonde Estuary over the six year period and only 42 large mesh and 31 small mesh seine hauls were made in the West Kleinemonde Estuary during the two year sampling period (Tables 3.2 and 3.3). A comparison of catches made over the same period (2008 and 2009) revealed that 19 species from 9 families were recorded from the East Kleinemonde Estuary (33 large and 31 small seine net hauls) and 22 species representing 11 families from the West Kleinemonde Estuary (42 large and 31 small seine net hauls).

In terms of families, Mugilidae and Sparidae were well represented during all sampling periods and in both estuaries (Table 3.3). A total of ten mugilid species and six sparids were recorded overall. Of these, eight mugilids and three sparids were present in the East Kleinemonde Estuary between 1998 and 2003 and ten mugilids and five sparids during the period 2004 to 2009, while seven mugilids and five sparids were recorded in the West Kleinemonde Estuary between 2008 and 2009.

Although CPUE for each species fluctuated between the different groupings of years (Tables 3.2 and 3.3), the only species that showed a significant change was the estuarine pipefish *Syngnathus watermeyeri*, with a significantly different CPUE (Wilcoxon matched pairs test, $P < 0.05$) between the macrophyte-dominated period (1998 – 2003) and the macrophyte-senescent period (2004 – 2009) in the East Kleinemonde Estuary. A number of species were present in one grouping but absent from its pair, such as the dusky kob *Argyrosomus japonicus* between sets of years in the East Kleinemonde Estuary. These species were, however, recorded with low CPUE values (when present) in only one of the six years and this change in abundance was therefore not statistically significant (Wilcoxon matched pairs test, $P > 0.05$).

Those species most consistently recorded in the large mesh seine net in both estuaries were the mugilids *Liza dumerili*, *Liza richardsonii*, *Mugil cephalus* and *Myxus capensis*, the sparids *Lithognathus lithognathus* and *Rhabdosargus holubi*, the moony *Monodactylus falciformis*, cichlid *Oreochromis mossambicus* and the haemulid *Pomadasys commersonnii* (Table 3.3). In the small mesh seine net, those species featuring in the catch in both estuaries for every year sampled were *Atherina breviceps*, *Gilchristella aestauria* and *Glossogobius callidus* (Table 3.2). In the large mesh seine *M. capensis* and *R. holubi* were numerically dominant, and in the small mesh seine the overwhelming contribution was from *A. breviceps* and *G. aestuaria* (Tables 3.2 and 3.3).

Table 3.1. Fish species recorded from the East Kleinemonde and West Kleinemonde estuaries. The number 1 indicates the presence of a species in the catch during the macrophyte-dominated periods in the East (Ei) Kleinemonde and West (Wi) Kleinemonde estuaries, and from the East Kleinemonde during the period of macrophyte senescence (Eii). Roman numerals refer to the categories of estuary-association (after Whitfield, 1994)

Family	Species	Common name	Category	Ei.	Eii.	Wi.
Ariidae	*Galeichthys feliceps*	White seacatfish	IIb		1	1
Atherinidae	*Atherina breviceps*	Cape silverside	Ib	1	1	1
Carangidae	*Caranx sexfasciatus*	Bigeye kingfish	IIb		1	
	Lichia amia	Garrick/Leervis	IIa	1	1	1
	Trachurus trachurus	Maasbanker	III			1
Cichlidae	*Oreochromis mossambicus*	Mozambique tilapia	IV	1	1	1
Clupeidae	*Gilchristella aestuaria*	Estuarine roundherring	Ia	1	1	1
Elopidae	*Elops machnata*	Ladyfish	IIa	1	1	1
Gobiidae	*Glossogobius callidus*	River goby	Ib	1	1	1
	Glossogobius giuris	Tank goby	IV		1	
	Psammogobius knysnaensis	Speckled sandgoby	Ib	1	1	1
Haemulidae	*Pomadasys commersonnii*	Spotted grunter	IIa	1	1	1
	Pomadasys olivaceum	Piggy	III		1	
Hemiramphidae	*Hyporhamphus capensis*	Cape halfbeak	Ia	1	1	
Monodactylidae	*Monodactylus falciformis*	Oval moony	IIa	1	1	1
Mugilidae	*Liza alata*	Diamond mullet	IIb		1	1
	Liza dumerili	Groovy mullet	IIb	1	1	1
	Liza macrolepis	Largescale mullet	IIa	1	1	1
	Liza richardsonii	Southern mullet	IIc	1	1	1
	Liza tricuspidens	Striped mullet	IIb	1	1	1
	Mugil cephalus	Flathead mullet	IIa	1	1	1
	Myxus capensis	Freshwater mullet	Vb	1	1	1
	Valamugil buchanani	Bluetail mullet	IIc		1	
	Valamugil cunnesius	Longarm mullet	IIa	1	1	
	Valamugil robustus	Robust mullet	IIa	1	1	
Paralichthyidae	*Pseudorhombus arsius*	Largetooth flounder	III	1		
Pomatomidae	*Pomatomus saltatrix*	Elf	IIc		1	
Sciaenidae	*Argyrosomus japonicus*	Dusky kob	IIa	1	1	1
Soleidae	*Heteromycteris capensis*	Cape sole	IIb	1	1	1
	Solea bleekeri	Blackhand sole	IIb	1	1	1
Sparidae	*Diplodus cervinus hottentotus*	Zebra	III			1
	Diplodus sargus capensis	Blacktail	IIc		1	1
	Lithognathus lithognathus	White steenbras	IIa	1	1	1
	Rhabdosargus globiceps	White stumpnose	IIc		1	1
	Rhabdosargus holubi	Cape stumpnose	IIa	1	1	1
	Rhabdosargus sarba	Tropical stumpnose	IIb	1	1	
Syngnathidae	*Syngnathus acus*	Greater pipefish	Ib		1	
	Syngnathus temmincki	Longsnout pipefish	Ia			1
	Syngnathus watermeyeri	Estuarine pipefish	Ia	1		1
Teraponidae	*Terapon jarbua*	Thornfish	IIa		1	

	East Kleinemonde												West Kleinemonde	
Species	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2008	2009
Atherina breviceps	75.75	74.92	94.83	41.21	295.80	149.09	217.23	90.44	229.79	22.35	656.82	191.43	146.16	220.67
Gilchristella aestuaria	105.00	587.62	790.25	293.93	642.30	720.27	293.31	1547.31	753.36	105.53	99.18	192.36	178.05	1359.75
Glossogobius callidus	30.13	7.46	13.67	6.43	36.80	214.64	51.08	1.88	60.43	4.12	11.88	25.29	8.21	40.42
Glossogobius giuris								0.06						
Heteromycteris capensis	0.88			0.71			0.62	0.56		0.24	0.41		0.26	0.58
Hyporhamphus capensis				0.07		0.27	0.62		0.07		0.29	0.50		
Oreochromis mossambicus	0.25		8.08	0.79	9	24.91	2.46	0.13	0.14				26.37	2.17
Psammogobius knysnaensis		0.31			0.10	0.91	1	0.19	1.21	0.12	0.06		0.11	
Solea bleekeri	0.88		0.08			1.82	2.46	0.38	1.43	0.29	0.41	1.29	0.05	0.83
Syngnathus acus									0.07					
Syngnathus watermeyeri	1	0.92	0.42	0.07		0.09								
Synganthus temmincki														0.08
Total CPUE	213.88	671.23	907.33	343.21	984	1112	568.77	1640.94	1046.50	132.65	769.06	410.86	359.21	1624.50
Total no. of hauls	8	13	12	14	10	11	13	16	14	17	17	14	19	12

Table 3.2 Mean annual CPUE (number of fish/haul) for individual species from the small mesh seine net in the East Kleinemonde Estuary between 1998 and 2009, and West Kleinemonde estuary for the years 2008 and 2009. Blank spaces indicate a CPUE of zero

	East Kleinemonde												West Kleinemonde	
Species	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2008	2009
Argyrosomus japonicus							0.06	0.05	0.05					
Caranx sexfasciatus								0.05						
Diplodus cervinus hottentotus														0.20
Diplodus sargus capensis							0.06						0.06	0.16
Elops machnata													0.06	0.04
Heteromycteris capensis		0.06	0.13	0.26	0.05			0.05				0.12	0.12	0.04
Hyporhamphus capensis			0.19											
Lichia amia								0.25	0.05	0.08				
Lithognathus lithognathus	2.75	2	1	0.37	0.26	1.80	2.11	3.10	4.84	1.58	3.25	2	0.12	0.32
Liza dumerili	13.25	0.47	0.94	1.84	1.68	1.60	6.56	3.85	7.47	7	21.13	7.65	1.41	2.40
Liza macrolepis					0.05	0.07		0.05	0.05					
Liza richardsonii	24.38	3.18		1.05	1.84	5.80	7	3.25	0.21	0.83	11	7.12	1.53	1.04
Liza tricuspidens				0.37	0.68	0.80	0.78	0.95				0.12	0.06	0.12
Monodactylus falciformis	9.63	13.24	7.25	0.79	0.58	2.20	4.06	2.70	0.16	0.54	3.75	4.47	0.71	2.36
Mugil cephalus		0.12	0.19	0.05	6.89	1.13	0.94	0.15	0.16	3.71	0.31	0.12	3.82	0.16
Myxus capensis	0.25	8.94	34.81	2.68	25.21	19.53	82.56	18.80	17.79	18.29	37.81	33.24	16.47	13.36
Oreochromis mossambicus	1.38	2.76	0.13	0.16	0.47	8.40		3.35	0.32	0.04	0.31	0.29	0.29	0.88
Pomadasys commersonnii	0.38	0.12		0.32	0.21	0.33	0.33	0.10	0.16	0.17				0.12
Pomadasys olivaceum								0.10						
Pomatomus saltatrix							0.06							
Pseudorhombus arsius						0.07								
Rhabdosargus globiceps									0.05		0.13		0.12	
Rhabdosargus holubi	135.63	88.65	40.19	21.32	22.79	264.40	54.11	30.20	48.11	12.67	62.06	106.47	17.94	23.08
Rhabdosargus sarba								0.05				0.06		
Solea bleekeri		0.06		0.05	0.05	0.33	0.56	0.25	0.05	0.08				
Syngnathus temmincki														0.16
Syngnathus watermeyeri	2.25													0.08
Terapon jarbua								0.05						
Valamugil cunnesius		0.06			0.05		0.17	0.05	0.16		0.06	0.06		
Valamugil robustus					0.05		1.22							
Total CPUE	189.88	119.65	84.81	29.26	60.89	306.47	160.56	67.40	79.63	45	139.81	161.71	42.71	44.52
Total no. of hauls	8	17	16	19	19	15	18	20	19	24	16	17	17	25

Table 3.3 Mean annual CPUE (number of fish/haul) for individual species from the large mesh seine net in the East Kleinemonde Estuary between 1998 and 2009, and West Kleinemonde Estuary for the years 2008 and 2009. Blank spaces indicate a CPUE of zero

3.2.1 Change within the East Kleinemonde Estuary following the loss of submerged macrophytes

As well as a change in catch composition, the relative contributions of individual species to total gear CPUE are different between the macrophyte-dominated (1998 – 2003) and macrophyte-senescent (2004 – 2009) periods in the East Kleinemonde Estuary (Figure 3.5), and reflect changes in individual species' CPUE (Tables 3.2 and 3.3). In the small mesh seine net catches, the three dominant species during both periods in the East Kleinemonde Estuary were *G. aestuaria*, *A. breviceps* and *G. callidus* (Figure 3.5). Although *G. aestuaria* dominated the small mesh seine net catches in both sets of years (75.6% and 65.3% of total CPUE respectively), the relative contribution to total catch by *A. breviceps* increased from 16.4% in 1998 – 2003 to 31.3% in 2004 – 2009. Similarly, the percentage contribution by *S. bleekeri* increased from 0.06% to 0.1% over the two periods, and that of *O. mossambicus* decreased from 1% in 1998 – 2003 to 0.05% in 2004 – 2009.

In the East Kleinemonde Estuary, the large mesh seine net catches were dominated by *R. holubi* during the 1998 – 2003 period (71.6% of total catch), followed by *M. capensis* (13.7% of total catch) (Figure 3.8). The 2004 – 2009 period witnessed an increased contribution by this mullet species (32.2%) to the detriment of that of *R. holubi* (47.4%). Similarly, the percentage contribution by other Mugilid species also increased in the latter period, these being *L. richardsonii* (3.5% to 4.3%) and *L. dumerili* (2% to 8.2%). These changes in annual CPUE however did not differ significantly between the two large groupings of years (Wilcoxon matched pairs test, $P < 0.05$).

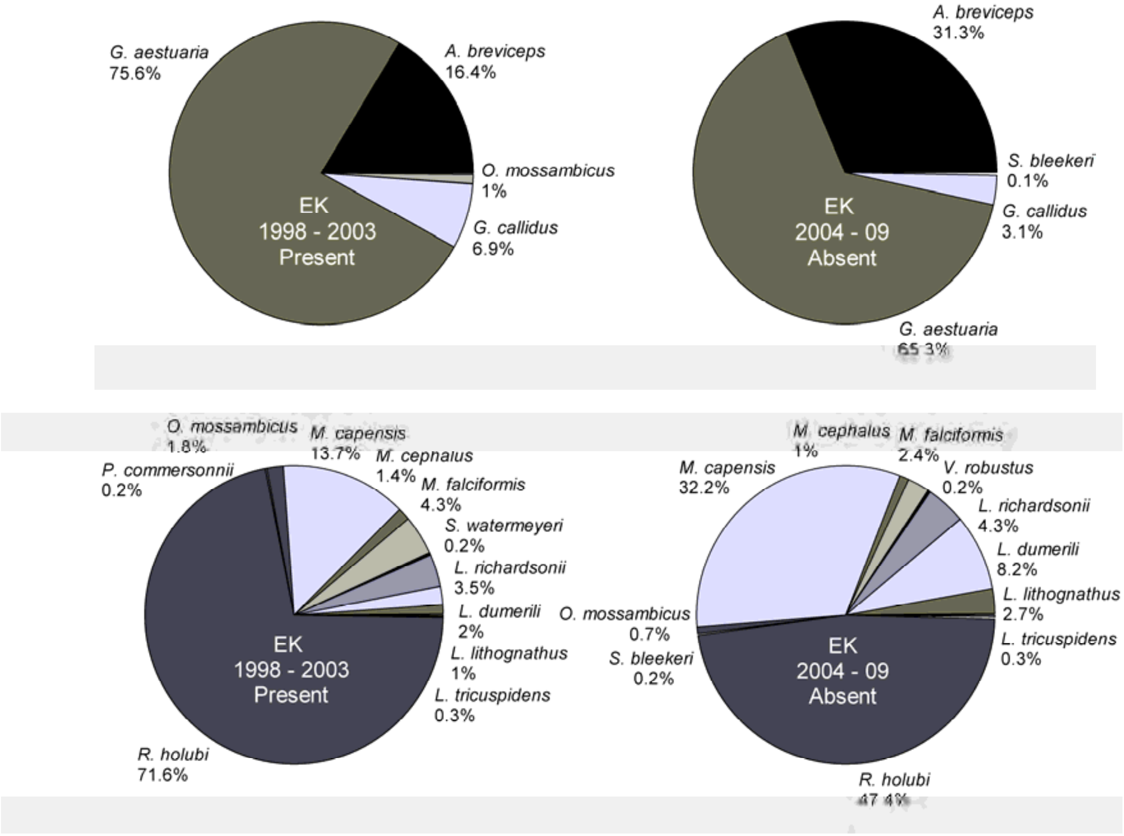

Figure 3.5 Percentage contribution of individual species to total small mesh (top), and large mesh (bottom) seine net CPUE in the East Kleinemonde Estuary over the different periods of macrophyte abundance. ‘Present’ and ‘absent’ refer to the status of beds of submerged macrophytes in the estuary

Ordination and classification tools in PRIMER may be used to test the significance of different groups determined *a priori*, or as a tool to identify patterns within data from which hypotheses may be formulated (Clarke & Warwick, 1994). In the first case, annual CPUE data from the East Kleinemonde Estuary were grouped by the presence (1998 – 2003) or absence (2004 – 2009) of submerged macrophyte beds (Figure 3.6). Although ANOSIM suggested a significant separation between these groups (Global R = 0.235, $P < 0.05$), ordination of this data did not reveal a very clear distinction between groups.

Indeed in some cases the distance between years with and without macrophyte beds (e.g. 1999 and 2009) was less than that between years within the same group (e.g. 1998 and 1999). As identified by SIMPER, four species made the most significant, and almost equal, contributions to dissimilarity between groupings. These were *M. capensis* (10.98%), *R. holubi* (10.73%), *L. richardsonii* (10.24%) and *L. dumerili* (9.18%). Of the 15 species included in the analysis, five contributed less than 5% respectively to the dissimilarity, these being *L. amia*, *L. lithognathus*, *A. japonicus*, *R. globiceps* and *R. sarba*.

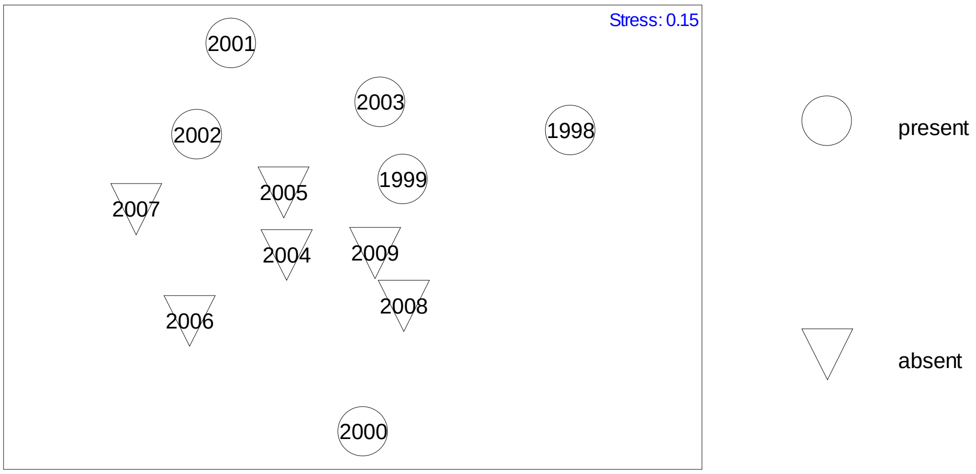

Figure 3.6 Ordination plot of annual large mesh seine net data from the East Kleinemonde Estuary for the period 1998 – 2009. Symbols represent macrophyte-dominated (circles) or macrophyte-senescent (triangles) years

Adopting the second approach, that is, allowing PRIMER to determine significant groupings within the data resulted in classification identifying the presence of two larger groupings of years (C and D) at approximately the 74% level of similarity, with 1998 (A) and 2000 (B) as outlying groups (Figure 3.7). ANOSIM reveals these groupings are significant (Global R = 0.729, P = 0.01). The species that most commonly made the largest contributions to between-group dissimilarity were the mugilid species *L.*

richardsonii, *L. tricuspidens*, *M. cephalus* and *M. capensis*, and the sparid *R. holubi*. In almost all cases these species contributed at least 60% of the dissimilarity between groupings. Other species making important contributions to the dissimilarity included *O. mossambicus, M. falciformis* and *P. commersonnii.*

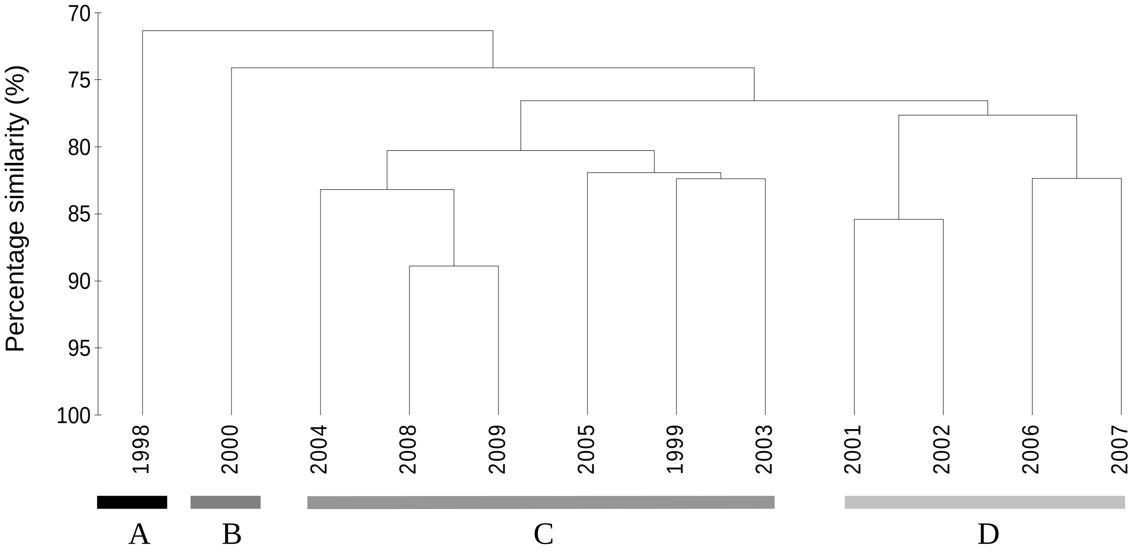

Figure 3.7 Classification of annual large mesh seine net CPUE data from the East Kleinemonde Estuary for the period 1998 – 2009. Separate groupings at the 74% level of similarity are identified by bars of different shades and the letters A, B, C and D

BIO-ENV was used to match the significant groupings identified in the dendrogram to the available environmental data. The best grouping of years in a manner consistent with that of the fish community groupings was presented by a combination of the presence/absence of macrophyte beds together with a spring open mouth event (ρ_w = 0.327). In fact, the closest matches involved combinations of mouth status (percentage of the year closed, open or overwash, and the presence/absence of spring open mouth events) or macrophyte presence/absence variables. The best correlation involving salinity was at ρ_w = 0.234, in combination with percentage of the year the mouth was open, and the presence/absence of both macrophyte beds as well as a spring mouth opening. The best match for annual rainfall was again in combination with these three variables together with the percentage of time the mouth was closed at $\rho_w = 0.108$.

The most obvious feature of the large mesh seine net catch composition is that the catch was dominated by the sparid *R. holubi* and mugilid *M. capensis* in all years and in both estuaries (Table 3.3). With the exceptions of 2000 and 2002, *R. holubi* alone accounted for almost 80% of the total annual catch within the macrophyte-dominated years, with the upper percentage contribution by this species during the macrophyte-senescent period decreasing to roughly 60% of total catch (Figure 3.8). In terms of actual numbers of individuals caught each year, *M. capensis* shows a peak in abundance in 2004 (CPUE = 83), although this peak is far smaller than the maximum abundance of *R. holubi* recorded in 2003 (CPUE = 264).

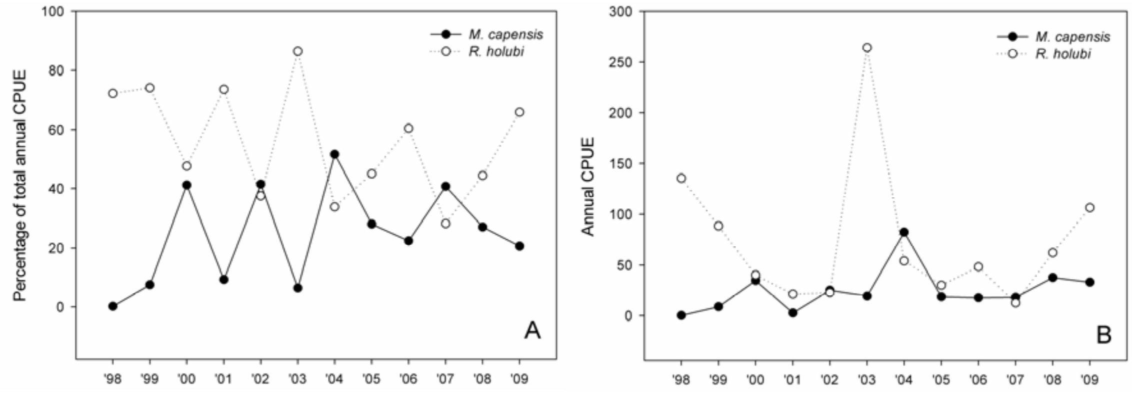

Figure 3.8 Annual CPUE for *M. capensis* and *R. holubi* from the East Kleinemonde Estuary (1998 - 2009), expressed as a percentage of total annual large mesh seine net CPUE (A) and as the total number of individuals caught (B)

An evaluation of the large mesh seine net fish community of the East Kleinemonde Estuary in terms of functional trophic guilds and habitat utilization groups are given in

Figure 3.9. It is evident that the patterns of abundance (total seine net CPUE and annual CPUE for each group) are dictated by the patterns of abundance of *R. holubi* (an omnivore and submerged vegetation-associated species) and *M. capensis* (a detritivore and bare sediment-associated species).

The detritivore and omnivore trophic guilds were numerically dominant, making up the largest proportion of the catch each year (Figure 3.9a). The piscivores (*A. japonicus* and *L. amia*), zooplanktivore (*M. falciformis*) and carnivores (*L. lithognathus* and *P. commersonnii*) were all present in low numbers. The only change in total CPUE between macrophyte-dominated and macrophyte-senescent phases that was significantly different was in the case of the detritivores (Wilcoxon matched pairs test, $P < 0.05$).

In terms of habitat utilization, those species associated with bare sediment substrata appeared to be present in a greater abundance than the vegetation-associated species in the macrophyte-senescent years (Figure 3.9b). In 2004 and 2007 this was especially marked, with the relative percentage contributions by vegetation-associated and sediment-associated species being 36.4% and 63.6% in 2004, and 29.4% and 70.6% in 2007 respectively. Statistically, only the change in CPUE of the sediment-associated species between the periods 1998 – 2003 and 2004 – 2009 was significant (Wilcoxon matched pairs test, $P < 0.05$).

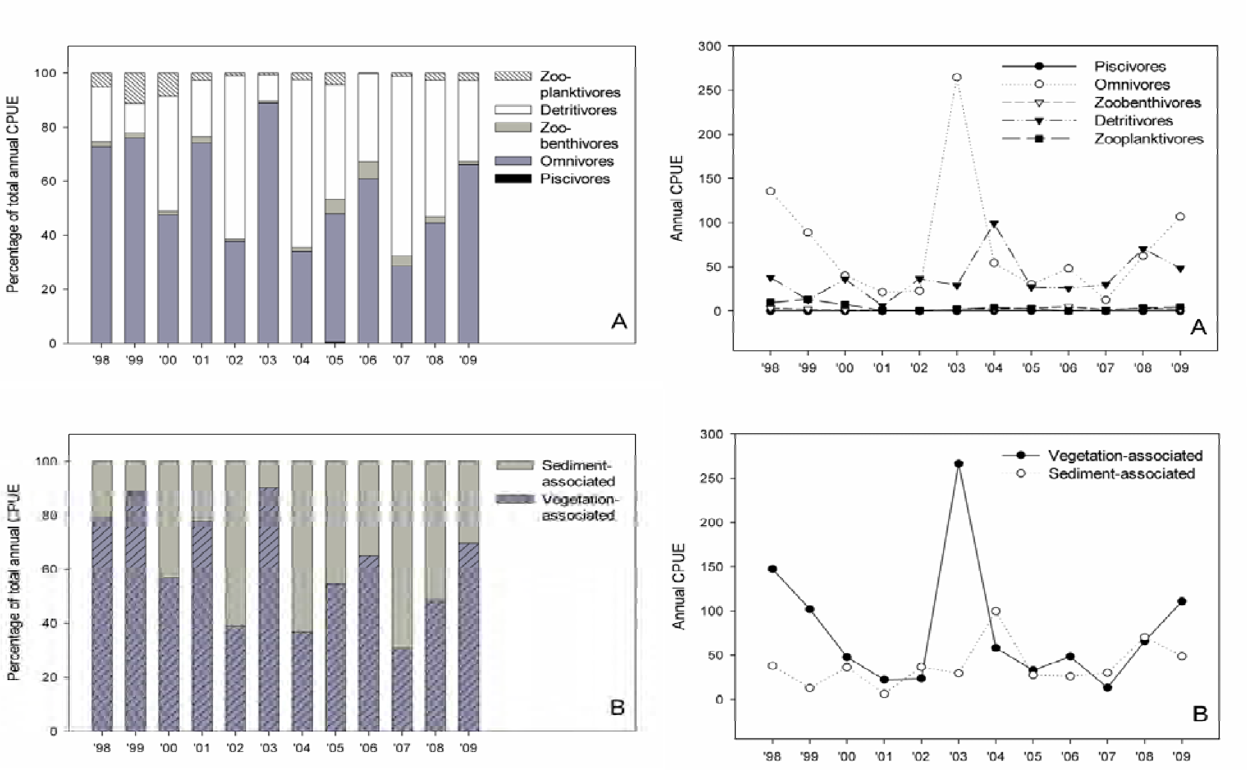

Figure 3.9 Annual large mesh seine net CPUE from the East Kleinemonde Estuary for A) respective trophic guilds and B) the vegetation-associated and sediment-associated species as a percentage of total annual CPUE and of individuals caught

3.2.2 Differences in fish assemblages between the East and West Kleinemonde estuaries

The relative abundance of species within the total small mesh seine net catches from the West Kleinemonde Estuary closely resembled that from the macrophyte-dominated period in the East Kleinemonde Estuary (1998 – 2003), but was dramatically different to the macrophyte-senescent period (2008 – 2009) (Figure 3.5). While *G. aestuaria* was the numerically dominant component within the West Kleinemonde Estuary (74.9% of total catch) *A. breviceps* dominated catches in the East Kleinemonde Estuary (73.5% of the total) in the 2008 – 2009 period. The relative abundance of *R. holubi* and *M. capensis* (the dominant large mesh seine net species) in 2008 and 2009 was different in the two estuaries. *R. holubi* contributed towards a higher percentage of the total catch in the East Kleinemonde Estuary (56.2%) than the West Kleinemonde Estuary (48%) (Figure 3.10). The opposite was true for *M. capensis* (33.4% compared with 23.5% of total catch in the West and East Kleinemonde estuaries respectively). The mullets *L. dumerili* and *L. richardsonii* were important contributors to the 2008 - 2009 total gear CPUE in the East Kleinemonde, collectively accounting for over 15% of total catch.

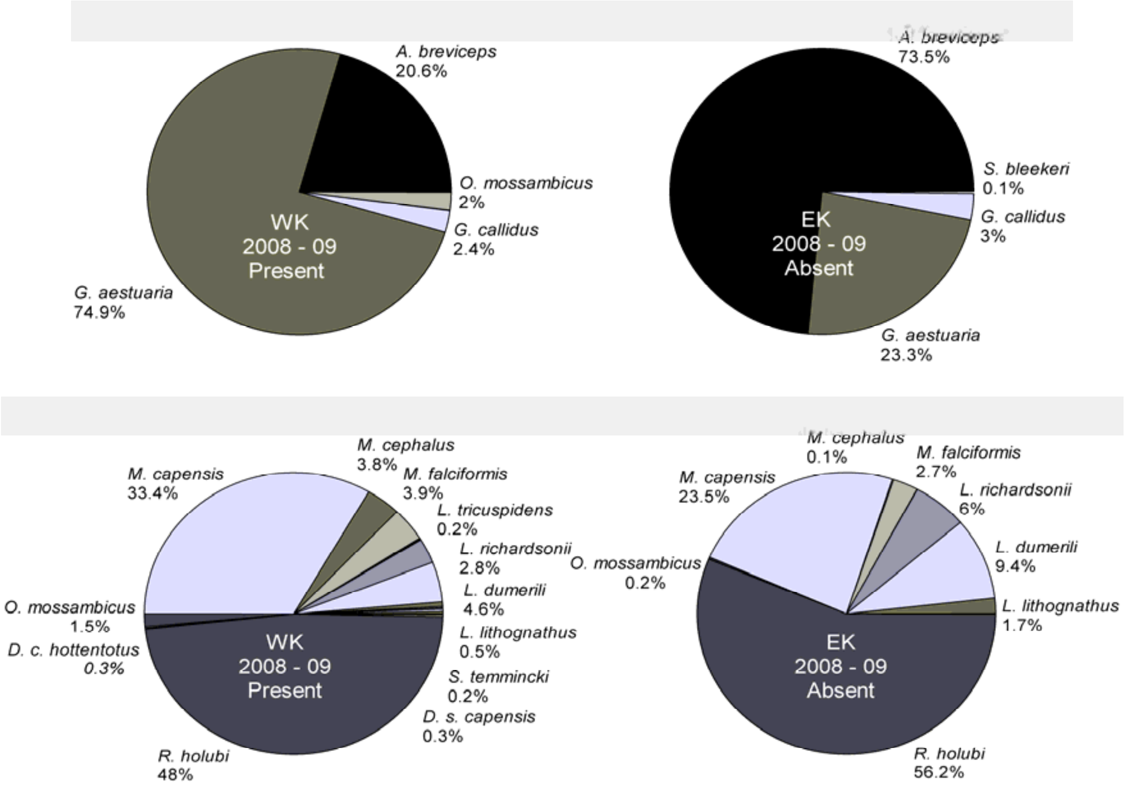

Figure 3.10 Percentage contribution of individual species to the total small mesh (top) and large mesh (bottom) seine net CPUE in the East Kleinemonde and West Kleinemonde estuaries over the different phases of macrophyte abundance. ‘Present’ and ‘absent’ refer to the status of beds of submerged macrophytes in each estuary

Classification and ordination of large mesh seine net data from the East and West Kleinemonde estuaries over the same time period revealed a very clear separation, with CPUE grouping by estuary (Figure 3.11). Superimposing macrophyte presence/absence upon this grouping yielded significant results (ANOSIM, global R = 1, $P < 0.05$). SIMPER analysis revealed that the CPUE of 16 species contributed 90% to the between-group dissimilarity. Once again, these species included *R. holubi* (10.7%), *L. dumerili* (8.9%), *L. richardsonii* (8.0%), *V. cunnesius* (6.0%) and *M. capensis* (5.7%). The higher abundance of *L. lithognathus* in catches from the East than from the West Kleinemonde in 2008 and 2009 led to it contributing 7.2% towards the total dissimilarity, and the absence of both *D. sargus capensis* and *H. capensis* from the East Kleinemonde Estuary meant that they contributed 6.8% and 6.3% of the dissimilarity respectively.

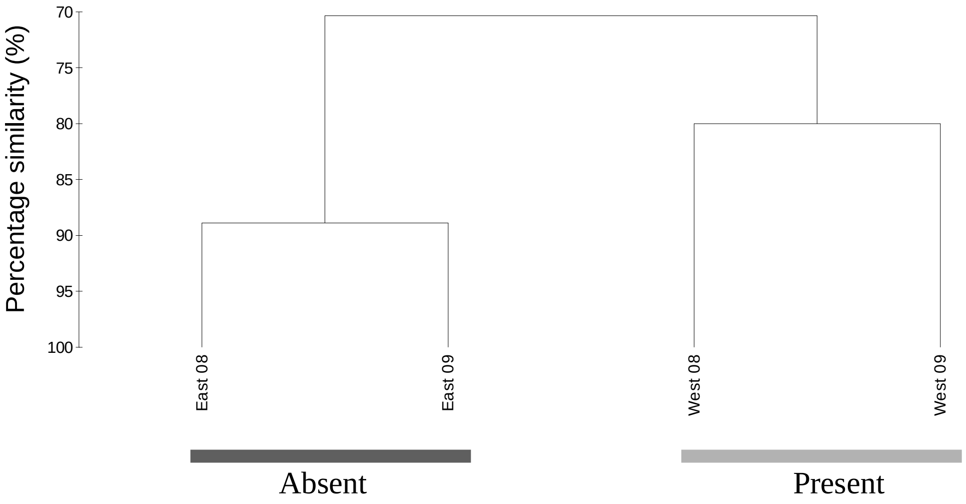

Figure 3.11 Classification of annual large mesh seine net CPUE data from the East Kleinemonde and West Kleinemonde estuaries during 2008 and 2009. ‘Present’ and ‘absent’ refer to the status of beds of submerged macrophytes in each estuary

As BIO-ENV correlates physical variables to the groupings of biological data (Clarke & Warwick, 1994), any variable that is categorical in nature and different between the two systems will result in a perfect correlation. The variables used in this study included the presence/absence of submerged macrophyte beds, estuary surface area and estuary catchment size. Indeed, results from BIO-ENV with these variables singly or in combination yielded a ρ_w of 1. The categorical nature of these variables suggests that such a result is of little value as it does not give any real indication of the potential importance of such variables in structuring fish assemblage.

When the categorical variables were excluded, the strongest correlations between groupings in physical and biological data were found using variations of estuary mouth status, these being (i) the percentage of days in the year that the estuary mouth was open, (ii) the occurrence (or not) of a spring opening event and (iii) the percentage of days that the estuary mouth was open during spring ($\rho_w = 0.265$).

When comparing functional groups between the two estuaries, only the piscivores showed an elevated CPUE in the West Kleinemonde Estuary (0.05 fish/haul) and were in fact absent from the East Kleinemonde catch during this period. The rest of the trophic guilds showed a lower abundance in the West Kleinemonde than the East Kleinemonde, these CPUEs (in fish/haul) being 21 and 85 for the omnivores, 0.4 and 2.7 for the carnivores, 20 and 59 for the detritivores and 1.7 and 4.1 for the zooplanktivorous fishes, respectively. Similarly, the total abundance of vegetation-associated species, as well as that of sediment-associated species were both higher in the East Kleinemonde than in the West Kleinemonde; CPUEs of 89 and 23 for the vegetation-associated species, and 59 and 20 for those sediment-associated species respectively.

4. DISCUSSION

Annual seine net catches from both the East and West Kleinemonde estuaries were characterized by the consistent presence of relatively few species (nine in total) and the numerical dominance of a subset of these taxa, namely *M. capensis* and *R. holubi* in the large mesh seine net and *A. breviceps* and *G. aestuaria* in the small mesh seine net. These findings were consistent with those of other studies conducted on TOCEs within the warm-temperate biogeographic region of South Africa (*inter alia* Cowley & Whitfield, 2001b; Vorwerk *et al.*, 2001; James *et al.*, 2007).

Changes within the fish assemblage of the East Kleinemonde Estuary were observed between the macrophyte-dominated and macrophyte-senescent sets of years (Figure 3.5). The dominant contribution by vegetation-associated species, in particular *R. holubi* and *M. falciformis* during the 1998 to 2003 macrophyte-dominated period, was replaced by sediment-associated species (especially Mugilidae) during the years 2004 to 2009 (macrophyte-senescent phase). In addition, the critically endangered *S. watermeyeri* was not recorded in catches from the 2004 to 2009 macrophyte-senescent period.

These findings are similar to those from other South African estuarine systems. An increased abundance of *R. holubi* and *M. falciformis* was documented for submerged *Zostera* beds in the Kromme Estuary when compared to adjacent bare sediment areas (Hanekom & Baird, 1984). The temporary disappearance of macrophyte beds in the permanently open Swartkops Estuary resulted in a loss of certain fish species (e.g. blacktail *Diplodus sargus capensis*), as well as a change in the composition of the estuarine fish assemblage (Beckley, 1983). Similarly, a temporary loss of submerged aquatic macrophytes from the littoral zone of the Swartvlei Estuary (an estuarine lake system) led to a change in numerical dominance from the vegetation-associated species (*R. holubi* and *M. falciformis*) to that of species associated with bare sediment environments (members of the family Mugilidae), as well as decreased condition of the former species (Whitfield, 1984, 1986).

Evidence from the Wilson Inlet, south-western Australia, revealed that both species richness and diversity were lower in dense beds of *Ruppia megacarpa* than over adjacent bare sand, although the opposite pattern was found for fish density and biomass, this probably linked to the shelter from predation offered by this plant to estuarine fishes (Humphries *et al.*, 1992). The presence of submerged macrophytes was highlighted as being one of a series of factors (including estuary mouth status and salinity) controlling fish composition within a range of estuaries of varying degrees of connectivity with the ocean along the south coast of Western Australia (Chuwen *et al*., 2009; Hoeksema *et al*., 2009).

Classification and ordination significantly separated the 2008 – 2009 fish catch data between the East and West Kleinemonde estuaries (Figure 3.11) but these differences in fish assemblages did not necessarily reflect differences in the availability of aquatic macrophyte habitat. Although the sediment-associated species did show much higher CPUE in the East Kleinemonde than the West Kleinemonde during 2008 - 2009, these were lower than the total CPUE of vegetation-associated species within the East Kleinemonde Estuary in this same period. In addition, the East Kleinemonde had a higher CPUE of vegetation-associated species overall but this could be related to poor catches in the West Kleinemonde middle reaches where steep sided banks and dense macrophyte beds reduced seine net efficiency. Both species of pipefish *S. temmincki* and *S. watermeyeri* were recorded in the West Kleinemonde seine net catches but not in those of the East Kleinemonde, thus indicating the importance of submerged macrophyte habitat to these species.

In the case of the catch data groupings within the East Kleinemonde Estuary, the presence/absence of submerged macrophytes together with the occurrence of a spring mouth opening provided the best agreement ($\rho_w = 0.327$). Estuary mouth state, more specifically the period of mouth opening events and occurrence of an opening event during the months of spring best correlated with the biological groupings in the comparison between East and West Kleinemonde estuaries ($\rho_w = 0.265$). The grouping of years within the East Kleinemonde Estuary by macrophyte-dominated/senescent periods, although significant, was not very strong and possibly indicated that other factors were involved. The very distinctive separation of catch data between the East and West Kleinemonde estuaries indicated that the fish assemblages were quite different. Whether this difference is due to the presence or absence of submerged macrophytes, or differences of other physical factors remains unclear, especially as patterns in the species composition and relative abundances of the catch data between estuaries did not appear to follow the differences expected from habitat variation alone (Figure 3.10).

It is well known that estuary mouth state is an important factor structuring fish assemblages within TOCEs (e.g. Wallace & van der Elst, 1975; Beckley, 1984; Whitfield, 1996; Vorwerk *et al*., 2003) and it is likely that this factor affected patterns in catch data within both estuaries during the sampling period. In an effort therefore to assess the influence on fish assemblages of the availability of macrophyte beds alone, catch data were compared between years in which estuary mouth condition was similar. Within the dataset available, mouth conditions were comparable in the years 1998 (macrophyte-dominated) and 2008 (macrophyte-senescent) in the East Kleinemonde Estuary, and 2008 (macrophyte-dominated) in the West Kleinemonde Estuary. These years were all linked to predominantly closed phases of the estuaries, with limited overwash events and no mouth opening events (Figure 3.3).

When catch data were compared between these years, certain patterns in the large mesh seine net catch data were apparent (Table 3.3). In the East Kleinemonde Estuary, respective CPUEs of the sediment-associated species *L. dumerili*, *L. richardsonii*, *M. capensis* and *H. capensis* were all much higher in the macrophyte-senescent year (2009) than in the macrophyte-dominated 1999. In addition, the catch of the vegetation-associated *M. falciformis* decreased in the East Kleinemonde Estuary following a loss of submerged macrophytes (East Kleinemonde 1999 to 2009), but the CPUE of this species was higher in the East Kleinemonde in 2009 than in the West Kleinemonde in the same year. As discussed previously, the catchability of this species may have been impaired by the thick macrophyte beds and the steep banks within the latter estuary.

The CPUE of the vegetation-associated *R. holubi* was higher in the East Kleinemonde during 2009 (macrophyte-senescent) than in any year when macrophyte beds were present in this system. Furthermore, although the abundance of *R. holubi* decreased in the 2004 – 2009 macrophyte-senescent period in the East Kleinemonde Estuary, it remained a dominant component of total catch (Figure 3.5). This pattern is unlikely to be linked to variability in recruitment success, as this species, a serial spawner that recruits successfully during mouth opening events and overwash conditions (Cowley & Whitfield, 2001b; Cowley *et al*., 2001; Kemp & Froneman, 2004) is not restricted by seasonal patterns in recruitment opportunities nor by low numbers of recruits available in the surf zone or by the type of estuarine access opportunity. Overwash events occured in both estuaries in all years except 1999 in the East Kleinemonde (Figure 3.3), and have been identified as the most important form of recruitment opportunity for the East Kleinemonde Estuary (Bell *et al*., 2001). Even a short overwash event can introduce large numbers of juvenile *R. holubi* into an estuary. Preliminary estimates by Kemp and Froneman (2004) for the West Kleinemonde Estuary suggest an hour-long period of overwash can introduce between 8 000 and 33 500 *R. holubi* larvae and postlarvae into a system.

Fishes have a well-developed sense of smell and it is therefore likely that olfactory cues from estuarine or freshwater sources can be detected by estuarine-dependent species such as *R. holubi* (James *et al*., 2005). The importance of such cues from estuarine water seeping through the sand bar of a TOCE in assisting these species to locate an estuarine habitat was first hypothesized by Whitfield (1998). The recorded accumulation of ichthyoplankton in the surf zone adjacent to estuary mouths (Harris *et al*., 2001), of which estuary-dependent species were the dominant component (Whitfield, 1989; Cowley *et al*., 2001), appears to further support the idea of the presence of a cue by which they orientate towards the estuary.

Confirmation of the role of olfaction for the larvae of selected marine fish species to locate estuaries was recently provided by choice chamber experiments conducted by James *et al*. (2005). Under experimental conditions, postflexion *R. holubi* selected estuarine and riverine water at a significantly higher frequency than that of seawater, thus indicating the ability of this species to respond to the presence of olfactory cues. Since submerged macrophyte beds are both important refuge and feeding areas for this species (Cowley & Whitfield, 2001b), it is likely (although untested) that they are sensitive to olfactory cues indicating the presence of these plants. The mouths of the East and West

Kleinemonde estuaries open to the sea in close proximity and at times in the past they have been joined. The presence of olfactory cues originating from the macrophyte-dominated West Kleinemonde Estuary in the nearshore marine environment adjacent to the East Kleinemonde Estuary mouth could be responsible for the continued dominance of *R. holubi* in the latter estuary following the loss of this habitat.

Although habitat change is implied as the causal factor when differences in vegetation-associated or sediment-associated species are recorded, relative CPUE for a range of species may in fact be correlated to the recent history of estuary mouth conditions, a factor that has been shown to be very important in structuring estuarine fish communities (Wallace & van der Elst, 1975; Beckley, 1984; Strydom *et al*., 2003) as well as influencing the abundance of individual species (Cowley & Whitfield, 2001a). Closed mouth conditions as a limitation to recruitment have been identified as a major reason for the reduced species richness within TOCEs as compared to permanently open systems where recruitment is not restricted to mouth opening or overwash events (Vorwerk *et al*., 2003).

Highest species richness in the East Kleinemonde large mesh seine net catches was found in the years 2004 to 2006 when a total of 15, 20 and 15 species were recorded in the catches, respectively (Table 3.3). Mouth condition data revealed that the years of 2003, 2005 and 2006 showed the lowest total percentage of closed conditions and a roughly equal occurrence of both overwash and mouth opening events (Figure 3.3). Mouth condition data for these years would affect large mesh seine catch data for 2004, 2006 and 2007. The high number of species encountered in the catch in 2005 therefore seems to be an anomaly, as during 2004 the estuary mouth was largely closed (88.8% of the year) and recruitment opportunities would therefore have been reduced.

The highest catch for the freshwater mullet *M. capensis,* which was recorded in 2004 (Table 3.3), may also be related to mouth condition. Although the East Kleinemonde mouth was open for a higher percentage of time during the years 2005 and 2006 than 2003, this was the year of the major flood event. *M. capensis* is a facultative catadromous species, and within TOCEs is commonly associated with the upper reaches where riverine influence is strongest (Whitfield & Bok, 1998). The presence of large volumes of riverine water in the coastal zone following the flood possibly provided the stimulus for exceptional recruitment during the open mouth phase with strong river flow.

Estuarine mouth conditions influence the physical environment within an estuary, which not only affect fish recruitment but also the growth and success of submerged macrophytes (Riddin & Adams, 2008a). It is important to note, therefore, that changes in the patterns of fish assemblages within the East Kleinemonde Estuary between 1998 - 2003 and 2004 – 2009 may be correlated but coincidental to changes in the abundance of submerged macrophyte habitat, with both biotic groups responding to the same variations in the physical environment, as illustrated in the following example.

Following a mouth opening, a drop in water level exposes submerged macrophytes which desiccate and die, while a closed mouth and stable water level conditions for a period of at least two months facilitate macrophyte growth (Riddin & Adams, 2008a). Similarly, such conditions during late spring or early summer are ideal for *O. mossambicus* to breed (Whitfield & Blaber, 1979; Ellender *et al.*, 2008). A mouth opening event during the spring/summer peak breeding season has been shown to be detrimental to the success of this species in the East Kleinemonde Estuary, leading to a subsequently reduced abundance of juveniles (Ellender, 2006). The higher abundance of *O. mossambicus* in catches from macrophyte-dominated years than in years when these plants were absent was also evident in both the small and large mesh seine net data, and in both intra- and inter-estuary comparisons. Both sets of macrophyte-dominated groupings, however, also corresponded to years in which the estuary water level was fairly stable, thus indicating that certain plant and fish species were responding directly to this abiotic driver.

5. CONCLUSION

Although estuarine mouth condition is probably the most important factor that influences the structure of fish assemblages within TOCEs, the presence of submerged macrophytes, or at least the promise of this habitat via olfactory cues to recruiting fishes appears to be important to certain species, such as *R. holubi*. In the case of this and other vegetation-associated species, it is unclear whether the role of submerged macrophyte beds lies in the fact that they provide a refuge and/or foraging area (Cowley & Whitfield, 2001b). However, for certain species (e.g. the pipefish *S. watermeyeri*) submerged macrophyte habitat is critical; this is clearly highlighted by the sudden absence of this species from catch records within the East Kleinemonde Estuary in all years during which submerged macrophytes were absent from this estuary.

The lack of any clear evidence on the importance or role of submerged macrophytes in structuring fish assemblages when catch data from the East and West Kleinemonde estuaries, with their differences in cover of submerged macrophytes, were compared during this study can be attributed to one or both of the following: (i) the short data series available with which to compare the systems, or (ii) the differences in physical characteristics between estuaries making such a comparison unfeasible.

In Chapter Six stable isotopes will be used to further explore the potential importance of submerged macrophytes to fishes within TOCEs; this time examining their role as a source of carbon. In so doing, it is hoped that further understanding as to the exact role of submerged macrophytes such as *R. cirrhosa* and *P. pectinatus* within TOCEs will be gained.

CHAPTER FOUR: CARBON AND NITROGEN ISOTOPE SAMPLE PREPARATION METHODOLOGY

1. INTRODUCTION

The natural variability in the ratio of heavy to light isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) within the tissues of animals (consumers) or plants (producers) has allowed scientists to make use of stable isotope analysis as a tool to address a range of ecological, physiological and archaeological questions (Peterson & Fry, 1987; Post *et al.*, 2007). Stable isotope analysis is commonly used in feeding studies where carbon traces energy flow from producer to consumer, while nitrogen is a useful indicator of the trophic level of an organism. It is well established that the isotopic signature of the tissue of an organism can vary according to tissue type and the content of lipids and/or carbonates (Arrington *et al.*, 2006; Sweeting *et al.*, 2006; Post *et al.*, 2007). Failure to address these sources of variation introduces bias that confounds the interpretation of trophic relationships as well as prevents inter-study comparability and integration (Jacob *et al.*, 2005; Carabel *et al.*, 2006; Logan *et al.*, 2008).

Lipids are the most ^{13}C-depleted of all the biochemical fractions within an organism, having stable isotopic carbon ratio ($\delta^{13}C$) values more negative than carbohydrates or proteins (up to 6 – 8 ‰, DeNiro & Epstein, 1978), resulting from fractionation during their synthesis (DeNiro & Epstein, 1978; Tieszen *et al.*, 1983; Michener & Schell, 1994; Pinnegar & Polunin, 1999). Samples rich in lipids may thus appear depleted in ^{13}C. The degree to which the tissue $\delta^{13}C$ is altered depends on the lipid content of sample tissues analysed (Post *et al.*, 2007).

The two most common approaches for normalizing $\delta^{13}C$ values skewed by lipids are the chemical extraction of lipids prior to analysis (Bligh & Dyer, 1959), or post-analysis correction using a mathematical model (McConnaughey & McRoy, 1979). The advantage of the mathematical approach is that it saves time prior to analysis and avoids the potential effect that chemical treatment may have on $\delta^{15}N$ values, as lipids can be bound to amino acids which may be leached during the chemical extraction process (Sotiropoulos *et al*., 2004; Bodin *et al*., 2007). The applicability of developed models to a broad range of taxa is, however, questionable, and the accuracy of many models in correcting for lipid content is largely untested (Post *et al*., 2007).

The carbon found in inorganic carbonate ($CaCO_3$) is from a different source to that of carbon incorporated into the tissue and so has a different $\delta^{13}C$ (Fry, 1988). Carbonate is non-dietary and its presence will complicate trophic analysis. Consequently, it must either be physically removed (e.g. separation of shell material from body tissue) or chemically treated to produce carbonate-free samples. Physical removal is sometimes not possible in cases where the small size of individuals prevents the removal of carbonated structures or where carbonate particles are embedded in the organic tissue (Carabel *et al*., 2006). Acidification by the addition of hydrochloric acid (HCl) is the most commonly used technique to remove carbonate from samples. Similar to the chemical extraction of lipids, the effect that acidification has on the nitrogen isotopic signature of the tissue is uncertain. Acidified samples have been reported to have an elevated C:N ratio due to the proportionally higher decrease in nitrogen than carbon tissue content caused by this process (Jacob *et al*., 2005). According to these authors acidification decreased $\delta^{15}N$ in invertebrate and fish samples, although the significance of this decrease was lessened by the large variation in $\delta^{15}N$ that exists between trophic levels.

Despite the wide use of stable isotope analyses, there remains no consensus on the need to account for the presence of lipids within sample tissues, nor a single standardized protocol to address this problem (Murry *et al*., 2006; Post *et al*., 2007; Logan *et al*., 2008). Similarly, ongoing debate remains around when and how samples should be acidified, as well as the effects acidification has on isotope ratios (Jacob *et al*., 2005; Carabel *et al*., 2006). Based on the variability in results between samples that have addressed the presence of lipids or carbonates, some researchers have recommended processing samples in duplicate; a treated sample for an estimation of $\delta^{13}C$ and an untreated sample for $\delta^{15}N$ (Sotiropoulos *et al*., 2004; Logan *et al*., 2008). However, this is not always practical due to time constraints, where funding is limited or body size or available amount of the sample is small. Consequently, there is a need for a better understanding of (i) when it is necessary to account for these compounds and (ii) the effects removal or correction methods have on the interpretation of stable isotope data.

The aims of this chapter were to (i) determine the effect sample treatment (carbonate removal and lipid extraction) has on the stable isotope ratios of carbon and nitrogen in selected estuarine producers (plants) and consumers (fish), and to (ii) assess the possible bias caused by the presence of carbonates in plant samples and lipids in fish samples in the context of the broader study.

2. METHODS

2.1 Sample collection

While different plant and fish species were collected from the East and West Kleinemonde estuaries, all individuals of the same species were sourced from the same system (Table 4.1). Plant leaf and stem tissue was rinsed in distilled water and cleared of epifauna or epiphytes prior to analysis. Fish muscle tissue free of skin, bones or scales was collected from the epaxial musculature of a range of fish species. All samples were kept frozen at -20°C prior to analysis in the laboratory.

Table 4.1. List of the plant and fish species included in this study (EK = East Kleinemonde Estuary, WK = West Kleinemonde Estuary)

Species	Family	Group	n	Location
Fish species				
Argyrosomus japonicus	Sciaenidae	Piscivore	6	WK
Myxus capensis	Mugilidae	Detritivore	7	WK
Rhabdosargus holubi	Sparidae	Omnivore	10	WK
Monodactylus falciformis	Monodactylidae	Omnivore	7	WK
Lithognathus lithognathus	Sparidae	Zoobenthivore	6	EK
Plant species				
Phragmites australis	Poaceae	Emergent reed	3	EK
Ruppia cirrhosa	Ruppiaceae	Submerged macrophyte	3	EK
Potamogeton pectinatus	Potamogetonaceae	Submerged macrophyte	3	EK
Caulacanthus ustulatus	Caulacanthaceae	Submerged alga	3	EK
Codium tenue	Codiaceae	Submerged alga	2	EK
Epiphytes		Submerged alga	3	EK

2.2 Sample treatment

Prior to carbonate removal treatment for the plants and lipid extraction treatment for the fishes, all samples were defrosted, dried at a temperature not exceeding 60°C (Carabel *et al.*, 2006) and then homogenized using either a pestle and mortar or an electronic grinding mill. Duplicate samples were taken from each individual; one for the treatment and one for a control against which the effect of the treatment on sample isotopic ratios could be compared.

2.2.1 Removal of carbonates

Carbonates are known to be absent from soft tissues such as fish muscle (Jacob *et al.*, 2005; Carabel *et al.*, 2006), and so only plant samples were acid washed. Hydrochloric acid (10%) was added to each sample drop-by-drop until all CO_2 had bubbled off (Jacob *et al.*, 2005; Carabel *et al.*, 2006; Logan *et al.*, 2008), according to the equation:

$CaCO_3 + 2HCl \rightarrow CO_2{}^{\uparrow} + CaCl_2 + H_2O$ (Serrano *et al.*, 2008).

Although subsequent rinsing with distilled water is not advised (Jacob *et al*., 2005; Carabel *et al*., 2006), the acidity of unrinsed samples damages the mass spectrometer during analysis for stable isotope ratios (N. Richoux. pers. comm.). As a consequence, all samples were thoroughly rinsed to remove acid residue before being re-dried and homogenized as detailed above.

2.2.2 Chemical extraction of lipids

Homogenized fish samples were immersed overnight in a solution of 2:1:0.8 chloroform : methanol : water according to the method of Bligh and Dyer (1959). Thereafter the solution of solvent and extracted lipids was pipetted off. Samples were dried to remove the remaining solvent, and re-ground where necessary.

2.3 Stable isotope analysis

Ground samples were sent to the Archaeology Department of the University of Cape Town for analysis of $\delta^{15}N$ and $\delta^{13}C$. Samples were combusted in a Flash EA 1112 series elemental analyzer (Thermo Finnigan, Italy), and the gases passed to a Delta Plus XP IRMS (isotope ratio mass spectrometer) (Thermo Electron, Germany), via a Conflo III gas control unit (Thermo Finnigan, Italy).

Isotope ratios for both C and N were calculated according to the equation:

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

where δX is the difference in isotopic composition between the sample and the international standard, expressed in parts per thousand (‰), and R is the ratio of heavy to light isotope ($^{15}N/^{14}N$ and $^{13}C/^{12}C$) of the sample or standard respectively (Peterson & Fry, 1987). The international standard for nitrogen is atmospheric nitrogen, and for carbon is Pee-Dee Belemnite.

2.4 Mathematical model

As an alternative to chemical extraction of lipids, $\delta^{13}C_{lipid\text{-}free}$ values were calculated for fish muscle samples using $\delta^{13}C$ and C:N ratios for the untreated (i.e. control) fish samples according to the equations of Post *et al.* (2007) and Fry (2002). The equations were as follows:

1. Post *et al*. (2007) for aquatic organisms as adapted from McConnaughey and McRoy (1979):

$$\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times C{:}N$$

2. Fry (2002) for muscle samples from generalist, benthic-feeding fishes:

$$\delta_{lipid\text{-}free\ muscle} = \delta + 6 - (22.2/R)$$

where δ is the measured C isotopic value, R is the measured C:N and lipids are assumed to be 6 ‰ depleted in C isotopic composition versus untreated muscle (Fry, 2002).

2.5 Statistical analysis

Differences in mean changes in C and N isotopes ($\delta^{13}C_{treated}$ - $\delta^{13}C_{untreated}$, and $\delta^{15}N_{treated}$ - $\delta^{15}N_{untreated}$) were tested for both treatments. The assumption of normality of samples was tested using a Shapiro-Wilk's test, and then mean change in samples was analysed using a paired *t* test ($P < 0.05$), both run on the STATISTICA Software package (version 8.0). Apart from the *C. tenue* samples which were all collected during winter, plant samples represent a mix of both summer and winter sampling seasons because of the change in availability of plants throughout the year. While it is possible that this effect of season may influence the results predicted here, the small sample sizes (n=3) prevented statistical qualification of that effect.

Using $\delta^{13}C$ and C:N ratios for the untreated fish samples, $\delta^{13}C_{lipid\text{-}free}$ values were calculated using the equations of Post *et al.* (2007) and Fry (2002). These values were then plotted against a 1:1 ratio of $\delta^{13}C_{predicted}$: $\delta^{13}C_{observed}$ values to which the model results were correlated using a linear regression. Modelling efficiency (EF; Mayer & Butler, 1993), a measure of agreement between observed and simulated values was calculated for each model according to the equation:

$$EF = 1 - \frac{\Sigma(u_i - \hat{u}_i)^2}{\Sigma(u_i - \bar{u}_i)^2}$$

where u_i is the observed and $\hat{u}_i$ the predicted value. EF indicates 'goodness of fit' of a model, with a perfect fit having a value of 1. Negative values indicate an unsuitable model (Mayer & Butler, 1993).

3. RESULTS

3.1 Removal of carbonates

Isotope values for carbon and nitrogen, together with C:N ratio for all the plant treatment and control samples are shown in Table 4.2. Although the small sample size (n = 2) for *C. tenue* prevents meaningful statistical analysis, data are presented for it nonetheless. Treatment of plant samples with HCl did not significantly affect either $\delta^{13}C$ or $\delta^{15}N$ values (paired *t* test, $P < 0.05$) (Table 4.3). This finding was consistent for all the plant species tested, except for *C. ustulatus*, where the positive change in $\delta^{15}N$ was significant ($P = 0.006$). Changes in C:N ratio indicates, however, that nitrogen was lost from *P. australis* and the submerged algae (*C. ustulatus* and *C. tenue*) and carbon from the submerged macrophytes (*R. cirrhosa* and *P. pectinatus*) and the epiphytes (Table 4.2).

Considering the accepted change of 3.4 ‰ between consecutive trophic levels and 1 ‰ between consumers for $\delta^{15}N$ and $\delta^{13}C$ respectively (DeNiro & Epstein, 1978; DeNiro & Epstein, 1980; Peterson & Fry, 1987), acidification with HCl did not result in ecologically significant changes. The only exception was the observed change in $\delta^{13}C$ for epiphytes, where $\Delta\delta^{13}C$ was -3.387 (Table 4.3) with mean $\delta^{13}C_{treated}$ being -28.874 ± 1.671 and mean $\delta^{13}C_{untreated}$ -25.486 ± 3.126 (Table 4.2).

Table 4.2. Plant samples prepared in duplicate for analysis of the effects of acidification with HCl on stable isotope ratios. Samples that were treated with acid are indicated by ‘t’

Species	n		$\delta^{15}N$ Avg. (±S.D.)	$\delta^{13}C$ Avg. (±S.D.)	C:N Avg. (±S.D.)
P. australis	3	t	5.479 ±2.498	-24.181 ±0.616	26.970 ±2.037
	3		5.518 ±2.36	-24.217 ±0.468	15.798 ±0.803
R. cirrhosa	3	t	6.466 ±2.307	-16.457 ±0.843	23.132 ±1.795
	3		6.465 ±1.942	-16.682 ±1.194	26.432 ±2.061
P. pectinatus	3	t	5.799 ±3.636	-16.185 ±1.664	18.584 ±5.758
	3		7.882 ±1.574	-16.148 ±1.345	18.807 ±5.494
C. ustulatus	3	t	8.219 ±0.379	-22.438 ±0.200	35.084 ±5.858
	3		7.733 ±0.417	-22.413 ±0.078	34.598 ±2.714
C. tenue	2	t	8.473 ±1.489	-29.286 ±1.249	19.190 ±1.830
	2		8.430 ±1.368	-29.034 ±1.808	17.989 ±0.667
Epiphytes	3	t	8.999 ±0.571	-28.874 ±1.671	7.086 ±0.202
	3		9.100 ±1.152	-25.486 ±3.126	9.594 ±1.036

Table 4.3. Mean change in isotope ratios (‰) of carbon and nitrogen ($\Delta\delta = \delta_{treated} - \delta_{untreated}$) following acidification with HCl, with results of a paired *t* test (dependent samples, $P < 0.05$). An asterisk indicates significant differences

Species	n	$\Delta\delta^{15}N$ Avg. (±S.D.)	P value	$\Delta\delta^{13}C$ Avg. (±S.D.)	P value
P. australis	3	-0.039 ±0.324	0.854	0.036 ±0.372	0.883
R. cirrhosa	3	0.001 ±0.379	0.996	0.225 ±0.950	0.721
P. pectinatus	3	-2.082 ±3.944	0.457	-0.037 ±0.320	0.859
C. ustulatus	3	0.486 ±0.067	0.006*	-0.025 ±0.171	0.825
C. tenue	2	0.043 ±0.121	0.706	-0.253 ±0.559	0.638
Epiphytes	3	-0.102 ±1.416	0.912	-3.387 ±1.547	0.063

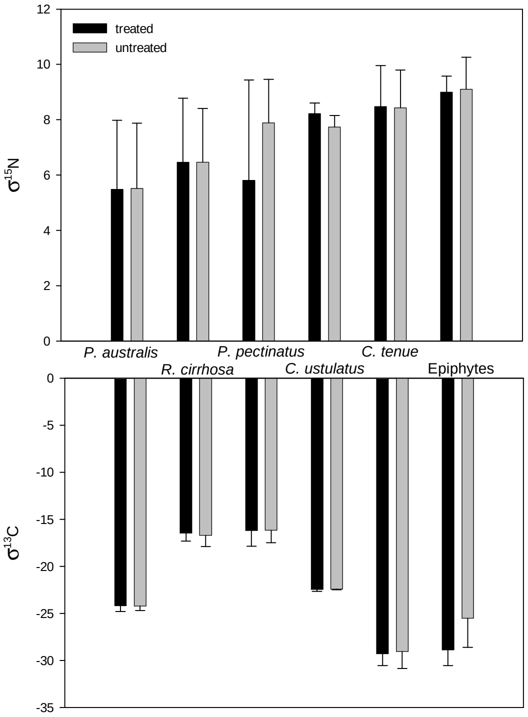

Figure 4.1. Mean $\delta^{13}C$ and $\delta^{15}N$ of samples before and after acidification with HCl. Error bars indicate standard deviation

3.2 Chemical extraction of lipids

Ecologically significant changes in isotope ratios following lipid removal with a chloroform/methanol solvent did not occur for either carbon or nitrogen for any of the fish species (Table 4.5, Figure 4.2). Lipid extraction resulted in a positive change in mean $\delta^{15}N$ for the all fish species except *M. capensis*, which showed a decrease in $\delta^{15}N$ (Table 4.4). Mean changes in $\delta^{13}C$ were positive for most fish species following lipid extraction, except for *M. capensis* and *L. lithognathus* which showed a decrease in $\delta^{13}C$ (Table 4.5). The changes were, however, only statistically significant for nitrogen in *R. holubi* and carbon in *M. falciformis* (paired *t* test, P = 0.016 and 0.037 respectively).

For all fish species analysed (n = 5), C:N ratios pre- and post-lipid removal changed marginally, with the average treatment effect being a slight decrease in the ratio, as is to be expected with the removal of carbon-rich lipids (Table 4.4). An exception to this pattern was *R. holubi* where 3 of the 10 flesh samples for this species showed an increased C:N ratio following extraction (average increase 0.238). Maximum sample C:N prior to lipid extraction was 3.794 (*M. falciformis*); however for 31 of the 34 total samples C:N was below 3.5 (range 3.024 to 3.422).

Table 4.4. Fish muscle samples analysed in duplicate for the effects of lipid removal on stable isotope ratios. Samples from which lipids were chemically extracted are indicated by ‘t’

Species	n		$\delta^{15}N$ Avg. (±S.D.)	$\delta^{13}C$ Avg. (±S.D.)	C:N Avg. (±S.D.)
A. japonicus	6	t	16.738±0.675	-24.320 ±1.095	3.128±0.103
	6		16.330±0.503	-24.434 ±0.977	3.180±0.113
M. capensis	7	t	15.424±0.792	-27.110 ±1.400	3.155±0.091
	7		15.489±0.584	-26.972 ±1.468	3.205±0.043
R. holubi	10	t	15.065±0.963	-23.460 ±1.359	3.327±0.159
	10		14.734±1.128	-23.758 ±1.296	3.332±0.146
M. falciformis	6	t	16.539±1.247	-21.942 ±1.434	3.227±0.110
	6		16.374±1.398	-22.255 ±1.402	3.459±0.189
L. lithognathus	5	t	16.064±0.885	-21.104 ±1.679	3.152±0.032
	5		16.043±0.780	-20.942 ±1.640	3.152±0.052

Table 4.5. Mean change in isotope ratios (‰) of carbon and nitrogen ($\Delta\delta = \delta_{treated} - \delta_{untreated}$) following chemical lipid extraction, with results of a paired *t* test (dependent samples, $P < 0.05$). An asterisk indicates significant differences

Species	n	$\Delta\delta^{15}N$ Avg. (±S.D.)	P value	$\Delta\delta^{13}C$ Avg. (±S.D.)	P value
A. japonicus	6	0.408 ±0.408	0.058	0.114 ±0.389	0.507
M. capensis	7	-0.065 ±0.328	0.620	-0.139 ±0.430	0.426
R. holubi	10	0.331 ±0.351	0.016*	0.299 ±0.419	0.051
M. falciformis	6	0.165 ±0.312	0.143	0.314 ±0.368	0.037*
L. lithognathus	5	0.021 ±0.353	0.900	-0.161 ±0.304	0.301

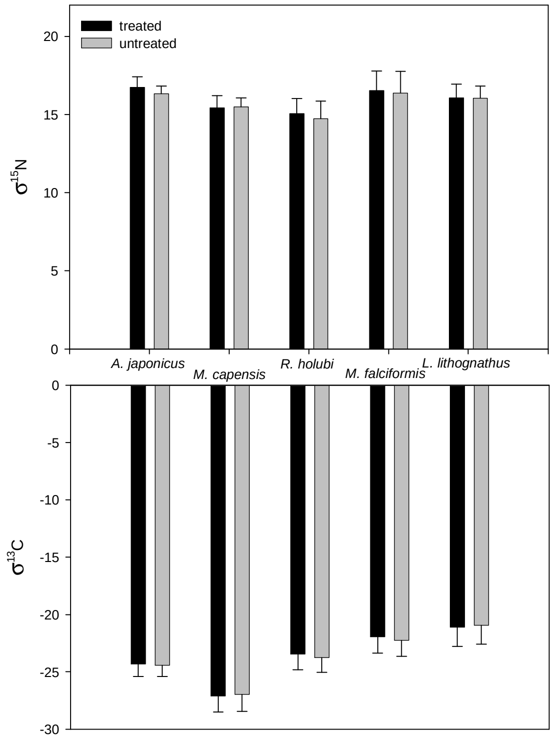

Figure 4.2. Mean $\delta^{13}C$ and $\delta^{15}N$ of samples before and after lipid extraction. Error bars indicate standard deviation

3.3 Mathematical model

A strong linear relationship existed between observed δ^{13}C values and those predicted by the models of both Post *et al.* (2007) ($R^2 = 0.975$) and Fry (2002) ($R^2 = 0.970$) (Figure 4.3). Modelling efficiency was also high for both models (Post *et al.* 2007 EF = 0.968; Fry 2002 EF = 0.829).

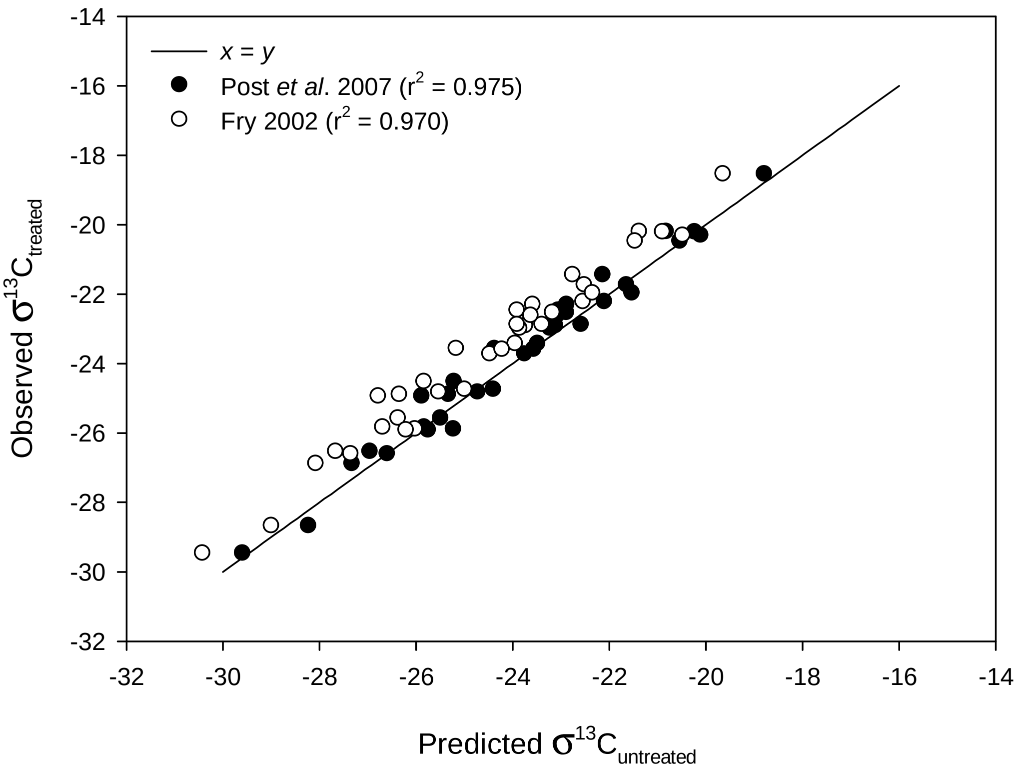

Figure 4.3 Observed (chemically extracted) and predicted (lipid normalization models of Post *et al.* (2007), and Fry (2002)) δ^{13}C values

4. DISCUSSION

4.1 Carbonate content of estuarine plants

The effect of treatment with HCl on the stable isotope ratios of a range of estuarine plants was variable, with a general lack of significant differences (Table 4.3). Half of the plant groups analysed showed a positive change in $\delta^{15}N$ following treatment (with the exceptions of *P. australis*, *P. pectinatus* and epiphytes), while the overall trend for $\Delta\delta^{13}C$ was negative (with increases in $\Delta\delta^{13}C$ for *P. australis* and *R. cirrhosa*). Except for *C. ustulatus,* standard deviations for the means of carbon and nitrogen isotope ratios, and the difference pre- and post-acidification were large, indicating variability between individuals. This may be due to the small number of replicates for each plant species ($n = 3$ or 2 in the case of *C. ustulatus*) or attributed to the untested effect of season.

The only statistically significant change in isotopic ratio with acidification was an increase in $\delta^{15}N$ (paired *t* test, $P = 0.006$) in the case of *C. ustulatus* (Table 4.3). This change (mean $\Delta\delta^{15}N = 0.486 \pm 0.067$) was, however, within the 3.4 ‰ fractionation between trophic levels and so was not ecologically significant (DeNiro & Epstein, 1980; Peterson & Fry, 1987), unlike that of the change in carbon isotopic ratio of the epiphytes (mean $\Delta\delta^{13}C = -3.387 \pm 1.574$) that was greater than the accepted 0-1 ‰ fractionation between food source and consumer (DeNiro & Epstein, 1978; Peterson & Fry, 1987). It is likely that the epiphyte sample was not adequately sorted prior to analysis and included bacteria, detrital particles or microscopic invertebrates. The arthropod exoskeleton has a high carbonate content (Serrano *et al.*, 2008) and the large $\Delta\delta^{13}C$ in the epiphytic sample could be due to the loss of exoskeleton carbonate rather than that of the plants. As these were the only significant results out of the test samples, it can be concluded that carbonate content of these estuarine plants is generally low, making acidification prior to isotope analysis unnecessary.

A review of available literature indicated that the measured effects of acidification on isotopic ratios are variable. A significant reduction in $\delta^{15}N$ was found after acid washing of seagrass leaves (Bunn *et al*., 1995), suspended organic matter (Carabel *et al*., 2006), and intertidal algae (Ng *et al*., 2007). A decrease in δ^{15}N was reported in grazing mollusc muscle tissue but this was not significant (Ng *et al*., 2007). Carabel *et al*. (2006) record a significant decrease in δ^{13}C in sedimentary organic matter, suspended particulate organic matter, plankton and crustaceans with a high carbonate content, while Serrano *et al*. (2008) found a non-significant reduction in whole beach arthropod (orders Araneae, Geophilomorpha, Coleoptera, Isopoda and Amphipoda) δ^{13}C. Acidification with HCl was found to have no effect on ratios of either C or N in penaid shrimp tissue (Bosley & Wainright, 1999, exoskeleton included; Bunn *et al*., 1995), fish muscle (Bosley & Wainright, 1999; Carabel *et al*., 2006), seaweeds (Carabel *et al*., 2006) and cephalopod tissue (Carabel *et al*., 2006).

It is difficult to draw a conclusion on the effect of acid washing on sample stable isotope ratios with any certainty. Although it would appear that certain groups have a low carbonate content and therefore do not need to be acidified (Carabel *et al*., 2006), even in the case of materials with a high carbonate content (e.g. invertebrate exoskeleton) the results of acid washing are not always predictable and are variable (Bunn *et al*., 1995; Bosley & Wainright, 1999; Carabel *et al*., 2006; Serrano *et al*., 2008). Furthermore, although carbonates do not contain nitrogen (Serrano *et al*., 2008), a decrease in δ^{15}N in some cases suggests that acidification removes dietary nitrogen such as proteins, chitin and polysaccharides (Ostle *et al*., 1999 in Ng *et al*., 2007).

In an attempt to overcome the problem of sample carbonate content it is recommended, where possible, to use material with known low carbonate content. This may involve the removal of exoskeletons or other calcareous structures from the animal tissue prior to analysis (Bunn *et al*., 1995). Alternatively, samples may be analyzed in duplicate with untreated samples giving an estimate of δ^{15}N. Jacob *et al*. (2005) offer correction factors that may be applied to non-acidified but carbonate-free samples to make them comparable to acidified samples. However, these factors were created for an Antarctic ecosystem and their applicability to other systems remains untested.

4.2 Chemical extraction of lipids

It is worth noting that corrections for lipid content by either chemical or mathematical means may in some cases be done erroneously. As a consumer will assimilate most of the lipid content of a prey item it is necessary to know the stable isotope value of prey tissues with lipids included, with lipid-corrected isotope values in this situation yielding invalid results. Lipid corrections should therefore not be indiscriminately applied to all samples.

In general, results of this study show an increase in $\delta^{15}N$ for all fish species except the mullet *M. capensis*, with increases only being significant for *R. holubi* (paired *t* test, P = 0.016) (Table 4.5). However, lipid extraction had far more variable effects on isotope values for carbon, with three of the fish species (*A. japonicus*, *R. holubi* and *M. falciformis*) showing an increase in $\delta^{13}C$ post-extraction and *M. capensis* and *L. lithognathus* a decrease in $\delta^{13}C$. The only significant result in terms of change in $\delta^{13}C$ was for *M. falciformis* (paired *t* test, P = 0.037). These results may imply that the Bligh and Dyer (1959) method of lipid extraction is inefficient, and that the absence of significant differences between treated and control samples is due to there effectively being minimal difference in treatment. Although possible, this conclusion is unlikely. The method was originally developed specifically for fish muscle tissue and is widely accepted as being highly effective at removing lipids from flesh samples (Iverson *et al.*, 2001), as well as being suitable for plant tissues (Fishwick & Wright, 1977).

A more probable reason for the non-significant change in fish muscle tissue $\delta^{13}C$ post-extraction is that the samples used in this test had low lipid content. In this study this could have been influenced by life history, as juvenile fishes direct all energy into somatic growth. In general though, the lipid content of fish muscle is low (Pinnegar & Polunin, 1999; Iverson *et al.*, 2001; Logan *et al.*, 2008) and varies seasonally due to the deposition of triacylglycerols (Arrington *et al.*, 2006). The primary site of lipid storage in fish is related to the life history of a species but is most commonly the liver rather than muscle tissue (Post *et al.*, 2007). Arrington *et al.* (2006) record primary sites of lipid storage as the liver and mesentery for *Serrasalmus manueli* and *Semaprochilodus kneri*, a piscivore and detritivore respectively, both of which exhibit no parental care. In comparison, lipids were stored in the liver, mesentery and muscle for *Cichlia temensis*, a piscivore that demonstrates extended brood care and for whom increased fat reserves are necessary.

Further indication of a low lipid content lies in the C:N ratios of the fish flesh samples (Table 4.4). As first predicted by, and subsequently observed in the mathematical model of McConnaughey and McRoy (1979), a strong relationship exists between C:N and lipid content in a wide variety of animal tissue (Bodin *et al*., 2007; Post *et al*., 2007). The C:N ratio is commonly used as a proxy for lipid content because lipids are composed mainly of carbon and almost no nitrogen, with the result that changes in lipid content are reflected in changes in the C:N ratio (Bodin *et al*., 2007; Post *et al*., 2007; Logan *et al*., 2008).

A tissue lipid content of 5% equates to a C:N ratio of 3.5, and for aquatic animals this is considered to be the threshold below which lipid normalization is unnecessary (Bodin *et al*., 2007; Post *et al*., 2007). Of all the fish species, the highest average muscle tissue C:N ratios pre-extraction were found in *R. holubi* (3.332) and *M. falciformis* (3.459) – both marginally lower than the 5% lipid content threshold. The absence of significant changes post lipid extraction for all but these two species suggests that C:N is a reliable indicator of the sample lipid content and thus the need to extract before processing.

Approximately a third of the samples of *R. holubi* flesh had increased C:N ratios post extraction (3 of 10; average increase 0.238), suggesting nitrogenous compounds were leached during the process of lipid removal. The significant change in $\delta^{15}N$ for this species post extraction (paired *t* test, $P = 0.016$) confirms the loss of such compounds. The chloroform/methanol solvent used in the Bligh and Dyer method is not lipid-specific, with the result that nitrogen may also be affected (Murry *et al*., 2006). Alternatively, structural polar lipids such as glycolipids or phospholipids are attached to amino acids which may be co-extracted during the removal process, leading to an enrichment of $\delta^{15}N$ when this class of lipids is present (Sotiropoulos *et al*., 2004; Bodin *et al*., 2007).

The findings of previous work examining the effect of lipid extraction on isotope ratios are variable, with a significant increase in $\delta^{13}C$ recorded post extraction for fish tissues in some studies, e.g. Murry *et al*. (2006) dorsal flesh tissue; Logan *et al*. (2008) liver, muscle and gonad; Mintenbeck *et al*. (2008) muscle and skin. However, in other studies no significant increase in $\delta^{13}C$ was recorded, e.g. Schlechtriem *et al*. (2003) entire fish body; Sotiropoulos *et al*. (2004) muscle. Effects on $\delta^{15}N$ also seem to be variable, with Murry *et al*. (2006) and Mintenbeck *et al*. (2008) recording a significant effect (dorsal flesh tissue, and muscle and skin respectively), and Logan *et al*. (2008) significant increases for only some fish muscle samples. Sotiropoulos *et al*. (2004) found a significant change in $\delta^{15}N$ for whole juvenile fish but not for flesh samples from adult specimens.

In terms of invertebrates, a significant increase in $\delta^{13}C$ was found following lipid extraction by Logan *et al*. (2008) (eight freshwater arthropod species and marine krill and shortfin squid), but not by Schlechtriem *et al*. (2003) (freshwater zooplankton and a free-living nematode). By examining individual tissue types of the spider crab (*Maja brachydactyla*), Bodin *et al.* (2007) were able to show an absence of significant differences in muscle tissue isotope ratios, but significant changes for $\delta^{13}C$ in gonad tissue, and for both $\delta^{13}C$ and $\delta^{15}N$ in hepatopancreatic tissue. This range of results is probably a consequence of the concentration dependence of the lipid bias (Post *et al*., 2007). Lipid-rich samples would have a $\delta^{13}C$ 3 – 4 ‰ more negative than an extracted sample, while little effect is seen following extraction where the lipid concentration is low (Bodin *et al*., 2007; Post *et al*., 2007). What is highlighted by the variability in the above results is that lipid extraction should not be applied indiscriminately to all samples (Logan *et al*., 2008).

4.3 Fit of the mathematical models

Lipid normalization models presented by Post *et al*. (2007) and Fry (2002) were both strongly linearly related to the 1:1 ratio between observed and predicted δ^{13}C. This combined with modelling efficiencies of almost 1 suggest both models are suitable to correct for the effect of lipids on the ratio of carbon isotopes within samples of estuarine fish muscle tissue. Of the two though, that of Post *et al*. (2007) was best, with both regression results and EF being closest to 1 (R^2 = 0.975 and 0.970, and EF = 0.968 and 0.829 for Post *et al*. 2007 and Fry 2002, respectively).

Model fit improves with specificity, and data are far better estimated using models with species- and tissue-specific parameters (Logan *et al*., 2008). The model of Fry (2002) was generated using fish flesh, while that of Post *et al*. (2007) was modified for aquatic organisms from the mathematical model proposed by McConnaughey and McRoy (1979) which has been found to underestimate $\Delta\delta^{13}$C (Sweeting *et al*., 2006; Post *et al*., 2007). Although Post *et al*. (2007) advised that tissue-specific studies should use their results with caution this model appears to be suitable for fish muscle tissue.

The mathematical models for lipid normalization used here are robust for fish muscle tissue and a suitable alternative for chemical lipid extraction. Relationships between C:N, % lipid and δ^{13}C vary however, depending on taxonomic family (Bodin *et al*., 2007). In addition, the applicability of the models to other groups of aquatic organisms, as well as their use for terrestrial animals is questionable, while plants show no relationship between C:N and δ^{13}C (Post *et al*., 2007).

5. CONCLUSION AND RECOMMENDATIONS

Chemical lipid extraction, although effective at reducing sample lipid content, appears to affect the nitrogen value of fish muscle tissue. Fish muscle tissue shows a strong relationship between C:N and lipid content, with C:N being an excellent indicator of lipid content as suggested by Post *et al*. (2007). Mathematical normalization using an appropriate model is therefore the preferred method for correcting for lipid content as it preserves the integrity of the nitrogen content and is cheaper and easier to apply than alternative methods.

Where C:N is low, as was the case in this study, chemical extraction should be avoided as nitrogen ratios may be affected by the process, as evidenced in the samples of *R. holubi*. Overall, fish muscle had a low and fairly uniform lipid content and plant tissue a low carbonate content, with a lack of significant results for both tests. Consequently, samples processed during this study were not subjected to either acid washing or lipid extraction as it has been shown to be unnecessary and would hinder direct comparison with isotope work already completed by researchers on other biotic components of the East Kleinemonde Estuary.

CHAPTER FIVE: THE IMPORTANCE OF SUBMERGED AQUATIC MACROPHYTES AS A FOOD SOURCE FOR SELECTED ICHTHYOFAUNA – AN INVESTIGATION USING STABLE ISOTOPES

1. INTRODUCTION

Estuarine food webs

It has been suggested that autochthonous- and allochthonous-derived detritus provide the major energy flow pathway in estuaries, since detritus is the major food source for most invertebrate prey of carnivorous fishes within these systems (Day, 1951; Odum, 1971; Marais, 1984; Mann, 1988; Bennett, 1989a). Detrital-derived energy flows directly to mugilids, via zooplankton to the clupeids and via detritivorous zoobenthos to sparids. Detritivores such as mugilids and zooplanktivores represented by clupeids are usually very abundant in South African estuaries (Whitfield, 1980; Harrison & Whitfield, 2006).

Both allochthonous and autochthonous carbon sources contribute to the detrital pool in an estuary, and detritus therefore represents a mixed carbon source (Richoux & Froneman, 2007). Allochthonous inputs include terrestrial plant matter that enters the system via the river or as material that is blown or falls into the water from surrounding vegetation, as well as marine sources such as wrack or phytoplankton. Autochthonous primary producers in Eastern Cape estuaries include submerged macrophytes, algae, phytoplankton, microphytobenthos, and salt marsh plants. Production by the various primary producers may be highly variable between estuaries, influenced by physical conditions such as freshwater input (Froneman, 2000a), turbidity and nutrient availability within each system (Baird, 1999).

Stable isotopes as a method for detrital food webs

Due to the small particle size and decayed nature of detritus, traditional feeding study methods such as gut content analysis are not suitable for identifying trophic relationships within estuaries. Stable isotopes of carbon and nitrogen have therefore become an increasingly common method in such studies. The ratio of carbon isotopes $^{13}C/^{12}C$ changes very little between producer and consumer, and tracing this ratio provides an indication of the energy flow pathways. On the other hand, $^{15}N/^{14}N$ is more dramatically altered by each consecutive consumer and is therefore used to indicate trophic level. The value of this method lies in the fact that it is based upon food that a consumer has assimilated and not just ingested, as other dietary methods do.

Interpretation of isotopic ratios in estuarine consumers is complicated by the variable nature of the isotope ratios of estuarine food sources. Inputs to the detrital pool include material from various geographic locations, different types of producers and at various stages of decomposition (Kreeger & Newell, 2000), and the relative contributions which change over space and time and may influence ratios of both $^{13}C/^{12}C$ and $^{15}N/^{14}N$ (Handley & Raven, 1992; Boon & Bunn, 1994). Detritus can therefore show wide variation in C and N isotope values between locations and seasons (Richoux & Froneman, 2007).

Aims of this study

Following a significant flood event in May 2003 the East Kleinemonde Estuary was largely macrophyte-free, while the West Kleinemonde Estuary, in contrast, retained both *Ruppia cirrhosa* and *Potamogeton pectinatus* beds during the post-flood event and were extensive for the duration of this study. The main aim of this investigation was to assess the importance of the submerged macrophytes *Ruppia cirrhosa* and *Potamogeton pectinatus* as carbon sources for selected fish species in a macrophyte-dominated West Kleinemonde and macrophyte-free East Kleinemonde estuaries, with the hypothesis being that the isotope signatures of the fishes will reflect the differential sources of carbon within each estuary.

2. METHODS

2.1 Field sampling

Sampling of the East Kleinemonde and West Kleinemonde estuaries took place on two consecutive days within a one week sampling period in February (summer) and July (winter) of 2008, and again in February (summer) of 2009. The sampling protocol was identical in each estuary. Based on physical characteristics, each system was divided into a lower, middle and upper reach and sampling effort divided almost equally amongst the areas. Fishes were sampled using a fleet of seine and gill nets, following a standard protocol that has been used in the East Kleinemonde for the last 14 years (James *et al*., 2008a, 2008b).

Marine-spawning fish species and those that spawn within the estuarine environment were targeted separately using seine nets of different mesh sizes. A small mesh seine (30 m x 2 m) with a 5mm bar mesh was used to sample the estuarine-spawning fishes and a large mesh seine net (50 m x 2 m) with a 15 mm bar mesh for the marine spawners. Six monofilament gill nets (10 m x 2 m) with sections of 45 mm, 75 mm and 100 mm stretch meshes were set overnight along the length of each estuary, with effort divided equally amongst the reaches of each system, in order to capture the larger species that actively avoid a seine net.

The seine nets were laid in a semi-circle from the shore using a motorized boat and hauled ashore by three to four people. From each seine haul individuals to be used in the fish feeding study were removed from the catch and placed on ice to be processed later. Individuals for isotope analysis were separated from the rest of the catch in the field and their stomach contents and flesh samples removed for later processing in the laboratory.

Flesh samples were frozen immediately after dissection and stomach contents preserved in 5% formalin.

During each sampling period plants were collected from the lower, middle and upper reaches of both estuaries. A variety of plants were selected based on their abundance within the study estuaries and potential to act as food (carbon) sources to the estuarine fauna. The plants included aquatic macrophytes, submerged algae and an emergent reed (Table 5.1). Three replicates per species were collected in each reach (where possible) and frozen for later analysis in the laboratory.

Table 5.1 Plant species collected and analysed from the East Kleinemonde and West Kleinemonde estuaries

Species	**Family**	**Group**
Phragmites australis	Poaceae	Emergent reed
Potamogeton pectinatus	Potamogetonaceae	Submerged macrophyte
Ruppia cirrhosa	Ruppiaceae	Submerged macrophyte
Caulacanthus ustulatus	Caulacanthaceae	Submerged alga
Codium tenue	Codiaceae	Submerged alga
Epiphytes		Submerged alga

2.2 Analysis of the stable isotope ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$

In an attempt to assess the relative importance of different plant groups as a source of carbon in each estuary, fish species that represented different trophic guilds were chosen, based on the classification system of Elliott *et al*. (2008) (Table 5.2).

Table 5.2 Fish species chosen to represent the range of trophic guilds (after Elliott *et al.*, 2008) within the East Kleinemonde and West Kleinemonde estuaries

Trophic guild	**Species**	**Family**	**Common name**
Detritivore	*Liza dumerili*	Mugilidae	Groovy mullet
	Liza richardsonii	Mugilidae	Southern mullet
	Myxus capensis	Mugilidae	Freshwater mullet
Opportunist (omnivore)	*Rhabdosargus holubi*	Sparidae	Cape stumpnose
Zooplanktivore	*Atherina breviceps*	Atherinidae	Cape silverside
	Gilchristella aestuaria	Clupeidae	Estuarine roundherring
	Monodactylus falciformis	Monodactylidae	Cape moony
Zoobenthivore	*Glossogobius callidus*	Gobiidae	River goby
	Lithognathus lithognathus	Sparidae	White steenbras
	Pomadasys commersonnii	Haemulidae	Spotted grunter
Piscivore	*Argyrosomus japonicus*	Sciaenidae	Dusky kob
	Elops machnata	Elopidae	Ladyfish
	Lichia amia	Carangidae	Leervis/Garrick

2.2.1 Laboratory preparation of samples

Fish flesh samples: Dorsal flesh is the most commonly used tissue in studies of stable isotopes in fish (Post *et al.*, 2007). Samples of dorsal tissue from large fishes were cleared under a microscope of all non-flesh components, namely bone, skin and scales. In the case of some smaller individuals, flesh was sampled from the entire trunk with all non-muscle material also being removed prior to processing. All samples were oven-dried at a temperature not exceeding 60°C (Carabel *et al.*, 2006) and then kept in a dessicator at room temperature, until such time as they were ground to a fine powder using a pestle and mortar.

Plants: Plant samples collected were washed in distilled water to remove salt as well as to ensure that no foreign items such as invertebrates or other matter was included in the samples. Following washing, the plants were dried as described for the fish flesh samples above and then ground to a fine powder using an electric grinding mill.

2.2.2 Stable isotope analysis

Ground samples were sent to the Archaeology Department of the University of Cape Town for analysis of $\delta^{15}N$ and $\delta^{13}C$. The dried samples were weighed to an accuracy of 1 µg on a Sartorius micro balance, and then combusted in a Flash EA 1112 series elemental analyzer. The gases were passed to a Delta Plus XP IRMS (isotope ratio mass spectrometer) via a Conflo III gas control unit.

The ratio of heavy to light isotope for each sample was assessed relative to a standard according to the equation:

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

where δX is the difference in isotopic composition between the sample and the standard, expressed in parts per thousand (‰), and R is the ratio of heavy to light isotope ($^{15}N/^{14}N$ and $^{13}C/^{12}C$) of the sample or standard respectively (Peterson & Fry, 1987).

In-house standards against which the sample isotope ratios were assessed had been calibrated against IAEA (International Atomic Energy Agency) standards. Nitrogen is expressed in terms of its value relative to atmospheric nitrogen, and carbon to Pee-Dee Belemnite.

2.3 Statistical analysis

Sample sizes for each species, estuary and sampling season were influenced by the availability (abundance) of specimens. For analysis of $\delta^{13}C$, sample sizes were small ($n \leq 16$) and non-parametric statistical tests were used in all cases. Where more than two groups were available, a Kruskal-Wallis was used to test variation in isotopic ratios according to region of the estuary and season. Where data were only available for two groups, a Mann-Whitney U test was used to test for significant variation. However, in the case of fish $\delta^{15}N$, seasonal values were grouped, thus making sample sizes large enough for the use of parametric statistics. A one-way ANOVA, followed by a Newman-Keuls post-hoc multiple range test, were used to identify significant differences in nitrogen isotope signatures between fishes and homogenous groupings therein. All tests were performed at the $P < 0.05$ level of significance using the STATISTICA Software package (version 8.0).

Although statistically significant, the degree of fractionation of isotope ratios between food source and consumer is usually very low and therefore unlikely to be ecologically significant. Trophic fractionation for $\delta^{13}C$ is commonly accepted at 0-1 ‰, and that for $\delta^{15}N$ is 3.4 ‰ (DeNiro & Epstein, 1978; DeNiro & Epstein, 1980; Peterson & Fry, 1987). Variation that is outside of this range for either isotope will affect interpretation of food web structure and is therefore considered to be of ecological significance (Murry *et al*., 2006).

3. RESULTS

3.1 Spatial/temporal variation

Statistical analysis (Kruskal-Wallis or Mann-Whitney U, $P < 0.05$) showed an overwhelming non-significant pattern of isotope ratio variation for site of collection of fishes from both the East and West Kleinemonde estuaries. Site samples were therefore grouped together, forming a single sample per species for each season in each estuary.

The effect of season introduced a significant variation in $\delta^{13}C$ for a number of plants and fishes in both estuaries. This temporal component was also significant in plant $\delta^{15}N$ but not for fish $\delta^{15}N$. Consequently, the analyses of fish $\delta^{15}N$ values in both the East and West Kleinemonde estuaries were averaged over the three sampling intervals. The analyses of plant and fish carbon, and plant nitrogen ratios were, however, kept separate between seasons.

3.2 Carbon

3.2.1 East Kleinemonde Estuary

The $\delta^{13}C$ values for plants in the East Kleinemonde Estuary were consistent for each species throughout the seasons and showed very little overlap between species (Figure 5.1 and Table 5.3). The most enriched species were the submerged macrophytes *R. cirrhosa* (-18.607 to -15.883 ‰) and *P. pectinatus* (-17.563 ‰), with the reed *P. australis* (-24.147 to -23.715 ‰) and all groups of submerged algae (-30.136 to -22.413 ‰) being more depleted. All submerged primary producers showed a high degree of seasonal variability in $\delta^{13}C$ values. These changes are both statistically and ecologically significant in the macrophyte *R. cirrhosa*, as well as in the alga *C. ustulatus*. A statistically significant seasonal change was found in *P. australis* (Kruskal-Wallis, $P < 0.05$).

Only nine fish species were present in the catches during all three sampling periods. Of these, the variation in $\delta^{13}C$ with season was found to be of both statistical (Kruskal-Wallis, $P < 0.05$) and ecological significance for only three species (Table 5.3). In the case of *A. breviceps*, summer and winter of 2008 values were significantly different, with the summer 2009 value being similar to both previous seasons ($P = 0.022$). For *M. capensis* the summer 2008 was similar to both winter 2008 and summer 2009 values, which were significantly different from each other ($P = 0.0001$). The summer and winter 2008 $\delta^{13}C$ values of *G. callidus* were similar, and both were significantly different to that of summer 2009 ($P = 0.0004$).

All of the fish species sampled show relatively depleted $\delta^{13}C$ values, ranging from -27.508 ‰ (*G. aestuaria*) to -19.865 ‰ (*L. richardsonii*) in summer 2008, -27.165 ‰ (*G. aestauria*) to -20.695 ‰ (*L. dumerili*) in winter 2008, and -27.002 ‰ (*G. aestuaria*) to -22.114 ‰ (*G. callidus*) in summer 2009 (Figure 5.1 and Table 5.3). In both the summer and winter of 2008 the $\delta^{13}C$ values for *G. aestuaria*, *L. dumerili* and *L. lithognathus* were quite different from those of the rest of the fishes (Figure 5.1). *G. aestuaria* (-27.508 to -27.002 ‰) consistently had the most depleted $\delta^{13}C$ values, while *L. lithognathus* (-22.427

to -20.942 ‰), *L. dumerili* (-20.695 to -20.640 ‰) and *L. richardsonii* (-19.865 ‰) generally had the most enriched values.

Table 5.3 Average (± SD) δ^{13}C (‰) values for plants and fish species from the East Kleinemonde Estuary over three sampling periods. Sample size for each sampling period is indicated by ‘n’. Statistically significant results are indicated by a single asterisk (Kruskal-Wallis, $P < 0.05$), with ecological significance ($\Delta\delta^{13}$C > 1 ‰) being denoted by two asterisks (U = upper reaches, M = middle reaches and L = lower reaches of the estuary)

					δ^{13}C (Avg ± SD)			
Species	Region	n	Summer '08	n	Winter '08	n	Summer '09	Significance
Phragmites australis	U	3	-24.147 ± 0.377	3	-24.109 ± 0.192	3	-23.715 ± 0.132	*
Ruppia cirrhosa	M	3	-15.833 ± 0.739	3	-16.660 ± 0.713	3	-18.607 ± 0.367	**
Potamogeton pectinatus	U	3			-17.563 ± 0.351			
Codium tenue	L	3	-30.001 ± 0.273	3	-27.829 ± 0.360	3	-26.082 ± 0.309	
Caulacanthus ustulatus	M	3			-22.413 ± 0.078		-30.136 ± 0.649	**
Green epiphytes	U	1					-21.322	
Liza dumerili		7	-20.640 ± 1.057	10	-20.695 ± 0.987	0		
Liza richardsonii		9	-19.865 ± 0.797	0		0		
Myxus capensis		7	-23.876 ± 1.646	15	-25.368 ± 1.540	13	-22.323 ± 1.261	
Rhabdosargus holubi		6	-25.097 ± 1.523	15	-23.783 ± 1.857	15	-22.692 ± 2.084	
Atherina breviceps		15	-23.296 ± 0.818	15	-24.117 ± 0.735	15	-23.648 ± 0.330	
Gilchristella aestuaria		15	-27.508 ± 0.544	15	-27.165 ± 0.526	14	-27.002 ± 0.389	
Monodactylus falciformis		9	-23.520 ± 0.889	11	-23.881 ± 0.946	15	-23.376 ± 0.984	
Glossogobius callidus		5	-23.476 ± 0.652	11	-23.743 ± 0.909	15	-22.114 ± 1.134	
Lithognathus lithognathus		5	-20.942 ± 1.640	8	-21.482 ± 0.618	1	-22.427	
Pomadasys commersonnii		9	-23.634 ± 1.273	0		0		
Argyrosomus japonicus		7	-24.678 ± 1.206	2	-25.827 ± 0.168	1	-25.224	
Elops machnata		1	-22.774	0		0		
Lichia amia		2	-23.288 ± 0.231	0		0		

When placed into different trophic guilds (Figure 5.1), the zooplanktivores (*G. aestuaria* range -27.508 to -27.002 ‰, *A. breviceps* -24.117 to -23.296 ‰, *M. falciformis* -23.881 to -23.376 ‰) and detritivores (*M. capensis* -25.368 to -22.323 ‰, *L. dumerili* -20.695 to -20.640 ‰, *L. richardsonii* -19.865 ‰) had the largest range of δ^{13}C values with an almost 4 ‰ difference between species within each season in both groups. *G. aestauria* and *M. capensis* were distinctively different to other members in their respective groups. The δ^{13}C values for representative carnivorous and piscivorous fishes were similar in the respective trophic groups. The carnivorous fishes were represented by *G. callidus* (-23.743 to -22.114 ‰), *P. commersonnii* (-23.634 ‰) and *L. lithognathus* (-22.427 to -20.942 ‰), and the piscivores by *A. japonicus* (-25.827 to -24.678 ‰), *E. machnata* (-22.774 ‰) and *L. amia* (-23.288 ‰).

3.2.2 West Kleinemonde Estuary

The pattern of δ^{13}C in plants was similar to that in the East Kleinemonde Estuary (Table 5.4). The submerged macrophytes *R. cirrhosa* (-20.036 to -15.956 ‰) and *P. pectinatus* (-19.823 to -16.416 ‰) were the most enriched, with their position relative to each other changing between summer and winter 2008. The seasonal changes in δ^{13}C of these two submerged macrophytes were ecologically significant. *P. australis* (-25.550 to -24.976 ‰) was marginally more depleted than in the East Kleinemonde Estuary but δ^{13}C values were fairly consistent across the seasons. The two groups of epiphytic algae had very different δ^{13}C values, with the epiphytic green algae (-24.474 ‰) being very similar to the value of *P. australis* from which it was collected. The brown filamentous algae (-29.557 ‰) collected from the lower reaches was more depleted in ^{13}C than the above green algae. The submerged alga *C. tenue* and *C. ustulatus* were not present within this estuary during the study.

Of the 13 fish species used in this study, ten were collected and examined for seasonal variation in δ^{13}C in the West Kleinemonde Estuary (Table 5.4). As in the East Kleinemonde Estuary, the $\Delta\delta^{13}$C for *M. capensis* and *G. callidus* was statistically and ecologically significant (P = 0.009 and 0.018 respectively), while the pattern of variation in the mullet was identical to that in the East Kleinemonde Estuary. In the case of *G. callidus*, there was a significant difference in δ^{13}C between winter 2008 and summer 2009, while the summer 2008 value was similar to both sampling seasons. *G. aestuaria* and *R. holubi* showed the same pattern of statistical and ecological significance, with summer 2008 and summer 2009 $\delta^{13}C$ values being significantly different, but the winter 2008 samples being similar to both seasons (P = 0.0001 for *G. aestuaria* and 0.001 for *R. holubi* respectively). When divided into trophic guilds, the average $\delta^{13}C$ values for all fishes (excluding piscivores) had larger standard deviations in winter 2008 when compared to the summer seasons (Figure 5.2).

Similar to the East Kleinemonde Estuary, the fishes had relatively depleted $\delta^{13}C$ values (Figure 5.2), which ranged from -27.144 ‰ (*M. capensis*) to -18.348 ‰ (*L. richardsonii*) in summer 2008, from -27.031 ‰ (*M. capensis*) to -20.287 ‰ (*M. falciformis*) in winter 2008 and from -24.726 ‰ (*M. capensis*) to -19.864 ‰ (*L. lithognathus*) in summer 2009. Apart from the more distinctive *M. capensis*, *G. aestuaria* and *L. richardsonii* $\delta^{13}C$ values, the summer 2008 fish values appear to form a continuum of similar values with no species being very distinctive from another. Although *M. capensis, G. aestuaria, R. holubi* and *E. machnata* appear to have distinctive $\delta^{13}C$ values in winter 2008, the large standard deviations indicate that there was considerable overlap between species. During summer 2009, *M. capensis* once again had the most depleted $\delta^{13}C$ value, while *A. japonicus*. *G. aestuaria* and *G. callidus* had intermediate values and *R. holubi* and *A. breviceps* had slightly enriched values. *L. dumerili, L. richardsonii, M. falciformis* and *L. lithognathus* consistently had the most enriched $\delta^{13}C$ values of all the fish species that were sampled (Table 5.4).

Table 5.4 Average (± SD) δ^{13}C (‰) values for plants and fish species from the West Kleinemonde Estuary over three sampling periods. Sample size for each sampling period is indicated by ‘n’. Statistically significant results are indicated by a single asterisk (Kruskal-Wallis, $P < 0.05$), with ecological significance ($\Delta\delta^{13}$C > 1 ‰) being denoted by two asterisks (U = upper reaches, M = middle reaches and L = lower reaches of the estuary)

					δ^{13}C (Avg ± SD)			
Species	Region	n	Summer '08	n	Winter '08	n	Summer '09	Significance
Phragmites australis	U	3	-24.976 ± 0.254	3	-25.148 ± 0.183	3	-25.550 ± 0.473	
Ruppia cirrhosa	M	3	-17.227 ± 0.250	3	-20.036 ± 0.152	3	-15.956 ± 0.600	**
Potamogeton pectinatus	U	3	-19.823 ± 0.092	3	-16.416 ± 0.126	3		**
Green epiphytes	U					1	-24.474	
Brown epiphytes	L					1	-29.557	
Liza dumerili		6	-20.917 ± 1.751	9	-20.879 ± 2.396	0		
Liza richardsonii		5	-18.348 ± 1.057	0		0		
Myxus capensis		10	-27.144 ± 1.257	16	-27.031 ± 2.905	9	-24.726 ± 1.277	
Rhabdosargus holubi		15	-24.566 ± 1.919	10	-23.687 ± 2.429	15	-21.977 ± 1.179	
Atherina breviceps		15	-22.339 ± 1.024	15	-21.648 ± 1.957	15	-21.741 ± 0.905	
Gilchristella aestuaria		15	-26.766 ± 2.306	12	-25.411 ± 2.622	15	-23.011 ± 0.878	
Monodactylus falciformis		8	-22.150 ± 1.507	11	-20.287 ± 2.290	13	-20.846 ± 0.903	
Glossogobius callidus		10	-20.944 ± 1.515	8	-20.785 ± 1.943	8	-22.170 ± 0.361	
Lithognathus lithognathus		1	-21.291	3	-21.265 ± 1.694	1	-19.864	
Pomadasys commersonnii		0		0		0		
Argyrosomus japonicus		8	-24.070 ± 1.101	0		2	-24.545 ± 1.293	
Elops machnata		2	-23.033 ± 2.552	5	-23.525 ± 0.706	0		
Lichia amia		7	-22.601 ± 1.986	0		0		

When divided into different trophic groups, there was considerable intra-guild variation within the lower trophic groups. For example, amongst the detritivores the δ^{13}C summer 2008 values for *M. capensis* were 6 and 9 ‰ more depleted than for *L. dumerili* and *L. richardsonii* respectively (Table 5.4). Similarly, the range in δ^{13}C values for *G. aestuaria* (-26.766 to -23.011 ‰) was larger than in the other zooplanktivores (*A. breviceps* -22.339 to -21.648 ‰ and *M. falciformis* -22.150 to -20.287 ‰). In contrast, the carbon isotopic values were tightly grouped within the omnivores, carnivores and piscivores (Table 5.4).

3.3 Nitrogen

3.3.1 East Kleinemonde Estuary

The $\delta^{15}N$ for most plants fell within a narrow range (Table 5.5). There was, however, large seasonal variation in $\delta^{15}N$ for *P. australis* that was of ecological significance. The variation for *C. tenue* was statistically but not ecologically significant as the values were within the 3.4 ‰ range of fractionation per trophic level.

Seasonal variation of $\delta^{15}N$ was statistically but not ecologically significant for two of the nine fish species examined (Table 5.5). For *G. aestuaria*, the summer 2008 (16.365 ± 0.514 ‰) and summer 2009 (15.557 ± 0.728 ‰) values were significantly different, with winter 2008 being similar to both ($P = 0.007$). For *M. capensis*, however, the summer 2009 $\delta^{15}N$ values (13.907 ± 1.143 ‰) were significantly different to those of both summer and winter of 2008 ($P = 0.001$).

Table 5.5 Average (± SD) δ^{15}N (‰) values for plants and fish species from the East Kleinemonde Estuary over three sampling periods. Sample size for each sampling period is indicated by 'n'. Statistically significant seasonal differences are indicated by a single asterisk (One-way ANOVA or Mann-Whitney U, $P < 0.05$), with ecological significance ($\Delta\delta^{15}$N > 3.4 ‰) being denoted by two asterisks (U = upper reaches, M = middle reaches and L = lower reaches of the estuary)

					δ^{15}N (Avg ± SD)			
Species	Region	n	Summer '08	n	Winter '08	n	Summer '09	Significance
Phragmites australis	U	3	5.215 ± 0.259	3	3.366 ± 0.168	3	-1.292 ± 0.680	**
Ruppia cirrhosa	M	3	7.727 ± 0.850	3	7.690 ± 0.398	3	7.146 ± 0.111	
Potamogeton pectinatus	U	3			8.464 ± 0.270			
Codium tenue	L	3	7.214 ± 0.551	3	9.515 ± 0.152	3	8.988 ± 0.062	*
Caulacanthus ustulatus	M	3			7.733 ± 0.417		8.899 ± 0.061	
Green epiphytes	U	1					7.496	
Liza dumerili		7	15.172 ± 1.074	10	13.783 ± 1.236	0		
Liza richardsonii		9	14.344 ± 0.446	0		0		
Myxus capensis		7	15.502 ± 1.318	15	15.797 ± 0.921	13	13.907 ± 1.143	
Rhabdosargus holubi		6	16.165 ± 1.406	15	14.932 ± 1.476	15	15.055 ± 0.895	
Atherina breviceps		15	14.945 ± 0.849	15	15.089 ± 0.819	15	15.281 ± 0.530	
Gilchristella aestuaria		15	16.365 ± 0.514	15	15.885 ± 0.703	14	15.577 ± 0.728	
Monodactylus falciformis		9	16.389 ± 1.227	11	16.121 ± 1.246	15	16.118 ± 1.081	
Glossogobius callidus		5	14.566 ± 0.513	11	13.178 ± 1.890	15	14.725 ± 0.447	
Lithognathus lithognathus		5	16.043 ± 0.780	8	16.269 ± 0.917	1	16.489	
Pomadasys commersonnii		9	15.666 ± 0.516	0		0		
Argyrosomus japonicus		7	17.703 ± 0.540	2	18.220 ± 0.134	1	18.224	
Elops machnata		1	17.638	0		0		
Lichia amia		2	18 ± 0.012	0		0		

Following a one-way ANOVA, the Newman-Keuls multiple range test ($P < 0.05$) identified three homogenous groups within the fish species (Table 5.6). The enriched nitrogen signatures of the three piscivores *A. japonicus*, *E. machnata* and *L. amia* formed a distinct group but the average δ^{15}N values for the remaining fish species were relatively similar, with considerable overlap between species within the omnivorous and carnivorous groups. Overall, the range of average δ^{15}N values was small, with just under 4 ‰ separating the lowest (13.725 ‰, *L. richardsonii*) from the highest (17.638 ‰, *E. machnata*) value (Table 5.6).

Table 5.6 Information on the δ^{15}N (‰) values of fishes in the East Kleinemonde Estuary from summer 2008 to summer 2009. Sample size for each species is indicated by ‘n’ and homogenous groupings, as indicated by the Newman-Keuls multiple range test ($P < 0.05$), are also included

	n	δ^{15}N (Avg ± SD)	Range	Groups		
L. dumerili	17	14.229 ± 1.286	10.887 - 16.640	x		
L. richardsonii	9	13.725 ± 1.219	11.104 - 14.484	x	x	
M. capensis	35	15.036 ± 1.380	11.910 - 16.988	x	x	
R. holubi	36	15.188 ± 1.294	12.290 - 17.547	x	x	
A. breviceps	45	15.105 ± 0.743	13.470 - 16.327	x	x	
G. aestuaria	44	15.951 ± 0.717	14.086 - 16.945	x	x	
M. falciformis	35	16.287 ± 1.150	13.459 - 17.984		x	
G. callidus	31	14.150 ± 1.364	10.257 - 15.458	x		
L. lithognathus	14	16.204 ± 0.812	14.329 - 17.176		x	
P. commersonnii	9	15.666 ± 0.516	14.643 - 16.365	x	x	
A. japonicus	10	17.849 ± 0.484	16.269 - 19.560			x
E. machnata	1	17.638				x
L. amia	2	18.0 ± 0.012	17.992 - 18.008			x

3.3.2 West Kleinemonde Estuary

All plant species that were collected from more than one season showed significant seasonal variation in δ^{15}N beyond the 3.4 ‰ value of enrichment (Table 5.7). In the submerged macrophytes, summer 2008 values were significantly depleted when compared to those of later seasons, while the reed *P. pectinatus* showed a seasonal change of > 12 ‰ between summer and winter of 2008. Apart from the depleted summer 2008 values, the δ^{15}N of all submerged plant species were similar.

Significant seasonal variation in δ^{15}N was found in only a few of the fish species sampled in the West Kleinemonde (see below), so this aspect was not pursued with further analyses and species-specific average δ^{15}N representing all seasons were used. As in the East Kleinemonde, statistically significant differences in δ^{15}N with season were found for both *G. aestuaria* and *M. capensis*, as well as for *R. holubi* (Kruskal-Wallis, $P < 0.05$) (Table 5.7). In the case of *G. aestuaria*, this difference was also ecologically significant, with the summers being significantly different to each other but similar to winter 2008 ($P = 0.0001$). For both *M. capensis* and *R. holubi* the summer 2008 and summer 2009 δ^{15}N values were significantly different, with winter 2008 also being significantly different from summer 2009 for *M. capensis* ($P = 0.001$) but similar to both summer 2008 and summer 2009 for *R. holubi* ($P = 0.0001$).

Table 5.7 Average (± SD) δ^{15}N (‰) values for plants and fish species from the West Kleinemonde Estuary over three sampling periods. Sample size for each sampling period is indicated by 'n'. Statistically significant seasonal differences are indicated by a single asterisk (One-way ANOVA or Mann-Whitney U, $P < 0.05$), with ecological significance ($\Delta\delta^{15}$N > 3.4 ‰) being denoted by two asterisks (U = upper reaches, M = middle reaches and L = lower reaches of the estuary)

					δ^{15}N (Avg ± SD)			
Species	Region	n	Summer '08	n	Winter '08	n	Summer '09	Significance
Phragmites australis	U	3	7.551 ± 0.451	3	-5.072 ± 0.387	3	4.657 ± 0.184	**
Ruppia cirrhosa	M	3	4.271 ± 0.150	3	11.233 ± 0.239	3	9.628 ± 0.363	**
Potamogeton pectinatus	U	3	5.634 ± 0.123	3	9.197 ± 0.187	3		**
Green epiphytes	U					1	12.280	
Brown epiphytes	L					1	9.817	
Liza dumerili		6	13.246 ± 0.563	9	13.065 ± 0.843	0		
Liza richardsonii		5	10.428 ± 1.158	0		0		
Myxus capensis		10	15.467 ± 0.661	16	14.590 ± 1.249	9	13.898 ± 0.633	*
Rhabdosargus holubi		15	15.056 ± 1.102	10	14.441 ± 1.280	15	14 ± 0.816	*
Atherina breviceps		15	13.856 ± 0.767	15	13.704 ± 1.331	15	13.499 ± 0.809	
Gilchristella aestuaria		15	15.106 ± 0.661	12	14.563 ± 0.969	15	13.465 ± 0.853	**
Monodactylus falciformis		8	16.079 ± 1.544	11	13.834 ± 2.079	13	14.849 ± 0.707	
Glossogobius callidus		10	12.945 ± 1.916	8	13.083 ± 2.192	8	12.771 ± 0.912	
Lithognathus lithognathus		1	12.877	3	13.022 ± 0.855	1	12.286	
Pomadasys commersonnii		0		0		0		
Argyrosomus japonicus		8	16.117 ± 0.731	0		2	17.087 ± 0.616	
Elops machnata		2	16.359 ± 0.054	5	16.843 ± 0.127	0		
Lichia amia		7	14.847 ± 1.321	0		0		

The δ^{15}N values for West Kleinemonde fish showed a wider distribution (species average range > 6 ‰) than in the East Kleinemonde Estuary and were more depleted (Table 5.8). Four distinctive homogenous groups were identified by a Newman-Keuls multiple range test ($P < 0.05$) following a one-way ANOVA. *L. richardsonii* had the lowest average δ^{15}N (10.428 ‰), a value that was quite separate from the other fish species. The piscivores *A. japonicus* and *E. machnata* with more enriched muscle δ^{15}N values formed a separate group. The piscivore *L. amia* was grouped together with the zooplanktivores *A. breviceps, G. aestuaria* and *M. falciformis*, the omnivore *R. holubi* and the detritivore *M. capensis*. The final group included *L. lithognathus, G. callidus, A. breviceps* and *L. dumerili*, with *A. breviceps* being the only fish species that was part of more than one group. A range of δ^{15}N values was characteristic of almost all the fish species sampled, with that of *G. callidus*, for example, being 9.630 to 16.005 ‰ (Table 5.8).

Table 5.8 Information of the δ^{15}N (‰) values of fish from the West Kleinemonde Estuary during summer 2008 to summer 2009. Sample size for each species is indicated by ‘n’ and homogenous groupings, as indicated by the Newman-Keuls multiple range test ($P < 0.05$), are also included

	n	δ^{15}N (Avg ± SD)	Range	Groups			
L. dumerili	15	13.137 ± 0.726	11.780 - 14.242		x		
L. richardsonii	5	10.428 ± 1.158	9.091 - 11.764				x
M. capensis	35	14.663 ± 1.116	11.711 - 16.237	x			
R. holubi	40	14.506 ± 1.126	12.195 - 16.908	x			
A. breviceps	45	13.687 ± 0.990	11.249 - 15.155	x	x		
G. aestuaria	42	14.365 ± 1.076	12.419 - 15.890	x			
M. falciformis	32	14.808 ± 1.697	10.775 - 17.716	x			
G. callidus	26	12.934 ± 1.707	9.630 - 16.005		x		
L. lithognathus	5	13.131 ± 0.901	12.267 - 13.951		x		
P. commersonnii	1	13.674					
A. japonicus	10	16.311 ± 0.791	14.643 - 17.522			x	
E. machnata	7	16.562 ± 0.496	16.321 - 17.044			x	
L. amia	7	14.744 ± 1.393	12.883 - 16.235	x			

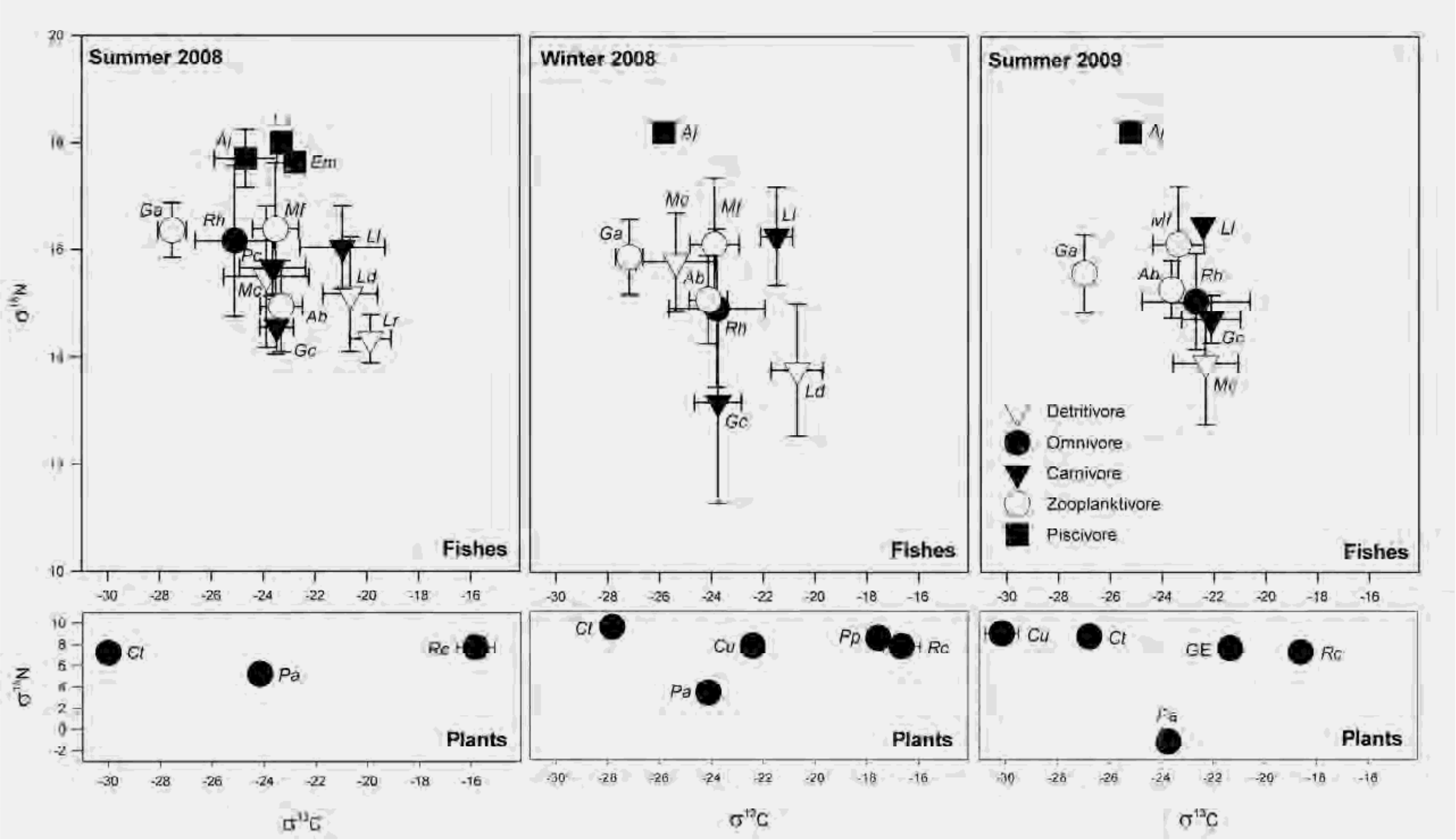

Figure 5.1 Seasonal δ^{13}C (‰) and δ^{15}N (‰) values of plants and fishes in the East Kleinemonde estuary. Values are averages with error bars indicating standard deviation. Abbreviated species names are as follows: Plants Ct = *C. tenue*, Cu = *C. ustulatus*, GE and BE = green and brown epiphytic algae respectively, Pa = *P. australis*, Pp = *P. pectinatus* and Rc = *R. cirrhosa*. Fishes Lr = *L. richardsonii*, Ld = *L. dumerili* Mc = *M. capensis*, Gc = *G. callidus*, Ll = *L. lithognathus*, Pc = *P. commersonnii*, Ab = *A. breviceps*, Ga = *G. aestuaria*, Mf = *M. falciformis*, Rh = *R. holubi*, La = *L. amia*, Aj = *A. japonicus* and Em = *E. machnata*

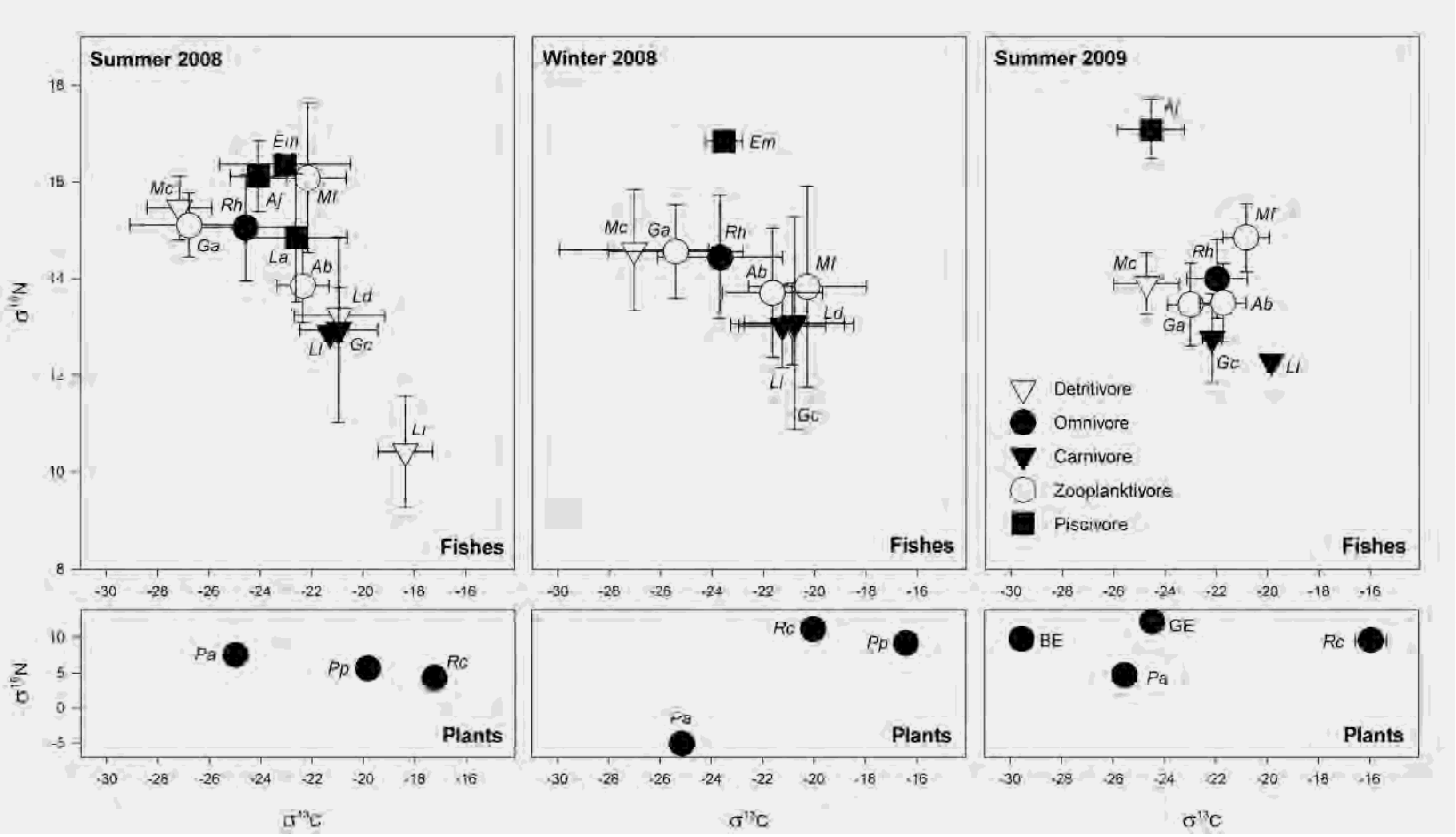

Figure 5.2 Seasonal δ^{13}C (‰) and δ^{15}N (‰) values of plants and fishes in the West Kleinemonde estuary. Values are averages with error bars indicating standard deviation. Abbreviated species names are as follows: Plants Ct = *C. tenue*, Cu = *C. ustulatus*, GE and BE = green and brown epiphytic algae respectively, Pa = *P. australis*, Pp = *P. pectinatus* and Rc = *R. cirrhosa*. Fishes Lr = *L. richardsonii*, Ld = *L. dumerili* Mc = *M. capensis*, Gc = *G. callidus*, Ll = *L. lithognathus*, Pc = *P. commersonnii*, Ab = *A. breviceps*, Ga = *G. aestuaria*, Mf = *M. falciformis*, Rh = *R. holubi*, La = *L. amia*, Aj = *A. japonicus* and Em = *E. machnata*

4. DISCUSSION

4.1 Spatial variation

Although several studies have shown that estuarine plants display spatial variation in their isotopic composition (Boon & Bunn, 1994; Deegan & Garritt, 1997; Guest *et al*., 2004), such trends within consumer tissues may however be confounded by several factors. For example, resident species may not feed along the whole length of an estuary, and hence rely heavily upon locally (point source) produced organic matter (Deegan & Garritt, 1997; Fry, 2002). Conversely, for mobile species, the signature of plants collected at a specific site may not represent that of the same plant at a different site where feeding takes place. This, combined with the time-integrated nature for vertebrate tissues to reflect new $\delta^{13}C$, could influence the interpretation of spatial variability (Vander Zanden *et al*., 1998). Despite the uncertainties of determining spatial variability, the findings of this study indicated that there was little or no spatial variation in the isotope ratio of fishes collected throughout the East Kleinemonde and West Kleinemonde estuaries.

There is evidence, however, to suggest that different species within the same trophic guild use different carbon sources. For example, the difference in $\delta^{13}C$ between that of *M. capensis* and the other mullet species sampled suggests that it utilizes a carbon source that is more depleted than that of the other species. This is not a unique result (Mbande *et al*., 2004) and *M. capensis* is often associated with the upper reaches of estuaries where riverine influences are strong (Bok, 1984). There is a possibility that in these areas *M. capensis* utilizes a carbon source that was not sampled and is different to sources available to fishes in the middle and lower reaches (Mbande *et al*., 2004). Similarly, the divergence of $\delta^{13}C$ signals from *G. aestuaria* when compared to *A. breviceps* and *M. falciformis* (Tables 5.3 and 5.4) probably indicates that the former species obtains its carbon from a more depleted source than that utilized by the other zooplanktivores.

4.2 Temporal variation

There was marked seasonal variation in $\delta^{13}C$ values for the plants in both estuaries, as well as $\delta^{15}N$ values in the West Kleinemonde Estuary. Where samples were collected during all three sampling periods, the most dramatic changes occurred between the two summer seasons, while the winter 2008 values were generally intermediate (Tables 5.3 and 5.4, and 5.7). The emergent reed (*P. australis*) showed the lowest variability in $\delta^{13}C$ between sampling periods, with a range of 0.4 ‰ in the East Kleinemonde Estuary and 0.5 ‰ in the West Kleinemonde Estuary. All of the other plant species collected during more than one sampling period showed change that was greater than the trophic level 1 ‰ degree of enrichment for ^{13}C and therefore were of ecological significance (DeNiro & Epstein, 1978). Values for the submerged alga (*C. ustulatus*) revealed the greatest $\delta^{13}C$ change, with a depletion of > 7 ‰ between the winter 2008 and summer 2009, in the East Kleinemonde Estuary. Patterns of spatial and temporal variation therefore appeared to vary between plant groups (e.g. epiphytes and macrophytes), and between species within a group, as recorded by Boon & Bunn (1994).

Several other studies have reported a similar high degree of spatio-temporal variability in carbon and nitrogen isotopic ratios for plants. For example, Boon & Bunn (1994) recorded variation of > 10 ‰ in C and N ratios within single species collected at different times of the year and from different sites within Australian floodplain lakes (billabongs). Similarly, Lowdon & Dyck (1974, in DeNiro & Epstein, 1978) found a difference of more than 5 ‰ during the growth season in maple leaves at one locality in Canada. These findings emphasize the importance of collecting samples from both producers and consumers at the same time when attempting to interpret trophic relationships.

Variation in isotope ratios of aquatic plants has been linked to changes in physical conditions with season. An increase in irradiance in summer for example, has commonly been reported as a reason for temporal variation in carbon ratios (Durako & Hall, 1992; Grice *et al.*, 1996). The volume of freshwater inflow may influence isotopic ratios of carbon in an estuary, and this in turn affects plant $\delta^{13}C$ (Paterson & Whitfield, 1997). Nitrogen availability determines the degree of fractionation that occurs; where it is limiting all the available nitrogen is taken up by plants. Consequently, very little to no fractionation takes place and plants have an isotopic composition very similar to that of atmospheric N_2 (Peterson & Fry, 1987).

Limited patterns of seasonal variability in $\delta^{13}C$ were found in some of the fish species sampled. Two species, namely *G. callidus* and *M. capensis* showed statistically and ecologically significant variation with season in both estuaries, with this variation also shown in *A. breviceps* and *L. richardsonii* in the East Kleinemonde Estuary (Table 5.3), and *G. aestuaria* and *R. holubi* in the West Kleinemonde Estaury (Table 5.4). However, most fish species showed limited seasonal variability in $\delta^{13}C$ and this can be ascribed to a number of reasons, most importantly the rate of tissue turnover in fishes. Several authors (*inter alia* Tieszen *et al.*, 1983 and Michener & Schell, 1994) have shown that the delay between a change in isotope value of a food source being reflected in the tissue of the consumer (as well as its persistence in the tissue) depends on the turnover rate of that tissue, with the more metabolically active tissues turning over more quickly. Different tissues will therefore reflect dietary intake over different time periods (Dalerum & Angerbjörn, 2005). Greater temporal variation is commonly reported in groups that have high turnover rates such as invertebrates (Goering *et al.*, 1990; Kang *et al.*, 1999) and fish with a short lifespan, which are generally small in size (Maruyama *et al.*, 2001; Vizzini & Mazzola 2002; Vizzini & Mazzola 2003). Of the three small-bodied fish species sampled during this study (i.e. *A. breviceps*, *G. aestuaria* and *G. callidus*) two showed marked seasonal variation in $\delta^{13}C$ in the East Kleinemonde Estuary (*A. breviceps* and *G. callidus*, Table 5.3) and two in the West Kleinemonde Estuary (*G. aestuaria* and *G. callidus*, Table 5.4). Richoux and Froneman (2007) found a seasonal change in carbon isotope signatures in plants was mirrored by a change in $\delta^{13}C$ for five species of invertebrate consumers in the permanently open Kariega Estuary.

Vertebrates generally though have low turnover rates, with the result that their isotopic signatures are integrated over a longer time period than that of plants or invertebrates

(Hesslein *et al.*, 1993; Vander Zanden *et al.*, 1998). Seasonal changes in plants, which occur over relatively short time periods, will therefore not always be mirrored in all fish species. Furthermore, there is evidence to suggest that fishes have discontinuous growth patterns (Perga & Gerdeaux, 2005) and do not lay down muscle tissue year-round. As fish are ectotherms, flesh is only laid down during the growth seasons of an individual and not as metabolic replacement (Maruyama *et al.*, 2001; Perga & Gerdeaux, 2005). However, Blaber (1974b) noted that although maximum growth rate of *R. holubi* in the West Kleinemonde Estuary occurs during summer, growth slows but does not cease during the winter months. Therefore the lack of seasonal changes in some fish species is probably because, on an annual basis, the isotopic ratio of flesh closely reflects diet during the summer months.

In terms of δ^{15}N, the general absence of a seasonal pattern was even more marked in the fishes. In the East Kleinemonde Estuary, three species (*G. aestuaria*, *M. capensis* and *L. richardsonii*) showed significant seasonal variation but these changes were within the 3.4 ‰ enrichment with trophic level and so were not considered to be of ecological importance (Table 5.5). Statistically significant seasonal variation was shown in three species (*R. holubi*, *M. capensis* and *G. aestuaria*) in the West Kleinemonde Estuary, but an ecologically significant change was only observed for *G. aestuaria* (Table 5.7). The lower number of ecologically significant results for seasonal variation in nitrogen isotope ratios compared to carbon may reflect a consistent diet of most of the sampled fish species. It might also be due to the larger degree of enrichment with trophic level for nitrogen (3.4 ‰ compared to 1 ‰), which allows for greater variation in isotopic ratios without these being ecologically significant. Additionally, the higher seasonal variation for carbon in both estuaries could indicate that although fishes are utilizing different sources of carbon with time, they are still feeding within their respective functional trophic guilds.

4.3 Flow of carbon

The use of isotopes in estuarine studies faces a number of limitations linked to the dominance within these food webs by generalist omnivores (Richoux & Froneman, 2008), reliance on a detritus-based food web which by its very nature is variable, and the wide variety of potential carbon sources available (Kreeger & Newell, 2000 in Richoux & Froneman, 2007). The estuarine carbon pool has inputs from a wide range of sources, including both plant and animal material. The plant matter may be from autochthonous production as well as allochthonous sources from the marine, freshwater and terrestrial environments, the relative contribution of which changes with time (Mbande *et al*., 2004), and physical conditions such as freshwater inflow and estuary mouth phase (Nozais *et al*., 2001; Froneman, 2002). Therefore estuarine consumers may be assimilating a wide range of food sources with separate and unknown carbon and nitrogen isotopic signatures. Secondly, detritus itself, as well as being a blend of organic matter from disparate sources, has associated colonies of microbes and fungi responsible for its degradation. Added to this is the largely unknown effect that the process of degradation has on the isotopic ratios within plant material.

Detrital $\delta^{13}C$ is generally assumed to reflect that of its composite fractions (Haines & Montague, 1979; Fry & Sherr, 1984; Zieman *et al*., 1984; Mann, 1988; Machás *et al*., 2006). For example, in the Kariega Estuary, detritus collected in a salt marsh was more enriched and similar to the $\delta^{13}C$ of the salt marsh plant *Spartina maritima*, when compared to detritus collected in the estuary channel which would have included more depleted sources such as phytoplankton (Paterson & Whitfield, 1997). Similarly, POM $\delta^{13}C$ in the Mngazana Estuary very closely resembled that of the mangrove plants from which it was probably largely derived (Mbande *et al*., 2004). Sediment detritus samples from the Gamtoos Estuary, which has no salt marshes or mangroves, closely resembled the isotopic composition of fringing reed vegetation (Schlacher & Wooldridge, 1996).

The effects of microbial degradation on stable isotope ratios of carbon and nitrogen in source material have received considerable research attention. Fellerhoff *et al*. (2003)

experimentally determined changes in stable isotope ratios of carbon and nitrogen in a range of macrophytes undergoing decomposition. Heterotrophs did not change macrophyte $\delta^{13}C$ during this process but increased the variability of $\delta^{15}N$ by about 6 ‰. Similarly, microbial action substantially increased $\delta^{15}N$ of terrestrial material in the Hudson River Estuary, USA (Caraco *et al.*, 1998) and caused a marked reduction in $\delta^{15}N$ but not $\delta^{13}C$ in mangrove material (Zieman *et al.*, 1984) and in standing dead *Spartina alterniflora* tissue (Currin *et al.*, 1995). Other studies that recorded a significant change in plant $\delta^{15}N$ with decomposition include Caraco *et al* (1998), Fourqurean and Schrlau (2003), Hill and McQuaid (2009) and under anoxic laboratory conditions by Lehmann *et al*. (2002). Little change was recorded in either $\delta^{13}C$ or $\delta^{15}N$ in seagrasses (Zieman *et al.*, 1984), *Zostera noltii* (Machás *et al.*, 2006), *Spartina alterniflora* (Haines, 1977) and other salt marsh plants (Haines & Montague, 1979). Degradation-related changes in $\delta^{13}C$ were found in both seagrasses and mangrove leaves (Fourqurean & Schrlau, 2003), in seagrass and macroalgae (Fenton & Ritz, 1988), macroalgae (Hill & McQuaid, 2009), diatoms (Lehmann *et al.*, 2002) and *Spartina alterniflora* (Peterson *et al.*, 1980; Benner *et al.*, 1991).

Suggested reasons for the above variation in results following degradation include the preferential loss of ^{13}C isotopically heavier polysaccharides when compared to lignin (Benner *et al.*, 1991), different modes of decomposition between plant species (Zieman *et al.*, 1984), and different starting fractions of labile or refractory material (Roman & Tenore, 1984; Fourqurean & Schrlau, 2003; Machás *et al.*, 2006;). The process of degradation itself may be affected by physical conditions such as temperature (Roman & Tenore, 1984), which varies seasonally, as well as available oxygen (Lehmann *et al.*, 2002) and other factors.

The role of decomposing microbes and fungi in the flow of energy through aquatic food webs is twofold. Firstly, degradation of refractory material changes its composition, thus making it available to consumers (Roman & Tenore, 1984; Norderhaug *et al.*, 2003). Secondly, the microbes themselves may serve as an important food source (Hill &

McQuaid, 2009), supplying a high proportion of the nitrogen requirements of detritivorous consumers (Newell *et al*., 1982; Currin *et al*., 1995; Caraco *et al*., 1998).

Despite the limitations of using an isotopic approach to study estuarine food webs, the information from the East Kleinemonde and West Kleinemonde estuaries does allow us to infer a number of things. Firstly, the submerged macrophytes *R. cirrhosa* and *P. pectinatus* do not appear to be a direct food source for any of the fish species sampled. Both macrophyte species are distinctive from the other estuarine plants collected, having $\delta^{13}C$ values throughout all seasons that are very enriched relative to those of the other plants, while fish muscle $\delta^{13}C$ values are more depleted. The only fish species that shows a $\delta^{13}C$ within the macrophyte range is *L. richardsonii*, but this was only observed in the West Kleinemonde Estuary during summer 2008 (Table 5.4).

Field observations made during this study revealed that the stomachs of *R. holubi* consistently contained remains of the most abundant macrophyte at the time, thus showing that this material is directly grazed. The species however lacks a cellulase in its digestive system and so only the epiphytic diatoms and other algae with fragile cellular walls can be digested, with the macrophytic component passing through the gut undigested (Blaber, 1974a; de Wet & Marais, 1990). The $\delta^{13}C$ of *R. holubi* muscle tissue was within the range of epiphytic algae, taking into account its spatial variation (Tables 5.3 and 5.4). The importance of epiphytic algae as a food source is well recognized (e.g. Sullivan & Moncreiff, 1990) and is also widely consumed by invertebrates (Kitting *et al*., 1984; Vizzini & Mazzola, 2003; Henninger *et al*., 2009).

Based on the above discussion, the contribution by macrophytic ^{13}C to fish consumers must be via a detrital pathway. Unfortunately, such an indirect contribution to the diet of consumers is difficult to measure as the effects of microbes and fungi on the isotope signature of detrital matter is largely unknown, as is the identity and relative contribution of different sources to the detrital pool.

The $\delta^{13}C$ values of fishes in both the East Kleinemonde and West Kleinemonde estuaries formed a continuum rather than distinct separate groupings, which is in agreement with the findings of Mbande *et al*. (2004) regarding fish assemblages in the Mngazi and Mngazana estuaries. The overlap in carbon resources for estuary-associated fishes in South African estuaries is probably linked to a direct or indirect reliance on detritus that is derived from multiple sources. Branch & Branch (1981) estimated that more than 80% of all estuarine invertebrates in South African estuaries are detritus feeders, including *Callianassa kraussi* that is a common prey item of some fish species in this study. Indeed, detritus has long been recognized as being the major pathway of energy flow in estuarine systems (Odum, 1971; Marais, 1984; Mann, 1988; Bennett, 1989a), with a high proportion of all estuarine consumers being detritivores.

The isotopic signatures of detritus are largely determined by the contributing sources and their proportions (Hill & McQuaid, 2009). It is well-established that macrophytes contribute significantly to the detrital pool of estuaries in which they are present (Carr & Adams, 1973; Branch & Grindley, 1979; Vizzini *et al*., 2002). The enriched submerged macrophyte $\delta^{13}C$ may change during breakdown such that detrital matter with a high percentage contribution of this material will not exactly match that of the fresh plants, but instead be similar to that of organisms consuming it.

Support for the presence of submerged macrophytic material in the detrital pool of the East Kleinemonde and West Kleinemonde estuaries may be found in a comparison of the range of $\delta^{13}C$ values in the fish species sampled. The $\delta^{13}C$ from the same fish species in each estuary indicated that those in the West Kleinemonde Estuary were more enriched, suggesting that the detritus had a higher contribution by a carbon-enriched component. During this study, the West Kleinemonde Estuary had extensive macrophyte beds, with expected relative contribution to detrital matter by macrophytes therefore being high. Mbande *et al*. (2004) reached a similar conclusion after recording more depleted carbon signatures in fishes from a mangrove-dominated estuary (Mngazana Estuary) when compared to more enriched signatures in one without mangroves (Mngazi Estuary) due to the input of mangrove material to the detrital pool.

The more depleted carbon isotope values of fishes from the East Kleinemonde Estuary probably reflect a high contribution to the POM by an alternative carbon source. Microalgae, including phytoplankton and microphytobenthic algae, have been shown to contribute significantly to total production within estuaries (Kitting *et al*., 1984) and arguments have been presented for it being the primary source of carbon in estuarine food webs (Peterson *et al*., 1986; Gearing, 1988 in Schlacher & Wooldridge, 1996). Due to the large area and volume they occupy, phytoplankton usually contribute more than macrophytes to the overall primary production of an estuary (Adams *et al*., 1999). In addition, benthic microalgae have been shown to be an important food source for Mugilidae in South African estuaries (e.g. Masson & Marais, 1975) thus further entrenching the role of algae as a carbon source for fishes.

Although not directly measured in this study, carbon isotope values for phytoplankton in Eastern Cape estuaries are generally depleted in ^{13}C. For example, POC values measured in the Sundays Estuary were in the range of -27 to -29.5 ‰ (Jerling & Wooldridge, 1995a), suspended particulate matter δ^{13}C in the Gamtoos Estuary was -31.2 ‰ (Schlacher & Wooldridge, 1996) and the 10-20 μm component of the suspended particulates within the Kariega Estuary was -24.6 ‰ (Paterson & Whitfield, 1997). According to Adams *et al*. (1999) phytoplankton production within Eastern Cape TOCEs is on average low, but may fluctuate widely (Perissinotto *et al*., 2000). Furthermore, TOCE values are generally below that of permanently open estuaries which tend to exceed 15 mg m^{-3} and may even attain > 100 mg m^{-3} at times (Adams *et al*., 1999). Chlorophyll *a* concentrations in the East Kleinemonde Estuary have been found to be low, with Gama (2008) recording a maximum concentration of 17.13 μg L^{-1} or 17.13 x 10^{-4} mg m^{-3}. Low concentrations (1.13 – 2.12 mg.m^{-3}) have also been recorded in the permanently open Kariega Estuary (Froneman 2000b), while the Nyara, Mpenjati and Mdloti TOCEs had maximum phytoplankton biomass levels of 1.3 mg.m^{-3}, 15.4 mg.m^{-3} and 8.4 mg.m^{-3}, respectively (Perissinotto *et al*., 2003).

Production by phytoplankton is generally dominated by the smaller size classes (< 20 μm) (Perissinotto *et al.*, 2002). Measurements in the East Kleinemonde Estuary four weeks after a mouth opening event (with five to six weeks estimated as being necessary for a buildup to maximum phytoplankton biomass) indicated that of the different size classes, the pico- (1.2 – 2.7 μm) and nanophytoplankton (2.7 – 20 μm) dominated total water column chlorophyll *a*, contributing 27 and 67%, respectively (Whitfield *et al.*, 2008). Large zooplankton such as mysids show reduced feeding efficiency on phytoplankton cells < 20 μm (Jerling & Wooldridge, 1995b; Froneman, 2000b). Much of this production would thus enter the microbial loop where, as with other POM material, the effects of degradation on isotope signatures are uncertain. Phytoplankton, due to its fluctuating abundance, generally low concentrations and dominance by small size classes, is thus unlikely to be an important direct food source in the East Kleinemonde Estuary.

Microphytobenthic algae have been recognized as an important food source in TOCEs (Masson & Marais, 1975; Perissinotto *et al.*, 2000; Perissinotto *et al.*, 2003). They generally have biomass values 2 – 3 orders of magnitude higher than phytoplankton (Adams *et* al., 1999; Nozais *et al.*, 2001; Perissinotto *et al.*, 2003) with limited seasonality when compared to phytoplankton. Benthic algae in TOCEs are not light limited because these systems are typically shallow with the euphotic depth exceeding total depth, with water currents and turbidity low during the dominant closed phase (Perissinotto *et al.*, 2000). Furthermore, unlike phytoplankton cells, which face nutrient deficiency during periods of mouth closure, the microphytobenthos obtain nutrients from a large pool present within the substratum (Nozais *et al.*, 2001; Perissinotto *et al.*, 2003). Microphytobenthic algae are therefore less vulnerable than phytoplankton to changing physical conditions related to the open and closed TOCE phases (Nozais *et al.*, 2001), although they do seem to show maximum biomass values that coincide with closed periods in some systems (Nozais *et al.*, 2001; Perissinotto *et al.*, 2006). During the day especially, when vertically-migrating zooplankton are found in close association with the substratum (Perissinotto *et al.*, 2000), microphytobenthic algae have been shown to be an important food source (Perissinotto *et al.*, 2003).

Assuming contributions from both carbon-depleted sources such as microalgae or other estuarine plants, and submerged macrophytes, the following fish species have δ^{13}C values that resemble detritus in the East Kleinemonde and West Kleinemonde estuaries (ranges reported are over all seasons and from both estuaries): *A. breviceps*, *G. callidus*, *L. lithognathus*, *L. dumerili*, *L. richardsonii*, *M. falciformis* and *P. commersonnii*. Both *A. breviceps* (-24.1 to -21.1 ‰) and *M. falciformis* (-23.9 to -20.3 ‰) are zooplanktivores (Bennett, 1979; Blaber, 1979; Coetzee, 1982b), whose prey feed directly on detritus. *G. callidus* (-23.7 to -20.8 ‰), *P. commersonnii* (-23.6 ‰) and *L. lithognathus* (-22.4 to -19.9 ‰) are benthic carnivores feeding on invertebrates that consume detritus. Lastly, *L. richardsonii* (-19.9 to -18.3 ‰) and *L. dumerili* (-20.9 to -20.6 ‰), which were the most enriched δ^{13}C of all the fish species studied are detritivores that feed directly on this material (van der Elst, 1993).

It must be stated that there is always a degree of uncertainty when working with isotopes. When more than two possible food sources exist it is impossible to make conclusions about the relative contribution of carbon sources to the diet of consumers without the use of mixing models (Phillips & Gregg, 2003; Phillips *et al*., 2005), although such models often do not adequately address the uncertainty associated with multiple sources and fractionation (Moore & Semmens, 2008). The observed δ^{13}C of the fish muscle samples could in fact reflect the importance of a single source that has not been measured. Because no allochthonous sources were considered in this study, further investigation that includes a wider range of sources may reveal more distinctive patterns of feeding. Allochthonous carbon could be important to localized food webs within estuaries and includes marine material such as wrack but also marine and freshwater phytoplankton, river-borne terrestrial detritus (Mann, 1988), wind-borne terrestrial plant material and faecal matter from birds and mammals.

4.4 Trophic structure

The inclusion of additional elements, such as nitrogen and sulphur in stable isotope analysis provides information at a higher resolution, and therefore offers an opportunity to validate food source utilization indicated by $\delta^{13}C$ alone (Peterson & Fry, 1987; Peterson, 1999). Unfortunately, the nitrogen isotope ratios of the suite of submerged plants collected from the East Kleinemonde, in particular, and West Kleinemonde estuaries were very similar (Tables 5.5 and 5.7). Consequently, it was not possible to distinguish between the relative contributions of different submerged estuarine plants to fish $\delta^{15}N$. Similarly, Smit *et al*. (2006) found the range of nitrogen isotope signatures of potential food items at the trophic base to be too small to make it a useful tracer of pathways of nitrogen flow in a Western Australian seagrass bed.

The only plant species with a distinctive $\delta^{15}N$ was the reed *P. australis*, which is semi-terrestrial and has its roots in fresh water. Although there are other sources of variation in plant $\delta^{15}N$ (Handley & Raven, 1992), these results probably indicate that *P. australis* utilizes a different nitrogen source from the submerged plants which all have similar signatures and are likely to be sharing the same source (Richoux & Froneman, 2007). Consequently, analysis of fish $\delta^{15}N$ can indicate whether nitrogen is being obtained from this emergent producer or the submerged plants, but cannot distinguish between the relative contributions of different submerged estuarine plants. This evaluation was further complicated in the West Kleinemonde Estuary where *P. australis* $\delta^{15}N$ was similar to that of the submerged plants. However, in the East Kleinemonde the fish reflected an enriched $\delta^{15}N$ source, which rules out *P. australis* as being a likely food source, assuming an enrichment of 3.4 ‰ per trophic level (DeNiro & Epstein, 1980) and analyzing change in $\delta^{15}N$ of up to two trophic levels for all fish species.

Nitrogen becomes enriched as it moves through the trophic levels, hence top predators should have the most enriched $\delta^{15}N$ values with lower trophic groups having increasingly depleted values. In both estuaries, the piscivores *A. japonicus* and *E. machnata*, as well as *L. amia* in the East Kleinemonde had the most enriched nitrogen isotopic ratios (Figures

5.1 and 5.2). The species with most depleted ratios included the detritivorous mugilid species, especially *L. richardsonii*, and the goby *G. callidus*.

Three homogenous groups of fish $\delta^{15}N$ were identified in the East Kleinemonde Estuary and four in the West Kleinemonde Estuary. There was a high degree of overlap of species between groups, excluding the piscivores in the East Kleinemonde Estuary, while the grouping was more distinctive in the West Kleinemonde Estuary (Tables 5.6 and 5.8). Within these groups, individual species showed large variation in nitrogen signatures. Certain species, for example *G. callidus* in both estuaries showed a range of $\delta^{15}N$ that encompassed one to 1.5 changes in trophic structure (Tables 5.6 and 5.8). Such variability is probably due to the complex nature of detritus that includes material from a wide range of sources together with associated micro-organisms (Richoux & Froneman, 2007), as well as the high degree of recycling that takes place in a detritus-driven food web, thus making interpretation of trophic information very difficult.

The range of average fish $\delta^{15}N$ values was narrower in the East Kleinemonde Estuary (about 4 ‰) than in the West Kleinemonde Estuary (about 6 ‰) (Tables 5.6 and 5.8). This, together with the overlap between species in homogenous groups, perhaps reflects a tighter clustering around similar or limited food sources within the East Kleinemonde Estuary. In the West Kleinemonde Estuary, it is possible that the greater diversity of available food sources allows for food item selection and trophic level expansion rather than an individual simply feeding on whatever is available at the time. Despite some separation (e.g. in the piscivores), the nitrogen isotope signatures of fishes in both estuaries showed a continuum rather than clear distinctions between trophic levels. A similar pattern was found by Richoux and Froneman (2007) with invertebrates in the Kariega Estuary and was ascribed to the presence of complex trophic pathways. Kaehler *et al*. (2000), on the other hand, attributed a similar pattern to a high degree of omnivory amongst marine consumers in the food web of the Prince Edward Islands. Richoux and Froneman (2008), using fatty acids, identified a dominance of generalist omnivores within the Kariega Estuary ecosystem and Alfaro *et al*. (2006) found that within a range of estuarine consumers (invertebrates and fish) none was dependent on a single food source in a New Zealand mangrove/seagrass estuary. In estuarine systems in general, indistinct groupings of nitrogen isotope signatures may be due to the dependence of consumers on the detrital food chain (Carr & Adams, 1973; Branch & Grindley, 1979; Hanekom & Baird, 1984; Vizzini *et al.*, 2002) and the high degree of interconnectedness between components (Marais, 1984; Heymans & Baird, 1995; Vizzini & Mazzola, 2003).

Authors using isotopes in estuarine and coastal studies have found $\delta^{15}N$ to be an unreliable source of information for identifying food sources. It does, however, have value in identifying the trophic position of fish consumers, as evidenced in this study. However, ranges of nitrogen isotopes are large within specimens of a single species, and signatures have often been found to overlap between consumers, thus reducing its value in trophic studies (Handley & Raven, 1992; Kaehler *et al.*, 2000; Smit *et al.*, 2006; Richoux & Froneman, 2007). Values of $\delta^{13}C$ are therefore the most commonly-used isotope in studies to identify the food sources of aquatic invertebrates and fishes (e.g. Fry & Parker, 1979; Fry *et al.*, 1982; Paterson & Whitfield, 1997; Froneman, 2001, 2002c; Mbande *et al.*, 2004).

5. CONCLUSION

The potential effects of spatial and temporal variability on isotopic ratios of carbon and nitrogen are often overlooked. Results of this and other studies, however, suggest that variability with season or sampling locality can be significant, particularly in plants, and can lead to a misinterpretation of trophic relationships. This highlights the importance of collecting both producer and consumer material from the same locality and at the same time when conducting isotope studies.

Carbon isotope signatures of fish consumers in the East and West Kleinemonde estuaries reflected the dominant input from available/abundant carbon sources in each system. Fish $\delta^{13}C$ in the West Kleinemonde Estuary indicates an increased influence of ^{13}C-enriched material derived from submerged macrophytes, while in the East Kleinemonde Estuary a greater contribution was derived from a more depleted source, probably microphytobenthic algae. Although macrophytes are not a direct food source, species such as *R. cirrhosa* and *P. pectinatus* make an important contribution to the carbon pool in TOCEs where they are abundant, with detritus being the probable trophic route to higher consumers.

Nitrogen isotope ratios generally showed high intra-species, and low inter-species variability within both plant and fish tissues. Such variability renders this element largely unsuitable for studies in detritus-based ecosystems such as estuaries, where original sources are indistinct and influenced by a high degree of recycling. The inclusion of other elements such as sulphur could increase the resolution of trophic studies within TOCEs, thereby promoting a greater understanding of the energy flow pathways within these estuarine systems.

CHAPTER SIX: GENERAL DISCUSSION AND CONCLUSIONS

According to current understanding, the value of submerged macrophyte beds to fishes within estuarine systems lies in their ability to provide refuge and foraging areas (Carr & Adams, 1973; Adams, 1976). These beds form a habitat that is structurally complex, thus providing small fishes and invertebrates shelter from predation (Adams *et al*., 2004; Henninger *et al*., 2009) as well as providing a large surface area for the attachment of epiphytes and invertebrates (Dame, 2008). Furthermore, submerged macrophytes slow the flow of water within estuaries (Carr & Adams, 1973), thereby improving their suitability as a nursery area for juveniles of a variety of fish species (Day, 1951; Whitfield *et al*., 1981; Day *et al*., 1989; Harrison, 2003). Fishes utilizing estuaries thus benefit both directly and indirectly from the presence of submerged macrophytes.

Results of this study, in brief, seem to suggest that (i) estuarine mouth conditions (i.e. recruitment opportunities) are more important in terms of structuring fish assemblages within temporarily open/closed estuaries (TOCEs) than the presence or absence of submerged macrophytes, and (ii) that although submerged macrophytes contribute to the detrital pool of a system in which they are abundant, these plants are not themselves utilized as a direct food source. Ichthyofaunal sampling in the East Kleinemonde Estuary for a period exceeding a decade indicated that the loss of the submerged macrophyte beds from this TOCE did not result in a decrease in species richness but did influence the relative abundance of certain species, with a shift in dominance from vegetation-associated species (e.g. *Rhabdosargus holubi*) to those associated with sandy environments, such as members of the family Mugilidae. The fish assemblage of the East Kleinemonde Estuary, and possibly other TOCEs, appears to be adaptable and therefore relatively resilient to such a habitat loss. The exception to this is the pipefish *Syngnathus watermeyeri* that was absent during the macrophyte-senescent phase; an important result as this species is critically endangered and the East and West Kleinemonde Estuaries represent the last known localities within which viable populations of this species are supported (Cowley & Whitfield, 2001b).

Both *Monodactylus falciformis* and *R. holubi* are closely associated with beds of submerged macrophytes, and a decline in macrophyte cover has often been linked to a decline in abundance of these species (Hanekom & Baird, 1984; Whitfield, 1984; Froneman & Henninger, 2009). Although they may be used as both refuge and foraging areas (Cowley & Whitfield, 2001b), the relative importance of these two functions for the above species is unclear (Hanekom & Baird, 1984; Cowley & Whitfield, 2001b). The effect of a loss of submerged macrophyte beds from the East Kleinemonde Estuary on these species could therefore be manifested as either a change in abundance due to increased vulnerability to predators, or in a change in diet.

Rather than the macrophyte material, it is the epiphytes or high density of invertebrates associated with beds of submerged macrophytes that are generally the important direct food source for estuarine fishes, with no species recorded in association with submerged macrophytes being exclusively herbivorous (Hanekom & Baird, 1984). It can be assumed that a loss of submerged macrophytes would be combined with a loss of epiphyte and invertebrate biomass (Kikuchi, 1974; Orth, 1975; Davies, 1982). Highest abundance and biomass of invertebrates within TOCEs are indeed associated with beds of submerged macrophytes (Bernard & Froneman, 2005; Froneman & Henninger, 2009), as these beds provide protection from predators (Emmerson, 1986) as well as a food-rich environment (Henninger *et al*., 2009). One of the reasons proposed for a decline in abundance and biomass of the caridean shrimp *Palaemon peringueyi* and isopod *Exosphaeroma hylocoetes* in the Kasouga Estuary, was a decline in extent of the beds of the dominant submerged macrophyte leaving these species vulnerable to predation together with reduced recruitment opportunities for *P. peringueyi* (Froneman & Henninger, 2009).

Both *R. holubi* and *M. falciformis* feed extensively on invertebrates associated with aquatic macrophyte beds (Whitfield, 1984). Unlike *M. falciformis*, which is carnivorous, *R. holubi* is omnivorous and able to switch between a diet dominated by epiphytic material to one that is carnivorous based on the relative availability of invertebrate food items (Bennett, 1979). The latter species commonly ingests macrophytic material but only digests the epiphytic component (Blaber, 1974a; de Wet & Marais, 1990). Although average δ^{15}N and δ^{13}C values for *R. holubi* were different between the East Kleinemonde and West Kleinemonde estuaries, this variation was not large enough to indicate a marked difference in diets between individuals in these two estuaries. Waltham & Connolly (2006) used a change in average δ^{13}C of 5.2 ‰ to indicate a shift in diet of the garfish *Arrhamphus sclerolepis* within artificial urban waterways of southeast Queensland, Australia, while in this study the change for *R. holubi* between estuaries was never greater than 0.7 ‰. Indeed, stomach contents of *R. holubi* individuals from the East Kleinemonde Estuary during 2008 and 2009 (personal observations), a period in which submerged macrophytes were absent from the system, did not contain *Ruppia cirrhosa* but did include the macroalgae *Codium tenue* which was abundant in the estuary at the time. In other words, despite the absence of macrophytes, individuals of this species continued to feed on epiphytic algae but relied on an alternative algal host.

In contrast to *R. holubi*, average δ^{13}C values of *M. falciformis* were consistently more enriched in the West Kleinemonde than in the East Kleinemonde Estuary (Table 6.1), with the degree of enrichment ranging from 1.3 ‰ (summer 2008) to 3.6 ‰ (winter 2008). Following macrophyte senescence in the Swartvlei Estuary, Whitfield (1984) noted a shift in the diet of *M. falciformis* from a dominance of invertebrates associated with the submerged plants to that of small fishes and zoobenthic invertebrates, thus indicating that this species is also able to obtain food from alternate sources when aquatic macrophytes disappear.

Table 6.1. Seasonal δ^{13}C (‰) values of *R. holubi* and *M. falciformis* for the East Kleinemonde (submerged macrophytes absent) and West Kleinemonde (submerged macrophytes present) estuaries

		δ^{13}C (Avg ± SD)	
	Season	E. Kleinemonde	W. Kleinemonde
Rhabdosargus holubi	Summer '08	-25.097 ± 1.523	-24.566 ± 1.919
	Winter '08	-23.783 ± 1.857	-23.687 ± 2.429
	Summer '09	-22.692 ± 2.084	-21.977 ± 1.179
Monodactylus falciformis	Summer '08	-23.520 ± 0.889	-22.150 ± 1.507
	Winter '08	-23.881 ± 0.946	-20.287 ± 2.290
	Summer '09	-23.376 ± 0.984	-20.846 ± 0.903

Besides dietary shifts, the loss of submerged macrophytes from a TOCE should affect the abundance of vegetation-associated fish taxa. In the East Kleinemonde Estuary, changes in the abundance of *R. holubi* and *M. falciformis* following loss of submerged macrophytes from the system were consistent with this change in habitat. Catch per unit effort (CPUE) of species most commonly associated with sandy environments (e.g. *M. capensis*) was highest in the East Kleinemonde Estuary during the period of macrophyte senescence (2004 – 2009), and those of the vegetation-associated *M. falciformis* and *R. holubi* highest when submerged macrophyte beds were present (1998 – 2003). A similar scenario was observed following loss of submerged macrophytes from the Swartvlei Estuary (see Figure 6.1 for comparison), with the percentage contribution to total catch of sediment-associated species of the family Mugilidae increasing by more than 20% and the abundance of the vegetation-associated *M. falciformis* decreasing by 21% from macrophyte to sand phases (Whitfield, 1986).

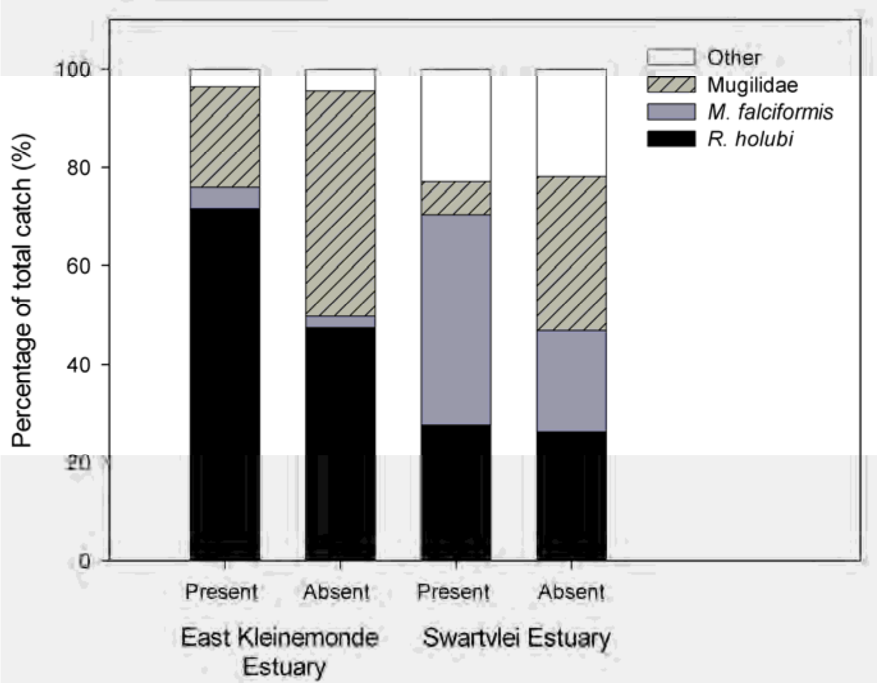

Figure 6.1. Changes in the catch composition of the vegetation-associated species *R. holubi* and *M. falciformis*, and certain species of the sediment-associated family Mugilidae (*L. dumerili*, *L. richardsonii*, *M. cephalus* and *M. capensis*) following a loss of submerged macrophytes from the East Kleinemonde and Swartvlei estuaries. Data for the Swartvlei Estuary were obtained from Whitfield (1986)

Such a clear pattern was not, however, visible in a comparison between the East Kleinemonde and West Kleinemonde estuaries. During the period 2008 – 2009, CPUE of sediment-associated species was highest in the East Kleinemonde Estuary during the macrophyte-senescent phase. Both *M. falciformis* and *R. holubi* though were more abundant in the catch from the East Kleinemonde during this period, although *M. falciformis* contributed a greater percentage of total catch in the West Kleinemonde Estuary. However, it is important to note that catch efficiency of the seine nets was considerably higher in the East Kleinemonde compared to the West Kleinemonde Estuary and this would have influenced CPUE comparisons between the two systems.

Ordination and classification by PRIMER using large mesh seine net catch data clearly separated the two estuaries; a result that was significant (ANOSIM, Global R = 1, $P < 0.05$). This grouping, however, could be due to any number of factors that show variation between the two systems (including estuary size, degree and proximity of urbanization to each system) and not necessarily the presence or absence of submerged macrophytes, although the two are correlated. In the same way, the fact that the nature of the changes in the East Kleinemonde seine net catch following a loss of submerged macrophytes was not strictly repeated in the comparison between systems brings into question the comparability of the two datasets, and suggests that the presence or absence of macrophyte beds is probably not the only factor influencing the different estuarine fish assemblages in this case.

Although habitat heterogeneity has been recognized as an important factor influencing fish assemblages within TOCEs (Hanekom & Baird, 1984; Whitfield, 1984; Russell, 1996; Whitfield, 1996), estuary mouth condition is regarded as being the single most important factor influencing TOCE fish assemblages (Beckley, 1984; Kok & Whitfield, 1986; Bennett, 1989b; Vorwerk *et al*., 2003; James *et al*., 2007). For example, Russell (1996) identified that although biomass of submerged macrophytes affected fish abundance in the Wilderness and Swartvlei estuaries, estuary mouth status was the stronger controlling factor. Furthermore, Hanekom and Baird (1984) attributed non-significant differences in a number of aspects of catch from *Zostera* and non-*Zostera* regions of the Kromme Estuary to the influence of a range of additional factors besides the presence or absence of seagrass beds. Further factors that may influence TOCE fish assemblages include estuary size, with larger TOCEs having higher species richness than smaller estuaries (Vorwerk *et al*. 2003), as well as salinity, temperature and turbidity (Harrison & Whitfield, 2006; Whitfield, 1996).

Most notably, the pipefish *Syngnathus watermeyeri*, which is only found in association with submerged macrophytes, was not encountered in the catches of either small or large mesh seine nets from the period in which submerged macrophytes were absent from the East Kleinemonde Estuary. It was however, together with *S. temmincki*, recorded in catches from both the West and East Kleinemonde estuaries when beds of these plants were present. The estuarine pipefish *S. watermeyeri* is dependent on estuarine plant beds for its entire life cycle whereas *S. temmincki* breeds both in estuaries and the sea (Whitfield, 1998) and can therefore readily recolonise systems once submerged macrophyte habitats have recovered.

The estuarine pipefish *S. watermeyeri* is critically endangered (Baillie & Groombridge, 1996), and was previously found in the Bushmans, Kariega and Kasouga estuaries (Dawson, 1986). This species appears to be highly sensitive to human perturbations of the aquatic environment (Whitfield, 1998). It disappeared from the Bushmans and Kariega estuaries following the construction of major impoundments in the catchments of these systems that severely reduced the amount of freshwater entering the estuaries (Whitfield & Wooldridge, 1994) and led to frequent hypersaline (> 40 ppt) conditions (Ter Morshuizen & Whitfield, 1994). The only viable remaining populations were recorded in the East Kleinemonde and West Kleinemonde estuaries (Cowley & Whitfield, 2001b) until recently when a population was reported from the Kariega Estuary, likely as a result of increased freshwater inflow (Vorwerk *et al*., 2007).

Current regulations of the Marine Living Resources Act (18 of 1998) prohibit (i) the capture of Syngnathids (pipefishes and seahorses), and (ii) the disturbance and removal of estuarine vegetation, algae and macrophytes. Several countries have legislation protecting so-called ‘critical habitats’ – areas which are essential to the conservation of a threatened species (Groombridge, 1992). Examples of these include the Endangered Species Act (September 1973) in the U.S.A., the Flora and Fauna Guarantee Act (1988) of Victoria, Australia and the French Nature Conservation Act (July 1976). Based on the sensitivity of *S. watermeyeri* to human disturbances and its highly limited distribution, the submerged macrophyte beds within the East Kleinemonde and West Kleinemonde estuaries need to be recognized as a very important habitat.

The inter-annual variability in fish catches from estuaries is a characteristic of estuarine systems in general (Nichols, 1985) and primarily reflects changing annual recruitment strength of individual species (James *et al*., 2007), influenced by biotic factors including spawning success as well as abiotic factors, most important of which is recruitment opportunity as determined by estuary mouth condition (Bell *et al*., 2001; Cowley *et al*., 2001; Vivier & Cyrus, 2001; Vorwerk *et al*., 2003; Kemp & Froneman, 2004; James *et al*., 2007). Cowley and Whitfield (2001a) conducted a series of mark-recapture studies in the East Kleinemonde Estuary and showed large inter-annual variability in the population size of most species. Population fluctuations were ascribed to estuary mouth conditions (recruitment opportunities) and levels of avian piscivory. The present study and that of James *et al*. (2008a) clearly illustrate the value of long-term datasets for the identification of trends in biological data (Flint, 1985), with patterns in fish assemblage related to habitat changes in the East Kleinemonde Estuary only becoming apparent over the long time period.

The response of individual fish species to biotic and abiotic changes is not usually the same. In the case involving *R. holubi* and *M. falciformis* in the East Kleinemonde Estuary, these two species did not respond to changes in the same manner. Whereas *M. falciformis* did undergo a decline in abundance in line with the loss of submerged macrophytes from the East Kleinemonde Estuary, *R. holubi* remained a dominant component of the seine net catch from this estuary throughout the study period. This result is similar to that recorded at Swartvlei when submerged macrophytes were lost but *R. holubi* remained a dominant component of the littoral fish fauna (Whitfield, 1986). *R. holubi* is ubiquitous and a prolific spawner such that there are always larvae available to recruit into estuaries. Another possible reason for the lack of a marked decline in the abundance of *R. holubi* during the period of macrophyte senescence may be linked to larval recruitment strategy in combination with local conditions. This species has been shown to use olfaction during recruitment (James *et al*., 2005) and, due to the proximity of the mouths of the two Kleinemonde estuaries, could be responding to West Kleinemonde macrophyte cues seeping through the joint sand bar and then migrating into whichever estuary provides an access opportunity.

Catch data is a function not only of recruitment by fishes into an estuary but also the survival of recruits within the system during the period preceding capture. While physical processes determine success of the initial recruitment process (Kemp & Froneman, 2004; Cowley *et al.*, 2001), biological processes within the estuary determine ultimate recruit survival and hence success (Kemp & Froneman, 2004). Predation by piscivorous birds for example has been shown to play a major role in controlling TOCE fish populations (Cowley & Whitfield, 2001a). Furthermore, mark-recapture estimates of the population size of *R. holubi* within the West Kleinemonde Estuary indicated overall mortality over a seven month closed period in 1971 was in the order of 80%, with mortality rates being density-dependent and attributed largely to predation by piscivorous birds, in particular *Ardea cinerea*, *Phalacrocorax carbo* and *P. africanus*, whose numbers seemed to be closely related to the density of this prey species (Blaber, 1973).

Submerged macrophytes mediate such biological interactions, reducing predation risk due to the structural complexity of the habitat (Adams *et al.*, 2004) as well as increasing habitat heterogeneity and fish food availability. The decline in abundance of the vegetation-associated *M. falciformis* and *R. holubi* during the period of macrophyte senescence, as well as the absence of the pipefish *S. watermeyeri* in the East Kleinemonde Estuary during these years, is testament to the role that the submerged macrophytes *R. cirrhosa* and *P. pectinatus* play in the provision of these functions.

A decline in both the abundance and body condition of *R. holubi* and *M. falciformis* was recorded from the Swartvlei Estuary following a loss of submerged macrophyte beds from that system (Whitfield, 1984). Although condition of both species was not measured in this study, the continued presence of both *R. holubi* and *M. falciformis* within the East Kleinemonde Estuary despite the absence of macrophyte beds suggests they are resilient and able to adapt to changes within the estuarine environment. Furthermore, stable isotope analyses and anecdotal gut contents of a range of fish species from the East Kleinemonde and West Kleinemonde (personal observation) did not suggest that the diets were dissimilar between these estuaries despite differing abundances of submerged macrophytes. However, fish species from the West Kleinemonde Estuary generally had

δ^{13}C values that were more enriched, thus reflecting the increased contribution of macrophyte material to the detrital pool within this system. There is no isotopic evidence from this study to suggest, however, that the submerged macrophyte species are being utilized as a direct food source by any of the species sampled, a result that is consistent with findings in the literature (e.g. Mann, 1988; Smit *et al*., 2006).

The suitability of stable isotope analysis as a tool for estuarine food web studies

Analysis of stable isotopes has proven to be a valuable tool for a range of disciplines, and is most effective where there are a limited number of end members with distinctive isotopic signatures (Peterson, 1999). Unfortunately, estuarine systems tend to have energy inputs from diverse sources, terrestrial, marine and autochthonous production (Richoux & Froneman, 2007), resulting in consumers with intermediate δ^{13}C values that cannot unambiguously identify their carbon sources. Food webs within estuaries are characterized by a high degree of detritivory (Day, 1951; Odum, 1971; Branch & Branch, 1981; Mann, 1988; Whitfield & Lubke, 1998), with material that is consumed being a mix of plant and animal origin as well as having potentially been recycled multiple times, and having a suite of decomposing bacteria and fungi associated with it (Kreeger & Newell, 2000; Norderhaug *et al*., 2003).

Although stable isotope analysis was able to indicate a difference in carbon pools between the East Kleinemonde and West Kleinemonde estuaries related to the presence or absence of extensive submerged macrophyte beds, the dietary source of carbon for individual species was not clearly elucidated, with the range of fish species tested showing overlapping values of δ^{13}C. In addition, the consumer fish species had large standard deviations for δ^{15}N, probably a consequence of the recycled and composite nature of detritus in estuaries (Richoux & Froneman, 2007). For this reason, carbon is generally the element most commonly used to ascertain primary energy sources in estuarine stable isotope studies (e.g. Fry & Parker, 1979; Fry *et al*., 1982; Paterson & Whitfield, 1997; Froneman, 2001, 2002c; Mbande *et al*., 2004).

The inclusion of additional elements, such as sulphur in stable isotope analyses could potentially help to elucidate estuarine trophic interactions by providing information at a higher resolution (Peterson & Fry, 1987; Peterson, 1999). Another practice is to combine the use of stable isotopes with analysis of fatty acids which, like ratios of stable isotopes can be used to trace the flow of primary producer-derived material through a system. These methods have successfully been used in combination in other South African estuaries (e.g. Richoux & Froneman, 2008).

Conclusions and potential areas for future research

The input of macrophyte material into the carbon pool of the West Kleinemonde Estuary is apparent in the enriched $\delta^{13}C$ values of fishes in this system relative to those in the East Kleinemonde Estuary. As this is via the detrital pathway the absence of the submerged macrophytes from the East Kleinemonde Estuary, although reflected in their depleted $\delta^{13}C$ values, does not appear to directly affect the feeding of the fishes in this system. It seems that in the absence of submerged macrophytes, fishes can find suitable alternative sources of food, as was the case for *R. holubi* that fed on epiphytic material on the macroalga *C. tenue* in the East Kleinemonde Estuary when *R. cirrhosa* was absent (personal observation). The decline in abundance of the vegetation-associated *R. holubi* and *M. falciformis* in this estuary was probably due to increased rates of predation associated with the loss of refuge offered by the beds of submerged macrophytes rather than a decreased availability of food. This suggests that of the two, the function of macrophyte beds as a refuge rather than foraging area is the more important to vegetation-associated species, with similar observations being made by Hanekom and Baird (1984), as well as Humphries *et al*. (1992).

Submerged macrophyte beds are therefore important habitats within TOCEs. Not only is the nursery function of an estuary closely related to aquatic plant communities (Wallace & van der Elst, 1975; Beckley 1983), but the presence of this habitat is essential to the success of specialized species such as the pipefishes *S. watermeyeri* and *S. temmencki*.

The vegetation-associated *R. holubi* and *M. falciformis* seem to be able to adapt to the loss of this habitat, but at an increased risk of predation.

The question of whether the *R. holubi* responds to macrophyte-based olfactory cues when recruiting into a TOCE, or whether there is another cue that is more important could be assessed by choice chamber experiments and would help to explain its continued high abundance in the East Kleinemonde Estuary despite the absence of these plants. Furthermore, a study investigating the condition of vegetation-associated species within the East Kleinemonde Estuary would give an indication of the actual success of these species in the absence of macrophyte beds.

While stable isotope analyses are a popular method for obtaining ecological information in trophic studies, much of the way results are interpreted is based on assumptions, in particular those regarding the degree of fractionation that takes place within material as it is transferred between system components. Isotope values reflect both source and process information and it is a lack of information regarding process effects that causes uncertainty as to the source information (Peterson & Fry, 1987). Furthermore, methodological inconsistencies, as highlighted in this thesis, hamper between-study comparisons. While numerous authors have called for a standardized methodology, none seems to have been adopted (see Chapter Four for details). There is thus a need for experimental, laboratory-based stable isotope studies to address these assumptions, as well as a standardized methodology that will enable comparisons between studies.

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