

**Phosphorus distribution among selected abiotic and biotic components of
two KwaZulu-Natal estuaries, South Africa**

by

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ABSTRACT

Phosphorus is an essential element since it controls primary productivity in aquatic ecosystems and its excess can lead to eutrophication in receiving systems. The aim of this project was to determine phosphorus distribution in biotic and abiotic nutrient pools of two KwaZulu-Natal estuaries.

Samples of dissolved inorganic phosphorus (DIP), particulate phosphorus (PP), phytoplankton, microphytobenthos, zooplankton, macrozoobenthos and sediment were collected in the temporarily open/closed Mpenjati (MP) and permanently open Mlalazi Estuary (ML) during May (ML), September (MP) and November (ML+MP) using standard methods. Chlorophyll a concentrations as well as species richness, abundance and biomass of zooplankton and macrozoobenthos were analysed. Living and non living nutrient pools were analysed for phosphorus and were compared between stations, sampling sessions, estuaries and taxa.

Zooplankton abundance and biomass in the Mlalazi Estuary was higher during May than November. In the Mpenjati Estuary highest zooplankton abundance and biomass was recorded during September than November. No significant differences were apparent in abundance ($p = 0.217$) and biomass ($p = 0.974$) of zooplankton between the two estuaries. Macrozoobenthos abundance and biomass in the Mlalazi Estuary was higher during May than November. In the Mpenjati Estuary macrozoobenthos abundance and biomass was higher during November than September. Significant differences in abundance ($p = 0.003$) and biomass ($p = 0.020$) were apparent between the estuaries.

Sediment to a depth of 10 cm comprised the highest phosphorus biomass than any other nutrient pool in both Mlalazi ($4871.1 \text{ mgP} \cdot \text{m}^{-2} \pm 5888.9 \text{ SD}$) and Mpenjati ($2578.6 \text{ mgP} \cdot \text{m}^{-2} \pm 1828.0 \text{ SD}$) estuaries followed by DIP ($120.5 \text{ mgP} \cdot \text{m}^{-2} \pm 177.7 \text{ SD}$ and $5.9 \text{ mgP} \cdot \text{m}^{-2} \pm 6.1 \text{ SD}$ respectively). In both estuaries, the lowest phosphorus biomass was contained in zooplankton with both estuaries containing zooplankton P biomass of $0.001 \text{ mgP} \cdot \text{m}^{-2} \pm 0.002 \text{ SD}$. Particulate phosphorus and DIP concentrations were higher in the upper reaches in both estuaries indicating that rivers were the main sources of this nutrient in these systems. The Mlalazi Estuary had higher nutrient levels than the Mpenjati Estuary. Such elevated nutrients can be enhanced by the continuous river flow into the permanently open estuary. In both estuaries, no significant differences were apparent in zooplankton and macrozoobenthos P content between different taxa.

As the candidate's supervisor I have/have not approved this thesis/dissertation for submission.

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PREFACE

The work described in this dissertation was carried out in the School of Life Sciences, University of KwaZulu-Natal, Durban, from April 2011 to September 2013, under the supervision of Dr. Ursula Scharler.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

DECLARATION - PLAGIARISM

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19 September 2013

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Chapter 1

Introduction

1.1. Definition of an estuary and estuarine characteristics

Estuaries are among the most productive ecosystems of the globe which are of economic and ecological value (McLusky 2004; Chuwen et al. 2009; Vasconcelos et al. 2010). Characteristics of these systems include a constantly changing mixture of salt and fresh water as well as fine and generally coarse sedimentary material received from rivers and the sea respectively. The distribution of sedimentary material in estuaries is controlled by the size of particles as well as speed of currents (Day 1981a; Levin et al. 2001; McLusky 2004). Horizontal and vertical gradients of salinity in estuaries are characteristic factors of most systems (McLusky 1993; Louw 2007). Levels of tidal mixing, local topography as well as freshwater inflow are the determinants of the extent of such salinity gradients (Boaden and Seed 1985; Louw 2007). Temperature regimes within these systems vary with depth, continental and marine climate as well as the input of water from adjacent systems with different temperatures (McLusky 2004).

The above contribute to a highly variable environment where organisms have to deal with the instability of habitat (e.g. sediment composition and distribution) and physiological stress (Perillo 1995; McLusky 1999; Harrison and Whitfield 2006; James and Harrison 2009). As a result, relatively few species have developed adaptations to live in these systems (Levin et al. 2001). Species richness in estuaries is therefore generally lower than the adjacent freshwater and marine environment, however often occur in high population densities (Levin et al. 2001; Elliott and McLusky 2002; McLusky 2004).

Pritchard (1967) defined an estuary as, “An estuary is a semi-enclosed coastal body of water which has a free connection with the open sea and within which sea water is measurably diluted with fresh water derived from land drainage”. Smaller temporarily open/closed estuaries and lagoons were not taken into account in this definition as it was largely based on features of large northern hemisphere estuaries. Day (1980, 1981) revised Pritchard’s (1967) definition to: “An estuary is a partially enclosed coastal body of water which is either permanently or periodically open to the sea and within which there is a measurable variation of salinity due to the mixture of sea water with fresh water derived from land drainage”. Fairbridge (1980) also proposed a definition of an estuary as,

“An estuary is an inlet of the sea reaching into a river valley as far as the upper limit of tidal rise, usually being divisible into three sectors: (a) a marine or lower estuary, in free connection with the open sea; (b) a middle estuary subject to strong salt and freshwater mixing; and (c) an upper or fluvial estuary, characterised by freshwater but subject to strong tidal action. The limits between these sectors are variable and subject to constant changes in the river discharges”.

Perillo (1995) and Elliott and McLusky (2002) argued that in Day's (1980, 1981) definition, tidal variation was left out and emphasis was on salinity. Tides play an important role by providing energy for the mixing mechanism in estuaries but sometimes wind can have a considerable effect regarding mixing (Perillo 1995). Tidal mixing does not only influence the salinity of estuaries, it is also associated with processes such as erosion and circulation. In the fluvial reaches the tidal action brings changes to river discharge, sediment as well as pollutants transport characteristics (Perillo 1995; McLusky 1999). After Perillo's (1995) argument and revision he proposed a new definition as, “An estuary is a semi-enclosed coastal body of water that extends to the effective limit of tidal influence, within which seawater entering from one or more free connections with the open sea, or any other saline coastal body of water, is significantly diluted with fresh water derived from land drainage, and can sustain euryhaline biological species for either part or the whole of their life cycle”. He further mentioned that this definition includes aspects which were omitted before i.e. (i) hierarchical estuaries that possess primary to tertiary tributaries such as the Chesapeake Bay, (ii) the existence of more than one free connection, hence coastal lagoons are also included in the definition, (iii) the coexistence of tidal action and invasion of sea water and (iv) the inclusion of biological aspects where the estuary can be a habitat for species that can feature a wide range of salinities.

The Water Framework Directive (WFD) of the European Union regards an estuary as a habitat on its own but habitats like salt marsh, reedbeds, sand and mud flats are also included (Elliott and McLusky 2002). Romao (1996) then gave a European habitat definition of an estuary as, “Downstream part of a river valley, subject to the tide and extending from the limit of brackish waters. River estuaries are coastal inlets where, unlike ‘large shallow inlets and bays’ there is generally a substantial freshwater influence. The mixing of freshwater and seawater and the reduced current flows in the shelter of the estuary lead to the deposition of fine sediments, often forming extensive intertidal sand and mud flats. Where the tidal currents are faster than the flood tides, most sediments deposit to form a delta at the mouth of an estuary. Although the above definition is rather

long, Elliot and McLusky (2002) considered this definition more realistic and accurate and they further regarded it as closer to the definitions of Prichard (1967) and Fairbridge (1980) than any other succeeding definitions which have considered estuaries as the “non-tidal brackish seas” or “river plumes extending into open seas”. The South African National Water Act 36 of 1998 define an estuary as, “a partially or fully enclosed body of water - (a) which is open to the sea permanently or periodically; (b) within which sea water can be diluted to an extent that is measurable, with freshwater drained from land”.

In Day’s (1980, 1981) definition, hypersaline conditions were omitted. According to Potter et al. (2010), formation of sandbars at the mouths of estuaries and increased salinity conditions were not included in the previous definition by Day (1980). Potter et al. (2010) modified Day’s (1980) definition to, “An estuary is a partially enclosed coastal body of water that is either permanently or periodically open to the sea and which receives at least periodic discharge from a river(s), and thus, while its salinity is typically less than that of natural sea water and varies temporally and along its length, it can become hypersaline in regions when evaporative water loss is high and freshwater and tidal inputs are negligible”. Pritchard’s (1967) and Day’s (1980) definitions have thus been extended to include small, temporarily open/closed estuaries (TOCEs), which are the main dominant type of estuaries in South Africa.

1.2. Importance of estuaries and major anthropogenic impacts

Estuarine ecosystems are of high ecological value because they provide suitable nursery grounds for many marine species (Potter and Hyndes 1999; Elliott and McLusky 2002; Beck et al. 2003; Nicolas et al. 2007; Vasconcelos et al. 2010). These species utilise the estuarine environment to benefit from appropriate conditions required for growth which include high food availability, suitable water temperature and sheltered habitat type, which contrasts with the inshore marine environment featuring heavy wave action, possible strong currents and lower levels of food availability (Pittman and McAlpine 2001; Vasconcelos et al. 2010; Wasserman and Strydom 2011). Such marine species remain in estuaries for part of their life or entire life cycle after which they join adult populations in the marine environment (Perillo 1995; Whitfield 1999b; Strydom and Whitfield 2000; Vasconcelos et al. 2010). Since many species rely on estuaries as nursery areas, the survival of their early life stages is dependent on these systems (Whitfield 1999b). Furthermore, many bird species depend on estuaries for their diet (Hockey and Turpie 1999).

Estuaries also serve as filters since they trap excess nutrients received from land drainage (Scharler and Baird 2005; Taljaard et al. 2009; Telesh and Khlebovich 2010). Compared to rivers and marine environments, these systems are generally richer in nutrients and organic matter production (de Villiers and Hodgson 1999; Turpie et al. 2002). The trapping property of estuaries allows nutrients to be retained and recycled. Unlike estuaries which receive nutrients both from the sea and rivers (Lohrenz et al. 1999), lakes are generally deficient in nutrients and have to recycle more (Lewis 1996). It has been stated that mixing is important for primary production in aquatic systems since it brings buried nutrients into the water column (Lewis 1996). Higher concentrations of nutrients in estuaries as well as mixing (which bring buried nutrients into the water column) enhanced by tidal action and river flow allow for high primary productivity in these systems compared to lakes.

During the past decades ecologists have shown that animals play a major role in nutrient cycling in marine and freshwater ecosystems (Arnot and Vanni 1996; Vanni et al. 1997; Vanni 2002; Hall et al. 2003; Moslemi et al. 2012). When river flow is higher, more nutrients are transported to the lower reaches of estuaries and the adjacent sea (Carić et al. 2012). The sorptive capacity of fine clay particles allows estuarine sediments to maintain high amounts of sorbed nutrients (Carić et al. 2012). Estuaries can contain phosphorus concentrations higher than those in rivers (Froelich 1988; Forsgren and Jansson 1992; Sundby et al. 1992). Clavero et al. (1999) stated that a balance exists between phosphorus in sediments and in the water column. During low nutrient supply, phosphorus is released to the water column from the pore water thus increasing nutrient concentrations in the overlying water. Other estuaries contribute to coastal ocean productivity through tidal exports of nutrients (Howarth 1988; Levin et al. 2001).

Estuarine ecosystems are susceptible to external perturbations and are considered to be among the most threatened ecosystems by anthropogenic impacts which degrade their ecological function, including their ability to act as nursery grounds (McLusky 2004; Nicolas et al. 2007). Increased human population settling close to the estuaries makes these systems more vulnerable to human impacts (Thomas et al. 2005; Nicolas et al. 2007; Perissinotto et al. 2010). Anthropogenic impacts include effluent discharges, introduction of invasive species, nutrient enrichment, water abstraction and overfishing (Allanson and Read 1995). Due to human impacts, estuaries experience changes in hydrodynamics, composition of biological communities and shifts in species diversity (which might also result from increased sediments loads as well as change in river, estuarine and sea water

temperature as a result of global climate change causing species to change their distribution) (Allanson and Read 1995; Nicolas et al. 2007; James and Paterson 2011). Changes in erosion and siltation can bring changes to estuarine morphology (Pontee et al. 2004). Reduction in freshwater input causes problems to these systems as they depend on freshwater flow to open the inlet, flush sediment, nutrients and pollutants from the estuary. Such reduction results in alteration of estuarine water quality (Pontee et al. 2004). Freshwater together with tidal exchange provide turbidity gradients in estuaries which are essential during the nursery period of selected fish species by providing olfactory cues for juveniles and reducing predatory rates by impairing visibility to predatory fish (Blaber and Blaber 1980; Allanson and Read 1995; Whitfield 2005). Reduced fresh water inflow may lead to prolonged estuarine mouth closure by sand bars, which inhibits marine species to migrate to the estuaries and back to the sea and this may reduce population size and species richness in the marine environment (Mann and Pradervand 2007; James and Paterson 2011).

1.3. South African estuaries

The coast of South Africa features 258 functional estuaries (Whitfield 1999a; Whitfield 2000). These systems together with their percentage contribution have been classified into five categories as: permanently open estuaries (POEs) (23 %), temporarily open/closed estuaries (TOCEs) (71 %), river mouths (5 %), estuarine lakes (3 %) and estuarine bays (2 %) (Whitfield 1992; Whitfield 2000).

1.3.1. Permanently open estuaries (POEs)

Permanently open estuaries have a permanent connection to the sea with moderate tidal prism which is typical to South African estuaries (Whitfield 1992). These systems are mostly dominant in the northern hemisphere e.g. European and North American coasts (Perissinotto et al. 2010; Potter et al. 2010). Generally these systems are characterised by large catchments (> 500 km²) and high runoffs throughout the year (Whitfield 1992; Whitfield and Bate 2007). Both tidal and river flows are the principal drivers of the water column mixing process in these systems with mean salinities fluctuating between 15 and 35 (Whitfield 2005). Headwaters of these systems experience oligohaline conditions while the mouth regions experience euhaline conditions. If there are major impoundments in these systems, base flows of fresh water are reduced and tidal mixing processes dominate such systems (Allanson and Read 1995). Hypersalinity has been reported in the head

waters of few permanently open estuaries that have limited freshwater supply (Whitfield 2005) e.g. in the Kariega Estuary where salinities of 42 and 35 have been measured in the upper and lower reaches respectively (Matcher et al. 2011) and in the Kromme Estuary where the upper reaches can become hypersaline (Wooldridge and Callahan 2000). However, during strong river flow periods, oligohaline conditions can sometimes be recorded in middle and lower reaches of POEs (Whitfield 2005). As a result of impoundments built in river catchments such events will become less common because most flood water (e.g. smaller to moderate floods) are captured in such structures (Whitfield 2005). Reduction in freshwater flow events will result in increased flood tidal deltas (accumulation of sediment near the inlet due to faster flood tidal currents) which narrow the lower reaches of an estuary and consequently reduce the tidal exchange between the sea and the estuary, and sediment may gradually extend further upstream (Grange et al. 2000; Cooper 2001; Whitfield 2005).

1.3.2. Temporarily open/closed estuaries (TOCEs)

Most South African TOCEs have small (< 500 km²) river catchments (Whitfield 1992; Whitfield 2005). Such estuaries also occur in Australia e.g. the Smiths Lake, Harbord and Coorong estuaries (Roy et al. 2001), on the south-eastern coast of Brazil and in Uruguay (Bonilla et al. 2005) and on the south-western coasts of India and Sri Lanka (Ranasinghe and Pattiaratchi 1998; Ranasinghe and Pattiaratchi 2003). Other TOCEs are found on the south and west coast of USA e.g. in Texas and California (Gobler et al. 2005; Kraus et al. 2008). During dry season and low river inflow these systems loose connection with the sea as a result of a sand bar that forms at the mouth (Perissinotto et al. 2010; Whitfield et al. 2012). Following high rainfall and high river inflow, the estuarine water level rises and equals or exceeds the sand bar after which the estuary breaches and an outflow channel is formed (Whitfield 1992; Whitfield 2000; Froneman 2002b; Perissinotto et al. 2010). Following this event the estuarine water level quickly drops and exposes large areas of the estuary bed which may have been colonised by rich communities of flora (e.g. macrophytes and microphytobenthos) and fauna (e.g. macrozoobenthos) since it has been submerged for extended periods (Whitfield 1992; Perissinotto et al. 2010). After an estuary empties, a short period of tidal exchange follows (Perissinotto et al. 2000; Froneman 2002b). The open phase ends because of re-formation of a sand bar as a result of reduction in freshwater inflow (Perissinotto et al. 2010).

Closed periods can vary from days to months and years depending on freshwater inflow and it is this dynamic opening and closing process that determines the physico-chemical processes, biological structure, hydrodynamics and ecological functioning of TOCEs (Perissinotto et al. 2010). There has been a decline in freshwater flow in these systems as a result of damming and water abstraction in several South African estuaries (Grange et al. 2000). This decline plays an essential role in opening and closing of the estuary mouth since these events depend largely on the amount of runoff from the inflowing rivers. In contrast, other South African estuaries are now opening more frequently because of increased water supply from waste water treatment works which also increases nutrient supply e.g. the Mhlanga Estuary (Thomas et al. 2005; Lawrie et al. 2010). A decrease in freshwater inflow will result in prolonged estuarine mouth closure and shorter open phases which may inhibit migration of fish and invertebrates between the estuary and the sea (Whitfield 2005; Mann and Pradervand 2007). If the mouth permanently closes there will be a reduction in species richness and marine species may be locally extinct. The estuary will then be dominated by estuarine and freshwater species. Hypersaline conditions may also develop under conditions of high evaporation rates and low rainfall (Whitfield 2005). The resulting low river inflow reduces the amount of nutrients entering the estuary which in turn reduce nutrients concentrations essential for primary production which supports zooplankton (Whitfield 1995; Whitfield 2005). Reduction in zooplankton abundance together with periodically recorded hypersalinity may result in decline of species diversity and abundance of zooplanktivorous fishes (Whitfield 1995; Whitfield 2005). Although the TOCEs are found in coastal environments around the world, they have been understudied relative to permanently open estuaries but South African TOCEs have received considerable attention (Perissinotto et al. 2000; Froneman 2001; Nozais et al. 2001; Froneman 2002b; Perissinotto et al. 2002; Perissinotto et al. 2003; Froneman 2004a).

1.3.3. River Mouths

River mouths in South Africa are characterized by permanently open mouths and large catchment areas ($> 10\,000\text{ km}^2$) (Whitfield 1992). However, their tidal prism is small ($< 1 \times 10^6\text{ m}^3$) (Whitfield 1992). Physical processes of these systems are generally controlled by the river rather than the tidal influence. During the events of moderate to high fresh water runoff, sea water is in general hardly ever recorded in the upper estuarine reaches, but a dilution of fresh and sea water is possible in the lower reaches during low flow periods. One characteristic of these systems is a very high silt load derived from the river. Regardless of high volume of water passing through river mouths, they are

generally shallow (< 2m deep) (Whitfield 1992) although depths of up to 15 meters can be recorded as a result of periodic floods (Swart et al. 1988). Water temperature of river mouths is generally controlled by freshwater inflow, however, the bottom water temperature in the lower reaches can sometimes be influenced by the sea (Whitfield 1992). Freshwater biota dominate such systems (Day 1981b). Examples of South African river mouths are Thukela, Mvoti, Mzimvubu and Storms River estuaries (Whitfield 2000).

1.3.4. Estuarine Bays

One feature of these systems is the frequent substitution of estuarine water by the sea water in the lower part of the channel as opposed to river mouths (Whitfield 1992). These systems generally have large tidal prism ($>10 \times 10^6 \text{ m}^3$). Such systems generally receive high amounts of sea water. The lower reaches of these systems normally have salinity levels greater than 25, e.g. the Knysna system (Largier et al. 2000), but salinities below this level are recorded in the lower reaches during heavy river flow (Grindley 1985; Whitfield 1992). The mixing process is mostly dominated by tides and wind. There is also a strong salinity stratification in the upper and middle reaches (Whitfield 1992; Largier et al. 2000). South African coast possesses estuarine bays which are natural (e.g. the Knysna system) and artificial (e.g. Richards Bay and Durban Bay estuarine systems) (Whitfield 1992; Whitfield 2000).

1.3.5. Estuarine lakes

Most South African estuarine lakes display a separation from the sea by vegetated sand dune systems (Whitfield 1992). The South African coast features only eight of these systems and these are Kleinmonde, Klein, Wilderness, Swartvlei, Nhlabane, St. Lucia, Mgobezeleni and Kosi estuarine system, (Whitfield 2000). Other estuarine lakes lose their estuarine character after they have been completely isolated from the sea for a couple of years and are then referred to as coastal lakes. These systems still possess remnant estuarine biota tolerant of freshwater conditions (Whitfield 1992). The Kosi system is one example of an estuarine lake which has a permanent connection to the sea while the Swartvlei is an example of an estuarine lake which has a temporal marine connection. Mixing of the water column is mostly driven by wind even in the deeper systems. During low water inflow or drought conditions, these systems can become hypersaline (Whitfield 1992). St Lucia is an example of an estuarine lake that can become hypersaline in the northern and middle reaches during drought conditions (Vivier and Cyrus 2009). Water

temperatures of such systems are less subjective to river flow or tidal exchange since the tidal prisms of such systems are generally smaller compared to their size as a result of constricted channels linking them to the sea. Other estuarine lakes receive about 50 % of their input from precipitation e.g. St. Lucia (Whitfield 1992; Vivier and Cyrus 2009). Generally, water temperatures of estuarine lake systems are directly influenced by solar heating (Whitfield 1992).

From the five types of estuaries on the South African coast, this study focuses on a permanently open (Mlalazi) and a temporarily open/closed (Mpenjati) Estuary.

1.4. Phosphorus as a macronutrient and its role in estuaries

1.4.1. Importance and sources of phosphorus

Phosphorus (P) is an essential nutrient for all life forms (Correll 1999; Elser 2012). It forms part of deoxyribonucleic acid (DNA) as well as ribonucleic acid (RNA) (Conley et al. 2009). Phosphorus also plays a significant role in cellular metabolism during the transmission of energy through the adenosine triphosphate (ATP) molecule (Sterner and Elser 2003). This nutrient occurs in organic and inorganic forms (e.g. orthophosphates, polyphosphates or metaphosphate) (Paytan and McLaughlin 2007). Approximately 5-10 % of P transported by rivers to coastal and estuarine waters is in dissolved form and the remainder is in particulate form (Froelich 1988; Follmi 1996; Fisher et al. 1999). In aquatic ecosystems orthophosphate (PO_4^{-3}) is the principal soluble inorganic form in which P is available and utilised by aquatic plants (Correll 1999; Paytan and McLaughlin 2007).

Weathering of rocks and leaching of phosphate salts from the soil are the main sources of biologically available phosphorus together with decomposition of organic matter (Huanxin et al. 1997; Paytan and McLaughlin 2007). The mobility, availability and spatial distribution of P within an estuary are determined by the flow regime. Higher concentrations are recorded during heavy rainfall and high flow events due to flushing and resuspension of the sediments (Gao et al. 2010). Particulate phosphorus that has entered the estuary may be deposited to the sediments after which microbial communities gradually consume organic components of the sediment, and sediment phosphorus is eventually released back to water column as orthophosphate (Correll 1999). Bottom sediment phosphorus can also be released back to the water column by bottom feeding fish as they disturb and stir up the sediment causing a release of phosphorus back to the water column

(Callender 1982). Dissolved organic nutrient sources in estuaries include freshwater inflow, tidal exchange, as well as debris and leaf litter falling from the surrounding flora. Dissolved inorganic nutrients in estuaries are received from the inflowing river, ground water, seepage and from marine waters during the open mouth phase (Callender 1982; Eyre 1998; Slomp 2011). In general higher amounts are received from the river compared to the adjacent sea and other sources (Callender 1982; Eyre 1998).

1.4.2. Phosphorus, primary productivity and eutrophication

River discharge is one component contributing towards nutrient transportation into estuaries (Eyre and Balls 1999; Loneragan 1999). Nitrogen (N) and Phosphorus (P) exported from rivers contribute greatly towards primary productivity in estuaries and the adjacent marine environment (Fisher et al. 1992; Statham 2012). Their availability largely determines the productivity of coastal environments (Fisher et al. 1992). These nutrients occur in varying stoichiometric ratios as a result of differences in the rate in which they are supplied, taken up, stored in living tissues and in non-living particulate and dissolved pools in the sediments and water column and made available through catabolic and anabolic processes (Howarth 1988; Elser and Hessen 2005). Few studies (Gobler et al. 2005; Snow and Adams 2007) found that TOCEs have higher macronutrient concentrations during the open compared to the closed phase. After a prolonged mouth closure the water column may experience macronutrient depletion due to persistent algal uptake (Perissinotto et al. 2010).

Phytoplankton chlorophyll a concentrations recorded in POEs can be lower than those recorded in TOCEs within the same biogeographic region (Adams and Bate 1999). For example, chlorophyll a concentration measured in the Great Brak Estuary (a TOCE in the warm temperate region) was $13 \mu\text{g}\cdot\text{l}^{-1}$ while chlorophyll a concentration measured in the Gourits Estuary (a POE in the warm temperate region) was less than $1 \mu\text{g}\cdot\text{l}^{-1}$ during 1992 (Adams and Bate 1999). Higher concentrations of microphytobenthic chlorophyll a has been measured in TOCEs compared to the POEs (Adams and Bate 1999). These high concentrations are often associated with low turbidity leading to increased light availability, calm current flow and high macronutrient concentrations in sediments (Froneman 2002b).

Although phosphorus loading is known to be a good predictor of primary production in estuaries, there is an argument whether nitrogen or phosphorus is the limiting nutrient in these systems

(Howarth and Marino 2006; Conley et al. 2009). Increasing number of studies concludes that there are limiting nutrients in marine ecosystems other than nitrogen (Herbland et al. 1998). Phosphorus limitation has been reported in marine, estuarine and nearshore systems such as the Chesapeake Bay (Taft and Taylor 1976), Pearl and Changjiang estuaries (Yin et al. 2000; Duan et al. 2008), Huanghe River Estuary (Liu et al. 2003), Mediterranean Sea (Krom et al. 1991), Xiamen Bay (Harrison et al. 1990), South Carolina salt marsh (Sundareshwar et al. 2003), estuaries along the north-eastern margin of the Gulf of Mexico (Myers and Iverson 1981) and few western Australian estuaries (McComb et al. 1981).

Phosphorus has been widely reported to control the degree of eutrophication in aquatic systems (Redfield 1958; Herbland et al. 1998; Wang et al. 2003; Wepener 2007; Lukkari et al. 2008).

An increase in human population settling near coastal areas has raised the amount of new anthropogenic nutrient inputs into catchments and estuaries (Puigserver et al. 2002; Paerl 2006). As a result, these aquatic systems receive large amounts of land based nutrients and other pollutants entering via surface run off (e.g. agricultural runoff), atmospheric deposition and outflows from waste water treatment works (Paerl 2006). Such conditions may lead to formation of algal blooms followed by depletion of oxygen in the estuarine water which may lead to invertebrate and fish kills (Whitfield 1995; Adams and Bate 1999).

In productive estuaries which sometimes experience phytoplankton blooms, a decline in phytoplankton concentration may result from cell shading through cell abundance which inhibits light penetration to the entire phytoplankton community e.g. in the Mhlanga and Mdloti estuaries (Thomas et al. 2005). High chlorophyll a concentrations exceeding $20 \text{ mg chl a} \cdot \text{m}^{-3}$ have been recorded in permanently open estuaries of the South African coast (Bate and Adams 2000). Chlorophyll a concentrations higher than $100 \text{ mg chl a} \cdot \text{m}^{-3}$ have also been measured in few South African POEs e.g. in the Sundays and Gamtoos estuaries (Hilmer and Bate 1990; Bate and Adams 2000; Snow et al. 2000). Phytoplankton bloom is defined as chlorophyll a concentration greater than $20 \mu\text{g} \cdot \text{l}^{-1}$ (Adams and Bate 1999), although Fielding et al. (1991) reported phytoplankton bloom in the St. Lucia Estuary with mean chlorophyll a concentration of $16 \mu\text{g} \cdot \text{l}^{-1}$. No dense algal blooms have been apparent in South African TOCEs (Perissinotto et al. 2000; Nozais et al. 2001; Perissinotto et al. 2003).

1.4.3. Phosphorus and zooplankton

Studies have confirmed that freshwater zooplankton display differences in P content between species, and these interspecific differences are higher for P compared to nitrogen and carbon (Andersen and Hessen 1991; Hassett et al. 1997; Vrede et al. 1999). Vrede et al. (1999) examined the distribution of phosphorus in three zooplankton species (*Daphnia magna*, *D. galeata* and *Eudiaptomus gracilis*) collected from Norwegian cultures, Lake Erken and Lake Norrviken respectively, where P content of *Daphnia magna* (1.5 %) and *D. Galeata* (1.4 %) were three-fold higher than that of *E gracilis* (0.5 %). Their results showed no variation in phosphorus:dry weight ratio across the size range for *D. magna* and *E. gracilis* but in *D. Galeata* they reported an increase in P content with an increase in body size. Their study also confirmed the low intraspecific variability in P:DW ratio in zooplankton. Interspecific and intraspecific (e.g. *Arcatia* spp) differences between zooplankton P content have also been reported in marine ecosystems such as the Baltic Sea (Walve and Larsson 1999). It was proposed that the variation in P content between *Arcatia* spp was due to developmental stages and seasons (Walve and Larsson 1999). Body P content (%) of organisms can vary among size classes with smaller organisms having higher P content (%) due to higher growth rate and higher P demands when compared to larger ones (Cross et al. 2003).

1.4.4. Phosphorus and macrozoobenthos

Phosphorus has been measured in macrozoobenthos from 35 streams in Indiana-Michigan and central Wisconsin (USA), and it was reported that P varies with taxon and site (Evans-White et al. 2005). It has been reported that body size of benthic organisms can generally explain very little variation in % P content of organisms and in addition, variability is generally higher for % P content than for % C and % N (Evans-White et al. 2005; Martinson et al. 2008).

Crustaceans have been reported to have higher % P content compared to molluscs and insects (Evans-White et al. 2005). In support of crustaceans being richer in P than nitrogen and carbon relative to molluscs and insects, concentrations of crustaceans (P = 0.9 %, C = 35 %, N = 7.4 %); insects (P = 0.6 %, C = 48 %, N = 10 %) and molluscs (without shell) (P = 0.8 %, C = 42 %, N = 9.6 %) have been recorded for 35 streams in the United States of America (Evans-White et al. 2005). This higher P content in crustaceans may perhaps be due to higher rRNA content in crustaceans compared to molluscs and insects (Evans-White et al. 2005). Another explanation may

be that P is associated with calcium in benthic crustacean carapaces (for moulting and growth processes) and this may account for higher % P and lower C:P and N:P ratios of crustaceans compared to molluscs and insects (Evans-White et al. 2005). A study conducted by Frost et al. (2003) in eight Canadian lakes revealed that there was a significant variation in % P content of benthic macroinvertebrates compared to % N and % C content. Body P content for all nine taxa varied 10-fold from 0.1% to 1.4 %, body N content varied 2-fold from 5.8 % to 13.6 % while body C content was less variable, ranging from 32.5 to 53.5 % (Frost et al. 2003). Significant variation in P content between taxonomic groups was apparent with little variation across the eight lakes (Frost et al. 2003).

Variations in % P content as well as C:P ratio between macrozoobenthic species have been reported in several lakes in Canada located in different regions i.e. central Alberta and north western Ontario, as well as in Lake Erkenin, Sweden (Frost et al. 2003; Evans-White et al. 2005; Liess and Hillebrand 2005). Phosphorus has been measured in six benthic species of Antarctic marine system where phosphorus content varied two-fold from 0.7 to 1.3 % while C and N content were less variable from 49 to 60 % and 10 to 14 % respectively. The growth rate hypothesis (which states that growth related demands essential for generation of organism's ribosomal RNA are determined by the organisms body P content) was generally not true for such system with low temperature because organisms body P contents were relatively higher despite the very slow growth rate experienced by polar marine macrozoobenthos (Clarke 2008).

1.4.5. Phosphorus and fish

It has been documented that fish and fisheries management can play an essential role in freshwater nutrient dynamics but information is scarce for marine ecosystems (Hjerne and Hansson 2002). Fish contribution to the recycling of phosphorus in lakes have been documented (Griffiths 2006). Phosphorus content (%) in fish has been measured in Lake Superior coastal wetland in North America (Tanner et al. 2000), Experimental Lakes area in northwest Ontario, Canada (Sterner and George 2000) and in Utrata River, Poland (Penczak 1985) where variation in P content (%) between species, size classes as well as sampling sites was reported. Fish body P content (%) has been reported to vary more among fish families than among species within families (Vanni et al. 2002).

1.4.6. Role of organisms in nutrient cycling

The degree of consumption and release of nutrients by organisms can determine if an organism is a nutrient net source or net sink in a given time (Vanni 2002). Estuarine fauna play an important role in recycling of nutrients in estuaries and in addition they excrete nutrients in remineralised form ready to be used by primary producers (Nalepa et al. 1991; Vanni 2002). The rate at which these estuarine organisms excrete nutrients are important for primary production and the ratio at which these nutrients are excreted can determine the degree of either nitrogen or phosphorus limitation in aquatic ecosystems (Vanni 2002). Longer lived animals with bigger body size can sequester large amounts of nutrients in their bodies and they serve as nutrient pools and consequently as a nutrient source to their predators or during decomposition (Vaughn and Hakenkamp 2001; Vanni 2002). Unlike in zooplankton and macrozoobenthos where more P is stored in ribosomes and other repositories, more phosphorus in fish is stored in bones (Vanni and Findlay 1990; Sterner and Elser 2002).

The phosphorus amount excreted by fish per body size can be higher than that excreted by zooplankton e.g. in the Lake 221 in north-western Ontario (Vanni and Findlay 1990). Sediment feeding fish populations of Lake Gjersjoen in Norway have been reported to contribute to phosphorus supply twice as high that of external loading (Brabrand et al. 1990). It has been argued that P amount released by fish is lower than the phytoplankton demands e.g. in the Lake Memphremagog situated between Canada and United States (Nakashima and Leggett 1980) while others argue that P excretion by fish is sufficient to support primary production e.g. in the Union Lake in USA and in few experiments conducted in tanks (Lamara 1975; Reinertsen et al. 1986; Threlkeld 1988). A scenario of a decrease in dissolved P concentration following a decline in abundance of benthivorous/planktivorous fish has been reported in Lake Vaeng, Denmark (Sondergaard et al. 1990). In this case, it is likely that the fish community can have an impact on primary production through control of the nutrient supply (Sondergaard et al. 1990). Benthic invertebrates waste can be an important nutrient source for benthic algae (Evans-White and Lamberti 2005).

1.4.7. Phosphorus balance between organisms and their food sources

Organisms can serve as nutrient sinks for elements in greatest shortage (Walve and Larsson 1999; Vanni 2002; Park et al. 2003). The growth of most freshwater and marine zooplankton has been

shown to be restricted by phosphorus (Hessen 1992; Park et al. 2003). It has been mentioned that zooplankton (Sterner 1997) and macrozoobenthos (Frost and Elser 2002; Elser et al. 2005) growth rates are related to concentrations of phosphorus within the algal food base. A balance must be kept between the intake of carbon and phosphorus with respect to the grazer's body demands in order for the grazer to maintain a homeostatic C:P ratio (Anderson et al. 1978).

Zooplankton have supported the growth rate hypothesis where it was found that 75-90 % of P content was contained in RNA, this explains that zooplankton invest very little P (as opposed to N) in biochemicals other than RNA (Elser 2012). Aquatic herbivores like zooplankton and macrozoobenthos are known to have low C:P ratio compared to that normally found in their food (Cross et al. 2005). This mismatch can alter food web dynamics by affecting nutrient release (e.g. excretion of nutrients of high C:P ratio), growth and reproduction of organisms (Andersen and Hessen 1991; Andersen 1997; Sterner and Elser 2002; Urabe et al. 2002). Zooplankton species like *Daphnia* spp. display a constraint in growth when they are fed on autotrophs with low P content (Sterner and Elser 2003). The high C:P ratio in algae that results from the blooms can cause a drop in zooplankton population abundance (e.g. in Lake Berryessa in California) (Park et al. 2002; Park et al. 2003).

The ambient P levels and photosynthetic active radiation (PAR) contribute to shifts in cellular C:P ratio of phytoplankton (Urabe and Sterner 1996; Andersen et al. 2007). High photosynthetic rate enhanced by high amount of PAR results in accumulation of C-rich macromolecules. This in turn leads to increased C:P ratio of autotroph biomass. Such condition is greatly enhanced when there is low P supply (Andersen et al. 2007). When light is limiting but P is saturated, autotroph biomass will be lower although algal cells will contain high P:C ratio which depicts high food quality for herbivores. Shifts in food quality versus quantity may cause changes in herbivores growth rate along the light gradient (Urabe and Sterner 1996). The herbivore growth rate is expected to increase along the gradient of increasing light with constant total P and it reaches maximum at an optimum light: nutrient balance. Finally the growth rate declines as a result of high light:P, and a decrease in food P:C ratio which depicts a low food quality (Urabe and Sterner 1996; Andersen et al. 2007). This relationship between food quantity and food quality limitation is essential for assessments of ecosystem productivity (Andersen et al. 2007).

1.4.8. Phosphorus and chemical processes in sediment

Understanding the transportation of P from terrestrial environment to coastal and marine environment is very important for quantifying global P cycling and to overcome problems associated with eutrophication (Follmi 1996). In lakes and estuaries, sediments are the principal drivers in regeneration of phosphate (Sundby et al. 1992; Anschutz et al. 1998). After the dissolved and particulate inputs of P has reached the receiving systems, the particles may release phosphate to the water column and P compounds get hydrolyzed either chemically or enzymically to form orthophosphate during periods of low river flow and low nutrient input (Sundby et al. 1992). Microbial communities in the sediments utilise much of the sediment organic material and they release it back to the water column as orthophosphate which in turn is utilised by the primary producers (Sundby et al. 1992; Hartzell and Jordan 2010). This process (named phosphate buffer mechanism) maintains a constant dissolved phosphate concentration in the water column through the influence of sediments regardless of biological removal and input effects (Pomeroy et al. 1965; Froelich 1988).

In estuarine ecosystems, short term and spatial shifts in limitation of nutrients can exist (Anschutz et al. 1998; Fisher et al. 1999). Phosphorus sorption in sediments can change with salinity gradients and this is explained by metal oxides carrying a net negative charge at seawater pH and a net positive charge at freshwater pH (Barrow et al. 1980; Sundareshwar and Morris 1999; Dunne et al. 2005). It is this change in salinity and pH that alter phosphorus binding potential along the estuary salinity gradient (Anschutz et al. 1998; Sundareshwar and Morris 1999). The inverse relationship between the salinity and phosphorus sorption potential may be due to a decrease in iron hydroxide content of sediment with increasing salinity (Sundareshwar and Morris 1999).

Phosphorus concentration in sediments has been measured in few estuarine systems e.g. in the Richmond River Estuary in Australia and Palmones River Estuary in Spain where concentrations of PO_4^{-3} adsorbed in sediment was increasing from the mouth towards the upper reaches (Clavero et al. 1999; Mckee et al. 2000).

1.5. Phosphorus dynamics in estuaries of South Africa and other parts of the world

Many estuaries along South African coast rely on river-derived nutrients for stimulation of primary production (Snow et al. 2000; Taljaard et al. 2009). Dissolved inorganic nutrient (e.g. dissolved inorganic phosphorus) concentrations during freshwater dominated states in estuaries are greatly determined by physical processes which involve the extent of mixing of freshwater and sea water (Taljaard et al. 2009). During freshwater dominated states in estuaries, nutrients like phosphorus entering the estuary are flushed into the adjacent sea without considerable transformation and utilisation leading to a very low primary production (Snow and Taljaard 2007; Taljaard et al. 2009).

Conditions prevailing during closed phase (e.g. limited water exchange and stable sediments) of South African TOCEs introduce favourable conditions for biochemical and biological processes to control nutrient cycling and transformation (Snow and Taljaard 2007; Taljaard et al. 2009). Supporting this statement, in the Great Brak Estuary the phosphorus concentrations were near-depleted after 80 days of mouth closure in 2007 indicating its considerable removal and utilisation from the water column (Taljaard et al. 2009). This event has also been apparent in the Mdloti Estuary where low phosphorus concentrations were measured during events of prolonged mouth closure (Perissinotto et al. 2004). Such low concentrations during closed phases give evidence that small and shallow South African TOCEs cannot support substantial primary production in the water column once the nutrients in the overlying waters are depleted, although significant benthic production can be apparent (Perissinotto et al. 2004).

When the TOCEs are in their semi-closed state with the outflow present but no tidal exchange, they receive a continuous riverine input of phosphorus and other nutrients. Although these may be in low supply in pristine catchments, they can be retained adequately long for stimulation of water column primary production (Snow and Taljaard 2007). When South African TOCEs reach their closed state, phosphorus and other nutrients may be received from the nutrient richer coastal waters through overwash, although this is limited to areas near the mouth (Snow and Taljaard 2007; Taljaard et al. 2009). High nutrient availability as well as adequate residence time near the mouth areas can stimulate phytoplankton blooms in the lower reaches (Snow and Taljaard 2007; Taljaard et al. 2009). Gama et al. (2005) reported a peak in phytoplankton production after an overwash event in the Van Stadens Estuary in the Eastern Cape. During a closed phase where there is very little or no

river flow, the estuary may rely on ground water and remineralisation as inputs of dissolved inorganic phosphorus (Snow and Taljaard 2007).

Dissolved inorganic phosphorus has been measured in many estuaries on the coast of South Africa e.g. in the Kariega, Swartkops, Kromme, Sundays, Knysna, Mdloti, Mhlanga and Mpenjati estuaries (Emmerson 1985; Allanson 1999; Allanson et al. 2000; Scharler and Baird 2003a; Thomas et al. 2005). In South African estuarine systems phosphate concentrations have been reported to be generally higher in the upper reaches decreasing towards the mouth, as in the Kariega and Great Fish estuaries (Bate et al. 2002). Scharler and Baird (2003) pointed out that the upper reaches of other South African estuaries are seen as nutrient sinks for inorganic dissolved nutrients. However, during the events of severe droughts where reverse salinity gradients prevail, phosphorus concentrations can be higher near the mouth (e.g. in the Kariega River Estuary in the Eastern Cape (Allanson 1999).

Phosphorus exchange is mostly influenced by the redox potential of the environment (Winter 1999). During anaerobic conditions in estuarine sediments, phosphorus release rate from the particulate matter has been reported to be higher when compared to aerobic conditions e.g. in the Swartvlei Estuary (Silberbauer 1982; Chambers et al. 1995). Macrophytes have been reported to play a vital role in phosphorus exchange through foliar release (McRoy et al. 1972; Winter 1999). Liptrot (1978) reported *Zostera capensis* beds of the Swartvlei Estuary as an active agent in the uptake and excretion of phosphorus. He also highlighted that the algal mat (*Enteromorpha spp.*) is among the compartments to which the phosphorus excreted by *Zostera* is transferred during the closed phase of the Swartvlei Estuary.

Dissolved inorganic phosphorus (DIP) has been reported to increase from the upper towards the lower reaches during the low river flow in few Australian estuaries e.g. in the Jardine, Annan and Daintree estuaries and in few estuaries in United Kingdom e.g. in the Inverness, Cromarty and Dornoch Firths estuaries (Eyre and Balls 1999). This pattern depicts their pristine condition (Eyre and Balls 1999). In the Scheldt Estuary, Europe, particulate organic phosphorus (POP), particulate inorganic phosphorus (PIP) as well as dissolved organic phosphorus (DOP) concentrations have been reported to decrease from the upper towards the lower reaches (van der Zee et al. 2007).

1.6. Aim

Phosphorus, an important macronutrient for all life forms has been measured in estuaries of South Africa in the form of dissolved inorganic phosphorus e.g. (Emmerson 1985; Allanson et al. 2000; Scharler and Baird 2003a; Thomas et al. 2005). In South African estuaries, no information is available on phosphorus content in biotic (e.g. phytoplankton, zooplankton and macrozoobenthos) and abiotic (e.g. sediment) components to highlight how this nutrient is distributed through estuarine food webs. Therefore the aim of this project was to determine phosphorus distribution in living and non-living nutrient pools of two KwaZulu-Natal estuaries.

1.7. Objectives

Looking at variations between low river flow (May and September in the Mlalazi and Mpenjati Estuary respectively) and high river flow period (November for both estuaries), objectives were the following.

To determine:

- Changes in abundance and biomass of the zooplankton and macrozoobenthos between the low (May and September for the Mlalazi and Mpenjati Estuary respectively) and high (November for both estuaries) river flow periods, stations and between the estuaries.
- Changes in standing stocks of all living and non-living nutrient pools in terms of phosphorus (P) by measuring the P content of biotic and abiotic nutrient pools including macrozoobenthos, zooplankton, total suspended solids and detritus, where macrozoobenthos and zooplankton were divided into various taxa.
- Shifts in P distribution among different taxa and along the estuarine salinity gradient.

1.8. Hypotheses

As outlined in the introduction, biogeochemical processes in estuaries are controlled by freshwater inflow and differing river flow patterns can cause changes in composition of biological communities and their distribution (Powell et al. 2002). It was hypothesised that the abundance and biomass of biotic (zooplankton, macrozoobenthos and phytoplankton) and abiotic (TSS)

components will change in low (May and September) and high (November) river flow and along the estuary length.

Phosphorus concentrations in estuaries were expected to vary with time as a result of changes in river flow patterns with higher concentrations expected during high river flow events due to higher nutrient supply from the elevated riverine inflow and resuspension from sediment (Gao et al. 2010). Since phosphorus is derived from the river, higher concentrations were expected in the upper reaches. It was therefore hypothesised that phosphorus standing stocks in abiotic (DIP, PP and sediment P) and biotic (phytoplankton) components will change with low (May and September) and high (November) river flow periods and/or along the estuary length.

Organism P content (%) can vary among size classes with smaller organisms having higher P content (%) due to higher growth rate and higher P demands when compared to larger organisms (Cross et al. 2003). In this study it was expected that the larger zooplankton species (i.e. *Pseudodiaptomus hessei*) will have higher P content than the smaller *Arcatia natalensis*. P content was also expected to differ between benthic taxa because crustaceans are known to have higher % P content compared to other macrozoobenthos groups because P is associated with calcium in benthic crustacean carapaces (Evans-White et al. 2005). It was then hypothesised that phosphorus standing stocks in biotic components (zooplankton and macrozoobenthos) will vary between taxa.

Chapter 2

Materials and Methods

Two estuaries (Mlalazi and Mpenjati) were sampled during May and September respectively and again in November 2011. Mlalazi was sampled in May (for the low river flow period). Sampling session representing low river flow period for the temporarily open/closed Mpenjati Estuary was supposed to be conducted when the mouth was closed, however, as a result of high precipitation in 2011, the inlet only closed in September and sampling had to be postponed until then. Sampling representing high river inflow period was conducted in November for both estuaries. In order to determine P distribution in biotic and abiotic nutrient pools in each estuary the following was sampled: Subsurface water for determination of dissolved inorganic nutrients, total suspended solids (TSS) and phytoplankton chlorophyll a. Sediment was sampled for determination of microphytobenthos chlorophyll a and sediment P content. Zooplankton and macrozoobenthos were also sampled. Living organisms were identified to species (zooplankton) and family (macrozoobenthos) level and enumerated. Wet and dry weight of animals was measured and animals were ground to powder. The TSS filters, sediment and animal tissue were digested to get the P content in these nutrient pools. Details of all methods are listed below.

2.1. Study areas

2.1.1. Mlalazi Estuary

The Mlalazi Estuary (28° 57' S; 31° 48' E) is a permanently open estuary (POE) on the north eastern coast of South Africa (Whitfield 2000) (Figure 2.1). The length of the Mlalazi River is approximately 54 km with a catchment area of approximately 415 km² (Day 1981b). The Mlalazi Estuary is 1-3 m deep and 100 m wide for most of its length but can extend to 200 m near the mouth (Day 1981b). The bottom substrate is sandy mud but consists of clay in the upper reaches (Day 1981b). According to Day (1951) subdivision of the Mlalazi Estuary, both banks of the upper (lake area and narrow section above it) and middle (channel stretching up to the southern tip of the lake) reaches of the estuary are fringed by mangrove forests dominated by two species; *Avicennia officinalis* and *Bruguiera gymnorhiza* (Hill 1966; Papadopoulos et al. 2002). Behind mangrove forest in the lake region is *Hibiscus tiliceus* forest (Hill 1966). The mouth region of the Mlalazi is covered by sand dune which is 10 m high and supporting small *Casuarina* forest at the mouth side

(Hill 1966). Mangroves started to colonize the estuary in the early 1950s. Since 1952 prolonged dry periods have been experienced intermittently and the mouth has been periodically closing (Hill 1966). The catchment land cover of the Mlalazi Estuary is not considered degraded, 53 % of it is natural and approximately 46 % of it is used for agricultural farming e.g. for sugarcane (Harrison et al. 2001). There are two bridges crossing the estuary in the upper reaches, the first one is part of the national road (N2) and the second one is the railway bridge (Figure 2.1).

2.1.2 Mpenjati Estuary

The Mpenjati Estuary (30° 58' S and 30° 17' E) is a temporarily open/closed system on the south coast of KwaZulu-Natal (KZN) (Whitfield 2000) situated approximately 165 km south west of Durban (Begg 1978) (Figure 2.1). The system has a catchment area of approximately 101 km² and it occupies an area of 11.6 ha with an axial length of 1.1 km (Begg 1978). The Mpenjati River is approximately 18 km long (Begg 1978). The bed of the estuary is composed of stones in the upper reaches and muddy substrata in the middle reaches while the substrata at the lower reaches is mainly sandy (Kibirige and Perissinotto 2003). In terms of anthropogenic impacts when compared to other estuaries, the Mpenjati Estuary is moderately impacted (Whitfield 2000). There are two road bridges crossing this system. The old bridge is located in the upper reaches and is part of a regional road (Louis Botha Ave). The second bridge is in the middle reaches and is part of a National Road (R 61) (Figure 2.1). The mouth of the Mpenjati Estuary is generally closed for over 65 % of the year (Perissinotto et al. 2002). The estuary mouth is closed by the formation of a sand bar during the period of either low or no rainfall which is usually May to September (dry season). The estuary gains its connection to the sea as a result of heavy rainfall which usually takes place from October to April (wet season) (Kibirige et al. 2002; Perissinotto et al. 2003). During the open phase of the estuary, hydrodynamics of the water column are greatly subjective to tidal and riverine input but during the closed phase hydrodynamics are mainly influenced by wind (Perissinotto et al. 2002; Whitfield 1992). The nearby catchment and the upper reaches of the Mpenjati Estuary are fringed by the agricultural plantations with sugar cane and banana being the predominant crops. There is Palm Beach Waste Water Treatment Works in the upper reaches discharging treated waste water into the estuary.

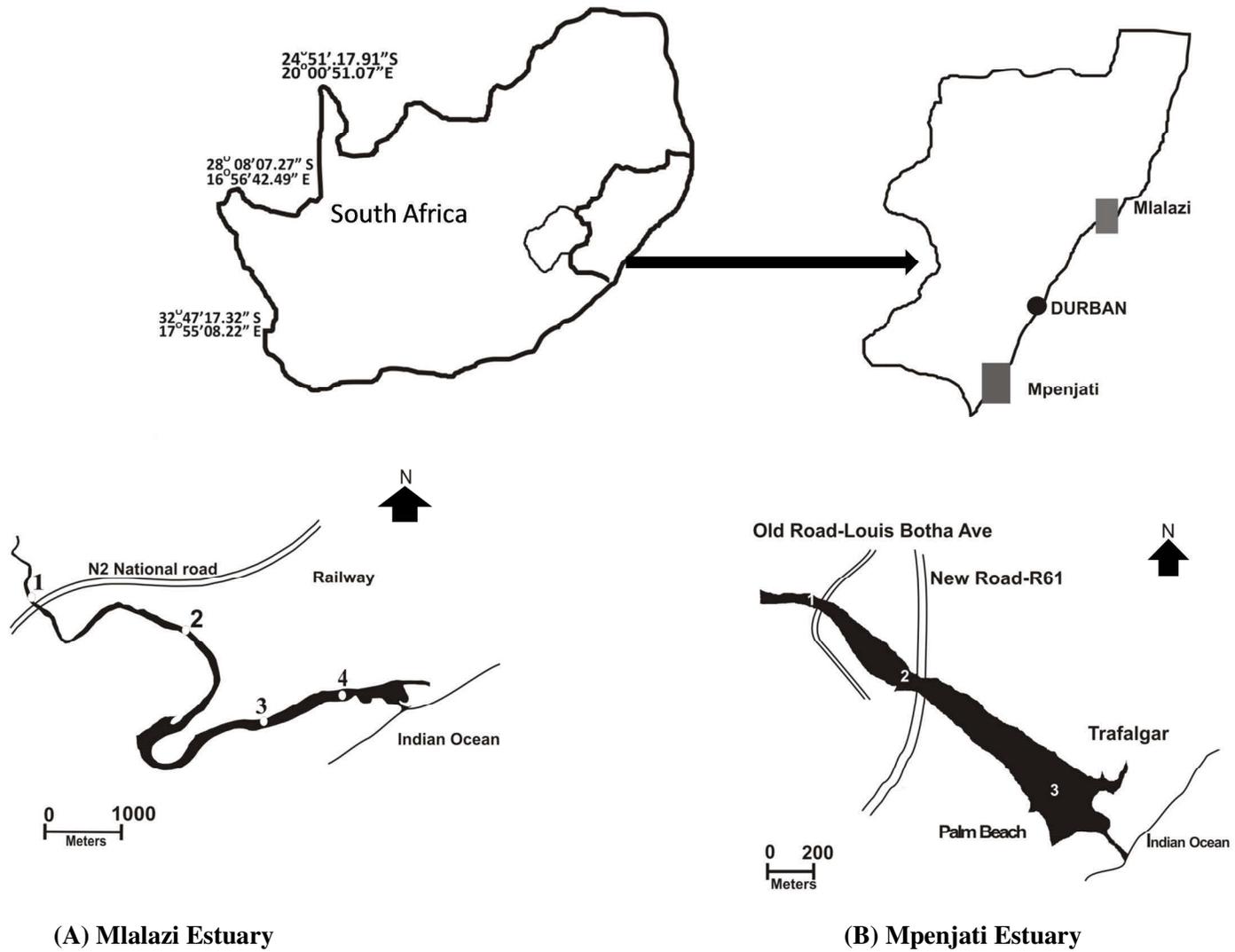


Figure 2.1: Map of the Mlalazi (A) and Mpenjati (B) estuaries with sampling stations.

2.2. Field work

Biological and environmental samples were collected from the Mlalazi (permanently open estuary) and Mpenjati (temporarily open/closed estuary) during the study period. Four stations were sampled in the Mlalazi (ML) and three stations in the Mpenjati Estuary (MP) (Figure 2.1). The first sampling session was conducted in May 2011 for the Mlalazi Estuary and September 2011 for the Mpenjati Estuary. The second sampling session was conducted in November 2011 for both estuaries. Biological and environmental samples were collected from both estuaries at all stations.

2.2.1. Environmental parameters

Physico-chemical parameters were measured in both estuaries at each station during each sampling period. Salinity, dissolved oxygen ($\text{mg}\cdot\text{l}^{-1}$), pH, temperature ($^{\circ}\text{C}$), conductivity ($\text{mS}\cdot\text{cm}^{-1}$) and oxidation reduction potential (ORP) (mV) were measured near the surface and bottom of the water column using YSI 556 MPS multiprobe system. Water depth was recorded at each station in both estuaries during each sampling session. The Mthunzini and Mzimkhulu rainfall gauges nearest to the Mlalazi and Mpenjati estuaries respectively were used to infer rainfall levels in these estuarine systems during the study period.

2.2.2. Abiotic samples

Water samples for dissolved inorganic nutrients, total suspended solids (TSS), and particulate phosphorus (PP) were collected from the sub-surface using 100 ml (for dissolved inorganic nutrients) and 1000 ml (for TSS and PP) acid washed polyethylene bottles. Three replicates were collected at all stations in both estuaries and kept in a cooler box with ice. For TSS and PP samples, depending on the amount of silt in each replicate, a volume between 600 and 1000 ml was filtered through a pre-combusted and weighed GF/F Whatman filter ($0.7\ \mu\text{m}$ pore size and 47 mm diameter). Filters were wrapped in foil and stored in the fridge over night for further analysis in the laboratory. Dissolved inorganic nutrient samples were obtained from each replicate filtrate and then stored in 100 ml bottles which were refrigerated overnight until they were taken back to the laboratory. All the filtration was done in the field. Three replicates of sediment samples for determination of phosphorus content were collected from each station in both estuaries. Sediment was collected using a 20 mm internal diameter corer. The first top 2.5 centimetres of the sediment

was cut off and stored in polyethylene bottles. Sediment samples were refrigerated overnight until they were oven dried at 60 °C in the laboratory.

2.2.3. Biotic samples

Three replicates for chlorophyll a, microphytobenthos and macrozoobenthos were collected at each station in both estuaries. Two replicates were collected for zooplankton at each station in both estuaries. Water samples for phytoplankton chlorophyll a determination were collected using acid washed 250 ml polyethylene bottles. Each replicate was filtered through 20, 2 and 0.7 micron filters to retain microplankton (> 20 µm), nanoplankton (2-20 µm) and picoplankton (< 2 µm) respectively. Filters were stored in polyethylene vials with 10 ml of 90 % acetone for extraction of pigments. Sediment samples for microphytobenthic chlorophyll a determination were collected using a 20 mm internal diameter corer. The first top centimeter of the sediment was cut off and stored in 100 ml polyethylene bottle with 10 ml of 90 % acetone for extraction of pigments. Phytoplankton and microphytobenthic chlorophyll a samples were stored in the dark and were refrigerated for 24 hours prior to analysis.

Macrozoobenthos samples were collected using a van Veen 12.110 grab (250 cm² in area, depth sampled down to 10 cm). Each replicate was stirred in a bucket and decanted five times through a 500 µm sieve to extract organisms. All organisms were stored in honey jars and preserved in 10 % formalin containing Rose Bengal dye to aid sorting in the laboratory. Grab samplers (e.g. van Veen grab) are generally used in standard protocols to collect soft bottom macrozoobenthos (Klemm et al. 1990; EPA 2001). This sampling tool samples up to 10 cm depth (Flach and Heip 1996). Therefore deep burrowing animals like *Callichirus kraussi* and *Upogebia africana* were under represented if this method alone was used. To account for deep burrowing prawns, data for abundance and biomass generated by the third year students who were involved in marine biology research project (BIOL 391) was used. All prawn samples were collected in the lower and middle reaches of the Mpenjati Estuary in March (2013) using the prawn pump (Ndaba and Joseph 2013). Fifteen stations (three transects) were sampled in the intertidal region of the lower reaches and five stations were sampled in the middle reaches. Some of the dried prawns obtained from the third year students were then analysed for phosphorus to get the idea of phosphorus content in these organisms. Zooplankton samples were collected during daytime using a 200 µm mesh plankton net attached to a hyperbenthic sled which was towed for 20 meters. Samples were stored in honey jars and preserved in 10 % formalin containing Rose Bengal dye to aid sorting in the laboratory.

2.3. Lab work

2.3.1. Abiotic samples

In the lab all water samples for dissolved inorganic nutrients were stored in the freezer until they were analysed for dissolved inorganic phosphorus (DIP as orthophosphate) and dissolved inorganic nitrogen (DIN) which included nitrate, nitrite and ammonia. Filters for TSS and PP were oven dried at 60 °C for 24 hours. All dried filters were weighed to obtain weights of suspended solids. Dry filters were kept in airtight plastic bags until they were analysed for P content.

2.3.2. Biotic samples

In the lab, phytoplankton and microphytobenthic chlorophyll a and phaeopigment concentrations were measured using a Turner Designs fluorometer (acidification method). Prior to running samples on the fluorometer, the instrument was calibrated using a pure chlorophyll a standard protocol already installed on the instrument. For determination of chlorophyll a and phaeopigment concentrations, the instrument was set to take into account the volume of solvent (extract volume) as well as the volume of water filtered so that the reading could represent chlorophyll a concentration in $\mu\text{g}\cdot\text{l}^{-1}$ of estuarine water.

All macrozoobenthos and zooplankton samples were washed with the purpose of removing formalin and mud from the samples. Macrozoobenthos and zooplankton samples were washed through a 500 μm and 63 μm sieves respectively. Plant material in the samples was carefully removed by hand and all organisms that clung on plant material were carefully removed. Macrozoobenthos samples were poured into a petri dish where they were classified into major groups (polychaetes, crustaceans and molluscs) under a dissecting microscope. Each major group was identified to family level using Day (1974), Day (1967), Griffiths (1976), Kensley(1978) and Steyn and Lussi (1998) as a guide. Identified organisms were counted, stored in eppendorf tubes and preserved in 10 % formalin. Organisms were weighed to obtain wet and dry weights. To obtain wet weight, all formalin was drained out of the eppendorf tubes and organisms were rinsed with distilled water after which they were blotted dry with a paper towel (except for polychaetes of very small body size). Wet organisms were then weighed on a 5 decimal digital balance (Shimadzu, AUW220D). After recording wet weights for each replicate, organisms were re-stored in eppendorf tubes and were

oven dried at 60 °C for 24 hours. Dry organisms were weighed on a 5 decimal digital balance (Shimadzu, AUW220D) to obtain dry weights for each replicate.

Organisms that were not blotted dry were weighed with eppendorf tubes. Excess water was carefully removed using a narrow glass pipette. After drying, organisms were weighed without eppendorf tubes to get dry weights. After recording dry weights for each replicate, each empty eppendorf tube in which organisms were kept was weighed and its value was subtracted from the initial wet weight to get the actual wet weight for the organisms in each replicate. All dry organisms were ground in an eppendorf tube using a glass rod and they were kept in eppendorf tubes for further analysis of phosphorus content.

Zooplankton samples were diluted in freshwater of 1 litre volume. Organisms were brought to suspension by a thorough stirring of the sample. Three subsamples were withdrawn from each sample using a 20 ml plastic scoop tied to a rod. The withdrawal of subsamples was done while stirring continuously to avoid settlement of organisms (Perissinotto and Wooldridge 1989; Jerling and Wooldridge 1995). Organisms in each subsample were identified to species level and enumerated under a dissecting microscope using Day (1974) as an identification guide. The coefficient of variation between subsamples was always below 10 %. Identified organisms were stored in eppendorf tubes and preserved in 10 % formalin. The most dominant taxa were weighed to obtain wet and dry weight. To obtain wet weight, all formalin was drained out of the eppendorf tubes and organisms were rinsed with distilled water. Zooplankton samples were not blotted dry due to their very small body size which made it difficult to remove them from and return to the tube. Excess water was then carefully removed using a narrow glass pipette. Wet organisms were weighed on a 5 decimal digital balance (Shimadzu, AUW220D) with eppendorf tubes. After wet weights were recorded, organisms were oven dried at 60 °C for 24 hours and dry organisms were weighed on a 5 decimal digital balance (Shimadzu, AUW220D) without eppendorf tubes. After recording dry weights for each replicate, each empty eppendorf tube in which organisms were kept was weighed and its value was subtracted from the initial wet weight to get the actual wet weight value for each replicate. Dry organisms were ground in an eppendorf tube using a glass rod and were kept in eppendorf tubes for further analysis of phosphorus content.

2.4. Phosphorus analysis

Water samples for dissolved inorganic nutrients were thawed overnight and were analysed for DIP as orthophosphate as reported by Gales et al. (1966). Filters (PP), sediment, zooplankton and macrozoobenthos samples were analysed for total phosphorus. Phytoplankton and microphytobenthos P content was estimated using conversion factors explained below. All filters, water and sediment samples were analysed. Fourteen samples of zooplankton were analysed for phosphorus in the Mlalazi Estuary from the May sampling session and eleven samples were analysed for phosphorus in the Mpenjati Estuary from the September sampling session. A total of 37 and 31 macrozoobenthos samples were analysed for phosphorus in the Mlalazi Estuary during May and November respectively while a total of 23 and 22 samples were analysed in the Mpenjati Estuary during September and November respectively. Samples were analysed for phosphorus content using the persulphate digestion method (Raimbault et al. 1999). Prior to digestion an oxidising reagent was prepared as follows: 21.6 g of disodium tetraborate (Merck) and 10.8 g potassium peroxodisulfate (Merck) were dissolved in 180 ml distilled water preheated at 60 °C and rapidly stirring using a glass rod. Since disodium tetraborate crystallizes in a few minutes when exposed to ambient temperature, only a specific quantity needed for one batch of samples was prepared at a time to avoid crystallisation.

Digestion was carried out using 40 ml Teflon autoclave bottles pre-washed in 10 % hydrochloric acid. Weight of ground organisms not exceeding 8 mg for macrozoobenthos, 5 mg for zooplankton and 30 mg for sediment was measured out using a 5 decimal digital balance (Shimadzu, AUW220D). After each sample had been weighed it was poured directly into the autoclave bottle. Pre weighed PP filters were directly inserted into autoclave bottles. Following weighing, the oxidising reagent was prepared and 4 ml was added into each bottle and 30 ml of distilled water was added. Autoclave bottles were closed until one screw less that tight and autoclaved for 30 minutes at 120 °C. Digested samples were poured into 100 ml volumetric flasks and distilled water was added to fill the flasks up to 100 ml. Undigested water samples as well as digested filters, sediment and animal samples were sent to the CSIR (Durban) for phosphorus (as orthophosphate) analysis.

The P concentrations from the CSIR laboratory were given in a form of PO_4^{-3} ($\text{mg}\cdot\text{l}^{-1}$) as P. Considering that all the digested samples were filled up to 100 ml before they were sent for P analysis, all the concentrations were multiplied by 0.1 L (a dilution volume) to have them in

mgP·100 ml. Phosphorus concentrations of zooplankton, macrozoobenthos, sediment as well as the biomass of the total suspended solids (TSS) expressed as particulate phosphorus (PP) were then divided by the digested sample weight to get mgP·mg sample. This quotient value (mgP·mg sample) was multiplied by 100 to get the percentage P content in animals, sediment and PP. To get the animals (zooplankton and macrozoobenthos) phosphorus biomass per area (per m²), the P biomass (mgP·mg sample) for digested animals was multiplied by the total biomass (dry weight) of animals in the whole area sampled. To calculate the sediment phosphorus mass per core, sediment P mass (mgP·mg sample) was multiplied by the total sediment dry weight per core. Following this conversion, P mass (mgP·core) was divided by the core area of 0.00031·m² to get the sediment P mass per area sampled. For determination of DIP mass, the P concentrations in mgP·l⁻¹ were converted to mgP·m⁻³ by multiplying by 1000. These concentrations were then multiplied by the station depth (which was measured at every station during all the sampling sessions to account for tidal influence) to have them in mgP·m⁻². The same conversions from mg·l⁻¹ to mg·m⁻² was applied on phytoplankton P biomass estimation. For determination of particulate P biomass, the volume filtered through the GFF (in ml) was converted to m³ by multiplying by 1000000 after which it was multiplied by the station depth (which was measured at every station during all the sampling sessions to account for tidal influence) to have it in m². The particulate phosphorus biomass (mgP·mg sample) was then divided by the calculated area (m²) to get PP per area (mgP·m⁻²).

Phytoplankton and microphytobenthos phosphorus content was estimated following a C:chlorophyll a ratio of 100:1 (Brown et al. 1991) after which a P:C ratio of 1:106 (Redfield 1958) was applied. The C:chlorophyll a ratio has been reported to vary widely between different ecosystems (Banse 1977) ranging from 30:1 for vigorous ecosystems e.g. (Lenz 1974) and several hundred to 1 for senescent ecosystems. This ratio does not vary randomly but it is greatly regulated in response to irradiance, nutrients levels and temperature (Cloern et al. 1995; Geider et al. 1997). As it is difficult to use individual ratio for each data point, the C:chlorophyll a ratio of 100:1 was chosen because it is considered by Brown et al. (1991) as the optimal ratio for estimating carbon biomass from chlorophyll a concentrations.

2.5. Data analysis

Univariate analyses were conducted using SPSS 19 for Windows. Data which did not satisfy the assumptions of a parametric test (i.e. normality and even distribution of residuals) were normalized using $\log(x+1)$ transformation, after which assumptions were satisfied. Two way analysis of variance (ANOVA) was applied to test for spatial and temporal differences in macrozoobenthos and zooplankton abundance as well as biomass within each estuary. Two way ANOVA was also applied to test for spatial and temporal differences in phytoplankton and microphytobenthic chlorophyll a as well as TSS concentrations within each estuary. Rather than performing three way ANOVA to detect differences between stations, sampling sessions and estuaries, one way ANOVA was performed to compare zooplankton and macrozoobenthos abundance and biomass as well as phytoplankton and microphytobenthos chlorophyll a and TSS concentrations between the two estuaries. One way ANOVA was performed to get simple differences between ML and MP and this was not added as a third factor because it could not provide valuable effect on the interaction output. Also, Underwood (1997) stated that any tests of hypothesis about the main effects can be violated by the higher order interaction.

Multivariate analysis was performed due to its ability to take into account multiple response variables (abundance and species composition) simultaneously unlike in univariate ANOVAs. This analysis helps in detecting levels of similarities and dissimilarities in species composition within and between sampling stations. Group differences on response variables considered simultaneously were determined. Multivariate analysis was conducted using PRIMER (Plymouth Routines In Multivariate Ecological Research) statistical package, version 6 (Clarke and Gorley 2006). Analysis was performed on abundance data and all data were square root transformed. Similarity of stations was calculated using Bray-Curtis similarity. Similarity Profile (SIMPROF) analysis was performed to determine groups of stations in a dendrogram that could not be significantly differentiated from each other.

Two way ANOVA was applied to test for differences in zooplankton P content between species and stations (note: only samples from May (ML) and September (MP) sampling sessions could be analysed for P, not enough material for P analysis was obtained during the November sampling session). One way ANOVA was performed to test for differences in zooplankton P content between estuaries. Three way ANOVA to test for differences in P content between species, stations and

estuaries was not performed because of the reasons stated above. Differences in phytoplankton P content between stations and sampling sessions within each estuary were also tested using the two way ANOVA.

Macrozoobenthos P content data of the Mlalazi Estuary could not satisfy the normality assumption even after several transformations. A non parametric two way ANOVA was then performed to test for differences in P content between macrozoobenthos groups and sampling sessions within the estuary. Macrozoobenthos P content data of the Mpenjati Estuary were square root transformed after which the assumptions were satisfied. Two way ANOVA was then performed to test for differences in macrozoobenthos P content between macrozoobenthos groups and sampling sessions within the estuary. Some macrozoobenthos families and groups were missing in some stations in both ML and MP, therefore three way ANOVA could not be performed to test for differences in macrozoobenthos P content between macrozoobenthos groups, stations and sampling sessions within each estuary because there was no full data points for all factors to be analysed. Non parametric One way ANOVA (Kruskal –Wallis Test) was then performed to get simple differences in macrozoobenthos P content between the two estuaries.

Chapter 3

Results

Physico-chemical data were compared along the salinity gradient and between the sampling sessions for both Mlalazi and Mpenjati estuaries. Biological data including chlorophyll a, macrozoobenthos and zooplankton were analysed and their concentration, abundance and biomass was compared along the salinity gradient, between sampling sessions and between estuaries. Because of the prawn (*Callichirus kraussi*) big body size, the data for the abundance-biomass as well as phosphorus content for these organisms were presented separately and not combined with other benthic crustaceans as this was going to mask the biomass of other benthic groups. Nutrient concentrations analysed as dissolved inorganic phosphorus, particulate phosphorus, phosphorus in sediment and biota were compared along the salinity gradient and between sampling sessions and estuaries. Phosphorus content in biota was also compared between taxa, stations, sampling sessions and estuaries.

3.1. Physico-chemical characteristics

Selected physico-chemical parameters measured in the Mlalazi and Mpenjati estuaries are presented in Table 3.1 and 3.2 respectively. Temperatures measured in the Mlalazi Estuary were higher during the November sampling when compared to the May sampling session (Table 3.1). Bottom temperatures of the Mlalazi estuary were generally higher (22.1 to 25.0 °C) than the surface temperatures (21.3 to 23.0 °C) during the May sampling session. During the November sampling session bottom temperatures were about the same (24.6 to 25.5 °C) as those of the surface (25.2 to 26.4 °C) (Table 3.1).

Temperatures measured in the Mpenjati Estuary were generally lower during the November sampling when compared to the September sampling session (Table 3.2). Bottom water temperatures recorded in the Mpenjati Estuary during the November sampling session were about the same (20.1 to 21.3 °C) as those recorded from the surface (20.2 to 20.9 °C). During the September sampling session, bottom temperatures were generally higher than the surface temperatures (Table 3.2).

In the Mlalazi Estuary, salinity values were higher during the May sampling when compared to the November sampling session (Table 3.1). During the May sampling session, salinities measured from the bottom waters (23.4 to 33.4) were higher than those measured from the surface (17 to 28) (Table 3.1). During the November sampling session of the Mlalazi Estuary, strong river flow resulted in salinities ranging from 0.1 to 0.2 throughout the estuary (Table 3.1). An estuarine salinity gradient was observed during May sampling sessions with salinity values increasing gradually from the upper towards the lower reaches (Table 3.1).

In the Mpenjati Estuary salinity values were higher during the September when compared to the November sampling session. Salinities measured during the September sampling session were higher in the bottom (31.2 to 31.4) when compared to the surface waters (4.4 to 25.8) (Table 3.2). Salinities recorded during the November sampling session were generally higher in the bottom when compared to the surface waters. During the November sampling session of the Mpenjati Estuary, strong river flow resulted in salinities ranging from 0.1 to 0.6 throughout the estuary. An estuarine salinity gradient was observed during both September and November sampling sessions with salinities increasing from the upper towards the lower reaches (Table 3.2).

Table 3.1: Surface and bottom measurements of temperature and salinity during May and November (2011) sampling sessions in the Mlalazi Estuary.

Estuary	Sampling session	Station	Parameter	Unit measurement	Surface	Bottom
ML	May	upper	temperature	°C	23.0	25.0
ML	May	middle	temperature	°C	21.3	23.3
ML	May	middle	temperature	°C	21.8	22.8
ML	May	lower	temperature	°C	22.5	22.1
ML	November	upper	temperature	°C	25.2	25.0
ML	November	middle	temperature	°C	25.4	25.2
ML	November	middle	temperature	°C	26.4	25.5
ML	November	lower	temperature	°C	26.0	24.6
ML	May	upper	salinity		17.0	23.4
ML	May	middle	salinity		17.1	28.0
ML	May	middle	salinity		21.9	31.2
ML	May	lower	salinity		27.9	33.4
ML	November	upper	salinity		0.1	0.1
ML	November	middle	salinity		0.2	0.2
ML	November	middle	salinity		0.2	0.2
ML	November	lower	salinity		0.2	0.2

Table 3.2: Surface and bottom measurements of temperature and salinity during September and November (2011) sampling sessions in the Mpenjati Estuary.

Estuary	Season	Station	Parameter	Unit measurement	Surface	Bottom
MP	September	upper	temperature	°C	21.7	21.5
MP	September	middle	temperature	°C	21.4	22.0
MP	September	lower	temperature	°C	22.3	22.6
MP	November	upper	temperature	°C	20.2	20.3
MP	November	middle	temperature	°C	20.4	20.1
MP	November	lower	temperature	°C	21.0	21.3
MP	September	upper	salinity		4.4	31.2
MP	September	middle	salinity		10.3	31.2
MP	September	lower	salinity		25.8	31.4
MP	November	upper	salinity		0.1	0.1
MP	November	middle	salinity		0.1	1.1
MP	November	lower	salinity		0.6	0.6

3.2. Rainfall patterns

Rainfall levels in the Mlalazi Estuary as inferred from the Mthunzini gauging station were 22.0 mm during May and 241.8 mm during November. Rainfall levels in the Mpenjati Estuary as inferred from the Mzimkhulu gauging station were 16.2 mm during September and 302.6 mm during November (Figure 3.1).

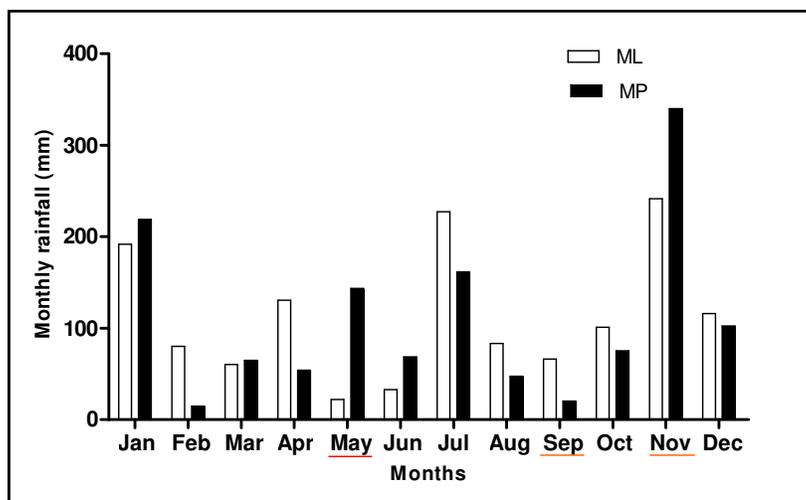


Figure 3.1: Rainfall pattern for the Mlalazi and Mpenjati Estuary during 2011. Data were obtained from www.sasa.org.za. Months in which sampling was conducted are underlined.

3.3. Total Suspended Solids (TSS)

In the Mlalazi Estuary, TSS concentrations were higher during November ($48.9 \text{ mg}\cdot\text{l}^{-1} \pm 44.3 \text{ SD}$) than May ($29.3 \text{ mg}\cdot\text{l}^{-1} \pm 0.9 \text{ SD}$) (Figure 3.2 A). Concentrations of TSS showed no significant differences between stations ($p = 0.413$) and between sampling sessions ($p = 0.098$) (Table 3.7). During both May and November sampling sessions, TSS concentrations showed a general increase from the upper to the lower reaches.

In the Mpenjati Estuary, TSS concentrations were higher during November ($28.1 \text{ mg}\cdot\text{l}^{-1} \pm 2.8 \text{ SD}$) than September ($24.8 \text{ mg}\cdot\text{l}^{-1} \pm 2.9 \text{ SD}$) (Figure 3.2 B). Total suspended solids concentrations showed significant differences between stations ($p < 0.0005$), and between sampling sessions ($p = 0.020$) (Table 3.7). Total suspended solids concentrations recorded in the Mpenjati Estuary during both November and September sampling sessions showed a general increase from the upper to the

lower reaches (Figure 3.2 B). Total suspended solids concentrations showed significant differences between the Mlalazi and Mpenjati estuaries ($p = 0.038$) (Table 3.7), with the Mlalazi Estuary having higher TSS concentrations (range = 8.9 - 100.0 $\text{mg}\cdot\text{l}^{-1}$) than the Mpenjati Estuary (range = 11.4 - 31.0 $\text{mg}\cdot\text{l}^{-1}$) (Figure 3.2).

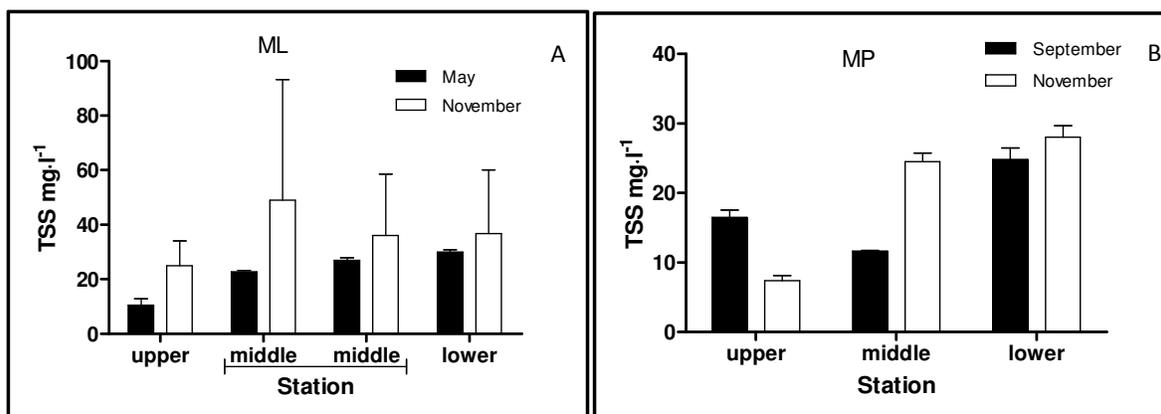


Figure 3.2: Total suspended solids (TSS) recorded in the Mlalazi (A) and Mpenjati (B) Estuary during May, September and November sampling sessions. Data represent mean (\pm SD, $n = 3$).

3.4. Nutrients

Nutrients concentrations ($\text{mg}\cdot\text{l}^{-1}$) were calculated into $\text{mg}\cdot\text{m}^{-3}$ and were multiplied by the depth of each station to have them in $\text{mg}\cdot\text{m}^{-2}$. In the Mlalazi Estuary the dissolved inorganic phosphorus (DIP) concentrations were higher during May sampling ($1696.1 \text{ mgP}\cdot\text{m}^{-2} \pm 273.2 \text{ SD}$) when compared to the November sampling session ($62.0 \text{ mgP}\cdot\text{m}^{-2} \pm 2.0 \text{ SD}$) (Figure 3.3 A). The highest DIP concentrations were recorded from the upper reaches during the May sampling (mean of $1696.1 \text{ mgP}\cdot\text{m}^{-2} \pm 273.2 \text{ SD}$), although a clear trend in DIP concentrations along the salinity gradient during both May and November sampling sessions was not observed. There were significant differences in DIP concentrations between stations ($p < 0.0005$) and sampling sessions ($p < 0.0005$) (Table 3.8).

In the Mpenjati Estuary DIP concentrations were higher during the September sampling ($52.3 \text{ mgP}\cdot\text{m}^{-2} \pm 2.9 \text{ SD}$) when compared to the November sampling session ($6.0 \text{ mgP}\cdot\text{m}^{-2} \pm 0.9 \text{ SD}$) (Figure 3.3 B). During the November sampling session DIP concentrations were increasing from the upper towards the lower reaches while there was no clear trend in DIP concentrations along the

salinity gradient during the September sampling session (Figure 3.3 B). There were significant differences in DIP concentrations between sampling stations ($p < 0.010$) and sampling sessions ($p < 0.0005$) (Table 3.8). There were significant differences in DIP concentrations between the Mlalazi and Mpenjati estuaries ($p < 0.009$), with the Mlalazi Estuary having higher (range = 24.0 – 2005.7 $\text{mgP}\cdot\text{m}^{-2}$) DIP concentrations than the Mpenjati Estuary (range = below detection limit – 62.0) (Table 3.8).

Concentrations of nitrate + nitrite combined recorded during the November sampling session in the Mlalazi Estuary were generally higher ($1103.3 \text{ mg}\cdot\text{m}^{-2} \pm 46.7 \text{ SD}$) than those recorded during the May sampling session ($752 \text{ mg}\cdot\text{m}^{-2} \pm 150.4 \text{ SD}$) (Figure 3.3. C). There was no clear trend in nitrate + nitrite concentrations along the salinity gradient during both sampling sessions but the highest concentration was recorded from the upper reaches during the May sampling session (Figure 3.3. C). There were significant differences in nitrate + nitrite concentrations between stations ($p < 0.0005$) and sampling sessions ($p < 0.0005$) (Table 3.8).

Concentrations of nitrate + nitrite were generally higher during September ($310 \text{ mg}\cdot\text{m}^{-2} \pm 119.2 \text{ SD}$) when compared to November sampling session ($207 \text{ mg}\cdot\text{m}^{-2} \pm 41.0 \text{ SD}$) in the Mpenjati Estuary (Figure 3.3 D). Highest concentrations were recorded in the middle reaches during both September and November sampling sessions (Figure 3.3 D). There were significant differences between sampling stations ($p < 0.0005$) but there were no significant differences between sampling sessions ($p = 0.210$) (Table 3.8). There were significant differences in nitrate + nitrite concentrations between the Mlalazi and Mpenjati estuaries ($p < 0.0005$), with the Mlalazi Estuary having higher concentrations (range = 34.9 - 1190.0 $\text{mg}\cdot\text{m}^{-2}$) than the Mpenjati Estuary (range = below detection limit – 421.0 $\text{mg}\cdot\text{m}^{-2}$) (Table 3.8).

Ammonia concentrations recorded in the Mlalazi Estuary during May sampling ($1696.1 \text{ mg}\cdot\text{m}^{-2} \pm 273.2 \text{ SD}$) were higher than those concentrations recorded during the November sampling session ($291.3 \text{ mg}\cdot\text{m}^{-2} \pm 42.6 \text{ SD}$) (Figure 3.3 E). Although there was no clear trend in ammonia concentrations in the Mlalazi Estuary along the estuary length during both May and November sampling sessions, highest ammonia concentrations were recorded from the upper reaches. There were significant differences in ammonia concentrations between stations ($p = 0.009$) and sampling sessions ($p = 0.003$) (Table 3.8).

Ammonia concentrations in the Mpenjati Estuary were generally higher during September ($61.5 \text{ mg}\cdot\text{m}^{-2} \pm 57.5 \text{ SD}$) when compared to November sampling session ($46.7 \text{ mg}\cdot\text{m}^{-2} \pm 18.4 \text{ SD}$) (Figure 3.3 F). There was no clear trend in ammonia concentrations along the salinity gradient during both September and November sampling sessions (Figure 3.3 F). There were no significant differences in ammonia concentrations between the Mpenjati sampling stations ($p = 0.969$) as well as sampling sessions ($p = 0.523$) (Table 3.8). There were significant differences in ammonia concentrations between the Mlalazi and Mpenjati estuaries ($p < 0.0005$), with the Mlalazi Estuary having higher ammonia concentrations (range = $126.0 - 1489.0 \text{ mg}\cdot\text{m}^{-2}$) than the Mpenjati Estuary (range = below detection limit - $70.5 \text{ mg}\cdot\text{m}^{-2}$) (Table 3.8).

In the Mlalazi Estuary the P biomass of the total suspended solids (TSS) expressed as particulate phosphorus (PP) was higher during the November sampling ($1.4 \text{ mg}\cdot\text{m}^{-2} \pm 0.1 \text{ SD}$) when compared to May sampling session ($0.9 \text{ mg}\cdot\text{m}^{-2} \pm 0.1 \text{ SD}$) (Figure 3.4 A). Particulate phosphorus concentrations recorded during May and November sampling sessions of the Mlalazi Estuary were generally decreasing from the upper towards the lower reaches (Figure 3.4. A). There were significant differences in PP biomass between stations ($p < 0.0005$) and sampling sessions ($p < 0.0005$) (Table 3.8). Phosphorus content (%) in TSS was higher during May ($0.1 \% \pm 0.02 \text{ SD}$) when compared to November ($0.05 \% \pm 0.005 \text{ SD}$) (Figure 3.4 C). The percentage P content was decreasing from the upper to the lower reaches during May while the opposite was observed during November (Figure 3.4 C).

Particulate phosphorus concentrations recorded in the Mpenjati Estuary was generally higher during the November sampling ($1.8 \text{ mg}\cdot\text{m}^{-2} \pm 0.5 \text{ SD}$) when compared to the September sampling session ($0.8 \text{ mg}\cdot\text{m}^{-2} \pm 0.03 \text{ SD}$) (Figure 3.4 B). There was no clear trend in PP concentrations along the salinity gradient during both September and November sampling sessions. There were significant differences in PP concentrations between stations ($p = 0.030$) as well as sampling sessions ($p < 0.0005$) (Table 3.8). There were no significant differences in PP concentrations between the Mlalazi and Mpenjati estuaries ($p = 0.460$) (Table 3. 8). Percentage phosphorus content was generally higher during September ($0.08 \% \pm 0.003 \text{ SD}$) than November ($0.07 \% \pm 0.02 \text{ SD}$). During November, phosphorus content (%) was decreasing from the upper to the lower reaches with no clear trend in TSS P content during September (Figure 3.4 D).

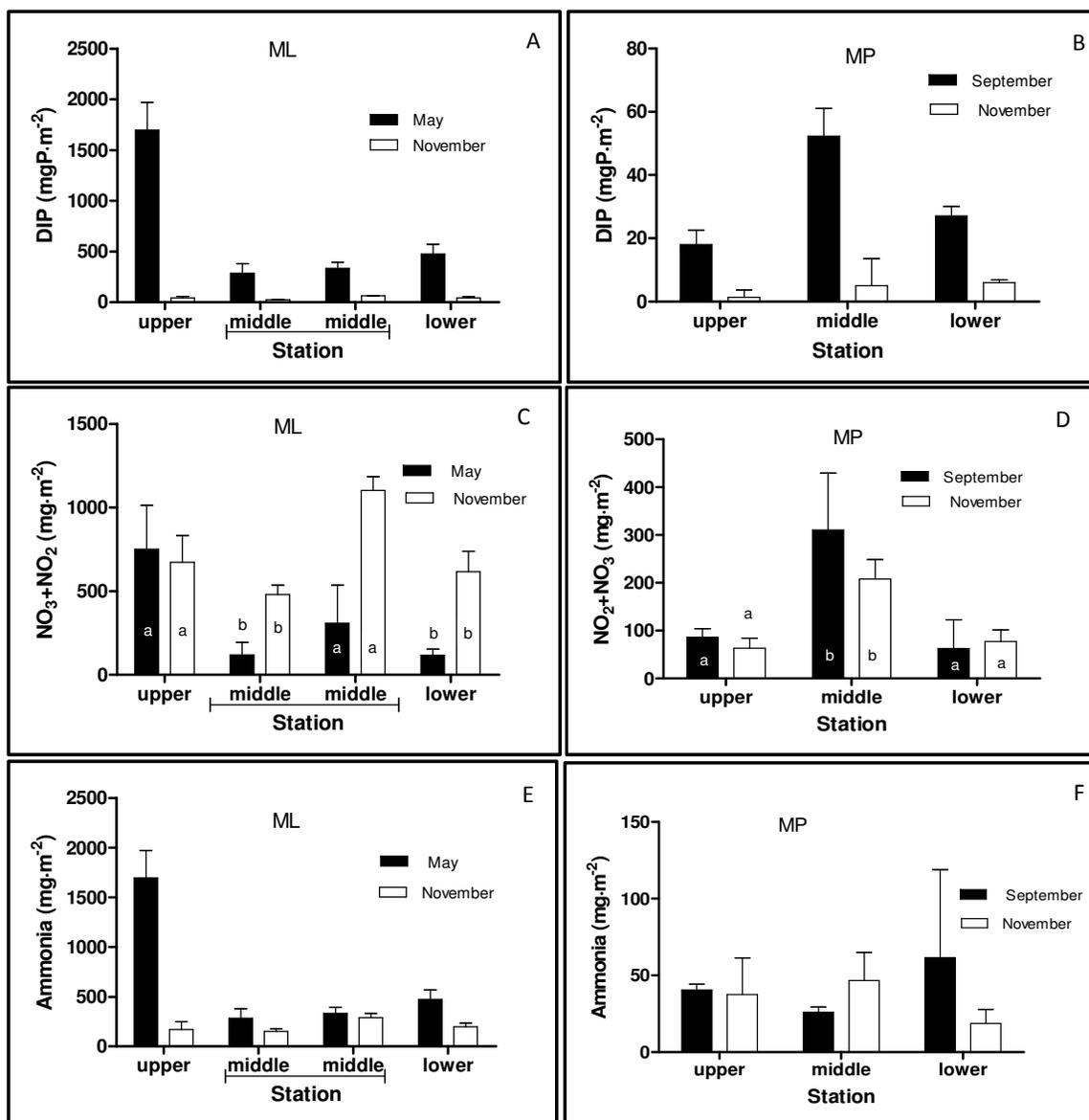


Figure 3.3: Dissolved inorganic phosphorus (DIP), NO₂ + NO₃ and ammonia concentrations recorded in the Mlalazi (ML) and Mpenjati (MP) estuaries during May, September and November sampling sessions of 2011. Data represent mean (\pm SD, n = 3).

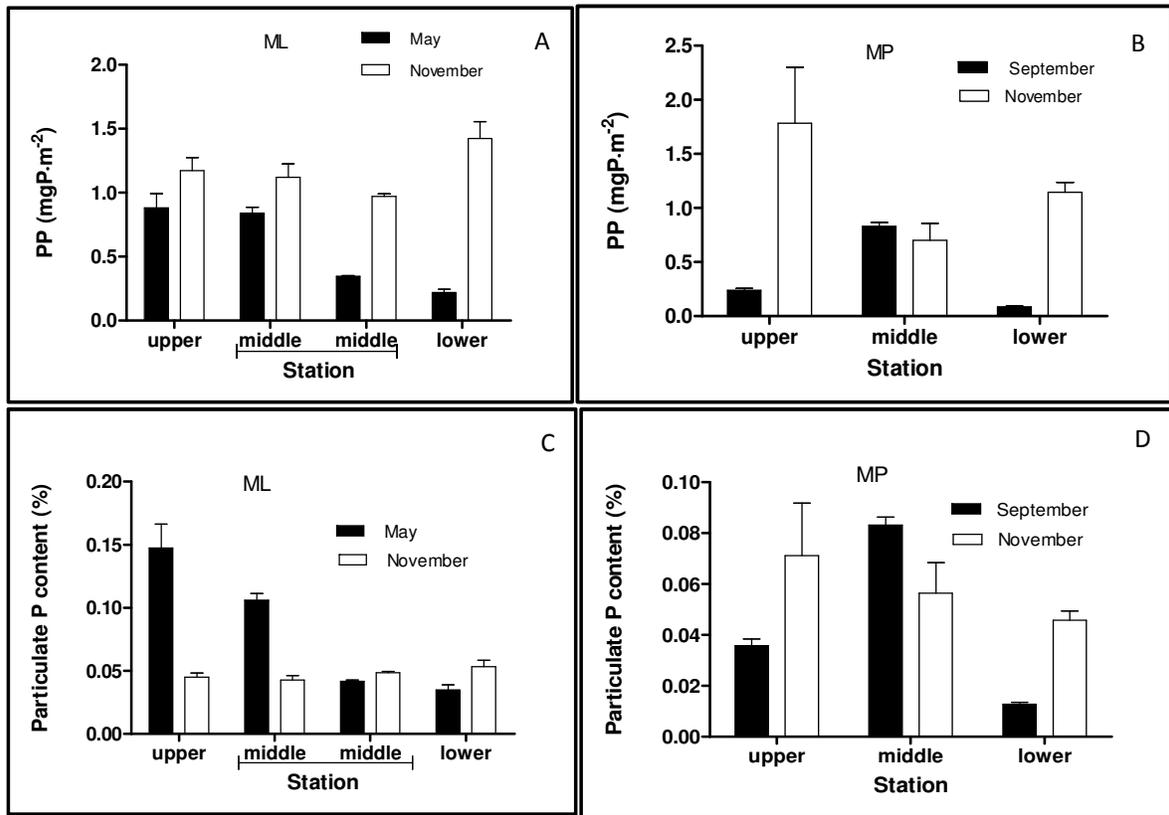


Figure 3.4: Particulate phosphorus (PP) concentrations (A and B) and percentage phosphorus content (C and D) measured in the water column of the Mlalazi (ML) and Mpenjati (MP) estuaries during May, September and November sampling sessions of 2011. In A and B, data represent mean (\pm SD, $n = 3$).

3.5. Phytoplankton and microphytobenthos

In the Mlalazi Estuary higher phytoplankton chlorophyll a concentrations were recorded during the May sampling ($1.1 \mu\text{g}\cdot\text{l}^{-1} \pm 0.1$ SD) when compared to the November sampling session ($0.3 \mu\text{g}\cdot\text{l}^{-1} \pm 0.1$ SD) (Figure 3.5 A). During November, the phytoplankton chlorophyll a concentration was generally increasing from the upper towards the lower reaches, however, there was no clear trend in phytoplankton chlorophyll a concentration along the salinity gradient during the May sampling session (Figure 3.5. A). There were significant differences in phytoplankton chlorophyll a concentrations of the Mlalazi Estuary between stations ($p < 0.0005$) and sampling sessions ($p < 0.0005$) (Table 3.7).

In the Mpenjati Estuary higher phytoplankton chlorophyll a concentrations were recorded during the September sampling ($0.8 \mu\text{g}\cdot\text{l}^{-1} \pm 0.1 \text{ SD}$) when compared to the November sampling session ($0.03 \mu\text{g}\cdot\text{l}^{-1} \pm 0.06 \text{ SD}$) (Figure 3.5. B). There was no clear trend in phytoplankton chlorophyll a concentration along the salinity gradient during both September and November sampling sessions. There were significant differences in phytoplankton chlorophyll a concentration between stations ($p = 0.004$) and sampling sessions ($p < 0.0005$) (Table 3.7). There were no significant differences in phytoplankton chlorophyll a concentrations between the Mlalazi and Mpenjati estuaries ($p = 0.304$).

In the Mlalazi Estuary higher microphytobenthic chlorophyll a concentrations were recorded during the November sampling when compared to the May sampling session (Figure 3.5 C). During the November sampling, microphytobenthic chlorophyll a concentrations in the upper reaches were below the detection limit. There were significant differences in the microphytobenthic chlorophyll a concentrations of the Mlalazi Estuary between sampling sessions ($p = 0.002$) (Table 3.7). Comparisons between the stations were not performed since data from the upper reaches were missing (Table 3.7).

In the Mpenjati Estuary, higher microphytobenthic chlorophyll a concentrations were recorded during the November sampling when compared to the September sampling session (Figure 3.5 D). During the November sampling, microphytobenthic chlorophyll a concentrations in the lower reaches were below the detection limit. There were significant differences in microphytobenthic chlorophyll a concentrations between sampling sessions ($p = 0.001$). Comparisons between the stations were not performed since data from the lower reaches were missing. There were no significant differences in microphytobenthic chlorophyll a concentrations between the Mlalazi and Mpenjati estuaries ($p = 0.875$) (Table 3.7).

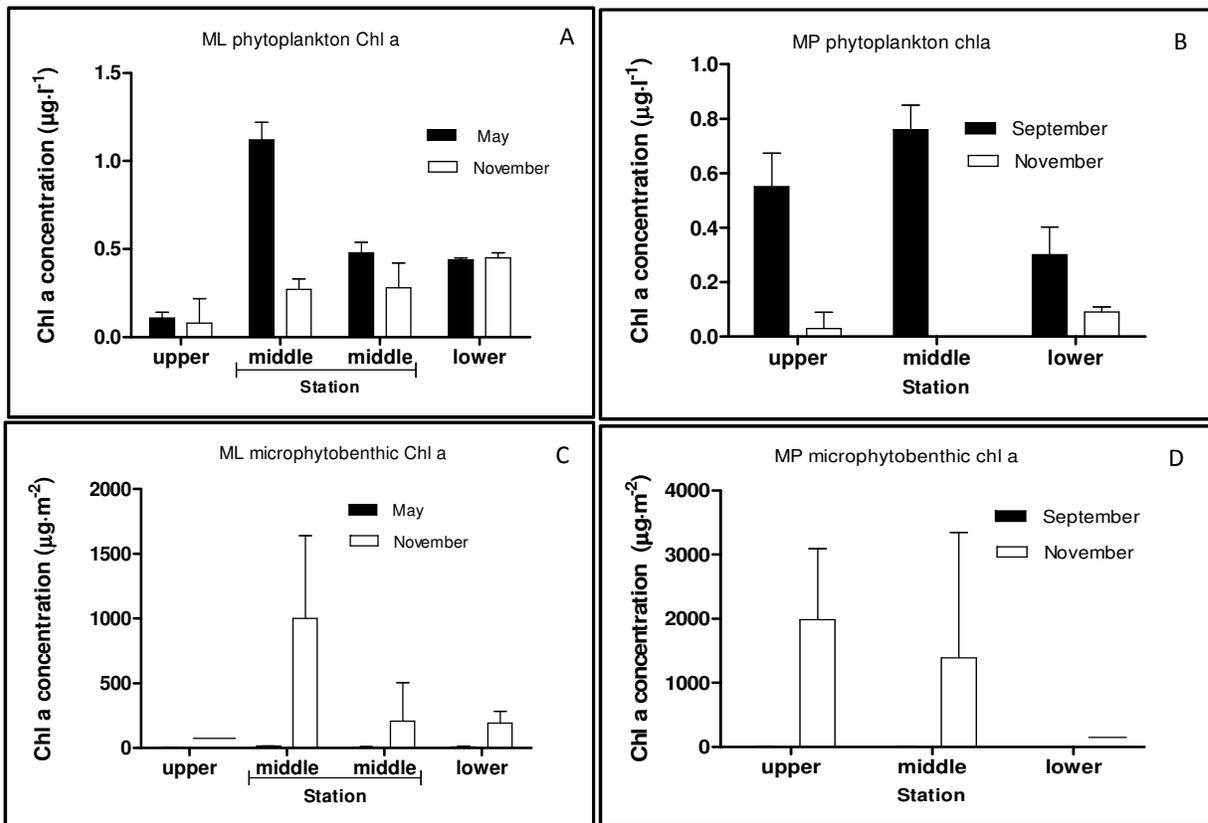


Figure 3.5: Chlorophyll a concentrations for phytoplankton (A and B) and microphytobenthos (C and D) in the Mlalazi (ML) and Mpenjati (MP) estuaries during May, September and November sampling sessions of 2011. Data represent mean (\pm SD, $n = 3$).

3.6. Zooplankton community analysis

3.6.1. Community composition and abundance

A total of six taxa were identified in the Mlalazi Estuary during May and three during November. Taxa recorded in the Mlalazi Estuary during May were *Arcatia natalensis*, *Pseudodiaptomus hessei*, *Mesopodopsis africana*, *Gastrosaccus psammodytes*, nauplii and jelly fish. Taxa recorded during November were *A. natalensis*, *P. hessei* and zoea larvae. No clear trend in number of taxa along the salinity gradient was apparent during both sampling sessions (Figure 3.6 A).

A total of five taxa were recorded in the Mpenjati Estuary during September and three during November. Taxa recorded during September were *A. natalensis*, *P. hessei*, *M. africana*, nauplii and jelly fish. Taxa recorded during November were *A. natalensis*, *P. hessei* and *M. africana*. During

the September sampling session, taxa in the Mpenjati Estuary were found throughout the estuarine system except for *M. Africana* which was recorded only in the upper reaches. The number of taxa was decreasing from the upper towards the lower reaches during September while the opposite was observed during November (Figure 3.6 B). Two taxa that numerically dominated the zooplankton community of the Mlalazi and Mpenjati estuaries during May, September and November sampling sessions were the copepods *A. Natalensis* and *P. hessei*. Combined, these two taxa contributed more than 90% of the total abundance from all stations in both estuaries.

In the Mlalazi Estuary zooplankton abundance was significantly higher during May ($23718.1 \text{ individuals}\cdot\text{m}^{-3} \pm 15689.6 \text{ SD}$) than November ($1041.4 \text{ individuals}\cdot\text{m}^{-3} \pm 506.4 \text{ SD}$) (Figure 3.6 C). Zooplankton mean abundance of the Mlalazi Estuary was generally increasing from the upper towards the lower reaches (Figure 3.6 C). No significant differences were observed in zooplankton abundance of the Mlalazi Estuary between stations ($p = 0.151$) but there were significant differences in abundance between sampling sessions ($p = 0.003$) (Table 3.7).

In the Mpenjati Estuary zooplankton mean abundance was higher during September ($8890.7 \text{ individuals}\cdot\text{m}^{-3} \pm 1769.6 \text{ SD}$) than November ($1786.4 \text{ individuals}\cdot\text{m}^{-3} \pm 366.7 \text{ SD}$) (Figure 3.6 D). During both September and November sampling sessions, highest mean abundances were recorded from the middle reaches (Figure 3.6 D). There were significant differences in zooplankton abundance in the Mpenjati Estuary between stations ($p = 0.007$) and sampling sessions ($p = 0.002$) (Table 3.7). There were no significant differences in zooplankton abundance between the Mlalazi and Mpenjati Estuary ($p = 0.217$) (Table 3.7).

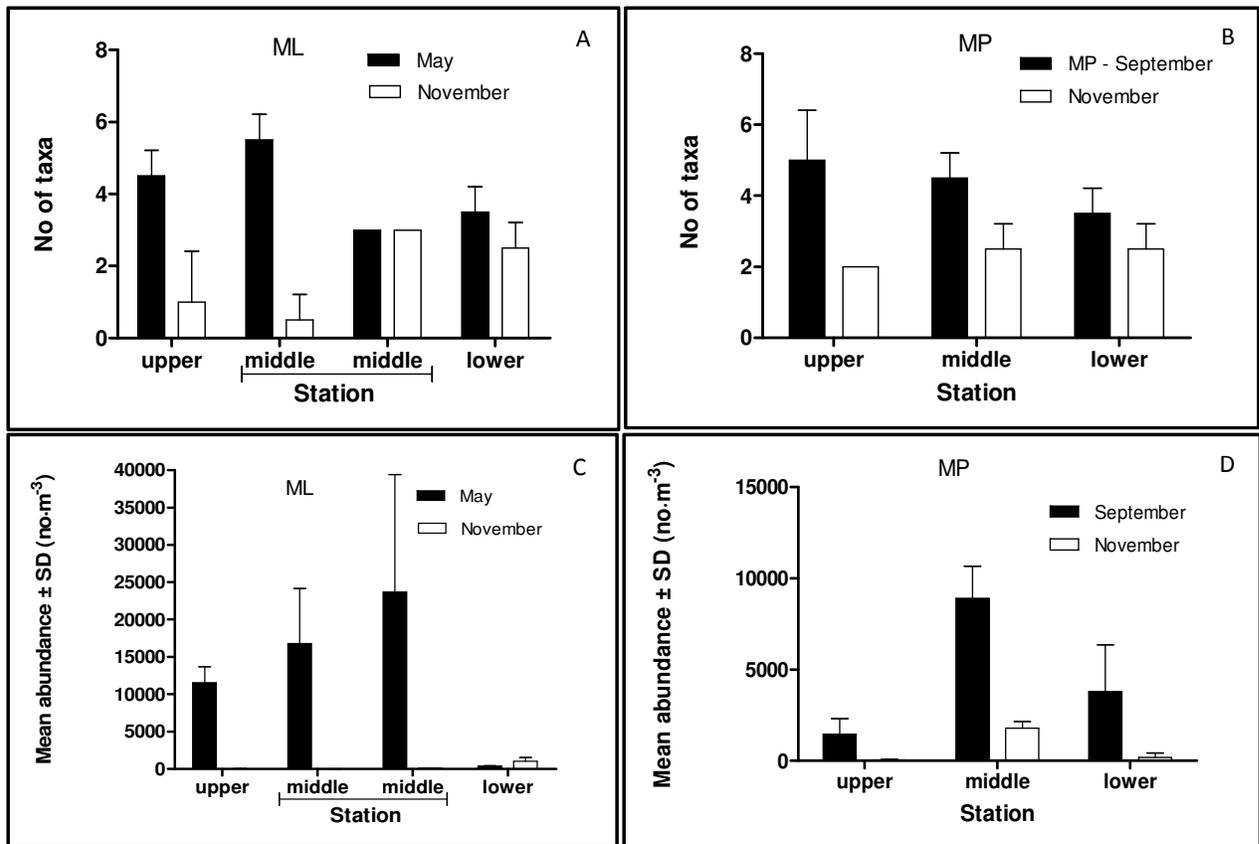


Figure 3.6: Number of taxa (A and B) and abundance (C and D) for zooplankton in the Mlalazi (ML) and Mpenjati (MP) estuaries during May, September and November sampling sessions of 2011. Data represent mean (\pm SD, $n = 2$).

3.6.2. Biomass

In the Mlalazi Estuary higher biomass was recorded in May ($8.2 \text{ mg dry weight}\cdot\text{m}^{-3} \pm 2.3 \text{ SD}$) than November ($0.2 \text{ mg dry weight}\cdot\text{m}^{-3} \pm 0.1 \text{ SD}$) (Figure 3.7 A). Zooplankton biomass was decreasing from the upper towards the lower reaches during the May sampling session (Figure 3.7 A). There were significant differences in zooplankton biomass of the Mlalazi Estuary between stations ($p = 0.034$) as well as sampling sessions ($p < 0.0005$) (Table 3.7).

In the Mpenjati Estuary higher zooplankton biomass was recorded during September ($8.9 \text{ mg dry weight}\cdot\text{m}^{-3} \pm 1.5 \text{ SD}$) than November ($0.4 \text{ mg dry weight}\cdot\text{m}^{-3} \pm 0.01 \text{ SD}$) (Figure 3.7 B). There were no significant differences in zooplankton biomass between stations ($p = 0.052$), however, there were significant differences in zooplankton biomass between sampling sessions ($p = 0.003$) (Table 3.7).

There were no significant differences in zooplankton biomass between the Mlalazi and Mpenjati estuaries ($p = 0.974$) (Table 3.7).

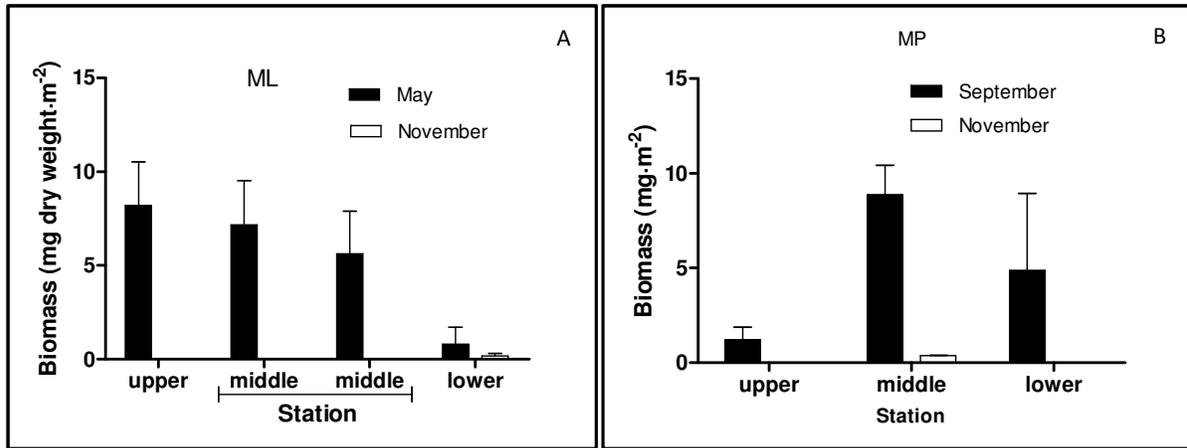


Figure 3.7: Zooplankton biomass (dry weight) of the Mlalazi (A) and Mpenjati (B) estuaries during May, September and November sampling sessions of 2011. Data represent mean (\pm SD, $n = 2$).

3.6.3. Abundance biomass relationship

In both Mlalazi and Mpenjati estuaries, zooplankton biomass was concurrently decreasing with abundance during May, September and November sampling sessions (Figure 3.8 and 3.9 A and B). Relatively low biomass was measured during November which was associated with low abundance recorded during this sampling session in both estuaries (Figure 3.8 and 3.9 A and B). Mysid *Mesopodopsis Africana* showed very low abundance with a significantly higher biomass in the Mlalazi Estuary during May (Figure 3.8 A). In the Mpenjati Estuary, biomass and abundance showed a similar pattern (Figure 3.6 and 3.7). In the Mlalazi Estuary, however, biomass and abundance showed an opposite pattern (Figure 3.6 and 3.7).

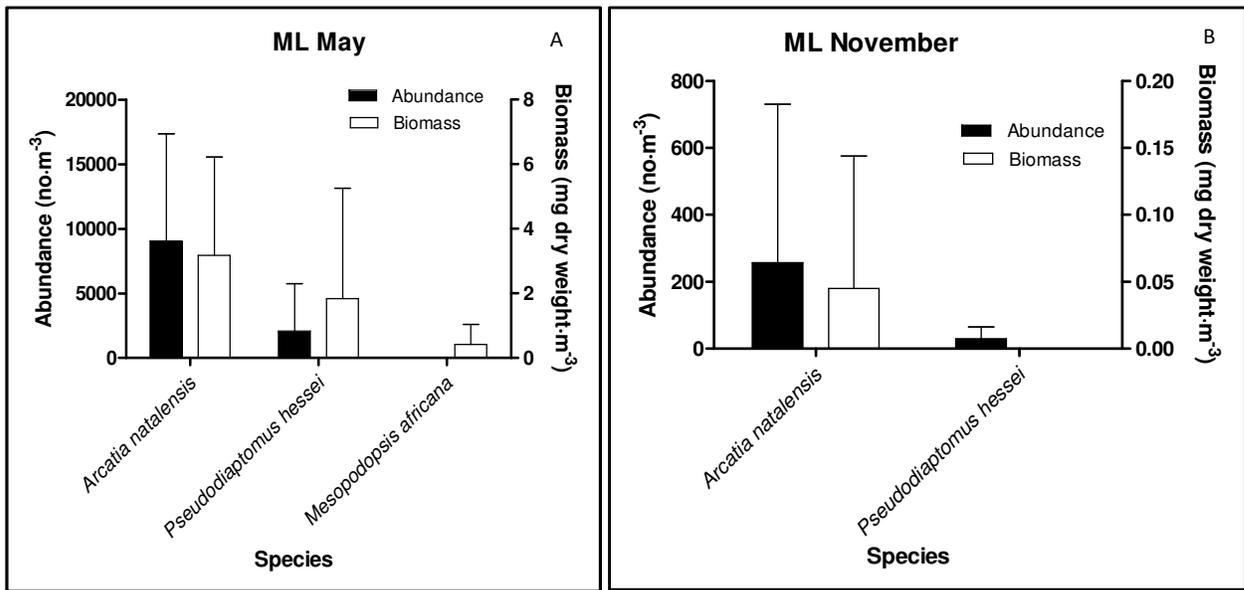


Figure 3.8: Relationship between zooplankton abundance and biomass in the Mlalazi Estuary during May (A) and November (B) sampling sessions of 2011.

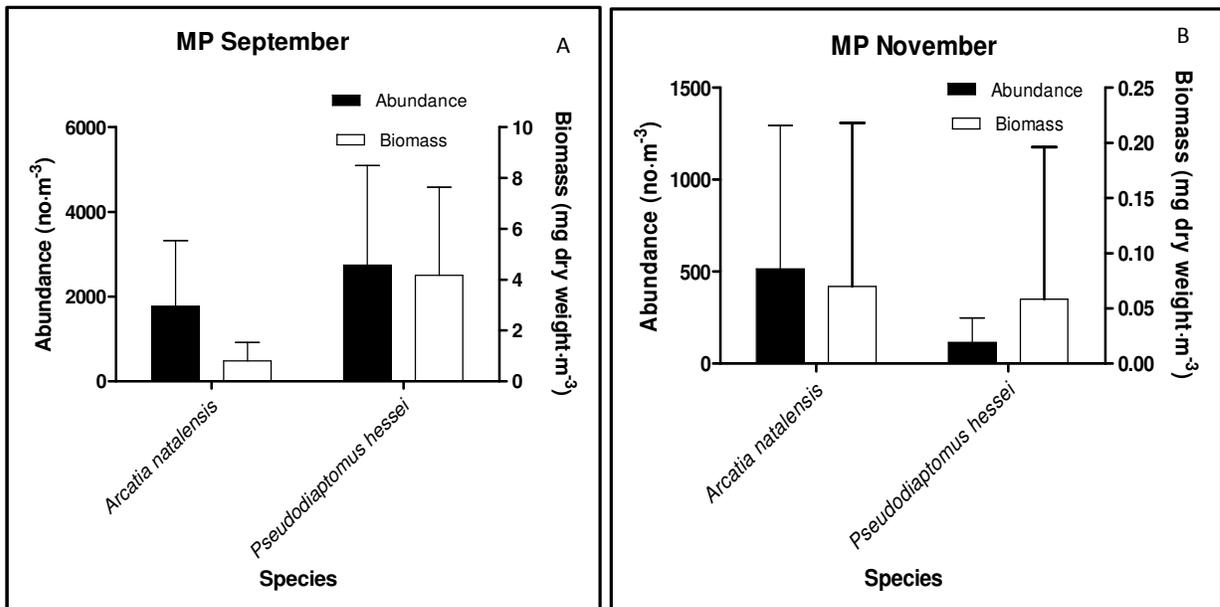


Figure 3.9: Relationship between zooplankton abundance and biomass in the Mpenjati Estuary during September (A) and November (B) sampling sessions of 2011.

3.7. Macrozoobenthos community analysis

3.7.1 .Community composition, abundance and community assemblage patterns

A total of 19 and 21 families were recorded in the Mlalazi Estuary out of 10563 and 3241 individuals during May and November sampling sessions respectively (Table 3.3 and 3.4). The most abundant orders during May were Polychaeta, Tanaidacea and Gastropoda contributing 67 %, 19 % and 9 % respectively while Amphipoda, Bivalvia and Polychaeta were the most dominant groups during November contributing 34 %, 29 % and 27 % respectively (Figure 3.10 A and B). The number of families was generally increasing from the upper towards the lower reaches during both May and November sampling sessions (Figure 3.11 A).

In the Mpenjati Estuary a total of 13 families were recorded out of 7862 and 10008 individuals during September and November sampling sessions respectively (Table 3.5 and 3.6). The most dominant orders during the September sampling session were Polychaeta, Amphipoda and Isopoda contributing 94 %, 3 % and 2 % respectively while the November sampling session was dominated by Polychaeta, Amphipoda and Isopoda contributing 76 %, 20 % and 3 % respectively (Figure 3.11 C and D). The number of families was generally decreasing from the upper towards the lower reaches of the estuary (Figure 3.11 B).

Macrozoobenthos taxa abundance was generally higher during the May sampling when compared to the November sampling session in the Mlalazi estuary (Figure 3.11 C). Mean abundance showed no significant difference between stations ($p = 0.615$), however, there was a significant difference between May and November sampling sessions ($p < 0.0005$) (Table 3.7).

In the Mpenjati Estuary, abundance of taxa was generally higher during the November sampling when compared to the September sampling session. There were no significant differences in abundance between the stations ($p = 0.683$) but there were significant differences between sampling sessions ($p = 0.0333$) (Table 3.7). During the September sampling session abundance was increasing from the upper towards the lower reaches while the opposite trend was apparent during the November sampling session (Figure 3.11 D). There was a significant difference ($p = 0.003$) in abundance between the Mlalazi and Mpenjati estuaries (Table 3.7). Mpenjati Estuary generally had a higher overall abundance than the Mlalazi Estuary during all sampling sessions (Figure 3.11 C and D).

The macrozoobenthos community of the Mlalazi Estuary separated into five groups after cluster analysis (Figure 3.12). Generally, there was a seasonal separation in the Mlalazi stations (Figure 3.12). Assemblages in the upper and middle reaches were closely clustered in May (Figure 12). During the May sampling session, average similarity within stations ranged from 59 - 80 %, with the upper reaches displaying highest similarity. Average dissimilarity between stations ranged from 43 - 74 %. Stations in the upper reaches had the lowest dissimilarity (43 %) while the highest dissimilarity was observed between stations in the upper and lower reaches. During the November sampling session, average similarity within stations ranged from 46 - 66 % with lower reaches displaying the highest similarity. Average dissimilarity between stations ranged between 58 – 86 %. Lowest average dissimilarity was observed from stations in the upper reaches while the highest dissimilarity was observed between stations in the upper and lower reaches.

The macrozoobenthos community of the Mpenjati Estuary separated into five groups after cluster analysis, with one outlier (Figure 3.13). There was a seasonal separation in all the groups identified (Figure 3.13). Average similarity within the stations ranged from 62 - 81 % during September, with the lower reaches having the highest similarity. Average dissimilarity between the stations was low, ranging from 35 - 40 %. The middle and lower reaches had the lowest dissimilarity while the upper and lower reaches had the highest dissimilarity. During November, average similarity within the stations ranged from 67 - 85 %, with the middle reaches having the highest similarity. Average dissimilarity between the stations ranged from 42 - 59 %. The upper and middle reaches had the lowest dissimilarity while the middle and lower reaches displayed the highest dissimilarity.

The multivariate analysis results did not differ from the ANOVA results in a sense that both analyses showed differences between the sampling sessions with ANOVA results showing significant differences between sampling sessions and the dendrogram showing seasonal separation in all groups identified from both the Mlalazi and Mpenjati estuaries.

3.7.2. Biomass

In the Mlalazi Estuary biomass was higher during May compared to November (Figure 3.14. A). There was no clear trend in biomass along the salinity gradient during both May and November. There were significant differences in biomass between stations ($p = 0.026$) but there was no significant difference between sampling sessions ($p = 0.68$) (Table 3.7).

In the Mpenjati Estuary biomass was higher during November than September (Figure 3.14. B). There was an increase in biomass from the upper towards the lower reaches during both September and November sampling sessions (Figure 3.14. B). There were significant differences in biomass between stations ($p = 0.003$) and sampling sessions ($p = 0.017$) (Table 3.7). There was a significant difference in biomass between the Mlalazi and Mpenjati estuaries ($p = 0.020$) (Table 3.7).

3.7.3. Abundance-biomass relationship

Abundance and biomass of selected macrozoobenthos families in the Mlalazi and Mpenjati estuaries is presented in Figure 3.15 (A, B, C and D). Other families had very high abundance with low biomass in terms of dry weight. For example, Spionidae in the Mlalazi Estuary during the May sampling session had a mean abundance of $1274.0 \text{ individuals}\cdot\text{m}^{-2} \pm 1224.0 \text{ SD}$ with the mean biomass of $1.4 \text{ mg dry weight}\cdot\text{m}^{-2} \pm 0.9 \text{ SD}$ (Figure 3.15). Other families had low abundance but contributed high biomass in terms of dry weight, e.g. Tellinidae in the Mlalazi Estuary during the May sampling session had the abundance of $13.0 \text{ individuals}\cdot\text{m}^{-2} \pm 17.9 \text{ SD}$ with mean biomass of $10.5 \text{ mg dry weight}\cdot\text{m}^{-2} \pm 20.1 \text{ SD}$ without shell (Figure 3.17). The polychaetes and molluscs generally contributed higher biomass than the crustaceans during both May and November sampling sessions of the Mlalazi Estuary (Figure 3.15 A and B) and during the November sampling session in the Mpenjati Estuary (Figure 3.15 D). Data generated by the third year students revealed that abundance and biomass of *Callichirus kraussi* prawns of the Mpenjati Estuary was higher at the lower when compared to the middle reaches (Figure 3.33 A) although there were no statistically significant differences in both abundance ($p = 0.077$) and biomass ($p = 0.239$) between the reaches. Overall, the prawn biomass obtained from the third year students data was 500 fold higher than that of other benthic groups combined (data of the present study) in this system.

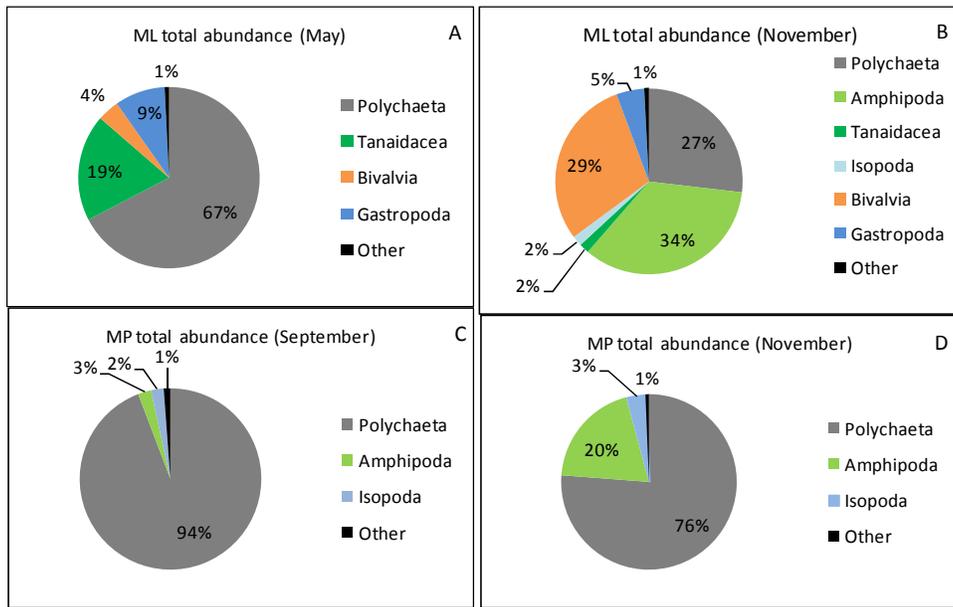


Figure 3.10: Dominant orders recorded in the Mlalazi and Mpenjati estuaries during the May, September and November sampling sessions of 2011. All taxa which contributed less than 2 % of the total abundance were grouped together as “Other”.

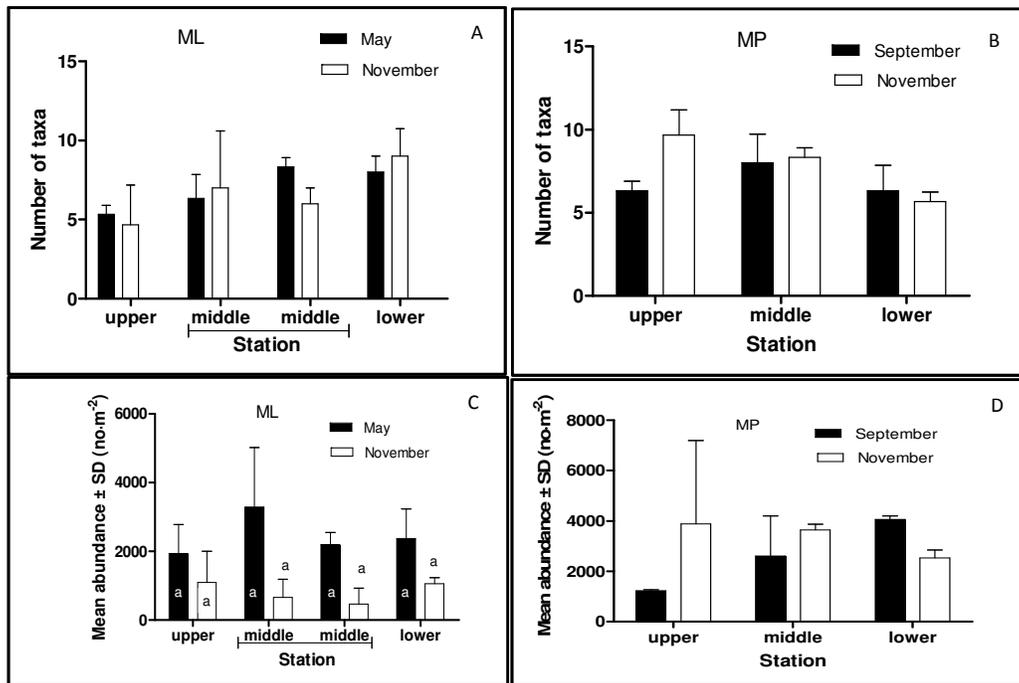


Figure 3.11: Number of taxa (A and B) and abundances (C and D) for macrozoobenthos of the Mlalazi (ML) and Mpenjati (MP) estuaries during May, September and November sampling sessions. Data represent mean (± SD, n = 3).

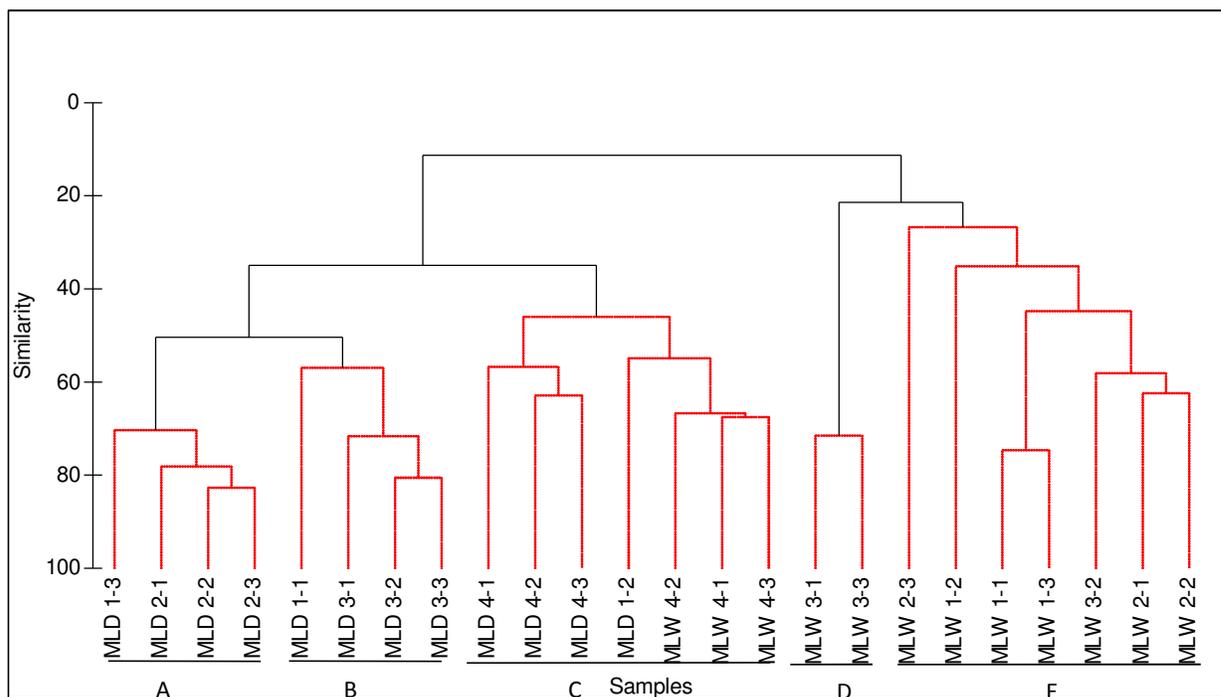


Figure 3.12: A classification (cluster) dendrogram showing five groups identified by a SIMPROF test in the Mlalazi Estuary during May and November sampling sessions. Letters (A, B, C, D and E) indicate stations that could not be significantly differentiated from each other ($p < 0.05$). Codes in the x-axis depict the estuary (ML), sampling session (D = May, W = November) and sampling station (1-3 = station one, replicate 3).

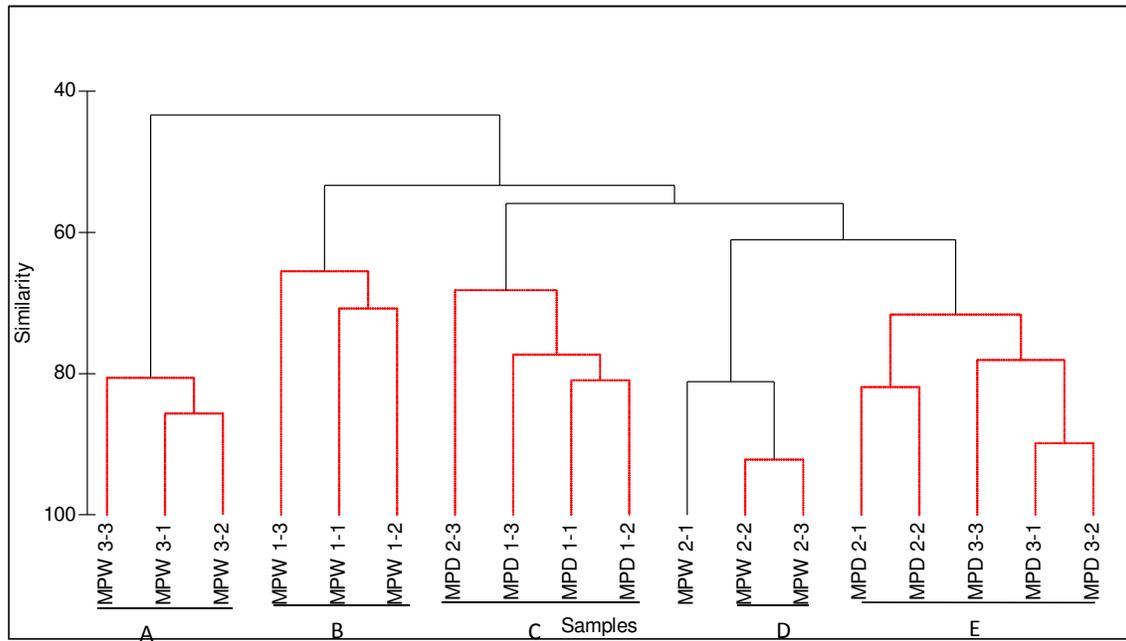


Figure 3.13: A classification (cluster) dendrogram showing five groups identified by a SIMPROF test in the Mpenjati Estuary during September and November sampling sessions. Letters (A, B, C, D and E) indicate stations that could not be significantly differentiated from each other ($p < 0.05$). Codes in the x-axis depict the estuary (MP), sampling session (D = September, W = November) and sampling station (1-3 = station one, replicate 3).

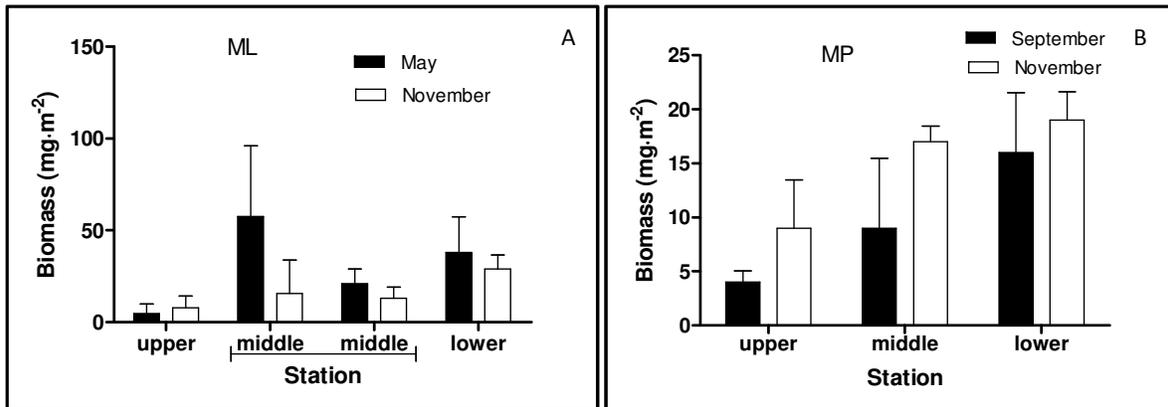


Figure 3.14: Macrozoobenthos biomass (dry weight) of Mlalazi (A) and Mpenjati (B) estuaries during May, September and November sampling sessions of 2011.

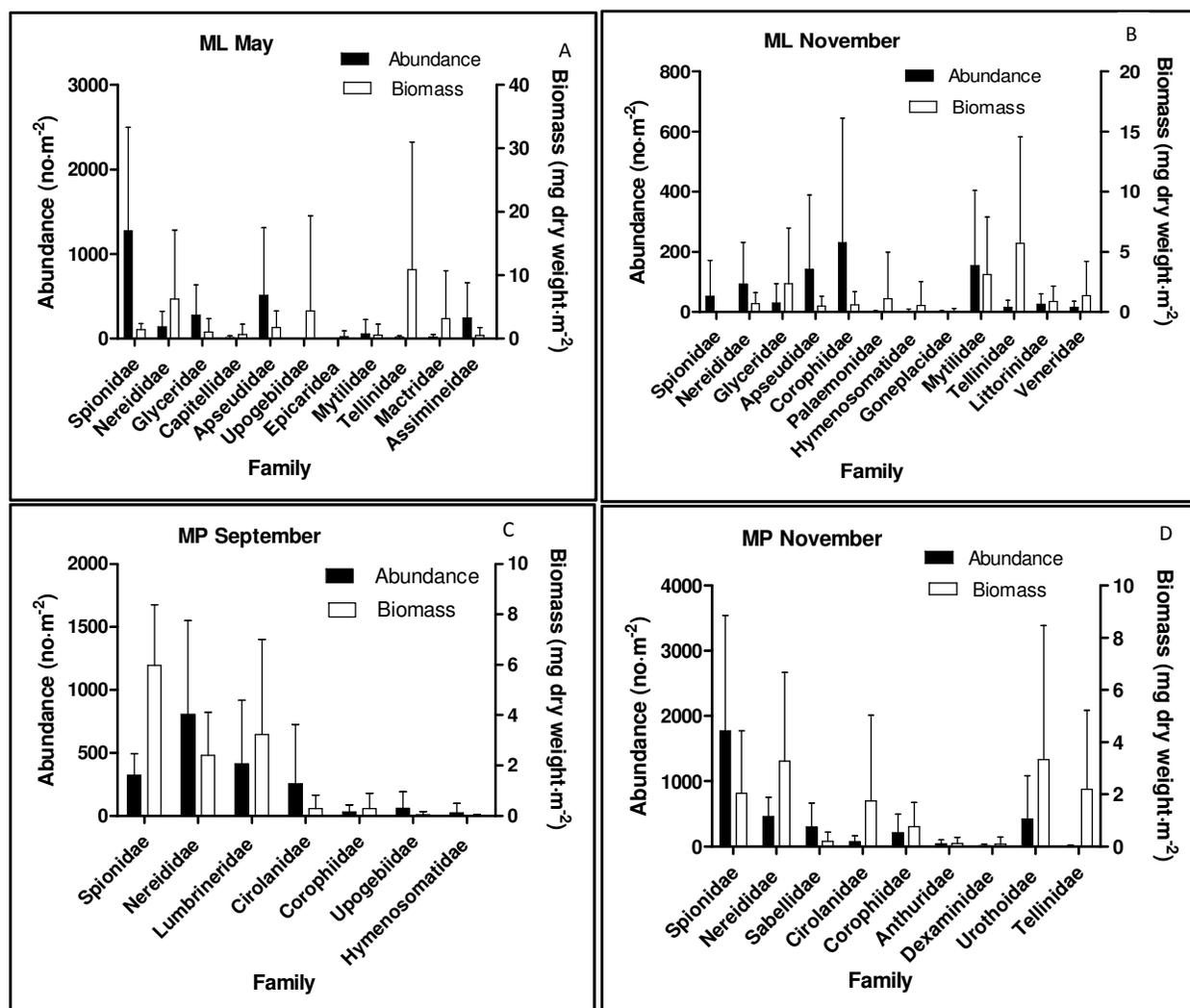


Figure 3.15: Abundance and biomass of macrozoobenthos of selected families (with dry weights which could be detected by the balance) in the Mlalazi (A and B) and Mpenjati (C and D) estuaries during May, September and November sampling sessions of 2011.

Table 3.3: Mean abundance (No·m⁻² ± SD, n = 3) of the benthic taxa recorded at four stations in the Mlalazi Estuary during May 2011. Five most abundant taxa in percentage are shown in bold.

TAXA	ML 1		ML 2		ML 3		ML 4		ML	ML
	Abundance	SD	Abundance	SD	Abundance	SD	Abundance	SD	TOTAL	% contrib.
POLYCHATA										
Spionidae	1120.0	669.9	3048.9	882.1	751.1	189.2	177.8	90.8	5097.8	48.3
Sabellidae	57.8	60.1	142.2	136.8	62.2	3.8			262.2	2.5
Nereididae	57.8	27.8	435.6	7.7	57.8	42.9	4.4	7.7	555.6	5.3
Glyceridae	226.7	294.8	608.9	480.1	222.2	362.1	35.6	27.8	1093.3	10.3
Cirratulidae					40.0	35.3	31.1	53.9	71.1	0.7
Lumbrineridae					4.4	7.7	22.2	27.8	26.7	0.3
Capitellidae							40.0	40.0	40.0	0.4
Syllidae							13.3	23.1	13.3	0.1
TANAIDACEA										
Apseudidae	248.9	385.1	26.7	23.1	48.9	7.7	1724.4	610.2	2048.9	19.4
AMPHIPODA										
Corophiidae			4.4	7.7					4.4	0.0
DECAPODA										
<i>Callichirus kraussi</i>							4.4	7.7	4.4	0.0
BIVALVIA										
Mytilidae	226.7	313.5							226.7	2.1
Tellinidae			26.7	23.1			26.7	13.3	53.3	0.5
Solenidae			4.4	7.7	4.4	7.7			8.9	0.1
Mactridae					40.0	23.1	31.1	31.1	71.1	0.7
Veneridae					4.4	7.7			4.4	0.0
Donacidae							4.4	7.7	4.4	0.0
GASTROPODA										
Assimineidae					924.4	162.9	48.1	48.1	972.5	9.2
Nassariidae							4.4	7.7	4.4	0.0
TOTAL	1937.8		4297.8		2160.0		2168.1		10563.6	
NO OF TAXA	6.0		8.0		11.0		14.0		19.0	

Table 3.4: Mean abundance (No·m⁻² ± SD, n = 3) of the benthic taxa recorded at four stations in the Mlalazi Estuary during November 2011. The five most abundant taxa in percentage are shown in bold.

TAXA	ML 1		ML 2		ML 3		ML 4		ML	ML
	Abundance	SD	Abundance	SD	Abundance	SD	Abundance	SD	TOTAL	% contrib.
POLYCHATA										
Spionidae			8.9	15.4			200.0	186.7	208.9	6.4
Sabellidae							4.4	7.7	4.4	0.1
Nereididae	222.2	192.9	137.8	129.5			13.3	13.3	373.3	11.5
Glyceridae							120.0	81.1	120.0	3.7
Cirratulidae							8.9	15.4	8.9	0.3
Lumbrineridae			13.3	13.3				0.0	13.3	0.4
Syllidae			4.4	7.7				0.0	4.4	0.1
TANAIDACEA										
Apseudidae	26.7	46.2					542.2	116.5	568.9	17.6
AMPHIPODA										
Corophiidae	724.4	643.0	155.6	136.8	40.0	23.1	4.4	7.7	924.4	28.5
ISOPODA										
Cirolanidae			4.4	7.7			4.4	7.7	8.9	0.3
Anthuridae	22.2	20.4							22.2	0.7
DECAPODA										
Hymenosomatidae	8.9	15.4							8.9	0.3
Palaemonidae					4.4	7.7			4.4	0.1
Goneplacidae							4.4	7.7	4.4	0.1
MYSIDA										
<i>Mesopodopsis africana</i>					4.4	7.7			4.4	0.1
BIVALVIA										
Mytilidae	75.6	73.4	293.3	257.2	248.9	431.1			617.8	19.1
Tellinidae			8.9	15.4	4.4	7.7	48.9	20.4	62.2	1.9
Veneridae			4.4	7.7	48.9	7.7	8.9	7.7	62.2	1.9
Lucinidae			8.9	15.4	17.8	15.4	31.1	42.9	57.8	1.8
GASTROPODA										
Nassariidae			4.4	7.7	22.2	20.4			54.7	1.7
Littorinidae			13.3	23.1	75.6	20.4	17.8	15.4	106.7	3.3
TOTAL	1080.0		657.8		466.7		1008.9		3241.4	
NO. OF TAXA	6.0		12.0		9.0		13.0		21.0	

Table 3.5: Mean abundance ($\text{No}\cdot\text{m}^{-2} \pm \text{SD}$, $n = 3$) of the benthic taxa recorded at three stations in the Mpenjati Estuary during September 2011. The five most abundant taxa in percentage are shown in bold.

TAXA	MP 1		MP 2		MP 3		MP TOTAL	MP % contrib.
	Abundance	SD	Abundance	SD	Abundance	SD		
POLYCHATA								
Spionidae	880.0	74.2	1226.7	773.0	1480.0	220.3	3586.7	45.7
Sabellidae	31.1	15.4	160.0	167.1	248.9	215.5	440.0	5.6
Nereididae	177.8	393.1	604.4	393.1	662.2	303.3	1444.4	18.4
Lumbrineridae	88.9	27.8	240.0	70.6	1613.3	406.0	1942.2	24.7
AMPHIPODA								
Corophiidae	4.4	7.7	160.0	186.7	8.9	15.4	173.3	2.2
Urothoidae			4.4	7.7			4.4	0.1
Lysianassidae	4.4	7.7					4.4	0.1
ISOPODA								
Cirolanidae	22.2	38.5	146.7	161.7	8.9	15.4	177.8	2.3
DECAPODA								
<i>Callichirus kraussi</i>			8.9	15.4	22.2	38.5	31.1	0.4
Hymenosomatidae			8.9	15.4			8.9	0.1
MYSIDA								
<i>Gastrosaccus psammodytes</i>			17.8	7.7	8.9	7.7	26.7	0.3
BIVALVIA								
Tellinidae	4.4	7.7			4.4	7.7	8.9	0.1
Donacidae	4.4	7.7					4.4	0.1
TOTAL	1217.8		2577.8		4057.8		7853.3	
NO OF TAXA	9.0		11.0		10.0		13.0	

Table 3.6: Mean abundance (No·m⁻² ± SD, n = 3) of the benthic taxa recorded at three stations in the Mpenjati Estuary during November 2011. The five most abundant taxa in percentage are shown in bold.

TAXA	MP 1		MP 2		MP 3		TOTAL	MP % contrib.
	Abundance	SD	Abundance	SD	Abundance	SD		
POLYCHATA								
Spionidae	2555.6	2738.3	2462.2	316.7	293.3	185.2	5311.1	53.3
Sabellidae	93.3	81.1	751.1	250.3	53.3	92.4	897.8	9.0
Nereididae	457.8	141.3	146.7	58.1	773.3	188.1	1377.8	13.8
Lumbrineridae			13.3	13.3			13.3	0.1
TANAIDACEA								
Apseudidae	57.8	27.8					57.8	0.6
AMPHIPODA								
Corophiidae	511.1	343.9	35.6	20.4	93.3	53.3	640.0	6.4
Urothoidae					1262.2	384.9	1262.2	12.7
CUMACEA								
Nannastacidae	4.4	7.7					4.4	0.0
ISOPODA								
Cirolanidae	13.3	13.3	164.4	108.6	44.4	20.4	222.2	2.2
Anthuridae	111.1	60.1	13.3	23.1			124.4	1.2
Idoteidae	8.9	7.7					8.9	0.1
BIVALVIA								
Mytilidae	13.3	23.1					13.3	0.1
Tellinidae	4.4	7.7	22.2	7.7			26.7	0.3
TOTAL	3831.1		3608.9		2520.0		9960.0	
NO OF TAXA	12.0		9.0		7.0		13.0	

Table 3.7: Summary of the results of analysis of variance (ANOVA) for total suspended solids (TSS), chlorophyll a concentrations as well as macrozoobenthos and zooplankton abundance and biomass in the Mlalazi and Mpenjati estuaries. All *p* values representing significant differences are highlighted in grey.

Variable	Comparisons	<i>F</i>	<i>p</i>	df
Total suspended solids (TSS) (mg·l ⁻¹)	Stations - ML	1.01	0.413	3
	Sampling sessions - ML	3.08	0.098	1
	Stations -MP	77.98	< 0.0005	2
	Sampling sessions - MP	7.23	0.020	1
	Estuaries (ML and MP)	4.68	0.038	1
Chlorophyll a (µg·l ⁻¹)	Stations - ML	50.93	< 0.0005	3
	Sampling sessions - ML	57.03	< 0.0005	1
	Stations - MP	8.81	0.004	2
	Sampling sessions - MP	177.92	< 0.0005	1
	Estuaries (ML and MP)	1.09	0.304	1
Microphytobenthos (µg·m ⁻²)	Sampling sessions - ML	13.48	0.002	1
	Sampling sessions - MP	19.19	0.001	1
	Estuaries (ML and MP)	0.03	0.875	1
Zooplankton abundance (no·m ⁻³)	Stations - ML	12.24	0.151	3
	Sampling sessions - ML	17.24	0.003	1
	Stations - MP	12.68	0.007	2
	Sampling sessions - MP	27.53	0.002	1
	Estuaries - (ML and MP)	1.62	0.217	1
Zooplankton biomass (mg dry weight·m ⁻³)	Stations - ML	4.76	0.034	3
	Sampling sessions - ML	59.92	< 0.0005	1
	Stations - MP	5.01	0.052	2
	Sampling sessions - MP	22.06	0.003	1
	Estuaries (ML and MP)	0.00	0.974	1
Macrozoobenthos abundance (no·m ⁻²)	Stations - ML	0.62	0.615	3
	Sampling sessions - ML	21.48	< 0.0005	1
	Stations - MP	0.39	0.683	3
	Sampling sessions - MP	1.02	0.333	1
	Estuaries (ML and MP)	12.54	0.003	1
Macrozoobenthos biomass (mg·m ⁻²)	Stations - ML	4.03	0.026	3
	Sampling sessions - ML	3.84	0.086	1
	Stations - MP	9.62	0.003	3
	Station - MP	7.74	0.017	1
	Estuaries (ML and MP)	5.94	0.020	1

Table 3.8: Summary of the results of analysis of variance (ANOVA) for dissolved inorganic nutrients, particulate phosphorus and phosphorus content (%) in biota for the Mlalazi and Mpenjati estuaries. All *p* values representing significant differences are highlighted in grey.

Variable	Comparisons	<i>F</i>	<i>p</i>	df
Dissolved inorganic phosphorus (DIP) (mg·m ⁻²)	Stations - ML	55.69	< 0.0005	3
	Sampling sessions - ML	212.13	< 0.0005	1
	Stations - MP	6.49	0.010	2
	Sampling sessions - MP	42.16	< 0.0005	1
	Estuaries (ML and MP)	7.43	0.009	1
Nitrate + Nitrite (mg·m ⁻²)	Stations - ML	12.99	< 0.0005	3
	Sampling sessions - ML	41.85	< 0.0005	1
	Stations - MP	20.02	< 0.0005	2
	Sampling sessions - MP	1.75	0.210	1
	Estuaries (ML and MP)	37.98	< 0.0005	1
Ammonia (mg·m ⁻²)	Stations - ML	5.11	0.009	3
	Sampling sessions - ML	11.64	0.003	1
	Stations - MP	0.32	0.969	2
	Sampling sessions - MP	0.43	0.523	1
	Estuaries (ML and MP)	133.67	< 0.0005	1
Particulate phosphorus (mg·m ⁻²)	Stations - ML	24.42	< 0.0005	3
	Sampling sessions - ML	319.48	< 0.0005	1
	Stations - MP	4.75	0.030	2
	Sampling sessions - MP	60.90	< 0.0005	1
	Estuaries (ML and MP)	0.56	0.460	1
Phytoplankton phosphorus content (mg·m ⁻²)	Stations - ML	45.60	< 0.0005	2
	Sampling sessions - ML	60.25	< 0.0005	1
	Stations - MP	3.34	0.073	2
	Sampling sessions - MP	80.60	< 0.0005	2
Phosphorus content (%) in zooplankton	Taxa - ML	1.70	0.236	2
	Stations - ML	3.52	0.062	3
	Taxa - MP	0.29	0.610	1
	Stations - MP	0.960	0.445	2
	Estuaries (ML and MP)	1.96	0.174	1
Phosphorus content (%) in macrozoobenthos	Taxa - ML	0.89	0.415	2
	Sampling sessions - ML	0.04	0.852	1
	Taxa - MP	2.83	0.660	2
	Sampling sessions - MP	2.73	0.104	1
Kruskal Wallis Test (N = 124 for ML, N = 69 for MP)	Estuaries (ML and MP)		0.001	1

3.8. Phosphorus distribution in biota and sediment

Macrozoobenthos, zooplankton and sediment phosphorus content was compared between different groups (macrozoobenthos), species (zooplankton), and stations (sediment, zooplankton and phytoplankton). Comparisons between sampling sessions and estuaries were also performed.

3.8.1. Phytoplankton

Phytoplankton phosphorus content roughly estimated from the Redfield ratio (C:P = 106:1) was higher during May ($1.7 \text{ mg}\cdot\text{m}^{-2} \pm 0.1 \text{ SD}$) than November ($0.1 \text{ mg}\cdot\text{m}^{-2} \pm 0.2 \text{ SD}$) in the Mlalazi Estuary (Figure 3.16 A). The phytoplankton P content was generally increasing from the upper towards the lower reaches during both May and November sampling sessions (Figure 3.16 A). There were significant differences in phytoplankton P content between stations ($p < 0.0005$) and sampling sessions ($p < 0.0005$) of the Mlalazi Estuary (Table 3.8).

In the Mpenjati Estuary, estimates of phytoplankton P content were higher during September ($0.7 \text{ mg}\cdot\text{m}^{-2} \pm 0.2 \text{ SD}$) when compared to the November sampling session ($0.04 \text{ mg}\cdot\text{m}^{-2} \pm 0.01 \text{ SD}$) (Figure 3.16 B). During the September sampling session, phytoplankton P content was decreasing from the upper towards the lower reaches with no clear trend in phytoplankton P content along the estuary length during the November sampling session (figure 3.16 B). There were no significant differences in phytoplankton P content between stations ($p = 0.073$) but there were significant differences between the sampling sessions ($p < 0.0005$) (Table 3.8). There were significant differences in phytoplankton P content between the Mlalazi and Mpenjati estuaries ($p = 0.042$) (Table 3.8).

In the Mlalazi Estuary estimates of microphytobenthos P content estimated from the Redfield ratio (C:P = 106:1) were higher during November ($0.9 \text{ mg}\cdot\text{m}^{-2} \pm 0.6 \text{ SD}$) than May ($0.01 \text{ mg}\cdot\text{m}^{-2} \pm 0.001 \text{ SD}$) (Figure 3.17 A). Microphytobenthos P content of the Mlalazi Estuary was generally decreasing from the upper towards the lower reaches during November but there was no clear trend along the estuary length during May (Figure 3.17 A).

Estimates of microphytobenthos P content of the Mpenjati Estuary were higher during November ($1.7 \text{ mg}\cdot\text{m}^{-2} \pm 0.9 \text{ SD}$) than September ($0.001 \text{ mg}\cdot\text{m}^{-2} \pm 0.001 \text{ SD}$) (Figure 3.17 B). Microphytobenthos P content was generally decreasing from the upper towards the lower reaches

during November but there was no clear trend along the salinity gradient during September (Figure 3.17 B). There was a significant difference in microphytobenthos P content between the Mlalazi and Mpenjati estuaries ($p = 0.022$) and between sampling sessions ($p < 0.0005$) (Table 3.8).

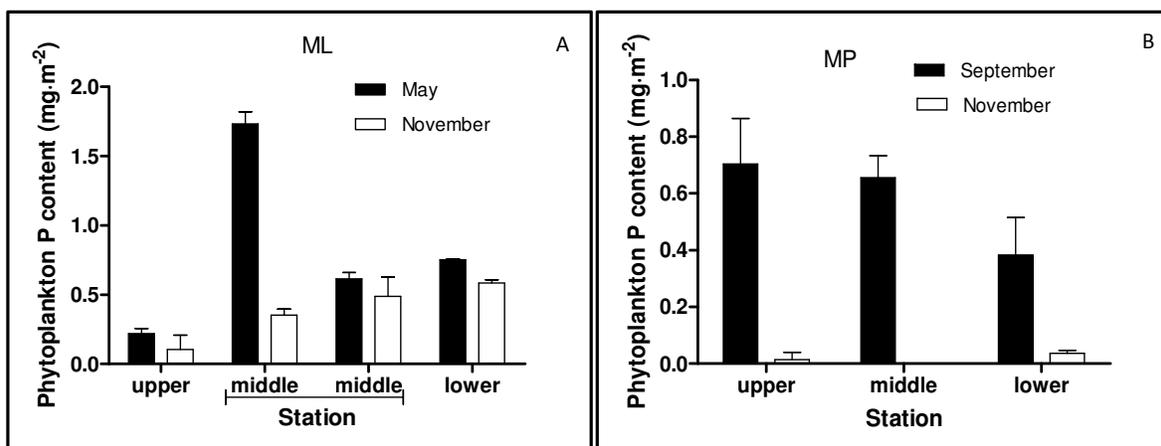


Figure 3.16: Phytoplankton phosphorus content estimated from the Redfield ratio for the Mlalazi (A) and Mpenjati (B) estuaries during May, September and November sampling sessions of 2011. Data represent mean (\pm SD, $n = 3$).

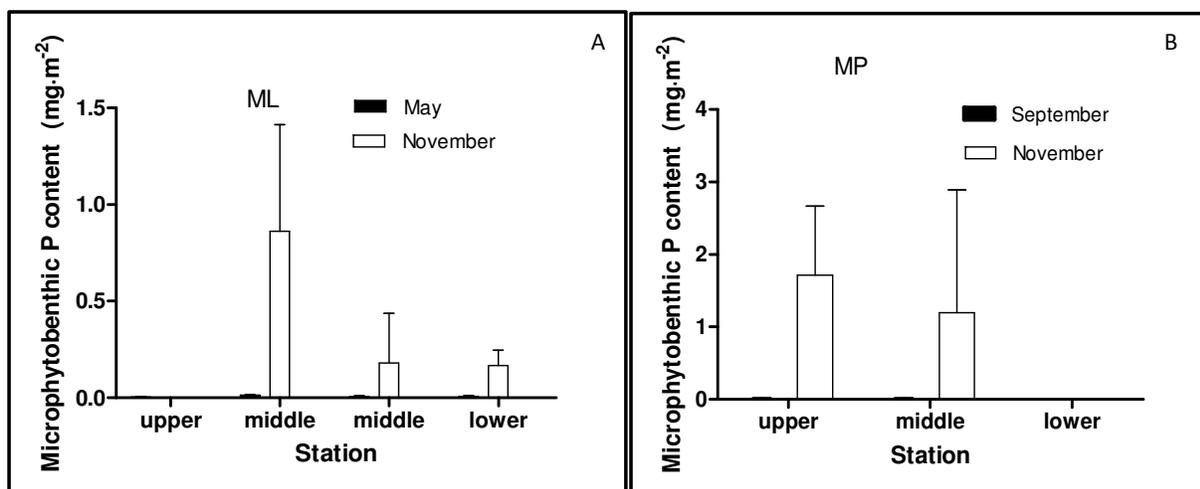


Figure 3.17: Microphytobenthos phosphorus content estimated from the Redfield ratio for the Mlalazi (A) and Mpenjati (B) estuaries during May, September and November sampling sessions of 2011. Data represent mean (\pm SD, $n = 3$).

3.8.2. Zooplankton

The copepod *A. Natalensis* comprised the highest mean percentage phosphorus content (1.7 %) of all zooplankton taxa in the Mlalazi Estuary during May (Figure 3.18 A). The copepod *P.hessei* had comparatively high biomass in terms of dry weight but displayed lower P biomass (Figure 3.19 A and B). There were no significant differences in zooplankton P content between taxa ($p = 0.236$) and stations ($p = 0.062$) (Table 3.8). Phosphorus content for zooplankton could not be measured during November sampling in both estuaries due to low zooplankton biomasses which lead to insufficient material for digestion. Consequently, P content could not be compared between different sampling sessions.

Pseudodiaptomus hessei comprised the highest mean percentage phosphorus content (0.6 %) of all taxa in the Mpenjati Estuary during September (Figure 3.18 B). *Pseudodiaptomus hessei* had the highest biomass in terms of dry weight and comprised the highest phosphorus biomass of all zooplankton taxa in the Mpenjati Estuary during September (Figure 3.20 A and B). There were no significant differences in P content between different zooplankton taxa ($p = 0.610$) and stations ($p = 0.445$) (Table 3.8). No significant differences were observed in zooplankton phosphorus content between the two estuaries ($p = 0.174$) (Table 3.8). In general, the percentage phosphorus content of zooplankton measured in the Mlalazi Estuary (range = 0.1-1.7 %) were higher than those measured in the Mpenjati Estuary (range = 0.2 - 0.6 %).

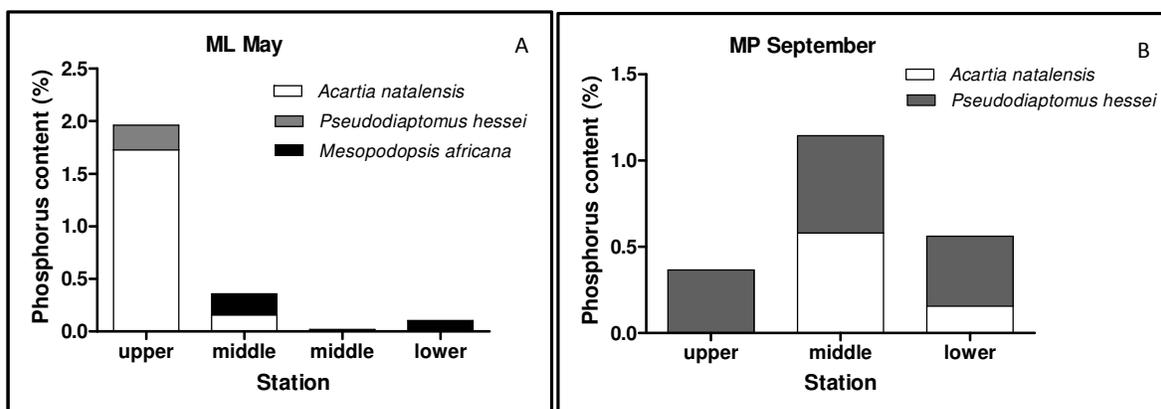


Figure 3.18: Phosphorus content (%) in different zooplankton taxa in the Mlalazi (A) and Mpenjati (B) estuaries during May and September.

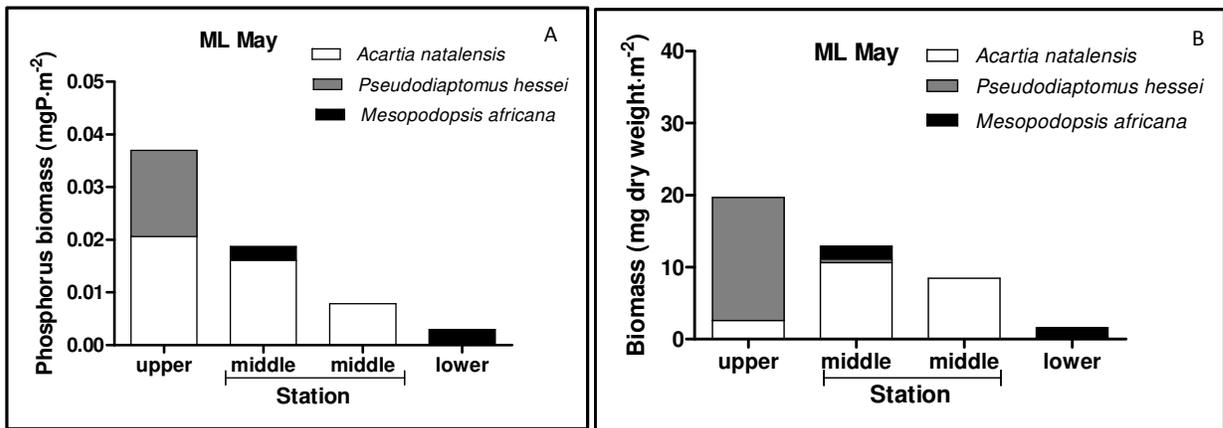


Figure 3.19: Phosphorus biomass and biomass in terms of dry weight in different zooplankton taxa in the Mlalazi Estuary during May.

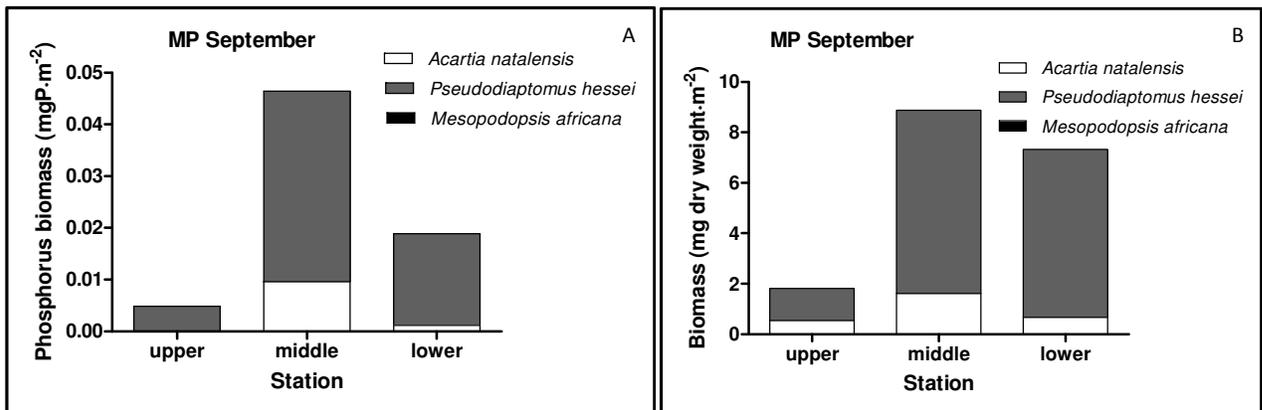


Figure 3.20: Phosphorus biomass and biomass in terms of dry weight in different zooplankton taxa in the Mpenjati Estuary during September.

3.8.3. Macrozoobenthos

In the Mlalazi Estuary, polychaetes comprised the highest mean percentage phosphorus content (0.4 %) of all macrozoobenthos taxa during May while molluscs (0.4 %) comprised the highest mean percentage P content during November (Figure 3.21 A and B). However, crustaceans comprised the highest mean percentage P content in the lower reaches in May and in the upper reaches in November (Figure 3.21 A and B). Overall, macrozoobenthos mean phosphorus content was higher in November (range = 0.2 - 0.8 %) than May (0.1 - 0.7 %). During May, polychaetes in the Mlalazi Estuary had low biomass in terms of dry weight but they displayed higher mean P biomass (Figure 3.22 A and B). Higher biomass in terms of dry weight together with high mean phosphorus biomass

was observed in molluscs during November (Figure 3.23 A and B). There were no significant differences in % P content between different taxa ($p = 0.415$) and sampling sessions ($p = 0.852$) (Table 3.8).

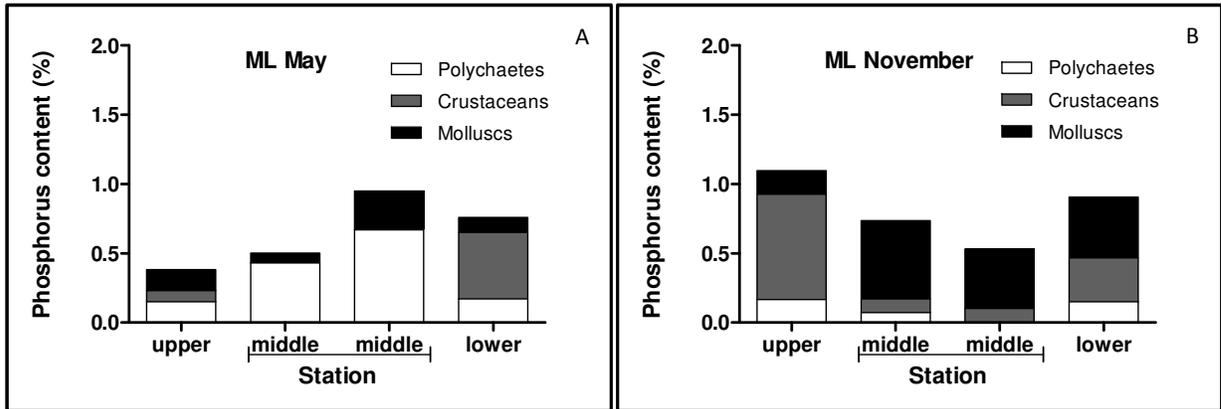


Figure 3.21: Phosphorus content (%) in different groups of macrozoobenthos in the Mlalazi Estuary during May and November.

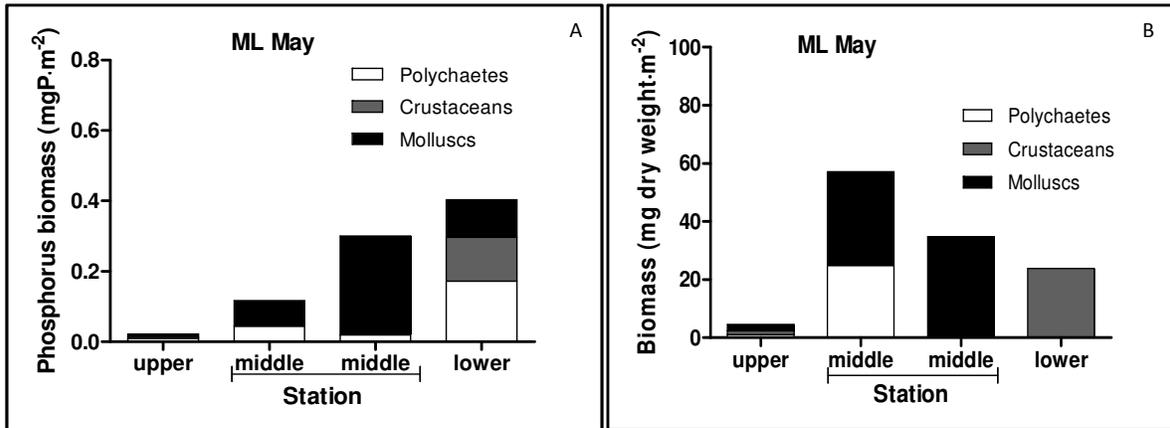


Figure 3.22: Phosphorus biomass and biomass in terms of dry weight in different groups of macrozoobenthos in the Mlalazi Estuary during May.

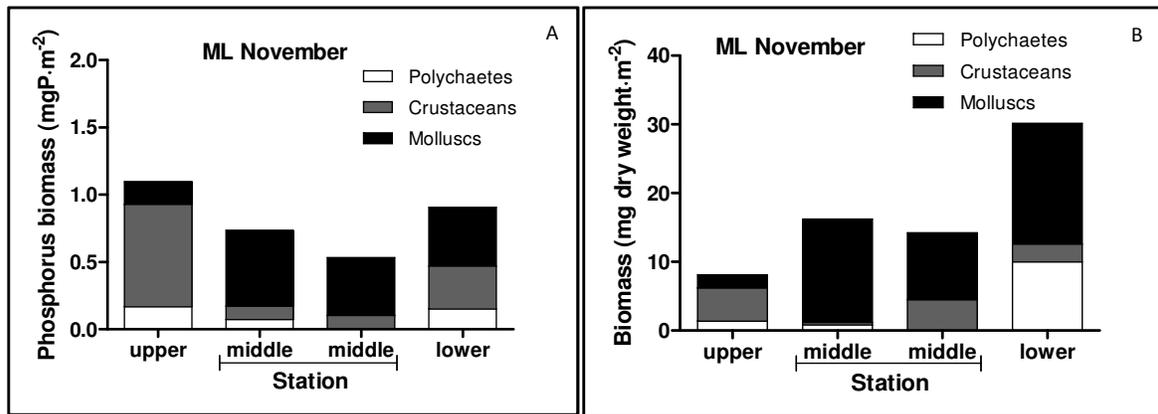


Figure 3.23: Phosphorus biomass and biomass in terms of dry weight in different groups of macrozoobenthos in the Mlalazi Estuary during November.

In the Mpenjati Estuary, polychaetes comprised the highest mean percentage phosphorus content of all macrozoobenthic taxa during September and November (1.2 and 0.3 % respectively) (Figure 3.24 A and B). No molluscs were caught in the Mpenjati Estuary during September. In November, molluscs comprised the lowest mean phosphorus content (0.2 %) (Figure 3.24 B). During September, polychaetes generally had high mean phosphorus biomass and high biomass in terms of dry weight except for the middle reaches where polychaetes had negligible biomass in terms of dry weight but displayed significantly high phosphorus biomass (Figure 3.25 A and B).

During November, molluscs in the Mpenjati Estuary showed relatively higher biomass in terms of dry weight but displayed lower P biomass (Figure 3.26 A and B). There were no molluscs recorded in the lower reaches of the Mpenjati Estuary during November. In the middle reaches, polychaetes had lower biomass in terms of dry weight but displayed relatively higher mean P biomass (Figure 3.26 A and B). There were no significant differences in P biomass between different benthic taxa ($p = 0.660$) and between sampling sessions ($p = 0.104$) (Table 3.8). There were significant differences in macrozoobenthos P biomass between the two estuaries ($p = 0.001$) (Table 3.8). Phosphorus biomass as well as percentage phosphorus content of *Callichirus kraussi* in the Mpenjati Estuary was higher in the lower when compared to the middle reaches (Figure 3.33 B). However no statistically significant differences were observed in P biomass ($p = 0.129$) and percentage P content ($p = 0.327$) of these prawns between the reaches. Although the percentage phosphorus content of the prawns was within the range of other benthic groups, the phosphorus biomass (mgP.m⁻²) was generally higher when compared to P biomass of other benthic groups (Figure 3.33 B).

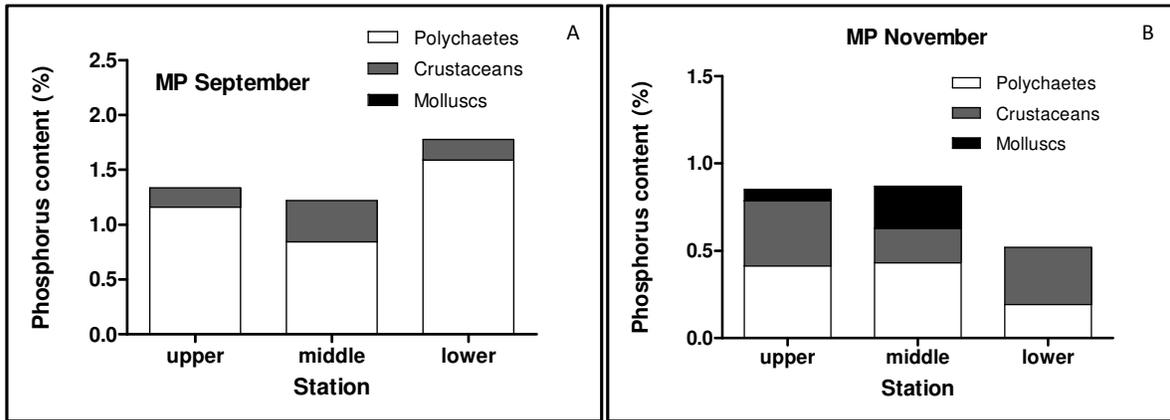


Figure 3.24: Phosphorus content (%) in different groups of macrozoobenthos in the Mpenjati Estuary during September (A) and November (B).

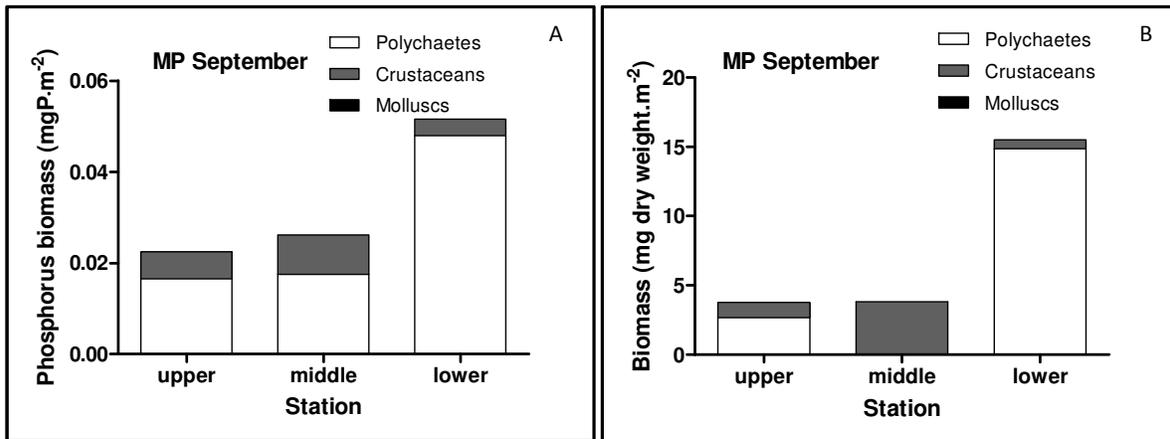


Figure 3.25: Phosphorus biomass and biomass in terms of dry weight in different groups of macrozoobenthos in the Mpenjati Estuary during September.

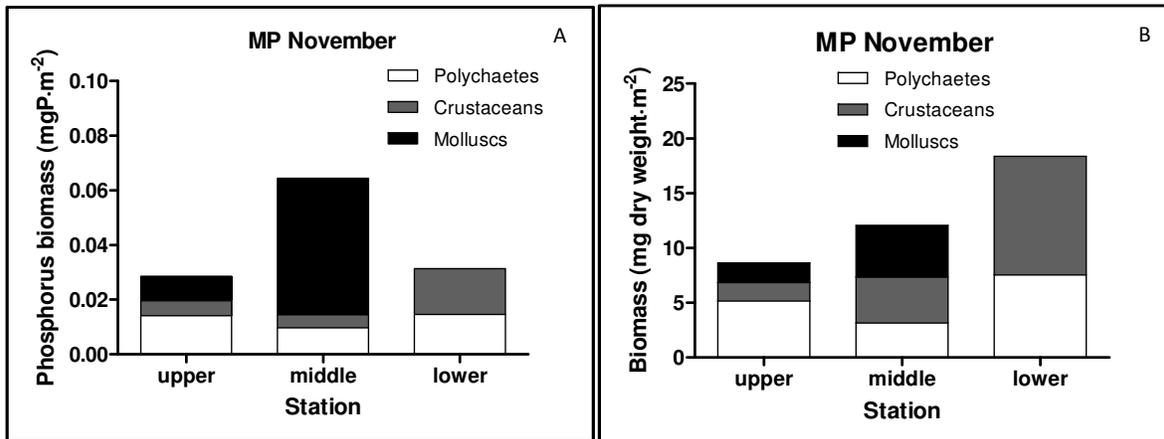


Figure 3.26: Phosphorus biomass and biomass in terms of dry weight in different groups of macrozoobenthos in the Mpenjati Estuary during November.

3.8.4. Sediment

In the Mlalazi Estuary, phosphorus concentrations in sediment (10 cm depth) were generally higher during May ($63943.7 \text{ mgP}\cdot\text{m}^{-2} \pm 38859.1 \text{ SD}$) than November ($15233.7 \text{ mgP}\cdot\text{m}^{-2} \pm 1297.8 \text{ SD}$) (Figure 3.27 A). During May, phosphorus mass in sediment of the Mlalazi Estuary was generally decreasing from the upper to the lower reaches while no clear pattern was observed during November (Figure 3.27 A).

In the Mpenjati Estuary phosphorus mass in sediment was higher during September ($17724.4 \text{ mgP}\cdot\text{m}^{-2} \pm 3836.3 \text{ SD}$) than November ($7080.5 \text{ mgP}\cdot\text{m}^{-2} \pm 0.00 \text{ SD}$) (Figure 3.27 B). During September P mass in sediment was decreasing from the upper towards the lower reaches with no clear trend during November (Figure 3.27 B). Overall, phosphorus mass in sediment was higher in the Mlalazi (range = $3226.0 - 95315.3 \text{ mgP}\cdot\text{m}^{-2}$) than the Mpenjati Estuary (range = $1378.7 - 21758.7 \text{ mgP}\cdot\text{m}^{-2}$) (Figure 3.27 A and B).

In the Mlalazi Estuary, sediment P content in percentage was generally higher during November than May except for the upper reaches (Figure 3.28 A). Phosphorus content (%) in the Mlalazi Estuary was generally decreasing from the upper towards the lower reaches during May with no clear trend in P content along the estuary length during November (Figure 3.28 A).

In the Mpenjati Estuary higher percentage P content was recorded during September than November (Figure 3.28 B). During September, sediment percentage P content was generally decreasing from the upper to the lower reaches but there was no clear trend in sediment P content along the salinity gradient during November (Figure 3.28 B). Overall, percentage phosphorus content was higher in the Mlalazi (range = 0.003 – 0.1 %) than the Mpenjati Estuary (range = 0.001 – 0.02 %) (Figure 3.28 A and B).

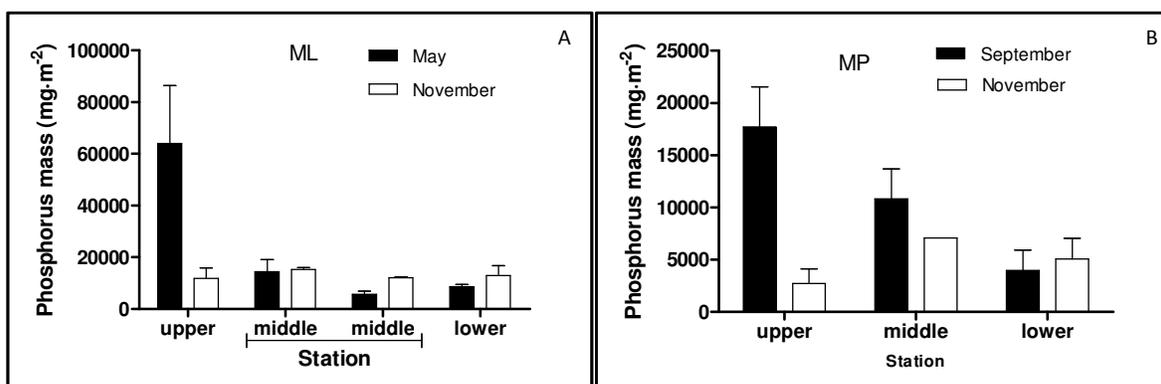


Figure 3.27: Phosphorus mass in sediment (for 10 cm depth) in the Mlalazi (A) and Mpenjati (B) estuaries during May, September and November sampling sessions. Data represent mean (\pm SD, $n = 3$).

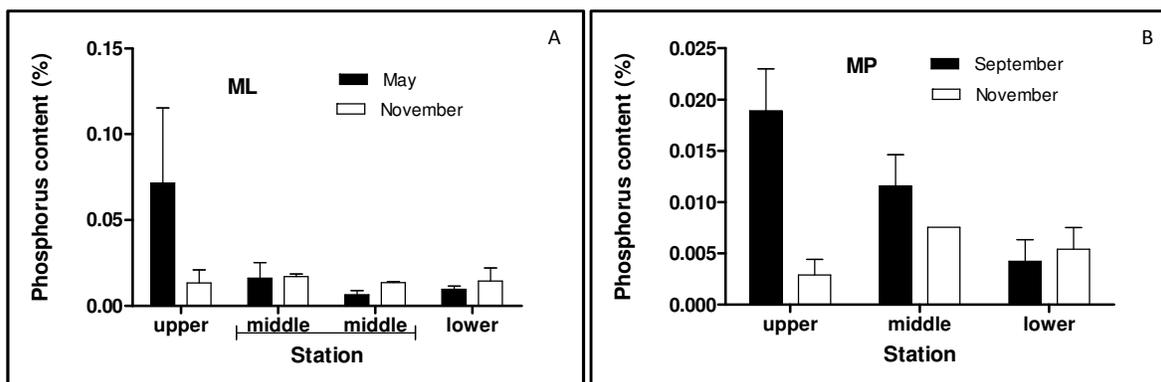


Figure 3.28: Phosphorus content (%) in sediment (for 10 cm depth) in the Mlalazi (A) and Mpenjati (B) estuaries during May, September and November. Data represent mean (\pm SD, $n = 3$).

3.8.5. Overall phosphorus distribution in Mlalazi and Mpenjati estuaries

In the Mlalazi estuary, sediment comprised the highest phosphorus mass ($18.1 \text{ gP}\cdot\text{m}^{-2} \pm 5.9 \text{ SD}$) than any other nutrient pool where P was measured (Figure 3.29). Zooplankton ($0.01 \text{ mgP}\cdot\text{m}^{-2} \pm 0.01 \text{ SD}$) and macrozoobenthos ($0.01 \text{ mgP}\cdot\text{m}^{-2} \pm 0.03 \text{ SD}$) comprised the lowest P biomass in the Mlalazi Estuary (Figure 3.19).

Sediment ($7.9 \text{ gP}\cdot\text{m}^{-2} \pm 5.6 \text{ SD}$) comprised the highest P mass in the Mpenjati Estuary with the lowest P biomass being measured in zooplankton ($0.01 \text{ mgP}\cdot\text{m}^{-2} \pm 0.01$) and macrozoobenthos ($0.01 \text{ mgP}\cdot\text{m}^{-2} \pm 0.01 \text{ SD}$) (Figure 3.20).

Overall, abiotic components had higher phosphorus mass when compared to biotic in both Mlalazi and Mpenjati estuaries (Figure 3.31). Phosphorus biomass for biotic components was about the same in both estuaries, however, overall phosphorus mass in abiotic components in the Mlalazi was higher than that of the Mpenjati Estuary (Figure 3.31). In both Mlalazi and Mpenjati estuaries overall phosphorus biomass in pelagic biota was higher than that measured in benthic biota (Figure 3.32).

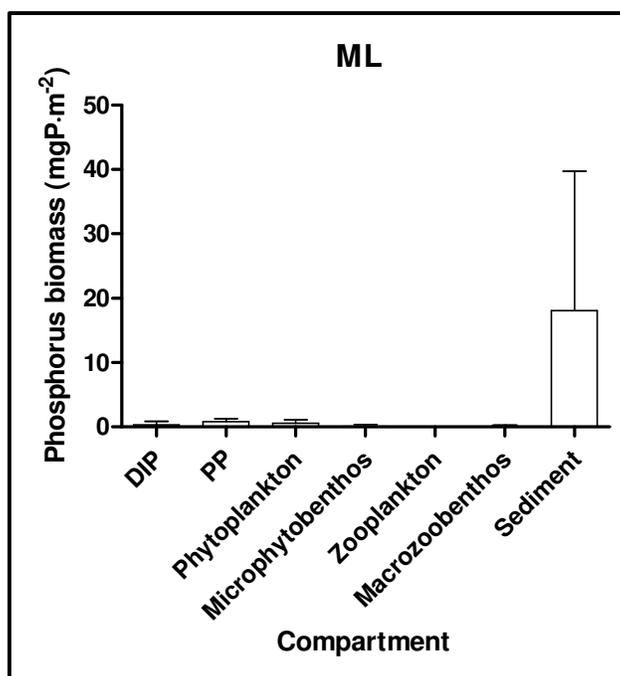


Figure 3.29: Overall distribution of phosphorus in living and non living nutrient pools of the Mlalazi Estuary. Phosphorus biomass is presented in $\text{mgP}\cdot\text{m}^{-2}$ except for the sediment and dissolved inorganic phosphorus (DIP) where P is presented in $\text{gP}\cdot\text{m}^{-2}$.

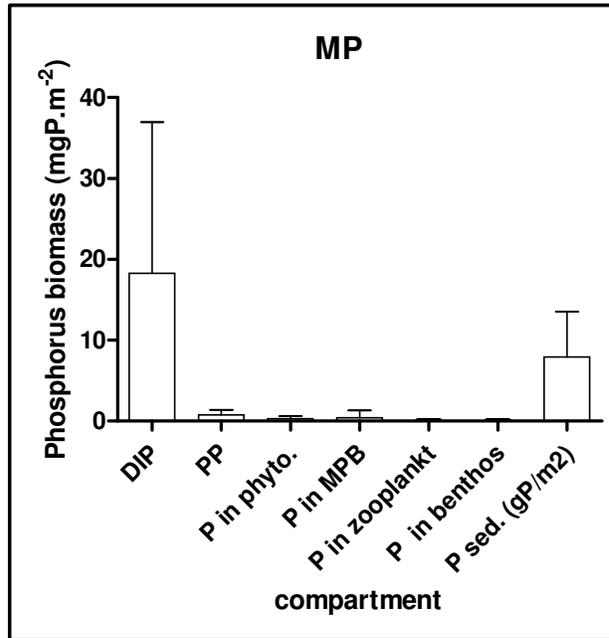


Figure 3.30: Overall distribution of phosphorus in living and non living nutrient pools of the Mpenjati Estuary. Phosphorus biomass is presented in $\text{mgP}\cdot\text{m}^{-2}$ except for the sediment where P is presented in $\text{gP}\cdot\text{m}^{-2}$.

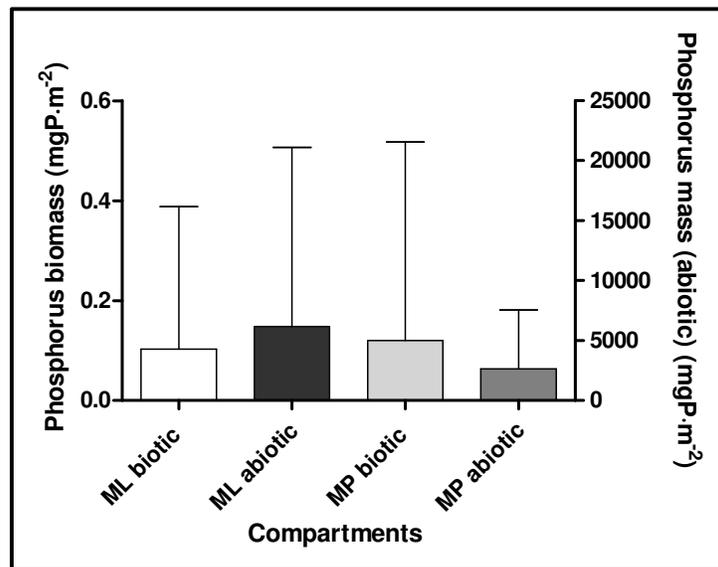


Figure 3.31: Overall phosphorus distribution in biotic and abiotic components of the Mlalazi and Mpenjati estuaries. Phosphorus biomass of the biotic components is presented on the left Y axes and phosphorus mass of the abiotic components is presented on the right Y axes.

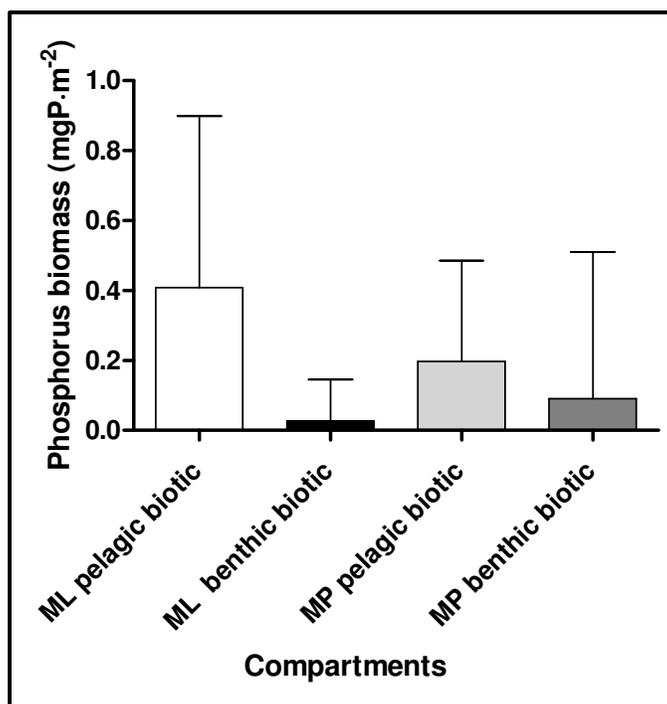


Figure 3.32: Overall phosphorus distribution in the pelagic biotic and benthic biotic components of the Mlalazi and Mpenjati estuaries.

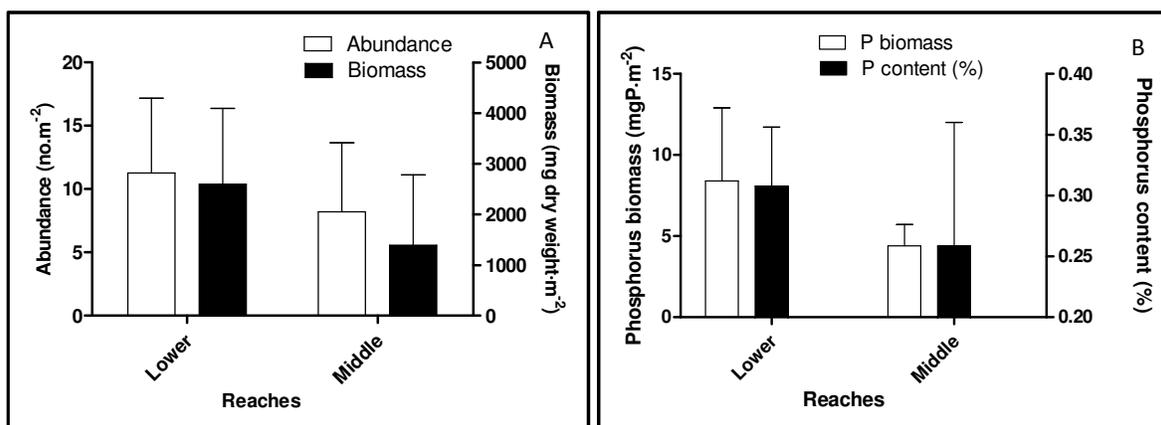


Figure 3.33: Abundance and biomass (dry weight) (A) as well as phosphorus biomass and percentage phosphorus content (B) of the *Callichirus kraussi* in the Mpenjati Estuary.

Chapter 4

Discussion

In this study, phosphorus content in living and non-living nutrient pools of the permanently open Mlalazi (ML) and temporarily open/closed Mpenjati (MP) Estuary was measured to give an insight of how P distribution changes with sampling sessions and along the estuarine salinity gradients. Prior to phosphorus determination, biological data including chlorophyll a, macrozoobenthos and zooplankton were analysed for concentrations, abundance and biomass. Values of abundance and biomass were compared along the salinity gradient and between sampling sessions and estuaries. Phosphorus content in biota was compared between species (zooplankton), families (macrozoobenthos) and stations (zooplankton and macrozoobenthos). Dissolved inorganic phosphorus, particulate phosphorus and phosphorus in sediment were also compared between stations, sampling sessions and between estuaries.

4.1. Physico – chemical characteristics

There was a clear evidence of change in temperature and salinity between May, September and November sampling sessions in the Mpenjati and Mlalazi estuaries. Whereas temperatures were higher during November as compared to May in the Mlalazi Estuary, they were lower during November in the Mpenjati Estuary when compared to the September sampling session. During the open phase of the Mpenjati Estuary, the efficient flushing by the tidal inflow of colder sea water could have resulted in a decreased water temperature in this system, a similar case has been reported for the Kasouga Estuary (Froneman 2002a). These results are contrary to previous records of the Mpenjati Estuary system (Kibirige and Perissinotto 2003; Anandraj et al. 2007).

Salinities were higher during May and September when compared to November in the Mlalazi and Mpenjati Estuary respectively. Rainfall levels recorded during November in both Mlalazi and Mpenjati estuaries were higher than those recorded during May and September in these estuaries (www.sasa.org.za). High rainfall during November lead to a decrease in salinity levels throughout the whole Mlalazi and Mpenjati estuarine systems. Similar to the Mlalazi and Mpenjati estuaries during the present study period, a decrease in salinity throughout the estuary following high rainfall has been generally apparent in most South African TOCEs and POEs (Froneman 2002b; Froneman 2002a; Kibirige and Perissinotto 2003; Thomas et al. 2005; Kibirige et al. 2006). Rainfall levels

during the present study period were similar to those previously reported by Perissinotto et al. (2002) in the Mpenjati Estuary where highest rainfall was recorded during December while the lowest was recorded during August.

4.2. Total suspended solids

Total suspended solids (TSS) concentrations were higher in November than May and September in both Mlalazi and Mpenjati estuaries. There was a general increase in TSS concentrations from the upper towards the lower reaches of both Mlalazi and Mpenjati estuaries during the present study. Similar to the Mpenjati Estuary, TSS concentrations of the Kasouga (Froneman 2002b) were higher during the open than the closed phase. An increase in TSS generally takes place following rainfall which results in sediment disturbance (Froneman 2002b). Such patterns have been observed in South African estuaries such as Kariega, Great Fish and Kasouga estuaries (Grange and Allanson 1995; Grange et al. 2000; Froneman 2002b; Froneman 2002a).

4.3. Nutrients

Dissolved inorganic phosphorus (DIP) concentrations were higher during May and September than November in the Mlalazi and Mpenjati estuaries respectively. In the Mlalazi Estuary, concentrations of nitrate + nitrite were higher during November than May but in the Mpenjati Estuary the opposite was observed with high concentrations recorded during September. Ammonia concentrations were higher in May and September than November in both Mlalazi and Mpenjati estuaries.

Increased DIP and nitrate + nitrite concentrations in the Mlalazi and Mpenjati estuaries during May and September respectively contradicts with the DIP and nitrate + nitrite concentrations previously recorded in most South African estuaries where elevated concentrations were experienced during high rainfall e.g. (Allanson and Read 1995; Nozais et al. 2001; Froneman 2002b; Scharler and Baird 2003a; Gama et al. 2005; Kibirige et al. 2006). Elevated nutrient concentrations (nitrate, nitrite and ammonia) in winter (August) sampling have been reported in the Mlalazi Estuary, except for DIP concentrations which were higher during summer (February) (Mabaso 2002). Following a strong river inflow, lower nutrient concentrations were recorded in Maitland Estuary in the Eastern Cape (Gama et al. 2005). It was suggested that nutrient retention was reduced together with low retention of water within the estuary as some water was flushed out of the estuary rapidly (Gama et al. 2005).

During the present study, it was suggested that the reduced concentrations of DIP and nitrate + nitrite in the Mpenjati Estuary and reduced concentrations of DIP in the Mlalazi Estuary during November were as a result of high rainfall which diluted these nutrients in the river.

The land cover at the head (above the railway bridge) of the Mlalazi Estuary is used for agricultural farming, e.g. for sugarcane. Higher nitrate + nitrite concentrations in the Mlalazi Estuary during the November sampling session were likely to have come from agricultural runoff. Compared to the Mpenjati Estuary, the Mlalazi Estuary had higher nutrient concentrations which are associated with the regular freshwater inflow which frequently brings in nutrients to this estuary. During May, all measured nutrients (DIP, nitrate + nitrite and ammonia) were highest in the upper reaches in the Mlalazi Estuary, a case reported for the Swartkops, Kromme and Sundays estuaries (Emmerson 1985; Scharler and Baird 2003a). This gives support that most of the nutrients in these estuaries are probably derived from their rivers as highest concentrations are recorded at their upper reaches. In the Mpenjati Estuary highest nutrient concentrations were generally recorded in the middle reaches during both sampling sessions. Although Perissinotto et al. (2002) stated that river flow together with Palm Beach waste water treatment discharge are major sources of DIN (dissolved inorganic nitrogen) and DIP in this system, higher nutrient concentrations would be expected from the upper reaches since the Palm Beach WWTW is located further upstream of national road bridge R61.

Particulate phosphorus concentrations were higher in November than in May and September in both Mlalazi and Mpenjati estuaries respectively. In the Mlalazi Estuary PP concentrations were generally decreasing from the upper towards the lower reaches of the estuary during both sampling sessions. Contrary to the Gamtoos Estuary, the Mlalazi Estuary had elevated PP concentrations during period of high river inflow while the Gamtoos Estuary displayed decreased PP concentrations during high river flow period (Scharler and Baird 2003b). In few South African POEs, PP concentrations have been reported to decrease from the upper towards the lower reaches e.g. in the Gamtoos Estuary (Scharler et al. 2002; Scharler and Baird 2003b), Kariega and Great Fish Estuary (Bate et al. 2002). The permanently open Mlalazi showed the similar pattern to that previously reported for other South African POEs with PP concentrations decreasing from the upper towards the lower reaches. This also gives evidence of a river being the main source of particulate phosphorus to the estuary. High concentrations of PP during November were suggested to be attributed from the efficient mixing from the river and tidal currents during November which could potentially disturb sediment, bringing the buried particulate phosphorus into the water column.

4.4. Phytoplankton and microphytobenthos

Phytoplankton chlorophyll a concentrations were higher during May and September than November sampling session in both Mlalazi and Mpenjati estuaries. In general, higher chlorophyll a concentrations during May and September in the Mlalazi and Mpenjati Estuary were associated with higher nutrients concentrations recorded in these systems during these sampling sessions.

Chlorophyll a concentrations recorded in the Mpenjati Estuary during the present study were lower than those previously recorded in this system e.g. (Perissinotto et al. 2003). Similar to the Mpenjati Estuary during the present study, few South African TOCEs have displayed high chlorophyll a concentrations during the closed when compared to the open phase, e.g. in the Mdloti, Mhlanga, Nyara, Maitland and Van Stadens Estuary (Perissinotto et al. 2003; Gama et al. 2005; Thomas et al. 2005). Contrary to the Mpenjati Estuary during the present study, high chlorophyll a concentrations during high river inflow have been reported in the Kasouga estuary (Froneman 2002b; Froneman 2002a). Generally, mean phytoplankton chlorophyll a concentrations recorded in the Mpenjati Estuary were lower than those recorded in other few South African TOCEs, e.g. in the Mdloti, Nyara, Mhlanga, Kasouga, Great Brak, Van Stadens and Maitland Estuary (Nozais et al. 2001; Froneman 2002b; Perissinotto et al. 2003; Gama et al. 2005; Thomas et al. 2005).

Mean phytoplankton chlorophyll a concentrations recorded in the Mlalazi Estuary during the present study were lower than those previously recorded in this system (Mabaso 2002). Similar to the Mlalazi Estuary during the November sampling, the Berg Estuary chlorophyll a concentrations were highest in the lower reaches (Adams and Bate 1999). However, these results contradicts with those previously reported for the Gamtoos, Sundays, Kromme and Swartkops Estuary where chlorophyll a concentrations were highest in the upper reaches decreasing down the estuary towards the mouth (Snow et al. 2000; Bate et al. 2002; Scharler and Baird 2003a). Generally chlorophyll a concentrations recorded in the Mlalazi Estuary during the study period were lower than the concentrations previously recorded in other South African permanently open estuaries e.g. in the Kromme, Sundays, Great Fish, Gamtoos and Berg estuaries (Snow et al. 2000; Bate et al. 2002; Scharler and Baird 2003a). Similar to the Mlalazi Estuary, very low ($< 1 \mu\text{g}\cdot\text{l}^{-1}$) chlorophyll a concentrations have been recorded in other South African permanently open estuaries including Palmiet and Gourits Estuary (Adams and Bate 1999).

The permanently open Mlalazi had higher chlorophyll a concentrations than the Mpenjati Estuary. This is in agreement with the reported higher chlorophyll a concentrations in POEs compared to TOCEs of South Africa as a result of continuous water inflow in the POEs which brings along nutrients in these systems. Consequently, these higher levels of nutrients in POEs promote phytoplankton production (Allanson and Read 1995; Grange and Allanson 1995; Froneman 2002b; Perissinotto et al. 2003). The low pelagic chlorophyll a concentrations in both Mlalazi and Mpenjati estuaries during high river inflow in November were suggested to have been caused by estuarine flushing which washed phytoplankton to the adjacent sea. It is also suggested that strong river flow during November caused a reduction in water residence time which is essential for nutrient utilisation for primary producers, hence phytoplankton was unable to trap the nutrients efficiently resulting in low chlorophyll a concentrations. It was suggested that higher chlorophyll a concentrations during May and September in both Mlalazi and Mpenjati estuaries were enhanced by the higher nutrient levels during these sampling sessions. Adams and Bate (1999) reported that nutrient availability regulates phytoplankton biomass in estuaries.

In the Mlalazi and Mpenjati estuaries the microphytobenthic chlorophyll a concentrations were higher during November when compared to May and September sampling sessions. Microphytobenthic chlorophyll a concentrations measured in the Mpenjati Estuary during the current study were higher than those previously recorded in this system (Perissinotto et al. 2002) and other South African TOCEs such as the Great Brak, Nyara and Mdloti estuaries (Adams and Bate 1999). Contrary to the present study, lower microphytobenthic chlorophyll a concentrations have been previously measured during the open phase of the Mpenjati Estuary compared to the closed phase (Perissinotto et al. 2002). Such pattern have been also apparent in Van Stadens and Maitland Estuary (Gama et al. 2005). Few South African TOCEs have shown a similar pattern to that of the present study where higher microphytobenthic chlorophyll a concentrations were recorded during the open compared to the closed phase e.g. in the Mdloti and Kasouga estuaries (Nozais et al. 2001; Froneman 2002b; Froneman 2002a). Microphytobenthic chlorophyll a concentrations recorded in the Mlalazi Estuary during the present study were lower than those previously recorded in few South African POEs such as the Berg, Goukou, Gourits, Gamtoos and Sundays estuaries (Adams and Bate 1999).

Chlorophyll a concentrations measured for microphytobenthos were higher than those of phytoplankton during the present study as reported for several South African estuaries (Froneman 2002b; Froneman 2002a). The temporarily open/closed Mpenjati had higher microphytobenthic chlorophyll a than the permanently open Mlalazi system. Supporting this, Adams and Bate (1999) and Perissinotto et al. (2003) stated that the adequate fresh water inflow in permanently open estuaries supports high biomass of phytoplankton, playing a minor role in controlling benthic microalgae. It has been reported that these TOCEs support high microphytobenthic biomass as a result of the suitable conditions existing in these systems which include low turbidity and low current speed, more stable sediment and high nutrient concentrations in the substratum (Perissinotto et al. 2003).

Highest benthic chlorophyll a concentrations recorded in November during this study were unexpected. According to Perissinotto et al. (2003), during periods of high rainfall which carries suspensoids into the estuary, most phytoplankton cells below the euphotic zone suffer from light limitation which inhibits their photosynthetic machinery. It has also been reported that increased fine sediment in shallow waters can lead to reduced microalgal production (Cahoon et al. 1999).

From the current study, it was speculated that nutrient concentrations in the water column are not the most important factor controlling microphytobenthic chlorophyll a concentration. Elevated November microphytobenthic chlorophyll a concentrations should have been made possible by nutrients buried in the sediment which escape to water column during nutrient recycling.

4.5. Zooplankton

4.5.1. Abundance

Zooplankton mean abundance was higher in May and September than November in both Mlalazi and Mpenjati estuaries respectively. During all sampling sessions copepods *A. natalensis* and *P. hessei* combined comprised more than 90 % of the zooplankton abundance in both Mlalazi and Mpenjati estuaries, a typical phenomenon for South African estuaries (Wooldridge 1999; Jerling 2005). In the Mlalazi Estuary, abundance was generally increasing from the upper towards the lower reaches during the May and November sampling sessions. In the Mpenjati Estuary the highest

mean abundance was recorded from the middle reaches. Highest abundance in the middle reaches of this system has also been reported by Kibirige and Perissinotto (2003).

Very low zooplankton abundance recorded during the high river inflow in both Mlalazi (salinity range of 0.1 - 0.2) and Mpenjati (salinity range of 0.1 - 0.6) estuaries could be a result of the outflow of estuarine water together with zooplankton into the marine environment. Temporal patterns of zooplankton abundance during two sampling sessions of the Mlalazi and Mpenjati Estuary were similar to those of Mhlathuze Estuary where very low abundances were recorded during strong fresh water inflow (Jerling 2008). Similar to the Mpenjati Estuary during the present study, higher zooplankton mean abundance during the closed phase has been previously reported in this system (Kibirige and Perissinotto 2003).

A different spatial distribution pattern to other South African POEs was apparent in the Mlalazi Estuary with lower abundance at the upper reaches. Abundance of copepods was highest in the upper reaches decreasing down the salinity gradient in the Olifants, Great Berg, Breede, Heuningnes, Goukou and Kromme estuaries (Wooldridge and Callahan 2000; Montoya-Maya and Strydom 2009). Zooplankton mean abundance recorded in the Mlalazi Estuary ($23718 \text{ individuals}\cdot\text{m}^{-3} \pm 15689 \text{ SD}$) during the present study was higher than those previously recorded in other South African permanently open estuaries e.g. in the Goukou (mean = $6175 \text{ individuals}\cdot\text{m}^{-3}$), Breede (mean = $4049 \text{ individuals}\cdot\text{m}^{-3}$), Heuningnes (mean = $3877 \text{ individuals}\cdot\text{m}^{-3}$), Great Berg (mean = $6841 \text{ individuals}\cdot\text{m}^{-3}$) and Olifants Estuary ($6269 \text{ individuals}\cdot\text{m}^{-3}$) (Montoya-Maya and Strydom 2009). Mean zooplankton abundance recorded in the Mpenjati Estuary ($8890 \text{ individuals}\cdot\text{m}^{-3} \pm 1769 \text{ SD}$) during the present study was three-fold higher than that previously reported for this system, two-fold higher than that previously recorded in the Diep Estuary and six-fold higher than that previously reported in the Mhlanga Estuary (Kibirige and Perissinotto 2003; Montoya-Maya and Strydom 2009). However, the Mpenjati zooplankton abundance of the present study was 28-fold lower than that previously reported in the Mdloti Estuary (Kibirige et al. 2006) and it was generally lower than that of Van Stadens Estuary (mean = $9278 \text{ individuals}\cdot\text{m}^{-3}$) (Gama et al. 2005).

The permanently open Mlalazi had higher zooplankton abundance compared to the temporarily open/closed Mpenjati Estuary. High zooplankton abundance in the POE is likely to be influenced by the constant freshwater inflow which constantly bring nutrients which promote phytoplankton

biomass (on which zooplankton graze) as reported by Grange et al. (2000) and Wooldridge (1999). Generally, zooplankton abundance was concurrently increasing with the phytoplankton chlorophyll a concentrations during all sampling sessions in both Mlalazi and Mpenjati estuaries. This relationship suggested that zooplankton abundance is controlled by phytoplankton. Such a pattern has been apparent in the permanently open Kariega, Great Fish and Sundays Estuary (Wooldridge and Bailey 1982; Jerling and Wooldridge 1991; Grange et al. 2000) and in a temporarily open/closed Kasouga Estuary (Froneman 2002b; Froneman 2004a).

Although the abundances recorded in the Mlalazi Estuary were higher than those previously recorded in other South African permanently open estuaries, it is in contrast with the statement made by Wooldridge (1999) that most South African estuaries exhibit minimum abundances during low river inflow and maximum abundances during high river inflow. Similar to the Mpenjati Estuary, few South African TOCEs attain their maximum abundance during the closed phase of the estuary (Kibirige and Perissinotto 2003; Montoya-Maya and Strydom 2009). It has been reported that this elevated abundance level is related to stability of an estuary during this period as a result of less freshwater inflow and limited exchange with the sea water (Perissinotto et al. 2003).

4.5.2. Biomass

Zooplankton biomass was higher during May and September compared to November in both Mlalazi and Mpenjati estuaries. The temporal zooplankton biomass pattern of the Mpenjati Estuary during the present study was similar to that previously reported for this system where highest biomass was recorded during the closed phase of the estuary (Kibirige and Perissinotto 2003). High zooplankton biomass values during the closed phase have also been reported in the Mhlanga and Mdloti estuaries (Whitfield 1980; Perissinotto et al. 2003; Kibirige et al. 2006). Contrary to the Mlalazi and Mpenjati Estuary during the current study, higher biomass has been recorded during high river inflow in the Kasouga, Kariega and Great Fish Estuary (Allanson and Read 1995; Grange et al. 2000; Froneman 2001; Froneman 2002a; Froneman 2004b).

It has been reported that zooplankton biomass is often higher in the upper reaches (Grindley 1981). This is in agreement with the zooplankton biomass values recorded in the Mlalazi Estuary during May. In the Mpenjati Estuary highest biomass was recorded in the middle reaches and the lowest was recorded from the upper reaches, a spatial pattern previously reported for the Nyara Estuary

(Perissinotto et al. 2000). This pattern was directly related to the zooplankton abundance which was highest in the middle reaches and lowest in the upper reaches. Mysid *Mesopodopsis africana* recorded in the Mlalazi Estuary had low abundance (11.8 individuals·m⁻³) than the copepods (11115 individuals·m⁻³) but it attained higher biomass in terms of dry weight which suggests that this species contribute more biomass within the system despite its low abundance. Mysids can therefore be a good food source for the secondary consumers which would ingest and accumulate more biomass from these organisms even if their abundance is low.

Zooplankton biomass values recorded in the Mpenjati Estuary (8.9 mg dryweight·m⁻³) during the present study were lower than those previously recorded in this system (280 mg dry weight·m⁻³) and other TOCEs of South Africa such as Nyara (150 mg dry weight·m⁻³), Kasouga (103.5 mg dry weigh·m⁻³), Mhlanga (51.6 mg dry weight·m⁻³) and Mdloti (126.5 mg dry weight·m⁻³) estuaries (Perissinotto et al. 2000; Froneman 2004a; Kibirige et al. 2006). Zooplankton biomass values recorded in the Mlalazi Estuary (8.2 mg dry weight·m⁻³) were lower than those previously recorded in few South African POEs such as the Swartkops (90 mg dry weight·m⁻³), Kariega (47 mg dry weight·m⁻³) and Great Fish estuaries (4253 mg dry weight·m⁻³) (Grindley 1981; Wooldridge 1999; Grange et al. 2000). Higher biomass in these POEs are likely to be attributed from the higher chlorophyll a concentrations reported in these systems, which depicts higher phytoplankton biomass to support higher zooplankton biomass.

4.6. Macrozoobenthos

4.6.1. Species richness and abundance

The number of taxa recorded in the Mlalazi estuary (21) during the study period was lower than that previously recorded in this system (28) (Mabaso 2002) and other South African POEs e.g. Gamtoos Estuary (35) (Schlacher and Wooldridge 1996), Swartkops (28) (Hanekom et al. 1989) and Great Berg Estuary (44) (Wooldridge and Deyzel 2009b). However, the number of taxa recorded in the Mlalazi Estuary during the current study was generally higher than that previously recorded in the Mfolozi-Msunduzi Estuary (17) (Ngqulana et al. 2010). In the Mlalazi Estuary number of taxa was increasing from the upper towards the lower reaches. This trend has been previously reported for South African POEs (Schlacher and Wooldridge 1996).

The numerically dominant group in the Mlalazi Estuary during May were polychaetes (67 %). From the study conducted by Mabaso (2002) in the Mlalazi Estuary, polychaetes also dominated this system during all sampling sessions of the study period. Contrary to the Mlalazi Estuary during the present study polychaetes dominated the Mfolozi-Msunduzi and Gamtoos estuaries during high river flow periods (Ngqulana et al. 2010). The November sampling session of the Mlalazi Estuary was mostly dominated by the amphipods (29 %), contradicting with the Gamtoos Estuary where amphipods dominated the system during low river flow period (Schlacher and Wooldridge 1996). Such change in dominant groups with river flow conditions has been reported in South African estuaries and has been explained as the dynamic nature of community change in the benthos (Wooldridge and Deyzel 2009b).

In the Mpenjati Estuary the most numerically dominant groups were polychaetes during September (94 %) and November (76 %) sampling sessions, a similar pattern previously reported for many South African TOCEs e.g. in the Zinkwasi (83 %), Mhlanga (62 %), Isipingo (81 %), Kandandlovu (67 %), Manzimtoti (87 %), Zotsha (67 %) and Uvuzana (82 %) estuaries (Stow 2011).

Mean abundance of macrozoobenthos was higher during May ($3287 \text{ individuals} \cdot \text{m}^{-2} \pm 1735 \text{ SD}$) than November ($1102 \text{ individuals} \cdot \text{m}^{-2} \pm 897 \text{ SD}$) in the Mlalazi Estuary while abundance was higher during November than September in the Mpenjati Estuary. Macrozoobenthos abundance recorded in the Mlalazi Estuary during the current study was 44-fold lower than that previously recorded in this system (Mabaso 2002). Macrozoobenthos abundance in the Mlalazi Estuary during the present study was 3-fold lower than that reported in the Gamtoos and Great Berg estuaries (Wooldridge and Deyzel 2009a). Contrary to the present study, Mlalazi lower macrozoobenthos abundances have been reported during period of low river flow (Mabaso 2002). Similar to the Mlalazi Estuary, low macrozoobenthos abundances have been reported during high river inflow in the Great Berg and Gamtoos estuaries (Wooldridge and Deyzel 2009a).

Mean macrozoobenthos abundance recorded in the Mpenjati Estuary during the current study was two-fold lower than that previously recorded in the Siyaya, Zinkwazi, Kandandlovu, and Zotsha estuaries and was 4-fold lower than that recorded in the Mhlanga, Isipingo, Manzimtoti, and Uvuzana estuaries (MacKay 1996; Stow 2011). Contrary to the Mpenjati estuary during the current study, highest abundance in the Siyaya Estuary was recorded during low river inflow (MacKay 1996). In both Mlalazi and Mpenjati estuaries, abundance was generally increasing from the upper

towards the lower reaches during May and September respectively, a case which has been reported for few South African estuaries (Schlacher and Wooldridge 1996).

4.6.2. Biomass

Information is scarce regarding the biomass of macrozoobenthos in South African estuaries. Most of the studies conducted in South African estuaries investigate more about macrozoobenthos abundance and community structure and very little attention has been given to biomass. In the Mlalazi Estuary macrozoobenthos biomass was higher during May ($57.6 \text{ mg dry weight}\cdot\text{m}^{-2} \pm 38.5 \text{ SD}$) compared to November ($28.9 \text{ mg dry weight}\cdot\text{m}^{-2} \pm 7.7 \text{ SD}$) while in the Mpenjati Estuary higher biomass was recorded during November ($19.0 \text{ mg dry weight}\cdot\text{m}^{-2} \pm 2.6 \text{ SD}$) compared to September ($16.0 \text{ mg dry weight}\cdot\text{m}^{-2} \pm 5.5 \text{ SD}$). Highest macrozoobenthos biomass during May in the Mlalazi Estuary and during November in the Mpenjati Estuary was associated with the highest abundance during these sampling sessions in these two estuaries.

The macrozoobenthos biomass recorded in the Mlalazi Estuary during the present study was 362-fold lower than that previously recorded in the Swartkops Estuary (Hanekom et al. 1989). The highest contribution of molluscs (49 %) to the macrozoobenthic biomass (although with the lowest abundance) of the Mlalazi Estuary contradicted with that of the Swartkops Estuary where crustaceans, mainly *Upogebia africana* (85 %) and *Callichirus kraussi* (10 %) contributed highest biomass (Hanekom et al. 1989). Overall, the macrozoobenthos biomass of the Mpenjati estuary was 4-fold lower than that of the permanently open Mlalazi Estuary during the current study and it was 1105-fold lower than that of the Swartkops Estuary. However the biomass per m^2 of the *Callichirus kraussi* of the temporarily open/closed Mpenjati Estuary was 1600 fold lower than that recorded for the permanently open Swartkops Estuary (Hanekom et al. 1989).

4.7. Phosphorus content of biota and sediment

4.7.1. Phytoplankton

There is scarce published information on the phytoplankton phosphorus content in South African estuaries. Few South African studies have estimated phytoplankton carbon content on few marine systems (Schleyer 1981; Brown et al. 1991). Phytoplankton P content ($\text{mgP}\cdot\text{m}^{-2}$) was higher during May and September when compared to November sampling session for both Mlalazi and Mpenjati

estuaries. The co-variation in phytoplankton chlorophyll a and phosphorus biomass was expected because same ratios were used for chlorophyll a to phosphorus conversion.

4.7.2. Zooplankton

During May, copepod *A. Natalensis* comprised the highest phosphorus content (1.7 %) of all zooplankton taxa in the Mlalazi Estuary. During September copepod *P. hessei* comprised the highest phosphorus content (0.6 %) of all zooplankton taxa in the Mpenjati Estuary. No other studies have been conducted in South African estuaries to examine P content of zooplankton.

Zooplankton P content has been examined in other parts of the world and most of these studies were conducted in lakes (Sternner 1990; Andersen and Hessen 1991; Hassett et al. 1997; Vrede et al. 1999; Dobberfuhl and Elser 2000). Phosphorus content measured in zooplankton of the Mlalazi Estuary during May was generally higher than that measured in zooplankton of Lake Erken in Sweden (1.4 %). Zooplankton P content in the Mpenjati Estuary was 4-fold lower than that of Lake Erken in Sweden. Differing food quality between these systems can explain such variation.

There were no significant differences in P content between zooplankton taxa in both Mlalazi and Mpenjati estuaries. Similar to the current study, Vrede et al. (1999) reported no significant variations in P content between three zooplankton taxa collected in different lakes. In contrast to the present study, interspecific and intraspecific differences in species P content was reported in Baltic Sea although it was stated that P content variation within species i.e. *Acartia* sp. was due to developmental stage (Walve and Larsson 1999).

4.7.3. Macrozoobenthos

Polychaetes comprised the highest phosphorus content of all macrozoobenthic taxa in the Mlalazi Estuary during May (0.4 %) and Molluscs comprised the highest P during November (0.4 %) sampling session. This elemental P content in molluscs for this study represents only soft tissue of the organisms. Shells were excluded because Evans-White et al. (2005) reported that the high calcium carbonate in molluscs shells reduces the overall content of P in these organisms. Polychaetes comprised the highest phosphorus content of all macrozoobenthic taxa in the Mpenjati Estuary during September (1.2 %) and November (0.4 %). There have been no studies examining macrozoobenthos phosphorus content in South African estuaries. However, macrozoobenthos P

content has been measured in other parts of the world in freshwater and marine systems (Frost et al. 2003; Cross et al. 2005; Evans-White et al. 2005; Martinson et al. 2008).

Contrary to the Mlalazi Estuary during the present study, higher P content (%) in crustaceans than molluscs was reported for few lakes in Canada and few streams in North America (Frost et al. 2003; Evans-White et al. 2005). Evans-White (2005) stated that higher P content (%) in crustaceans is associated with calcium in carapaces of benthic crustaceans. The highest P content (%) of molluscs in the Mlalazi Estuary was recorded during November. This high P content was suggested to be related to the high PP concentrations measured during this sampling session, which elevated phosphorus nutrient levels for filter feeding bivalves of this system. Contrary to the current study, significant variations in P content (%) among different benthic taxa have been reported in few streams of North America (Evans-White et al. 2005; Liess and Hillebrand 2005). Molluscs phosphorus content measured in the Mlalazi Estuary was two-fold higher than that recorded in 35 streams in North America (Evans-White et al. 2005). It is speculated that the elevated P content in molluscs of Mlalazi Estuary was subject to relatively higher phosphorus concentrations in the estuaries than the fast flowing streams. Overall, the P content of the macrozoobenthos of the Mlalazi Estuary was similar to that of eight Canadian lakes where highest P content of 1.6 % was reported out of 9 taxa combined from all lakes investigated (Frost et al. 2003). Contrary to the present study, variations in P content within and between species have been reported in marine systems (Clarke 2008). High phosphorus biomass in the prawns during the current study was suggested to be a result of high biomass (dry weight) of these organisms.

Chapter 5

Summary and conclusions

This study aimed at determining phosphorus distribution in biotic and abiotic nutrient pools of the permanently open Mlalazi and temporarily open/closed Mpenjati estuaries. Biological data including chlorophyll a, macrozoobenthos and zooplankton were analysed and their abundance and biomass (zooplankton and macrozoobenthos) as well as concentration (chlorophyll a) was compared along the estuarine salinity gradients, between sampling sessions and between estuaries. It was hypothesised that the abundance and biomass of the fauna will change with stations, different river inflows (sampling sessions) and along the estuarine salinity gradient. It was also hypothesized that standing stocks of living and non living nutrient pools in terms of phosphorus content change with low (May and September for Mlalazi and Mpenjati Estuary respectively) and high (November for both estuaries) river flow and along the length of the two KZN estuaries. The current study was the first to examine phosphorus content of biota in South African estuaries.

Dissolved inorganic phosphorus concentrations were higher during May and September than November in the Mlalazi and Mpenjati Estuary respectively. In the Mlalazi and Mpenjati estuaries, there were significant differences in DIP concentrations between sampling sessions and between stations. Particulate phosphorus concentrations were higher during November than May and September in both estuaries with highest concentrations recorded from the upper reaches. Significant differences in PP concentrations between sampling sessions and stations were apparent in both estuaries.

Phytoplankton chlorophyll a concentrations were higher during May and September than November in both Mlalazi and Mpenjati Estuary respectively. Contrary to phytoplankton chlorophyll a concentrations, microphytobenthos chlorophyll a concentrations were higher during November when compared to May and September. Chlorophyll a temporal patterns observed in the Mpenjati Estuary were similar to those of few South African estuaries e.g. (Perissinotto et al. 2003; Gama et al. 2005; Thomas et al. 2005) where high chlorophyll a concentrations were recorded during the closed compared to the open phase. Distribution of phytoplankton chlorophyll a concentrations along the Mlalazi Estuary length during November was dissimilar to that of few South African POEs where concentrations were decreasing from the upper towards the lower reaches e.g. (Snow et al. 2000; Bate et al. 2002; Scharler and Baird 2003a).

Zooplankton abundance was higher during May and September when compared to the November sampling session in both Mlalazi and Mpenjati estuaries respectively. Spatial distribution of zooplankton abundance in the Mlalazi Estuary with the increasing abundance from the upper to the lower reaches differed from that of other South African estuaries e.g.(Wooldridge and Callahan 2000; Montoya-Maya and Strydom 2009). Zooplankton biomass in this system showed a different trend from that of abundance with the biomass decreasing from the upper towards the lower reaches.

Macrozoobenthos abundance of the Mlalazi estuary was higher during May than November while abundance in the Mpenjati Estuary was higher during November than September. Macrozoobenthos abundance from the Mlalazi Estuary during the present study was similar to those of other South African POEs e.g. (Wooldridge and Deyzel 2009a). Macrozoobenthos biomass in the Mpenjati Estuary during September was concurrently increasing with the abundance. However, in November, biomass was increasing with a decrease in abundance, indicating that species recorded during November had low abundance with relatively high biomass.

In zooplankton, copepod *A. Natalensis* comprised the highest phosphorus content in the Mlalazi Estuary while copepod *P. hessei* comprised the highest P content in the Mpenjati Estuary. In macrozoobenthos of the Mlalazi Estuary, polychaetes comprised the highest P content during May and molluscs comprised the highest P content in November. Polychaetes comprised the highest P content in the Mpenjati Estuary during both sampling sessions. The current study demonstrated that phosphorus biomass does not always correlate with biomass in terms of dry weight, although *P. hessei* in the Mpenjati Estuary and *M. africana* in the Mlalazi Estuary showed similar levels of phosphorus biomass to those of biomass in terms of dry weight. During November, Molluscs displayed highest P biomass as well as highest biomass in terms of dry weight in the Mlalazi Estuary. Other benthic groups (e.g. polychaetes) had higher biomass in terms of dry weight but displayed relatively low P biomass. Overall, macrozoobenthos showed no significant differences in P content between taxa, sampling sessions and between estuaries. Zooplankton also showed no significant differences between taxa, stations and between the two estuaries.

Overall, the highest phosphorus biomass was contained in sediment in both Mlalazi and Mpenjati estuaries. Although the percentage P levels in sediment were more or less similar to those of the biota and PP, the ubiquity of sediment contributed to high P biomass. Following sediment, higher P

content was in the form of dissolved inorganic phosphorus. In both estuaries the lowest phosphorus was contained in zooplankton. Phytoplankton P biomass was higher than that of microphytobenthos in the Mlalazi Estuary while the opposite was observed in the Mpenjati Estuary.

The shortcomings of the study lied on sampling (sampling months/sessions). The sampling months of the Mlalazi and Mpenjati Estuary were different for representation of the low river flow, although this was only determined by the levels of rainfall to choose a proper sampling month for the temporarily open/closed Mpenjati. This led to two different months (May and September) to be compared for the two estuaries unlike the period of high river flow represented by November for both estuaries. Additionally, increasing the size of the sample i.e. the number of sampling sessions in both Mlalazi and Mpenjati would greatly enhance comparability between estuaries and sampling sessions. However, this attribute was limited by the length of the study period. In the future it is recommended that more components e.g. fish, meiofauna and bacteria are included to get a better understanding of phosphorus distribution in estuarine food webs. Classification of macrozoobenthos to feeding guilds may also give a better resolution to the P content comparison for the future studies to see if the diet of certain groups brings any changes to organisms P content.

Chapter 6

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