POPULATION DYNAMICS OF SELECTED ICHTHYOFAUNAL COMPONENTS IN THE TEMPERATE, TEMPORARILY OPEN/CLOSED KASOUGA ESTUARY, SOUTH AFRICA

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ABSTRACT

The spatial and temporal pattern of ichthyofaunal community composition in relation to selected physico-chemical (temperature and salinity) and biological variables (chlorophyll-*a* and zooplankton) was investigated at ten stations in the temperate temporarily open/closed Kasouga estuary. In addition, the food web structure in the estuary was investigated using stable carbon isotope analysis.

Results of the 5 metre seine net survey indicated that ichthyofaunal composition and biomass in the Kasouga estuary was largely determined by seasonality and mouth condition. Maximum abundance and biomass of ichthyofauna was recorded during summer or during those periods when overtopping occurred. Overtopping coincided with the recruitment of marine estuarine dependant species, which dominated the catches both numerically and in biomass. The recruitment of these species resulted in an increase in diversity of the ichthyofaunal community.

There were no significant spatial patterns in the distribution of smaller ichthyofauna (<50mm SL) identified in Bray-Curtis similarity matrices using cluster analysis (Primer 5 v5.2.4). Spearman's correlation analysis revealed that there were no significant correlations between abundance and biomass of ichthyofauna and selected physico-chemical and biological variables other than salinity ($P>0.05$ in all other cases). There were two distinct spatial patterns in the distribution of the larger ichthyofauna (>50mm SL). These corresponded to a grouping associated with the mouth region and a grouping associated with the remaining regions of the estuary.

Stable isotope analysis indicated that the primary source of carbon utilised by the ichthyofauna of the Kasouga estuary was derived from the channel, most likely microphytobenthic algae. The contributions of the riparian and salt marsh vegetation to the total carbon flow appear to be minimal.

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CHAPTER 1

GENERAL INTRODUCTION

The important role of estuaries as nursery grounds for a variety of marine and estuarine ichthyofauna is now well documented (Whitfield, 1998; Harrison, 1998; Vorwerk *et al*., 2003). The nursery function of estuaries can be attributed to several factors; including increased food availability, high turbidity and the presence of refuge areas against predation (Whitfield, 1998).

The utilisation of estuaries by southern African fish has been classified into a number of categories according to the dependency of the species on the estuary (Whitfield, 1994, 1998). Category I comprises those species that are, primarily, estuarine spawners. Category II includes marine species whose juveniles utilise the estuaries as nursery areas. Category III fish are those marine species that may be found in the estuary but are not dependent on the estuaries for their survival. Freshwater species, whose ranges extend into the estuarine environment in varying degrees as salinity tolerances allows, fall under Category IV. Category V includes all those migratory species that use the estuarine environment as a transition zone between the marine and freshwater environment (Whitfield, 1994, 1998). Each category typically comprises sub-categories (Table 1.1).

Table 1.1: The five major categories of estuarine associated fish species in southern African estuaries (after Whitfield, 1998).

Categories	Description of categories
I	Estuarine species which breed in southern African estuaries. Further subdivided into: Ia. Resident species which have not been recorded spawning in marine or freshwater environments. Ib. Resident species which also have marine or freshwater breeding populations.
II	Euryhaline marine species which usually breed at sea with the juveniles showing varying degrees of dependence on southern African estuaries. Further subdivided into: IIa. Juveniles dependant on estuaries as nursery areas. IIb. Juveniles occur mainly in estuaries, but are also found at sea. IIc. Juveniles occur in estuaries but are usually more abundant at sea.
III	Marine species which occur in estuaries in small numbers but are not dependent on these systems.
IV	Freshwater species, whose penetration into estuaries is determined primarily by salinity tolerance. This category includes some species which may breed in both freshwater and estuarine systems.
V	Catadromous species which use estuaries as transit routes between the marine and freshwater environments but may also occupy estuaries in certain regions. Further subdivided into: Va. Obligate catadromous species which require a freshwater phase in their development. Vb. Facultative catadromous species which do not require a freshwater phase in their development.

A number of biotic and abiotic factors (Figure 1.1) have been identified as being important in determining estuarine utilisation by fish species (Whitfield, 1998; Vorwerk, 2001). Temperature, salinity, catchment size, turbidity, estuary size, mouth condition and habitat availability have been identified as important abiotic factors (Whitfield, 1998; Vorwerk, 2001). Food availability, predation and competition are deemed important biotic factors. The abiotic and biotic variables do not act individually but synergistically (Whitfield, 1998).

1.1 Temporarily Open/Closed Estuaries (TOCEs)

Temporarily open/closed systems account for 71% of all estuaries along the South African coastline (Whitfield, 1998, 2000). These systems are typically characterised by having catchment areas of <500 km^2 (Whitfield, 1992, 1998). As a consequence, freshwater runoff is often not enough to prevent deposition of sand across the mouth of the estuary. Consequently, these systems are separated from the sea for extended periods of time. All but one of the TOCEs along the South African coastline are found in the warm temperate and subtropical biogeographical regions. There are 84 TOCEs in the warm temperate zone and 93 in the subtropical zone (Whitfield, 2000; Harrison *et al*., 2000) (Figure 1.2).

1.2 Physico-chemical environment of TOCEs

Two types of TOCEs are found along the southern African coastline, perched and non-perched closed estuaries (Harrison *et al*., 2000). In perched estuaries (mainly a feature of small KwaZulu-Natal systems) water levels in the estuary exceed that of the marine environment. (Harrison *et al*., 2000). In non-perched systems water levels in the estuary are in the same range of the marine waters.

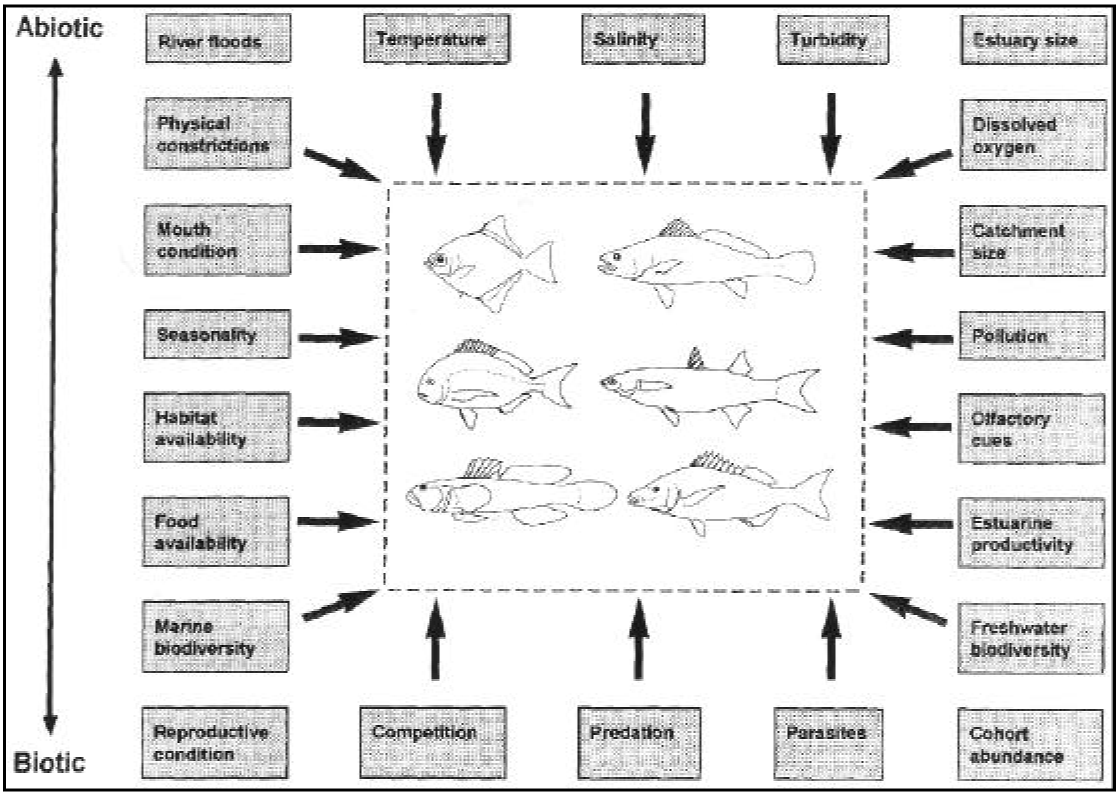


Figure 1.1: Biotic and abiotic factors influencing fish species in southern African estuaries. The scale on the left-hand side illustrates the trend from predominantly abiotic variables in the top of the diagram to biotic variables at the bottom. (after Whitfield, 1998).

Perched estuaries (Figure 1.3) often experience lower salinities than non-perched estuaries (Harrison *et al*., 2000). The lower salinity values in the perched systems can be attributed to a reduction in overtopping events due to the presence of a high sandbar at the mouth. The primary water source for these systems is freshwater influx (Harrison *et al*., 2000). In times of breaching, the estuary may drain in a matter of hours resulting in some cases of a large surface area being dramatically reduced to a single channel (Harrison *et al*., 2000). Non-perched systems (Figure 1.4) experience overtopping events more frequently than the perched systems resulting in higher salinities in these systems (Harrison *et al*., 2000). In cases where there is a marked reduction of freshwater inflow, hypersaline conditions may be experienced (Harrison *et al*., 2000; Whitfield, 1992, 1998).

Temporarily open/closed estuaries are generally small and often very shallow (<2 m in depth) (Whitfield, 1992, 1998; Froneman, 2002a, 2002b). As a result the estuaries are usually thoroughly mixed with little or no stratification, as the wind driven mixing is sufficient to facilitate both horizontal and vertical mixing of the water column (Whitfield, 1992, 1998; Froneman, 2002a, 2002b). Froneman (2002a, 2002b) showed that the water column in the Kasouga estuary (Eastern Cape) was well mixed with no clear stratification evident. In the larger TOCEs (e.g. East Kleinemonde estuary along the Eastern Cape coast) horizontal and vertical stratification may be prominent during the open phase, however, during the closed phase the estuaries exhibit uniform conditions (Whitfield, 1992, 1998).

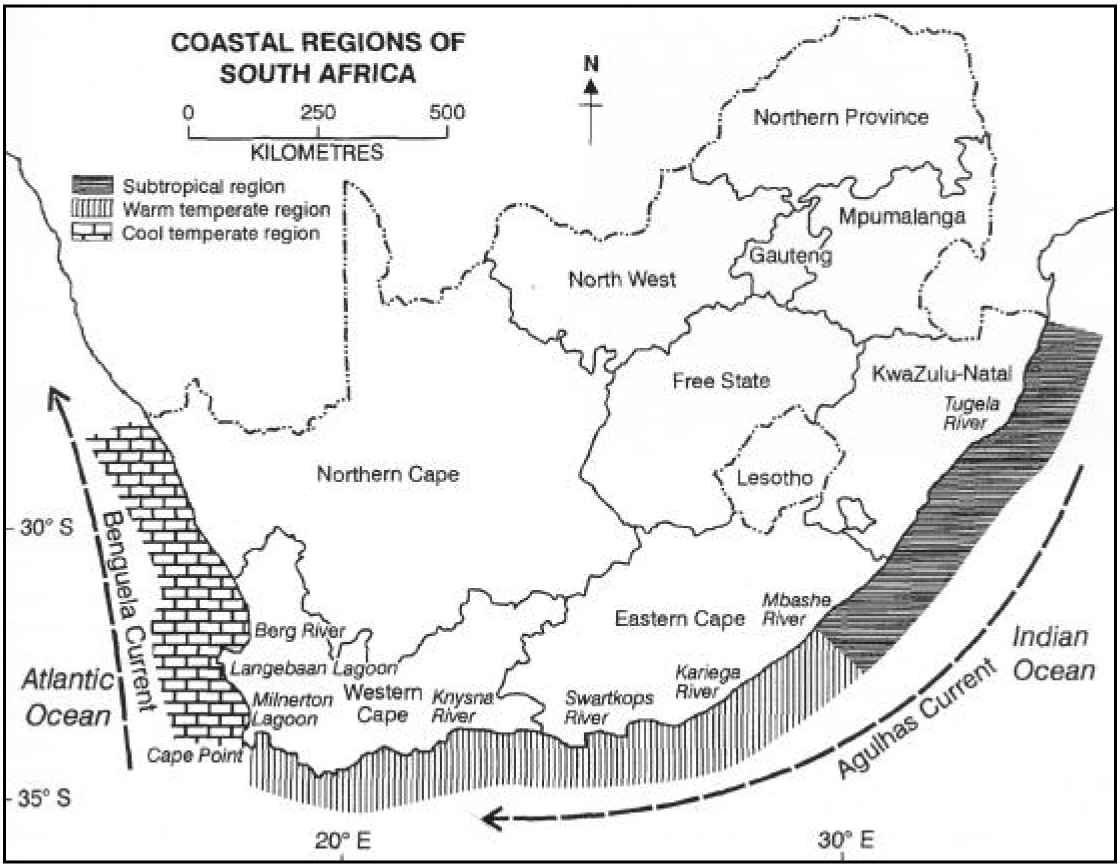


Figure 1.2: Biogeographical regions along the South African coastline (after Whitfield, 1994).

The water temperatures in temporarily open closed systems are determined primarily by seasonality and the regional climate (Whitfield, 1992, 1998). In the cool temperate zone, annual water temperatures ranged from 11°C and 32°C (Day, 1981; Harrison 2004). Estuarine water temperatures in the subtropical zone range between 18°C and 32°C and between 19°C and 28°C in the warm temperate zone (Day, 1981; Perissinotto *et al.*, 2000, 2002).

Variations in salinity in TOCEs take place over weeks rather than the hourly variations recorded in permanently open estuaries (Whitfield, 1992, 1998). In cases when overtopping occurs, the influx of seawater drastically affects the salinity of the estuary (Whitfield, 1992, 1998; Froneman, 2002a, 2002b). Combinations of freshwater seepage and overtopping may create changes by as much as 30‰ (parts per thousand) over periods of less than two weeks (Whitfield, 1992, 1998; Froneman, 2002a, 2002b).

Mesohaline conditions (5-18‰) generally predominate during the closed phase of temporarily open/closed estuaries. However limnetic (0.1-0.5‰) and hypersaline (>40‰) conditions may also be experienced in TOCEs (Whitfield, 1992, 1998). Limnetic conditions are experienced during periods of high rainfall (Nozais *et al.*, 2001), while during periods of drought or high evaporation, the water loss results in hypersaline conditions prevailing throughout the system (Whitfield, 1998).

1.3 Sediment

Bottom sediment distribution in TOCEs is similar to that of permanently open systems. Primarily the sediments in the upper and middle reaches of TOCEs are comprised of mud/clay and silt and organic derived sediments whereas, coarse to medium sand dominates in the region of the mouth (Day, 1981). When the estuaries are closed, the distribution patterns of various sediments remains the same, as the water currents in closed systems are generally insufficient to allow sediment movement.

1.4 Light Environment

Light penetration in TOCEs is highly variable, reflecting changing mouth condition and the freshwater inflow into the estuary (Whitfield, 1998; Hecht & van der Lingen, 1992; Perissinotto *et al*., 2000, 2002; Nozais *et al*., 2001; Froneman, 2002b). During the open phase or following freshwater inflow into the estuary, turbidity measurements of 75-90 NTU (Nephelometric Turbidity Units) may be reached (Cooper *et al*., 1993; Froneman, 2002b). During the periods when the mouth is closed, however, turbidity is very much reduced with readings of <10 NTU common (Froneman, 2002a, 2002b).

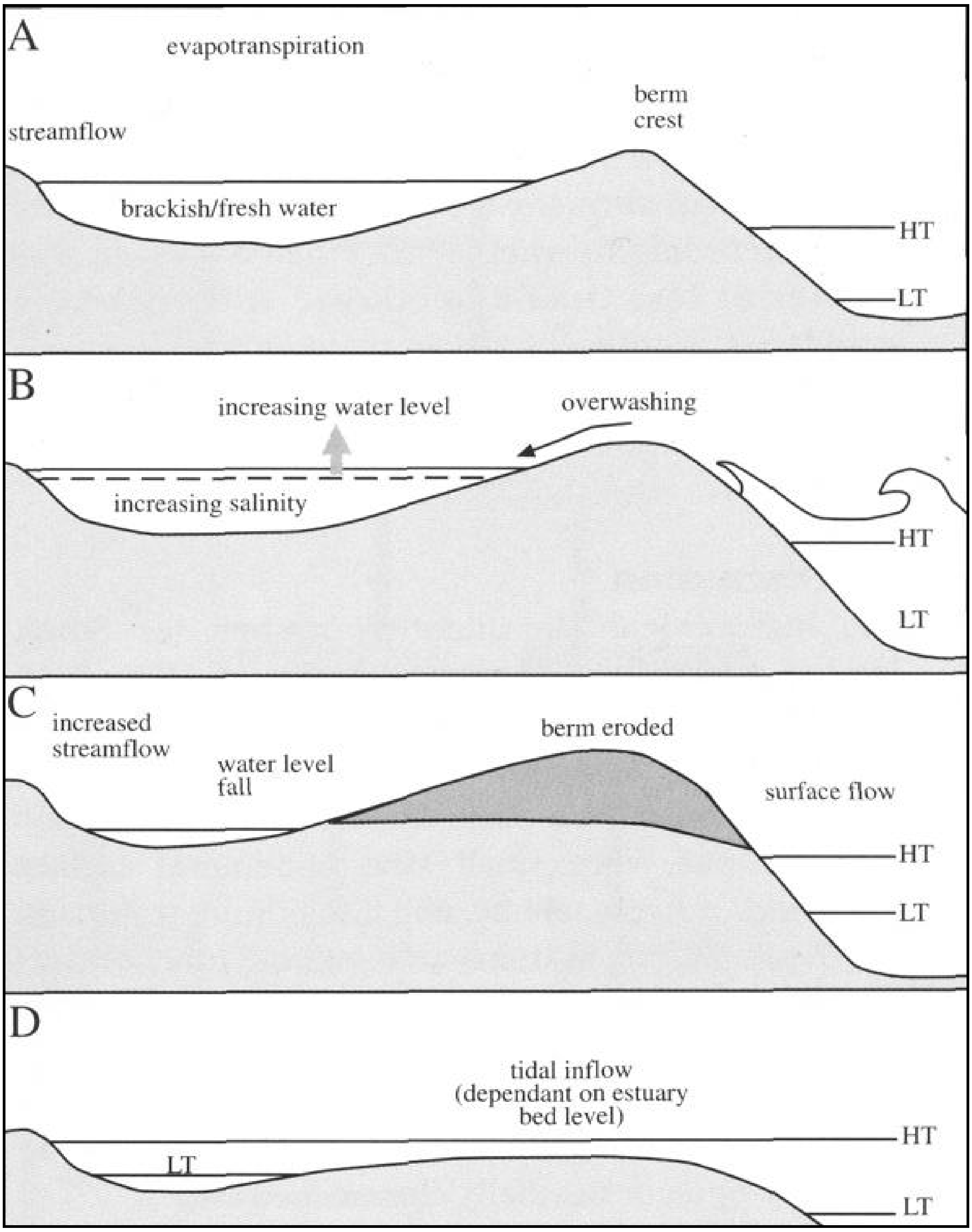


Figure 1.3: Diagram of perched normally closed estuary showing cross-sectional (A-D). Under balanced conditions (A), the stream inflow is matched by evapotranspiration and seepage. Overwashing (B) may elevate water levels and salinity. Increased streamflow (C) may promote breaching. When breached (D), the water levels are lowered and tidal flow may take place if the berm level is sufficiently low (after Harrison *et al*., 2000).

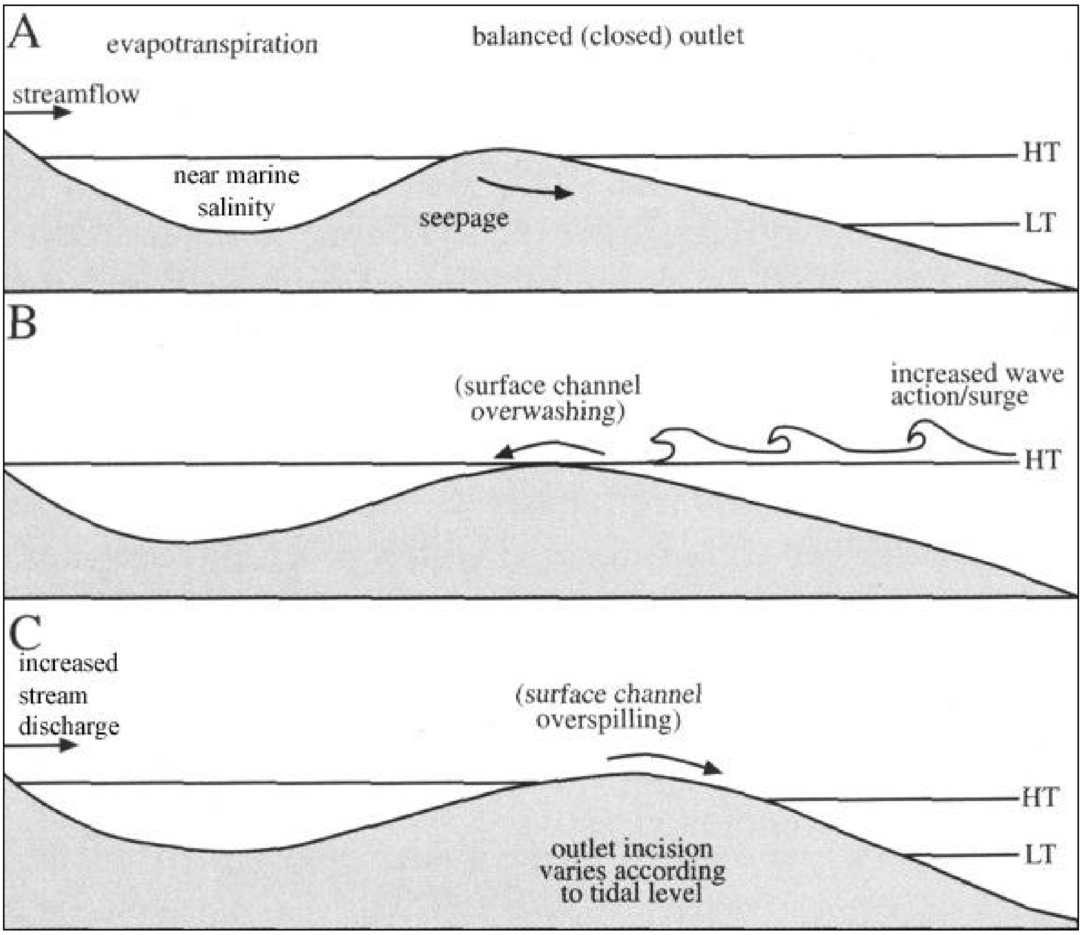


Figure 1.4: Diagram of non-perched and normally closed estuary in cross-section (A-C). Under balanced conditions (A) streamflow is balanced by losses through evapotranspiration and seepage. Under high wave energy (B) overwashing introduces marine water into the system. Under enhanced inputs from overwashing (B) or streamflow (C), the system may breach. The depth of incision is low since the estuary water level is so close to sea level (after Harrison *et al.*, 2000).

1.5 Biology

1.5.1 Phytoplankton and Microphytobenthic algae

The phytoplankton biomass values in TOCEs are generally lower than those reported for permanently open systems within the same geographical region (Adams & Bate, 1999; Perissinotto *et al.*, 2000; Nozais *et al.*, 2001; Perissinotto *et al.*, 2002). In TOCEs, phytoplankton biomass has been shown to vary between 0.09 mg chl-*a* m^{-3} and 15.4 mg chl-*a* m^{-3}, while in permanently open estuaries phytoplankton biomass values exceeding 20 mg chl-*a* m^{-3} and up to 100 mg chl-*a* m^{-3} are not uncommon (Adams & Bate, 1999; Froneman, 2002a, 2002b). The low phytoplankton biomass in TOCEs is thought to reflect low primary production rates due to reduced macronutrient concentrations resulting from reduced freshwater inflow (Adams *et al.*, 1999; Froneman, 2002a, 2002b). In contrast to phytoplankton, microphytobenthic algae concentrations in TOCEs typically exceed those found in the open systems (Adams & Bate, 1994; Nozais *et al.*, 2001; Froneman, 2002a). Indeed, in TOCEs the microbenthic algae have been shown to attain levels 2 - 3 orders of magnitude higher than phytoplankton biomass (Nozais *et al.*, 2001; Froneman, 2002a; Perissinotto *et al.*, 2003). Low turbidity, high concentrations of macronutrients in the sediments and reduced current flow are the likely reason for the high contribution of the microphytobenthic algae to the total primary production in the temporarily open/closed systems (Adams & Bate, 1999).

1.5.2 Macrophytes

Macrophyte distribution and composition in estuarine environments has been linked to geographic position of the system and mouth condition (Day, 1981). Mangroves are absent in TOCEs (Day, 1981). The macrophytes found in the TOCEs are generally those species that are tolerant to salinity variations. Although *Zostera capensis* is mainly found in marine dominated systems, its high salinity tolerance (Iyer & Barnabus, 1993) allows it to persist in many temporarily open/closed estuaries where mesohaline conditions pervail e.g. Swartvlei (Howard-Williams & Liptrot, 1980). The primary species found in TOCEs in the warm temperate zone are *Phragmites australis*, *Ruppia* spp, *Sarcocornia perennis*, *Juncus kraussii*, *Chenolea diffusa* and along the banks *Acacia karoo* (Lubke & de Moor, 1998).

Salt marshes are a prominent feature in TOCEs in the warm temperate and cold temperate zones but are virtually absent in the sub-tropical estuaries. Salt marshes are areas of high nutrient production (Taylor, 1988) and when inundated are important as nursery areas for many of the juvenile fish in the estuaries and good feeding grounds for predatory fish and other animals (Paterson & Whitfield, 1996, 1997).

1.5.3 Zooplankton

Studies on zooplankton communities in TOCEs are limited to a few studies in the sub-tropical and warm temperate zones (Froneman, 2004b; Kibirige & Perissinotto, 2003). Zooplankton diversity in TOCEs has been shown to be much lower than that of permanently open estuaries particularly during extended periods of mouth closure (Grindley, 1981; Kibirige & Perissinotto, 2003). The low diversity can be attributed to the reduction in recruitment of marine species and the virtual absence of typical estuarine fauna within these systems (Froneman, 2004b). The zooplankton in TOCEs typically comprises copepods, isopods, mysids and amphipods (Connell *et al.*, 1981;

Grindley, 1981; Blaber *et al*., 1984; Schlacher & Wooldridge, 1995; Perissinotto *et al*., 2000; Kibirige, 2002). Copepods generally comprise >95% of zooplankton abundance and biomass (Froneman, 2002a, 2002b; Kibirige & Perissinotto, 2003). Several recent studies have shown that the zooplankton biomass in TOCEs may attain levels far in excess of those found in the larger permanently open estuaries, particularly during the closed phase (Kibirige & Perissinotto, 2003; Froneman, 2004b). The high zooplankton biomass recorded within the TOCEs are sustained by the elevated microphytobenthic stocks that are generally recorded within these systems (Perissinotto *et al*., 2000).

1.5.4 Ichthyofauna

The study of ichthyofaunal assemblages in warm temperate TOCEs has largely been restricted to specific target species e.g. *Rhabdosargus holubi* (Blaber, 1973a, 1973b, 1973c, 1974a, 1974b) or has a limited temporal coverage. For example a study conducted by Vorwerk *et al*. (2003) in 10 Eastern Cape estuaries, was restricted to a one survey during each of the winter and summer seasons. A notable exception is the study conducted by Cowley (1998) in the East Kleinemonde Estuary.

The ichthyofaunal community structure within TOCEs is largely determined by mouth status (Cowley, 1998; Vorwerk, 2001) (Figure 1.5). In the absence of any link to the marine environment, ichthyofauna are dominated by estuarine dependent species including *Atherina breviceps, Glossogobius callidus* and *Gilchristella aestuaria* both numerically and by biomass (Cowley, 1998; Vorwerk, 2001; Vorwerk *et al*., 2003). The establishment of a link to the marine environment following breaching or overtopping coincides with an increased contribution of marine breeding species. This has the net affect of increasing the ichthyofaunal diversity within these systems (Cowley, 1998; Vorwerk, 2001).

Cowley (1998) identified two distinct species assemblages in the East Kleinemonde estuary. The first group was associated with the mouth area, while the second assemblage extended along the entire length of the estuary. The presence of these two groupings was ascribed to the distribution of sediments within the estuary.

Recruitment of juveniles into estuaries usually occurs during periods when the estuary has breached or during overtopping events (Whitfield, 1998; Cowley, 1998; Cowley & Whitfield, 2001; Vivier & Cyrus, 2001; Vorwerk, 2001). When the adults or older juveniles were leaving the estuary there is a recruitment of the small juveniles of marine estuarine-dependent species (Whitfield, 1998).

Despite the numerical importance of TOCEs along the southern African coastline, few studies have investigated the temporal and spatial variations in ichthyofaunal assemblages and food web structure within these systems.

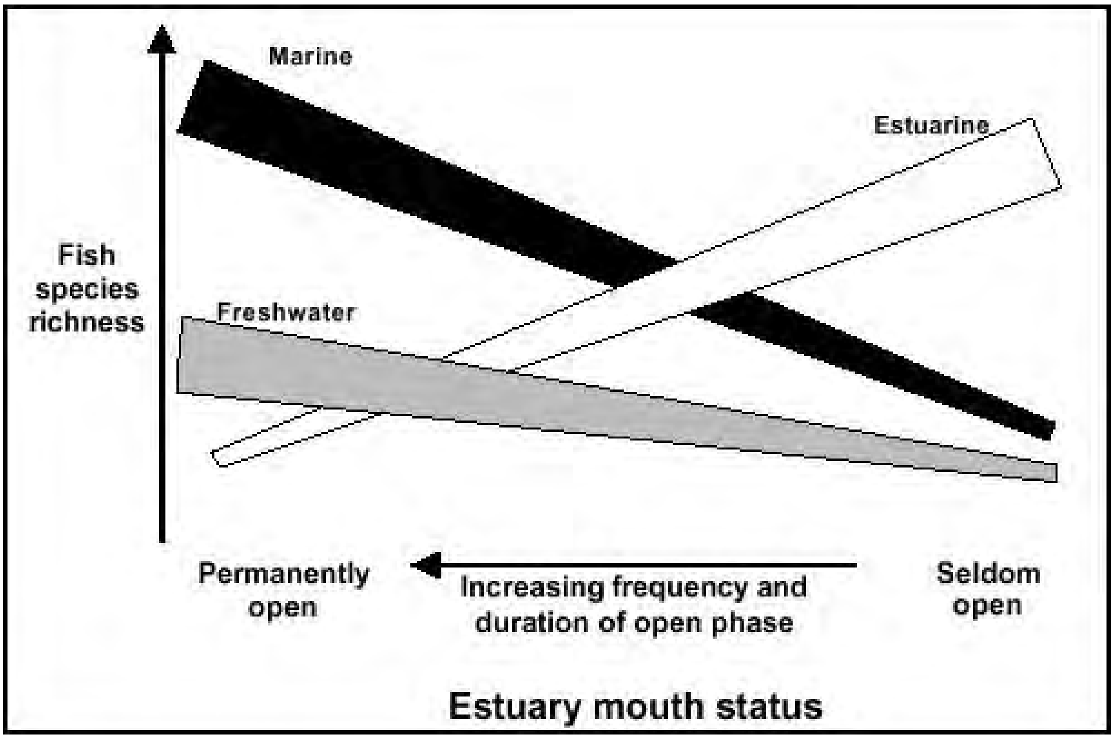


Figure 1.5: Diagrammatic representation of contributions by marine, estuarine and freshwater species to the composition of fish communities in estuaries subject to differing mouth status (from Vorwerk, 2001).

1.6 Aims

The aims of this study are as follows:

- To assess the spatial and temporal variations in the selected components of the ichthyofaunal community structure in the temporarily open/closed Kasouga Estuary.

- To identify the main sources of carbon utilised by the resident fishes of the temporarily open/closed Kasouga Estuary using stable isotope analysis.

CHAPTER 2

STUDY SITE

The temporarily open/closed Kasouga estuary (mouth coordinates: 33° 39’S; 26° 44’E) lies approximately halfway between Port Alfred and Kenton-on-Sea and is reached along a dirt farm road from the coastal road (R72). Residential development is limited to the eastern bank of the estuary (Figure 2.1). Access to the beach is from a gravel parking lot close to the mouth of the estuary. A wooden boom restricts vehicle access to the beach.

The catchment size of the Kasouga estuary has been estimated at 39 km^2 (Froneman, 2002a, 2002b, 2004a). The majority of the catchment area is agricultural land that is in varying states of degradation. The gently sloping higher ground is primarily employed in cattle farming, although some chicory farming occurs near the east bank between the R72 bridge and the salt marsh (Figure 2.1). The small valleys and tributary streams are mainly lined with valley bushveld vegetation.

The surface area of the estuary covers approximately 22 hectares, the total surface area of the estuary may be as high as 28 hectares during periods of high water level (Froneman, 2002a).

The estuary is navigable for approximately 2.5 km up river. The average depth in the channel at high water ranges from <0.5 m to 2 m. During breaching events, mean depth of the estuary is generally <0.5 m (Froneman, 2002a, 2002b).

A total of 14 ichthyofauna sample sites were selected along the length of the estuary during the study. Ten of the sites were sampled monthly using the 5 m seine net while the remaining 4 stations were sampled using the 50 m seine net. The co-ordinates and a brief description of the 14 stations are listed in Table 2.1 and Table 2.2.

Table 2.1: Global Positioning System co-ordinates for the sample sites for the 5 metre seine net and the substrate type and submerged vegetation cover (+ <10%, ++ <50%, +++ >50%) at each of the stations. Vegetation cover was estimated visually.

Station Number	Longitude	Latitude	Substrate	Vegetation
1	33° 39 . 18’ S	026° 44 . 09’ E	Marine sediments	-
2	33° 39 . 14’ S	026° 44 . 01’ E	Marine sediments	+
3	33° 39 . 17’ S	026° 43 . 94’ E	Muddy sediments	+
4	33° 39 . 09’ S	026° 43 . 84’ E	Muddy sediments	+
5	33° 39 . 94’ S	026° 43 . 79’ E	Rocky substrate	+
6	33° 38 . 79’ S	026° 43 . 63’ E	Rocky/muddy	+
7	33° 38 . 87’ S	026° 43 . 51’ E	Muddy sediments	++
8	33° 38 . 86’ S	026° 43 . 25’ E	Muddy sediments	+++
9	33° 38 . 72’ S	026° 43 . 11’ E	Muddy sediments	++
10	33° 38 . 53’ S	026° 42 . 89’ E	Muddy sediments	++

Table 2.2: GPS co-ordinates for the stations occupied using the 50 metre seine net and the substrate type and submerged vegetation cover (+ <10%, ++ <50%, +++ >50%) at each of the stations. Vegetation cover was estimated visually.

Station Number	Longitude	Latitude	Substrate	Vegetation
1	33° 39 . 18’ S	026° 44 . 09’ E	Marine sediments	-
2	33° 39 . 17’ S	026° 43 . 94’ E	Muddy sediments	+
3	33° 38 . 87’ S	026° 43 . 51’ E	Muddy sediments	++
4	33° 38 . 90’ S	026° 43 . 35’ E	Muddy sediments	++

Brief descriptions of the various sampling sites are listed below.

Stations 1, 2 and 3 in the lower reaches were characterised by the predominance of marine sediments and the virtual absence of aquatic macrophytes (see next chapter). Water depth within the lower reaches of the estuary was generally <1 m.

Sediments of the middle reaches of the estuary (stations 4 through to 7) comprised mainly of mud, which was typically covered by submerged macrophytes (particularly *Ruppia* spp.). The eastern bank was characterised by an extensive bed of *P. australis*. In the upper region of the middle reaches, salt marshes were found on both the eastern and western banks of the estuary.

The upper reaches of the estuary (stations 8-10) were characterised by a narrow channel (depth 1-1.8 m), generally not exceeding 10 m in width. The substrate was typically comprised of fine mud, which was periodically covered by submerged macrophytes (*Ruppia* spp.). An extensive bed of *P. australis* was located on the western bank of the estuary.

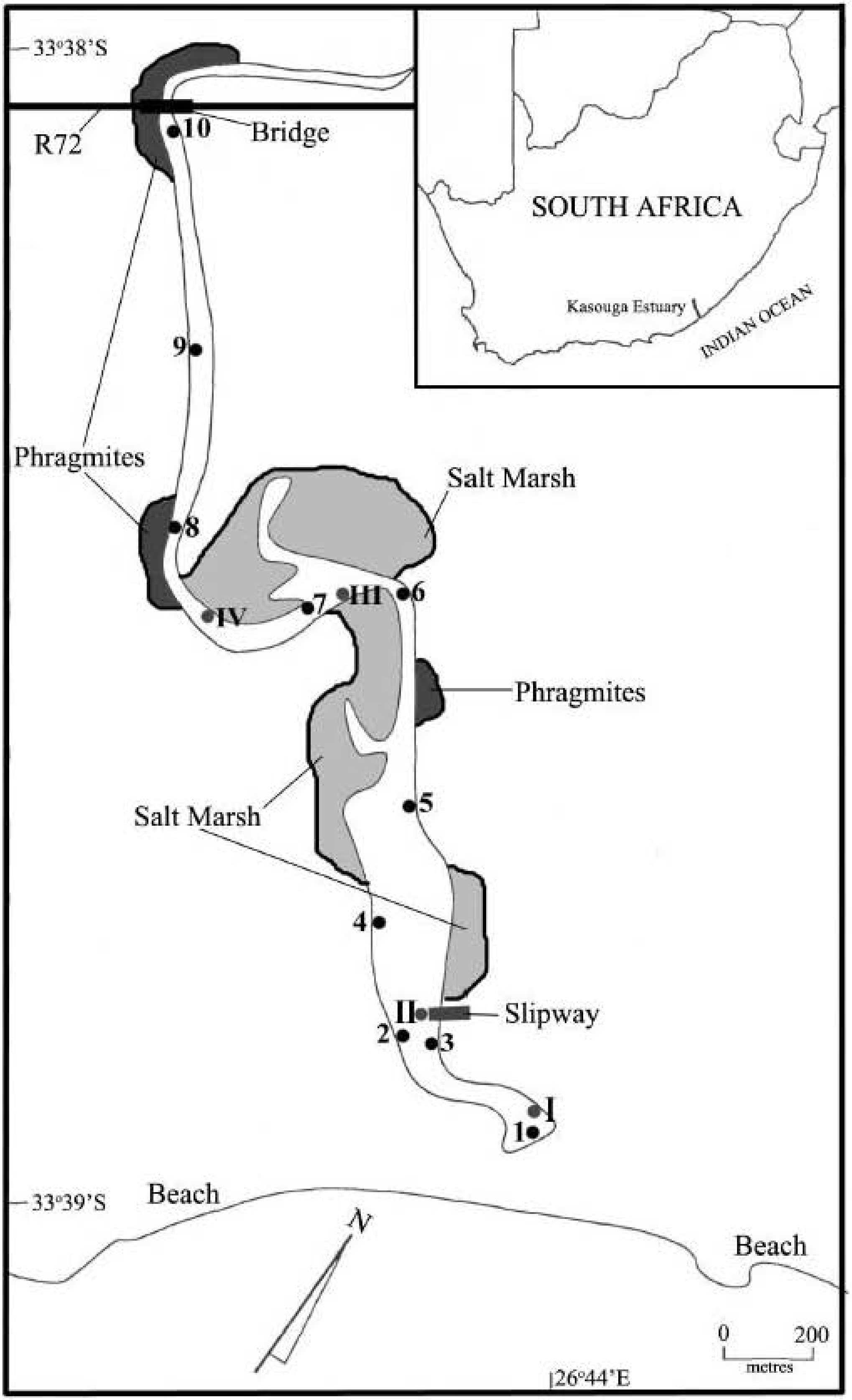


Figure 2.1: Stations occupied in the Kasouga estuary for the period March 2002 to May 2003. Stations 1-10 were sampled with the 5 m seine net and Station I-IV with the 50 m seine net.

CHAPTER 3

SPECIES COMPOSITION, SPATIAL AND TEMPORAL DISTRIBUTION OF ICHTHYOFAUNA USING THE 5 METRE AND 50 METRE SEINE NETS

3.1 Introduction

Previous ichthyofaunal work on the Kasouga estuary has been limited to collection by Blaber (1973a, 1973c, 1974a) of *R. holubi* and the inclusion of Kasouga estuary as a system inhabited by the endangered *Syngnathus watermeyeri* Smith, 1963 (Smith & Heemstra, 1991; Cowley, 1998). Other work recently conducted on the Kasouga estuary has primarily been on physico-chemical and plankton components and their relation to aspects such as overtopping or breaching events (Froneman, 2002a, 2002b, 2004).

The aim of this study was to identify spatial and temporal patterns in ichthyofaunal species. The environmental and biological parameters were studied to identify possible factors influencing the presence and distribution of species within the system.

3.2 Materials and Methods

3.2.1 Physico-chemical Variables

Surface temperature (at 0.5 m depth), salinity and turbidity were measured at each sampling site. Temperature (°C) was recorded on site using an electronic hand held thermocouple (Hama Instrument). Salinity (‰) was measured using a refractometer either on site or from water samples transported back to the laboratory. Turbidity (Nephelometric Turbidity Units - NTU) was recorded from water samples returned to the laboratory using a turbidimeter (Hach model 2100A). Surface values were used as previous studies conducted in the estuary showed that the water column is well mixed with little or no stratification evident (Froneman, 2002a, 2002b).

3.2.2 Phytoplankton

Water samples (250 ml) were taken from each station (n = 3 for each station) to determine *in situ* chlorophyll-*a* (Chl-*a*) concentrations. Water samples were filtered through a GF/C filter (vacuum, <5 cmHg) and extracted in the dark for 24 hr in 90% acetone. Chl-*a* concentrations were determined fluorometrically, before and after acidification according to the method of Holm-Hansen & Riemann (1978). Data were expressed as mg Chl-*a* m^{-3}.

3.2.3 Zooplankton

Zooplankton tows were conducted at night using a modified WP-2 net with a mouth area of 0.05 m^2 and a mesh size of 60 µm. The net was fitted with a flowmeter (General Oceanics) to determine the volume of water filtered during each tow. The net was towed obliquely at the surface (depth 0.75 m). Samples were immediately preserved in 10% buffered formalin (Hexamine). In the laboratory subsamples (one eighth to one sixteenth sub-sample) were then gently filtered (vacuum <5 cmHg) through a pre-weighed GF/F filter for the determination of zooplankton dry weight. The samples were dried in an oven for 24 hrs at 60°C and expressed as mg dwt m^{-3}.

3.2.4 *Vegetation*

Vegetation was estimated as percentage coverage by estimating the area covered by visual assessment as described in Cowley (1998).

3.2.5 *Fish (5 m seine net)*

The early developmental stages and smaller (Standard Length {SL} <50 mm) ichthyofauna of the Kasouga estuary was sampled using a 5 metre by 1 metre seine net with a mesh size of 500 ìm. Two people pulled the net parallel to the bank for a pre-determined length of 10 metres (kept consistent using a rope of 10 m laid out along the bank) and the pull is ended with the person in the deeper section coming into the bank in a semicircular fashion and then the net is pulled up on to the shore by both people, trapping the fish in the net; the area sampled by the net varied between 45 m^{2} and 50 m^{2}. The bottom of the net was kept in contact with the bottom. The fish caught were immediately preserved in 10% formaldehyde for later analysis in the laboratory. Fish were identified using Smith & Heemstra (1991) and SL of individuals was measured to the nearest 0.01 mm using a digital Vernier calliper. Abundances and biomass of each species were then determined for each station. Data were expressed as individuals m^{-2} (ind m^{-2}) or biomass as g wwt m^{-2}.

3.2.6 *Fish (50 m seine net)*

Sampling of larger ichthyofauna (SL >50 mm) was undertaken employing a seine net measuring 50 m by 2 m with 30 mm stretched mesh and a bunt of 10 mm box mesh. The net was laid in a semi-circle from the bank by boat or wading then pulled ashore.

Netting was undertaken monthly during the day from June 2002 to May 2003. This did not coincide with the 5 m seine netting due to the unavailability of the net for the first three months. The area sampled during each tow was estimated at 450 m^2. The study sites were selected to limit the possibility of obstruction to the net during the retrieval process. The net was pulled by two to three individuals, ensuring the foot-rope was always kept in contact with the ground.

Fish caught were immediately placed into plastic carboys filled with estuarine water. Fish were identified, measured to the nearest mm SL and the biomass of each species determined using a handheld fisherman’s spring scale (accurate to 20 g). After measurements, fish were returned to the estuary. Where field identification was not possible, specimens were preserved and returned to the laboratory for identification.

3.2.7 Analysis

The spatial patterns and distribution of the fish in the Kasouga Estuary were analysed employing a non-parametric multivariate analysis from the PRIMER 5 (version 5.2.4 for Windows) software package (Clarke & Warwick, 1998). The ichthyofaunal abundance data were combined into stations for spatial comparison, into months for the temporal scale and combined as a whole to give an overall indication of the richness and diversity of the estuary. A similarity matrix of the data was produced by transforming {log(x+1)} the abundance data. A cluster analysis of the data was performed with a complete linkage hierarchical sorting strategy. Dendograms and non-metric multidimensional scaling (MDS) were used to determine spatial and temporal relationships. The SIMPER analysis (PRIMER 5) was run on the different reaches and seasons to identify species that are the main contributors to the similarity in each of the groups (season or reaches) identified with the numerical analysis. The richness and diversity of the ichthyofauna in the Kasouga estuary was expressed using both the Margalef's Richness Index and Shannon Wiener's Diversity index. These were calculated using the PRIMER 5 (version 5.2.4 for Windows) computer package.

Margalef's Richness Index

$$d=(S-1)/\log N$$

Where d is Margalef's index, S is the number of species and N is the number of individuals. This index calculates the number of species in the sample relative to the number of individuals. This reduces the sample size bias (Clarke & Warwick, 1994)

Shannon-Wiener's Diversity Index

$$H'=-S_i\, p_i\, (\log p_i)$$

Where H' is the diversity index, i is the sample number and p_i is the percentage of the ith species contributing to the total count.

3.3 Results

The Kasouga estuary breached twice during the study period opening first on the 18th of August and closing on the 22nd of October 2002. The second breaching occurred in November 2002 from the 11th to the 20th. There were also three major overtopping events during the period of study; one on May 25th, one on June 30th and one at the beginning of December (2nd).

Newman-Keuls tests performed after a One-Way Analysis of Variance (ANOVA) test indicated that there were no significant spatial differences in the biological and physico-chemical variables during each month ($P>0.05$ in all cases). As a consequence, values for these variables for each month have been pooled.

3.3.1 Temperature

Water temperatures exhibited distinct seasonality with maximum values recorded during summer (average value 28.72°C, maximum value 33.90ºC) and minimum values during winter (average value 15.04°C, minimum value 8.20ºC). Intermediate water temperatures were recorded in spring and autumn (range 19ºC - 27ºC) (Figure 3.1).

3.3.2 Salinity

There were no distinct seasonal patterns in salinity during the investigation (Figure 3.2). Rather salinity values during the study varied in accordance to the overtopping and breaching events. Increases in salinity values in May and June were associated with the overtopping events that occurred over those periods. After the first breaching event, the salinity values dropped from ~30‰ to ~8‰ and remained low in August and September. Thereafter, values increased up to ~25‰. During the second breaching event, salinity values decreased although the drop in values (decreased by ~1‰) were not as dramatic as that recorded during the first breaching event (Figure 3.2). During the months of April and May 2003 the estuary was open and the salinity levels dropped accordingly. The decrease in salinity values was, however, not as extreme as those recorded during August and September 2002.

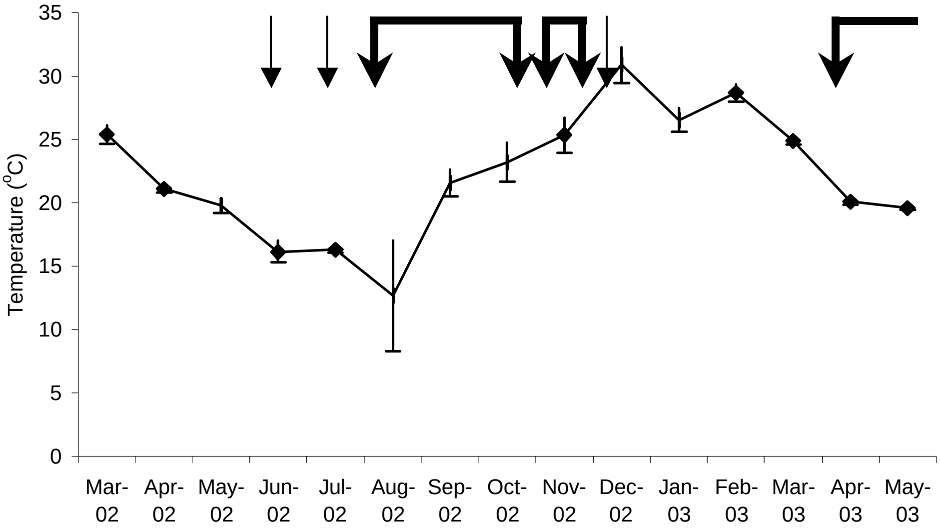


Figure 3.1: The mean monthly water temperatures (°C) (± standard deviation) in the Kasouga estuary for the period, March 2002 to May 2003. The thicker connected arrows indicate the period when the estuary was breached and the thin arrows indicate large overtopping events.

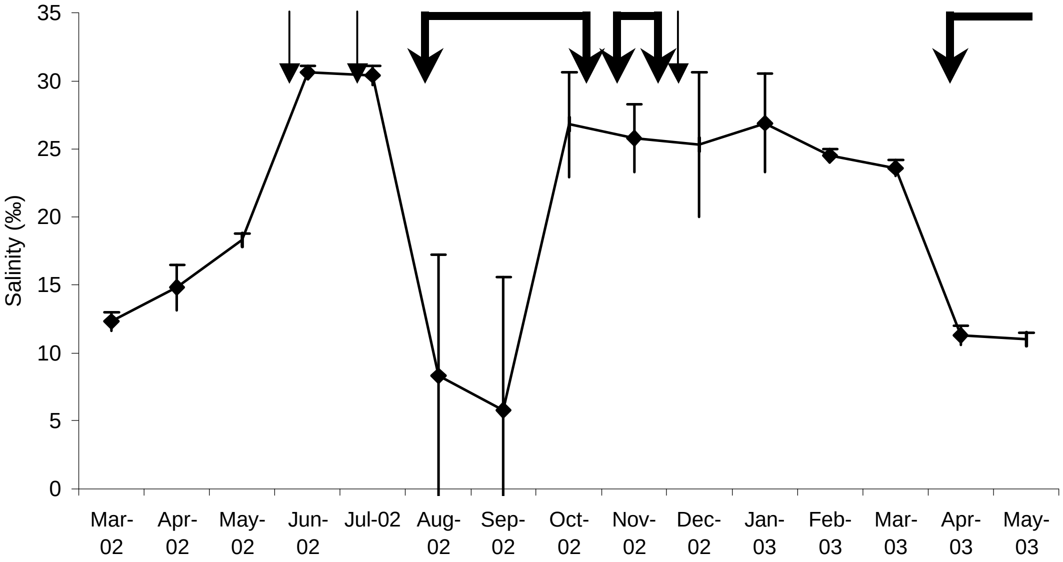


Figure 3.2: The mean monthly salinity (± standard deviation) in the Kasouga estuary for the study period, March 2002 to May 2003. The thicker connected arrows indicate the period when the estuary was breached and the thin arrows indicate large overtopping events.

3.3.3 Turbidity

Turbidity values during the study period were highly variable and ranged from 8 NTU to 61 NTU (Figure 3.3). Fluctuations in turbidity were generally linked to the inflow of freshwater into the system (as is evident from the decrease in salinity) and overtopping. The highest values (50-59 NTU) were recorded after the breaching event in August.

3.3.4 Phytoplankton

Total chlorophyll-*a* concentration during the study ranged between 0.14 mg chl-*a* m^{-3} and 3.05 mg chl-*a* m^{-3} (Figure 3.4). The highest chlorophyll-*a* concentrations were generally recorded following freshwater inflow into the estuary (as is evident from the decrease in salinity). Indeed, total chl-*a* concentration was negatively correlated to salinity (r^2= 0.51; $P<0.05$). During these periods, total chlorophyll-*a* concentrations were primarily >1.5 mg chl-*a* m^{-3}. Exceptions were recorded during August and September where the total chl-*a* concentrations were <1.5 mg chl-*a* m^{-3}. In the absence of freshwater inflow the total chlorophyll- *a* concentrations were usually below 1.5 mg chl-*a* m^{-3} (Figure 3.4).

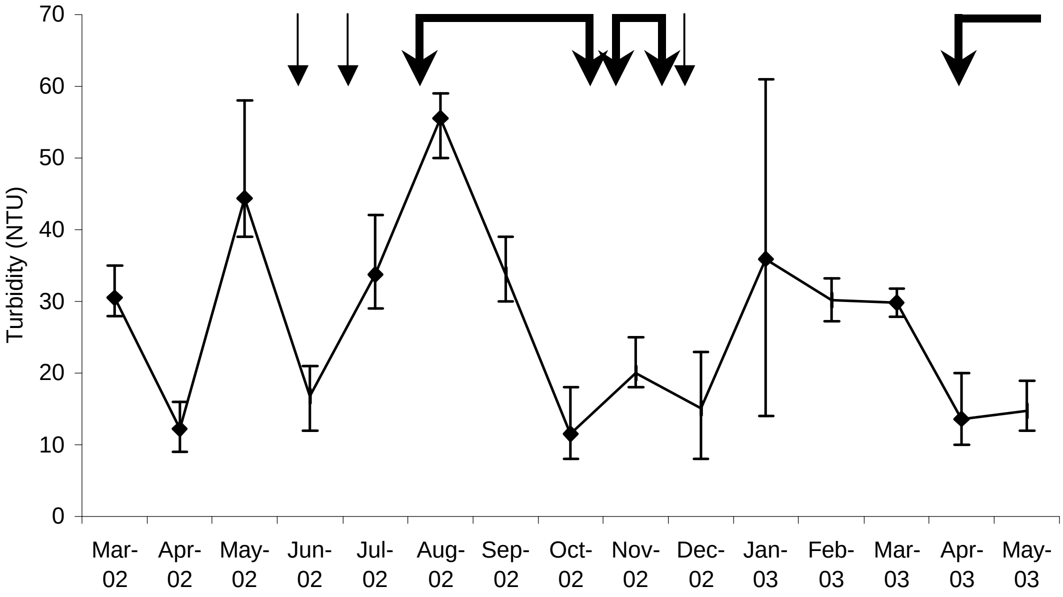


Figure 3.3: Mean turbidity and the maximum and minimum values (error bars) of the Kasouga estuary for the study period, March 2002 to May 2003. (Small arrows indicate overtopping and thicker connected arrows indicate periods when the mouth is open to the sea).

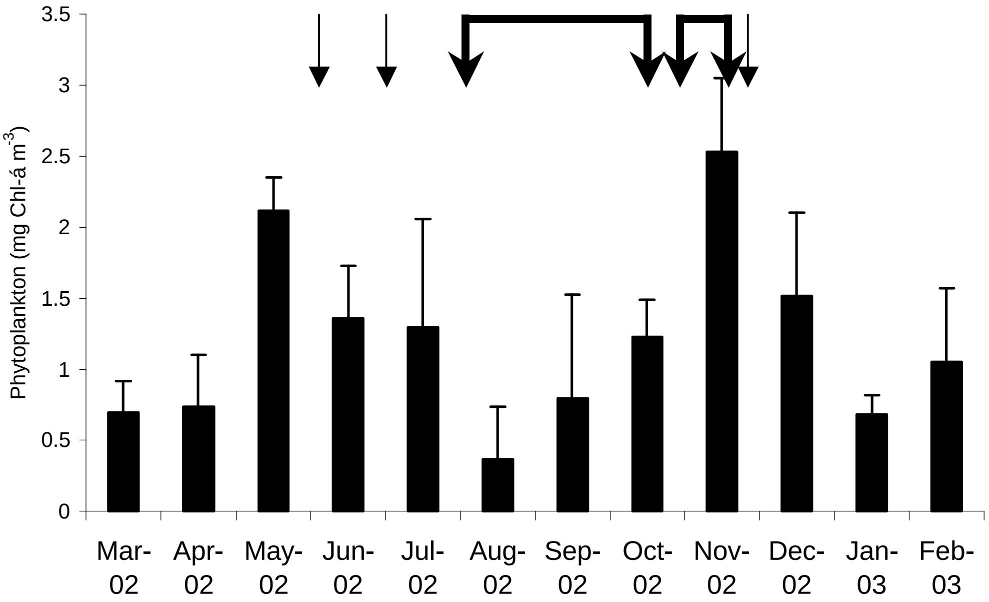


Figure 3.4: Mean monthly phytoplankton biomass (mg chl-*a* m^{-3}) in the Kasouga Estuary during the study period, March 2002 to February 2003. Error bars are standard deviation. (Small arrows indicate overtopping and thicker connected arrows indicate periods when the mouth is open to the sea).

3.3.5 Zooplankton

No zooplankton data were collected over the period August 2002 to October 2002 (Figure 3.5) because of the shallow depth. Total zooplankton biomass during the study ranged from 20.19 mg dwt m^{-3} to 60.06 mg dwt m^{-3} and showed a strong temporal pattern. The highest biomass values were normally recorded in summer (>35 mg dwt m^{-3}) and the lowest in winter (<30 mg dwt m^{-3}). An exception occurred in January 2003, when the total zooplankton biomass was estimated at 28.7 mg dwt m^{-3} (Figure 3.5). Intermediate zooplankton biomass values were recorded in the autumn and winter months (range 21 mg dwt m^{-3} and 50 mg dwt m^{-3}). Total zooplankton was significantly correlated to the total chl-*a* concentration (r^2= 0.71; $P<0.05$).

3.3.6 Vegetation Cover

Submerged aquatic vegetation cover at the various sampling stations during the study was variable on a monthly basis ranging from 0% to 100% cover. Over the entire sampling period the percentage vegetation cover was greatest during winter (July 2002 – August 2002) with a minor peak also recorded in January 2003 (Figure 3.6). The upper reaches of the estuary generally exhibited the highest average vegetation cover (Figure 3.7). The station nearest the mouth region of the estuary was very sparse in submerged vegetation with cover generally <10% of the total surface area (Figure 3.7).

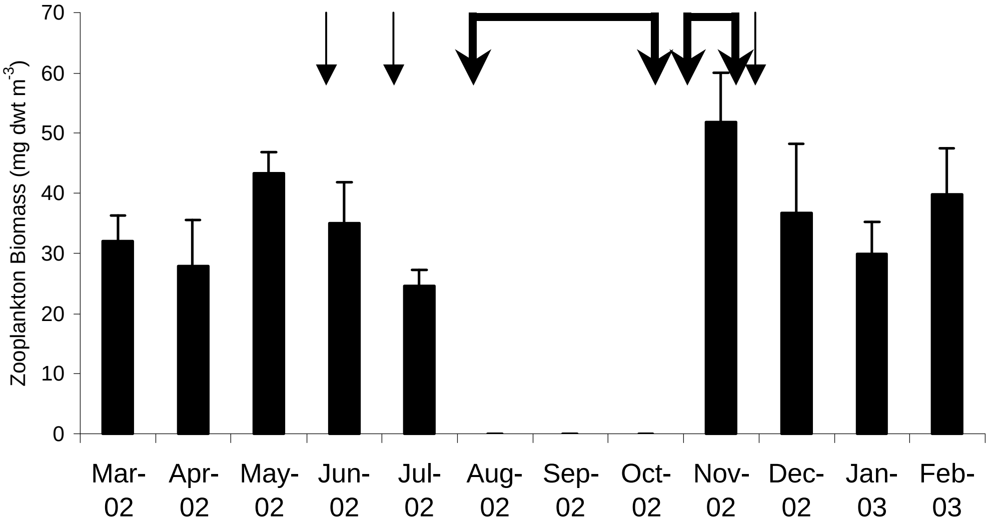


Figure 3.5: Mean monthly biomass of zooplankton (mg dwt m^{-3}) in the Kasouga estuary over the period of March 2002 to February 2003 in the Kasouga Estuary. Error bars are standard deviation. No data were collected over the period August to October due to the shallow depth of the estuary. (Small arrows indicate overtopping and thicker connected arrows indicate periods when the mouth is open to the sea).

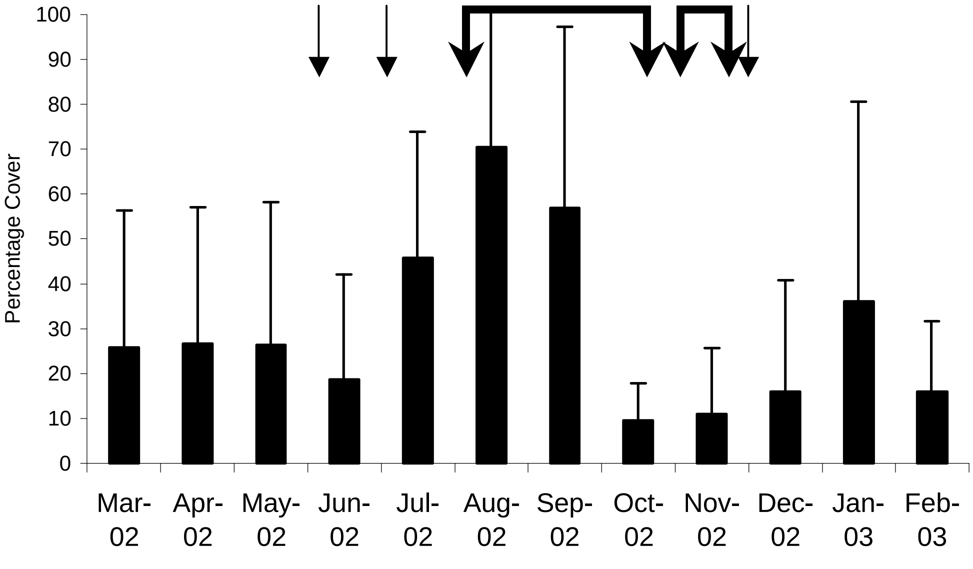


Figure 3.6: Mean monthly vegetation cover of the sample sites in the Kasouga Estuary for the period March 2002 to February 2003 with standard deviation (error bars). (Small arrows indicate overtopping and thicker connected arrows indicate periods when the mouth is open to the sea).

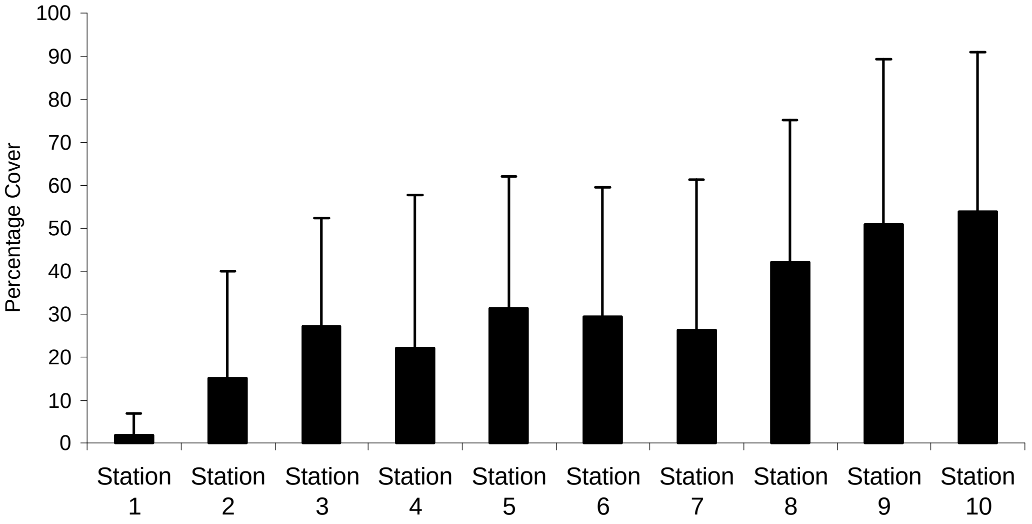


Figure 3.7: Mean vegetation cover (%) for the sampling stations in the Kasouga Estuary for the sampling period of March 2002 to February 2003 with standard deviation (error bars). Station 1 is at the mouth and station 10 is in the upper reaches near the coastal road bridge.

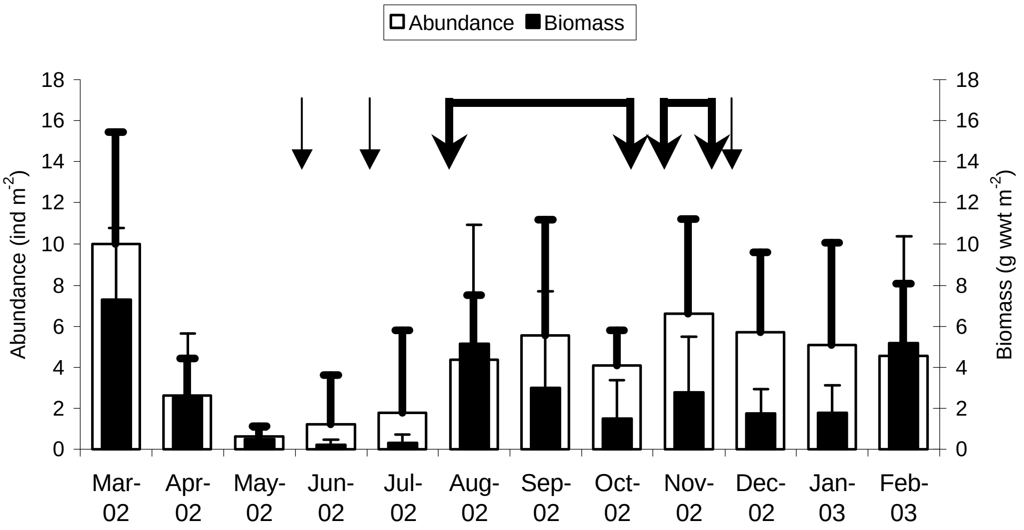


Figure 3.8: Mean abundance and biomass of ichthyofauna in the Kasouga estuary for the period of study with standard deviation (error bars). (Small arrows indicate overtopping and thicker connected arrows indicate periods when the mouth is open to the sea).

3.3.7 *Fish (5 m seine net)*

3.3.7.1 Abundance and Biomass

A total of 20 036 fish comprising 15 species from 9 families (Table 3.1) were sampled using the 5 metre seine net for the period March 2002 to February 2003. Total fish abundance during the study period ranged between 0 and 24.1 ind m^{-2} and exhibited a weak seasonal pattern with the lowest values generally recorded during the winter months (June, July and August) (average 2.45 ind m^{-2} ± 1.67 ind m^{-2}) and the highest abundances in spring (September, October and November) (average 5.42 ind m^{-2} ± 1.27 ind m^{-2}). Intermediate values were recorded during summer (December, January and February) (average 5.11 ind m^{-2} ± 0.59 ind m^{-2}) and autumn (March, April and May) (average 4.41 ind m^{-2} ± 4.92 ind m^{-2}). Total ichthyofaunal abundance increased following the overtopping events (May and June) and doubled from the previous month (July) when the estuary breached in August. Abundances once again increased after the second breaching event in November (Figure 3.8).

The ichthyofaunal biomass throughout the study period was significantly correlated to the fish abundance ($r^2 = 0.29$; $P < 0.05$) (Figure 3.8). Total ichthyofaunal biomass during the study ranged between 0 and 20.25 g wwt m^{-2} with maximum values recorded in autumn (average 3.44 g wwt m^{-2} ± 3.49 g wwt m^{-2}). Intermediate values were recorded in spring (average 2.40 g wwt m^{-2} ±0.79 g wwt m^{-2}) and summer (average 3.03 g wwt m^{-2} ± 1.98 g wwt m^{-2}), while the lowest values were recorded in winter (average 1.90 g wwt m^{-2} ± 2.81 g wwt m^{-2}). An exception was recorded in August 2002, where the third highest average monthly biomass (5.11 g wwt m^{-2})

during the entire investigation was recorded (Figure 3.8). Total ichthyofaunal biomass increased following breaching and overtopping events.

3.3.7.2 Estuarine Utilisation

During the first 5 months of the survey, estuarine dependent species (Category I) (*G. callidus* and *A. breviceps*) numerically dominated the catch comprising between 38% and 99% of all fish collected. Category II species (marine species with estuarine dependent juveniles) represented the second largest contributor to catch with contributions of between 0% and 54% of the total. Following the breaching of the estuary in early August 2002, more category II species (4-7 species) dominated the catch than category I species (2-5 species) (Figure 3.9). In September 2002 category I and II species had similar contributions to the percentage of the catch (50.8% and 49.2% respectively) and in October 2002 category II dominated the catch numerically (54.4%). Category III and V species were not caught using the 5 m seine net and category IV species, namely *Oreochromis mossambicus*, contributed <5% of the catch for all months except in November 2002, December 2002 and January 2003 (13.1%, 9.4% and 7.8% respectively).

Glossogobius callidus (Figure 3.11) dominated the small ichthyofauna catch (Figure 3.10) of the Kasouga Estuary both numerically and by biomass comprising on average 33% (range 10% to 47%) of the total abundance and 67% (range 46% to 89%) of the total biomass. The contribution of this species was highest in the upper reaches of the estuary, where it contributed between 33% and 57% of abundance and 55% to 85% of the total biomass. *Glossogobius callidus* contributed between 25% to 47% of the entire catch (abundance) for each month except during June and July where their contribution to the total catch was less than 20% (15.08% and 10.08% respectively) (Figure 3.11). Abundances of *G. callidus* displayed a distinct temporal pattern with the highest values recorded in spring (average 2.14 ind.m^{-2}, 1.6 g wwt m^{-2}) and the lowest in winter (average 0.8 ind m^{-2}, 1.1 g wwt m^{-2}). Intermediate values were recorded in summer (average 1.6 ind m^{-2}, 1.7 g wwt m^{-2}) and autumn (average 1.22 ind m^{-2}, 1.8 g wwt m^{-2}).

Atherina breviceps (Figure 3.12) was the second highest contributing species (Figure 3.10) with maximum values recorded (average 1.97 ind m^{-2}) in the lower reaches of the estuary (Figure 3.12). An exception was recorded at station 8, where *A. breviceps* contributed almost 50% of all fish caught at that station for the entire year. Peaks in the abundance and biomass of *A. breviceps* were recorded in the late summer months (January and February 2003) and after overtopping events (June and July 2002).

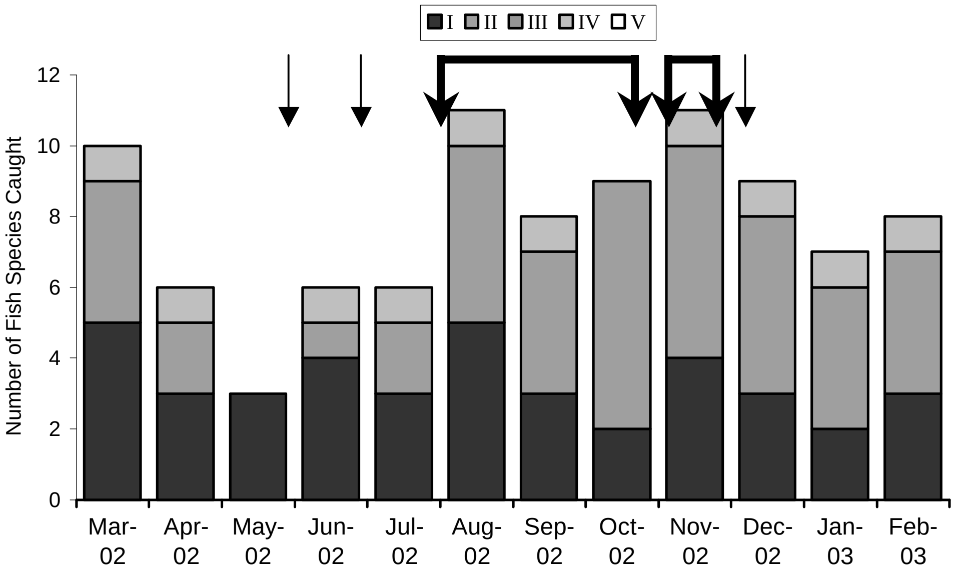


Figure 3.9: Contribution of fish in the different estuarine utilisation categories for each month of the sampling period. (Small arrows indicate overtopping and thicker connected arrows indicate periods when the mouth is open to the sea).

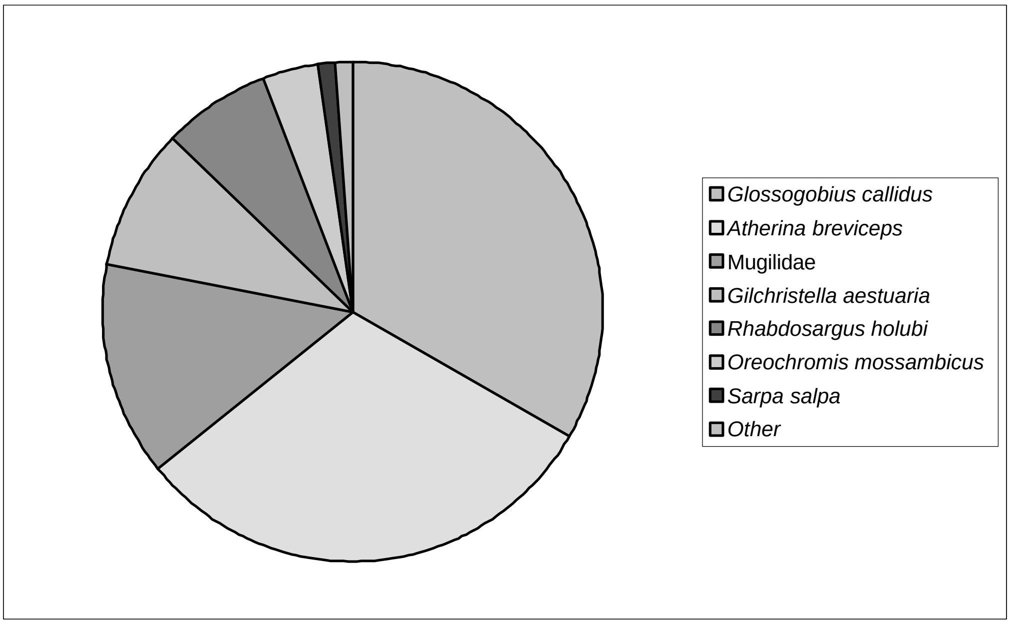


Figure 3.10: Species contribution to the overall catch of 20036 fish sampled with the small seine net. Other comprises 1.20% of catch and is made up of: *Heteromycteris capensis, Monodactylus falciformis, Psammogobius knysnaensis, Diplodus sargus capensis, Solea bleekeri, Lithognathus lithognathus, Pandaka silvana* and *Omobranchus woodi*.

Table 3.1: Total abundance and biomass of fish (SL <50 mm) caught and the subsequent percentage contribution of each species to the total catch of the 5 metre seine net for the study period March 2002 to February 2003. (The question mark in the estuarine utilisation category indicates an uncertain utilisation).

Family	Species	Common Name	Estuarine Utilisation Category	Abundance Total Numbers	Abundance Percentage Contribution	Biomass Total Biomass (g wwt)	Biomass Percentage Contribution
Atherinidae	*Atherina breviceps* Valenciennes, 1835	Cape silverside	**Ib**	6159	30.74	1879.86	15.03
Cichlidae	*Oreochromis mossambicus* (Peters, 1852)	Mozambique tilapia	**IV**	764	3.81	1261.95	10.09
Clupeidae	*Gilchristella aestuaria* (Gilchrist, 1914)	Estuarine roundherring	**Ia**	1819	9.08	867.32	6.93
Gobiidae	*Glossogobius callidus* (Smith, 1937)	River goby	**Ib**	6698	33.43	7247.15	57.93
	Psammogobius knysnaensis Smith, 1936	Speckled sandgoby	**Ib?**	29	0.14	15.61	0.12
	Pandaka silvana (Barnard, 1943)	Dwarf goby	**Ia?**	1	0.00	0.41	0.00
Monodactylidae	*Monodactylus falciformis* Lacepède, 1800	Oval moony	**IIa**	65	0.32	66.43	0.53
Mugilidae	Juvenile Mugilidae	Juvenile mullet	**II?**	2787	13.91	727.67	5.82
Soleidae	*Heteromycteris capensis* Kaup, 1858	Cape sole	**IIb**	113	0.56	34.00	0.27
	Solea bleekeri Boulenger, 1898	Blackhand sole	**IIb**	11	0.05	28.42	0.23
Sparidae	*Diplodus sargus capensis* (Smith, 1844)	Blacktail	**IIc**	17	0.08	6.85	0.05
	Lithognathus lithognathus (Cuvier, 1830)	White steenbras	**IIa**	3	0.01	0.69	0.01
	Rhabdosargus holubi (Steindachner, 1881)	Cape stumpnose	**IIa**	1379	6.88	366.55	2.93
	Sarpa salpa (Linnaeus, 1758)	Strepie	**IIc**	190	0.95	3.73	0.03
Blenniidae	*Omobranchus woodi* (Gilchrist & Thompson, 1908)	Kappie blenny	**Ia**	1	0.00	3.33	0.03
			Total	**20036**		**12509.97g**	

Although Mugilidae (Figure 3.13) were recorded throughout the length of the estuary, the majority were caught in the lower reaches (mean abundance: lower reaches 0.88 ind m^{-2} (±SD 0.32); middle reaches 0.51 ind m^{-2} (±SD 0.22); upper reaches 0.42 ind m^{-2} (±SD 0.08)). For juvenile Mugilidae, minimum values were recorded in autumn and winter (0.03 and 0.1 ind m^{-2}, respectively) and maximum values in spring and summer (1.49 and 0.89 ind m^{-2}, respectively). Biomass values showed this same trend except that peak biomass for juvenile mullet occurred in February 2003.

Gilchristella aestuaria (Figure 3.14) showed no pattern in distribution in the estuary. Although a few *G. aestuaria* were caught at stations 2 through 4 with an average of 0.03 ind m^{-2} (±SD 0.02), the majority were caught in the upper reaches of the estuary (11% of total catch ±SD 1.9%) with the exception of station 1 (13% of total catch). Maximum abundance of *G. aestuaria* was recorded at the end of winter and early spring 1.35 to 0.69 ind m^{-2} and maximum biomass was recorded in spring (0.22 ind m^{-2} ±SD 0.23). Outside these periods, values were generally <0.3 ind m^{-2} and <0.01 g wwt m^{-2}.

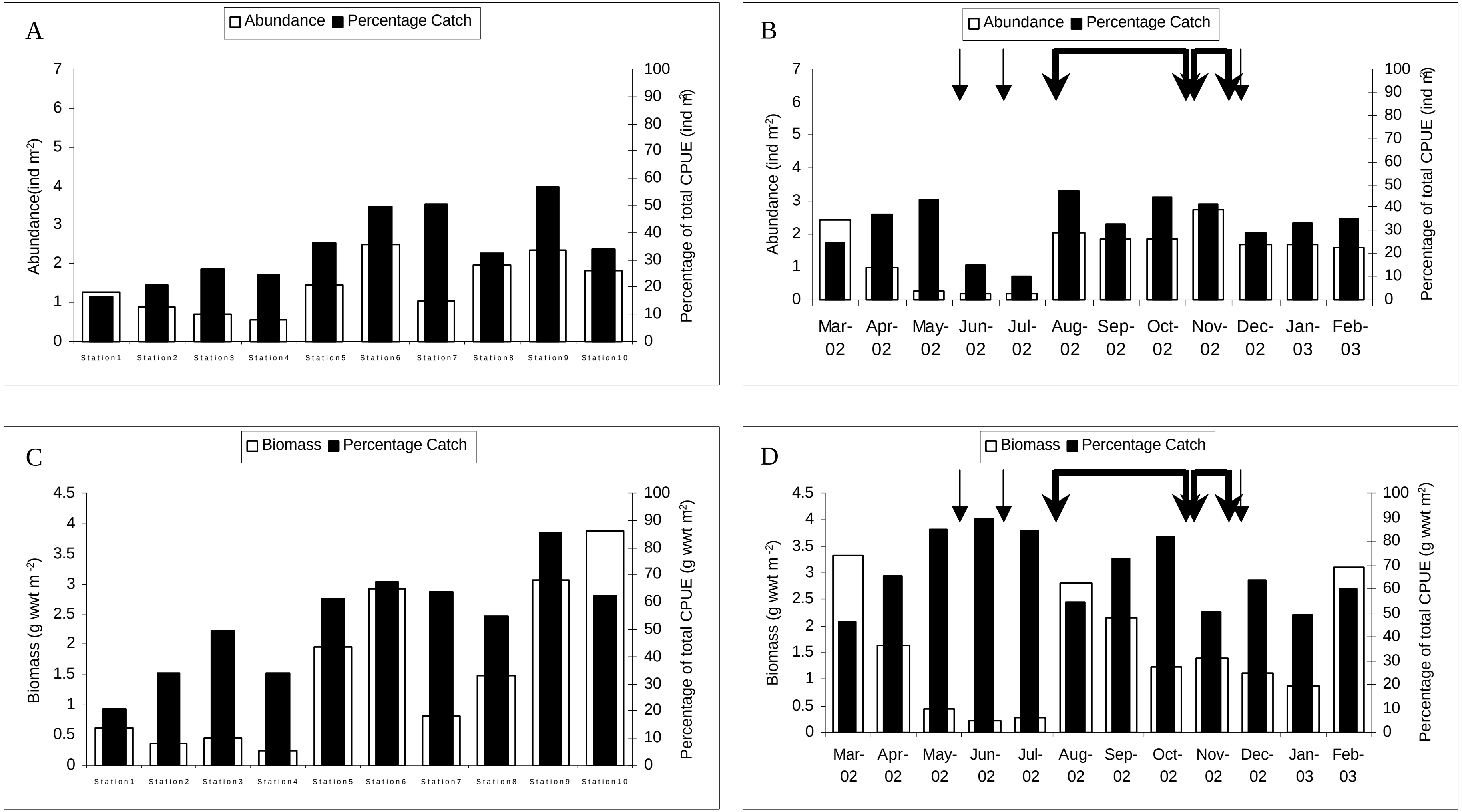


Figure 3.11: Total abundance (A & B) of *Glossogobius callidus* showing the abundance spatially throughout the estuary (A) and seasonally (B) from March 2002 to February 2003. Total biomass (C & D) of *G. callidus* showing biomass distributed spatially throughout the estuary (C) and (D) seasonally for the same period as abundance. The thin arrows indicate overtopping and the thick connected arrows indicate breached mouth conditions.

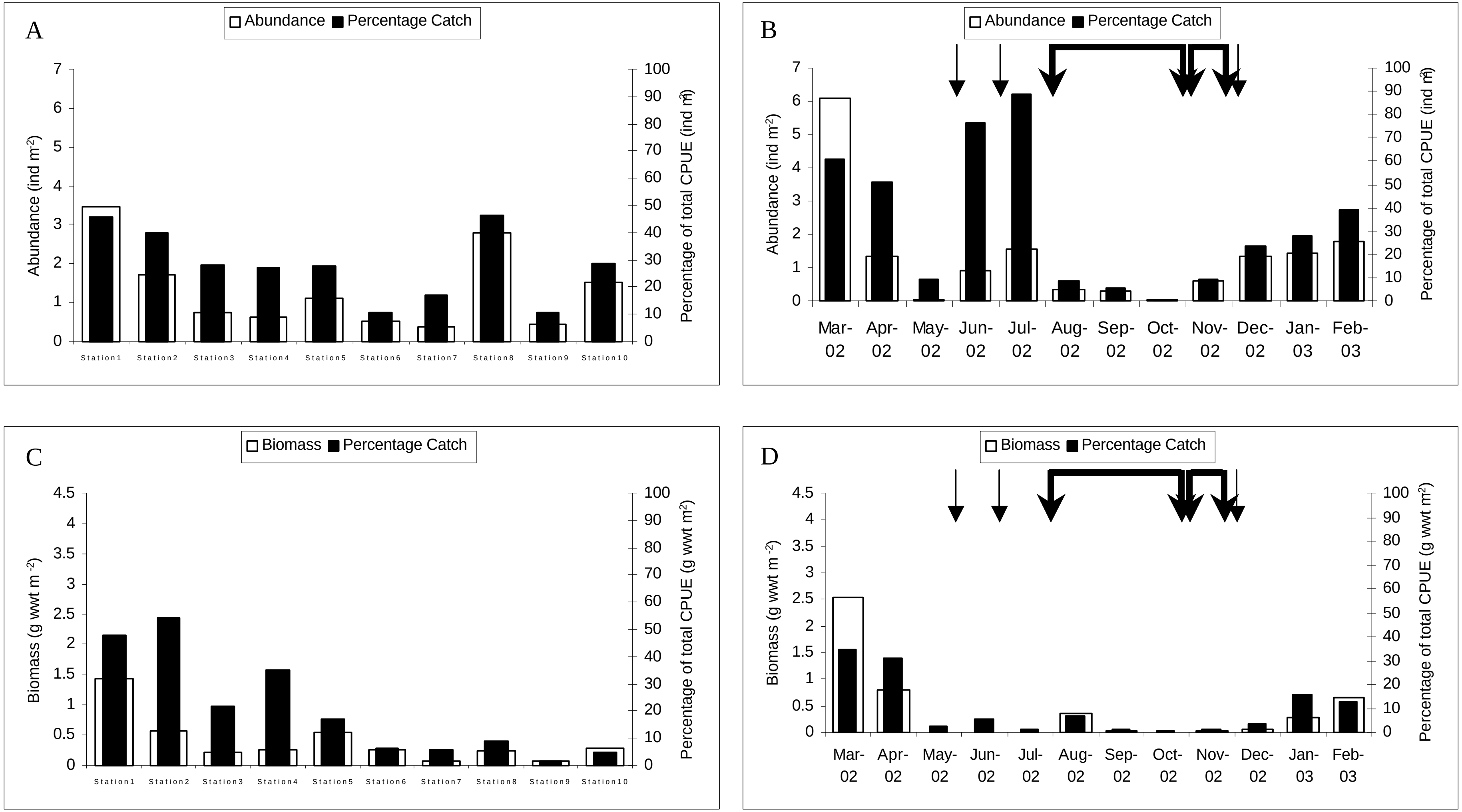


Figure 3.12: Total abundance (A & B) of *Atherina breviceps* showing the abundance spatially throughout the estuary (A) and seasonally (B) from March 2002 to February 2003. Total biomass (C & D) of *A. breviceps* showing biomass distributed spatially throughout the estuary (C) and (D) seasonally for the same period as abundance. The thin arrows indicate overtopping and the thick connected arrows indicate breached mouth conditions.

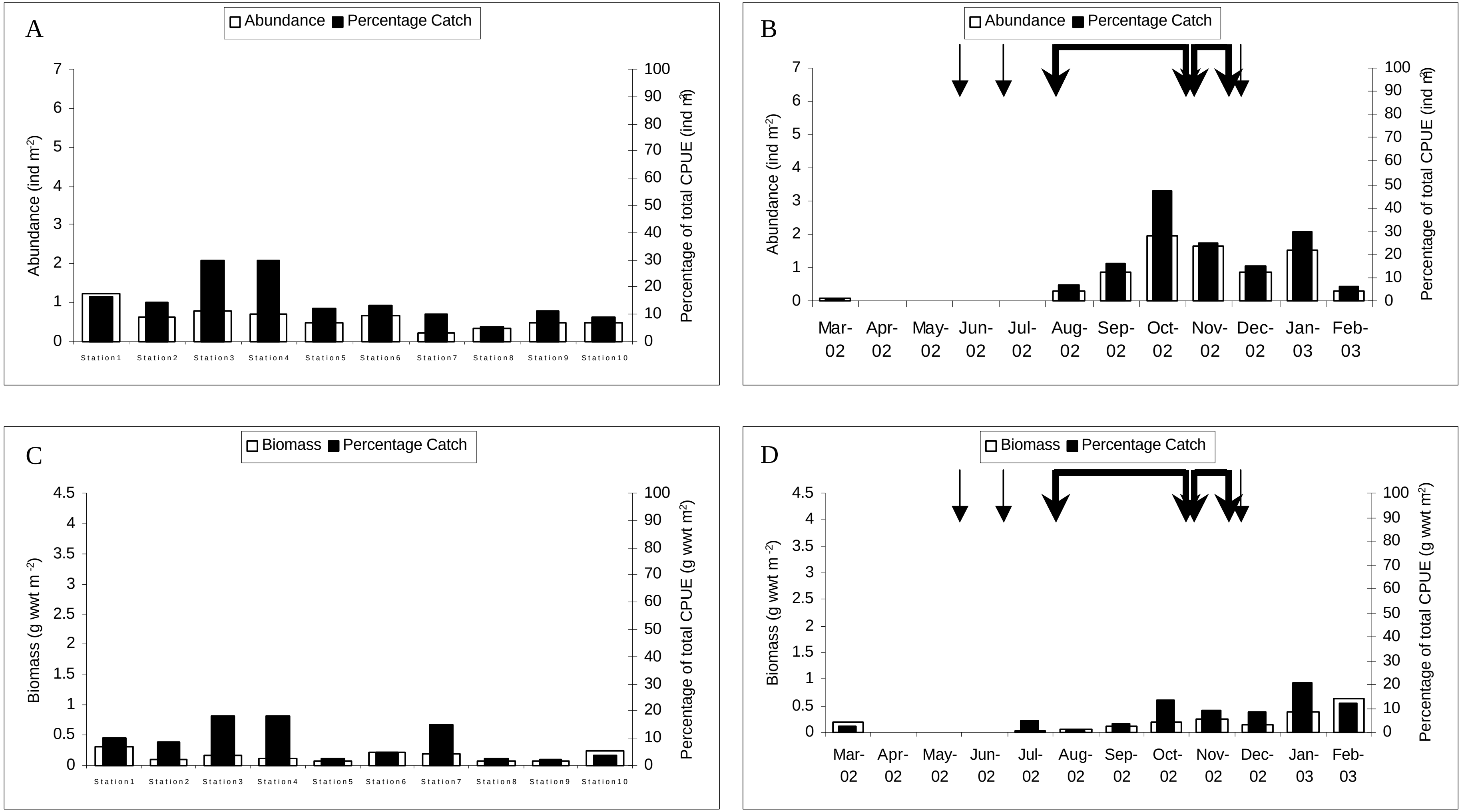


Figure 3.13: Total abundance (A & B) of juvenile Mugilidae showing the abundance spatially throughout the estuary (A) and seasonally (B) from March 2002 to February 2003. Total biomass (C & D) of juvenile Mugilidae showing biomass distributed spatially throughout the estuary (C) and (D) seasonally for the same period as abundance. The thin arrows indicate overtopping and the thick connected arrows indicate breached mouth conditions.

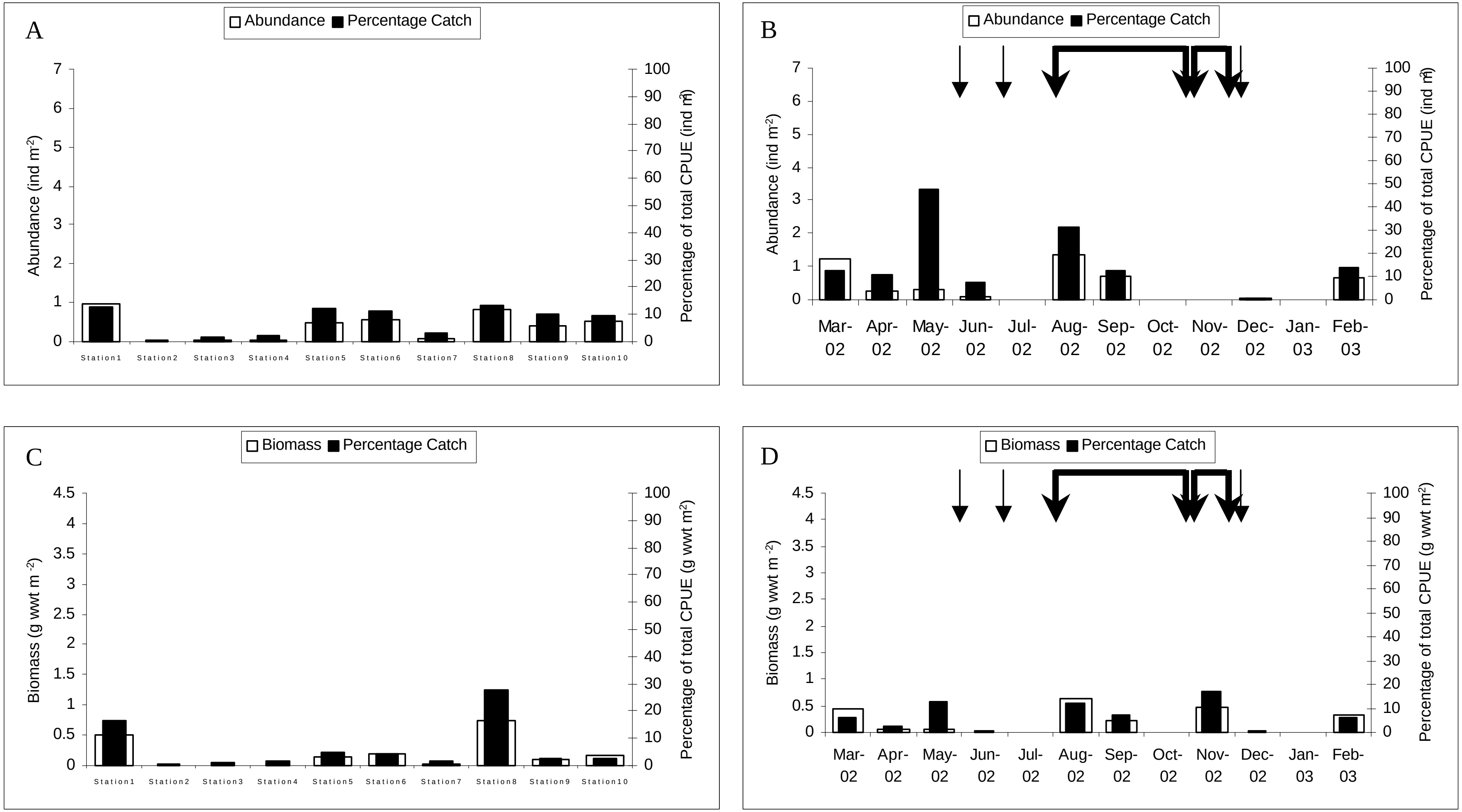


Figure 3.14: Total abundance (A & B) of *Gilchristella aestuaria* showing the abundance spatially throughout the estuary (A) and seasonally (B) from March 2002 to February 2003. Total biomass (C & D) of *G. aestuaria* showing biomass distributed spatially throughout the estuary (C) and (D) seasonally for the same period as abundance. The thin arrows indicate overtopping and the thick connected arrows indicate breached mouth conditions.

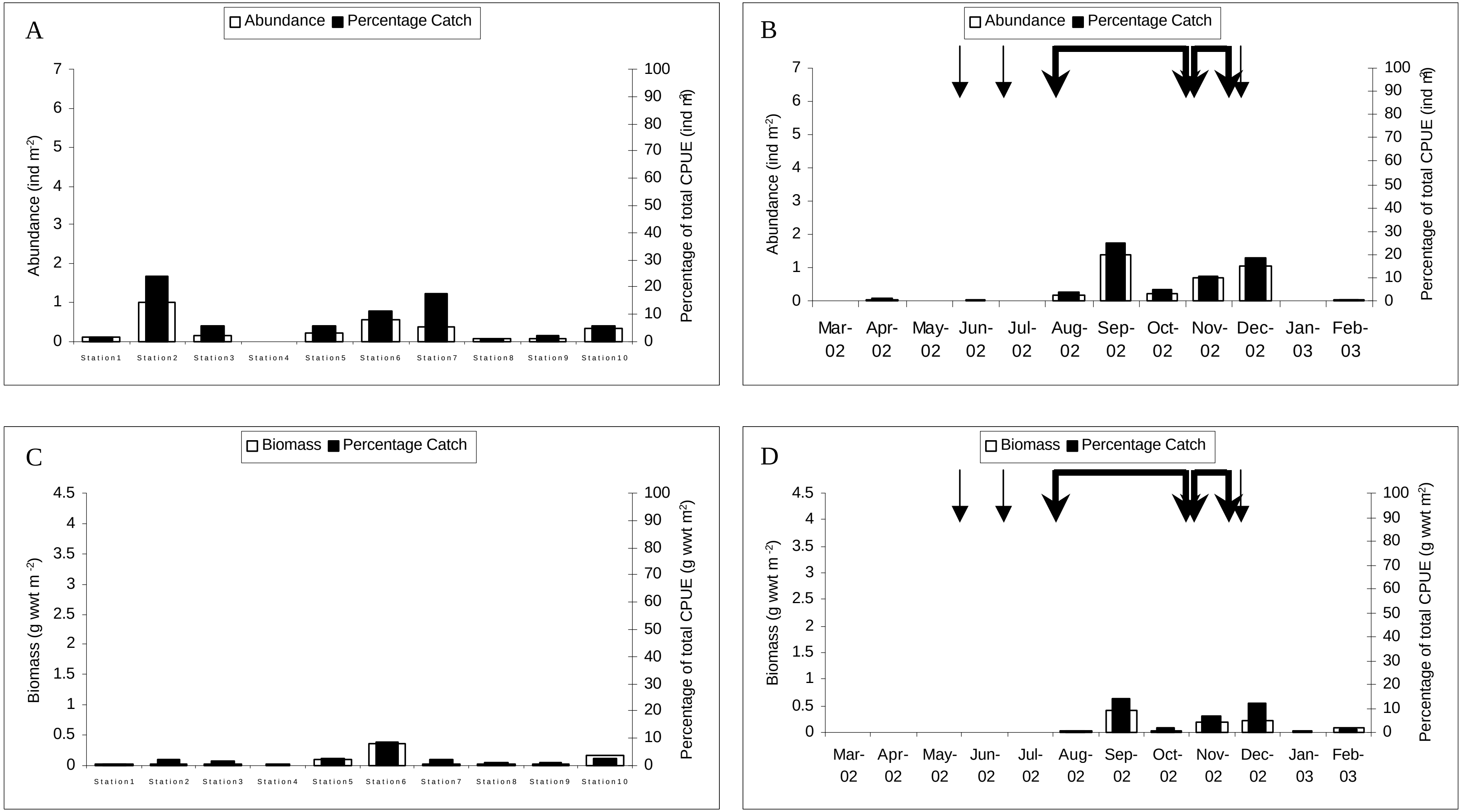


Figure 3.15: Total abundance (A & B) of *Rhabdosargus holubi* showing the abundance spatially throughout the estuary (A) and seasonally (B) from March 2002 to February 2003. Total biomass (C & D) of *R. holubi* showing biomass distributed spatially throughout the estuary (C) and (D) seasonally for the same period abundance. The thin arrows indicate overtopping and the thick connected arrows indicate breached mouth conditions.

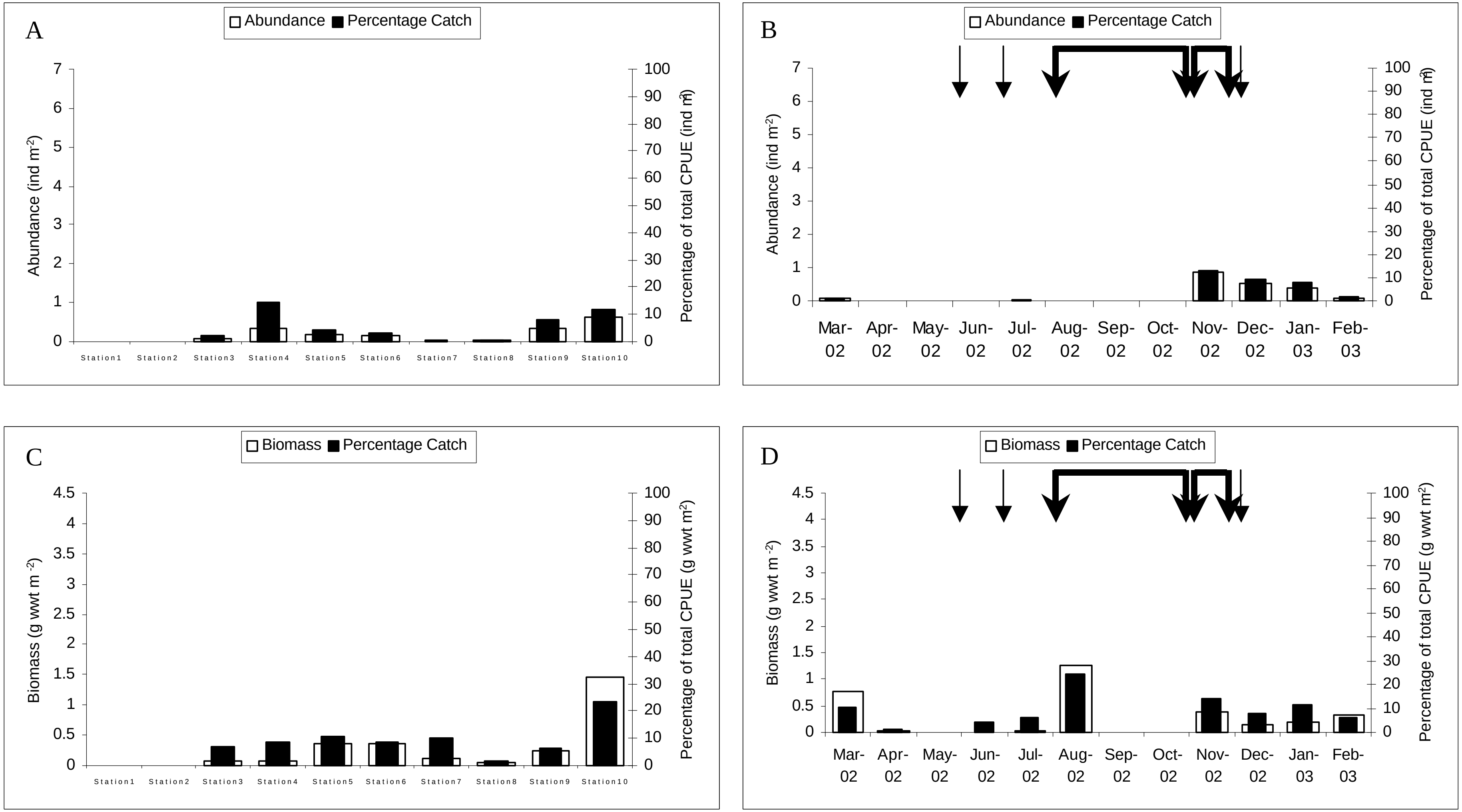


Figure 3.16: Total abundance (A & B) of *Oreochromis mossambicus* showing the abundance spatially throughout the estuary (A) and seasonally (B) from March 2002 to February 2003. Total biomass (C & D) of *O. mossambicus* showing biomass distributed spatially throughout the estuary (C) and (D) seasonally for the same period as abundance. The thin arrows indicate overtopping and the thick connected arrows indicate breached mouth conditions.

Juvenile *R. holubi* (SL <50 mm) (Figure 3.15) were caught along the entire length of the estuary, with no single reach more prominent than another, although the average abundance decreased from the lower to the upper reaches (10.3% to 8.8% to 3.1% of total catch). Although variable, a temporal pattern in the abundance and biomass of *R. holubi* was evident. The highest biomass and abundance of *R. holubi* was recorded over the period August 2002 to December 2002 when the abundance values were always >0.1 ind m^{-2} and the biomass values >0.02 g m^{-2}. In the winter months of June 2002 and July 2002 the abundance and biomass were always <0.01 CPUE (ind m^{-2} or g m^{-2}). The highest average percentage contribution was recorded in spring with 13.5% of total abundance and 7.4% of the total biomass. This coincided with the estuary being open. The next highest values with 6.5% of abundance and 4.7% of biomass occurred in summer. The lowest values were recorded in autumn with slightly higher contributions in winter that coincided with the occurrence of overtopping events (Figure 3.15).

Oreochromis mossambicus (Figure 3.16) was the only species caught that was of freshwater origin. It was recorded at all stations along the estuary with the exception of stations 1 and 2 located close to the mouth. The presence of *O. mossambicus* was most prominent during the spring (September to November) and summer (December to February) months of 2002 and 2003. The high biomass (24% of the catch) during August was due to large individuals caught in the upper and middle reaches, which were brought down stream during the increased freshwater flow.

3.3.7.3 Species Diversity

Margalef's richness index values ranged between 0.66 (Station 2) and 1.26 (Station 3) and between 1.27 (Station 8) and 1.62 (Station 10) for Shannon-Wiener diversity index (Figure 3.17). Highest values were recorded when overtopping had occurred or when the estuary had breached (Figure 3.18). An exception was recorded following the first overtopping event where values for both indices remained low 0.76 (Margalef) and 0.41 (Shannon-Wiener).

3.3.7.4 Numerical Analysis

The ichthyofauna abundance data did not reveal any significant spatial patterns (Figure 3.19). Spatial and/or temporal patterns may have been masked by the extreme variability in the abundance and biomass of early developmental stages of the ichthyofauna in the estuary during the study (Figure 3.19).

To assess temporal abundances and biomass patterns, numerical analyses were conducted on the mean CPUE for each month (Figure 3.20). Results of the analysis indicated the presence of two distinct groupings (ANOSIM $P<0.05$). Group 1 comprised stations occupied during autumn and winter and Group 2 those stations sampled during the spring and summer months. Group 1 is thus designated as the Cold Water Group (CWG) and Group 2, the Warm Water Group (WWG). Exceptions were in August (late winter), which was placed in the WWG, and February (a summer month), which was placed in the CWG.

The MDS plot (Figure 3.21) showed the distinct grouping between the cold water group and warm water group with a stress value of 0.04.

Differences between the WWG and the CWG were ascribed to differences in the relative contribution of the dominant species rather than presence/absence of individual species (SIMPER analysis). The ichthyofaunal species accounting for >90% of the dissimilarity between each group is shown in Table 3.2.

There were no significant correlations between ichthyofaunal abundance and biomass and the selected physico-chemical and biological variables measured during the study (Spearman correlation analysis).

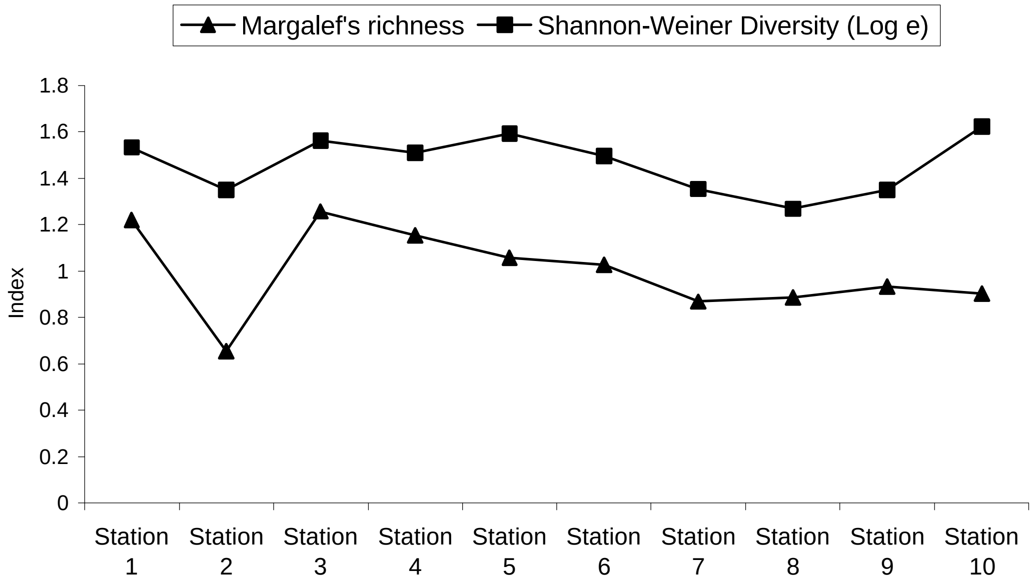


Figure 3.17: Graphical representation of the richness and diversity indices for the combined data for the sampling stations of the Kasouga estuary.

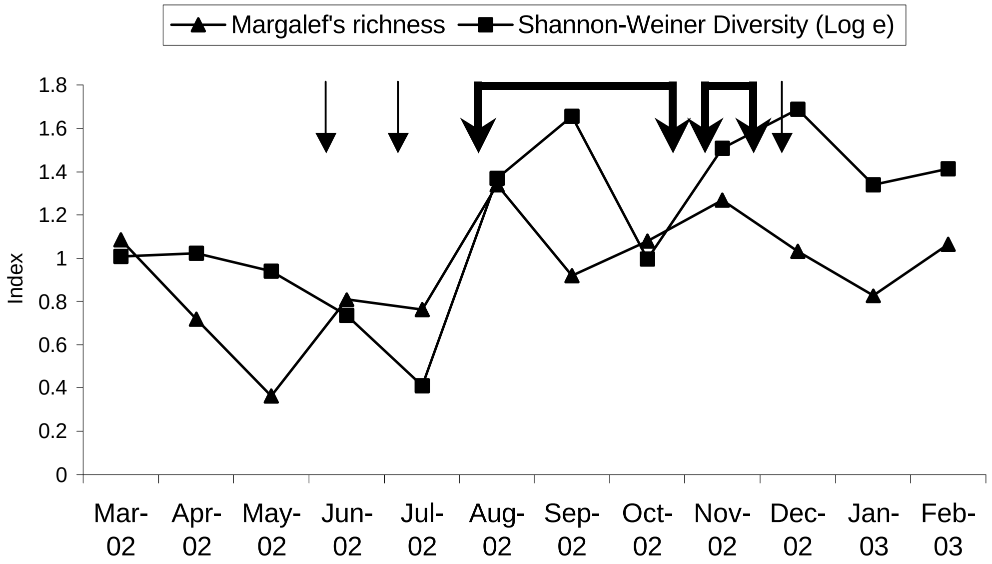
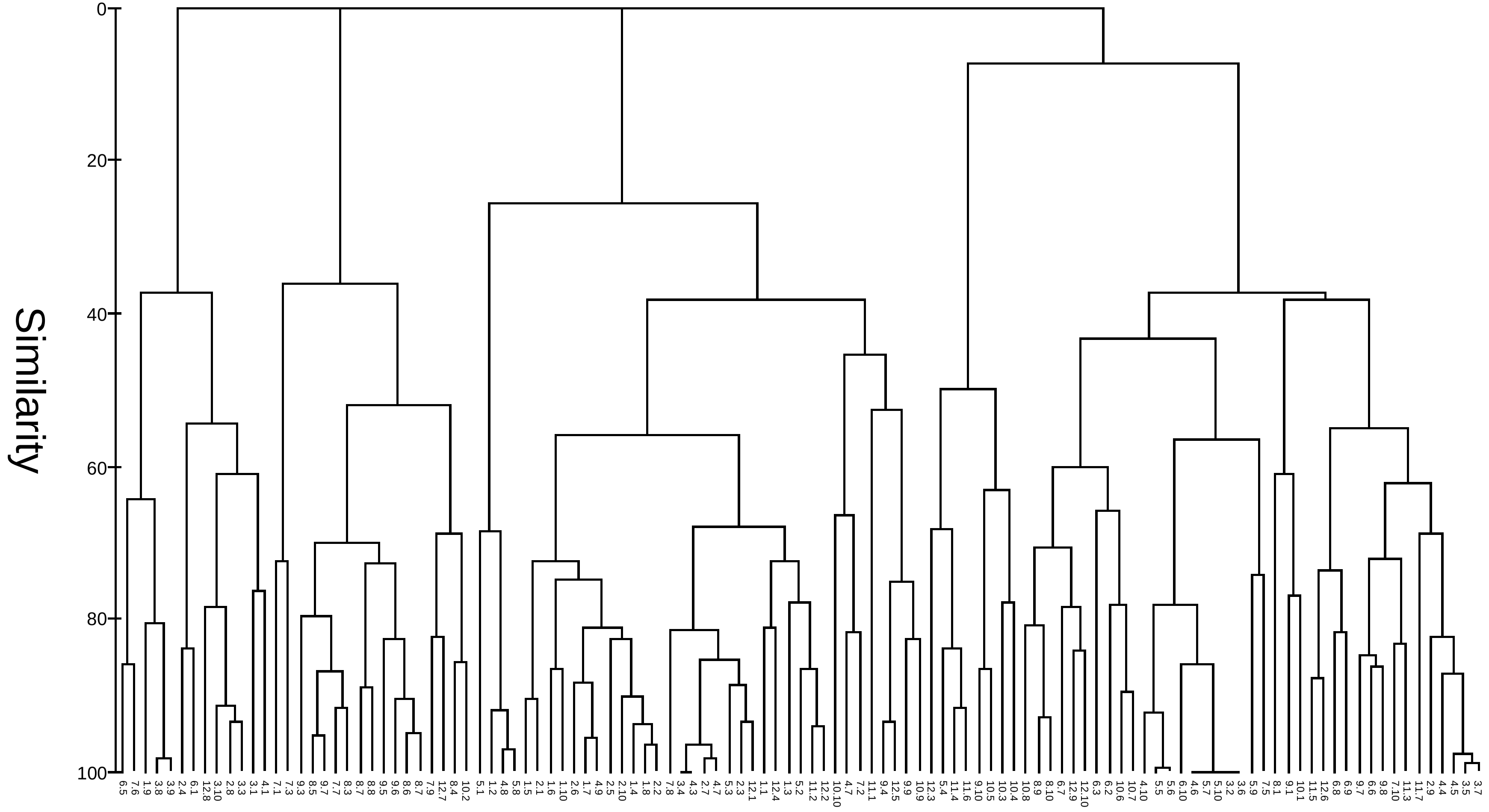


Figure 3.18: Graphical representation of the richness and diversity indices for the combined data for the individual months.



gure 3.19: Dendogram of entire data set (5 metre seine net) for the study period March 2002 to February 2003. On the x -axis, the number in front of the mal point shows the station number (1-10).

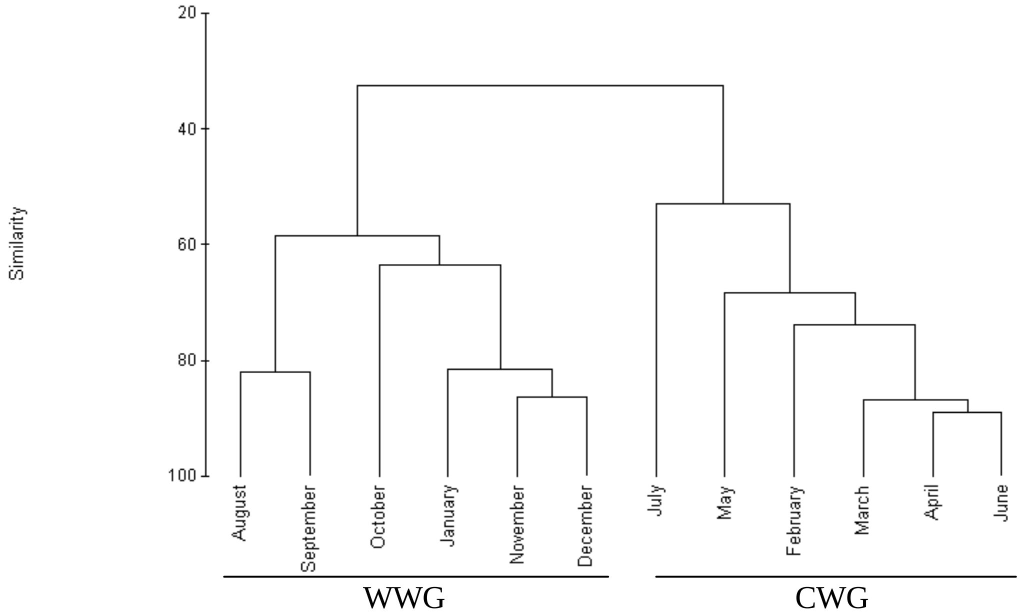


Figure 3.20: Hierarchal cluster analysis of ichthyofauna collected using the 5 m seine net (Cold Water Group: CWG and Warm Water Group: WWG).

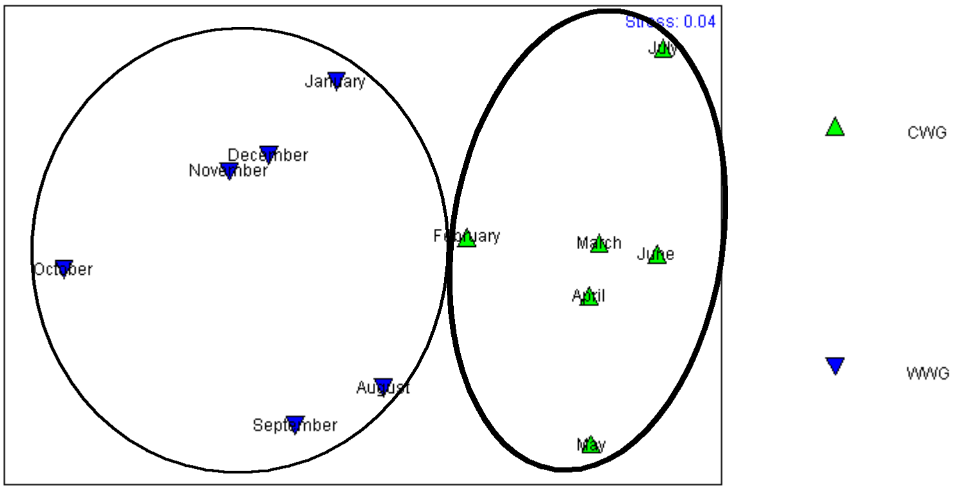


Figure 3.21: MDS plot of fish abundance data for species caught during the study analysed on a monthly basis showing the two groups (Cold Water Group: CWG and Warm Water Group: WWG) during the period of study.

Table 3.2: Species contributing up to 90% of the similarity within the Cold Water Group (CWG) and Warm Water Group (WWG) identified by the SIMPER analysis.

Cold vs. Warm 44.42 dissimilarity	CWG	WWG			
Species	Average Abundance (ind m^{-2})	Average Abundance (ind m^{-2})	Average Dissimilarity	Percentage contribution (%)	Cumulative percentage contribution (%)
Juvenile Mugilidae	0.06	1.19	10.33	23.25	23.25
Gilchristella aestuaria	0.42	0.35	7.15	16.09	39.34
Rhabdosargus holubi	0.01	0.58	6.91	15.56	54.90
Atherina breviceps	1.96	0.68	6.80	15.30	70.20
Oreochromis mossambicus	0.04	0.30	4.51	10.16	80.35
Sarpa salpa	0.00	0.09	2.10	4.74	85.09
Glossogobius callidus	0.94	1.97	2.10	4.72	89.81
Heteromycteris capensis	0.00	0.05	1.73	3.89	93.70

3.3.8 Fish (50 m seine net)

3.3.8.1 Abundance and Biomass

A total of 1548 fish (>50mm SL) representing 17 species from 9 families were caught over the period June 2002 and May 2003 using the large 50 m seine net (Table 3.3). The number of species caught in each month varied between two and nine species and the abundance of individuals ranged from 0 to 0.5 ind m^{-2}. An inverse seasonal pattern was exhibited in the abundance of the larger fish caught, with autumn and winter values (autumn: 0.16 ind m^{-2}; winter: 0.08 ind m^{-2}) exceeding those recorded in spring and summer (spring: 0.06 ind m^{-2}; summer: 0.05 ind m^{-2}).

Average monthly ichthyofaunal biomass caught in the 50 m seine net during the study ranged between 0.28 and 11.18 g wwt m^{-2} with maximum values recorded in winter (average 5.35 g wwt m^{-2} ± 11.51 g wwt m^{-2}). Intermediate values were recorded in spring (average 2.80 g wwt m^{-2} ± 3.61 g wwt m^{-2}) and in autumn (average 4.36 g wwt m^{-2} ± 3.22 g wwt m^{-2}) while the lowest values were recorded in summer (average 2.11 g wwt m^{-2} ± 2.20 g wwt m^{-2}).

3.3.8.2 Estuarine Utilisation

Throughout the study, category II species (Table 3.3, Figure 3.23) dominated the 50m seine net catch numerically comprising between 82% and 100% of all ichthyofauna sampled. The remaining categories generally contributed <5% of the total ichthyofauna sampled each month. An exception was recorded in June 2002, where these categories comprised 18%.

The sparid, *R. holubi* was identified as the single most abundant species accounting for between 10% and 98% of all fish sampled. Also well represented were the Mugilidae (*Liza richardsonii* and *Liza dumerilii*) and *O. mossambicus*. The monthly contribution of these species was, however, always <15% of the total. Exceptions were recorded in April 2003 and May 2003. In April 2003 *L. dumerilii* accounted for 86% of the total catch, *L. richardsonii* contributed 48% of the total abundance in May 2003.

Table 3.3: Total abundance and biomass of fish (SL >50 mm) caught and the subsequent percentage contribution of each species to the total catch of the 50 metre seine net for the study period of June 2002 to May 2003.

Family	Species	Common Name	Estuarine Utilisation Category	Abundance		Biomass	
				Total Numbers	Percentage Contribution	Total Biomass (g wwt)	Percentage Contribution
Carangidae	*Lichia amia* (Linnaeus, 1758)	Garrick/Leervis	**IIa**	5	0.32	1820	2.65
Cheilodactylidae	*Chirodactylus brachydactylus* (Cuvier, 1830)	Twotone fingerfin	**III**	1	0.06	10	0.01
Cichlidae	*Oreochromis mossambicus* (Peters, 1852)	Mocambique tilapia	**IV**	44	6.46	2270	3.31
Gobiidae	*Glossogobius callidus* (Smith, 1937)	River goby	**Ib**	7	0.45	1305	1.90
Haemulidae	*Pomadasys commersonnii* (Lacepède, 1801)	Spotted grunter	**IIa**	2	0.13	1300	1.89
Monodactylidae	*Monodactylus falciformis* Lacepède, 1800	Oval moony	**IIb**	67	4.33	1470	2.14
Mugilidae	*Liza dumerilii* (Steindachner, 1869)	Groovy mullet	**IIb**	222	14.34	5520	8.05
	Liza macrolepis (Smith, 1846)	Largescale mullet	**IIa**	29	1.87	4290	6.25
	Liza richardsonii (Smith, 1846)	Southern mullet	**IIc**	59	3.81	625	0.91
	Liza tricuspidens (Smith, 1935)	Striped mullet	**IIb**	10	0.65	199	0.29
	Mugil cephalus Linnaeus, 1758	Flathead mullet	**IIa**	8	0.52	294	0.43
	Myxus capensis (Valenciennes, 1836)	Freshwater mullet	**Vb**	2	0.13	286	0.42
Soleidae	*Solea bleekeri* Boulenger, 1898	Blackhand sole	**IIb**	3	0.193	25	0.04
Sparidae	*Diplodus sargus capensis* (Smith, 1844)	Blacktail	**IIc**	17	1.10	330	0.48
	Lithognathus lithognathus (Cuvier, 1830)	White steenbras	**IIa**	20	1.29	4150	6.05
	Rhabdosargus holubi (Steindachner, 1881)	Cape stumpnose	**IIa**	1048	64.08	44672	65.11
	Sarpa salpa (Linnaeus, 1758)	Strepie	**IIc**	4	0.26	40	0.06
			Total	**1548**		**68606g**	

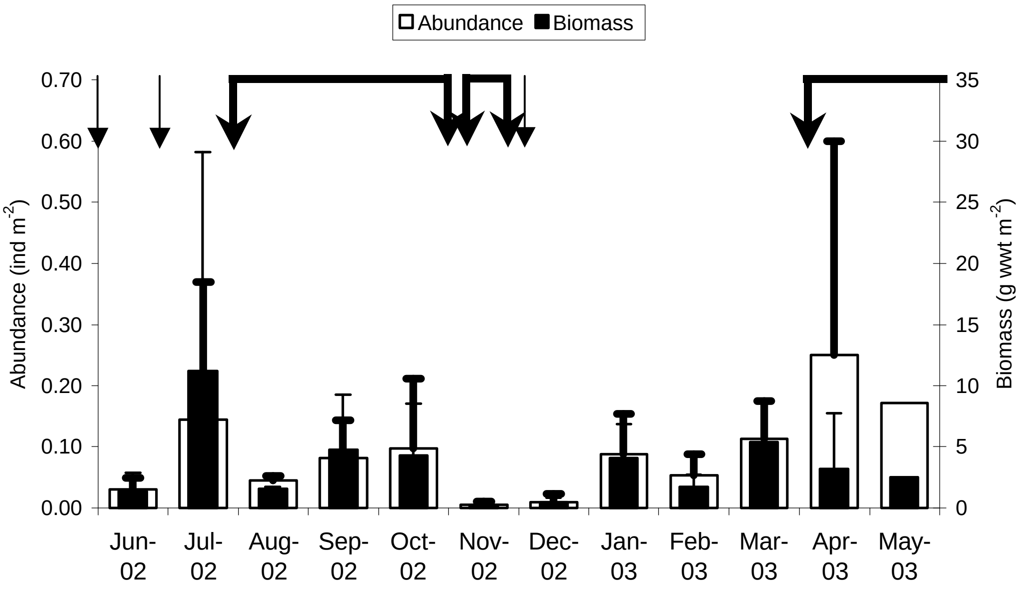


Figure 3.22: Mean abundance and biomass of ichthyofauna (SL>50 mm) in the Kasouga Estuary for the period of study (± standard deviation). (Small arrows indicate overtopping and thicker connected arrows indicate periods when the mouth was open).

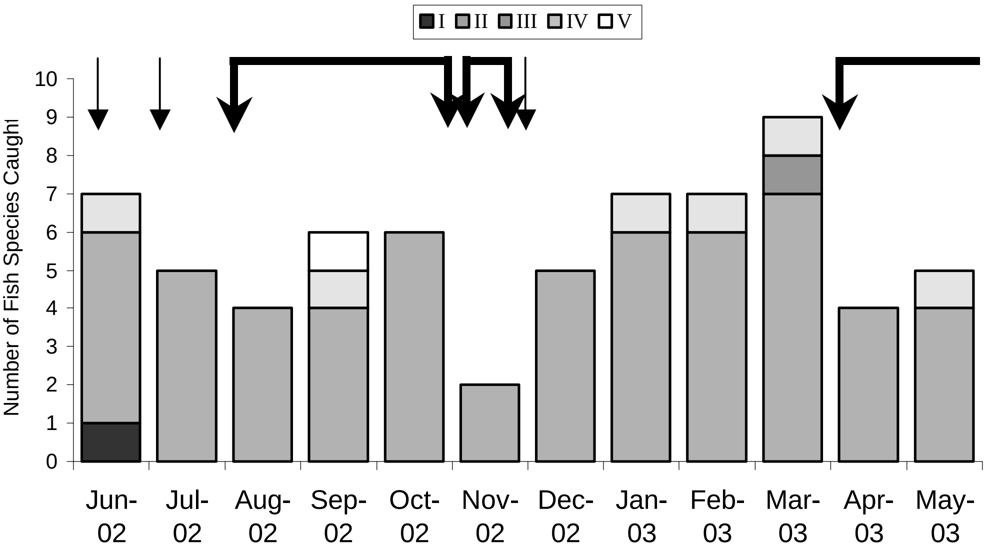


Figure 3.23: Contribution of species (SL>50 mm), in each estuarine utilisation category, to the total number of species caught for each month of the study.

Changes in abundance and biomass of *R. holubi* in the Kasouga Estuary were linked to the establishment of a link to the marine environment (Figure 3.24). The highest abundance (from 0.02 to 0.14 ind m^{-2}) and biomass values (0.90 to 9.60 g wwt m^{-2}) were recorded when the estuary had overtopped. Conversely, abundance (average 0.06 ind m^{-2} ± 0.03 ind m^{-2}) and biomass (average 2.07 g wwt m^{-2} ±0.78 g wwt m^{-2}) of *R. holubi* declined when the estuary breached. The spatial distribution of *R. holubi* in the estuary was fairly uniform with the exception of station 3 where a peak in the *R. holubi* abundance and biomass was recorded (0.10 ind m^{-2} and 5.35 g wwt m^{-2}, respectively).

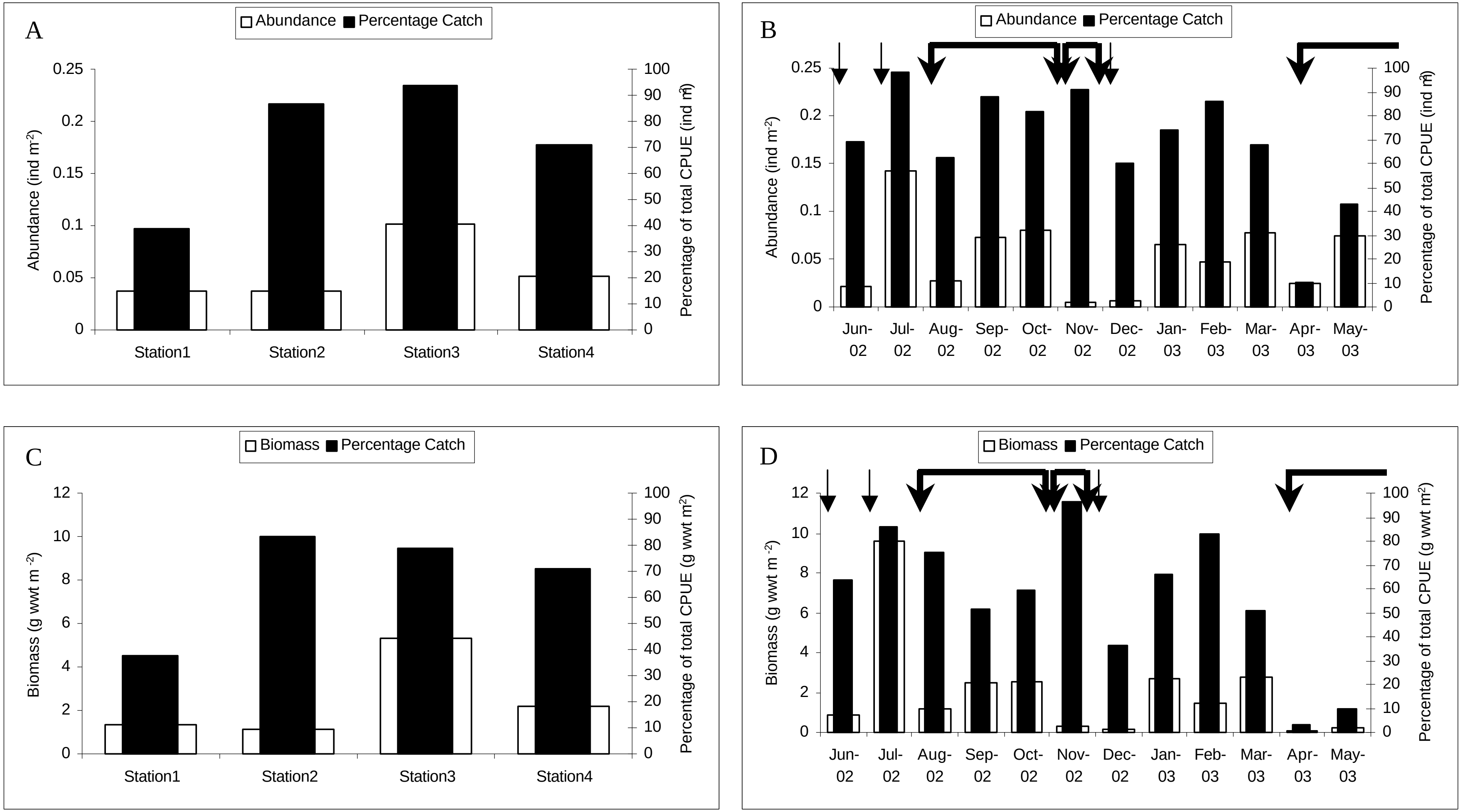


Figure 3.24: Total abundance (A & B) of *Rhabdosargus holubi* showing the abundance spatially throughout the estuary (A) and seasonally (B) from June 2002 to May 2003. Total biomass (C & D) of *R. holubi* showing biomass distributed spatially throughout the estuary (C) and (D) seasonally for the same period as abundance. The thin arrows indicate overtopping and the thick connected arrows indicate breached mouth conditions.

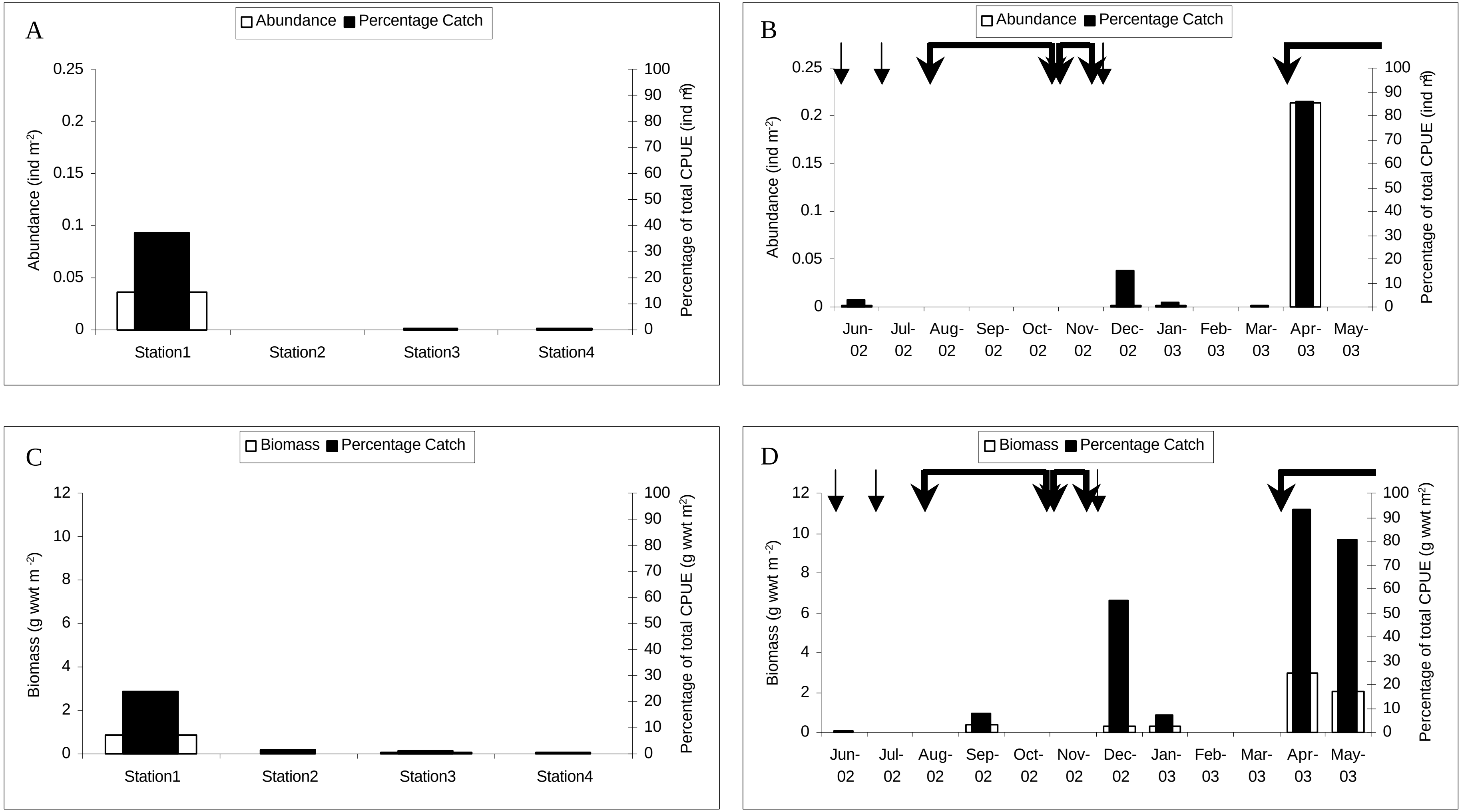


Figure 3.25: Total abundance (A & B) of *Liza dumerilii* showing the abundance spatially throughout the estuary (A) and seasonally (B) from June 2002 to May 2003. Total biomass (C & D) of *L. dumerilii* showing biomass distributed spatially throughout the estuary (C) and (D) seasonally for the same period as abundance. The thin arrows indicate overtopping and the thick connected arrows indicate breached mouth conditions.

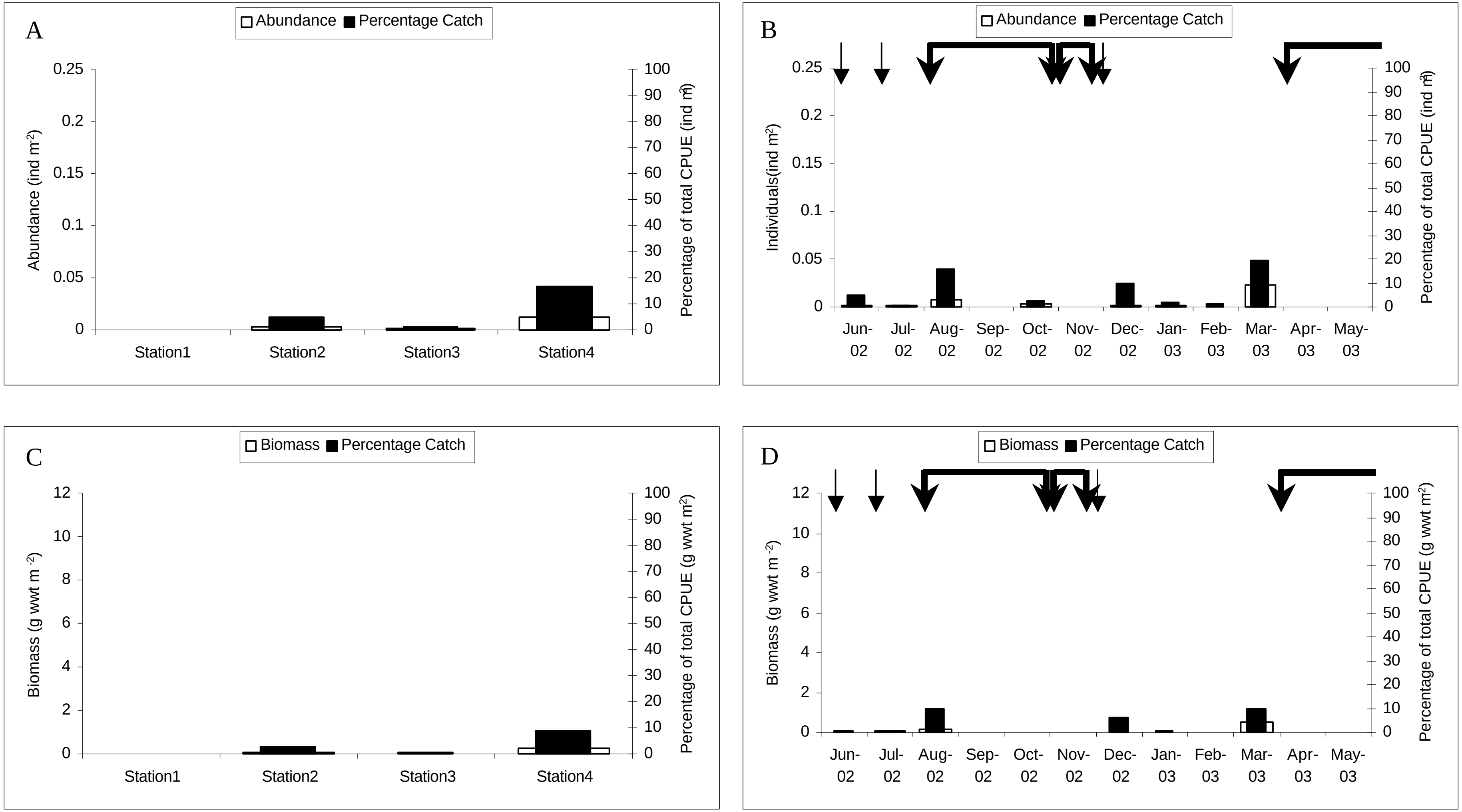


Figure 3.26: Total abundance (A & B) of *Monodactylus falciformis* showing the abundance spatially throughout the estuary (A) and seasonally (B) from June 2002 to May 2003. Total biomass (C & D) of *M. falciformis* showing biomass distributed spatially throughout the estuary (C) and (D) seasonally for the same period as abundance. The thin arrows indicate overtopping and the thick connected arrows indicate breached mouth conditions.

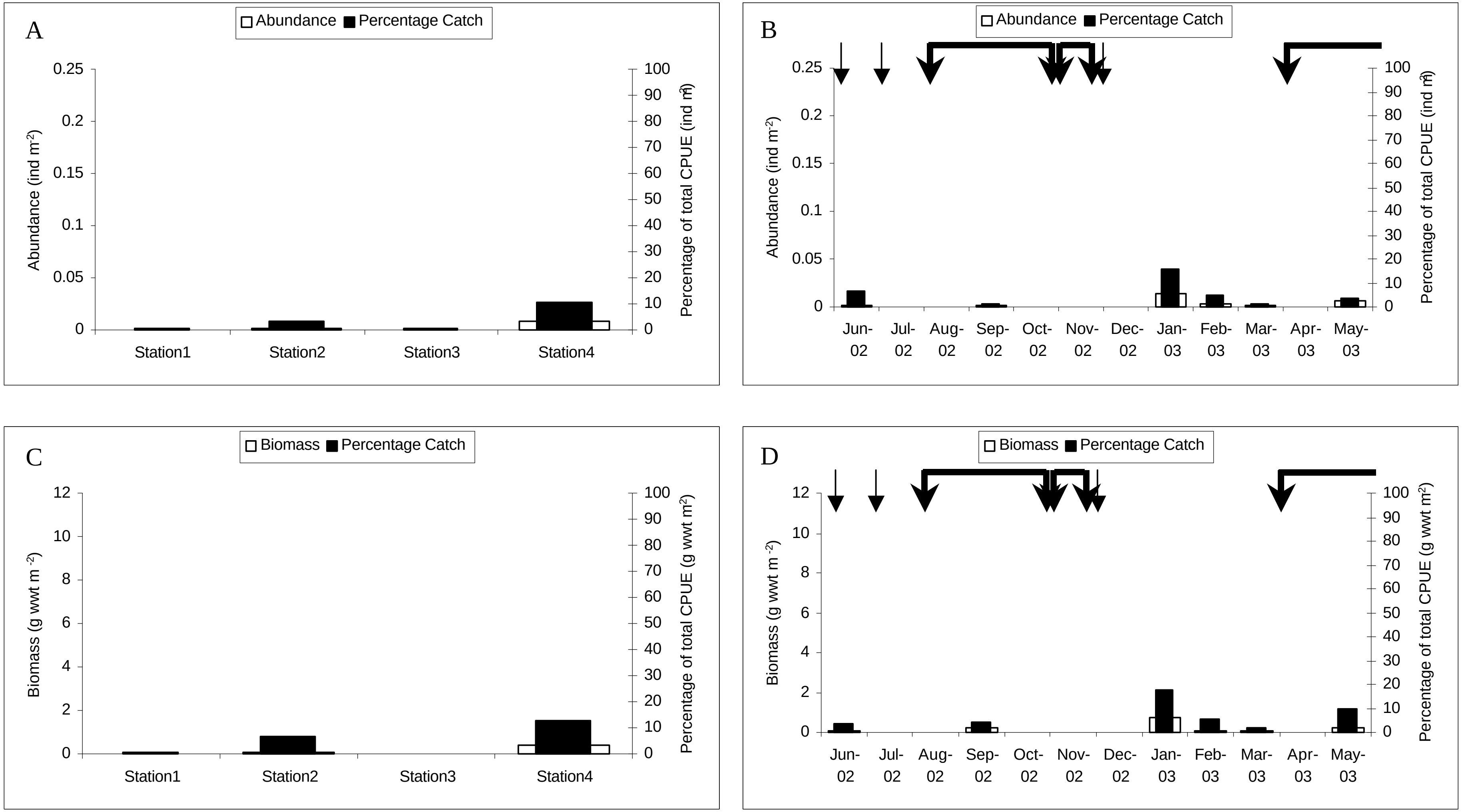


Figure 3.27: Total abundance (A & B) of *Oreochromis mossambicus* showing the abundance spatially throughout the estuary (A) and seasonally (B) from June 2002 to May 2003. Total biomass (C & D) of *O. mossambicus* showing biomass distributed spatially throughout the estuary (C) and (D) seasonally for the same period as abundance. The thin arrows indicate overtopping and the thick connected arrows indicate breached mouth conditions.

The remaining species contributed <5% (total catch = 60 individuals) of the total ichthyofauna collected with the 50 m seine. Species that contributed more than 60 individuals to the total catch were *L. dumerilii* (Figure 3.25), *O. mossambicus* (Figure 3.27) and *M. falciformis* (Figure 3.26). *Liza dumerilii* was most abundant in the mouth region during the last breaching event. In total, 44 *O. mossambicus* were caught during the study, the majority of which were recorded in the upper stations during the summer months of January 2003 to March 2003. *Monodactylus falciformis* was the fourth highest contributing species with 67 individuals caught. The majority of *M. falciformis* were caught in the upper reaches during March 2003 (42 at station 4).

3.3.8.3 Species Diversity

Margalef's species index values during the study ranged between 0.54 and 1.48 (combined value of 2.18) and the Shannon-Wiener diversity index values between 0.11 and 1.04 (combined value of 1.24). For both indices, the highest values were recorded in March 2003 (Figure 3.28). Species diversity did not appear to be related to mouth condition or to overtopping events. The general trend for both the Margalef's and Shannon's indices was a decrease in winter with an increase in diversity in August which droppped off in November 2002 and then rose quickly in December 2002 where it stayed above 1 for Margalef and above 0.6 for Shannon. Thereafter values decreased in April 2003 only to increase slightly in May 2003.

A distinct spatial pattern in both Margalef's species richness and Shannon-Wiener's diversity index values was observed (Table 3.4). The highest values were recorded nearest the mouth (station 1). The index values at stations in the upper and middle reaches of the estuary revealed no distinct patterns.

3.3.8.4 Numerical Analysis

Results of numerical analysis indicated that there were no significant temporal patterns in the community structure of the ichthyofauna >50 mm SL (Figure 3.29 and Figure 3.30). As a result, analysis was conducted using mean monthly values of ichthyofaunal abundances from the four stations. Four groupings were observed (Figure 3.29 and Figure 3.30).

Hierarchical cluster analysis revealed the presence of two distinct groupings. Group 1 comprised the station at the mouth and Group 2 the stations in the middle and upper reaches of the estuary. Group 1 and Group 2 were significantly different from one another (ANOSIM, $P<0.05$). Distinction of the two groupings could be attributed to both the presence/absence of specific species and relative abundances of the numerically dominant species (SIMPER analysis). In particular, abundances of *L. dumerilii* where average abundance were 0.04 ind m^{-2} at the mouth and <0.01 ind m^{-2} in the remaining reaches (Table 3.5). Also Group 1, was characterised by the presence of the mugilids, *Liza tricuspidens* and *Liza macrolepis*. These two species were absent from stations in the upper/middle reaches of the estuary.

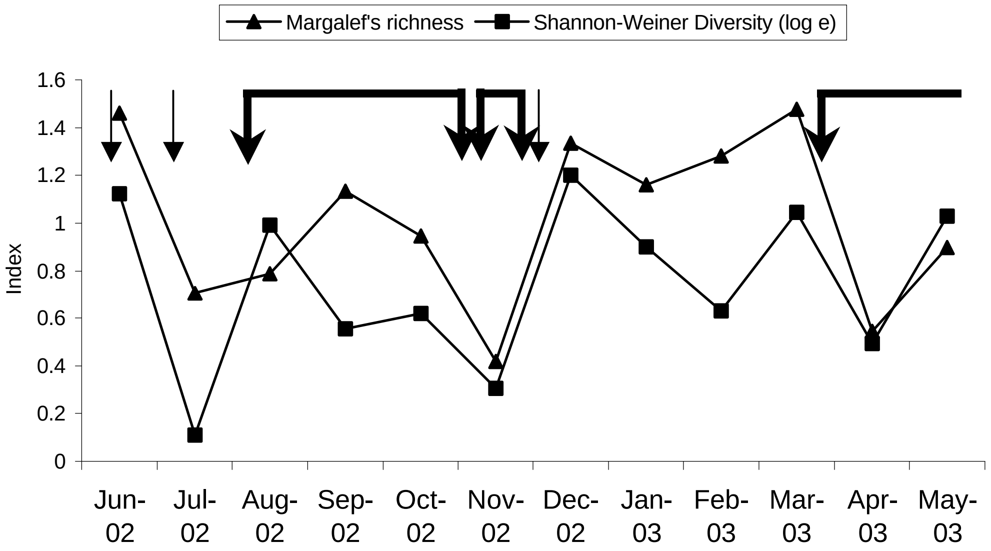


Figure 3.28: Richness and diversity indices for the mean monthly data for the study period using the 50 metre seine net.

Table 3.4: Total number of species caught, number of fish caught and the indices of richness and diversity calculated using the PRIMER 5 software package.

Station	Total number of Species caught	Total number of fish caught	Margalef's Species Richness (d)	Shannon-Wiener diversity (Log e)
Station 1 (Mouth)	14	587	2.039	1.509
Station 2 (Slipway)	8	237	1.28	0.6202
Station 3 (opposite Salt Marsh)	11	432	1.648	0.3544
Station 4 (opposite cormorants roost)	7	292	1.057	0.869

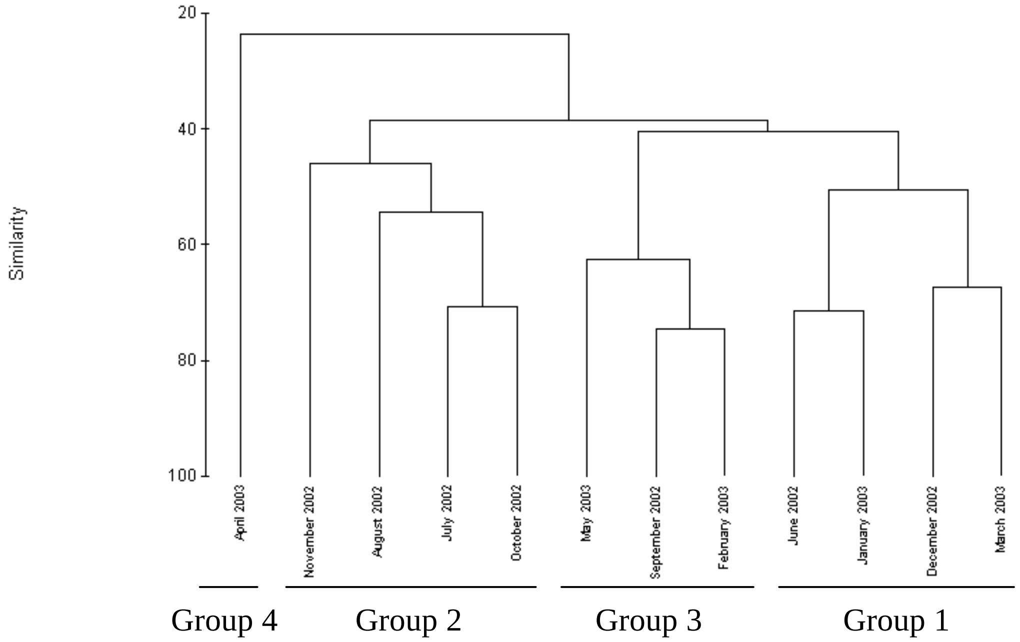


Figure 3.29: **Hierarchal cluster analysis of ichthyofauna collected using the 50 m seine net.**

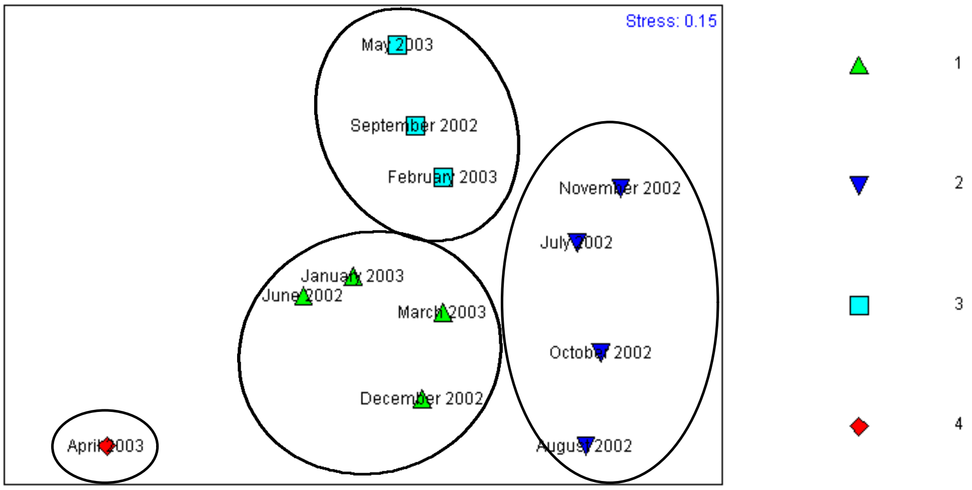


Figure 3.30: **MDS plot of fish abundance data for species (SL>50 mm) caught during the study period and analysed on a monthly basis, identified four groups.**

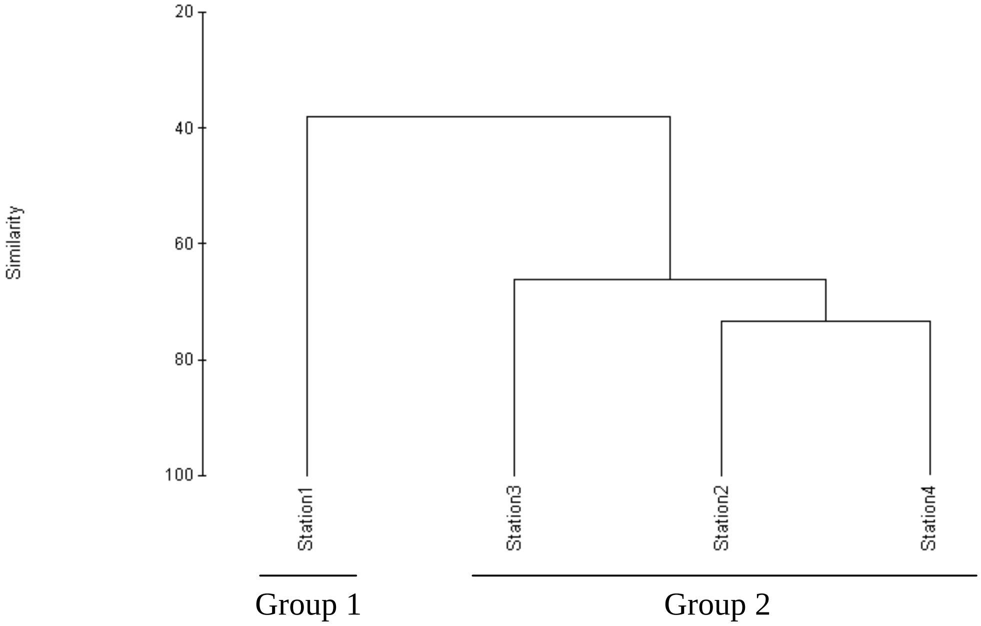


Figure 3.31: Cluster dendogram of fish abundance data for species (SL >50 mm) caught during the study period analysed according to station.

Table 3.5: Species contributing to 90% of the dissimilarity between the 2 groups of the stations identified by SIMPER analysis.

Group 1 vs. Group 2 57.94 dissimilarity	Group 1	Group 2			
Species	Average Abundance (ind m^{-2})	Average Abundance (ind m^{-2})	Average Dissimilarity	Percentage contribution (%)	Cumulative percentage contribution (%)
Liza dumerilii	0.04	<0.01	12.39	21.38	21.38
Liza richardsonii	0.01	<0.01	6.85	11.82	33.20
Liza macrolepis	<0.01	-	6.46	11.15	44.35
Monodactylus falciformis	<0.01	0.01	6.03	10.40	54.75
Oreochromis mossambicus	<0.01	<0.01	3.70	6.38	61.13
Lithognathus lithognathus	<0.01	<0.01	3.67	6.33	67.47
Liza tricuspidens	<0.01	-	3.61	6.22	73.69
Diplodus sargus capensis	<0.01	<0.01	3.03	5.23	78.92
Rhabdosargus holubi	0.04	0.06	2.71	4.69	83.61
Sarpa salpa	<0.01	<0.01	1.88	3.25	86.86
Glossogobius callidus	<0.01	<0.01	1.79	3.09	89.96
Mugil cephalus	<0.01	<0.01	1.75	3.02	92.97

3.4 Discussion

3.4.1 Environmental parameters

Water temperatures in the Kasouga estuary exhibited a strong seasonal pattern with maximum values (>25ºC) recorded in summer and minimum values in winter (<18ºC) (Figure 3.1). The observed pattern agrees with a previous study in the temporarily open/closed East Kleinemonde estuary within the same geographical region (Cowley, 1998). The absence of any seasonal variability in salinity is not surprising as salinity is governed by a variety of factors including inflow of fresh or marine water into the estuary and evaporation (Froneman, 2002b). Variations in salinity within the Kasouga estuary were generally linked to the inflow of freshwater (reducing salinity values) and the establishment of a link with the marine environment following overtopping or breaching events. The initial increase in salinity values during the first three months of sampling can probably be attributed to evaporation because of the gradual increase in values (Whitfield, 1998). The overtopping events in May and June 2002 resulted in the salinity values in the estuary being equivalent to seawater (Figure 3.2). In August 2002 the rains resulted in an increase in freshwater runoff in the catchment area and this, combined with the already high level of the estuary due to topping up by the overtopping events resulted in the first breaching event of the study.

The temporal patterns in total chlorophyll-*a* concentrations and zooplankton biomass observed during this study can be attributed to the strong seasonal pattern in the water temperature and its influence on the growth of plankton (Froneman, 2002a). Within each season, changes in chlorophyll-*a* concentration and zooplankton biomass were strongly linked to freshwater inflow and mouth status (Figures 3.4 and 3.5). The increase in chlorophyll-*a* following breaching events in early spring and late summer (December 2002) is not surprising as freshwater inflow represents the primary source of nutrients necessary to sustain the growth of phytoplankton (Campbell *et al.*, 1991; Froneman, 2002b). The subsequent increase in zooplankton biomass following breaching events could be related to increased chlorophyll-*a* and the subsequent food availability (Froneman, 2004a).

3.4.2 5 m seine net

The abundance and biomass of small ichthyofauna demonstrated a strong seasonal pattern (Figure 3.8) with maximum values recorded in summer and minimum values during winter as with the plankton (Figure 3.4). The observed pattern can probably be attributed to the influence of temperature on the growth and reproduction of fish species. Within each season, the abundance and biomass of the early developmental stages of ichthyofauna (SL <50 mm) were strongly affected by the mouth status of the estuary and overtopping events. The increase in abundance and biomass of ichthyofauna in temporarily open/closed estuaries following overtopping events is now well documented and can be attributed to the recruitment of marine breeding species (e.g. *A. breviceps*, *R. holubi,* Mugilidae and *M. falciformis*) into the estuary during overtopping (Cowley *et al.*, 2001; Vivier & Cyrus, 2001; Kemp & Froneman, 2004). These species showed an increase in abundance in the Kasouga estuary after periods of overtopping. The recruitment of marine breeding species into the estuary, following the overtopping and breaching events, resulted in an increase in ichthyofaunal species diversity within the estuary and a shift in the estuarine utilisation categories from a category I dominant community to a system dominated by category II species (Figure 3.18). This agrees with previous studies in TOCEs within the same geographic region (Vorwerk, 2001; Cowley, 1998).

The main component of the increased abundance of ichthyofauna following overtopping events (May and June) was *A. breviceps* (Figure 3.12). Although the peak in the breeding season of *A. breviceps* is from September to January, the larvae (5-8mm BL) are abundant in the top layer of the water column from September to March (Whitfield, 1989; Melville-Smith & Baird, 1980). The majority of *A. breviceps* caught during the study were very small (<10 mm, post flexion larvae) (G. Tweddle, unpublished data), suggesting that the larger fish are either consumed by predators such as piscivorous birds or larger fish, or move into the marine environment during breaching events.

The most numerically dominant fish species caught in the Kasouga estuary using the 5 m seine net was the goby *G. callidus* which was caught throughout the year and was present at all of the stations along the length of the estuary. *Glossogobius callidus* is an estuarine spawning species that has been shown to be prominent in estuaries that are characterised by muddy sediments (Cowley, 1998). While *G. callidus* was found throughout the estuary, the sandy sediments near the mouth region yielded fewer *G. callidus* than upstream (Figure 3.11). The higher abundance of *G. callidus* in the middle reaches of the estuary can likely be attributed to increased vegetation cover, which offers a refuge area for the fish (Figure 3.7).

Cowley (1998) showed that *G. callidus* and *P. knysnaensis* were spatially segregated in the East Kleinemonde estuary. *Glossogobius callidus* dominated the upper reaches and *P. knysnaensis* were more prominent nearer the mouth. He attributed the observed to the preference of *P. knysnaensis* for sandy sediments. In this study a similar pattern was observed, with *P. knysnaensis* only being recorded near the mouth.

The “inverse distributional trends” (Cowley, 1998 p.48) of the two zooplanktivorous species, *A. breviceps* and *G. aestuaria* observed during this study are in agreement with a number of previous investigations conducted in a variety of estuaries along the southern African coast (Cowley, 1998; Vorwerk, 2001). This trend can probably be attributed to competition as both species are reported to feed on the same prey type (mainly copepods) (Froneman & Vorwerk, 2003).

The majority of species caught in the Kasouga estuary were category II species (estuarine dependent marine species). In terms of abundance, however, the majority of fish caught were estuarine spawners. *Glossogobius callidus*, *A. breviceps* and *G. aestuaria* were most numerous, comprising almost three quarters of all fish sampled using the 5 m seine net. Similar contributions of *A. breviceps* and *G. aestuaria* were found in other estuaries (e.g. Vorwerk, 2001; Cowley, 1998). The underlying reasons for this pattern are uncertain, but may be due to a number of factors including the size of the estuary, accessibility from the sea and duration that the mouth is open. Alternatively the use of the smaller 5 m seine net with a finer mesh may have resulted in more smaller fish (SL <50 mm) being caught than in the larger mesh size seine nets

(3 cm stretch mesh in the wings and 1 cm stretch mesh in the bag) used in the other studies (Cowley, 1998; Vorwerk, 2001). The Kasouga estuary also had more sampling sites and was more frequently sampled and this gives a more thorough picture of species composition and distribution throughout the estuary.

Factors affecting recruitment may also affect the composition of species in the Kasouga estuary. The small size of the estuary and the small catchment could reduce the factors involved in cueing specific species for recruitment. The lower number of overtopping events that occurred in the Kasouga estuary as opposed to the frequency of these events occurring in the other systems studied by Cowley (1998) and Vorwerk (2001) may have been a deciding factor in the lower species richness of the Kasouga estuary.

Results of the numerical analysis did not identify any significant spatial patterns in the ichthyofaunal community structure. The observed spatial pattern can likely be related to the virtual absence of any horizontal gradient in temperature and salinity within the estuary. The marked temporal pattern identified (Cold Water Group and Warm Water Group) with the numerical analysis can probably be attributed to the influence of the colder autumn and winter temperatures on the reproductive activities of the ichthyofauna in the estuary. Indeed the maximum abundance and biomass of the ichthyofauna (SL<50 mm) were recorded in summer (Figure 3.8).

3.4.3 50 m seine net

The estimates of ichthyofaunal abundance and biomass obtained using the large seine net during this study are in the range reported for a number of TOCEs within the same geographic region (Cowley, 1998; Vorwerk, 2001; Vorwerk *et al.*, 2003). The values presented here are, however, substantially lower than those recorded in large permanently open systems. The higher values recorded for permanently open systems may be related to increased habitat and food availability within the larger systems (Cowley, 1998; Whitfield, 1998; Vorwerk, 2001). The lack of seasonal patterns in the ichthyofaunal abundance and biomass in this study agrees with a study of ten estuaries along the Eastern Cape coastline (Vorwerk *et al.*, 2003).

Category II species were the main contributors to the total ichthyofaunal catches, as in Vorwerk (2001) and Cowley (1998). The predominance of category II species within TOCEs over Category III species can be attributed to the low affinity of the Category III species for these types of systems and the limited recruitment due to the estuary being separated from the marine environment for extended periods (Vorwerk, 2001). Furthermore, TOCEs have smaller catchments and thus the cues available to marine species (Category III) that are likely to enter estuaries are much reduced.

The small size of the estuary and the repeated breaching affected the larger ichthyofauna of the Kasouga estuary. For example *R. holubi* was initially recorded in low numbers throughout the estuary, however, over the period June to July 2002 (corresponding to periods of overtopping), it increased by ≈300%. *Rhabdosargus holubi*, even the larger juveniles, recruit during periods of overtopping

(Cowley, 1998; Kemp & Froneman, 2004). This species declined in abundance in August following the breaching as juveniles and adults left the estuary. Following the breaching event, juvenile *R. holubi* recruited into the estuary causing a subsequent increase in numbers.

The overall ichthyofaunal diversity of the 50 m seine net was 2.18 (d) for the Margalef species richness and 1.33 (H') for the Shannon-Wiener. The Margalef diversity index expresses the overall numbers of fish caught resulting in a low value for the Kasouga due to the large contribution of *R. holubi* to the catches. The Shannon-Wiener, however, provides a good overall composition of the diversity thus reducing the bias attributed to *R. holubi*. Throughout the study period, the diversity fluctuated with very little pattern. There was a slight increase in diversity from August to October when the estuary was open, but during the closed period of December to March the diversity was higher. The slightly lower diversity and species richness recorded during those periods when the estuary was breached can likely be attributed to the migration of fish out of the estuary into the marine environment. Alternatively, the observed pattern can be related to absence of data from the upper reaches of the estuary which due to low water levels, were not sampled.

Numerical analysis indicated the presence of 2 distinct ichthyofaunal assemblages, one characteristic to the mouth region and the other extending throughout the upper and middle reaches of the estuary. This result is in agreement with a study conducted of the East Kleinemonde Estuary within the same geographic region (Cowley, 1998). The presence of a mouth community is attributed to the presence of marine sediments within the region with an associated distinctive ichthyofaunal community. *Chirodactylus brachydactylus*, *Sarpa salpa*, *L. macrolepis*, *L. richardsonii*, *L. dumerilii*, *L. tricuspidens*, *Mugil cephalus* and *Myxus capensis* were primarily recorded at the mouth station with few specimens caught elsewhere.

CHAPTER 4

INVESTIGATION OF THE FEEDING ECOLOGY OF SELECTED FISH SPECIES USING STABLE ISOTOPE ($\delta^{13}C$) ANALYSIS

4.1 Introduction

The feeding ecology of fish in estuaries has traditionally been studied using gut content analysis (e.g. De Wet & Marais, 1990; Coetzee, 1982). There are, however, problems associated with this approach. There may be an over-representation of indigestible food items (De Wet & Marais, 1990; Coetzee, 1982) and highly digestible food items may be under-represented (Hecht & van der Lingen, 1992). Stomach content analysis provides only an instantaneous indication of prey consumed by fish and not time integrated assessment. Finally because of the wide range of potential food sources available to organisms within estuaries, a substantial knowledge on the biota within the system is required (De Wet & Marais, 1990; Coetzee, 1982; Blaber, 1974a). More recently, studies on the food web dynamics of aquatic systems have employed stable isotope analysis. The analysis of stable isotopes is based on the fractionation of certain predictable isotopes as they pass through the food webs (Fry & Sherr, 1984). The use of carbon (C), particularly the ratio of ^{13}C:^{12}C, allows a researcher to study the sources of energy available to the organism as the isotope ratio of an organism reflects the C ratio of its prey/food source (Fry & Sherr, 1984; Peterson & Fry, 1987).

Perry *et al*. (1999) used stable isotope analysis along south-western Vancouver Island to show that there were distinct differences in carbon utilisation between the slope/deep ocean and the continental shelf food webs and that these differences were carried up the food web from the particulate organic matter (POM) to zooplankton and to larval fishes. Stable isotopes analysis has been used by other authors (Creach *et al*., 1997; Harvey & Kitchell, 2000) to study the food web structure in a variety of ecosystems, including estuaries and lakes.

Locally, Paterson and Whitfield (1997) and Froneman (2001a) have used stable carbon isotope analysis to study the food web structure in the temperate permanently open Kariega Estuary on the Eastern Cape coastline. These authors found there were two main carbon pathways within the estuary. The so called “littoral pathway” was derived from *Z. capensis* (and its associated epiphytes) and *Spartina maritima*, while the second pathway occurred in the channel where the main contributors to the carbon flow were macrophytes, phytoplankton and terrestrial derived detritus.

To date, no attempt has been made to assess the food web structure of ichthyofauna in temporarily open/closed estuaries. The aim of this study was to identify the primary source of carbon utilised by the detritivorous and omnivorous ichthyofauna in the temporarily open/closed Kasouga estuary.

4.2 Materials and Methods

4.2.1 Sampling

Sampling of fish, plankton, plant and particulate organic matter (POM) was undertaken to obtain samples for stable isotope analysis. Particulate Organic Matter (POM) was collected by filtering estuarine water (10 L) through a pre-combusted GF/F filter. Any zooplankton were manually removed using a Heerenberg dissecting microscope operated at 40x magnification.

Terrestrial and aquatic plant material was collected by removal of the leafy components by hand. The samples were washed in distilled water to remove any POM. Microphytobenthic samples were collected using a 5 cm diameter corer. Following the collection, the top 2 mm of the sediment was removed and frozen for later analysis. In the laboratory samples were defrosted and the algal mat separated from the detritus by washing in filtered seawater. The sample was then oven dried at 60°C for 24 hours.

Collection of zooplankton took place at night in a series of oblique tows using a WP-2 net with a mesh size of 60 μm and a mouth area of 0.05 m^2. Samples were immediately frozen in liquid nitrogen and taken to the laboratory for analysis. The individual zooplankton were combined (200-500 copepods, single individual for larger zooplankton) to give enough dry weight to allow stable isotope analysis.

The fish used for stable isotope analysis caught in the 5 m and 50 m seine nets, were kept in filtered estuarine seawater overnight to allow gut evacuation. After 12 hours, fish were sacrificed and a section of the dorsal muscle dissected out.

4.2.2 Preparation for Stable Isotope Analysis (SIA)

All samples for the SIA were oven dried for 24 hours at 60°C. To reduce any inaccuracy in the SIA due to different fatty tissue proportions in zooplankton and fish samples, the samples were defatted in a solution of chloroform, methanol and water (2:1:0.8) according to Froneman, 2001a.

4.2.2.1 Stable Isotope Analysis

Samples were combusted in an on-line Carlo-ebre preparation unit at the University of Cape Town's Archaeology Department. The internal standard used was Merk gelatine calibrated for several IAEA reference materials. ∂X (X is Carbon) was determined using a Finnigan-MAT stable isotope mass spectrometer.

The results are expressed in ∂ values that have units of ‰ relative to the Pee Dee belemnite (PDB) for the carbon samples (Peterson and Fry 1987).

$$\partial X = [(R_{sample}/R_{standard})-1] \times 10^3$$

Where R is the ratio of heavy isotope over light isotope (^{13}C:^{12}C).

4.2.3 Statistical analysis

An one-way ANOVA (Analysis of Variance) test was employed, followed by a multiple range test (Newman-Keuls Post-hoc Test) to determine whether there were any significant differences in the $\delta^{13}C$ values of the fish.

4.3 Results

4.3.1 Vegetation and Particulate Organic Matter (POM)

The vegetation samples exhibited δ^{13}C values ranging from -27.50‰ to -18.08‰ (Figure 4.1 and Table 4.1). The POM and the benthic algae had intermediate δ^{13}C values (-18.15‰ and -19.58‰ respectively) and *J. kraussii, A. karoo, S. perennis* and *P. australis* had moderately depleted δ^{13}C values ranging between -23.5‰ and -24.7‰ (see Figure 4.1 and Table 4.1). *Chenolea diffusa* also has depleted values (-25.8‰ to -24.90‰) but does not group with *J. kraussii, A. karoo, S. perennis* and *P. australis*. Among the vegetation sampled *Ruppia* spp. was the most depleted with a mean δ^{13}C value of -26.90‰.

4.3.2 Primary Consumers

Copepods, isopods and mysids had intermediate δ^{13}C values ranging from -16.30‰ to -18.55‰ (see Figure 4.1 and Table 4.2). Mysids had an average δ^{13}C value of -16.38‰ and were significantly different ($P<0.05$) from the copepods and isopods that had an average δ^{13}C value of -18.34‰ and -18.01‰, respectively (see Figure 4.1 and Table 4.2). The δ^{13}C values of copepods and isopods were not significantly different from one another ($P>0.05$). *Palaemon peringueyi* juveniles and adults were not significantly different from one another and were the most depleted of the invertebrate species with the juveniles having a lowest value of -24.50‰. *Upogebia africana*'s range of values was slightly higher and overlapped slightly with the adult *P. peringueyi* but it was found to be significantly different ($P<0.05$).

4.3.3 Ichthyofauna

Collectively, the fish analysed during the study accounted for >90% of the total ichthyofauna abundance and biomass in the estuary (see previous chapter). The δ^{13}C values of the fish in the Kasouga ranged from intermediate to moderately depleted. Three significantly different ($P<0.05$) assemblages were identified (Newman-Keuls post-hoc test). The major group of fish, comprising *M. cephalus*, *D. sargus capensis*, *A. breviceps*, *G. aestuaria*, *S. salpa* and juvenile *R. holubi*, had intermediate δ^{13}C values ranging from -18.36‰ to -15.80‰ (see Figure 4.1 and Table 4.3). The moderately depleted group comprising *R. holubi* (adults), *O. mossambicus* and *M. falciformis*, had respective mean δ^{13}C values of -21.38‰, -21.27‰ and -21.15‰ (see Figure 4.1 and Table 4.3). *Glossogobius callidus* had the most depleted δ^{13}C value of all the ichthyofauna sampled with a mean value of -23.39‰ (± 1.41‰) (see Figure 4.1 and Table 4.3).

Table 4.1: $\delta^{13}C$ values (‰) of the vegetation and Particulate Organic Matter (POM) in the Kasouga estuary.

Plant Species	Habitat	Mean $\delta^{13}C$ Values (‰)	Range	Grouping by Newman-Keuls Test (α=0.05)
Ruppia spp.	Aquatic	-26.90	-27.50 to -26.30	A
Chenolea diffusa	Littoral	-25.35	-25.80 to -24.90	B
Phragmites australis	Littoral	-24.67	-25.02 to -24.32	C
Sarcocornia perennis	Salt Marsh	-24.09	-24.36 to -23.82	C D
Acacia karoo	Terrestrial	-24.06	-24.35 to -23.77	C D
Eragrostis spp.	Terrestrial	-23.81	-24.84 to -22.78	C D
Juncus kraussii	Terrestrial	-23.45	-23.56 to -23.34	D
Microphytobenthic algae	Sediment	-19.58	-19.96 to -19.20	E
POM	Channel	-18.15	-18.22 to -18.08	F

Table 4.2: $\delta^{13}C$ values (‰) of the invertebrate species in the Kasouga estuary.

Invertebrate Species	Mean $\delta^{13}C$ Values (‰)	Range	Grouping by Newman-Keuls Test (α=0.05)
Palaemon peringueyi (Juvenile) (Stebbing, 1915)	-24.30	-24.50 to -24.10	A
Palaemon peringueyi (Adult) (Stebbing, 1915)	-23.95	-24.20 to -23.70	A
Upogebia africana Ortmann	-23.20	-23.80 to -22.60	B
Copepods	-18.34	-18.55 to -18.13	C
Isopods	-18.01	-18.29 to -17.73	C
Mysids	-16.38	-16.46 to -16.30	D

Table 4.3: $\delta^{13}C$ values (‰) of the ichthyofauna in the Kasouga estuary. Common names are listed in brackets.

Fish Species	Estuarine Category	Mean $\delta^{13}C$ Values (‰)	Range	Grouping by Newman-Keuls Test ($\alpha=0.05$)
Glossogobius callidus (River goby)	Ib	-23.39	-24.80 to -21.97	A
Rhabdosargus holubi (Adult Cape stumpnose)	IIa	-21.38	-21.56 to -21.20	B
Oreochromis mossambicus (Mozambique tilapia)	IV	-21.27	-21.36 to -21.18	B
Monodactylus falciformis (Oval moony)	IIa	-21.15	-21.50 to -20.80	B
Liza richardsonii (Southern mullet)	IIc	-18.15	-18.80 to -17.50	C
Mugil cephalus (Flathead mullet)	IIa	-18.06	-18.36 to -17.76	C
Diplodus sargus capensis (Blacktail)	IIc	-17.90	-18.20 to -17.60	C
Atherina breviceps (Cape silverside)	Ib	-17.81	-17.92 to -17.70	C
Gilchristella aestuaria (Estuarine roundherring)	Ia	-17.51	-17.83 to -17.19	C
Sarpa salpa (Strepie)	IIc	-17.00	-17.20 to -16.80	C
Rhabdosargus holubi (Juvenile Cape stumpnose)	IIa	-16.95	-18.10 to -15.80	C

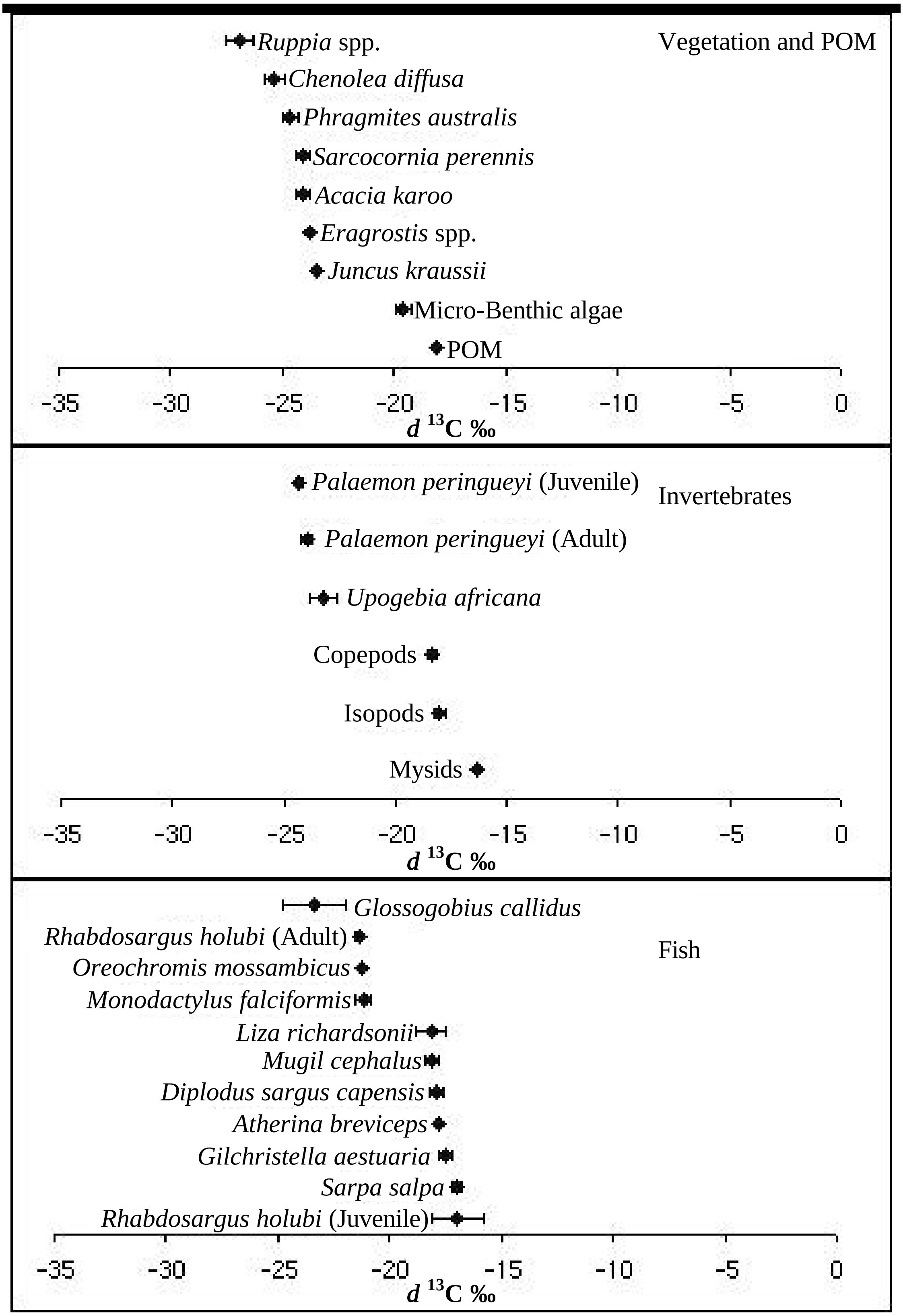


Figure 4.1: ***d*** **^{13}C (‰) values and standard error of vegetation, invertebrates and fish in the Kasouga Estuary.**

4.4 Discussion

This study was conducted when the estuary was closed and typically exhibited low chlorophyll-*a* concentrations (generally <2.0 mg chl-*a* m^{-3}, Froneman, 2002b). The low chl-*a* concentrations were due to low macronutrient concentrations resulting from the reduced/no freshwater inflow, which limits growth of the phytoplankton. It should be noted that various carbon sources available to the consumers of the estuary may demonstrate a high degree of temporal variability. Under conditions when the estuary has breached, the oceanic environment may contribute to carbon pools through inflow of POM or recruitment of both invertebrates (zooplankton) and vertebrates (ichthyofauna). Similarly during freshwater inflow into the estuary, the increase in macronutrients availability would promote phytoplankton production. Not only is there an import of carbon during these times but also carbon export in the system both through flushing out of organic matter and through the migration of invertebrates and ichthyofauna out into the marine environment during the periods when the estuary has breached.

Studies conducted in permanently open estuaries within the same geographic region have demonstrated that the isotopic values of consumers are closely linked to the region where they were sampled (Froneman, 2002c; Paterson & Whitfield, 1997). Analysis of $\delta^{13}C$ for *P. peringueyi* indicated values closely linked to *Ruppia* spp., suggesting the primary source of carbon utilised by *P. peringueyi* is derived from *Ruppia* spp. Whether *Ruppia* spp. is consumed directly or via the detrital food web is unknown. In addition to providing a potential food source, the *Ruppia* spp. probably also provides a refuge area for *P. peringueyi* against predation by the large ichthyofauna (e.g. *M. falciformis*).

The copepods and isopods in the Kasouga Estuary had mean isotopic values of -18.34‰ and -18.01‰, respectively. Riparian, salt marsh vegetation and *Ruppia* spp. can be excluded as an important carbon source as these sources are too depleted in ^{13}C. Possible alternative carbon sources include phytoplankton and microphytobenthic algae. Phytoplankton typically bracketed the δ^{13}C value of -18‰ which is higher than those reported by Froneman (2002b) and Fry and Sherr (1984) who reported isotopic values between -30‰ and -25‰, which is also similar to the range of -29.5‰ to -24.5‰ for suspended particulate organic matter as found by Jerling & Wooldridge (1995). Phytoplankton can thus be excluded as an important carbon source for the zooplankton in the estuary. Recent studies conducted in the Kasouga estuary indicated that microphytobenthic algae stocks are 2-3 orders of magnitude higher than phytoplankton stocks (Froneman, 2002c). The similar isotopic values obtained for zooplankton and microphytobenthic algae suggest that microphytobenthic algae represent the primary carbon source for the zooplankton. The isotopic value of the mysid was moderately more depleted than the zooplankton. This result is not surprising as recent studies indicate that mysids consume copepods (Froneman, 2001b).

The invertebrate feeders *R. holubi*, *O. mossambicus* and *M. falciformis* had mean δ^{13}C values ranging between -21.15‰ and -21.38‰. The moderately depleted values obtained for these species suggest that water column zooplankton (copepods, isopods and mysids) are poorly utilised as a carbon source. The carbon signatures from these species suggest that these taxa are utilising carbon derived from a carbon depleted source, probably from the shrimp, *P. peringueyi* and the mud prawn *U. africana*. Unfortunately, isotope analysis cannot determine the various contributions of the different carbon sources to total carbon intake. Isotopic values for *R. holubi* demonstrate a shift with development, suggesting a change in diet with an increase in size. Juvenile *R. holubi* had $\delta^{13}C$ values in the same range as those species consuming zooplankton. In contrast, adults appear to feed on larger invertebrates.

Oreochromis mossambicus are herbivorous/omnivorous species (Whitfield, 1998). Based on the $\delta^{13}C$ values, it is apparent that *O. mossambicus* is consuming carbon derived mainly from a combination of littoral and terrestrial vegetation. Unfortunately, isotopic analysis does not provide any indication of what proportions the plants from different groups contribute to the diet of this species. As larvae (10-30mm) *Rhabdosargus holubi* feed mainly on copepods while the juveniles (SL>30mm) feed on epiphytes (Blaber, 1974a) and epibenthic invertebrates (Whitfield, 1998). Assuming an enrichment factor of $1^{o}/_{oo}$ per trophic level, the $\delta^{13}C$ value of the larger *R. holubi* does not correspond to any particular food source sampled. This suggests that some of the components of the food web of the Kasouga estuary were not sampled during the present investigation.

Monodactylus falciformis had similar $\delta^{13}C$ values to *O. mossambicus* and larger *R. holubi* but they are primarily invertebrate feeders, feeding on crabs, isopods, amphipods and insects (Whitfield, 1998). Considering the enrichment factor, their diet in the Kasouga could be comprised of mud prawns and swimming prawns or from some species that were not sampled.

The two Mugilidae species sampled during this study (*L. richardsonii* and *M. cephalus*) had mean $\delta^{13}C$ values of -18.15‰ and -18.06‰, respectively. Mugilidae in southern African estuaries have been shown to feed primarily on detritus, microphytobenthic algae and meiofauna (Whitfield, 1998). Assuming a stepwise enrichment of 1‰ per trophic level, the most likely source of carbon consumed by both mullet species is the microphytobenthic algae that had a $\delta^{13}C$ value of -19.58‰. Unfortunately, no isotopic values of meiofauna were made during the present study. As a consequence, it is not possible to determine to what extent the meiofauna contribute to the diet of the two mullet species.

The two zooplanktivorous ichthyofaunal species, *A. breviceps* and *G. aestuaria* had isotopic values (ranging from -17.83‰ to -17.19‰) in the range reported for the copepods and isopods, agreeing with numerous feeding studies conducted in a variety of estuaries along the southern African coast (Whitfield, 1998; Froneman & Vorwerk, 2003). Mean isotopic $\delta^{13}C$ values for *D. sargus capensis* (-17.90‰) and *S. salpa* (-17.00‰) show that these species also consume a combination of copepods and isopods.

Diplodus sargus capensis consume isopods and amphipods as well as polychaetes, ostracods and chironomid larvae and the fish larger than 50mm are often herbivorous (Whitfield, 1998). Their isotope ratio corresponds to that of the isopods and amphipods sampled in the Kasouga. They also exhibited a slightly lower isotope ratio than *G. aestuaria* and *A. breviceps*, thus it is unlikely they were consuming mysids or prawns, which had higher or lower isotope values, respectively. *Sarpa salpa* fell into the same grouping (group C, Table III) and is also an invertebrate feeder (Whitfield 1998).

The most depleted δ^{13}C values observed for ichthyofauna were recorded for the goby, *G. callidus*. Insects dominate the food source for this species, but copepods, branchipods and ostracods also contribute to the diet (Whitfield, 1998). Although the values are similar to those of *R. holubi*, *O. mossambicus* and *M. falciformis,* it does not group with these three species, suggesting a different source of carbon.

Previous studies conducted in permanently open estuaries within the same geographic region indicated the presence of two distinct trophic pathways (Paterson & Whitfield, 1997; Froneman, 2001a, 2002c). The first pathway was based on the carbon-enriched eelgrass and the salt marsh vegetation (littoral pathway), while the second pathway was based on the carbon depleted terrestrial vegetation, phytoplankton and estuarine macrophytes (the so called channel pathway). The littoral pathway was shown to be largely absent from the Kasouga system. Although Kasouga estuary is characterised by the presence of extensive salt marshes, these are only inundated following freshwater influx into the estuary or during extreme overtopping events (Froneman, 2002b). Production from the salt marshes is thus only available to the heterotrophic organisms in the estuary for short periods of time. In contrast in many of the permanently open systems that are strongly influenced by tidal regimes, salt marshes are only inundated properly during spring high tides. Production of carbon within the salt marshes is, therefore, readily exported and made available to heterotrophic organisms in the estuary. There is thus a marked difference in the trophic pathways between permanently open estuaries and the temporarily open/closed Kasouga estuary.

CHAPTER 5

GENERAL DISCUSSION

There is an increasing awareness of the fragile nature of South African estuarine ecosystems. It therefore is important to identify and understand the possible impacts that the encroaching development of adjacent land or freshwater abstraction is having or will have on these systems (Avis, 1998). Vorwerk (2001) suggested that the lack of knowledge attributed to the smaller estuaries is detrimental to the management and handling of such systems. Temporarily open/closed estuaries should not have the same management strategies as large permanently open systems as the anthropogenic impacts on the two types of estuarine systems is likely to differ.

In agreement with a number of previous studies conducted in TOCEs within the same geographic region, there were no distinct horizontal gradients in selected physico-chemical and biological variables during the study. The lack of gradients can be attributed to the small catchment size which results in limited freshwater inflow, while strong coastal winds facilitate the horizontal and vertical mixing of the water column (Froneman, 2002a, 2002b). Temperature displayed a strong seasonal pattern. Increases in chlorophyll-*a* concentrations and zooplankton biomass following the inflow of freshwater into the estuary during the study, agrees with investigations carried out in both permanently open and temporarily open/closed estuaries within the same geographic region (Campbell *et al.*, 1991; Adams & Bate, 1999; Froneman, 2001a, 2002a; Wooldridge, 1999). Freshwater inflow into estuaries is the primary source of macronutrients necessary to sustain growth of phytoplankton (Campbell *et al*., 1991; Adams & Bate, 1999; Froneman, 2002b). The increase in total chlorophyll-*a* concentration following freshwater inflow into the Kasouga estuary can likely be linked to increases in growth rates of phytoplankton due to elevated macronutrient concentration. The increase in zooplankton stocks in the Kasouga estuary following freshwater inflow can be linked to increased food availability namely chlorophyll-*a* (Froneman, 2002a).

The biomass and species composition of the early developmental stages (SL<50 mm) of ichthyofauna in the Kasouga was linked to both seasonality and overtopping or breaching events (Figure 3.8 and 3.9). This agrees with previous studies conducted in TOCEs in the same geographic region (Vorwerk *et al*., 2001; Cowley, 1998; Kemp & Froneman, 2004). The increase in biomass (SL<50 mm) following overtopping events was largely attributed to the recruitment of marine breeding estuarine fish including *A. breviceps*, juvenile *R. holubi* and Mugilidae into the estuary (Figures 3.12 and 3.15). This recruitment coincided with a change in the estuarine utilisation classes of the ichthyofauna, from a dominance of category I species (Estuarine species) to category II species (Estuarine dependant marine species). The overtopping events were also associated with an increase of the ichthyofaunal (SL<50 mm) diversity within the estuary.

In contrast to the smaller ichthyofauna, the biomass of the larger fish (SL>50 mm) demonstrated a decline when the estuary breached, which could largely be attributed to the emigration of the larger fish into the marine environment. These facts highlight the importance of the mouth status in determining the community composition of the larger ichthyofaunal component of the Kasouga estuary.

The species composition of the Kasouga estuary was primarily dominated by marine breeding species (category II) with a poor contribution of the typical estuarine species. The low contribution of these species can likely be attributed to the experimental design and sampling gear employed which would have under-sampled the smaller fish in the channel waters.

Ichthyofaunal community in permanently open estuaries are influenced by temperature, salinity and dissolved oxygen (Whitfield, 1998). In the absence of any significant horizontal gradients in physico-chemical variables the distribution of the smaller ichthyofauna did not demonstrate any significant spatial patterns. Preliminary data suggest that biological interactions (competition between the zooplanktivorous species, *A. breviceps* and *G. aestuaria*), particularly during the closed phase, play an important role in determining the distribution of some of the fish in the Kasouga Estuary. However, the presence of a distinct ichthyofaunal community at the mouth of the estuary where marine sediments dominate suggests sediment type and may partially contribute to the spatial heterogeneity of ichthyofauna in the temporarily open/closed Kasouga estuary. It is worth noting, the lack of any spatial pattern in the distribution of smaller component of the ichthyofaunal assemblage of the Kasouga estuary may be linked to the sampling procedure. Due to the presences of numerous submerged obstacles (tree trunks and rock outcroppings) no otter trawls were undertaken during the study. Further sampling may have shown more discreet spatial patterns in the distribution of the smaller ichthyofauna. These patterns may be linked to amongst others habitat availability (e.g. submerged macrophyte beds) or substrate type.

Stable isotope studies conducted in the permanently open Kariega estuary within the same geographic region identified two distinct carbon pathways; the so-called "channel pathway" and the "littoral pathway" (Paterson & Whitfield, 1997; Froneman, 2001a, 2002c). The channel pathway comprised carbon derived mainly from phytoplankton and submerged macrophytes, while the carbon in the littoral pathway was derived from extensive salt marshes and riparian vegetation. During the present study, stable isotope analysis indicated the presence of only one main pathway, the "channel pathway" which was largely sustained by the extensive microphytobenthic algal stocks within the system. According to Nozais *et al.* (2001), the elevated microphytobenthic stocks found in TOCEs can be linked to favourable light environment confirmed by the virtual absence of currents (principally tidal) within the system. The difference in the food web structure between the permanently open Kariega estuary and the Kasouga estuary appear to be linked to mouth status. The extensive saltmarshes within the Kasouga estuary are only inundated during flood events or during those periods when large overtopping events occur (Froneman, 2002b). It is therefore not surprising that carbon derived from the extensive salt marshes of the Kasouga estuary appeared to make only a minor contribution to total carbon flow. In contrast, the salt marsh vegetation in the Kariega estuary is inundated extensively during spring high tides. The inundation of the salt marshes would facilitate the transfer of carbon from the terrestrial environment to the channel (Froneman, 2002b).

In conclusion, results of the study indicate that mouth status and overtopping events at the Kasouga estuary plays an important role in determining the ichthyofaunal species composition and biomass. Furthermore, the absence of the tidal influence due to the presence of the sand bar at the mouth promotes the growth of microphytobenthic algae, which largely sustains either directly or indirectly the food web of the estuary.

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