Zooplankton dynamics and ecophysiology in the St. Lucia Estuary, with emphasis on the dominant mysid *Mesopodopsis africana*

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ABSTRACT

The St. Lucia Estuary, Africa's largest estuarine lake, is currently experiencing an unprecedented crisis related to freshwater deprivation. This has resulted in a reversed salinity gradient and drastically reduced water levels. These harsh environmental conditions, combined with the limited connection with the open ocean have lead to a loss of biodiversity in the system. The dominant zooplankton taxa include the copepods *Pseudodiaptomus stuhlmanni* and *Acartia natalensis* and the mysid *Mesopodopsis africana*. In March 2007, the closed-mouth state was briefly interrupted by an open-mouth phase, induced by a unique combination of extreme climatic events. With the incoming seawater, previously excluded marine taxa re-entered the system, increasing its diversity significantly. Salinity and temperature have been referred to as driving forces in aquatic ecosystems. The tolerance limits of the key mysid species were, therefore, investigated. Results showed that *M. africana* has some of the highest recorded upper salinity and temperature tolerances for a mysid. Because of their high biomass, mysids have the potential to affect microalgal standing stocks. Their grazing dynamics (in relation to autotrophic food availability) were investigated in two contrasting environments within the estuary. Ingestion rates and subsequently population grazing impacts on the total microalgal standing stocks were higher at the Mouth than at Charters Creek. This was attributed to the harsh environmental conditions in the latter region. Despite the lower ingestion rates exhibited here, these mysids seem capable of meeting their energetic requirements from a microalgal diet alone. Stable isotope data, though, show that they also utilise a heterotrophic diet. Results of the mixed model SIAR v 4 revealed the contribution of the different carbon sources to the diet of *M. africana*. Most unique was this mysid's ability to modify its diet on both short temporal and spatial scales. Resource utilization between the dominant taxa was also compared. All three taxa appear to be opportunistic feeders, capable of incorporating a number of food items in their diet. Between food partitioning, predator avoidance strategies, and their common ability to survive in highly dynamic environments, these species are capable of co-existing, and together contribute to the overall resilience so far shown by the system.

PREFACE

The experimental work described in this PhD thesis was carried out in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Westville, from January 2007 to November 2010, under the supervision of Professor Renzo Perissinotto.

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.

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I, **Nicola Kim Carrasco** declare that:

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DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis are as follows:

Publication 1

Carrasco NK, Perissinotto R, Pillay D (2010) Zooplankton of the St. Lucia Estuary during the current drought cycle: a comparison between open- and closed-mouth conditions. Mar Ecol Prog Ser 399: 157 – 171

Publication 2

Carrasco NK, Perissinotto R (2011a) Temperature and salinity tolerance of *Mesopodopsis africana* in the freshwater-deprived St. Lucia Estuary, South Africa. J Exp Mar Biol Ecol 399: 93 – 100

Publication 3

Carrasco NK, Perissinotto R (2010a) *In situ* feeding rates and grazing impacts of the mysid *Mesopodopsis africana* O Tattersall in the St. Lucia Estuary, South Africa. J Exp Mar Biol Ecol 396: 61 – 68

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For whatever we lose (like a you or a me),
It's always our self we find in the sea.
~E.E. Cummings

For Merron Martin,

In life you taught me to love, have faith and always persevere; and in death, you taught me the importance of living each moment to the full and embracing each given opportunity to experience the new and follow our dreams.

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Abbreviations and Notations

b	Gut Pigment Destruction
Chl *a*	Chlorophyll *a*
DO	Dissolved Oxygen
DW	Dry Weight
G	Gut Pigment Content
GRT	Gut Residence Time
I	Ingestion Rate
k	Gut Evacuation Rate
MAB	Microalgal Biomass
MDS	Multi Dimensional Scaling
MPB	Microphytobenthic Biomass
NTU	Nephelometric Turbidity Units
PP	Primary Production
PPL	Phytoplankton Biomass
POM	Particulate Organic Matter
R	ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$
RPM	Revolution Per Minute
SD	Standard Deviation
SE	Standard Error
SOM	Sedimentary Organic Matter
SPM	Suspended Particulate Matter
TL	Trophic Level
TOCE	Temporarily Open/Closed Estuary
X	^{13}C or ^{15}N
$\delta^{15}N_c$	nitrogen isotopic composition of the consumer

$\delta^{15}N_{base}$ nitrogen isotopic composition of the food base

λ trophic level of the base ($\lambda = 1$ for primary producers)

Δ_n estimate of the average increase in $\Delta^{15}N$ per trophic level

GENERAL INTRODUCTION

Estuaries are dynamic ecosystems characterized by rapid and intense fluctuations in physico-chemical factors. These ecosystems usually support rich biotic communities, which are often spatially and seasonally variable (McLusky and Elliott 2004). The St. Lucia Estuary forms part of the iSimangaliso (formerly Greater St. Lucia) Wetland Park. iSimangaliso is South Africa's first UNESCO World Heritage Site and has been designated a Ramsar Wetland of international importance (Begg 1978, Cyrus and Vivier 2006). It is the largest estuarine lake in Africa (Fielding *et al*. 1991, Cyrus and Vivier 2006). Covering an area of 325 km^2, it represents approximately 80% of KwaZulu-Natal's total estuarine area (Begg 1978).The lake system is governed by cyclical wet and dry periods, each lasting between four and ten years (Begg 1978). St. Lucia is currently experiencing severe freshwater deprivation, with below average rainfall persisting since 2002. Drought conditions have been exacerbated by the canalisation of the Mfolozi River, and the subsequent diversion of its freshwater away from the St. Lucia Estuary, in order to avoid the perceived threat of siltation (Whitfield and Taylor 2009). The present drought has led to a drastic reduction in water levels and concomitant salinity increases. A reversed salinity gradient has persisted, with the occurrence of hypersaline conditions in the northern regions of the lake. The hypersaline conditions (70 to 90) that developed in the North Lake between 1969 and 1971, as a direct result of a previous drought, led to a number of extraordinary changes in some of the basic trophic relations. These involved mainly: (1) a bloom of dinoflagellates (Grindley and Heydorn 1970); (2) the dominance of chironomid larvae and harpacticoid copepods in bentho-pelagic samples (Grindley 1982); (3) a population explosion of aerial spiders; and (4) the loss of most of the plankton present, leaving only few species with high salinity tolerance (Grindley 1981).

The central focus of this PhD study is to investigate aspects of zooplankton ecology in the St. Lucia Estuary and set these in a regional and global context. A range of studies have highlighted the importance of zooplankton as indicators of change in marine systems (e.g. Bonnet and Frid 2004, Beaugrand 2005). Their rapid response to ecosystem perturbations (Kiørboe and Nielsen 1994, Hays *et al.* 2005) is achieved through strong coupling between environmental change and plankton dynamics (Roemmich and McGowan 1995). In temporarily open/closed estuaries, maximum zooplankton abundance and biomass is generally recorded during closed-mouth conditions (Whitfield 1980, Kibirige and Perissinotto 2003a, Kibirige *et al*. 2006), with communities usually dominated by a few euryhaline taxa (Froneman 2004a). Breaching events lead to the loss of zooplankton and their food resources, leaving low densities and biomass during open-mouth conditions. Species richness, on the other hand, is typically greater during

open phases, due to recruitment of marine neritic species within the estuary (Froneman 2004a). Increases or decreases in precipitation and runoff may create extreme events, floods or droughts respectively, and are increasing in frequency worldwide (Gleick 2003, Mirza 2003). The effects of such events remain unknown for estuarine communities, and are probably crucial for naturally variable systems such as the St. Lucia Estuary. Breaching in temporarily open/closed estuaries usually occurs because of increased precipitation in the catchment and greater head pressure in the lower estuary, resulting in a break of the sandbar (Whitfield 1992). However, after remaining closed since June 2002, on the 3rd of March 2007, the St. Lucia Estuary breached from the seaward side, due to the combined effects of Cyclone Gamede and a spring equinox. This resulted in exceptional wave energy development in the surf zone and the consequent breakage of the ~100 m sand berm (R Taylor, pers. comm.). This breaching event allowed sea water to enter the estuary for the first time in approximately five years, thus providing the unique opportunity to determine the responses of zooplankton to open- and closed-mouth conditions, in terms of community structure, abundance and biomass. This research component is addressed in detail in Chapter 1.

Zooplankton communities in St. Lucia, like most estuarine zooplankton assemblages in southern Africa (Wooldridge 1999), are dominated by typical estuarine taxa, such as *Pseudodiaptomus stuhlmanni*, *Acartia natalensis* and *Mesopodopsis africana,* which often collectively contribute over 90% of the total zooplankton abundance (Carrasco *et al.* 2010: Chapter 1). While copepods are often numerically abundant, mysids usually dominate zooplankton biomass (Carrasco *et al*. 2010: Chapter 1, Wooldridge 1999). Mysids are relatively adaptive species, capable of enduring the rapid and intense fluctuations in physico-chemical factors often associated with estuarine ecosystems. Salinity and temperature are major driving forces in aquatic ecosystems, especially in St. Lucia, where freshwater deprivation has fractionated the system into a variety of different habitats. Grindley (1976, 1982) earlier recorded salinity ranges for many of the common zooplankton taxa inhabiting the estuary, and suggested that *M. africana* could tolerate salinity levels up to 60. However, this conclusion was based on the occurrence of adults at ranges of salinity in the natural environment, rather than on tolerance limits defined through laboratory experimentation. Chapter 2, therefore, aims to determine the temperature and salinity tolerance of *M. africana* both through field collections and experimental studies.

Mysids play an important role in food webs as both consumers and producers (Mauchline 1980). As consumers, mysids are generally regarded as omnivores, capable of successfully exploiting a variety of food resources. Diet may include phytobenthos, phytoplankton, detritus, sedimentary organic matter, microzooplankton, mesozooplankton and small benthic

invertebrates (Wilhelm *et al*. 2002, Kibirige and Perissinotto 2003a, Lehtiniemi and Nordström 2008, Vilas *et al*. 2008). Mysids may feed on phytoplankton, detritus and smaller zooplankton by means of a suspension-feeding current or, alternatively, actively prey on moving zooplankton by means of raptorial or ambush feeding (Mauchline 1980). The ability of mysids to feed selectively on prey of different sizes can modify the structure of estuarine zooplankton (Fulton 1982, Hansson *et al*. 1990, Kouassi *et al*. 2006) and phytoplankton (Lindén and Kuosa 2004). Mysids link primary and secondary production with higher trophic levels (Mees and Jones 1997, Viherluoto and Viitasalo 2001, Lehtiniemi *et al*. 2009), being important in the diets of a number of estuarine resident and juvenile fish species (Whitfield 1998). In Chapter 3 the trophic role of *Mesopodopsis africana* within the St. Lucia Estuary is investigated. This study compares the feeding rates and grazing impact of *M. africana* on microalgal biomass and production at two different stations within this unique lake complex: Charters Creek, which has been severely affected by the desiccation process; and the Mouth, which remains virtually unchanged.

While *M. africana* plays a major role in the trophic functioning of the system, it has disappeared from large sections of the estuarine lake due to the ongoing drought, currently occurring only in its mouth region and in parts of the Narrows and South Lake (Fig. 1.1). Knowledge of food web properties, such as trophic processes, enables inferences to be made about species interactions and ecosystem structure and function. The use of stable isotopes in ecological research has been recognized as an important tool towards this end. Since stable carbon isotopes ($\delta^{13}C$) are known to fractionate little between energy transfers, they are commonly used to quantify food sources and energy flow in aquatic systems. Stable nitrogen isotopes ($\delta^{15}N$) fractionate more and are typically used to infer trophic positions of consumers in a food chain (DeNiro and Epstein 1978, Peterson and Fry 1987). Models may allow simulation of possible diets and assessment of the relative importance of various sets of organic matter sources to consumers (Phillips and Eldridge 2006). Knowledge of trophic linkages would be especially relevant information for management, since certain food chain links may disappear due to environmental stresses, such as those experienced in the St. Lucia Estuary. The aim of Chapter 4 was, therefore, to assess the spatial and temporal feeding preferences of this key zooplankton species under different environmental conditions. Stable $\delta^{13}C$ and $\delta^{15}N$ isotopes were thus used to gain insight into the trophic interactions and diet of *Mesopodopsis africana* within the St. Lucia Estuary. Additionally, the contribution of different carbon sources to the diet of the other two co-dominant zooplankton species found in the St. Lucia Estuary, *Pseudodiaptomus stuhlmanni* and *Acartia natalensis*, are compared with those of *M. africana*, in order to better understand the

ecosystem functioning of the estuary during this period of crisis. This is dealt with in the final Chapter 5.

Overall, this study documents the changes that occur in the zooplankton during different mouth conditions and investigates the role which dominant species, such as *Mesopodopsis africana*, play in linking primary production with higher trophic levels, as well as regulating the availability of primary producers. Outcomes of this study will not only add to the available information on functioning of the St. Lucia estuarine lake, but also better inform its management during periods of extreme stress such as the present one.

In summary, the main objectives of the study were as follows:

1) to determine the responses of zooplankton to open- and closed-mouth conditions, in terms of community structure, abundance and biomass;
2) to investigate the temperature and salinity tolerances of *M. africana,* both through experimental studies and collection of field data;
3) to compare the feeding rates and grazing impact of *M. africana* on microalgal biomass and production in two regions of the estuary that have been impacted differently by the desiccation process;
4) to assess the spatial and temporal feeding preferences of *M. africana* in the St. Lucia Estuary under different environmental conditions, with the use of stable $\delta^{13}C$ and $\delta^{15}N$ isotopes;
5) to compare the diet composition of the three dominant taxa normally found within the estuary, *M. africana, P. stuhlmanni* and *A. natalensis*, under different environmental conditions.

Zooplankton of the St. Lucia Estuary during the current drought cycle: a comparison between open- and closed-mouth conditions

ABSTRACT

The St. Lucia Estuary is currently experiencing a drought-induced crisis, resulting in the system being closed off from the sea for approximately eight years. This closure was interrupted by a brief open-mouth phase, induced by a unique combination of extreme climatic events. The primary aim of the present study was to compare zooplankton dynamics during open- and closed-mouth conditions. Sampling was undertaken during quarterly surveys from February 2006 to November 2008. During the closed-mouth phase, up to 70% of the lake bed was dry and salinity levels in the northern lakes often exceeded 90, making these areas largely uninhabitable for zooplankton. However, in the lower regions where drought effects were less harsh, zooplankton was characterized by high densities and biomass of typical estuarine taxa such as the copepods *Pseudodiaptomus stuhlmanni*, *Acartia natalensis* and the mysid *Mesopodopsis africana*. Of the 69 taxa recorded during the study period, only 27 were present during the closed-mouth phase. Under open-mouth conditions, previously excluded marine taxa (e.g. the prawn *Penaeus indicus*), once again re-entered the system, increasing its diversity significantly. A unique occurrence after mouth re-closure, was the colonisation of the mouth area by swarms of the tunicate *Oikopleura dioica* (> 10^3 ind.m^{-3}) while, previously dominant zooplankton grazers virtually disappeared from there. These findings emphasize the complexity of the system and stress the need for further research into the potential impacts of environmental and climate changes on this key African estuarine lake.

Keywords: drought, extreme events, *Oikopleura dioica*, St. Lucia Estuary, zooplankton

1.1 INTRODUCTION

The St. Lucia Estuary, on the eastern seaboard of South Africa, is a system that experiences periodic isolation from the Indian Ocean. This normally occurs during periods of low or no river inflow. Under such conditions, a sand berm may form across the estuary mouth (Taylor 2006). Similar estuaries are present in a variety of regions worldwide, e.g. Australia (Roy *et al*. 2001), Brazil and Uruguay (Bonilia *et al.* 2005), India and Sri Lanka (Ranasinghe and Pattiaratchi 2003) and the United States of America, particularly in California and Texas and occasionally

as far north as Long Island, New York (Gobler *et al*. 2005). Within South Africa, approximately 70% of the currently functional estuaries are of this nature, with most of them being specifically classified as temporarily open/closed estuaries (TOCEs, Whitfield 1992).

The St. Lucia Estuary forms part of the iSimangaliso (formerly Greater St. Lucia) Wetland Park. iSimangaliso is South Africa's first UNESCO World Heritage Site and has been designated a Ramsar Wetland of international importance (Begg 1978, Cyrus and Vivier 2006). It is the largest estuarine lake in Africa (Fielding *et al*. 1991, Cyrus and Vivier 2006). Covering an area of 325 km^2, it represents approximately 80% of KwaZulu-Natal's total estuarine area (Begg 1978).The lake system is governed by cyclical wet and dry phases, each lasting between four and ten years (Begg 1978). St. Lucia is currently experiencing a drought cycle, with below average rainfall persisting since 2002. Prior to 1920, the Mfolozi River discharged into the St. Lucia Estuary and would buffer water loss during periods of drought, but in the 1930s a canal was excavated through the Mfolozi flats for agricultural purposes (Begg 1978). The natural filtration system of the swamps was, therefore, destroyed and the two systems have since been artificially maintained separate in an attempt to avoid the perceived threat of siltation from the Mfolozi. This alteration of the system's catchment has further exacerbated the severity of the current drought cycle. The present drought has led to a drastic reduction in water levels and concomitant salinity increases. A reversed salinity gradient has persisted, with the occurrence of hypersaline conditions in the northern regions of the lake. The hypersaline conditions (70 to 90) that developed in the North Lake between 1969 and 1971 as a direct result of a previous drought, led to a number of extraordinary changes in some of the basic trophic relations. These involved mainly: (1) a bloom of dinoflagellates (Grindley and Heydorn 1970); (2) the dominance of chironomid larvae and harpacticoid copepods in bentho-pelagic samples (Grindley 1982); (3) a population explosion of aerial spiders; and (4) the loss of most of the plankton present, leaving only few species with high salinity tolerance (Grindley 1981).

Breaching in temporarily open/closed estuaries usually occurs because of increased precipitation in the catchment and greater head pressure in the lower estuary, resulting in a break of the sandbar (Whitfield 1992). On the 3rd of March 2007, however, the St. Lucia Estuary breached from the seaward side, due to the combined effects of Cyclone Gamede and a spring equinox. This resulted in exceptional wave energy development in the surf zone and the consequent breakage of the ~100 m sand berm (R Taylor, pers. comm.). This breaching event allowed sea water to enter the estuary for the first time in approximately five years and was unique in that the estuary was still experiencing drought conditions in the region.

A range of studies have highlighted the importance of zooplankton as indicators of change in marine systems (e.g. Bonnet and Frid 2004, Beaugrand 2005). Their rapid response to ecosystem perturbations (Kiørboe and Nielsen 1994, Hays *et al.* 2005) is achieved through strong coupling between environmental change and plankton dynamics (Roemmich and McGowan 1995). In temporarily open/closed estuaries, maximum zooplankton abundance and biomass is generally recorded during closed-mouth conditions (Whitfield 1980, Kibirige and Perissinotto 2003a, Kibirige *et al.* 2006), with communities usually dominated by a few euryhaline taxa (Froneman 2004a). Breaching events lead to the loss of zooplankton and their food resources, leaving low densities and biomass during open-mouth conditions. Species richness, on the other hand, is typically greater during open phases, due to recruitment of marine neritic species within the estuary (Froneman 2004a). Increases or decreases in precipitation and runoff may create extreme events, floods or droughts respectively, and are increasing in frequency worldwide (Gleick 2003, Mirza 2003). The effects of such events remain unknown for estuarine communities, and are probably crucial for naturally variable systems such as the St. Lucia Estuary. From a zooplankton perspective, no comprehensive survey has been conducted in the St. Lucia Estuary, aside from the relatively outdated work by Grindley (1976, 1982). This study, therefore, aims to determine the responses of zooplankton to open- and closed-mouth conditions, in terms of community structure, abundance and biomass. This knowledge would contribute significantly to the synthesis of information on the secondary producers of the St. Lucia Estuary, enhancing understanding of the ecology of this system, especially in response to environmental fluctuations.

1.2 MATERIALS AND METHODS

Quarterly surveys were undertaken at five representative stations within the St. Lucia Estuary, from February 2006 to October 2008. These stations included: the Mouth, Esengeni, Catalina Bay, Charters Creek and Listers Point (Fig. 1.1). The study period covered three different hydrological phases: a closed phase (February 2006 to February 2007), an open phase (March 2007 to August 2007), and a re-closed period (November 2007 to October 2008). Zooplankton, phytoplankton and microphytobenthic samples together with physico-chemical data were collected at each site on each sampling occasion.

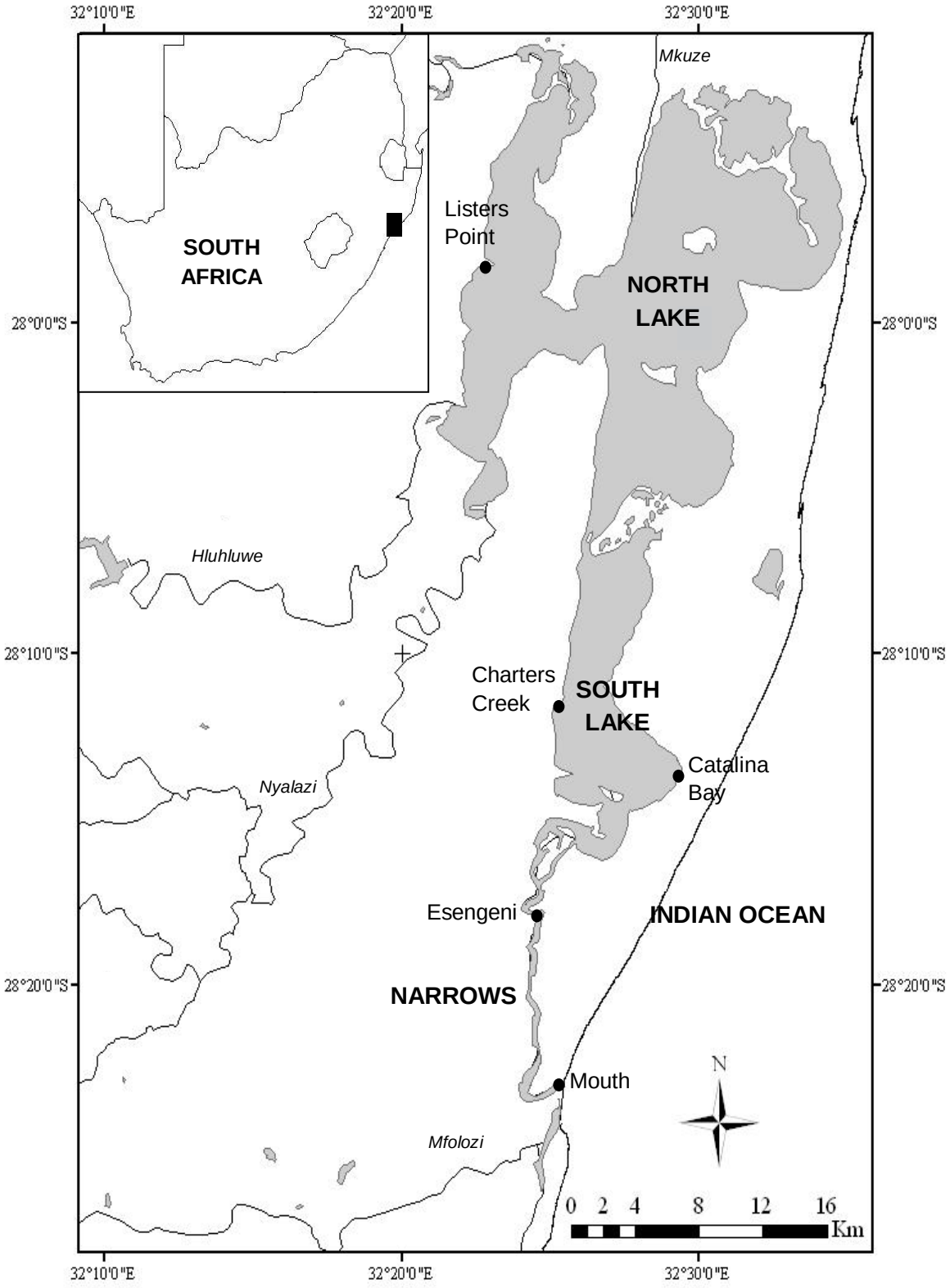

Fig. 1.1: Map of the St. Lucia Estuary showing sampling sites and its geographical position within South Africa.

1.2.1 Physico-chemical variables

Physico-chemical measurements were taken with a YSI 6920 water quality logger, fitted with temperature (˚C), depth (m), dissolved oxygen ($mg.L^{-1}$), pH and turbidity (Nephelometric Turbidity Units or NTUs) probes. Where possible, measurements were made at both the surface and the bottom of the water column. In cases where the water level was less than 10 cm deep, the probe was placed horizontally so that all the sensors were submerged.

1.2.2 Pelagic and benthic microalgae

Subsurface water samples were collected at each of the five stations during each sampling season. Near-bottom water samples were collected with a pop bottle at sites where water levels were deep enough. One 250 mL subsample was filtered through a GF/F filter to determine the total concentration of chlorophyll *a* (chl *a*). Phytoplankton biomass (mg $pigm.m^{-3}$) was determined fluorometrically (Turner Designs 10-AU) after cold extracting chl *a* and phaeopigments from filters in 10 mL 90% acetone for 48 h in the dark. Microphytobenthic cores (2 cm internal diameter, n = 3, depth = 1cm) were collected on each occasion at each station and placed in 100 mL polyethylene bottles containing 30 mL 90% acetone for microphytobenthic chl *a* extraction (Nozais *et al*. 2001). Biomass was again determined fluorometrically and expressed as mg $pigm.m^{-2}$.

1.2.3 Zooplankton

Single daytime mesozooplankton samples were collected using an epibenthic sled fitted with 200 µm mesh (open area ratio ≈ 3:1). This method was employed due to the shallow water levels and the diel migration pattern of many of the zooplankton taxa present in the system. The sled was towed in the shallow waters near-shore at all stations, except at Esengeni, where a boat was used. The mouth of the net was semi-circular in shape (radius = 18.5 cm) and was mounted on a sled such that the net was raised 7.5 cm above the sediment surface. The volume of water filtered (≈ 1.43 m^3) was calculated by multiplying the area of the sled mouth by the distance towed (27 m). The sample was emptied into a 500 mL bottle containing 4% phloxine-stained formaldehyde. In regions where water depth was too shallow for the sled to be used, a hand held D-net (radius = 28 cm) with the same mesh size was driven over a 20 m transect. Many of the zooplankton taxa in the St. Lucia Estuary exhibit diel vertical migration, however, given that the water depth at most of the stations sampled was < 0.5 m, sampling with the epibenthic sled

filtered water from almost the entire water-column. In cases where water depth exceeded 1 m (i.e. the Mouth and Esengeni), the use of the hyperbenthic sled would have ensured the suitable collection of demersal zooplankton during the daytime (cf. Kibirige *et al*. 2006).

In the laboratory, samples were suspended in 0.5 to 5 L solutions, depending on the density of organisms. The main sample was then stirred vigorously so that all the organisms remained in a homogenous suspension. A 20 mL plastic vial attached to a metal rod was then used to withdraw 3 to 6 subsamples from mid-depth (Perissinotto and Wooldridge 1989, Jerling and Wooldridge 1995). Zooplankton within the samples was identified and counted with a dissecting microscope (400 x). In all cases, the coefficient of variation between subsamples was less than 10%. Zooplankton density was then calculated as ind.m^{-3}.

Biomass was estimated as the total dry weight (mg DW.m^{-3}) of the sample. The sample was divided into two equal portions with a Folsom Plankton Splitter (McEwen *et al*. 1954). Large detritus and sediment particles were removed from the sample under a dissecting microscope (400 x) and biomass was measured by oven drying half of each sample at 60˚C for 24 h. For those samples that contained too much detritus, between 10 and 50 individuals of each species were oven-dried (24 h, 60˚C) in pre-weighed tin capsules. Triplicate weights were recorded for each species. The average weight for each species was then multiplied by the respective abundance so as to obtain the average dry weight per sample.

1.2.4 Statistical analyses

Univariate statistical analyses were conducted with SPSS version 15 for Windows. Data which did not satisfy the assumptions of parametric testing (i.e. normality and even distribution of residuals) were normalised using a log (x+1) transformation. Two-way analysis of variance (ANOVA) without replication (Zar 1996) was applied to test for possible spatial and temporal differences in total zooplankton abundance, biomass and diversity. Results from these tests showed no seasonal variation within each mouth state, allowing one-way ANOVA with post hoc Tukey's tests to be used to test for differences (in phytoplankton and microphytobenthic biomass as well as zooplankton abundance, biomass and diversity) between stations and between mouth states. Spearman's correlations were used to test for relationships between environmental variables and the three univariate community parameters.

Multivariate analysis was conducted on abundance data using the PRIMER package (version 6.0). All data were square-root transformed. ANOSIM showed no significant seasonal

difference in zooplankton community structure within each hydrological phase (closed, open and re-closure). For this reason, densities of taxa during each season were averaged to give the mean abundance of each taxon at the different stations during each of the mouth phases. A Bray-Curtis similarity matrix was then calculated from the five stations sampled during the different mouth phases. Cluster analysis (group averaged) and multidimensional scaling (MDS) was used to visually assess spatio-temporal differences in zooplankton assemblages, and then tested using analysis of similarity (ANOSIM). Where differences were found, the SIMPER routine determined the relative contribution of individual species to community structure between mouth states. The BIOENV function (using Harmonic Spearman Correlation) was then used to relate environmental variables to the zooplankton communities.

1.3 RESULTS

1.3.1 Physical environment

During the closed-mouth phase, > 50% of the estuary was dry and the greatest depth recorded was 2.48 m at the Mouth in April 2006. During the open-mouth phase, all sites were submerged or inundated and, while most northern lake stations were initially very shallow, by the time the Mouth re-closed in August 2007, water levels approached 2003 levels (~ 0.9 m average).

During the closed phase, a reversed salinity gradient existed throughout the estuary (Table 1.1). Salinity levels ranged from 1.8 at Esengeni in February 2007, to 86.9 at Listers Point in February 2006. During the open phase, salinity levels were generally marine (35), but the reversed salinity gradient re-established once again after mouth re-closure.

Water temperature values ranged between 15.2 and 34.8˚C in summer, 19.7 and 25.2˚C in autumn, 17.2 and 24.4˚C in winter and 22.5 and 30.5˚C in spring. Dissolved oxygen was highly variable throughout the survey, with values ranging from 1.92 $mgO_2.L^{-1}$ at Listers Point in November 2006 to 11.9 $mgO_2.L^{-1}$ at Esengeni in November 2006. Turbidity values ranged from 1 NTU at Catalina Bay to 341 NTU at Charters Creek. The highest turbidity levels were generally recorded at Esengeni, Charters Creek and Listers Point (Table 1.1).

1.3.2 Phytoplankton and microphytobenthic biomass

Phytoplankton biomass ranged from 1.49 to 421 mg $pigm.m^{-3}$ during the study period. Biomass peaked during the closed-mouth phase, with maximum phytoplankton biomass recorded in the

northern regions of the lake. Biomass at the Mouth and Narrows was substantially lower. During the open and re-closed mouth phases, biomass was generally uniform across all stations (Table 1.1).

Microphytobenthic biomass ranged from 0.47 to 451 mg pigm.m^{-2} during the study period. There was a significant difference between mouth states (ANOVA, $F_{2,58} = 6.75$, $p < 0.01$). Like phytoplankton biomass, microphytobenthic biomass was also greater in the closed-mouth phase and was also generally greater in the northern regions of the lake (Table 1.1). These variations were, however, neither spatially nor seasonally significantly different ($p > 0.05$).

1.3.3 Zooplankton

Total recorded zooplankton abundance in the St. Lucia Estuary ranged from 53.5 to 13.4 x 10^4 ind.m^{-3} during the study period (Table 1.2). Abundance varied between mouth states (ANOVA, $F_{2,61} = 5.04$, $p < 0.01$, Fig. 1.2a), peaking after mouth re-closure. Abundance was greatest at the Mouth and Esengeni during the closed phase. During the open-mouth phase, zooplankton abundance was low throughout the lake system, showing no spatial variation (ANOVA, $F_{4,9} = 0.579$, $p > 0.05$). After mouth re-closure, densities escalated to a maximum of 13.4 x 10^4 ind.m^{-3} at Esengeni. While zooplankton was recorded at Charters Creek and Listers Point after the mouth breached, these densities diminished by October 2008 when hypersaline conditions again persisted (Fig. 1.2, 1.3a).

Abundance of the mysid *Mesopodopsis africana* was greatest at the Mouth and Esengeni during the closed-mouth phase, but was virtually zero throughout the system during the open-mouth phase. After mouth re-closure, high densities of *M. africana* were recorded in the South Lake stations. Small populations were also recorded at Listers Point in November 2007 (Fig. 1.3b). *Pseudodiaptomus stuhlmanni* was present at all stations except Listers Point during the closed phase. Densities decreased during the open-mouth period and picked up again after mouth re-closure at all stations except the Mouth. Densities during the re-closure period were higher than those previously recorded, especially at Esengeni (Fig. 1.3c). Abundance of *Acartia natalensis* was greatest at the Mouth and Esengeni during the closed phase and increased substantially to a maximum of 5.6 x 10^4 ind.m^{-3} in July 2008 (Fig. 1.3d).

Mean zooplankton biomass recorded by Grindley (1982) for the five main areas of the St. Lucia system during the period 1967 to 1974, are compared with biomass data generated from this study in Fig. 1.4. There was an increase in biomass from 1967 through to 1974 corresponding

with an increase in fresh water flow. Although 2006 was during the peak of the drought, biomass during this year was within the range of that in 1974.

Data from the present study confirmed a significant difference in biomass between mouth states (ANOVA, $F_{2,60}$ = 7.21, p < 0.05, Fig. 1.2b). Biomass during the re-closed phase was significantly greater than in the closed and open phases (post hoc Tukey's test, p < 0.05). Biomass was also generally greatest at the Mouth and Esengeni and lower in the lakes during the closed phases (post hoc Tukey's test, p < 0.05). The opposite was true for the open phase (Fig. 1.2b); however, the differences were not significant (post hoc Tukey's test, p > 0.05). Following mouth re-closure, average biomass ranged from 81.6 to 676 mg DW.m^{-3}, but spatial variation was not significant (ANOVA, $F_{4,20}$ = 2.01, p > 0.05).

Zooplankton species richness was significantly higher during open-mouth conditions (post hoc Tukey's test, p < 0.05, Fig. 1.2c). During the closed phase, diversity at the Mouth and Esengeni was significantly greater than that at Charters Creek and Listers Point (post hoc Tukey's test, p < 0.05). In the open phase, peaks in species richness were recorded at the Mouth and Esengeni. Following mouth re-closure, species richness was roughly uniform throughout the system, ranging from 5 taxa at Charters Creek, to 13 taxa at the Mouth (Table 1.2).

Table 1.1: Physico-chemical variables, phytoplankton (PPL) and microphytobenthic (MPB) biomass measured at each station during the different mouth states (mean ± SE). NTU: Nephelometric Turbidity Units.

Mouth state	Station	MPB (mg pigm.m^{-2})	PPL (mg pigm.m^{-3})	Depth (m)	Oxygen (mg.L^{-1})	Salinity	Temperature (˚C)	Turbidity (NTU)
Closed	**Mouth**	54.1 ± 24.2	22.3 ± 9.96	1.34 ± 0.60	8.99 ± 4.02	13.15 ± 5.88	25.5 ± 11.4	6.76 ± 3.02
	Esengeni	43.7 ± 19.6	25.0 ± 11.2	0.83 ± 0.37	7.75 ± 3.47	6.45 ± 2.88	24.5 ± 11.0	28.7 ± 12.8
	Catalina Bay	189 ± 84.7	26.7 ± 11.9	0.38 ± 0.17	9.06 ± 4.05	14.5 ± 6.49	24.8 ± 11.1	42.8 ± 19.1
	Charters Creek	176 ± 78.7	40.9 ± 18.3	0.09 ± 0.04	8.38 ± 4.19	24.0 ± 12.0	29.2 ± 14.6	41.4 ± 18.5
	Listers Point	143 ± 63.9	116 ± 52.0	0.12 ± 0.05	4.96 ± 2.48	44.0 ± 22.0	29.8 ± 14.9	85.8 ± 38.4
Open	**Mouth**	19.9 ± 11.5	13.4 ± 7.75	0.13 ± 0.08	7.57 ± 4.37	34.6 ± 20.0	23.1 ± 13.3	24.4 ± 14.1
	Esengeni	23.0 ± 16.3	24.7 ± 17.5	0.83 ± 0.58	6.78 ± 4.79	33.9 ± 24.0	21.4 ± 15.1	41.2 ± 29.1
	Catalina Bay	86.2 ± 49.7	7.09 ± 4.09	0.72 ± 0.42	7.50 ± 4.33	29.1 ± 16.8	23.7 ± 13.7	8.37 ± 4.83
	Charters Creek	154 ± 89.0	26.7 ± 15.4	0.29 ± 0.17	8.51 ± 4.92	29.7 ± 17.2	25.5 ± 14.7	20.7 ± 12.0
	Listers Point	62.0 ± 35.8	23.8 ± 13.8	0.29 ± 0.17	6.91 ± 3.99	35.1 ± 20.2	23.8 ± 13.7	32.8 ± 18.9
Re-closed	**Mouth**	23.2 ± 10.4	15.9 ± 7.10	0.58 ± 0.26	5.24 ± 2.62	24.5 ± 11.0	21.6 ± 9.7	20.9 ± 10.5
	Esengeni	12.6 ± 5.61	23.7 ± 10.6	1.58 ± 0.70	6.00 ± 3.00	27.2 ± 12.2	21.3 ± 9.52	49.6 ± 24.8
	Catalina Bay	26.1 ± 11.7	1.96 ± 0.88	1.52 ± 0.68	5.04 ± 2.52	37.1 ± 16.6	24.1 ± 10.8	10.6 ± 5.28
	Charters Creek	10.4 ± 4.65	7.25 ± 3.24	0.22 ± 0.10	7.16 ± 3.58	49.4 ± 22.1	23.7 ± 10.6	186 ± 93.1
	Listers Point	5.81 ± 2.60	22.2 ± 9.95	0.24 ± 0.11	7.57 ± 3.78	49.1 ± 22.0	21.0 ± 9.37	234 ± 117

Table 1.2: Total zooplankton abundance, biomass and species richness recorded across the five representative stations of the St. Lucia Estuary during the study period. N/W: stations without water.

		Abundance (ind.m^{-3})				Biomass (mg DW.m^{-3})				Species richness (no. taxa)			
Mouth state	**Station**	**Mean**	**SE**	**Min**	**Max**	**Mean**	**SE**	**Min**	**Max**	**Mean**	**SE**	**Min**	**Max**
Closed	**Mouth**	10108	1954	5270	16768	145	67.9	24.5	391	8.8	1.2	6	13
	Esengeni	39093	16676	6619	10269	151	49.8	22.8	294	8	0.8	5	10
	Catalina Bay	9017	5890	0	30408	287	254	0	1302	4.8	1.7	0	10
	Charters Creek	11649	11553	0	57861	71.8	62.8	0	321	2.6	1.6	0	7
	Listers Point	N/W	N/W	N/W	N/W	N/W	N/W	N/W	N/W	N/W	N/W	N/W	N/W
Open	**Mouth**	1113	499	188	1901	10.7	3.3	4.1	14.9	21	4.5	16	30
	Esengeni	1045	859	186	1904	10.3	8.6	1.8	18.9	17	5	12	22
	Catalina Bay	625	196	404	1017	5.8	1.1	4.7	7.9	4.7	1.2	3	7
	Charters Creek	1612	900	623	3409	25	16.2	5.1	57	8	3.6	3	15
	Listers Point	6223	3589	223	12636	294	274	2	841	4	1.5	2	7
Re-closed	**Mouth**	20674	10914	2313	62394	114.5	40.9	23.2	253	9	1.1	7	13
	Esengeni	60270	22261	17372	133675	454	143	104	871	7.6	0.5	6	9
	Catalina Bay	20350	11585	782	50076	340	233	8.2	1215	7.6	0.5	6	9
	Charters Creek	21870	10224	1184	52213	676	257	16.7	1363	8	1	5	10
	Listers Point	2055	628	0	3419	81.6	47.5	0	260	5.6	1.6	0	9

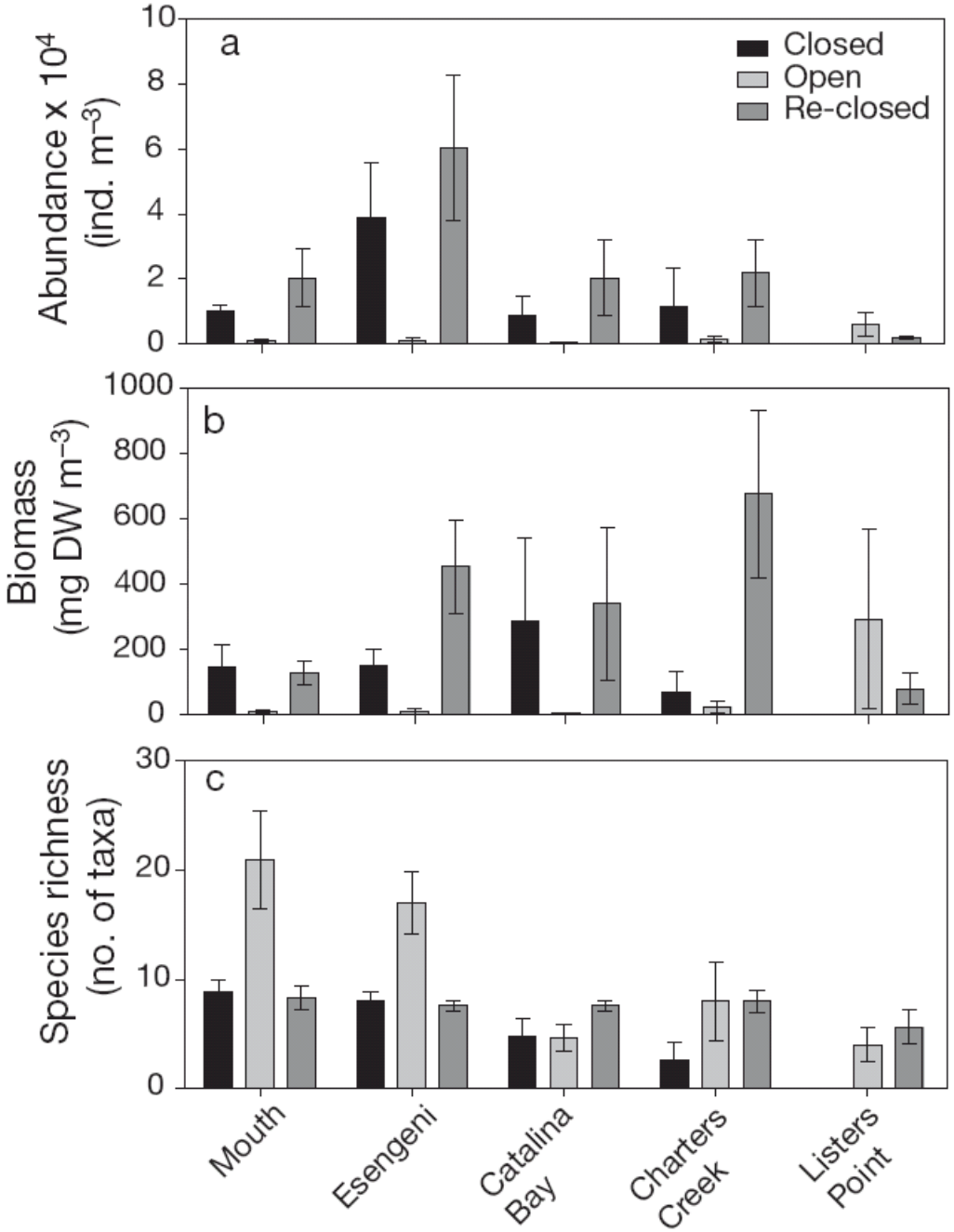

Fig. 1.2: Average (± SE) (a) total zooplankton abundance (x 10^4), (b) biomass and (c) species richness during the closed, open and re-closed mouth phases of the St. Lucia Estuary.

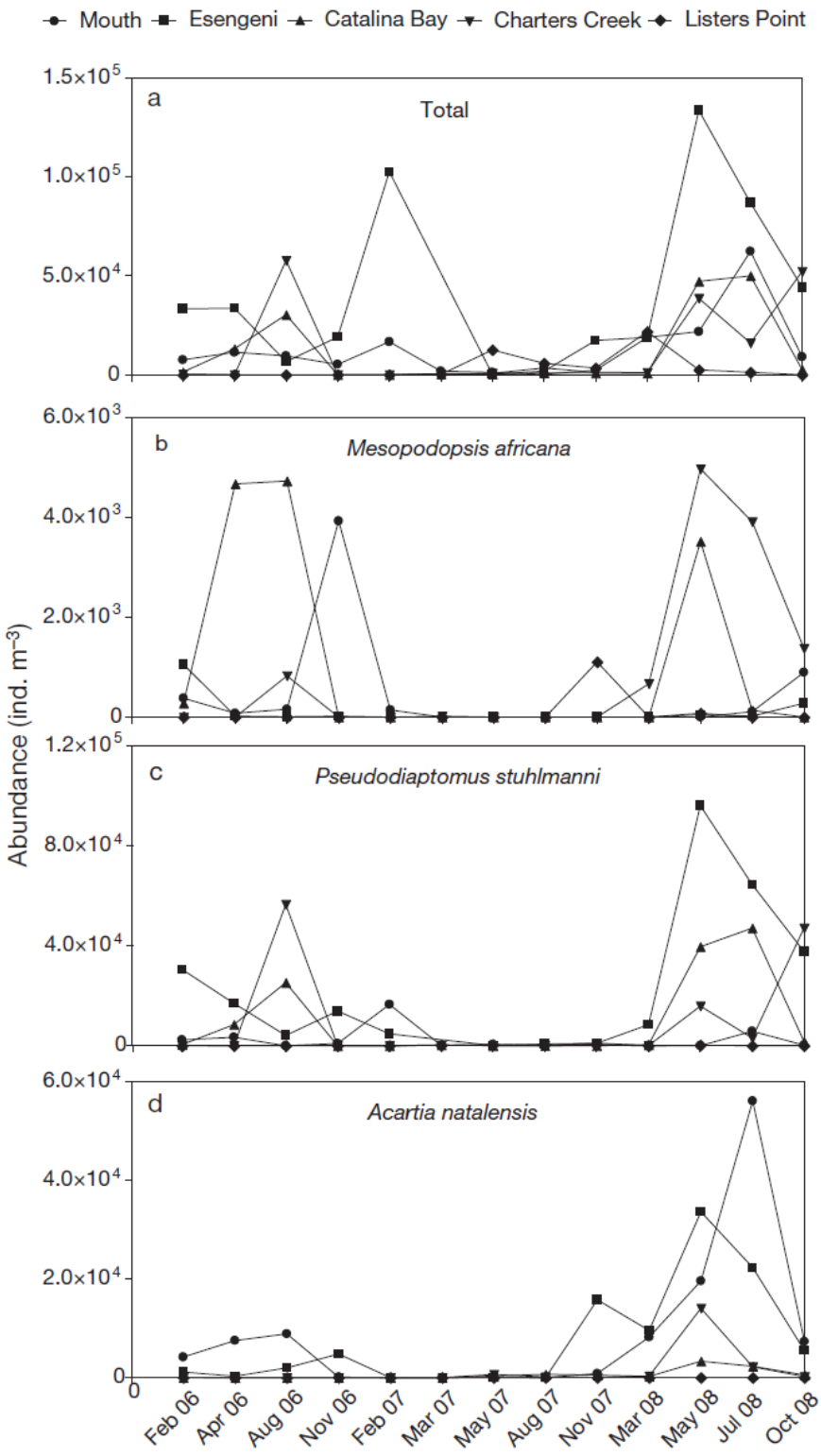

Fig. 1.3: Average temporal densities of the total zooplankton and of the three most dominant taxa in the system throughout the study period.

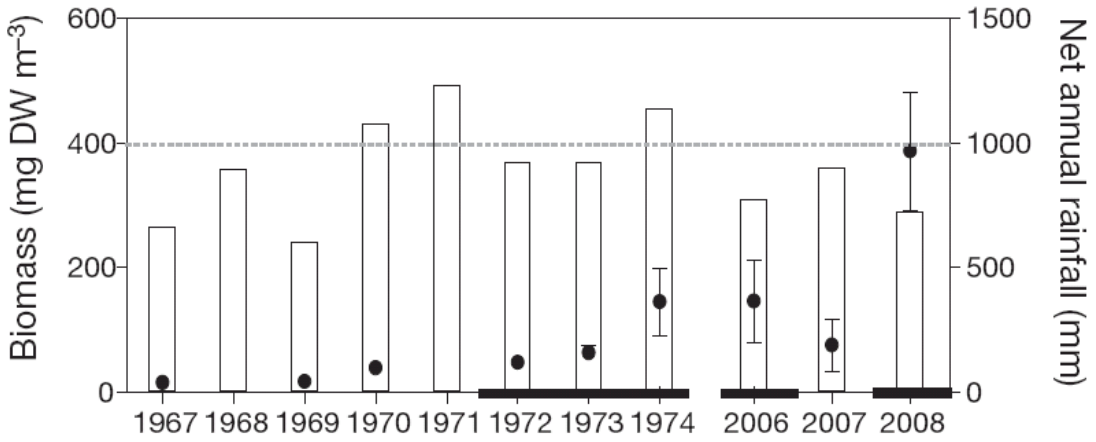

Fig. 1.4: Time series of average (± SE) zooplankton biomass (●) from 1967 to 1974 (Grindley 1982) and from 2006 to 2008. White bars show the net annual rainfall and the dotted line represents the average net annual rainfall for St. Lucia. Periods of thickening along the x-axis indicate closed-mouth conditions.

1.3.4 Community structure

A total of 69 different zooplankton taxa was recorded during the entire study period, 27 of which were present during the closed phase, 51 during the open phase and 33 during the re-closed phase (Table 1.3). A number of taxa was introduced when the estuary mouth breached. These included various harpacticoid copepods, such as *Macrosetella* sp., *Euterpina acutifrons* and *Metis* sp., calanoids such as *Eucalanus* sp. and *Mesocalanus* sp. as well as poecilostomatoids, *Oncaea* spp. and *Corycaeus* spp. Other marine recruits which were previously excluded, such as the prawn *Penaeus indicus,* were also recorded following the breaching event.

Cluster analysis and MDS plots (Fig. 1.5) showed that zooplankton communities differed between the open- and closed-mouth states, and was statistically confirmed by ANOSIM ($p < 0.01$; $R = 0.838$). Sites within the closed-mouth cluster showed between 40 and 80% similarity, while those in the open-mouth cluster were more dissimilar, showing only 15 to 55% similarity. The zooplankton community present at Listers Point subsequent to the re-closure of the estuary's mouth was an anomalous data point, as it grouped in the "open-mouth" cluster.

Table 1.3: Spatial variations in average zooplankton density* during the different mouth phases at the five representative stations within the St. Lucia Estuary. M: mouth, E: Esengeni, CB: Catalina Bay, CC: Charters Creek, LP: Listers Point, Unid.: unidentified

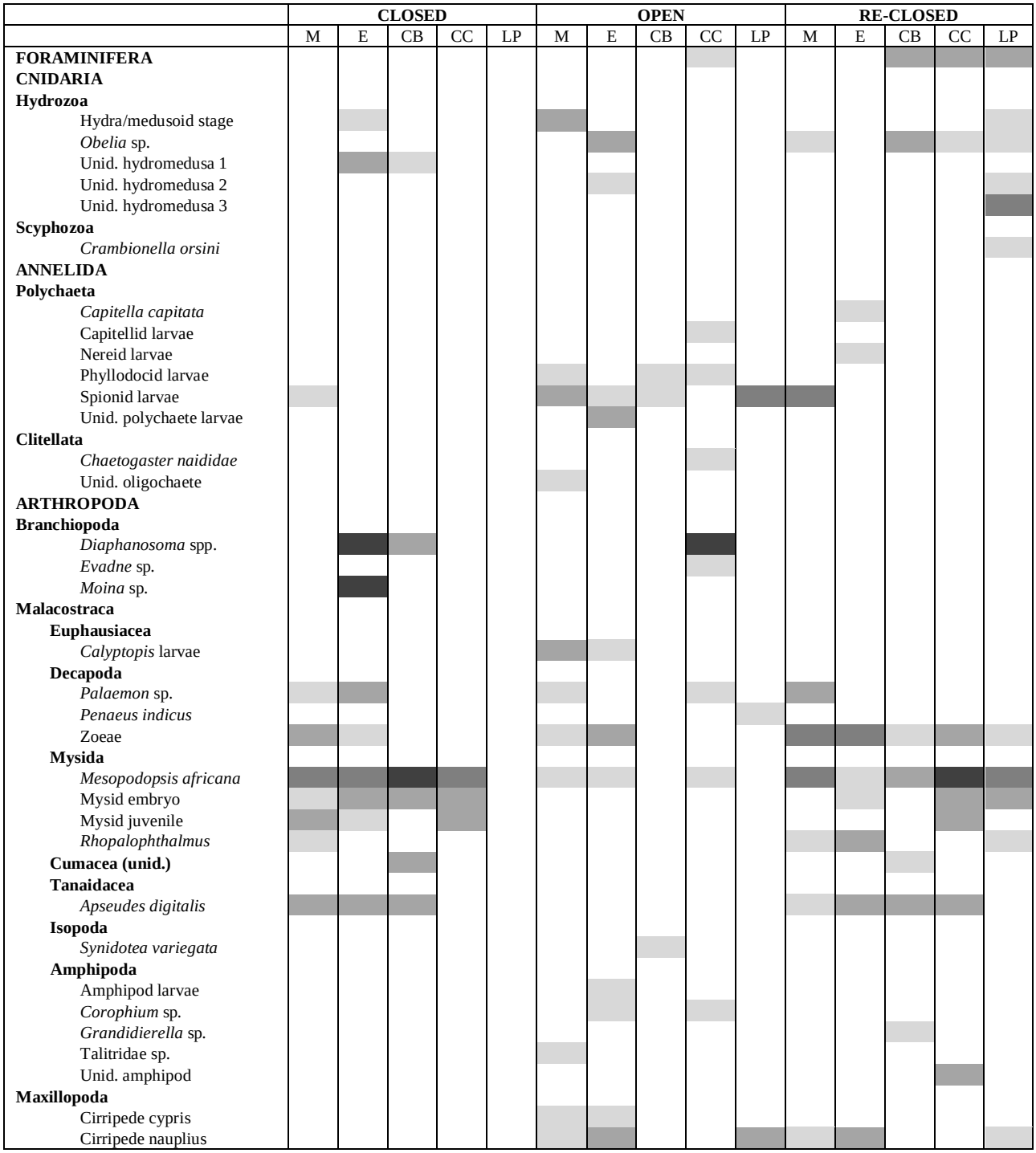

Table 1.3 cont.	CLOSED					OPEN					RE-CLOSED				
	M	E	CB	CC	LP	M	E	CB	CC	LP	M	E	CB	CC	LP
Copepoda															
Nauplii															
Calanoida															
Acartia natalensis															
Eucalanus sp.															
Mesocalanus sp.															
Paracalanus spp.															
Pseudodiaptomus stuhlmanni															
Temora discaudata															
Temora turbinata															
Cyclopoida															
Halicyclops spp.															
Oithona spp.															
Poecilostomatoida															
Corycaeus spp.															
Oncaea spp.															
Harpacticoida															
Cletocamptus spp.															
Euterpina acutifrons															
Harpacticoid nauplii															
Macrosetella sp.															
Metis sp.															
Unid. harpacticoid copepod															
Ostracoda (unid.)															
Chelicerata															
Hydracarina sp.															
Insecta															
Chironomid larvae															
Dragonfly larvae															
Tricopteran larvae															
Unid. dipteran larvae															
MOLLUSCA															
Gastropoda															
Gastropod veligers															
Bivalvia															
Bivalve larvae															
ECTOPROCTA															
Cyphonautes larvae															
CHAETOGNATHA (unid.)															
CHORDATA															
Ascidiacea															
Ascidian larvae															
Appendicularia															
Oikopleura dioica															
Osteichthyes															
Ambassis ambassis (juvenile)															
Fish eggs															
Fish larvae															

*					
Density (ind. m^{-3})	< 9	10 - 99	100 – 999	1000 – 9999	10 000 > 100 000

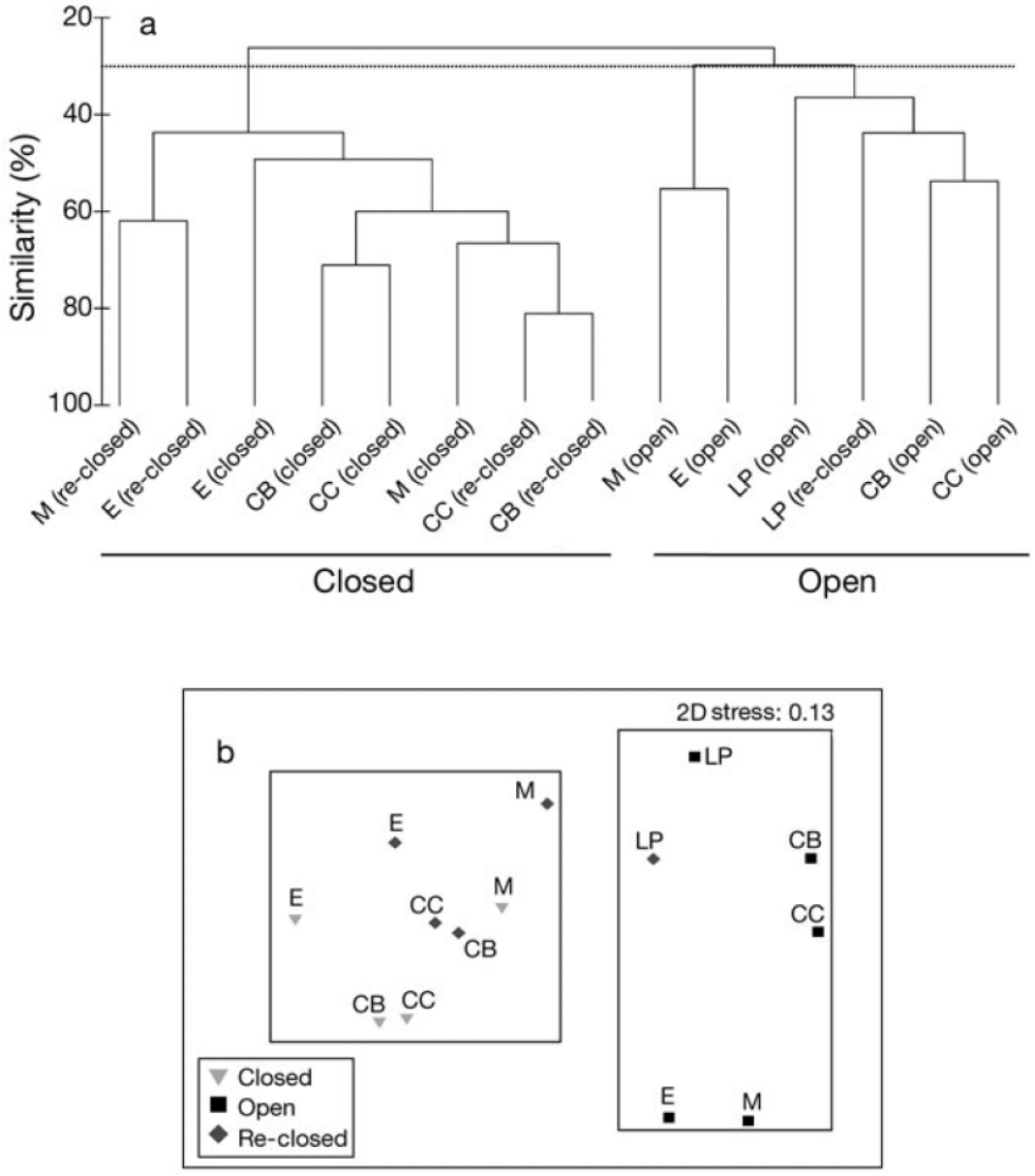

Fig. 1.5: (a) Dendrogram and (b) multidimensional scaling ordination showing the grouping of zooplankton communities during closed, open and re-closed mouth phases in the St. Lucia Estuary. The dotted line in (a) represents 30% similarity. M: Mouth, E: Esengeni, CB: Catalina Bay, CC: Charters Creek, LP: Listers Point.

The dominant taxa during the closed phases were the calanoid copepods, *Pseudodiaptomus stuhlmanni* and *Acartia natalensis*, contributing on average 74.1 and 17.9%, to total zooplankton abundance, respectively. These taxa made a cumulative contribution of 92% to total zooplankton density. Although the abundance of *P. stuhlmanni* and *A. natalensis* decreased significantly during the open-mouth phase, they still accounted for 74% of the total zooplankton assemblage. The remaining contributors were *Euterpina acutifrons* (11.2%), gastropod veligers (3.07%) and copepod nauplii (2.9%).

Data analysis using the SIMPER routine revealed that 90.7% of the dissimilarity between the open and closed phases was due to eight different taxa (Table 1.4). These included *Pseudodiaptomus stuhlmanni* (48.3%), *Acartia natalensis* (25%), *Mesopodopsis africana* (5.18%), *Halicylops* spp. (3.77%), *Cletocamptus* spp. (3.27%), gastropod veligers (2.23%), polychaete larvae (1.54%) and *Euterpina acutifrons* (1.4%).

Table 1.4: Zooplankton taxa accounting for 90.7% of the dissimilarity between the two clusters (viz. open and closed) identified using dendrograms and multidimensional scaling ordinations from SIMPER.

Species	Closed Average abundance ($ind.m^{-3}$)	Open Average abundance ($ind.m^{-3}$)	% Contribution	% Cumulative contribution
P. stuhlmanni	9883	261	48.3	48.3
A. natalensis	4061	1121	25.0	73.3
M. africana	612	1.54	5.18	78.5
***Halicyclops* spp.**	1287	16.3	3.77	82.2
Cletocamptus	3.98	213	3.27	85.5
Gastropod	490	33.9	2.23	87.7
Polychaete	29.3	185	1.54	89.3
E. acutifrons	0.93	72.6	1.40	90.7

With the exception of Listers Point during the closed phase, cluster analysis showed all stations to be > 30% similar during each of the mouth phases. Zooplankton communities at Catalina Bay and Charters Creek as well as at the Mouth and Esengeni consistently grouped together during the open- and reclosed-mouth phases. During the closed-mouth phase, zooplankton communities were more

similar to those at Catalina Bay and Charters Creek than Esengeni. In all mouth states, however, communities at Listers Point were always separated from any of the above (Fig. 1.6).

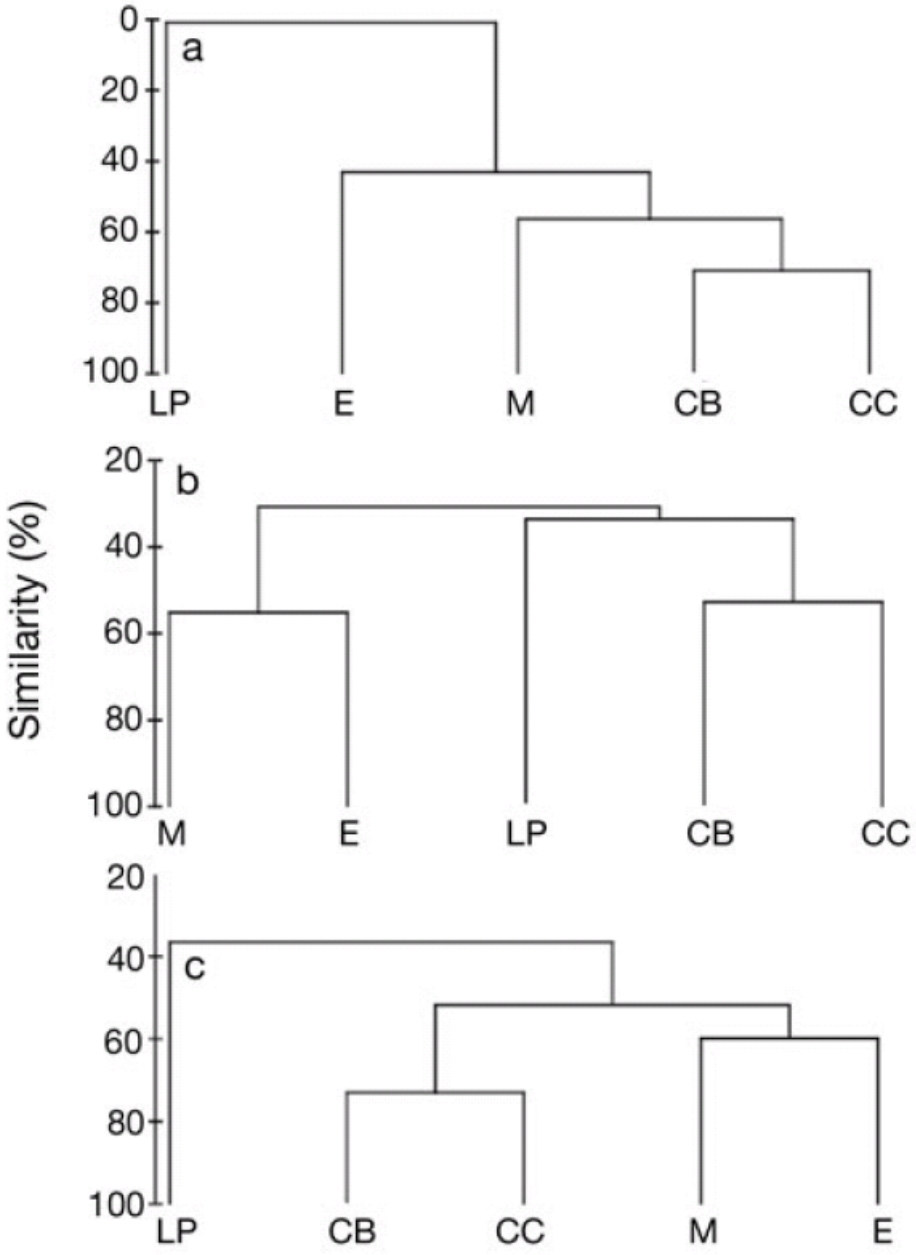

Fig. 1.6: Dendrograms showing spatial variation in community structure during the (a) closed, (b) open and (c) re-closed mouth phases of the St. Lucia Estuary during the study period. Station codes are as in Fig. 1.5.

ANOSIM also confirmed significant spatial variability in zooplankton assemblages within each mouth state. Most notable was the difference between the Mouth and all other stations after mouth re-closure (R = 0.29, p < 0.05). While the zooplankton communities at all other stations were characterised by typical St. Lucia zooplankton, the Mouth was dominated by *Oikopleura dioica* and *Acartia natalensis*. *O. dioica* entered the estuary during the open-mouth state and upon re-closure appeared to flourish in co-existence with *A. natalensis*, reaching a maximum abundance of 10^4 ind.m^{-3} up to five months after mouth closure. However, in May 2008, the *O. dioica* population dwindled, leaving *A. natalensis* as the single dominant species in the area. Only in July 2008, did the zooplankton community structure at the Mouth start resembling that observed prior to mouth breaching, with *Mesopodopsis africana* and *Pseudodiaptomus stuhlmanni* once again making a substantial contribution to the total zooplankton abundance (Fig. 1.7).

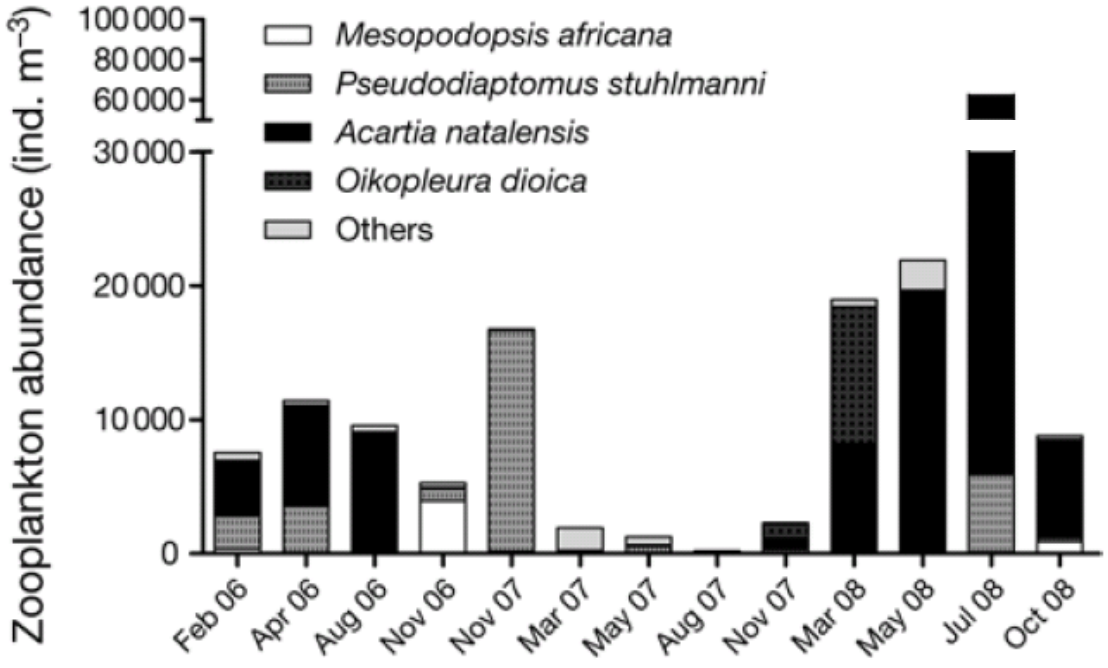

Fig. 1.7: Average abundance of the dominant zooplankton taxa at the Mouth of the St. Lucia Estuary during the study period.

The BIOENV procedure identified different environmental variables influencing the zooplankton communities. Overall, interactions between microphytobenthic biomass, phytoplankton biomass, salinity and temperature best explained the patterns observed in the zooplankton assemblages (R = 0.37). Within the closed phase, interactions between microphytobenthic biomass, phytoplankton, dissolved oxygen, salinity and temperature best explained zooplankton distribution (R = 0.31). Microphytobenthic biomass and dissolved oxygen were the primary determinants during the open-mouth phase, while after mouth re-closure, water depth, salinity and temperature explained most of the variation in zooplankton (R = 0.38).

Spearman Rank correlation between environmental parameters and zooplankton diversity, density and biomass showed few significant correlations (Table 1.5). On the whole, zooplankton abundance, biomass and species richness were inversely related to microphytobenthic biomass. Zooplankton abundance and species richness correlated positively with water depth and negatively with temperature. Additionally, a negative relationship existed between zooplankton abundance and salinity. Within the first closed phase, zooplankton biomass and species richness was also inversely related to microphytobenthic biomass, while negative correlations existed between zooplankton abundance and salinity as well as temperature. During the open mouth and re-closure phases, microphytobenthic biomass was the only parameter to significantly correlate with zooplankton species richness, positively during the closed-mouth phase and negatively during the open-mouth phase (Table 1.5).

Table 1.5: Spearman's rank correlation between physico-chemical parameters and diversity, abundance and biomass of zooplankton during the different mouth states of the St. Lucia Estuary. MPB: Microphytobenthic biomass, PPL: Phytoplankton biomass, *: $p < 0.05$, **: $p < 0.01$.

Mouth Status	Parameter	MPB	PPL	Depth	Oxygen	Salinity	Temperature	Turbidity
Closed	**Biomass**	**-0.58	-0.07	0.06	0.09	-0.37	-0.33	-0.09
	Abundanc	-0.40	0.13	0.15	0.21	*-0.46	*-0.45	-0.03
	Diversity	**-0.58	0.03	0.48	0.22	-0.50	-0.41	-0.22
Open	**Biomass**	-0.34	0.18	-0.06	-0.05	-0.20	-0.32	-0.08
	Abundanc	-0.35	0.13	-0.09	-0.10	-0.19	-0.33	-0.05
	Diversity	*-0.58	-0.01	0.24	-0.04	0.02	-0.47	0.16
Re-closed	**Biomass**	-0.27	0.15	0.09	-0.01	-0.18	0.15	0.26
	Abundanc	-0.21	0.26	0.33	0.11	-0.34	0.01	0.06
	Diversity	*0.57	-0.12	0.18	-0.19	-0.19	0.17	-0.29
Overall	**Biomass**	**-0.44	0.04	0.16	0.07	-0.17	-0.22	0.10
	Abundanc	**-0.37	0.12	*0.27	0.16	*-0.27	*-0.31	0.06
	Diversity	**-0.38	-0.01	*0.36	0.01	0.01	*-0.33	-0.05

1.4 DISCUSSION

As has been previously observed in most South African estuaries, maximum zooplankton abundance and biomass in the St. Lucia Estuary was recorded during the closed-mouth phase (Whitfield 1980, Kibirige and Perissinotto 2003a, Kibirige *et al.* 2006). This is owed to a combination of stable mouth conditions, limited freshwater input and minimal exchange of water with the sea (Gaughan and Potter 1995, Perissinotto *et al.* 2000). Low zooplankton abundance and biomass during open-mouth conditions are consistent with findings reported in the literature and are linked to the export of biomass-rich estuarine water into the ocean (Froneman 2004a).

In Grindley's (1982) study of zooplankton biomass in the St. Lucia system (1967 to 1974), which covered both open and closed mouth phases and extreme salinity fluctuations, peaks in biomass corresponded with fresh water pulses (Grindley 1982). It is, therefore, not surprising to find no seasonal variation in total zooplankton biomass during this study, since the survey took place during harsh drought conditions, with little freshwater input. Biomass was generally highest at the Mouth and Narrows and substantially lower in the lakes during the closed phase. This is because the northern regions were most severely affected by the drought, culminating in reduced water depth and greater temperatures and salinity levels. Conversely, zooplankton biomass was greater in the lakes during open-mouth conditions. These findings correspond with those of Grindley (1982) and can be attributed to the lakes being a more stable environment under open-mouth conditions. Biomass levels recorded at the peak of the drought (2006) during this study were generally within the range of those recorded by Grindley in 1974, despite the above average rainfall which the area received during that year. Comparisons between these studies must, however, be taken with caution for the following reasons. Firstly, Grindley (1982) used two different sampling methods, a ring-net (30 cm diameter, 90 µm mesh) and a Clarke Bumpus sampler (12.5 cm diameter, 125 µm). Secondly, all samples were taken at the surface (hand-held) and next to the boat gunnels, thereby creating substantial disturbance (T Wooldridge, pers. comm.) and lastly, sampling frequency and intensity was not consistent.

In this study, biomass peaked after mouth re-closure, exceeding levels which were recorded both during this study and that of Grindley (1982). Inflowing marine water may have aided the build-up in zooplankton biomass during the subsequent closed phase, as was previously observed in the Kasouga Estuary for instance (Froneman 2004a). On a regional scale, biomass records were generally within the range of those reported by Wooldridge (1999) for South

African estuaries. In fact, even some of the lowest values recorded during this study exceeded those documented in temperate north systems, such as the Patuxent River, Narragansett Bay and Westerschelde (Heip *et al*. 1995 and references therein).

Prolonged periods of mouth closure in temporarily open/closed are generally associated with low levels of zooplankton taxonomic diversity (Froneman 2004a, Perissinotto *et al*. 2004). In the current study, the lake zooplankton was, like most estuarine zooplankton assemblages in southern Africa (Wooldridge 1999), dominated by typical estuarine taxa, such as *Pseudodiaptomus stuhlmanni*, *Acartia natalensis* and *Mesopodopsis africana*, which often collectively contributed over 90% of the total zooplankton abundance. The zooplankton assemblages of St. Lucia, as described by Grindley (1982), were generally made up of: (1) a stenohaline marine component, consisting of copepods such as *Corycaeus* spp., which tidally accessed the mouth; (2) a euryhaline marine component, including species of *Paracalanus*, which were able to penetrate a little further; and (3) a freshwater component, consisting of species such as *Diaptomus* spp. and *Cyclops* spp., found at the mouths of inflowing rivers. Only 27 of the 95 previously recorded taxa (Grindley 1976) were observed in the system during the closed-mouth phase. Missing components included marine and freshwater taxa, which were absent because of prolonged reduced freshwater input (Allanson and Read 1995), and the closed-mouth state which prevented tidal exchange. Primo *et al.* (2009) showed that dry periods in the downstream stations of the permanently open Mondego Estuary (Portugal) were also characterised by a lack of seasonality, low taxonomic diversity and a high density of *Acartia* spp. However, in contrast to the St. Lucia system, marine taxa dominated the Mondego Estuary, probably due to its open mouth (Primo *et al.* 2009). The opening of the St. Lucia Estuary mouth in March 2007 led to a great increase in species richness. Marine taxa, such as the copepods *Corycaeus* spp., *Paracalanus* spp., juvenile *Penaeus indicus*, and estuarine dependent ichthyoplankton recruited into the system. The timing of the mouth breaching allowed for the autumn cohort of *P. indicus* to enter the estuary. This cohort would usually over-winter in the estuary and return to sea the next spring (Benfield *et al.* 1989), but was prevented from doing so due to the reformation of the beach berm by 24 August 2007.

Because this breaching event took place during a drought, it is not surprising that there was still a general lack of freshwater zooplankton. Groundwater seepage points along the eastern shores have been hypothesised to form microhabitats of reduced salinity, capable of acting as reservoirs during dry cycles and providing refugia for estuarine species, for later re-colonisation of the estuary (Taylor *et al.* 2005). However, data from an allied study carried out in 2005 (S

Singh, unpubl.), showed that the zooplankton communities of these refugia are significantly different from those of neighbouring sites and, therefore, probably not the primary source of re-colonisation and recruitment. It is more likely that some species of zooplankton may be able to produce resting stages/eggs capable of surviving dry/hypersaline conditions for long periods of time (e.g. Uye 1985, William-Howze 1997, Engel 2005). Re-colonisation could also have occurred from adjacent parts of the estuary that had not dried out completely during the drought (e.g. the Narrows or the southern part of South Lake).

Abundance of the mysid *Mesopodopsis africana* decreased drastically after the system breached, becoming almost entirely absent within a month. Possible explanations may include reduced water levels in the mouth region as a result of sediment deposition, populations being flushed out to sea (e.g. Wooldridge 1986, Kibirige and Perissinotto 2003a), or increased predation pressure by fish that entered the estuary from the ocean. In this study, mysid swarms were mostly restricted to the mouth region during the closed phase, but were recorded in low numbers, along with *Pseudodiaptomus stuhlmanni* and *Acartia natalensis* at Charters Creek and Listers Point after the estuary breached. It is likely that the more favourable conditions (viz. increased water level and decreased salinity) allowed for the development of these communities in the North Lake, which was probably a more stable environment when compared to the Mouth and the Narrows at the time.

Multivariate correlations showed weak associations of zooplankton with environmental parameters in the estuary. This is not uncommon in estuaries (Hastie and Smith 2006) and is probably a product of the highly dynamic nature of this system. Overall, the interaction between microphytobenthic biomass, phytoplankton biomass, salinity and temperature best explained the variation in community structure. In terms of univariate variables, microphytobenthic biomass was inversely related to zooplankton biomass, abundance and species richness. Reduced grazing pressure could have allowed for microphytobenthos proliferation, since biomass was generally greatest at Charters Creek and Listers Point, where conditions were often not favourable for the survival of zooplankton. Similar findings were reported by Pillay and Perissinotto (2008, 2009) for the benthic communities of the St. Lucia Estuary. Overall, zooplankton abundance and species richness correlated positively with water depth. Such a relationship is expected, since habitat for zooplankton increases with water depth. Additionally, the Mouth and Narrows are deeper stations, partly protected from the effects of drought due to the fresh water inflow at Makakatana, Mfolozi and Mpate rivers. Zooplankton abundance and salinity were negatively correlated, reflecting the negative impact of hypersaline conditions on zooplankton abundance.

Additionally, zooplankton abundance and species richness were greater in cooler waters. This could be a product of the shallower waters (e.g. Charters Creek and Listers Point) being more susceptible to heating. This negative correlation is, therefore, probably more related to depth than temperature itself, since water temperature decreases with depth. High temperature is nevertheless known and expected to negatively affect the physiology of many zooplankton species (e.g. Moore *et al.* 1996, Norberg and De Angelis 1997).

Zooplankton dynamics at the mouth of the St. Lucia Estuary followed a very unique pattern, particularly after mouth re-closure. High abundances of the appendicularian, *Oikopleura dioica* ($>10^4$ ind.m^{-3}) were found. These tunicates live in individual mucous cases, which efficiently concentrate and collect particulate matter from the water (Deibel 1998). While appendicularians commonly occur in permanently open estuaries, there are no reports of *O. dioica* swarms thriving in estuaries under closed-mouth conditions. Studies have shown that other planktonic grazers are often rare or absent in swarms of planktonic tunicates (Frazer 1962, Berner 1967, Deibel 1980). Several factors may contribute to this apparent exclusion of other herbivores. Firstly, high reproductive rates enable planktonic tunicates to exploit food sources more rapidly (Heron 1972, Deibel 1980). Also, appendicuarians are food generalists, capable of ingesting a wide size spectrum of naturally occurring particles (Diebel 1998). Particle consumption by tunicates may, therefore, reduce the food available to juvenile stages of other herbivores, decreasing recruitment of competing zooplankton. Finally, the exceptionally high filtering rates of most tunicates could reduce phytoplankton standing stocks to levels that would not support other grazers. In older swarms, both copepods and appendicularians may co-exist in high densities, suggesting that, given enough time and sufficient food, herbivores with longer generation times could also exploit the same phytoplankton stocks (Alldredge and Madin 1982).

With the arrival of *Oikopleura dioica* in the St. Lucia Estuary, the zooplankton community structure in the mouth area shifted drastically from being dominated by *Mesopodopsis africana* and *Pseudodiaptomus stuhlmanni*, which used to make up the bulk of the biomass, to being dominated by *O. dioica* and *Acartia natalensis*. There are several possible explanations for the apparent exclusion of these larger taxa. Firstly, it is possible that changes in the physical environment associated with the re-closure of the estuary mouth (e.g. a decrease in turbidity and an increase in water level) favoured *O. dioica*, to the exclusion of the other grazers. Secondly, *O. dioica*, with its efficient feeding mode (Alldredge 1981, Deibel 1998) may have outcompeted *P. stuhlmanni.* Thirdly, the slower regeneration times of copepods such as *P. stuhlmanni* (e.g. *Paracalanus crassirostris* versus. *O. dioica* ratio of ≈ 1/10, Hopcroft and Roff 1995) may have

made it vulnerable to predation, with the opposite being true for *O. dioica*. Lastly, heavy fish predation may have limited populations of *M. africana* and *P. stuhlmanni*. Both of these taxa are important components in the diet of a number of zooplanktivorous fish in the St. Lucia Estuary (Blaber 1979). While *Oikopleura* spp. are the preferred food type for many fish species because of their high carbon and nitrogen content, as well as their lack of a carapace and slow reaction times (Gorsky and Fenaux 1998), they also exhibit extremely fast generation rates (Deibel 1998). So, within the St. Lucia Mouth, the effect of fish predation on *O. dioica* abundance may have been counterbalanced by their fast generation rates.

This community shift, however, lasted only for about two months, as *Oikopleura dioica* disappeared completely from the estuary by the end of May 2008. A number of factors may have contributed to this. Firstly, the salinity dropped down to 23 in May 2008, which, according to Uye and Ichino (1995), is outside its range of tolerance. Secondly, in the absence of *Pseudodiaptomus stuhlmanni* and *Mesopodopsis africana*, *O. dioica* may have become the primary target of fish predation. Lastly, it has been shown that copepods in high abundance may have a significant impact on the abundance of appendicularians by ingesting their eggs and juveniles (López-Urrutia *et al*. 2004). Whichever combination of factors it may be, the zooplankton community at the mouth of the estuary, two months subsequent to mouth re-closure, was dominated by *A. natalensis*, with an average individual size of about 800 µm. Only in October 2008, did the zooplankton community at the Mouth start resembling that of its pre-breaching state. Large numbers of immature *M. africana* were once again recorded on this occasion, along with small populations of *P. stuhlmanni*.

Shifts in zooplankton community structure, especially the opportunistic dominance of *Oikopleura dioica* under closed mouth conditions, emphasize the complexity and the erratic nature of the system in response to environmental variability. The unpredictable and extreme changes recorded in this study concur with those reported by Grindley and Heydorn (1970), where blooms of spiders, midges or dinoflagellates signalled major environmental change. The only major difference between the two studies was the types of organisms that flourished. In spite of severe environmental perturbations, the St. Lucia Estuary has been reported as being extremely resilient in the past (Begg 1978, Taylor 2006), but it is now critical to monitor the extent and the time scale of its recovery from the current crisis, once a new wet cycle sets in.

Temperature and salinity tolerance of *Mesopodopsis africana* in the freshwater-deprived St. Lucia Estuary, South Africa

ABSTRACT

Mesopodopsis africana is a key species in the St. Lucia Estuary, Africa's largest estuarine lake. This system is currently undergoing an unprecedented crisis due to freshwater deprivation. A reversed salinity gradient has persisted with hypersaline conditions (> 300) occurring in the upper regions of the estuarine lake. In the context of climate change, rising temperatures will not only push the thermal tolerance limits of estuarine organisms, but increased evaporation from this lakes large surface area will lead to further salinity increases. The present study aims to determine the temperature and salinity tolerance of *M. africana*, both through *in situ* studies and the use of laboratory experiments. Results indicate that *M. africana* is a broad euryhaline species. Mysids were recorded at salinity levels ranging from 2.55 to 64.5 *in situ*. While experiments revealed a narrower salinity tolerance, acclimation resulted in a significant increase in the tolerance range of this species. It is probable, however, that slower acclimation times may increase survival rates even further, particularly in the higher salinity treatments. *M. africana* was especially tolerant of the lower salinity levels. In the 20ºC acclimation experiment, LS_{50} at 1 and 2.5 was only reached after 8 and > 168 h, respectively. Survival at 10 and 40ºC was negligible at all salinity levels. This concurs with field results which documented mysids at temperatures ranging from 16.2 to 30.9ºC. Salinity and temperature increases associated with global climate change may, therefore, have significant implications for these mysid populations, with cascading effects on the higher trophic levels which they support.

Keywords: drought, hypersalinity, iSimangaliso Wetland Park, *Mesopodopsis africana*, temperature.

2.1. INTRODUCTION

Zooplankton is critical to the functioning of aquatic food webs because of their sheer abundance and vital ecosystem roles (Richardson 2008). Not only do they represent the link between primary and secondary producers with higher trophic levels, but their rapid response to

ecosystem alterations makes them an effective predictor of climate change (Hays *et al*. 2005). Extreme events such as droughts, storms and heat waves have become more common in recent decades (Easterling *et al*. 2000) and are predicted to continue and intensify (IPCC 2007). Ecosystems which are particularly sensitive to the effects of climate change are shallow estuarine lakes.

The St. Lucia Estuary, Africa's largest estuarine lake (Fielding *et al*. 1991, Cyrus and Vivier 2006), forms part of the iSimangaliso (formerly Greater St. Lucia) Wetland Park. iSimangaliso is South Africa's first UNESCO World Heritage Site and has been designated a Ramsar Wetland of international importance (Begg 1978, Cyrus and Vivier 2006). The lake system is governed by cyclical wet and dry phases, each lasting between four and ten years (Begg 1978). St. Lucia is currently experiencing a drought cycle, with below average rainfall persisting since 2002. Prior to 1920, the Mfolozi River discharged into the St. Lucia Estuary and would buffer water loss during periods of drought, but in the 1930s a canal was excavated through the Mfolozi flats for agricultural purposes (Begg 1978, Whitfield and Taylor 2009). The natural filtration system of the swamps was, therefore, destroyed and the two systems have since been artificially maintained separate in an attempt to avoid the perceived threat of siltation from the Mfolozi. This alteration of the system's catchment has further exacerbated the severity of the current drought cycle.

A reversed salinity gradient has persisted virtually uninterrupted since 2002, with values ranging from 1.8 in the Narrows to > 300 in the upper reaches of the estuary. Due to shallow lake levels, water temperatures in summer can sometimes exceed 40ºC. The biotic communities here are, therefore, also characterised by a strong spatial heterogeneity. Temperature and salinity are both considered dominant "ecological master factors," which may act either singly or collectively to modify the structure, function, and distribution of estuarine organisms (Kinne 1971, Alderdice 1972, Dorgelo 1976). The survival of individuals and the success of their populations are, in part, dependent upon their ability to tolerate the highly variable conditions that typically characterise this environment. Temperature and salinity are also at the mercy of global climate change. According to the latest IPCC report (2007), temperatures are rising by 0.2˚C per decade. Additionally, the frequency and intensity of extreme weather events is forecast to increase (Planton *et al*. 2008). Rising temperatures will not only push the thermal tolerance limits of estuarine organisms, but increased evaporation from the large surface area of this estuarine lake will lead to concomitant salinity increases, which would further influence the biotic communities present in the system.

Zooplankton communities in St. Lucia, like most estuarine zooplankton assemblages in southern Africa (Wooldridge 1999), are dominated by typical estuarine taxa, such as *Pseudodiaptomus stuhlmanni*, *Acartia natalensis* and *Mesopodopsis africana*, which often collectively contribute over 90% of the total zooplankton abundance (Carrasco *et al*. 2010: Chapter 1, Jerling *et al*. 2010). While copepods are often numerically abundant, mysids generally show more importance in terms of biomass. In the St. Lucia Estuary, *M. africana* is a key species, playing a major role in the trophic functioning of the system (Carrasco and Perissinotto 2010b: Chapter 4, 2011b: Chapter 5). Grindley (1976, 1982) earlier recorded salinity ranges for many of the common zooplankton taxa inhabiting the estuary, and suggested that *M. africana* could tolerate salinity levels up to 60. However, this conclusion was based on the occurrence of adults at ranges of salinity in the natural environment, rather than on tolerance limits defined through laboratory experimentation. The present study, therefore, aims to determine the temperature and salinity tolerances of *M. africana* both through *in situ* studies and the use of laboratory experiments.

2.2. MATERIALS AND METHODS

2.2.1 Study area

The St. Lucia Estuary is situated in northern KwaZulu-Natal and lies between 27 ˚ 52'0" S to 28˚ 24'0" S and 32˚ 21'0"E to 32˚ 34'0"E. The system is made up of three shallow lakes connected to the Indian Ocean by a 21 km channel known as the Narrows (Fig. 1.1). In total, the St. Lucia estuarine lake covers an area between 300 and 350 km^2, depending on water levels (Begg, 1978). The estuary mouth was closed off from the sea for the entire study period, apart from a brief six month open-mouth phase, when a combination of extreme conditions resulted in the breaching of the estuary mouth from the sea side.

2.2.2 *In situ* sampling

Quarterly surveys were undertaken at five representative stations within the St. Lucia Estuary, from February 2005 through to May 2010. These stations included: the Mouth, Esengeni, Catalina Bay, Charters Creek and Listers Point (Fig. 1.1). The study period covered three different hydrological phases, viz. a closed-mouth phase (February 2005 to February 2007), an

open-mouth phase (March 2007 to August 2007), and a re-closed period (November 2007 to May 2010). Zooplankton, phytoplankton and microphytobenthic samples, together with physico-chemical data, were collected at each site on each sampling occasion. Data from 2005 was collected by S. Singh.

2.2.2.1 Physico-chemical variables

Physico-chemical measurements were taken with a YSI 6920 water quality logger, fitted with temperature (˚C), depth (m), dissolved oxygen ($mg.L^{-1}$), pH and turbidity (Nephelometric Turbidity Units or NTUs) probes. Where possible, measurements were made at both the surface and the bottom of the water column. In cases where the water level was less than 10 cm deep, the probe was placed horizontally, so that all the sensors were submerged.

2.2.2.2 Mysid sampling

Single daytime mesozooplankton samples were collected using an epibenthic sled fitted with 200 µm mesh. This method was employed due to the shallow water levels and the diel migration pattern of *Mesopodopsis africana* in the system (Carrasco and Perissinotto 2010a: Chapter 3). The sled was towed in the shallow waters near-shore at all stations, except at Esengeni, where a boat was used. The mouth of the net was semi-circular in shape (radius = 18.5 cm) and was mounted on a sled such that the net was raised 7.5 cm above the sediment surface. The volume of water filtered (≈ 1.43 m^3) was calculated by multiplying the area of the sled mouth by the distance towed (27 m). Samples were emptied into 500 mL bottles containing 4% phloxine-stained formaldehyde. In regions where water depth was too shallow for the sled to be used, a hand held D-net (radius = 28 cm) with the same mesh size was driven over a 20 m transect.

In the laboratory, samples were suspended in 0.5 to 5 L solutions, depending on the density of organisms. The main sample was then stirred vigorously so that all the organisms remained in a homogenous suspension. A 20 mL plastic vial attached to a metal rod was then used to withdraw three subsamples from mid-depth (Perissinotto and Wooldridge 1989, Carrasco *et al*. 2010: Chapter 1). Mysids within the samples were identified and counted with a Kyowa SDZ dissecting microscope (400 x). Mysid density was then calculated as $ind.m^{-3}$.

2.2.3 Experimental study

2.2.3.1 Collection and maintenance of mysids

Mysids for experiments were collected using the same epibenthic sled mentioned above, which was towed across the mouth of the St. Lucia Estuary during the daytime. On return to the laboratory, mysids were held briefly (≈1 h) in aerated estuarine water at room temperature and thereafter, transferred in sets of ten to be used in shock and acclimation experiments, as explained below. Experiments were conducted in June 2009 at four different temperatures (10, 20, 30 and 40ºC) and under natural light conditions. Two types of experiments were conducted: mysids held at a salinity of 14 (salinity at the Mouth) were either directly transferred to 0, 2.5, 5, 25, 40, 50 and 60 (hereafter referred to as shock experiments); or were acclimated to these salinity levels at a rate of up to 2 per hour for 24 h until target salinity treatments were reached. Temperatures were similarly gradually changed over a period of 24 h until target temperatures were reached (hereafter referred to as acclimation experiments). Since the goal of the acclimation experiments was to ascertain whether the tolerance limits of the mysids seen in the shock experiments could be stretched, the following range of salinity levels was used in the acclimation experiments: 0, 1, 2.5, 45, 55 and 65.

2.2.3.2 Salinity and temperature tolerance experiments

Dilute experimental media were prepared by adding filtered (0.45 µm) dam water (0.31) to estuarine water (14), while high salinity media were prepared by adding "Instant Marine" aquarium salts to estuarine water. Dam water was used for the freshwater exposure. Physico-chemical measurements were again measured with a YSI 6920 water quality logger and salinity and temperature were monitored using a refractometer and mercury thermometer respectively. Ten mysids were exposed to each experimental salinity and temperature treatment in individual plastic containers filled with 500 mL of medium. The water media were made up to the 7 different salinity treatments in 7 different 5 L buckets. There were four replicates for each salinity-temperature combination. The 500 mL plastic containers were capped with 200 µm mesh and immersed in each of the aerated buckets, which were subsequently submerged in the different temperature water baths. The shock experiments lasted 72 h, with survival monitored at 1, 2, 4, 8, 16, 24, 24, 48 and 72 h intervals, observing, counting and removing dead individuals. Four replicates for the control were held at ambient room temperature in natural estuarine water from the study site. In the acclimation experiments, monitoring of survival only

proceeded after mysids had been acclimated to target salinity levels. Data presented take into account mortalities that occurred during the acclimation process. Acclimation experiments lasted 168 h and monitoring proceeded as above for the first 72 h and every 24 h thereafter.

In order to provide mysids with their naturally occurring food, microplankton was collected by towing a 20 µm net across the estuary mouth for a distance of 27 m. The sample was then placed in a 20 L bucket and gently stirred. 500 mL of this was then filtered out and placed into each experimental treatment. Fresh food was added to the experimental chambers daily.

2.2.4 Statistical analysis

Multivariate analysis was conducted on mysid abundance data using the PRIMER package (version 6.0). All data were square-root transformed. The BIOENV function (using Harmonic Spearman Correlation) was then used to relate environmental variables to mysid abundance. The environmental variables which best explained the variation in mysid abundance were then plotted against mysid abundance, and a regression co-efficient was generated in SPSS version 15 for Windows.

Differences between mortality in experimental treatments were analysed using two-way repeated measures (RM)-ANOVA in SPSS. Data for this test could not be normalised through transformation. A parametric test was carried out despite the data displaying uneven distribution of the residuals. It has, however, been stated that ANOVA tests are robust enough to perform analyses on nonparametric data (Zar 1996). Additionally, the post hoc test, Dunnets T3 was used for inter-treatment comparisons since this test assumes unequal distribution of the residuals. A two-way RM-ANOVA was, therefore, used to determine whether mortality was significantly affected by temperature and salinity, and the interaction between these variables. An independent samples t-test was also used to determine whether mysid survival was significantly different between shock and acclimation experiments. Only those salinity treatments which were the same in both experiments (i.e. 2.5 and 40) were compared in this analysis.

2.3. RESULTS

3.1 Physico-chemical environment

During the closed-mouth phase, > 50% of the estuary was dry and the greatest water depth recorded was 2.48 m at the Mouth in April 2006. During the open-mouth phase, all sites were submerged or inundated. Salinity values ranged from 1.8 at Esengeni in February 2007 to 141 at Listers Point in August 2009. During the open-mouth phase, the salinity across the lake was generally marine (35), but the reversed salinity gradient re-established once again after mouth re-closure. Water temperatures ranged between 20.1 and 41.0°C in summer, 15.2 and 30.6°C in autumn, 17.2 and 26.1°C in winter and 22.3 and 30.5°C in spring. Dissolved oxygen was highly variable throughout the survey, with values ranging from 0.5 mg $O_2.L^{-1}$ at Listers Point in February 2005 to 12.8 mg $O_2.L^{-1}$ at Esengeni in August 2005. Turbidity ranged from 1 NTU at Catalina Bay to 341 NTU at Charters Creek. The highest turbidity levels were generally recorded at Esengeni, Charters Creek and Listers Point (Table 2.1).

2.3.2 Mysid abundance

Mysid abundance ranged from complete absence to 3.7 x 10^4 ind. m^{-3}. During the closed phases (February 2005 to February 2007 and November 2008 to May 2010), maximum mysid abundance was recorded in the lower reaches of the estuary such as the Mouth, Esengeni and Catalina Bay. During the open-mouth phase, highest mysid densities were recorded in the northern lakes (Fig. 2.1).

The BIOENV procedure identified different environmental variables influencing mysid abundance. Overall, the combination of temperature and salinity best explained the patterns observed in the mysid assemblages (R = 0.21). Spearman Rank correlation between environmental parameters and mysid density showed no significant correlations. On the whole, mysid abundance correlated negatively with salinity and temperature and positively with water depth. During field collections, mysids were found inhabiting areas with salinity values ranging from 2.55 to 64.4 and temperatures from 16.2 to 30.92˚C (Fig. 2.2).

Table 2.1: Physico-chemical variables, microphytobenthic (MPB) and Phytoplankton (PPL) biomass measured at each station during the different mouth phases (mean ± SE). DO: Dissolved Oxygen, NTU: Nephelometric Turbidity Units.

Mouth state	Station	MPB (mg pigm. m^{-2})	PPL (mg pigm.m^{-3})	Depth (m)	DO (mg.L^{-1})	Salinity	Temperature (°C)	Turbidity (NTU)
closed	**Mouth**	50.0 ± 19.4	21.6 ± 5.57	1.19 ± 0.54	8.38 ± 0.74	13.0 ±1.02	24.5 ± 1.36	8.4 ± 3.24
	Esengeni	61.0 ± 12.1	25.8 ± 3.96	0.66 ± 0.24	8.18 ± 0.95	7.21 ± 1	24.0 ± 1.52	28.2 ± 2.55
	Catalina Bay	130 ± 52.0	16.5 ± 8.03	0.11 ± 0.03	7.81 ± 0.72	17.5 ± 2.68	24.3 ± 1.56	31.7 ± 15.9
	Charters Creek	146 ± 45.3	27.4 ± 9.89	0.11 ± 0.02	6.38 ± 0.85	26.1 ± 3.46	27.3 ± 1.91	33.2 ± 7.31
	Listers Point	139 ± 27.3	104 ± 49.5	0.07 ± 0.01	4.91 ± 0.88	53.4 ± 12.5	28.5 ± 1.26	33.5 ± 9.17
open	**Mouth**	101 ± 86.1	13.4 ± 3.09	0.05 ± 0.03	7.57 ±0.63	34.6 ± 1.05	23.1 ± 1.6	24.4 ± 15.8
	Esengeni	23.0 ± 22.4	24.7 ± 2.19	0.33 ± 0.23	6.78 ±0.57	33.9 ± 1.78	21.4 ± 2.45	41.2 ± 21.7
	Catalina Bay	344 ± 258	7.09 ± 2.58	0.25 ± 0.1	7.5 ±1.26	29.1 ± 3.26	23.7 ± 3.4	8.37 ± 4.18
	Charters Creek	1117 ± 1046	26.7 ± 10.3	0.26 ± 0.16	8.51 ±1.15	29.7 ± 3.17	25.5 ± 2.78	20.7 ± 10.4
	Listers Point	495 ± 475	23.8 ± 4.85	0.28 ± 0.14	6.91 ±0.91	35.1 ± 4.25	23.8 ± 2.73	32.8 ± 17.7
Re-closed	**Mouth**	24.8 ± 7.93	24.1 ± 7.24	2.3 ± 0.43	6.31 ±1.5	15.6 ± 3.5	22.9 ± 2.42	23.2 ± 5.04
	Esengeni	22.5 ± 7.26	38.3 ± 13.3	1.4 ± 0.19	6.66 ±1.44	13.9 ± 4.47	22.9 ± 2.41	45.8 ± 7.95
	Catalina Bay	55.8 ± 15.3	15.1 ±7.55	0.21 ± 0.05	5.81 ±1.52	43.4 ± 6.98	24.4 ± 2.75	43.4 ± 13.2
	Charters Creek	127 ± 92.3	10.4 ± 3.59	0.19 ± 0.05	7.33 ±0.77	50.0 ± 7.74	26.2 ± 2.85	133 ± 35.6
	Listers Point	37.7 ±20.5	48.4 ± 18.1	0.24 ± 0.06	7.17 ±0.92	79.5 ± 11.8	26.4 ±3.32	166 ± 54.8

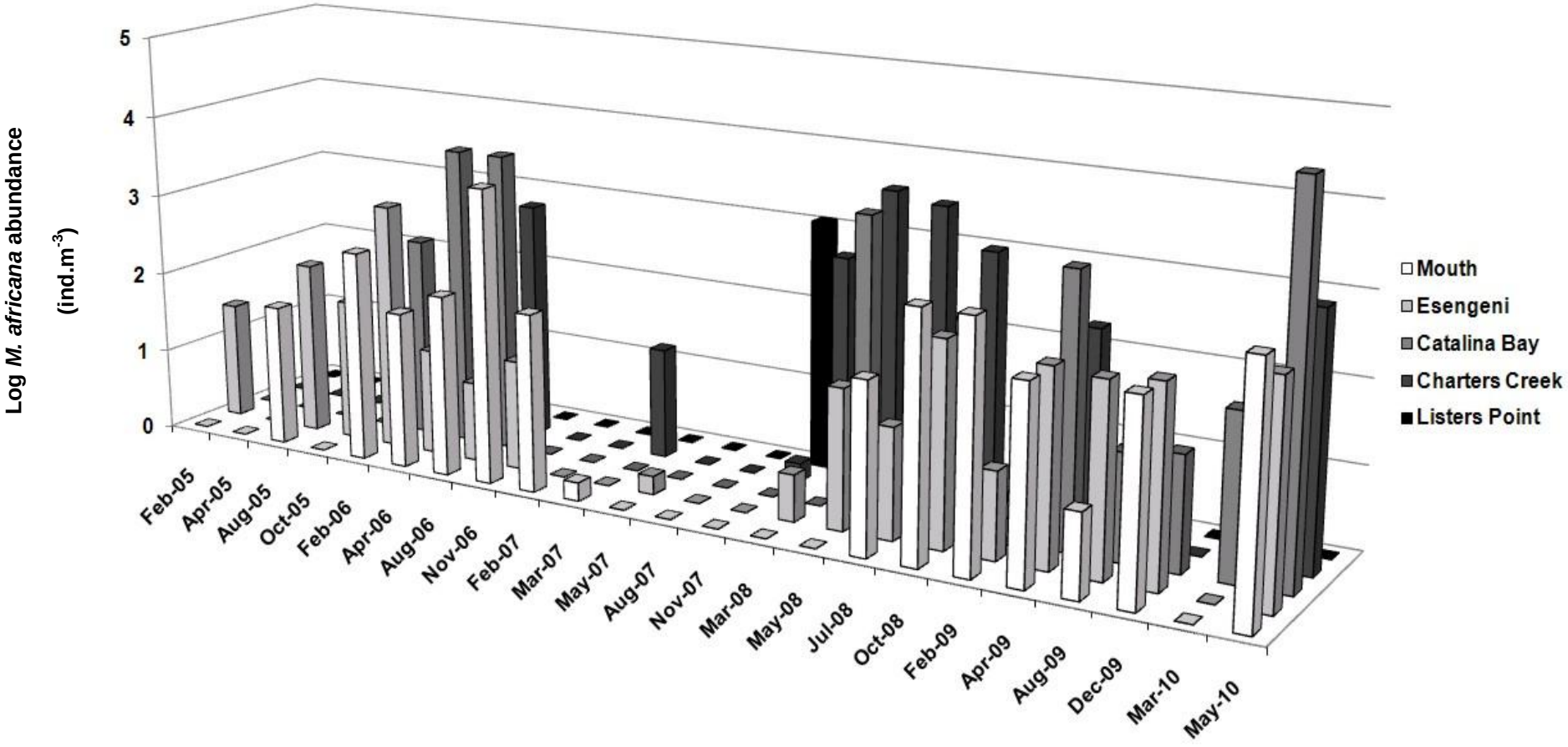

Fig.2.1: *M. africana* abundance at the 5 representative stations in the St. Lucia Estuary from February 2005 to May 2010. The maximum abundance value (3.7×10^4) is excluded due to compression of the y-axis.

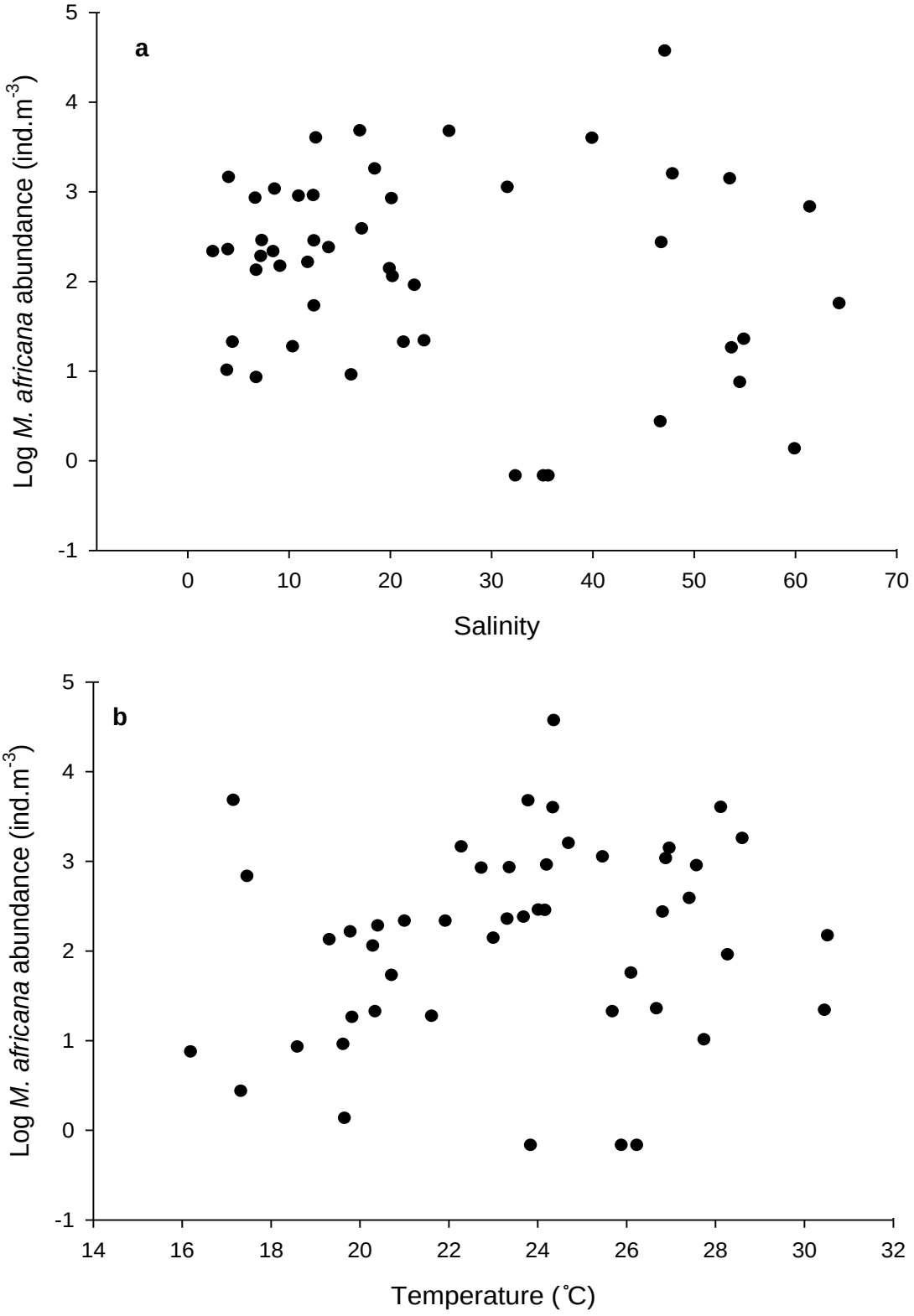

Fig.2.2: *M. africana* abundance correlated against *in situ* (a) salinity and (b) temperature at time of collection.

2.3.3 Temperature and salinity shock and acclimation experiments

The range of tolerance for *Mesopodopsis africana* was narrower under experimental conditions than under field conditions. In the shock experiments, temperature and salinity, as well as the interaction between these variables, had a significant effect on mortality (RM-ANOVA, F_{18} = 60.7, $p < 0.001$). For temperature, mortality in the 20 and 30ºC treatments was significantly different to that in the 10 and 40 ºC temperature treatments (post hoc Dunnets T3, $p < 0.001$). Mortality at 10 and 40º C was extremely high, such that no mysids survived past the first hour. Mortality was significantly higher at 0, 50 and 60 than at 5 and 25 (post hoc Dunnets T3, $p < 0.001$). At 20˚C, LS_{50} was not reached in the 2.5, 5 and 25 salinity treatments. At a salinity of 40, LS_{50} was reached after 8 h. In the 30˚C treatments, LS_{50} was reached after 8 h at a salinity of 2.5 , after 24 h at 5 and 25 and after only 2 h at 40 (Fig. 2.3).

Survival improved significantly in the acclimation experiments (t = 3.14, $p < 0.01$). Both temperature and salinity, and the interaction between these variables, had a significant effect on mysid mortality (RM-ANOVA, F_{15} = 18.2, $p < 0.001$). As in the shock experiment, mortality in the 20 and 30ºC temperature treatments was significantly less than that in the 10 and 40ºC temperature treatments (post hoc Dunnets T3, $p < 0.001$). Mortality was significantly greater at 55 and 65 than at 1, 2.5 and 40 (post hoc Dunnets T3, $p < 0.001$). At 10˚C, LS_{50} for 2.5 was reached after 4 h. At 20˚C LS_{50} for 1 was reached after 2 h. LS_{50} for 2.5 was not reached. LS_{50} for 40 was reached after 8 h. At 30˚C, LS_{50} for 1, 2.5 and 40 treatments were reached after 1, 72 and 4 h respectively. For all other treatments, survival was negligible (Fig. 2.4).

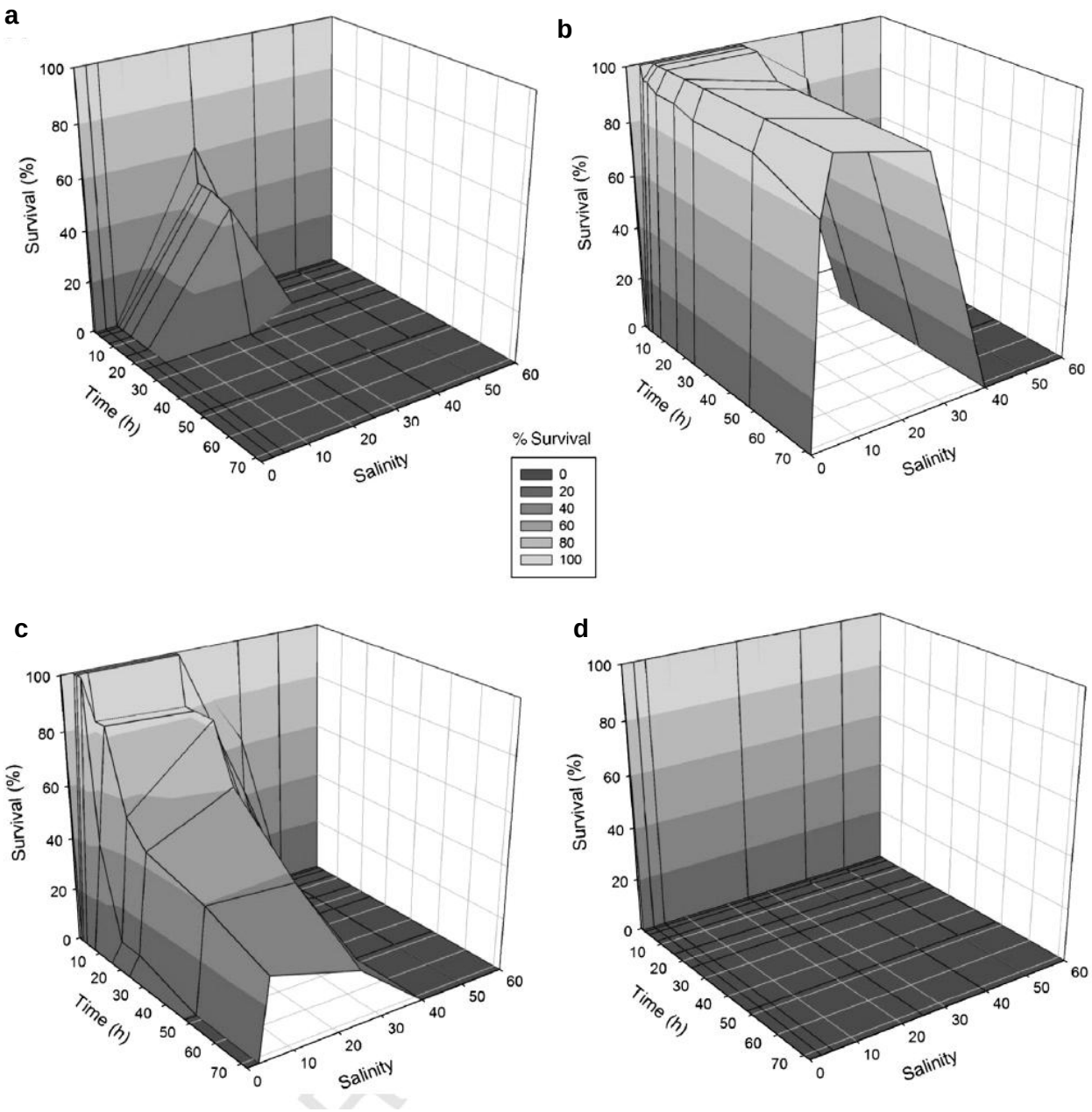

Fig.2.3: 3-D plot showing the percent survival of *M. africana* at each of the salinity treatments for the duration of the shock experiment at (a) 10ºC, (b) 20ºC, (c) 30ºC and (d) 40ºC.

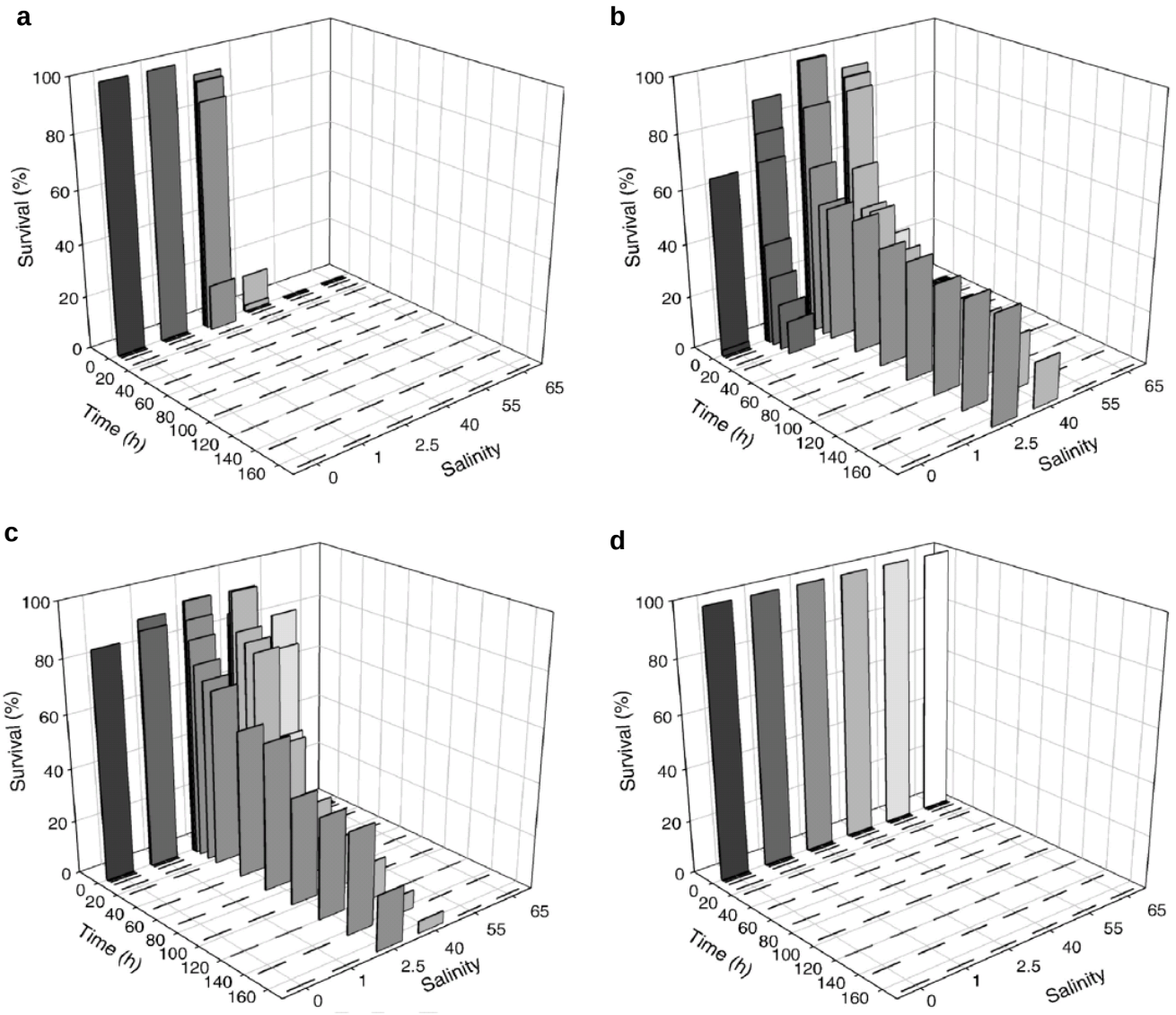

Fig.2.4: 3-D plot showing the percent survival of *M. africana* at each of the salinity treatments for the duration of the acclimation experiment at (a) 10ºC, (b) 20ºC, (c) 30ºC and (d) 40ºC.

2.4. DISCUSSION

Temperature and salinity are arguably the two most important variables influencing estuarine inhabitants. Natural abundances of *Mesopodopsis africana* recorded over the last five years were plotted against the temperatures and salinity levels at which they occurred. Mysids were found at temperatures ranging from 16.2 to 30.9ºC and at salinity levels ranging from 2.55 to 64.4. Grindley (1982) recorded salinity ranges for many of the common zooplankton taxa in the St. Lucia Estuary and documented the euryhaline nature of *M. africana*, stating that this mysid can tolerate salinity levels up to 60. However, this conclusion was based on the occurrence of adults at ranges of salinity in the natural environment, rather than on tolerance limits defined through experimentation. The present study utilized an integrated approach, observing both *in situ* and *in vitro* responses of *M. africana* to varying temperature and salinity levels.

In this study, *Mesopodopsis africana* populations were generally restricted to the Mouth and Narrows during closed-mouth conditions, presumably because these areas were partly protected from the drought due to the freshwater input from the Mpate and Mfolozi Rivers. However, after the mouth breaching event of March 2007, mysid populations established themselves in the Northern lakes. This region was probably a more stable environment than the Mouth at the time, since it was not subjected to the same degree of regular tidal flushing that was experienced at the Mouth (Carrasco *et al*. 2010: Chapter 1). After mouth re-closure, the salinity levels in the Northern Lakes exceeded the tolerance limits of *M. africana*, resulting in their re-appearance in the lower reaches of the lake. Multivariate correlations, however, showed weak associations of *M. africana* densities with environmental parameters in the estuary. This is not uncommon in estuaries (Hastie and Smith 2006) and is probably a product of the highly dynamic nature of the system. The most important environmental variables affecting the distribution of *M. africana* were temperature and salinity. Mysid abundance and salinity were negatively correlated, reflecting the overall negative impact of hypersaline conditions. Additionally, mysid abundance was generally greater in cooler waters. This could be a product of the shallower waters (e.g. Charters Creek and Listers Point) being more susceptible to heating and evaporation. High temperatures are also known and expected to negatively affect the physiology of most zooplankton species (e.g. Moore *et al*. 1996, Norberg and DeAngelis 1997). In this study, the experimental results for temperature tolerance of *M. africana* mirrored those recorded in the field. *M. africana* was recorded surviving at temperatures up to 31.4ºC, which is one of the

highest documented temperature tolerances for a mysid (Bhattacharya 1982, McKenny and Celestial 1995).

In this study, mortality increased significantly when the salinity change was accompanied by a change in temperature, a scenario not unlikely under natural conditions. McKenney and Celestial (1995) also documented the significant interaction effect between temperature and salinity on the growth of *Mysidopsis bahia* collected from Santa Rosa Sound (Florida). Overall, the results for *Mesopodopsis africana* show that it is a broad euryhaline species. *M. orientalis*, for instance, tolerates a salinity range of about 3 to 31 when exposed to temperatures from 20 to 30ºC (Bhattacharya 1982). Adult *Neomysis americana*, acclimated to 20, tolerates salinity levels from 4 to 34 (Miller 1964). The only other species confirmed to be as tolerant of hyperhaline conditions as *Mesopodopsis africana* is *Neomysis intermedia*, which is capable of surviving salinity levels from 0.06 to 45 (Murano 1966, Roast *et al*. 2001). It was apparent that *M. africana* was more tolerant of the lower salinity levels. In the 20ºC acclimation experiments, the LS_{50} at 1 and 2.5 was only reached after 8 and > 168 h respectively. It must be noted, though, that the mysid population used for these experiments was taken from the mouth region of the St. Lucia Estuary, which at the time exhibited a salinity of 14. Mysids were, therefore, acclimated to a much lower salinity.

Acclimation is an important factor in establishing salinity tolerance of organisms (Bhattacharya 1982, Delisle and Roberts 1986, Kefford *et al*. 2007). Research has shown that stepwise transfer of individuals to different salinity levels may increase the upper and lower tolerance limits of organisms (Baylon and Suzuki 2007, Bhattacharya 1982, Delisle and Roberts 1986). Repeated salinity changes may, however, also have a cumulative negative effect and increase mortality at salinity levels that are otherwise tolerated (Vilas *et al*. 2009). In this study, acclimation was shown to significantly increase survival. During shock experiments, the LS_{50} for mysids in the 40 treatment was reached after 8 h while in the acclimation experiment, LS_{50} was only reached after 24 h. A key outcome of this study is that while acclimation does indeed increase salinity tolerance, the rate of acclimation is also of critical importance. Should one be logistically capable of employing a slower acclimation rate of weeks or even months, it is possible that the salinity tolerance observed in the field would mimic those recorded under experimental conditions. Mysids introduced to salinity levels of 50 and 60 did not survive past the first hour during the *in vitro* experiments, but were recorded living at 64 during field acclimated conditions. To our knowledge, this is the highest recorded salinity tolerance threshold for mysids. It must be noted though, that on occasions when mysids were collected at 60 to 64 in

the field, they were visibly physiologically stressed and probably experiencing sub-lethal effects, being highly inactive and not particularly responsive to mechanical stimulus (NK Carrasc, pers. observ.).

Although not studied statistically in this experiment, it was evident that juvenile mysids were more tolerant of extreme salinity levels when compared to their adult counterparts. Similar results are supported by other studies (Vilas *et al.* 2009). It is thought that, while macrozooplankton may control their estuarine position by vertical tide-related migrations, the movement of smaller zooplankton is probably insufficient to totally control their estuarine distribution (Kimmerer *et al.* 1998). High osmoregulation abilities in earlier developmental stages may, therefore, be an important adaptive value of organisms inhabiting highly variable systems, allowing their successful survival during hatching (Charmantier and Anger 1999, Charmantier *et al*. 2002).

Mesopodopsis africana is a key species in the St. Lucia Estuary and plays a vital role in linking primary and secondary production with higher trophic levels (Carrasco and Perissinotto 2010b: Chapter 4, 2011b: Chapter 5). Results presented in this study may, therefore, be useful in predicting how mysid populations could be affected by future environmental changes induced by anthropogenic climate changes. This is particularly important, since environmental changes in this system have been additionally aggravated by the artificial diversion of the main freshwater input source of the estuary, the Mfolozi River. While *M. africana* does have some of the highest recorded upper salinity and temperature tolerances recorded for a mysid, temperatures above 31ºC and salinity levels above 65 respectively, which are often recorded in this shallow estuarine lake, will influence the distribution of this species. Because of the importance of mysids in estuarine trophic food webs, the understanding of their community ecology is crucial in aiding the development of ecosystem-based management plans for estuaries.

In situ feeding rates and grazing impact of *Mesopodopsis africana* in the St. Lucia Estuary, South Africa

ABSTRACT

Mesopodopsis africana is an important mysid in southern African coastal zooplankton and a key species in the St. Lucia estuarine lake, which is currently undergoing severe desiccation owing to freshwater deprivation. *M. africana* populations through much of the system are consequently under severe environmental stress. This study investigates the grazing dynamics of this mysid species, in relation to autotrophic food availability and other environmental constraints in two contrasting areas of the St. Lucia Estuary, Charters Creek, heavily affected by the desiccation process and the Mouth, virtually under unchanged conditions. Gut evacuation experiments were conducted once each during the day and the night. Evacuation rates were consistently higher during the night, ranging from 0.27 to 0.33 h^{-1} at Charters Creek and from 1.13 to 1.24 h^{-1} at the Mouth. Ingestion rates were, therefore, higher at the Mouth resulting in population grazing impacts of 2.5% of the total microalgal biomass, while the grazing impact at Charters Creek was only 0.5%. The spatial variation in ingestion rates could be attributed to seasonal differences in gut evacuation rates, differences in the mean size of mysids used, or the physico-chemical conditions present at the two stations. It is suggested that mysid populations at Charters Creek are predominantly driven by bottom-up forces, initiated by the harsh environmental conditions. Despite the lower ingestion rates exhibited at Charters Creek, results indicate that these mysids are capable of meeting all their energetic requirements from a microalgal diet alone, although they may also utilise a heterotrophic diet.

Keywords: feeding, grazing impact, *Mesopodopsis africana*, salinity, St. Lucia Estuary, turbidity.

3.1 INTRODUCTION

Estuaries are dynamic ecosystems characterized by rapid and intense fluctuations in physico-chemical factors. These ecosystems usually support rich biotic communities, which are often spatially and seasonally variable (McLusky and Elliott 2004). Mysid shrimps are a common and key constituent of estuarine ecosystems, playing an important role in food webs, as both consumers and producers (Mauchline 1980). As consumers, mysids are generally regarded as omnivores, capable of successfully exploiting a variety of food resources. Diet may include phytobenthos, phytoplankton, detritus, sediment, microzooplankton, mesozooplankton and

small benthic invertebrates (Wilhelm *et al.* 2002, Kibirige and Perissinotto 2003a, Lehtiniemi and Nordström 2008, Vilas *et al.* 2008). Mysids may feed on phytoplankton, detritus and smaller zooplankton by means of a suspension-feeding current or, alternatively, actively prey on moving zooplankton by means of raptorial or ambush feeding (Mauchline 1980). The ability of mysids to feed selectively on prey of different sizes can modify the structure of estuarine zooplankton (Fulton 1982, Hansson *et al.* 1990, Kouassi *et al.* 2006) and phytoplankton (Lindén and Kuosa 2004). Mysids link primary and secondary production with higher trophic levels (Mees and Jones 1997, Viherluoto and Viitasalo 2001, Lehtiniemi *et al.* 2009), being important in the diets of a number of estuarine resident and juvenile fish species (Whitfield 1998). The characteristic of diel-vertical migration present in some species is generally accepted as a mechanism by which mysids reduce their risk of predation (Gliwicz 1986, Boscarino *et al.* 2009). This characteristic also gives some species the potential to link energy transfer between pelagic and benthic environments (Taylor 2008, Vilas *et al.* 2008, Lehiniemi *et al.* 2009).

The St. Lucia Estuary forms part of the iSimangaliso (formerly Greater St. Lucia) Wetland Park. iSimangaliso is South Africa's first UNESCO World Heritage Site and has been designated a Ramsar Wetland of international importance (Begg 1978). It is the largest estuarine lake in Africa (Cyrus and Vivier 2006, Fielding *et al.* 1991). Covering an area of ~325 km^2, it represents approximately 80% of KwaZulu-Natal's total estuarine area (Begg 1978). The lake system is governed by cyclical wet and dry phases, each lasting between four and ten years (Begg 1978). St. Lucia is currently experiencing a freshwater starvation-induced crisis. Drought conditions have been exacerbated by the canalisation of the Mfolozi River, and the subsequent diversion of its freshwater away from the St. Lucia Estuary, in order to avoid the perceived threat of siltation (Whitfield and Taylor 2009). The present drought has led to a drastic reduction in water levels and concomitant salinity increases. A reversed salinity gradient has persisted, with the occurrence of hypersaline conditions in the northern regions of the lake.

Zooplankton communities in St. Lucia, like most estuarine zooplankton assemblages in southern Africa (Wooldridge 1999), are dominated by typical estuarine taxa, such as *Pseudodiaptomus stuhlmanni*, *Acartia natalensis* and *Mesopodopsis africana*, which often collectively contribute over 90% of the total zooplankton abundance (Carrasco *et al.* 2010: Chapter 1). While copepods are often numerically abundant, mysids show more importance in terms of biomass. In the St. Lucia Estuary, *M. africana* plays a major role in the trophic functioning of the system and is generally among the dominant components of its zooplankton community (Carrasco *et al.* 2010: Chapter 1, Jerling *et al.* 2010). However, due to the ongoing drought it has disappeared from

large sections of the estuarine lake, currently occurring only in its mouth region and in parts of the Narrows and South Lake (Fig. 1.1). The present study compares the feeding rates and grazing impact of *M. africana* on microalgal biomass and production at two different stations within this unique lake complex: Charters Creek, which has been severely affected by the desiccation process; and the Mouth, which remains virtually unchanged. The overarching aim of the study was to establish the trophic role of this key species within the St. Lucia Estuary, in order to better inform the sustainable management of this ecosystem during periods of extreme stress, such as the current one.

3.2 MATERIALS AND METHODS

3.2.1 Study site and mysid collections

The St. Lucia Estuary is situated in northern KwaZulu-Natal and lies between 27˚ 52'0" S to 28˚ 24'0" S and 32˚ 21'0"E to 32˚ 34'0"E. The system is made up of three shallow lakes connected to the Indian Ocean by a 21 km channel known as the Narrows (Fig. 1.1). In total, the St. Lucia estuarine lake covers an area between 300 and 350 km^2, depending on water levels (Begg 1978). *In situ* grazing experiments were conducted at Charters Creek in September 2008 and at the Mouth in February 2009. On both occasions, physico-chemical measurements were taken with a YSI 6920 water quality logger, fitted with temperature, depth, conductivity, dissolved oxygen, pH and turbidity probes. Mysids were collected by towing a hyperbenthic sled (200 µm mesh, open area ratio ≈ 3:1) along the bottom of the estuary for 27 m. The mouth of the net was semi-circular in shape (radius = 18.5 cm) and was mounted on a sled such that the net was raised 7.5 cm above the sediment surface.

3.2.2 Pelagic and benthic microalgae

Five 250 mL subsurface water samples were collected from the Mouth and Charters Creek in February 2009 and September 2008, respectively. This water was filtered through a GF/F filter to determine the total concentration of chlorophyll *a* (chl *a*) and phaeopigments. Phytoplankton biomass was determined fluorometrically (Turner Designs 10-AU) after cold extracting chl *a* and phaeopigments from filters in 10 mL 90% acetone, for 48 h in the dark. Microphytobenthic

cores (2 cm internal diameter, n = 3, depth = 1 cm) were collected at each station and placed in 100 mL polyethylene bottles containing 30 mL 90% acetone for chl *a* extraction (Nozais *et al*. 2001). Biomass was again determined fluorometrically and expressed as mg pigm.m^{-2}.

3.2.3 Zooplankton grazing experiments

For the estimation of diel variations in gut pigment content, mysids were collected at 4 h intervals for a period of 24 h at both stations. Variability in feeding activity was assessed during winter (early September 2008) at Charters Creek and in summer (February 2009) at the Mouth. Ten replicates, each containing ten mysids, were used for each time interval. After being rinsed in filtered estuarine water, the mysids were placed in 6 mL 90% acetone and stored at 4°C for 48 h in the dark for chlorophyll extraction. The pigment content of the acetone solution was measured with a Turner Designs 10-AU fluorometer and expressed as chl *a* equivalents per individual (ng pigm.ind^{-1}). Differences between day and night gut pigment levels were verified using independent samples t-test in SPSS version 15 for Windows, after checking for normality and homogeneity of variance of the residuals.

A further zooplankton sample was collected at each time period to assess changes in community structure and abundance. These samples were fixed with 4% phloxin-stained formalin. In the laboratory, samples were suspended in 1 to 5 L solutions, depending on the density of organisms. The main sample was then stirred vigorously, so that all the organisms remained in a homogenous suspension. A 20 mL plastic vial attached to a metal rod was used to withdraw three subsamples from mid-depth (Perissinotto and Wooldridge 1989, Carrasco *et al*. 2010: Chapter 1). Zooplankton within the samples was identified and counted with a dissecting microscope (400 x). In all cases, the coefficient of variation between subsamples was less than 10%.

Grazing rates of *Mesopodopsis africana* were estimated using the *in situ* gut fluorescent technique. To calculate the gut evacuation rate, freshly caught zooplankton were gently placed in 20 L plastic buckets. Mysids had to be carefully removed from this master sample and rinsed in filtered estuarine water before being placed in 250 mL polyethylene bottles containing filtered seawater and non-fluorescent corn starch. Ten mysids were placed in each bottle and strapped to a plankton wheel (1 rpm) for their designated time period. Mysids were removed every ten minutes for the first hour and 20 minutes thereafter. For each time interval

measurement, five replicates were used. The ten individuals were pooled in plastic tubes (10 mL) containing 6 ml 90% acetone for chlorophyll extraction as above. The gut evacuation rate was derived from the slope of the regression of the change in gut pigment versus time (Perissinotto and Pakhomov 1996). This experiment was repeated twice at each station, once during the day and again during the night.

An estimate of the gut pigment destruction rate was determined using the two-compartment approach (Perissinotto 1992). Prior to the experiments, mysids were allowed to empty their guts for 24 h in particle free water to which corn starch had been added. Five replicate bottles, each containing 10 mysids, were incubated for 1 h in naturally occurring phytoplankton concentrations. This estuarine water was first passed through a 100 μm mesh so as to remove larger metazoan grazers. A further five replicates were incubated without grazers (control). A comparison of pigment budgets in the control and experimental treatments was then carried out. Any decrease in the pigment concentrations in the grazing bottles was attributed to gut pigment destruction, as no faecal pellets were observed at the bottom of the containers or on the collecting sieve at the end of the experiment (Perissinotto 1992, Kiørboe and Tiselius 1987). Daily ingestion rates (I, ng pigm.ind^{-1}.day^{-1}) were estimated from the equation:

$$I = k.G/(1- b)$$

Where, k is the gut evacuation rate (h^{-1}), G is an integrated value (over 24 h) of gut pigment contents and b is a non-dimensional index of pigment destruction (Wang and Conover 1986). Ingestion rates were calculated using both (day-night) evacuation rates.

3.2.4 Grazing impact and daily ration

Mysid population grazing impact was calculated as the product of mysid abundance and individual ingestion rates. Due to the large variability in zooplankton abundance and feeding activity during the diurnal cycle, integrated values (over a 24 h period) of both gut pigment contents and abundance were used to calculate feeding impact (Perissinotto 1992). Grazing impact was expressed as a percentage of the microalgal standing stock consumed per day as well as impact on the primary production. Microalgal standing stock was calculated by combining phytoplankton biomass with microphytobenthic biomass. To do this, microphytobenthos concentrations first needed to be multiplied by the water depth at each

station.While primary production was not assessed during this study, estimates generated by van der Molen and Perissinotto (2011) were used to approximate the percent impact of *Mesopodopsis africana* grazing on the total primary production (benthic and pelagic combined).

Daily rations for *Mesopodopsis africana* were estimated using the relationship of carbon content versus dry weight of Beers (1966) and Parsons *et al.* (1984): C = 35 to 43% DW. To determine individual dry weights, 10 to 20 individuals were placed in pre-weighed tin capsules and oven-dried at 60˚C for 24 h. Dry weights were then calculated by subtracting the initial weight from the final. Weights were determined using a Sartorius microbalance with resolution at the 0.0001 mg level.

3.3 RESULTS

3.3.1 Physico-chemical environment and microalgae

Water temperatures ranged between 19 and 21°C at Charters Creek during winter and between 26 and 28°C at the Mouth during summer. Salinity ranged from 6.3 at the Mouth to 49.9 at Charters Creek. The average water depth at Charters Creek was 0.5 m. These shallow depths, combined with the fine sandy (< 63 μm) substratum, resulted in high turbidity levels manifesting at this station (Table 3.1). Here, during the winter phase phytoplankton biomass varied between 4.27 and 5.72 mg pigm.m^{-3}. Microphytobenthic biomass ranged from 6.90 to 35.9 mg pigm.m^{-2}. Conversely, the Mouth was characterized by deep pools (± 5 m) with relatively low turbidity (average = 36.3 Nephelometric Turbidity Units or NTUs). At this station, in summer (February 2009), phytoplankton biomass ranged from 10.5 to 15.3 mg pigm.m^{-3}. Microphytobenthic biomass ranged from 21.2 to 35.1 mg pigm.m^{-2} (Table 3.1).

Table 3.1: Physico-chemical variables, phytoplankton (PPL) and microphytobenthic (MPB) biomass measured at Charters Creek and the Mouth. Mean ± SE.

	Date	Depth (m)	Salinity	Temp (ºC)	Turbidity (NTU)	PPL (mg pigm.m^{-3})	MPB (mg pigm.m^{-2})
Charters Creek	3-4/ 09/ 08	0.7	49.9 ± 0.02	19	167 ± 29.4	4.98 ± 0.41	17.9 ± 4.97
Mouth	27-28/ 02/ 09	3.0	6.3 ± 1.07	27	36.3 ± 2.94	12.6 ± 0.33	20.1 ± 8.34

3.3.2 Zooplankton abundance and biomass

Total zooplankton abundance ranged from 1.9 x 10^4 to 1.5 x 10^5 ind.m^{-3} at Charters Creek during September 2008 and from 2.8 x 10^3 to 2.3 x 10^4 ind.m^{-3} at the Mouth in February 2009. The dominant taxa at both stations were *Pseudodiaptomus stuhlmanni, Acartia natalensis* and *Mesopodopsis africana*, collectively contributing more than 90% of the total zooplankton abundance. While the abovementioned copepods were always numerically dominant, *M. africana* was more important than copepods in terms of biomass at the Mouth, but not at Charters Creek. At the Mouth, *M. africana* was only responsible for about 30% of total zooplankton abundance; it accounted for 92% of total zooplankton biomass. At Charters Creek, *M. africana* was responsible for less than 1% of total zooplankton abundance and made up 13% of total zooplankton biomass, with *P. stuhlmanni* contributing 84% towards total the total zooplankton biomass (Fig. 3.1). Interestingly, there was an inverse relationship between the diurnal abundance of *A. natalensis* and *P. stuhlmanni*, and that of *M. africana*. While mysid densities decreased during the dark/night-time intervals, copepod densities increased during the same period (Fig. 3.2).

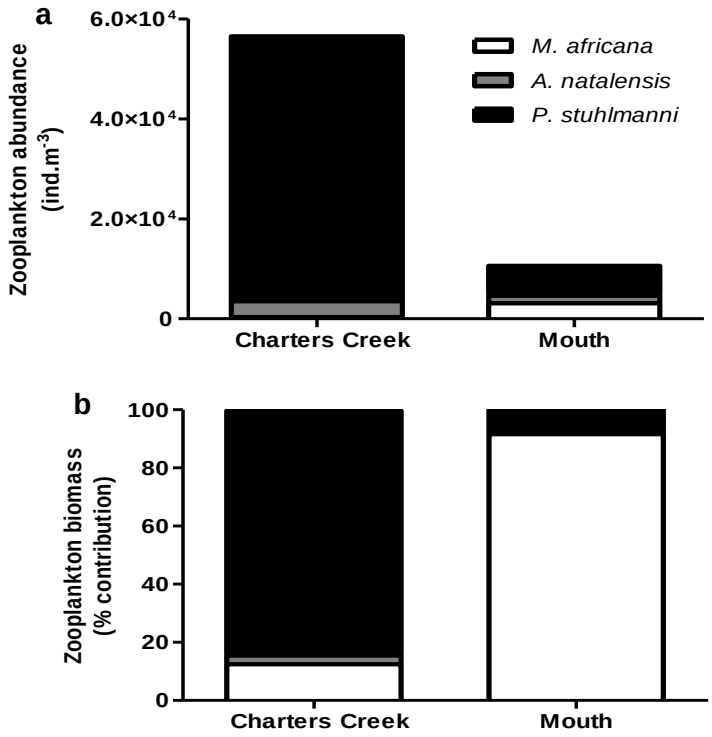

Fig. 3.1: Average contribution of the dominant taxa to the (a) total zooplankton abundance and (b) biomass at Charters Creek and the Mouth during the study period.

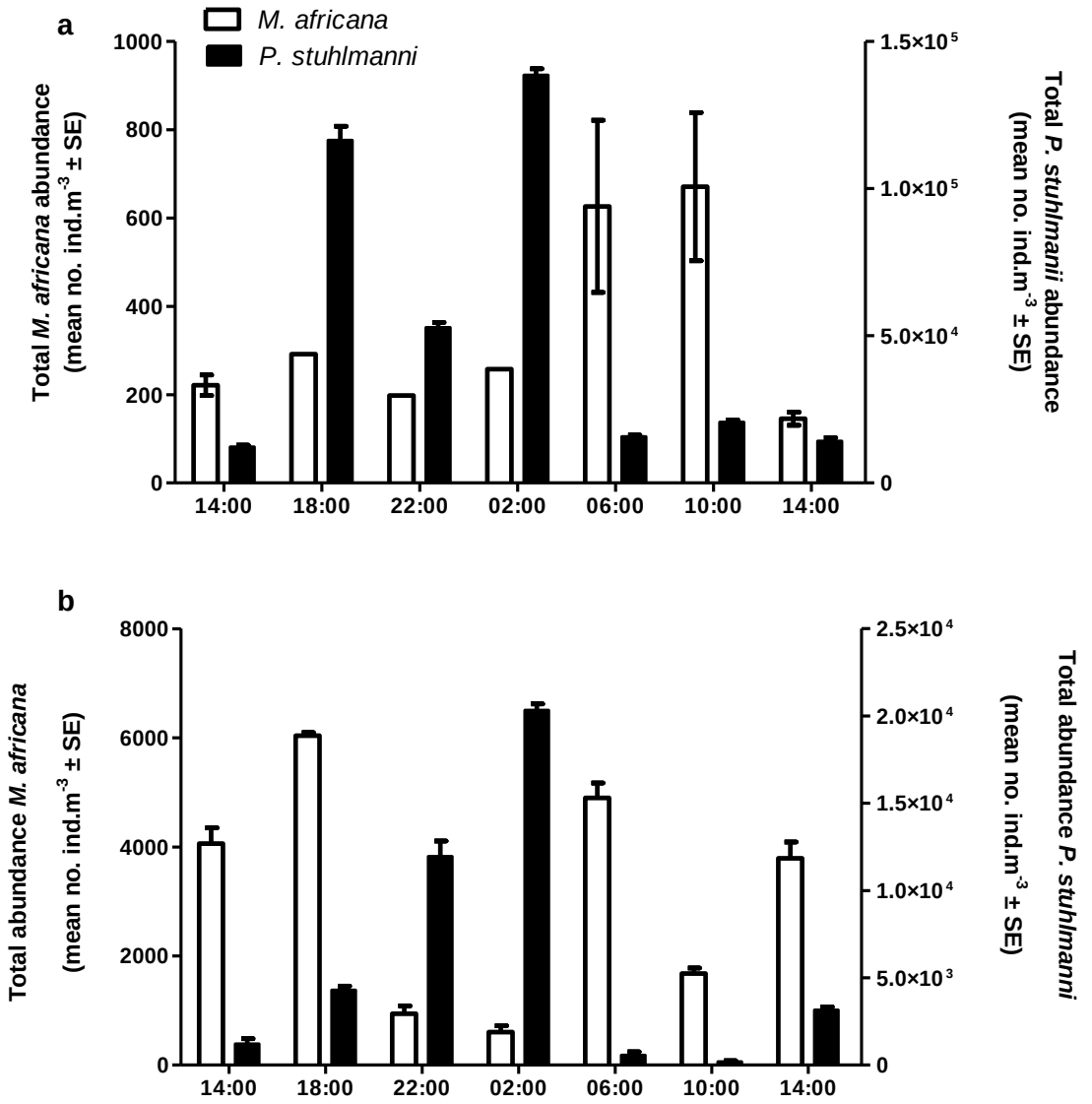

Fig. 3.2: Diurnal variations in zooplankton abundance at (a) Charters Creek in September 2008 and (b) the Mouth in February 2009.

3.3.3 Mysid grazing experiments

The average length (mean ± SD) of individual mysids used for these experiments was 6.66 ± 0.63 mm at Charters Creek and 5.26 ± 1.04 mm at the Mouth. Gut evacuation rates in *Mesopodopsis africana* were greater during the night than during the day (Fig. 3.3). Significant day/night differences were also recorded in the gut pigment contents of *M. africana* at both stations. Night values at the Mouth were on average twice as high as daytime values (t = -7.441,

$p < 0.001$) (Fig. 3.4). Gut pigment contents (mean ± SE) averaged 0.3 ± 0.02 ng pigm.ind^{-1} during the day and 0.53 ± 0.02 ng pigm.ind^{-1} at night. While diel differences in gut pigment content at Charters Creek were less evident, day and night values were still significantly different (t = -3.27, $p < 0.001$). Here, night-time values averaged 0.51 ± 0.42 ng pigm.ind.$^{-1}$, while daytime values averaged 0.31 ± 0.04 ng pigm.ind.$^{-1}$.

Evacuation rates at Charters Creek ranged from 0.27 h^{-1} during the day, to 0.33 h^{-1} at night. Gut pigment destruction averaged 99.1% of the total pigment ingested. At the Mouth, gut evacuation rates were 1.13 h^{-1} during the day and 1.24 h^{-1} at night, with a gut pigment destruction of 98.2% of the total pigment ingested. *In situ* grazing rates at the Mouth were, therefore, greater than those recorded at Charters Creek. Grazing rates of *Mesopodopsis africana* at Charters Creek approximated 16.4 ng pigm.ind^{-1}.h^{-1}, while those at the Mouth were 23.2 ng pigm.ind^{-1}.h^{-1}. Taking into account the integrated values of gut pigment content and abundance at each time interval throughout the 24 h sampling period, the mysid grazing impact on the total microalgal biomass was estimated at 0.146 mg pigm.m^{-3}.d^{-1} at Charters Creek and 1.58 mg pigm.m^{-3}.d^{-1} at the Mouth. Using the ingestion rates obtained with the *in situ* grazing method, the daily carbon rations were calculated to be 31.6% at the Mouth in summer and 22.4% at Charters Creek in winter (Table 3.2).

Table 3.2: Estimates of gut evacuation rates (k), gut pigment destruction (b^1), ingestion rate (I) and impact of mysid population grazing on total microalgal biomass (MAB) and primary production (PP) as well as the estimated contribution towards the daily carbon ration (DR) at Charters Creek and the Mouth in the St. Lucia Estuary.

	k (h^{-1})	b^1 (%)	I (ng pigm.ind^{-1}.hr^{-1})	MAB (%)	PP (%)	DR (%)
Charters creek	0.27 (day) - 0.33 (night)	99.1	16.4	0.48	3.05	22.4
Mouth	1.13 (day) - 1.24 (night)	98.2	23.2	2.52	11.0	31.6

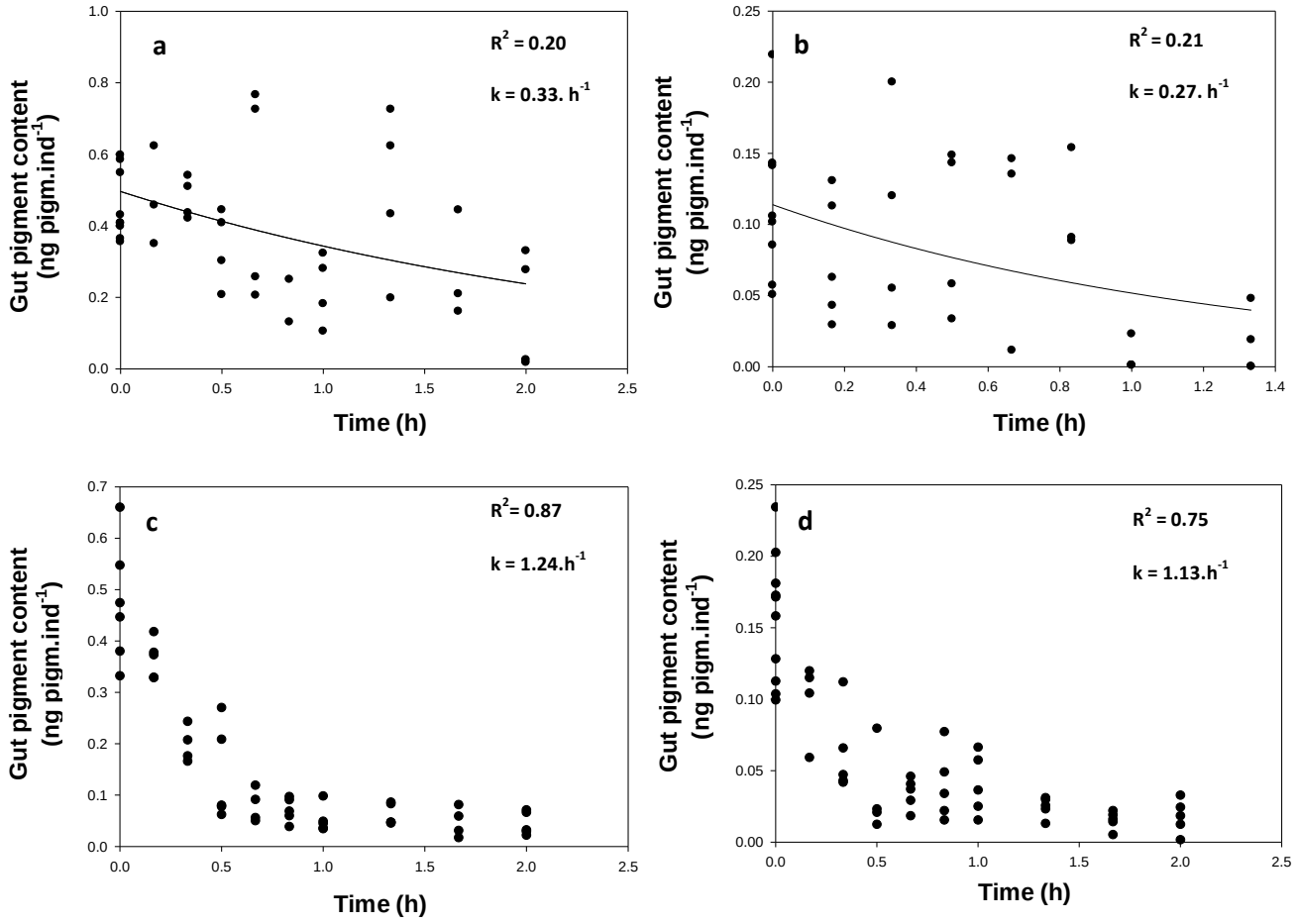

Fig. 3.3: Gut evacuation rates of *M. africana* in September 2008 at Charters Creek during (a) night and (b) day, and in February 2009 at the Mouth during (c) night and (d) day.

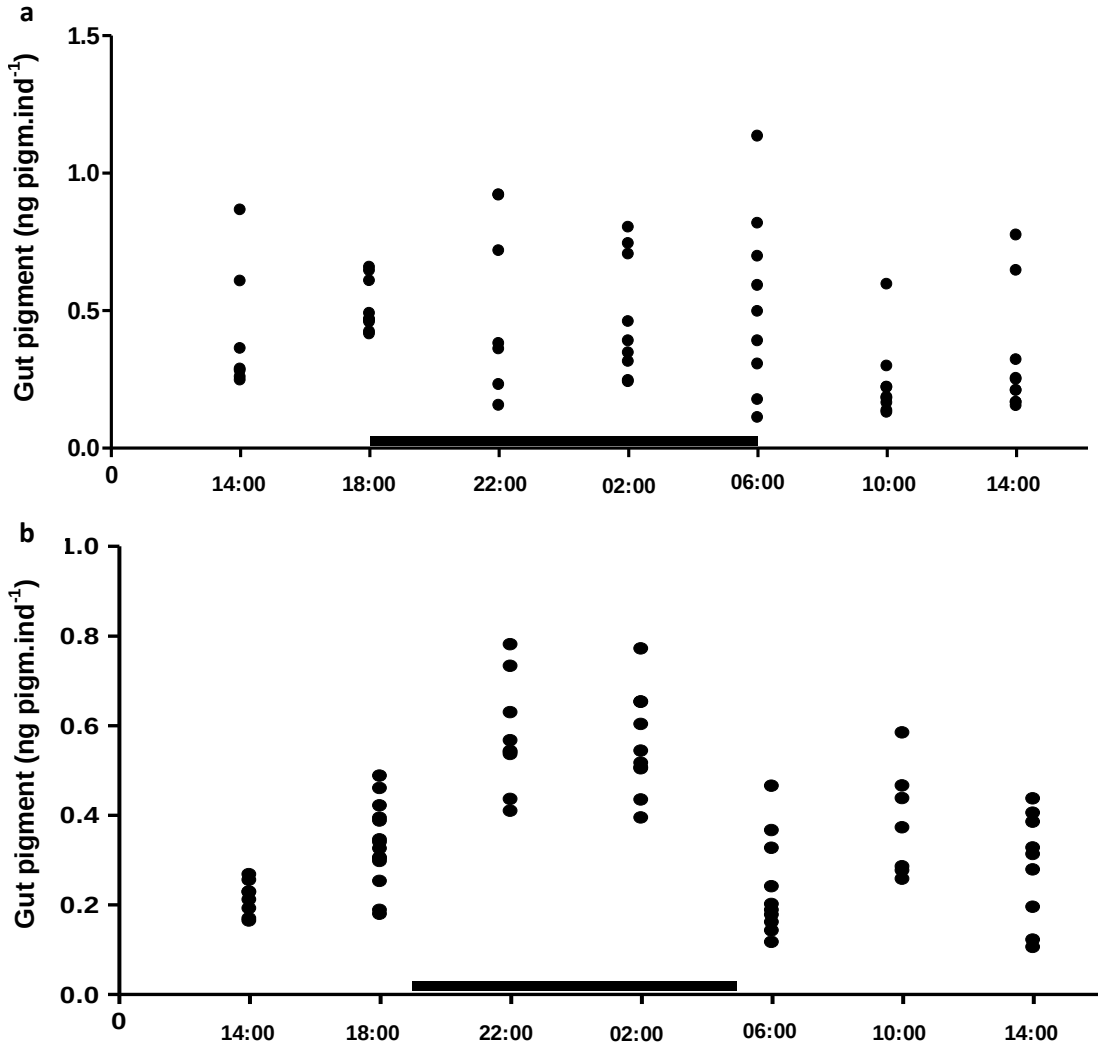

Fig. 3.4: Diurnal variations in total gut pigment levels per individual mysid at (a) Charters Creek in September 2008 and (b) the Mouth in March 2009. Period of darkness is indicated by thickening of the horizontal axis.

3.4 DISCUSSION

Mysids are a ubiquitous component of many aquatic ecosystems and represent an important link between producers and higher trophic levels (Mees and Jones 1997, Viherluoto and Viitasalo 2001, Lehtiniemi *et al.* 2009). The plasticity of their feeding mode gives them the potential to structure communities (e.g. Fulton 1982, Hansson *et al*. 1990, Kouassi *et al*. 2006). Mysids may also indirectly affect phytoplankton biomass as their excretion contributes towards regenerated production (Lindén and Kuosa 2004). While *Mesopodopsis africana* is not numerically dominant in the St. Lucia Estuary, it often makes a substantial contribution towards total zooplankton biomass and its presence is probably integral to the functioning of food webs within the estuary. Despite the importance of lower trophic level organisms, information on the grazing impact of *M. africana* on the pelagic and benthic primary production is lacking. The present study represents the first account of the feeding ecology of this species.

The gut evacuation rates of *Mesopodopsis africana* recorded during this study are in the same range as those reported for other mysid species in South African estuaries. The gut evacuation of *Mesopodopsis wooldridgei* in the Kariega Estuary ranged from 0.9 to 1.38 h^{-1} (Froneman 2001), while in Algoa Bay this was estimated at 1.5 h^{-1} (Webb *et al*. 1987). Gut evacuation rates of *Gastrosaccus bevifissura* in the Mpenjati Estuary ranged from 0.62 to 0.68 h^{-1} (Kibirige and Perissinotto 2003b). Certain mysid species undergo diel vertical migration, rising to the top of the water column to feed at night and descending at dawn in order to reduce the risk of predation. Several abiotic (illumination, water depth and the presence of thermoclines: Mauchline 1980) and biotic factors (predator and prey distribution: Boscarino *et al*. 2009) are known to affect the diel migration of mysid species. In this study, diel patterns in mysid gut pigments were evident both at Charters Creek and the Mouth, with higher gut pigment concentrations recorded during the night. Froneman (2001) did not observe diel patterns in gut pigment content in *M. wooldridgei* in the Kariega Estuary, substantiating that the well-mixed nature of the system (and hence the even distribution of chl *a* in the water column) revoked the need for mysids to feed only at night in the water column. Charters Creek is situated on the west coast of South Lake, which is known to be a turbid location (Cyrus 1988). Here, shallow waters combined with a fine sandy substratum frequently lead to turbidity values exceeding 300 NTU. Since vulnerability of mysids to predation decreases with decreasing water transparency, it was thought that the mysid populations at Charters Creek may no longer need to undergo diel vertical migrations to reduce the risk of predation. However, even though Charters Creek at the time was a shallow turbid station, and hence chl *a* probably evenly distributed throughout the

water column, mysids here still exhibited diel vertical migration. Not only were higher mysids densities recorded at the bottom during the day, but significant day/night differences in gut pigment content of mysids were also recorded. These differences were, however, less evident at Charters Creek than at the Mouth. Turbidity seemed to play a more important role in the presence of *M. africana* in the water column at Charters Creek. The increased mysid density from 06:00 to 10:00 was noticeable and these times incidentally corresponded with still calm conditions. Another interesting finding was the observed negative relationship between the abundance of the copepods *Pseudodiaptomus stuhlmanni* and *Acartia natalensis* and that of *M. africana* on both sampling occasions. Mysids may have migrated to surface waters at night, explaining the lower densities caught during sampling with the epibenthic sled. Abundance of *P. stuhlmanni*, however, increased despite the fact that this genus is also known to undergo diel-vertical migration (Kibirige and Perissinotto 2003b, Froneman 2004b). This anomaly could be a strategy to avoid predation by mysids. Fulton (1982) found mysid predation to have a significant effect on copepod densities. The effects of mysid predation on species composition appeared to depend on the relationship between their prey preferences and the dominant copepod species present in the communities. Additionally, Viitasalo *et al*. (1998) reported that the copepods *Eurytemora affinis* and *Temora longicornis* were able to detect the mysid *Neomysis integer* from a further distance than they were able to detect the three-spined stickleback *Gasterosteus aculeatus*.

Estimations of ingestion rates were corrected for pigment destruction due to digestion or degradation of chl *a* to non-fluorescent end products. Gut pigment destruction during this study ranged from 97.0 to 99.4% of the total pigment ingested. These values are high in comparison to those recorded for *Mesopodopsis wooldridgei* in the Kariega Estuary (53 to 84%: Froneman 2001), but similar to those recorded for *Gastrosaccus brevifissura* in the Mpenjati Estuary (99.6%: Kibirige and Perissinotto 2003b). There has been some controversy regarding the use of the pigment destruction coefficient in the gut pigment method for measuring ingestion rates (Durbin and Campbell 2007). These authors argue that tracers in the gut may be either digested and assimilated, or evacuated, and that both processes are taken into account when an evacuation rate curve is determined. However, when ingestion rates and subsequently daily rations were calculated in this study without correcting for gut pigment destruction, values were unrealistically low (< 0.1%)(cf: Mauchline 1998), especially given that microalgae (POM and MPB combined) supposedly contribute on average ~60% of this mysid's diet, as confirmed by isotope analysis (Carrasco and Perissinotto 2010b: Chapter 4, 2011b: Chapter 5). With the

inclusion of the gut pigment destruction coefficient, daily carbon rations ranged from 22.4 to 31.6% of the total body carbon. This is assuming that microalgae contribute 100% of the mysid diet. However, it has been shown that microalgae often only contribute ~60% of this mysid's diet (Carrasco and Perissinotto 2010b: Chapter 4). The daily ration of microalgae in mysid body carbon would then be in the range of 13.4 to 18.9%. Assuming that mysids require 10% body carbon per day to meet their basic metabolic requirements (Froneman 2000), these results indicate that mysids may be able to meet all their energetic requirements for moulting, reproduction and growth from a microalgal diet alone at both Charters Creek and the Mouth (Froneman 2000). However, the observed negative relationship between copepod and mysid densities suggests that *M. africana* may also utilize a heterotrophic diet. Indeed stable isotope data have confirmed their omnivorous nature, specifically at the Mouth, where a large portion of the mysid diet was made up by *Pseudodiaptomus stuhlmanni* (Carrasco and Perissinotto 2010b: Chapter 4). In a situation where microalgal biomass may be insufficient to sustain mysids' diet, they are likely to switch to zooplankton prey (Froneman 2001, Jerling and Wooldridge 1995, Kouassi *et al*. 2006). Metillo *et al*. (2007) also documented the broadly omnivorous nature of three co-occurring Tasmanian mysid species.

Gut evacuation rates were greater at night than during the day for both stations. This is consistent with the finding that most feeding activity takes place during the night, as indicated by the diel variation in gut pigments recorded in this study. Viherluoto and Viitasalo (2001) also found that the feeding of the pelagic Baltic Sea mysid, *Mysis mixta*, was significantly higher during dark conditions. They attributed this to the mysid's adaptation to avoid visual predation by pelagic fish. In this study, ingestion rates at the Mouth of the St. Lucia Estuary were significantly higher than those at Charters Creek. There are several possible explanations for this. Firstly, grazing experiments at Charters Creek were carried out during winter, while those at the Mouth were carried out in summer. Gut evacuation rates are highly variable, being affected by factors such as temperature, prey type, food concentration and feeding history (Chipps 1998, Dam *et al*. 1991, Ritz 2008). High temperatures, specifically, are known to increase metabolic rates and thus organisms in warmer waters tend to have higher gut evacuation rates than in cooler waters (e.g. Chipps 1998, Froneman 2004b). Secondly, high turbidity levels, such as those recorded at Charters Creek, have been shown to negatively affect the ingestion rates of *Mesopodopsis africana* (Carrasco *et al*. 2007). Thirdly, salinity values at Charters Creek were significantly higher than those at the Mouth. Although *M. africana* is believed to have a salinity tolerance up to 60 (Grindley 1982), it is possible that feeding rates

were lower at Charters Creek due to osmotic stress. Lastly, the average size of mysids used for experiments at Charters Creek was slightly larger than those used at the Mouth. The increase in gut evacuation rate for the smaller *M. africana* can probably be related to the higher metabolic rates of the smaller individuals (Pakhomov *et al*. 1997, Gurney 2000, Froneman, 2001). Additionally, Ritz (2008) tested the relationship between gut residence time (GRT) and mysid size and found that, under continuous feeding conditions, gut residence time is linearly and positively correlated with body length. This concurs with the findings of this study, as mysid body size was smaller at the Mouth than at Charters Creek. While mysids at the Mouth fitted Ritz's graph well for their size, those from Charters Creek had much longer GRT. This might have been due to the harsh conditions experienced at Charters Creek (salinity, turbidity etc), or a difference in diet at the two stations.

Mysid abundance was also lower at Charters Creek than at the Mouth. The main zooplanktivorous fish at Charters Creek were *Gilchristella aestuaria* and *Oreochromis mossambicus*. At the Mouth, these were *A. ambassis*, *G. aestuaria*, *O. mossambicus*, *Monodactylus argenteus* and *Gerres acinaces* and they were more abundant than at Charters Creek (Carrasco and Perissinotto in review). This suggested that fish predation was the main limiting factor for mysid abundance at the Mouth, since at the time the Mouth region was not subjected to the hypersaline conditions, high turbidity and low water levels experienced at Charters Creek. High turbidity levels have not only been shown to interfere with the filter-feeding capability of some zooplankton taxa, but suspended particles also increase the back scattering of light in the water column, reducing phytoplankton productivity (Kirk 1985).

Assuming that all pigments recorded in the gut of the mysids were of microalgal origin, the impact of the mysid population would be 0.146 mg pigm.m^{-3}.d^{-1} at Charters Creek and 1.58 mg pigm.m^{-3}.d^{-1} at the Mouth. Mysids at Charters Creek and the Mouth could, therefore, account for the clearance of up to 0.48 and 2.58% of the available microalgal biomass at each respective station. Wind-induced turbidity in shallow waters can often affect the exchange of microalgal biomass between benthic and pelagic subsystems (Scheffer 1998). One can, therefore, not be entirely certain that the primary production measured in the water column is only a product of phytoplankton, or whether it is the sum of this and that of re-suspended benthic microalgae (van der Molen and Perissinotto 2011). Van der Molen and Perissinotto (2011) estimated primary production for five different regions in the St. Lucia Estuary. Using these estimates, the percent

impact of *Mesopodopsis africana* grazing on the total primary production (benthic and pelagic combined) was calculated to be 3.05% at Charters Creek and 11.0% at the Mouth.

While *Mesopodopsis africana* was only responsible for about 30% of total zooplankton abundance at the Mouth, it accounted for 92% of its total zooplankton biomass. At Charters Creek, however, *M. africana* only made up 13% of total zooplankton biomass, with *Pseudodiaptomus stuhlmanni* contributing 84%. This indicates that *P. stuhlmanni* may be the main taxon responsible for the consumption of primary production in this region. Froneman (2004) provided estimates of gut evacuation rates for all seasons for *P. hessei* and *Acartia longipatella*, in the temporarily open/closed Kasouga Estuary on the east coast of South Africa. Using these values to estimate the feeding impact of *P. stuhlmanni* at Charters Creek, up to 6.72% of the local phytoplankton biomass could be utilized by this copepod species in winter and up to 47% during the summer (assuming that abundance and biomass of both *P. stuhlmanni* and phytoplankton remain relatively stable).

The role of mysid species, such as *Mesopodopsis africana*, in structuring food webs is widely known (e.g. Fulton 1982, Hansson *et al*. 1990, Kouassi *et al*. 2006). Their high biomass, relative to the other dominant taxa, grants them the potential to have substantial impacts on ecosystem functioning. This study has provided some detail on the feeding rates of *M. africana* at two different habitats within the St. Lucia estuarine lake. The mysid populations at these stations seem to be sculpted by different factors. While mysid populations at the Mouth appear to be mainly driven by top-down forces in the form of fish predation, at Charters Creek they are predominantly driven by bottom-up forces, initiated by harsh environmental conditions, such as high salinity and turbidity. The effects of this bottom-up forcing are evident in the smaller mysid population size, depressed feeding rates and subsequently, a relatively minor impact on primary production. In fact, these harsh conditions were responsible for the temporary disappearance of *M. africana* from Charters Creek during this study and also for its current absence from all other shallow lake areas of the system (Carrasco *et al*. 2010: Chapter 1). While the Charters Creek area is currently still functioning, continued drought conditions may lead to the permanent disappearance of the mysid populations in this region. This would restrict the distribution of this key species to the Mouth/Narrows portion of the system, i.e. less than 5% of the total lake surface

Spatial and temporal variations in the diet of the mysid *Mesopodopsis africana* in the St. Lucia Estuary, South Africa

ABSTRACT

This study presents one of the few known examples where a mysid species has been observed modifying its diet rapidly and under natural conditions, in response to environmental changes. *Mesopodopsis africana* is a dominant mysid in many estuaries along the east coast of South Africa, and a key species in the St. Lucia Estuary, Africa's largest estuarine lake. St. Lucia is currently undergoing severe desiccation owing to freshwater deprivation. Lack of freshwater input has dampened the effect of temporal variations, while different regions have become more spatially heterogeneous. The mixed model SIAR v 4.0 (Stable Isotope Analysis in R) was used to determine the likely contribution of each of the available carbon sources to the diet of *M. africana*. The copepod *Pseudodiaptomus stuhlmanni* made a significant contribution to *M. africana'*s diet in the Mouth region. At Catalina Bay, mysids mostly utilized particulate organic matter, while at Charters Creek they were most closely associated with the macroalga *Cladophora* sp. The sensitivity of Charters Creek to drought effects is emphasized here, as well as the important role *M. africana* plays in this habitat as an omnivore, increasing the connectance and, hence, sustaining its food web. While the Mouth and Narrows are partly protected from drought effects, the northern lakes have experienced further increases in salinity during the past decade, forcing the periodical exclusion of this mysid from much of the system. This has lead to severe effects on the food webs that the mysid supports under normal conditions.

Keywords: iSimangaliso Wetland Park, key species, South Africa, stable isotopes, trophic relations

4.1 INTRODUCTION

Mysid shrimps are a common and key constituent of estuarine ecosystems, playing an important role in food webs, as both consumers and producers (Mauchline 1980). As consumers, mysids are generally considered omnivorous, feeding on a wide range of items. Diet may include phytobenthos, phytoplankton, detritus, sediment, microzooplankton, mesozooplankton and small benthic invertebrates (Wilhelm *et al.* 2002, Kibirige and Perissinotto 2003a, Lehtiniemi and Nordström 2008, Vilas *et al.* 2008). The ability of mysids to feed selectively on prey of

different sizes can modify the structure of estuarine zooplankton (Fulton 1982, Hansson *et al.* 1990, Kouassi *et al.* 2006) and phytoplankton (Lindén and Kuosa 2004) assemblages. The potential for omnivory in mysids allows them to modify their diet in response to changes in food quality and abundance (Hughes 1980). Mysids are also relatively adaptive species, capable of enduring the rapid and intense fluctuations in physico-chemical factors often associated with estuarine ecosystems.

The St. Lucia Estuary, Africa's largest estuarine lake (Fielding *et al.* 1991, Cyrus and Vivier 2006), forms part of the iSimangaliso (formerly Greater St. Lucia) Wetland Park. This system is currently undergoing unusually harsh conditions. The area characteristically experiences cyclical wet and dry phases, each lasting between four and ten years (Begg 1978). Past agricultural practices, combined with the below average rainfall the area has received since 2002, has resulted in the estuary being cut off from the ocean. Low freshwater input and high evaporation rates have led to the persistence of a reversed salinity gradient, with hypersaline conditions in the upper reaches of the estuarine lake. These harsh conditions combined with the closed-mouth state, have resulted in only the most adaptive species remaining (Carrasco *et al.* 2010: Chapter 1). Dominant zooplankton within the St. Lucia Estuary include the mysid *Mesopodopsis africana* and the calanoid copepods *Pseudodiaptomus stuhlmanni* and *Acartia natalensis* (Carrasco *et al*. 2010: Chapter 1, Jerling *et al*. 2010). While the copepods are numerically dominant, mysids tend to be more important in terms of biomass (Wooldridge 1999). Due to the ongoing drought, *M. africana* has disappeared from large sections of the estuarine lake, currently occurring only in its mouth region and in parts of the Narrows and South Lake (Fig. 1.1).

This study examines the implications of harsh environmental conditions on the key zooplankton species *Mesopodopsis africana*. Knowledge of food web properties, such as trophic processes, enables inferences to be made about species interactions and ecosystem structure and function. The use of stable isotopes in ecological research has been recognized as an important tool towards this end. Since stable carbon isotopes ($\delta^{13}C$) are known to fractionate little between energy transfers, they are commonly used to quantify food sources and energy flow in aquatic systems. Stable nitrogen isotopes ($\delta^{15}N$) fractionate more and are typically used to infer trophic positions of consumers in a food chain (DeNiro and Epstein 1978, Peterson and Fry 1987). Models may allow simulation of possible diets and assessment of the relative importance of various sets of organic matter sources to consumers (Phillips and Eldridge 2006). Knowledge of trophic linkages would be especially relevant information for management, since certain food

chain links may disappear due to environmental stresses, such as those experienced in the St. Lucia Estuary.

In the subtropical climate of KwaZulu-Natal there are essentially two seasons, one characterized by regular rainfall (October to April) and the other with virtually no rain at all (May to September). The current drought crisis has also fractionated the system into very diverse habitats in relatively close range to one another. The overarching aim of this study is, therefore, to assess the spatial and temporal feeding preferences of this key zooplankton species under different environmental conditions. Stable $\delta^{13}C$ and $\delta^{15}N$ isotopes are thus used to gain insight into the trophic interactions and diet of *Mesopodopsis africana* within the St. Lucia Estuary.

4.2 MATERIALS AND METHODS

4.2.1 Physico-chemical environment and microalgal biomass

Physico-chemical measurements were taken on each sampling occasion with a YSI 6920 water quality logger, fitted with temperature, depth, conductivity, dissolved oxygen, pH and turbidity probes. For phytoplankton biomass, subsurface water samples were collected from each sampling station and filtered through a GF/F filter to determine the total concentration of chlorophyll *a* (chl *a*) and phaeopigments. Phytoplankton biomass (mg pigm.m^{-3}) was determined fluorometrically (Turner Designs 10-AU) after cold extracting chl *a* and phaeopigments from filters in 10 mL 90% acetone, for 48 h in the dark. Microphytobenthic cores (2 cm internal diameter, n = 3, depth = 1 cm) were collected at each station and placed in 100 mL polyethylene bottles containing 30 mL 90% acetone for chl *a* extraction (Nozais *et al*. 2001). Biomass was again determined fluorometrically and expressed as mg pigm.m^{-2}.

4.2.2 Sample collection and processing

For stable isotope analysis, microphytobenthos (MPB), particulate organic matter (POM), macroalgae (*Cladophora* sp.), detritus, sedimentary organic matter (SOM), and zooplankton samples were collected from stations within the St. Lucia Estuary, depending on their availability. For temporal comparisons, samples were collected from Charters Creek in September 2008 (late winter, dry season) and April 2009 (early autumn, wet season). The latter was used as a wet season comparison, since mysids were not available at Charters Creek until

that time. Further samples were collected from the Mouth in February 2009 (summer, wet season) and June 2009 (winter, dry season). In addition, mysids and their potential dietary items were collected simultaneously from four different stations within the estuary (Mouth, Esengeni, Catalina Bay and Charters Creek; Fig. 1.1) in April 2009, to explore the possibility of spatial differences in the proportion of different carbon sources contributing to the diet of *Mesopodopsis africana*. The spatial extent of each station was roughly 100 m^2 and each station would take about one day to sample.

To obtain an estimate of SOM, the upper 1 cm layer of sediment was collected and treated with excess 2% hydrochloric acid (HCl) in order to remove carbonates. The sediment was then rinsed thoroughly with distilled water, dried in the 60˚C for 24 h and subsequently crushed with a pestle and mortar and packaged in micro-centrifuge tubes (Eppendorf) for isotope analysis (e.g. Smit *et al.* 2005). Detritus (or decomposing plant organic matter) was collected within the floating foam which was often found at the surface near the water's edge. Detritus was similarly treated with 2% HCl to remove carbonates, rinsed with distilled water, dried at 60˚C for 24 h and subsequently crushed with a pestle and mortar and packaged in tin capsules for isotope analysis. Triplicate samples were taken for both SOM and detritus on each sampling occasion.

For the extraction of MPB, dense algal mats were collected by scraping the upper 1 cm of sediment. This sediment was then re-suspended in filtered estuarine water and stirred, so that the MPB remained in suspension while the heavier sediment settled to the bottom of the container. For stations where the sediment was too fine for this protocol to be used, a procedure similar to that of Couch (1989) was employed. Sediment was collected as above by scraping the upper 2 mm in areas of dense microalgal mats. Within 4 h, the sediment was brought back to the laboratory where it was spread out onto trays with a depth of about 1 cm. A 63 μm nylon sheet was then placed over this, followed by a further nylon sheet. This was then covered with a thin layer of pre-combusted sediment (400˚C, 6 h). The trays were held under light and the sediment was kept moist by spraying it with filtered estuarine water from the sampling site. After 12 h, the top 2 mm of the sediment into which the motile algae had migrated, was collected and sieved through a 100 μm mesh so as to retain the sediment. The microalgae were then filtered onto previously combusted Whatman GF/F filters. Water samples for POM were filtered onto pre-combusted (450°C, 6 h) Whatman GF/F filters. POM and MPB samples were acid-treated with 2% HCl to remove any inorganic carbon in the form of calcium carbonate ($CaCO_3$) that may have been present. Filters containing POM and MPB (in triplicate) were placed into aluminium foil envelopes and frozen prior to laboratory-based processing.

Samples of the dominant macroalgae (*Cladophora* sp.) were collected and rinsed thoroughly with distilled water to ensure the removal of any attached sediment particles. The alga was subsequently dried at 60˚C in an air-circulated oven for 24 h and thereafter homogenized into a fine powder using a pestle and mortar. Approximately 1 mg of tissue was then placed into 3 to 5 replicate tin capsules for isotope analysis.

An epibenthic sled was used for the collection of zooplankton (200 µm mesh). While *Mesopodopsis africana* does undergo diel vertical migration (Carrasco and Perissinotto 2010a: Chapter 3), the water depth at most stations was < 0.5 m. Sampling with the epibenthic sled (radius = 18.5 cm) filtered water from almost the entire water-column. Where water depth exceeded 1 m (i.e. the Mouth and Esengeni), the use of the hyperbenthic sled would have ensured the suitable collection of epibenthic mysids during the daytime (cf. Kibirige *et al.* 2006). Zooplankton was concentrated on 200 µm Nitex mesh, placed in aluminium foil envelopes and frozen prior to laboratory sorting into the dominant taxa. These were *M. africana* (Mysidacea), *Pseudodiaptomus stuhlmanni* and *Acartia natalensis* (Copepoda). Due to the size of these taxa, whole animals needed to be pooled together in order to get enough tissue. About 20 and 200 individuals were needed for each sample for the mysids and copepods respectively. Three to five replicates were used for each species. Zooplankton was first defatted in a solution of methanol: chloroform: distilled water (2:1:0.8; Bligh and Dyer 1959), before being acid treated (2% HCl) to remove $CaCO_3$. After being treated for lipids and carbonates, zooplankton samples were dried in an air-circulated oven at 60°C for 24 h.

4.2.3 Stable isotope analysis

Samples were weighed into 5 x 8 mm tin capsules and analysed by the Stable Light Isotope Unit (Department of Archaeology, University of Cape Town, South Africa). The samples were combusted in a Flash EA 1112 series elemental analyzer (Thermo Finnigan, Italy). The gases were passed to a Delta Plus XP IRMS (isotope ratio mass spectrometer) (Thermo electron, Germany), via a Conflo III gas control unit (Thermo Finnigan, Germany). All stable isotope ratios are reported in the conventional delta (δ) notation as parts per thousand (‰) deviation from the international standard, where:

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

X is ^{13}C or ^{15}N and *R* is the corresponding ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$

The global standard for the carbon isotope was Vienna Pee Dee Belemnite and atmospheric nitrogen for the nitrogen isotope.

4.2.4 Statistical analysis, trophic level and source contribution to diet

Statistical analyses were conducted using SPSS version 15 for Windows. Data were checked for normality and even distribution of the residuals. One-way ANOVA was then applied to test for significant temporal ansd/or spatial differences in carbon and nitrogen stable isotope ratios of the primary carbon sources (POM, MPB, *Cladophora* sp., SOM and plant detritus).

The mixed model SIAR v 4.0 (Stable Isotope Analysis in R) of Parnell *et al.* (2010) was used to determine the likely contribution of each of the potential food items to the diet of the mysids on both spatial and temporal scales. Prior to modeling, $\delta^{15}N$ and $\delta^{13}C$ values of all food categories were corrected for trophic enrichment using respective fractionation factors of 3.6 for $\delta^{15}N$ and 0.4 for $\delta^{13}C$, which are appropriate for mysids according to Gorokhova and Hansson (1999). The observation that animal tissues are enriched in nitrogen relative to their diets (DeNiro and Epstein 1981) has been used in the estimation of an animal's trophic level. This can be done using the following equation modified by Post (2002):

$$TL = \lambda + (\delta^{15}N_c - \delta^{15}N_{base}) / \Delta_n$$

Where $\delta^{15}N_c$ is the nitrogen isotopic composition of the consumer, $\delta^{15}N_{base}$ is that of the food base and was chosen on the basis of its proximity to the consumer under analysis, λ is the trophic level of the base ($\lambda = 1$ for primary producers) and Δ_n is the estimate of the average increase in $\Delta^{15}N$ per trophic level (Post 2002).

4.3 RESULTS

4.3.1 Autotrophic sources and physico-chemical characteristics

4.3.1.1 Temporal variations

At the Mouth, microphytobenthic biomass was greatest during the dry season, while phytoplankton biomass was greatest during the wet season. At Charters Creek,

microphytobenthic and phytoplankton biomass did not change substantially between wet and dry seasons. Salinity increased from 11 during the wet season to 16.2 during the dry season at the Mouth and increased from 46.8 during the wet season to 53.6 during the dry season at Charters Creek. Temperature was additionally always higher during the wet season (Table 4.1).

4.3.1.2 Spatial variations

In terms of spatial differences, phytoplankton biomass was generally greatest at the Mouth and Esengeni, decreasing further north in the system. Conversely, microphytobenthic biomass was generally greater in the lakes than at Esengeni and the Mouth (Table 4.1). The percentage of organic matter in the sediment increased towards the north. Percent SOM ranged from 0.84% at the Mouth to 3.58% at Charters Creek. *Cladophora* sp. was only present at Charters Creek and was found in both the wet and the dry season (Table 4.1). The St. Lucia estuarine lake was characterized by a reversed salinity gradient. Salinity levels ranged from 2.55 at Esengeni to > 46.8 at Charters Creek. Water temperatures ranged from 19.6 to 27.6˚C. Turbidity varied between stations, being lowest at Esengeni and highest at Charters Creek. Water depths were greatest at the Mouth and Esengeni, while those at Catalina Bay and Charters Creek were much shallower (Table 4.1).

4.3.2 Stable isotopes

4.3.2.1 Temporal variations

Despite increased rainfall and temperatures in the wet season, there was no significant temporal difference in the $\delta^{13}C$ values of the primary carbon sources at the Mouth (ANOVA, $F_{1,20} = 1.61$, $p > 0.05$). The $\delta^{13}C$ signatures of all the primary carbon sources at the Mouth were significantly different to each other (ANOVA, $F_{3,18} = 78.7$, $p < 0.05$) and ranged from -13.81 to -29.25 ‰. In both wet and dry seasons, MPB was most enriched in $\delta^{13}C$, while POM was most depleted (Fig. 4.1a and b). $\delta^{13}C$ signatures of the primary carbon sources at Charters Creek differed significantly between seasons (ANOVA, $F_{1,20} = 13.7$, $p < 0.01$). All sources of organic carbon were significantly different at Charters Creek (ANOVA, $F_{2,19} = 10.2$, $p < 0.01$) with the exception of POM and MPB (post hoc Tukey's test, $p > 0.05$). Overall, $\delta^{13}C$ signatures of primary carbon sources at Charters Creek ranged from -15.7 to -24.2 ‰, with *Cladophora* sp.

having the most enriched $\delta^{13}C$ signature, while POM and SOM $\delta^{13}C$ signatures were the most depleted (Fig. 4.1c and d). At both the Mouth and Charters Creek, $\delta^{13}C$ signatures of the primary carbon sources, particularly POM, were generally more enriched (+ 1 to 2 ‰) during the wet season (Table 4.2).

For the primary consumers, *Pseudodiaptomus stuhlmanni* and *Mesopodopsis africana*, $\delta^{13}C$ signatures at the Mouth during the wet season were significantly more enriched than those during the dry season (ANOVA, *M. africana*: $F_{1,3} = 211$, $p < 0.001$, *P. stuhlmanni*: $F_{1,3} = 5065$, $p < 0.001$). Conversely, $\delta^{13}C$ signatures of these two species at Charters Creek were significantly more enriched during the dry season compared to the wet season (ANOVA, *M. africana*: $F_{1,7} = 21.5$, $p < 0.001$, *P. stuhlmanni*: $F_{1,5} = 289$, $p < 0.001$) (Fig. 4.1, Table 4.2).

While there was no significant temporal variation in $\delta^{15}N$ signatures of the primary carbon sources (ANOVA, Mouth: $F_{1,20}$ 0.41, $p > 0.05$; Charters Creek: $F_{1,20} = 0.35$, $p > 0.05$), the sources were significantly different to each other (ANOVA, Mouth: $F_{3,18} = 32$, $p < 0.001$; Charters Creek: $F_{2,19} = 7.45$, $p < 0.001$). $\delta^{15}N$ signatures of the primary carbon sources ranged from 0.5 ‰ for SOM to 7.7 ‰ POM at the Mouth (Fig. 4.1, Table 4.2). At the Mouth and Charters Creek, $\delta^{15}N$ signatures for *Mesopodopsis africana* were higher during the wet season. For *Pseudodiaptomus stuhlmanni*, however, values were relatively stable between seasons (Fig. 4.1, Table 4.2).

4.3.2.2 Spatial variations

$\delta^{13}C$ values of the primary carbon sources at the Mouth and Esengeni were significantly more depleted than those at Charters Creek (ANOVA, $F_{3,33} = 6.27$, $p < 0.01$). While the $\delta^{13}C$ values of the primary carbon sources at the Mouth, Esengeni and Catalina Bay were all significantly different from each other (ANOVA, $p < 0.01$), SOM and detritus $\delta^{13}C$ values were not significantly different to those of POM at Charters Creek (post hoc Tukey's test, $p > 0.05$). As for the primary consumers, $\delta^{13}C$ signatures became less depleted with distance from the estuary mouth (ANOVA, *Mesopodopsis africana*: $F_{3,15} = 1065$, $p < 0.001$, *Pseudodiaptomus stuhlmanni*: $F_{2,5} = 932$, $p < 0.001$). $\delta^{13}C$ signatures ranged from -30.3 to -22.3 ‰ for *P. stuhlmanni* and from -27.3 to -18.4 ‰ for *M. africana* (Fig. 4.2, Table 4.2).

The $\delta^{15}N$ signatures of the primary produces were fairly similar between sites (ANOVA, $F_{3,33} = 2.52$, $p > 0.05$), ranging from ~4 to ~9 ‰. Among the primary consumers, *Mesopodopsis*

africana was most enriched in nitrogen at the Mouth, with a $\delta^{15}N$ of 14.1 ‰. $\delta^{15}N$ signals at Esengeni, Catalina Bay and Charters Creek were 10, 11.4 and 11.3 ‰ respectively. For *Pseudodiaptomus stuhlmanni*, $\delta^{15}N$ signatures at the Mouth (11.8 ‰) were slightly higher than those at Esengeni and Charters Creek, with values of 7.0 and 10.5 ‰ respectively (Fig. 4.2, Table 4.2).

Table 4.1: Physico-chemical parameters and autotrophic variables measured on each of the sampling occasions during wet and dry seasons in the St. Lucia Estuary. +/- : present/absent; N/S: no sample taken; PPL: phytoplankton biomass; MPB: microphytobenthic biomass; DO: dissolved oxygen; Temp: temperature.

	Season	Station	MPB (mg pigm.m^{-2})	PPL (mg pigm.m^{-3})	Depth (m)	DO (mg.L^{-1})	Salinity	Temp (ºC)	Turbidity (NTU)	Detritus (+/-)	*Cladophora* sp. (+/-)
Spatial study	wet	Mouth	5.15	61.5	2	5.23	8.52	21.9	62.6	+	-
		Esengeni	24.5	51.2	1.2	6.74	2.55	21	17.5	-	-
		Catalina Bay	151	11	0.2	5.69	18.6	28.6	43	-	-
		Charters Creek	34.2	4.94	0.2	7.69	46.8	26.8	102	-	+
Temporal study	wet	Mouth	5.15	68.5	5	7.16	11	27.6	21.9	+	-
	dry	Mouth	72.5	23.9	4	7.63	16.2	19.6	10.1	+	-
	wet	Charters Creek	34.2	4.94	0.2	7.69	46.8	26.8	102	+	+
	dry	Charters Creek	17.9	4.98	0.1	8.45	53.6	21.5	341	N/S	+

Table 4.2: Average $\delta^{13}C$ and $\delta^{15}N$ values (± SD) of the primary carbon sources and consumers during wet and dry seasons in the St. Lucia Estuary. -: absent, N/S: no sample taken, POM: particulate organic matter, MPB: microphytobenthic biomass, SOM: sedimentary organic matter, wet/dry: wet/dry season.

				POM	MPB	SOM	Detritus	*Cladophora*	*P. stuhlmanni*	*M. africana*
Spatial study		**M**	$\delta^{13}C$	-25.3 ± 0.2	-15.5 ± 0.01	-24.95 ± 0.1	N/S	-	-30.3 ± 0.2	-27.3 ± 0.41
			$\delta^{15}N$	8.5± 0.2	5.56 ± 0.45	5.01 ±0.08	N/S	-	11.8 ± 1.0	14.1 ± 0.2
		E	$\delta^{13}C$	-24.1 ± 0.41	-20.1 ±0.18	-21.62 ± 0.06	N/S	-	-27.9 ± 0.17	-25.9 ± 0.27
	wet		$\delta^{15}N$	4.9 ± 0.1	4.07 ± 0.08	5.7 ± 0.08	N/S	-	7.0 ± 0.25	10.0 ± 0.31
		CB	$\delta^{13}C$	-19.4 ± 0.21	N/S	-16.95 ± 0.02	N/S	-	-	-18.4 ± 0.17
			$\delta^{15}N$	8.84 ± 0.83	N/S	4.16 ± 0.02	N/S	-	-	11.3 ± 0.27
		CC	$\delta^{13}C$	-19.1 ± 0.13	-17.0 ± 0.11	-20.19 ± 0.83	-19.7 ± 0.64	-15.7 ± 0.07	-22.3 ± 0.26	-18.4 ± 0.37
			$\delta^{15}N$	6.86 ± 1.36	5.86 ± 1.38	4.05 ± 1.21	6.12 ± 0.02	6.2 ± 0.22	10.5 ± 0.22	11.3 ± 0.29
Temporal study	**wet**	**M**	$\delta^{13}C$	-27.6 ± 1.18	-17.3 ± 0.23	-26.33	-22.7 ± 0.25	-	-26.3 ± 0.09	-26.2 ± 0.01
			$\delta^{15}N$	5.84 ± 1.28	3.08 ± 0.63	0.5	5.8 ± 0.46	-	10.6 ± 0.18	14.4 ± 0.25
	dry	**M**	$\delta^{13}C$	-29.3 ± 0.04	-13.8 ± 0.03	-23.6 ± 0.83	-19.8 ± 0.05	-	-31.1 ± 0.02	-27.3 ± 0.13
			$\delta^{15}N$	7.70 ± 1.16	1.67 ± 0.22	1.63 ± 0.47	5.20 ± 0.04	-	10.8 ± 0.31	12.6 ± 0.53
	wet	**CC**	$\delta^{13}C$	-19.1 ± 0.13	-17.0 ± 0.11	-20.2 ± 0.83	-19.6 ± 1.07	-15.7 ± 0.08	-22.3 ± 0.26	-18.4 ± 0.37
			$\delta^{15}N$	6.86 ± 1.36	5.86 ± 1.38	4.05 ± 1.21	6.13 ± 0.02	6.20 ± 0.22	10.5 ± 0.22	11.3 ± 0.29
	dry	**CC**	$\delta^{13}C$	-20.0 ± 0.12	-20.1 ± 0.04	-	-	-17.9 ± 0.06	-20.2 ± 0.03	-17.4 ± 0.3
			$\delta^{15}N$	4.43 ± 0.76	2.68 ± 0.28	-	-	8.08 ± 0.12	10.2 ± 0.25	10.4 ± 0.37

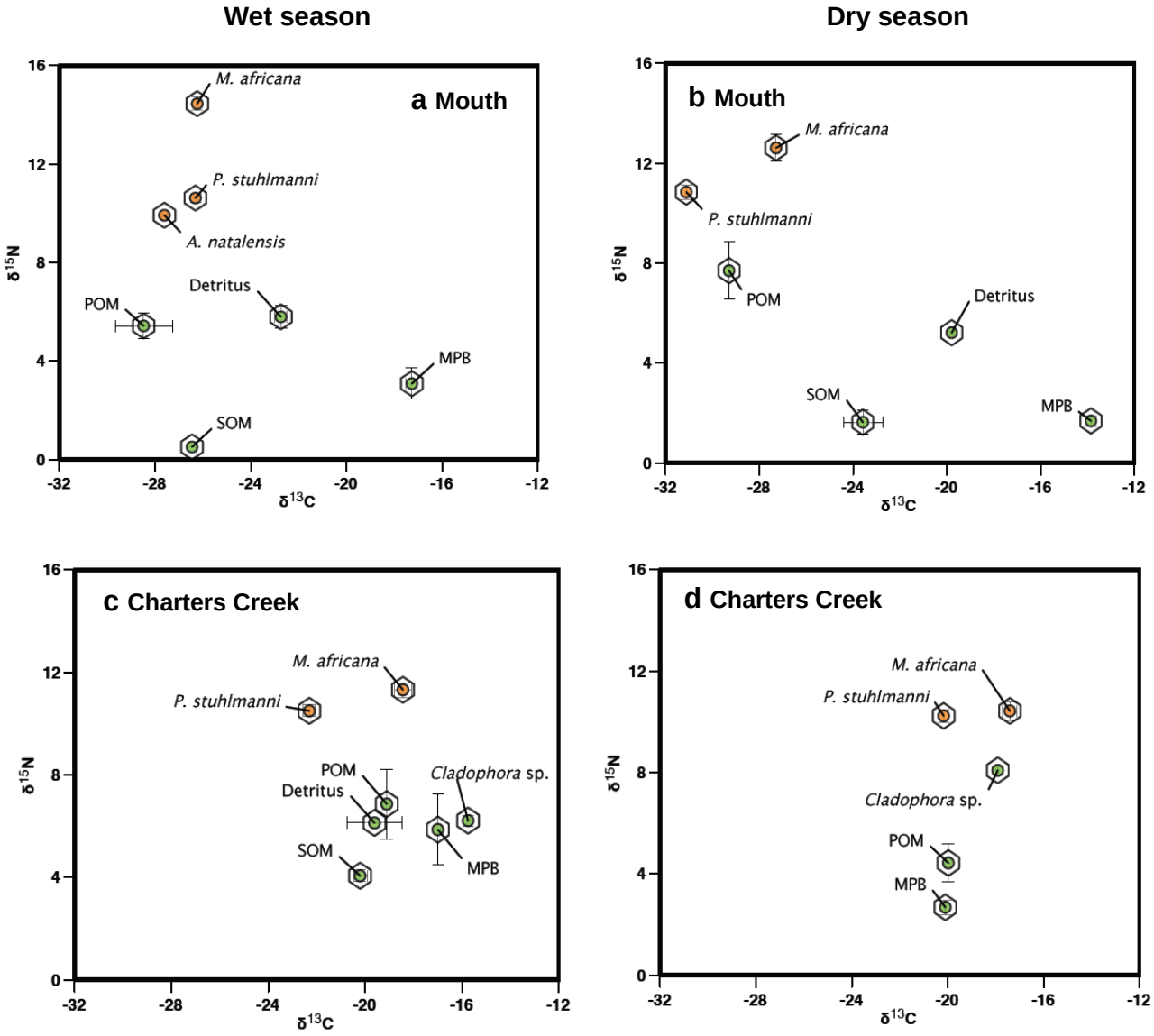

Fig. 4.1: Plots of $\delta^{13}C$ and $\delta^{15}N$ of primary carbon sources and consumers sampled at the Mouth during (a) wet and (b) dry seasons and at Charters Creek in (c) wet and (d) dry seasons. Error bars show SD. POM: particulate organic matter, SOM: sedimentary organic matter, MPB: microphytobenthos.

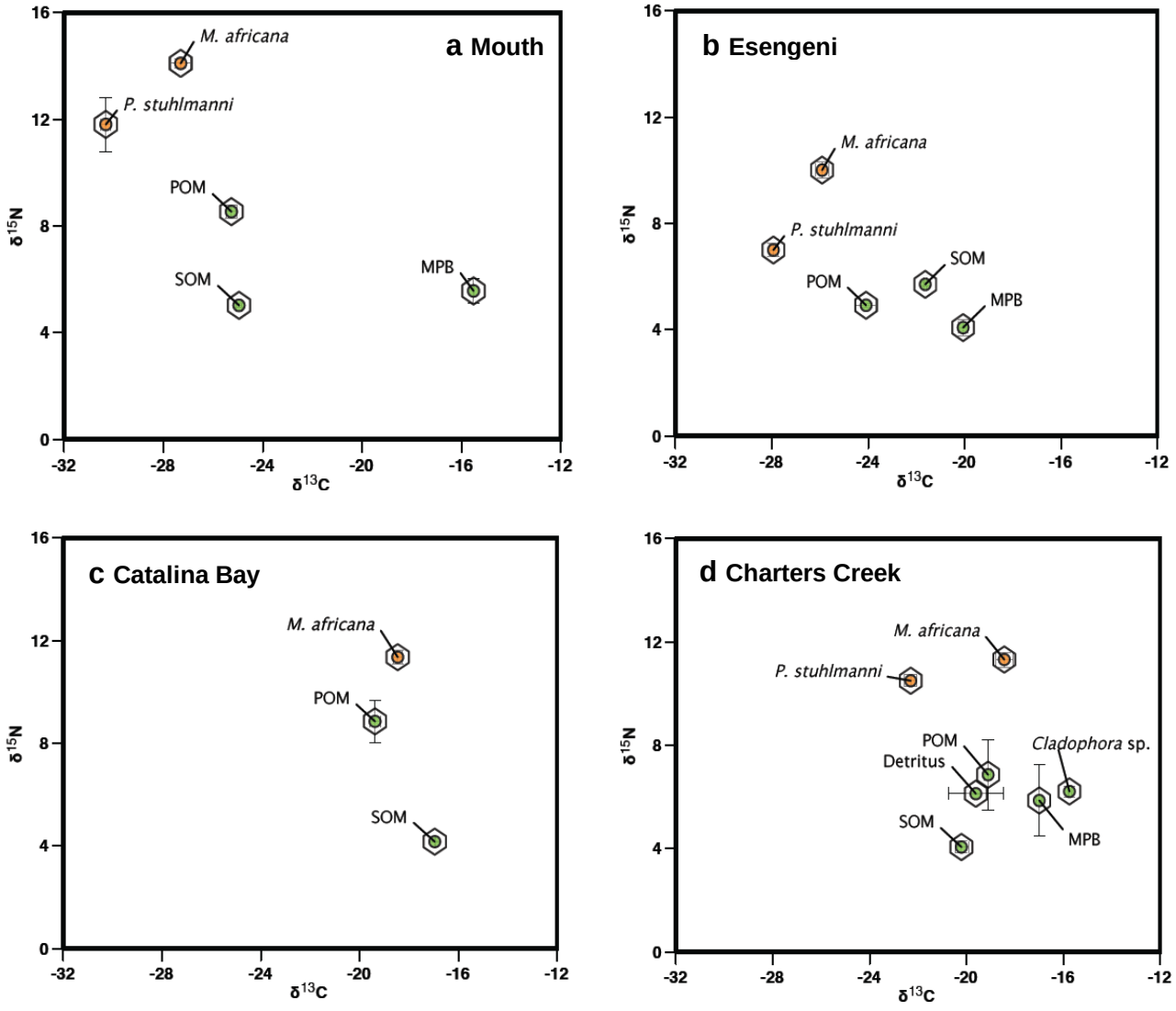

Fig. 4.2: Plots of $\delta^{13}C$ and $\delta^{15}N$ of primary carbon sources and consumers sampled at (a) the Mouth, (b) Esengeni, (c) Catalina Bay and (d) Charters Creek. Error bars show SD. Abbreviations see Fig. 4.1.

4.3.2.3 Trophic level and source contribution to diet

The trophic levels of the primary consumers varied slightly between wet and dry seasons. There were no significant temporal differences in trophic level at the Mouth (ANOVA, *Mesopodopsis africana*: $F_{1,3}$ = 4.00, $p > 0.05$: *Pseudodiaptomus stuhlmanni*: $F_{1,3}$ = 0.38, $p > 0.05$). *M. africana* was most enriched in $\delta^{15}N$ during the wet season and, therefore, occupied the highest trophic position (3.39) followed by *P. stuhlmanni* (2.35) and *Acartia natalensis* (2.2) at the Mouth. During the dry season at the Mouth, *M. africana* occupied the highest trophic level (2.71), followed by *P. stuhlmanni* (2.21). The trophic levels of primary consumers at Charters Creek did vary significantly from wet to dry seasons (ANOVA, *M. africana* : $F_{1,7}$ = 87.5, $p < 0.001$, *P. stuhlmanni*: $F_{1,5}$ = 143, $p < 0.001$). Among the primary consumers at Charters Creek, *M. africana* had the highest trophic level (2.23), followed closely by *P. stuhlmanni* (2.07) in the wet season, but in the dry season it was *Rhopalophthalmus tropicalis* that was identified as the highest trophic level (2.89), followed by *P. stuhlmanni* (2.61) and lastly *M. africana* (1.65). The trophic levels of primary consumers varied significantly between stations (ANOVA, *M. africana*: $F_{3,15}$ = 126, $p < 0.001$, *P. stuhlmanni*: $F_{2,5}$ = 7.74, $p < 0.05$). These differences were more pronounced for *M. africana*, which exhibited a significantly higher trophic level at the Mouth and Esengeni than at Catalina Bay and Charters Creek (post hoc Tukey's test, $p < 0.01$).

The SIAR v 4.0 mixed model resolved proportions of different food sources in the diet of *Mesopodopsis africana*. Contribution of different carbon sources to the diet of *M. africana* was relatively constant between wet and dry seasons at the Mouth (Fig. 4.3). In the wet season (Fig. 4.3a), the copepods *Pseudodiaptomus stuhlmanni* and *Acartia natalensis* were the most important components of the mysid diet, contributing up to 52 and 46% respectively. Next important was SOM (up to 34%), POM and plant detritus, contributing up to ~ 29% of the mysid diet. In the dry season (Fig. 4.3b), *P. stuhlmanni* contributed between 13 to 74% of the diet, followed by POM. At Charters Creek, the relative proportions of the carbon sources in the diet of *M. africana* changed slightly from wet to dry seasons, with *Cladophora* sp. being in both cases the most important dietary item, followed by *P. stuhlmanni* and POM (Fig. 4.3c and d).

Contribution of different carbon sources to the diet of *Mesopodopsis africana* also varied spatially (Fig. 4.4). At the Mouth and Esengeni, *Pseudodiaptomus stuhlmanni* contributed the greatest proportion to the mysid diet, with 41 to 76% of the diet at the Mouth and 48 to 69% at Esengeni.

Next important in terms of diet contribution was POM, contributing up to 47% of the diet at the Mouth and 32% at Esengeni. SOM and MPB made minor contributions to their overall diet. The copepod *P. stuhlmanni* was almost completely absent during this survey at Catalina Bay. At this station, POM made the greatest contribution to the mysid diet, followed by SOM and MPB. At Charters Creek, *Cladophora* sp. made the most substantial contribution to the mysid diet, accounting for up to 52% of the diet, while MPB and *P. stuhlmanni* contributed between 0 and 40% of the diet (Fig. 4.4).

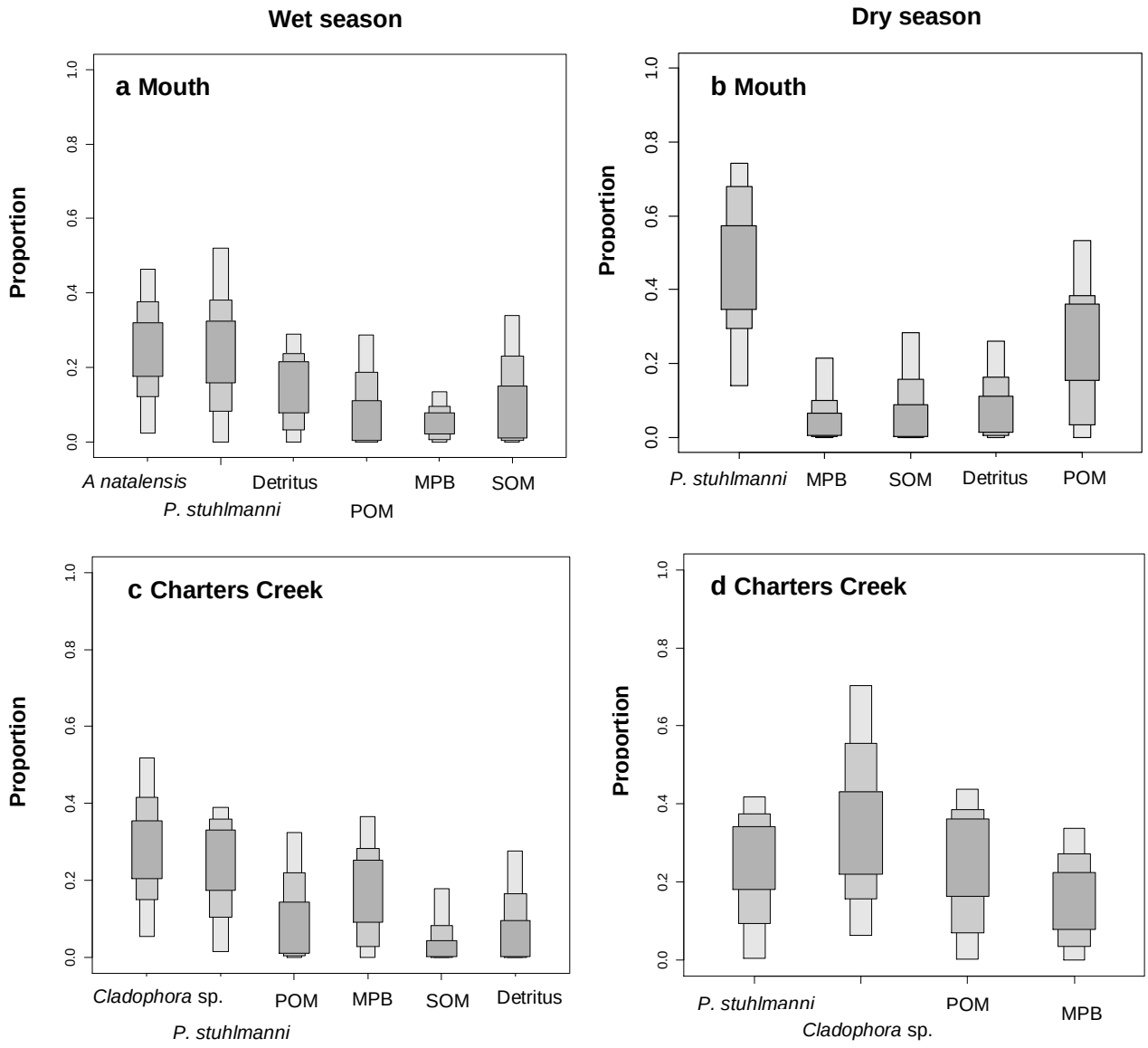

Fig. 4.3: Contribution of different primary carbon sources to the diet of *M. africana* using SIAR at the Mouth in (a) wet and (b) dry seasons and at Charters Creek during (c) wet and (d) dry season. Abbreviations see Fig 4.1.

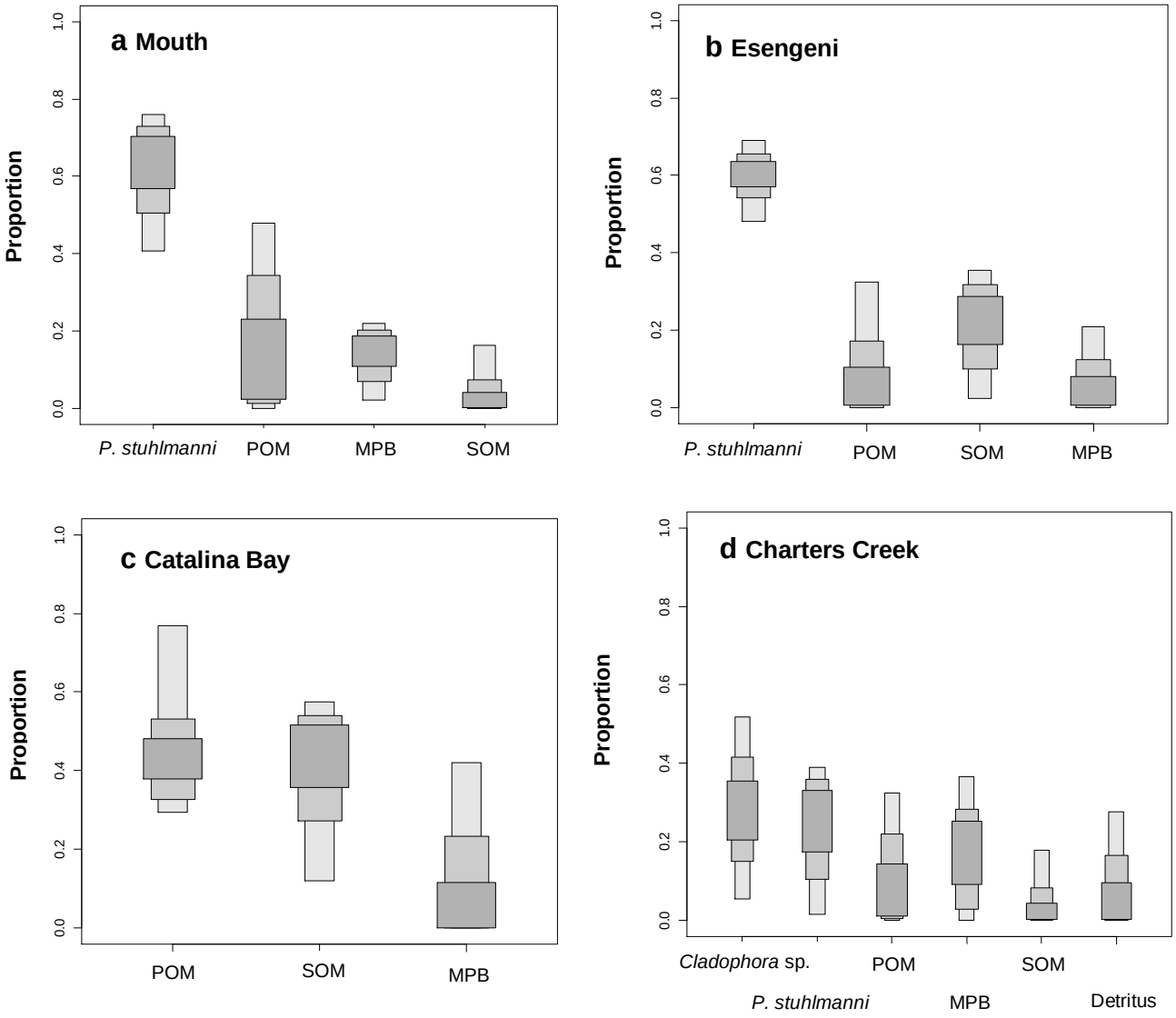

Fig. 4.4: Contribution of different carbon sources to the diet of *M. africana* using SIAR at four stations within the St. Lucia Estuary, (a) the Mouth, (b) Esengeni, (c) Catalina Bay and (d) Charters Creek. Abbreviations see Fig. 4.1.

4.4 DISCUSSION

The present study demonstrates the ability of *Mesopodopsis africana* to modify its diet in response to different environmental conditions on short temporal and spatial scales within the same system. The nature of freshwater deprivation in the St. Lucia Estuary has resulted in a northward gradient of drought effects. While regions in the South have recently been relatively protected from the drought due to freshwater input from the Mpate and Mfolozi Rivers through the link canal (Whitfield and Taylor 2009), hypersalinity and low water levels have become increasingly more severe towards the north. This profile has fractionated the system into a variety of different habitats in relatively close range to one another. The potential omnivorous feeding habits of mysids (cf. Mauchline 1980, Lehtiniemi and Nordström 2008, Vilas *et al.* 2008) have thus allowed this species to modify rapidly its diet in accordance with changes in food type and abundance.

Spatial differences in isotope signatures seemed to be greater than temporal variations. This was not surprising given the vast differences between sites due to habitat fragmentation. In terms of seasonality, on the eastern seaboard of South Africa, the rainy season extends from October to April and the dry season from May to September. However, recent estimates indicate that only approximately 24% of the normal freshwater inflow is currently entering the St. Lucia Estuary from the catchments (D Stretch, pers. comm.). During the study, $\delta^{13}C$ values were slightly raised during the wet season. Allanson and Read (1987, 1995) demonstrated that increased freshwater input to a system promotes phytoplankton primary production through macronutrient availability and water-column stability. The slightly raised $\delta^{13}C$ values observed in the wet season in the St. Lucia Estuary may, therefore, be a reflection of the increase in primary production. Goering *et al.* (1990) found that plankton $\delta^{13}C$ values increased by about 2 ‰ during a phytoplankton bloom. It has been suggested that changes in phytoplankton assemblages can affect the $\delta^{13}C$ composition of the marine ecosystem upwards of 10 ‰ (Fogel *et al.* 1992).

$\delta^{13}C$ signatures of POM at the Mouth and Esengeni were more depleted than those at Charters Creek and Catalina Bay. Signatures in the mouth region were closer to $\delta^{13}C$ signatures of mangrove leaves (Kao and Chang 1998, Bouillon *et al.* 2004). An explanation for this could be that the particulate organic matter contained material that originated from senescent leaves of mangroves (Govender *et al.* in review), a habitat which is located near and around the Mouth and Narrows region (Begg 1978). Additionally, the Mouth and Esengeni have recently received a steady riverine

input (Whitfield and Taylor 2009). Depleted carbon signatures could, therefore, also reflect allocthonous input upstream. $\delta^{13}C$ signatures of terrestrial fringing vegetation such as *Salicornia* sp. at Catalina Bay and *Phragmites* sp. at Esengeni ranged from -29.01 to -29.4 ‰ and from -28 to -19 ‰ respectively. These values are similar to those recorded for POM in the Mouth region, suggesting that allochthonous input most probably contained $\delta^{13}C$-depleted terrestrial detritus. The trend of an increase in $\delta^{13}C$ values of POM along an estuarine gradient was recorded by Fontugne and Jouanneau (1987) and confirmed by similar studies on other hydrosystems (Riera and Richard 1996, Bardonnet and Riera 2005, Suzuki *et al*. 2008). It can be attributed to the gradual mixing together of sources of fluvial (light $\delta^{13}C$) and marine (heavy $\delta^{13}C$) organic matter (Fontugne and Jouanneau 1987).

Mysids are generally regarded as omnivores, capable of successfully exploiting a variety of food resources. Diet may include phytobenthos, phytoplankton, detritus, sediment, microzooplankton, mesozooplankton and small benthic invertebrates (Wilhelm *et al.* 2002, Kibirige and Perissinotto 2003a, Lehtiniemi and Nordström 2008, Vilas *et al.* 2008). $\delta^{15}N$ signals for *Mesopodopsis africana* in this study ranged from 10 ‰ to 14 ‰. These values are similar to those recorded by Rolff *et al.* (1993) and Rolff (1998) for *Mysis mixta* in the northern Baltic Sea, and within the range of those recorded for *M. stenolepsis* and *Neomysis americana* in the St. Lawrence Estuary in Canada and suggest that mysids may be feeding on mesozooplankton (Winkler *et al.* 2007). Diet analysis using SIAR v 4.0 showed that mysids at the Mouth and Esengeni occupied higher trophic levels, feeding mainly on the calanoid copepod *Pseudodiaptomus stuhlmanni*. POM was, however, the main food source for *M. africana* at Catalina Bay. This could be because these copepods were almost completely absent from this station at the time. Mysid predation has been shown to be linked to food availability. Jerling and Wooldridge (1995) found that the mysids *M. wooldridgei* and *Rhopalophthalmus terranatalis* in the Sundays River Estuary (South Africa) preferentially fed on nauplii as opposed to copepodites, due to the high abundance of nauplii in the water column. Diet shifts according to food availability have also been previously recorded in other studies (Viherluoto *et al.* 2000, Winkler *et al.* 2007, Vilas *et al.* 2008). Feeding on POM may, therefore, be a result of mysids feeding on the most abundant prey, rather than selective feeding. The flexibility in the diet of *M. africana* is also shown on a temporal scale. While the dominant food items for *M. africana* did not change significantly from wet to dry seasons, the proportions of the different sources in the mysid diet varied. This again may be linked to food availability.

The diet of *Mesopodopsis africana* at Charters Creek was also slightly more difficult to quantify, since it appeared as though all sources were making similar contributions to the diet. This could be because of the greater number of autotrophic sources available or it could be a reflection of not all the $\delta^{13}C$ sources being significantly different. Wahl *et al.* (2007) stated that lack of separation of several primary food sources made identification of the sources of organic matter that supported secondary production in the main channel of the Mississippi River, difficult. The general lack of one dominant food source at Charters Creek may also be a reflection of the harsh environmental conditions present in this area at the time. Charters Creek is situated on the western shores of South Lake, which is known to be a turbid location (Cyrus 1988). Here, shallow waters combined with a fine sandy substratum frequently lead to turbidity values exceeding 300 NTU. Additionally, zooplankton communities at Charters Creek may have been more stressed on account of freshwater starvation and the resultant hypersalinity and low water levels. It could be assumed that under stressful conditions, zooplankton may become less selective in their prey choice. Diet shifts in mysids have previously been reported and related to food availability (Viherluoto *et al.* 2000, Winkler *et al.* 2007, Vilas *et al.* 2008). That is, in order to complete their diet, mysids show a more opportunistic behaviour when resources are more limited. Additionally, Tackx *et al.* (2003) demonstrated the limited ability of *Eurytemora affinis* to feed selectively under high suspended particulate matter loads.

While *Mesopodopsis africana* is a euryhaline species, capable of surviving salinity values from 2.5 to 65. (Carrasco and Perissinotto 2011a: Chapter 2), continued drought effects could force the exclusion of this species. *M. africana* is an important connecting species in the St. Lucia Estuary, linking primary and secondary production with higher trophic levels (Mees and Jones 1997, Viherluoto and Viitasalo 2001, Lehtiniemi *et al.* 2009). Blaber (1979) documented their importance in the diet of zooplanktivorous fish such as *Gilchristella aestuaria, Hilsa kelee* and *Thryssa vitrirostris* in the St. Lucia Estuary. In particular, *M. africana* was second in importance in terms of calorific contribution after *Pseudodiaptomus stuhlmanni* (Blaber 1979). Its loss would, therefore, have substantial impacts on food web functioning in different regions of this key estuarine lake. The potential though for this mysid to alter its diet in response to a dynamic environment, such as St. Lucia, and it's persistence through the many past hydrological phases, is a prime example of its adaptive nature and re-enforces the resilience so far shown by the system.

The comparative diet of the dominant zooplankton species in the St. Lucia Estuary, South Africa

ABSTRACT

The copepods *Pseudodiaptomus stuhlmanni*, *Acartia natalensis* and the mysid *Mesopodopsis africana* are the dominant zooplankton taxa in the St. Lucia Estuary, Africa's largest estuarine lake. This system is currently undergoing severe desiccation, owing to freshwater deprivation. Carbon and nitrogen stable isotopes of the main primary and secondary producers were analysed temporally at two contrasting habitats, Charters Creek, heavily affected by desiccation, and the Mouth, virtually under unchanged conditions. The present study aimed to compare the diet composition of these species under different environmental conditions. The mixed model SIAR (Stable Isotope Analysis in R) v 4.0 was used to determine the contribution of each food item to the diet of these taxa. Copepods made a substantial contribution to the diet of *M. africana* in the Mouth region, while those at Charters Creek utilized an even mixture of the available sources. At the Mouth, *P. stuhlmanni* utilized mostly particulate organic matter (POM), while feeding on an even mixture of available sources at Charters Creek. Limited data were available for *A. natalensis,* due to its short seasonal occurrence. Results do, however, indicate that this species feeds primarily on POM at the Mouth. Overall, results indicate that diet composition of all three species is more selective at the Mouth. This can be attributed either to the harsh environmental conditions prevailing at Charters Creek, or the poor separation of $\delta^{13}C$ signatures of primary producers in this region. The food partitioning abilities shown by these species may facilitate the utilisation of resources available in the estuary.

Keywords: *Acartia natalensis,* diet, iSimangaliso Wetland Park, *Mesopodopsis africana, Pseudodiaptomus stuhlmanni*

5.1 INTRODUCTION

Estuaries are dynamic ecosystems characterized by rapid and intense fluctuations in physico-chemical factors. Their biotic communities are, therefore, also often characterized by strong spatial and seasonal variability (McLusky and Elliott 2004). Across South Africa, however, and in many other estuaries worldwide, copepods of the genera *Pseudodiaptomus* and *Acartia* and mysids of the genus *Mesopodopsis* are often found co-occurring in such high densities that they are considered the dominant zooplankton taxa (e.g. Wooldridge 1999). This is also true in the St. Lucia Estuary (Carrasco *et al.* 2010: Chapter 1, Jerling *et al*. 2010), Africa's largest estuarine lake (Fielding *et al.* 1991, Cyrus and Vivier 2006). The St. Lucia Estuary forms part of the iSimangaliso (formerly Greater St. Lucia) Wetland Park. iSimangaliso is South Africa's first UNESCO World Heritage Site and has three designated Ramsar Wetlands of international importance since 1992 (Begg 1978, Cyrus and Vivier 2006). The lake system is governed by cyclical wet and dry periods, each lasting between four and ten years (Begg 1978). St. Lucia is currently experiencing a freshwater starvation-induced crisis. Drought conditions have been exacerbated by the canalisation of the Mfolozi River, and the subsequent diversion of its freshwater away from the St. Lucia Estuary, in order to avoid the perceived threat of siltation (Carrasco *et al.* 2007, Whitfield and Taylor 2009). The present drought has led to a drastic reduction in water levels and concomitant salinity increases. A reversed salinity gradient has persisted, with the occurrence of hypersaline conditions in the northern regions of the estuarine lake. These low water levels and hypersaline conditions now threaten the integrity of the biotic communities present in the system.

The mysid *Mesopodopsis africana* and the calanoid copepods *Pseudodiaptomus stuhlmanni* and *Acartia natalensis*, are the dominant zooplankton taxa in the St. Lucia Estuary and as such, play a vital role in linking primary and/or secondary production with higher trophic levels (Mees and Jones 1997, Viherluoto and Viitasalo 2001, Lehtiniemi *et al.* 2009). They are important in the diets of a number of estuarine resident and juvenile marine fish species (Whitfield 1998). Blaber (1979) documented their importance in the diet of zooplanktivorous fish, such as *Gilchristella aestuaria*, *Hilsa kelee* and *Thryssa vitrirostris* in the St. Lucia Estuary. In this assessment, *P. stuhlmanni* was first in importance in terms of calorific contribution, followed by *M. africana* (Blaber 1979). Mysid shrimps are important in the food webs of aquatic ecosystems, as both consumers and producers (Mauchline 1980). As consumers, mysids are generally considered omnivorous, feeding on a wide

range of items. Diet may include phytobenthos, phytoplankton, detritus, sediment, microzooplankton, mesozooplankton and small benthic invertebrates (Wilhelm *et al.* 2002, Kibirige and Perissinotto 2003a, Lehtiniemi and Nordström 2008, Vilas *et al.* 2008). Copepods are also capable of utilizing a wide range of diets (Kleppel 1993). Like mysids (Mauchline 1980), copepods may feed by means of a suspension-feeding current or, alternatively, actively prey on moving zooplankton by means of raptorial or ambush feeding (Jiang and Osborn 2004). Strong selectivity of grazers tends to decrease the total impact on the seston or phytoplankton biomass (Mazumder *et al.* 1990). Food selectivity is also a significant mechanism of control for zooplankton communities, affecting the adaptive strategies of organisms. Additionally, selectivity supports food partitioning and thus decreases competition for food resources between species (Rotthaupt 1990).

The use of stable isotopes as a tool in ecological research is increasing rapidly. Since stable carbon isotopes ($\delta^{13}C$) are known to fractionate little between energy transfers, they are commonly used to quantify food sources and energy flow in lake systems. Stable nitrogen isotopes ($\delta^{15}N$) fractionate more and are typically used to infer trophic positions of consumers in a food chain (DeNiro and Epstein 1978, Peterson and Fry 1987). Models may allow simulation of possible diets and assessment of the relative importance of various sets of organic matter sources to consumers. This study aims to compare the contribution of different carbon sources to diets of the copepods *Pseudodiaptomus stuhlmanni* and *Acartia natalensis* and the mysid *Mesopodopsis africana*. Stable $\delta^{13}C$ and $\delta^{15}N$ isotopes are used to gain insight into the spatial and temporal feeding preferences of these taxa at two unique habitats within the St. Lucia Estuary, Charters Creek, heavily affected by the desiccation process, and the Mouth, virtually under unchanged conditions.

5.2 MATERIALS AND METHODS

5.2.1 Study site

The St. Lucia Estuary is situated in northern KwaZulu-Natal and lies between 27 ˚ 52'0" S to 28˚ 24'0" S and 32˚ 21'0"E to 32˚ 34'0"E. The system is made up of three shallow lakes connected to the Indian Ocean by a 21 km channel known as the Narrows (Fig. 1.1). In total, the St. Lucia estuarine lake covers an area between 300 and 350 km^2, depending on water levels (Begg 1978). Samples were collected from two regions within the estuarine complex, the Mouth and Charters Creek (Fig.

1.1). The spatial extent of each station was roughly 100 m^2. The east coast of KZN typically experiences two climatic seasons, one characterized by regular rain (October to April) and the other with virtually no rain at all (May to September). For temporal comparisons, samples were collected from Charters Creek in September 2008 (late winter, dry season) and April 2009 (early autumn, wet season) and at the Mouth in February 2009 (summer, wet season) and June 2009 (winter, dry season). Physico-chemical measurements were taken with a YSI 6920 water quality logger, fitted with temperature (˚C), depth (m), dissolved oxygen ($mg.L^{-1}$), pH and turbidity (Nephelometric Turbidity Units or NTUs) probes.

5.2.2 Pelagic and benthic microalgae

Three 250 mL subsurface water samples were collected at each station on each sampling occasion and filtered through a Whatman GF/F filter to determine the total concentration of chlorophyll *a* (chl *a*) and phaeopigments. A further 250 mL subsample was then serially filtered through 20 µm, 2 µm and GF/F filters for the determination of size fractionated chl *a* and phaeopigments. Phytoplankton biomass was determined fluorometrically (Turner Designs 10-AU) after cold extracting chl *a* and phaeopigments from filters in 10 mL 90% acetone for 48 h in the dark. Microphytobenthic cores (2 cm internal diameter, n = 3, depth = 1 cm) were collected on each occasion at each station and placed in 100 mL polyethylene bottles containing 30 mL 90% acetone for microphytobenthic chl *a* extraction (Nozais *et al.* 2001). Biomass was again determined fluorometrically and expressed as mg $pigm.m^{-2}$. Phytoplankton community composition in the water column was measured with a Fluoroprobe (BBE-Moldaenke), an instrument which fluorometrically determines the relative composition of a variety of microalgal classes (Gregor and Maršálek 2004).

5.2.3 Sample collection and processing

For stable isotope analysis, microphytobenthic biomass (MPB), particulate organic matter (POM), macroalgae (*Cladophora* sp.), sedimentary organic matter (SOM), plant detritus and zooplankton samples were collected (depending on their availability) from Charters Creek and the Mouth during both wet and dry seasons.

To obtain an estimate of SOM, the upper 1 cm layer of sediment was collected and treated with excess 2% hydrochloric acid (HCl) in order to remove carbonates. The sediment was then rinsed thoroughly with distilled water, dried at 60˚C for 24 h and subsequently crushed with a pestle and mortar and packaged in microcentrifuge tubes until further processing by the laboratory in Cape Town (e.g. Smit *et al.* 2005). Detritus (or decomposing plant organic matter) was collected within the floating foam which was often found at the surface near the water edge. Detritus was similarly treated with 2% HCl to remove carbonates, rinsed with distilled water, dried at 60˚C for 24 h and subsequently crushed with a pestle and mortar and packaged in tin capsules for isotope analysis. Triplicate samples were taken for both SOM and detritus on each sampling occasion.

For the extraction of MPB, dense algal mats were collected by scraping the upper 1 cm of sediment. This sediment was then re-suspended in filtered estuarine water and stirred so that the MPB remained in suspension while the heavier sediment settled to the bottom of the container. At Charters Creek, the sediment was too fine for this protocol to be used, so a procedure similar to that described by Couch (1989) was employed instead. Sediment was collected as above by scraping the upper 2 mm in areas of dense microalgal mats. The benthic microalgae were then separated from the sediment on the basis of their vertical migration properties and subsequently filtered onto previously combusted (450°C, 6 h) Whatman GF/F filters. Water samples for POM were similarly filtered onto pre-combusted Whatman GF/F filters. POM and MPB samples were then acid treated with 2% hydrochloric acid (HCl) to remove any inorganic carbon in the form of calcium carbonate ($CaCO_3$) that may have been present. Filters containing POM and MPB (in triplicate) were placed into aluminium foil envelopes, and frozen prior to laboratory-based processing.

Samples of the dominant macroalga (*Cladophora* sp.) were collected and rinsed thoroughly with distilled water. Algae were subsequently dried at 60˚C in an air-circulated oven for 24 h and thereafter homogenized into a fine powder using a pestle and mortar. Approximately 1 mg of tissue was then placed into 3 to 5 replicate tin capsules for further processing.

An epibenthic sled was used for the collection of zooplankton (200 µm mesh). In cases where water depth exceeded 1 m (i.e. the Mouth and Esengeni), the use of the hyperbenthic sled during the daytime ensured the suitable collection of zooplankton exhibiting migration characteristics (cf. Kibirige *et al.* 2006). Zooplankton was concentrated on 200 µm Nitex mesh, placed in aluminium foil envelopes and frozen prior to laboratory sorting into the dominant taxa. These were

Mesopodopsis africana, Pseudodiaptomus stuhlmanni and *Acartia natalensis*. About 20 and 200 individuals were needed for each of the 3 to 5 replicates prepared for the mysids and copepods respectively. Zooplankton was first defatted in a solution of methanol: chloroform: distilled water (2:1:0.8; Bligh and Dyer 1959), before being acid treated (2% HCl) to remove $CaCO_3$. After being treated for lipids and carbonates, zooplankton samples were dried in an air-circulated oven at 60°C for 24 h.

5.2.4 Stable isotope analysis

Samples were weighed into 5 x 8 mm tin capsules and analysed by the Stable Light Isotope Unit (Department of Archaeology, University of Cape Town, South Africa). The samples were combusted in a Flash EA 1112 series elemental analyzer (Thermo Finnigan, Italy). The gases were passed to a Delta Plus XP IRMS (isotope ratio mass spectrometer) (Thermo electron, Germany), via a Conflo III gas control unit (Thermo Finnigan, Germany). All stable isotope ratios are reported in the conventional delta (δ) notation as parts per thousand (‰) deviation from the international standard, where:

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

X is ^{13}C or ^{15}N and R is the corresponding ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$

The global standard for the carbon isotope was Vienna Pee Dee Belemnite and atmospheric nitrogen for the nitrogen isotope.

5.2.5 Statistical analysis, trophic level and source contribution to diet

Statistical analyses were conducted using SPSS version 15 for Windows. Data were checked for normality and even distribution of the residuals. Since the data satisfied all the assumptions for parametric testing, one-way ANOVA was used to confirm whether or not carbon isotope ratios of primary producers (POM, MPB, *Cladophora* sp., SOM and plant detritus) and secondary producers

(*Mesopodopsis africana, Pseudodiaptomus stuhlmanni* and *Acartia natalensis*) differed significantly between seasons.

The mixed model SIAR v 4.0 (Stable Isotope Analysis in R) of Parnell *et al.* (2010) was used to determine the likely contribution of each of the potential food items to the diet of the zooplankton. Prior to modelling, $\delta^{15}N$ and $\delta^{13}C$ values of all food categories were corrected for trophic enrichment using respective fractionation factors of 3.6 for $\delta^{15}N$ and 0.4 for $\delta^{13}C$, which are appropriate for mysids according to Gorokhova and Hansson (1999). These correction factors were also applied for the copepods, since no data are available for these taxa. The observation that animal tissues are enriched in nitrogen relative to their diets (DeNiro and Epstein 1981) has been used in the estimation of an animal's trophic level. This can be done using the following equation modified by Post (2002):

$$TL = \lambda + (\delta^{15}N_c - \delta^{15}N_{base}) / \Delta_n$$

Where $\delta^{15}N_c$ is the nitrogen isotopic composition of the consumer, $\delta^{15}N_{base}$ is that of the food base and was chosen on the basis of its proximity to the consumer under analysis, λ is the trophic level of the base ($\lambda = 1$ for primary producers) and Δ_n is the estimate of the average increase in $\Delta^{15}N$ per trophic level (Post 2002).

5.3 RESULTS

5.3.1 Physical characteristics

At the time of the study, the St. Lucia estuarine lake was characterized by a reversed salinity gradient. Salinity ranged from 4 at the Mouth to > 55 at Charters Creek. Water temperature differed from wet to dry seasons, ranging from 19.6˚C at the Mouth in July 2009 to 27.6˚C at the Mouth in February 2009. Turbidity varied between sites, being lowest at the Mouth (10.1 (Nephelometric Turbidity Units or NTUs) and highest at Charters Creek (> 300 NTUs). Water depth was greatest at the Mouth (max 3 to 4 m), while levels at Charters Creek were much shallower (max 0.1 to 0.7 m) (Table 5.1).

5.3.2 Autotrophic sources

Phytoplankton biomass ranged from 4.86 to 68.5 mg pigm.m^{-3}. Biomass was lowest at the Mouth during the dry season and highest at the Mouth during the wet season (Table 5.1). Phytoplankton biomass remained relatively unchanged from dry to wet seasons at Charters Creek. Size fractionated phytoplankton revealed minor differences in size distribution, with the majority of the phytoplankton made up by nanoplankton, followed by picoplankton and microplankton (Table 5.1). In terms of microalgal class contribution, at Charters Creek in the wet season 82.5% of the pelagic microalgae was made up by diatoms, dinoflagellates and chlorophytes and the remainder by cryptophytes and cyanobacteria. Unfortunately no results are available for the dry season. At the Mouth, the microalgae were dominated by chlorophytes and cryptophytes, which together contributed up to 97% of the total microalgae present in both wet and dry seasons. Microphytobenthic biomass ranged from 5.15 to 34.2 mg pigm.m^{-2}. Biomass was generally greater at Charters Creek when compared to the Mouth region (Table 5.1). The percentage of organic matter in the sediment increased towards the north within the system. Percent organic matter ranged from ~0.84% at the Mouth to ~3.58% at Charters Creek. *Cladophora* sp. was only ever present at Charters Creek and was found in both the wet and the dry season (Table 5.1).

5.3.3 Stable isotope analysis: temporal and spatial variations

Although there was no significant temporal difference in the $\delta^{13}C$ signatures of the primary producers at the Mouth (ANOVA, $F_{1,20} = 1.61$ $p < 0.05$), the $\delta^{13}C$ signatures of all the primary producers were significantly different from each other (ANOVA, $F_{3,18} = 78.7$, $p < 0.05$), ranging from -13.81 for MPB to -29.25 ‰ for POM (Fig. 5.1a and b). Temporal variations in $\delta^{13}C$ signatures of the primary producers at Charters Creek were significantly different (ANOVA, $F_{1,20} = 13.7$, $p < 0.05$). With the exception of POM, MPB and detritus, all sources were significantly different from each other (post hoc Tukey's, $p < 0.05$). Overall, $\delta^{13}C$ signatures of primary producers at Charters Creek ranged from -15.7 for *Cladophora* sp. to -24.2 ‰ for SOM (Fig. 5.1c and d). At both the Mouth and Charters Creek, carbon isotope signatures were slightly more enriched (+ 1 to 2 ‰) during the wet season (Table 5.2). While there were no significant temporal variations in $\delta^{15}N$ signatures of the primary producers (ANOVA, Mouth: $F_{1,20} = 0.408$, $p > 0.05$;

Charters Creek: $F_{1,20}$ = 0.35, p > 0.05), the sources were significantly different to each other (ANOVA, Mouth: $F_{3,18}$ = 32, p < 0.001; Charters Creek: $F_{2,19}$ = 7.45, p < 0.001). $\delta^{15}N$ signatures of the primary producers ranged from 0.5 ‰ for SOM to 7.7 ‰ POM at the Mouth (Fig. 5.1, Table 5.2).

Table 5.1: Physico-chemical parameters and autotrophic variables measured on each of the sampling occasions during wet and dry seasons at Charters Creek and the Mouth in the St. Lucia Estuary. +/- : present/absent. N/S: no sample taken; MPB: Microphytobenthic biomass, PPL: Phytoplankton biomass.

	Charters Creek		Mouth	
	Wet	**Dry**	**Wet**	**Dry**
Date	27-Apr-09	3-Sep-08	27-Feb-09	05-Jul-09
Temperature (˚C)	26.8	21.5	27.6	19.6
Salinity	46.8	53.6	11.1	12.5
Turbidity (NTU)	102	341	21.5	10.1
Water depth (m)	0.2	0.1	1.00	3.0
MPB (mg pigm.m^{-2})	34.2	17.9	5.15	28.1
PPL (mg pigm.m^{-3})	4.94	4.98	68.5	23.9
Microplankton (%)	9.07	22.7	0.78	53.5
Nanoplankton (%)	72.6	76.1	85.5	7.68
Picoplankton (%)	18.4	1.23	13.8	38.8
Detritus (+/-)	+	N/S	+	+
***Cladophora* sp. (+/-)**	+	+	-	-

Table 5.2: Average $\delta^{13}C$ and $\delta^{15}N$ values (‰ ± SD) of the primary producers and consumers during wet and dry seasons at Charters Creek and the Mouth in the St. Lucia Estuary.

	Charters Creek				Mouth			
	Wet		Dry		Wet		Dry	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
***Cladophora* sp.**	-15.7 ± 0.08	6.20 ± 0.22	-17.9 ± 0.06	8.08 ± 0.12	-	-	-	-
POM	-19.1 ± 0.13	6.86 ± 1.36	-20.0 ± 0.12	4.43 ± 0.76	-27.6 ± 1.18	5.84 ± 1.28	-29.3 ± 0.04	7.70 ± 1.16
MPB	-17.0 ± 0.11	5.86 ± 1.38	-20.1 ± 0.04	2.68 ± 0.28	-17.3 ± 0.23	3.08 ± 0.63	-13.8 ± 0.03	1.67 ± 0.22
SOM	-20.2 ± 0.83	4.05 ± 1.21	N/S	N/S	-26.33	0.5	-23.6 ± 0.83	1.63 ± 0.47
Detritus	-19.6 ± 1.07	6.13 ± 0.02	N/S	N/S	-22.7 ± 0.25	5.8 ± 0.46	-19.8 ± 0.05	5.20 ± 0.04
M. africana	-25.9 ± 0.27	10.0 ± 0.31	-17.4 ± 0.3	10.41 ± 0.37	-26.2 ± 0.01	14. 5 ± 0.25	-27.3 ± 0.13	12.6 ± 0.53
P. stuhlmanni	-22.3 ± 0.26	10.5 ± 0.22	-20.2 ± 0.03	10.2 ± 0.25	-26.3 ± 0.09	10.6 ± 0.18	-31.1 ± 0.02	10.8 ± 0.31
A. natalensis	-	-	-	-	-27.6 ± 0.1	9.92 ± 0.06	-	-

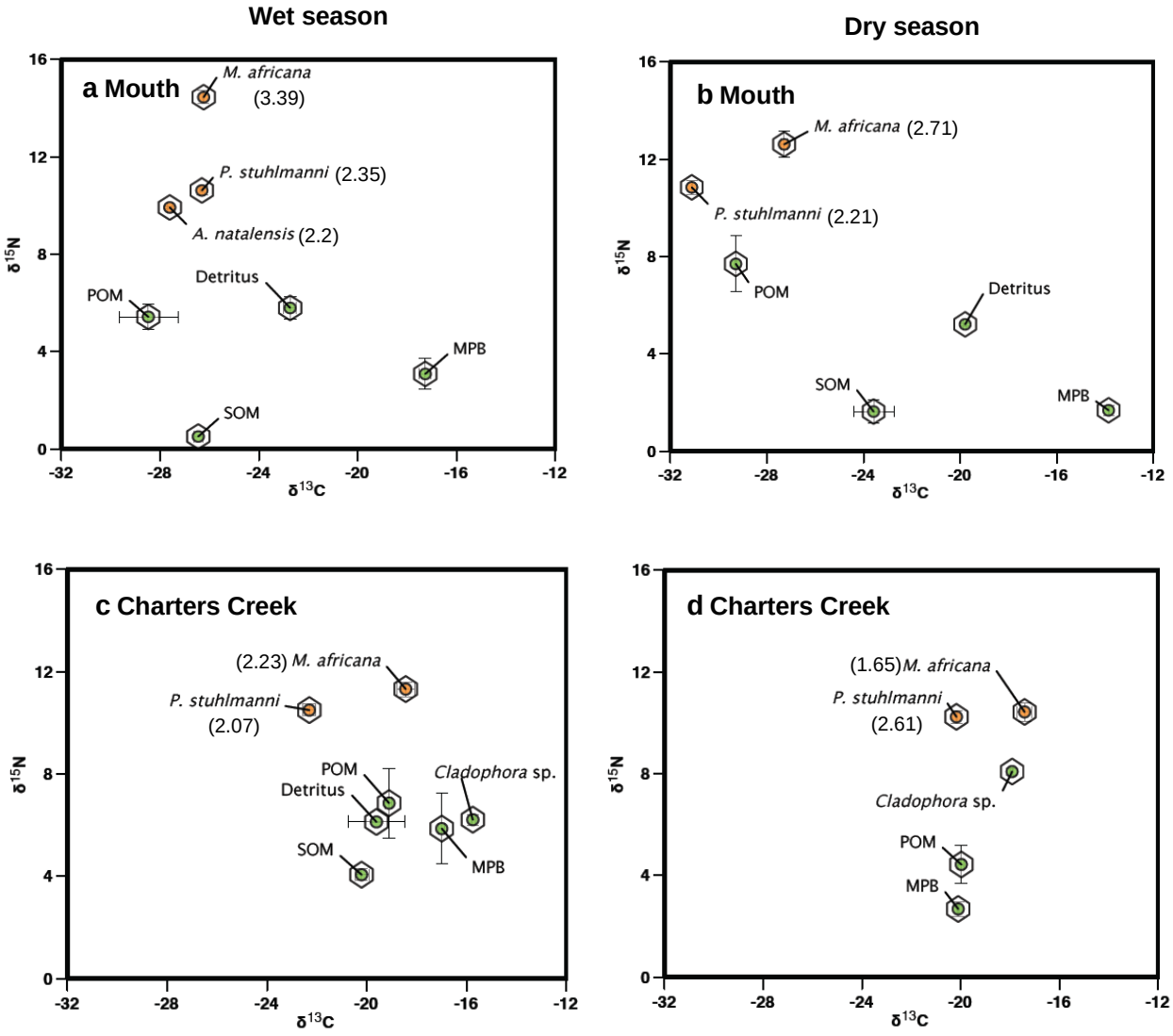

Fig. 5.1: Plots of $\delta^{13}C$ and $\delta^{15}N$ of primary carbon sources and consumers sampled at the Mouth in (a) wet and (b) dry seasons, and at Charters Creek in (c) wet and (d) dry seasons. Error bars show SD. The numbers in brackets associated with each of the species names are the respective trophic levels. POM: particulate organic matter, SOM: sedimentary organic matter, MPB: microphytobenthos.

5.3.4 Trophic level and source contribution to diet

The mysid *Mesopodopsis africana* was usually most enriched in $\delta^{15}N$ (Table 5.2) and, therefore, occupied the highest trophic position, followed by the copepods *Pseudodiaptomus stuhlmanni* and *Acartia natalensis*. Trophic positions were higher at the Mouth than at Charters Creek. While the trophic level of *P. stuhlmanni* remained relatively unchanged in the wet and dry seasons at both Charters Creek and the Mouth, the trophic level of *M. africana* was higher at the Mouth than at Charters Creek (Fig. 5.1).

Copepods were the most important in the diet of *Mesopodopsis africana* at the Mouth. In the wet season (Fig. 5.2a), *Pseudodiaptomus stuhlmanni* and *Acartia natalensis* contributed up to 52 and 46% of the diet respectively. In the dry season (Fig. 5.2b), *P. stuhlmanni* was the most important in this mysid diet contributing between 13 and 74% of the diet. Next important in terms of diet contribution was POM, while SOM and MPB made minor contributions to its overall diet (Fig. 5.2b). The proportion of different carbon sources to the diet of *M. africana* at Charters Creek was relatively even. *Cladophora* sp., POM, MPB and SOM made similar contributions. In the wet season (Fig. 5.2c), *Cladophora* sp. made the most substantial contribution of up to 49% of the diet, while MPB, SOM, POM, detritus and *P. stuhlmanni* all contributed between 0 and 40% of the diet. In the dry season (Fig. 5.2d) *Cladophora* sp. also played the greatest role in the mysid diet (6 to 71%), followed by POM, *P.stuhlmanni* and MPB.

At the Mouth, *Pseudodiaptomus stuhlmanni* utilized mostly POM (up to 66%) with SOM, plant detritus and MPB contributing up to 45, 60 and 19% of the diet respectively in the wet season (Fig. 5.3a). In the dry season, POM contributed up to 74% of the overall diet with MPB, SOM and detritus only contributing up to ~45% of the diet (Fig. 5.3b). At Charters Creek, *P. stuhlmanni* appeared to be feeding on a relatively even mixture of the available carbon sources, with each dietary item contributing on average between 0 and 48 % of the diet during the wet season (Fig. 5.4c). During the dry season, POM and MPB made the greatest contribution (up to ~80%), followed by *Cladophora* sp. (Fig. 5.3d). Limited data are available for *Acartia natalensis*, due to its short seasonal occurrence at the Mouth station only. Results do, however, indicate that in this area the species feeds primarily on POM, followed by SOM, detritus and lastly, MPB.

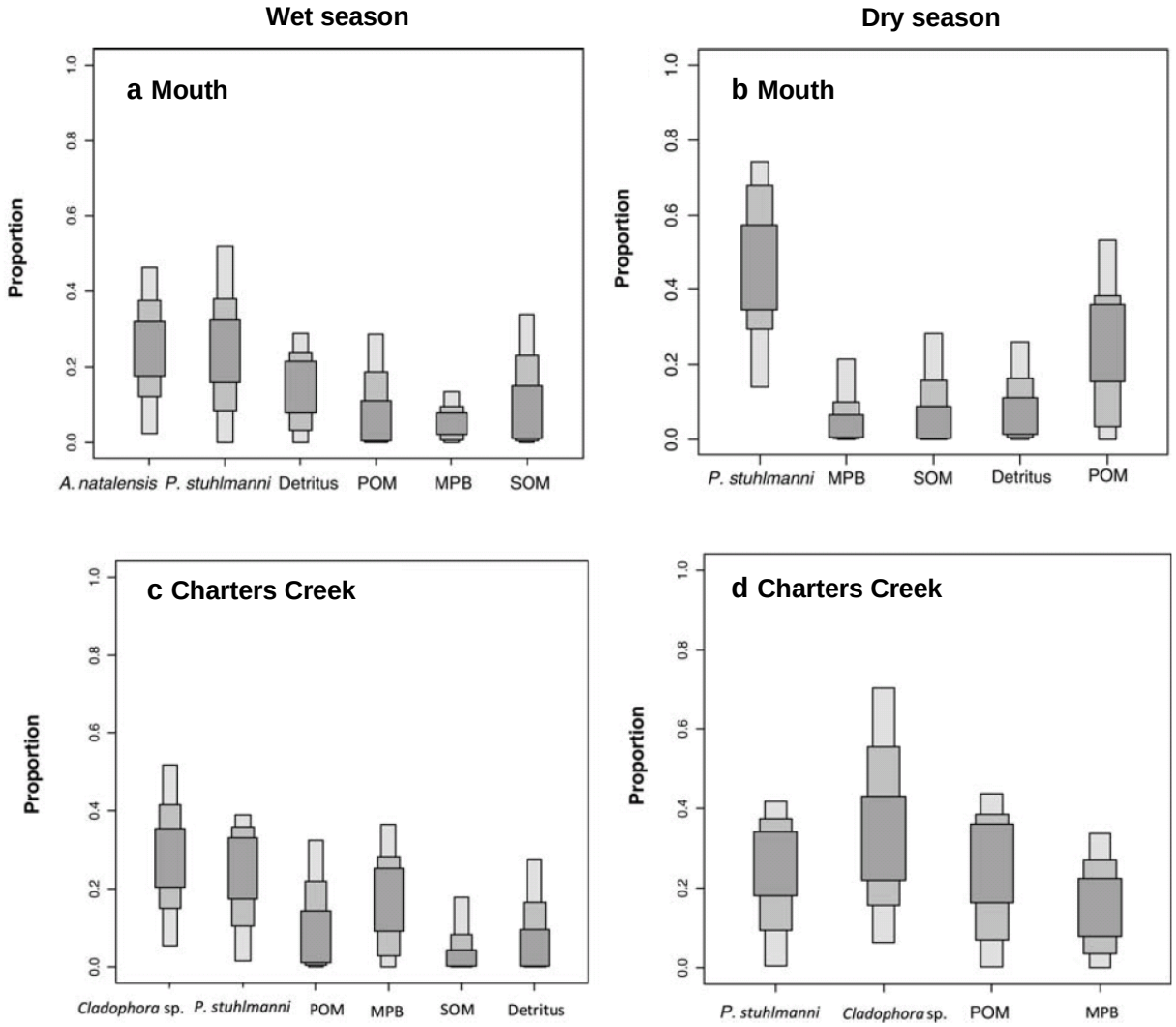

Fig. 5.2: Bar graph showing contribution of different primary carbon sources to the diet of *M. africana* using SIAR at the Mouth in (a) wet and (b) dry seasons and at Charters Creek during (c) wet and (d) dry seasons. Abbreviations see Fig. 5.1.

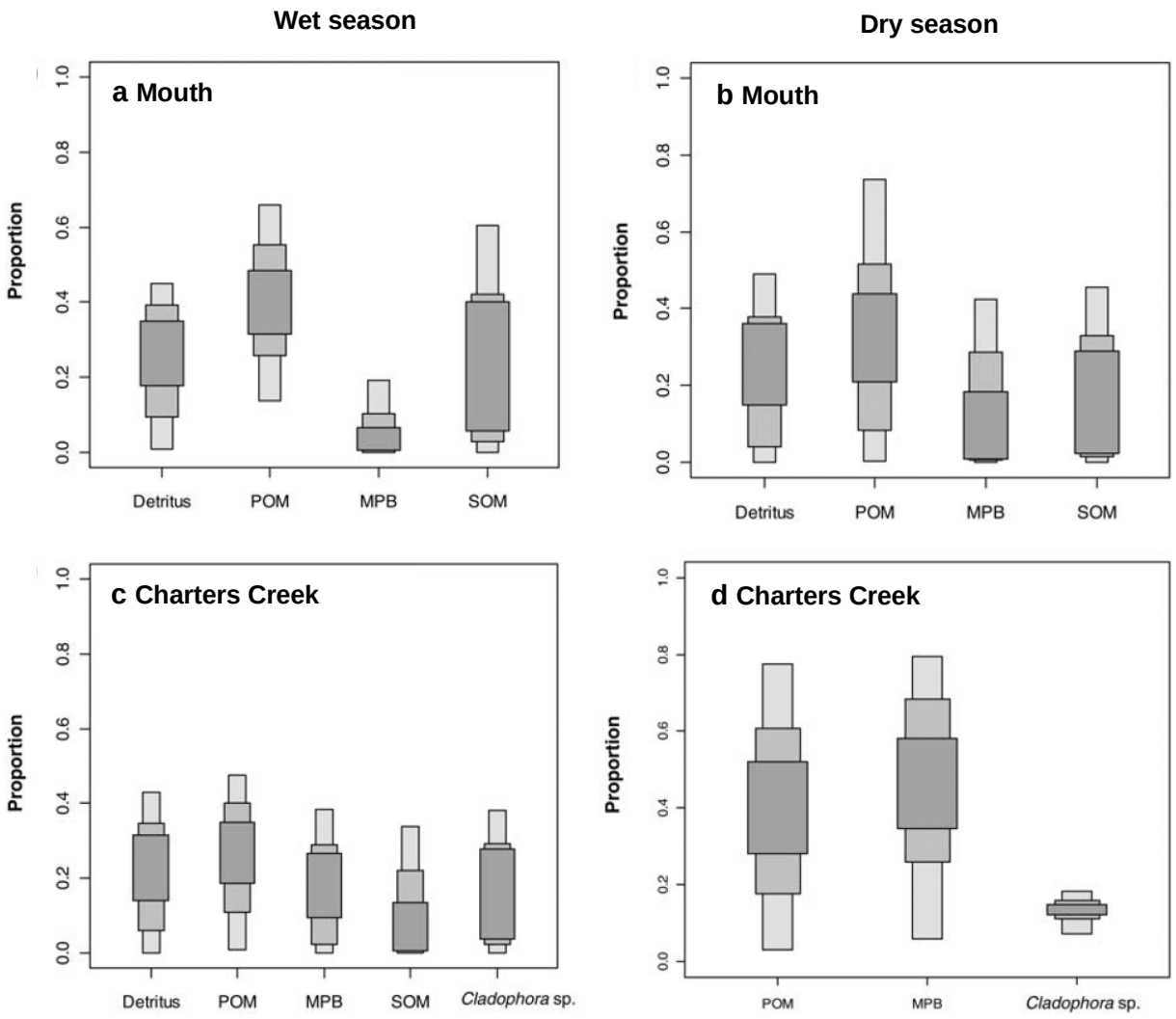

Fig. 5.3: Bar graph showing contribution of different primary carbon sources to the diet of *P. stuhlmanni* using SIAR at the Mouth in (a) wet and (b) dry seasons and at Charters Creek during (c) wet and (d) dry seasons. Abbreviations see Fig. 5.1.

5.4 DISCUSSION

Across South Africa, and in many other estuaries worldwide, copepods of the genera *Pseudodiaptomus* and *Acartia* as well as mysids of the genus *Mesopodopsis* often co-occur in such high densities that they constitute the dominant zooplankton (cf: Wooldridge 1999, Montoya-Maya and Strydom 2009, Ramaiah *et al.* 1996). This is also true for the St. Lucia Estuary. Here, while copepods are often numerically dominant, mysids tend to contribute more in terms of biomass (Carrasco *et al.* 2010: Chapter 1). The role of zooplankton in linking primary and/or secondary producers with higher trophic levels is widely documented. While three key species, i.e. *M. africana, P. stuhlmanni* and *A. natalensis*, are known to dominate the zooplankton community of in the St. Lucia Estuary (Carrasco *et al.* 2010: Chapter 1, Jerling *et al.* 2010), not much is known about their comparative feeding preferences and resources utilization.

The $\delta^{13}C$ signals of POM at the Mouth were more depleted during the wet season. It is possible that POM contained material that originated from senescent leaves of mangroves (Govender *et al.* in review) a habitat which is located near and around the Mouth and Narrows region (Begg 1978). Additionally, the Mouth region now receives a steady riverine input through the Mfolozi River (Whitfield and Taylor 2009). Depleted carbon signatures could, therefore, also be reflective of allocthonous input upstream. Alternatively, $\delta^{13}C$ signatures of POM at Charters Creek were more depleted during the dry season. A possible explanation for this could be a potential shift in community structure of the primary producers in response to the development of the hypersaline conditions at this station during the dry season. The same does not apply to the Mouth because this area remains remarkably stable, due to its more regular freshwater input. In this region, zooplankton abundance followed the well-documented trend of having a higher abundance in the wet season due to the increased phytoplankton productivity brought about by freshwater input and its nutrient load. At Charters Creek, however, the diversity and abundance of higher trophic levels, such as fish and zooplankton itself, had already decreased from September 2008 to April 2009, despite the latter being the wet season, stressing the severity of the current freshwater-starvation crisis (Carrasco and Perissinotto in review).

Attempts to isotopically evaluate pelagic food web structure are generally hindered by the difficulty of physically separating the similarly sized components at the base of the food web: phytoplankton,

bacteria, protozoa and detrital particles (Jones *et al.* 1998). Results should, therefore, be analysed with caution due to the limited number of sources used. Additionally, while size fractionated isotope results would have offered valuable insight into the selective feeding of these zooplankton species, technical difficulties prevented their collection. Nevertheless, data were collected on the pelagic microalgal size composition as well as the different microalgal groups present at each station during each season. The phytoplankton present at the time at the Mouth and Charters Creek was dominated by nano-plankton, with micro- and pico-plankton contributing relatively minor percentages. Additionally, while the Mouth was characterized by chlorophytes and cryptophytes, Charters Creek was characterized by diatoms, dinoflagellates and chlorophytes and only to a lesser extent by cryptophytes and cyanophytes.

Mysids are generally regarded as omnivores, capable of successfully exploiting a variety of food resources. $\delta^{15}N$ signals for *Mesopodopsis africana* in this study ranged from 10 to 14 ‰, suggesting that mysids may be feeding mainly on mesozooplankton. Diet analysis using SIAR v 4.0 showed that in both wet and dry seasons, *M. africana* at the Mouth consistently consumed a greater proportion of copepods relative to the other available carbon sources. Next in importance in the diet of this mysid was POM. At Charters Creek, mysids generally consumed all available carbon sources in relatively even proportions. However, the important contribution of the macroalgae *Cladophora* sp. to the mysids diet during the dry season was noted. The importance of *Cladophora* sp. in the diet of *M. africana* in the St. Lucia Estuary was also noted by Govender *et al.* (in review). The comparatively high proportion of this carbon source in the mysids diet (especially during the dry season) could be a reflection of the seasonal abundance of this food item. Diet shifts according to food availability have also been previously recorded in other studies (Viherluoto *et al.* 2000, Winkler *et al.* 2007, Vilas *et al.* 2008).

Copepods are also capable of utilizing a wide range of diets (Kleppel 1993). In the St. Lucia Estuary, $\delta^{15}N$ signatures for *Pseudodiaptomus stuhlmanni* and *Acartia natalensis* ranged from 9.92 to 10.8 ‰. These values are similar to those reported for *P. hessei* in the Kariega Estuary, South Africa (Richoux and Froneman 2007) and for *A. omorii* in the Chikugu River estuary, Japan (Suzuki *et al.* 2008). In this study, *P. stuhlmanni* fed proportionally more on POM at the Mouth, both in wet and dry seasons. Limited data were available for *A. natalensis*, due to its short seasonal

occurrence. However, available results show that *A. natalensis* also preferentially fed on POM, although SOM and MPB also played a significant, albeit minor role in its diet.

Among the species investigated, *Mesopodopsis africana* occupied the highest trophic level, followed by *Pseudodiaptomus stuhlmanni* and *Acartia natalensis*. The trophic position of *M. africana* at Charters Creek was, however, lower than at the Mouth. This can be attributed to the greater proportion of primary producers in its diet as opposed to secondary producers. The trophic position of *P. stuhlmanni* on the other hand, was relatively consistent throughout stations and seasons. Kibirige *et al.* (2002) identified potential, alternative food sources of the dominant zooplankton species of the Mpenjati Estuary (South Africa). Results indicated that the mysid *Gastrosaccus brevifissura* and the copepods *Pseudodiaptomus hessei* and *Acartia natalensis* during the dry season derived their highest food contribution from MPB, POM and detritus respectively. In this study however, *Mesopodopsis africana* fed proportionally more on copepods and POM at the Mouth and Charters Creek respectively, while the copepods consumed mainly POM at both stations. Kibirige *et al.* (2002) suggested that the dominant zooplankton taxa, while occupying the same tropic level, derived most of their energy requirements from different food sources, thus minimizing inter-specific competition for resources and improving the utilization of resources available in the estuary. Similarly, in the St. Lucia Estuary, while the dominant taxa did not all belong to the same trophic level, the omnivorous nature and food partitioning abilities shown, appeared to regulate the availability of primary producers, and also, to some extent, allow the co-habitation of these three species.

At Charters Creek, there was seldom a dominant food source. It appeared as though all sources were making similar contributions to the diet. Shallow water levels coupled with windy conditions often result in the resuspension of fine sandy substratum, possibly explaining why $\delta^{13}C$ values for some of the primary carbon sources at this station were not significantly different from each other. This creates some difficulty in identifying the major primary carbon source to a species diet. The lack of a dominant food source may also be a reflection of the harsh environmental conditions present in this area at the time. Charters Creek is situated on the west coast of South Lake, which is known to be a turbid location (Cyrus 1988). Here, shallow waters combined with a fine sandy substratum frequently lead to turbidity values exceeding 300 NTU. Additionally, zooplankton communities at

Charters Creek may have been more stressed on account of freshwater starvation and the resultant hypersalinity and low water levels. It could be assumed that under stressful conditions, zooplankton may become less selective in their prey choice. Tackx *et al.* (2003) demonstrated the ability of *Eurytemora affinis* to feed selectively on phytoplankton, and showed that limitation of selective feeding occurred under high suspended particulate matter (SPM) loads. This concurs with the findings of Gasparini *et al.* (1999) who also showed that phytoplankton uptake by *E. affinis* seemed to be hampered at SPM concentrations of the order of hundreds $mg.L^{-1}$. Gasparini and Castel (1997) showed that, in the Gironde Estuary, *E. affinis* can also feed to a substantial degree on heterotrophic nanoplankton, especially at high SPM concentrations. This would, however, depend to a large degree, on whether or not phytoplankton was a limiting food source. Carrasco *et al.* (2007) also documented the negative effect of increased silt loads on feeding and mortality rates of *M. africana*.

The presence of diel-vertical migration in some zooplankton species is generally accepted as a mechanism to reduce their risk of predation (Gliwicz 1986, Boscarino *et al.* 2009). This characteristic also gives some species the potential to link energy transfer between pelagic and benthic environments (Taylor 2008, Vilas *et al.* 2008, Lehiniemi *et al.* 2009). Diel vertical migration in *Mesopodopsis africana* in the St. Lucia Estuary has been recorded by Carrasco and Perissinotto (2010a: Chapter 3), where higher gut pigment concentrations were documented during night hours compared to daytime. Kouassi *et al.* (2001) also demonstrated the ability of *Acartia clausi* and *Pseudodiaptomus hessei* to migrate vertically in the water column in the oligohaline area of the Ebrié Lagoon (Côte d'Ivoire). There, *P. hessei* fed mostly on benthic algal particles during the day and on seston particles at night, allowing it to actively contribute towards the benthic food chain. While *A. clausi* also displayed clear diel vertical migration, it covered lower amplitudes and fed mostly during the night on seston particles. The food partitioning between these species explained their ability to cohabit at high biomass in the oligohaline area of the Ebrié Lagoon (Kouassi *et al.* 2001). An earlier study by Carrasco and Perissinotto (2010a: Chapter 3) documented a negative relationship between the abundance of the copepods *P. stuhlmanni* and *A. natalensis* and that of *M. africana* both at Charters Creek and the Mouth. Mysids may have migrated to surface waters at night, explaining the lower densities observed during sampling with the epibenthic sled. Abundance of *P. stuhlmanni*, however, increased despite the fact that this genus is also known to undergo diel-vertical migration (Kibirige and Perissinotto 2003b, Froneman 2004b). This anomaly could be a strategy to avoid predation by mysids. Fulton (1982) found mysid predation to have a

significant effect on copepod densities. The effect of mysid predation on species composition appears to depend on the relationship between their prey preferences and the dominant copepod species present in the community. This inverse relationship may have evolved as a mechanism by which copepods can avoid mysid predation and thereby live in co-existence with *M. africana*.

Overall, results indicate that the diet composition of all three species is more selective at the Mouth. This could be attributed to either the harsh environmental conditions prevailing at Charters Creek, or the greater number of autotrophic sources available in this region, compared to the Mouth. These three taxa are the key zooplankton species in the St. Lucia Estuary. The high proportion of *Pseudodiaptomus stuhlmanni* in the diets of *Mesopodopsis africana* at the Mouth could explain the apparent inverse relationship in the abundance of the copepods in relation to that of the mysids. The omnivorous nature of *Mesopodopsis africana* may also aid in relieving the grazing pressure on primary producers by feeding directly on copepods. *M. africana*, *P. stuhlmanni* and *A. natalensis* all appear to be opportunistic feeders, capable of incorporating a number of food items in their diet. Between food partitioning, predator avoidance strategies, and their common ability to survive in highly dynamic environments, these species are capable of thriving in the same area together.

CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

A total of 258 estuaries are currently found along the South African coastline, with the vast majority (~70%) currently listed as temporarily open/closed estuaries (TOCEs, Whitfield 1992). As has been previously observed in most temporarily open/closed South African estuaries, maximum zooplankton abundance and biomass in the St. Lucia Estuary was recorded during the closed-mouth phase (Whitfield 1980, Kibirige and Perissinotto 2003a, Kibirige *et al.* 2006). This is owed to a combination of stable mouth conditions, limited freshwater input and minimal exchange of water with the sea (Gaughan and Potter 1995, Perissinotto *et al.* 2000). Low zooplankton abundance and biomass during open-mouth conditions are consistent with findings reported in the literature and are linked to the export of biomass-rich estuarine water into the ocean (Froneman 2004a).

Prolonged periods of mouth closure in temporarily open/closed estuaries are generally associated with low levels of zooplankton taxonomic diversity (Froneman 2004a, Perissinotto *et al.* 2004, Jerling *et al*. 2010). In the current study, the lake zooplankton was, like most estuarine zooplankton assemblages in southern Africa, including those in permanently open estuaries (Wooldridge 1999), dominated by typical estuarine taxa, such as *Pseudodiaptomus stuhlmanni*, *Acartia natalensis* and *Mesopodopsis africana*, which often collectively contributed over 90% of the total zooplankton abundance. Only 27 of the 95 previously recorded taxa (Grindley 1976) were observed in the system during the closed-mouth phase. Missing components included marine and freshwater taxa, which were absent because of prolonged reduced freshwater input (Allanson and Read 1995), and the closed-mouth state, which prevented tidal exchange (Jerling *et al*. 2010). Primo *et al*. (2009) showed that dry periods in the downstream stations of the permanently open Mondego Estuary (Portugal) were also characterised by a lack of seasonality, low taxonomicdiversity and a high density of *Acartia* spp. However, in contrast to the St. Lucia system, marine taxa dominated the Mondego Estuary, probably due to its open mouth (Primo *et al*. 2009). The opening of the St. Lucia Estuary mouth in March 2007 led to a significant increase in species richness. Marine taxa, such as the copepods *Corycaeus* spp., *Paracalanus* spp., juvenile *Penaeus indicus*, and estuarine dependent ichthyoplankton recruited into the system.

Multivariate correlations showed weak associations of zooplankton with environmental parameters measured in the in the St. Lucia Estuary. This is not uncommon in estuaries (Hastie and Smith 2006) and is probably a product of the highly dynamic nature of this system. Overall, the interaction between microphytobenthic biomass, phytoplankton biomass, salinity and temperature best explained the variation in community structure. In terms of univariate analysis, microphytobenthic biomass was inversely related to zooplankton biomass, abundance and species richness. Reduced grazing pressure could have allowed for microphytobenthos proliferation, since biomass was generally greatest at Charters Creek and Listers Point, where conditions were often not favourable for the survival of zooplankton. Overall, zooplankton abundance and species richness correlated positively with water depth. Such a relationship is expected, since habitat for zooplankton increases with water depth. Additionally, the Mouth and the Narrows are deeper regions, partly protected from the effects of drought due to the fresh water inflow at Makakatana, Mfolozi and Mpate rivers. Zooplankton abundance and salinity were negatively correlated, reflecting the negative impact of hypersaline conditions on zooplankton abundance. Additionally, zooplankton abundance and species richness were greater in cooler waters. High temperatures are known and expected to negatively affect the physiology of many zooplankton species (e.g. Moore *et al*. 1996, Norberg and De Angelis 1997).

Temperature and salinity are arguably the two most important variables influencing estuarine inhabitants. Natural abundances of *Mesopodopsis africana* recorded from February 2007 to May 2010 were plotted against the temperature and salinity at which they occurred. Mysids were found at temperatures ranging from 16.2 to 30.9ºC and at salinity levels ranging from 2.55 to 64.4. These are some of the highest documented upper salinity and temperature thresholds for a mysid species (Murano 1966, Roast *et al*. 2001). It must be noted though that mysids found at salinity levels from 60 to 64.4 were visibly physiologically stressed and probably experiencing sub-lethal effects, being highly inactive and not particularly responsive to mechanical stimulus (NK Carrasco, pers. observ.). Acclimation was found to be an important factor in establishing salinity tolerance of mysids. However, while acclimation was shown to significantly increase survival of *M. africana*, it was evident that acclimation rate is of critical importance. Recent measurements at Listers Point in False Bay show that under conditions of high rainfall in the field, salinity levels can drop by ~ 180 in 3.5 weeks. Similarly, during periods of low freshwater input, salinity levels may rise by ~ 75 in 2 weeks. On average, this is a decrease and increase in salinity of ~ 7 and ~ 6 per day, respectively.

These decreases and increases are still significantly lower than those implemented in the acclimation experiments conducted during this study. Should one be logistically capable of employing a slower acclimation rate of weeks or even months, it is possible that the salinity tolerance observed in the field would mimic those recorded under experimental conditions. Results presented in this study may be useful in predicting how mysid populations could be affected by future environmental changes induced by anthropogenic climate changes. This is particularly important, since environmental changes in this system have been aggravated by the artificial diversion of the main freshwater input source of the estuary, the Mfolozi River. While *M. africana* does have some of the highest recorded salinity and temperature tolerances recorded for a mysid, temperatures above 31ºC and salinity levels above 65, which are often recorded in this shallow estuarine lake, will have a marked influence on the distribution of this species.

The role of mysid species, such as *Mesopodopsis africana*, in structuring food webs is widely known (e.g. Fulton 1982, Hansson *et al.* 1990, Kouassi *et al.* 2006). Their high biomass, relative to the other dominant taxa, grants them the potential to have substantial impacts on ecosystem functioning. Ingestion rates were higher at the Mouth of the St. Lucia Estuary, resulting in population grazing impacts of 2.5% of the total microalgal biomass, while the grazing impact at Charters Creek was only 0.5%. The spatial variation in ingestion rates could be attributed to seasonal differences in gut evacuation rates, differences in the mean size of mysids used, or the physico-chemical conditions present at the two stations. It is suggested that mysid populations at Charters Creek are predominantly driven by bottom-up forces, initiated by harsh environmental conditions. Despite the lower ingestion rates exhibited at Charters Creek, results indicate that these mysids are capable of meeting all their energetic requirements from a microalgal diet alone, although they may also utilise a heterotrophic diet (Carrasco and Perissinotto 2010b: Chapter 4, 2011b: Chapter 5). Given the the turbid nature of Charters Creek and the most likely even distribution of chl *a* in the water column, it was surprising that mysid populations at this station still exhibited diel vertical migration (however to a lesser degree to that at the Mouth). Additionally, there was a negative relationship between the abundance of the copepods *Peudodiaptomus stuhlmanni* and *Acartia natalensis* and that of *M. africana* on both sampling occasions. Mysids may have migrated to surface waters at night, explaining the lower numbers caught with the epibenthic sled. Abundance of *P. stuhlmanni*, however, increased despite the fact that this genus is also known

to undergo diel-vertical migration (Kibirige and Perissinotto 2003, Froneman 2004). This anomaly could represent a strategy to avoid predation by mysids. It was later shown through diet analysis using SIAR version 4.0 that the calanoid copepod *P. stuhlmanni* was in most cases the main prey item of *M. africana*. Mysids at Catalina Bay fed mainly on POM, probably because the copepods were almost completely absent from this station at the time. Feeding on POM may, therefore, be a result of mysids feeding on the most abundant prey, rather than selective feeding. The flexibility in the diet of *M. africana* was also shown on a temporal scale. While the dominant food items for *M. africana* did not change significantly from wet to dry seasons, the proportions of the different sources in the mysid diet varied. This again may be linked to food availability.

Copepods are also capable of utilizing a wide range of diets (Kleppel 1993). In the St. Lucia Estuary, *Pseudodiaptomus stuhlmanni* fed proportionally more on POM at the Mouth, both in wet and dry seasons. Limited data were available for *Acartia natalensis*, due to its short seasonal occurrence. However, available results show that *A. natalensis* also preferentially fed on POM, although SOM and MPB also played a significant, albeit minor role in its diet. Among the species investigated, *Mesopodopsis africana* occupied the highest trophic level, followed by *P. stuhlmanni* and *A. natalensis*. In this study, *M. africana* fed proportionally more on copepods and POM at the Mouth and Charters Creek respectively, while the copepods consumed mainly POM at both stations. The omnivorous nature and food partitioning abilities shown by these species may regulate the availability of primary producers, and also, to some extent, allow the co-habitation of these three species. Overall, the diet composition of these species was more selective at the Mouth. This could be attributed to either the harsh environmental conditions prevailing at Charters Creek, or the greater number of autotrophic sources available in this region, compared to the Mouth. The high proportion of *P. stuhlmanni* in the diets of *M. africana* at the Mouth could explain the apparent inverse relationship in the abundance of the copepods in relation to that of the mysids. *M. africana* regulates the abundance of the copepods *P. stuhlmanni* and *A. natalensis* and, therefore, also helps relieve the grazing pressure on the main primary producers.

M. africana, P. stuhlmanni and *A. natalensis* all appear to be opportunistic feeders, capable of incorporating a number of food items in their diet. Between food partitioning, predator avoidance strategies, and their common ability to survive in highly dynamic environments, these species are capable of thriving in the same area together. Although these taxa are all euryhaline, capable of

withstanding wide fluctuations in salinity (Grindley 1976), continued drought effects could force their exclusion. Because of the importance of zooplankton in estuarine food webs, their loss would have substantial impacts on food web functioning in different regions of this key estuarine lake. The potential though for these species to alter their diet in response to a dynamic environment, and their persistence through the many past hydrological phases, is a prime example of their adaptive nature and re-enforces the resilience so far shown by the system (Begg 1978, Jerling *et al*. 2010).

Recommendations for future research

At St Lucia, pelagic communities are currently undergoing major losses in biodiversity (e.g. a decline of 60-70% in zooplankton taxa and 40% in fish species) and dominance shifts, with species adapted to extreme conditions taking over large sections of the system. Recently, a halophilic community has been documented in the system. In particular, the northern hypersaline (> 100) lakes have exhibited a 1½ year-old orange bloom of cyanobacteria (*Cyanothece* sp.). These cyanobacteria are consumed by a ciliate (*Fabrea* cf. *salina*; W Petz, pers comm), which is in turn consumed by the cyclopoid copepod *Apocyclops* cf. *dengizicus*. This copepod is then the main dietary item for lesser and greater flamingos which reside in this region (R Taylor, pers comm). *Apocyclops* cf. *dengizicus* is unique in that it has an extremely high salinity tolerance, only disappearing from the region once salinity exceeds 135. It is thought, though, that this copepod is capable of forming resting stages or spores capable of enduring unusually harsh conditions. Future research could investigate aspects of this species ecophysiology, as well as that of others which have also been found in the system. Two species of hydromedusa have recently been recorded at Esengeni and Catalina Bay and are possibly also undescribed (M Gibbons, pers comm). Because of the partial isolation of the system from other estuarine lakes, some of its species are probably micro-endemics and, therefore, restricted to St. Lucia itself. A thorough taxonomic analysis of all the invertebrates of the estuary and a full biodiversity audit for the system is, therefore, necessary. Secondly, research in this thesis has focused on the feeding habits of the dominant mysid *Mesopodopsis africana*, but it is also essential to understand the role which zooplankton play in the diet of the dominant fish species as well as other higher trophic levels commonly found in the system. Lastly, and of critical importance, would be to identify the key features of the recovery dynamics of the St. Lucia Estuary, should it shift from a dry to a wet state. The situation so far recorded in this estuarine lake shows that the system

has experienced a dramatic deterioration during the past eight years, as a result of the combination of climatic events and human interventions. The system has been widely reported as extremely resilient in the past, but it remains to be seen to what extent and within what time scale it will be able to recover from the current crisis, once a new wet cycle sets in.

The above aims can be further sumarised into the following specifics:

1) to investigate further the role of zooplankton in the diet of higher trophic level organisms, food web pathways could be investigated using stable isotope ($\delta^{13}C$ and $\delta^{15}N$) techniques;

2) to undertake a comprehensive biodiversity audit for the estuary (as a UNESCO World Heritage Site);

3) to collect and describe the taxa that are still new to science and identify those which are endemic to the system;

4) to continue with quarterly and ad-hoc monitoring surveys, in order to capture the critical events that will unfold when the region transitions from dry to wet conditions.

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