Terrestrial-aquatic transfers by hippopotamus (*Hippopotamus amphibius*): effects on food web and benthic community structure of the St Lucia Estuary, iSimangaliso Wetland Park, World Heritage Site, South Africa.

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Plagiarism Declaration

The data described in this thesis were collected in the St Lucia Estuarine system, from July 2013 to March 2015, under a research agreement with the iSimangaliso Wetland Park Authority. This thesis, submitted for the degree of Doctor of Philosophy in the Department of Biological Sciences, Science Faculty, University of Cape Town, represents original work by the author and has not otherwise been submitted for any degree at any other University.

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March 2019

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Abstract

Africa's last extant aquatic megaherbivore, the hippopotamus, facilitates linkages between terrestrial and aquatic systems at scales, frequencies and intensities that are probably unmatched by any other natural process. Through defaecation of terrestrial grasses into aquatic habitats, hippos disproportionately enhance boundary permeability across the aquatic-terrestrial divide. Little, however, is known about the ecological ramifications of these transfers for recipient communities and broader functioning in aquatic ecosystems, with equivalent knowledge for estuaries being virtually non-existent. Using a combination of *in situ* (1) experiments manipulating hippo dung inputs, (2) assessments of carbon and nitrogen stable isotope ratios and (3) fatty acid analyses, I aimed to quantify the influence of hippo dung on food web and benthic community structure in the St Lucia Estuary - a subtropical estuarine lake on the east coast of South Africa. It was hypothesized that experimental dung enrichment at high levels would result in significant declines in benthic community metrics and that food web components in biotopes with contrasting hippo numbers would differ in isotopic and fatty acid signatures. Results from experiments revealed that effects of hippo dung on benthic assemblages were assemblage specific. Microphytobenthic biomass was reduced by up to 70 %; macrobenthic abundance, biomass and richness declined by 76, 56 and 27 % respectively, while meiofauna were negligibly impacted by experimental dung enrichment. Results therefore suggest a greater resilience of meiofauna to high dung inputs relative to microphytobenthos and macrofauna. Comparisons of food web components from biotopes with contrasting hippo numbers (the Narrows: hippos dense; Charter's Creek: hippos rare) indicated distinct consumer isotopic and fatty acid profiles, suggesting different dietary sources. Contrary to expectations, stable isotope mixing models revealed a greater reliance on hippo dung as a food source by consumers in Charter's Creek (i.e. where hippos were rare). Fatty acid biomarkers suggested that in the presence of heavy dung loading, consumer diets incorporated

less benthic diatoms, more bacteria, and generally reflected stronger dependence on terrestrial food sources. Overall, this study demonstrates the potential for hippo dung to influence consumers and trophic interactions due to its role as a trophic resource and modifier of abiotic conditions. However, findings of *in situ* experiments also show that in high amounts, dung inputs can lead to declines in benthic metrics. Apart from enhancing understanding of the broader roles hippos play in aquatic ecosystems, this study highlights considerations relevant to managing hippo populations and dung inputs, especially under drought conditions. This is central to maintenance of ecological functioning in a system that is regarded as a biodiversity hotspot and key tourist attraction. Specifically, it is important that water levels are managed to prevent dung accumulation and deleterious effects, particularly on the benthos.

CHAPTER 1:

INTRODUCTION

1.1 Global change

For the duration of its roughly 4.5-billion-year history, the earth has constantly been changing. Historically the change was gradual, enabling life on earth to evolve and adapt, however, in their relatively short-lived history, humans have caused rates of global change to accelerate dramatically (Levine et al. 1995, Schlesinger & Bernhardt 2013). These global changes include: (1) altered biogeochemical cycling of nitrogen, oxygen and other elements, (2) increased concentrations of atmospheric carbon dioxide (CO_2), (3) global habitat fragmentation, changes in land use and land cover, and (4) climate change (Vitousek et al. 1997, Walther et al. 2002, Johnson et al. 2011).

Anthropogenic changes are driven by increasing human populations and *per capita* resource use, especially since the onset of the industrial age and the development of commercial agriculture (Vitousek et al. 1997, Occhipinti-Ambrogi 2007). A prominent example of human induced change on a planetary scale is the increase in global temperatures by approximately 0.65 – 0.71°C over the past century (Hawkins et al. 2017). Accompanying this change are multiple large-scale alterations to planetary properties and processes, such as a reduction in sea-ice extent, rising sea levels, increased ocean acidification and altered patterns of precipitation and ocean currents (Doney et al. 2012). These abiotic changes have strongly altered ecological and biological processes, leading to concerns that rapid rates of environmental change could exceed evolutionary capacities of organisms to adapt, resulting in substantial biodiversity loss (Hughes et al. 2003, Doney et al. 2012). This idea is supported by a wave of increased global extinctions, local extirpations and populations being threatened with extinction over the past few centauries (Barnosky et al. 2011, Dirzo et al. 2014, McCauley, Pinsky, et al. 2015, Young et al. 2016).

Key global change processes threatening global ecology include habitat modification, resource exploitation, species invasions, pollution, disease/pathogens, and climate change - all of which are human-induced or strengthened by human activities (Vitousek et al. 1997, Barnosky et al. 2011, Young et al. 2016). A commonly reported response to these processes are alterations in species abundance and distributions, with concomitant indirect ecosystem-level changes due to altered biological interactions, community stability, composition and dynamics (Dukes & Mooney 1999, Walther et al. 2002, Johnson et al. 2011). It has been shown using modelling techniques that 80 % of initial extinctions in hypothetical and natural food webs occur indirectly due to primary species loss or declines in functions provided (Säterberg et al. 2013, Young et al. 2016). Research elsewhere has shown that the effects of species and biodiversity loss on ecosystem functioning is significant and comparable with more commonly cited drivers of global change (Cardinale et al. 2012, Hooper et al. 2012).

In light of the above, it is important for ecologists to understand the direct and indirect influences of global change, particularly when keystone species are involved. Keystone engineers are especially relevant in this regard, since they create, modify and maintain habitats through their activities, over large spatial scales (Jones et al. 1994, Wright & Jones 2006). Shifts in the abundance and distribution of these species are thus likely to have significant ecological consequences across multiple trophic levels due to the intensity and scales at which they modulate resources for other species (Hooper et al. 2005, Moore 2006, Mosepele et al. 2009, Estes et al. 2011). In addition, if affected species are critical players in ecosystem linkages, (through migration or engineering habitat corridors), then ecological changes caused by distributional shifts can manifest across multiple ecosystems.

1.2 Cross-system transfers and animals as vectors of transfers

Traditionally, ecosystems were considered to be closed, self-contained entities that were influenced by processes and interactions occurring within them (Vanni et al. 2004, Witman et al. 2004, Richardson et al. 2010). However, over the past decades, ecologists have come to realise that ecosystems are open and linked by the movement of nutrients, materials and organisms across boundaries. It has also become clear that this connectivity is critical to ecosystem dynamics and functioning (Polis et al. 1997, Cadenasso et al. 2003, Strayer et al. 2003, Marczak et al. 2007). This movement of biotic and abiotic resources among ecosystems is variously referred to as cross-system trophic transfers, subsidies or allochthonous resources, in reference to the latter originating outside of the recipient ecosystem. These transfers have been shown to influence recipient ecosystems directly, by influencing top-down or bottom-up processes. They can also indirectly affect ecosystems by changing the physico-chemical conditions in receiving environments (Polis et al. 1997, Huxel et al. 2004, Holt 2008, Richardson et al. 2010).

Recent reviews have shown that cross-system transfers are diverse, pervasive across all habitats, and act over multiple spatial and temporal scales (Polis et al. 2004, Marczak et al. 2007). Transfers can occur between habitats within an ecosystem, (e.g. between benthic and pelagic environments) or between ecosystems of different types. Salmonid movement from the ocean to rivers and then indirectly to land is a commonly cited example of a between-ecosystem transfer (Cederholm et al. 1999, Naiman et al. 2002, Vanni 2002, Vanni et al. 2004). Transfers are generally thought to elicit positive effects on a range of taxonomic groups, resulting in enhanced growth and productivity within recipient environments (Cadenasso et al. 2003, Marczak et al. 2007, Richardson et al. 2010). However, the net effect of a transfer within a given ecosystem is contextually dependent, and determined by multiple factors including (1) traits of the transfer (e.g. its lability, quantity and quality); (2) whether it is actively or passively incorporated into food webs as well as the trophic level at which it enters the food web, (3)

functional traits of consumers, and (4) the productivity of the recipient ecosystem (Marczak et al. 2007, Marcarelli et al. 2011). For example, herbivorous consumers are unlikely to benefit substantially from transfers of invertebrates. Generally, effects are strongest when the transfer is of high quality or is moving from a high- to low-productivity system (Polis et al. 1997, Cadenasso et al. 2003, Anderson & Polis 2004, Cole et al. 2006, Marczak et al. 2007, Savage et al. 2012).

Allochthonous resources can be transported across boundaries by abiotic (water and wind) or biotic (mobile consumers) vectors. Water or wind driven movements such as diffusion, runoff, groundwater flow and advection are examples of abiotic vectors (Polis et al. 1997, Cadenasso et al. 2003). In contrast, transfer by mobile consumers typically involves the consumption of resources in one ecosystem followed by defecation or consumer death in the recipient ecosystem. Examples of these include the spawning and subsequent death of salmonids when moving from marine to freshwater systems or the movement of water birds between aquatic feeding grounds and island nesting sites where they excrete nutrient rich guano (Polis et al. 1997, Vanni 2002, Cadenasso et al. 2003, Vanni & Headworth 2004). The movement of these animals can form important linkages between ecosystems, especially in view of animal-mediated transfers frequently being of higher quality, as well as more spatially and temporally aggregated than abiotically-mediated transfers (McClain et al. 2003, Marcarelli et al. 2011). In aquatic systems with limited water exchange and minimal discharge, the movement of organisms acts as the dominant vector of allochthonous transfer, making this amongst the most important mechanisms for cross-system connectivity in semi-arid regions (Abrantes & Sheaves 2008, Howe & Simenstad 2015).

Animal-mediated transfers have been shown to affect food web structure and stability (Sabo & Power 2002, Leroux & Loreau 2008, Richardson & Sato 2015, Masese et al. 2018), ecosystem productivity, (Cole et al. 2006, Menninger et al. 2008, Marcarelli et al. 2011,

Subalusky et al. 2018) and nutrient cycling (Kitchell et al. 1999, Vanni 2002, Moyo et al. 2017). However, the magnitude and direction of the resultant effects is dependent on numerous factors including transfer quality, quantity, timing and duration (Richardson et al. 2010, Marcarelli et al. 2011, Subalusky et al. 2015). These in turn can be affected by individual characteristics of the animal vector, such as body size, life cycle and movement patterns (Vanni 2002, Menninger et al. 2008, Richardson et al. 2010). For example, it is generally accepted that while bigger animals transfer larger quantities of allochthonous material, the quality of the transfer decreases with increasing body size (Vanni 2002). On a similar note, due to the long life cycles of cicadas, transfers mediated by them are rare, occurring every 17 years in some cases (Menninger et al. 2008), while in comparison, transfers driven by aquatic insects and amphibians are more regular, occurring seasonally, due to their short life cycles (Nakano & Murakami 2001, Richardson et al. 2010, Larsen et al. 2016). Similarly, migratory animals such as geese, salmonids and even whales also mediate pulsed transfers that are heterogeneous in space and time (Polis 1998, Cederholm et al. 1999, Kitchell et al. 1999, Nicol et al. 2010, Roman & McCarthy 2010, Levi et al. 2013). In contrast, transfers mediated by animals that make daily foraging trips between ecosystems, such as bats, beavers and semi-aquatic birds, occur in one general location throughout the year, thereby allowing daily transfers to accumulate in the recipient ecosystem (Polis 1998, Duchamp et al. 2010, Anderson et al. 2015). Such transient consumers passing through system corridors are particularly important in modulating the strength of ecosystem linkages, but they are also capable of initiating multi-layered alterations to recipient ecosystems through various pathways, including the (1) modification of trophic interactions by virtue of their memberships in food webs, (2) physical engineering of abiotic and biotic characteristics of ecosystems through their activities and (3) transfer of trophic resources through defecation. In line with this, ecologists have investigated the effect of herbivores as vectors for cross-systems transfers (Wolf et al. 2013, Doughty et al. 2016) and

recent research has highlighted the profound influence larger aquatic herbivores (>10 kg) can have on ecosystem structure, functioning and associated species within aquatic ecosystems (Bakker et al. 2016, Chritz et al. 2016).

1.3 Hippopotamus as ecosystem engineers and vectors of trophic transfers

The hippopotamus (*Hippopotamus amphibius* – hereafter referred to as hippo) is one of only five extant megaherbivores (> 1000 kg) remaining in Africa, along with elephant, black and white rhinos, and giraffe (Owen-Smith 1988). Hippos are large, squat mammals weighing approximately 1300 – 2600 kg and have a shoulder height not exceeding 1.4 m (Owen-Smith 1988). Formerly widespread in rivers and lakes throughout sub-Saharan Africa, hippo populations have been declining (Fig. 1.1) due to habitat loss and human-hippo conflicts, which has resulted in the species being listed as 'vulnerable' on the IUCN Red list since 2006 (Grey & Harper 2002, Lewison 2007, Kanga et al. 2012, Taylor 2013a, Lewison & Pluháček 2017). Of the 38 countries in which hippos currently reside, population sizes are declining in 16 countries, stable in nine countries (of which South Africa is one) and increasing in four, while the remaining nine countries are data deficient (Lewison & Pluháček 2017). The overall global decline in hippo numbers is cause for alarm, given that large herbivore declines since the late Pleistocene - as a result of hunting and anthropogenic activity - have been linked to significant changes in the structure and functioning of ecosystems (Bakker et al. 2016).

Hippos are strongly dependant on water, spending the daytime wallowing or being submerged in aquatic habitats to protect their sensitive skin and avoid heat stress (Owen-Smith 1988, Eltringham 1999, Cerling et al. 2008). However, after nightfall, hippos emerge onto land to feed on the surrounding grasslands, generally remaining within 3 km of the water, although records show that individuals may travel up to 10 km away during drought conditions (Owen-Smith 1988, Eltringham 1999). This semi-aquatic lifestyle makes hippos unique among

African megaherbivore species. It is the combination of large body size, selective grazing habits and movement between terrestrial and aquatic ecosystems that has given hippos a reputation as major ecosystem engineers (Naiman & Rogers 1997, Moore 2006, Jacobs et al. 2007, Mosepele et al. 2009, Kanga et al. 2013).

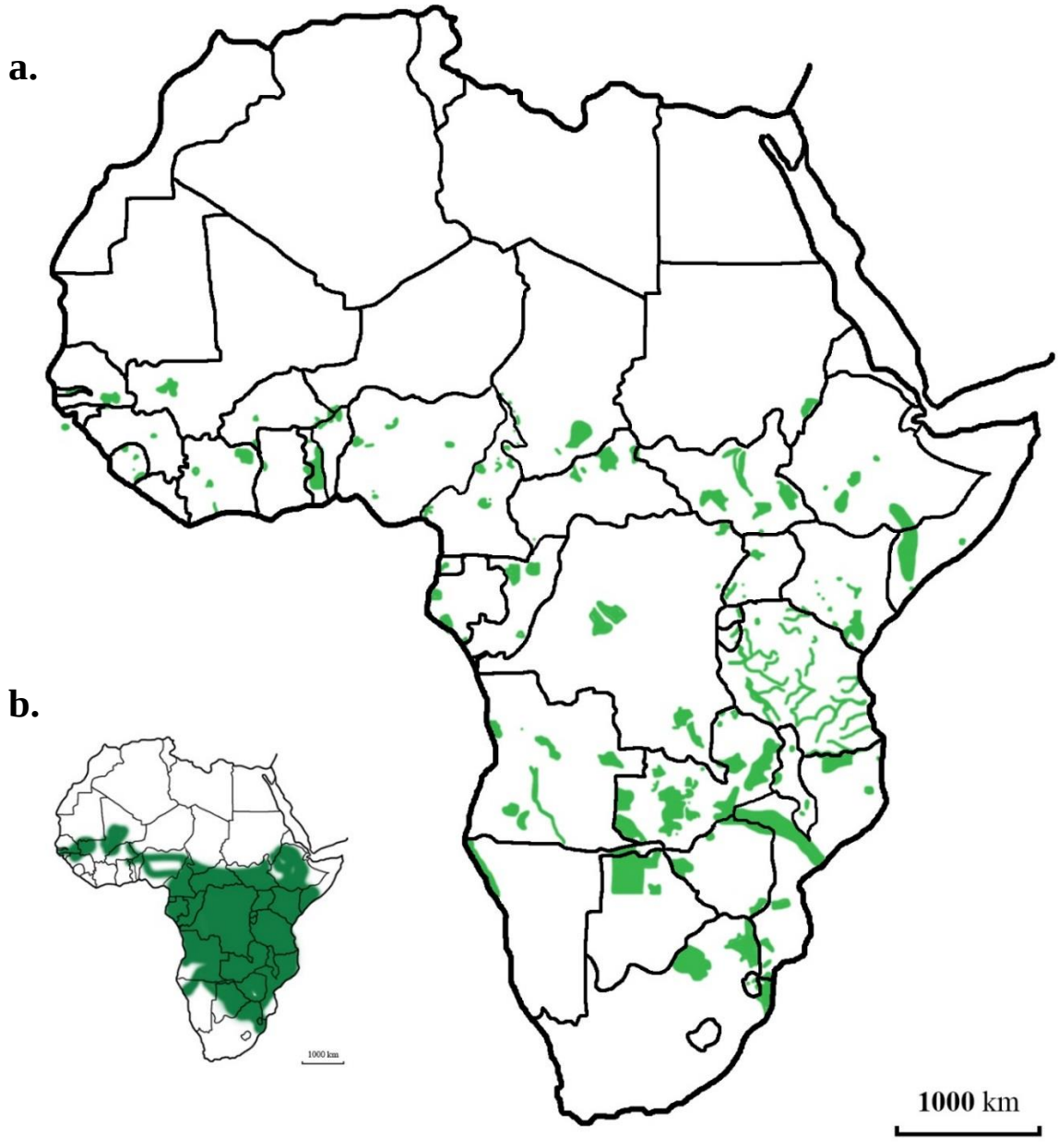


Figure 1.1: Estimated a) present (± 2017) and b) 1959 hippo population distribution within sub-Saharan Africa. It is important to note that hippo populations in 1959 were likely already in decline. (Adapted from Lewison 2007, Lewison & Pluháček 2017).

Research on the effects of hippos as engineers has typically focused on two major driving mechanisms: (1) their ability to modify terrestrial vegetation and (2) the geomorphological/physical changes caused by the movement of these large animals. The preference of hippos for short grasses, which do not slip between their lips, and the fact that they frequently return to the same grazing sites, often leads to the formation of short-grass grazing lawns, which has been shown to attract and increase the abundance of other grazers (Field 1970, Lock 1972, McNaughton 1984, 1985, Owen-Smith 1988, Eltringham 1999, Kanga et al. 2013, Chritz et al. 2016). Similarly, hippos physically modify their aquatic habitats by creating wallows in their aquatic refuges, which through repeated usage, deepens pools or river beds. This increases the habitat persistence during drought periods, which in turn facilitates other species, such as fish and crocodiles. In addition, their behaviour of walking on the benthos and not swimming, increases sediment re-suspension, and can therefore elevate turbidity (Naiman & Rogers 1997, McCarthy et al. 1998, Eltringham 1999, Coughlin & Fish 2009). During repeated nocturnal foraging movements, hippos create networks of incised, vegetation-free, pathways or trails that increase connectivity between terrestrial and aquatic landscapes (Figure 1.2). These pathways can act as channels that facilitate abiotic and biotic connectivity in the form of water/material flows and the movement of organisms (fish and invertebrates) across ecosystems (Naiman & Rogers 1997, McCarthy et al. 1998, Deocampo 2002, Mosepele et al. 2009, Bakker et al. 2016).

These engineering impacts of hippos have the ability to significantly affect both terrestrial and aquatic habitats, however, the direct transfer of basal trophic resources through defecation, is arguably the most potent mechanism by which hippos mediate connectivity and material flows across terrestrial-aquatic corridors. As megaherbivores, hippos consume large amounts of grass by night, which are then excreted into aquatic systems by day

Figure 1.2: Pathways created by hippos through movement between the water and grazing lawns (a & b). Paths can increase erosion and sediment deposition at the water's edge (c) and act as channels connecting habitat patches, thereby facilitating material transport (d).

(Field 1970, Naiman & Rogers 1997, Masese et al. 2015, Subalusky et al. 2015). Unlike most other forms of cross-system transfers that are temporally pulsed (Polis et al. 1997, Nakano & Murakami 2001, Naiman et al. 2002, Richardson et al. 2010, Subalusky et al. 2017), defecation by hippos occurs daily (Taylor 2013a, Subalusky et al. 2015), resulting in unprecedented quantities of trophic resources being transferred across terrestrial-aquatic boundaries. Adult hippos are capable of ingesting approximately 40 kg of short grasses during a single 5 – 6 hour nocturnal feeding foray (Grey & Harper 2002, Coughlin & Fish 2009). Grey & Harper (2002) estimated that each hippo defecates at least once a night, expelling an approximate weight of 8 kg of dung on land. Therefore, the remaining, larger portion of dung is likely expelled into the water. It has been estimated that the entire hippo population of the Mara River (Kenya) transfers roughly 36 tonnes of grasses into the river system daily – based on defecation of roughly 8.7 kg of grass (wet weight) per hippo, per day (Subalusky et al. 2015). A gross annual input of approximately 5,840 tonnes has been estimated for Lake Naivasha (Kenya, Grey & Harper 2002). In light of these values, it is doubtful whether any other natural processes could replicate hippo-mediated resource transfers from terrestrial to aquatic ecosystems.

While the importance of hippo dung for the functioning of aquatic systems has been alluded to by researchers (Naiman & Rogers 1997, McCarthy et al. 1998, Taylor 2013a), it is only recently that these effects have been investigated rigorously. The majority of the studies on the topic have focused on the biochemical effects of dung on water quality (Gereta & Wolanski 1998, Wolanski & Gereta 1999, Subalusky et al. 2015, Dutton et al. 2018, Stears et al. 2018) or the impact of dung on particular species or groups in their aquatic habitats (McCauley, Dawson, et al. 2015, Stears et al. 2018, Subalusky et al. 2018). However, very little has been done on the effect of this allochthonous transfer at the level of whole food webs or communities (Masese et al. 2015, 2018), or the relative importance of the dung as a trophic resource within aquatic systems (Jacobs et al. 2007, Jackson et al. 2012), despite the fact that

food web and community structure is critically important for ecosystem functioning (Pimm et al. 1991, Pimm 2002). In addition, research on the effects of hippos as vectors of allochthonous transfer has been undertaken in freshwater lakes or riverine ecosystems, with little insight into the effects of these megaherbivores in estuarine ecosystems.

1.4 The St Lucia Estuary

The St Lucia Estuarine system (described in detail in Chapter 2, Fig. 2.1), located on the eastern coast of South Africa, is the largest estuarine lake in Africa and is home to a population of approximately 1000 hippos, reported to transfer approximately 2000 tonnes (dry mass) into the system annually (Taylor 2013a). Although the system is one of the most studied estuaries in the country, little is known about its resident hippo population (Taylor 1980, 2013a, Perissinotto, Stretch, et al. 2013, Prinsloo 2016). Historical records show hippos present throughout the system, however, drought conditions, human conflict and anthropogenic manipulation have resulted in the displacement of hippos southwards, with roughly 50 % of the population now occurring in a section known as the Narrows (Taylor 2013a). Due to the lack of natural predators and the protection offered within the iSimangaliso Wetland Park, the hippo population of the St Lucia Estuary has been growing at a rate of roughly 2-3 % per year (20-30 individuals, Taylor 2013a). This population growth may have important ecological repercussions, given findings elsewhere that detrimental effects, as severe as the collapse (> 90 % decline over baseline abundance) of entire ecosystems, could occur when populations of large herbivores grow beyond a certain threshold (Bakker et al. 2016).

St Lucia has historically been governed by cyclical droughts and flooding events, each lasting between 4 and 10 years, however the most recent drought period was the longest, most severe event on record, lasting in excess of 12 years (Whitfield & Taylor 2009, Cyrus et al. 2011, Humphries et al. 2016). During this period, hippo dung was seen to accumulate, and

dense mats of dung were observed forming on the benthos in areas heavily populated with hippos (Fig. 1.3). This observation formed the core basis for the research underpinning this thesis, which broadly aims to investigate the effects of hippo-mediated transfers on benthic communities and the effects of dung as a trophic resource on food webs in biotopes experiencing contrasting amounts of hippo dung inputs.

The structure of this thesis is outlined in Figure 1.4. Proceeding this general introduction and literature review, Chapter 2 provides a brief description of philosophical and conceptual considerations that underpin the questions and hypotheses presented, while outlining in detail the methods followed in this study. Two distinct approaches formed the foundation of this thesis. The first was based on a manipulative field experiment to quantify the effects of hippo dung on functionally distinct benthic communities (microphytobenthos, macrofauna and meiofauna). Results from this component are reported in Chapters 3 and 4. The second foundational approach employed was that of comparative, field surveys aimed at determining the importance of hippo dung as a trophic resource for food webs. This was based on *in situ* tracer techniques (stable isotopes and fatty acids) which were applied to food web components from biotopes that had contrasting hippo densities. Results of stable isotope analyses are reported in Chapter 5 and fatty acid analyses in Chapter 6. Chapter 7 concludes the thesis by synthesizing key findings emerging from this study.

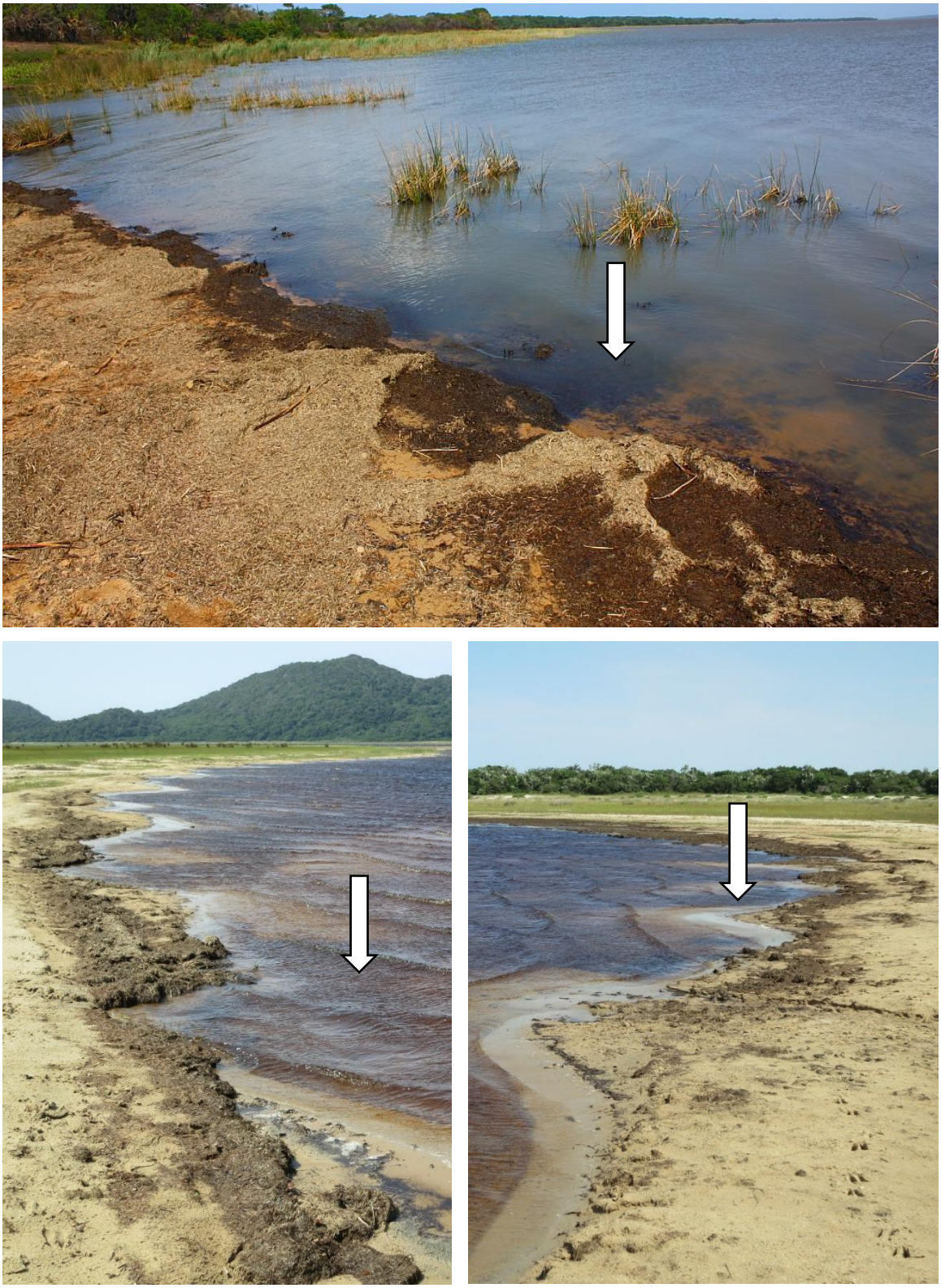
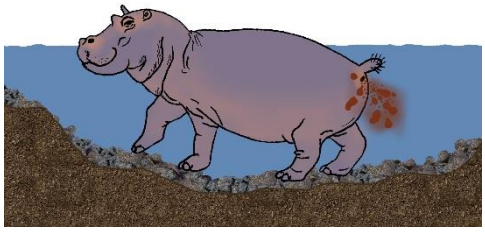


Figure 1.3: Photos taken within the St Lucia Estuarine system showing the accumulation of hippo dung at the water's edge as well as within the water, where it forms mats on the benthos (indicated by the arrows). Top photo credit: Xander Combrink.



HIPPO-MEDIATED TROPHIC TRANSFERS

CHAPTER 1
Introduction & literature review

CHAPTER 2
Methodology

CHAPTER 3
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FIELD EXPERIMENT
Dung impacts on benthic communities

CHAPTER 4
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CHAPTER 5
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Dung as a food source: fatty acid approach

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Synthesis

Figure 1.4: Thesis outline illustrating the chapter contents relative to the methodological approaches used.

CHAPTER 2:

METHODOLOGY

2.1 Approaches to answering questions in estuarine ecology

2.1.1 The scientific method

Developing a mechanistic understanding of processes and phenomena occurring in nature has its foundations in the scientific method. This framework is essentially based on deductive reasoning, which involves developing explanations for observed phenomena. The foundation of this framework is built on observation of a pattern or a change in condition within a given system. Sometimes this may involve a brief "observational study" (Manly 1992) to provide a quantitative and robust description of the pattern (Quinn & Keough 2002). Next, a model is developed, i.e. an explanation of the observed pattern (Underwood 1990, Ford 2000). The researcher then uses a combination of existing knowledge about the process or system under investigation, previous observations, existing theory or research, belief and insight to form a statement/s that explain why the observation occurred (Peters 1991, Quinn & Keough 2002). This is generally described as a hypothesis, which is falsified using various approaches, which are either experimental in nature or reliant upon field mensurative approaches. Both of these approaches form the bases of this PhD thesis, and are thus elaborated upon below, while emphasising their strengths and weaknesses in testing hypotheses.

2.1.2 Field comparisons and ecological experiments

The techniques for testing hypotheses can be represented by two distinct approaches: (1) field comparisons or natural experiments, which are non-manipulative and rely on the opportunistic use of the natural variation within a system to provide a test for a hypothesis, and (2) interventionist or manipulative experiments in which the researcher alters or controls the specific factor they wish to investigate (Connell 1974, Peterson 1980, Virnstein 1980, Reise

1985). The latter consists of both field and lab experiments and all three approaches mentioned here have distinct advantages and disadvantages.

The most natural of these techniques is that of the non-manipulative natural experiments - these include systematic sampling or observations made in an ecosystem, using nature's variability to supply "built-in" experiments (Peterson 1980). Although these "outdoor laboratories" preserve the scale and timing of events or organism responses, it is difficult to find proper controls for natural experiments (Connell 1974, Underwood 1990). The reasons for this being that ecosystems are subject to numerous fluctuations and consist of many co-interacting factors that generate confounding effects. In addition, any event that changes one factor often permeates through the entire system, leaving little opportunity available for comparative controls (Connell 1974, Peterson 1980, Carpenter et al. 1995). Moreover, there are distinct variations between natural ecosystems that prevent researchers using neighbouring or similar systems as controls (Reise 1985, Carpenter et al. 1995, Alfaro et al. 2006). The consequence of this lack of controls means that natural experiments often only offer partial tests of hypotheses and are therefore unable to "speak unambiguously to the question of mechanism" (Peterson 1980, p293). In reality, researches therefore only make inferences about causality using this technique (Connell 1974, Peterson 1980, Virnstein 1980, Quinn & Keough 2002). In contrast, manipulative experiments offer the researcher greater control, however, these involve making artificial changes to nature and therefore come with their own drawbacks (Quinn & Keough 2002).

The greatest level of control in terms of manipulative experiments generally occurs in laboratory experiments, where all variables are in theory capable of being held constant, with the exception of the one under investigation. (Connell 1974, Quinn & Keough 2002). The use of laboratory experiments enables a researcher to better observe organisms, their interactions, behaviour and responses to induced changes, thereby providing robust insight into causality

and confirmation of presumed or inferred driving mechanisms (Connell 1974, Peterson 1980). However, a major drawback is that these experiments are often conducted indoors, in cages or aquaria, making it difficult to replicate the variability and complexity of entire ecosystems. In addition, laboratory/aquaria size and available funds tend to restrict the spatial and temporal scale of experiments to smaller areas and shorter time periods than natural systems. Similarly, they tend to eliminate the natural structure of a system (Peterson 1980) as these experiments often exclude small, fragile organisms that cannot be handled easily such as microbiota and meiofauna, which in turn alters the physical and chemical contexts in which experiments are conducted (Connell 1974, Peterson 1980). In addition, the small scale of lab experiments means that researchers are less likely to be able to extrapolate results to natural systems (Virnstein 1980, Quinn & Keough 2002). These experiments are useful for questions posed of individual species responses or for low levels of organisation (molecular, cellular and organismal). However, when questions relate to responses of entire populations or communities, field experiments are more effective (Connell 1974).

Field experiments are conducted *in situ* i.e. within the ecosystem of interest. As such, all factors, except the one being investigated and manipulated, vary naturally, allowing these experiments to more adequately reflect natural variability (Connell 1974, Peterson 1980, Quinn & Keough 2002). For this reason, the results of field experiments can be more confidently extrapolated to natural ecosystems relative to laboratory experiments, provided that appropriate controls and adequate replication is used (Connell 1974). The disadvantages of this technique are that (1) changing one factor often causes others to change too and (2) the need for artificial structures or cages to facilitate the change can introduce problems such as, shading, reduction of water flow, restriction of mobile animals and increased algal growth on the new substrate/surface (Connell 1974, Virnstein 1980, Reise 1985, Quinn & Keough 2002). The controls for field experiments must therefore include cages in which no changes are made,

allowing a comparison that eliminates as many of these procedural artefacts as possible (Quinn & Keough 2002). Also important is the randomisation of the location of cages, as well as randomised allocation of treatments and controls (Quinn & Keough 2002).

Many researchers select manipulative experiments as the favoured approach to test hypotheses (Underwood 1990, Quinn & Keough 2002). When comparing the two, Connell (1974) suggested that field experiments are always better than lab experiments - if the requirements for a proper experiment could be met i.e. controls, replicates and minimised incidental disturbances. However, he did admit that in some cases this is not possible, and lab experiments therefore become more appropriate. In reality however, it is commonly accepted and suggested that ecological research includes a combination of natural and manipulative experiments as each technique tells only part of the whole story (Virnstein 1980, Quinn & Keough 2002). By combining these approaches, complementary information can be gained on natural phenomena. Virnstein (1980 p281) summed it up well by saying that no single approach "…is both controlled enough to be interpreted without question and natural enough to be extrapolated to the field".

2.2 The study site

The St Lucia estuarine system, which is the focal study area of this PhD thesis, is located in Northern Kwa-Zulu Natal, on the eastern coast of South Africa (27°52′S and 28°24′S and 32°21′E and 32°34′E, Fig. 2.1). It is the largest estuarine lake in Africa (Cyrus et al. 2011) and boasts the title of "oldest formally protected estuary" worldwide - first receiving protection in 1895 (Whitfield & Taylor 2009, Porter 2013). The system forms part of the iSimangaliso Wetland Park and was declared a RAMSAR Wetland of International Importance in 1986 and recognised as an UNESCO World Heritage site in 1999 in appreciation of its long history of protection, rich biodiversity, rare and threatened species and its considerable conservation and

tourism value (Porter 2013). In 2002 the St Lucia Estuarine system was assigned a Conservation Importance Rating of 5th out of South Africa's then 246 recognised estuaries and was reported to hold 44.9 % of the calculated South African estuarine biodiversity (Turpie et al. 2002). In addition, St Lucia is an important nursery ground for many estuarine-associated fish and invertebrate species (Cyrus et al. 2011) and is the largest protected estuarine ecosystem for a high diversity and abundance of aquatic birds (Turpie et al. 2013) as well as for two IUCN red-listed species - the Nile Crocodile (Combrink et al. 2013, Warner et al. 2016) and the Hippopotamus. The hippo population in the St Lucia Estuary is one of the largest in Southern Africa (Whitfield & Taylor 2009, Taylor 2013a).

Estuaries are constantly changing, highly dynamic systems that are sensitive to alterations in water and sediment supply - these aspects are especially relevant for St Lucia (Humphries et al. 2016). Historically, the estuary and adjacent Mfolozi River merged shortly before the Indian Ocean forming a single outlet into the sea (Whitfield & Taylor 2009, Taylor 2013b; see Fig. 2.1 for mouth configuration during 2013 - 2015 field period). The combined St Lucia –Mfolozi Mouth, like the majority of southern African estuarine systems, naturally experienced periodic closure and isolation from the ocean during drought conditions, thus allowing fresh water from the Mfolozi to flow up the St Lucia Estuary and causing water levels to rise (Whitfield 1992, Whitfield & Taylor 2009). Unfortunately, the build-up of a berm combined with increased rising water levels at the start of the wet season resulted in flooding that threatened the livelihoods of sugarcane farmers, first established along the Mfolozi flood plains in 1911 (Searle 2013). St Lucia therefore has a relatively complex and extensive management history, starting back in 1932 – when the first manually assisted beach overtopping was conducted by a single farmer and his staff (Taylor 2013b). In time, the farmers also altered the Mfolozi by constructing canals along portions of the river, causing high sediment loads to enter and accumulate in the St Lucia system and the decision to artificially

separate the combined mouth into two independent outlets (Whitfield & Taylor 2009, Taylor 2013b). This act deprived the estuary of its largest supply of freshwater and caused mouth closures to last for more extended periods of time (Whitfield & Taylor 2009, Taylor 2013b).

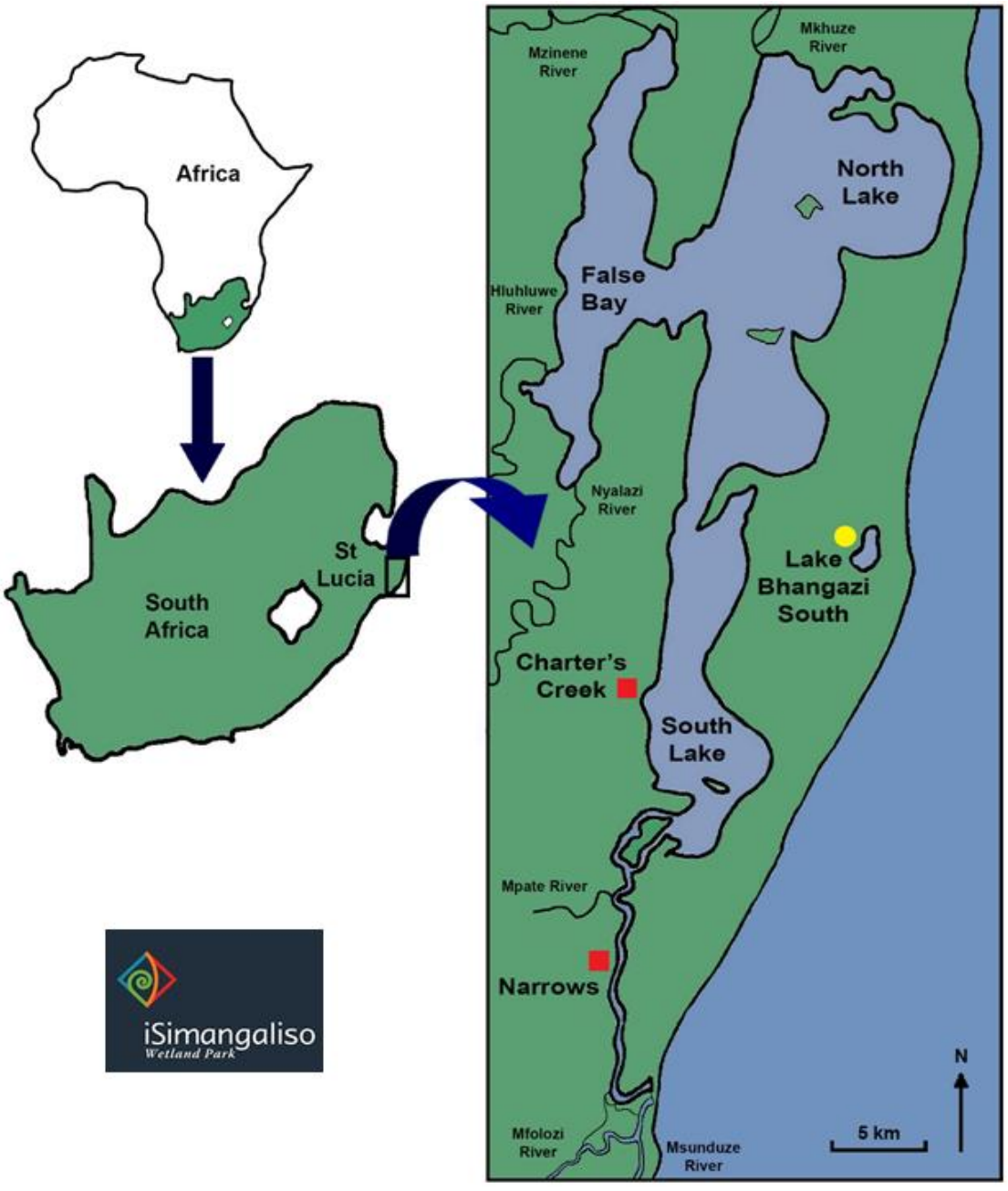


Figure 2.1: Map showing the geographical location within Africa and South Africa of the St Lucia System, the locations of the two biotopes at which sampling was conducted (Charter's Creek and Narrows – red squares) and the collection site for dung used in the experiment (Lake Bhangazi South – yellow circle). Note, the estuary map shows the system boundaries and not water levels at the time of the study.

This highly variable system is governed by cyclical, quasi-decadal dry and wet phases that mirror the local climatic regime (Begg 1978), however, the most recent mouth closure persisted for over a decade. The manipulation of freshwater inflow from the Mfolozi river, combined with increasing abstraction from the system's remaining four tributaries (Mkhuze, Mzinene, Hluhluwe and Nyalazi rivers; see Fig. 2.1) have stressed the estuary forcing it into an extreme state (Perissinotto, Stretch, et al. 2013). The recent drought events extending from 2002–2012 and 2015–2016 have been the most severe in recorded history, with water levels in the system dropping to only 10 % of the total surface area (Whitfield & Taylor 2009, Cyrus et al. 2011, Humphries et al. 2016) causing exaggerated salinities as high as 200 ppt (Cyrus et al. 2011, Perissinotto, Carrasco, et al. 2013) and severely affecting biodiversity (Pillay & Perissinotto 2008, Whitfield & Taylor 2009, MacKay et al. 2010, Cyrus et al. 2010).

Whitfield (1992) classified the system as an estuarine lake, made up of three interconnected lakes (False Bay, South and North Lake) connected to the temporarily open/closed mouth by a 21 km long meandering channel (known as the Narrows), which discharges into the Indian ocean (Fig. 2.1). The lakes and estuary encompass a total surface area of 328 km^2 (Perissinotto, Stretch, et al. 2013, Zikhali et al. 2015), however water levels fluctuate dramatically. Average water depth of the system is 0.9 m however, this too fluctuates depending on the prevailing climatic conditions as well as location within the system. While the Narrows varies between one to two meters deep, the lakes are usually shallow - averaging 0.2 m and areas of North Lake and False Bay, became completely desiccated during the recent drought (Carrasco & Perissinotto 2012).

The salinity of the system varies dramatically depending on (1) the state of the mouth, (2) the location within the system and (3) current climate - which affect rates of freshwater input as well as rates of evaporation. Generally, when the system is closed and isolated from the ocean a reverse salinity gradient forms, with salinities at the mouth varying between 1.8

and 36.1, while areas in the north range from 18.3 to 216.0 (Perissinotto, Carrasco, et al. 2013). Water temperature monitoring conduced from 2004 to 2011 reported an annual range from 15.2 to 41.2 °C throughout the system, with temperatures in shallow northern waters exhibiting a wider range than areas in the deeper Narrows and Mouth regions (Perissinotto, Carrasco, et al. 2013).

For this project, conducted towards the tail end of an extended drought, data were collected at two sites within the system: Charter's Creek (Fig. 2.2) and the Narrows (Fig. 2.3). Charter's Creek, located on the western shore of South Lake, is infrequently visited by small numbers of Hippopotamus. However, the research area within the Narrows, located between the Mpate stream inlet towards the north and the limit of the recreational fishing section towards the south, is densely populated with numerous resident hippo pods. Ariel surveys conducted in 2013 recorded densities of 1.37 and 20.62 hippos per km of shoreline at Charter's Creek and the Narrows respectively (Prinsloo 2016). In addition, although the Narrows constitutes only 2 % of the system's total surface area, it is home to roughly 53 % of the hippo population recorded in 2013 (Prinsloo 2016).

Charter's Creek is dominated by medium (500–250 μm) and fine sand (250–125 μm), contributing 39.8 % and 46.2 % to total sediment composition while sediment in the Narrows is dominated by mud/silt (< 63 μm, 75 % of total Perissinotto, Carrasco, et al. 2013). The size and shallow average water depth in St Lucia, combined with high concentrations of total suspended solids and wind driven wave action lead to generally high turbidities (Zikhali et al. 2015). Highly variable turbidity fluctuations are recorded throughout the system ranging from 1 NTU (Nephelometric Turbidity Units) to 951 NTU, with the highest readings generally recorded at Charter's Creek and the Narrows (Perissinotto, Carrasco, et al. 2013). Similarly, the system experiences dynamic nutrient loading, with Dissolved Inorganic Phosphorus (DIP)

ranging between 0.0001 and 15.14 μM and Dissolved Inorganic Nitrogen (DIN) fluctuating between 0.001 and 770 μM (Perissinotto, Carrasco, et al. 2013).

Vegetation types found surrounding the St Lucia estuarine system include savannah, thicket, woodlands, grasslands, coastal forests and wetlands (Scott-Shaw & Escott 2011). A recent study on the spatial and behavioural ecology of hippos in the St Lucia system identified "proximity to wetland vegetation" as one of the primary factors influencing the spatial distribution of hippos, along with water depth, and proximity to humans (Prinsloo 2016). In addition, the Narrows was shown to be surrounded by more suitable grazing vegetation for hippos than the area surrounding Charter's Creek (Perissinotto, Stretch, et al. 2013, Prinsloo 2016). Hippos predominantly feed on short, C4 photosynthesising grasses from the surrounding shoreline (Field 1970). Although it has been observed that hippos may feed on macrophytes within aquatic systems, contributions of these are generally minor compared to C4 grasses (Grey & Harper 2002).

2.3 Experimental design (Chapters 3 & 4)

In situ mesocosm experiments, which form the bases of Chapters 3 and 4, were conducted at two sites (150 m apart; water depth 40-50 cm) within Charter's Creek in the St Lucia Estuary (27°52'S & 28°24'S and 32°21'E & 32°34'E, Fig. 2.2). Ten randomly interspersed (2-3 m apart) inclusion/exclusion cages (height = 1 m, width and length = 50 cm) were deployed at each of the two experimental sites from the start of October to the end of November 2014. The two treatments employed were dung inclusion and dung exclusion (n = 5 each per site). Cages were composed of a frame made of four 1 m pine rods (2 cm diameter) surrounded by 3mm shade mesh and hammered 30 cm into the sediment. Cage tops were uncovered and had a clearance of 10 cm above the water's surface (Fig. 2.2c). Cages were left

unmanipulated for two days post-installation, allowing the sediment to resettle and to ensure cages remained in place, without shifting, or sinking.

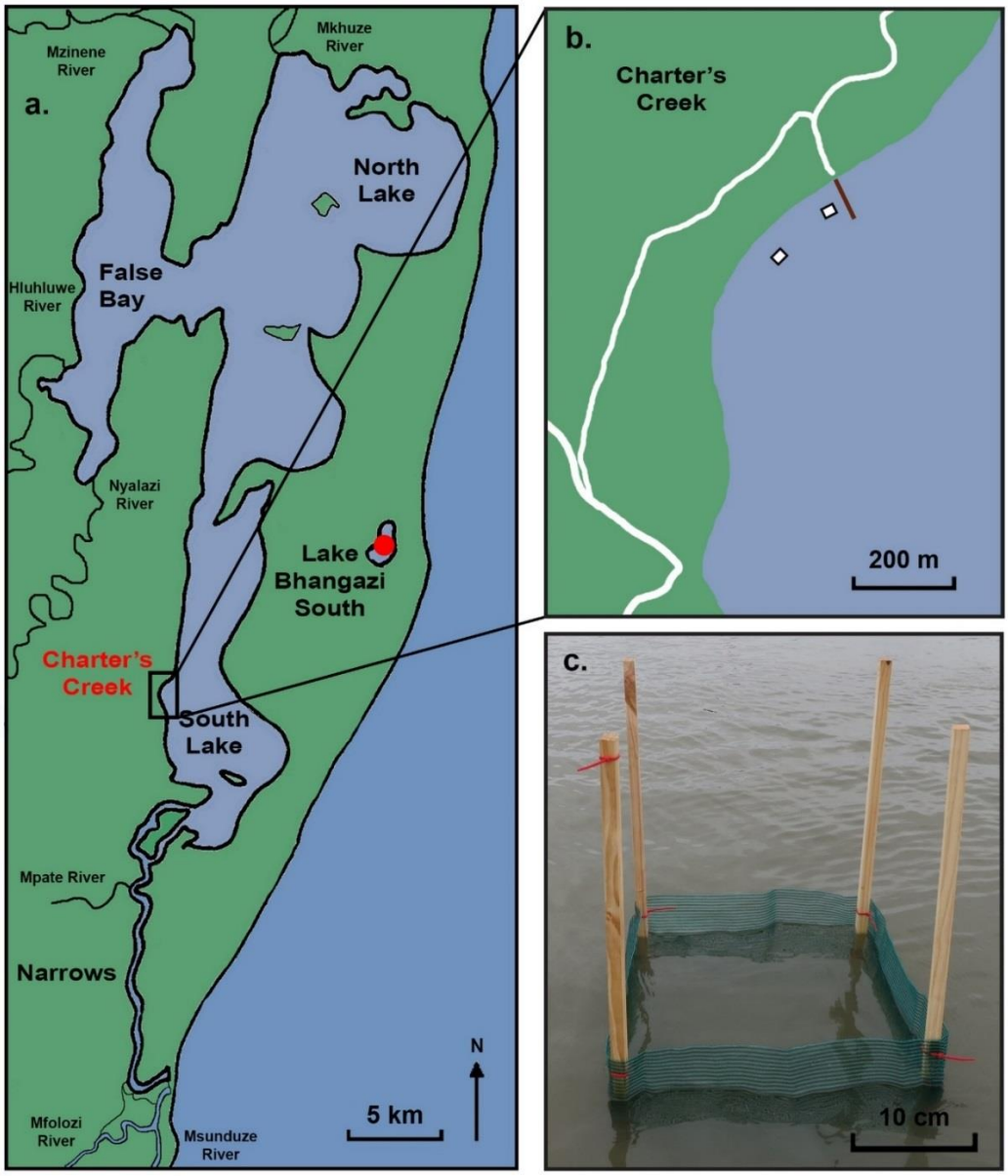


Figure 2.2: Map showing a) location of the experimental site, Charter's Creek – within the St Lucia Estuarine System and the area where fresh dung was collected (Lake Bhangazi South circle), b) the location of the two sample sites (1 and 2) within Charter's Creek relative to the management jetty and c) a close up of an experimental cage.

Fresh hippo dung, voided within 24 hours, was collected weekly by walking with a park ranger along hippo paths adjacent to Lake Bhangazi South, on the eastern shore of the estuary

(Fig. 2.2a). Dung samples from 3-5 individual middens were pooled and homogenised, a subset of roughly one cup (300 ml) was added to dung inclusion cages, once per week for a period of six weeks. Inspection of cages prior to weekly dung additions, indicated that previously added dung did not accumulate – possibly indicating rapid disintegration by microorganism activity and wind mixing. The amount of dung added to inclusion cages was determined from prior sampling of sites within the Narrows where hippos were abundant. This involved quantifying mean volume of dung recorded in benthic grab samples (n = 2 per site, area = 0.026 m^2, depth = 20 cm) collected at nine sites along a 2-3 km section and then scaling up to the area of each experimental cage. For this component, grab samples were collected no further than 150 m from three resident hippo pods ranging in size from 15 to 30 individuals per pod. Within 8 hours of collection, replicate grabs from each site were combined in buckets, sieved (2 mm) and the volume of remaining dung was measured in a sample jar. Exclusion cages were left unmanipulated, with no dung added.

Sample collection and processing: Upon termination of the experiment, the response of microphytobenthic biomass (as chl-*a*) was assessed using sediment cores (n = 2 per cage, depth = 1 cm, diameter = 2 cm). Samples were stored in 30 ml of 90 % acetone and refrigerated for 48 hours before chl-*a* concentrations were determined using a Turner Designs Trilogy fluorometer. In addition, sediment cores for assessing the response of benthic macrofaunal (n = 2 per cage, diameter = 10 cm, depth = 15 cm) and meiofaunal (n = 3 per cage, diameter = 2 cm, depth = 1 cm) assemblages were collected. Macrofaunal cores were sequentially washed through a 500 μm sieve five times before being passed through a 2 000 μm sieve - the material captured within the sieves was combined and preserved in an ethanol (70 %) and Rose Bengal (5 %) solution. Meiofauna samples were sieved in the laboratory through a 400 μm mesh to remove any macrofauna and then again through a 40 μm mesh and preserved (ethanol-Rose Bengal solution). Macro- and meiofaunal organisms were identified to the lowest possible

taxonomic level and enumerated. The biomass of each macrofaunal taxon was determined per sample using a Mettler ToledoMX5 balance (precision = 1 µg). The size of discriminating macrofaunal and meiofaunal taxa, identified by SIMPER to cumulatively account for 90 % of the community dissimilarity between treatments at each site, was determined using photographs input into ImageJ (an open source image processing program). Macrofauna were photographed using a Leica dissecting microscope and meiofauna using a Leica DM 500 compound microscope, each fitted with a Leica ICC50 camera.

Statistical analyses. All multivariate analyses were run using PRIMER v6.1 (unstandardized and transformed Log (x + 1) data). Spatial variability in macro- and meiofaunal community structure (based on abundance and biomass data for macrofauna and abundance data for meiofauna) was visually assessed using non-metric multidimensional scaling ordinations (nMDS), with PERMANOVA (permutational analysis of variance) providing quantitative support for groupings, based on Bray-Curtis similarity matrices. For PERMANOVA analyses, dung treatments (dung exclusion/inclusion) were nested within site (the highest spatial factor of a nested hierarchical design). Multivariate dispersion of samples within treatments was calculated using the PERMDISP function.

For macrofauna, the DOMINANCE function was used to construct ABC (abundance biomass comparison) curves and calculate W statistics, in order to investigate the effect of dung enrichment on ranked species abundance versus biomass. SIMPER (similarity percentages) was used to identify the discriminating taxa that cumulatively contributed 90 % to community dissimilarity between dung treatments within sites. Macrofaunal community descriptors (total abundance, biomass, taxonomic richness, evenness and diversity) and meiofaunal community descriptors (total abundance, richness, evenness and diversity) were calculated using the DIVERSE function. Nested ANOVA (analysis of variance) was employed to determine the influence of site and dung enrichment on benthic chl-*a* levels, as well as macro- and meiofaunal

community descriptors. The effects of site and dung treatment on the abundance and size of individual macro- and meiofaunal taxa, identified by SIMPER, were also determined using Nested AVOVA. Tests for normality (Q-Q plots) and homogeneity of variances (Bartlett Tests) were conducted to meet the assumptions required for parametric testing. Where necessary, data were transformed (Log (x + 1), square root or 4^{th} root) preceding any parametric testing. The statistical programming language, R, was used to conduct all univariate statistical tests.

2.4 Stable isotope and fatty acid analyses: (Chapters 5 & 6)

Four seasonal sets of food web samples were collected from two biotopes (the Narrows and Charter's Creek) within the St Lucia estuary; (n = 4, i.e. March, July, November 2014 and February 2015, hereafter referred to as Seasons 1 – 4). These samples were subjected to stable isotope and fatty acid analyses, which form the bases of Chapters 5 and 6 of this thesis.

Three sites, located between the upstream limit of the recreational fishing boats and the Mpate River inlet (covering a total distance of 2-3 km), were sampled in the Narrows. Each site was located next to a resident hippo pod (between 15 to 30 hippos per pod) and comprised three subsites, with two being ±50 m upstream and downstream of the pod and one being opposite the pod (50 m away). The layout for sampling Charter's Creek was similarly comprised of three subsites (spanning ±100 m each) sampled for each of the three sites located north of the management jetty. The total distance across sampling sites was 2-3 km (Fig. 2.3).

Physico-chemical measurements were collected at each of the nine subsites per biotope to provide environmental data across all four sampling seasons. Two exceptions occurred at Charter's Creek however. Firstly, in season 3, there was a single biotope measurement collected - due to instrument malfunction. Secondly, low rainfall levels at Charter's Creek during season 4 left sites C1 and C2 desiccated and inaccessible resulting in three

measurements being taken at site C3 (Fig. 2.3). Water temperature, salinity, turbidity and dissolved oxygen were recorded using an YSI 6600-V2 Multisystem probe. Water depth was determined using an incrementally marked wooden pole (precision = 0.1 m).

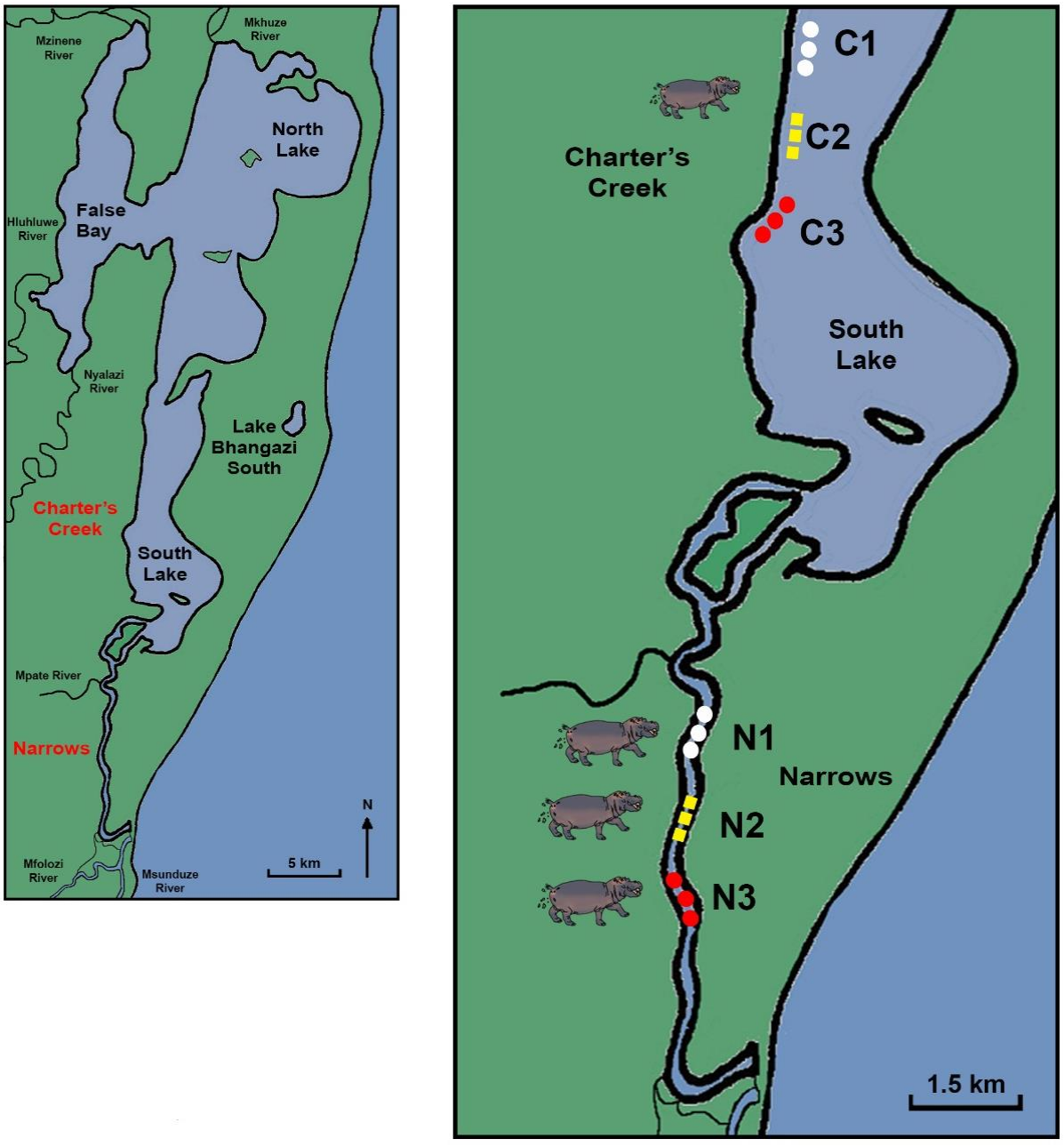


Figure 2.3: Map showing all sampled subsites (circles) within each site (numbered and colour coded) at the two biotopes, Charter's Creek (C) and The Narrows (N), and their location within the Estuary.

Food web components for fatty acid (FA) and stable isotope analysis (SIA) were collected from multiple trophic levels at each subsite (microphytobenthos – MPB, sediment organic matter – SOM, particulate organic matter – POM, zooplankton, benthic macrofauna and dominant fish species). Sediment samples (n = 3 pooled cores per subsite), for determination of MPB and SOM, were collected from the top 1 cm of sediment using a 2 cm (inner diameter) corer. MPB was separated from SOM by making use of the phototactic migration of diatoms (Riera & Richard 1996). Samples were treated in a procedure similar to that described by Couch (1989) as used by Carrasco & Perissinotto (2011). Briefly, moist sediment cores were spread in small trays, with a fine mesh (500 µm) placed upon the sediment surface and covered by an evenly distributed layer (3-5 mm) of sterilized sand (autoclaved at 450°C). Samples were exposed to a direct florescent light source for 6-8 hours, while being kept moist by adding small amounts of filtered estuary water (Whatman glassfibre filters - GF/Fs). Following illumination, the mesh was lifted along with the top layer of sand and migrated MPB, while the remaining sample was kept for SOM determination. Sediment samples were stored in foil envelopes and frozen at -10°C in the field and at -80°C upon returning to the laboratory. If 2 % hydrochloric acid (HCl) dropped onto the sediment with a dropper caused the sample to bubble vigorously, samples were acidified with 2 % HCl, then rinsed in distilled water to remove any potential inorganic calcium carbonate ($CaCO_3$). Sediment samples were lyophilized at -60 °C (VirTis Benchtop K) for 24-48 h. MPB samples were first sieved though a 20 µm sieve to remove the autoclaved sand before being lyophilized. For SIA, approximately 65 mg of SOM sample and 45 mg of MPB sample were packaged separately into 12 x 6 mm pressed tin capsules. For FA analysis, roughly 1500 mg of SOM and all of the remaining MPB sample were weighed (Mettler Toledo XP205 balance) and stored in lipid-cleaned 10 ml test tubes for further analysis (see below). This method may favour the

extraction of the more mobile, phototactic diatoms relative to other groups of microflora, however, it is the only method available for field extraction of MPB from SOM.

A one litre water sample was collected at each subsite for analysis of POM. Samples were homogenised and divided into two 500 ml portions which were filtered, first through a 100 µm mesh - to removed zooplankton and detritus, then onto pre-combusted Whatman glassfibre filters (GF/Fs), one each for SIA and FA analysis. If necessary, filters were acidified with 2 % HCl to remove inorganic $CaCO_3$. Zooplankton samples were collected using an epibethic D-sled (radius = 18 cm, mesh = 100 µm) trawled for ± 30 m (n = 1 drag per subsite). Samples were filtered onto 100 µm Nitex mesh, enclosed in foil envelopes and frozen. In the laboratory, POM and zooplankton filters were freeze dried (lyophilized). One POM filter per subsite was scraped and weighed into 12 x 6 mm pressed tin capsules for SIA and one into a lipid-cleaned 10 ml test tube for FA. Zooplankton samples were ground into a fine powder using a mortar and pestle - a 1 - 1.2 mg sample was weighed into tin capsules for SIA and a 25 mg sample stored in test tubes for FA. Due to the fact that fatty acid tissue samples degrade if not frozen, it was not feasible to sort the zooplankton samples into dominate taxa, as the time needed to do this would ruin the samples. Therefore, it was only possible to obtain an overall, combined zooplankton sample for both SIA and FA signature.

A Zablocki Type Ekman grab (area = 0.026 m^2) was used to collect benthic macrofauna. Two grabs per subsite were combined and washed through a 500 µm sieve (x5) and a 2000 µm sieve and temporarily stored in jars. Individuals of each macrobenthic taxon were sorted within 12 hours of collection and frozen in foil envelopes for further processing in the lab. While all taxa present within each season were collected only dominant taxa, that occurred across all four seasons, were used for comparative analyses. These were the amphipod *Grandidierella bonnieroides* and the isopod *Cyathura estuaria*. The small size of both species meant that in order to have enough tissue for both stable isotope and fatty acid analysis, all individuals within

a taxon were pooled and crushed using a mortar and pestle, creating a single subsite value for each taxon when present. A 1 - 1.2 mg sample of both species was encapsulated for SIA and all remaining tissue put into test tubes for FA analysis.

Dominant fish species (n = max 3 per species) at each subsite within Charter's Creek, were collected using a purse-seine net, dragged by boat from the shore line. Due to the inaccessibility of the shoreline within the Narrows, fish samples were collected using a castnet (radius = 2 m) operated from a small boat. Dominant species were defined by Kon et al. (2015) as species represented by three or more individuals per site per sampling season. Fish samples were identified and measured (total length). For larger species a 2 x 2 cm sample of muscle tissue was cut from below the dorsal fin before being frozen in foil envelopes, while smaller species were frozen whole. Once in the laboratory, tissue samples were lyophilized for 24-48 hours and homogenised to powder using a lipid cleaned mortar and pestle. A 1 - 1.2 mg sample was encapsulated for SIA and roughly 30 mg were stored in test tubes for FA. Dominant fish species common across both biotopes and multiple seasons included tilapia - *Oreochromis mossambicus*, mullet - *Chelon (Liza) dumerili* and glassy - *Ambassis ambassis*.

Under the supervision of an Ezemvelo KZN Wildlife game ranger, hippo dung samples from five fresh (voided within 24 hours) dung middens, were collected along hippo pathways adjacent to the Narrows shoreline (Fig. 2.4). As a safety precaution, I was not allowed to conduct dung sample collections alone and a shortage of available game rangers during sample periods meant that only two seasonal dung samples were collected. The isotopic (T-test: $\delta^{13}C$ $p = 0.125$, $\delta^{15}N$ $p = 0.857$) and fatty acid (PERMANOVA: $p = 0.260$) signatures for these dung samples did not differ significantly between the two seasonal samples and therefore the samples were pooled to produce a single dung signature used for all analyses.

In order to determine the extent to which the dung could be traced within higher trophic positions, I attempted to obtain tissue samples of two apex predators within the St Lucia Estuary – the bull shark (*Carcharhinus leucas*; from fisherman) and Nile crocodile (*Crocodylus niloticus*). No Bull Shark tissue samples were collected due to there being no catches by local fisherman during the study period.

Figure 2.4: Fresh dung samples (a) collected along hippo pathways adjacent to the Narrows (b) in the presence of a park ranger (c).

Crocodile tissue was obtained from live captures as part of an ongoing specialist study by Dr Xander Combrink (Ezemvelo Wildlife). Ethical clearance and permits were covered as part of Dr Combrink's research. Nile Crocodiles were captured using noosing and "short game" techniques outlined in Combrink et al. (2012). Briefly, crocodile capture occurred at night when a spotlight could be used to find the animals. For noosing, capture took place from a boat using a self-locking cable, opened to form a noose, connected to the end of a Kevlar rope and attached to a long pole (Fig. 2.5a). Upon approach, the noose was slipped over the head of the crocodile and closed (Fig. 2.5b), tethering the crocodile to the rope (which in turn was secured to the boat to prevent escape). Alternately for "short game", the preferred sampling method for

the shallower waters of the back channel where boat access was limited, an 8/0 weighted treble hook (with barbs removed) attached to a Kevlar rope, was thrown beyond a crocodile and then quickly drawn back in over the animal to hook into the epidermal scales (Fig. 2.5c-e). Once drawn closer to the shoreline or boat, the crocodile could be noosed as mentioned above and manoeuvred on shore. Special care was taken to ensure that the noosed animal's head was kept above water when being moved towards the shoreline. The mouth of the animal was secured closed using one or two large cable ties, set in place by a rope fed through a hollow PVC pipe (1.5 m), and then further secured with duct tape. The animal's eyes were covered to reduce visual stimuli and stress, and the back legs of larger individuals were restrained with rope (done carefully to minimise risk of injury to the limbs and joints - Fig. 2.6).

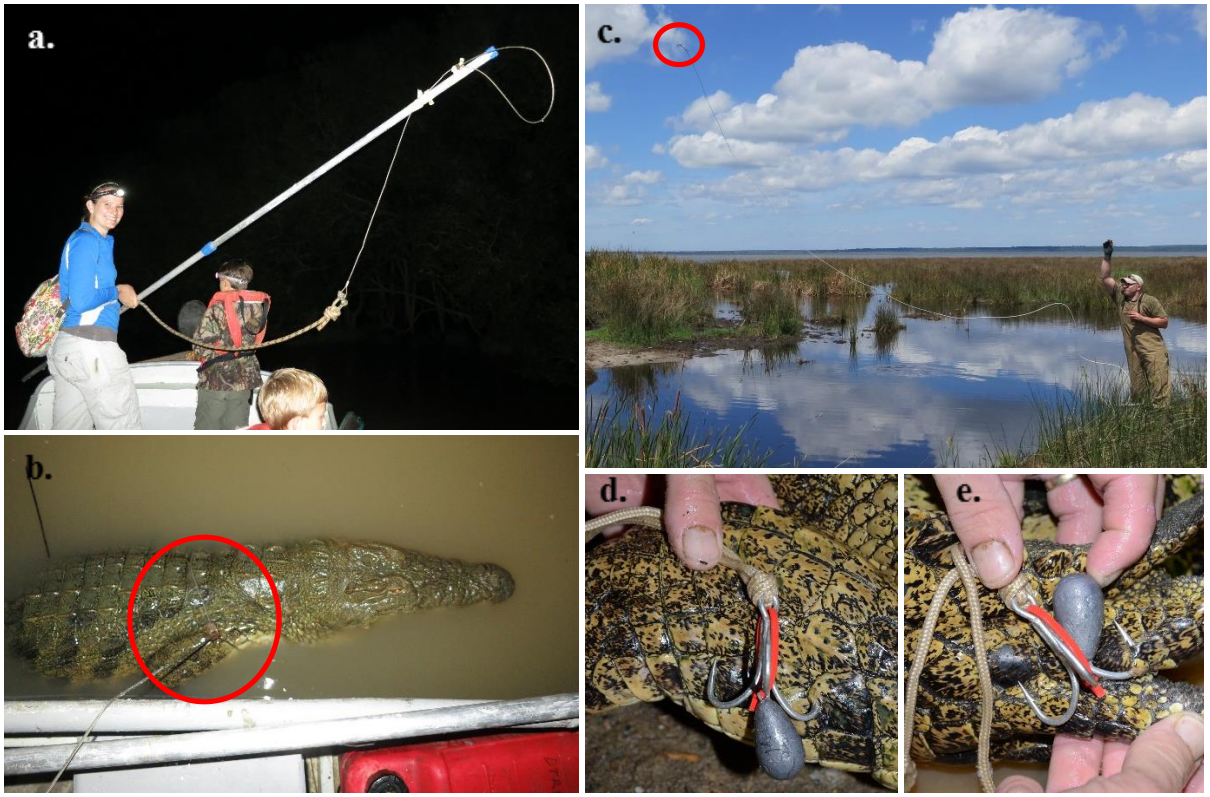


Figure 2.5: Crocodile capture techniques; a) noose with self-locking cable, rope and pole; b) example where self-locking cable was closed/secured behind crocodile's head, c) throwing "short game" from shoreline - circle indicating the location of treble hook attached to end of the Kevlar line, and d-e) weighted treble hook caught in epidermal scales.

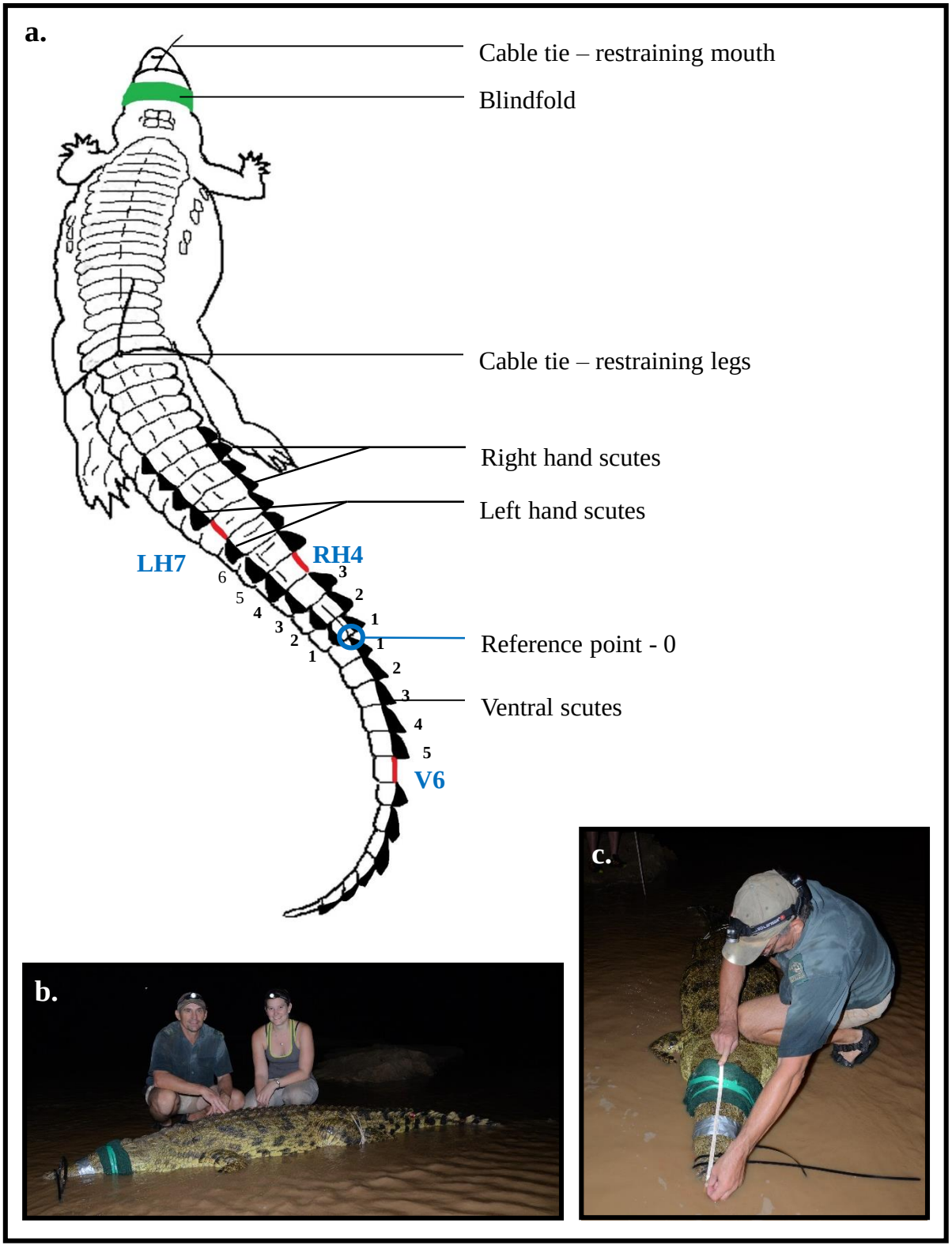


Figure 2.6: a) How to mark a Crocodile: The blue circle marks the reference point from which to start numbering scutes. Individual named LH7-RH4-V6 – hence the removal of scutes marked in blue, b and c) the correct method for restraining a captured crocodile.

For the purposes of this research, only tissue samples were needed, however for the research done by Dr Combrink, data collection included morphometric measurements, sexing the animal, as well as marking the individual with a unique tail scute cutting - the latter being the method by which tissue samples were acquired for this PhD project. The predetermined tail scutes from numbered locations (Fig.2.6a) were cut as quickly and efficiently as possible – removing one scute from the left, right and ventral ridge of the tail. Tissue samples were stored in foil envelopes and kept frozen at -80 °C. In the laboratory samples were pulverised into powder using a mortar and pestle and aliquots of 1 - 1.2 mg were encapsulated for SIA and roughly 50 - 60 mg stored in test tubes for FA analyses.

Stable isotope analysis. Samples were processed at the Stable Light Isotope Laboratory (Archaeology Department, University of Cape Town, South Africa) combusted in a Flash 2000 organic elemental analyser and passed through a Delta V Plus isotope ratio mass spectrometer (IRMS) via a Conflo IV gas control unit (all supplied by Thermo Scientific, Bremen, Germany) to determine stable carbon (^{13}C and ^{12}C) and nitrogen (^{15}N and ^{14}N) concentrations and ratios. SI values were adjusted using in-house[1] and certified (Sigma Valine and Merck Gel) standards, calibrated against IAEA (International Atomic Energy Agency) standards. Isotope ratios were expressed using the delta (δ) notation, represented as the relative (parts per mille, ‰) difference between samples and the international standards (Pee-Dee Belemnite limestone for carbon and atmospheric N_2 for nitrogen):

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

[1] UCT In-house standards: ‘Choc’ - a commercial chocolate/egg mixture sourced from a colleague in USA and ‘Seal’ – crushed seal bone, demineralized and dissolved in acid, then reconstituted in gel form, made by UCT.

where δ(‰) is $\delta^{13}C$ or $\delta^{15}N$ and R_{sample} and $R_{standard}$ are the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratios of the sample and standard, respectively. The precision of all measurements was ±0.09‰ and ±0.12‰ for carbon and nitrogen, respectively.

Biplots of $\delta^{13}C$ vs $\delta^{15}N$ values for each biotope across all four sampling seasons were constructed to show the relative positions of the food web components, common to both biotopes, in bivariate isotope space (Fig. 5.1). Plots showing separately the seasonal $\delta^{13}C$ and $\delta^{15}N$ differences/shifts between biotopes were composed for all individual components of the food web: basal resources - POM, SOM and MPB (Fig. 5.2) and primary consumers – *G. bonnieroides, C. estuaria* and zooplankton (Fig. 5.3). The fish data was assessed both as pooled samples of all individuals of the same species and with data separated into size classes for the dominant species occurring in both biotopes in more than one season (Fig. 5.4 – 5.8). A Nested-ANOVA (analysis of variance) – designed with season as the highest hierarchical factor, followed by biotope (Narrows/Charter's Creek) within which site was nested – was employed to test the effects of season, biotope and site on the $\delta^{13}C$ and $\delta^{15}N$ values of the various food web components.

To determine the proportion of hippo dung contributing to the diets of consumers within the two biotopes, SIA data were analysed using R and JAGS software and the 'MixSIAR' package (Plummer 2003, R Core Team 2016, Stock & Semmens 2016a). Trophic enrichment factors (TEFs) for $\delta^{15}N$ were specified as 2.5 ± 1.0 SD for invertebrates and 4.0 ± 1.0 SD for fish species, and TEFs for $\delta^{13}C$ were set at 1.0 ± 0.5 SD for both. These values reflect a compromise between generalised published fractionation values (Vander Zanden & Rasmussen 2001, Post 2002) and calculated site-specific values (Caut et al. 2009, Bird et al. 2016). Furthermore, MixSIAR is robust towards a reasonable level of uncertainty in the trophic enrichment factor estimates by accommodating this within a residual error term (Parnell et al. 2010, Stock & Semmens 2016a), hence the conservative fractionation values used.

Consumer diets were assessed using MixSIAR models run with Residual*Process error term to improve model estimates and accounting for variability in consumer tracer data (Stock & Semmens 2016b, a). Bayesian mixing models, such as those used in MixSIAR, can now assign diet composition with greater certainty than simple linear mixing models. These models also allow for a greater number of sources to be included in the model than the previously accepted n + 1, where n is the number of tracers used (Phillips & Gregg 2003, Parnell et al. 2010). An example of this can be seen in Rishworth et al. (2017) where MixSIAR was used to determine the proportional contribution of eight sources to the diets of macrofaunal consumers.

However, it is important to ensure that the isotopic signatures of sources are distinct (Vander Zanden & Rasmussen 2001). Source signatures that are not distinct should be combined if the following criterion are met; 1) Isotopic signatures should be clustered and not significantly different and 2) the sources must be logically related - it must make biological sense to combine the sources (Phillips et al. 2005). For this reason, the variability among source isotopic signatures, within a given season and biotope, was visually assessed using isoscape plots produced in R and statistically by running an ANOVA to assess whether $\delta^{13}C$ and $\delta^{15}N$ values were significantly different. Any sources that did not differ significantly in both carbon and nitrogen isotopic values were combined into a single source. Of these, only those that could logically be pooled (i.e. both sources pelagic or benthic in origin) were combined prior to running models. Markov Chain Monte Carlo (MCMC) run length was determined by assessing the model convergence with the Gelman-Rubin and Geweke diagnostic (Stock & Semmens 2016b, a). Posterior probability distributions of the available food sources were assessed for dietary composition and the most likely proportions of sources contributing to the diets of species were plotted using the medians (50 % quantiles).

Fatty acid analysis. For FA analyses all samples were stored at -80 °C until they could be lyophilized at -60 °C (Freeze dried, VirTis Benchtop K) for 24-48 h. Tissue samples were

homogenised in a lipid washed mortar and pestle and filter samples were peeled, before being weighed for dry mass determination (Mettler Toledo XP205 balance). Lipids were extracted and fatty acid methyl esters (FAMEs) derived using established protocols with one-step method modified from Indarti et al. (2005, see also Richoux et al. 2010). Aliquots of tissue (specific dry mass dependant on tissue type as indicated above) were added to thrice lipid cleaned 10 ml test tubes containing 2 ml chloroform ($CHCl_3$) and 0.01 % butylated hydroxytoluene (BHT). A small measure of fatty acid internal standard (10-20 µl of 6-8 mg of nonadecanoic acid standard (19:0) per 10 ml of $CHCl_3$) was added to each sample before test tubes were flushed with nitrogen, sealed with Teflon tape and stored at -20°C. A mixture of sulphuric acid (H_2SO_4) and anhydrous methanol [(MeOH) ratio: 0.3:1.7] was added to samples before being re-flushed with nitrogen and teflon caps, vortexed, sonicated in an ice bath for 5 min and heated for 30 min at 100°C. Once cooled to room temperature, samples were diluted with 1 ml ultrapure (milliQ) water and centrifuged for 3 min at 3000 rpm. The upper aqueous layer of the stratified sample was removed and discarded, the lower lipid layer containing the FAMEs was dried using sodium sulphate (Na_2SO_4) and rinsed through a drying filter before being concentrated under nitrogen gas and then suspended in Hexane. For crocodile tissue samples, polar (structural) and neutral (reserve) lipids were separated following total lipid extractions (Christie 2003).

Gas chromatographic (GC) analysis of FAMEs suspended in hexane was performed using an Agilent 7890 equipped with a ZB-Waxplus 320 column (30 m long x 0.32 mm internal diameter) and a flame ionization detector with helium as the carrier gas. Aliquots of sample were injected using a G7683 auto-injector into GC oven under the following temperature programme: 70°C for 1 min, raised to 170°C at 40°C/min for 4 min, finally raised to 250°C for 4.5 min – total run time = 40 min. Chemstation (version B.04.02) was used to integrate and calibrate FAME peaks produced by the flame ionisation detector (FID). Representative

samples of each species/sample type were analysed using a gas chromatography/mass spectrometer (GC/MS; Agilent Technologies 7000 GCMS-QQQ running Masshunter version 5.00 and the NIST 08 MS library) equipped with an identical column type and temperature protocol as the GC analyses. These samples, combined with comparisons of retention times produced by know external standards, were used to confirm the identity of peaks produced by FID. Comparing FAME peak areas with the peak area of the know concentration of internal standard (19:0), the amount of FAME in each sample could be quantified as a fatty acid weight (mg/g dry mass of sample). Quantitative values were then transformed into qualitative (proportional) data, which were expressed as a percentage of the total proportion of fatty acids within each sample.

All multivariate analyses were run using PRIMER v6.1 with unstandardized, qualitative fatty acid abundance data. The spatial variability of the total fatty acid profiles of species from the two biotopes was visually assessed using non-metric multidimensional scaling ordinations (nMDS) and statistically assessed using PERMANOVA (permutational analysis of variance) based on Bray-Curtis similarity matrices. Specifically, nMDS was used to plot fatty acid profiles of basal resources and consumers, of each season, in two-dimensional space. Basal resources were plotted together with the dung fatty acid profile to visually assess if all sources were different. Alternately, the primary and secondary consumers were all plotted in separate ordinations for each season as ordinations across seasons contained too many data points to allow spatial discrimination. For fish species, only pooled data were plotted due to low sample numbers of some size classes. For PERMANOVA analyses, a nested hierarchical design was used with season as the highest spatial factor, within which biotopes (Narrows/Charter's) were nested. SIMPER (Similarity percentages) was used to determine which fatty acids contributed to 90 % (cumulatively) of the dissimilarity between the two biotopes and the similarity between the food web profiles within each biotope and the dung profiles.

The mean proportions (as a percentage of total fatty acids) of recognised biomarkers were used to compare the fatty acid profiles of all food web components between the two biotopes. These biomarkers included (1) a terrestrial marker: sum 18:2ω6 + 18:3ω3 (Budge & Parrish 1998, Budge et al. 2001, Dalsgaard et al. 2003), (2) a bacterial marker: sum of 15:0, 17:0, and all iso- and anteiso-branched chain fatty acids (Haddad et al. 1992, Harvey 1994, Budge & Parrish 1998, Brett et al. 2006), (3) the sum of Essential Fatty Acids (EFA – sum 20:4ω6, 20:5ω3 and 22:6ω3, Brett et al. 2006, Hixson et al. 2015, Moyo et al. 2017) and (4) a diatom marker: Σ16/Σ18 (sum of all acids containing 16 carbon atoms/sum of all acids containing 18 carbon atoms, Parrish et al. 2000, Budge et al. 2001, Dalsgaard et al. 2003). The values were plotted in bar graphs for visual assessment and Nested ANOVAs (analysis of variance) conducted using R, were employed to determine the effects of biotope and season.

CHAPTER 3:

BENTHIC MACROFAUNAL RESPONSES TO EXPERIMENTAL ENRICHMENT BY HIPPO DUNG[2]

[2] Results from this chapter have been published in:
Dawson, J., Pillay, D., Roberts, P.J. and Perissinotto, R., 2016. Declines in benthic macroinvertebrate community metrics and microphytobenthic biomass in an estuarine lake following enrichment by hippo dung. Scientific Reports, 6, p.37359.

3.1 Introduction

3.1.1 Ecosystem stress and benthic macrofauna

Growing human populations and development along coastlines is threatening the integrity and resilience of marine and estuarine ecosystems globally (Loreau et al. 2001). Global change and associated concerns about our ability to monitor, manage and mitigate stressor effects on our natural environments (Rodil et al. 2013) has led to increased attention and interest in whole ecosystem research, with a focus on biodiversity loss and consequences for ecosystem functioning (Loreau et al. 2001, Cardinale et al. 2012, Hooper et al. 2012, Tilman et al. 2014). Observations of community responses is one of the methods commonly used to detect stressor effects, describe the consequences of natural and anthropogenic disturbances and infer possible causes and consequences of ecosystem change (Rodil et al. 2013).

Benthic macrofauna, typically defined as bottom substrate-dwelling organisms that are larger than 500 µm (Warwick et al. 1986, Whomersley et al. 2009), are often used as indicators of stressor impacts and associated impairment of ecosystem functioning. Indeed, some research has shown that benthic macrofauna are more sensitive to stressor impacts than other benthic organisms (Josefson & Widbom 1988, Whomersley et al. 2009), resulting in macrofauna being widely used as environmental indicators (Pearson & Rosenberg 1978). This is also partially because their larger sizes and ease of handling means that the biology of macrofaunal species is generally well-known and taxonomic expertise is more readily available compared to meio- and microfauna (Gray et al. 1988, Pollack et al. 2011). Macrofauna are ideal bio-indicators because of their generally sedentary nature and relative longevity (> 2 years for some species), which enables them to reflect local conditions over an extended period of time, thus making them useful indicators in long term studies (Gray et al. 1988, Josefson & Widbom 1988, Whomersley et al. 2009, Pollack et al. 2011).

Macrofauna are critically important functional components of benthic ecosystems (Gaston et al. 1998). Their intermediate trophic position allows them to exert both consumer-induced top-down and resource-induced bottom-up controls on food web components (Pollack et al. 2011, Huang et al. 2013). In addition, bioturbation by macrofauna affects physical and biogeochemical contexts at the sediment-water interface (Gaston et al. 1998, Lohrer et al. 2004, Lee 2008, Van Colen et al. 2009), by (1) increasing sediment oxygenation, porosity and penetrability (Lohrer et al. 2004, Pillay et al. 2011), (2) facilitating the transport of deep particulate organic matter (Herman et al. 1999, Needham et al. 2011, Gladstone-Gallagher et al. 2017) and (3) increasing concentrations of extracellular polymeric substances (EPS) through mucus addition or bacterial stimulation (Dawson & Pillay 2011). Other important functions of benthic macrofauna include: microphytobenthic control via grazing (Lohrer et al. 2004, Huang et al. 2013), nutrient recycling (Gaston et al. 1998, Lee 2008, Needham et al. 2011), decomposition of organic matter (Gaston et al. 1998, Lohrer et al. 2004), supporting fisheries by acting as trophic resources (Gaston et al. 1998, Cyrus & Vivier 2006, Pillay et al. 2013) and acting as bio-indicators of pollution and environmental stress (Pearson & Rosenberg 1978, Pollack et al. 2011, Dittmann et al. 2015).

3.1.2 Estuaries and hippopotamus

Estuaries are highly dynamic ecosystems that display strong connections to the atmosphere, oceans, freshwater catchments and land. Such connectivity is achieved by complex processes occurring across ecosystem boundaries, and is critical for the ecological functioning of estuarine and neighbouring ecosystems (Polis et al. 1997, Cadenasso et al. 2003, Elliott & Whitfield 2011, Whitfield et al. 2012). However, this level of connectivity, combined with the high human reliance on these systems, imposes a greater risk of impairment of ecological function in estuaries, as they are affected by natural and anthropogenic perturbations

occurring in both aquatic and adjacent ecosystems (Lotze et al. 2006, Elliott & Whitfield 2011). Estuaries are for example, heavily affected by high levels of nutrient and pollutant inputs from urban, agricultural and industrial effluents that are of marine, freshwater and terrestrial origin (Dolbeth et al. 2007). In this regard, numerous studies have therefore quantified the effects of pollution (Pearson & Rosenberg 1978, Warwick et al. 1987, 1990, Gray et al. 1990, Gaston et al. 1998) and eutrophication (Kemp et al. 2005, Smith & Schindler 2009, Dolbeth et al. 2011, Greig et al. 2012, Douglas et al. 2017) on estuarine ecosystems and their associated benthic macrofauna. Similarly, hydrological disturbances such as, floods, droughts and alterations to freshwater inputs are not solely a product of changes within estuaries, but are impacted by processes occurring at much larger spatial scales across fringing systems. Such events are known stressors of estuarine environments and have often been linked to changes in macrofaunal community structure (Hastie & Smith 2006, Pillay & Perissinotto 2008, 2013, Pollack et al. 2011, Dittmann et al. 2015). Other stressors impacting benthic invertebrates include: sedimentation and burial (Whomersley et al. 2009), hypoxia (Josefson & Widbom 1988, Van Colen et al. 2009), habitat loss/change and fragmentation (Thrush et al. 2003, Hewitt et al. 2008), salinity (Boltt 1975, Blaber et al. 1983, Owen & Forbes 1997, 2002, Lawrie & Stretch 2011) and mouth state (Whitfield et al. 2008). Although any one of these individual stressors may not be calamitous, combinations of these stressors could result in habitat loss or fragmentation, and catastrophic changes in biodiversity associated with the removal of functionally important, site-specific species (Thrush et al. 2017).

While it is generally accepted that most estuaries are characterised by a high degree of connectivity with fringing ecosystems, the majority of southern African estuaries (along with several others from arid or semi-arid climates e.g. Australia, the southeastern coasts of Brazil and Uruguay and southwestern coasts of India and Sri Lanka) are temporarily open/closed systems, characterised by an intermittent separation from the ocean (Whitfield 1992,

Perissinotto, Stretch, et al. 2010, Whitfield & Elliott 2012). This separation from marine inputs, combined with limited freshwater inflow, results in the systems frequently becoming water stressed (Whitfield 1992, Whitfield et al. 2008, MacKay et al. 2010, Whitfield & Elliott 2012).

The St Lucia Estuarine system is an excellent example of such a periodically water stressed system, as it undergoes long-term cycling between droughts and flooding (Begg 1978, Cyrus et al. 2011). Ecological functioning in this system is greatly dependant on freshwater inflow, rates of evaporative water loss and the frequency and duration of marine connectivity (Cyrus & Vivier 2006, Whitfield & Taylor 2009, Pillay & Perissinotto 2013). In this regard, numerous studies have quantified the effects of droughts on the benthic invertebrates within the system, with the main mechanisms reported to drive changes in the benthos being hypersalinity, sedimentation, habitat compartmentalisation and desiccation (Pillay & Perissinotto 2008, 2013, MacKay et al. 2010, Perissinotto, Pillay, et al. 2010, Pillay et al. 2013). However, other incidental mechanisms by which droughts impact ecological processes and biotic assemblages have rarely been investigated, with limited consideration of the broader relevance of these phenomena. In the St Lucia Estuary for example, bioengineering by hippos is likely a major driver of ecological processes in the system, but is likely to be altered in strength and direction by droughts, since this modifies background contexts in which interactions occur. This is also likely given broader theoretical recognitions that engineering activity is contingent upon environmental contexts that determine the nature of biological interactions (Jones et al. 1994, Wright & Jones 2006, Hastings et al. 2007, Romero et al. 2015).

Due in part to their dangerous reputation, studies conducted on the ecological roles of hippos have been scarce in the wild (Pennisi 2014). Therefore, very little is known about ecosystem-level impacts of these iconic megaherbivores (Bakker et al. 2016). Most studies on hippos have focused on impacts of pathway formation on land (McCarthy et al. 1998, Deocampo 2002, Mosepele et al. 2009) and their ability to alter terrestrial (Lock 1972, Kanga

et al. 2013) and aquatic vegetation (Bakker et al. 2016). However, one of the most significant ecological roles played by hippos - their facilitation of trophic transfers from terrestrial to aquatic systems through defecation (Grey & Harper 2002, Jacobs et al. 2007, Masese et al. 2015, McCauley, Dawson, et al. 2015, Subalusky et al. 2015) is a particularly poorly understood mechanism by which hippos impact aquatic ecosystems.

The potential for such hippo-mediated trophic transfers (i.e. defecation) to impact aquatic communities and processes across multiple trophic levels has often been alluded to (Jackson et al. 2012, Taylor 2013a, Pennisi 2014, Masese et al. 2015) and a few studies have quantified their effects on water quality and chemistry (Gereta & Wolanski 1998, Wolanski & Gereta 1999, Subalusky et al. 2015, Dutton et al. 2018, Stears et al. 2018), and on individual food web components (McCauley, Dawson, et al. 2015, Dutton et al. 2018, Stears et al. 2018). Initially, a general theme emerging in the literature on hippo defecation was the notion that community and food web effects are likely to be stimulatory (i.e. having positive bottom-up impacts on food web components) however, recent research has challenged this idea (McCauley, Dawson, et al. 2015, Subalusky et al. 2015, Dutton et al. 2018, Stears et al. 2018).

Generally lacking, however, is an appreciation that net effects resulting from hippo defecation are likely to be density-dependant and non-linear, as reported for other mega-herbivore activities (Bakker et al. 2016). At low population sizes, mega-herbivores may induce stimulatory responses; however, beyond some hypothetical threshold, increasing population densities are likely to generate inhibitory outcomes. Such a unimodal model should in theory be equally applicable in predicting responses to dung enrichment, as illustrated in Figure 3.1. At low inputs and accumulation rates, dung can subsidize recipient ecosystems, strengthening resource-induced bottom-up interactions, thereby causing positive effects on ecosystem functioning and community responses such as, productivity, nutrient cycling, consumer biomass and/or abundance (Polis et al. 1997). However, if transfer rates are high and dung

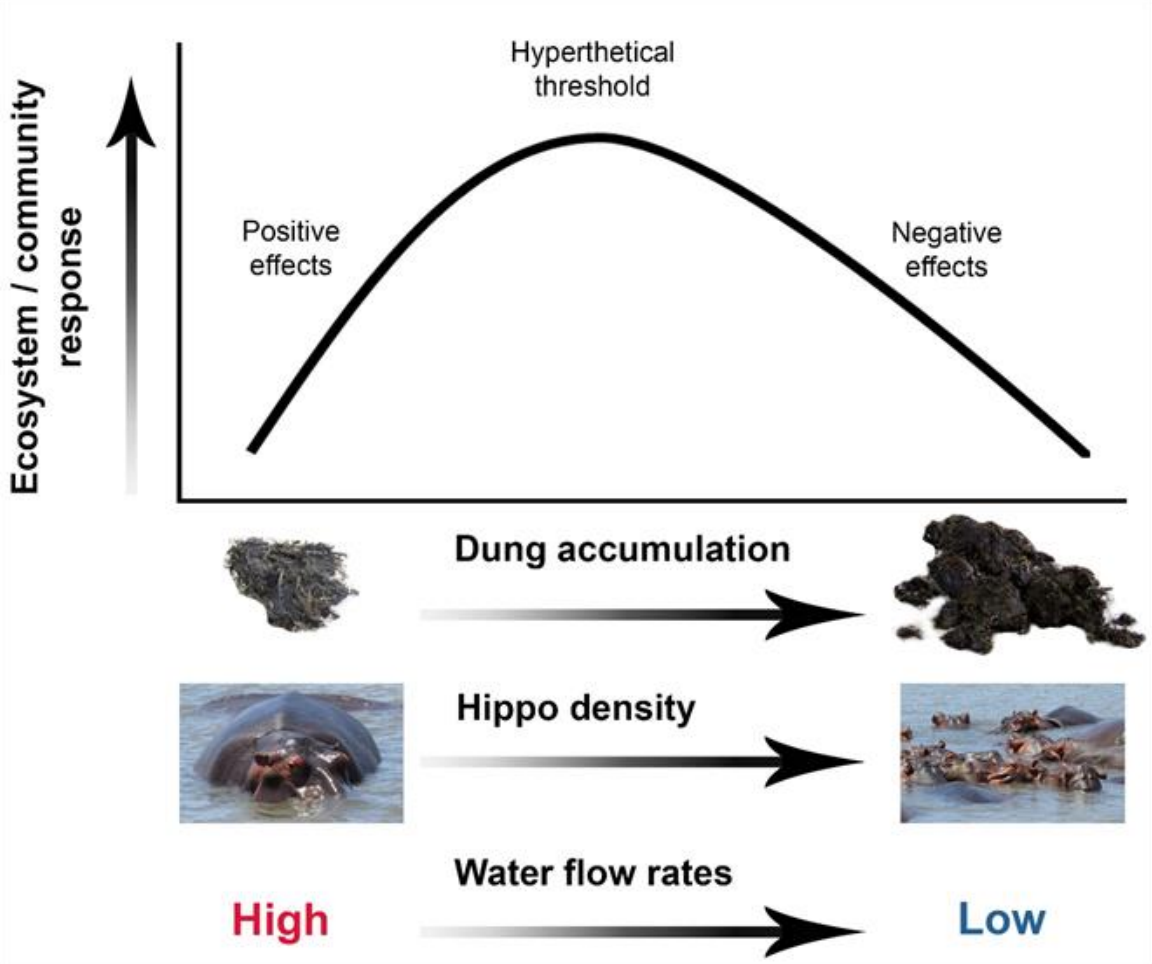


Figure 3.1: Schematic showing expected unimodal responses of ecosystem processes and benthic community metrics to increasing hippo dung inputs, hippo aggregation and decreased water flow rates.

accumulates, negative ecosystem affects could result, such as, declining water quality caused by anoxia and physiological stress being imposed on communities to the point of individual mortality (Wolanski & Gereta 1999, Pennisi 2014, Subalusky et al. 2015, Bakker et al. 2016). High persistence and accumulation rates of dung in turn are influenced by two factors viz. (1) the number of hippos resident within the system that cumulatively contribute to dung inputs and (2) local hydrodynamics, which determines dung residence times. Rapidly moving water potentially increases dispersal and transport of dung while low flow promotes dung accumulation and retention (Pennisi 2014, McCauley, Dawson, et al. 2015, Dutton et al. 2018).

Therefore, densely hippo populated systems that experience minimal hydrodynamic forcing will likely be more susceptible to the deleterious impacts of dung accumulation.

In the St Lucia estuary during the latest drought, which persisted for over a decade, reductions in water levels along with contraction and compartmentalisation of aquatic habitats have caused hippos to aggregate (Pillay & Perissinotto 2008, Taylor 2013a). By extension, the implication is also that dung concentrations may have increased under the drought conditions. This has been supported by the observation of dense dung layers forming on the benthos and increased quantities of dung present in benthic grab samples taken within sections of the estuary with dense hippo populations (Fig. 3.2).

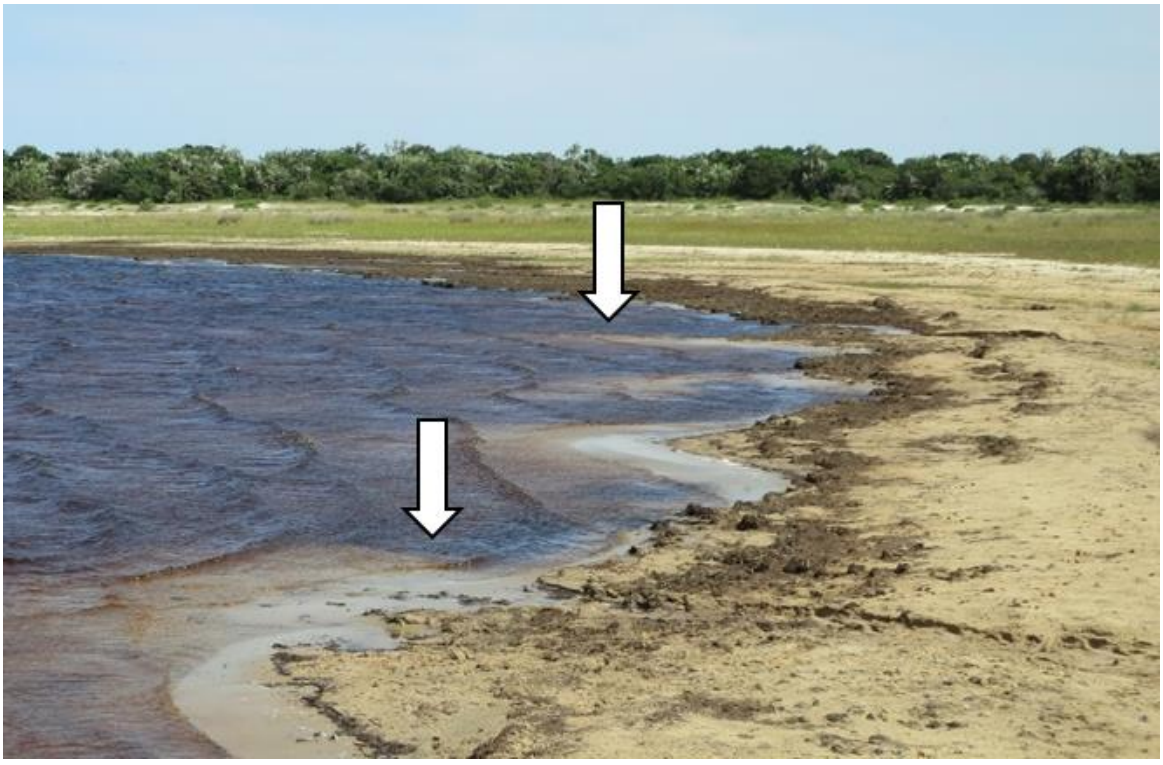


Figure 3.2: Photo taken within the St Lucia Estuarine system showing the accumulation of hippo dung, which forms thick mats on the benthos (indicated by the arrows).

With the above observations in mind, the central aim of this chapter was to use *in situ* inclusion/exclusion experiments to investigate the effects of hippo dung inputs and

accumulation on the macrobenthic communities of the St Lucia Estuarine system. More specifically, the aim was to determine whether the direction and magnitude of macrofaunal community responses (i.e. abundance, diversity, biomass and size) to the experimental enrichment of benthic plots were spatially consistent. Levels of dung used in experimental plots mimicked those recorded at sites in the Narrows, (Fig. 2.1) where approximately 50 % of the hippo population occurs (Taylor 2013a). Based on the level of dung recorded and/or observed in heavily populated areas (mats roughly 1 cm thick), it was hypothesized that enrichment of plots with hippo dung would result in significant shifts in benthic community structure. More specifically, it was hypothesised that microphytobenthic biomass and macrofaunal community metrics would be reduced following dung enrichment. An additional hypothesis was that while richness may decrease, opportunistic and more resilient species would increase in size due to greater levels of trophic resources.

3.2 Results

3.2.1 Community response

The addition of hippo dung resulted in a reduction of microalgal (chl-*a*) biomass by roughly half at both experimental sites relative to controls (Table 3.1, Fig. 3.3; Nested ANOVA, Dung Treatment: $F_{2,31} = 4.994$; $p = 0.013$). Chl-*a* biomass also differed between sites (Fig. 3.3; Nested ANOVA, $F_{1,31} = 7.488$; $p = 0.010$), generally being greater at Site 2 than at Site 1.

Table 3.1: Results of nested ANOVA testing for differences in macrofaunal community descriptors, macrofaunal biomass and microalgal biomass between sites and dung treatments. Bold *p*-values indicate statistically significant results. *F* = F-statistic, *p* = significance level, DF = degrees of freedom.

	Nested ANOVA					
	Site			Treatment		
	F	DF	*p*	*F*	DF	*p*
Microalgal Biomass	7.488	(1,31)	**0.010**	4.994	(2,31)	**0.013**
Macrofaunal Abundance	5.820	(1,32)	**0.022**	14.433	(2,32)	**<0.001**
Macrofaunal Biomass	0.122	(1,32)	0.729	1.963	(2,32)	0.157
Macrofaunal Species Richness	0.001	(1,32)	0.975	4.810	(2,32)	**0.015**
Macrofaunal Evenness	0.209	(1,32)	0.651	3.500	(2,32)	**0.042**
Macrofaunal Diversity	0.013	(1,32)	0.909	3.891	(2,32)	**0.031**

The community structure of macrofaunal assemblages differed statistically between dung inclusion and dung exclusion treatments (PERMANOVA pseudo $F_{2,39} = 2.45$; $p = 0.014$), while site differences were insignificant (PERMANOVA pseudo $F_{1,39} = 1.75$; $p = 0.334$). Results of PERMANOVA are visually supported by nMDS ordinations (Fig. 3.4), which show a spatial separation of macrofaunal community structure between dung inclusion and exclusion plots at both sites. Multivariate dispersion tests showed that the enrichment of plots with hippo dung increased variability in macrofaunal assemblages relative to exclusions, with the response being stronger in Site 2 (Table 3.2, PERMDIST, dung present: 38.072 ± 3.1 SE; dung absent: 17.809 ± 1.8 SE) than Site 1 (PERMDIST, dung present: 23.731 ± 2.6 SE; dung absent: 22.194 ± 2.9 SE).

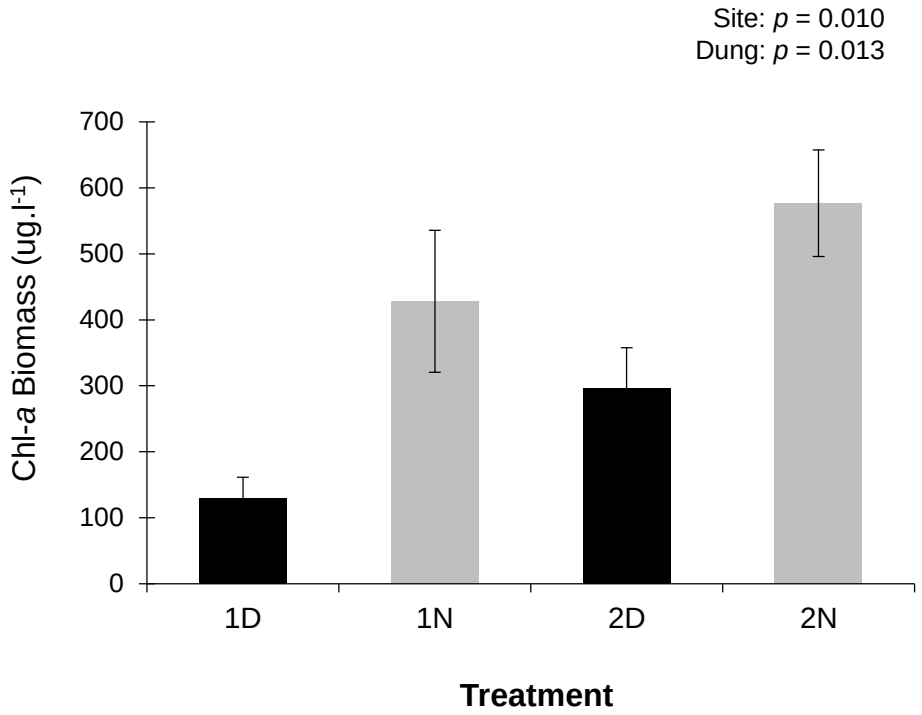


Figure 3.3: Variation in mean microalgal biomass (± 1 SE) at the two experimental sites in response to dung addition (D - black) and exclusion (N - grey). Numbers in treatment name = site number. Results of Nested ANOVA are shown.

Table 3.2: Summary statistics for multivariate dispersion tests showing average variability (+ SE) of macrofaunal communities, based on abundance and biomass data, between dung treatments at each site.

PERMDISP							
Macrofauna Abundance				**Macrofauna Biomass**			
Site	**Treatment**	**Average Dispersion**	**SE**	**Site**	**Treatment**	**Average dispersion**	**SE**
1	Dung	23.731	2.58	1	Dung	32.608	4.10
1	No Dung	22.194	2.93	1	No Dung	35.374	4.51
2	Dung	38.072	3.09	2	Dung	43.704	4.77
2	No Dung	17.809	1.79	2	No Dung	33.872	3.67

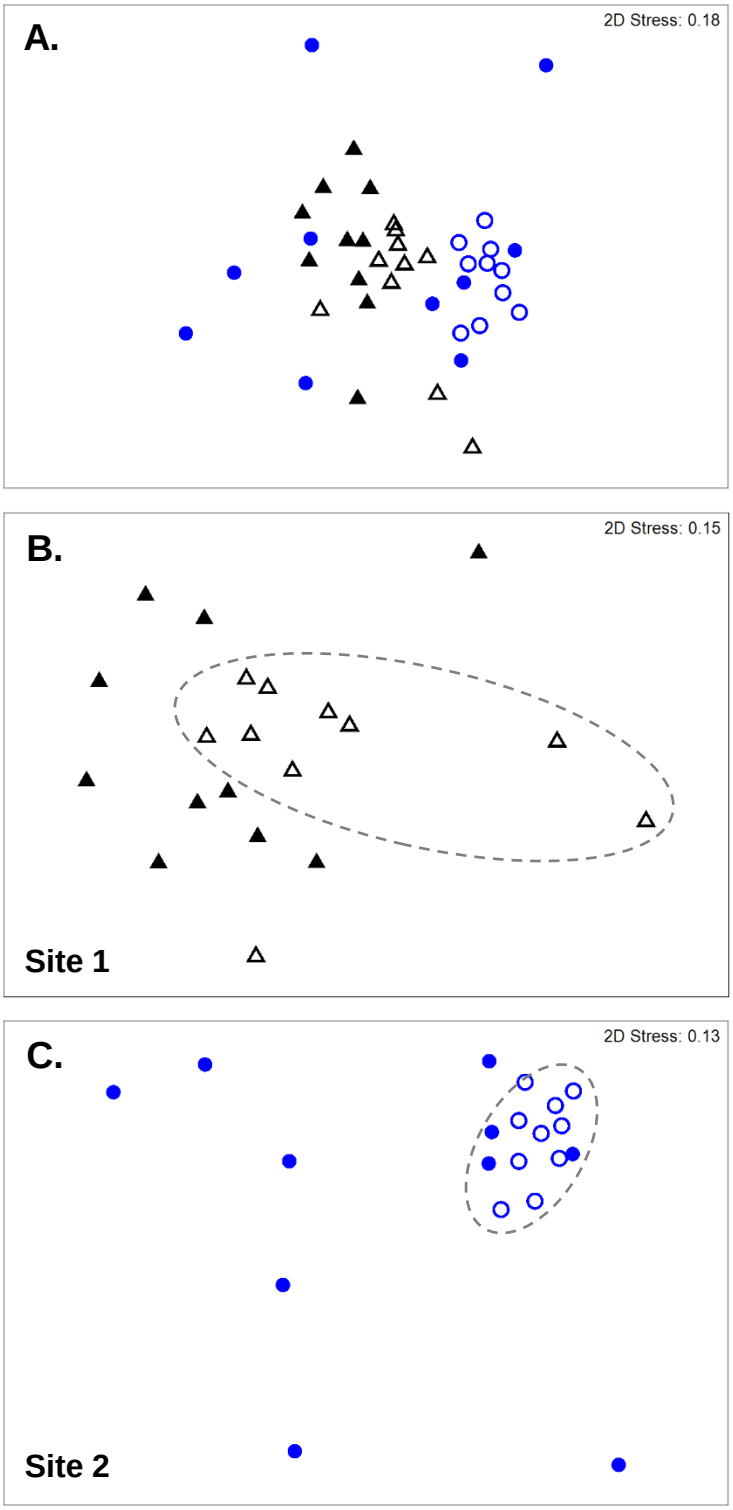


Figure 3.4: Non-metric multidimensional scaling ordination (nMDS) showing spatial variation in macrofaunal community structure, based on abundance data, between sites and dung treatments. A. shows samples from dung inclusion (filled symbols) and exclusion (unfilled symbols) treatments at Site 1 (black symbols) & 2 (blue symbols). B. shows dung inclusion and exclusion treatments at Site 1, while C. shows dung inclusion and exclusion treatments at Site 2.

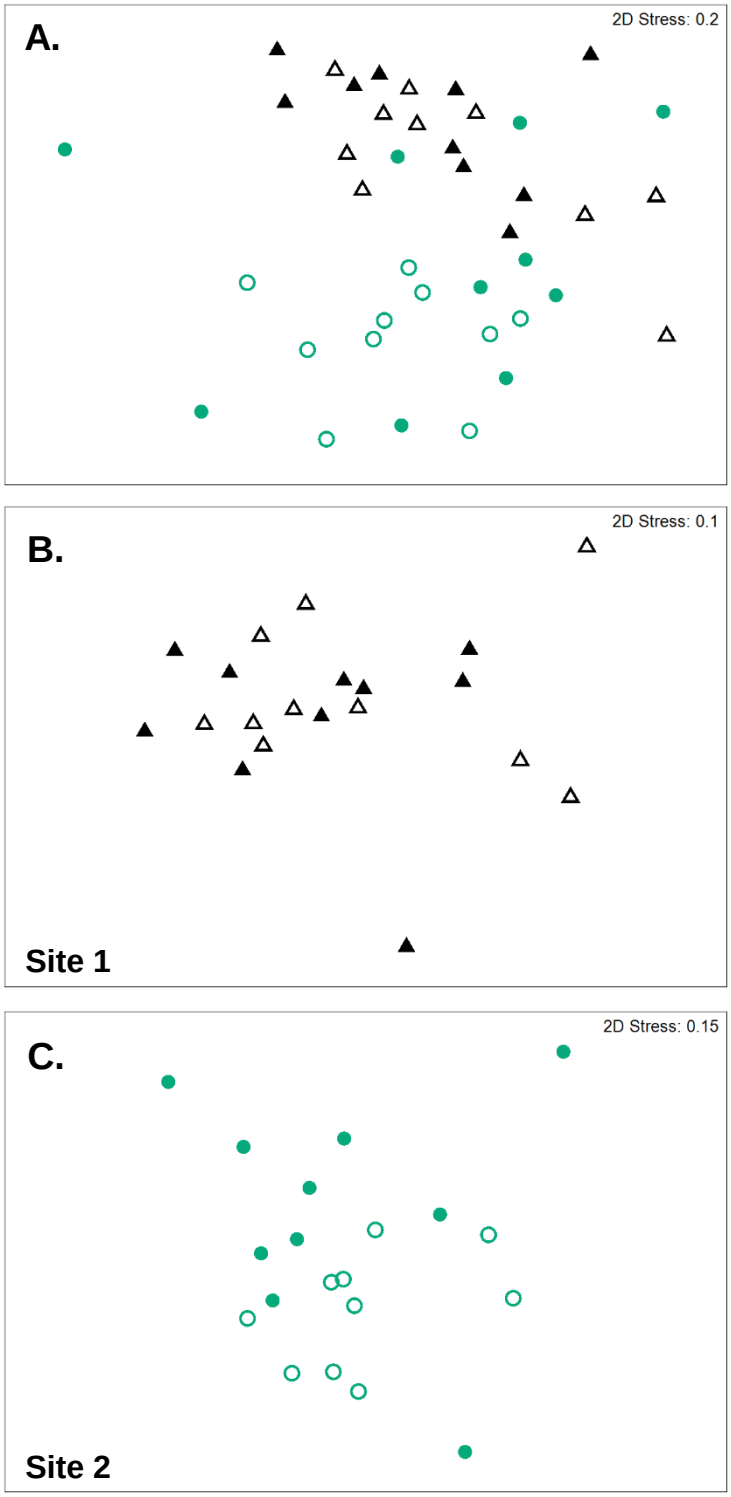


Figure 3.5: Non-metric multidimensional scaling ordination (nMDS) showing spatial variation in macrofaunal community structure, based on biomass data, between sites and dung treatments. A. shows samples from dung inclusion (filled symbols) and exclusion (unfilled symbols) treatments at Site 1 (black symbols) & 2 (green symbols). B. shows dung inclusion and exclusion treatments at Site 1 while C. shows dung inclusion and exclusion treatments at Site 2.

Non-metric multidimensional scaling ordinations (nMDS) based on biomass data show a slight separation between sites and between treatments in Site 2, however this was not statistically supported as results of PERMANOVA showed no difference between sites (Fig. 3.5, PERMANOVA pseudo $F_{1,39}$ = 4.22; p = 0.334) or between dung treatments (PERMANOVA pseudo $F_{2,39}$ = 1.47; p = 0.136). Multivariate dispersion based on biomass data indicated more community variability in plots enriched with hippo dung relative to dung exclusions at Site 2 (Table 3.2, PERMDIST, dung present: 43.704 ± 4.8 SE; dung absent: 33.872 ± 3.7 SE) whereas dung inclusion reduced variability at Site 1 (PERMDIST, dung present: 32.608 ± 4.1 SE; dung absent: 35.374 ± 4.5 SE).

Macrofaunal abundance differed significantly between sites (Table 3.1, Fig. 3.6; Nested ANOVA, $F_{1,32}$ = 5.820, p = 0.022) and more strongly between dung exclusion and inclusion treatments (Nested ANOVA, $F_{2,32}$ = 14.433, $p < 0.001$), with the addition of dung resulting in a decline in abundance by 32 and 70 % at Sites 1 2 respectively. Macrofaunal biomass was decreased by 44 and 56 % at Site 1 and Site 2 respectively. As a result of high variance in the data, this trend was not statistically supported (Table 3.1, Fig. 3.6; Nested ANOVA, $F_{2,32}$ = 1.963, p = 0.157).

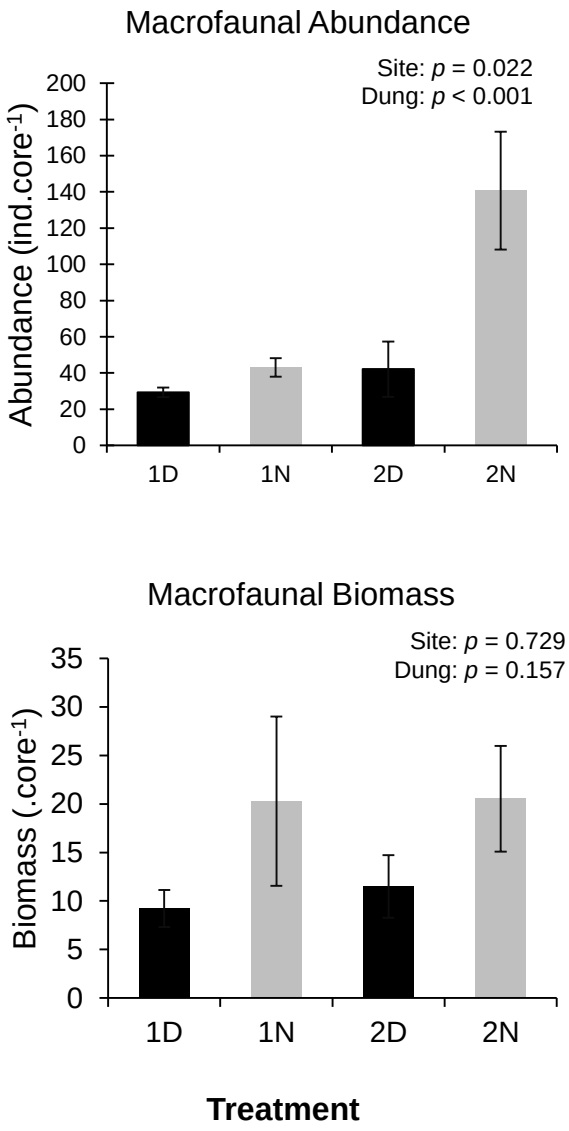


Figure 3.6: Variation in mean macrofaunal abundance and biomass (± 1 SE) between dung addition (D – black) and dung exclusion (N – grey). Numbers in treatment name = site number. Results of Nested ANOVA are shown for each panel.

Site differences in species richness were statistically insignificant (Table 3.1, Fig. 3.7; Nested ANOVA, $F_{1,32}$ = 0.001, p = 0.975), however, dung treatment differences were significant (Nested ANOVA, $F_{2,32}$ = 4.810, p = 0.015). Generally, the addition of dung to experimental plots caused a depression of macrofaunal richness relative to controls. This was greater at Site 1 where richness decreased by 27 % relative to an 8 % decrease at Site 2.

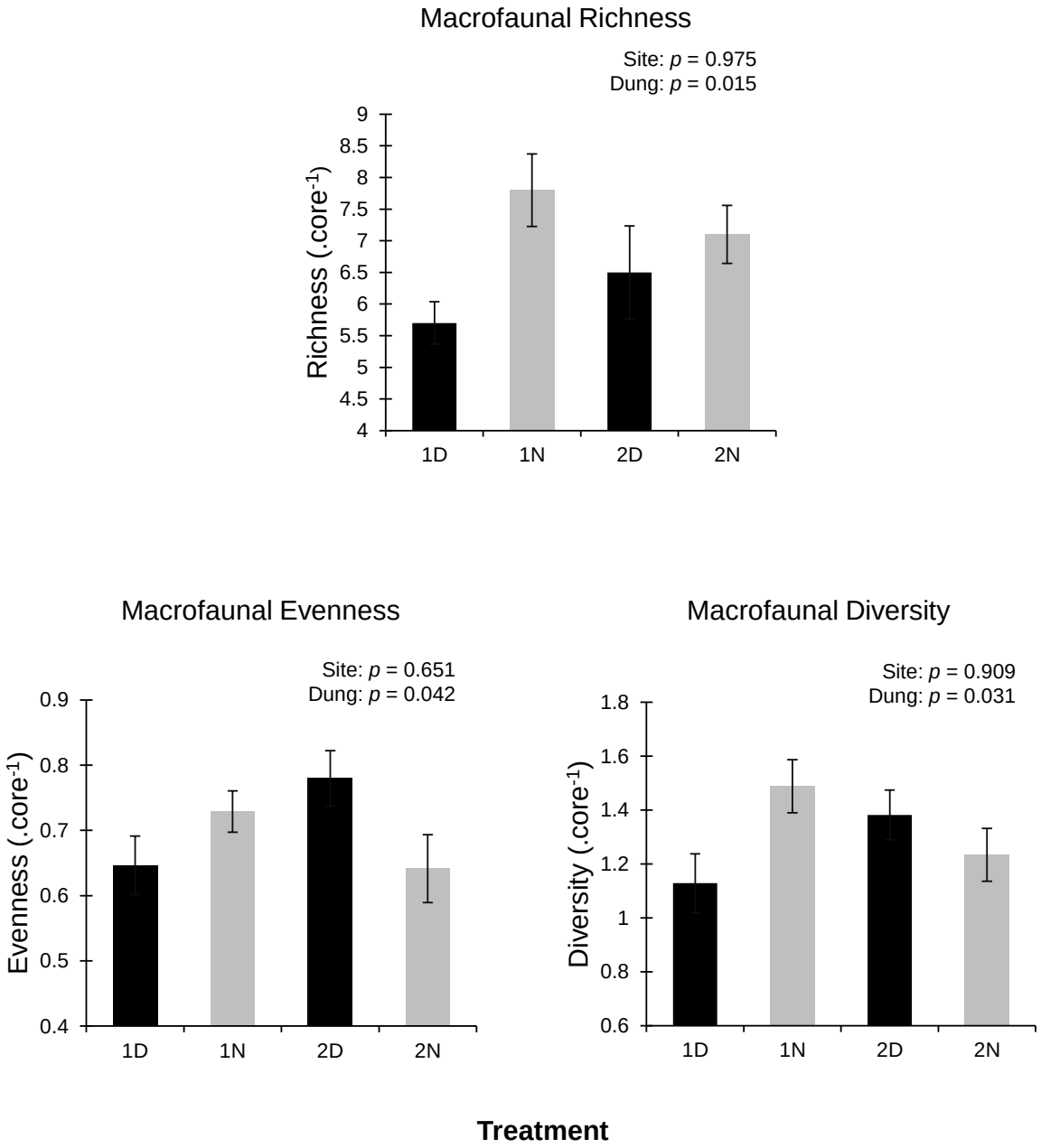


Figure 3.7: Spatial variation in mean macrofaunal community metrics (± 1 SE) at the two experimental sites in response to dung addition (D - black) and exclusion (N - grey). Numbers in treatment name = site number; results of Nested ANOVA are shown.

Macrofaunal evenness was statistically indistinguishable between sites (Table 3.1, Fig. 3.7; Nested ANOVA, $F_{1,32} = 0.209$, $p = 0.651$) but was significantly different between dung treatment (Nested ANOVA, $F_{2,32} = 3.500$, $p = 0.042$). Interestingly, the direction of the response to dung treatment differed between sites, with evenness decreasing in dung addition plots at Site 1 but increasing in dung addition plots at Site 2. Similarly, macrofaunal diversity was not significantly affected by site (Table 3.1, Fig. 3.7; Nested ANOVA, $F_{1,32} = 0.013$, $p = 0.909$) but was affected by dung treatment (Nested ANOVA, $F_{2,32} = 3.891$, $p = 0.031$) with a response pattern similar to that recorded for community evenness.

Hippo dung inclusion caused interesting responses of cumulative abundance-biomass plots of macrobenthic communities (Fig. 3.8). In Site 2, the abundance of dominant species decreased relative to biomass when dung was added. This is supported by an increase in the W-statistic from dung exclusions (W = -0.168) to dung inclusions (W = 0.031). This response was reversed at Site 1, where W-statistics decreased from 0.081 in dung exclusions to 0.016 in dung inclusion plots.

3.2.2 Individual taxon responses

At the species level, seven and five taxa were identified by SIMPER to cumulatively account for 90 % of the dissimilarity between dung exclusions and inclusions at Site 1 and Site 2 respectively, based on abundance data (Table 3.3). The abundance of all dominant taxa identified decreased with the addition of hippo dung, with the exception of one taxon at Site 1 - *Composetia keiskama* (polychaete). The largest observed declines in abundance caused by dung addition were 85 and 80 % at Site 1 and Site 2, while the increase in abundance of *Composetia keiskama*, at Site 1, was 13 % (Table 3.3).

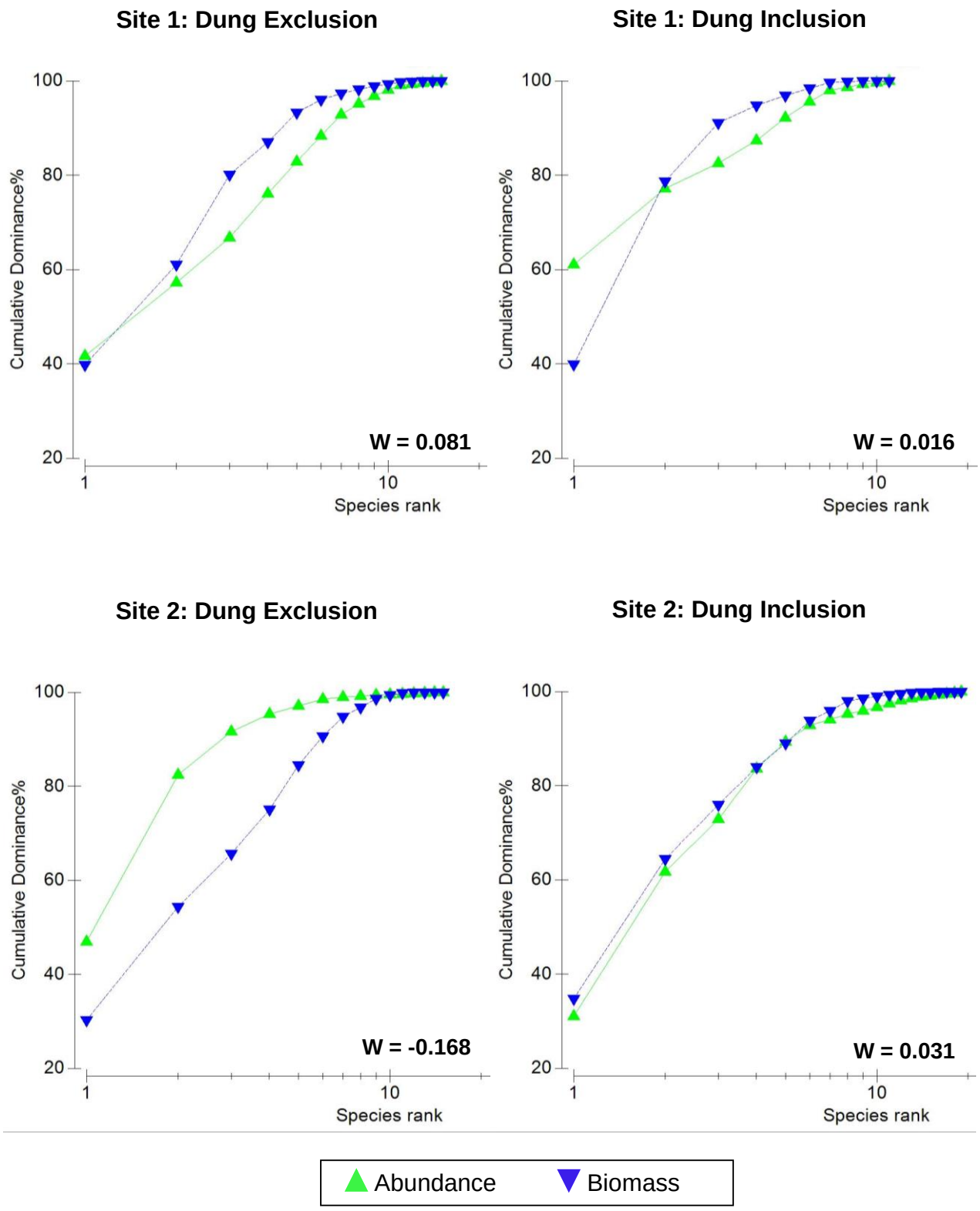


Figure 3.8: Cumulative dominance plots showing ranked species abundance (green symbols) and biomass (blue symbols) for macrofaunal assemblages in dung exclusion and inclusion treatments at Sites 1 & 2.

Table 3.3: Macrofaunal species identified by SIMPER to cumulatively account for 90 % of the community dissimilarity between treatments at each site based on abundance and biomass data. Bold text highlights the taxa showing an increase in abundance or biomass with the addition of hippo dung. P: polychaete, D: decapod, B: bivalve, I: isopod, M: mysid, C: cumacea.

	Site 1			Site 2	
	Dung	No Dung		Dung	No Dung
Dominant Taxa (abundance data)	**Average abundance**		**Dominant Taxa (abundance data)**	**Average abundance**	
Polydora sp. (P)	17.90	18.00	*Mesopodopsis africana* (M)	12.90	66.10
Crab zoea (D)	1.00	6.70	Crab zoea (D)	13.10	49.90
Brachidontes virgiliae (B)	0.70	4.00	*Polydora* sp. (P)	4.70	12.90
***Composetia keiskama* (P)**	**4.70**	**4.10**	*Composetia keiskama* (P)	4.50	5.20
Cyathura estuaria (I)	1.60	2.90	Cumacea (C)	2.40	2.50
Mesopodopsis africana (M)	1.40	2.40			
Cumacea (C)	1.40	1.90			
Dominant Taxa (biomass data)	**Average Biomass**		**Dominant Taxa (biomass data)**	**Average Biomass**	
Cyathura estuaria (I)	3.68	4.31	*Mesopodopsis africana* (M)	4.00	6.24
Polydora sp. (P)	3.57	3.85	Crab zoea (D)	0.92	4.93
Meretrix morphina (B)	0.00	8.09	***Cyathura estuaria* (I)**	**3.42**	**0.86**
Composetia keiskama (P)	1.14	1.28	*Composetia keiskama* (P)	1.31	1.94
Brachidontes virgiliae (B)	0.19	1.39	*Dendronereis arborifera* (P)	0.55	1.94
Dendronereis arborifera (P)	0.14	0.55	*Polydora* sp. (P)	0.58	2.32
			Meretrix morphina (B)	0.00	1.26

SIMPER identified six and seven discriminating taxa accounting for the 90 % dissimilarity in macrofaunal community structure at Site 1 and 2 respectively, based on biomass data (Table 3.3). The biomass of six out of six taxa decreased in dung inclusion plots at Site 1, while at Site 2, six out of seven taxa decreased as a result of dung addition. The only taxon to increase in biomass (*Cyathura estuaria* - isopod) did so by 75 % (Site 2). The largest decrease in biomass recorded was 100 % for *Meretrix morphina* (bivalve), which disappeared in the presence of dung at both Site 1 and 2 (Table 3.3).

Table 3.4: Results of nested ANOVA testing for differences in individual macrofaunal taxa abundance and size between sites and dung treatments. Bold *p*-values indicate statistically significant results. *F* = F-statistic, *p* = significance level, DF = degrees of freedom. P: polychaete, D: decapod, B: bivalve, I: isopod, M: mysid, C: cumacea.

Nested ANOVA						
Individual taxa abundance						
	Site			Treatment		
Taxa	*F*	DF	*p*	*F*	DF	*p*
Mesopodopsis africana (M)	54.709	(1,32)	**<0.001**	24.802	(2,32)	**<0.001**
Crab Zoea (D)	14.952	(1,32)	**<0.001**	16.414	(2,32)	**<0.001**
Polydora sp. (P)	9.442	(1,32)	**0.004**	2.538	(2,32)	0.095
Composetia keiskama (P)	0.09	(1,32)	0.766	0.309	(2,32)	0.736
Cumacea (C)	0.946	(1,32)	0.338	0.458	(2,32)	0.637
Brachidontes virgiliae (B)	0.009	(1,32)	0.927	0.738	(2,32)	0.486
Cyathura estuaria (I)	6.486	(1,32)	**0.016**	1.859	(2,32)	0.172
Individual taxa size						
	Site			Treatment		
Taxa	*F*	DF	*p*	*F*	DF	*p*
Mesopodopsis africana (M)	0.932	(1,21)	0.345	6.924	(2,21)	**0.005**
Crab Zoea (D)	0.055	(1,23)	0.817	1.521	(2,23)	0.234
Polydora sp. (P)	5.010	(1,28)	**0.033**	1.635	(2,28)	0.213
Composetia keiskama (P)	0.055	(1,31)	0.817	1.521	(2,31)	0.234
Cumacea (C)	3.018	(1,22)	0.096	0.297	(2,22)	0.746
Brachidontes virgiliae (B)	18.360	(1,7)	**0.004**	6.551	(2,7)	**0.025**
Cyathura estuaria (I)	0.019	(1,23)	0.891	3.447	(2,23)	**0.049**

Of the seven macrofaunal taxa identified by SIMPER to account for 90 % dissimilarity between dung treatments (Table 3.4), site and dung treatment had a significant effect on the abundance of two taxa: *Mesopodopsis africana* (mysid) and crab zoea (decapod, Table 3.4, Fig. 3.9; Nested ANOVA, Site: $p < 0.001$ and dung treatment: $p < 0.001$ for both taxa). The addition of hippo dung caused a decline in the abundance of both taxa, with a greater decline evident at Site 2 than at Site 1. *Polydora* sp. (polychaete) was significantly affected by site (Table 3.4, Fig. 3.9; Nested ANOVA, $F_{1,28} = 9.442$, $p = 0.004$). Treatment effects on this polychaete were marginally insignificant (Nested ANOVA, $F_{2,28} = 1.635$, $p = 0.095$), at Site 1

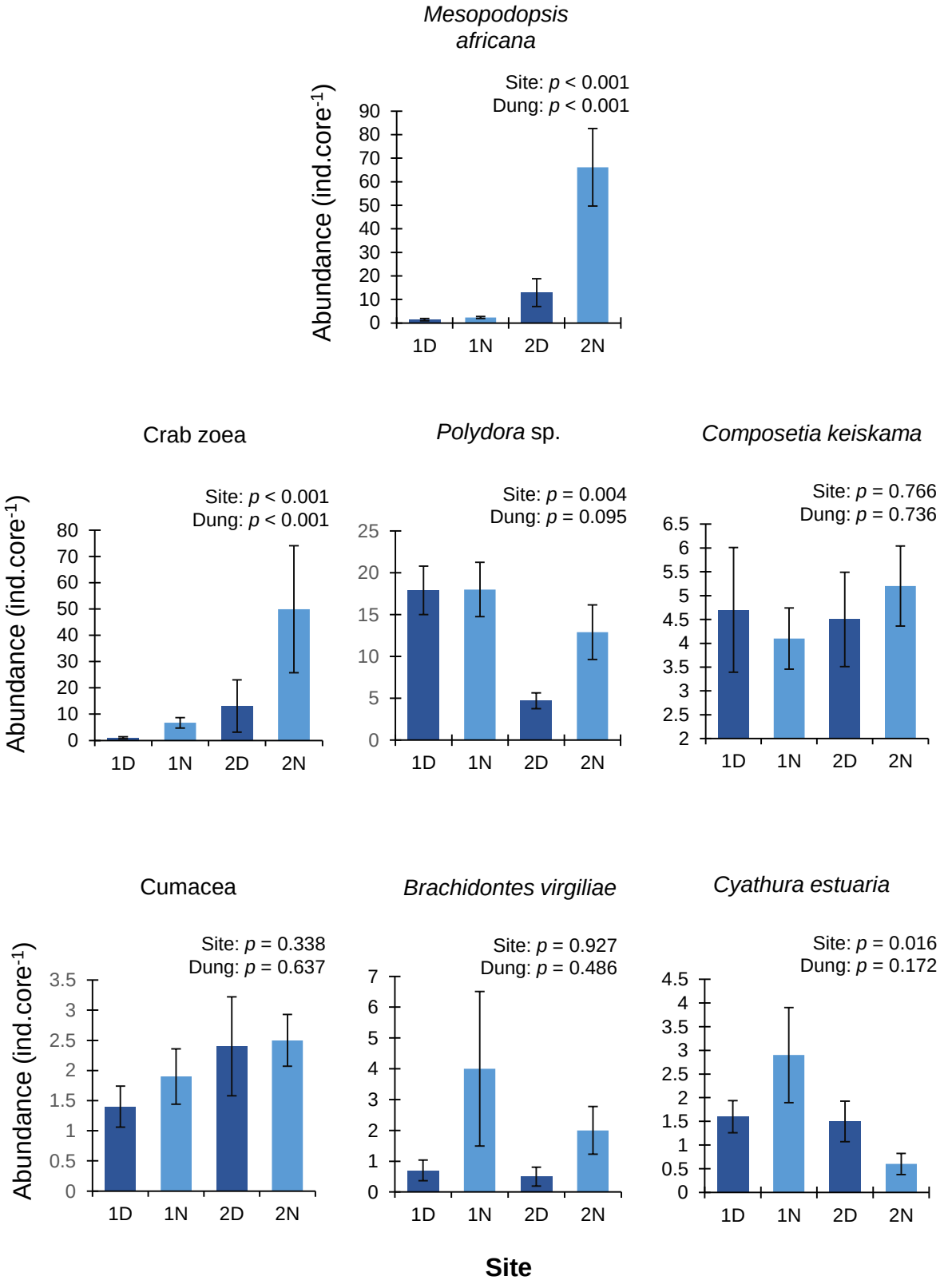


Figure 3.9: Mean abundance (± 1 SE) of individual macrofaunal taxa in response to dung treatment: dung addition (D – dark blue) and dung exclusion (N – light blue). Results of Nested ANOVA are shown for each panel.

abundance between treatments was similar however, at Site 2 abundance decreased by more than 50 % in the presence of dung. *Composetia keiskama*, Cumacea and *Brachidontes virgiliae* (bivalve) were not statistically affected by site or dung treatment (Table 3.4, Fig. 3.9; Nested ANOVA, Site: $p > 0.05$ and dung treatment: $p > 0.05$ for all). Site had a statistical effect on abundance of *Cyathura estuaria* (Table 3.4, Fig. 3.9; Nested ANOVA, $F_{1,32} = 6.486$, $p = 0.016$) however, treatment effects were not significant (Nested ANOVA, $F_{2,32} = 1.859$, $p = 0.172$).

With regards to size of the dominant taxa, two taxa were significantly affected by site viz. *Polydora* sp. and *Brachidontes virgiliae* (Table 3.4, Fig. 3.10; Nested ANOVA, Site: $p < 0.05$), with both taxa larger in Site 1. Three of the taxa exhibited a significant dung treatment response: *B. virgiliae* increased size in the presence of dung at Site 1, but size was similar between dung treatments at Site 2 (Table 3.4, Fig. 3.10; Nested ANOVA, Dung treatment: $p = 0.025$); *Mesopodopsis africana* and *Cyathura estuaria* both grew larger in dung inclusion plots at both sites (Table 3.4, Fig. 3.10; Nested ANOVA, Dung treatment: $p = 0.005$ and $p = 0.049$), though for *C. estuaria* differences at Site 2 were minor.

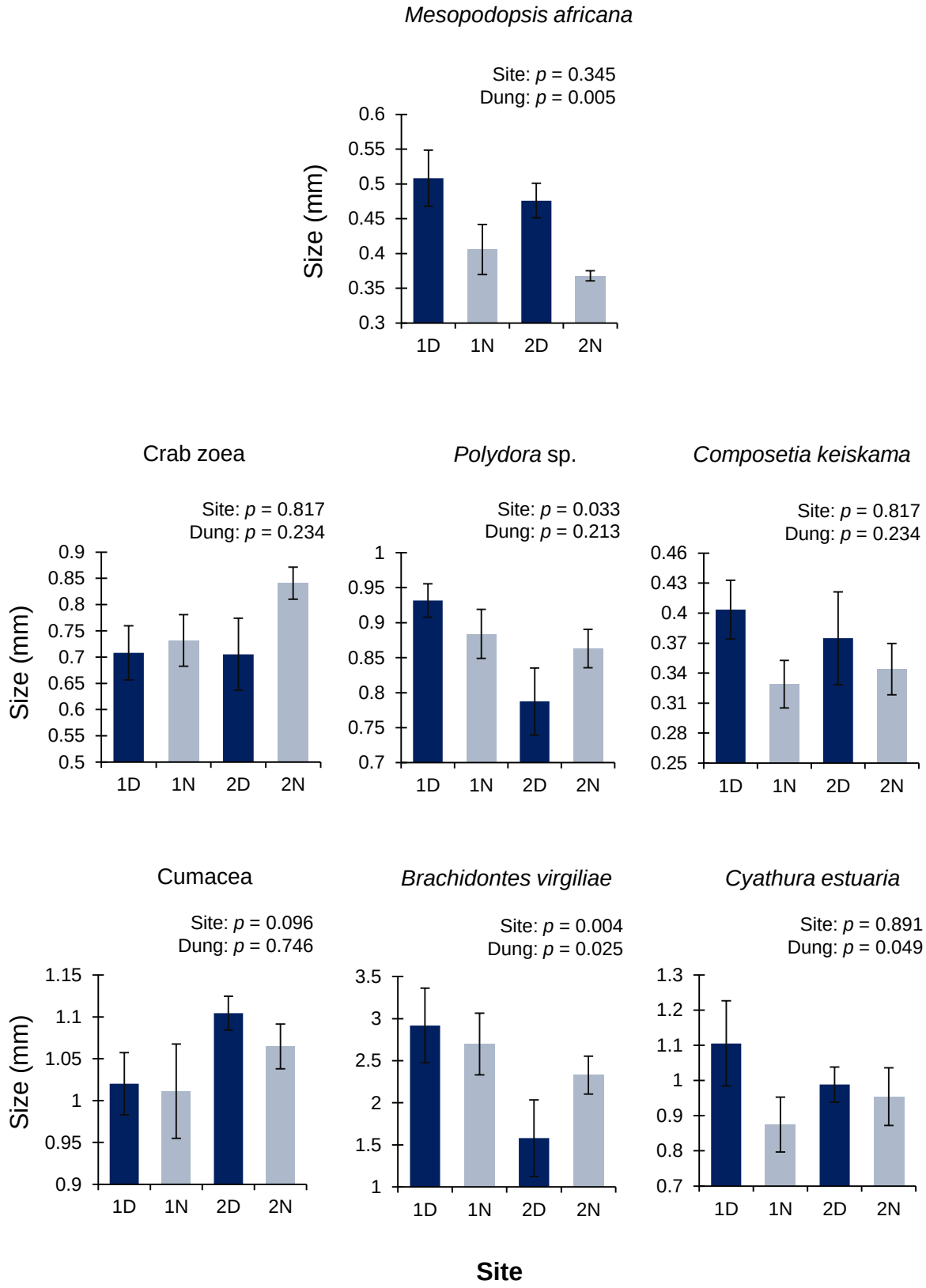


Figure 3.10: Mean taxon size (± 1 SE) at two experimental sites in response to dung inclusion (D - navy) and no dung (N - grey). Results of Nested ANOVA are shown for each panel.

3.3 Discussion

Given the observation of dung forming mats over the benthos during the latest drought, the goal of this chapter was to quantify its effect on macrobenthic communities of St Lucia. The second objective was to determine whether outcomes were spatially consistent. It was hypothesised that dung accumulation would alter macrofaunal assemblages, causing negative effects on community metrics, with an increase in size of remaining opportunistic species. Results from experimental inclusion/exclusion plots showed that regular inputs of hippo dung, at concentrations observed during drought conditions, had significant effects on macrobenthic community structure. Generally, the hypotheses posed were supported, with dung addition causing depressions of microphytobenthic biomass and macrobenthic community metrics including abundance, species richness and biomass.

3.3.1 Community response in space

As hypothesized, macrofaunal community structure in dung inclusions visually and statistically differed from those in dung exclusion plots at both sites. There was, however, no spatial variation at the community level between the two sites. In addition, of the six community metrics examined in this chapter, only two (microalgal biomass, macrofaunal abundance) displayed spatial variation at the site level. The decline in microalgal biomass was greatest at Site 1, while the decline in macrofaunal abundance was greatest at Site 2. The remaining four metrics (macrofaunal biomass, richness, evenness and diversity) all had non-significant spatial variation, suggesting that at the spatial scale examined here (150 m), responses of community metrics to hippo dung enrichment tended to be spatially consistent. With regards to the response of community metrics to dung treatments, four of the six metrics showed similar patterns to dung enrichment – exhibiting a decline in the presence of dung at both sites (microalgae biomass, macrofaunal abundance, richness and biomass). Of these, all

except macrofaunal biomass, which showed high variance, were statistically significant declines. In contrast, while macrofaunal evenness and diversity also displayed significant treatment effects, the direction of the responses varied between sites. At Site 1, macrofaunal evenness and diversity both decreased in dung enrichment plots in accordance with patterns for other community metrics, but at Site 2 patterns were reversed.

The responses of dominant individual macrofauna taxa revealed that four (*Mesopodopsis africana,* crab zoea, Cumacea and *Brachidontes virgiliae*) of the seven taxa exhibited a similar trend of decreased abundance in dung-enrichment plots at both sites. Individual taxa showed more spatial variation than community metrics, with four of the seven dominant taxa (*M. africana,* crab zoea, *Polydora* sp. and *Cyathura estuaria*) varying significantly in abundance between sites. Interestingly, only *M. africana* and crab zoea displayed significant treatment responses, with both decreasing in the presence of dung, especially at Site 2. With regards to sizes of dominant taxa, three (*M. africana, B. virgiliae* and *C. estuaria*) out of seven had significant responses to dung enrichment, with the hypothesis of larger individuals within dung enrichment plots being upheld at both sites for *M. africana* and *C. estuaria*. For *B. virgiliae*, however, individuals were larger in dung plots at Site 1 and smaller in dung plots at Site 2. For size, only two taxa (*Polydora* sp. and *B. virgiliae)* displayed significant spatial variation between sites.

Overall, findings point to between site variability being weak at the community metric level and becoming stronger, but not dominant at the level of individuals. In the few cases where site variability was detected, these could be explained by observed variation in wind-driven wave action, with greater wave action observed at Site 1 than at Site 2.

3.3.2 Mechanisms underlying macrofaunal responses to dung

There are a number of potential mechanisms that could drive the responses of communities to dung enrichment, as summarised in Figure 3.11. It is important to note these mechanisms are hypothetical given that no data were collected to support them. However, given the importance of developing a mechanistic understanding of ecological processes, possible pathways are suggested to explain emergent patterns in this chapter, based on relevant published work.

The depression of benthic microalgal biomass by 50 to 70 %, is likely caused by a decrease in available incident light as a result of hippo dung shading the benthos and increasing turbidity. Wolanski & Gereta (1999) attributed an increase in turbidity and resultant reduction in photic zone depth and light penetration to a combination of animal dung, suspended sediment by hippo bioturbation and decaying vegetation, when conducting a study on the oxygen cycle within a hippo pool in Serengeti National Park, Tanzania. Other than this study, the potential for hippo dung to cause shading has rarely been explored, however, studies elsewhere have shown that terrestrial organic matter transfers can reduce primary production through shading (Jones et al. 2012, Kelly et al. 2014). Similarly, increased levels of suspended organic matter have been shown to cause a reduction in incident light to the benthic environment which results in decreased productivity (Kemp et al. 2005, Bilotta & Brazier 2008, Smith & Schindler 2009).

During this experiment, dung was observed to settle on the benthos shortly after addition to cages. This may suggest another mechanism by which dung can affect microalgal biomass: any flow-induced movement of this matter over the sediment surface could result in abrasion and resuspension of microphytobenthos. While there is no direct evidence of the abrasive effects of hippo dung in the literature, it can be inferred from a review conducted by Bilotta & Brazier (2008), who reported that suspended solids (inorganic and organic matter) can abrade and damage photosynthesising organisms, as well as scrub microalgae off the benthos.

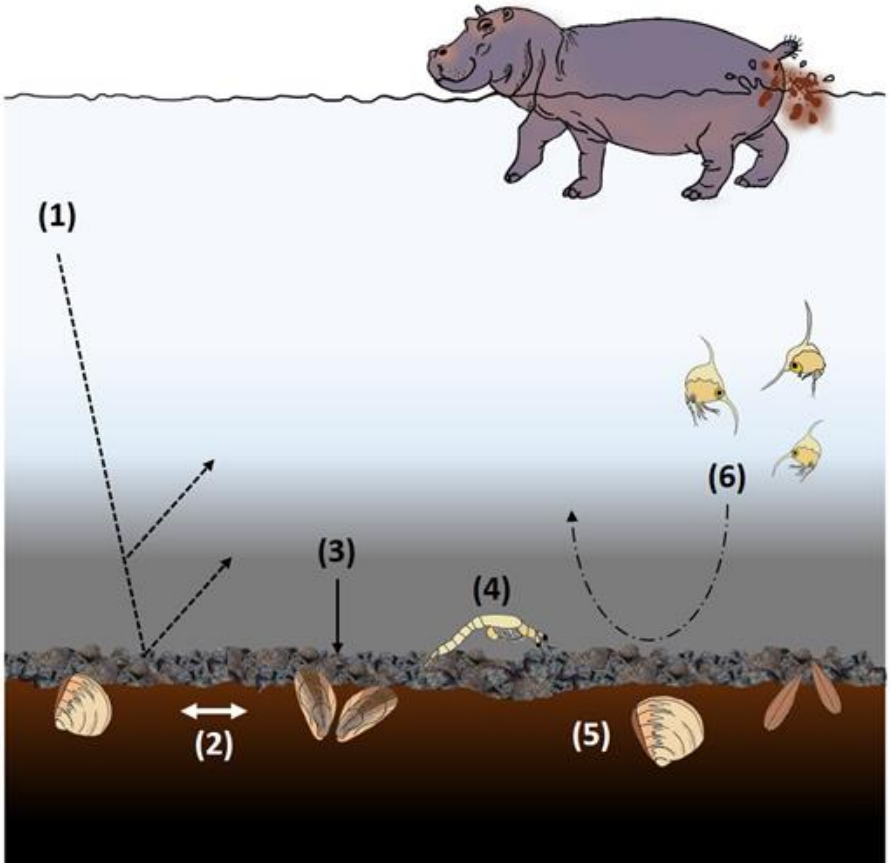


Figure 3.11: Schematic showing hypothetical mechanisms by which hippo dung accumulation can impact benthic macrofaunal communities. When inputs accumulate, dung can (1) increase turbidity, thereby reducing light penetration and depress microphytobenthic biomass; (2) disrupt, damage or dislodge surface-dwelling organisms by increasing sediment surface abrasion and scouring; (3) enhance total suspended organic matter, thereby stressing filter-feeding taxa; (4) reduce recruitment by acting as a physical barrier between recruits and the sediment surface; (5) increase anoxia and hydrogen sulphide flux; (6) act as a negative settlement cue for larval macrofauna.

The recorded decline in benthic microalgal biomass in the presence of dung could generate important bottom-up effects on macrofaunal communities (Huang et al. 2013). Primary producers are recognised as important trophic resources for benthic invertebrates (Haines & Montague 1979, Miller et al. 1996). Therefore, reductions in microalgal biomass subsequent to dung enrichment, may negatively impact benthic consumers, by reducing the

availability of high quality autochthonous resources (Jones et al. 2012). This could explain the observed declines in macrofaunal abundance and dominant taxa. Indirect consequences may also arise from reductions in food resource availability. For example, studies have shown that benthic invertebrates emigrate from patches with low food availability, or avoid settling in these patches (Kohler 1985, Ruetz & Stephens 2003).

Dung accumulation can have strong effects on bentho-pelagic biogeochemistry. It has been shown that hippo dung can increase dissolved and particulate nutrient concentrations, thereby inducing eutrophication and subsequent algal blooms. The latter has been speculated to reduce incident light reaching the benthos and cause anoxic conditions (Gereta & Wolanski 1998, Wolanski & Gereta 1999, Pennisi 2014, Stears et al. 2018). Similarly, an increased abundance of bacterial decomposers, and accompanying biochemical oxygen demand associated with decaying faecal matter, results in anoxia at the sediment-water interface (Wolanski & Gereta 1999, Dutton et al. 2018). In addition, the decomposition of hippo dung has been shown to generate heat, thereby causing temperatures on the sediment surface to increase (Wolanski & Gereta 1999). These dung-induced alterations to physico-chemical conditions could impose physiological stresses on benthic macrofauna, leading to diminished abundance of sensitive taxa.

The majority of macrofaunal species have a planktonic larval stage in their life cycle. Studies have shown that larvae of various species are capable of accepting or rejecting specific substrates based on specific physical and chemical cues. Therefore any process that alters larval settlement requirements will likely impact adult assemblages (Eckman 1996, Pillay & Perissinotto 2008). This idea forms the basis for another mechanism by which hippo dung can affect macrobenthic community structure: the alteration of larval settlement and recruitment by induced physico-chemical change. Firstly, dung mats may act as a physical barrier preventing the movement of recruits from the pelagic to benthic environment. In addition,

abrasion of these dung mats may cause the resuspension of newly settled recruits. Secondly, dung may negatively impact larvae by creating low oxygen, high temperature and ammonia conditions, which can act as negative settlement cues (Marinelli & Woodin 2004). Evidence supporting the ability of hippo dung to impair larval settlement is the observed declines in abundance of crab zoea in dung inclusion plots, especially at Site 2.

In addition, water and wind driven waves could cause dung mats to scour the sediment surface, exposing benthic organisms to deleterious effects such as damage to respiratory organs and dislodgement (Bilotta & Brazier 2008). Resuspension of macrofauna as a result of scouring may also increase predation risk, as organisms dislodged from the benthos and the shelter it provides, drift un-protected in the water column (Ruetz & Stephens 2003, Bilotta & Brazier 2008). Even if predation is avoided, dung mats may obstruct re-settlement of organisms and/or result in emigration to avoid unfavourable conditions (Brittain & Eikeland 1988, Bilotta & Brazier 2008). These mechanisms may explain the diminished contributions of surface-dwelling taxa (*Cyathura estuaria*, *Mesopodopsis africana* and crab zoea) to community composition in dung enrichment plots. Similarly, increased turbidity and concentrations of suspended solids can negatively impact filter-feeding species. This is based on prior research showing that high levels of water column particulates can stress or kill filter-feeding organisms by clogging feeding structures and reducing filtration efficiency (Rhoads & Young 1970, Bilotta & Brazier 2008). This mechanism may contribute to the decline and exclusion of filter-feeding bivalves (*Brachidontes virgiliae* and *Meretrix morphina*) from dung enrichment plots.

3.3.3 Functional responses to dung

Ecologists have long recognised the importance of population, life-history and ecological attributes in influencing responses to environmental change (Haddad et al. 2008, Williams et al. 2010). These attributes are referred to as functional traits (Williams et al. 2010,

Mouillot et al. 2013, Rodil et al. 2013) and are important in understanding response directions and magnitudes of benthic assemblages to hippo dung accumulation. Generally, surficial macrofaunal taxa contribute significantly to community shifts resulting from disturbances (e.g. Rodil et al. 2013). This notion is relevant to understanding functional responses to hippo dung, particularly because dung disturbance of the benthos is largely surficial. This suggests that organisms most likely to be susceptible to dung accumulation would be those living on or close to the sediment surface. In addition, it is generally accepted that while most of the benthic abundance occurs within 5 cm of the sediment surface, the greater proportion of benthic biomass is found more than 5 cm below the sediment surface (Weston 1990). Collectively, these factors can be used to hypothesise that smaller, surface-associated taxa are more vulnerable to dung accumulation. This hypothesis was for the most part supported in this chapter.

At Site 2, two small, surface-dwelling taxa (*Mesopodopsis africana* and crab zoea) numerically dominated the assemblage in dung exclusion plots. The largest response magnitude to dung addition was also demonstrated by these two taxa, with average abundance dropping by 80 and 74 % respectively. The declines of *M. africana* and crab zoea could account for the observed peak in community evenness seen at dung enrichment plots in Site 2, as the site is no longer dominated by these two species. Further support for the hypothesis that traits such as, body-size and habitat position, affect the sensitivity of taxa to environmental change comes from ABC curves (abundance/biomass comparisons) at Site 2, where dung inclusion resulted in a reduction in taxa with low biomass (viz *M. africana* and crab zoea).

Benthic macrofaunal assemblages exposed to increased organic loading are known to undergo the following responses: (1) a depression of species richness, (2) a decline in biomass, (3) an increase in total number of individuals caused by large numbers of a few opportunistic species, (4) changes in the relative dominance of functional groups and (5) a decline in

community body size but an increase in individual body size (Pearson & Rosenberg 1978, Weston 1990). In this study, increased organic loading in the form of hippo dung additions, caused macrobenthic communities to respond as expected with regards to (1) and (2), with both richness and biomass declining in the presence of hippo dung. During prolonged drought conditions and/or mouth closure, estuarine benthic macrofaunal communities shift to *r*-selected, opportunistic generalists (Hastie & Smith 2006, Pillay et al. 2013). The decline in macrofaunal abundance and lack of a 'peak of opportunists' ((3) Pearson & Rosenberg 1978) in the presence of dung may be explained by the fact that during the present drought and extended mouth closure, the St Lucia Estuary may already comprise opportunists or *r*-selected species (MacKay et al. 2010). The additional stress imposed by dung inputs may therefore exceed tolerance levels of existing *r*-selected taxa. With regards to (4), the reduced dominance of surface-associated taxa has already been discussed.

Weston (1990) suggested that "the relationship between enrichment and body size is complex". The size responses of individual taxa to dung addition recorded in this experiment (Fig. 3.10) supports this assertion. Of the dominant taxa identified by SIMPER, two showed neutral or insignificant responses to dung addition, two show a decrease in size at dung inclusion plots in Site 2 (*Polydora* sp. and *Brachidontes virgiliae*), while others conformed to the hypothesised increase in individual body size with hippo dung inclusions (viz. *Mesopodopsis africana, Composetia keiskama* and *Cyathura estuaria*). It is possible that the enhanced organic enrichment, as a result of dung inputs, provides an additional food source allowing these opportunistic individuals to grow larger.

3.3.4 Implications

Under natural conditions, inputs of hippo dung in the St Lucia Estuary occur consistently on a daily basis, over decadal time scales, and over tens of kilometres in spatial

scale. In this context, the experimental findings likely underestimate hippo dung impacts given the (1) restricted spatial and temporal scale over which the study was conducted and (2) the low frequency of dung additions to experimental plots. In addition, the larger surface area and shallow water depth of the lake-like environment at Charter's Creek means that wind-driven wave action can stir up the bottom sediment (Zikhali et al. 2014, 2015), thereby increasing dispersal of dung. In contrast, the greater depth and reduced surface area of the Narrows, where hippos are highly abundant, increases dung retention, resulting potentially in stronger effects of dung accumulation.

Results suggest that dung inputs may have large effects, predominantly by reducing primary and secondary productivity. In addition, higher order indirect effects could also occur through bottom-up processes. Declines in macrofaunal abundance (by up to 70 %) and individual taxa (by 80 - 90 % for dominant taxa) could filter up to negatively influence higher trophic levels, including those occurring in pelagic ecosystems, based on prior studies documenting how changes at lower trophic levels can induce shifts at higher trophic positions (Loreau et al. 2001). In this context, it is noteworthy that some of the taxa negatively affected by dung enrichment in this experiment are known to be key trophic resources for fish in St Lucia (Blaber 1979, Carrasco & Perissinotto 2010). For example, mysids are recognised as important dietary resources for fish species and aquatic birds such as, flamingos (Blaber 1979, Carrasco et al. 2012, 2013, Turpie et al. 2013). Furthermore, studies on eutrophication have highlighted the potential for declines in macro-invertebrate production associated with persistent organic inputs to shift fish assemblages from benthic to pelagic dominance (Kemp et al. 2005). These lines of reasoning suggest a need to understand potential consequences of impacts on consumers arising from dung related impacts to the benthos, particularly in light of research by Govender et al. (2011) in the St Lucia Estuary highlighting the reliance of consumers on benthic trophic resources under drought conditions.

A major driver of estuarine functioning is water flow and sediment movement - known generally as hydromorphology (Elliott & Whitfield 2011). Research has shown that the effects of suspended solids on aquatic biota are dependent on the concentration, duration and timing of exposure (Bilotta & Brazier 2008). The effects of hippo dung should also in theory be dependent on concentration and duration. Effects observed in this study will therefore likely be strengthened by conditions that enhance dung accumulation and persistence over the benthos. This will probably be strongly determined by the volumes of dung defecated and the rates of flow, with the combination of high dung inputs coupled with low water flow likely to magnify dung effects (Pennisi 2014, Stears et al. 2018).

Two important processes affecting flow rates within aquatic ecosystems are (1) anthropogenic manipulations of freshwater inputs as a result of land use alterations and abstractions (Snoussi et al. 2007, Datry et al. 2014) and (2) droughts, which are a common feature of arid and semi-arid climates (Pillay & Perissinotto 2008) and which are likely to become more frequent and severe with climate change (Dolbeth et al. 2011). Both of these can cause significant flow declines within aquatic systems and both are major determinants of functioning in the St Lucia Estuarine system. Human-induced flow manipulations within the St Lucia catchment areas include damming, water abstraction, land use in the form of sugar cane farming and forestry and the artificial diversion of the Mfolozi River – the largest tributary into the system (Van Niekerk 2004, Whitfield & Taylor 2009, Searle 2013, Stretch & Maro 2013).

The St Lucia system is typified by cyclical wet and dry phases and mouth closures however, the recent drought saw the mouth closed to the ocean for upwards of a decade and water levels drop to as low as 10 % of the lakes surface area, resulting in fragmentation and the formation of lentic pools of water. The decline in availability of water bodies large enough for hippos to wallow in has resulted in the concentration of hippos in pools and in the estuary

(Taylor 2013a, Stommel et al. 2016). Due to the largely impermeable nature of their boundaries, isolated pools or closed ponds that are frequently inhabited by hippos (Taylor 2013a, Pennisi 2014, McCauley, Dawson, et al. 2015), are most susceptible to dung retention. These conditions, combined with the fact that the estuary currently supports a large (highest density in past 60 years) and growing (3 % per annum) hippo population (Taylor 2013a), are likely to enhance dung concentration and retention, which, based on the findings of this chapter, would act over-and-above background stresses to deleteriously impact microalgal biomass and macrobenthic community metrics.

Importantly, as shown by studies elsewhere (Dolbeth et al. 2011), stressor-induced changes to benthic assemblages can lead to an overall decline in environmental quality and ecosystem resilience. This is particularly relevant when numerous stresses interact in imposing additive or synergistic pressure on assemblages. The intolerance of benthic macrofauna and microalgae to hippo dung addition during this comparatively short-lived experiment, raises concerns that persistent faecal inputs and accumulation over an extended time period may threaten the success of key ecological interactions and functions of these groups. Therefore, awareness of the potential for hippo dung accumulation to function as an important indirect or secondary stressor for benthic communities needs to be flagged, particularly in light of climate change and during drought conditions, due to the potential for dung to simultaneously interact with known primary drought stressors such as, habitat loss and desiccation, hypersalinity and recruitment limitations through extended mouth closure (see Figure 3.12, Pillay & Perissinotto 2008, Carrasco & Perissinotto 2012).

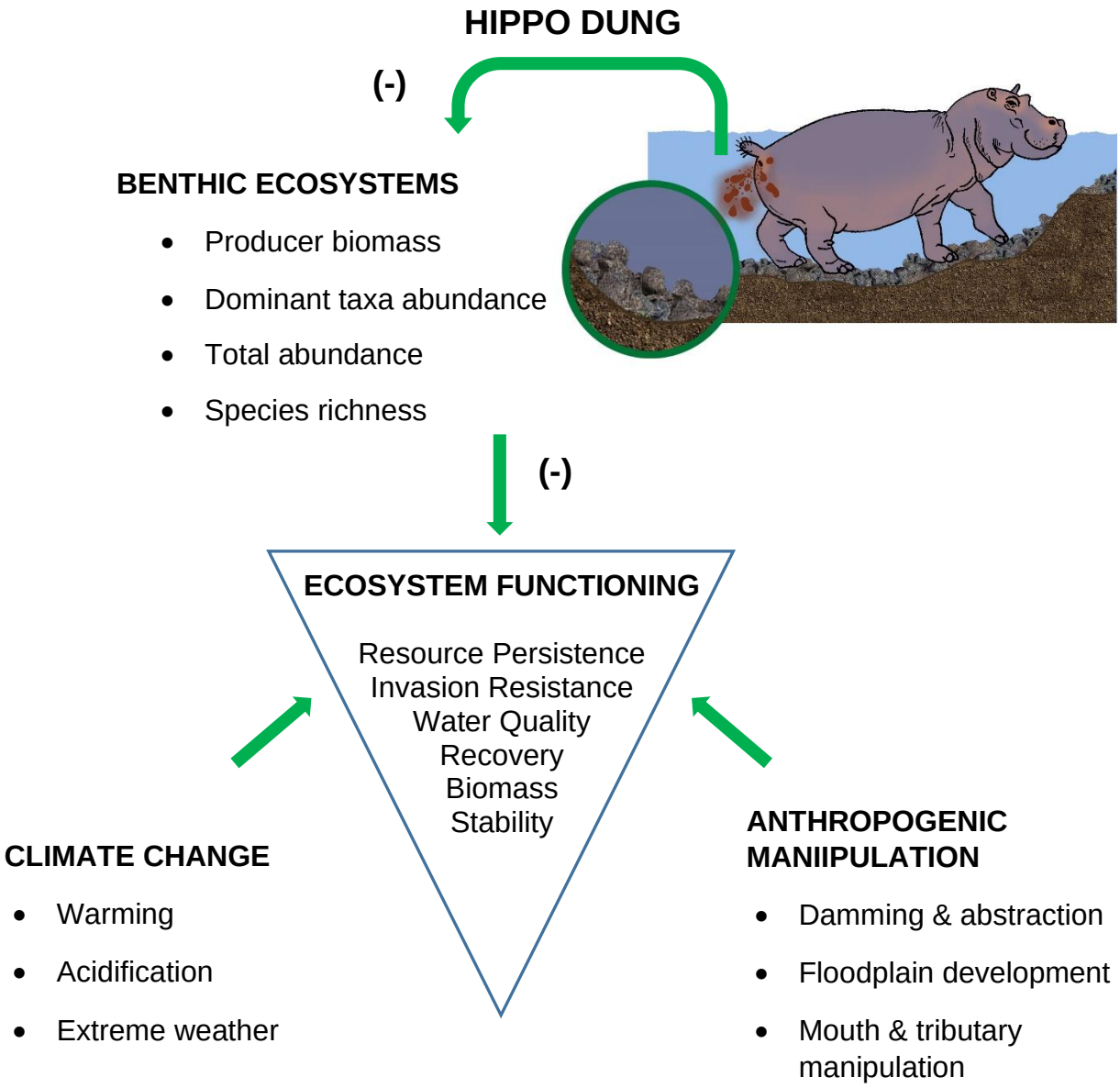


Figure 3.12: Schematic overview of repercussions of persistent dung inputs by dense hippo aggregates under low flow rates on benthic ecosystems and potential impacts on broader ecosystem functioning. Faecal inputs depress producer biomass, species richness and community abundance as well as that of dominant taxa. In combination, the latter weakens processes such as resource persistence, ecosystem stability, ability to resist invasion and potential to recover from disturbances. Persistent and intense dung inputs potentially act in concert with climate change and anthropogenic manipulation of aquatic systems to impair ecological functioning, thus requiring management intervention.

CHAPTER 4:

BENTHIC MEIOFAUNAL RESPONSES TO EXPERIMENTAL ENRICHMENT BY HIPPO DUNG

4.1 Introduction

4.1.1 Ecosystem functioning and functional traits

Understanding the link between biodiversity and ecosystem functioning has been one of the major challenges in ecological research studies for over two decades (Loreau et al. 2001, Cardinale et al. 2012, Hooper et al. 2012, Tilman et al. 2014). In this regard, several studies have explored the link between biodiversity and ecosystem functioning, emphasising that biodiversity loss can lead to a decline in ecosystem functions and processes such as (1) resistance (the ability to withstand change); (2) resilience or recovery potential (the ability to recover post change); (3) stability (the ability to withstand recurrent change/disturbance); (4) nutrient cycling; (5) water quality and (6) productivity (Worm & Duffy 2003, Hooper et al. 2005, Worm et al. 2006).

Increased biodiversity has been shown to enhance multi-functionality i.e. species rich ecosystems sustain multiple functions compared to depauperate ecosystems, providing ecosystem services that are of higher quality and less temporally variable (Yachi & Loreau 1999, Worm et al. 2006, Lefcheck et al. 2015). Therefore, species richness can provide a buffer against change, with the presence of numerous species ensuring that some will continue to persist and function even if others fail (the insurance hypothesis, Naeem & Li 1997, Yachi & Loreau 1999). However, a number of studies have illustrated that ecosystem functioning may be strongly influenced by species-specific traits and functional diversity rather than species richness alone (Loreau et al. 2001, Hooper et al. 2005, Norling et al. 2007, Cardinale et al. 2012). These studies highlight that while increased diversity does affect ecosystem processes, the observed responses are underpinned by species identity, density and functional traits (Ieno et al. 2006, Gagic et al. 2015). Therefore, using functional traits to investigate ecosystem functioning can be a powerful approach rather than the traditional approaches of using

community metrics (e.g. richness and abundance) within a particular organism group (Mouillot et al. 2013, Gagic et al. 2015). As such, functional traits are now considered key determinants of the way assemblages influence ecosystem functioning (de Bello et al. 2010).

A functional trait is defined as a characteristic of an organism that influences its performance and has demonstrable links to its function. In animals, functional trait examples include morphology, physiology, life history, behaviour and feeding habits (de Bello et al. 2010, Mouillot et al. 2013). It has been recognised that different assemblages, or functional groups, can have a different suite of traits that determine the magnitude and direction of their response to change/disturbance (Yachi & Loreau 1999, de Bello et al. 2010). Therefore, after a disturbance event, species with traits that allow them to withstand stressors or recover quickly will dominate the post-event population and will therefore determine extant trait characteristics and ecosystem functioning (Haddad et al. 2008, Mouillot et al. 2013). Schmitz et al. (2004) provided evidence that trophic cascades (the indirect effect of predators on producers mediated by consumers) were underpinned by trait-mediated effects. This illuminates the importance of recognising, and explicitly considering, organismal/community traits when assessing stressor impacts.

In the previous chapter, experimental techniques were used to quantify the effects of hippo dung inputs on macrofaunal and microalgal communities, which demonstrated a largely deleterious effect of dung on both. Recognising that ecosystem responses to perturbations are contingent upon biological traits of resident assemblages, the aim of this chapter was to experimentally test the effects of dung on benthic meiofauna, an assemblage with very different functional and biological traits to those of macrofauna. This is in line with the advice provided by Piot et al. (2014), who suggested that greater attention should be given to meiofaunal communities when conducting research on ecosystem functioning.

4.1.2 Benthic meiofauna and their use as indicators

Benthic meiofauna are generally defined as organisms that pass through a 0.5 or 1 mm mesh sieve, but are retained on a 63 µm mesh sieve, making them generally invisible to the naked eye (Heip et al. 1988, Schratzberger & Ingels 2017). This organismal group is characterised by having (1) relatively short life cycles, (2) an absence of a planktonic larval phase and (3) high abundance and diversity - even in systems with naturally high variability, such as estuaries (Heip et al. 1988, Somerfield et al. 2006, Alves et al. 2013). Meiofauna are considered important components of estuarine ecosystems and play a vital, and arguably more substantial role than macrofauna, in linking benthic primary producers and higher trophic levels (Castel 1992, Coull 1999, Nozais et al. 2005), simultaneously acting as critical food resources for predatory meiobenthos, benthic macrofauna, fish and even aquatic birds (Gee 1989, Gaston 1992, Nozais et al. 2005, Carpentier et al. 2014, Schratzberger & Ingels 2017).

Despite their small size, meiofauna have been recognised as important bioturbators and ecosystem engineers that modify physical, chemical and biological resource flows and properties in sediment (Schratzberger & Ingels 2017). Their movements within the benthos can influence multiple processes, including (1) sediment stability and erodibility, (2) fluxes of dissolved particles between overlying water and sediment and (3) sediment oxygenation (Coull 1999, Schratzberger & Ingels 2017). In addition, similar to the effects caused by extracellular polymeric substances (EPS) produced by macrofauna (Dawson & Pillay 2011), the production of sticky mucus by meiofauna enhances sediment stability, promotes bacterial and microalgal abundance, and facilitates nutrient trapping. This stimulatory effect on microbial community structure and activity, in turn influences rates of primary production and decomposition of organic matter (Riemann & Schrage 1978, Schratzberger & Ingels 2017).

Despite being ubiquitous and abundant in marine systems worldwide, meiofauna have only recently been included in research on ecosystem functioning and relatively few studies have used meiofauna as bio-indicators (Herman & Heip 1988, Whomersley et al. 2009, Alves et al. 2013, Zeppilli et al. 2015, Schratzberger & Ingels 2017). Heip et al. (1988) provides detailed arguments against and in favour of using meiofauna as bio-indicators. Briefly, the drawbacks include that they are taxonomically difficult to identify by inexperienced researchers, therefore requiring experts and extended time for identification (Herman & Heip 1988, Warwick 1988). In addition, preservation of samples (usually in alcohol or formalin) renders many "soft-bodied" meiofaunal species virtually unrecognisable (Heip et al. 1988). In spite of these drawbacks, the use of meiofauna as indicators have some advantages. Firstly, meiofaunal assemblages are more temporally stable than those of macrofauna, due to their accelerated growth rate, which allows them to recover from ecosystem change more rapidly (Worm et al. 2006), resulting in less variability both qualitatively and quantitatively. This makes it easier to monitor temporal changes from a stable, non-fluctuating baseline (Heip et al. 1988, Haddad et al. 2008). Secondly, meiofauna are smaller and therefore require less processing in the field (e.g. they can be preserved or fixed without a need for sieving). Lastly, meiofauna are abundant and diverse, occupying all habitats and their rapid generation times yield faster response times to perturbations (months not years).

Even with recent increases in popularity of meiofauna as bio-indicators (Alves et al. 2013) and the potential for comparisons of macrofaunal and meiofaunal responses to provide robust information on ecosystem responses to ecological perturbations, studies generally rarely make use of comparative approaches incorporating both meio- and macrofauna (Warwick et al. 1990, Somerfield et al. 2006, Whomersley et al. 2009). The community structure of both assemblages is likely determined by different processes. In the case of meiofauna for example, rapid growth rates to maturity enable then to develop a greater degree of feeding specialisation

on particles of different shape, size and quality, thereby allowing a more diverse group to co-exist within the same niche (Warwick 1984, Heip et al. 1988).

Conversely, macrofauna, which are less selective feeders, are dependent on spatial partitioning of habitats to maintain diversity (Warwick 1984). Due to this evolutionary diversification, with each group displaying distinct biological traits and therefore different levels of tolerance or sensitivity to disturbance, macrofaunal and meiofaunal communities often exhibit differential responses to disturbance (Haddad et al. 2008, Whomersley et al. 2009, Mouillot et al. 2013). In this regard, research has shown that while meiofauna are more susceptible to stressors such as pollutants, they are less sensitive to physical disturbance such as, hypoxia and burial (Josefson & Widbom 1988, Warwick et al. 1990, Somerfield et al. 2006). Therefore, when using both assemblages as indicators together, differential responses of macrofaunal and meiofaunal communities under the same disturbance regime could tell us more about the nature of the disturbance (Gray et al. 1990, Whomersley et al. 2009).

In the previous chapter, a schematic showing a hypothetical model predicting unimodal responses and response thresholds of benthic macrofaunal community metrics to increasing dung inputs was presented (Fig. 3.1). It is important to recognise however, that the threshold at which dung inputs shift from eliciting promotive to inhibitory responses is not fixed and is likely fluid, being dependant on local ecosystem features and biological traits of recipient assemblages (Hoffmann et al. 2012). The implication therefore is that the form of response curves will differ depending on the functional traits of the assemblages being investigated, as illustrated in Figure 4.1. In the context of responses to hippo dung, assemblages that express traits and/or life history strategies that negatively predispose them to dung are likely to display relatively sharp thresholds at which increasing dung inputs switch from inducing positive to negative responses (assemblage A). In contrast, assemblages with traits that enhance resistance to change will display a higher response threshold to increasing dung inputs (assemblage B).

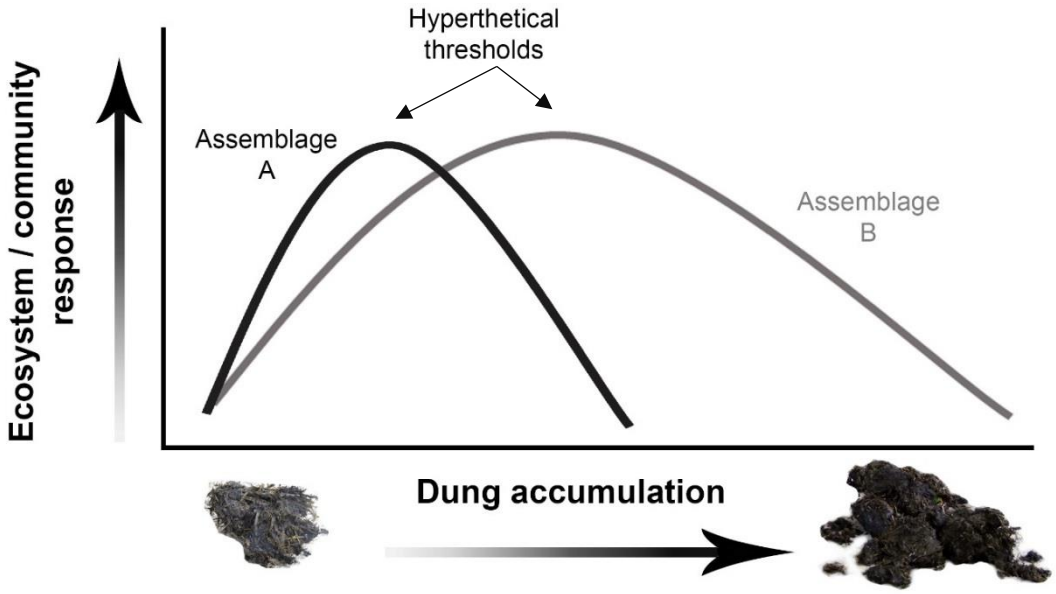


Figure 4.1: Schematic showing the hypothetical responses of two assemblages to increasing dung inputs. Assemblage A is more sensitive to disturbance and therefore has a lower tolerance threshold at which a positive response becomes negative. Assemblage B is more resistant to effects of hippo dung accumulation, and thus has a higher tolerance to dung inputs.

In Chapter 3, it was observed that, at current levels, hippo dung inputs in St Lucia can lead to declines in microalgal biomass and macrofaunal community metrics. The primary objective of this chapter was to investigate the response of meiofauna to the same experimental enrichment of hippo dung, thus enabling comparisons in responses between these functionally distinct, but ecologically important benthic assemblages. Specifically, the aim was to quantify the magnitude and direction of meiofaunal community responses to experimental dung enrichment and to determine whether responses were spatially consistent. Given previous studies showing that meiofauna have a greater resistance and resilience to disturbance (Josefson & Widbom 1988, Warwick et al. 1990, Haddad et al. 2008, Whomersley et al. 2009), it was predicted that meiofauna would be less sensitive to dung addition. The hypothesis

proposed was therefore, that meiofaunal responses would be weaker than macrofaunal responses and that responses would be taxon specific, with some taxa exhibiting a higher tolerance to hippo dung accumulation. Ultimately, the findings of this chapter will be compared to those of Chapter 3 to make inferences about benthic ecosystem functioning in response to hippo dung inputs under drought conditions.

4.2 Results

4.2.1 Community responses to dung

Meiofaunal community structure was statistically indistinguishable between sites (Fig. 4.2, PERMANOVA pseudo $F_{1,59}$ = 0.726; p = 0.684) and dung treatments (PERMANOVA pseudo $F_{2,59}$ = 1.717; p = 0.136). The latter is visually supported by nMDS ordinations, showing overlap of samples between different sites and dung treatments (Fig. 4.2). Multivariate dispersion was greater in meiofaunal assemblages following addition of hippo dung at Site 2 (Table 4.1, Fig. 4.2, PERMDIST, dung present: 26.637 ± 3.7 SE; dung absent: 14.762 ± 1.4 SE). In contrast, dung addition reduced meiofaunal assemblage variability in Site 1 (Table 4.1, Fig. 4.2, PERMDIST, dung present: 15.836 ± 2.7 SE; dung absent: 23.715 ± 3.6 SE).

Table 4.1: Summary statistics for multivariate dispersion tests showing average variability (+ SE) of meiofaunal communities (based on abundance data) between dung treatments at each site.

PERMDISP			
Site	**Treatment**	**Average Dispersion**	**SE**
1	Dung	15.836	2.65
	No Dung	23.715	3.57
2	Dung	26.637	3.7
	No Dung	14.762	1.44

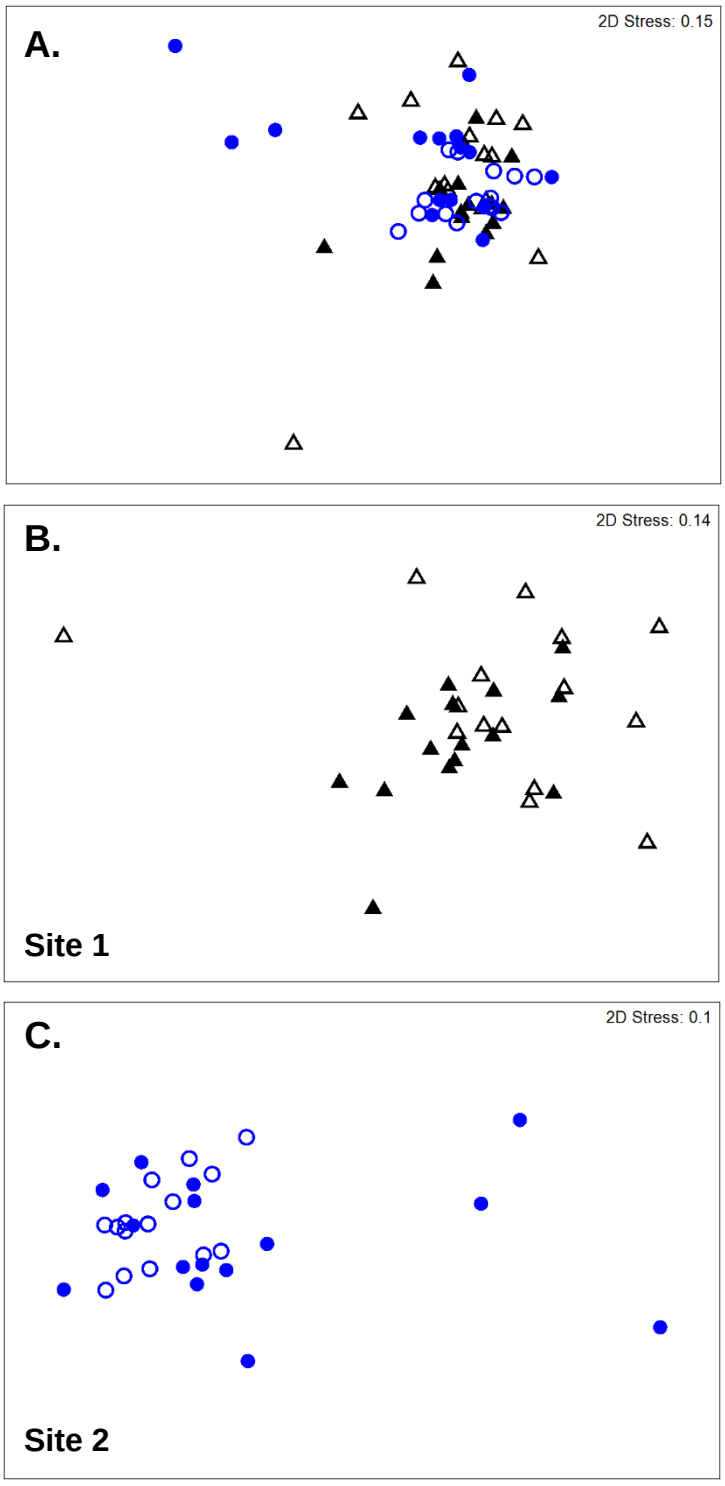


Figure 4.2: Non-metric multidimensional scaling ordination (nMDS) showing spatial variation in meiofaunal community structure, based on abundance data, between sites and dung treatments. A. shows samples from dung inclusion (filled symbols) and exclusion (unfilled symbols) treatments at Sites 1 (black symbols) & 2 (blue symbols). B. shows dung inclusion and exclusion treatments at Site 1, while C. shows dung inclusion and exclusion treatments at Sites 2.

Meiofaunal abundance generally decreased following hippo dung enrichment, with abundance decreasing by 26.8 % at Site 1 and 37.5 % at Site 2. However, due to high variance in the data, this trend was not statistically supported. Neither dung treatment nor site significantly affected meiofaunal abundance (Table 4.2, Fig. 4.3, Nested ANOVA, Site: $F_{1,52}$ = 1.235, p = 0.271, Dung Treatment: $F_{2,52}$ = 1.832, p = 0.170). Meiofaunal species richness was not significantly affected by site (Fig. 4; Nested ANOVA, $F_{1,52}$ = 0.012, p = 0.913), but was affected by dung treatment (Nested ANOVA, $F_{2,52}$ = 5.083, p = 0.010). At Site 1, richness increased with the addition of dung, whereas the reverse was evident at Site 2.

Table 4.2: Results of Nested ANOVA testing for differences in meiofaunal community descriptors between sites and dung treatments. Bold *p*-values indicate statistically significant differences. *F* = F-statistic, *p* = significance level, DF = degrees of freedom.

	Nested ANOVA					
	Site			Treatment		
	F	DF	*p*	*F*	DF	*p*
Meiofaunal Abundance	1.235	(1,52)	0.271	1.832	(2,52)	0.170
Meiofaunal Species Richness	0.012	(1,52)	0.913	5.083	(2,52)	**0.010**
Meiofaunal Evenness	8.384	(1,52)	**0.006**	2.202	(2,52)	0.121
Meiofaunal Diversity	1.628	(1,52)	0.208	6.238	(2,52)	**0.004**

Meiofaunal evenness was statistically distinguishable between sites, with values being lower in Site 1than Site 2 (Table 4.2, Fig. 4.3, Nested ANOVA, $F_{1,52}$ = 8.384, p = 0.006). Evenness was not significantly different between dung treatments (Table 4.2, Fig. 4.3, Nested ANOVA, $F_{2,52}$ = 2.202, p = 0.121). Conversely, meiofaunal diversity did not differ between experiment sites (Table 4.2, Fig. 4.3, Nested ANOVA, $F_{1,52}$ = 1.628, p = 0.208) but was significantly affected by dung treatment (Table 4.2, Fig. 4.3, Nested ANOVA, $F_{2,52}$ = 6.238, p = 0.004). At Site 1, diversity increased with dung enrichment but decreased with dung addition at Site 2. This pattern mirrored that observed for richness.

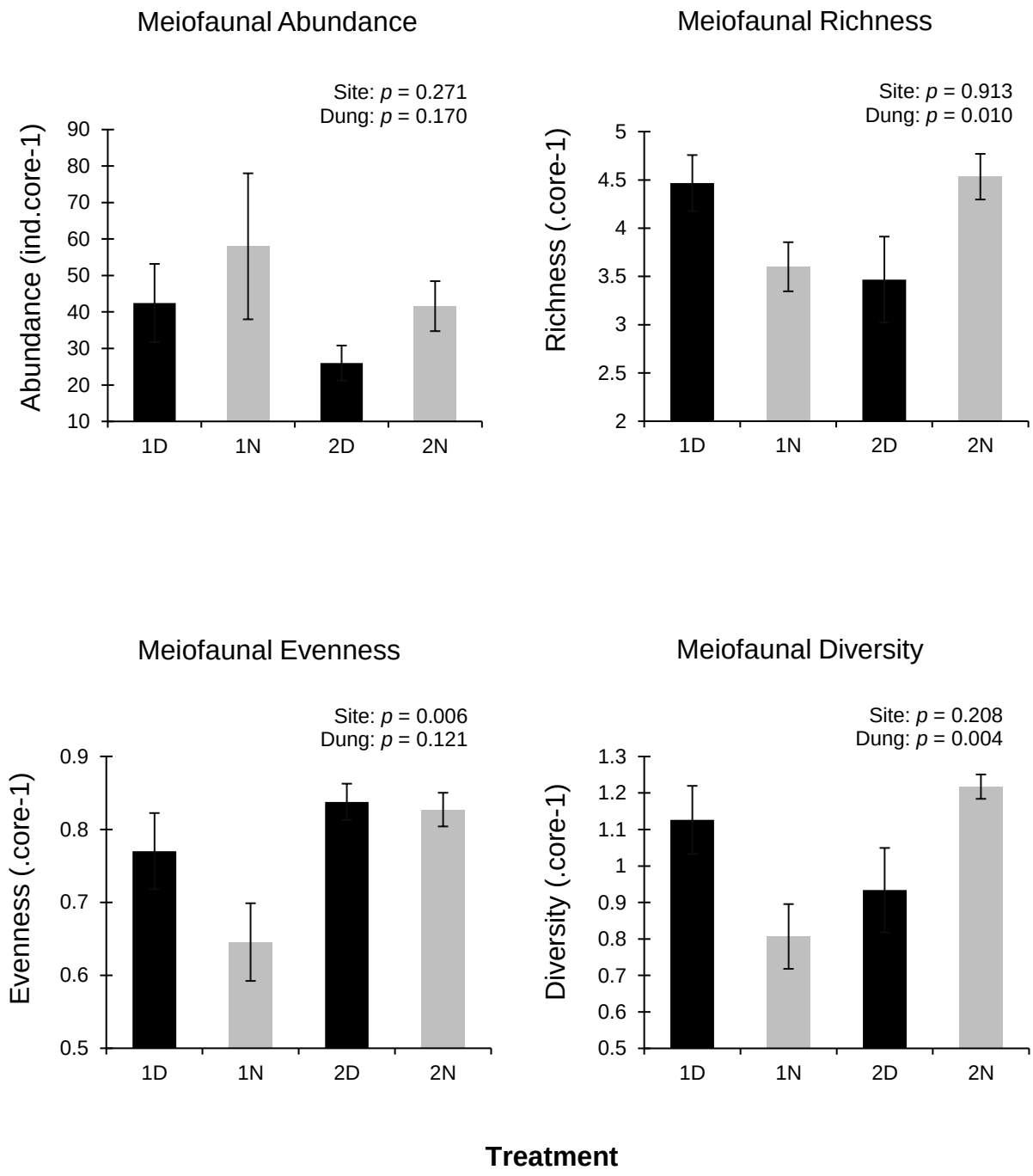


Figure 4.3: Spatial variation in mean (± 1 SE) meiofaunal community metrics at the two experimental sites in response to dung addition (D - black) and exclusion (N - grey). Numbers in treatment name = site number; results of Nested ANOVA are shown.

4.2.2 Responses of individual taxa to dung

At an individual taxon level, SIMPER analyses identified four meiofaunal taxa accounting for 90 % dissimilarity between dung treatments at Site 1 and Site 2 (Table 4.3). Unlike trends in macrofaunal taxa (Table 3.3), which showed predominantly a decrease in abundance in the presence of dung, meiofaunal taxa exhibited both positive and negative responses to dung enrichment, depending on the site. At Site 1, the abundance of one taxon decreased and 3 taxa increased to varying degrees, whereas the abundance of all four dominant taxa at Site 2 were depressed following dung enrichment.

Table 4.3: Meiofaunal taxa identified by SIMPER to cumulatively account for 90 % of the community dissimilarity between dung exclusion and inclusion treatments at Site 1 and 2, based on abundance data. Bold text highlights taxa showing an increase in abundance with the addition of hippo dung. F: Foraminifera, N: Nemata, G: Gastropoda, P: Polychaeta.

	Site 1			Site 2	
	Dung	No Dung		Dung	No Dung
Dominant Taxa (Abundance data)	**Average abundance**		**Dominant Taxa (Abundance data)**	**Average abundance**	
Foraminifera (F)	24.53	44.80	*Assiminea* cf. *capensis* (G)	8.13	13.33
Nematode sp. (N)	**8.00**	**7.53**	Nematode sp. (N)	8.20	14.00
***Assiminea* cf. *capensis* (G)**	**5.93**	**3.47**	Foraminifera (F)	6.87	11.07
***Polydora* sp. (P)**	**2.53**	**1.20**	*Polydora* sp. (P)	1.20	1.33

Of the four taxa identified by SIMPER to account for 90 % dissimilarity between dung treatments, only one taxon (*Assiminea* cf. *capensis*) was significantly affected by both site and dung treatment (Table 4.4, Fig. 4.4; Nested ANOVA, site: $F_{1,52}$ = 10.467, p = 0.002; dung treatment: $F_{2,52}$ = 3.478, p = 0.038). At Site 1, dung enrichment increased the abundance of *A. capensis*, whereas at Site 2, abundance decreased in dung treatment plots compared to un-enriched plots. The abundance of one taxon, Foraminifera, was marginally non-significantly

different between sites (Table 4.4, Fig. 4.4; Nested ANOVA, site: $F_{1,52}$ = 3.554 p = 0.065). Both the Nematode sp. and *Polydora* sp. displayed marginally non-significant dung treatment responses (Nested ANOVA p = 0.072 and p = 0.067 respectively). However, responses were erratic, as Nematode sp. abundance decreased at Site 2 with dung addition, but at site 1 *Polydora* sp. abundance increased (Fig. 4.4).

Table 4.4: Results of Nested ANOVA testing for differences in individual meiofaunal taxa abundance and size between sites and dung treatments. Bold *p*-values indicate statistically significant differences. Taxon: F: Foraminifera, N: Nemata, G: Gastropoda, P: Polychaeta. *F* = F-statistic, *p* = significance, DF = degrees of freedom.

Nested ANOVA						
Individual taxa abundance						
	Site			Treatment		
Taxa	*F*	DF	*p*	*F*	DF	*p*
Foraminifera (F)	3.554	(1,52)	0.065	1.364	(2,52)	0.265
Nematode sp. (N)	2.246	(1,52)	0.140	2.754	(2,52)	0.072
Assiminea cf. *capensis* (G)	10.467	(1,52)	**0.002**	3.478	(2,52)	**0.038**
Polydora sp. (P)	2.282	(1,52)	0.137	2.846	(2,52)	0.067
Individual taxa size						
	Site			Treatment		
Taxa	*F*	DF	*p*	*F*	DF	*p*
Foraminifera (F)	0.473	(1,44)	0.495	1.171	(2,44)	0.192
Nematode sp. (N)	5.361	(1,46)	**0.025**	16.316	(2,46)	< **0.001**
Assiminea cf. *capensis* (G)	1.186	(1,45)	0.282	0.554	(2,45)	0.578
Polydora sp. (P)	0.816	(1,11)	0.386	0.300	(2,11)	0.746

When considering the size of dominant taxa, only Nematode sp. displayed significant responses to site and dung treatment (Table 4.4, Fig. 4.5; Nested ANOVA, site: $F_{1,46}$ = 5.361, p = 0.025; dung treatment: $F_{2,46}$ = 16.316, p < 0.001). At Site 1, Nematodes sp. were larger in dung enrichment plots, but conversely at Site 2, individuals were smaller in enriched plots. Foraminifera, *Assiminea* and *Polydora* sp. all had non-significant responses to both site and treatment (p > 0.05 for all).

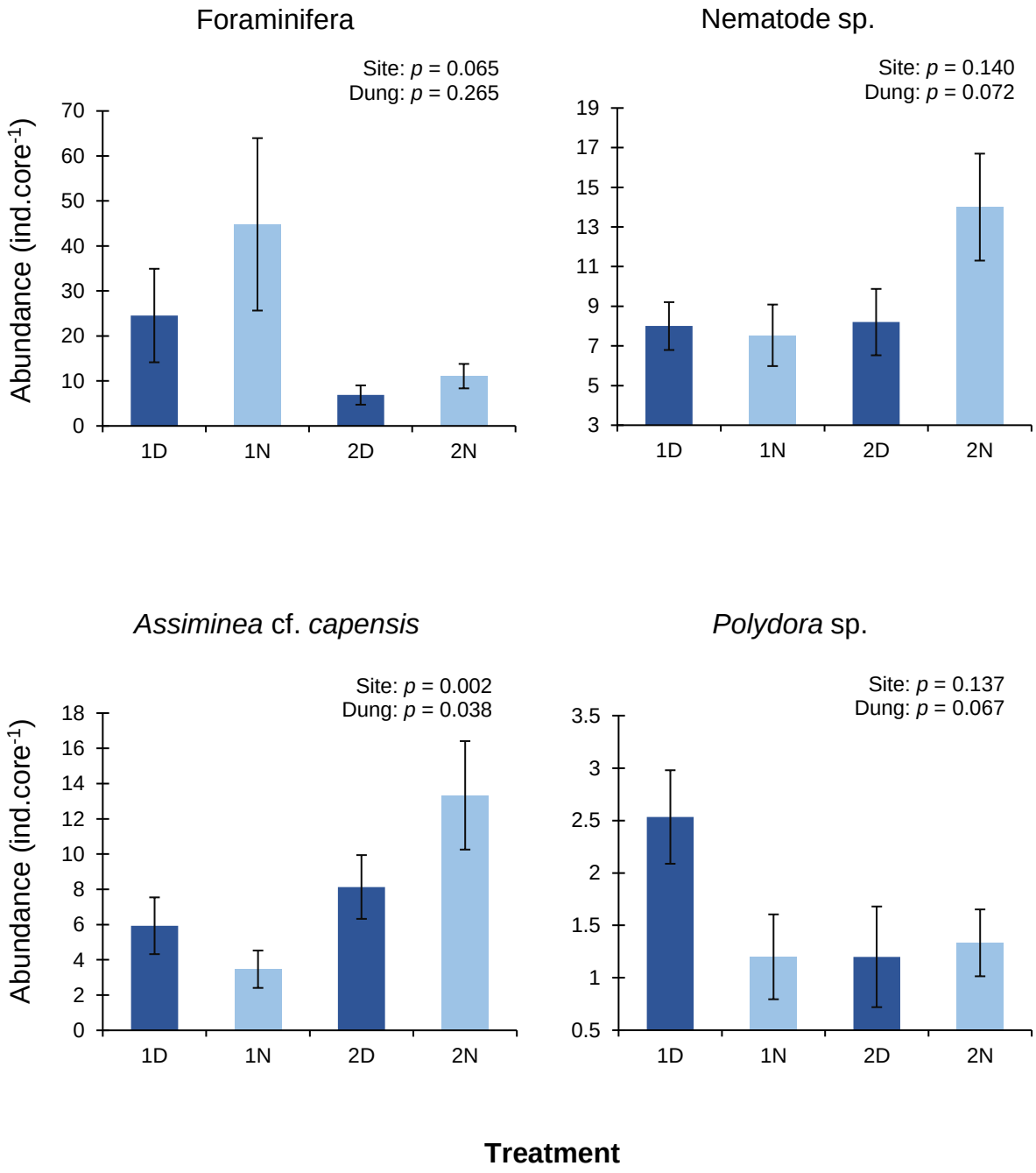


Figure 4.4: Spatial variation in mean abundance (± 1 SE) of dominant meiofauna taxa at the two experimental sites in response to dung addition (D – dark blue) and exclusion (N – light blue). Numbers in treatment name = site number; results of Nested ANOVA are shown.

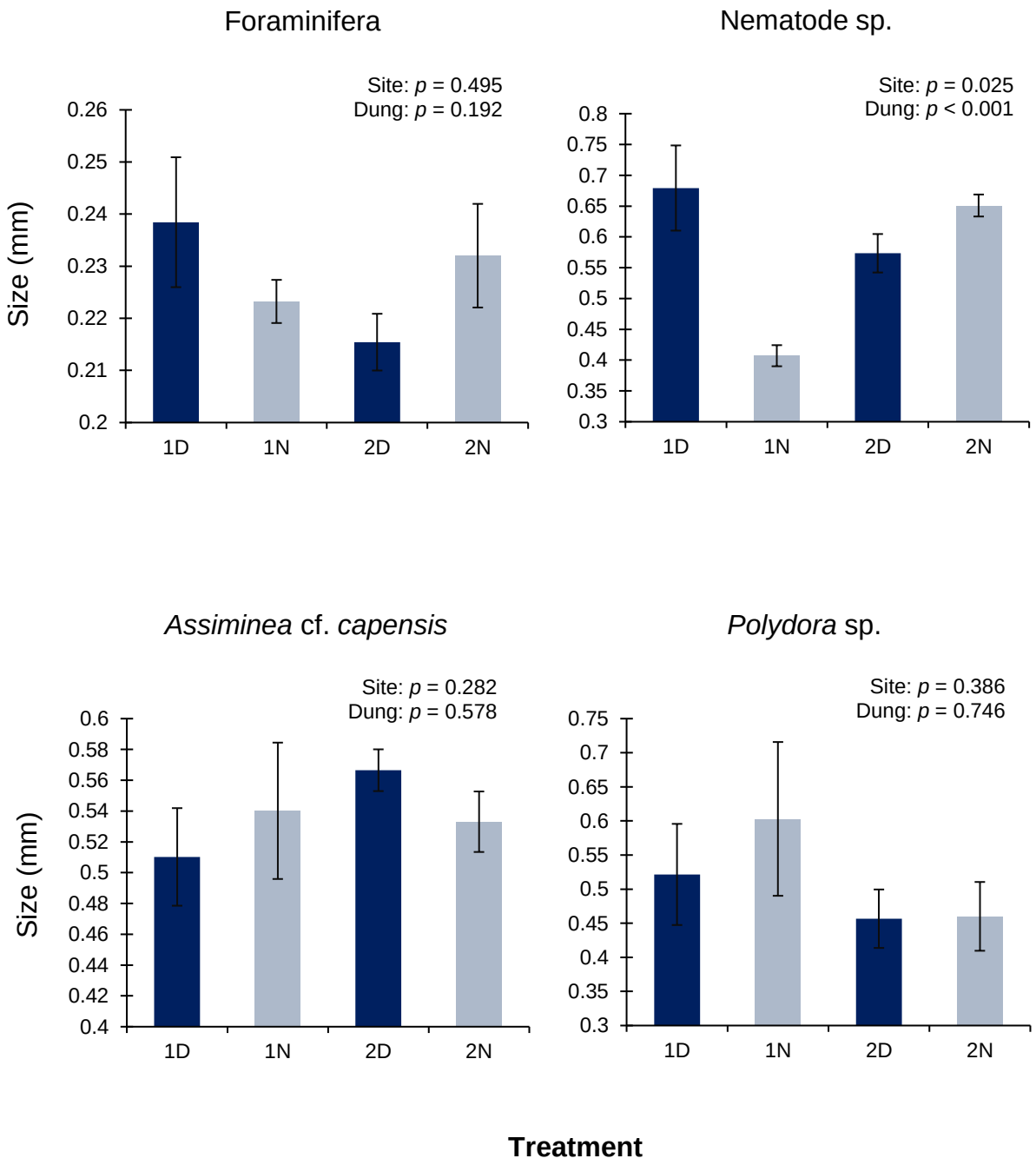


Figure 4.5: Spatial variation in mean size (± 1 SE) of dominant individual meiofauna taxa at the two experimental sites in response to dung addition (D - navy) and dung exclusion (N - grey). Numbers in treatment name = site number; results of Nested ANOVA are shown.

4.3 Discussion

Comparing the responses of functionally distinct communities to ecosystem change can increase our understanding of mechanisms propagating change, and improve our ability to mitigate or manage them in future (Warwick et al. 1990, Rodil et al. 2013). With this notion in mind, the central aim of this chapter was to quantify the magnitude and direction of meiofaunal responses to experimental hippo dung accumulation in the St Lucia Estuary and determine whether responses were spatially consistent. The secondary objective was to compare these responses to those of macrofauna recorded in Chapter 3, to determine if dung inputs elicited the same responses from two functionally distinct benthic groups.

It was predicted that meiofauna would be more resilient to dung accumulation than macrofauna and would therefore show weaker responses to dung inputs. Based on levels of dung used and environmental contexts under which the experiment was conducted, the data indicate differential responses of macro- and meiofaunal assemblages to enrichment by hippo dung. The (1) neutral responses of meiofaunal community structure, total abundance and evenness, the (2) positive and negative responses of meiofaunal richness, diversity and individual taxa and the (3) prevalence of declining trends in macrofaunal community and individual metrics together with a significant shift in the structure of this assemblage, point to benthic macrofauna being more vulnerable to hippo dung accumulation. Meiofauna in contrast, appear more robust in resisting negative effects of dung inputs, even responding positively in some instances.

4.3.1 Meiofaunal community responses in space

Meiofaunal community structure did not differ significantly (visually or statistically) between dung treatments, with no spatial variation at the community level between the two

sites. In addition, of the four community metrics examined in this chapter, only one (meiofaunal evenness) displayed spatial variation at the site level. Mean evenness at Site 1 was lower than at Site 2. Two of the four community metrics (species richness and diversity) had significant treatment effects. In both cases, richness and diversity were higher in dung enrichment plots at Site 1, but lower in enrichment plots at Site 2. Wind-driven wave action was observed to be noticeably greater at Site 1 than at Site 2 - therefore, the opposing responses of meiofaunal richness and diversity suggest that the direction of dung effects on meiofauna are site specific, potentially determined by wave action.

The responses of dominant meiofauna taxa were erratic, with no common trends evident between sites. With regards to treatment effects, SIMPER showed that at Site 1, all but one dominant taxon increased in abundance, while at Site 2, all dominant taxa decreased with dung enrichment. One of the four taxa (*Assiminea* cf. *capensis*) showed a statistically significant difference between sites, although Foraminifera had marginally non-significant spatial differences. Similarly, only *Assiminea* cf. *capensis* varied significantly between treatments. With regard to sizes of dominant meiofaunal taxa, responses were also erratic with patterns being reversed spatially, although this was only statistically supported in the Nematode sp., with larger individuals present in dung inclusion plots in Site 1 and smaller individuals in dung inclusion plots in Site 2. The opposing, site-specific responses of individual taxa is further support for the notion that wave action may cause spatial variations in meiofaunal responses.

Overall, the results point to a more variable response of meiofauna at the community metric level and the level of individual taxa abundance and size. In many cases, trends were not supported statistically, potentially indicating a high level of resilience of meiofauna to dung inputs. Placing the findings of this chapter into context of existing knowledge is difficult due to the lack of comparable studies of a similar nature. However, studies elsewhere that have differentiated responses of macro- and meiofauna to other stressors have shown that meiofauna

can be more robust than macrofauna (e.g. (Josefson & Widbom 1988, Warwick et al. 1990, Somerfield et al. 2006), which supports findings of this and the previous chapter.

4.3.2 Hypothesized mechanisms underlying meiofaunal responses to dung

Research has shown that interactions between meiofauna, macrofauna and microbiota are complex (Schratzberger & Ingels 2017). In Chapter 3 it was evident that microalgal biomass and macrofaunal abundance declined by up to 50 and 76 % respectively with the addition of dung, which is suggestive of dung indirectly supressing macrobenthic abundance through bottom-up mechanisms (amongst others), i.e. trophic resource limitation. In this chapter however, despite the decline in microphytobenthic biomass, meiofaunal abundance showed a weak response to dung enrichment. Studies have shown that meiofauna can apply low microalgal grazing pressure because they are not solely reliant on microphytobenthos, but also consume bacteria and other meiofaunal species (Nozais et al. 2005). As such, although the addition of dung evidently reduces microalgal abundance, it also potentially increases the abundance of benthic bacteria; firstly, by the addition of hippo gut bacteria present in dung, and secondly, by providing more substrate (dung) for bacterial decomposers to colonise (Jones 1992). In addition, meiofauna are known to stimulate bacterial abundance as their consumption/grazing of these microbiota keeps the population in the active growth phase, and their production of nitrogen rich mucus stimulates and traps additional microbes (Riemann & Helmke 2002, Schratzberger & Ingels 2017). Therefore, although microphytobenthic biomass may be negatively affected by dung addition, bacterial biomass may be positively affected. The mismatch in microalgal biomass and meiofaunal abundance aligns with the notion that meiofauna are not food limited (Nozais et al. 2005), and therefore not susceptible to bottom-up controls (Coull 1999).

Coull (1999) suggested that there is little evidence that top-down controls influence meiofaunal abundance and diversity. The implication is that meiofauna are able to resist predation pressure by macrofaunal species resulting in little evidence of consumer induced meiofaunal declines (Schratzberger & Ingels 2017). In the context of this study, the latter finding may explain the fact that reductions in macrofaunal abundance (and potentially a drop in top-down pressure) as a result of dung addition, did not result in increases in their abundance. Macrofauna do however play an important role in structuring benthic communities through bioturbation or physical disturbances and changes to sediment biochemistry (Braeckman et al. 2011, Schratzberger & Ingels 2017).

By altering sediment physico-chemical conditions such as porosity, penetrability and oxygen content, macrofauna increase habitat complexity and hence the available habitat for meiofauna (Hall & Bell 1988, Braeckman et al. 2011). Detritus has also been shown to increase habitat complexity (Langellotto & Denno 2004), which in turn promotes species density, diversity and persistence (Hall & Bell 1988, Moyle et al. 2010). Thus, with all these interacting processes occurring with positive and negative effects, it is possible that the lack of significant and consistent patterns in meiofaunal responses to enrichment with hippo dung may be due to opposing processes negating each other and therefore generating null responses. Of course, the resilience of meiofauna to dung inputs, by virtue of their inherent biological traits such as rapid turnover rates (Schratzberger & Ingels 2017), should not be discounted as an explanation for generally neutral findings.

An alternative explanation for the neutral response of meiofauna abundance to dung inputs may be related to methodological artefacts. It is possible that due to the presence of dung on the sediment surface, along with potential deteriorating abiotic conditions (as suggested in Chapter 3), intolerant meiofauna may have migrated deeper into the sediment, with tolerant ones remaining in the upper sediment layer. Studies elsewhere have shown that meiofauna may

undergo vertical migration in response to fish predation and physical disturbance (Coull et al. 1989, Palmer et al. 1992, Johnson et al. 2007, Schratzberger & Ingels 2017) therefore, it can be speculated that dung induced disturbance may similarly cause meiofauna to migrate deeper into the sediment. In this study, meiofauna samples were collected from the upper 1 cm sediment layer; this is a technique commonly used to sample meiofauna, including in the St Lucia Estuary (Carmen & Fry 2002, Nozais et al. 2005, Pillay & Perissinotto 2009, Bownes & Perissinotto 2012). This strategy however, would have meant that any potential meiofaunal shifts deeper into the sediment would have been undetected. This is an area that could be examined in future studies to test whether dung addition induces vertical shifts in meiofaunal communities within the sediment.

Interestingly, responses of individual meiofaunal taxa to dung addition were site specific, with dung eliciting increases in abundance in three species at Site 1 but decreases in abundance in all taxa at Site 2. This could be the result of variable abiotic conditions between the two sites such as, increase wave action observed at Site 1 relative to Site 2. When wave action is higher, water flow is similarly increased, and dung does not accumulate. Under these conditions, dung addition seemingly induces effects that are less detrimental and meiofaunal taxa may respond positively, possibly due to increased trophic resource availability in the form of organic matter from dung or associated bacteria. In contrast, low flow conditions and stagnation may cause negative effects by potentially reducing water quality (Gereta & Wolanski 1998, Dutton et al. 2018, Stears et al. 2018, Subalusky et al. 2018).

4.3.3 Comparisons with macrofauna

The results of this and the previous chapter indicate that while responses of meio- and macrofaunal assemblages to dung inputs were different, they were not always spatially consistent, even at the relatively small spatial scale (150 m) over which the experiment was

conducted. In the case of benthic macrofauna, response directions were generally spatially uniform, with dung largely inducing reductions in community and individual metrics. However, magnitudes of macrofaunal responses were highly variable spatially. Conversely, meiofauna exhibited responses that were inconsistent in both direction and magnitude. Collectively, these trends indicate that even at small spatial scales within aquatic biotopes that are usually considered relatively uniform, such as lakes, meso-scale processes may be important in mediating responses of particular assemblages to hippo dung inputs. As mentioned earlier, *in situ* observations indicated greater wave action in Site 1, suggesting that at scales of hundreds of meters, variability in flow can be significant in determining responses of assemblages to dung inputs, with low flow intensifying negative impacts in the case of benthic macrofauna, and meiofaunal richness and diversity (Stears et al. 2018, Subalusky et al. 2018).

The differential susceptibilities of macro- and meiofauna to hippo dung recorded in this and the previous chapter are likely driven by the unique morphological and life-history traits these groups express, such as, growth rate and colonization ability (Loreau et al. 2001). Macrofaunal life-cycles typically incorporate planktonic larval stages, which can be affected by hippo dung (1) forming a physical barrier that restricts larval settlement and/or (2) causing negative settlement cues as a result of altered water and sediment biogeochemistry (through decomposition, Fig. 3.11). In contrast, the majority of meiofaunal taxa do not have larval stages, but instead undergo direct development within sediments (Dahms & Qian 2004); therefore a physical barrier of dung would not necessarily inhibit recruitment onto the sediment surface. In addition, meiofauna may conceivably be positively affected by the abrasive effects of hippo dung as their lack of a mobile larval phase makes them partially dependant on recolonization through resuspension of sediment into the water column (Josefson & Widbom 1988). The decline in abundance of crab zoea (table 3.3) following enrichment supports the idea that dung presence can dampen macrofaunal recruitment, while the significant increase in

Nematode sp. in dung treatments at Site 1 (Fig. 4.5) partially supports the hypothesized positive effect on meiofaunal colonization.

Given existing evidence of low oxygen states selecting against large-bodied benthic organisms (Pearson & Rosenberg 1978), the greater size of macrofauna is also a trait that negatively predisposes this group to low oxygen levels associated with decomposition of dung. Warwick (1993) demonstrated that under stable conditions or low levels of disturbance, larger, long-lived species (*K*-selected) contributed significant biomass, if not abundance, to the community. However, in a disturbed ecosystem, opportunistic, smaller, short-lived species (*r*-selected) become dominant in both biomass and abundance. The experimental findings showing a decline in larger individuals (macrofauna) and subsequent persistence of smaller individuals (meiofauna) suggest that the addition of hippo dung is causing a biological shift to smaller sized benthic assemblages by functioning as a disturbance agent. This supports the notion that organic enrichment, regardless of its origin, can cause a disturbance which results in a shift in the state of a benthic community, by selecting for smaller, rapidly growing and reproducing species (Pearson & Rosenberg 1978, Kemp et al. 2005, Smith & Schindler 2009).

4.3.4 Concluding perspectives

Findings from this chapter show that meiofauna respond differentially to hippo dung enrichment relative to macrofauna, in which meiofaunal responses are neutral or site-specific, but also opposing in direction to those of macrofauna. Therefore, meiofauna appear to be more robust than macrofaunal assemblages. The implication of this finding is that in addition to meiofauna being resilient, the ecosystem functions that they provide (e.g. nutrient recycling and mineralisation, bioturbation) are also likely to be more resilient to dung inputs. This contrasts with the situation for macrofauna, where high dung inputs generate largely negative impacts, with similar effects therefore likely for the functions they provide.

CHAPTER 5:

STABLE ISOTOPE SIGNATURES OF FOOD WEB COMPONENTS IN AREAS WITH CONTRASTING HIPPO DUNG INPUTS: A COMPARATIVE APPROACH

5.1 Introduction

In the previous data chapters, the effects of experimental dung enrichment on meio- and macrobenthic communities were examined. This was motivated by observations of hippo dung accumulating over the benthos. However, apart from having community levels implications, hippo dung may also have complex consequences for entire food webs, spanning both aquatic and benthic habitats, by functioning as a trophic resource for consumers. This aspect of the ecology of hippo dung forms the basis of this and the next chapter.

5.1.1 Food webs & stable isotopes

Stuart Pimm (2002) famously stated that "To protect nature, we must have some understanding of her complexities, for which the food web is the basic description". Indeed, food webs and food web interactions have become a central focus of ecological research, reflecting the importance of these areas in unravelling ecological complexity (Polis & Strong 1996, Pimm 2002, Polis et al. 2004, Sanders et al. 2014). Food webs can be conceptualised as hierarchical models or maps that depict the flow of energy and materials within communities. Simplistically, they describe who eats who, and what (Pimm et al. 1991, Power & Dietrich 2002, Schindler & Lubetkin 2004), but are in reality composed of multiple resource and consumer modules linked by a network of reticulate connections (Polis & Strong 1996).

Several processes determine the topology of food webs and internal diversity, but these can be distilled into (1) top-down processes i.e. consumer induced regulation of lower trophic levels, including primary productivity; and (2) bottom-up forces, which involves regulation of higher trophic levels by basal trophic resources (Power & Dietrich 2002, Huang et al. 2013). Apart from shedding light on trophic relationships and interactions, research has shown the importance of including food webs in the understanding of broader ecosystem processes, since

food web interactions are central to governing ecosystem multi-functionality (Hall & Raffaelli 1991, Pimm 2002, Pasquaud et al. 2007, Bouillon et al. 2011). In this regard, several studies have demonstrated the utility of food web dynamics in developing a predictive understanding of disturbances and hence ecosystem stability, resistance and resilience (Pimm et al. 1991, Pimm 2002, Bird et al. 2016).

While it is possible to infer food web interactions using observations or experimental manipulations, this may be logistically difficult in aquatic ecosystems (Eggers & Jones 2000). Early food web studies used stomach content analysis (SCA) to determine consumer diets, however, there are several drawbacks to this technique. It often requires that focal consumers be sacrificed, and identification and enumeration of stomach contents are difficult, particularly when prey items are small and amorphous (e.g. algae and detritus). In addition, variable digestion rates of prey results in abundance estimates being compromised (Schindler & Lubetkin 2004, Pasquaud et al. 2007, Polito et al. 2011). Lastly, SCA provides a snapshot of consumer diets, as gut contents typically contain only the most recently consumed meal (Schindler & Lubetkin 2004).

Stable isotope analysis (SIA) provides a technique for ecologists to more accurately disentangle food web structure and function, due to the high precision at which the isotopic composition of food web components can be measured (Peterson & Fry 1987, Deines et al. 2009). According to Eggers and Jones (2000) “You are what you eat”, and therefore, the isotopic composition of consumer body tissue closely resembles that of its diet. The use of these tissues, which record prey assimilation over periods of weeks or months, provides a longer term, time-integrated measure of feeding history than the use of SCA (Schindler & Lubetkin 2004, Masese et al. 2015). Therefore, SIA has become more popular and isotopes have routinely been used as environmental tracers to assess trophic relationships and energy

flow within marine and aquatic ecosystems (Peterson & Fry 1987, Post 2002, Bouillon et al. 2011).

An important process underling SIA is that the isotopic composition of natural materials changes in predicable ways once consumed by an organism (Peterson & Fry 1987). This is known as fractionation, and refers to the preferential release of lighter elemental isotopes in biochemical reactions such as, respiration and excretion (Peterson & Fry 1987, Eggers & Jones 2000, Alfaro et al. 2006). As a result, in trophic interactions, consumers tend to become isotopically heavier than the food sources on which they feed (Eggers & Jones 2000). The ratio of ^{13}C to ^{12}C isotopes, commonly reported as $\delta^{13}C$, is used to differentiate between organic carbon sources and to infer energy flow within food webs because distinct $\delta^{13}C$ values can be ascribed to different sources and there is little fractionation (0-1 %) between sources and consumers (Eggers & Jones 2000, Vander Zanden & Rasmussen 2001, Post 2002, Masese et al. 2015).

Alternatively, due to their characteristically higher fractionation values (~3.4 %), nitrogen isotope ratios (^{15}N:^{14}N, reported as $\delta^{15}N$) are used to identify inorganic nitrogen sources and the relative trophic position of organisms (Peterson & Fry 1987, Vander Zanden & Rasmussen 2001, Post 2002). In this way, source material can be traced and identified within consumers. However, when stable isotopes are used to investigate the proportional contributions of multiple sources to a consumer (also known as mixture), it is essential that the sources have statistically distinguishable isotopic ratios (Phillips & Gregg 2001, 2003, Schindler & Lubetkin 2004). Therefore, the combination of both carbon and nitrogen isotope ratios allows for greater differentiation between food sources, especially if there is an overlap of one of the isotope ratios (Peterson et al. 1985, Peterson & Fry 1987, Masese et al. 2015). This duel isotope approach enables ecologists to understand and construct intricate food webs based on relatively accurate dietary information (Pasquaud et al. 2007).

5.1.2 Ecosystem connectivity, trophic transfers and hippos

Until about two decades ago, most food web studies focused on 'local spatial scales', treated ecosystems as closed and only considered interactions within systems, not between them (Marczak et al. 2007). However, it is now widely recognised that ecosystems are open and connected to several adjacent habitats. Such connectivity is mediated by cross-system transfers, including the movement of organic matter, nutrients, pollutants, prey and consumers across ecosystem boundaries, forming critical corridors between ecosystems (Polis et al. 1997, Cadenasso et al. 2003, Carpenter et al. 2005). Also referred to as allochthonous inputs (originating from external sources), these transfers have been shown to influence food webs by altering population dynamics and community interactions, and to affect ecosystem processes and functioning (Polis & Hurd 1996, Polis et al. 1997, Nakano et al. 1999, Leroux & Loreau 2008).

In aquatic ecosystems, numerous studies have highlighted the importance of terrestrially derived carbon for aquatic consumers and its subsequent incorporation into food webs (Carpenter et al. 2005, Cole et al. 2006, Abrantes & Sheaves 2008, Jones et al. 2012). In this way, terrestrial transfers can support multiple trophic levels in both benthic and pelagic food webs (Cole et al. 2006, Jones et al. 2012, Karlsson et al. 2012). Entry and incorporation of carbon of terrestrial origin into aquatic food webs can occur through two distinct mechanisms: (1) via direct ingestion of inputs, or (2), via the consumption of micro-organisms/bacteria that decompose the inputs (Cole et al. 2006, Karlsson et al. 2012, Tanentzap et al. 2017).

Hippos, like many large animals, disproportionally affect cross system transfers (Doughty et al. 2016). By transporting prodigious amounts of material into aquatic systems, hippos have the potential to significantly influence and potentially alter food web structure and

trophic interactions (Naiman & Rogers 1997, Masese et al. 2015, Subalusky et al. 2015, Bakker et al. 2016). Grey and Harper (2002), were the first to use stable isotopes to identify allochthonous hippo dung inputs, demonstrating that hippo dung isotopic ratios could be clearly distinguished from those of other aquatic resources. Since then, stable isotope analyses in hippo-dominated systems have shown that dung can subsidise riverine fish and invertebrates during the dry, low flow season (McCauley, Dawson, et al. 2015) and act as an important food source for an introduced crayfish species (Jackson et al. 2012). In addition, a limited number of studies have shown that hippo dung inputs can adversely affect riverine fish and invertebrate assemblages (Dutton et al. 2018, Stears et al. 2018). Absent, however, are studies investigating the relative importance of hippo dung for components of entire food webs. Scarcity of information of this nature limits the development of a general understanding of the relevance of hippo dung in aquatic ecosystems and hence its role in broader ecosystem function.

The St Lucia Estuarine system is one of the most studied estuarine systems in South Africa, due to its complexity, high rating as a conservation area, substantial size (roughly 50 % of South Africa's estuarine area) and its value as a tourist attraction (Turpie et al. 2002, Whitfield & Taylor 2009, Porter 2013, Scharler & MacKay 2013). In this regard, numerous studies have been conducted on physical estuarine properties, individual species and population groups within a specific trophic level (see Perissinotto et al. 2013 and references within) however, studies on entire food webs are rare (Scharler & MacKay 2013). In addition, studies that have used stable isotope analyses to assess food web dynamics and trophic functioning of St Lucia Estuary across different hydrological states, have not considered the potential role of hippo dung (Govender et al. 2011, Bird et al. 2016). It is against this backdrop that this chapter seeks to investigate the relative importance of hippo dung as a food source for consumers in the St Lucia Estuary. The chapter is based on *in situ* comparisons of stable isotope ratios of dominant food web components common to two biotopes that have different densities of hippos

and therefore potentially different levels of dung loading, viz. the Narrows (high hippo density) and Charter's Creek (low hippo density). Based on previous studies showing increased dependence by consumers on transferred resources with increasing allochthonous input rates (Solomon et al. 2011, Jones et al. 2012, Wilkinson et al. 2013), it was hypothesised that (1) food web components in the Narrows and Charter's Creek would differ in isotopic signatures and (2) hippo dung would have a greater proportional contribution to consumer diets in the Narrows relative to Charter's Creek.

5.2 Results

5.2.1 Physico-chemical data

Abiotic conditions in the Narrows were generally distinct from those in Charter's Creek. The Narrows was consistently deeper, with mean seasonal water depths ranging from 1.24 ± 0.07 to 2.10 ± 0.05 m, whereas depth ranged between 0.43 ± 0.03 and 1.20 ± 0.04 m in Charter's Creek (Table 5.1). The Narrows generally also had lower pH, salinity (except in season 3) and dissolved oxygen (except season 3) relative to Charter's Creek. Water temperature was similar between biotopes in season 1 and 2, but increased at Charter's Creek during season 4. Turbidity was seasonally variable between the two biotopes (Table 5.1).

5.2.2 Isotopic signatures

Stable isotope bi-plots revealed a distinct shift in the food web signatures between the Narrows and Charter's Creek throughout the study (Figure 5.1). Generally, $\delta^{13}C$ and $\delta^{15}N$ isotope ratios were lower in the Narrows relative to Charter's Creek. This negative shift was consistent across all four sampling seasons but was most pronounced in seasons 1 and 4.

Table 5.1: Summary of physico-chemical data collected in March, July, November 2014, and February 2015 in the St Lucia Estuarine system. Mean values (± 1SE) of nine subsites per biotope and per season are shown. Season 3 data for Charter's Creek comprise a single set of readings due to instrument malfunction. Season 4 data for Charter's Creek are based on 3 readings from Site 3 only, due to complete desiccation of Sites 1 and 2.

	Season 1		Season 2		Season 3		Season 4	
	Narrows	Charter's	Narrows	Charter's	Narrows	Charter's	Narrows	Charter's
Depth (m)	2.10 ± 0.05	0.95 ± 0.03	1.90 ± 0.07	1.20 ± 0.04	1.24 ± 0.07		1.64 ± 0.07	0.43 ± 0.03
Temperature (°C)	27.10 ± 0.10	26.87 ± 0.19	19.26 ± 0.18	19.35 ± 0.15	23.89 ± 0.04	23.72	27.61 ± 0.17	35.56 ± 0.11
Salinity	2.70 ± 0.10	16.13 ± 1.38	8.73 ± 0.16	14.71 ± 0.11	13.39 ± 0.24	9.07	5.51 ± 0.32	27.39 ± 0.11
pH	8.05 ± 0.02	8.52 ± 0.01	8.19 ± 0.03	8.66 ± 0.02	9.71 ± 0.01	9.89	8.27 ± 0.01	8.59 ± 0.04
Turbidity (NTU)	38.59 ± 3.97	15.32 ± 1.45	23.64 ± 2.47	30.83 ± 0.44	26.16 ± 2.36	20.80	19.70 ± 1.02	115.73 ± 17.51
Dissolved O_2 (%)	79.46 ± 1.54	98.61 ± 0.56	83.71 ± 3.06	98.90 ± 0.54	78.82 ± 1.18	74.70	91.30 ± 0.74	106.37 ± 0.59

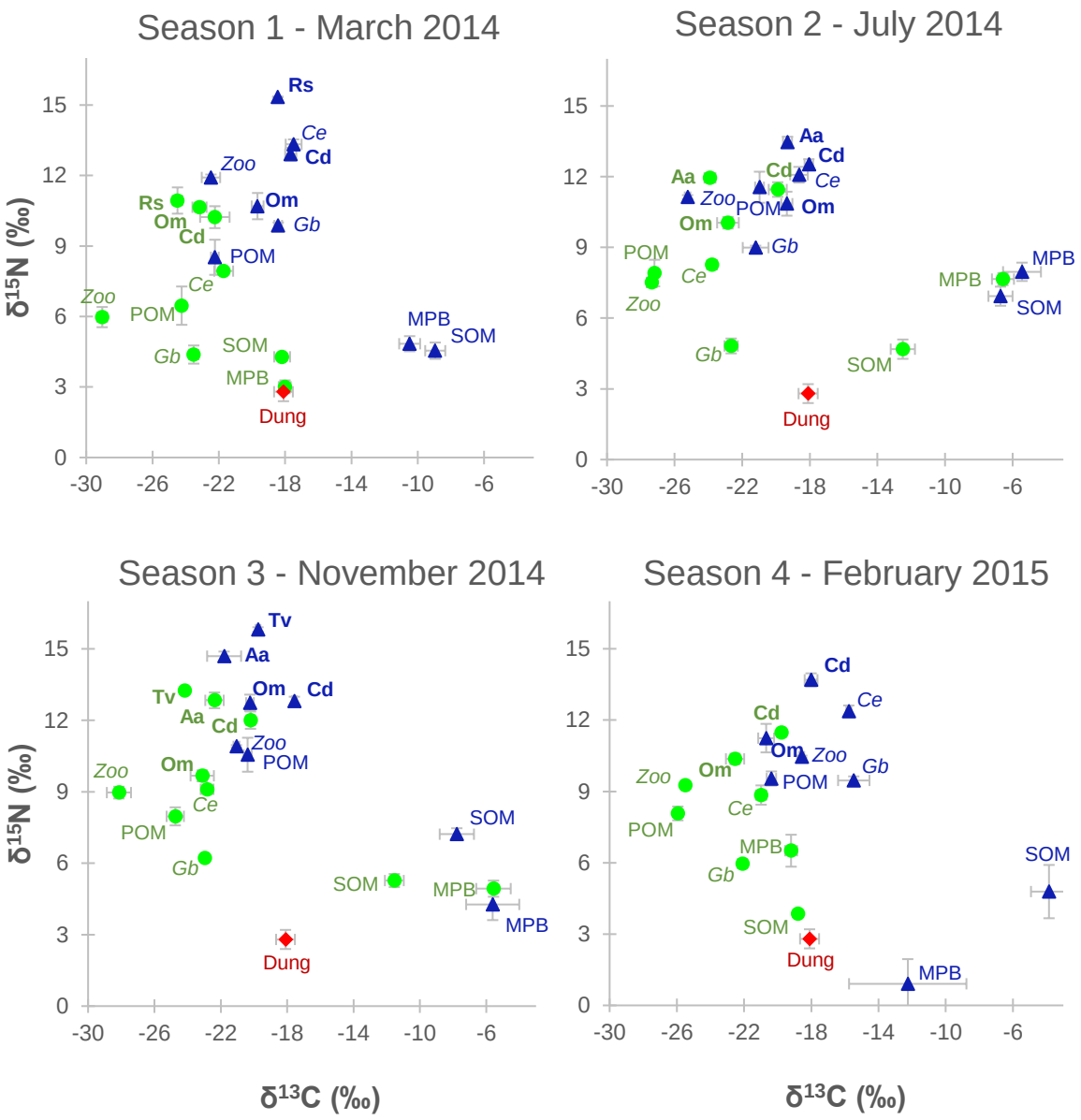


Figure 5.1: Mean isotopic values (mean ± 1SE) of food web components common in both biotopes; Narrows (green) and Charter's Creek (blue) as well as allochthonous sources in the form of hippo dung (red) for all four sampling seasons. Basal food source categories are in normal font, primary consumers are in italics and secondary consumers in **bold**. Basal sources: SOM = sediment organic matter; MPB = microphytobenthos; POM = particulate organic matter, and dung. Primary consumers: *Gb* = *Grandidierella bonnieroides* (amphipod); *Ce* = *Cyathura estuaria* (isopod) and *Zoo* = zooplankton. Secondary consumers (fish): **Om** = *Oreochromis mossambicus* (tilapia); **Cd** = *Chelon (=Liza) dumerili* (mullet); **Aa** = *Ambassis ambassis* (glassy); **Tv** = *Thryssa vitrirostris* (boney) and **Rs** = *Rhabdosargus sarba* (stumpnose).

No seasonal shift in isotopic signatures of food web components was evident within either the Narrows or Charter's Creek (Figure 5.2), despite large seasonal changes in salinity (2.70 ± 0.10 to 13.39 ± 0.24 for the Narrows and 9.07 to 27.39 ± 0.11 for Charter's Creek; Table 5.1).

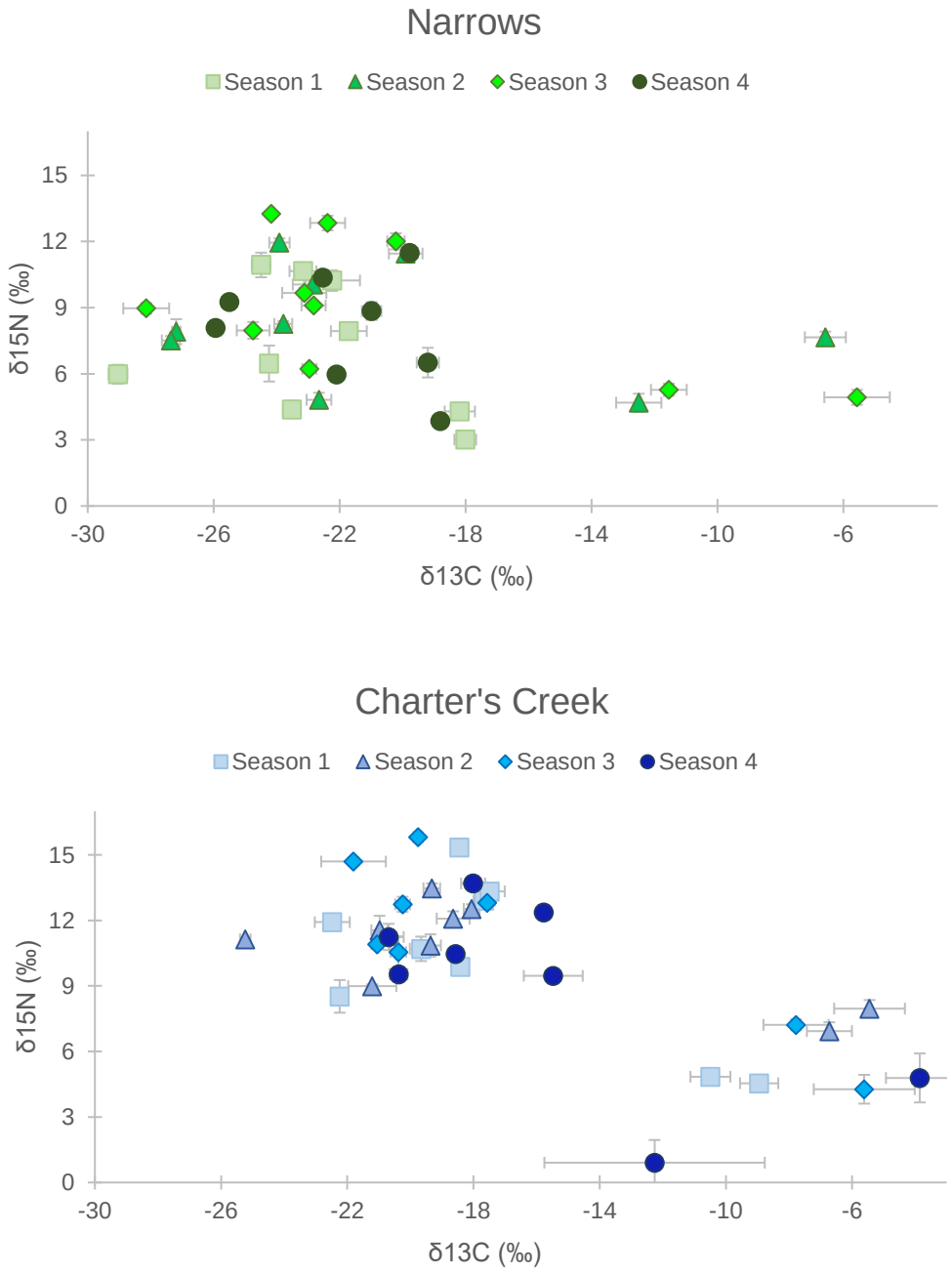


Figure 5.2: Mean isotopic values (mean ± 1SE) of food web components in the Narrows (top - green) and Charter's Creek (bottom - blue) for all four sampling seasons.

The $\delta^{13}C$ and $\delta^{15}N$ values of individual food web components showed a consistent pattern of more negative ($\delta^{13}C$) or lower ($\delta^{15}N$) values in the Narrows relative to Charter's Creek (Figure 5.3 – Figure 5.9). For the basal resources, $\delta^{13}C$ and $\delta^{15}N$ values for all three resources had significant seasonal variation (Table 5.2, Figure 5.3, Nested ANOVA, season: $p < 0.001$ for all). Similarly, all values for basal resources were significantly different between biotopes (Table 5.2, Figure 5.3, Nested ANOVA, biotope: $p < 0.001$), generally being reduced in the Narrows. All but the sediment organic matter (SOM) $\delta^{15}N$ values ($p = 0.240$) had significant site variation within biotopes (Table 5.2, Nested ANOVA, site: $p = 0.036$ for all). Trends in isotopic values for microphytobenthos (MPB) were not as consistent, with $\delta^{13}C$ values for season 2 and 3 being similar between the two biotopes and $\delta^{15}N$ values in season 2 being similar. In seasons 3 and 4, $\delta^{15}N$ values were lower in Charter's Creek relative to the Narrows. The average $\delta^{15}N$ value for hippo dung (2.80 ± 0.40 ‰) was constantly lower than all basal resources in both biotopes with the single exception being Season 4 Charter's Creek MPB. SOM and MPB $\delta^{13}C$ values in the Narrows were generally closer to that of hippo dung (-18.10 ± 0.57 ‰), however Charter's Creek particulate organic matter (POM) $\delta^{13}C$ values were closer to the average $\delta^{13}C$ of hippo dung (Figure 5.3).

Table 5.2: Results of nested ANOVA testing for differences in carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) ratios of all basal resources between seasons, biotopes and sites. Bold p-values indicate statistically significant results. POM = particulate organic matter, SOM = sediment organic matter, MPB = microphytobenthos, *F* = F-statistic, *p* = significance, DF = degrees of freedom.

		Nested ANOVA basal resources								
		Season			Biotope			Site		
Sample		*F*	DF	*P*	*F*	DF	*p*	*F*	DF	*p*
POM 	$\delta^{13}C$	41.86	3,22	**<0.001**	299.82	4,22	**<0.001**	2.54	14,22	**0.024**
	$\delta^{15}N$	13.26	3,22	**<0.001**	21.89	4,22	**<0.001**	4.22	14,22	**0.001**
SOM 	$\delta^{13}C$	66.02	3,22	**<0.001**	155.01	4,22	**<0.001**	5.33	14,22	**<0.001**
	$\delta^{15}N$	22.54	3,22	**<0.001**	13.59	4,22	**<0.001**	1.38	14,22	0.240
MPB 	$\delta^{13}C$	349.55	3,22	**<0.001**	59.37	4,22	**<0.001**	15.45	14,22	**<0.001**
	$\delta^{15}N$	39.40	3,22	**<0.001**	16.66	4,22	**<0.001**	2.34	14,22	**0.036**

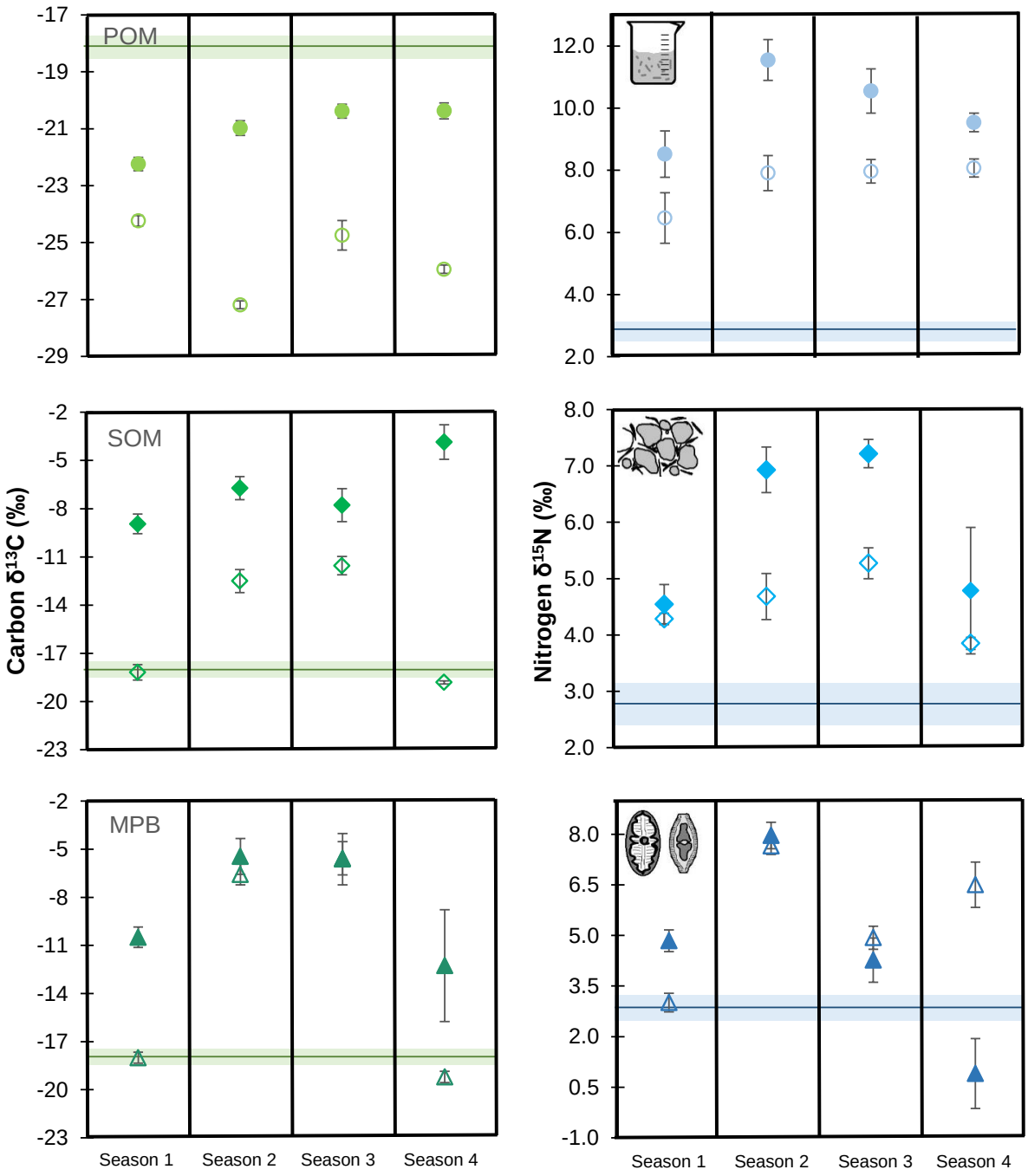


Figure 5.3: Carbon (green) and nitrogen (blue) stable isotope values ($\delta^{13}C$ and $\delta^{15}N$; mean ± 1SE) of basal resources: particulate organic matter (POM), sediment organic matter (SOM) and microphytobenthos (MPB) sampled in the Narrows, where dense hippo populations occur (hollow symbols) and at Charter's Creek, where hippos are rare (solid symbols). The $\delta^{13}C$ and $\delta^{15}N$ values of hippo dung are plotted (mean green and blue line respectively, with ± SD plotted in light green and blue).

The pattern of lower $\delta^{13}C$ and $\delta^{15}N$ values in the Narrows relative to Charter's Creek were also evident in all three primary consumers: the amphipod (*Grandidierella bonnieroides)*, the isopod (*Cyathura estuaria*) and the pooled zooplankton samples (Figure 5.4). The $\delta^{13}C$ and $\delta^{15}N$ values for all three consumers differed significantly between seasons (Table 5.3, Figure 5.4, Nested ANOVA, season: $p < 0.05$ for all) and between biotopes (Table 5.3, Figure 5.4, Nested ANOVA, biotope: $p \leq 0.001$ for all). *G. bonnieroides* and zooplankton $\delta^{13}C$ and $\delta^{15}N$ values had significant site differences (Table 5.3, Nested ANOVA, site: $p < 0.05$), however *C. estuaria* values were not significantly different between sites ($p > 0.05$). When comparing the isotopic ratio values of primary consumers to that of the hippo dung, $\delta^{15}N$ values of dung (2.80 ± 0.40 ‰) were lower than values from both biotopes however, $\delta^{13}C$ values of primary consumers within Charter's Creek were closer to average $\delta^{13}C$ value of hippo dung (-18.10 ± 0.57 ‰).

The tilapia (*Oreochromis mossambicus*), mullet (*Chelon (=Liza) dumerili*), glassy (*Ambassis ambassis*), stumpnose (*Rhabdosargus sarba*) and boney (*Thryssa vitrirostris*) from the Narrows had lower $\delta^{13}C$ and $\delta^{15}N$ values relative to those from Charter's Creek (Figure 5.5). Statistically, only *C. dumerili* had significant seasonal variation for both isotope values (Table 5.4, Figure 5.5, Nested ANOVA, season: $p < 0.05$), while both $\delta^{13}C$ and $\delta^{15}N$ values for *O. mossambicus*, *C. dumerili* and *A. ambassis* were different between biotopes (Table 5.4, Figure 5.5, Nested ANOVA, biotope: $p < 0.001$ for all). The $\delta^{15}N$ values for *O. mossambicus* and $\delta^{13}C$ values for *C. dumerili* were different between sites within biotopes (Table 5.4, Nested ANOVA, site: $p < 0.001$ for both) while $\delta^{13}C$ values for *O. mossambicus* and *A. ambassis*, and $\delta^{15}N$ values for *C. dumerili* and *A. ambassis* displayed non-significant site variation.

Table 5.3: Results of nested ANOVA testing for differences in carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) ratios of primary consumers between seasons, biotopes and sites. Bold p-values indicate statistically significant results. *F* = F-statistic, *p* = significance, DF = degrees of freedom.

		Nested ANOVA Primary consumers								
		Season			Biotope			Site		
		F	DF	*p*	*F*	DF	*p*	*F*	DF	*p*
Grandidierella bonnieroides	$\delta^{13}C$ 	21.945	2,14	**<0.001**	147.21	3,14	**<0.001**	7.804	10,14	**<0.001**
	$\delta^{15}N$	8.079	2,14	**0.005**	326.97	3,14	**<0.001**	3.995	10,14	**0.009**
Cyathura estuaria	$\delta^{13}C$ 	12.99	2,4	**0.018**	65.253	3,4	**<0.001**	2.718	6,4	0.176
	$\delta^{15}N$	8.081	2,4	**0.039**	47.239	3,4	**0.001**	0.866	6,4	0.584
Zooplankton	$\delta^{13}C$ 	31.66	3,21	**<0.001**	205.14	4,21	**<0.001**	3.41	14,21	**0.006**
	$\delta^{15}N$	55.24	3,21	**<0.001**	773.67	4,21	**<0.001**	16.60	14,21	**<0.001**

Table 5.4: Results of nested ANOVA testing for differences in carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) ratios of all fish species between seasons, biotopes and sites. Bold p-values indicate statistically significant results. *F* = F-statistic, *p* = significance, DF = degrees of freedom.

		Nested ANOVA Secondary consumers - pooled sizes								
		Season			Biotope			Site		
Sample		*F*	DF	*p*	*F*	DF	*p*	*F*	DF	*p*
Oreochromis mossambicus	$\delta^{13}C$ 	0.89	3,137	0.449	15.96	4,137	**<0.001**	0.84	14,137	0.630
	$\delta^{15}N$	2.31	3,137	0.080	13.92	4,137	**<0.001**	3.56	14,137	**<0.001**
Chelon (=Liza) dumerili	$\delta^{13}C$ 	7.54	3,65	**<0.001**	37.39	4,65	**<0.001**	3.77	11,65	**<0.001**
	$\delta^{15}N$	4.71	3,65	**0.005**	18.65	4,65	**<0.001**	1.94	11,65	0.050
Ambassis ambassis	$\delta^{13}C$ 	0.53	1,55	0.472	37.22	2,55	**<0.001**	1.46	7,55	0.203
	$\delta^{15}N$	1.82	1,55	0.183	10.71	2,55	**<0.001**	0.96	7,55	0.469

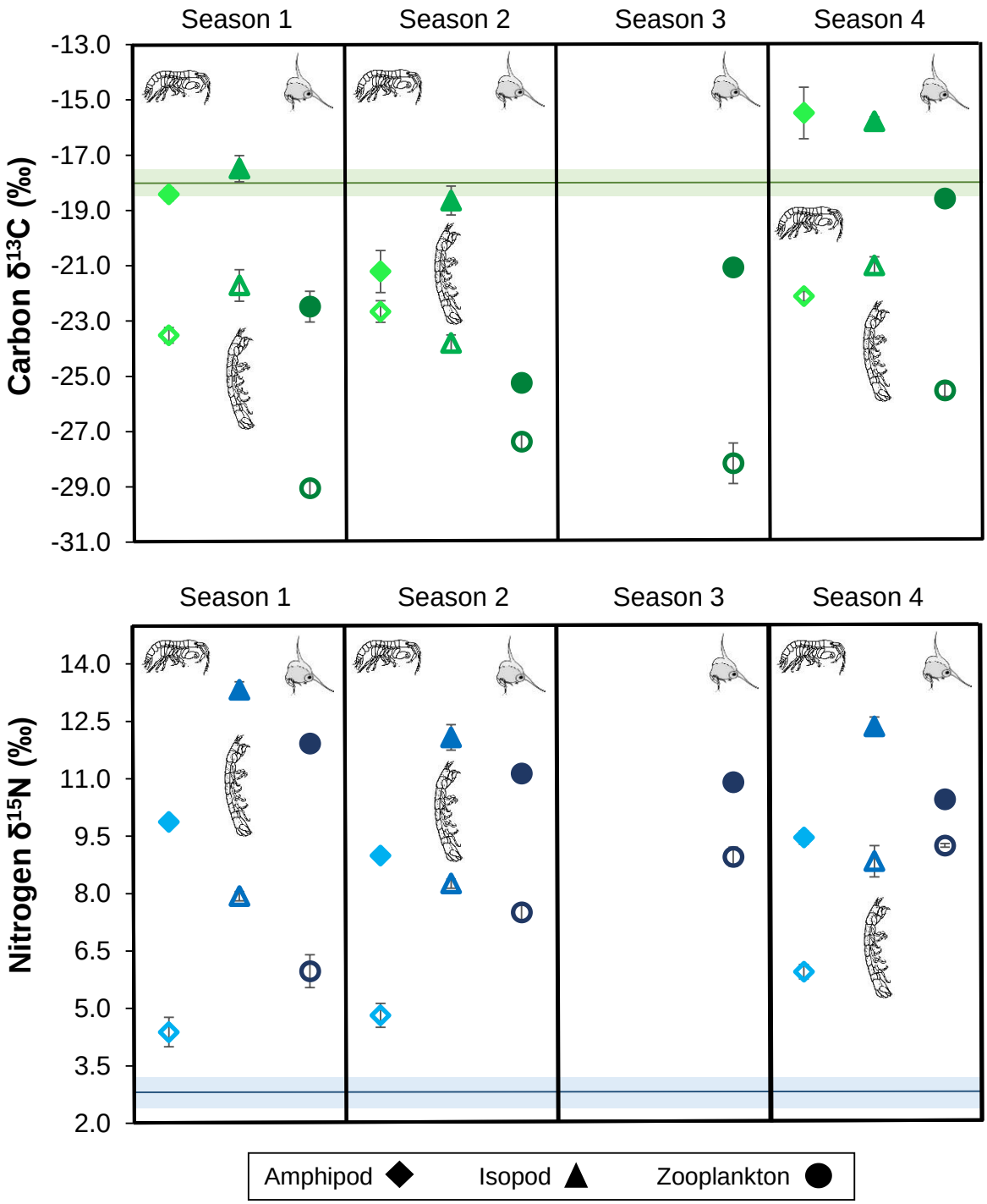


Figure 5.4: Carbon (green) and nitrogen (blue) stable isotope values ($\delta^{13}C$ and $\delta^{15}N$; mean ± 1SE) of primary consumer species: amphipod (*Grandidierella bonnieroides*) and isopod (*Cyathura estuaria*) and zooplankton sampled in the Narrows (hollow symbols) and at Charter's Creek (solid symbols). The $\delta^{13}C$ and $\delta^{15}N$ values of hippo dung are plotted (mean green and blue line respectively with ± SE plotted in light green and blue).

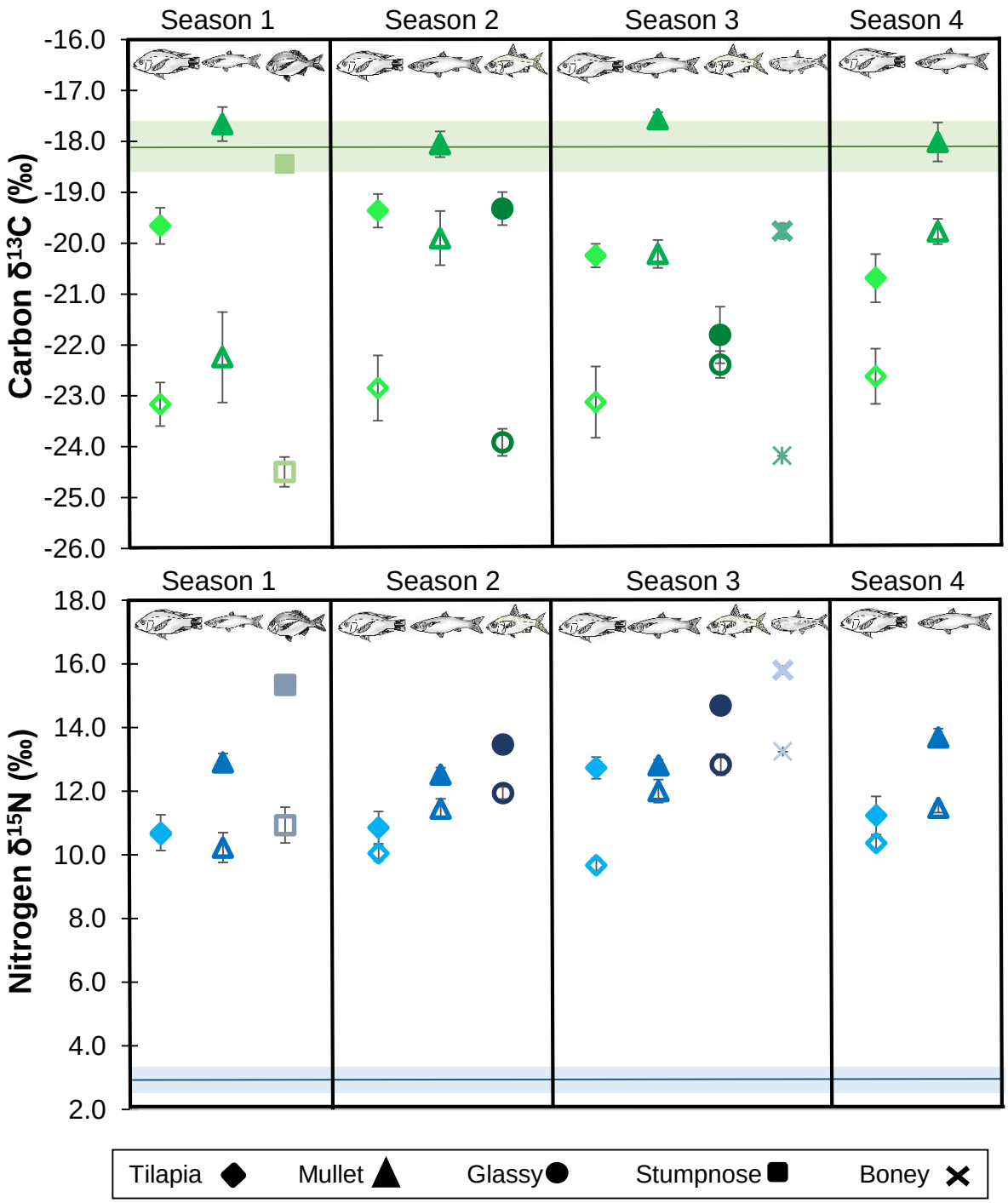


Figure 5.5: Carbon (green) and nitrogen (blue) stable isotope values ($\delta^{13}C$ and $\delta^{15}N$; mean ± 1SE) of fish found in both biotopes; Narrows (hollow symbols) and at Charter's Creek (solid symbols). All fish samples were pooled regardless of size, tilapia (*Oreochromis mossambicus*), mullet (*Chelon (=Liza) dumerili*), glassy (*Ambassis ambassis*), stumpnose (*Rhabdosargus sarba*) and boney (*Thryssa vitrirostris*). The $\delta^{13}C$ and $\delta^{15}N$ values of dung are plotted (mean green and blue line respectively with ± SE plotted in light green and blue).

When secondary consumers were separated into size classes (Figure 5.6 – Figure 5.8), only the $\delta^{15}N$ value for *A. ambassis* size category 1 (7.1 - 9.0 cm, Figure 5.8) was significantly different between seasons (Table 5.5, Nested ANOVA, season: $p = 0.030$). Significant biotope differences in $\delta^{13}C$ and $\delta^{15}N$ values were evident for *O. mossambicus* size category 2 (16.1-20.0 cm, Figure 5.6), *C. dumerili* size category 1 (16.1 - 19.0 cm, Figure 5.7) and *A. ambassis* size category 2 (9.1 - 11.0 cm), and in $\delta^{13}C$ values only for *C. dumerili* size category 2 (19.1 - 21.0 cm) and *A. ambassis* size category 1 (7.1 - 9.0 cm, Table 5.5, Nested ANOVA, biotope: $p < 0.05$). Except for *A. ambassis* size category 1, (7.1 - 9.0 cm, Table 5.5, Nested ANOVA, site: $p = 0.014$), all other isotope ratio values were statistically indistinguishable between sites ($p > 0.05$). The isotopic ratio values were generally lower in the Narrows than in Charter's Creek across all sizes of fish. When compared with average dung ratio values, $\delta^{13}C$ values of secondary consumers within Charter's Creek were closer to the average $\delta^{13}C$ value of hippo dung, while dung $\delta^{15}N$ was substantially lower than values from both biotopes.

Table 5.5: Results of nested ANOVA testing for differences in carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) ratios of fish species of different size classes between seasons, biotopes and sites. Bold p-values indicate statistically significant results. *F* = F-statistic, *p* = significance, DF = degrees of freedom.

Sample		Nested ANOVA Secondary consumers - size classes								
		Season			Biotope			Site		
		F	DF	*p*	*F*	DF	*p*	*F*	DF	*p*
O. mossambicus 1 	Tilapia 1 $\delta^{13}C$	0.62	3,13	0.616	0.81	4,13	0.544	0.88	7,13	0.545
12.1-16.0 cm	Tilapia 1 $\delta^{15}N$	1.55	3,13	0.248	0.55	4,13	0.705	1.72	7,13	0.188
O. mossambicus 2	Tilapia 2 $\delta^{13}C$	0.84	3,34	0.481	3.40	4,34	**0.019**	0.99	10,34	0.473
16.1-20.0 cm	Tilapia 2 $\delta^{15}N$	2.55	3,34	0.072	18.45	4,34	**<0.001**	1.29	10,34	0.277
O. mossambicus 3	Tilapia 3 $\delta^{13}C$				3.11	1,1	0.328	0.32	2,1	0.780
20.1-24.0 cm	Tilapia 3 $\delta^{15}N$				8.97	1,1	0.205	2.87	2,1	0.385
C. dumerili 1 	Mullet 1 $\delta^{13}C$	4.17	2,26	0.027	10.99	3,26	**<0.001**	1.42	8,26	0.235
16.1 - 19.0 cm	Mullet 1 $\delta^{15}N$	1.83	2,26	0.181	5.41	3,26	**0.005**	0.94	8,26	0.500
C. dumerili 2	Mullet 2 $\delta^{13}C$				1131.46	1,1	**0.019**	45.30	3,1	0.109
19.1 - 21.0 cm	Mullet 2 $\delta^{15}N$				25.29	1,1	0.125	184.99	3,1	0.054
A. ambassis 1 	Glassy 1 $\delta^{13}C$	0.03	1,25	0.859	31.85	2,25	**<0.001**	3.57	5,25	**0.014**
7.1 - 9.0 cm	Glassy 1 $\delta^{15}N$	5.33	1,25	**0.030**	3.37	2,25	0.051	0.96	5,25	0.460
A. ambassis 2	Glassy 2 $\delta^{13}C$				33.31	1,9	**<0.001**	2.70	3,9	0.109
9.1 - 11.0 cm	Glassy 2 $\delta^{15}N$				24.66	1,9	**<0.001**	1.05	3,9	0.419

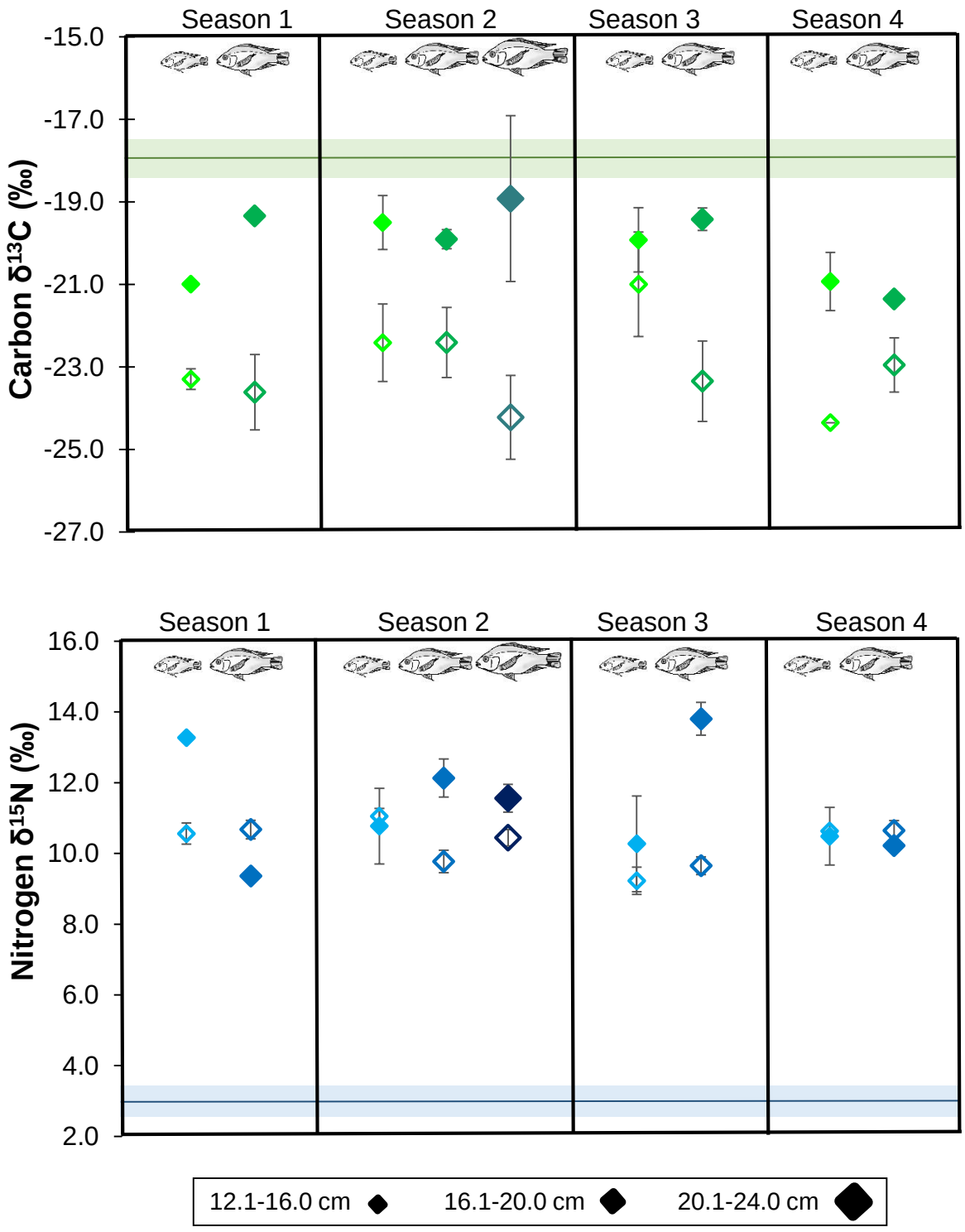


Figure 5.6: Carbon (green) and nitrogen (blue) stable isotope values ($\delta^{13}C$ and $\delta^{15}N$; mean ± 1SE) of tilapia (*Oreochromis mossambicus*) of varying size classes sampled in the Narrows (hollow symbols) and at Charter's Creek (solid symbols). The $\delta^{13}C$ and $\delta^{15}N$ values of hippo dung are plotted (mean green and blue line respectively with ± SE plotted in light green and blue).

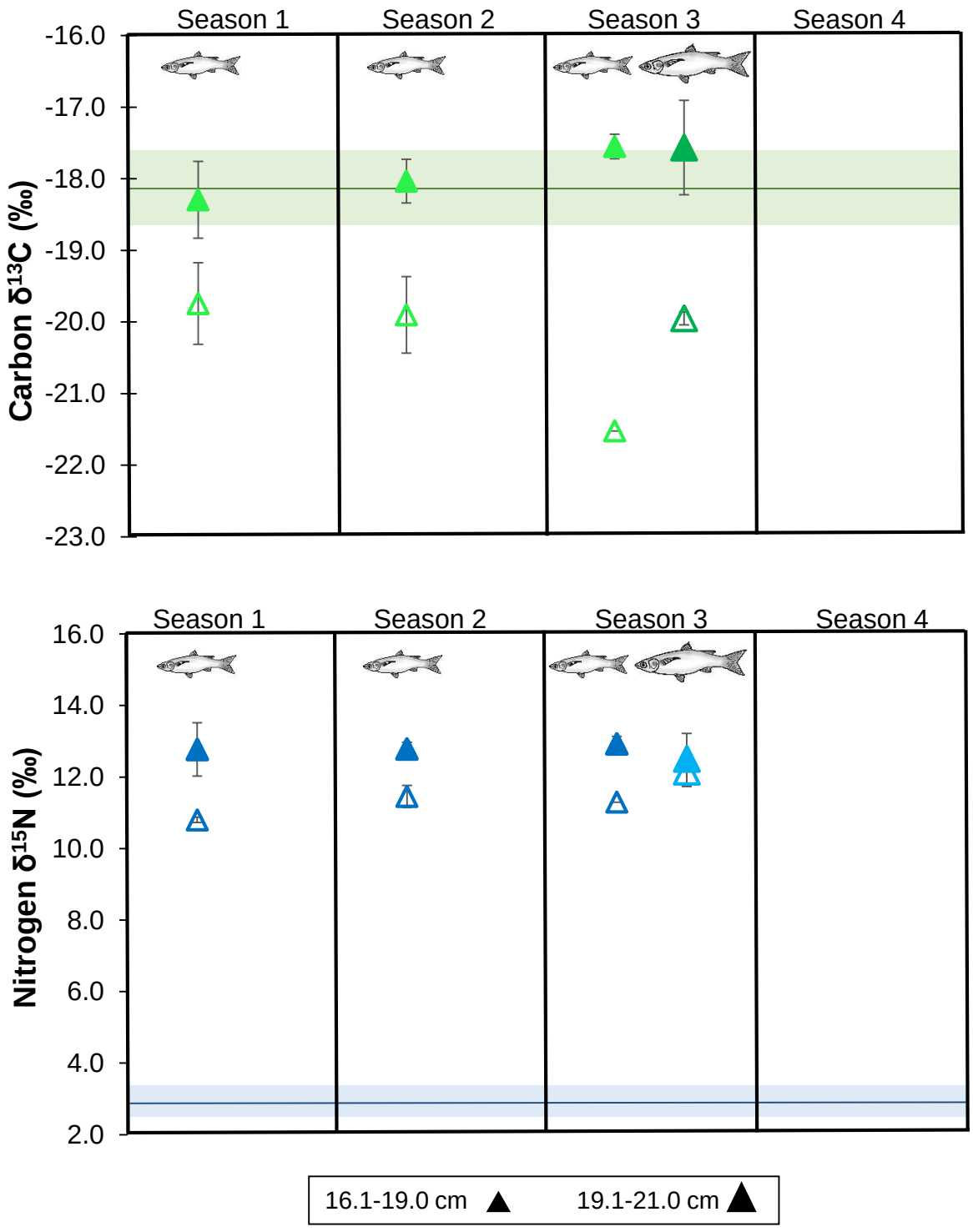


Figure 5.7: Carbon (green) and nitrogen (blue) stable isotope values ($\delta^{13}C$ and $\delta^{15}N$; mean ± 1SE) of mullet (*Chelon (=Liza) dumerili*) of varying size classes sampled in the Narrows (hollow symbols) and at Charter's Creek (solid symbols). The $\delta^{13}C$ and $\delta^{15}N$ values of hippo dung are plotted (mean green and blue line respectively with ± SE plotted in light green and blue).

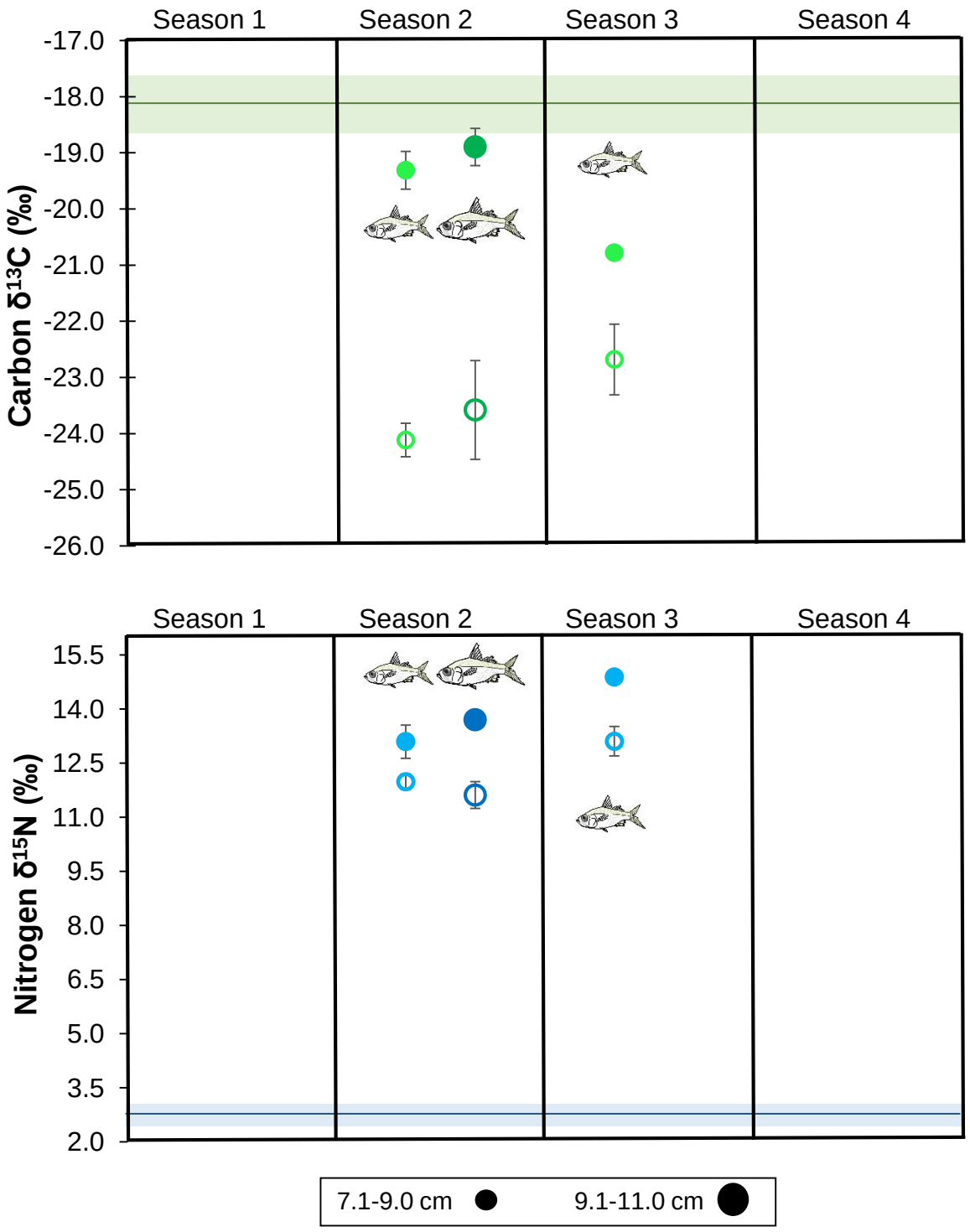


Figure 5.8: Carbon (green) and nitrogen (blue) stable isotope values ($\delta^{13}C$ and $\delta^{15}N$; mean ± 1SE) of glassy (*Ambassis ambassis*) of varying size classes sampled in the Narrows (hollow symbols) and at Charter's Creek (solid symbols). The $\delta^{13}C$ and $\delta^{15}N$ values of hippo dung are plotted (mean green and blue line respectively with ± SE plotted in light green and blue).

Isotopic values of samples of the apex predator, the Nile crocodile (*Crocodylus niloticus,* Figure 5.9) were lower in those collected from St Lucia estuary, relative to those outside of the estuary.

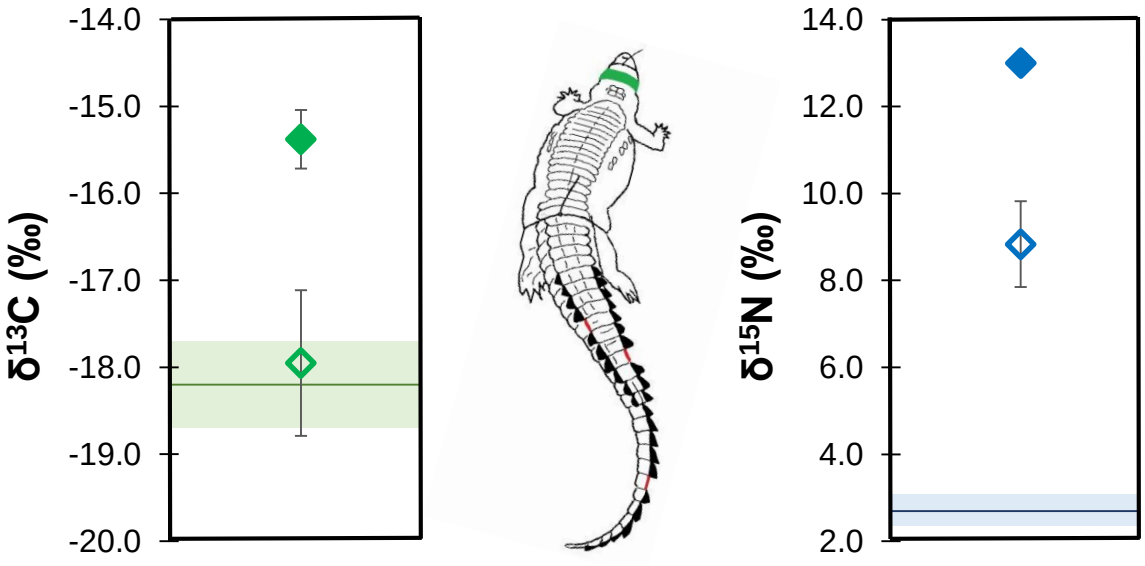


Figure 5.9: Carbon (green) and nitrogen (blue) stable isotope values ($\delta^{13}C$ and $\delta^{15}N$; mean ± 1SE) of the Nile crocodile (*Crocodylus niloticus*), sampled in the St Lucia Estuary, a system containing hippo dung (hollow symbols) and outside the estuary, areas assumed to have little hippo dung (solid symbols). Statistical tests were not performed due to low sample numbers (n = 2 for crocodiles sampled outside the St Lucia Estuary). The $\delta^{13}C$ and $\delta^{15}N$ values of hippo dung are plotted (mean green and blue line respectively with ± SE plotted in light green and blue.

5.2.3 Bayesian mixing models

Results from Bayesian mixing models indicated that hippo dung in the Narrows contributed between 2 to 65 % (based on median values) to the diets of primary consumers and 1 to 42 % to the diets of secondary consumers. At Charter's Creek, dung contributed between 3 to 54 % in primary consumers and 5 to 49 % in secondary consumers (Table 5.6).

Table 5.6: The percentage (median with minima and maxima in parentheses) of hippo dung contributing to the diets of dominant consumers in the Narrows and Charter's Creek for all four seasons as determined by Bayesian mixing models. A = Amphipoda; I = Isopoda; P = Perciformes.

Consumer	Season 1		Season 2		Season 3		Season 4	
	Narrows	Charter's	Narrows	Charter's	Narrows	Charter's	Narrows	Charter's
G. bonnieroides (A)	**15** (1 - 82)	**34** (20 - 45)	**32** (3 -65)	**54** (44 - 63)	**65** (54 - 77)	-	**64** (10 - 79)	**15** (1 - 38)
C. estuaria (I)	**10** (0 - 49)	**3** (0 - 9)	**18** (1 - 41)	**17** (4 - 29)	**17** (5 - 31)	-	**22** (4 - 50)	**3** (0 - 16)
Zooplankton	**49** (0 - 91)	**14** (4 - 23)	**2** (0 - 10)	**29** (17 - 40)	**14** (4 - 28)	**23** (5 - 36)	**10** (1 - 22)	**10** (0 - 30)
O. mossambicus 1 (P)	**4** (0 - 16)	**15** (2 - 33)	**3** (0 - 17)	**49** (33 - 62)	**42** (25 - 56)	**43** (4 - 60)	**4** (0 - 17)	**15** (1 - 43)
O. mossambicus 2 (P)	**2** (0 - 13)	**42** (1 - 72)	**1** (0 - 40)	**31** (15 - 45)	**34** (6 - 54)	**12** (2 - 23)	**2** (0 - 13)	**12** (0 - 49)
O. mossambicus 3 (P)	-	-	**2** (0 - 23)	**39** (15 - 66)	-	-	-	-
C. dumerili 1 (P)	**4** (0 - 19)	**15** (1 - 41)	**2** (0 - 19)	**22** (10 - 35)	**16** (3 - 36)	**16** (7 - 25)	-	-
C. dumerili 2 (P)	-	-	-	-	**12** (3 -26)	**26** (5 - 47)	-	-
A. ambassis 1 (P)	-	-	**1** (0 - 6)	**23** (9 - 37)	**4** (1 - 10)	**5** (1 - 15)	-	-
A. ambassis 2 (P)	-	-	**1** (0 - 12)	**15** (5 - 27)	-	-	-	-

More specifically, mixing models indicated seasonal and taxon-specific variability in the median proportional contributions of basal resources to primary consumers diets (Figure 5.10). The amphipod, *G. bonnieroides,* in Charter's Creek incorporated proportionally more dung into its diet in seasons 1 and 2 (34 and 54 %) relative to those in the Narrows (15 and 32 %). In contrast, dung contribution was greater in the Narrows during season 4 (64 % vs 15 % in Charter's Creek). In the Narrows, dung contributed more to the diet of the isopod *C. estuaria,* in seasons 1 and 4 (10 and 22 % relative to 3 % for both seasons in Charter's Creek), but dung contributions to the isopod in season 2 were similar (18 and 17 %). Dung contributions for all seasons and biotopes were relatively low for *C. estuaria,* never exceeded 22 %. In season 1, dung contributions to Narrows zooplankton were greater than in Charter's Creek (49 % compared to 14 %), but this trend was reversed in season 2 (2 compared to 29 %), and 3 (14 compared to 23 %). Dung contribution to zooplankton diets were the same at Charter's Creeks' and the Narrows in season 4 (10 % for both).

Contrary to expectations, mixing models revealed that hippo dung generally contributed more to secondary consumer diets in Charter's Creek, where hippos were rare, than in the hippo-dominated Narrows. With the exception of season 3, this trend was temporally consistent across all fish sizes (Figure 5.11). During seasons 1, 2 and 4, dung contributions to fish diets in the Narrows never exceeded 4.2 %, while contributions in Charter's Creek ranged from 12 % to 49 %. It is noteworthy that the isopod contributed more to the diets of fish in the Narrows than in Charter's Creek.

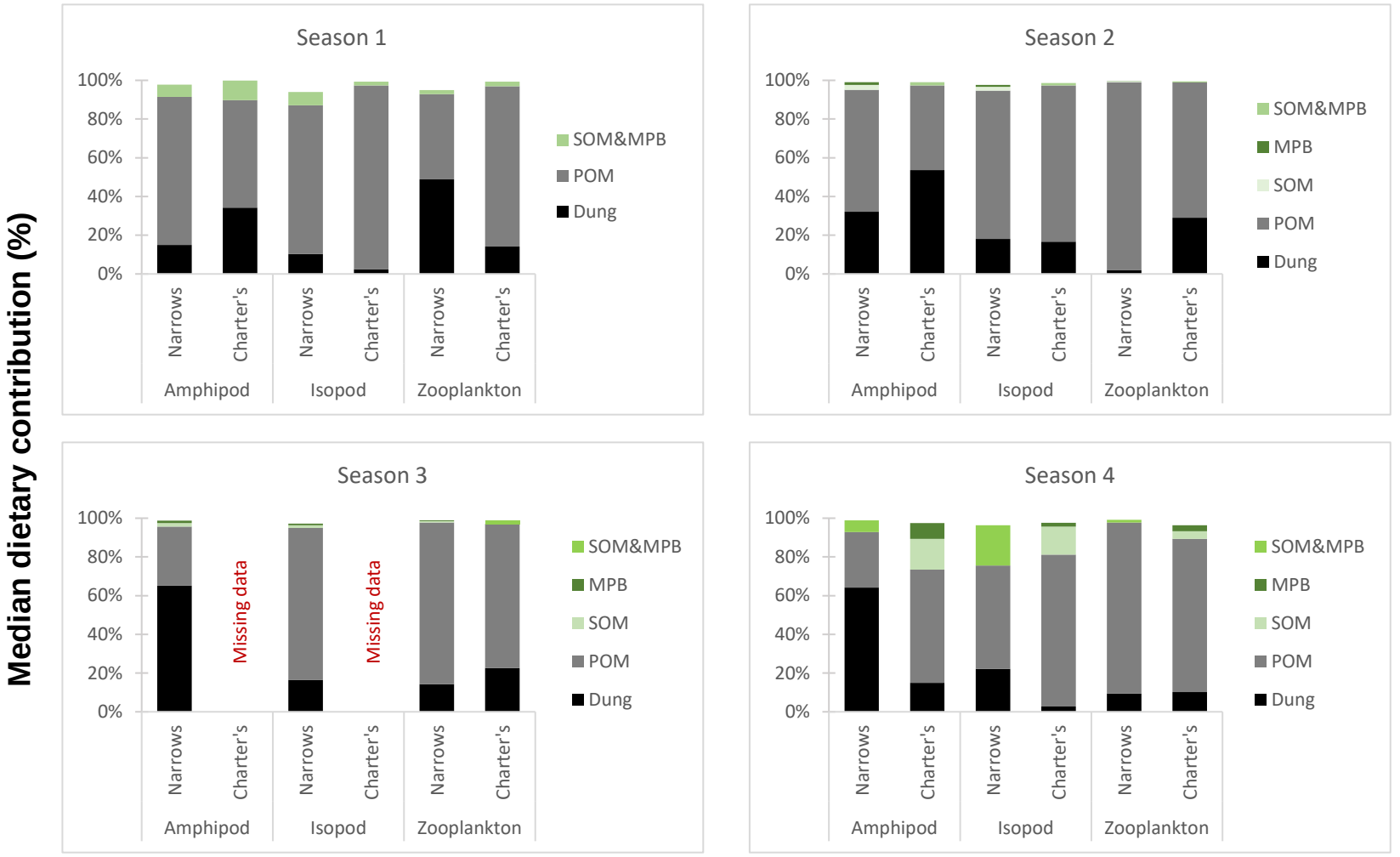


Figure 5.10: Results of Bayesian mixing models conducted in MixSIAR, showing the estimated proportions of source contributions to the diets of primary consumers: amphipod (*Grandidierella bonnieroides*), isopod (*Cyathura estuaria*), and zooplankton in the Narrows and Charter's Creek for all four seasons. Sources were combined if both nitrogen and carbon ratios were not significantly different.

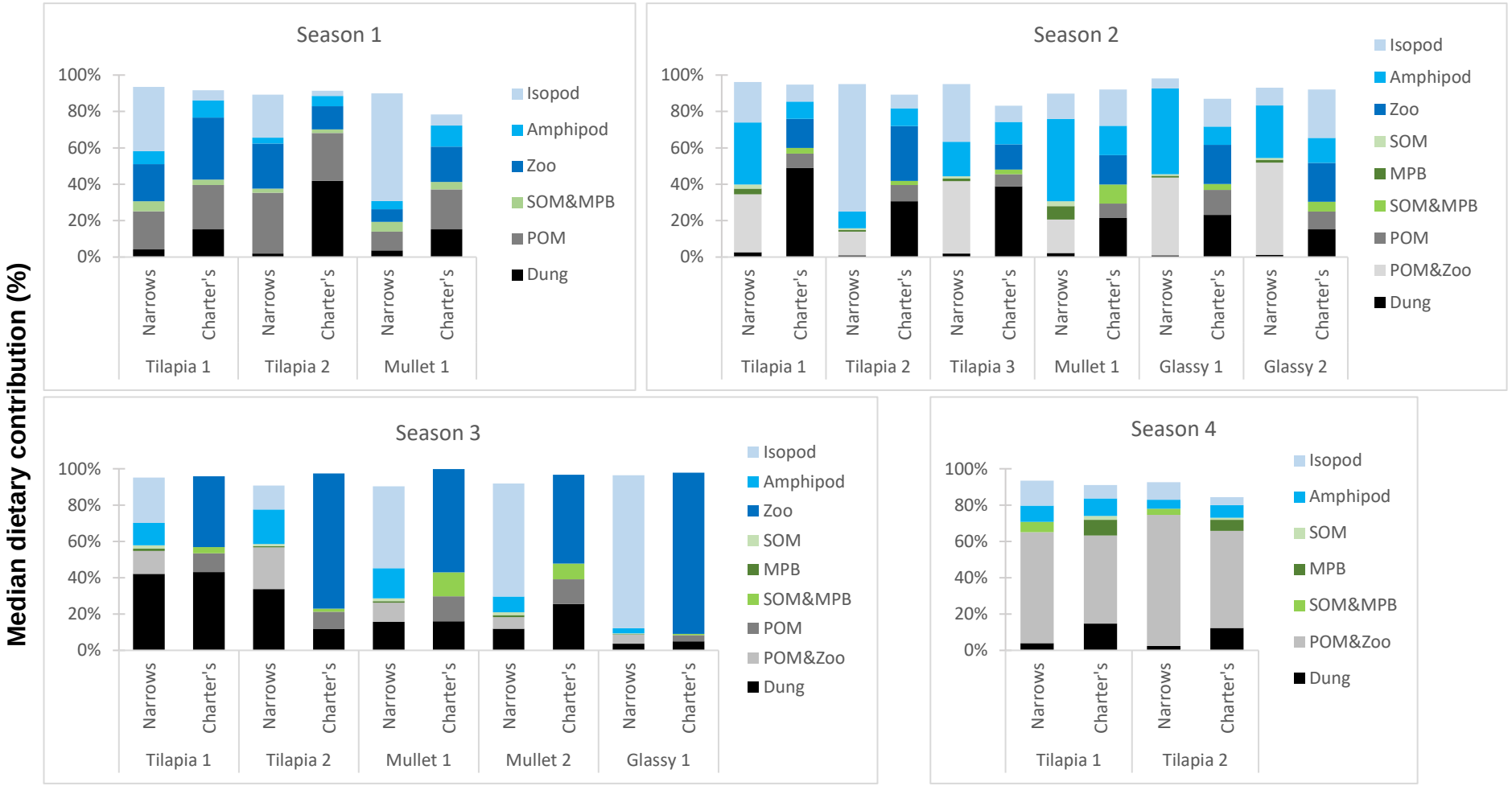


Figure 5.11: Results of Bayesian mixing models conducted in MixSIAR, showing the estimated proportions of source contributions to the diets of dominant fish species: tilapia (*Oreochromis mossambicus*), mullet (*Chelon (=Liza) dumerili*) and glassy (*Ambassis ambassis*) in the Narrows and Charter's Creek for all four seasons. Sources were combined if both nitrogen and carbon ratios were not significantly different. *Note season 3 Charter's Creek models are missing macrofaunal (isopod and amphipod) samples.

5.3 Discussion

The overarching goal of this chapter was to determine the relative importance of hippo dung as a food source within the St Lucia Estuarine system and to compare the isotopic signatures (in the form isotopic bi-plots) of dominant food web components within two biotopes that theoretically experience contrasting amounts of hippo dung inputs. The hypotheses proposed were that high and low dung inputs in the Narrows and Charter's Creek respectively would result in: (1) isotopic signatures of food web components being differentiated between the two biotopes and (2) that hippo dung would contribute proportionally more to consumer diets in the Narrows than Charter's Creek. To test these hypotheses, seasonal sampling of common food web components was carried out, followed by application of stable carbon and nitrogen isotopic analysis. These data were in turn used to run Bayesian mixing models, which quantified the proportional contribution of dung to consumer diets. Results generally supported the hypothesis that isotopic signatures of food webs would be distinct between the Narrows and Charter's Creek. However, contrary to the second hypothesis, mixing models showed a greater contribution of hippo dung to the diets of consumers from Charter's Creek than those from the Narrows (Fig. 5.10 & 5.11).

5.3.1 Stable isotope bi-plots

Stable isotope bi-plots showed that isotopic signatures of food web components were consistently differentiated between the Narrows and Charter's Creek across the four sampling seasons. More specifically, the trend of a decrease in both $\delta^{13}C$ and $\delta^{15}N$ isotope ratios in the Narrows relative to Charter's Creek was constant, regardless of season. While the trends recorded are consistent with the hypothesized effect of contrasting hippo dung inputs between the Narrows and Charter's Creek, it is important to recognise that emergent patterns may also have been related to abiotic differences between these biotopes. For example, studies have

shown that food sources from marine (more saline) habitats can be more enriched in the heavier ^{13}C isotope than less saline habitats, suggesting that salinity may influence isotopic signatures (Fry 2002). In the context of the St Lucia Estuary, salinity was one of the strongest differentiators of abiotic conditions between the Narrows and Charter's Creek (Table 5.1), with salinity being greater in the latter biotope. However, even though salinity varied substantially among seasons within each biotope (2.70 to 13.39 for the Narrows and 9.07 to 27.39 for Charter's Creek), food web bi-plots did not show any seasonal separation, either in the Narrows or Charter's Creek. This would suggest that salinity variations may not have played a significant role in observed differences in isotopic signatures between biotopes. However, in the absence of direct causal evidence, it would be difficult to exclude abiotic forcing as an explanation for isotopic differences observed, either in part of wholly.

Results indicated that the $\delta^{15}N$ ratio of hippo dung was low (2.8 ± 0.40 ‰) relative to other food web components sampled. In parallel, $\delta^{15}N$ ratios of food web components from the Narrows were constantly lower than those from Charter's Creek. These findings would suggest that lower nitrogen signatures recorded in the Narrows may be influenced by dung inputs. Similarly, the $\delta^{13}C$ values of basal sources in the Narrows (i.e. sediment organic matter (SOM) and microphytobenthos (MPB)) were more similar to hippo dung values than were those of Charter's Creek (Fig. 5.3). This would again provide circumstantial evidence that dung inputs may influence isotopic signatures of benthic basal resources.

In contrast, $\delta^{13}C$ values of particulate organic matter (POM) from Charter's Creek were more similar to dung values than in the Narrows. This trend can be explained by the fact that POM samples were typically collected from the upper 0.5 m of the water column in both biotopes. However, given that Charter's Creek is much shallower (0.98 ± 0.11 m) than the Narrows (1.72 ± 0.10 m), and prone to wind-driven wave action, it is likely that dung inputs, albeit in small quantities, remain entrained in the water column in this biotope. This situation

is unlikely in the Narrows, given its deeper nature and smaller surface area, which likely leads to dung settlement and hence weak contribution to POM (See Table 5.1, Perissinotto et al. 2013, Zikhali et al. 2015). In addition, POM carbon to nitrogen (C:N) ratios were higher at Charter's Creek than the Narrows for three of the four sampling seasons (9.4 vs 6.6; 7.5 vs 6.2 and 13.8 vs 9.7 for seasons 2, 3 and 4 respectively). Given that terrestrial plants have a higher C:N ratio than aquatic producers (Elser et al. 2000), the higher C:N values recorded at Charter's Creek likely reflect a greater terrestrial plant contribution and supports the idea that dung suspension in Charter's Creek contributes more to POM.

It is also noteworthy that $\delta^{13}C$ values of all primary and secondary consumers in Charter's Creek were similar to the $\delta^{13}C$ value of hippo dung, suggesting a greater reliance of consumers on hippo dung in Charter's Creek relative to the Narrows. Finally, the apex predator within the St Lucia Estuary, the Nile crocodile, had $\delta^{13}C$ values more similar to hippo dung values than crocodiles found outside of the estuary, where hippos are assumed to be absent or present in lower densities than within the estuary. However, caution needs to be exercised when interpreting these data given the very low sample sizes and that actual dung levels in systems outside of the St Lucia Estuary where crocodiles were captured are unknown.

The results from Nested ANOVA show that the signatures of basal resources and primary consumers were temporally and spatially variable, with statistical differences occurring at the site level. This suggests that within a given biotope, substantial heterogeneity exists at the level of basal resources and primary consumers. In the context of primary consumers, previous studies have shown that resident benthic species, such as crabs and slugs, derive dietary carbon from their immediate vicinity, and that in estuaries, organic matter is variable at scales of 5-10 m (Hsieh et al. 2002, Guest & Connolly 2005) while the microphytobenthos can vary spatially at scales as low as 1 cm (Jesus et al. 2005, Brito et al. 2009, Chennu et al. 2013). Therefore, the signatures of the small, sedentary primary consumers

that have restricted home ranges likely reflect variability in signatures of basal resources within their immediate vicinity. This could explain the significant temporal and spatial variability in isotopic signatures of primary consumers. In contrast, secondary consumers such as fish are more mobile and are likely to move between sites within a given biotope. The diets and hence isotopic signatures, therefore, are not influenced by availability and variability in food sources in their vicinity; they potentially have greater dietary choice. In support of this, isotopic data for fish in this study varied significantly between biotopes, but not between seasons or sites.

5.3.2 Stable isotope mixing models

Stable isotope mixing models are frequently used to determine the relative proportion a particular food source contributes to the diet of a consumer. The introduction of Bayesian techniques has improved the applicability and robustness of mixing models, mainly through greater flexibility with regard to the number of food sources that can be used and by using residual error terms to better account for uncertainties (Parnell et al. 2010, Phillips et al. 2014). In the context of this study, even though sampled food webs comprise multiple food sources, the outputs of mixing models are likely robust representations of the composition of consumer diets, and thus provide valuable insights on the potential contribution of hippo dung to consumer diets.

It was hypothesized that consumers in the Narrows would have a higher proportion of dung in their diets than those of Charter's Creek due to the greater hippo density in the Narrows and hence higher dung input. However, results generally did not agree with this hypothesis. In the case of benthic primary consumers (amphipod, *Grandidierella bonnieroides* and isopod, *Cyathura estuaria*), dung contributions to their diets varied as a function of season and consumer identity, with no consistent pattern evident. As discussed previously, it has been shown in research elsewhere that small primary consumers are affected by spatio-temporal

variability, including that of trophic resources (Carmen & Fry 2002, Galván et al. 2008). In the present study, variability in isotopic signatures of primary consumers thus reflects variation in basal resource signatures (Figure 5.3).

One possible driver of isotopic variability in primary consumers is the dynamics between hippo-dung and benthic algae availability. Prior work has shown that terrestrial organic matter becomes increasingly important in the diet of consumers when algae is limiting (McMeans et al. 2015). This could indicate that when dung is present in the vicinity of the consumer, benthic primary consumers could (directly or indirectly) incorporate dung into their diets, due to dung-induced shading causing declines in benthic algal biomass (based on experimental work of Chapter 3 and research by Jones et al. (2012)). However, when dung is scarce, and benthic microalgae abundant, the latter may be preferentially consumed. Evidence for this idea stems from results for amphipods in the Narrows in season 4 indicating a low proportional contribution of MPB in their diets and a higher contribution of dung. In contrast, the low contribution of dung to the diet of amphipods in Charter's Creek during season 4 could be explained by the significantly higher contribution of MPB in this season relative to other seasons.

Turbidity levels may explain to some degree the variability in dung contributions to zooplankton diets observed in this study (Carrasco & Perissinotto 2012, Carrasco et al. 2013). When turbidity is high (of which suspended dung is a contributor), a decline in phytoplankton production due to rapid light attenuation is likely (Gameiro et al. 2011, Cloern et al. 2014, Kelly et al. 2014). Under these conditions, zooplankton may increase reliance on dung as a trophic resource. This could explain the trend observed in season 1 for zooplankton in the Narrows. During season 1 in this biotope, turbidity was at its highest (38.59 NTU), and dung contributions to zooplankton diets were greater there than in Charter's Creek, where turbidity was roughly eight times lower (5.32 NTU). The higher contributions of dung to zooplankton

diets in the more turbid Charter's Creek during seasons 2 and 3 relative to the Narrows supports the idea that dung dependence in zooplankton is related to or influenced by turbidity. However, the similar contributions of dung in season 4 to zooplankton diets at both biotopes despite very high turbidity at Charter's Creek suggests that turbidity is unlikely to be a singular determinant of dung importance to zooplankton.

The variability in contribution of dung to primary consumer diets was not evident in secondary consumers (fish), which instead exhibited a strong and consistent pattern of higher contributions in Charter's Creek than in the Narrows. Prior research has shown a general trend of increased incorporation of allochthonous material to consumer diets with increased availability (Cole et al. 2011, Wilkinson et al. 2013), however results from mixing models for fish species within the St Lucia Estuary did not follow this trend. While the quantity of hippo dung was likely greater in the Narrows than in Charter's Creek, due to the high densities of hippos in this biotope, the dung contributed less to fish diets in the Narrows than in Charter's Creek. Conversely, primary consumers contributed more to fish diets in the Narrows than in Charter's Creek.

Pillay and Perissinotto (2008) recorded a northward (i.e. from the Narrows to Charter's Creek and beyond) decrease in macrofaunal richness and diversity within St Lucia. In addition, research elsewhere has shown that terrestrial organic matter has a greater impact on secondary production in systems with low food web productivity and few trophic resources (Pace et al. 2004, Jones et al. 2012, Wilkinson et al. 2013, Tanentzap et al. 2017). The trend of increasing dung contributions to fish species in Charter's Creek could possibly relate to reduced availability of alternative food sources. This idea is supported in part by the generally reduced contribution of the isopod, *C. estuaria* to fish diets from Charter's Creek relative to the Narrows. It is possible that in deeper conditions, dung settles onto the benthos and is therefore not incorporated into the diets of fish, since they are largely pelagic. In Charter's Creek

however, due to the shallow depth, dung may be prone to resuspension and thus incorporation into the pelagic food web and hence fish diets. This is supported by results showing that in Charter's Creek, POM $\delta^{13}C$ values were closer to dung values than was the case in the Narrows. In addition, the resuspension of dung may also reduce visibility and impair the hunting efficiency of some fish (Utne-Palm 2002, De Robertis et al. 2003, Granqvist & Mattila 2004), thereby explaining reduced contributions of primary consumers and increased contributions of dung to fish diets.

Based on existing knowledge of the system, the major food groups have been sampled in the present study following protocols of Govender et al. (2011). However, the possibility does exist that food sources were not sampled and therefore not included in the mixing models. For example, meiofauna, which was not included in the stable isotope analyses, have been shown elsewhere to contribute to the diets of estuarine consumers (Gee 1989, Castel 1992, Carpentier et al. 2014, Schratzberger & Ingels 2017). Therefore, while the major food web components of the system were sampled in the current study, findings must be interpreted in the context of potentially unsampled food web groups. The latter aspect therefore needs to be considered in future studies quantifying contributions of hippo dung to consumers diets.

In conclusion, findings of this chapter indicate a significant shift in isotopic signatures of food web components between two biotopes with different hippo densities and therefore likely different amounts of hippo dung inputs. This suggests that dung may have an impact on the diets of consumers within these habitats. Results from Bayesian mixing models support this idea, but in different ways for the assorted consumers. Dung made variable contributions to primary consumer diets in both biotopes, which likely reflected the recorded temporal variation in basal resources. For secondary consumers, patterns of dung contributions were consistent, being greater for fish in Charter's Creek than in the Narrows. This finding contradicts the hypothesis posed that dung would contribute more to consumer diets in the Narrows. This

would suggest that even though dung may be highly abundant in a system, this does not necessarily imply greater incorporation into consumer diets. This is in line with previous studies that show that although terrestrial transfers to aquatic ecosystems are often large, consumers do not select dietary resources purely on availability, but instead choose high-quality resources regardless of their origin (Marcarelli et al. 2011). Therefore, the contribution of hippo dung as a dietary resource within St Lucia is habitat specific, likely being dependant on water depth and availability of alternative trophic resources.

CHAPTER 6:

USING FATTY ACID PROFILES AND BIOMARKERS TO INVESTIGATE THE IMPORTANCE OF HIPPO DUNG IN CONSUMER DIETS

6.1 Introduction

In our relatively short existence on the earth, humans have greatly diminished biodiversity both on land and in the oceans (McCauley, Pinsky, et al. 2015). This is troubling considering that key ecosystem functions, such as, productivity, stability, resilience, resistance and nutrient cycling are highly dependent on, and are influenced by, biodiversity (Lohrer et al. 2004, 2010, Hooper et al. 2012, Tilman et al. 2014). Although ecosystem functioning has been studied for over 55 years (Odum 1968, Lohrer et al. 2012), an increase in species extinctions, the prevalence of anthropogenic disturbances and global change have made ecosystem functioning a hot topic in current research (Meyer et al. 1999, Loreau et al. 2001, Lohrer et al. 2012, Tilman et al. 2014, Bakker et al. 2016). In addition, recent approaches that quantify the economic value of ecosystem services have raised greater awareness of the importance and magnitude of these services relative to human built services, forcing humans to look at natural assets as crucial for their health, wealth and existence (Costanza et al. 1997, 2014). As a result, research has morphed from investigating individual organism groups, to instead focusing on the management and conservation of entire communities and ecosystems (Pasquaud et al. 2007 cf Petitgas 2002, Lohrer et al. 2012).

Megaherbivores (> 1000 kg) can act as agents of both maintenance and change, making them critical for ecosystem functioning and health (Chritz et al. 2016, Malhi et al. 2016). Ecosystem services provided by these large bodied, plant-consuming animals include nutrient cycling, geomorphic engineering, bush clearance and the maintenance of open systems or grazing lawns, enhancing net primary productivity, increasing connectivity and altering the organisation of trophic guilds (McNaughton 1985, Owen-Smith 1988, Naiman & Rogers 1997, Augustine et al. 2003, Bakker et al. 2016, Chritz et al. 2016). Although the effects of megaherbivores on land are reasonably well investigated and understood, little has been done on ecosystem-wide effects of these animals on aquatic systems and food webs (Bakker et al.

2016). Africa's last remaining semi-aquatic megaherbivore, the hippopotamus, is one of these megaherbivores that is able to drastically impact both terrestrial and aquatic systems (Jones et al. 1994, Bakker et al. 2016). The importance of hippos as vectors of terrestrial transfers has been alluded to in the previous chapter, which has partially shed light on the role of hippo dung in estuarine food webs.

6.1.1 Conditions in St Lucia

The St Lucia Estuarine system has until recently undergone a drought that began in 2002. This phase persisted for a longer period than previous dry cycles and has been regarded as one of the system's most severe droughts in history (Perissinotto, Stretch, et al. 2013). Associated changes in biophysical conditions have dramatically influenced species within the system and consequently caused changes to food webs (Govender et al. 2011, Scharler & MacKay 2013), resulting in reductions in the species pool by selecting those that are able to withstand extreme conditions. Superimposed upon this is the fact that resilience of remaining communities are likely to be compromised by additional anthropogenic water abstraction and associated changes in freshwater inflow (MacKay et al. 2010).

The significant drought-induced declines in water levels and flow within the estuary and the subsequent aggregation of hippos, has resulted in the formation of layers of dung covering the benthos (Taylor 2013a, Dawson et al. 2016). Despite the fact that organic matter/detritus is recognised as an important food source in estuaries, even when microalgal production is high (Whitfield 1983, Scharler & MacKay 2013), and that hippos are adding material to the detrital pool at rates unmatched by other natural processes, food web research within the system to date, has neglected the possible consequences and significance of hippo dung as a trophic resource. This omission is particularly relevant given that studies elsewhere have reported declines in abundance and diversity through dung inputs, and that the influence

of hippos on ecosystem functioning can be magnified by shifts in hydrological conditions caused by human activity (Masese et al. 2018, Stears et al. 2018, Subalusky et al. 2018).

6.1.2 Study techniques in food web ecology

The complexity of physical and biological processes occurring in estuaries makes food web studies and diet analysis challenging (Alfaro et al. 2006, Scharler & MacKay 2013, Antonio & Richoux 2014). Food web studies have traditionally used *in situ* field observations in conjunction with gut content analyses, however, constraints associated with these methods (described in Chapter 5) and improvements in laboratory techniques and equipment, have recently led to an increase in popularity of stable isotopes (i.e. $\delta^{15}N$ and $\delta^{13}C$) and lipid biomarkers (i.e. fatty acids) as quantitative tools in food web studies (Kharlamenko et al. 2001, Herman et al. 2005, Alfaro et al. 2006, West et al. 2006, Phillips et al. 2014 - Fig.1).

Although stable isotopes have shed significant light on food web interactions, this technique becomes problematic when source signatures overlap. This is especially relevant to detritus-based food webs (Pasquaud et al. 2007). In addition, even though it is possible to identify dietary sources for consumers using stable isotopes, it is difficult to determine the pathway by which sources reached consumers. In effect, direct and indirect consumptive pathways cannot be distinguished. Stable isotope analyses are also ineffective in assessing the contribution to consumer diets of heterotrophic microorganisms (bacteria), which are a dominant component in detrital food webs (Kharlamenko et al. 2001). These limitations can to some degree be addressed through the use of complementary fatty acid analysis, which can expand understanding of the complexity of food web processes occurring in aquatic ecosystems (Kharlamenko et al. 2001, Alfaro et al. 2006, Richoux & Froneman 2008). Fatty acid analysis also has limitations, but in combination with stable isotope analyses, both techniques provide different and yet complementary information required to understand trophic interactions

(Kharlamenko et al. 2001, Herman et al. 2005, Alfaro et al. 2006, Pasquaud et al. 2007, Richoux et al. 2010, Belicka et al. 2012).

6.1.3 Fatty acids and biomarkers

Fatty acids (FA) form part of a diverse group of carbon-rich molecules that encompass the majority of lipids present in all organisms and are responsible for membrane structure and energy storage (Iverson et al. 1997, Napolitano 1999, Budge et al. 2006). Many FAs are metabolically stable, meaning that even after being consumed, their structure remains unchanged (Napolitano 1999, Pasquaud et al. 2007). This transfer of the molecular properties of their diet to a consumer's tissue enables researchers to trace prey items and determine their dietary importance (Iverson et al. 1997). Like isotopes, and contrary to gut content analyses, FAs provide information on the long term diet of an organism, not just that of the most recently consumed meal (Dalsgaard et al. 2003, Pasquaud et al. 2007). Iverson (1993) first used the term "fatty acid signature" to refer to the complete collection of all the FAs present in an organism. Examining how these signatures or profiles change can provide valuable information about spatial and temporal dietary variations within and between populations (Iverson et al. 1997, Budge et al. 2006).

Particular FAs present within a given environment are fairly ubiquitous within food webs, making it difficult to trace the movement of trophic resources. However, certain fatty acids are only newly synthesized at low trophic levels by plants and specific bacteria and are then transferred, unchanged, to higher trophic levels when consumed. Therefore, the presence, combinations, and ratios of fatty acids like these, can be characteristic of and attributed to, specific food web sources (Budge & Parrish 1998, Dalsgaard et al. 2003). These are known as fatty acid trophic markers (FATM) or biomarkers (Budge & Parrish 1998, Dalsgaard et al. 2003, Budge et al. 2006) and are commonly used to determine trophic relationships. Although

FA values can only be used semi-quantitatively because of selective breakdown and consumer's internal biochemical conversion of some FA structures into others, the use of biomarkers does provide more qualitative information about source material than stable isotopes (Pasquaud et al. 2007).

A number of biomarkers have been suggested and/or used in the literature to determine allochthonous and autochthonous resource groups within aquatic environments (reviewed in Dalsgaard et al. 2003) - the most successful of which are those that are unique to a food source group within the specific environment (Dalsgaard et al. 2003, Richoux & Froneman 2008). For example, since certain FAs can only be produced *de novo* by plants but are assimilated and retained by animals, the ratios of all 16 chain carbons to all 18 chain carbons ($\Sigma C16/\Sigma C18$)[3] can be used as a general diatom marker (Parrish et al. 2000, Budge et al. 2001, Dalsgaard et al. 2003). Similarly, the sum of 15:0, 17:0, iso- and anteiso-branched chain FAs is used as a marker for bacteria, which commonly produce odd carbon-numbered and branched-chain fatty acids (Haddad et al. 1992, Harvey 1994, Budge & Parrish 1998, Brett et al. 2006). The sum of essential fatty acids: Arachidonic acid (ARA, 20:4ω6), Eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3), provide an indication of the origin of resources because aquatic primary producers, such as those found in the microphytobenthos, have greater quantities of these essential fatty acids (EFA) relative to terrestrial material (Brett et al. 2006, Hixson et al. 2015, Moyo et al. 2017). Lastly, the sum of the polyunsaturated fatty acids (PUFA) Linoleic acid (LIN, 18:2ω6) and α-Linolenic acid (ALA, 18:3ω3) are employed to indicate terrestrial material, a value higher than a 2.5 % of the total FA content constitutes significant terrestrial carbon input in a consumers diet (Budge & Parrish 1998, Budge et al. 2001).

[3] FA nomenclature is shown in Table 1

In Chapter 5, stable isotope analyses were used to compare the relative contribution of dung to the diets of consumers within two biotopes, each experiencing contrasting hippo densities and therefore theoretically different degrees of dung loading. This chapter extends the food web analyses undertaken in the previous chapter, by utilising complementary FA analysis to determine if (1) profiles of allochthonous hippo dung could be distinguished from autochthonous aquatic resources, (2) FA profiles of dominant consumers common to the Narrows and Charter's Creek differ and (3) FA biomarkers can provide information on the relative importance of hippo dung as a basal resource within these biotopes. It was hypothesised that food web components of the two biotopes would have disparate FA profiles, and that FA biomarker values would differ between biotopes, due primarily to differential dung loading. More specifically, it was predicted that there would be (1) greater terrestrial and bacterial biomarker values in the Narrows due to high dung input rates, but (2) a reduction in diatom biomarker values in the Narrows due to shading caused by dung, and (3) reduced EFA values in the Narrows as declines in microphytobenthos limit the available EFAs.

6.2 Results

Table 6.1 provides the names, chemical structures and abbreviations for the most common fatty acids described in the results section and the fatty acid trophic markers used to determine the relative importance of hippo dung within two biotopes.

6.2.1 Fatty acid profiles

The fatty acid profiles of microphytobenthos (MPB) and particulate organic matter (POM) differed significantly between seasons and biotopes (Nested PERMANOVA, MPB: season - pseudo $F_{3,62}$ = 4.082; p = 0.010; biotope - pseudo $F_{3,62}$ = 8.375; p = 0.001 and POM:

season - pseudo $F_{3,65}$ = 2.871; p = 0.010; biotope - pseudo $F_{4,65}$ = 8.375; p = 0.001). Sediment organic matter fatty acid profiles differed significantly between biotopes (Nested PERMANOVA, biotope - pseudo $F_{4,61}$ = 27.007; p = 0.001), while seasonal differences were insignificant (Nested PERMANOVA, season - pseudo $F_{3,61}$ = 1.326; p = 0.279). Non-metric multidimensional scaling ordinations (nMDS) indicated consistent spatial separation of fatty acid profiles of basal trophic resources between the Narrows and Charter's Creek (Fig. 6.1). The plots demonstrate that resources were generally distinct, both within and between biotopes across all four seasonal sample sets. Hippo dung fatty acid profiles generally differed from all other resources.

Table 6.1: The fatty acid nomenclature, structure and common abbreviations used in this study.

Type	Abbreviation	Structure
16 chain carbons	C16	16:0, 16:1, 16:2ω6 …
18 chain carbons	C18	18:0, 18:1, 18:1ω7 …
Saturated FAs	SAFA	14:0 - 28:0
Branched FAs	i- & ai-	i-15:0, i-16:0, i-17:0 & ai-15:0, ai-17:0
Monounsaturated FAs	MUFA	16:1, 18:1ω6/ω9, 18:1ω7, 20:1ω7, 20:1ω9
Linoleic acid	LIN	18:2ω6
α-Linolenic acid	ALA	18:3ω3
Eicosapentaenoic acid	EPA	20:5ω3
Arachidonic acid	ARA	20:4ω6
Docosahexaenoic acid	DHA	22:6ω3
Polyunsaturated FAs	PUFA	≥ 2 double bonds, e.g. 18:2ω6, 22:6ω3
Highly unsaturated FAs	HUFA	EPA, DHA, ARA
Fatty acid trophic markers	FATM	Biomarkers
Terrestrial	-	18:2ω6 + 18:3ω3
Bacterial	-	Σ 15:0, 17:0 and branched iso- and anteiso-
Essential fatty acids	EFA	20:4ω6 + 20:5ω3 + 22:6ω3
Diatom	Σ16/Σ18	ratio of all C16 and C18 fatty acids

For primary consumers, which included zooplankton, amphipods (*Grandidierella bonnieroides*) and isopods (*Cyathura estuaria*), nMDS ordinations indicated consistent spatial

separation of fatty acid profiles between Narrows and Charter's Creek samples. This was statistically supported by Nested PERMANOVA, which showed significant biotope differences for all three consumers (zooplankton, Fig. 6.2, pseudo $F_{4,64} = 30.511$; $p = 0.001$; *G. bonnieroides,* Fig. 6.3, pseudo $F_{3,40} = 13.107$; $p = 0.001$; *C. estuaria,* Fig. 6.4, pseudo $F_{2,11} = 11.192$; $p = 0.001$). However, seasonal differences in primary consumer fatty acid profiles were insignificant (zooplankton, pseudo $F_{3,64} = 1.500$; $p = 0.125$; *G. bonnieroides,* pseudo $F_{2,40} = 1.092$; $p = 0.338$; *C. estuaria*, pseudo $F_{1,11} = 0.982$; $p = 0.495$).

Similarly, for secondary consumers (fish), including tilapia (*Oreochromis mossambicus*), mullet (*Chelon (=Liza) dumerili*) and glassy (*Ambassis ambassis*), nMDS ordinations indicated a visual separation of consumer fatty acid profiles between the Narrows and Charter's Creek. Nested PERMANOVA analyses supported this, indicating statistically different profiles between biotopes for all three species (*O. mossambicus*, Fig. 6.5, pseudo $F_{4,175} = 14.879$; $p = 0.001$; *C. dumerili*, Fig. 6.6, pseudo $F_{3,77} = 8.672$; $p = 0.001$; *A. ambassis,* Fig. 6.7, pseudo $F_{2,70} = 5.215$; $p = 0.001$). With regard to seasonal differences in fish fatty acid profiles, *O. mossambicus* FA profiles were significantly different (pseudo $F_{3,175} = 2.816$; $p = 0.013$) however, *C. dumerili* and *A. ambassis* FA profiles did not differ seasonally (*C. dumerili*, pseudo $F_{2,77} = 1.041$; $p = 0.426$; *A. ambassis*, pseudo $F_{1,70} = 2.072$; $p = 0.165$). The fatty acid profiles of crocodiles did not differ between samples collected in the estuary and those collected outside the estuary (Fig. 6.8). This was statistically supported by PERMANOVA ($F_{1,6} = 0.419$; $p = 0.501$).

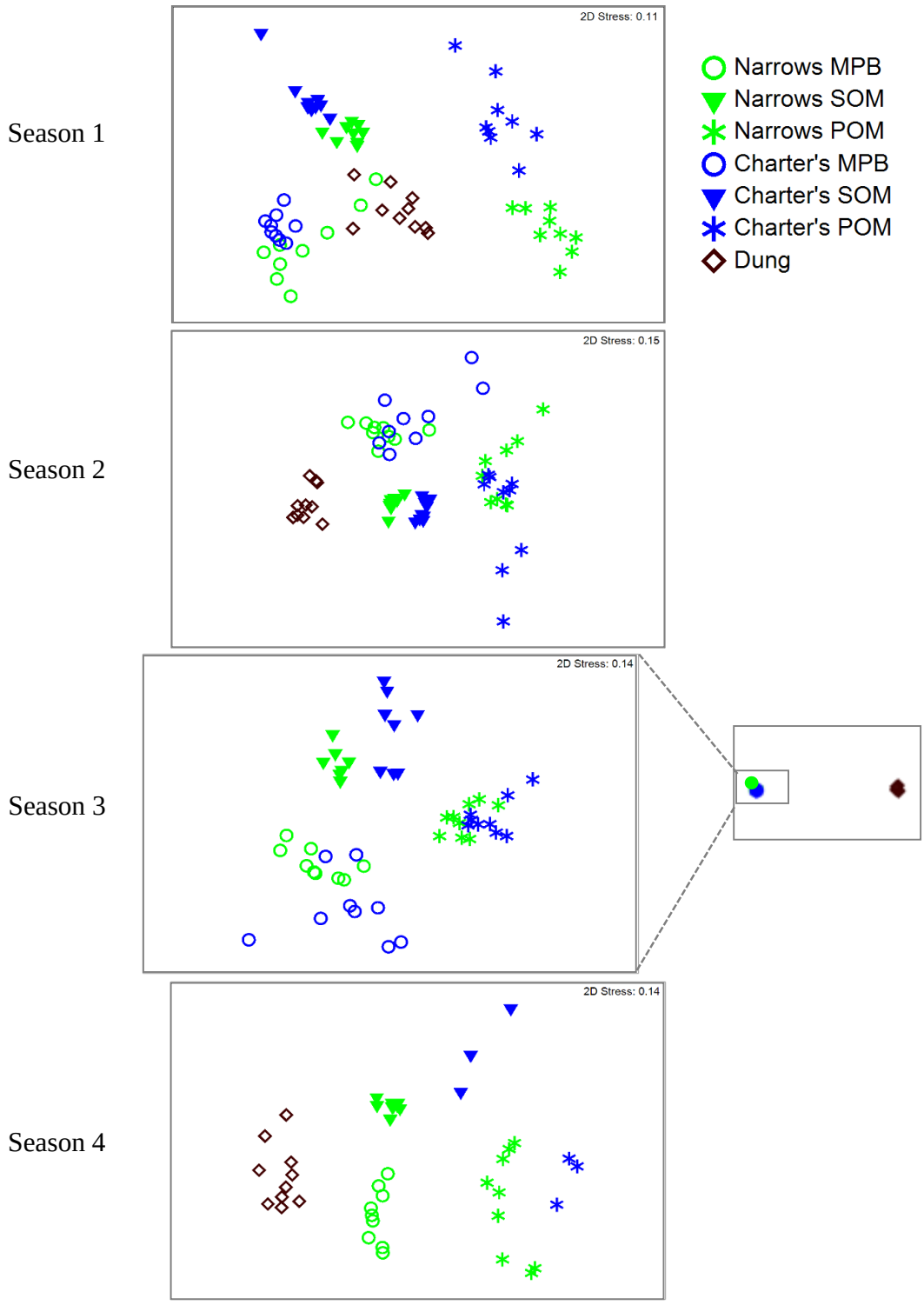


Figure 6.1: Non-metric multidimensional scaling ordinations (nMDS) showing spatial differences in basal resource fatty acid profiles (as a percentage of total fatty acid content; microphytobenthos (MPB - circle), sediment organic matter (SOM - triangle) and particulate organic matter (POM - asterisk) and hippo dung (diamond). Samples of dung (brown) and basal resources from the Narrows (green) and Charter's Creek (blue) for all four sampling seasons are shown. Inset for 3 shows the ordination with dung samples included.

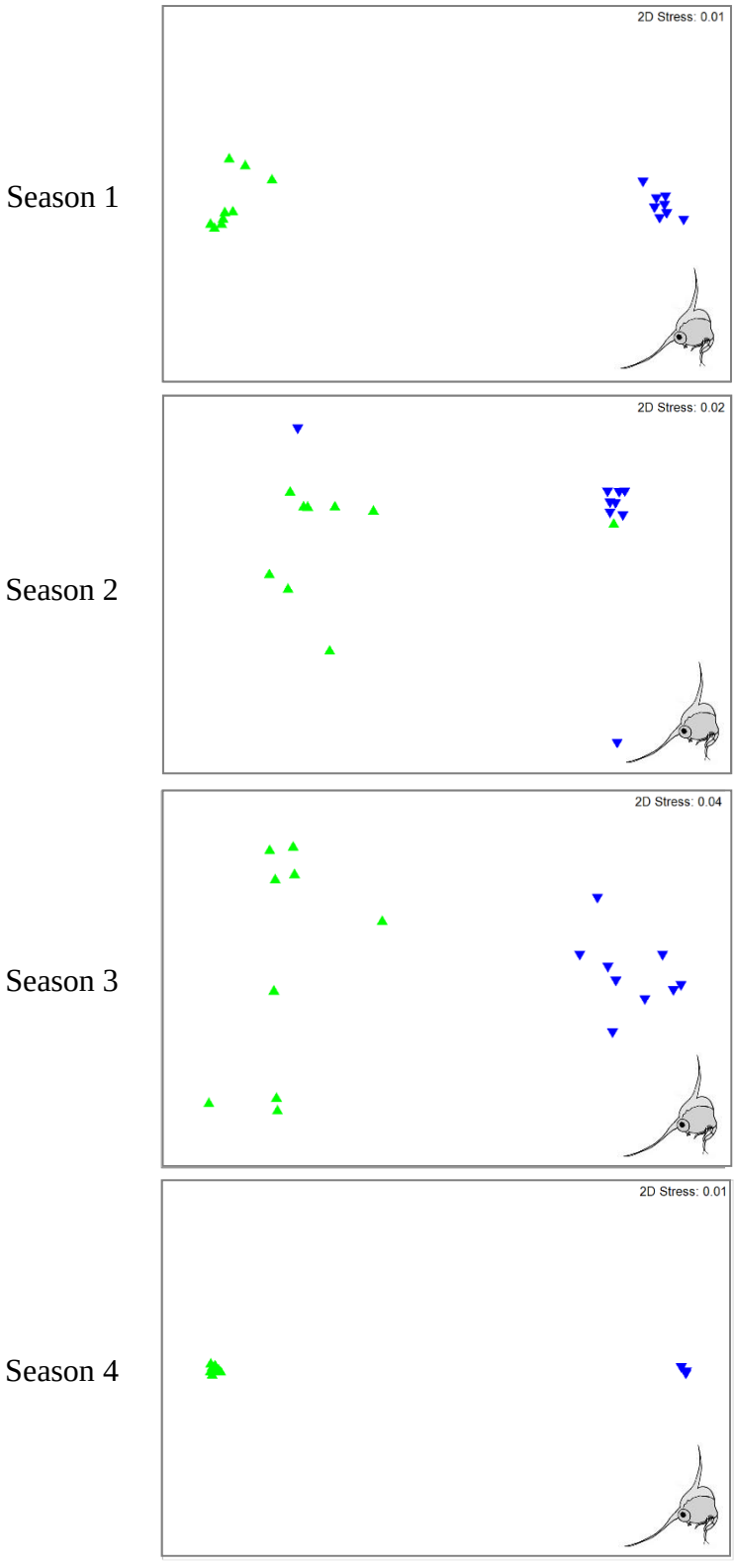


Figure 6.2: Non-metric multidimensional scaling ordinations (nMDS) based on the fatty acid profiles (as a percentage of total fatty acid content) of zooplankton showing a distinct separation between samples from the Narrows (green) and Charter's Creek (blue) for all four sampling seasons.

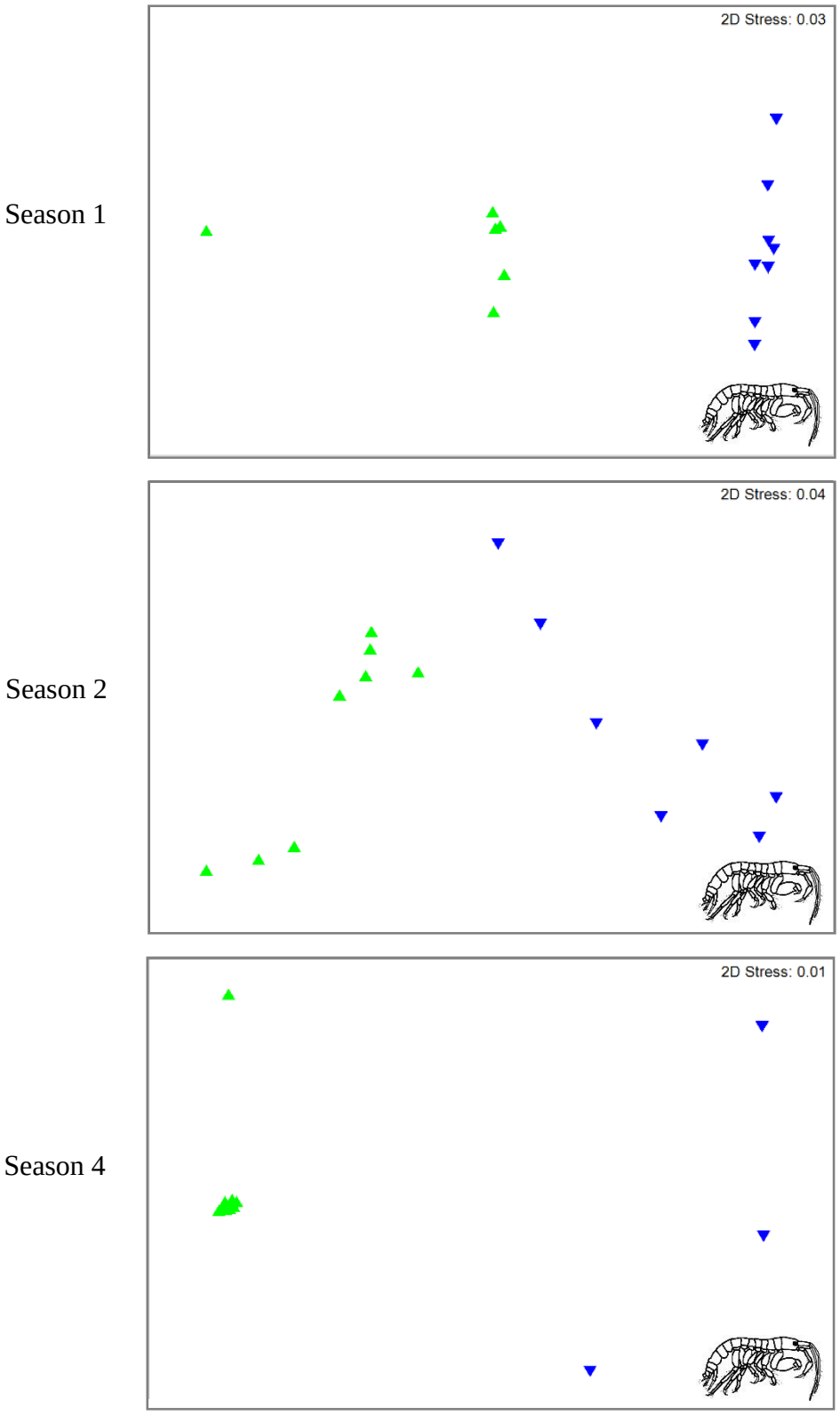
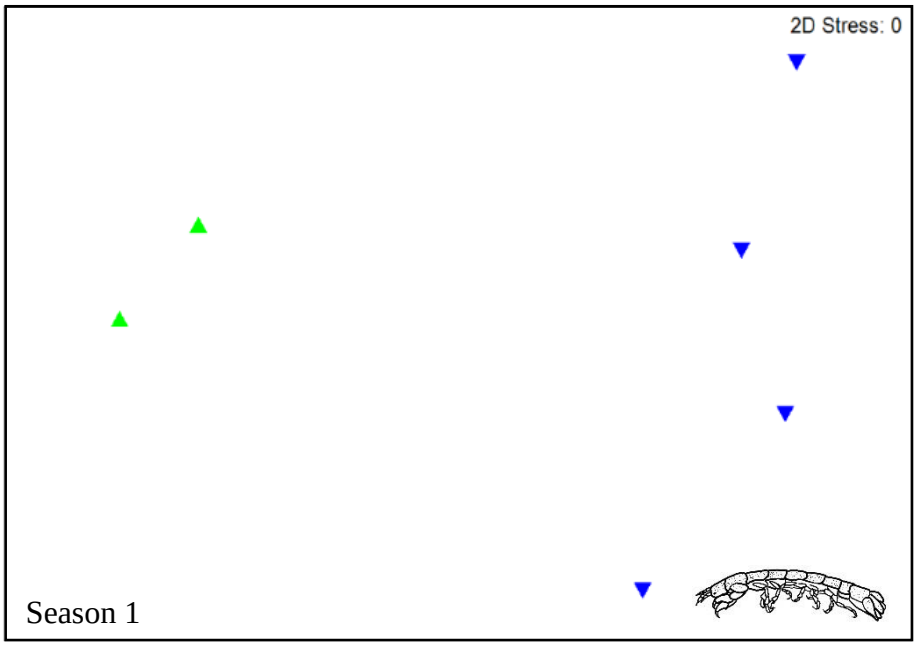


Figure 6.3: Non-metric multidimensional scaling ordinations (nMDS) based on the fatty acid profiles (as a percentage of total fatty acid content) of the amphipod (*Grandidierella bonnieroides*) showing a distinct separation between samples from the Narrows (green) and Charter's Creek (blue) for three sampling seasons.



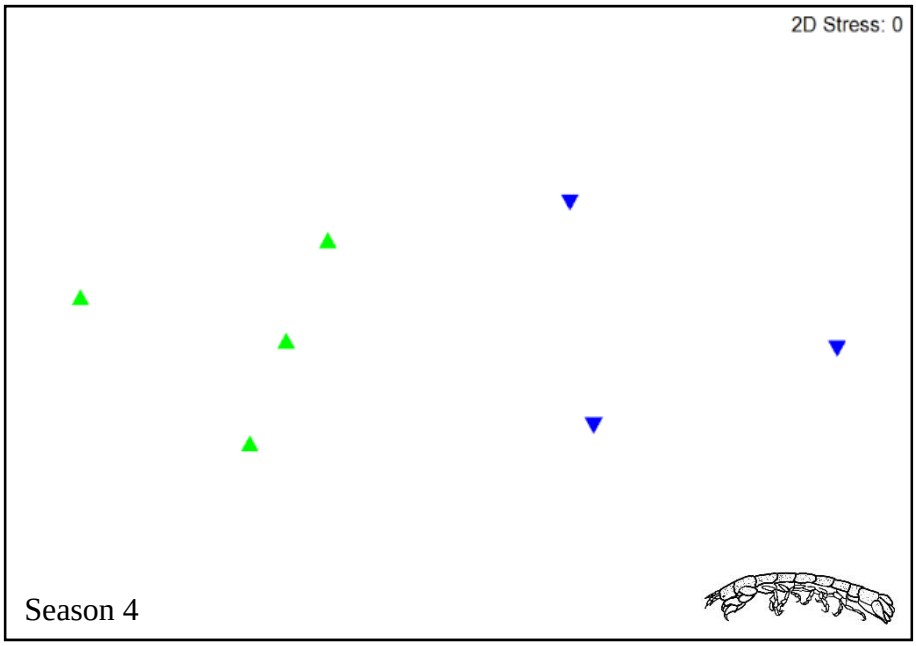


Figure 6.4: Non-metric multidimensional scaling ordinations (nMDS) based on the fatty acid profiles (as a percentage of total fatty acid content) of the isopod (*Cyathura estuaria*) showing a distinct separation between the samples from the Narrows (green) and Charter's Creek (blue) for two sampling seasons.

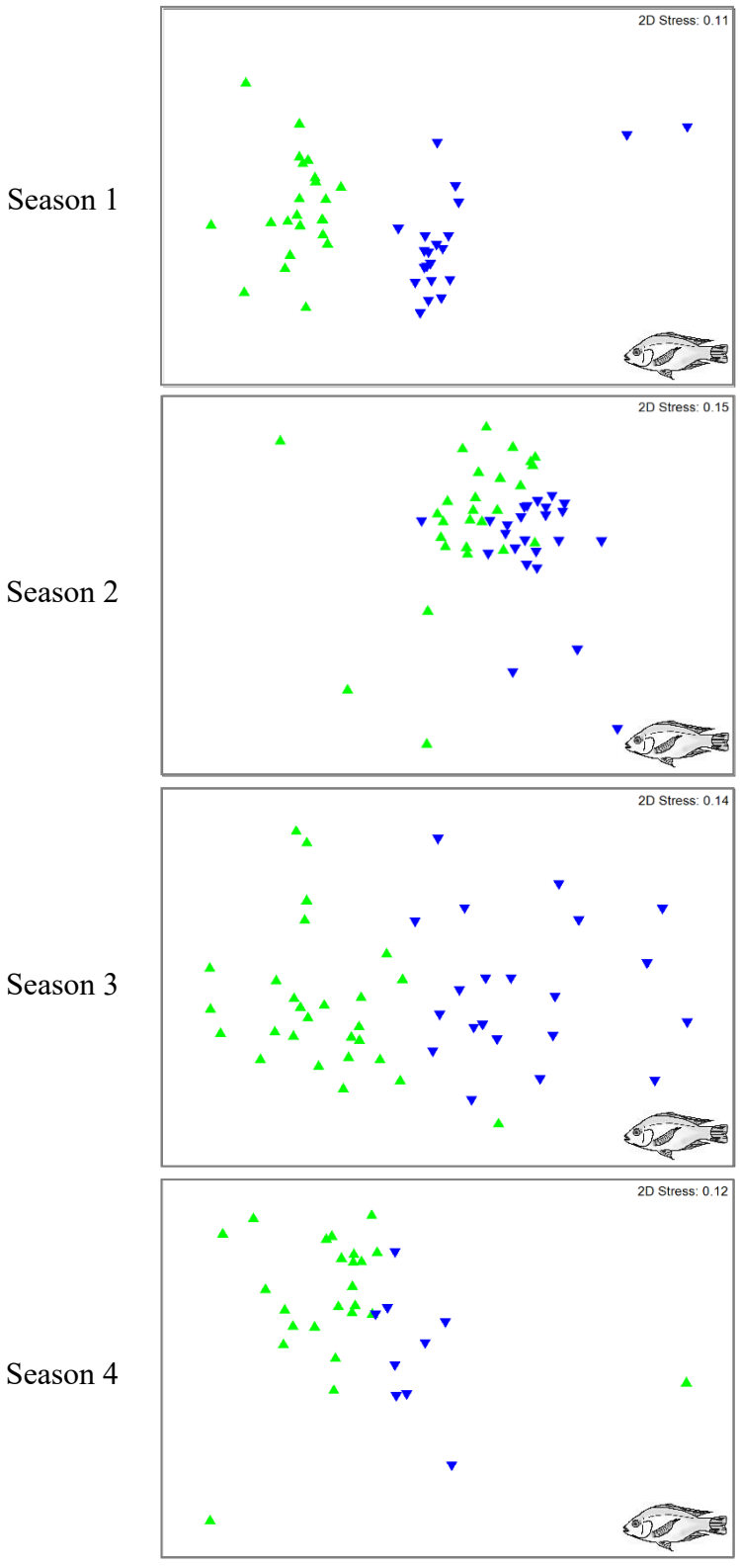


Figure 6.5: Non-metric multidimensional scaling ordinations (nMDS) based on the fatty acid profiles (as a percentage of total fatty acid content) of tilapia (*Oreochromis mossambicus*) showing a distinct separation between fish from the Narrows (green) and Charter's Creek (blue) for all four sampling seasons.

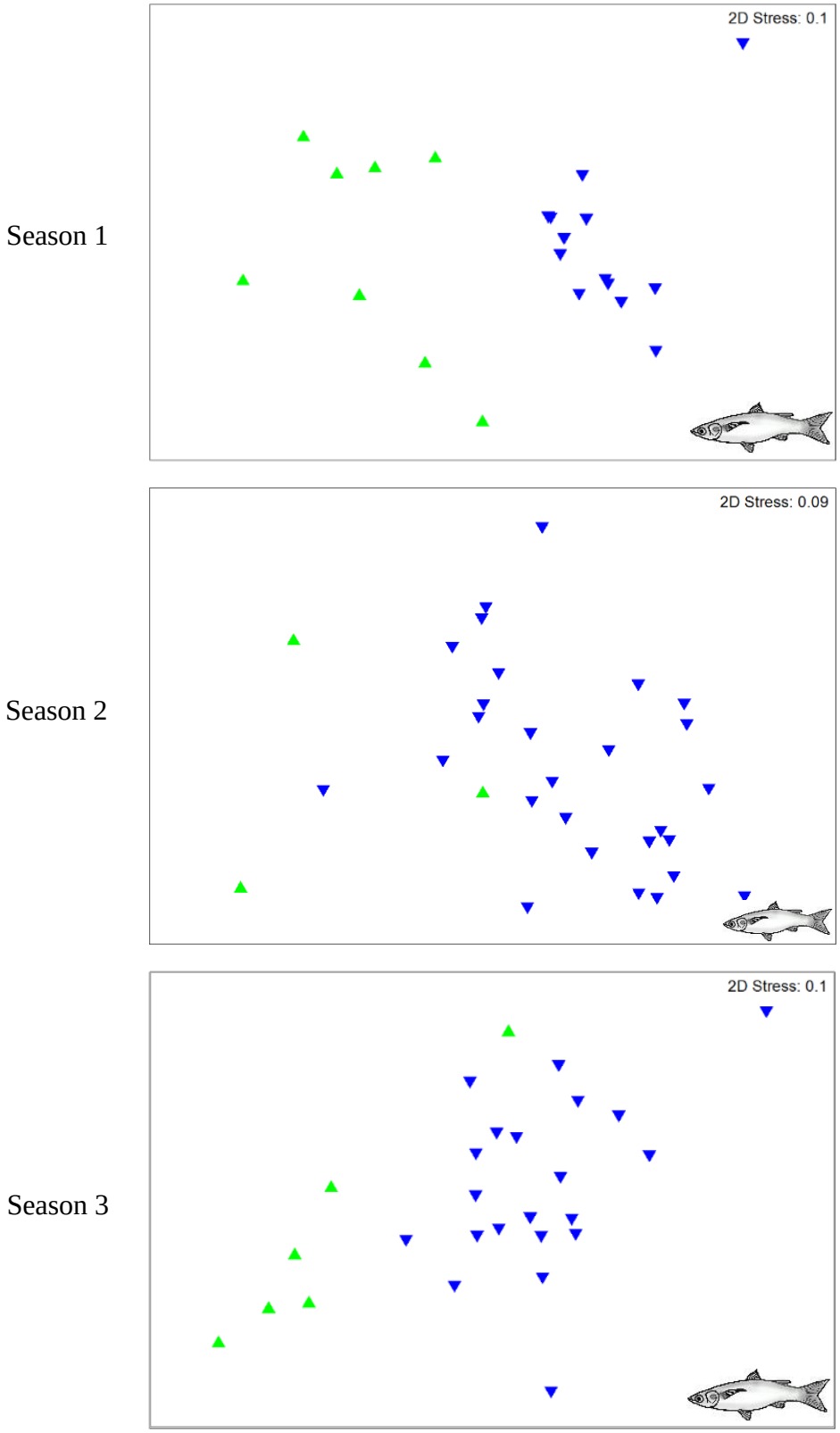


Figure 6.6: Non-metric multidimensional scaling ordinations (nMDS) based on the fatty acid profiles (as a percentage of total fatty acid content) of mullet (*Chelon (=Liza) dumerili*) showing a distinct separation between fish from the Narrows (green) and Charter's Creek (blue) for three sampling seasons.

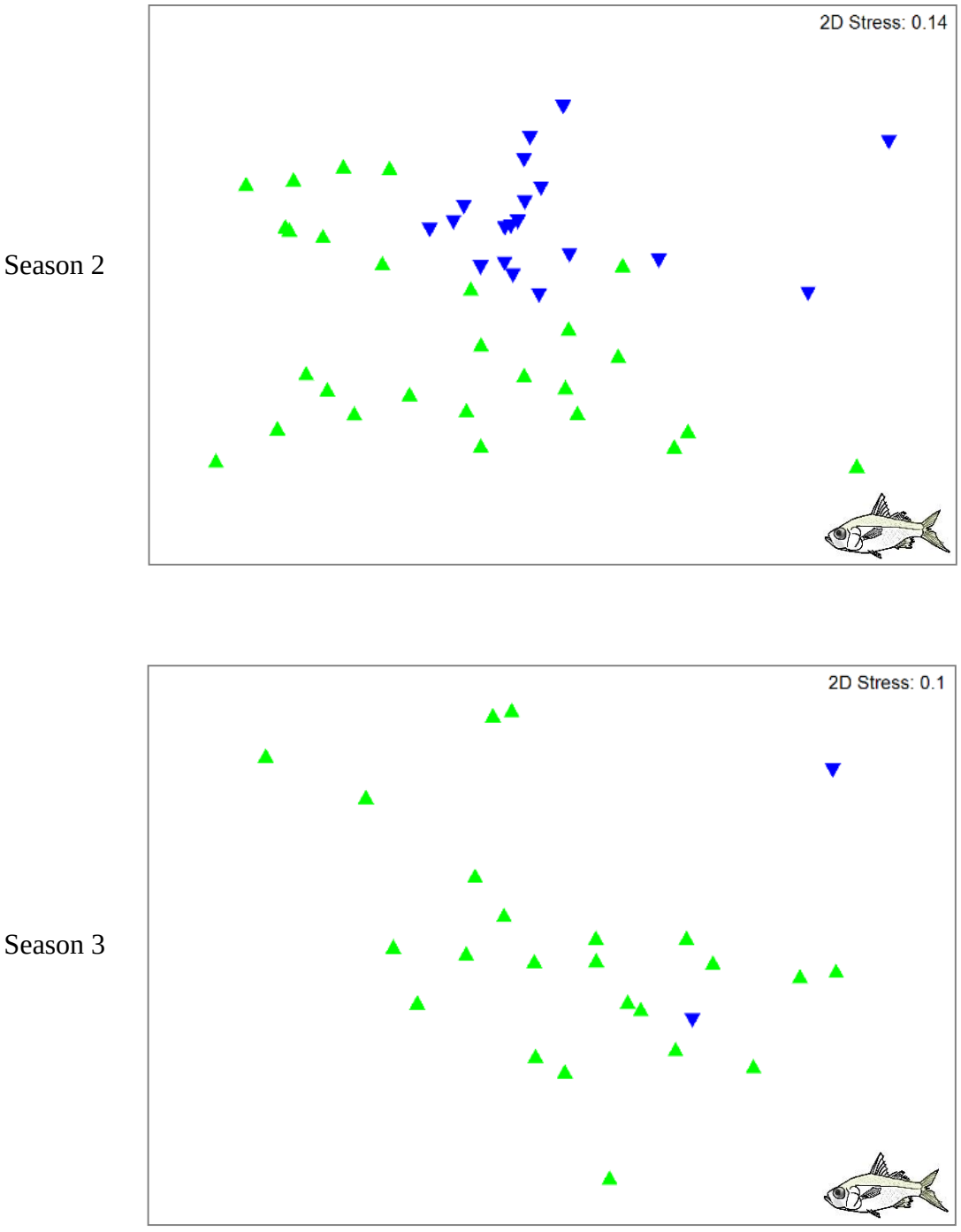


Figure 6.7: Non-metric multidimensional scaling ordinations (nMDS) based on the fatty acid profiles (as a percentage of total fatty acid content) of glassy (*Ambassis ambassis*) showing separation between fish from the Narrows (green) and Charter's Creek (blue) for all two sampling seasons.

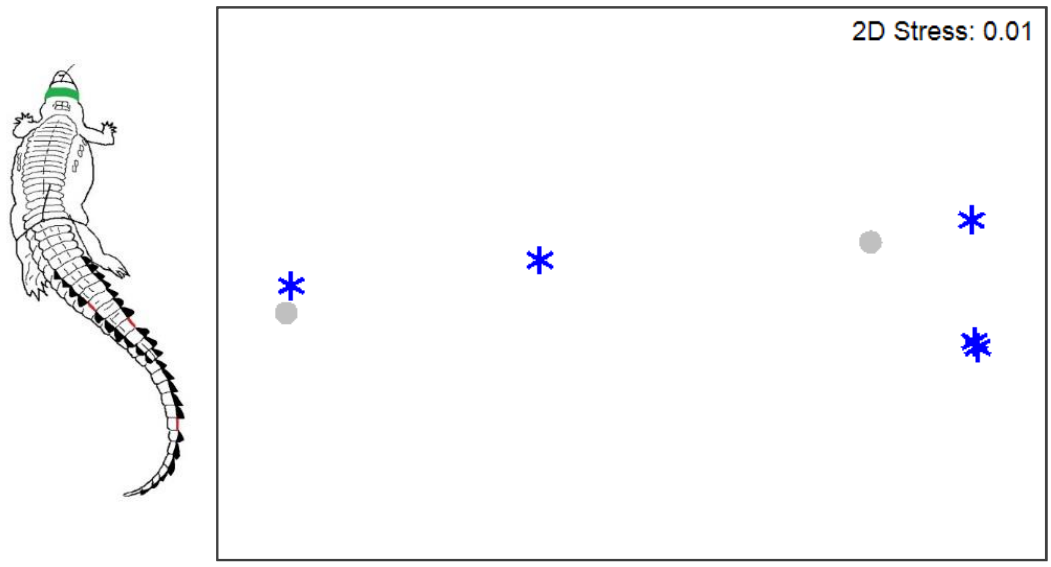


Figure 6.8: Non-metric multidimensional scaling ordinations (nMDS) based on the fatty acid profiles (as a percentage of total fatty acid content) of Nile crocodile (*Crocodylus niloticus*) tissue samples taken from within the St Lucia Estuary, a system containing large amounts of hippo dung (blue) and outside the estuary, areas containing low to no hippo dung (grey).

6.2.2 SIMPER analysis of food web fatty acid profiles

Similarity analyses undertaken in SIMPER showed that fatty acid profiles of the Narrows food web components were generally more similar to hippo dung profiles, than those in Charter's Creek (Tables 6.2 & 6.3). More specifically, in 21 out of the 29 comparisons, similarities to dung were greater in the Narrows than Charter's Creek, with similarity to dung in the Narrows being from 1.23 to 15.30 % greater than those of Charter's Creek. In the eight cases where Charter's Creek samples was more similar to dung, the differences ranged from 0.17 to 6.37. In terms of basal resource fatty acid profiles, exceptions to the general increase in similarity relative to hippo dung were recorded in season 3 MPB and POM in seasons 1 and 2. For consumers, exceptions were recorded for tilapia and mullet in season 3, glassy in season 2 and zooplankton in seasons 2 and 4. In these cases, Charter's Creek samples were more similar to dung.

Table 6.2: Results from SIMPER analyses showing the seasonal average dissimilarity in fatty acid profiles of basal resources between biotopes (Narrows and Charter's Creek) and the similarities of each resource's FA profile relative to the hippo dung profile, in each biotope. Difference = Narrows % similarity to dung – Charter's Creek % similarity to dung.

Basal resource	Season	Biotope	Dissimilarity between biotopes	Similarity to dung	Difference
Microphytobenthos MPB	1	Narrows Charter's	23.26	60.38 57.35	3.03
	2	Narrows Charter's	30.95	54.17 52.57	1.60
	3	Narrows Charter's	29.61	18.84 22.14	**-3.30**
	4	Narrows Charter's	-	65.33 -	
Sediment organic matter SOM	1	Narrows Charter's	27.83	65.59 56.64	8.95
	2	Narrows Charter's	18.73	61.54 50.65	10.89
	3	Narrows Charter's	34.15	20.99 18.91	2.08
	4	Narrows Charter's	33.12	63.26 47.96	15.30
Particulate organic matter POM	1	Narrows Charter's	39.76	51.89 55.19	**-3.30**
	2	Narrows Charter's	33.23	41.01 42.23	**-1.22**
	3	Narrows Charter's	20.31	22.73 20.55	2.18
	4	Narrows Charter's	30.22	52.58 43.49	9.09

6.2.3 Fatty acid biomarkers for food web components

Hippo dung had high terrestrial (Fig. 6.9, 9.13 % ± 0.73) and bacterial values (8.49 % ± 0.57), but very low values for both EFAs and diatom markers (0.85 % ± 0.18 and 0.48 % ± 0.04, respectively).

Table 6.3: Results from SIMPER analyses showing the seasonal average dissimilarity in fatty acid profiles of consumers between biotopes (Narrows and Charter's Creek) and the similarities of each consumer's FA profile relative to the hippo dung profile, in each biotope. Difference = Narrows % similarity to dung– Charter's Creek % similarity to dung.

Species	Season	Biotope	Dissimilarity between biotopes	Similarity to dung	Difference
Tilapia *Oreochromis mossambicus*	1	Narrows Charter's	17.15	43.18 38.25	4.93
	2	Narrows Charter's	14.60	43.22 40.53	2.69
	3	Narrows Charter's	15.18	45.29 47.05	**-1.76**
	4	Narrows Charter's	14.83	42.58 41.23	1.35
Mullet *Chelon (=Liza) dumerili*	1	Narrows Charter's	22.78	40.76 38.70	2.06
	2	Narrows Charter's	22.12	40.10 37.98	2.12
	3	Narrows Charter's	17.71	41.55 41.94	**-0.39**
Glassy *Ambassis ambassis*	2	Narrows Charter's	14.41	41.82 41.99	**-0.17**
	3	Narrows Charter's	14.45	43.93 42.17	1.76
Amphipod *Grandidierella bonnieroides*	1	Narrows Charter's	26.71	55.32 51.10	4.22
	2	Narrows Charter's	27.65	49.43 46.57	2.86
	4	Narrows Charter's	17.66	51.01 49.69	1.32
Isopod *Cyathura estuaria*	1	Narrows Charter's	30.11	56.11 46.89	9.22
	4	Narrows Charter's	21.66	51.93 49.83	2.10
Zooplankton	1	Narrows Charter's	37.82	52.38 39.91	12.47
	2	Narrows Charter's	33.79	32.40 38.77	**-6.37**
	3	Narrows Charter's	16.54	42.01 34.75	7.26
	4	Narrows Charter's	11.31	34.31 35.03	**-0.72**

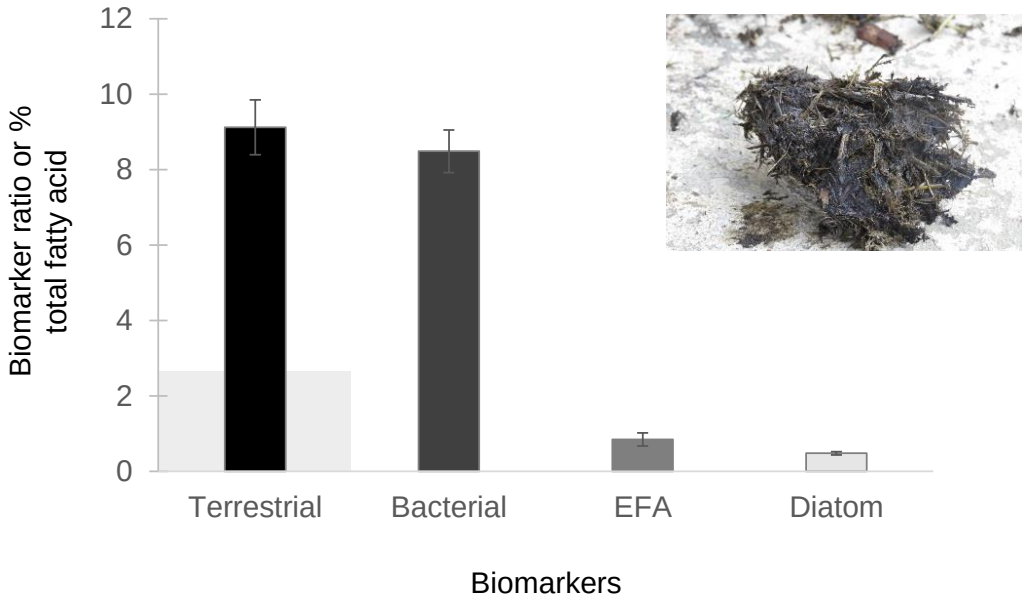


Figure 6.9: Mean fatty acid biomarker values (± 1SE) for fresh, seasonally pooled hippo dung samples. Shaded area indicates the 2.5 % value above which terrestrial inputs are considered significant. Terrestrial, bacterial and essential fatty acids (EFA) markers represent a percentage of the total fatty acids. Diatom values given as a ratio of the sum of 16 carbon FA/sum of 18 carbon FA.

All four fatty acid biomarkers in microphytobenthos (MPB) samples showed statistically significant seasonal and biotope variation (Table 6.4, Fig. 6.10, Nested ANOVA; season: $p < 0.001$ and biotope: $p < 0.001$). The MPB FA biomarkers did not show any strong trends in response to biotope, with the exception of diatom values being higher in Charter's Creek in two out of the three seasons where data were available. For sediment organic matter (SOM), terrestrial, bacterial and EFA biomarkers were significantly different between seasons and biotopes (Table 6.4, Fig. 6.11, Nested ANOVA; season: $p \leq 0.032$ and biotope: $p < 0.001$). The SOM diatom marker differed significantly only between biotopes ($p < 0.001$). Bacterial and diatom biomarkers strongly supported the hypothesis posed of there being higher bacterial and lower diatom values in Narrows vs Charter's Creek for SOM samples.

Table 6.4: Results of Nested ANOVA showing comparisons for biomarkers of food web basal resources between seasons, biotopes and sites. Significant values shown in bold. *F* = F-statistic, *p* = significance, DF = degrees of freedom.

Basal resources	Biomarker	Nested ANOVA Basal resources								
		Season			Biotope			Site		
		F	DF	*p*	*F*	DF	*p*	*F*	DF	*p*
Microphytobenthos MPB	Terrestrial	18.43	3,21	**<0.001**	9.48	3,21	**<0.001**	2.315	14,21	**0.040**
	Bacterial	25.04	3,21	**<0.001**	9.32	3,21	**<0.001**	1.384	14,21	0.243
	EFA	205.47	3,21	**<0.001**	733.12	3,21	**<0.001**	10.304	14,21	**<0.001**
	Diatom	11.49	3,21	**<0.001**	8.21	3,21	**<0.001**	4.547	14,21	**<0.001**
Sediment organic matter SOM	Terrestrial	52.56	3,18	**<0.001**	12.84	4,18	**<0.001**	2.789	14,18	**0.022**
	Bacterial	3.65	3,18	**0.032**	58.81	4,18	**<0.001**	1.476	14,18	0.216
	EFA	7.05	3,18	**0.002**	80.09	4,18	**<0.001**	4.867	14,18	**0.001**
	Diatom	2.62	3,18	0.083	50.91	4,18	**<0.001**	0.721	14,18	0.730
Particulate organic matter POM	Terrestrial	4.18	3,22	**0.017**	10.25	4,22	**<0.001**	3.172	14,22	**0.008**
	Bacterial	127.84	3,22	**<0.001**	126.46	4,22	**<0.001**	1.527	14,22	0.182
	EFA	42.12	3,22	**<0.001**	18.43	4,22	**<0.001**	8.414	14,22	**<0.001**
	Diatom	27.86	3,22	**<0.001**	20.81	4,22	**<0.001**	1.865	14,22	0.092

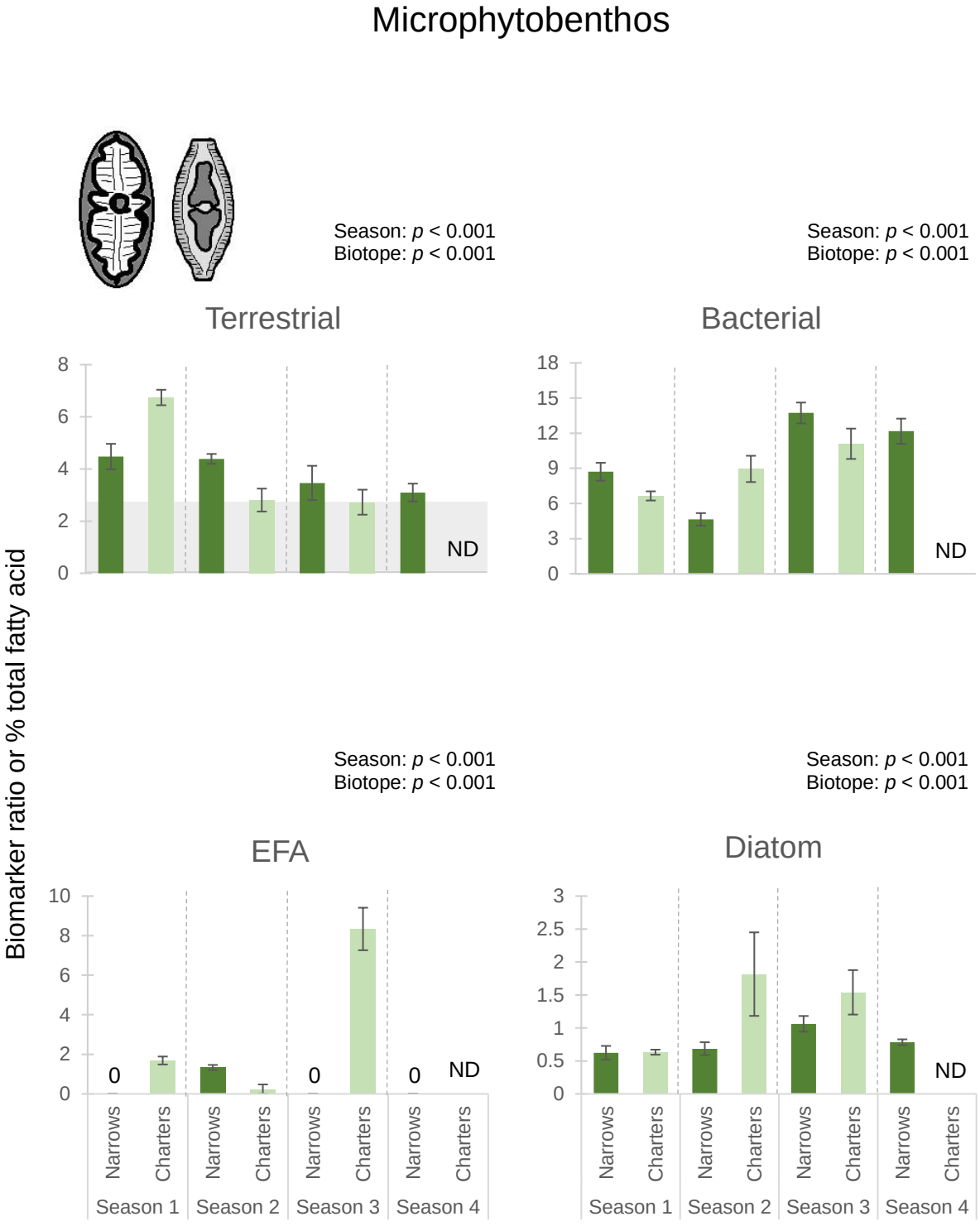


Figure 6.10: Mean biomarker values (± 1 SE) for microphytobenthos (MPB) of Narrows (dark) and Charter's Creek (light). Shaded area indicates the 2.5 % value above which terrestrial inputs are considered significant. ND = No data.

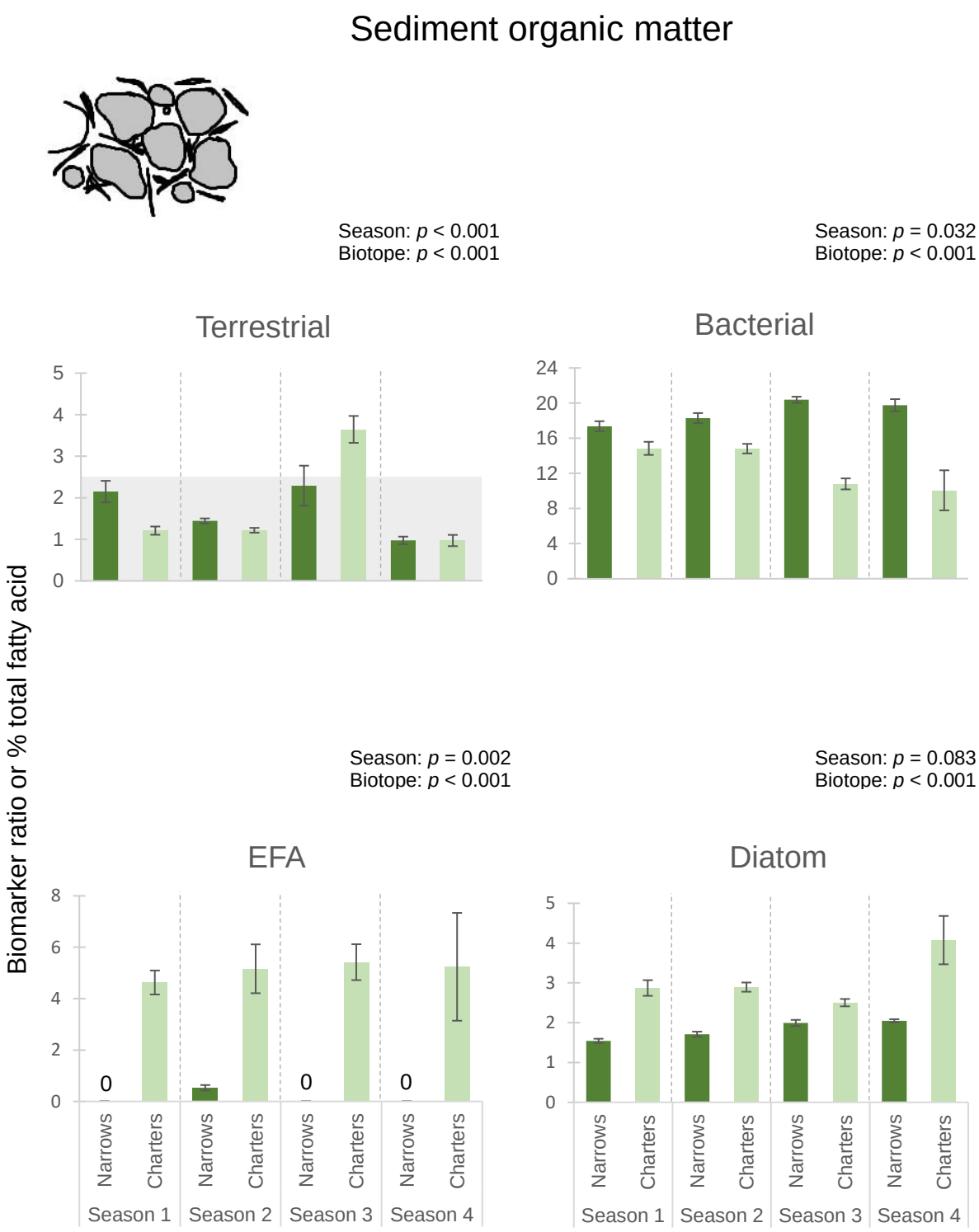


Figure 6.11: Mean biomarker values (± 1 SE) for sediment organic matter (SOM) of Narrows (green) and Charter's Creek (blue). Shaded area indicates the 2.5 % value above which terrestrial inputs are considered significant.

All four biomarkers for particulate organic matter (POM) had significant seasonal and biotope variations (Table 6.4, Fig. 6.12, Nested ANOVA; season: $p \leq 0.017$ and biotope: $p < 0.001$). For POM, Terrestrial biomarker values displayed the hypothesized trend in all four seasons, bacterial biomarkers in two of the three seasons for which data was available and diatom markers in three of the four seasons (Fig. 6.12). The essential fatty acid biomarker values were inconsistent for MPB, SOM and POM.

For primary consumers, all four zooplankton biomarkers had statistically significant seasonal and biotope variations (Table 6.5, Fig. 6.13, Nested ANOVA; season: $p < 0.001$ and biotope: $p < 0.001$, for all). Zooplankton biomarker values showed the hypothesized trends in two out of four seasons for both terrestrial and diatom biomarkers, and in three out of four seasons for the bacterial biomarker. For the amphipod, *Grandidierella bonnieroides*, seasonal variations were non-significant for terrestrial, bacterial and EFA biomarkers (Table 6.5, Fig. 6.14, Nested ANOVA; season: $p = 0.054$, $p = 0.063$ and $p = 0.070$, respectively), the diatom marker was significantly different between seasons ($p < 0.001$), and all four biomarkers showed significant biotope differences (Table 6.5, Fig. 6.14, Nested ANOVA; Biotope: $p < 0.5$). *G. bonnieroides* biomarker values displayed the hypothesized trends in all three of the seasons with data available for the terrestrial biomarker and in two out of 3 seasons for both bacterial and diatom markers. The isopod *Cyathura estuaria* had non-significant seasonal and biotope variations for all four biomarkers (Table 6.5, Fig. 6.15, Nested ANOVA; season: $p > 0.1$ and biotope: $p > 0.1$). Despite the lack of statistically significant variations between biotopes, potentially due to low sample numbers, patterns for terrestrial and diatom biomarkers conform to the hypothesized trends in two of the two seasons with data available, while this was true in only one season for the bacterial biomarker values.

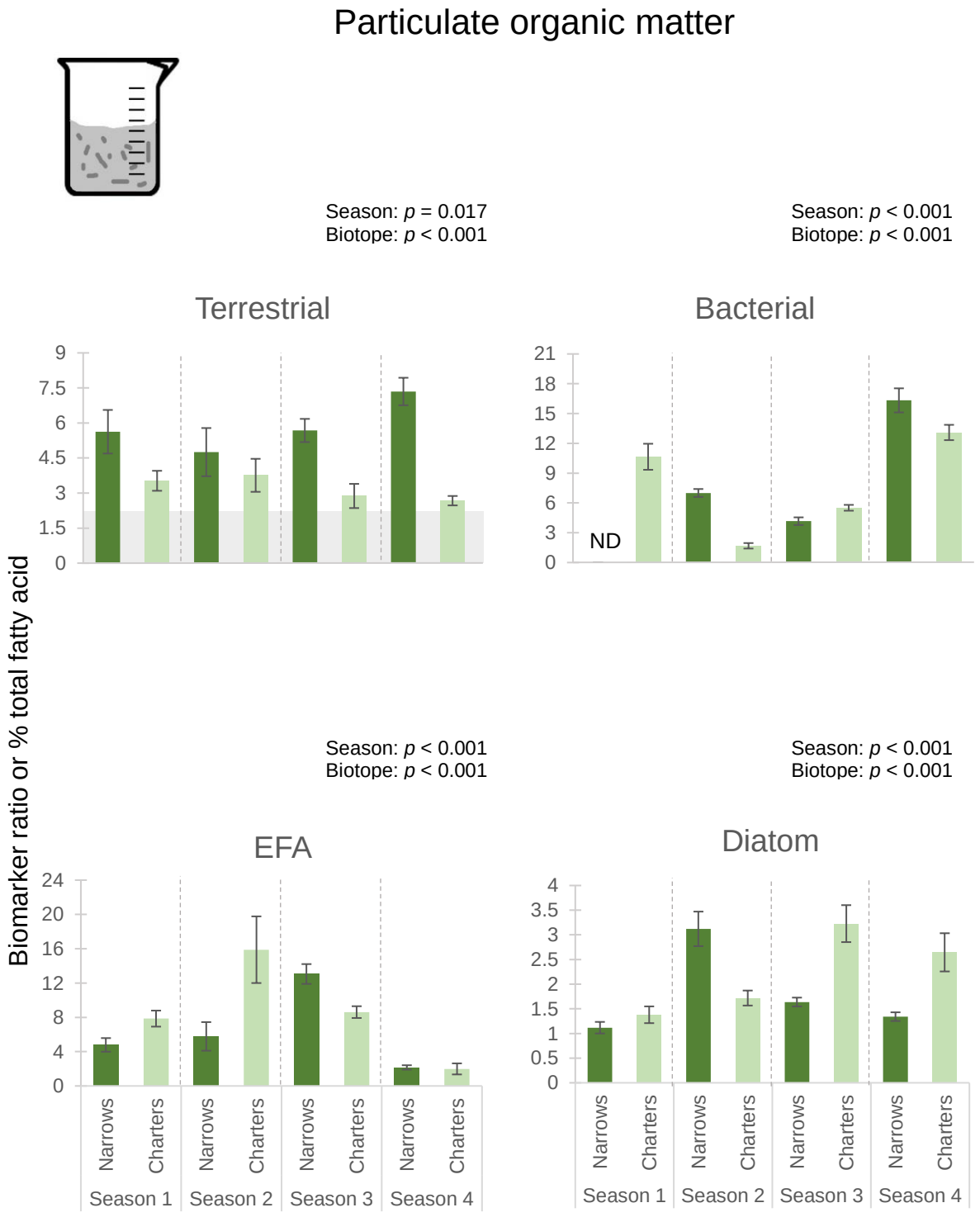
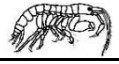
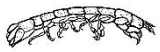


Figure 6.12: Mean biomarker values (± 1 SE) for particulate organic matter (POM) of Narrows (dark green) and Charter's Creek (light green). Shaded area indicates the 2.5 % value above which terrestrial inputs are considered significant

Table 6.5: Results of Nested ANOVA showing comparisons for biomarkers of food web primary consumers between seasons, biotopes and sites. Significant values shown in bold. *F* = F-statistic, *p* = significance, DF = degrees of freedom.

Primary consumer Species	Biomarker	Nested ANOVA Primary producers								
		Season			Biotope			Site		
		F	DF	*p*	*F*	DF	*p*	*F*	DF	*p*
Zooplankton 	Terrestrial	313.69	3,22	**<0.001**	191.60	4,22	**<0.001**	4.161	14,22	**0.002**
	Bacterial	77.41	3,22	**<0.001**	347.37	4,22	**<0.001**	8.926	14,22	**<0.001**
	EFA	28.86	3,22	**<0.001**	17.93	4,22	**<0.001**	1.104	14,22	0.408
	Diatom	99.92	3,22	**<0.001**	61.20	4,22	**<0.001**	4.442	14,22	**0.001**
Amphipod *Grandidierella bonnieroides* 	Terrestrial	3.98	2,10	0.054	69.22	3,10	**<0.001**	7.237	10,10	**0.002**
	Bacterial	3.71	2,10	0.063	76.66	3,10	**<0.001**	2.516	10,10	0.081
	EFA	3.52	2,10	0.070	6.02	3,10	**0.013**	1.353	10,10	0.321
	Diatom	26.37	2,10	**<0.001**	17.49	3,10	**<0.001**	1.181	10,10	0.399
Isopod *Cyathura estuaria* 	Terrestrial	1.30	1,1	0.458	18.25	2,1	0.163	0.585	4,1	0.739
	Bacterial	0.16	1,1	0.760	25.01	2,1	0.140	6.554	4,1	0.284
	EFA	10.02	1,1	0.195	3.13	2,1	0.371	1.113	4,1	0.603
	Diatom	0.64	1,1	0.571	5.55	2,1	0.287	0.294	4,1	0.861

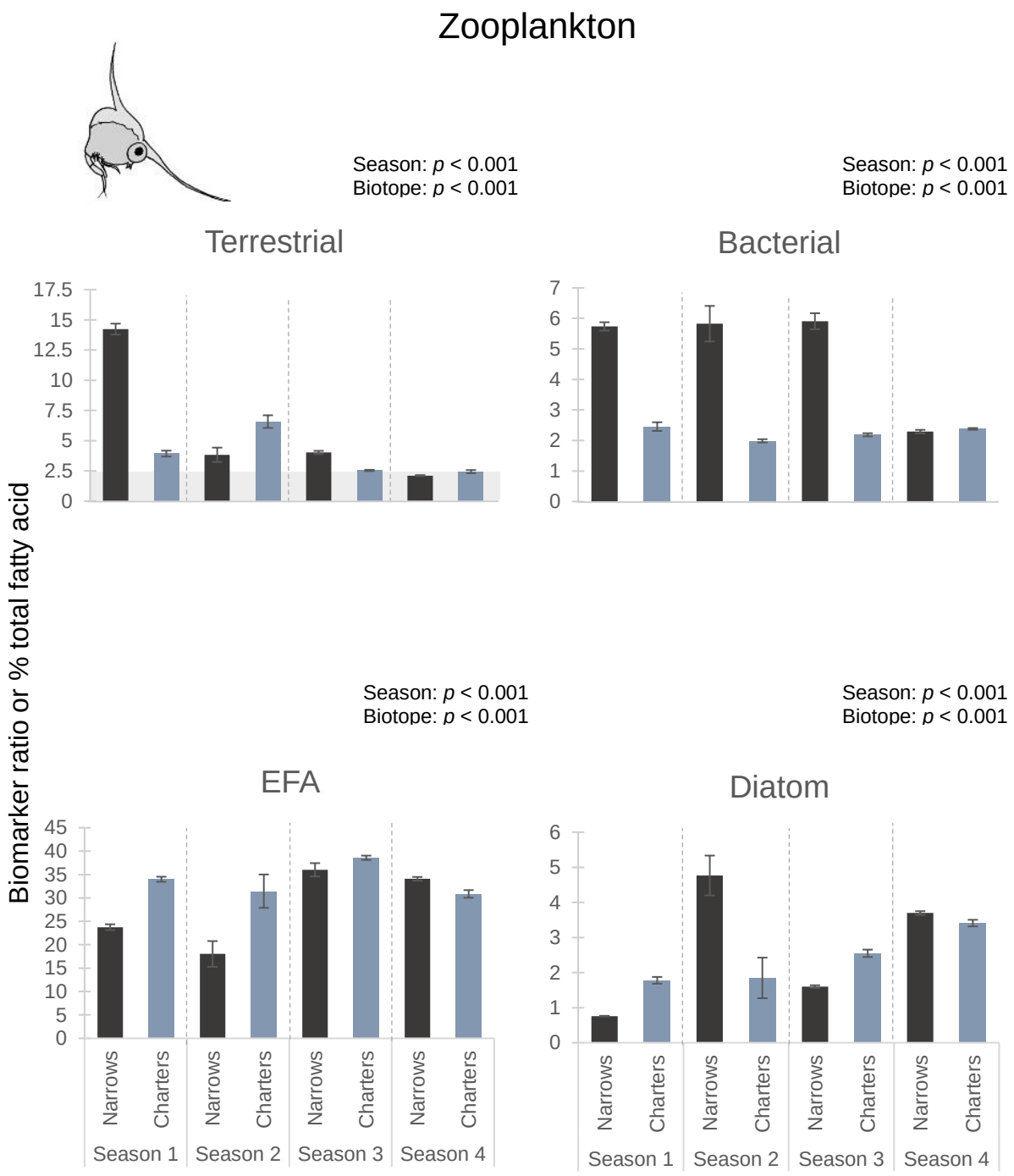


Figure 6.13: Mean biomarker values (± 1 SE) for zooplankton at the Narrows (black) and Charter's Creek (grey). Shaded area indicates the 2.5 % value above which terrestrial inputs are considered significant.

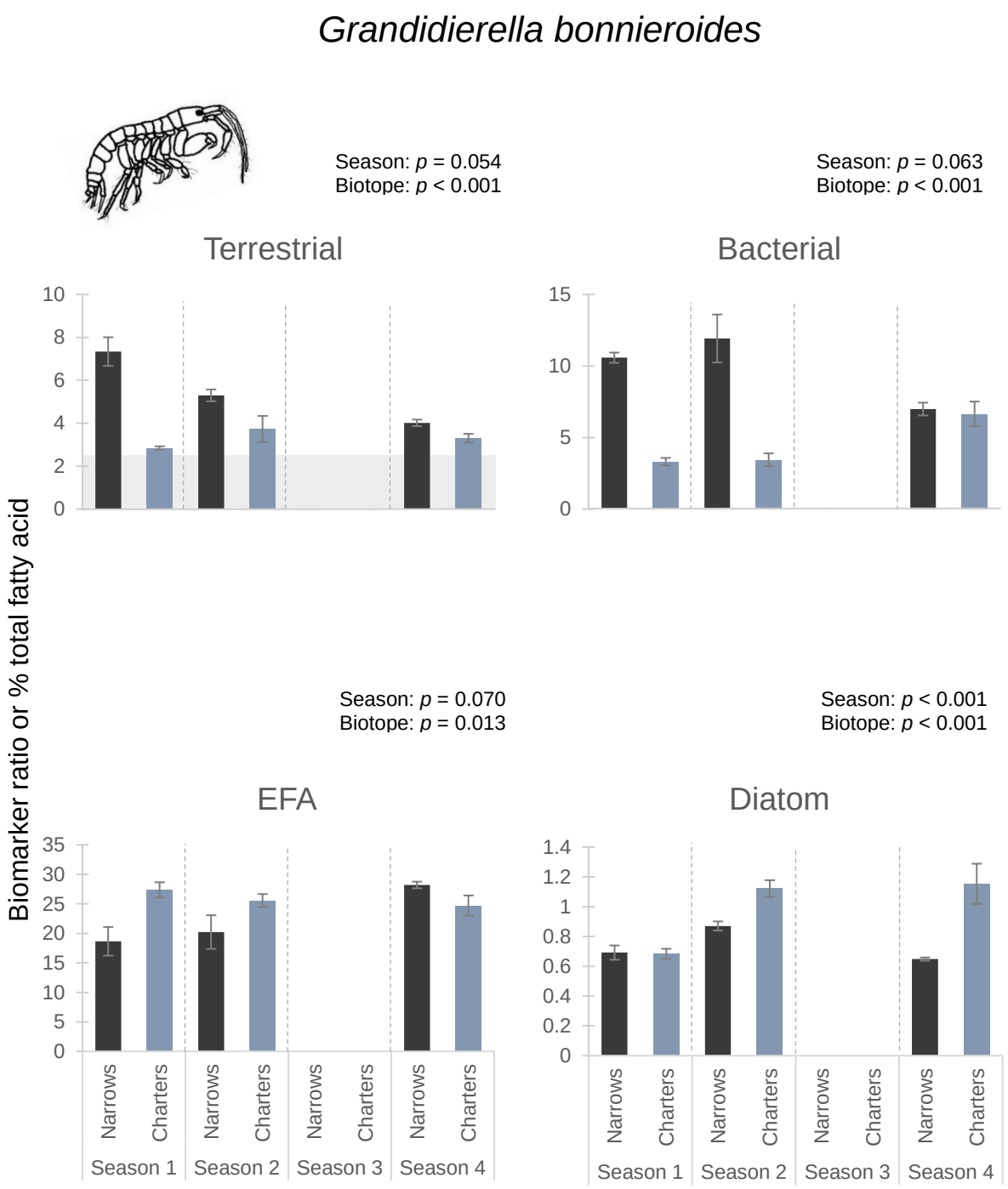


Figure 6.14: Mean biomarker values (± 1 SE) for amphipod, *Grandidierella bonnieroides*, at the Narrows (black) and Charter's Creek (grey). Shaded area indicates the 2.5 % value above which terrestrial inputs are considered significant.

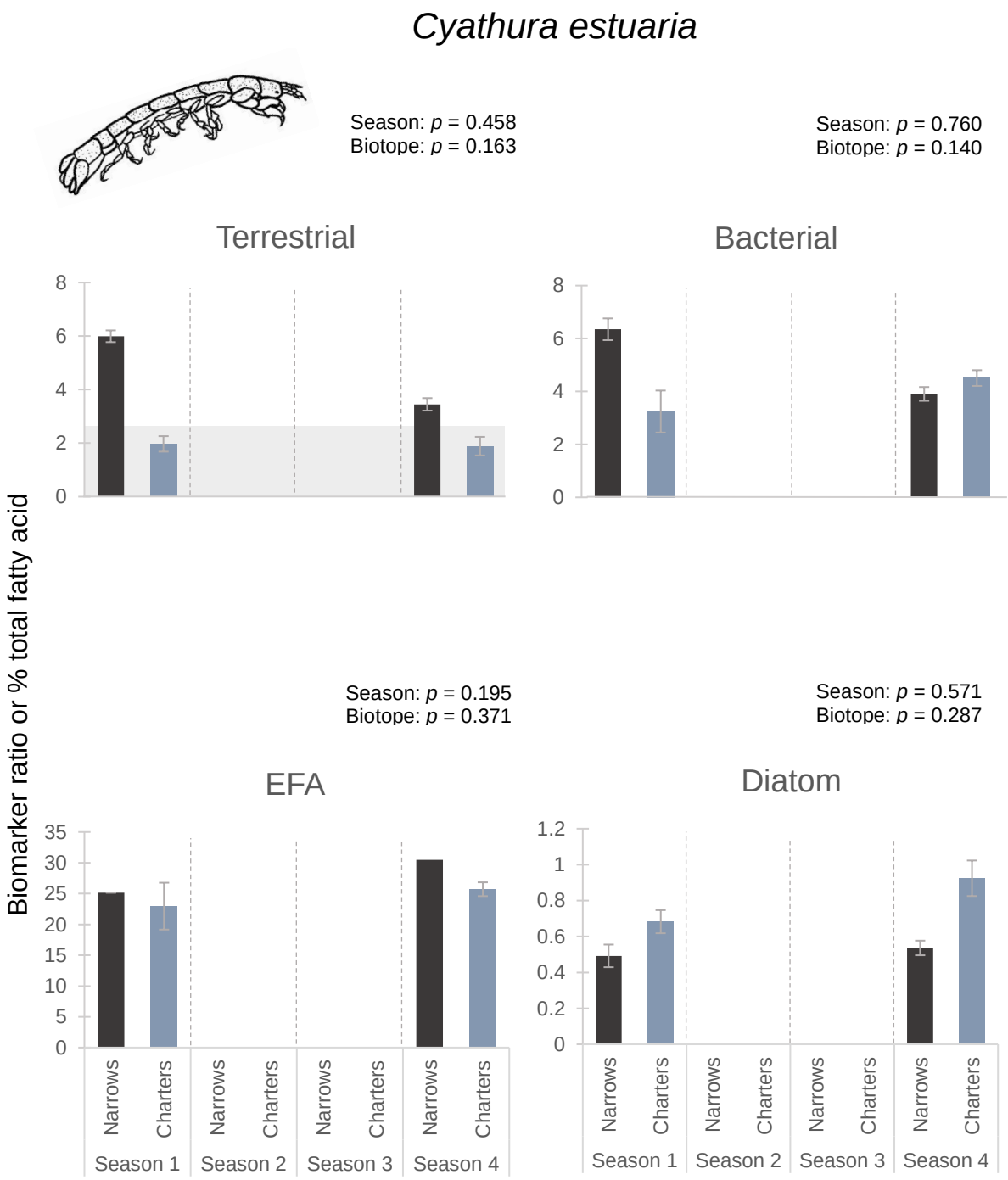


Figure 6.15: Mean biomarker values (± 1 SE) for the isopod, *Cyathura estuaria*, at the Narrows (black) and Charter's Creek (grey). Shaded area indicates the 2.5 % value above which terrestrial inputs are considered significant.

For fish pooled from all size classes, seasonal biomarker variations were significant for six out of 12 comparisons (Table 6.6, Fig. 6.16 – 6.18, *Oreochromis mossambicus*: bacterial and EFA markers; *Chelon (=Liza) dumerili:* terrestrial, bacterial and diatom markers and *Ambassis ambassis:* bacterial marker, season $p < 0.05$ for all). All four biomarkers for *O. mossambicus* and *C. dumerili* had statistically significant biotope variation (Table 6.6, Fig. 6.16 – 6.18, biotope $p \leq 0.05$ for all), while *A. ambassis* had significant biotope differences for terrestrial and bacterial markers (Table 6.6, Fig. 6.16 – 6.18, biotope $p = 0.019$ and $p = 0.001$). The biomarkers for the pooled size classes of all three fish species showed greater support of the proposed hypotheses than those of basal resources and primary consumers. In *O. mossambicus* (Fig. 6.16), terrestrial and bacterial biomarkers supported the hypotheses in all four seasons, while diatom marker values displayed the hypothesized trend in three of the four seasons. For *C. dumerili* (Fig. 6.17), the predicted pattern of higher terrestrial and bacterial marker values and lower diatom markers values in the Narrows relative to Charter's Creek was seen in all three seasons with available data, although the difference between biotopes was not always large. Similarly, terrestrial, bacterial and diatom biomarkers for *A. ambassis* (Fig. 6.18) showed patterns in support of the hypotheses for all available seasonal data.

In terms of fish within separate size classes, the patterns obtained were not as clear, mainly due to lower sample sizes and hence higher variability. For fish size classes, seasonal biomarker values were significantly different for 11 out of 16 comparisons (Table 6.7, Fig. 6.19 – 6.21, season $p < 0.05$). Eight out of 28 biomarker comparisons showed significant biotope variation (Table 6.7, Fig. 6.19 – 6.21, biotope $p < 0.05$). Terrestrial biomarker values met the hypothesized trend of higher values in the Narrows compared to Charter's Creek in five out of nine comparisons for *O. mossambicus* (Fig. 6.19), one out of four comparisons for *C. dumerili* (Fig. 6.20) and two out of three for *A. ambassis* (Fig. 6.21). Bacterial biomarkers

showed more support of the hypothesis, being higher in the Narrows compared to Charter's Creek in seven out of nine comparisons for *O. mossambicus*, three out of four comparisons for *C. dumerili* and all available comparisons for *A. ambassis.* Diatom marker values for *O. mossambicus* were very similar in all available comparisons, however the hypothesis, of lower diatom marker values in the Narrows relative to Charter's Creek was supported in three out of four comparisons for *C. dumerili* and one out of three comparisons for *A. ambassis.*

Table 6.6: Results of Nested ANOVA showing comparisons for biomarkers of pooled fish sizes between seasons, biotopes and sites. Significant values shown in bold. *F* = F-statistic, *p* = significance, DF = degrees of freedom.

Fish species	Biomarker	Nested ANOVA All Fish								
		Season			Biotope			Site		
		F	DF	*p*	*F*	DF	*p*	*F*	DF	*p*
All tilapia *Oreochromis mossambicus* 	Terrestrial	2.23	3,133	0.087	9.54	4,133	**<0.001**	6.259	14,133	**<0.001**
	Bacterial	10.50	3,133	**<0.001**	12.00	4,133	**<0.001**	1.759	14,133	0.051
	EFA	21.47	3,133	**<0.001**	13.31	4,133	**<0.001**	5.427	14,133	**<0.001**
	Diatom	1.86	3,133	0.140	5.53	4,133	**<0.001**	1.296	14,133	0.218
All mullet *Chelon (=Liza) dumerili* 	Terrestrial	6.75	2,49	**0.003**	3.82	3,49	**0.015**	7.183	11,49	**<0.001**
	Bacterial	13.75	2,49	**<0.001**	59.71	3,49	**<0.001**	3.162	11,49	**0.003**
	EFA	1.02	2,49	0.369	3.41	3,49	**0.025**	1.29	11,49	0.258
	Diatom	16.62	2,49	**<0.001**	8.84	3,49	**<0.001**	1.259	11,49	0.276
All glassy *Ambassis ambassis* 	Terrestrial	0.30	1,52	0.584	4.29	2,52	**0.019**	6.601	7,52	**<0.001**
	Bacterial	46.12	1,52	**<0.001**	7.85	2,52	**0.001**	0.939	7,52	0.485
	EFA	0.03	1,52	0.858	0.36	2,52	0.697	1.145	7,52	0.351
	Diatom	2.53	1,52	0.118	2.78	2,52	0.071	2.912	7,52	**0.012**

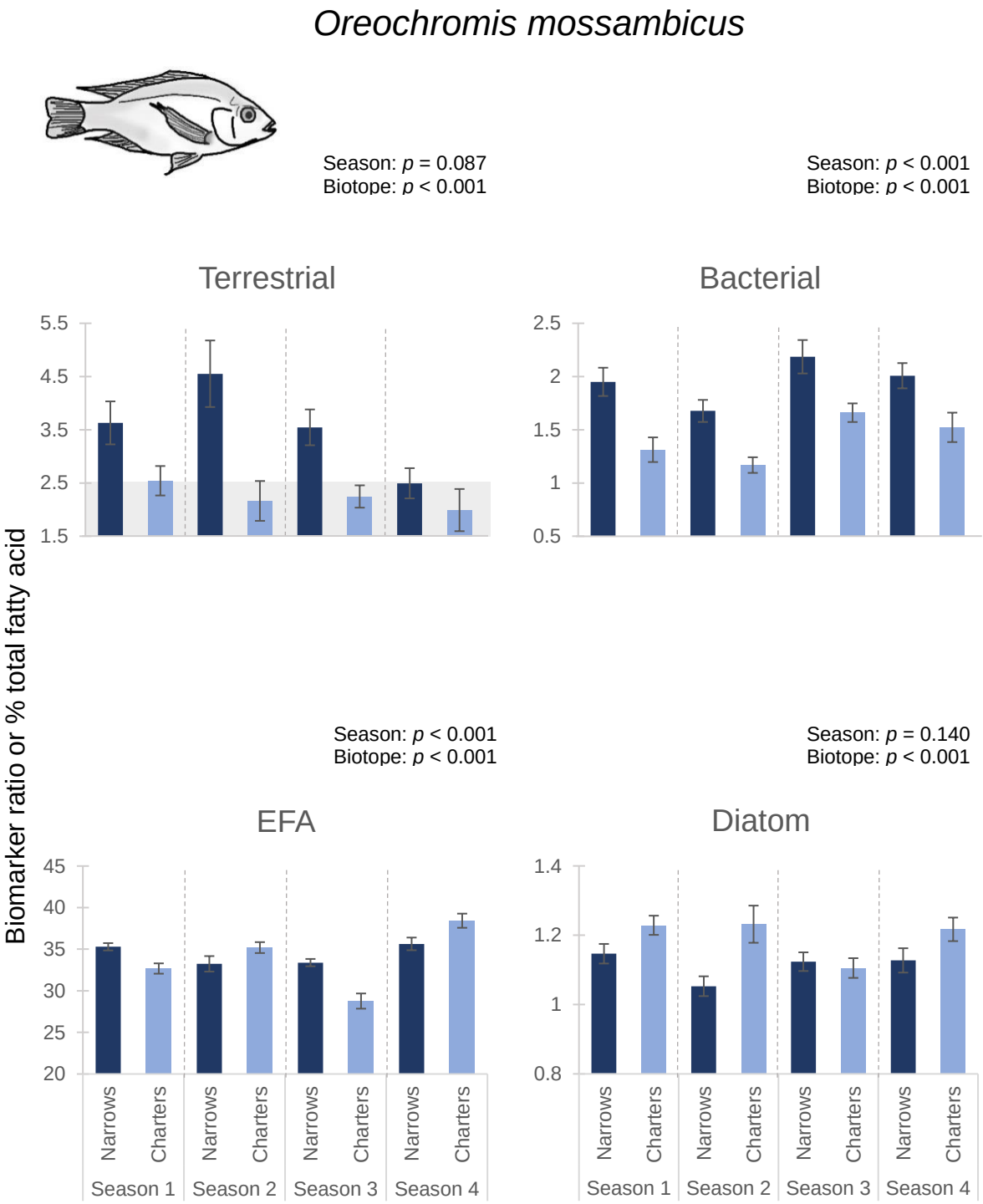


Figure 6.16: Mean biomarker values (± 1 SE) for all size classes of tilapia, *Oreochromis mossambicus*, Narrows (navy) and Charter's Creek (blue). Shaded area indicates the 2.5 % value above which terrestrial inputs are considered significant.

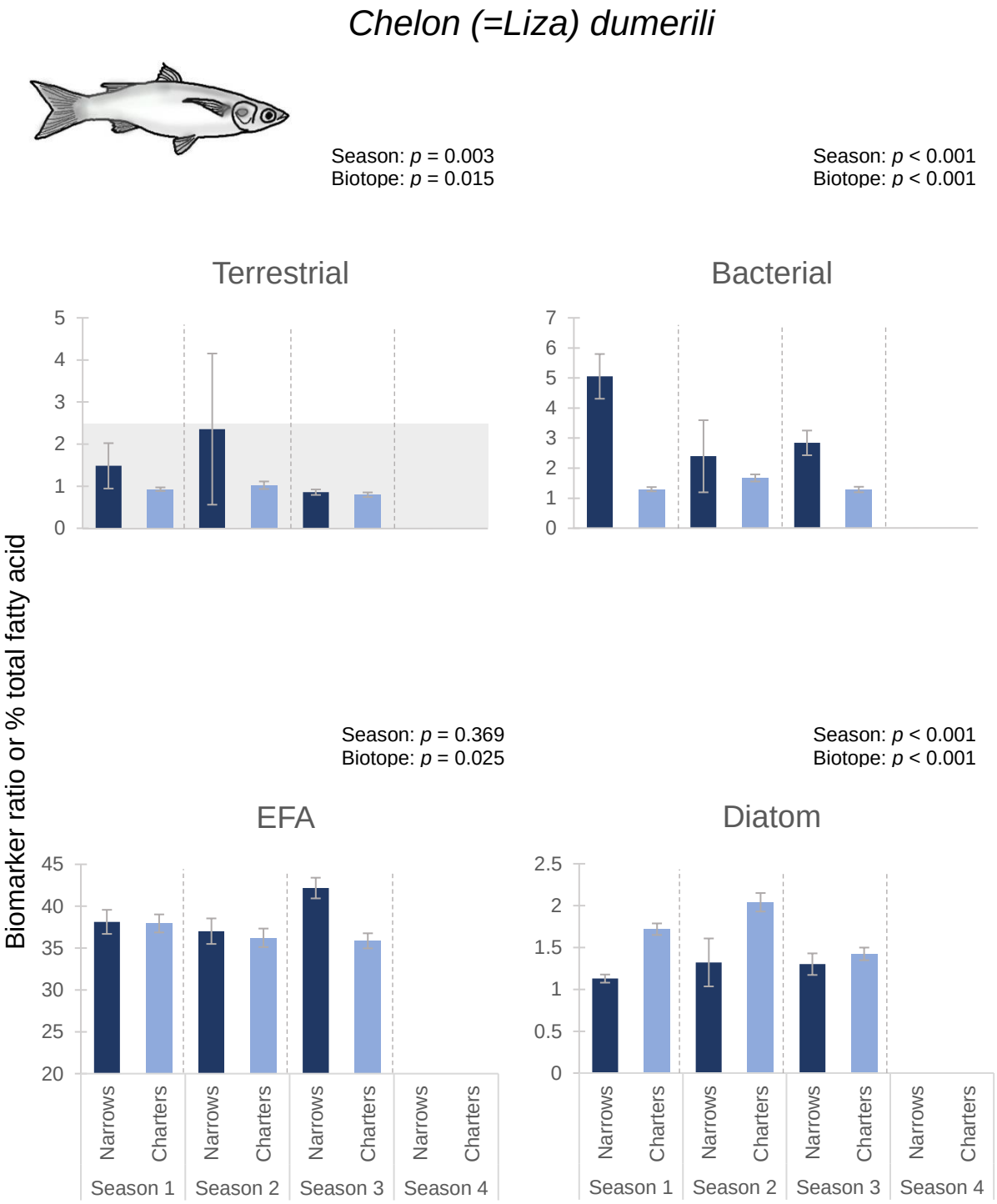


Figure 6.17: Mean biomarker values (± 1 SE) for mullet, *Chelon (=Liza) dumerili*, at the Narrows (navy) and Charter's Creek (blue. Shaded area indicates the 2.5 % value above which terrestrial inputs are considered significant.

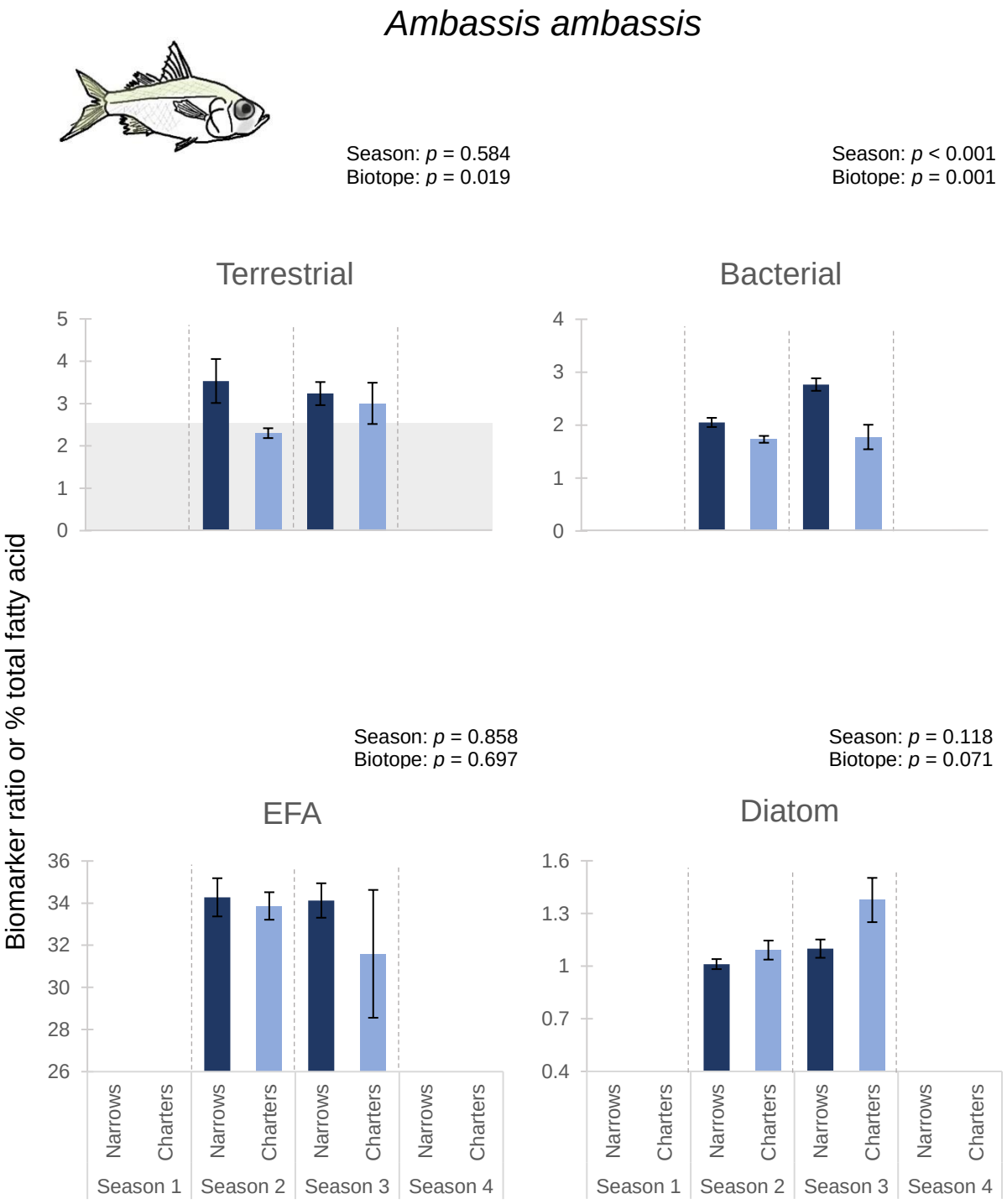


Figure 6.18: Mean biomarker values (± 1 SE) for glassy, *Ambassis ambassis*, at the Narrows (navy) and Charter's Creek (blue. Shaded area indicates the 2.5 % value above which terrestrial inputs are considered significant.

Table 6.7: Results of Nested ANOVA showing comparisons for biomarkers of fish size classes between seasons, biotopes and sites. Significant values shown in bold. *F* = F-statistic, *p* = significance, DF = degrees of freedom.

Fish species size classes	Biomarker	Season			Biotope			Site		
		F	DF	*p*	*F*	DF	*p*	*F*	DF	*p*
Tilapia 1	Terrestrial	10.45	3,13	**<0.001**	1.26	4,13	0.334	1.075	6,13	0.425
Oreochromis	Bacterial	3.78	3,13	**0.038**	1.51	4,13	0.257	0.624	6,13	0.709
mossambicus	EFA	17.64	3,13	**<0.001**	6.04	4,13	**0.006**	1.231	6,13	0.352
12.1-16.0 cm	Diatom	3.42	3,13	**0.050**	0.23	4,13	0.916	3.375	6,13	**0.031**
Tilapia 2	Terrestrial	9.09	3,33	**<0.001**	9.83	4,33	**<0.001**	3.403	10,33	**0.004**
Oreochromis	Bacterial	7.63	3,33	**<0.001**	3.51	4,33	**0.017**	1.337	10,33	0.252
mossambicus	EFA	4.30	3,33	**0.012**	1.69	4,33	0.175	1.932	10,33	0.076
16.1-20.0 cm	Diatom	1.42	3,33	0.256	2.44	4,33	0.066	0.933	10,33	0.516
Tilapia 3	Terrestrial				3.07	1,1	0.330	7.096	2,1	0.257
Oreochromis	Bacterial				25.98	1,1	0.123	0.458	2,1	0.722
mossambicus	EFA				0.09	1,1	0.815	0.219	2,1	0.834
20.1-24.0 cm	Diatom				0.34	1,1	0.664	2.057	2,1	0.442
Mullet 1	Terrestrial	9.31	2,24	**0.001**	14.92	3,24	**<0.001**	5.89	8,24	**<0.001**
Chelon (=Liza) dumerili	Bacterial	12.22	2,24	**<0.001**	25.07	3,24	**<0.001**	2.399	8,24	**0.047**
	EFA	0.24	2,24	0.788	0.29	3,24	0.835	1.326	8,24	0.278
16.1 - 19.0 cm	Diatom	7.82	2,24	**0.002**	4.72	3,24	**0.010**	1.239	8,24	0.320
Mullet 2	Terrestrial				0.76	1,1	0.544	9.963	3,1	0.228
Chelon (=Liza) dumerili	Bacterial				4.72	1,1	0.275	1.752	3,1	0.495
	EFA				162.25	1,1	**0.050**	14.811	3,1	0.188
19.1 - 21.0 cm	Diatom				118.77	1,1	0.058	31.162	3,1	0.131
Glassy 1	Terrestrial	0.19	1,25	0.669	0.57	2,25	0.575	5.581	5,25	**0.001**
Ambassis ambassis	Bacterial	26.71	1,25	**<0.001**	3.44	2,25	**0.048**	0.352	5,25	0.876
	EFA	0.09	1,25	0.773	0.76	2,25	0.478	1.000	5,25	0.438
7.1 - 9.0 cm	Diatom	4.08	1,25	0.054	1.95	2,25	0.163	0.22	5,25	0.949
Glassy 2	Terrestrial				0.11	1,9	0.748	26.33	3,9	**<0.001**
Ambassis ambassis	Bacterial				1.86	1,9	0.206	0.622	3,9	0.618
	EFA				0.53	1,9	0.487	0.384	3,9	0.767
9.1 - 11.0 cm	Diatom				0.82	1,9	0.388	0.389	3,9	0.764

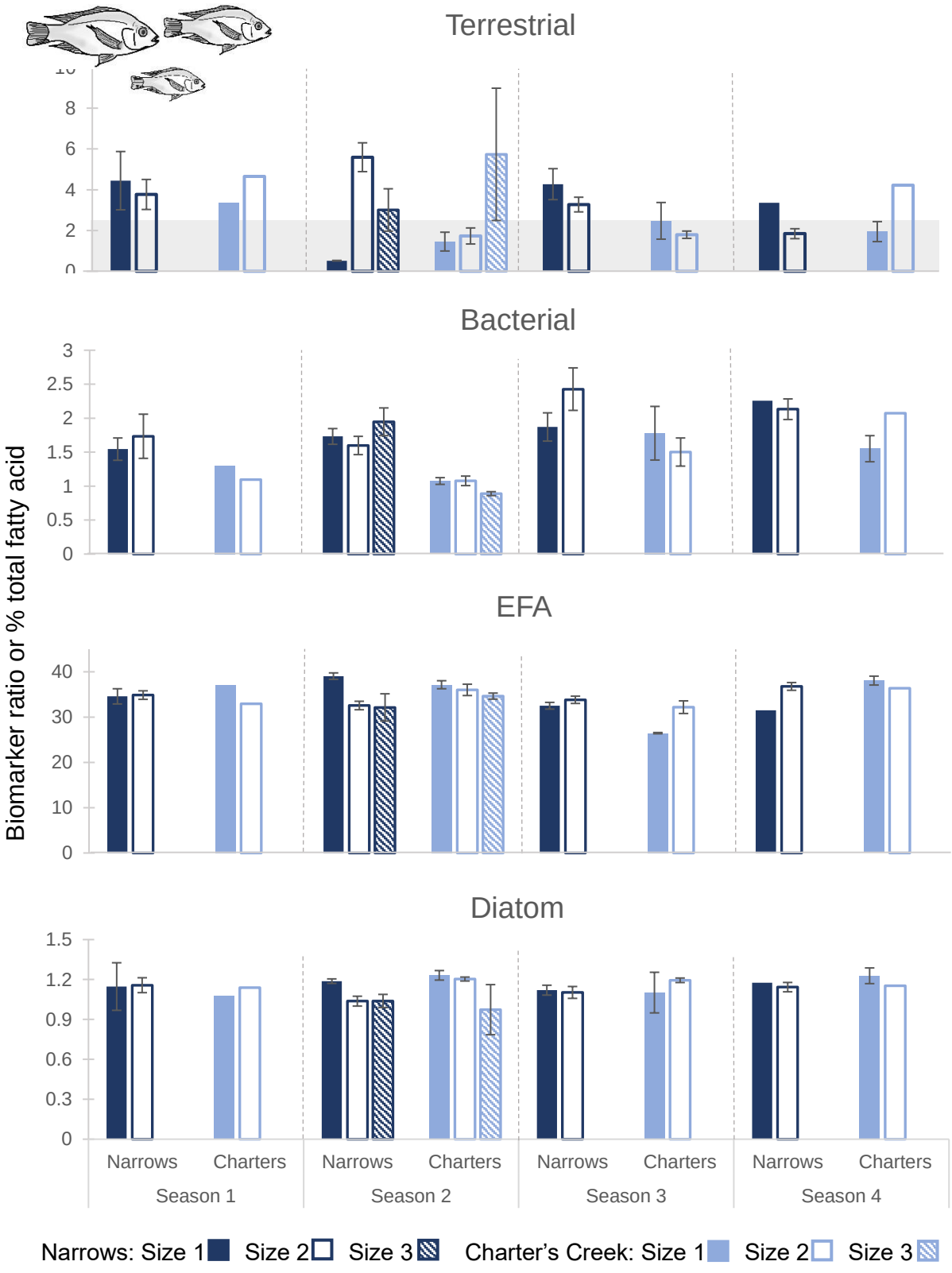


Figure 6.19: Mean biomarker values (± 1 SE) for three size classes of tilapia, *Oreochromis mossambicus*, of Narrows (navy) and Charter's Creek (blue). Shaded area indicates the 2.5 % value above which terrestrial inputs are considered significant.

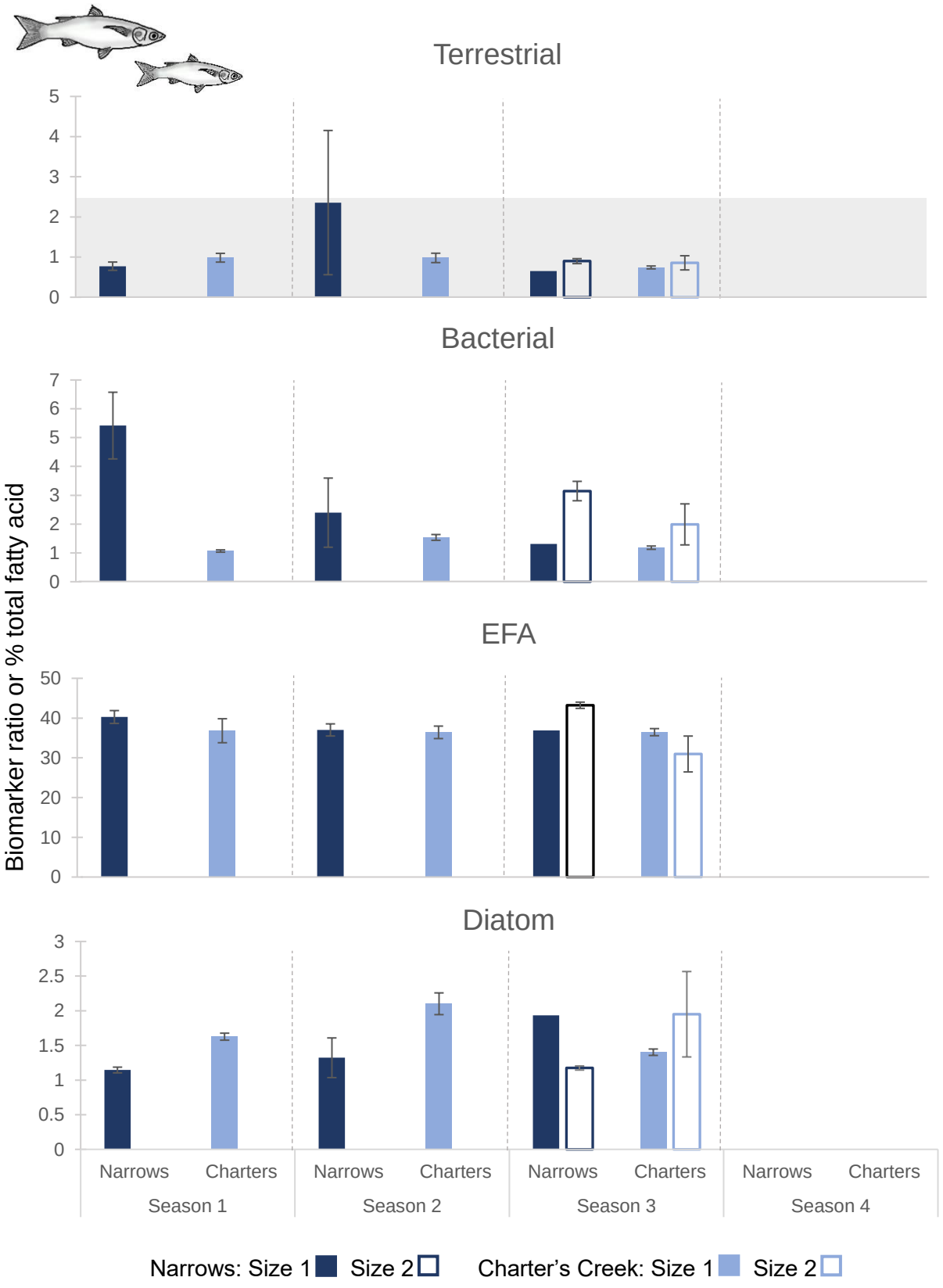


Figure 6.20: Mean biomarker values (± 1 SE) for two size classes of mullet, *Chelon (=Liza) dumerili*, at the Narrows (navy) and Charter's Creek (blue). Shaded area indicates the 2.5 % value above which terrestrial inputs are considered significant.

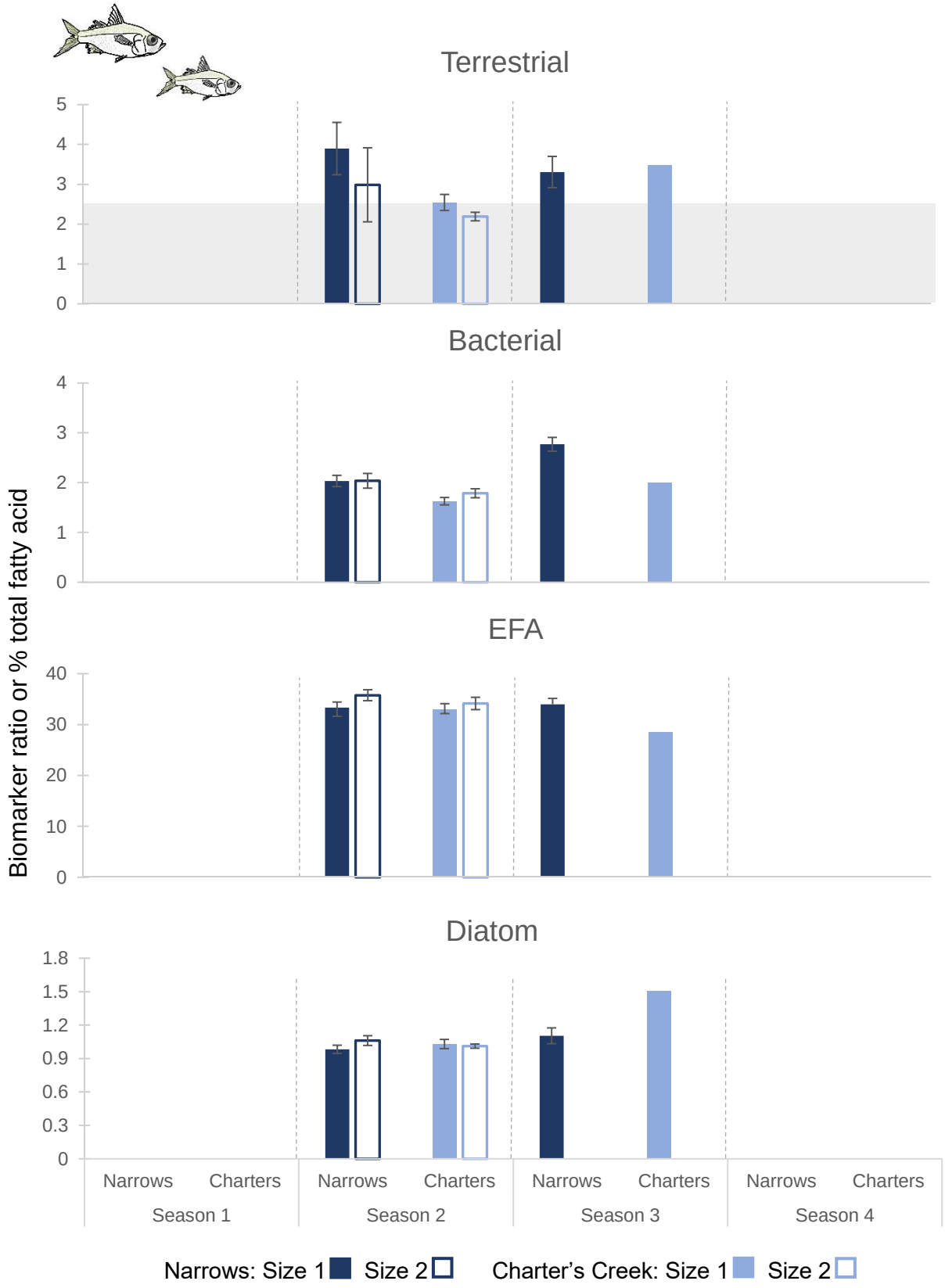


Figure 6.21: Mean biomarker values (± 1 SE) for two size classes of glassy, *Ambassis ambassis*, at the Narrows (navy) and Charter's Creek (blue). Shaded area indicates the 2.5 % value above which terrestrial inputs are considered significant.

Fatty acid biomarkers for the crocodiles sampled within the St Lucia Estuary compared to samples collected outside the estuary where only significantly different for the terrestrial biomarker (Fig. 6.22, t-test, p = 0.044,), with terrestrial values higher for samples collected within the Estuary. Bacterial, EFA and diatom markers were not significantly different between habitats (Fig. 6.22, t-test, p = 0.436, p = 0.209, p =0.319, respectively).

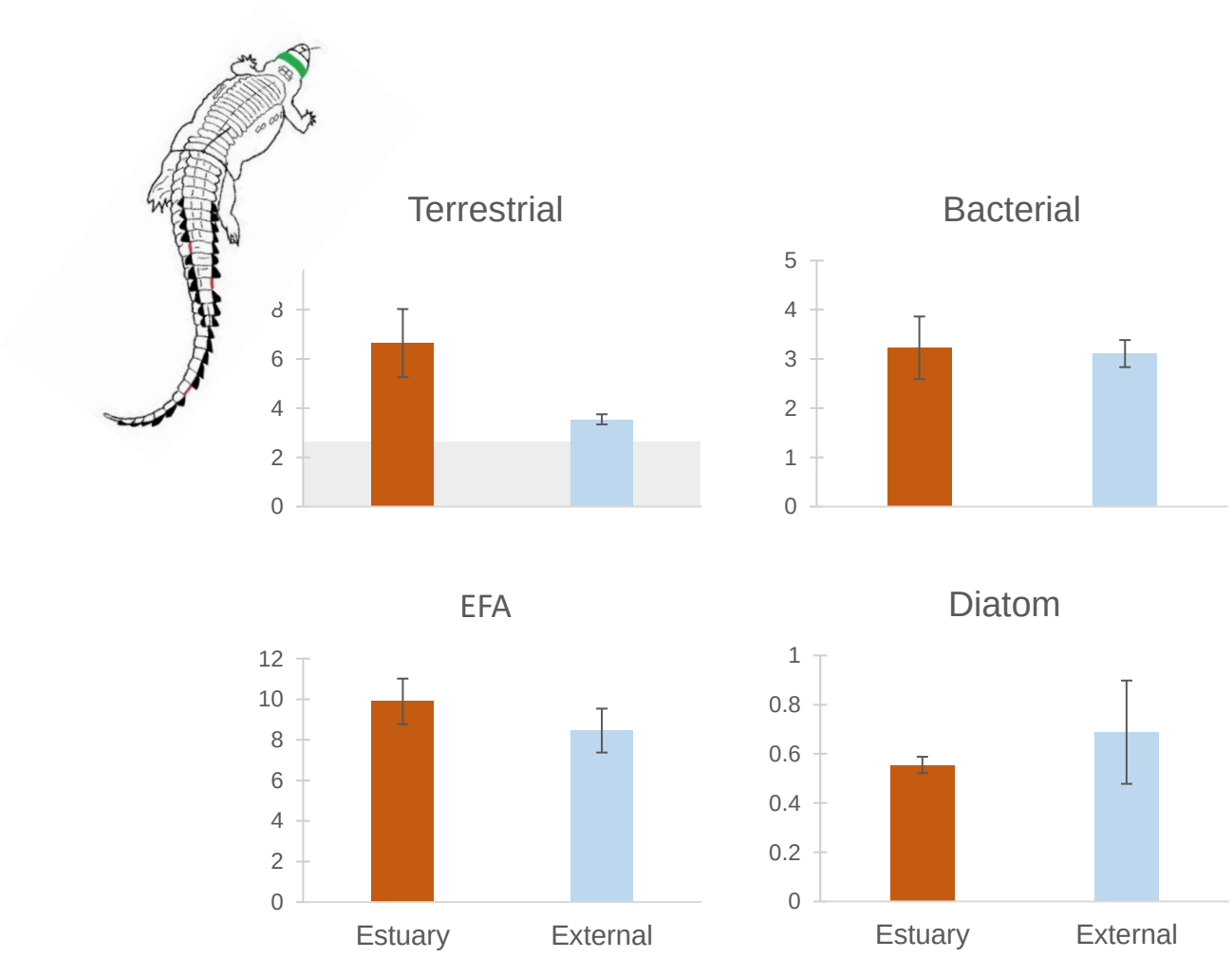


Figure 6.22: Mean biomarker values (± 1 SE) for the Nile crocodile, *Crocodylus niloticus*, sampled in the St Lucia Estuary, a system containing large amounts of hippo dung (brown) and outside the estuary, areas containing low amounts or no hippo dung (blue). Shaded area indicates the 2.5 % value above which terrestrial inputs are considered significant.

6.3 Discussion

This chapter provides novel analyses of fatty acid data that could shed new light on the potential influence of hippo-mediated terrestrial transfers (in the form of dung) for consumers and trophic interactions in the St Lucia Estuary. As was evident in stable isotope bi-plots in Chapter 5, ordination techniques indicated a distinct separation of fatty acid profiles of consumers and basal resources between the Narrows and Charter's Creek, which respectively have high and low hippo densities, and hence theoretically contrasting dung loading. This trend supports the hypothesis posed that profiles of food web components between the Narrows and Charter's Creek would differ. Fatty acid biomarker analyses also suggested that hippo dung inputs may influence food webs by decreasing and increasing the contribution of diatoms and bacteria respectively to consumer diets.

6.3.1 Fatty acid profiles

Previous studies have successfully utilised variability in the fatty acid profiles of food web components to infer food web linkages and trophic relationships within aquatic and terrestrial ecosystems (Koussoroplis et al. 2008, Lam et al. 2013, Moyo et al. 2017). Results of this chapter indicate that fatty acid (FA) profiles for aquatic basal resources were spatially distinct. This, together with the finding that the hippo dung FA profile differed significantly from that of basal aquatic resources within each biotope (Fig. 6.1), allowed for comparisons of consumer FA profiles to be made with those of specific basal resources, effectively allowing for consumer diets to be traced and the relative role of hippo dung to be assessed. The distinction between FA profiles of hippo dung and other basal resources is likely driven by the higher levels of terrestrial FAs (18:2ω6 and 18:3ω3, Fig. 6.8) and low proportion of essential fatty acids (EFA) in hippo dung. These patterns are expected, given that terrestrial carbon

resources are known to be of lower quality than aquatic ones (Jones et al. 2012), and therefore typically have less EFAs as a percentage of total FA.

Fatty acid profiles of consumers common to the Narrows and Charter's Creek were distinct between these biotopes regardless of the season. This pattern was visually evident for all primary consumers (zooplankton, *Grandidierella bonnieroides* and *Cyathura estuaria*) and fish (*Oreochromis mossambicus, Chelon (=Liza) dumerili* and *Ambassis ambassis*), and was generally statistically supported, except in cases where samples numbers were very low. This finding offers circumstantial evidence that the diets of consumers differ between areas with contrasting levels of dung. The split in FA profiles between biotopes for the majority of consumers may be due, to some degree, to contrasting hippo dung inputs. Statistical support for the above emanates from results of SIMPER analysis, which show that food web components within the Narrows were more similar to dung samples than those from Charter's Creek.

Hughes et al. (2005) similarly used multivariate analysis of fatty acid profiles to show that the diets of sea urchins from different sites and depths differed. While FA profiles have been used as dietary indicators in marine benthic food webs (see review by Kelly & Scheibling 2012), they have rarely been used to examine estuarine food web ecology (Richoux & Froneman 2008). The apparent shift in consumer FA profiles recorded in the current study between biotopes with contrasting dung inputs is consistent with a prior study which showed shifts in consumer isotopic composition between riverine sites with and without hippo dung inputs (Masese et al. 2018). In addition, other stable isotope studies have reported that transfers of terrestrially derived organic matter by animals such as geese (Kitchell et al. 1999) and hippos (Subalusky et al. 2018) can alter the composition of consumer tissues, which is suggestive of influences on consumers diets directly or indirectly.

6.3.2 Fatty acid biomarkers in basal resources

Assessing trends in specific fatty acid biomarkers can shed light on the possible causes of the shift in FA profiles recorded between the Narrows and Charter's Creek and therefore, the potential for hippo dung to influence the food web. The first major trend that emerged for basal resources was that there was no clear pattern in the four biomarkers investigated for microphytobenthic samples. This finding is somewhat expected, given that microphytobenthic organisms are photoautotrophs, and thus do not consume resources for metabolic purposes. Therefore, it is not entirely surprising that the fatty acid profiles of these groups did not conform to the hypotheses posed.

With regards to sediment organic matter (SOM), it would have been expected that samples from the Narrows would have had a significant terrestrial signature (i.e. > 2.5 % of total fatty acids, Dalsgaard et al. 2003) but, this was not the case. In addition, terrestrial biomarker values for SOM did not always support the hypothesised trend of higher values in the Narrows relative to Charter's Creek. However, these results contrast with those obtained for particulate organic matter (POM), in which terrestrial biomarker values supported the hypothesis of higher terrestrial values in the Narrows than Charter's Creek. These findings may suggest that once voided, hippo dung is not necessarily incorporated into the sediment. This could be achieved through settled dung being rapidly decomposed into smaller, lighter particles that are either entrained and/or consumed in the water column.

Bacterial biomarker values for POM did not show any strong trends between seasons or biotopes, and were on average lower than values for SOM. This finding suggests that bacteria are more abundant in sediment than in the water column, which likely reflects greater colonisation of dung settled on the benthos relative to dung particles in the water column. Bacterial biomarker values for SOM were constantly higher in the Narrows than in Charter's

Creek, although values recorded in the latter biotope were relatively high when compared to other studies (Budge & Parrish 1998, Meziane & Tsuchiya 2000, Budge et al. 2001, Kharlamenko et al. 2001). SOM is generally known to have high bacterial values however, levels recorded in the Narrows were almost two-fold higher than those reported in sediments from Northern hemisphere lakes (Budge & Parrish 1998, Budge et al. 2001), higher than intertidal sandflats in Southern Japan (Meziane & Tsuchiya 2000) and similar to, although still higher than sediments from a detritus rich inlet in the Sea of Japan (Kharlamenko et al. 2001).

Results from this study show that recently voided hippo dung has a strong bacterial FA signature (Fig. 6.8), probably due to colonisation of voided material by gut bacteria. In addition, post voiding colonisation by free-living bacteria may increase bacterial loads on dung, based on studies elsewhere reporting increased bacterial colonisation rates associated with plant and animal detritus (Benner & Hodson 1985, Jones 1992, Mudge et al. 1998). Therefore, it is likely that inputs of hippo dung increase the abundance of bacteria/microbes both directly, via the addition of gut bacteria, and indirectly by the addition of an organic substrate/trophic resource for free-living bacteria.

6.3.3 Fatty acid markers in consumers

Essential fatty acids (EFA) biomarkers can shed light on the physiological condition/fitness of consumers based on the rationale that those that consume high quality trophic resources have higher quantities of EFA (Brett et al. 2006, Torres-ruiz et al. 2007, Guo et al. 2016, Moyo et al. 2017). In the current study, although the EFA biomarker values were generally different between biotopes, the responses were inconsistent, with no distinct pattern emerging. It is generally understood that primary consumers gain more essential FAs through the consumption of aquatic primary producers than terrestrial matter (Hixson et al. 2015, Guo et al. 2016). In this regard, primary consumers such as zooplankton, are known to have a high

nutritional value, attributed to a high proportion of essential fatty acids in tissue (EFA; specifically 20:5ω3 and 22:6ω3), which in turn is obtained from consumed algae and diatoms (Brett & Muller-Navarra 1997, Müller-Navarra et al. 2000, Brett et al. 2009).

In Charter's Creek microphytobenthic biomass is generally high (Pillay & Perissinotto 2008). This is likely due to the shallow nature of this part of the system but the scarcity of hippo dung in this region may also contribute to the high microphytobenthic biomass, based on results from the experiment in Chapter 3, which showed a reduction in microphytobenthic biomass by up to 70 % in the presence of hippo dung. Therefore, it would be expected that consumers from Charter's Creek would display higher quantities of EFA than those from the Narrows. Although this trend was evident in a few consumer taxa, it did not manifest consistently, therefore offering little support for the hypothesis posed of reduced EFA values in the Narrows relative to Charter's Creek. The remaining FA markers for primary consumers generally supported the hypotheses posed and displayed similar patterns to those of basal resources, with higher terrestrial and bacterial values, and lower diatom values in the Narrows relative to Charter's Creek. With the exception of the isopod *C. estuaria* in Charter's Creek, primary consumers had terrestrial biomarker values greater than 2.5 % of the total FAs, indicating that the majority of primary consumers sampled are reliant upon terrestrial trophic resources, which is likely driven by dung inputs.

Biomarker values for fish strongly supported the hypotheses posed, with increased terrestrial and bacterial markers values, and decreased diatom values in the Narrows relative to Charter's Creek. Interestingly, terrestrial values for pooled *C. dumerili* (mullet) were lower in both biotopes than values for *O. mossambicus* (tilapia) and *A. ambassis* (glassy). The work of Hall et al. (2006) may shed light on this difference. In a three trophic level feeding study involving a predatory swimming crab, an herbivorous shore crab (the primary consumer) and mangrove leaves as the primary producer, it was demonstrated that terrestrial FAs investigated

(18:2ω6 and 18:3ω3) were successfully traced within the tissues of the shore crabs. However, only 18:3ω3 could be traced in the tissues of the predatory swimming crab that fed on shore crabs. The point emerging from this study is that transfer of particular terrestrial FAs may be dependent on the ability of a consumer to assimilate those FAs into body tissue. Therefore, although a consumer may indirectly be supported by terrestrial resources, it may not be evident in FA analyses.

In the context of the present study, the lower terrestrial biomarker signature in mullet may be due to it being unable to assimilate and therefore express this biomarker. At the same time, feeding trait differences may also account for the lower terrestrial signatures recorded in mullet. Both mullet and tilapia are iliophagous fish that consume both detritus and small benthic and endobenthic animals (Blaber 2000, Cyrus 2013). Although their diets do display some overlap, tilapia are known to have a greater reliance on detritus (Blaber 2000). Given this observation, it is possible that lower terrestrial signatures in mullet indicate that they are less reliant on dung directly or indirectly, supporting assertions that mullet are instead reliant on microphytobenthos and POM (Whitfield & Blaber 1978). Similarly, higher terrestrial signatures of the tilapia in the Narrows could indicate that this species, a known detritivore, is more dependent on dung directly or indirectly. High terrestrial biomarker values recorded for the glassy in the Narrows can be attributed to the fact that this species is primarily a zooplanktivore (Blaber 2000, Cyrus 2013), which in the current study, displayed higher terrestrial biomarker values in the Narrows.

In other studies, elevated levels of bacterial signatures within food web components have been attributed to the ingestion of particulate matter derived from POM and mangrove material (Meziane et al. 1997, Meziane & Tsuchiya 2000). In addition, the support of the microbial food web through bacterial colonization of leaf litter has been shown to impact a diverse range of species (Woodroffe 1982, 1985, Alfaro et al. 2006, Guo et al. 2016). In St

Lucia, from 1980 to 1982, a period when the estuary mouth was artificially kept open and mangroves were thriving, the estimated total mangrove leaf litter production was 1322.7 tonnes dry matter per year (Steinke & Ward 1988, Taylor 2013a). However, this is a very different picture to the drought-induced, closed mouth phase during which the current study was conducted. Since 2002, the near-decadal mouth closure resulted in lowered salinity within the Narrows and consequently caused mangrove populations to decline. Additional mangrove declines were recorded following a mortality event in 2013/2014, caused by the reconnection of the Mfolozi river, which increased water levels and resulted in the inundation and suffocation of mangrove aerial roots (Adams & Human 2016). Therefore, throughout the duration of this study, mangrove contributions to POM would have been reduced significantly relative to values reported in the 80s by Steinke & Ward (1988). In addition, studies investigating the diets of ichthyofauna have shown that even when abundant, mangrove leaf litter is not the primary contributor to the total assimilated carbon in fish, with macro- and microalgae being more important dietary contributors (Mbande et al. 2004, Whitfield 2017).

Currently, hippo populations within the system are increasing and transferring an estimated 2000 tonnes of dry matter per year (Taylor 2013a). Hippo dung inputs are therefore likely the dominant contributor to the detrital pool in the St Lucia Estuary, and the most likely explanation of the greater levels of bacterial biomarkers recorded in Narrows food web components, particularly for the fish. This is supported by studies elsewhere showing that hippo dung accumulation under low flow conditions can lead to the development of anoxic water layers (Stears et al. 2018), which have been showed to cause the replacement of algal-derived FAs with bacterial derived FAs (Wakeham & Canuel 1990).

6.3.4 Hippo dung in the St Lucia food web

The structure of food webs can be altered by changing environmental drivers, species interactions or a combination of the two, making them mutable in both space and time (Zeug & Winemiller 2008). The results of this study, show that the FA profiles and biomarkers of dominant food web components in the St Lucia Estuarine system display both temporal and spatial variation, differing significantly amongst the four sample seasons and between the two biotopes. Results indicate that in the Narrows, where hippos are dense and dung inputs likely high, food web components (1) have FA signatures more similar to hippo dung; (2) show increased levels of terrestrial and bacterial biomarkers and (3) reduced levels of diatom biomarkers. The latter is consistent with the idea presented in Chapter 4, that dung inputs can reduce microphytobenthic production, probably through shading and abrasion.

Given the strong trend of increased bacterial biomarker values in the Narrows relative to Charter's Creek, which is observable across all trophic levels, shifts of food webs from a microphytobenthic to a bacterial base may be a significant mechanism by which hippo dung influences aquatic food webs and communities. This change at the base of the food web could have significant implications for higher trophic levels and is supported by research showing that when they dominate, bacteria play a significant role in nutrient transfer to higher trophic levels, although only in the short term (Wenzel et al. 2012). Similarly, studies elsewhere have shown that food webs dependent on bacterial bases display increased food chain length, with a loss of energy at each level due to respiration and excretion. Such effects in turn can result in lowered fish production and reduce overall food web efficiency (Jones 1992, Degerman et al. 2018). This aspect may be worth investigating in future studies.

CHAPTER 7:

SYNTHESIS

7.1 Background

Anthropogenic developments and growing human populations are altering natural environments and threatening ecosystem functioning (Meyer et al. 1999, Dolbeth et al. 2007). This precipitous and pervasive dimension of global change makes it vital for ecologists to develop a predictive understanding of these change from the level of individual organisms to entire ecosystems (Dolbeth et al. 2011, Zeppilli et al. 2015). One particularly relevant aspect of global change and anthropogenic forcing are the changes induced in the distribution of key ecological engineers or keystone species, since direct changes are likely to result in far-reaching indirect ecological consequences across multiple organisational levels. This idea has been put into perspective by Estes et al. (2011), who showed that losses of apex predators across the globe is one of mankind's most pervasive influences. Importantly, such losses have been accompanied by cascading indirect alterations at lower trophic levels in marine, freshwater and terrestrial ecosystems. In addition, such changes have led to unanticipated influences on key ecological processes including disease dynamics, carbon sequestration and biogeochemical cycling amongst others.

The points raised in the preceding paragraph regarding human and global change, species distributions and indirect ecosystem effects are all relevant to hippopotamus (*Hippopotamus amphibius*), which are amphibious megaherbivores that are endemic to sub-Saharan Africa. They are classified as Vulnerable on the IUCN Red List and in need of greater research efforts, particularly in relation to their ecological influences and their susceptibility to global change and anthropogenic stressors. The distribution and abundance of hippos has been dramatically reduced (Fig. 1.1) over recent decades by habitat loss and fragmentation, exploitation (for ivory and meat), human-wildlife conflicts and civil unrest (Kanga et al. 2012, Lewison, R. & Pluháček 2017).

Hippo populations are currently considered stable overall, with conservation strongholds for the species in eastern and southern Africa. However, the key threats for this species remain human induced disturbances (habitat loss/fragmentation and hunting), climate change and severe weather events (Lewison & Pluháček 2017). Hippos are recorded as having "restricted distributions" in 33 of the 38 countries in which they reside and are either under total or partial protection in 26 of the countries (Lewison & Pluháček 2017). However, protection from humans, confinement within restricted areas and the lack of natural predators can result in hippo populations growing in conservation areas. In the St Lucia Estuary for example, which is the focal system in this study, the hippo population has been estimated to be increasing by between 2 – 3 % per year (Taylor 2013a). However, in this system, climate change and anthropogenic water abstraction has resulted in a contraction of water bodies, thus increasing densities (per km of shoreline) and the sizes of aggregations (MacKay et al. 2010, Taylor 2013a). Densities of up to 20.62 hippos/km of shoreline have been recorded within some areas of the St Lucia system (Prinsloo 2016).

Despite their substantial size and being an iconic African species, relatively little is known about the biology and ecology of hippos at a broad level, in part due to their "reputation for inflicting painful forms of death" (Pennisi 2014, pg 803). As such, studies on hippos have been limited. The majority of studies have been autecological in nature, focusing on feeding habits and additional behaviours, while others have explored impacts on terrestrial vegetation or of their movement across ecosystem boundaries (Field 1970, Lock 1972, McNaughton 1984, 1985, Naiman & Rogers 1997, McCarthy et al. 1998, Chritz et al. 2016). More recent studies have started to examine impacts of hippo dung on their environments, with a strong focus on modifications of biochemical properties and processes (Gereta & Wolanski 1998, Wolanski & Gereta 1999, Subalusky et al. 2015, Dutton et al. 2018, Stears et al. 2018).

Following research conducted by Grey and Harper (2002), which illustrated the use of stable isotopes to distinguish hippo dung from other resources, studies have used this technique to investigate the effects of dung on aquatic species, typically from within single trophic groups (McCauley, Dawson, et al. 2015, Stears & McCauley 2018, Stears et al. 2018). Consequences of hippo dung for food web structure have been rarely studied, though there have been some recent contributions (Masese et al. 2015, 2018). In addition, studies have generally been conducted in freshwater ecosystems in East Africa. Within southern Africa, hippo research has been limited to university dissertations (Taylor 1980, Prinsloo 2016), broad discussions in book chapters (Eltringham 1999, Taylor 2013a) or investigations conducted for the purpose of the IUCN Red List (Lewison & Pluháček 2017). This thesis thus addresses knowledge gaps identified by quantifying community and food web impacts of hippo dung using both comparative and experimental techniques. The study was carried out in the St Lucia Estuary on the east coast of South Africa, which is home to one of the largest hippo populations of South Africa (1000 hippos; Taylor 2013a).

7.2 Field experiment – community effects of dung

Chapters 3 & 4 focused on quantifying the magnitude, direction and spatial consistency of microalgal, meiofaunal and macrofaunal community responses to experimental hippo dung inputs. This approach enabled comparisons to be drawn between responses of functionally distinct communities and inferences to be made about benthic resilience. Dung inclusion and exclusion cages were set up at two sites within Charter's Creek - an area where hippo densities are known to be low. The hypotheses posed were that (1) microphytobenthic biomass and macrofaunal community metrics would be reduced following dung enrichment, (2) the individuals of opportunistic/resilient species would be larger in dung enrichment plots due to

added trophic resources, and (3) that meiofaunal responses would be weaker than macrofaunal responses and that responses would be taxon specific.

Findings from Chapters 3 and 4 generally supported the hypotheses posed, although, macrofaunal size weakly followed hypothesised effects, with two out of seven dominant taxa being larger within dung inclusions plots. The addition of hippo dung resulted in a significant decline in microphytobenthic biomass, by as much as 49 and 70 % in experimental treatments relative to controls (Fig. 3.3). This finding aligns with studies elsewhere that show reductions in benthic primary production with the addition of terrestrially derived organic matter, due mainly to an attenuation of incident light levels (Kemp et al. 2005, Smith & Schindler 2009, Jones et al. 2012, Kelly et al. 2014, Subalusky et al. 2018). In addition, studies elsewhere have postulated that suspended solids can potentially impact microphytobenthos by causing re-suspension or cellular damage (Bilotta & Brazier 2008).

Macrofaunal community structure, based on abundance (Fig. 3.4) and biomass (Fig. 3.5) data, differed between dung exclusion and inclusion treatments. This response was supported both visually and statistically for the former, but only visually for the latter, suggesting that macrofaunal biomass may not be as strongly impacted by dung additions as macrofaunal abundance. In contrast, meiofaunal community structure did not differ significantly (visually or statistically, Fig. 4.2) between dung treatments. The differential response of macrofaunal and meiofaunal communities matches research elsewhere that point to greater resistance of meiofauna to disturbances than macrofauna (Josefson & Widbom 1988, Warwick et al. 1990)

Macrofaunal abundance, biomass and richness declined by up to 76, 56 and 27 % following experimental enrichment by hippo dung (Fig. 3.6 & Fig. 3.7). A number of possible driving mechanisms for this were suggested (Fig. 3.11), including 1) bottom-up effects

associated with declines in microphytobenthos 2) dung abrasion scouring surface-dwelling taxa 3) impairment of filter-feeding by suspended faecal matter, 4) dung acting as a recruitment barrier at the sediment-water interface and 5) abiotic stress caused by dung decomposition (oxygen depletion and increased hydrogen sulphide flux). In contrast, community metrics for meiofauna exhibited non-significant responses to dung addition (Fig. 4.3) or where spatially idiosyncratic, adding further support to the notion that this group is more tolerant to dung inputs than macrofauna.

Response directions of macrofauna were generally spatially uniform, with dung largely inducing reductions in community (Fig. 3.6 & Fig. 3.7) and individual metrics (Table 3.4). However, magnitudes of responses were highly variable spatially. In the case of meiofauna, community responses were generally neutral (Fig. 4.3) and individual species exhibited responses that were inconsistent in both direction and magnitude (Table 4.3). Despite the two experimental sites being only 150 m apart, findings suggest that abiotic variability at this spatial scale is significant enough to influence benthic responses. *In situ* observations suggest that the most likely driver of response variability is wave action. In the case of macrofauna, community and individual responses were strongest at the site with low wave action.

Taken collectively, findings indicate that even at small spatial scales within aquatic biotopes, meso-scale processes may be important in mediating responses of particular assemblages to hippo dung inputs. This notion is supported by research elsewhere that shows that the effect of hippo dung is contextually dependant, with the magnitude and direction of responses dependent on water flow and the magnitude of dung inputs. High dung inputs and/or low flow rates can result in dung accumulation and declines in water quality as well as primary and secondary production, whereas low dung inputs and/or high flow rates could induce positive effects on production (Masese et al. 2015, 2018, McCauley, Dawson, et al. 2015, Stears et al. 2018, Subalusky & Post 2018, Subalusky et al. 2018).

Results indicate that persistent inputs of hippo dung, at quantities experienced in the Narrows of the St Lucia Estuary during a drought, can have differential impacts on functionally distinct benthic communities. The autotrophic nature of microphytobenthic communities and need for sunlight, makes this group particularly susceptible to dung-induced shading (Jones et al. 2012, Kelly et al. 2014, Subalusky et al. 2018). This, coupled with the larger sizes of macrofauna and planktonic larval reproductive stages, suggests a vulnerability of this group to persistent hippo dung inputs. Traits such as smaller size, direct benthic development, rapid growth rate and greater dietary breadth may contribute to increased resilience of meiofauna to dung inputs.

Future experimental studies might consider investigating the effects of a range of dung enrichment levels in order to determine the point at which hippo dung inputs switch from inducing positive to negative responses. Indeed, it is possible that at low levels, dung may induce stimulatory effects on benthic metrics. In addition, a study on the impact of dung addition on the vertical distribution of benthic organisms may provide further explanations for the differential responses of macro- and meiofaunal communities observed here.

7.3 Field survey – stable isotope and fatty acid analyses

Chapters 5 and 6 investigated potential food web consequences of hippo dung based on comparisons of two biotopes with contrasting hippo density. Four seasonal sets of food web samples were collected from the Narrows, where hippos were dense, and Charter's Creek, where hippos are rare. It was assumed that dung levels between these biotopes would reflect hippo densities. Specifically, food web components across multiple trophic levels were collected from each biotope, including microphytobenthos (MPB), sediment organic matter (SOM), particulate organic matter (POM), zooplankton, benthic macrofauna and dominant fish

species. Samples were subjected to fatty acid (FA) and stable isotope analyses (SIA) to make inferences about potential impacts of hippo dung inputs.

In Chapter 5, SIA was used to determine the relative contribution of hippo dung as a trophic resource to consumer diets from each biotope. Based on previous studies showing increased dependence by consumers on allochthonous resources with increasing input quantities (Solomon et al. 2011, Jones et al. 2012, Wilkinson et al. 2013), the hypotheses posed were that (1) food web components in the Narrows and Charter's Creek would differ in isotopic signatures and (2) hippo dung would have a greater proportional contribution to consumer diets in the Narrows relative to Charter's Creek.

The first hypothesis posed was supported as stable isotope bi-plots showed a distinct separation of food web components between the two biotopes (Fig. 5.1). More specifically, there was a consistent trend of decreased $\delta^{13}C$ and $\delta^{15}N$ isotope ratios of food web components in the Narrows relative to Charter's Creek, regardless of season. Within biotope comparisons of seasonal food web bi-plots showed no significant separations despite a broad range in seasonal salinity values (Fig. 5.2). This would suggest it was not variations in salinity (or other abiotic conditions), but differences in dung inputs between the two biotopes that played a role in the isotopic shifts observed.

Results indicated that $\delta^{13}C$ values of basal resources in the Narrows (i.e. sediment organic matter (SOM) and microphytobenthos (MPB)) were more similar to signatures of hippo dung than were those of Charter's Creek (Fig. 5.3), providing circumstantial evidence that dung inputs may influence carbon isotopic signatures of benthic basal resources. Particulate organic matter $\delta^{13}C$ values in Charter's Creek were more similar to dung than they were in the Narrows. This may be linked to water depth differences between biotopes, with shallow conditions in Charter's Creek causing POM to generally consist of resuspended dung,

whereas in the Narrows, deeper states prevent resuspension of dung. With regards to consumers, $\delta^{13}C$ values of all primary and secondary consumers in Charter's Creek were similar to the $\delta^{13}C$ value of hippo dung. Isotopic signatures of basal resources and primary consumers varied temporally and spatially (Table 5.2 & Table 5.3), while those of secondary consumers were more seasonally consistent, with variation displayed only between biotopes. It would therefore seem that small, sedentary primary consumers with limited spatial distributions reflect variability in isotopic signatures of basal resources in space and time. In contrast, secondary consumers, being mobile, are potentially unaffected by small-scale variability in trophic resources due to greater diet choice.

Interestingly, the hypothesis that there would be a greater proportional contribution of hippo dung to consumer diets in the Narrows relative to Charter's Creek was refuted. Results from Bayesian mixing models revealed that in primary consumers (Fig. 5.10), dung contributions to their diets varied as a function of season and consumer identity, with no consistent pattern evident. As indicated above, the diets of primary consumers were likely influenced by small-scale variability of basal resources in their immediate surroundings. In contrast, the diets of fish exhibited a strong trend of consistently higher proportional dung contributions in Charter's Creek than in the Narrows (Fig. 5.11). This counter-intuitive finding may be explained by the fact that macrofaunal richness and diversity in Charter's Creek is low relative to the Narrows (Pillay & Perissinotto 2008), possibly indicating reduced availability of alternative resources that could be of higher nutritional quality. Secondly, as argued previously, shallow conditions in the Charter's Creek may increase dung resuspension and hence its availability to fuel pelagic food webs. Results from this chapter thus indicate that the relative contribution of hippo dung as a dietary resource is not necessarily determined simplistically by input quantities, but is likely habitat dependant, with factors such as water depth and trophic resource diversity being important contextual variables.

In Chapter 6, fatty acid analysis was used as a tool to supplement the previous chapter, and to determine specifically if aquatic autochthonous basal resources and allochthonous hippo dung had divergent fatty acid (FA) profiles and if the profiles of dominant consumers common to the Narrows and Charter's Creek differ. The relative importance of hippo dung as a basal resource within these biotopes was also investigated using FA biomarkers. The hypotheses posed were that (1) food web components of the two biotopes would have disparate FA profiles suggestive of different diets, (2) high dung levels in the Narrows would result in greater terrestrial and bacterial biomarker values, (3) a decrease in primary production in response to shading by dung would result in a reduction in diatom biomarker values relative to Charter's Creek, and 4) essential fatty acid (EFA) marker values would be lower in the Narrows as a result of declines in the availability of EFA rich microphytobenthos. The first three hypotheses were strongly supported by FA results, however the fourth was weakly supported.

Results showed a distinct separation of hippo dung FA profile from profiles of all aquatic basal resources (Fig. 6.1). This is an expected outcome given that terrestrial organic matter is known to be of poorer quality than aquatic resources and therefore composed of different fatty acids (Torres-ruiz et al. 2007, Guo et al. 2016). Similarly, the FA profiles of all consumers showed clear distinctions between the Narrows and Charter's Creek, supporting the notion that the diets of consumers between the two biotopes were different and suggesting that hippo dung contributes to the shift in consumer diets between areas with contrasting levels of dung. Further support for this notion were results from similarity analyses, which showed that fatty acid profiles of the Narrows food web components were generally more similar to hippo dung profiles than those in Charter's Creek (Tables 6.2 & 6.3).

Fatty acid biomarker values provide interesting information regarding the influence of hippo dung within the St Lucia estuary. Results show that fresh hippo dung samples had high terrestrial biomarker values (9.13 ± 0.73 %), which was greater than the 2.5 % of the total FA

content that is typically used as a benchmark to denote a significant terrestrial carbon signature (Budge & Parrish 1998, Budge et al. 2001). This value was also substantially higher than all other terrestrial biomarker values with the exception being zooplankton in season 1. In addition, freshly voided hippo dung had high bacterial values (8.49 % ± 0.57, Fig. 6.9), which is probably a product of dung acting as a resource/substrate for bacterial colonization but reflecting also the presence of hippo gut biota.

The diatom biomarker hypothesis of greater values in Charter's Creek than in the Narrows was well supported. Although not consistent, values were generally lower in the Narrows than in Charter's Creek suggesting a lower diatom presence and likely lower overall microphytobenthic biomass in areas with high levels of dung inputs. This result aligns with that reported in Chapter 3, in which microphytobenthic biomass declined significantly in response to experimental hippo dung enrichment. Taken collectively, these findings offer support for the notion that hippo dung inputs can have deleterious effects on benthic primary production.

It was hypothesised that bacterial biomarker values would be higher in the Narrows given higher hippo densities and presumed greater dung levels than in Charter's Creek. Bacterial values of sediment organic matter (SOM, Fig. 6.11) provide strong evidence in support of this, as bacterial values in the Narrows (avg. - 18.94 ± 0.69 %) were consistently higher than in Charter's Creek (avg. - 12.62 ± 1.28 %). This potentially indicates that hippo dung settling onto the benthos becomes colonised or consumed by bacteria, a notion that is supported by previous research showing that bacteria colonise and decompose excreted organic matter (Jones 1992). It is noteworthy that the bacterial marker values in the Narrows were higher than those found in several other studies including from detritus rich habitats (Budge & Parrish 1998, Meziane & Tsuchiya 2000, Budge et al. 2001, Kharlamenko et al. 2001). The

bacterial marker values of consumers from the Narrows were generally significantly higher than those from Charter's Creek.

Results for terrestrial biomarker values provided support for the hypothesis that greater dung inputs in the Narrows would result in higher terrestrial values in this biotope relative to Charter's Creek. Although not ubiquitous across all seasons, terrestrial biomarker values were generally higher in the Narrows than in Charter's Creek. This may appear interesting in light of the results of the stable isotope mixing models, which suggested that dung contributed more to the diets of secondary consumers in Charter's Creek than in the Narrows. There are various potential explanations for this seemingly counter-intuitive finding of greater terrestrial and bacterial biomarkers in the Narrows but dung making low contributions to secondary consumer diets.

Firstly, it must be borne in mind that results from stable isotope mixing models are based on proportional dietary contributions. Thus, low contributions of dung to fish diets in the Narrows may simply reflect greater contributions of food sources that are rare/absent or not consumed in Charter's Creek for reason unknown. This is supported by data that in the Narrows, the isopod *Cyathura estuaria* made important contributions to fish diets, but not in Charter's Creek. In combination, isotope and fatty acid data suggest that in the Narrows, consumers may preferentially select high quality trophic resources. This is plausible given that hippo dung comprises grasses with high cellulose contents and is thus potentially of low quality (Field 1970, McNaughton 1985). However, in Charter's Creek, where it is hypothesised that shallow conditions increase dung resuspension, consumers may be more reliant on dung (directly or indirectly) due to its prevalence in the water column and potentially the scarcity of other trophic resources.

Secondly, colonisation of dung by bacteria and their subsequent consumption may have caused increases in terrestrial biomarkers in consumers, without dung actually being consumed. This idea is supported by Torres-ruiz et al. (2007), who postulated that increased levels of the fatty acid 18:2ω6 in benthic organic matter of streams could be explained by bacterial and fungal colonization of terrestrial transfers. This fatty acid was one of the two used in the current study as terrestrial biomarkers. Given the higher bacterial biomarker values in the Narrows, it is plausible that the high terrestrial signatures in Narrows consumers is due to their reliance of bacteria as a food source, which in turn inflates terrestrial signatures, despite proportionally less dung being consumed directly or indirectly.

Future studies might consider investigating the quality or stoichiometry of hippo dung as a trophic resource. In addition, given the observed shift of the food web in a heavily dung-loaded biotope from a microphytobenthic to a bacterial base, future studies might consider using phospholipid-derived fatty acids (PLFA) which are widely used in microbial ecology as taxonomic markers of bacteria. The use of PLFAs could increase understanding of the role of bacteria in food webs supplemented by high concentrations of hippo dung. Further studies may also benefit from a higher resolution of hippo dung sampling, which may provide information of how, if at all, hippo dung isotope and fatty acid signatures vary seasonally – especially in areas where predictable wet and dry seasons occur.

7.4 Concluding perspectives

Despite being one of the most conspicuous members of aquatic ecosystems in Africa, little is known about the influence of hippos as structuring agents of communities and food webs. In addition, despite transferring massive amounts of dung into aquatic systems, little is known on ecological ramifications for biotic communities and food webs. This thesis thus expands existing understanding of the role hippos play in aquatic ecosystems broadly, but

specifically sheds light on the ecological consequences of dung inputs. This study also highlights areas of management concern/relevance that may be pursued in future studies.

Findings from the experimental component of this thesis highlight potentially important ecosystem-level consequences of dung inputs. Declines in microphytobenthic biomass and macrofauna community metrics following experimental enrichment not only highlight their susceptibility to high dung inputs, but also raise potential concerns that losses of these groups could have broader ecological consequences, given the ecological functions they provide including functioning as trophic resources for higher consumers, stabilisation of sediment, biofilm formation, and sediment bioturbation and oxygenation (Lohrer et al. 2004, Needham et al. 2011, Pillay et al. 2011, Gladstone-Gallagher et al. 2017). Thus, there is the potential for persistent dung inputs to impair benthic functioning, leading to ecosystem degradation in the long term. Future work could thus expand the experiments conducted in this thesis by explicitly testing benthic responses to multiple levels (i.e. not presence/absence alone) so that theoretical thresholds at which dung switches from eliciting positive to negative responses could be identified. In addition, threshold information coupled with community response data and ecosystem function measurements (e.g. productivity, nutrient fluxes) can provide important information to managers on hippo dung effects that can drive decision making.

Results from fatty acid analyses suggest that increased hippo dung inputs can induce secondary consumer diets to shift and exhibit (1) higher terrestrial signatures, which is likely indicative of increased reliance on dung as a trophic source, (2) reduced microphytobenthic contributions to diets, and (3) a greater reliance on bacterial resources. This trio of dung-induced changes to the diets of consumers could have important repercussions for consumer performance in the long-term. Microphytobenthos is known to be a high quality resource because of low C:N and C:P ratios and enrichment in essential fatty acids. In contrast, bacteria and terrestrial organic matter are considered to be nutritionally inadequate, poor quality dietary

resources (Torres-ruiz et al. 2007, Taipale et al. 2012, 2014, Wenzel et al. 2012, Guo et al. 2016). Although aquatic consumers can maintain reasonably high growth rates while on a diet dominated by bacteria and/or terrestrial organic matter, they do require higher quality microalgae for certain functions like reproduction (Brett et al. 2009, Taipale et al. 2012, 2014). Elsewhere, it has been shown that while growth of individual consumers can be supported by terrestrial organic matter, it depressed biomass production at the population level (Karlsson et al. 2015). Therefore, although allochthonous resources and the associated increased bacterial abundance may be able to support aquatic ecosystems when nutrient rich resources are limited, they cannot completely replace autochthonous resources and there is an upper limit to this support (Karlsson et al. 2012, Wenzel et al. 2012).

In itself, this aspect can form the basis of further work, by using controlled feeding trials for example, in which consumer responses are tested against dung- or microalgal-dominated diets. Such work would assist in further clarifying the role of hippo dung in the context of global/anthropogenic change pressures that the St Lucia Estuary may face in future. It has been suggested that following droughts, unaffected areas the lakes function as important species pools to enable recovery (Pillay & Perissinotto 2008, MacKay et al. 2010, Govender et al. 2011, Scharler & MacKay 2013). However, if these areas become refuges for hippos, dung-induced shifts to bacterial dominance may reduce food web efficiency and weaken resilience. This potential effect could act in concert with the negative effects of dung on benthic primary and secondary production that were recorded in experiments in this study to depress ecosystem performance.

Taken collectively, findings of this thesis demonstrate that declines in primary and secondary production at high levels of dung input and a shift in food webs to bacterial-dominated bases are significant mechanisms by which hippos influence aquatic food webs and communities. As a whole, this study provides a useful platform to further quantify the impacts

of hippo-dung inputs for ecological processes in aquatic ecosystems. This is very relevant in protected ecosystems where rising hippo numbers and dung inputs can have significant effects on ecological processes. It is hoped that this thesis stimulates further research on the topic so that a predictive and mechanistic understanding of hippo-dung inputs can be advanced in aquatic ecosystems.

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