Dietary dynamics of two key fish species in the St Lucia estuarine system, South Africa

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ABSTRACT

Among the 155 species of fish recorded so far in the St Lucia estuarine lake, *Oreochromis mossambicus* and *Ambassis ambassis* are the two most prominent. Although originally endemic to southern Africa, *O. mossambicus* is now one of the most widely distributed exotic fish species worldwide. Together with *A. ambassis*, they have become the dominant fish species in the St Lucia estuarine lake since the closure of the mouth in 2002 and are, therefore, a crucial component of the food webs throughout the system. After a decade dominated by dry and hypersaline conditions, the St Lucia system has changed dramatically in terms of prevailing environmental conditions, as a result of higher than average rainfall at the end of 2011 and the onset of a new wet phase at the start of 2012. In response, *A. ambassis*, which prefers lower salinity regimes, has expanded its distribution range throughout the estuarine lake. Stable $\delta^{15}N$ and $\delta^{13}C$ isotope analysis was used in conjunction with gut content analysis to elucidate the diet of these species at sampling localities spanning the geographical range of the system and determine whether these species shift their diet in response to environmental or climatic shifts. From both studies it is evident that from a temporal and spatial scale these two species adopt similar, yet very different, dietary tactics. *Oreochromis mossambicus* was shown to adopt a generalist feeding strategy, opportunistically feeding on dietary items that are available thus allowing this species to alter its diet according to the environment that it inhabits. Trophic positioning of this species was found to be controlled by salinity in St Lucia as dietary composition differed greatly between sites. In contrast, *Ambassis ambassis* displayed a more specialist dietary composition, feeding predominantly on zooplankton. However, this species also opportunistically supplements its diet with additional sources when available. Trophic position of *A. ambassis* was higher in the dry season owing to the increased productivity of the system during the wet season. The success and dominance of both species in the St Lucia system can therefore be attributed to their dietary strategies. Under extreme environmental conditions, *O. mossambicus* has the added advantage of its wide tolerance of different environmental conditions, particularly salinity, thus allowing it to proliferate.

PREFACE

The fieldwork described in this study was conducted in the iSimangaliso Wetland Park in KwaZulu-Natal, South Africa. Laboratory analyses were conducted at the School of Life Sciences, University of KwaZulu-Natal, Westville campus under the supervision of Prof. Renzo Perissinotto and Dr. Nicola Carrasco.

These studies represent original work by the author and have not otherwise been submitted in any form for any degree to any other tertiary institution. Where work of others has been used they have been duly acknowledged in the text.

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DECLARATION 2 – PUBLICATIONS

Details of contribution to publications that form part and/or include research presented in this thesis (include publications in preparation, submitted, in press and published and give details of the contributions of each author to the experimental work and writing of each publication)

Publication 1

Dyer DC, Perissinotto R, Carrasco NK (2013) Post-flood dietary variation in the Mozambique Tilapia (*Oreochromis mossambicus*) in the St Lucia Estuary, South Africa. Marine Ecology Progress Series 476:199–214

Author contributions:

DCD conceived the study with RP and NKC. DCD carried out sample collections, laboratory processing, statistical analyses and wrote the manuscript. RP and NKC contributed valuable comments to the manuscript.

Publication 2

Dyer DC, Perissinotto R, Carrasco NK (Submitted) Temporal and spatial dietary dynamics of the Longspine Glassy (*Ambassis ambassis*) in the St Lucia Estuary, South Africa. Marine & Freshwater Research.

Author Contributions:

DCD conceived the study with RP and NKC. DCD carried out sample collections, laboratory processing, statistical analyses and wrote the manuscript. RP and NKC contributed valuable comments to the manuscript.

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David Clifford Dyer

February 2014

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Fieldwork is one of the most exciting aspects of any ecology based project, but the collection of samples can be gruelling and dangerous, particularly when working amongst crocodiles and hippos in a world heritage site. Nevertheless, there were always people willing to help. Special thanks go to all the students working in St Lucia from UKZN for their help with the collection of samples. On some occasions it was necessary to call in specialist reinforcements, these people threw their cast net until their arms and backs were sore, regardless of whether it was pouring with rain, really windy or a beautiful day. For this I am truly grateful to Michael Dyer, Allen Connell and Roy Jackson.

The relationship a student has with his/her supervisor is critical in the success of their project. I was very fortunate to have a passionate, enthusiastic and driven supervisor who has influenced me immensely. Renzo, thank you for giving me every opportunity to pursue a research project on a topic that really interests me, fish. Thank you for always having time to listen and assist me, whether its work related or dealing with administration. To my co-supervisor Nicola, thank you for always assisting me with sample identifications and for always giving me valuable advice. Your experience with reviewing manuscripts and helping with the publication process is greatly appreciated.

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GENERAL INTRODUCTION

The St Lucia Estuary, the largest estuarine lake in Africa (Figure 1.1), is an integral part of the iSimangaliso (formerly Greater St Lucia) Wetland Park which is South Africa's first UNESCO World Heritage Site and a RAMSAR Wetland of International Importance (Taylor 2006). Characteristically, the system experiences alternating wet and dry phases, which can persist for 4 to 10 year periods (Begg 1978). Following a decade-long dry phase (2002-2012), the system has recently entered a wet phase which commenced at the beginning of 2012. Prior to this, the system was characterized by below average rainfall and limited freshwater input. This resulted in much of the surface area of the lake system exhibiting very low water levels (<30 cm) or complete desiccation. A persistent reverse salinity gradient formed within the system with the upper reaches of the estuarine system exhibiting considerably higher salinities (40 – 200) than its lower reaches and mouth area (10 – 15) (Taylor 2006, Carrasco et al. 2012). Higher than average rainfall in the catchment of the system in late 2011 resulted in large amounts of freshwater entering the estuary, subsequently raising water levels substantially and diluting salinities throughout the system (Taylor et al. 2013). In addition, the Mfolozi River system has recently been linked to the estuary via an artificial canal, providing freshwater and inconsistent marine input into the system. Despite this, the St Lucia Estuary still remains largely closed off from the Indian Ocean (Taylor et al. 2013).

Due to the extended closure of the mouth (since 2002), there has been an approximate 40% loss in fish species richness throughout the estuarine system (Vivier et al. 2010, Whitfield 1980), with *Oreochromis mossambicus* dominating the fish community (Vivier et al. 2010). Its dominance throughout the system seems to be indicative of the extent to which the fish community has been affected, as this species is usually less dominant than other marine associated species (Whitfield & Blaber 1978). The primary reason for this is that under hypersaline conditions *O. mossambicus* is able to out-compete estuarine species as well as marine species, which are dependent on the estuary (Whitfield & Blaber 1978). *Oreochromis mossambicus* is highly tolerant of a wide range of environmental conditions, surviving in fresh-, brackish and marine waters and is capable of breeding in salinity levels up to 120 (Whitfield et al. 2006, Cyrus & Vivier 2006). In St Lucia, this species has been documented to be extremely euryhaline, surviving and breeding successfully in salinities of 70 – 120 within False Bay and in parts of the North Lake (Whitfield et al. 2006, Cyrus & Vivier 2006).

Skelton (1993) stated that *O. mossambicus* is predominantly herbivorous, feeding on algae (especially diatoms) and detritus, with larger individuals occasionally feeding on insects and other invertebrates. It has also been shown that this species is an opportunistic forager, feeding on a wide array of dietary components (Whitfield & Blaber 1979, Bruton & Boltt 1975). In addition, *O. mossambicus* also forms a vital food source for many key species, such as the Great White Pelican, crocodiles and other piscivorous fish and bird species, thus forming an important link in the food webs of this ecosystem (Whitfield 1998, Cyrus & Vivier 2006, Bowker & Downs 2008). Chapter 1 of this thesis will therefore focus on the effect of changes in environmental conditions on the dietary composition of this species, with particular emphasis on the shift from hypersaline toward more oligohaline/mesohaline conditions.

The family Ambassidae has historically been well represented in the St Lucia Estuary, with three different species being recorded since the first comprehensive fish survey conducted by Whitfield in 1980. In the most recent survey conducted by Vivier et al. (2010a) only *Ambassis ambassis* and *A. natalensis* were present in the system in 2008. Despite its occurrence during the survey, *A. natalensis* was only recorded in small numbers and in a limited area of the Narrows. Vivier et al. (2010a) indicated that these two estuarine species were among the dominant fish in the St Lucia system, with *A. ambassis* accounting for 12.7% of the total catch during this survey and around 10% in the survey conducted by Cyrus and Vivier (2006). Thus, in recent years, *A. ambassis* is the second most commonly recorded fish species after the Mozambique Tilapia, *Oreochromis mossambicus*.

Ambassis ambassis is distributed along the east coast of South Africa, and ranges northward to Kenya. This species is also found on the islands of Madagascar, Mauritius and Reunion (Skelton 1993, Whitfield 1998). *Ambassis ambassis* has a wide (17-32°C) temperature tolerance range (Martin 1988), but relatively low (2-35) salinity tolerance (Skelton 1993, Whitfield 1998). Because of this low tolerance to salinity, the distribution of this species is often limited to areas of an estuary where the salinity is below 10 (Martin 1988, Martin 1989, Whitfield 1998). Previous studies have reported that the species feeds at night and early in the morning on planktonic crustaceans, fish fry and larvae, as well as insects (Martin & Blaber 1983, Skelton 1993, Whitfield 1998). This species has been classified as a resident species in estuaries and it has been known to breed under estuarine conditions in the St Lucia Estuary (Whitfield 1998, Cyrus 2013). Population densities of *A. ambassis* were historically lower than the other Ambassidae species in St Lucia, as most of the system had a salinity exceeding

15 (Martin 1983). Under freshwater-dominated conditions within the St Lucia system, *A. ambassis* is among the dominant fish species, as it is able to tolerate oligohaline conditions (Cyrus 2013). Apart from being popular live bait used by recreational fishermen, *A. ambassis* is also a key prey species for many of the estuarine piscivorous bird and fish species found in the St Lucia system, forming an important link in its food webs (Martin 1983). Therefore, information regarding the dietary composition of a critical link in estuarine food webs, such as *Ambassis ambassis*, is vital in understanding the functioning of the system. Chapter 2 of this thesis will therefore examine the effect of changes in the environmental conditions on the dietary preferences of this species, with particular emphasis on the onset of the wet phase.

Stable isotope analysis has become an indispensable and informative tool for predicting the flow of organic matter through food webs in a variety of habitats (Peterson & Fry 1987, Michner & Lajtha 1994, Cabana & Rasmussen 1996, O'Reily et al. 2002, Bouillion et al. 2011, Wada et al. 2013). The use of stable isotopes has proven to provide a better long term representation of the diet of an organism, rather than the more temporally subjective view which would be provided by gut content analysis alone (Hesslein et al. 1993, Van der Zanden et al. 1997). As it considers assimilated material and not only ingested material, stable isotope analysis also gives a more accurate estimate of dietary composition over longer periods of time (Gearing 1991, Pinnegar & Polunin 1999). This is particularly relevant for fish muscle tissue, where turnover rates can be as slow as 0.1 to 0.2% per day (Hesslein et al. 1993). Stable carbon isotopes have been shown to fractionate very little between energy transfers and thus they can be used to confidently quantify food sources of an individual in aquatic systems (Gannes et al. 1998, Van der Zanden & Rasmussen 2001). Trophic positioning of consumers within a food web can be determined using stable nitrogen isotopes, as these are known to have a higher rate of fractionation between energy transfers (DeNiro & Epstein 1978, Van der Zanden et al. 1997). Gut content analysis is however still an accurate method of determining actual dietary composition over short periods of time, to gain a 'snapshot' of the diet for a particular period (Hyslop 1980). There are however obvious differences in the two methods; stable isotope analysis provides insight into the possible primary food sources on which a species may depend, whereas gut content analysis provides a definitive image of what a species is consuming, but over a much shorter period of time. Therefore, it is advantageous to use stable isotope analysis in conjunction with gut contents analysis to determine the dietary composition of an organism (Davis et al. 2012).

An understanding of the movement of organic matter through an estuarine food web, via the many links and pathways, is imperative for the management of such systems (Stephenson & Lyon 1982).

In summary, the main aims of this study were as follows:

1. To determine whether any evidence exists to suggest that *Oreochromis mossambicus* changes its diet as a result of environmental forcing, in particular the effect of different salinity levels.
2. To determine whether the dietary composition of *Ambassis ambassis* in the St Lucia Estuary varies as a result of different environmental conditions present at spatial and temporal scales.

CHAPTER 1

Post-flood dietary variation in the Mozambique Tilapia (*Oreochromis mossambicus*) in the St Lucia Estuary, South Africa

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ABSTRACT

Although originally endemic to southern Africa, the Mozambique Tilapia *Oreochromis mossambicus* is now among the most widely distributed exotic fish species worldwide. It has become the dominant fish species in the St Lucia estuarine lake (South Africa) since the closure of the mouth in 2002 and is therefore a crucial component of the food webs throughout the system. Following a decade-long drought phase, the estuary has received a large amount of freshwater inflow since 2011, resulting in a salinity decrease throughout the system. We compared dietary composition of *O. mossambicus* among three sites across a salinity gradient between the hypersaline and diluted stage to determine whether environmental conditions influence the diet of this species. Stable isotope analysis of carbon and nitrogen were used in conjunction with gut content analysis to elucidate dietary composition. A wide range of dietary sources was found during the hypersaline stage, with all sources contributing similar proportions to the diet. However, during the diluted stage that currently prevails in the system, specific dietary sources such as sediment organic matter were more dominant in the diet. Trophic position and salinity showed a significant negative relationship, indicating the adaptability of this species to salinity changes. A high degree of variability in the stomach contents of these fish was identified, with clear differences among sites and between seasons. This is an indication of the trophic plasticity which this species exhibits, which aids its ability to adapt to different environmental conditions and to dominate the fish community throughout the St Lucia estuarine system.

Keywords: *Oreochromis mossambicus*, iSimangaliso Wetland Park, stable isotopes, diet

1.1 INTRODUCTION

Oreochromis mossambicus (Peters 1852) is endemic to southern Africa, inhabiting coastal lakes, river systems and estuaries from the lower Zambezi River System in Mozambique to the Bushmans River in South Africa (Whitfield & Blaber 1979, Skelton 1993, Whitfield 1998). This species is considered one of the most widely distributed exotic fish species across the world, having successfully colonised 90 countries into which it has been introduced (De Silva *et al*. 2004, Canonico *et al*. 2005, Casal 2006). This expansion has threatened native fish populations globally (Canonico *et al*. 2005, Casal 2006). Understanding the dietary response of this species to changes in environmental conditions is thus of importance, not only to ecosystems within its native range, but to all systems where it has been introduced.

The St Lucia Estuary is the most extensive estuarine lake in Africa, forming a vital part of the iSimangaliso (formerly Greater St Lucia) Wetland Park, which is South Africa's first UNESCO World Heritage Site (Taylor 2006). Until recently, the system has experienced an exceptional dry-phase, which started in 2002 and ended with the onset of the latest La Niña event in 2011. The St Lucia estuarine system experiences a characteristic alternation of wet and dry phases, each persisting between four and ten years at a time (Begg 1978). The estuary mouth has also been closed naturally since June 2002, with a brief interruption in March-August 2007 caused by cyclonic activity over the ocean. Since 2002 the area has recorded below average rainfall and thus there has been limited freshwater input, with the main source of freshwater being the Mpate River which flows into the Narrows (see Figure 1.1). This lack of freshwater has resulted in much of the surface area of the lake exhibiting low water levels (<30 cm) or complete desiccation. All of these conditions have led to the formation of a persistent reverse salinity gradient within the system, with the upper reaches of the estuarine lake exhibiting much higher salinity levels (40 – 200) than its lower reaches and the mouth region (10 – 25) (Taylor 2006, Carrasco *et al.* 2012). Salinity is the most important factor affecting fish community structure within the estuarine environment (Blaber & Blaber 1980, Harrison & Whitfield 2006, Whitfield *et al*. 2006, Vivier *et al.* 2010). A period of high rainfall during December 2010/January 2011 resulted in high freshwater inflow into the system, raising water levels and diluting salinities throughout.

Since the closure of the mouth in 2002, there has been an approximate 40% loss in fish species richness throughout the estuarine system (Cyrus *et al.* 2010, Vivier *et al*. 2010), with *Oreochromis mossambicus* dominating the fish community (Vivier *et al.* 2010). Its

dominance throughout the system is considered indicative of the extent to which the fish community has been affected, as in the St Lucia system, this species was traditionally less dominant than other marine-associated species (Whitfield & Blaber 1978). The primary reason for this dominance is that under hypersaline conditions *O. mossambicus* is able to out-compete estuarine species as well as marine species that use the estuary as a nursery area (Whitfield & Blaber 1978). *Oreochromis mossambicus* is highly tolerant of a wide range of environmental conditions, surviving in fresh-, brackish and marine waters and is capable of breeding in salinity levels up to 120 (Cyrus & Vivier 2006, Whitfield *et al.* 2006). This highly adaptable species can tolerate temperatures below 15°C to above 40°C (Allanson *et al*. 1971, Whitfield & Blaber 1979, Behrends *et al*. 1990, Skelton 1993, Whitfield 1998, Ellender *et al*. 2008, Doupe *et al*. 2010). In St Lucia, this species has been documented to be extremely euryhaline, surviving and breeding successfully in salinities of 70 to 120 in False Bay and parts of the North Lake (Cyrus &Vivier 2006, Whitfield *et al.* 2006). Skelton (1993) stated that *O. mossambicus* is predominantly herbivorous, feeding on algae (especially diatoms) and detritus, with larger individuals occasionally feeding on insects and other invertebrates. This species is an opportunistic forager, feeding on a wide array of dietary components (Bruton & Boltt 1975, Whitfield & Blaber 1979). Epiphytic pennate diatoms are the main dietary food source for this fish, but the availability of this food source is unknown under the hypersaline conditions experienced in recent years in St Lucia (Vivier *et al.* 2010). *Oreochromis mossambicus* also forms a vital food source for many key species, such as the Great White Pelican, crocodiles and piscivorous fish and bird species, thus forming an important link in the food webs of this ecosystem (Whitfield 1998, Cyrus & Vivier 2006, Bowker & Downs 2008).

Understanding the trophic links and pathways of organic matter in estuarine food webs has critical implications for management of such systems (Stephenson & Lyon 1982). Therefore, information regarding the dietary composition of an important link in estuarine food webs, such as *Oreochromis mossambicus*, across a salinity gradient, is vital. Stable isotope analysis has proven to be an essential companion to gut content analysis, as it provides a better temporal indication of the diet of an organism, rather than a "snapshot" or temporally biased view which would be obtained from gut content analysis alone (Hesslein *et al.* 1993, Van der Zanden *et al*. 1997). Pinnegar and Polunin (1999) have shown that not all dietary material will be well represented in gut contents, whereas isotope analysis will give a more accurate estimate of dietary composition over a longer period of time. This is especially true in fish

muscle where turnover times per day can be as slow as 0.1 to 0.2% (Hesslein *et al.* 1993). Since stable carbon isotopes fractionate very little between energy transfers, they can be confidently used to quantify food sources in aquatic systems (Gannes *et al.* 1998). Stable nitrogen isotopes are known to fractionate more and are thus used to determine trophic positions of consumers in food webs (DeNiro & Epstein 1978).

The area around the St Lucia estuarine system is likely to become warmer and wetter as we approach the year 2100, as a result of climate change (Vaeret & Sokolic 2008). Understanding how the diet of a dominant fish species within the system may change based on changes in the environmental conditions can be vital in predicting how the system functioning could respond to these natural changes in the future. We therefore aimed to determine whether any evidence exists to suggest that *O. mossambicus* changes its diet as a result of environmental forcing, in particular the effect of different salinity levels. To achieve this, a temporal (seasonal) and spatial comparison was made between the diet of this species during hypersaline conditions and diluted (less saline) conditions.

1.2 MATERIALS & METHODS

1.2.1 Habitat characteristics

A YSI 6029 multi-probe system with 650 MDS data logger was used to measure *in situ* physico-chemical variables such as salinity, temperature, dissolved oxygen, pH and turbidity. Measurements were made at the sediment-water interface at all sites. Seasonal variation in the northern region of KwaZulu-Natal is characterized by wet periods when rainfall is regular, and dry periods when rainfall is almost absent. Therefore, sampling was carried out in February 2011 (summer, wet season) and again in May 2011 (winter, dry season), in order to assess the effects of this seasonal variation. Data from this study were then compared with those obtained from an earlier study in 2008/2009 by Carrasco *et al.* (2012), under prevailing hypersaline conditions. Three representative sites were chosen along the western shore of the lake system, each exhibiting varying susceptibility to environmental change: the mouth, which remains fairly stable in terms of salinity change; Charter's Creek, which is subject to large changes in salinity; and Lister's Point, which exhibits extreme changes in salinity (Figure 1.1).

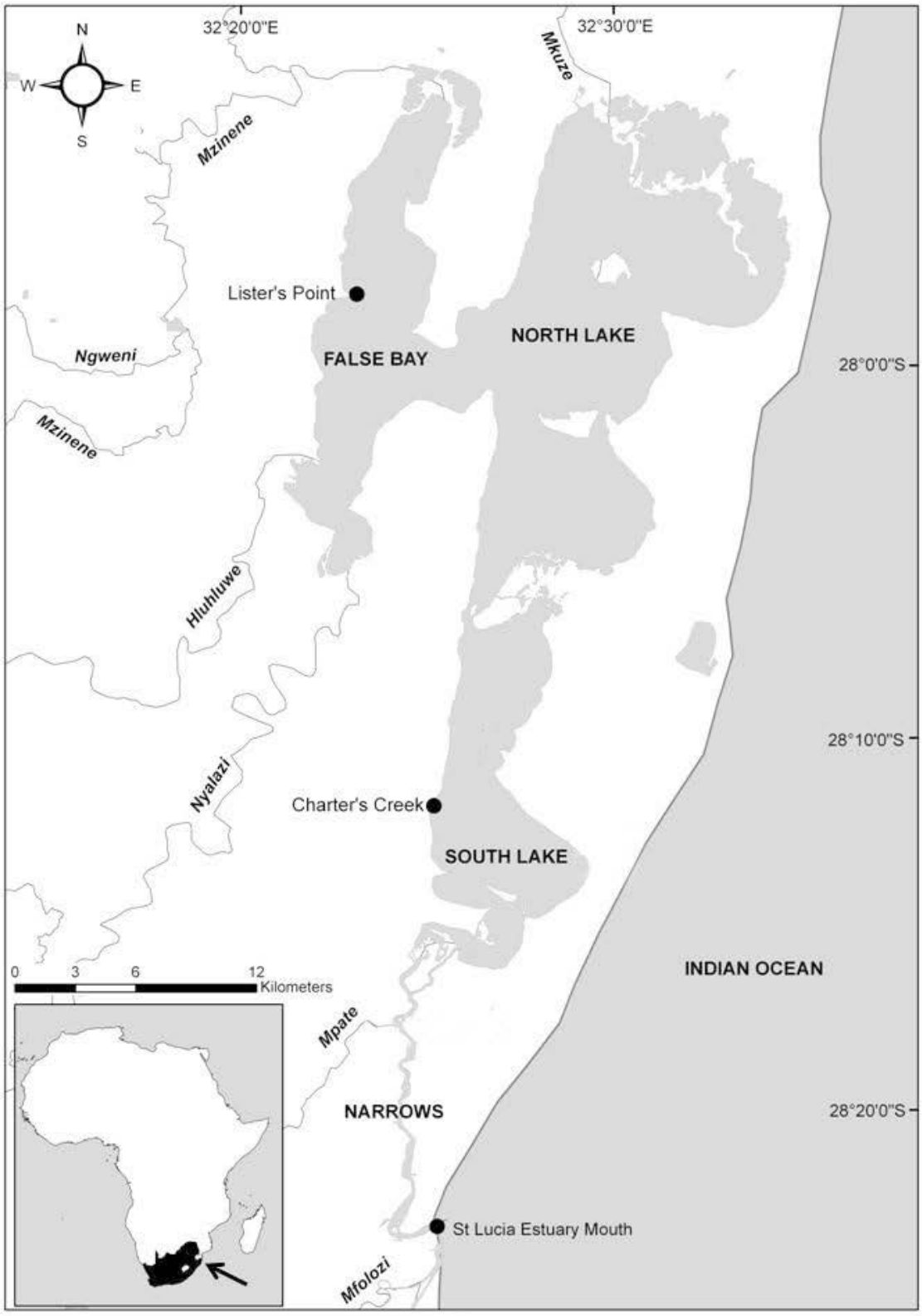

Figure 1.1: Map of the St Lucia estuarine system indicating the three sampling stations chosen for this study (•) and geographic position within South Africa.

1.2.2 Field sampling and laboratory processing

Isotope material was collected from *Oreochromis mossambicus* as well as from any of its potential food sources which were present at the study sites at the time of sampling. Samples of sediment organic matter (SOM), detritus, microphytobenthos (MPB), particulate organic matter (POM), zooplankton, benthic macrofauna and dominant macroalgae (such as *Cladophora* sp.), as well as fringing vegetation were collected at the three study sites, when present.

Fish samples were collected using a cast net with a diameter of 2.44 m, operated from the shore. Hand nets were also used to collect individuals which were able to evade the cast net. A total of 52 fish were collected from the three study sites during the wet and dry seasons, ranging in size from 30 mm to 210 mm. These were subsequently frozen (-20°C) prior to laboratory processing. Total length (in mm) for each fish collected was measured and recorded. Dorsal muscle samples were extracted from each individual, lipid treated (Bligh & Dyer 1959) and dried at 60°C for 24 hours. Using dorsal or other white muscle tissue for stable isotope analysis limits the variability in $\delta^{15}N$ values which can be associated with other tissue types (Pinnegar and Polunin 1999). Due to the fractionation of the St Lucia estuarine system and the territorial nature of this fish species, it was assumed that the fish sampled at the various sites were resident in these areas of the lake and that there was restricted movement between sites (Turner 1986, Oliveira & Almada 1998).

Zooplankton samples were collected with a hand-towed epibenthic sled (200 µm mesh, 37 cm radius). Samples were concentrated onto 20 µm mesh filters, placed into Petri dishes and frozen (-20°C) prior to laboratory processing. In the laboratory, each sample was sorted into the dominant taxa which were present at the time of collection. Depending on the taxa, 20 – 200 whole individuals were used per replicate. Dominant taxa included the copepods *Pseudodiaptomus stuhlmanni*, *Acartiella natalensis* and *Oithona* sp. as well as the mysid *Mesopodopsis africana*. Samples were then defatted for two hours in a solution of methanol, chloroform and distilled water in the ratio 2:1:0.8 respectively (Bligh & Dyer 1959). Thereafter samples were treated with 2% HCl to remove any carbonates (Lorrain *et al.* 2003, Carabel *et al.* 2006, Soreide *et al.* 2006) and then rinsed in excess distilled water before being dried at 60°C for 24 hours in an air-circulated oven.

Benthic macrofauna samples were collected using a Zabalocki-type Ekman grab. Three replicate samples were collected at each site, with each sample comprising three grabs.

Replicate grabs were collected 2 to 3 m apart. Samples were emptied into buckets to which water was added and stirred vigorously, thereby suspending benthic invertebrates. The supernatant was washed through a 500 µm sieve. After repeating this process five times, any material retained on the sieve was emptied into a plastic jar, while the remaining sediment was washed through a 2000 µm sieve, in order to collect larger and heavier organisms, such as bivalves, gastropods or crustaceans. Samples were subsequently frozen (-20°C) to preserve them for laboratory processing. In the laboratory, the organisms were sorted into their dominant taxa. These were nereid polychaetes, the bivalves *Solen cylindraceus*, *Macoma littoralis* and *Brachidontes virgiliae* as well as the gastropod snail *Assiminea* cf. *capensis* and the crab *Varuna litterata*. Single tissue samples were excised from larger bivalves and gastropods, as well as crab specimens. For replication, samples were collected from three different individuals. These samples were treated with excess 2% HCl for 24 hours, in order to remove any shell fragments. Smaller gastropods and polychaetes were used whole. These were also treated with excess 2% HCl for 24 hours to dissolve the shells of the gastropods and to ensure there was no carbonate material clinging to the polychaete appendages. Triplicate samples of these organisms were prepared where possible, using whole animals to achieve the desired weight of the sample for processing.

Detritus material, from the foam layer near the water edge, was collected at each of the study sites when present and kept frozen at -20°C until laboratory processing. After treating the samples with excess 2% HCl to remove possible biogenic carbonates, the samples were rinsed with distilled water and dried at 60°C for 24 hours.

Samples of the dominant macroalgae, such as *Cladophora* sp. and *Ulva* sp., macrophytes as well as any fringing vegetation were collected, where available. After thoroughly rinsing the samples with distilled water they were dried at 60°C for 24 hours.

Particulate organic matter (POM) was sampled by collecting triplicate water samples from each study site. These were then filtered onto pre-combusted (450°C for 6 hours) glass fibre filters (GF/F) using a vacuum filtration manifold. Once in the laboratory, filters were treated with excess 2% HCl to remove any inorganic carbon and placed into an air circulated oven at 60°C to dry for a period of 24 hours.

Triplicate sediment organic matter (SOM) samples were collected from each of the three study sites by removing the surface centimetre of sediment from a 20 mm diameter core and freezing it at -20°C prior to laboratory processing. Cores were collected at distances of 10 to

50 cm apart. Once in the laboratory, each sediment sample was treated with excess 2% HCl for a minimum of 24 hours, in order to remove any carbonates. Samples were thoroughly rinsed with distilled water and later dried at 60°C for 24 hours in an air-circulated oven.

Microphytobenthos (MPB) samples were collected in triplicate from each site by scraping the upper centimetre of sediment in areas of dense algal coverage, using the same coring method as for SOM collection. The samples were re-suspended in filtered estuarine water, stirring them in order to keep the MPB in suspension while the heavier sediment sunk. The supernatant was then filtered onto a pre-combusted (450°C for 6 hours) glass fibre filter (GF/F) and frozen (-20°C) prior to laboratory analysis. In areas of fine sediment where the coring method was not successful, a specialised method similar to that described by Couch (1989) was used to extract MPB. In the laboratory, the filters were treated with excess 2% HCl to remove any inorganic carbon in the form of calcium carbonate ($CaCO_3$). Filters were then dried again at 60°C for 24 hours and frozen (-20°C) for storage.

1.2.3 Stable isotope analysis

Once dry, sediment samples were milled and placed into Eppendorf™ microcentrifuge tubes, while filters were placed into tin foil envelopes. Animal, plant and algal tissues were ground into a homogenous mixture using a sterilized mortar and pestle, after which they were weighed into 5 x 8 mm tin capsules. For animal tissues, 0.6 mg of the homogenised tissue was weighed and packaged for isotope analysis. Plant and algal tissues required a larger sample to attain a signature and thus 2.0 mg of homogenised tissue was used. All samples were then sent to the Stable Light Isotope Unit, Department of Archaeology, University of Cape Town, South Africa, where they were analyzed using a Thermo Finnigan Flash EA 1112 series elemental analyzer equipped with a Thermo Finnigan Delta Plus XP isotope ratio mass spectrometer. Ratios were expressed as the parts per thousand deviation from the standard (atmospheric nitrogen for nitrogen and Vienna Pee Dee Belemnite for carbon) in delta (δ) notation according to:

$$\Delta X = [(R_{Sample}/R_{Standard}) - 1] \times 1000$$

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

Based on the valine internal standard, a precision of 0.03‰ and 0.05‰ was attained with this method for carbon and nitrogen, respectively.

1.2.4 Dietary composition and trophic positioning

Gut content analysis was conducted on each of the tilapia specimens collected to gain a better perspective of the short-term dietary composition for the species. Specimens were gutted, and the entire gut was preserved in 10% phloxin-stained formalin solution prior to laboratory analysis. Each gut was analysed under a dissecting microscope and the contents were identified as far as possible. Visible material was classified into the most likely dietary sources. Results should be interpreted with caution as using the entire gut may bias towards material which is not easily digested. Two methods were employed to quantify the contents following Hyslop (1980). A numerical abundance method was used for all dietary items which could be counted (zooplankton and other invertebrates); while an occurrence method was used to assess the proportion of fish which consumed specific dietary items. Where dietary items could not be counted, such as detritus and macroalgae, the proportion these contributed to the total gut content was estimated on a counting grid and expressed as a percentage. In order to combine the results from the two gut content analysis methods, the proportion of countable constituents was calculated from a corrected maximum from which the major uncountable dietary items had been removed. Thus, if uncountable material accounted for 90 % of the gut contents, the results of the numerical abundance method would be calculated out of a maximum of 10 %.

Trophic position was calculated using the following formula outlined by Post (2002):

$$\text{Trophic Position} = \lambda + (\delta^{15}N_{consumer} - \delta^{15}N_{base})/\Delta_n$$

where $\delta^{15}N_{consumer}$ is the nitrogen isotope signature of the fish and $\delta^{15}N_{base}$ is that of the food base chosen based on its relative position to the consumer, λ refers to the trophic level of the base ($\lambda = 1$ for primary producers) and Δ_n is the average trophic enrichment nitrogen (3.4‰). This provided an estimate of how far the consumer is from the trophic base, which was selected separately for each site and season.

1.2.5 Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 19 for Windows. Stable carbon and nitrogen isotope ratios of all possible food sources were analysed using a Two-way analysis of variance (ANOVA), in order to determine if there were differences between the sources in the wet and dry seasons and among the study sites. Tukey's HSD *post-hoc* comparisons were performed to identify specific differences between sources. Data

were checked for normality and the residuals of the ANOVA were checked for homoscedasticity to ensure that the assumptions of the test were met. Although the assumption of homoscedasticity was not met for any of the data, Zar (1996) states that ANOVA is robust enough to overcome this.

Following Parnell *et al*. (2010), Stable Isotope Analysis in R (SIAR) version 4.0 was used to create a mixing model, based on the standard corrected carbon and nitrogen ratios, in order to determine the likely contribution of each potential food item to the diet of *Oreochromis mossambicus* at the three study sites during the wet and dry season. A trophic enrichment factor (TEF), using values of 3.4‰ for $\delta^{15}N$ and 1‰ for $\delta^{13}C$ (Smit 2001, Carrasco *et al*. 2012), was incorporated in the model to account for fractionation between trophic levels. A standard deviation of 1‰ was used for both carbon and nitrogen signatures, to remove any bias in the variability of trophic fractionation among the sources (Caut *et al*. 2009, Inger *et al.* 2010). All possible dietary items collected at the sampling sites during the different seasons were used to run the models. If samples were not collected this source was excluded from the model, as no signature was available.

The Primer (version 6) multivariate statistics package (Clarke & Gorley, 2006) was used to test for dietary differences among the three sampling sites based on the gut contents. An analysis of similarity (ANOSIM) was performed and a non-metric multidimensional scaling (NMDS) plot of the data from all three sampling locations, taking both the wet and dry season into account was constructed. The ANOSIM calculates a Global R value using randomization to determine the average of the ranked dissimilarities within and among groups. An R value of 1 indicates the greatest dissimilarity possible and a value of 0 indicates no dissimilarity. A one-way ANOSIM was used to test for differences among the different sampling sites. The data was log transformed in order to minimise the effect of dietary items which were dominant, thus accounting for total dietary composition. A Bray-Curtis similarity coefficient was used to calculate the similarity of the data among sites. This similarity matrix was then used to create the NMDS plots, using 100 iterations in order to generate the most likely outcome. A 2-dimensional plot was generated from the output of the analysis. In order to gain the most accurate image of the groupings, obvious outliers were removed.

A linear regression was performed on the $\delta^{15}N$ and $\delta^{13}C$ signatures of *O. mossambicus* against the measured lengths of the fish sampled, in order to determine whether there was a

relationship between fish size and the relative isotope ratios. The assumption of normality of data was checked prior to running the test and it was satisfied.

A linear regression was also performed on the calculated trophic level of *O. mossambicus* and the physico-chemical parameters which were measured at the sampling sites, in order to test whether these affect the trophic level of the fish at the sampling sites. The assumptions of this test were met.

1.3 RESULTS

1.3.1 Physico-chemical characteristics of study sites

From the physico-chemical data (Figure 1.2) it is evident that the condition of the system has changed between the hypersaline stage in 2009 and the diluted stage in 2011. The salinity at the mouth remained stable over both periods, with values remaining in a narrow range around 10 to 15. During the hypersaline period, the salinity at Charter's Creek was 53.6 in the dry season (Carrasco *et al.* 2012), compared to 16.4 for the dry season during the diluted period. Salinities of 44.7 and 57.2 for the wet and dry season respectively were recorded at Lister's Point during the diluted period. During the hypersaline period, salinities of 141 and 85.5 were recorded for the wet and dry season respectively, which gives an indication of the extreme nature of this area.

During the diluted stage, temperature ranged from 22.1°C at Lister's Point in the wet season to 31.3°C at during the dry season. Turbidity was highest at Charter's Creek in the dry season (106 NTU) and lowest at the mouth during the wet season (7.8 NTU). Depth ranged from 0.15 m at Charter's Creek in the dry season to 0.46 m at the Mouth in the wet season. These conditions were markedly different from those recorded by Carrasco *et al.* (2012) for the hypersaline stage (Figure 1.2).

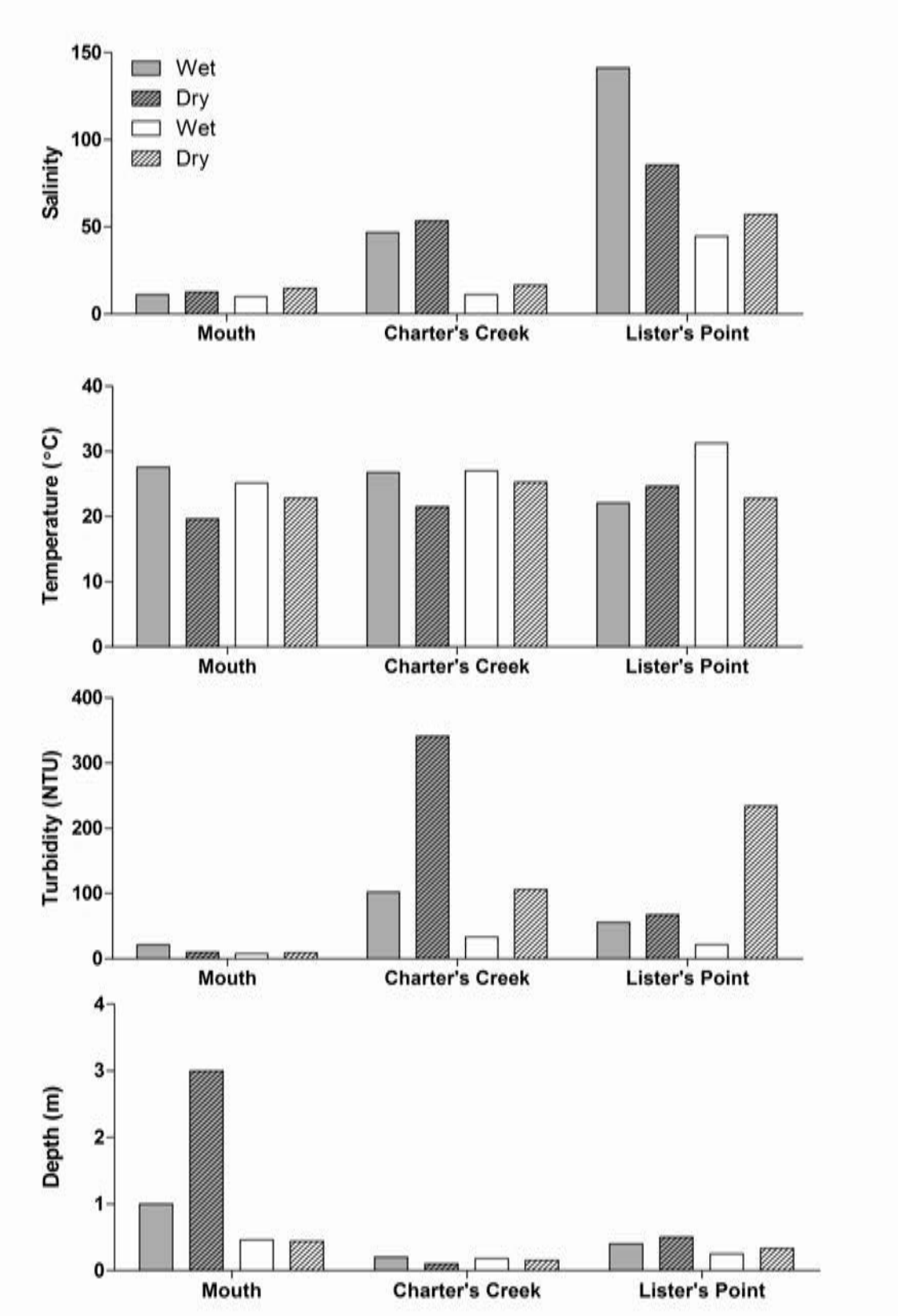

Figure 1.2: Physico-chemical parameters for the wet and dry seasons at the three study sites (Mouth, Charter's Creek and Lister's Point), during the 2011 diluted phase (white and white-slashed bars) and the previous (2008/9) hypersaline stage (grey and grey-slashed bars) (data for hypersaline stage from Carrasco *et al.* 2012).

1.3.2 Temporal and spatial variation in stable isotope signatures of dietary sources

Mouth

We found a significant difference in the $\delta^{13}C$ signatures of the source items between the wet and dry season ($F = 12.7$, $p < 0.05$). However, no significant difference was evident in the $\delta^{15}N$ signatures between the seasons ($F = 2.20$, $p > 0.05$). The different sources were significantly different from each other in both their $\delta^{15}N$ ($F = 10.3$, $p < 0.05$) and $\delta^{13}C$ signatures ($F = 19.3$, $p < 0.05$). The $\delta^{13}C$ signatures of the sources showed a wide range, with values ranging between -32.8‰ for POM and -15.4‰ for detritus. The $\delta^{15}N$ signatures were also found to vary greatly, with values ranging between 2.3‰ for detritus and 12.8‰ for *Mesopodopsis africana* (Figure 1.3, Table 1.1).

Charter's Creek

The results of the two-way ANOVA indicated no significant difference in $\delta^{15}N$ ($F = 0.1$, $p = 0.749$) and $\delta^{13}C$ ($F = 2.51$, $p = 0.119$) signatures between the wet and dry seasons. However, most sources significantly differed in both their $\delta^{15}N$ ($F = 23.5$, $p < 0.05$) and $\delta^{13}C$ ($F = 32.5$, $p < 0.05$) signatures. Where sources were found to be statistically similar ($p > 0.05$), they were combined to give a group signature. This was the case for the bivalves, which incorporated *Solen cylindraceus* and *Brachidontes virgiliae,* and the macroalgae which includes *Cladophora* sp. and *Ulva* sp. The $\delta^{15}N$ signatures ranged from 1.6‰ for MPB to 12.6‰ for the nereid polychaetes (Figure 3, Table 1). The $\delta^{13}C$ signature values ranged from -25.6‰ for detritus to -11.9‰ for the macroalgae group (Figure 1.3, Table 1.1).

Lister's Point

There was a significant difference in the $\delta^{13}C$ ($F = 7.76$, $p < 0.05$) and $\delta^{15}N$ ($F = 14.3$, $p < 0.05$) values between the wet and dry seasons. Significant differences were also found between the different sources for both $\delta^{13}C$ ($F = 13.8$, $p < 0.05$) and $\delta^{15}N$ ($F = 53.7$, $p < 0.05$). The $\delta^{13}C$ of the sources was found to range between -22.5‰ for POM and -12.3‰ for the submerged vegetation (Figure 3, Table 1). The $\delta^{15}N$ signature values had a smaller range, between 2.3‰ for POM and 7.6‰ for the submerged grass (Figure 1.3, Table 1.1).

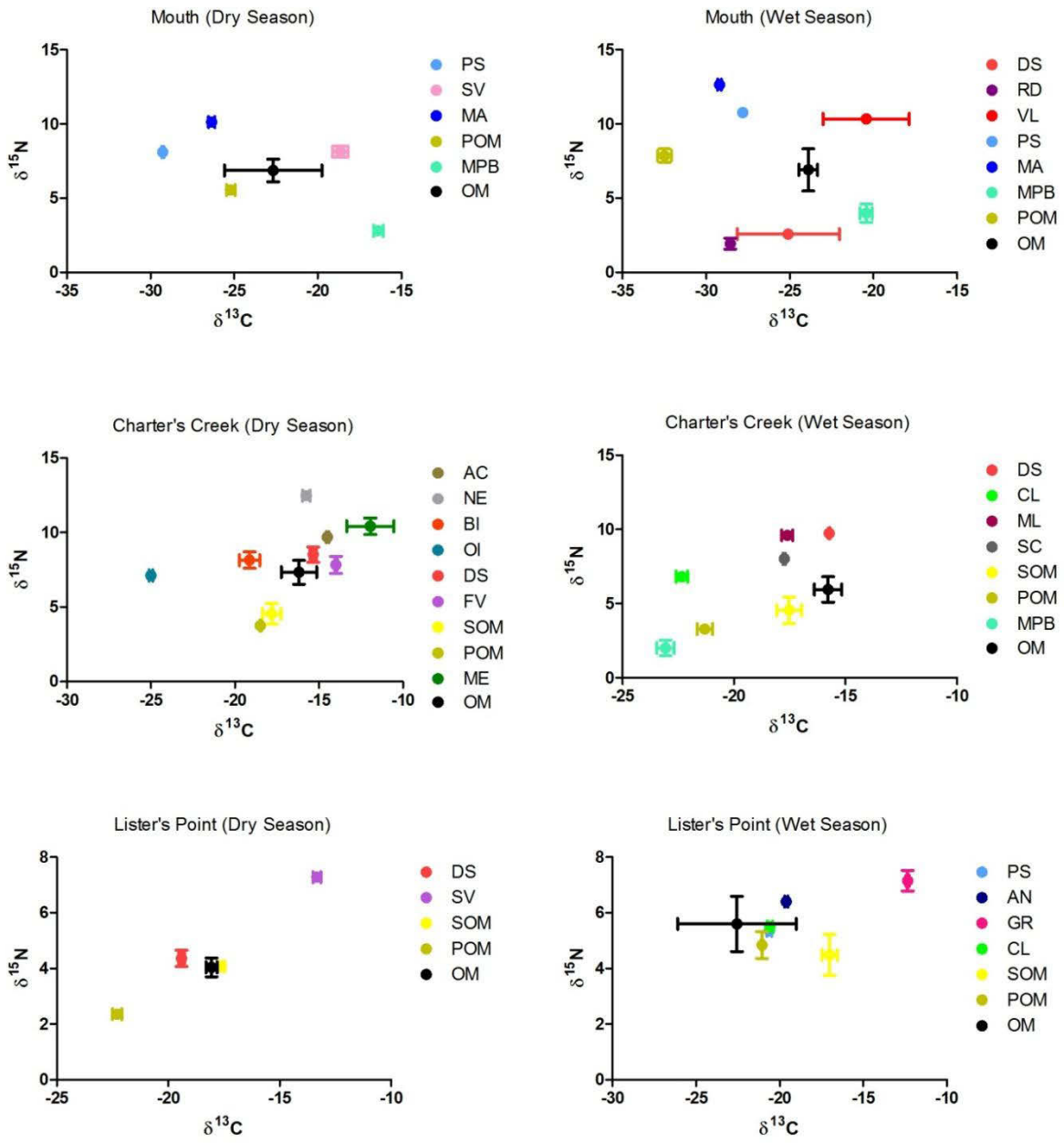

Figure 1.3: Mean (± SD) $\delta^{13}C$ and $\delta^{15}N$ signatures of *Oreochromis mossambicus* and the different food sources during the wet and dry seasons at the three sampling sites. The *O. mossambicus* signatures were corrected for trophic enrichment using standard fractionation values of 3.4‰ for $\delta^{15}N$ and 1‰ for $\delta^{13}C$, with error bars representing standard deviation for $\delta^{15}N$ and $\delta^{13}C$ signatures. Codes for dietary items: AN, *Acartiella natalensis*; AC, *Assiminea* cf. *capensis*; BI, Bivalves; CL, *Cladophora* sp.; DS, Detritus; FV, Fringing vegetation; GR, Grass; MA, *Mesopodopsis africana*; ME, Macroalgae; ML, *Macoma littoralis*; MPB, Microphytobenthos; NE, Nereid polychaetes; OI, *Oithona* sp.; OM, *Oreochromis mossambicus*; POM, Particulate organic matter; PS, *Pseudodiaptomus stuhlmanni*; RD, Reeds; SC, *Solen cylindraceus*; SOM, Sediment organic matter; SV, Submerged vegetation; VL, *Varuna litterata*.

Table 1.1: Mean stable isotope ratios of carbon and nitrogen of *Oreochromis mossambicus* and dietary sources for the three sampling sites in the St Lucia Estuary during both the wet and dry season. Codes for dietary items as in Figure 1.3.

		Dietary Items	AN	AC	BI	CL	DS	FV	GR	MA	ME	ML	MPB	NE	OI	OM	POM	PS	RD	SOM	SC	SV	VL
Mouth	Wet Season	δ13C ± SD	-	-	-	-	-25.1 ± 3.1	-	-	-29.2 ± 0.1	-	-	-20.4 ± 0.4	-	-	-23.9 ± 06	-32.5 ± 0.4	-27.8 ± 0	-28.6 ± 0.1	-	-	-	-20.4 ± 2.6
		δ15N ± SD	-	-	-	-	2.6 ± 0.3	-	-	12.6 ± 0.3	-	-	4.0 ± 0.6	-	-	6.9 ± 1.4	7.9 ± 0.5	10.8 ± 0	1.9 ± 0.4	-	-	-	10.3 ± 0.3
		C:N	-	-	-	-	26.2	-	-	3.7	-	-	6.9	-	-	3.2	5.7	5.2	11.9	-	-	-	3.4
		n	-	-	-	-	3	-	-	3	-	-	3	-	-	7	3	1	3	-	-	-	3
	Dry Season	δ13C ± SD	-	-	-	-	-	-	-	-25.2 ± 0.3	-	-	-13.5 ± 0.1	-	-	-22.7 ± 2.9	-16.4 ± 0.3	-29.26 ± 0.1	-	-	-	-18.64 ± 0.5	-
		δ15N ± SD	-	-	-	-	-	-	-	10.1 ± 0.0	-	-	8.6 ± 0.3	-	-	6.9 ± 0.8	2.8 ± 0.2	8.1 ± 0.0	-	-	-	8.1 ± 0.4	-
		C:N	-	-	-	-	-	-	-	4.5	-	-	7.3	-	-	3.1	5.3	5.4	-	-	-	14.2	-
		n	-	-	-	-	-	-	-	2	-	-	3	-	-	9	3	2	-	-	-	3	-
Charter's Creek	Wet Season	δ13C ± SD	-	-	-	-22.3 ± 0.3	-15.7 ± 0.0	-	-	-	-	-17.6 ± 0.3	-23.1 ± 0.4	-	-	-15.8 ± 0.6	-23.3 ± 0.3	-	-	-17.5 ± 0.6	-17.8 ± 0.1	-	-
		δ15N ± SD	-	-	-	6.8 ± 0.2	9.7 ± 0.3	-	-	-	-	9.6 ± 0.1	2.0 ± 0.5	-	-	6.0 ± 0.9	3.3 ± 0.3	-	-	4.6 ± 0.9	8.0 ± 0.1	-	-
		C:N	-	-	-	38.9	23.1	-	-	-	-	3.7	7.5	-	-	3.3	4.9	-	-	7.7	3.5	-	-
		n	-	-	-	6	3	-	-	-	-	3	3	-	-	12	2	-	-	3	3	-	-
	Dry Season	δ13C ± SD	-	-14.5 ± 0.1	-19.1 ± 0.6	-	-15.3 ± 0.2	-14.0 ± 0.1	-	-	-11.9 ± 1.4	-	-	-15.8 ± 0.2	-25.0 ± 0.1	-16.2 ± 1.1	-18.5 ± 0.0	-	-	-17.8 ± 0.5	-	-	-
		δ15N ± SD	-	9.7 ± 0.1	8.2 ± 0.5	-	8.5 ± 0.5	7.8 ± 0.6	-	-	10.4 ± 0.6	-	-	12.5 ± 0.2	7.1 ± 0.0	7.34 ± 0.8	3.8 ± 0.3	-	-	4.6 ± 0.7	-	-	-
		C:N	-	4.3	3.9	-	17.2	31.7	-	-	20.3	-	-	4.2	4.9	3.2	7.0	-	-	7.8	-	-	-
		n	-	3	6	-	3	3	-	-	9	-	-	3	2	8	3	-	-	3	-	-	-
Lister's Point	Wet Season	δ13C ± SD	-19.6 ± 0.1	-	-	-20.6 ± 0.2	-	-	-12.4 ± 0.1	-	-	-	-	-	-	-22.56 ± 3.5	-21.1 ± 0.0	-20.6 ± 0.1	-	-17.0 ± 0.5	-	-	-
		δ15N ± SD	6.4 ± 0.1	-	-	5.5 ± 0.0	-	-	7.2 ± 0.4	-	-	-	-	-	-	5.6 ± 1.0	4.8 ± 0.5	5.4 ± 0.1	-	4.5 ± 0.5	-	-	-
		C:N	4.7	-	-	9.7	-	-	11.7	-	-	-	-	-	-	3.2		5.7	-	8.0	-	-	-
		n	2	-	-	3	-	-	3	-	-	-	-	-	-	7	2	3	-	3	-	-	-
	Dry Season	δ13C ± SD	-	-	-	-	-19.4 ± 0.1	-	-	-	-	-	-	-	-	-18.1 ± 0.2	-22.3 ± 0.2	-	-	-17.6 ± 0.2	-	-13.3 ± 0.2	-
		δ15N ± SD	-	-	-	-	4.4 ± 0.3	-	-	-	-	-	-	-	-	4.0 ± 0.3	2.4 ± 0.2	-	-	4.1 ± 0.0	-	7.3 ± 0.2	-
		C:N	-	-	-	-	6.2	-	-	-	-	-	-	-	-	3.3	6.6	-	-	7.0	-	19.4	-
		n	-	-	-	-	3	-	-	-	-	-	-	-	-	9	3	-	-	3	-	3	-

1.3.3 Temporal and spatial variation in Oreochromis mossambicus signatures and trophic positioning

The C:N ratios of *Oreochromis mossambicus* were consistently low, with all values falling in the 3.0 to 3.4 range. We found a significant difference in $\delta^{13}C$ signatures of *O. mossambicus* among the three study sites ($F = 19.3$, $p < 0.05$), but the $\delta^{13}C$ signatures were not significantly different between seasons ($F = 1.65$, $p = 0.227$) or between the different sized individuals ($F = 1.62$, $p = 0.215$). The $\delta^{15}N$ signatures were also significantly different among the three study sites ($F = 18.6$, $p < 0.05$). Similar to $\delta^{13}C$, no significant difference was evident in the $\delta^{15}N$ signatures between the wet and dry season ($F = 0.578$, $p = 0.464$) or between the different sized individuals ($F = 0.966$, $p = 0.556$).

At Lister's Point, there was a significant negative relationship between the size of the fish sampled and both their $\delta^{13}C$ ($R^2 = 0.44$, $p = 0.003$, $n = 16$) and $\delta^{15}N$ signatures ($R^2 = 0.49$, $p = 0.002$, $n = 16$). At Charter's Creek, we noted a significant positive relationship between fish size and $\delta^{13}C$ signatures ($R^2 = 0.198$, $p = 0.049$, $n = 20$), and a significant, albeit very weak, relationship between the $\delta^{15}N$ signatures and the size of the fish sampled ($R^2 = 0.043$, $p = 0.043$, $n = 20$). No significant relationship between fish size and $\delta^{13}C$ ($R^2 = 0.06$, $p = 0.76$, $n = 16$) and $\delta^{15}N$ ($R^2 = 0.24$, $p = 0.26$, $n = 16$) signatures were found at the mouth. From a seasonal perspective, a significant negative relationship between both carbon ($R^2 = 0.54$, $p = 0.006$, $n = 12$) and nitrogen ($R^2 = 0.35$, $p = 0.041$, $n = 12$) signatures and the size of the fish collected at Charter's Creek during the wet season was identified. No significant relationships were identified at either of the other sites.

Trophic position was found to be significantly different among the three sampling sites ($F = 32.74$, $p < 0.05$), but not between the wet and dry season at each site ($p > 0.05$). Trophic position was significantly related to salinity ($R^2 = 0.56$, $p < 0.05$, $n = 52$) as well as turbidity ($R^2 = 0.29$, $p < 0.05$, $n = 52$) among the three sampling sites. Both relationships indicate an impact on the trophic positioning of *O. mossambicus*, with position decreasing with an increase in both salinity and turbidity. Together, salinity and turbidity account for most of the variability in trophic position among the sites. Temperature and depth were not found to be significantly related to trophic position.

1.3.4 Dietary contribution: stable isotopes

Mouth

At the mouth, all the dietary items contributed a similar proportion to the diet, to a maximum of 40% during the dry season (Figure 1.4). Microphytobenthos (MPB) showed a slightly higher overall proportion, contributing between 10 and 35% of the total diet during this season. A similar pattern was observed for the wet season, where two main items which appeared to play a greater role in the diet of *Oreochromis mossambicus*. *Varuna litterata*, a species of crab found here during the wet season, contributed between 5and 35% of the total diet. MPB was again important, contributing the highest proportion to the diet, up to 50%. All other dietary items contributed similar proportions of up to 25% of the total diet (Figure 1.4).

Charter's Creek

At Charter's Creek, all dietary items contributed similar proportions to the diet of *Oreochromis mossambicus* during the dry season, with most contributing around 20% of the diet (Figure 1.4). Nereid polychaetes and the copepod *Oithona* sp. were found to be of lesser importance, contributing less than 18%. Sediment organic matter (SOM) and particulate organic matter (POM) were the only items contributing higher proportions, up to 28% of the diet. The results from the wet season differ drastically. SOM was a dominant food source, contributing up to 62% of the diet. Detritus was also important, contributing up to 35% of the diet. All other food items contributed minor amounts (up to 20%) to the total diet (Figure 1.4).

Lister's Point

At Lister's Point, SOM and POM were identified as the dominant food items for tilapia during the dry season, potentially contributing up to 55 and 40% of the total diet, respectively (Figure 1.4). Detritus was found to contribute up to 37% of the total diet. Submerged vegetation was also found to be important, contributing up to 35% to the total diet. During the wet season, however, all dietary items contributed similar proportions to the diet (up to 30%), and no single food source appeared to play a dominant role in the diet. Grass and SOM were the two sources of least importance, contributing less than 30% to the diet (Figure 1.4).

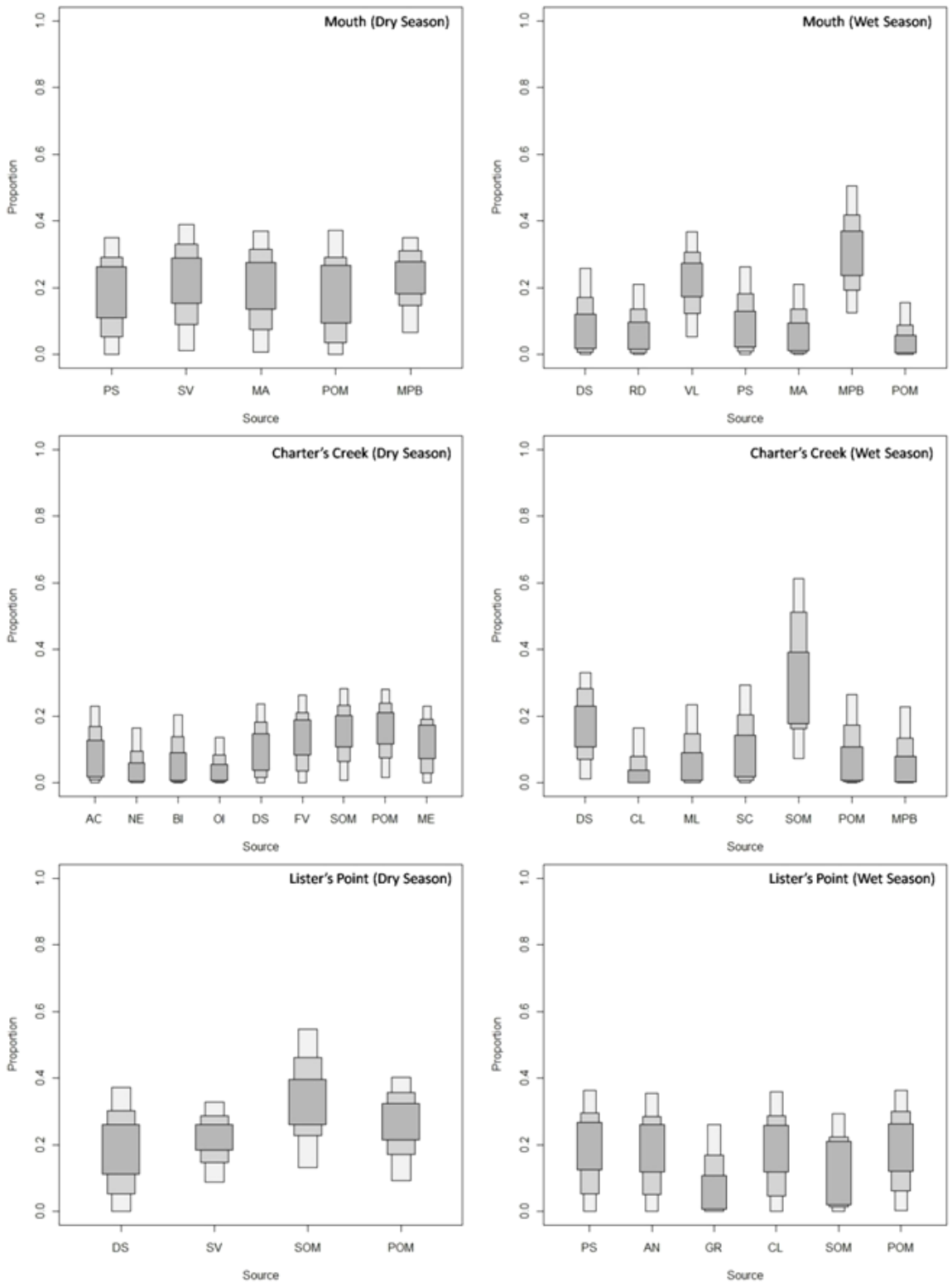

Figure 1.4: Stable Isotope Analysis in R (SIAR) mixing model box plots indicating the contribution of dietary items to the diet of *Oreochromis mossambicus* at the three sampling sites during the wet and dry seasons. 95, 75 and 25% credibility intervals are plotted for each item. Codes for dietary items as in Figure 1.3.

1.3.5 Dietary composition: gut contents

Mouth

At the mouth, a high degree of similarity was evident between the dietary composition of *Oreochromis mossambicus* during the wet and dry seasons. The most common sources found in the gut contents were detrital matter, contributing 77 – 80%, and sand grains, contributing 12 – 14% of the gut contents (Figure 1.5). Fish sampled during the dry season exhibited a higher proportion of sand material when compared to those sampled during the wet season. During the wet season, fish consumed macroalgae as this contributed 7% of the total gut contents. Shell fragments were found in the gut contents of fish sampled in the dry season but were absent in those collected during the wet season. Fish scales, ostracods, diatoms, copepods (*Acartiella natalensis*) and plant matter were found in the gut contents of fish sampled in both the wet and dry seasons (Table 1.2). The bivalve *Brachidontes virgiliae* was found in the stomach contents of fish collected in the dry season, but was absent during the wet season (Table 1.2). Macroalgae were detected in the gut contents of the largest individuals, but only during the dry season. Fish scales were only identified in the larger individuals collected in both the wet and dry seasons.

Charter's Creek

The greatest variation in gut content composition was found at Charter's Creek, with a large difference in the number of dietary components between the wet and dry season (Table 1.2). Detrital matter was found to be the most dominant food source during the wet season, whereas macroalgae were dominant in the dry season, contributing 42% of the gut contents (Figure 1.5). Fish scales, amphipods and sand grains were identified in conjunction with the plant matter from the fish sampled during the wet season. The dry season exhibited a markedly different result, with a greater degree of variety in the gut contents of these fish. Copepods (*Acartiella natalensis*), gastropod snails (*Assiminea* cf. *capensis*), ostracods, diatoms, fish bones & scales, sand grains and plant matter were identified from the gut contents of the fish sampled during this season (Table 1.2). During the dry season, 2 of the 9 fish collected contained only zooplankton in the gut contents. These were also the smallest individuals collected at the site. Fish scales were again only identified in the gut contents of the larger individuals. The bivalve *Solen cylindraceus* was only identified in the gut contents of fish collected in the dry season (Table 1.2).

Lister's Point

Lister's Point showed a similar trend to that observed at the mouth, with a high degree of similarity between the dietary composition of fish sampled in the wet and dry seasons. Here, the dominant food item found in the guts was detritus, which was found in high concentrations during the wet and dry seasons, contributing 67 and 83% of the total content, respectively. During the wet season, zooplankton such as the copepod *Acartiella natalensis* and cladocerans were found in all of the guts analysed. Ostracods were also identified from all the guts analysed (Table 1.2). Both of these items were completely absent from the gut contents during the dry season. Fish scales, copepods (*A. natalensis*), ostracods, detritus and diatoms were all identified from the gut contents of fish collected during the wet season, while foraminiferans, diatoms, detritus, sand grains and fish scales were identified from fish collected during the dry season (Table 1.2).

The results of the ANOSIM indicate a significant difference in the dietary composition among the different sampling locations ($R = 0.872$ $p = 0.001$). The non-metric multidimensional scaling procedure was considered to be adequately described in two dimensions with a stress value of 0.14 (Clarke & Warwick, 2001). From Figure 1.6, it is evident that the fish collected from the mouth separated from those collected at Charter's Creek, but were more similar to the individuals from Lister's Point. The specimens from Charter's Creek clustered separately from both the Lister's Point and mouth individuals based on the composition of their gut contents. We also noted some separation in clusters between the fish collected in the wet and dry seasons. This was particularly evident at Lister's Point and Charter's Creek, with the clusters for the wet and dry seasons exhibiting no overlap (Figure 1.6). At the mouth, there was some degree of overlap between the wet and dry seasons.

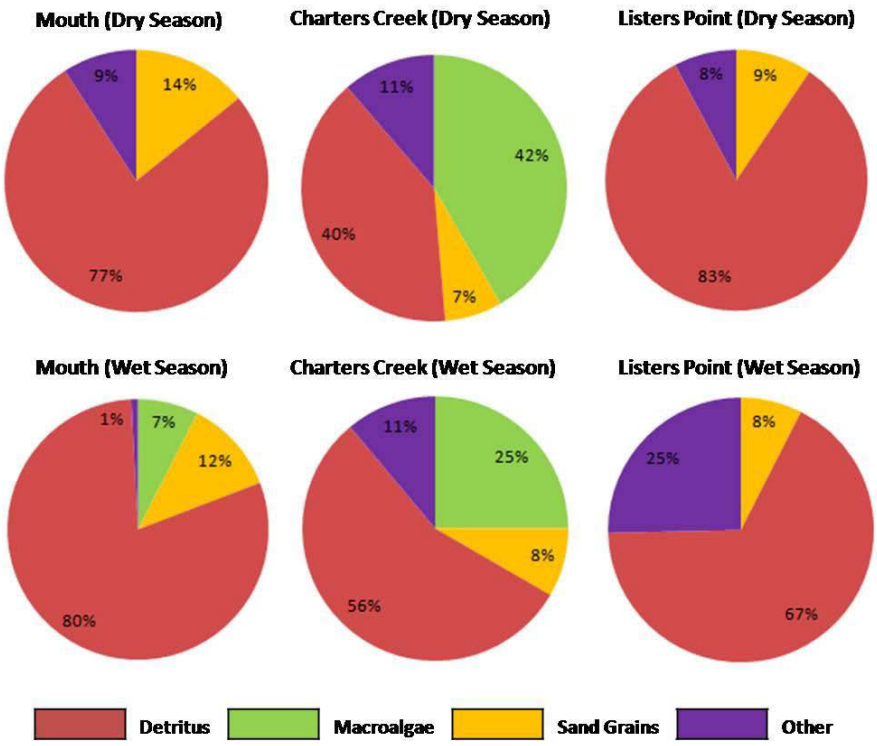

Figure 1.5: Major dietary items found in the gut contents of *Oreochromis mossambicus* collected at the different sampling sites in the St Lucia Estuary during the wet and dry seasons.

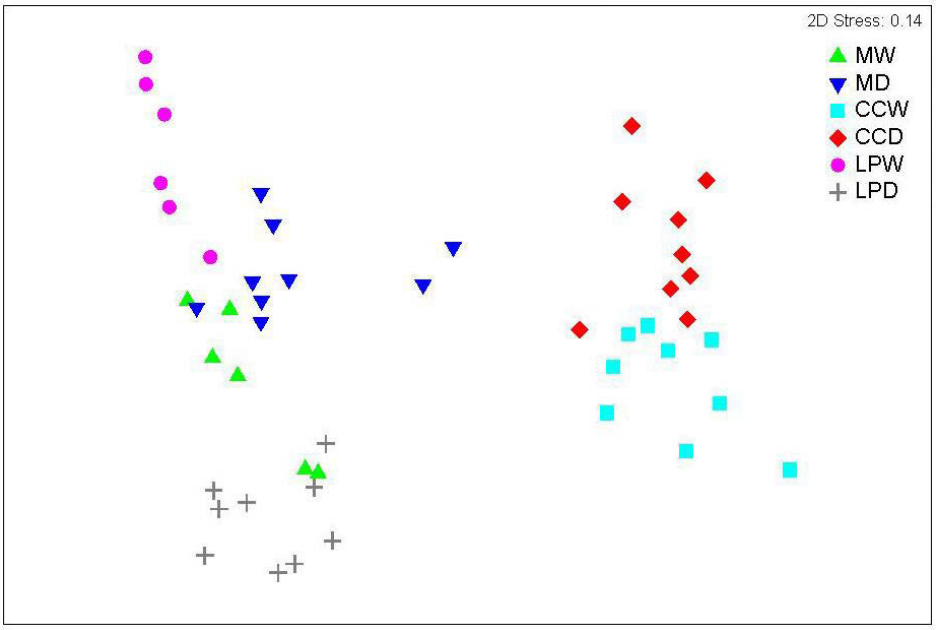

Figure 1.6: Non-metric multidimensional scaling (NMDS) plot of all the log transformed gut contents data of *Oreochromis mossambicus* from three sampling locations in the St Lucia Estuary (M: Mouth, CC: Charter's Creek, LP: Lister's Point) with (W) and (D) indicating the wet and dry seasons respectively.

Table 1.2: Occurrence frequency and Mean Number of individuals found in the gut contents of *Oreochromis mossambicus* at the different sites during the wet and dry season with all items falling into the "Other" category in Figure 1.5. Numbers in parenthesis indicate occurrence frequency (number of guts in which the item occurred).

	Mouth (Dry Season) n = 9	**Mouth (Wet Season)** n = 6	**Charters Creek (Dry Season)** n = 9	**Charters Creek (Wet Season)** n = 10	**Listers Point (Dry Season)** n = 9	**Listers Point (Wet Season)** n = 7
Dietary Item	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
A. cf. *capensis*	-	-	2.50 ± 2.14 (8)	5.50 ± 2.98 (8)	-	-
A. natalensis	-	-	1.00 ± 0.00 (1)	-	-	30.14 ± 21.95 (7)
Amphipoda	1.75 ± 0.96 (4)	1.00 ± 0.00 (2)	1.67 ± 0.58 (3)	9.00 ± 0.00 (1)	-	-
B. virgiliae	3.75 ± 1.83 (8)	-	2.63 ± 1.60 (8)	-	-	-
Cladocera	-	-	-	-	-	25.29 ± 23.26 (7)
Detritus	N/A (9)	N/A (6)	N/A (9)	N/A (10)	N/A (9)	N/A (7)
Diatoms	1.00 ± 0.00 (1)	1.00 ± 0.00 (1)	-	2.50 ± 0.71 (2)	1.80 ± 0.84 (5)	-
Sipuncula	2.75 ± 2.06 (4)	1.75 ± 0.96 (4)	1.00 ± 0.00 (2)	1.83 ± 0.75 (6)	4.80 ± 5.17 (5)	1.00 ± 0.00 (1)
Fish Egg	-	-	1.00 ± 0.00 (1)	1.50 ± 0.71 (2)	-	5.67 ± 2.52 (3)
Fish Scales	1.80 ± 0.84 (5)	2.50 ± 0.71 (2)	1.00 ± 0.00 (1)	1.33 ± 0.52 (6)	1.50 ± 0.58 (4)	3.80 ± 0.84 (5)
Foraminifera	-	-	-	-	2.00 ± 1.29 (7)	-
Insect Wing	-	-	-	-	-	1.00 ± 0.00 (1)
Macroalgae	N/A (2)	-	N/A (9)	N/A (9)	-	-
Nematoda	1.00 ± 0.00 (3)	2.00 ± 0.00 (1)	1.00 ± 0.00 (3)	2.50 ± 1.29 (4)	1.67 ± 1.15 (3)	1.00 ± 0.00 (1)
Ostracoda	9.56 ± 3.94 (9)	4.25 ± 2.75 (4)	-	1.00 ± 0.00 (2)	-	15.86 ± 15.06 (7)
P. stuhlmanni	-	-	-	-	-	-
S. cylindraceus	-	-	1.50 ± 1.00 (4)	-	-	-
Sand Grains	N/A (9)	N/A (6)	N/A (9)	N/A (10)	N/A (9)	N/A (7)
Shell Fragments	4.25 ± 1.67 (8)	1.00 ± 0.00 (1)	N/A (1)	-	-	-
Tanaid	-	-	-	11.00 ± 0.00 (1)	-	-

1.4 DISCUSSION

Previous studies conducted in the St Lucia system during the hypersaline period recorded salinities substantially higher than those recorded during the current study; this was particularly evident at Charter's Creek and at Lister's Point (Whitfield *et al.* 2006, Carrasco *et al*. 2012). These high salinities are indicative of the freshwater deprivation and persistent reverse salinity gradient which was present at that time. Although this reverse salinity gradient was still present in 2011, the heavy rainfall and consequent freshwater inflow during the wet season of 2010/2011 caused substantial dilution, with a marked decrease in the salinity at all the sampling stations (e.g. Charter's Creek was almost 75% less saline than during the study of Carrasco *et al.* 2012). At the Mouth however, there has been little effect in terms of salinity, with levels remaining between 9 and 15. At Lister's Point, salinity levels of over 140 were recorded during the hypersaline stage (Carrasco & Perissinotto 2012). Levels in this region in 2011 were within the range of 40 and 60, almost 60% less saline than the hypersaline stage (Figure 1.2). From these figures it is evident that the system in 2011 was much less saline than it has been for the past 8-9 years (Whitfield & Taylor 2009).

In terms of dietary composition, Carrasco *et al.* (2012) found that in the St Lucia system, *Oreochromis mossambicus* had a diverse diet, feeding on a variety of different food items. It was also documented that there was generally no dominant food source for this species, with all dietary constituents contributing similar proportions to its diet at both the mouth and Charter's Creek. In contrast, our study indicates that, under certain conditions, this species will target a specific food source, which will then constitute the majority of its diet. This was evident at Lister's Point during the dry season, where *O. mossambicus* consumed sedimentary organic matter (SOM) as its main food source, with this source constituting up to 55% of its total diet. At Charter's Creek during the wet season the diet was again dominated by two food sources, SOM (up to 62%) and detritus (up to 35%). The gut content analysis gave a different picture, with detritus being the dominant food source at all sites during both wet and dry seasons. Algal sources were also found to be dominant at Charter's Creek, but this was not reflected in the mixing models. At Lister's Point, both the gut contents and mixing models indicate that these fish have a more varied diet during the wet season, with a wider variety of sources than those recorded in the dry season. Inconsistencies between gut content analysis and mixing model results may occur because fish are feeding in different areas from where the sampling was carried out and thus not all food source signatures were obtained. Because the mixing models give a more long term average of the dietary intake, it could also

be possible that the results from these analyses represent relics from the weeks before sampling occurred, thus not reflecting the recent addition of some sources to the diet. This may have been the case at Charter's Creek, as plant/algal matter was the dominant food item from the gut contents of *O. mossambicus* during both seasons, which was however not reflected in the mixing models. This is probably due to the fact that *O. mossambicus* does not possess the cellulase enzyme which is required to break down cellulose (Dioundick & Stom 1990, Boschung & Mayden 2004). Gut contents did indicate a shift in food preference with size, with smaller individuals feeding on zooplankton and larger individuals consuming sources such as fish scales and higher proportions of detritus, which is concordant with reports by Whitfield (1998) and Skelton (1993). Considering both the gut contents and mixing model results, it is still evident that *O. mossambicus* changes its diet both seasonally and spatially. Diets did differ among the sampling sites as well as between seasons. These findings highlight the importance of combining isotope and gut content analyses to obtain an accurate picture of the diet of these fish.

Previous studies have reported that *O. mossambicus* preferentially feeds on plant/algal material and detritus (De Silva *et al.* 1984, Skelton 1993, Doupe *et al.* 2010). Our results contradict these findings to some extent, providing evidence that this fish species also feeds on benthic invertebrates and zooplankton, thus suggesting a more omnivorous diet. The occurrence of zooplankton in the gut contents of fish at Lister's Point during the wet season coincides with a bloom event which occurred here prior to sampling (*pers. obs*.). Zooplankton samples collected at the time of sampling indicate a concentration of around 600 000 ind.m^3, which is higher than average for this site (Carrasco *et al.* 2010). Both the gut contents and mixing models indicate a shift in diet to incorporate this abundant food source, which is indicative of the opportunistic nature of *O. mossambicus*. Different aged individuals of this species are known to feed on different food sources, with adults feeding preferentially on detritus and juveniles feeding more readily on benthic microfauna and zooplankton (Bruton & Boltt 1975, Whitfield & Blaber 1978). This was supported by the results of our study, which showed that smaller individuals at Charter's Creek and Lister's Point had zooplankton in their gut contents, while this was absent from the larger individuals. Pennate diatoms were thought to be the most important food sources during hypersaline conditions (Vivier *et al.* 2010), but this was not supported by results of our study either. The high concentrations of silt and sand grains found in the guts supports the observations of Piet and Guruge (1997), who showed that *O. mossambicus* is found in close proximity to the

substratum when actively feeding. The presence of fish scales in the gut contents of the larger specimens supports previous observations that this species feeds on other fish species, as well as its own young (Whitfield 1998). Doupe *et al*. (2009) provided field and laboratory evidence showing that *O. mossambicus* is capable of predatory behaviour, actively feeding on other native fish species, with larger individuals capturing more prey species. This could have great impacts for a system such as the St Lucia Estuary, which these fish inhabit, and could affect the already heavily impacted population structure (40% loss in diversity since mouth closure) of other native fish species.

The regression analysis provided an interesting outcome, particularly for Lister's Point, where a significant relationship between the size of the fish and the $\delta^{15}N$ signatures was obtained. Skelton (1993) indicated that juvenile tilapias are known to congregate in shoals in shallower water. This is further support for the likelihood that juveniles feed on different food sources, compared to their adult counterparts, as supported by other authors (e.g. Bruton & Boltt 1975, Whitfield & Blaber 1978). At Lister's Point, there is a very gentle gradient in the substrate as it enters the water, resulting in a broad region of very shallow water (up to 20 cm deep) along the banks. This provides a refuge for juvenile tilapia and could explain the relationship between fish size and $\delta^{15}N$ signatures. At Charter's Creek, there is also an extensive area of shallow water along the bank region and the relationship between fish size from this site and their $\delta^{13}C$ signatures indicates that these fish may be feeding in different areas. It is well-established that nitrogen signatures can be used to determine the relative trophic position of consumers (e.g. DeNiro & Epstein 1978). The regression analysis performed on trophic position of this species indicated that at higher salinities *O. mossambicus* feeds at a lower trophic level, when compared to lower salinity areas. This was evident as the mean trophic level at Lister's Point was found to be lower than at both Charter's Creek and the mouth. Salinity has been shown to affect trophic positioning within a food web (Post *et al.* 2000, Govender *et al.* 2011).

Balcombe *et al.* (2005) showed that fish species native to Australia tend to switch their diets depending on season, with fish sampled in dry and wet periods having significantly different diets due to changes in availability of food sources. Doupe *et al.* (2010) showed through experimental feeding of *O. mossambicus* that plant sources were not sufficient to sustain its metabolic requirements and that only fish fed a protein source were able to maintain body weights during the experiments. Thus, it was suggested that despite this fish being predominantly herbivorous, it requires some form of protein in its diet in order to survive

(Bowen 1979, Doupe *et al*. 2010). Our results suggest that alternative food sources, such as bivalves and zooplankton may be important in the diet of *O. mossambicus* in the St Lucia Estuary. Maddern *et al*. (2007) and Doupe *et al*. (2010) showed that fish exposed to different environmental conditions exhibit some degree of trophic plasticity, with *O. mossambicus* in different systems feeding on different dietary sources. Tilapias are in general able to adapt to changes in environmental conditions through changes in their life-history traits, as well as through facultative feeding (Maitipe & De Silva 1985, McKaye *et al.* 1995). Our study also shows that seasonal effects can cause a change in the diet of this fish; this was particularly evident at Lister's Point, as environmental conditions at this site can vary greatly between the wet and dry season.

Other invasive fish species, such as the Round Goby (*Neogobius melanostomus*), have been shown to exhibit similar properties with respect to their diet, in areas that they have invaded. Corkum *et al.* (2004) outline that this goby has a varied diet which consists of zooplankton, benthic invertebrates and molluscs. They also showed that this species undergoes an ontogenetic shift in diet, with adults becoming molluscivores. This is similar to *O. mossambicus,* which experiences a shift in diet from zooplankton and benthic invertebrates during juvenile stages to a diet dominated by detritus and macroalgae as adults (Whitfield 1998). Brush *et al*. (2012) also showed that the diet of this species of goby differed spatially, temporally as well as with body size. The reason for its invasion success can thus be attributed to its broad diet, as well as its ability to survive a broad range of environmental conditions (Corkum *et al.* 2004). Gido & Franssen (2007) showed that fish such as the blue tilapia (*Oreochromis aurens*) succeed as an invasive species in Central America due to their feeding preferences. These fish are omnivorous and have the ability to feed on lower-quality trophic sources, which are easily available in the habitats they have been introduced into, thus aiding in invasion success (Gido & Franssen 2007). In Australia, the ability and success of *O. mossambicus* to invade native river systems can in part be attributed to its dietary characteristics (Leveque 2002, Doupe & Burrows 2008). Leveque (2002) also suggests that the success of tilapia species in Africa and throughout the world can be attributed to their broad diet and their tolerance of a wide variety of environmental conditions. The invasion success of *O. mossambicus* also had detrimental impacts on the native fish and other fauna in numerous places including Australia, Southern U.S.A. and Madagascar (McKaye *et al*. 1995, Leveque 2002, Canonico *et al*. 2005, Gido & Franssen 2007, Maddern *et al*. 2007, Doupe & Burrows 2008). The dominance of this species in the St Lucia Estuary can thus be attributed

to the broad dietary preferences of these fish, which is vital in a system where food availability varies greatly.

One of the most pivotal requirements for future studies of this nature is the quantification of the different food sources in order to determine the relative abundances at the different sites during the wet and dry seasons. This will provide an estimate of the availability of these sources, which can also help to establish potential electivity indexes for the fish species at different periods. Forage ratio and Ivlev's electivity index are two methods which can be used to quantify food selection and have been modified to take into account food availability (Jacobs 1974).

In conclusion, we have shown that *Oreochromis mossambicus* in the St Lucia Estuary exhibits some degree of trophic plasticity, as it has the ability to alter its diet according to the prevailing conditions. During hypersaline conditions these fish fed on a variety of different sources, which included both plant/algal and animal material. However, there were no dominant sources under these conditions, with all dietary constituents contributing similar proportions. The current study revealed that there is some variability in this observation, with fish feeding predominantly on specific sources which dominate their diets during the less saline period. This variability was supported by both the mixing models and the gut content analysis. The main reason for this dominance is that it is likely that these food items are more abundant during the less saline conditions. The isotope analysis also indicated a variation in the trophic positioning of different sized individuals, where there are suitable habitats for juveniles to use as refuges (e.g. shallow water shore regions). The analysis of trophic positioning of *O. mossambicus* also revealed that salinity and turbidity are the driving factors responsible for the trophic position of this species in the system, either directly by influencing the fish themselves or indirectly by affecting their dietary sources. The hypothesis that there would be dietary variation between the hypersaline and diluted stages is therefore supported; as is the hypothesis that fish size can determine dietary composition. Overall, the success of this species and its subsequent dominance of the estuarine system can be attributed not only to its remarkable tolerance of salinity variations, but also in its ability to alter its diet accordingly.

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CHAPTER 2

Temporal and spatial dietary dynamics of the Longspine Glassy (*Ambassis ambassis*) in the St Lucia Estuary, South Africa

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ABSTRACT

Among the 155 species of fish recorded so far in the St Lucia estuarine lake, *Ambassis ambassis* is one of the most prominent. After a decade dominated by dry and hypersaline conditions, the St Lucia system has changed dramatically in terms of prevailing environmental conditions, as a result of higher than average rainfall at the end of 2011 and the onset of a new wet phase at the start of 2012. In response, *A. ambassis* has expanded its distribution range throughout the estuarine lake. Stable $\delta^{15}N$ and $\delta^{13}C$ isotope analysis was used in conjunction with gut content analysis to elucidate the diet of this species at five sampling localities spanning the geographical range of the system. Zooplankton species, such as *Pseudodiaptomus stuhlmanni*, *Mesopodopsis africana* and *Acartiella natalensis* were predominant in the diet of this fish. *Ambassis ambassis* is also thought to opportunistically supplement its diet with terrestrial and aquatic insects as these were prevalent in the gut contents. Results of a non-metric multidimensional scaling (NMDS) analysis revealed that there was considerable dietary overlap for the fish collected at the different sampling localities. Seasonally, trophic position was found to differ significantly, with the dry season having consistently higher isotopic signatures at all five sampling sites. A significant relationship was identified between trophic position and salinity and temperature, indicating the effect of these variables on the diet. *Ambassis ambassis*, therefore, occupies a vital role in the food webs of the St Lucia system, as it provides a critical link in the pathway of energy from lower to higher trophic levels.

Keywords: *Ambassis ambassis*, iSimangaliso Wetland Park, stable isotopes, gut content analysis, fish diet

2.1 INTRODUCTION

The St Lucia Estuary is the largest estuarine lake in Africa and has historically been considered the most important fish and prawn nursery along the southeast coast of Africa. The system is an integral part of the iSimangaliso (formerly Greater St Lucia) Wetland Park, which is South Africa's first UNESCO World Heritage Site and a RAMSAR Wetland of International Importance (Taylor 2006). With a recorded fish diversity of 155 species, it is one of the most important lakes in the world (Cyrus 2013). However, the system is exposed to drastic spatio-temporal changes in response to climatic shifts in the region. Characteristically, the system experiences alternating wet and dry phases, which can persist for 4 to 10 year periods (Begg 1978). Following a decade-long dry phase, the system has recently entered a wet phase which commenced at the beginning of 2012. Higher than average rainfall in the catchment of the system in late 2011 resulted in large amounts of freshwater entering the estuary, subsequently raising water levels substantially and diluting salinities throughout the system (Taylor et al. 2013). In addition, the Mfolozi River system has recently been linked to the estuary via an artificial canal, providing freshwater and inconsistent marine input into the system. Despite this, the St Lucia Estuary still remains largely closed off from the Indian Ocean (Taylor et al. 2013).

The family Ambassidae has historically been well represented in the St Lucia Estuary, with three different species being recorded since the first comprehensive fish checklist compiled by Whitfield in 1980. Three species, A*mbassis ambassis* (formerly *Ambassis productus)*, *A. natalensis* and *A. dussumieri* (formerly *A. gymnocephalus)* have subsequently been recorded in the St Lucia and Mfolozi River systems by various authors (Whitfield 1980, Vivier et al. 2010a, Vivier et al. 2010b, Cyrus et al. 2011). In the most recent survey conducted by Vivier et al. (2010a) only *A. ambassis* and *A. natalensis* were present in the system in 2008. Despite its occurrence during the survey, *A. natalensis* was only recorded in a limited area of the Narrows and in small numbers. Vivier et al. (2010a) indicated that these two estuarine species were still among the dominant fish in the St Lucia system, with *A. ambassis* accounting for 12.7% of the total catch during this survey and around 10% in the survey conducted by Cyrus and Vivier (2006). Thus, *A. ambassis* is the second most dominant fish species after the Mozambique Tilapia, *Oreochromis mossambicus*.

Ambassis ambassis is distributed along the east coast of South Africa extending northward to Kenya. This species is also found on the islands of Madagascar, Mauritius and Reunion (Skelton 1993, Whitfield 1998). *Ambassis ambassis* has a wide (17-32°C) temperature

tolerance range (Martin 1988), but relatively low (2-35) salinity tolerance (Skelton 1993, Whitfield 1998). Because of this low tolerance to salinity, the distribution of this species is often limited to areas of an estuary where the salinity is below 10 (Martin 1988, Martin 1989, Whitfield 1998). Previous studies have reported that the species feeds at night and early in the morning on planktonic crustaceans, fish fry and larvae, as well as insects (Martin & Blaber 1983, Skelton 1993, Whitfield 1998). *Ambassis ambassis* is the largest of the Ambassidae reaching sexual maturity between the length of 40 and 50 mm, probably owing to the rich composition of their diet, with a large proportion of its energy derived from fish fry and larvae, and crustaceans (Martin & Blaber 1983, Martin 1988, Whitfield 1998). This species has been classified as a resident species in estuaries and it has been known to breed under estuarine conditions in the St Lucia Estuary (Whitfield 1998, Cyrus 2013). Population densities of *A. ambassis* have historically been lower than the other Ambassidae species in St Lucia, as most of the system had a salinity exceeding 15 (Martin 1983). Under freshwater-dominated conditions within the St Lucia system, *A. ambassis* is among the dominant fish species, as it is able to tolerate oligohaline conditions (Cyrus 2013). Cyrus (1983) showed that in St Lucia, *A. ambassis* prefers turbid water (>50 NTU) during the day and lower turbidities (<10 NTU) at night. Apart from being a popular live bait used by recreational fishermen, *A. ambassis* is also a key prey species for many of the estuarine piscivorous bird and fish species found in the St Lucia system, forming an important link in the food web (Martin 1983). Therefore, information regarding the dietary composition of an important link in estuarine food webs, such as *Ambassis ambassis*, is vital in understanding the functioning of the system.

An understanding of the movement of organic matter through an estuarine food web, via the many links and pathways, is imperative for the management of such systems (Stephenson & Lyon 1982). Stable isotope ecology has become an indispensable and informative tool for predicting the flow of organic matter through food webs in a variety of habitats (Peterson & Fry 1987, Michner & Lajtha 1994, Cabana & Rasmussen 1996, O'Reily et al. 2002, Bouillion et al. 2011, Wada et al. 2013). The use of stable isotopes has proven to provide a better long term image of the diet of an organism, rather than the more temporally-biased view which would be provided by gut content analysis alone (Hesslein et al. 1993, Van der Zanden et al. 1997). As it considers assimilated material and not only ingested material, stable isotope analysis also gives a more accurate estimate of dietary composition over longer periods of time (Gearing 1991, Pinnegar & Polunin 1999). This is particularly relevant for fish muscle tissue, where turnover rates can be as slow as 0.1 to 0.2% per day (Hesslein et al. 1993).

Stable carbon isotopes have been shown to fractionate very little between energy transfers and thus they can be used to confidently quantify food sources of an individual in aquatic systems (Gannes et al. 1998, Van der Zanden & Rasmussen 2001). Trophic positioning of consumers within a food web can be determined using stable nitrogen isotopes, as these are known to have a higher rate of fractionation between energy transfers (DeNiro & Epstein 1978, Van der Zanden et al. 1997). It is however advantageous to use a combination of stable isotope analysis and gut content analysis to determine dietary composition.

The aim of this study was to determine the dietary dynamics of *A. ambassis* across spatial and temporal scales within the St Lucia Estuary. Understanding how this species responds to changes in environmental conditions, in terms of its diet, will provide information on the functioning of the food webs in the estuary, which is critical for conservation and management.

2.2 MATERIALS & METHODS

2.2.1 Habitat characteristics

Physico-chemical parameters such as temperature, salinity, pH, dissolved oxygen and turbidity were measured *in situ* with a YSI 6600V2-D multi-probe system coupled with a 650 MDS data logger. Measurements were taken at the sediment-water interface with the exception of the mouth and Esengeni where surface and bottom measurements were taken due to the water depth. The northern region of KwaZulu-Natal is characterised by two seasonal periods, wet periods with frequent rainfall and dry periods when rainfall is virtually absent. In order to account for the slow turnover rate of carbon and nitrogen within the tissue of the fish, sampling was carried out late in the respective seasons. Therefore, sampling was carried out in October/November 2012 (end of dry season) and February/March 2013 (end of wet season), in order to determine the effects of seasonal variation. Five representative sampling sites were chosen around the St Lucia estuarine system, each representing a different area and thus different environmental conditions within the system (Perissinotto et al. 2013a). Lister's Point in False Bay, Charter's Creek and Catalina Bay on the Western and Eastern Shores of South Lake respectively, Esengeni in the Narrows and the mouth itself were sampled during the wet and dry seasons (Figure 2.1).

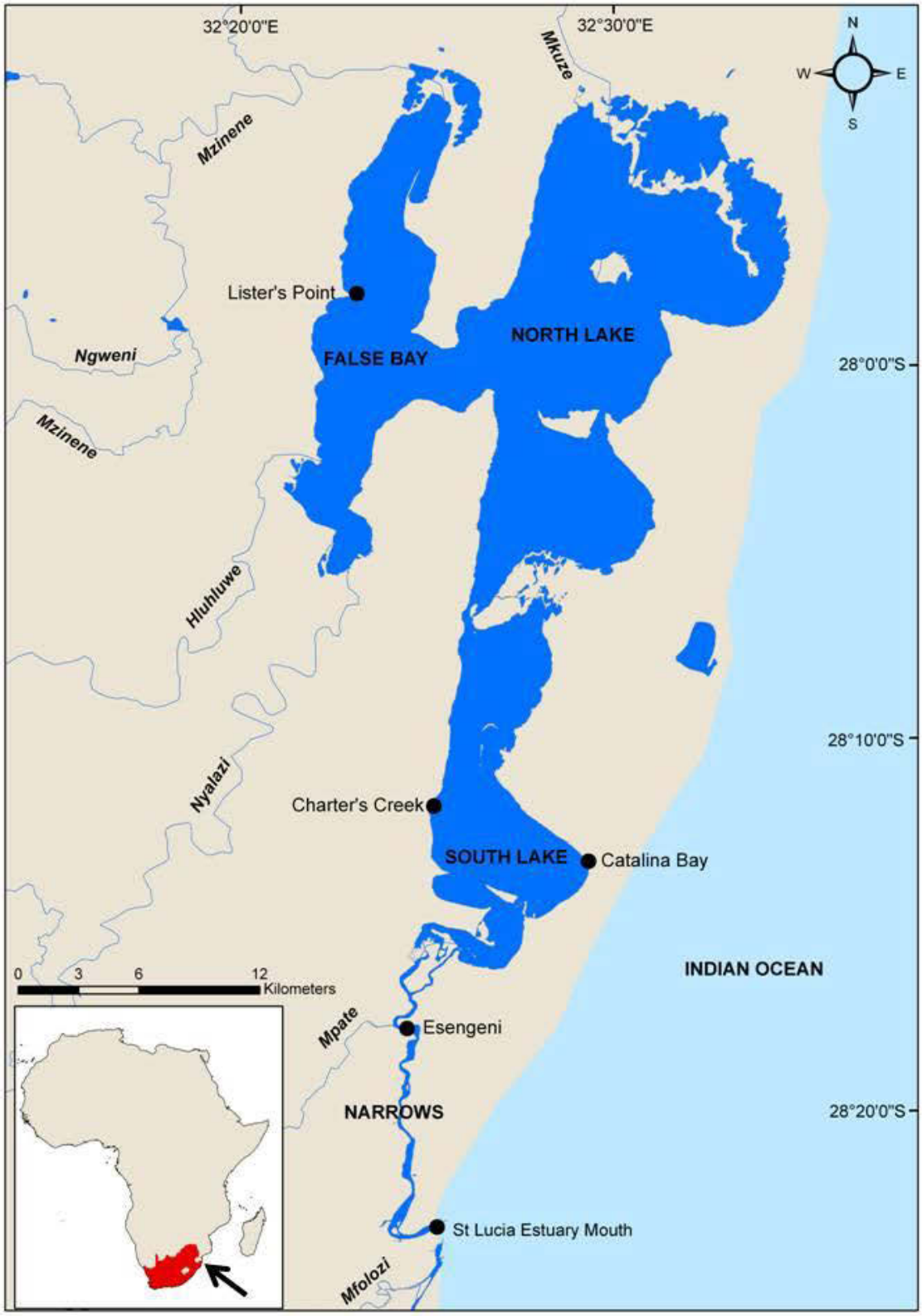

Figure 2.1: Map of the St Lucia Estuary showing the five sampling sites which were chosen for this study (•) and the geographic position of the system within South Africa.

2.2.2 Sample collection and preparation

Isotope material from ~20 *Ambassis ambassis* individuals per site were collected along with any potential food sources which were present at each of the sampling sites during the wet and dry season. Samples of detritus, sediment organic matter (SOM), particulate organic matter (POM), zooplankton, benthic macrofauna, dominant macroalgae (such as *Cladophora* sp.), macrophytes (such as *Phragmites* sp.), as well as fringing and recently submerged vegetation were collected at the five study sites, where available.

Fish were collected using a fine mesh cast net with a diameter of 2.44 m, operated from the shore or from a ski boat. Samples were also obtained from illegal gill nets which had been confiscated by the Ezemvelo KZN Wildlife authority. A total of 200 *Ambassis ambassis* specimens were collected at the five study sites during the wet and dry seasons between March 2012 and March 2013 and subsequently frozen (-20°C) prior to laboratory processing. Standard length (SL) was measured in mm for each fish collected. The fish collected ranged in size from 45 to 135 mm , which covers the size range from size at sexual maturity (40 – 50 mm) to near the maximum size recorded for this species in southern African waters (140 mm) (Whitfield 1998). For stable isotope analysis, 98 of the fish collected were used. Dorsal, white muscle tissue samples were excised from each individual and lipid treated in a solution of methanol, chloroform and distilled water in the ratio 2:1:0.8, respectively (Bligh & Dyer 1959). Thereafter, the samples were dried at 60°C for 24 hours in an air-circulated oven. White muscle tissue is used for stable isotope analysis to limit the variability in $\delta^{15}N$ values, which can arise when using other tissue types (Pinnegar & Polunin 1999). The fish used for isotope analysis ranged in size from 40 to 90 mm.

Zooplankton samples were collected either with an epibenthic sled (200 µm, 37 cm diameter) or with a hand net (55 µm, 26 cm diameter) both operated from the shore or from a ski boat where necessary. Samples were concentrated onto 20 µm mesh nylon filters, placed into Petri dishes and frozen (-20°C) prior to laboratory processing. Once thawed, each sample was sorted into the dominant taxa present at the time of sampling. Where possible, 20 to 200 whole individuals of the dominant taxa were used per replicate for the analysis. These taxa included the copepods *Pseudodiaptomus stuhlmanni*, *Acartiella natalensis*, the mysid *Mesopodopsis africana* and gastropod larvae. Samples were first lipid-treated for 2 hours (Bligh & Dyer 1959), then acid-treated to remove any carbonates (Lorrain et al. 2003, Carabel et al. 2006, Soreide et al. 2006) and finally rinsed in excess distilled water before being oven-dried at 60°C for 24 hours.

Samples of benthic macrofauna were collected using a Zabalocki-type Ekman grab. Three replicate samples were collected at each of the five study sites with each sample comprising three grabs. Each replicate grab was taken 2 to 3 m apart. Grabs were emptied into 20 L buckets; water was added and stirred vigorously, thereby suspending any benthic invertebrates in the samples. The supernatant was poured through a 500 µm sieve. This process was repeated five times and any material retained on the sieve was emptied into a plastic jar, while the remaining sediment was washed through a 2000 µm sieve to collect any larger and heavier organisms. Samples were subsequently frozen (-20°C) prior to laboratory processing. Once in the laboratory, organisms were sorted into the dominant taxa. Single tissue samples were extracted from larger organisms such as the bivalve *Solen cylindraceus*. This material was treated with excess 2% HCl for 24 hours, in order to remove any shell fragments. Smaller gastropods and bivalves such as *Assiminea* cf. *capensis*; *Coriandria durbanensis* and *Brachidontes virgiliae* were used whole. Gastropods were treated with 2% HCl for 24 hours to dissolve their shells. The smaller bivalves were dried and tissue was then scraped from the inside of the shells. Triplicate samples were prepared where possible, using as many individuals as necessary to attain the desired weight of 1.0 mg required for the analysis.

Detritus material was collected from the water's edge as well as any decaying material which was trapped in the epibenthic sled upon which the zooplankton net was mounted and frozen (-20°C) prior to laboratory processing. After treating the samples with excess 2% HCl to remove possible biogenic carbonates, samples were rinsed with distilled water and oven-dried at 60°C for 24 hours.

Dominant macroalgae, such as *Cladophora* sp. and *Ulva* sp., were collected where available along with any dominant macrophytes and any fringing and submerged vegetation present at the study sites. This material was thoroughly rinsed with distilled water and oven-dried at 60°C for 24 hours.

POM was collected by taking triplicate water samples at each of the study sites. These samples were filtered onto pre-combusted (450°C for 6 hours) glass fibre filters (GF/F) with the aid of a vacuum filtration manifold. Once in the laboratory, the filters were treated with excess 2% HCl to remove any inorganic carbon and dried in an air circulated oven at 60°C for 24 hours.

Triplicate SOM samples were collected from each of the five study sites by collecting the upper centimetre of sediment from a 20 mm diameter core and frozen (-20°C) prior to

laboratory processing. Cores were collected between 50 cm and 1 m apart. In the laboratory, sediment samples were treated with excess 2% HCl for a minimum period of 24 hours in order to remove any carbonates. Samples were thoroughly rinsed with distilled water and subsequently oven-dried at 60°C for a period of 24 hours.

2.2.3 Stable isotope analysis

Once dried, sediment samples were ground with a mortar and pestle and placed into Eppendorf™ micro centrifuge tubes. Treated filters were packaged into tin foil envelopes once dry. Animal, plant and algal samples were homogenised into a fine powder with the aid of a sterilised mortar and pestle and weighed out into 5 x 8 mm microanalysis tin capsules. For animal tissues, 1 mg of the homogenised powder was packaged into the capsules for analysis, while plant, algal and detritus required a larger sample to attain an accurate signature. For these, 2 mg of the homogenised powder was weighed out and packaged for the analysis.

All samples were then sent to IsoEnvironmental cc based in the Botany Department at Rhodes University, Grahamstown, South Africa, for analysis. Here the samples were analysed using a Europa Scientific Integra isotope ratio mass spectrometer linked to an ANCA SL elemental analyser. Ratios were expressed as the parts per thousand deviation from the standard (atmospheric nitrogen for nitrogen and Vienna Pee Dee Belemnite for carbon) in delta (δ) notation according to:

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

Where X = ^{13}C or ^{15}N and R = corresponding ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Based on the results of the analysis an accuracy of 0.08‰ and 0.11‰ was measured for carbon and nitrogen respectively.

2.2.4 Dietary composition and trophic positioning

Gut content analysis was conducted on each of the 200 *A. ambassis* specimens collected in order to gain a better perspective of the short term dietary composition of this species. Once thawed, specimens were gutted, and the entire gut was extracted and preserved in a 10% phloxine-stained formalin solution. Each gut was analysed under a dissecting microscope, and the visible contents were classified into the most likely dietary sources. Following Hyslop (1980), two methods were used to quantify the contents. A numerical abundance method was used for all dietary items which could be counted (e.g. zooplankton and other

invertebrates) and a frequency of occurrence method was used to determine the proportion of fish which consumed a particular dietary item. In the event that dietary items could not be counted, such as detritus and macroalgae, the proportion of the total gut content was estimated on a counting grid and estimated as a percentage. In order to combine the results from the two gut content analysis methods, the proportion of countable constituents was calculated from a corrected maximum from which the major uncountable dietary items had been removed. Thus, if uncountable material accounted for 10 % of the gut contents, the results of the numerical abundance method would be calculated out of a maximum of 90 %.

Following Post (2002), trophic position was calculated with the following formula:

$$\text{Trophic position} = \lambda + (\delta^{15}N_{consumer} - \delta^{15}N_{base})/\Delta_n$$

where $\delta^{15}N_{consumer}$ is the nitrogen signature of the consumer, $\delta^{15}N_{base}$ is that of the base of the food chain chosen based on its position relative to the consumer, λ refers to the trophic level of the base (for primary producers, $\lambda = 1$), and Δ_n is the average trophic enrichment of nitrogen (3.4‰). The trophic base was selected separately for each of the sample sites in order to give an accurate estimation of how far the consumer is from the trophic base.

2.2.5 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 21 for Windows. Stable carbon and nitrogen isotope ratios of all possible food sources were analysed using a Two-way ANOVA. Potential differences between the sources in the wet and dry seasons and among the study sites were therefore determined and Tukey's HSD *post-hoc* comparisons identified specific differences between sources. To ensure that the assumptions of the tests were met, the data were checked for normality and the residuals of the ANOVA were checked for homoscedasticity. The assumption of homoscedasticity was not met for any of the data, but Zar (1996) states that ANOVA is robust enough to overcome this.

In order to determine the likely contribution of each potential food item to the diet of *Ambassis ambassis* at the five study sites during the wet and dry season, SIAR (Stable Isotope Analysis in R) version 4.0 was used to create mixing models, based on the standard corrected carbon and nitrogen ratios (Parnell et al. 2010). A trophic enrichment factor (TEF) was incorporated in the model, using values of 3.4‰ for $\delta^{15}N$ and 1‰ for $\delta^{13}C$ (Smit 2001, Carrasco et al. 2012, Dyer et al. 2013). A standard deviation of 1‰ was used for both carbon and nitrogen signatures, to remove any bias in the variability of trophic fractionation among the sources (Caut et al. 2009, Inger et al. 2010). Dietary items collected at the sampling sites

during the different seasons were used to run the models. Items which were not collected were excluded from the model as no signature was available. For the SIAR modelling, it is necessary to assume that there is restricted movement of these fish between the five sampling sites.

Primer version 6 multivariate statistics package (Clarke & Gorley, 2006) was used to test for dietary differences among the five sampling sites based on the gut contents of *Ambassis ambassis*. A one-way analysis of similarity (ANOSIM) was used to test for differences among the five different sampling sites, taking wet and dry season into account. The data were log-transformed in order to minimise the effect of dietary items which were dominant, thus accounting for total dietary composition. A Bray-Curtis similarity coefficient was used to calculate the similarity of the data among sites. The ANOSIM calculates a Global R value using randomization to determine the average of the ranked dissimilarities within and among groups. An R value of 1 indicates the greatest dissimilarity possible (i.e. the biggest difference) and a value of 0 indicates no dissimilarity (i.e. the most similar). The similarity matrix was then used to create the NMDS plots, using 100 iterations in order to generate the most likely outcome. A 2-dimensional plot was generated from the output of the analysis. In order to gain the best image of the groupings, obvious outliers were removed when the plot was generated.

In order to determine whether there was a significant relationship between fish size and the relative isotope ratios, a simple linear regression was performed on the $\delta^{15}N$ and $\delta^{13}C$ signatures of *A. ambassis* against the measured lengths of the fish sampled. The assumption of normality of data was checked prior to running the test and was satisfied.

To test whether the environmental variables affect the trophic level of the fish at the sampling sites, a simple linear regression was performed on the calculated trophic level of *A. ambassis* and the physico-chemical parameters which were measured at each sampling site. The assumptions of this test were met.

2.3 RESULTS

2.3.1 Habitat characteristics

The physico-chemical data (Figure 2.2) show a distinct difference between the wet and dry seasonal periods during which the present study was carried out. Salinity throughout the

system exhibited a marked decrease in the wet season when compared to those of the dry season. A salinity of 35.1 was recorded at Lister's Point during the dry season, compared to a salinity of only 17.9 during the wet season. At Charter's Creek the salinity also decreased from 7.8 during the dry season to 3.4 during the wet season. Catalina Bay showed a less drastic decrease, from 3.1 in the dry season to 2.3 in the wet season. Salinities at Esengeni also decreased by 1 unit during the wet season, when compared to the dry season. The salinities at the mouth also showed a decrease from 11.4 in the dry season to 5.4 in the wet season.

Temperatures ranged from 31.8°C at the mouth during the wet season to 22.9°C at Esengeni during the dry season. Turbidity data showed that Catalina Bay had the lowest turbidity in the wet and dry seasons, with 5.2 NTU and 0.7 NTU, respectively. The highest turbidity recorded during the wet and dry seasons was at the mouth, with values of 151 NTU and 250 NTU, respectively. The depth data shows that Esengeni was the deepest site sampled, with a maximum depth of 1.82 m recorded during the wet season, whereas Charter's Creek was the shallowest site sampled with a maximum depth of 0.21 m during the wet season (Figure 2.2).

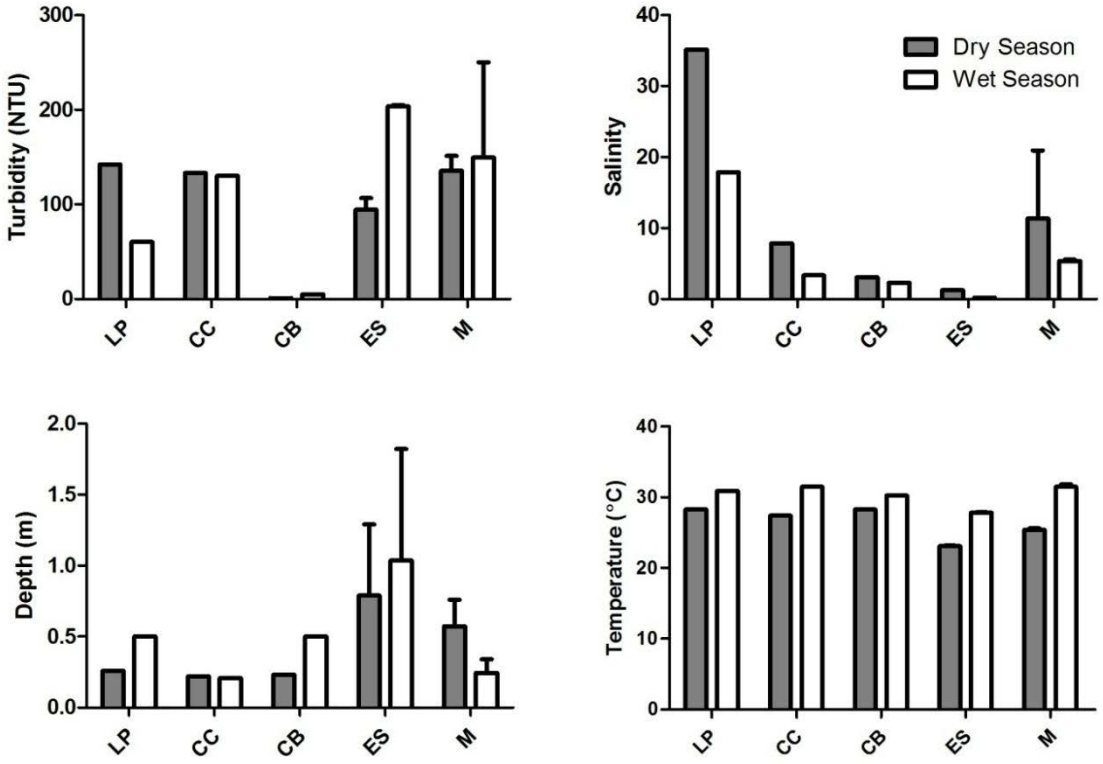

Figure 2.2: Physico-Chemical parameters measured during the dry (white bars) and wet (grey bars) seasons at the five study sites (LP – Lister's Point, CC – Charter's Creek, CB – Catalina Bay, ES – Esengeni, M – Mouth) in the St Lucia Estuary. Error bars for Esengeni and the mouth represent the variability between surface and bottom measurements.

2.3.2 Temporal and spatial variation in stable isotope signatures of dietary sources

Lister's Point

All dietary sources were found to be significantly different from each other in terms of their $\delta^{13}C$ ($F = 25.97$, $p = 0.001$) and $\delta^{15}N$ ($F = 26.16$, $p = 0.001$) signatures. There was, however, no significant difference in the $\delta^{13}C$ ($F = 0.002$, $p = 0.95$) and $\delta^{15}N$ ($F = 0.005$, $p = 0.96$) signatures of the dietary sources between the wet and dry seasons. The $\delta^{13}C$ signatures varied greatly among the different sources, with values between -26.71‰ for POM to -12.86‰ for submerged grass. The $\delta^{15}N$ signatures also varied greatly, with values ranging from 4.82‰ for SOM to 12.81‰ for the shrimp *Palaemon palaemon* (Figures 2.3 & 2.4; Table 2.1 & 2.2).

Charter's Creek

There was a significant difference between the different dietary sources, both in terms of their $\delta^{13}C$ ($F = 42.98$ $p = 0.001$) and $\delta^{15}N$ ($F = 31.22$ $p = 0.001$) signatures. The $\delta^{15}N$ signatures were statistically similar for the wet and dry seasons ($F = 2.332$ $p = 0.13$), whereas the $\delta^{13}C$ signatures were significantly different between seasons ($F = 11.44$ $p = 0.001$). The $\delta^{13}C$ signatures of the sources ranged from -22.91‰ for *Brachidontes virgiliae* to -13.02‰ for submerged grass. The $\delta^{15}N$ sources had a wide range, with values ranging from 1.21‰ for SOM to 12.59‰ for *Mesopodopsis africana* (Figures 2.3 & 2.4; Table 2.1 & 2.2).

Catalina Bay

Dietary sources collected at Catalina Bay were found to be significantly different in their $\delta^{15}N$ ($F = 36.91$ $p = 0.001$) and $\delta^{13}C$ ($F = 15.46$ $p = 0.001$) signatures. No significant difference between the wet and dry season was found for either $\delta^{13}C$ ($F = 0.13$ $p = 0.72$) or $\delta^{15}N$ ($F = 1.45$ $p = 0.24$) signatures. The range of $\delta^{13}C$ signatures was from -25.64‰ for *Brachidontes virgiliae* to -13.50‰ for *Stuckenia pectinata*. The $\delta^{15}N$ signatures ranged from 1.60‰ for SOM to 10.02‰ for *Pseudodiaptomus stuhlmanni* (Figures 2.3 & 2.4; Table 2.1 & 2.2).

Esengeni

All dietary sources were significantly different in terms of both $\delta^{13}C$ ($F = 46.60$ $p = 0.001$) and $\delta^{15}N$ ($F = 39.71$ $p = 0.001$) signatures. There were, however, no significant differences in source $\delta^{13}C$ ($F = 0.42$ $p = 0.52$) and $\delta^{15}N$ ($F = 0.58$ $p = 0.45$) signatures between the wet and dry season at Esengeni. The $\delta^{15}N$ signatures ranged from 2.29‰ for the reed *Scirpus littoralis* to 9.71‰ for the copepod *Acartiella natalensis*. The $\delta^{13}C$ signatures ranged from -30.05‰

for the bladderwort *Utricularia* sp. to-19.18‰ for SOM (Figures Figures 2.3 & 2.4; Table 2.1 & 2.2).

Mouth

At the mouth, dietary sources were significantly different in terms of the $\delta^{13}C$ ($F = 27.89$ $p = 0.001$) and $\delta^{15}N$ ($F = 18.41$ $p = 0.001$) signatures. Seasonally there was no difference in the $\delta^{15}N$ ($F = 1.12$ $p = 0.29$) and $\delta^{13}C$ ($F = 0.69$ $p = 0.41$) signatures of the dietary sources. The $\delta^{13}C$ signatures ranged from -27.82‰ for *Pseudodiaptomus stuhlmanni* to -20.68‰ for SOM. The $\delta^{15}N$ signatures ranged from 3.44‰ for SOM to 10.83‰ for *P. stuhlmanni* (Figures 2.3 & 2.4; Table 2.1 & 2.2).

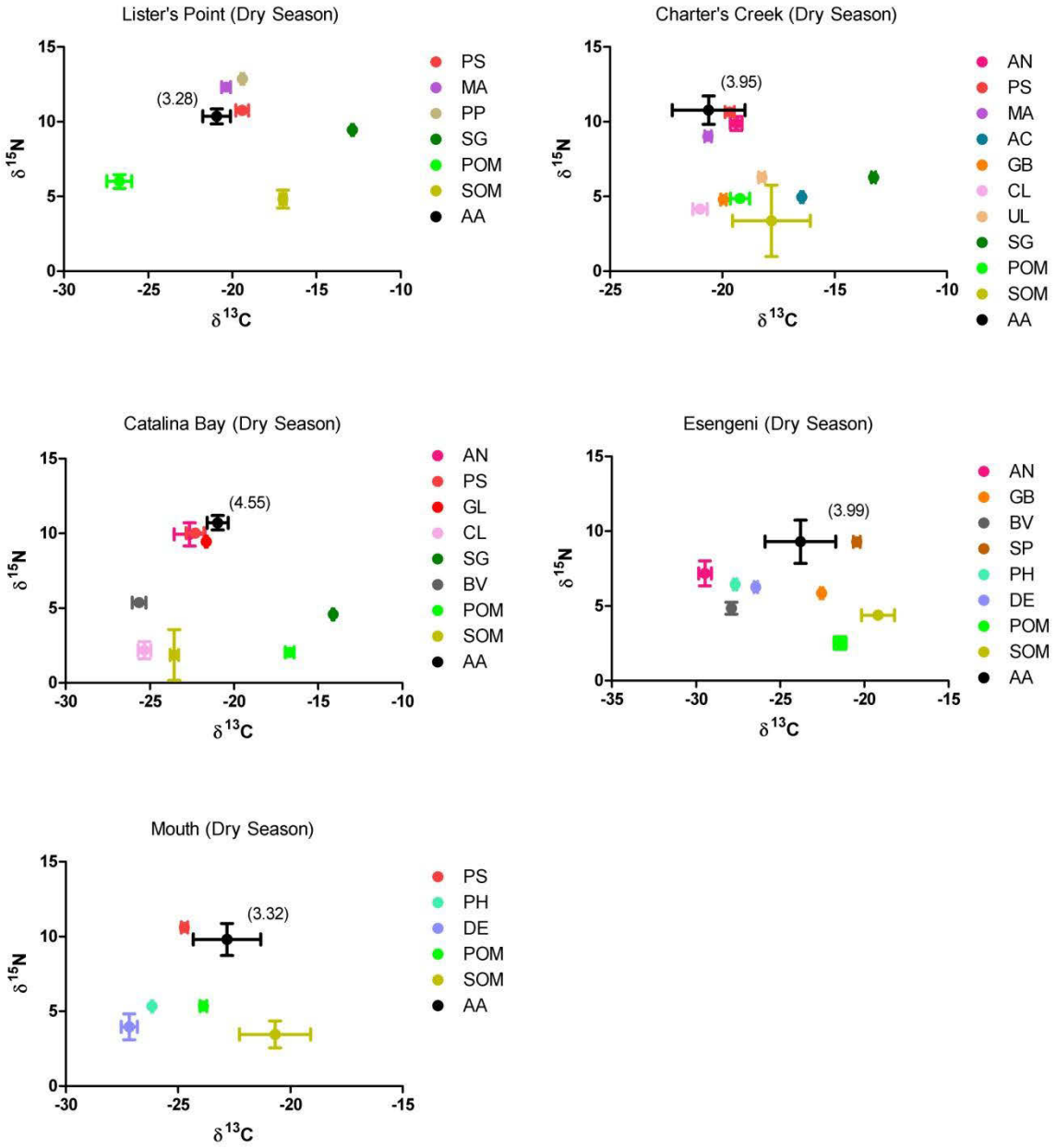

Figure 2.3: Mean (± SD) $\delta^{13}C$ and $\delta^{15}N$ signatures of *Ambassis ambassis* and the different food sources during the dry season at the five sampling sites throughout the St Lucia system. The *A. ambassis* signatures were corrected for trophic enrichment using standard fractionation values of 3.4‰ for $\delta^{15}N$ and 1‰ for $\delta^{13}C$. Values in parenthesis indicate the mean calculated trophic position of *A. ambassis*. Note different axis scales. Codes for dietary items: AA, *Ambassis ambassis*; AC, *Assiminea* cf. *capensis*; AN, *Acartiella natalensis*; BV, *Brachidontes virgiliae*; CL, *Cladophora* sp.; DE, Detritus; GB, *Grandidirella bonnieroides*; GL, Gastropod larvae; MA, *Mesopodopsis africana*; PH, *Phragmites* sp.; POM, Particulate Organic Matter; PP, *Palaemon palaemon*; PS, *Pseudodiaptomus stuhlmanni*; SG, Submerged grass; SOM, Sediment organic matter; SP, *Stuckenia pectinata*; UL, *Ulva* sp.

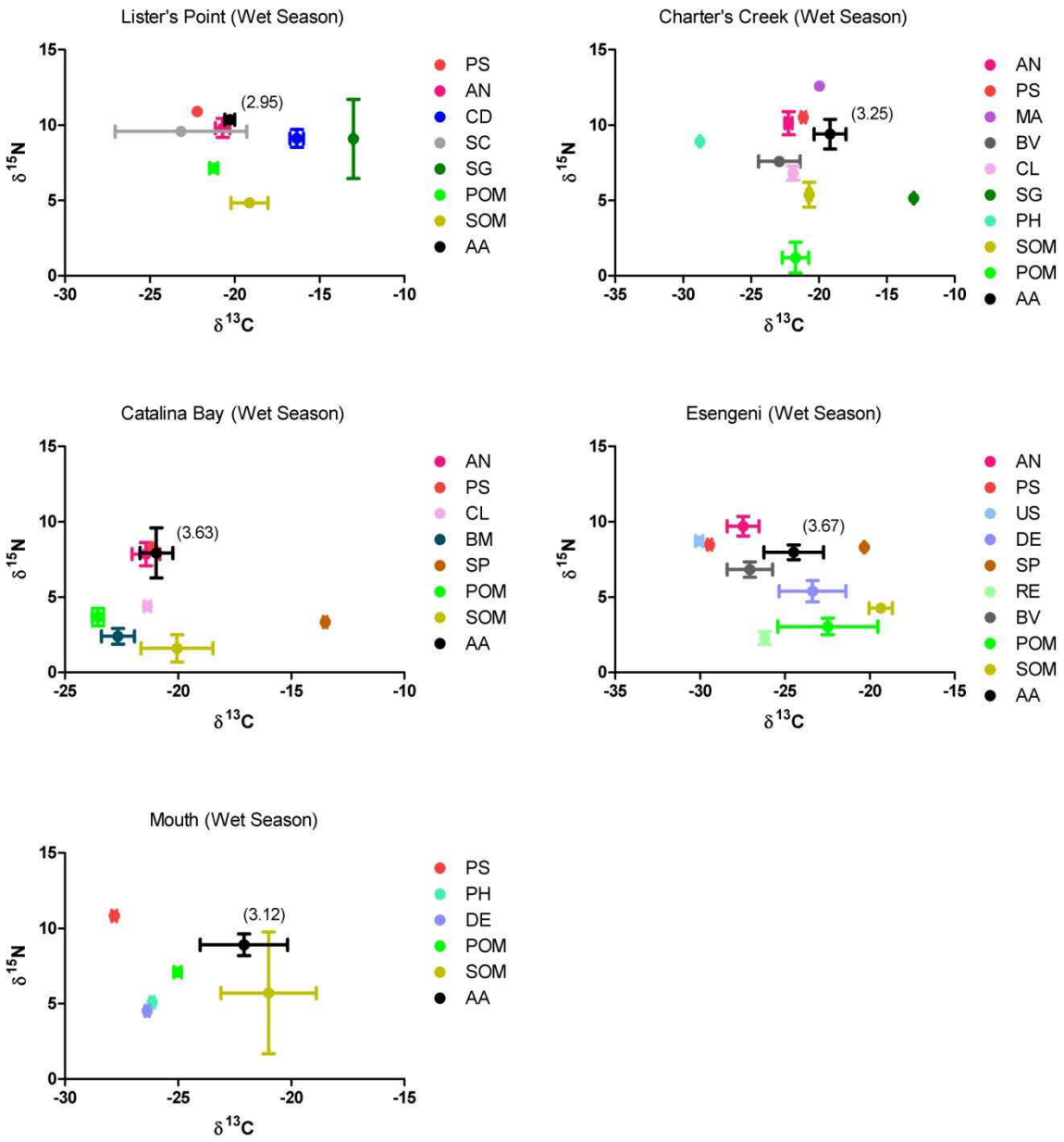

Figure 2.4: Mean (± SD) $\delta^{13}C$ and $\delta^{15}N$ signatures of *Ambassis ambassis* and the different food sources during the wet season at the five sampling sites throughout the St Lucia system. The *A. ambassis* signatures were corrected for trophic enrichment using standard fractionation values of 3.4‰ for $\delta^{15}N$ and 1‰ for $\delta^{13}C$. Values in parenthesis indicate the mean calculated trophic position of *A. ambassis.* Note different axis scales. Codes for dietary items: AA, *Ambassis ambassis*; AN, *Acartiella natalensis*; BM, *Bacopa monnieri*; BV, *Brachidontes virgiliae*; CD, *Coriandria durbanensis*; CL, *Cladophora* sp.; DE, Detritus; MA, *Mesopodopsis africana*; PH, *Phragmites* sp.; POM, Particulate Organic Matter; PS, *Pseudodiaptomus stuhlmanni*; RE, Reeds; SC, *Solen cylindraceus*; SG, Submerged grass; SOM, Sediment organic matter; SP, *Stuckenia pectinata*; US, *Utricularia* sp.

Table 2.1: Mean (± SD) $\delta^{13}C$ and $\delta^{15}N$ signatures of *Ambassis ambassis* and the different food sources during the dry season at the five sampling sites throughout the St Lucia system. Codes for dietary items: AA, *Ambassis ambassis*; AC, *Assiminea* cf. *capensis*; AN, *Acartiella natalensis*; BV, *Brachidontes virgiliae*; CL, *Cladophora* sp.; DE, Detritus; GB, *Grandidirella bonnieroides*; GL, Gastropod larvae; MA, *Mesopodopsis africana*; PH, *Phragmites* sp.; POM, Particulate Organic Matter; PP, *Palaemon palaemon*; PS, *Pseudodiaptomus stuhlmanni*; SG, Submerged grass; SOM, Sediment organic matter; SP, *Stuckenia pectinata*; UL*, Ulva* sp.

	Lister's Point			**Charter's Creek**			**Catalina Bay**			**Esengeni**			**Mouth**		
Dietary Items	C	N	n	C	N	n	C	N	n	C	N	n	C	N	n
AA	-19.61 ± 1.62	14.17 ± 0.95	10	-19.61 ± 1.62	14.17 ± 0.95	10	-19.97 ± 0.63	14.11 ± 0.48	10	-22.80 ± 2.10	12.69 ± 1.44	10	-21.82 ± 1.50	13.20 ± 1.07	10
AN	-	-	-	-19.38 ± 0.26	9.88 ± 0.46	3	-22.64 ± 0.91	9.93 ± 0.78	3	-29.47 ± 0.37	7.17 ± 0.83	3	-	-	-
AC	-	-	-	-16.46 ± 0.06	4.96 ± 0.27	3	-	-	-	-	-	-	-	-	-
BV	-	-	-				-25.64 ± 0.41	5.37 ± 0.25	3	-	-	-	-	-	-
CL	-	-	-	-21.00 ± 0.32	4.14 ± 0.20	3	-25.33 ± 0.38	2.18 ± 0.57	3	-	-	-	-	-	-
DE	-	-	-	-	-	-	-	-	-	-26.45 ± 0.10	6.25 ± 0.17	3	-27.18 ± 0.36	3.95 ± 0.87	3
GL	-	-	-	-	-	-	-21.65 ± 0.06	9.44 ± 0.13	3	-	-	-	-	-	-
GB	-	-	-	-19.95 ± 0.13	4.79 ± 0.20	3	-	-	-	-22.54 ± 0.09	5.85 ± 0.25	3	-	-	-
MA	-23.17 ± 3.87	9.58 ± 0.04	3	-20.64 ± 0.15	9.02 ± 0.32	3	-	-	-	-	-	-	-	-	-
PP	-13.01 ± 0.03	9.09 ± 2.63	3	-	-	-	-	-	-	-	-	-	-	-	-
POM	-26.71 ± 0.74	6.00 ± 0.46	3	-19.21 ± 0.43	4.86 ± 0.18	3	-16.70 ± 0.26	2.04 ± 0.11	3	-21.44 ± 0.35	2.52 ± 0.42	3	-23.87 ± 0.15	5.35 ± 0.20	3
PH	-	-	-	-	-	-	-	-	-	-27.67 ± 0.09	6.45 ± 0.10	3	-26.16 ± 0.03	5.33 ± 0.10	3
PS	-19.42 ± 0.38	6.74 ± 0.12	3	-19.67 ± 0.21	10.65 ± 0.17	3	-22.31 ± 0.53	10.02 ± 0.14	3	-	-	-	-24.72 ± 0.14	10.06 ± 0.19	3
RE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SG	-16.34 ± 0.40	9.12 ± 0.59	3	-13.26 ± 0.09	6.27 ± 0.10	3	-14.11 ± 0.06	4.58 ± 0.08	3	-	-	-	-	-	-
SOM	-16.99 ± 0.74	4.82 ± 0.60	3	-17.81 ± 1.73	3.37 ± 2.39	3	-23.55 ± 0.25	1.87 ± 1.70	3	-19.18 ± 0.99	4.38 ± 0.13	3	-20.68 ± 1.58	3.44 ± 0.90	3
SP	-	-	-	-	-	-	-	-	-	-20.45 ± 0.20	9.28 ± 0.14	3	-	-	-
UL	-	-	-	-18.24 ± 0.14	6.28 ± 0.24	3	-	-	-	-	-	-	-	-	-

Table 2.2: Mean (± SD) $\delta^{13}C$ and $\delta^{15}N$ signatures of *Ambassis ambassis* and the different food sources during the wet season at the five sampling sites throughout the St Lucia system. Codes for dietary items: AA, *Ambassis ambassis*; AN, *Acartiella natalensis*; BM, *Bacopa monnieri*; BV, *Brachidontes virgiliae*; CD, *Corandria durbanensis*; CL, *Cladophora* sp.; DE, Detritus; MA, *Mesopodopsis africana*; PH, *Phragmites* sp.; POM, Particulate Organic Matter; PS, *Pseudodiaptomus stuhlmanni*; RE, Reeds; SC, *Solen cylindraceus*; SG, Submerged grass; SOM, Sediment organic matter; SP, *Stuckenia pectinata*; US, *Utricularia* sp.

	Lister's Point			Charter's Creek			Catalina Bay			Esengeni			Mouth		
Dietary Items	**C**	**N**	**n**	**C**	**N**	**n**	**C**	**N**	**n**	**C**	**N**	**n**	**C**	**N**	**n**
AA	-19.29 ± 0.29	13.76 ± 0.32	9	-18.18 ± 1.18	14.17 ± 0.95	10	-19.96 ± 0.73	11.33 ± 1.66	10	-22.48 ± 1.76	11.38 ± 0.48	10	-21.09 ± 1.93	12.31 ± 0.72	9
AN	-20.71 ± 0.43	9.81 ± 0.63	3	-22.23 ± 0.27	10.13 ± 0.76	3	-21.42 ± 0.61	7.86 ± 0.77	3	-27.46 ± 0.93	9.71 ± 0.65	3	-	-	-
BM	-	-	-	-	-	-	-22.66 ± 0.73	2.40 ± 0.52	3	-	-	-	-	-	-
BV	-	-	-	-22.91 ± 1.53	7.60 ± 0.12	3	-	-	-	-27.07 ± 1.34	6.83 ± 0.51	3	-	-	-
CL	-	-	-	-21.90 ± 0.19	6.81 ± 0.47	3	-21.36 ± 0.15	4.40 ± 0.08	3	-	-	-	-	-	-
CD	-16.34 ± 0.40	9.12 ± 0.59	3	-	-	-	-	-	-	-	-	-	-	-	-
DE	-	-	-	-	-	-	-	-	-	-23.37 ± 1.97	5.39 ± 0.70	3	-26.15 ± 0.09	5.09 ± 0.04	3
MA	-	-	-	-19.95 ± 0.00	12.59 ± 0.00	1	-	-	-	-	-	-	-	-	-
POM	-21.33 ± 0.24	7.14 ± 0.16	3	-20.72 ± 0.16	5.38 ± 0.83	3	-23.55 ± 0.25	3.68 ± 0.58	3	-22.47 ± 2.95	3.05 ± 0.54	3	-25.02 ± 0.17	7.10 ± 0.10	3
PH	-	-	-	-28.76 ± 0.05	8.92 ± 0.01	3	-	-	-	-	-	-	-26.15 ± 0.09	5.09 ± 0.04	3
PS	-22.21 ± 0.00	10.91 ± 0.00	1	-21.14 ± 0.17	10.52 ± 0.31	3	-21.11 ± 0.13	8.27 ± 0.31	3	-29.44 ± 0.12	8.48 ± 0.18	3	-27.82 ± 0.11	10.83 ± 0.25	3
RE	-	-	-	-	-	-	-	-	-	-26.19 ± 0.07	2.29 ± 0.42	3	-	-	-
SG	-13.01 ± 0.03	9.09 ± 2.63	3	-13.02 ± 0.02	5.16 ± 0.13	3	-	-	-	-	-	-	-	-	-
SOM	-19.13 ± 1.09	4.85 ± 0.11	3	-21.72 ± 0.98	1.21 ± 1.02	3	-20.05 ± 1.59	1.60 ± 0.91	3	-19.35 ± 0.68	4.27 ± 0.14	3	-21.00 ± 2.10	5.71 ± 4.04	
SC	-23.17 ± 3.87	9.58 ± 0.04	3	-	-	-	-	-	-	-	-	-	-	-	-
SP	-	-	-	-	-	-	-13.50 ± 0.08	3.34 ± 0.11	3	-20.33 ± 0.03	8.30 ± 0.35	3	-	-	-
US	-	-	-	-	-	-	-	-	-	-30.05 ± 0.21	8.72 ± 0.09	3	-	-	-

2.3.3. Temporal and spatial variation in Ambassis ambassis signatures and trophic positioning

The C:N ratios of *Ambassis ambassis* were consistently low, with values ranging between 3.20 and 3.48. There was a significant difference in the $\delta^{13}C$ ($F = 29.60$ $p = 0.001$) and $\delta^{15}N$ ($F = 9.81$ $p = 0.001$) signatures of *A. ambassis* among the five sampling sites in the St Lucia system. Seasonally, $\delta^{15}N$ signatures were significantly different among the study sites ($F = 42.25$ $p = 0.001$) but $\delta^{13}C$ signatures were statistically similar ($F = 2.32$ $p = 0.13$). The $\delta^{13}C$ ($F = 3.70$ $p = 0.001$) and $\delta^{15}N$ ($F = 2.23$ $p = 0.01$) signatures of *A. ambassis* were significantly different for different sized individuals collected. Charter's Creek was the only site to yield a significant relationship between $\delta^{13}C$ signatures and fish size ($R^2 = 0.31$, $p = 0.01$, $n = 20$) with all other sites yielding non-significant relationships ($p > 0.05$). Charter's Creek ($R^2 = 0.34$, $p = 0.01$, $n = 20$), Catalina Bay ($R^2 = 0.56$, $p = 0.001$, $n = 20$) and Esengeni ($R^2 = 0.20$, $p = 0.04$, $n = 20$) all yielded significant relationships between $\delta^{15}N$ signatures and fish size, with Lister's Point and the mouth yielding non-significant results ($p > 0.05$).

Trophic position was significantly different among the five sampling sites ($F = 19.79$ $p = 0.001$), as well as significantly different between the wet and dry seasons ($F = 24.63$ $p = 0.001$). A significant, albeit very weak relationship was identified between trophic position and the size of the fish collected ($R^2 = 0.051$, $p = 0.026$, $n = 98$). A significant relationship was also found for trophic position and salinity ($R^2 = 0.15$, $p = 0.001$, $n = 98$), as well as trophic position and temperature ($R^2 = 0.087$, $p = 0.003$, $n = 98$). There was, however, no significant relationship between trophic position and turbidity or depth measured at the sampling sites ($p > 0.05$).

2.3.4 Dietary contribution: stable isotopes

At Lister's Point during the dry season, *Mesopodopsis africana* was the dominant dietary item, contributing between 10 and 55% of the total diet. *Pseudodiaptomus stuhlmanni* was also of dietary importance, contributing up to 40%. *Palaemon palaemon* and particulate organic matter (POM) were also important, both contributing up to 30% of the diet. Submerged grass and sediment organic matter (SOM) were not of great importance to the diet, contributing less than 10% (Figure 2.5). During the wet season, *P. stuhlmanni* was the most important dietary constituent, contributing up to 60% of the total diet. *Acartiella natalensis* was also of importance, contributing up to 40%. All other sources were of lesser importance, contributing less than 25% each (Figure 2.6).

At Charter's Creek, zooplankton dominated the diet with *P. stuhlmanni*, *M. africana* and *A. natalensis* making up the top three dominant sources, respectively, during the dry season. All other sources were of lesser importance, contributing less than 20% to the total diet (Figure 2.5). During the wet season, zooplankton was again dominant but this time *M. africana* was the main food source, contributing up to 70% of the total diet, while *P. stuhlmanni* and *A. natalensis* contributed up to 30 and 22%, respectively. Submerged grass was also found to play a role in the diet of these fish, contributing up to 25% of the total diet. All other sources were found to be of minor importance, contributing less than 20% (Figure 2.6).

Catalina Bay displayed a very similar trend to Charter's Creek during the dry season, with zooplankton dominating the diet. *Pseudodiaptomus stuhlmanni*, *A. natalensis* and gastropod larvae were the top three dietary items respectively. Again submerged grass was important to the diet, contributing up to 25% of the total. All other sources were less important, contributing less than 20% (Figure 2.5). During the wet season, *P. stuhlmanni* was the most dominant dietary item contributing up to 60% of the total diet. *Acartiella natalensis* was also dominant, contributing up to 50%. All other sources were of lesser importance, contributing less than 30% (Figure 2.6).

During the dry season, the aquatic plant *Stuckenia pectinata*, contributed up to 45% of the total diet of the fish collected at Esengeni. All other sources were of minor importance, contributing less than 25% (Figure 2.5). During the wet season, *S. pectinata* was again the dominant food source contributing up to 50%. *Acartiella natalensis* was also important to the diet, contributing up to 40%. All other dietary sources were of minor importance to the diet of these fish, contributing less than 30% (Figure 2.6).

At the mouth, *P. stuhlmanni* was the most dominant dietary item during both seasons, contributing up to 90% of the total diet during the dry season and up to 70% during the wet season. POM and SOM were also of importance during both the dry and wet seasons at the mouth. Detritus and *Phragmites* sp. were of minor importance to the diet of these fish, contributing less than 20% in both seasons (Figures 2.5 & 2.6).

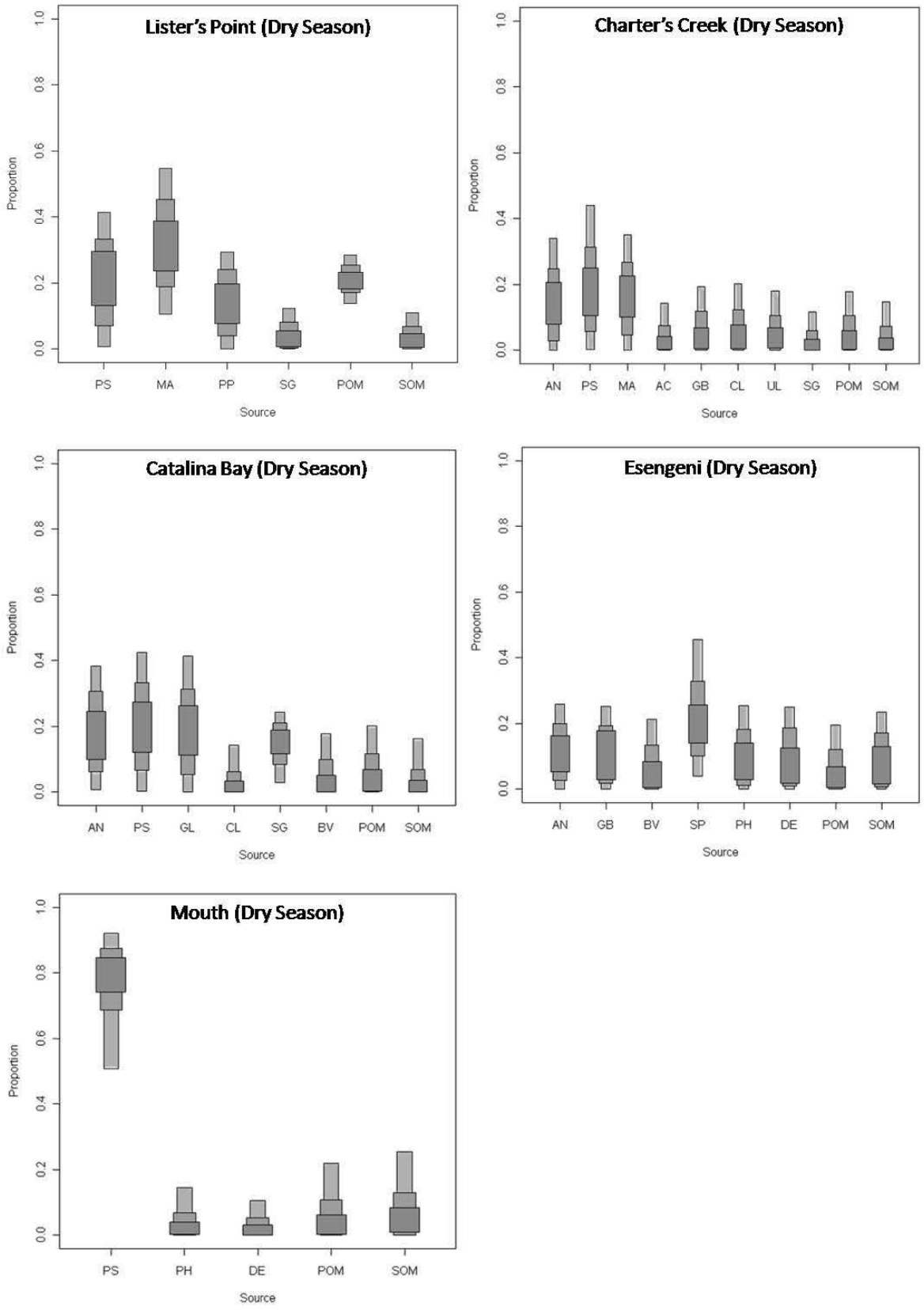

Figure 2.5: Contribution of dietary items to the diet of *Ambassis ambassis* at the five sampling sites in the St Lucia system during the dry season (based on Stable Isotope Analysis in R mixing models). For each item 25, 75 and 95% credibility intervals are plotted (light, medium and dark grey respectively). Codes for dietary items as in Figure 2.3.

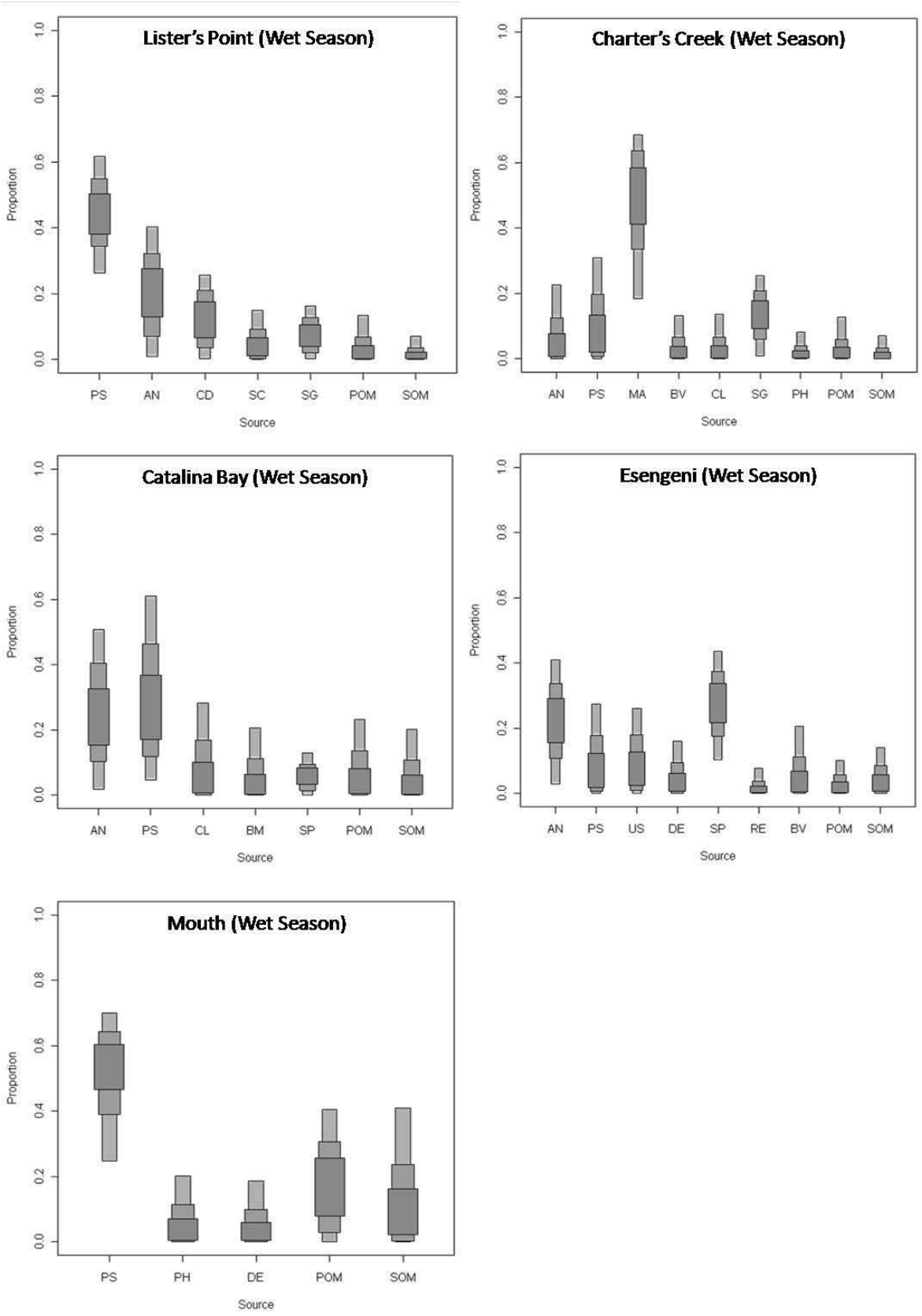

Figure 2.6: Contribution of dietary items to the diet of *Ambassis ambassis* at the five sampling sites in the St Lucia system during the wet season (based on Stable Isotope Analysis in R mixing models). For each item 25, 75 and 95% credibility intervals are plotted (light, medium and dark grey respectively). Codes for dietary items as in Figure 2.4.

2.3.5 Dietary composition: gut contents

Gut content analyses proved to be difficult for this species, as the guts of the majority of the fish collected were almost empty. Nevertheless, fish gut contents revealed that the short-term dietary composition of this species was in general similar for the five study sites, but there was however some difference in the composition between sites (Tables 2.3 & 2.4). At all sites, terrestrial and aquatic insects and insect larvae were present in the diet during both the wet and dry seasons. Diptera was the dominant order found in the gut contents as far as insects are concerned, with three families represented. Mosquito larvae were found in the gut contents from all sites, except the mouth, and were found in up to 70% of the fish collected at Charter's Creek during the dry season. During the wet season these larvae were only identified in the gut contents from fish collected at Lister's Point. *Pseudodiaptomus stuhlmanni* was identified from the gut contents from all sites during the wet season but only from the gut contents of the fish at the mouth and Lister's Point during the dry season. Gastropod larvae were identified from Catalina Bay and Esengeni and occurred in gut contents of 80 and 20% of the fish collected during the dry season at these places, respectively. The benthic gastropod, *Assimenea* cf. *capensis*, contributed to the diet of 50% of the fish collected at Charter's Creek during the wet season. *Mesopodopsis africana* were identified in the gut contents of fish at all sites but there was seasonal variation in their occurrence in the gut contents. Macroalgae were identified in the gut contents of fish at all sites except Esengeni, contributing to the diet of 55% of the fish collected at Lister's Point during the dry season. The greatest variety in gut contents was found at Esengeni during the dry season, with a total of twelve distinct dietary items identified here. The lowest diversity in gut contents was obtained from the mouth during the wet season, where only six distinct items were identified (Tables 2.3 & 2.4).

When dissecting fish, it was noticed that all the fish collected at Charter's Creek and the mouth during the dry season had enlarged gonads, indicating that these fish were in spawning condition. From the wet season samples, fish collected at Charter's Creek and the mouth again displayed mature gonads but some fish collected at Lister's Point also indicated signs of maturing gonads. Fish collected at Esengeni and Catalina Bay displayed no signs of advanced gonad development in either season.

The result of the ANOSIM indicates that there is a high degree of similarity between the five sampling sites based on the gut contents of the fish collected ($R = 0.379$, $p = 0.001$). The plot generated from the NMDS procedure is considered to be sufficiently described in two

dimensions with a stress value of 0.16 (Clarke & Warwick 2001). From Figure 2.7 it is evident that there is a high degree of similarity among the different sampling localities, with a large amount of overlap of the different clusters. This means that the fish from the five sampling sites are similar with respect to their dietary composition. It is also clear from Figure 2.7 that the fish collected at Charter's Creek, Catalina Bay and Esengeni separate out on a seasonal level with the respective seasons grouping together forming clusters, thus indicating variability in dietary composition among fish from the same site during the wet and dry seasons. At Lister's Point and the mouth there was however no indication of seasonal differences in the diet as there was a large degree of overlap in the data for these sites.

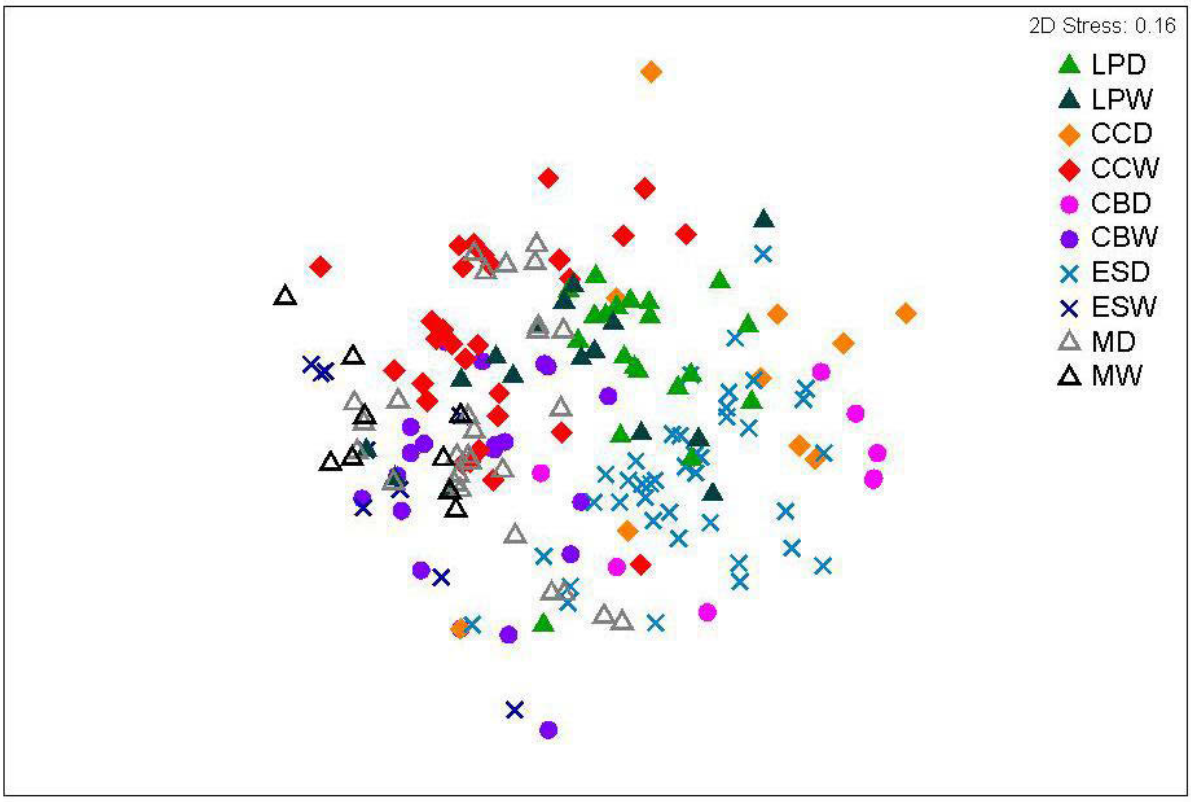

Figure 2.7: Non-metric multidimensional scaling (NMDS) plot of the log-transformed *Ambassis ambassis* gut content data from the five sampling localities in the St Lucia Estuary. M: Mouth, CC: Charter's Creek, CB: Catalina Bay, ES: Esengeni, LP: Lister's Point, D: Dry season, W: Wet season.

Table 2.3: Mean ± SD number of individuals found in the gut contents of *Ambassis ambassis* at the five sampling sites for the dry season as well as occurrence frequency (%) for each dietary item. Numbers in parenthesis indicate the sample size for each sampling location. (-): not found in gut contents.

Dietary Item	Lister's Point		Charter's Creek		Catalina Bay		Esengeni		Mouth	
	Mean ± SD	% of guts	Mean ± SD	% of guts	Mean ± SD	% of guts	Mean ± SD	% of guts	Mean ± SD	% of guts
Acrididae (Orthoptera)	1.00	05 (22)	-	-	-	-	-	-	-	-
Amphipoda	-	-	1.50 ± 0.71	20 (10)	-	-	1.41 ± 0.51	40 (42)	1.60 ± 0.89	17 (30)
Brachidontes virgiliae	-	-	-	-	-	-	1.21 ± 0.43	33 (42)	-	-
Calliphoridae (Diptera)	1.80 ± 0.84	23 (22)	1.67 ± 0.58	30 (10)	1.00 ± 0.00	30 (10)	1.67 ± 0.71	21 (42)	-	-
Corixidae (Hemiptera)	-	-	-	-	-	-	1.50 ± 0.71	05 (42)	-	-
Culicidae (Diptera)	-	-	12.00	10 (10)	-	-	-	-	-	-
Culicidae Larvae (Diptera)	1.80 ± 1.08	68 (22)	1.14 ± 0.38	70 (10)	1.00 ± 0.00	60 (10)	2.44 ± 1.45	64 (42)	-	-
Detritus (%)	-	-	-	-	15.00	10 (10)	-	-	7.50 ± 4.27	47 (30)
Formicidae (Hymenoptera)	4.00	05 (22)	-	-	10.00	10 (10)	-	-	-	-
Gastropoda Larvae	-	-	-	-	983.13 ± 845.98	80 (10)	1.00 ± 0.00	07 (42)	-	-
Gobiidae	1.00 ± 0.00	18 (22)	0.67 ± 0.58	30 (10)	-	-	-	-	-	-
Fish (Unknown)	-	-	-	-	-	-	2.00	02 (42)	-	-
Macroalgae (%)	12.50 ± 10.11	55 (22)	5.00	10 (10)	-	-	-	-	11.67 ± 8.29	30 (30)
Mesopodopsis africana	1.00 ± 0.00	27 (22)	-	-	-	-	1.00 ± 0.00	05 (42)	1.33 ± 0.50	30 (30)
Ostracoda	1.13 ± 0.35	36 (22)	-	-	1.00 ± 0.00	30 (10)	1.54 ± 0.88	31 (42)	1.71 ± 0.91	47 (30)
Pseudodiaptomus stuhlmanni	1.00	05 (22)	-	-	-	-	-	-	1.58 ± 0.79	40 (30)
Tipulidae (Diptera)	-	-	-	-	-	-	1.50 ± 0.71	05 (42)	-	-
Vespidae (Hymenoptera)	-	-	-	-	-	-	1.75 ± 0.89	19 (42)	-	-
Zygoptera Larvae	1.54 ± 0.78	59 (22)	-	-	1.00 ± 0.00	20 (10)	1.47 ± 0.62	40 (42)	1.17 ± 0.41	20 (30)

Table 2.4: Mean ± SD number of individuals found in the gut contents of *Ambassis ambassis* at the five sampling sites for the wet season as well as occurrence frequency (%) for each dietary item. Numbers in parenthesis indicate the sample size for each sampling location. (-): not found in gut contents.

Dietary Item	Lister's Point		Charter's Creek		Catalina Bay		Esengeni		Mouth	
	Mean ± SD	% of guts	Mean ± SD	% of guts	Mean ± SD	% of guts	Mean ± SD	% of guts	Mean ± SD	% of guts
Amphipoda	1.00 ± 0.00	25 (16)			1.10 ± 0.32	48 (21)	1.50 ± 0.71	20 (10)	1.00	11 (9)
Assimenea cf. *capensis*	-	-	1.80 ± 1.01	50 (30)	-	-	-	-	-	-
Brachidontes virgiliae	-	-	2.50 ± 2.17	33 (30)	-	-	-	-	-	-
Calliphoridae (Diptera)	1 ± 0.00	13 (16)	1.00 ± 0.00	10 (30)	-	-	-	-	-	-
Cladocera	-	-	52.67 ± 45.61	10 (30)	-	-	10.33 ± 4.51	30 (10)	2.00 ± 0.00	22 (9)
Culicidae (Diptera)	1 ± 0.00	19 (16)	-	-	-	-	-	-	-	-
Culicidae Larvae (Diptera)	2.00 ± 1.73	31 (16)	-	-	-	-	-	-	-	-
Detritus (%)	10.83 ± 5.85	38 (16)	7.81 ± 3.64	53 (30)	7.5 ± 2.61	57 (21)	7.50 ± 2.67	80 (10)	22.14 ± 16.80	77 (9)
Formicidae (Hymenoptera)	-	-	-	-	3.07 ± 2.40	67 (21)	3.00 ± 0.00	20 (10)	-	-
Gobiidae	1.25 ± 1.26	25 (16)	1.00 ± 0.00	10 (30)	1.00 ± 0.00	14 (21)	-	-	-	-
Macroalgae (%)	9.44 ± 4.64	56 (16)	8.00 ± 4.14	50 (30)	11.25 ± 2.50	19 (21)	-	-	-	-
Mesopodopsis africana	1.20 ± 0.45	31 (16)	1.50 ± 0.71	07 (30)	1.33 ± 0.52	29 (21)	-	-	-	-
Ostracoda	1.5 ± 0.58	25 (16)	1.71 ± 1.11	23 (30)	-	-	-	-	2.67 ± 2.08	33 (9)
Pseudodiaptomus stuhlmanni	2.00	06 (16)	4.22 ± 1.92	30 (30)	1.25 ± 0.50	19 (21)	11.17 ± 8.20	50 (10)	3.40 ± 2.30	56 (9)
Vespidae (Hymenoptera)	-	-	-	-	1.67 ± 0.58	14 (21)	1.00	10 (10)	-	-
Zygoptera Larvae	1 ± 0.00	25 (16)	-	-	1.43 ± 0.79	33 (21)	1.00	10 (10)	1.00	11 (9)

2.4 DISCUSSION

From the physico-chemical data presented, it is clear that the reverse salinity gradient that has been persistent in the St Lucia system since 2002 (Perissinotto et al. 2013a) was still largely in effect, the only difference is that salinities throughout the system have now decreased, indicative of the shift from a dry to a wet phase (Perissinotto et al. 2013a). The salinity at Lister's Point is evidently lower than what has been documented in the past at this site, with the maximum salinity recorded during this study at around 35. This is a marked decrease from past studies, such as those of Carrasco et al. (2012) and Dyer et al. (2013) who recorded salinities close to 150 and over 50 for this site, respectively. This decrease in salinity is evident at the other sampling sites as well. Charter's Creek has experienced a noticeable reduction in salinity, with the highest value falling below 10, compared to the 16.4 recorded earlier by Dyer et al. (2013). Catalina Bay has always experienced lower salinities than Charter's Creek, despite both sites being located in south lake; this is due to the freshwater seepage from the surrounding dunes along the eastern shores of the system (Taylor et al. 2006). Due to the freshwater inflow from the Mpate River, the salinity recorded at Esengeni was expected to be the lowest for this study (Perissinotto et al. 2013a). Here salinities were noticeably less than the other sites with the maximum value recorded only being 1.26, indicating a virtually limnetic state. Despite the freshwater dominance throughout the system, salinities have not changed dramatically at the mouth when compared to those recorded by Dyer et al. (2013). Values recorded at this site ranged from 5 to 18 for the wet and dry seasons respectively. The values are slightly higher than those recorded by Dyer et al. (2013) but this may be attributed to the inconsistent marine water influx into the system, via the artificially constructed Mfolozi channel (Taylor et al. 2013). It is now evident how the influx of freshwater and the onset of the wet phase have influenced the system and changed it to one which is now dominated by freshwater, distinctly different to what has prevailed for the last decade (Whitfield & Taylor 2009, Perissinotto et al. 2013a).

As a result of the changes in salinity throughout the system, *Ambassis ambassis* has extended its distribution range from that which has been historically documented for this species in St Lucia. Vivier et al. (2010a) indicated that this species was limited in terms of its distribution within the system during the 2006-2008 surveys. The most recent of these surveys showed that this species was only found in the Narrows, at Charter's Creek and at Fani's Island (Vivier et al. 2010a). The current study shows that it has now expanded its distribution northward and southward, with fish also occupying habitats around Lister's Point and the mouth respectively, thus being now effectively distributed throughout the system. This shift

in distribution can be positively related to the decrease in salinity, as this species has been shown to only tolerate a salinity range of 2 to 35, preferring to occupy areas with a salinity less than 15 (Martin 1988, Whitfield 1998). Blaber & Cyrus (1981) reported that the species also survived extended periods in salinities below 1 in Lake Nhlange of the Kosi system, therefore indicating that it is able to survive in freshwater habitats as well (Skelton 1993). Although this species was present at Lister's Point and Esengeni, the two extremes in salinity for this study, their numbers appeared at these sites as it required greater netting effort to collect the specimens, when compared to the other sites. The advanced developmental state of the gonads of the fish collected at Charter's Creek and the mouth may indicate that these sites are favourable for breeding or spawning. Little is known about the reproductive biology of *A. ambassis,* but it is believed that these fish breed within estuaries (van der Elst 1988). Connell (1996) identified eggs from the mouth region in the St Lucia Estuary during a survey in 1993, which resembled those of the Ambassidae and were thought to match closely to *A. dussumieri*. Identification and protection of these breeding areas for this species could be of vital importance for conservation efforts.

From a stable isotopes perspective, the diet of *A. ambassis* was dominated by zooplankton at the majority of the sites during both the wet and dry seasons, with the exception of Esengeni where the aquatic plant *Stuckenia pectinata* was also important in its diet. The presence of this aquatic plant and others in the models is however believed to be as a result of this source being transported through the food web, and therefore not directly preyed upon by *A. ambassis*. Our findings are thus congruent with the historical studies of the diet of these fish, which have shown that zooplankton, particularly fish larvae and crustaceans are of vital importance to the diet (Martin 1983, Martin & Blaber 1983, Martin 1989, Skelton 1993, Whitfield 1998). Martin & Blaber (1983) suggest that the principal reason for the larger size of *A. ambassis*, when compared to other Ambassidae, can be attributed to the high proportion of energy-rich crustaceans in their diet. Past studies were, however, based on gut content analyses alone. The present study is the first to complement gut content analysis with stable isotope analysis for a species of this family in southern Africa. The results from the gut contents analyses revealed a similar trend to that observed for the isotope mixing models. However, terrestrial and aquatic insects, as well as benthic invertebrates such as amphipods and bivalves, are also included in the dietary composition, which is congruent with the findings of Martin (1989), who found that this species can supplement its diet with insects of terrestrial and aquatic origin. As it is difficult to predict which insects will be preyed upon by these fish, insects were not included in the isotope sampling protocol for this study. *Ambassis*

ambassis preferentially feeds near the water surface and it is thought that fish opportunistically feed on insects that fall into the water (Martin 1989). In this study, insects were found in the gut contents of *A. ambassis* at all five sampling sites, but were particularly prevalent at Esengeni. The $\delta^{13}C$ signatures of the fish collected at Esengeni were the most depleted of the five sampling sites, thus indicating the importance of terrestrial input to the diet at this site. The order Diptera was the most commonly identified insects in the gut contents, as well as Hymenoptera and Orthoptera. Mosquito larvae, which are commonly found near the water surface, were preyed upon by *A. ambassis* at several sites. Another species of Ambassid, native to the Indian sub-continent, has been shown to be an effective bio-control vector by lowering larval mosquito numbers in freshwater environments (Chandra et al. 2008). Juvenile fish belonging to the Gobiidae family were also identified in the gut contents. These fish are small enough for *A. ambassis* to ingest whole and have been previously recorded in the gut contents of this species (Nhleko 2011). It is now evident that this species feeds at the surface (insect), in the water column (zooplankton) and along the substrate (Gobiidae).When it comes to the selection of prey items, Martin (1989) suggested that the species composition of ingested organisms by these fish is not as important as the size and availability of the dietary items. Therefore, any prey item which is small enough for the fish in question to eat will be targeted. This is indicative of an opportunistic foraging species, which is not exclusively targeting select organisms but merely supplementing its diet with the most energetically rich item it comes into contact with. Similarly, under flood conditions in Australian estuaries, Balcombe and Bunn (2005) showed that *Ambassis* sp. increase their dietary breadth and supplement their zooplanktivorous diet with insects and fish.

Nitrogen signatures of *Ambassis ambassis* were significantly different between the wet and dry seasons. Carbon signatures were, however, not statistically different. Perga & Gerdeaux (2005) showed that there is strong seasonal fluctuation in nitrogen isotope signatures for the Whitefish (*Coregonus lavaretus*) that were studied in a western European lake. Olin et al. (2013) also suggest strong seasonal variation in isotope signatures, with a marked increase in nitrogen values in the summer (wet) period. Nitrogen signatures were also shown to be higher in winter (dry) periods for *Ambassis jacksoniensis* in mangrove environments in southern Australia (Mazumder et al. 2008). Carbon and nitrogen isotope signatures were found to be significantly different for different-sized individuals; this can be related to the diet of these fish as it has been shown that there is a shift in diet of this species between juveniles and adults, with adults targeting food items in suspension (Martin 1989). Carbon isotope

signatures were positively related to the size of the fish collected only at Charter's Creek, which implies that different-sized fish are feeding in different areas at this site. Following the freshwater influx into the system, large areas of vegetation have been inundated at this site resulting in the creation of new 'safe' habitats, particularly for juveniles. Paxton et al. (1989) have shown that *Ambassis argrammus* form shoals which congregate amongst submerged vegetation. It is therefore likely that *A. ambassis* will behave in a similar manner, with juveniles and potentially adults utilising these habitats. Smaller fish using these areas would therefore be exposed to different dietary items, when compared to larger fish utilizing deeper water. Nitrogen signatures were positively related to fish size at Esengeni only, but negatively related to fish size at Charter's Creek and Catalina Bay. Nitrogen isotopes can give an indication of trophic level, as this element is known to fractionate between energy transfers (DeNiro & Epstein 1978). Therefore, it is likely that the larger fish at Esengeni are feeding at a higher trophic level compared to the smaller fish. Vinagre et al. (2012) suggests that as fish increase in size, their ability to eat larger and higher trophic level prey increases due to the increase in mouth gape. At Catalina Bay and Charter's Creek, this trend is reversed, with smaller fish feeding at a higher trophic level. This can also be associated with the inundated vegetation at these two sites. Smaller individuals may use the flooded vegetation as shelter and may thus come into contact with terrestrial insects which fall into the water and are preyed upon by the smaller fish.

Based on the calculated trophic positioning of *A. ambassis*, it was shown that this differed significantly between the five sampling sites, with the highest trophic position of 4.55 recorded at Catalina Bay during the dry season. The lowest trophic position was recorded at Lister's Point during the wet season with a value of 2.95. This gives an indication that the dietary composition of this species at the different sampling sites may differ, with fish targeting prey at a higher trophic position during the dry season. There was also a significant difference in the mean trophic level of these fish from a seasonal perspective, with the dry season having consistently higher values at all five sampling sites. This follows the trends observed by Vinagre et al. (2012), who found that trophic levels of numerous estuarine organisms are generally lower during the summer (wet) season. In their study, this was attributed to the higher abundances of micro- and macroalgae which will be preyed upon more extensively by lower trophic level organisms, thus resulting in a cascade up the food chain (Vinagre et al. 2012). In the St Lucia system, pelagic primary productivity has been shown to be higher during the wet season, when higher nutrient levels are recorded in the lakes as a result of increased freshwater inflow from the rivers in the catchment (Perissinotto

et al. 2013a; Perissinotto et al. 2013b). When linked to physico-chemical parameters, trophic position showed a significant, albeit very weak, relationship with temperature and salinity. This could explain the differences in trophic position between the different sampling sites, as salinity is a primary determinant for explaining the differences between sites.

From the results of the NMDS analysis, it is evident that there is clear overlap between the different sampling sites from a dietary composition perspective. All the sites grouped together with no clear clusters forming, indicating that the diet of the fish at the different sites was fairly similar. Seasonally there was a difference in dietary composition at three of the five sampling sites, Charter's Creek, Catalina Bay and Esengeni. At the mouth and Lister's Point, there was a large degree of overlap in the dietary composition, thus both the wet and dry seasons clustered together. It can therefore be assumed that these fish do not change their diet under the different environmental conditions which are present at a seasonal scale at these two sites. At the other three sites, seasonal changes in the trophic positioning, which coincides with the separation of the data on the NMDS plot, indicates a change in diet of this species between the wet and dry seasons.

In conclusion, *Ambassis ambassis* has been shown to be largely planktivorous, feeding predominantly on zooplankton throughout the St Lucia system. In addition, *A. ambassis* opportunistically supplements its diet with terrestrial and aquatic insects as well as juvenile fish when available. Varying environmental conditions, as witnessed at the different sampling localities, do not seem to have an effect on the dietary composition of this species as diet varied little between sites. The use of stable isotopes and gut content analysis has been shown to produce similar results with regard to the dietary composition of this fish species. There is however merit in using gut content analyses to determine the contribution of items which are opportunistically preyed upon which may be otherwise impossible to attain using stable isotope analysis. This fish species therefore proves to be a vital link in the estuarine food web at times, both as a predator and a prey item, providing a pivotal link in the pathway of energy between trophic levels. The success of *A. ambassis* within the St Lucia system is however governed by its tolerance to salinity rather than its dietary preferences. Thus the expansion of its geographical distribution in the system will only see this species become more common during the wet phase, and therefore more influential in the food webs of the system at this time.

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CONCLUSIONS & RECOMMENDATIONS FOR FUTURE WORK

Climate experts have predicted that the area around St Lucia is likely to become warmer and wetter as we move towards 2100, as a result of global climate change (Schulze 2000, Schulze 2005, Vaeret & Sokolic 2008, Lumsden et al. 2009). This will result in higher than average rainfall in this area and consequently more freshwater flow into the system (Lumsden et al. 2009). It is also predicted that there will be more frequent and bigger extreme weather events, such as droughts and floods, which will alter the St Lucia system (Mather et al. 2013). Under extreme drought conditions, Whitfield & Taylor (2009) and Cyrus et al. (2010) documented large changes to the physical environments in St Lucia, with dramatic losses in habitat as a result of low water levels. During this period, the trophic structuring of the St Lucia system has been observed to undergo large changes (Lawrie 2012, Mather et al. 2013). Due to the heterogeneity of the system, many different food webs occur, with different links and pathways in different areas (Govender et al. 2011, Scharler & MacKay 2013).Therefore, species which are able to adapt to the changing conditions, such as *Oreochromis mossambicus*, are likely to succeed in the system and become dominant in the trophic structures. Less environmentally tolerant species, such as *Ambassis ambassis*, rely on favourable environmental conditions to succeed and become dominant. When conditions become unfavourable, their distribution is limited to refuge areas in the Narrows where conditions are more stable. Dietary responses to changing environmental conditions have been reported for numerous species in varying habitats (Balcombe et al. 2005, Perga & Gerdeaux 2005, Xu et al. 2005, Vinagre et al. 2012). Understanding how these species respond to changing environmental conditions within the St Lucia Estuary, from a dietary perspective, is therefore crucial to our understanding of the trophic functioning of the St Lucia system as a whole.

In the St Lucia Estuary, *O. mossambicus* exhibits some degree of trophic plasticity, as it has the ability to alter its diet according to the prevailing conditions. During hypersaline conditions, these fish fed on a variety of different sources, which included both plant/algal and animal material (Carrasco et al. 2012). However, there were no dominant sources, with all dietary constituents contributing similar proportions. This study revealed that there is some variability in this trend under more freshwater-dominated conditions, with fish feeding predominantly on specific sources which thus dominate their diets. This was supported by

both the mixing models and the gut content analyses. The main reason for this is that it is likely that these food items are more abundant during the less saline conditions. From the results of the isotope analysis it can be predicted that this species derives most of its carbon from epiphytic algae and macrophyte detritus. This was evident by the dependence on microphytobenthos (MPB) and sediment organic matter (SOM) from the isotope modelling as well as from the prevalence of sand particles and detrital material in the gut contents of this species. The isotope analysis also indicated a variation in the trophic positioning of different-sized individuals, where there are suitable habitats for juveniles to use as refuges (e.g. shallow-water shore regions). The analysis of trophic positioning of *O. mossambicus* also revealed that salinity and turbidity are the driving factors responsible for the trophic position of this species in the system, either directly by influencing the fish themselves or indirectly by affecting their dietary sources. The hypothesis that there would be dietary variation between the hypersaline and diluted stages is therefore supported, as well as showing that fish size can determine dietary composition.

In contrast to *O. mossambicus* which shows a high degree of dietary plasticity, *Ambassis ambassis* has been shown to be largely planktivorous, feeding predominantly on zooplankton throughout the St Lucia system. In addition, *A. ambassis* opportunistically supplements its diet with terrestrial and aquatic insects as well as juvenile fish when available. These findings are very similar to those of Martin (1983) and Martin (1989) who showed that this fish is largely zooplanktivorous, with crustaceans and fish larvae being important in the diet. Martin (1989) also documented the importance of terrestrial and aquatic insects in the diet of this species which was evident in the findings of the current study as well. Past and present studies therefore indicate that the diet of *A. ambassis* has remained largely the same in the St. Lucia system. The stable isotope analysis conducted suggests that it is likely that this species derives the majority of its carbon from phytoplankton and particulate organic matter (POM). This is evident due to the high prevalence of zooplankton in the diet which rely on these sources as their primary carbon sources. Detrital material from macrophytes may also be important as this was found to be prevalent in the diet at Catalina Bay and Esengeni, but this does however require further investigation.

The trophic position of this species was shown to be higher during the dry season, compared to the wet season. It is thought that this is linked to the increased primary productivity in the system during the wet season, which will result in greater abundances of lower trophic level prey species, thus altering the trophic positioning of the food web as a whole. Varying

environmental conditions, as witnessed at the different sampling localities, do not seem to have an effect on the dietary composition of this species as diet varied little between sites. This fish therefore proves to be a vital link in the estuarine food web, both as a predator and a prey item, providing a pivotal link in the pathway of energy between trophic levels. The success of *A. ambassis* within the St Lucia system is however governed by its tolerance of salinity rather than its dietary preferences. Thus the expansion of its geographical distribution in the system will only see this species become more common during the wet phase, and therefore more influential in the food webs of the system.

From both studies it is evident that from a temporal and spatial perspective these two species adopt similar, yet very different, dietary tactics. *Oreochromis mossambicus* was shown to adopt a generalist feeding strategy, opportunistically feeding on dietary items that are available, allowing this species to alter its diet according to the environment which it inhabits and the food sources which are available. In contrast, *Ambassis ambassis* displayed a more specialist dietary composition, feeding predominantly on zooplankton. However, this species also opportunistically supplements its diet with additional sources when available. The success and dominance of both species in the St Lucia system can therefore be attributed to their dietary strategies. Under extreme environmental conditions, such as hypersalinity, *O. mossambicus* has the added advantage of its wide tolerance of different environmental conditions, thus allowing this species to proliferate in the St Lucia system. With the recent shift to a freshwater-dominated system, the two species are predicted to flourish, as a result of environmental conditions becoming more favourable to both.

Recommendations for future research

The trophic interactions and structuring of the St Lucia system have been well documented for the system under hypersaline conditions (Govender et al. 2011, Scharler & MacKay 2013). However, with the recent shift of the system to an oligohaline state, there is a need to document the trophic structuring of the system under these new conditions, as it is very likely that there have been fundamental changes in the faunal composition in response to the environmental changes.

There is also a need to investigate the effects of changing environmental conditions on the higher trophic level piscivorous fauna of the system. The present study has shown the effects of environmental conditions on the common prey items of larger fish, such as springer (*Elops machnata*), kob (*Argyrosomus japonicus*) and kingfish (Carangidae). Apart from fish,

piscivorous birds such as the great white pelican (*Pelicanus onocrotalus*), herons, cormorants and kingfishers as well as crocodiles rely on fish as a dietary source. Will any changes in trophic structure be reflected in these higher trophic level species as well?

With the onset of the oligohaline/mesohaline phase, and the already established reconnection with the Mfolozi River system, the fish community in the St Lucia system is likely to change to one in which freshwater-tolerant species become dominant (Cyrus 2013). Will the dominance of *O. mossambicus* continue as other freshwater tolerant species, such as *A. ambassis*, *Gilchristella aestuaria* and members of the Muglidae are now able to proliferate in the system? If not, will the effects of the shift in dominance of prey species solicit a dietary response from larger fish species who could shift their diet from less common fish prey to those that are more readily available?

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