PREDATION BY ALIEN LARGEMOUTH BASS, *Micropterus salmoides* LACEPÉDE 1802 (CENTRARCHIDAE: PERCIFORMES), ON INDIGENOUS MARINE FISH SPECIES IN THE KOWIE SYSTEM, SOUTH AFRICA

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ABSTRACT

Estuaries serve as nursery areas for a large number of estuary-associated fish species. Some of these taxa also use river catchments as nursery areas. During the upstream migration of this latter group, the juveniles are prone to predation by native and alien predatory fish inhabiting the system. The rate of invasion of ecosystems by alien organisms can be directly linked to anthropogenic influences, including both intentional and unintentional introductions by alien organisms into new regions. The largemouth bass, *Micropterus salmoides,* is a facultative piscivorous fish that has been successfully introduced worldwide for the main purpose of sport fishing. Where introduced, it has been found to negatively impact native fish and invertebrate species through predation, competitive exclusion and displacement of indigenous fish species. The aim of this thesis was to investigate the predatory impact of largemouth bass on the estuary-associated Cape moony *Monodactylus falciformis,* Cape stumpnose *Rhabdosargus holubi* and freshwater mullet *Myxus capensis* in the lower Kowie River of the Eastern Cape Province, South Africa. A combination of approaches was employed during this study. Stomach contents, stable isotopes and fatty acid analyses were employed for the reconstruction of the diet of largemouth bass. Acoustic telemetry was used to elucidate largemouth bass movements, particularly their ability to enter the upper reaches of the Kowie Estuary. Stomach contents and stable isotopes analyses showed that *M. salmoides* exhibit an ontogenic shift in diet, with small and medium sized individuals consuming the higher proportion of fish prey, while large sized individuals mostly consumed invertebrates such as crabs (*Potamonautes sidneyi*) and Odonata larvae, while consuming only a small proportion of estuary-associated fish. Fatty acid analysis only showed a direct connection between the fatty acid profiles of largemouth bass and those of *M. capensis* and *M. falciformis*. The acoustic telemetry results indicated that some *M. salmoides* individuals periodically move into the upper reaches of the estuary following river flood events. The results obtained from all these approaches highlight the risk posed by largemouth bass introductions on indigenous fish species, particularly those that enter the areas occupied by these top predators.

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DECLARATION

I, Mandla Leon Magoro, hereby declare that the work contained herein is my original work and has not been submitted before for the award of any other degree at any other university.

CHAPTER 1

GENERAL INTRODUCTION

1. INTRODUCED ORGANISMS

Human-mediated biological invasions have been occurring since early human history, with rates of invasion substantially increasing with advances in modern civilization, which has resulted in a higher frequency of worldwide human migrations (Carlton, 1996; Mack et al., 2000; Gozlan, 2008). One demonstration of how modern advances can aid the process of biological invasions is that ballast water used to stabilise ships often contains marine organisms which are then carried from one port to another (Hallegraeff and Bolch, 1991 and 1992; Picker and Griffiths, 2011). Amongst the numerous harmful impacts that introduced invasive organisms can pose, perhaps of most interest to the general public is that they can be detrimental to both a country's biota and economy (Vermeij, 1996; Pimentel et al., 2000; Van Wilgen et al., 2001; Pimentel et al., 2005; Picker and Griffiths, 2011). In the United States of America, nonindigenous species cause more than $100 billion in environmental damage annually (Pimentel et al., 2000 and 2005).

The economic impacts can further be compounded by the monetary costs involved in executing programs directed at controlling and eradicating invasive alien organisms. More effort has thus been directed at studies which seek to tackle the persistent issue of invasive aliens by understanding the mechanisms behind their spread, establishment and integration (Vermeij, 1996; Mack et al., 2000; Cambray, 2003; Gaither et al., 2013). This is all in stark contrast to the positive benefits derived from introduced organisms such as naturalized food crops, income generated from the sport fishing industry, as well as plants intended for timber production (Van Wilgen et al., 2001; Picker and Griffiths, 2011).

The level of invasion by non-indigenous fish species in South Africa is amongst the highest in the world. For example, Leprieur et al. (2008) identified this country as one of the top global freshwater fish invasion "hotspots". These hotspots are defined as areas where more than

25% of the extant species are non-indigenous. The introduction of alien fish species can threaten the survival of indigenous ichthyofauna and aquatic invertebrates through, amongst others, predation, competition and transfer of pathogens (Mack et al., 2000; Woodford et al., 2005; Weyl et al., 2010; Ellender et al., 2011; Gaither et al., 2013).

Exotic fish are mainly introduced into new regions for aquaculture, fisheries or ornamental purposes (McDowall, 1968; Weyl and Hecht, 1999; Cambray, 2003; Gaither et al., 2013). The issue of intentional fish introductions always stirs up debate as to whether a country's demand for economically important alien fish is worth risking the ecological implications that may result from such actions (Jackson, 2002; Gozlan et al., 2010). Therefore, prior to introduction of new fish species, investigations of the possible ecological impacts on indigenous aquatic communities, as well as the envisaged economic benefits, are required in order to make an informed decision (McDowall, 1968).

2. LARGEMOUTH BASS

2.1. Biology and ecology

The largemouth bass, *Micropterus salmoides* Lacepéde 1802 (Centrarchidae, Perciformes) is a facultative piscivorous freshwater fish (Weyl and Hecht, 1999; García-Berthou, 2002), which has a preference for clear lentic or slow-flowing waters with floating and submerged vegetation. It has a temperature tolerance range of 10 to 32°C and has been reported spawning during spring and summer in water temperatures of 15 to 18°C (Crass, 1964; Jubb, 1967; McDowall, 1968; Scott and Crossman, 1973; De Moor and Bruton, 1988; Skelton, 2001; García-Berthou, 2002).

Partly due to its popularity as a sport fish, a large number of studies have been conducted on its biology (e.g. Scott and Crossman, 1973; Bennet, 1974; Lee et al., 1980; Ludsin and DeVries, 1997; Garvey et al., 2002; Jackson, 2002; Parkos and Wahl, 2002). Various studies have shown that *M. salmoides* changes its diet with increase in age and body size, with juveniles normally feeding on small crustaceans, before switching to insects and then finally fish prey as adults (Clady, 1974; Keast, 1985; Olson, 1996; Weyl and Hecht, 1999; García-Berthou, 2002). This ontogenic shift in diet appears to occur in both indigenous and alien populations of *M. salmoides*. Another facet of largemouth bass feeding behaviour is cannibalism, wherein juveniles are consumed by older fish of larger size classes (Crass, 1964; Clady, 1974; Weyl and Hecht, 1999).

The largemouth bass is indigenous to North America, but authorised and unauthorised introductions have expanded its distribution to such an extent that it now inhabits freshwater bodies in parts of Europe, southern Africa, South America, Asia and numerous oceanic islands (Scott and Crossman, 1973; Lee et al., 1980; Migdalski and Fichter, 1987; García-Berthou, 2002; Jackson, 2002; Braun and Walser, 2011). *M. salmoides* was introduced into South Africa for the purpose of recreational angling in 1928 (Crass, 1964; De Moor and Bruton, 1988; Gozlan, 2008) and its presence has since been reported in several water bodies across the country (De Moor and Bruton, 1988; Skelton, 1993; Weyl and Lewis, 2006; Ellender et al., 2011).

2.2. Impacts of Largemouth bass

There are both positive and negative aspects associated with the introduction of *M. salmoides* into new regions. The positive aspect can be in the form of the contribution it makes to a country's economic development through, for example, the financial gains made from the fisheries industry and sport fishing. The ability of largemouth bass to feed on a variety of indigenous fish and invertebrates greatly increases its chances of survival when introduced into foreign water bodies. Ontogenic diet shifts, coupled with high fecundity and an ability to endure wide-ranging environmental conditions, enables *M. salmoides* to survive and proliferate in foreign waters (McDowall, 1968). The threat of bass predation can be acute in systems with highly threatened endemic species, possibly resulting in extinctions and severely impacting ecosystem functioning (Gozlan et al., 2010).

Consequently there are several examples of cases where the presence of *M. salmoides* has had negative impacts on indigenous fish and invertebrate species. Largemouth bass was attributed as negatively affecting the diversity, abundance and distribution of indigenous fish species in Zimbabwe's upper Manyame River (Gratwicke and Marshall, 2001) and a similar case was reported within the headwaters of the Swartkops River system in South Africa (Ellender et al., 2011). Weyl and Lewis (2006) and Wasserman et al. (2011) found that *M. salmoides* also preys on indigenous estuary-associated fish as well as indigenous terrestrial and aquatic invertebrates in the lower Kowie River, Eastern Cape, South Africa.

3. ESTUARY-ASSOCIATED FISH

Estuaries are important nursery areas characterised by fluctuating salinity, water temperature, dissolved oxygen and turbidity with an abundant food supply for fish (Beckley, 1984; Whitfield, 1998; Whitfield, 1999). Despite the rich food resources present in estuaries, the juveniles of some marine fish species (e.g. the freshwater mullet, *Myxus capensis*) migrate from the estuary into the upstream freshwater areas as part of their life history cycle (Whitfield, 1998; Beck et al., 2001; Dolbeth et al., 2008). Any opportunistic predation by alien freshwater piscivorous fishes on these juveniles can potentially reduce the chances of surviving till reproductive age and thus subsequently threaten the success of these species in riverine environments.

There have been preliminary scientific accounts of largemouth bass predation on South Africa's estuary-associated fish (Weyl and Lewis, 2006; Wasserman et al., 2011), however there is currently no general consensus concerning the exact impacts of largemouth bass on these indigenous species. The movements and migration of fish between marine, estuarine and freshwater areas is seen as an illustration of connectivity between adjacent ecosystems, and will result in the transfer of nutrients and organic matter (Gillanders et al., 2003; Ray, 2005). As such, predation by largemouth bass on migrating indigenous fish could represent a barrier to this natural energy transfer route.

4. STUDY AREA

The study area selected for this project was the lower reaches of the perennial Kowie River and the associated estuary located in the Eastern Cape Province, South Africa (Figure 1.1). The Kowie River originates in the Grahamstown hills and its main tributaries are the Bloukrans River, Brakrivier and Lushington River (Heydorn and Grindley, 1982). It is 70 km long, has an erratic flow pattern and catchment area that varies between 580 and 769 km^2 (Heydorn and Grindley, 1982; Watling and Watling, 1983; Whitfield, 2000, Sale et al., 2009) . A total of 10 fish species are known to occur in the area between the weir and the ebb and flow region of the river-estuary interface (Wasserman et al., 2011). The river flows into the permanently open Kowie Estuary, which drains into the Indian Ocean off the south-eastern coast of South Africa (Whitfield, 2000).

With its mouth located in the town of Port Alfred, formerly as Port Kowie and Port Frances, the Kowie Estuary is characterised by rapid diurnal water temperature fluctuations ranging between 21°C to 29°C during summer and 11°C to 16°C during winter (Hill and Allanson, 1971; Heydorn and Grindley, 1982; Kruger and Strydom, 2011). Rainfall in this warm-temperate region mainly takes place during summer, with an annual precipitation of 640 mm (Watling and Watling, 1983; Whitfield, 2000). The lower reaches and mouth of the estuary have undergone numerous anthropogenic alterations since British settlement in the Eastern Cape, initially aimed at developing it into a port (Heydorn and Grindley, 1982) and currently features artificial walls along the banks as well as commercial and residential buildings (Kruger and Strydom, 2011). Such anthropogenic developments put pressure on the integrity of a system which has a relatively high marine fish diversity (Heydorn and Grindley, 1982).

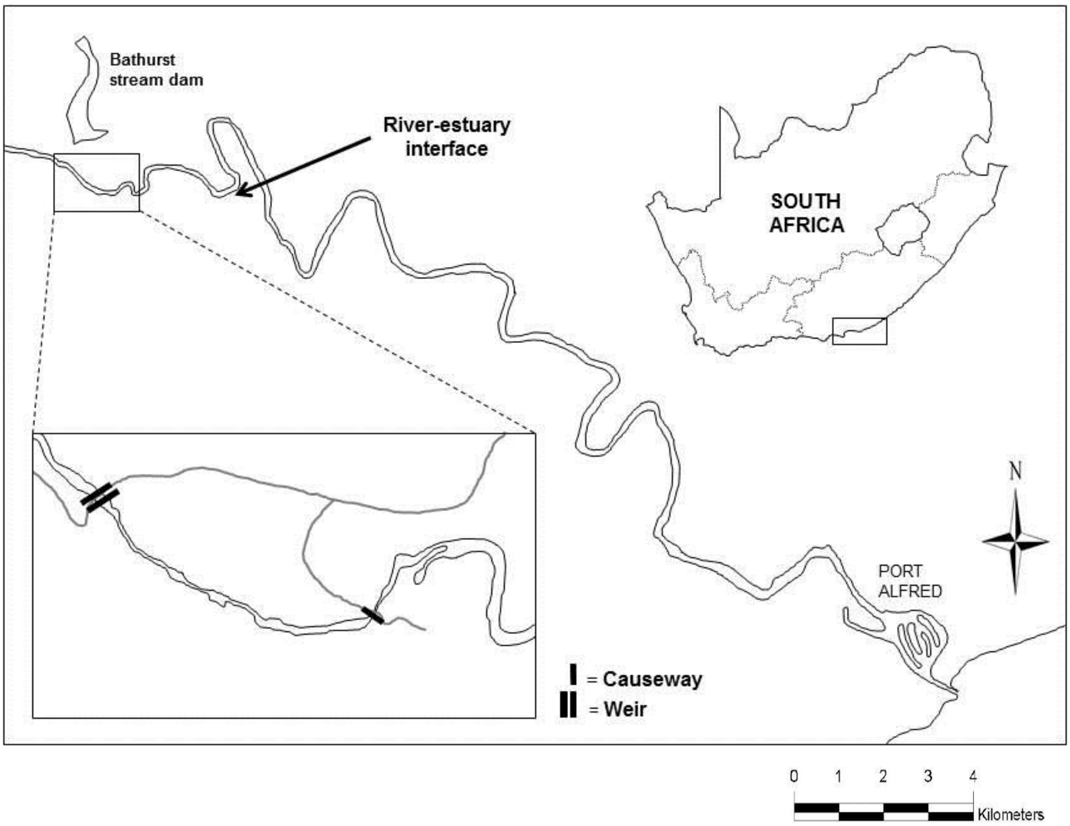

Figure 1.1: The location of the Kowie River and Estuary, Eastern Cape, South Africa showing the locations of the weir and the causeway. Also shown is the river-estuary interface.

5. OBJECTIVES AND HYPOTHESES

The primary objectives of the project were as follows:

Objective A

Determine whether *M. salmoides* preys on the juveniles of indigenous estuary-associated fish species, which migrate from the brackish into the freshwater section of the Kowie River. In essence, this was an attempt to establish if there are trophic links between *M. salmoides* and other fish species within the river.

Objective B

Determine how far individuals of *M. salmoides* move down the lower Kowie River towards the estuary in search of prey. Changes in the movement patterns of largemouth bass in response to salinity levels in the Kowie River were also monitored.

Objective C

Demonstrate the connectivity that exists between the Kowie's riverine and estuarine ecosystems as fish move between the two systems, and how this is impacted upon by alien *M. salmoides* populations.

The following hypotheses were suggested and tested:

Hypothesis A

Wasserman et al. (2011) found only a low occurrence of fish prey in the diet of small sized largemouth bass in the Kowie River, this occurrence increasing with size and depending on the availability of alternative prey such as invertebrates. An earlier dietary analysis conducted in this river, however, suggested a higher consumption of indigenous fish species by largemouth bass, with *Monodactylus falciformis* (Cape moony), *Mugil cephalus* (Flathead mullet) and *Myxus capensis* commonly recorded in largemouth bass stomachs (Weyl and Lewis, 2006). Weyl and Lewis (2006) and Wasserman et al. (2011) found that largemouth bass consume estuary-associated fish such as *Rhabdosargus holubi* (Cape stumpnose)*, M. falciformis, M. capensis and M. cephalus*. All these species migrate between the freshwater and estuarine sections of the river during their life cycle (Whitfield, 1998) and are vulnerable to bass predation during such times.

It was therefore hypothesised that *M. salmoides* acts as a potential barrier to the movement of indigenous estuary-associated fish species into the river, and can also pose a potential threat to those indigenous fish species occupying the river-estuary interface zone. It is, however, worth noting that largemouth bass inhabiting the reservoir upstream of the artificial weir are only able to effectively prey on the migratory estuary-associated fish species when the Kowie River flow is sufficient to activate the fish ladder, which permits the fish to migrate upstream and downstream with minimal restrictions. At low flow, when the fish ladder is non-functional, the diet of *M. salmoides* in the reservoir is usually dominated by invertebrates, mainly amphipods, dipterans, odonates and brachyurans (Weyl and Lewis, 2006; Wasserman et al., 2011)

Hypothesis B

A second hypothesis was that the population of largemouth bass which inhabits a section of the river downstream of the weir will consume higher quantities of indigenous estuary-associated fish. This is based upon the population's proximity to the upper reaches of the estuary and the probable greater abundance of marine and estuarine fish species in this section of the river.

Hypothesis C

Largemouth bass move towards the upper reaches of the Kowie Estuary during periods of high river flow, which is linked to the lowering of salinity levels as more freshwater enters the headwaters of the Kowie Estuary.

This project is part of a broader multidisciplinary programme led by Dr N.B. Richoux (Department of Zoology and Entomology, Rhodes University), which aims at elucidating the routes of organic matter transfer between the marine, estuarine, freshwater and terrestrial ecosystems within the Kowie catchment system. This involves the collection and analysis of diverse groups of organisms from terrestrial and aquatic ecosystems. These include phytoplankton, zooplankton, benthic aquatic invertebrates, insects, spiders, dragonflies, amphibians, birds and fish, *i.e.*, representatives of all major groups of organisms involved in energy transfers between adjacent terrestrial and aquatic habitats. Stomach contents, stable isotopes and fatty acid analysis were used for reconstructing the diet of largemouth bass. Acoustic telemetry was employed to elucidate the movement pattern of *M. salmoides*.

CHAPTER 2

CONTRIBUTION OF ESTUARY-ASSOCIATED FISH TO THE DIET OF LARGEMOUTH BASS IN THE LOWER KOWIE RIVER, AS DETERMINED BY STOMACH CONTENT AND STABLE ISOTOPE ANALYSES

1. INTRODUCTION

1.1. Stomach contents analysis

Various quantitative and qualitative methods have been implemented in fish diet studies, each with its own inherent advantages and disadvantages. Traditionally, methods employed in describing the diet of fish predators involve either the study and identification of the stomach and/or intestine contents, or direct observation of feeding habits (Hynes, 1950; Hyslop, 1980; Iverson et al., 2004). Gut content analysis refers to the method of food items identification within the gut, in order to qualitatively and quantitatively determine the dietary preferences of an organism (Liao et al., 2001). This method operates on the premise that the most abundant prey in the gut of a consumer represents the overall dietary habits of the population, and therefore also assumes that the prey is essential to the predator's morphological development and wellbeing (Liao et al., 2001).

Several methods can be used in gut content analysis, such as frequency of occurrence, gravimetric and volumetric techniques, as well as the points method (Swynnerton and Worthington, 1940; Pinkas et al., 1971; Hyslop, 1980). However, when used independently, these component indices methods have been found to be biased in favour of, or against, prey items of a particular size (Cortes, 1997). A such, it has been suggested that the use of compound indices can be a more reliable tool for investigating dietary preferences (Pinkas et al., 1971; Assis, 1996; Cortes, 1997; Liao et al., 2001). These compound indices include, but are not limited to, the Index of Relative Importance (IRI), percentage Index of Relative Importance (%IRI) and percentage Modified Index of Relative Importance (%MIRI). Several authors have discussed the aforementioned methods and indices as well as illustrating the advantages and drawbacks of each (Swynnerton and Worthington, 1940; Hynes, 1950; Hyslop, 1980; Cortes, 1997; Liao et al., 2001).

For this particular project, due to increased difficulty in identifying comparably more digested intestinal contents (Hynes, 1950) and to allow for comparisons with the results obtained by both Weyl and Lewis, (2006) and Wasserman et al. (2011), it was deemed appropriate to focus solely on the contents of the stomach; hence the name change to Stomach Contents Analysis (SCA).

1.2. The use of stable isotope analysis in ecological studies

The traditional method of stomach content analysis can only reveal dietary preferences based on the food items consumed by a fish shortly before it was captured ("last meal") with such items needing to be at a state of digestion that will still permit proper identification (Hansson et al., 1997). This means that food items which have passed through the stomach into the intestines are usually not analysed, and this can lead to inferences about dietary habits which neglect other potentially important prey items (Hynes, 1950; Vander Zanden et al., 1997; Vander Zanden et al., 1998; Vander Zanden and Rasmussen, 2002; Iverson et al., 2004). Assessments of fish diet based solely on the stomach contents are therefore be regarded as restricted to a short temporal scale. However, it is worth noting that stomach contents analysis can serve as an important reference for modern techniques when used to reveal the consumer's potential food sources.

Advances in modern technology have given rise to new ways of elucidating predator diets. One of the alternative methods to stomach content analysis is one that employs stable isotopes as diet tracers (Fry, 1991; Post, 2003; Plass-Johnson et al., 2012; Sheppard et al., 2012). This approach holds the advantage of incorporating and highlighting the importance of items which may have possibly been assimilated at an earlier stage in the consumer's life-span, and also provides evidence of the origin of particulate matter that cannot be properly identified from the stomach contents. It thus yields results which can more reliably reflect temporal and spatial variations in diet (Michener and Schell, 1994; Allan et al., 2010;

Layman et al., 2012). Stable isotope analysis (SIA) relies on the measurement of isotope ratios of a predator and its potential prey to determine the linkages in food webs or to trace flow pathways of organic matter from primary producers to top level predators (DeNiro and Epstein, 1978 and 1981; Hansson et al., 1997; Fry et al., 1999)

When elucidating the relationships between predator and prey, it is often advisable to use a minimum of two types of isotopes (Peterson et al., 1985). Most commonly employed in ecological studies are measurements of the stable isotope ratios of $^{13}C/^{12}C$ ($\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$) in consumer and prey tissues in comparison with international standards (Peterson and Fry, 1987; Vander Zanden et al., 1999; Cucherousset et al., 2012; Layman et al., 2012). These standards are PeeDee limestone (PDB) for $^{13}C/^{12}C$, and atmospheric air for $^{15}N/^{14}N$ (Peterson and Fry, 1987; Post, 2002; Barrow et al., 2008; Bond and Jones, 2009). The isotopic ratio of a consumer is related to that of its prey (DeNiro and Epstein, 1978). However, metabolic processes such as respiration can also affect a consumer's isotopic ratio via the retention of heavier isotopes and excretion of the lighter isotopes, resulting in enriched isotope values (DeNiro and Epstein, 1978; Alfaro et al., 2006).

For instance, nitrogen undergoes step-wise enrichment of the heavy isotope (^{15}N) and simultaneous fractionation of the light isotope (^{15}N) from prey to predator [ranging between two to four parts per thousand (‰)] (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Peterson and Fry, 1987; Hansson et al., 1997; Vander Zanden et al., 1997). The $\delta^{15}N$ value can thus be used for identification of trophic position of an organism in a food web. This requires the establishment of a baseline $\delta^{15}N$ signature, preferably from long-lived sedentary primary consumers which will also reflect the frequent temporal shifts in the $\delta^{15}N$ signatures of primary producers (Vander Zanden et al., 1997; Post, 2002; Arcagni et al., 2013). Post (2003) used $\delta^{15}N$ together with stomach contents analysis and otolith analysis to investigate the variation in the timing of ontogenetic niche shifts in largemouth bass. This approach has also been used to assess the migration of estuarine fishes by determining their dietary history as exhibited through location-specific isotope signatures (Herzka, 2005). $\delta^{15}N$analysis can also be useful for the elucidation of trophic relationships between native and non-native fish species (Cucherousset et al., 2012).

The δ^{13}C value of consumers is approximately 1‰ higher than that of their food (DeNiro and Epstein, 1978; Peterson and Fry, 1987; Bergamino et al., 2011). Therefore, this value can be utilised for identification of the predator's food sources within an ecosystem (Vander Zanden et al., 1997; Vander Zanden et al., 1998; Grey, 2006; Bond and Jones, 2009; Cucherousset et al., 2012). For example, δ^{13}C values can be used to trace the spatial origin of food sources used by consumers in aquatic systems (Bertrand et al., 2011). When used collaboratively, carbon and nitrogen isotopes are considered to be reliable tools for determining dietary habits of a species over an extended time scale (Cucherousset et al., 2012; Grey, 2006; Rybczynski et al., 2008). The temporal scale assessed using Stable isotope analysis varies, mostly depending on the type of tissue extracted for analysis (DeNiro and Epstein, 1978; Fry et al., 1999; Hill and McQuaid, 2009).

Stable isotopes analysis is capable of reflecting shifts in a consumer's preferred food sources (which may arise when fish migrate from one habitat to another, or from ontogenic changes in feeding habits) through isotopic turnover, the rate of which is dependent on the tissue type (e.g. blood, muscle or feathers), as well as the consumer's growth and metabolic rate (Fry and Arnold, 1982; Fry et al., 1999; Herzka, 2005; Layman et al., 2012). Turnover rates are higher in blood plasma compared to muscle tissue (Layman et al., 2012). For example, Buchheister and Latour (2010) found that carbon and nitrogen half-life ranged between 10 to 20 days in the liver, 22 to 44 days in blood, and 49 and 107 days in muscle tissue of the summer flounder (*Paralichthys dentatus*). The rate of isotopic turnover is also higher in young, fast-growing individuals, rather than in large mature specimens (Herzka, 2005).

For this particular project, stable isotope ratios of *M. salmoides* and its possible food sources in the lower Kowie River were obtained in collaboration with other researchers who were conducting their research in the same system. There was a particular focus on the three main indigenous estuary-associated fish species (*M. falciformis, R. holubi and M. capensis*) which were identified by both Weyl and Lewis (2006) and Wasserman et al. (2011) as the main prey of the bass in the Kowie River. Other prey such as the fish *Glossogobius callidus,* the crab *Potamonautes sidneyi* and members of the insect families Aeshnidae, Libellulidae and Coenagronidae, were also collected as these were observed in *M. salmoides* stomachs in this project.

2. MATERIALS AND METHODS

2.1. Sampling sites for *M. salmoides*

All the samples for this study were collected from the lower Kowie River, Eastern Cape, South Africa. The four sampling sites are shown in Figure 2.1. The first site (FW0) is located the furthest upstream, situated adjacent to a road causeway. It consists of a deep pool with overhanging trees on either bank (Figure 2.2). The second site (FW1) is located upstream of a five metre high concrete weir which has a fish ladder constructed on its downstream side to permit juvenile fish movement up into the catchment (Figure 2.3). The site consists of a deep pool with overhanging trees on either bank. A second deep pool is located immediately downstream of the weir. Site FW2 is located directly above a road causeway which is completely submerged during periods of spring high tide. This site comprised a longitudinal pool with emergent macrophytes on both banks (Figure 2.4). The fourth and final site (FW3) is located approximately 500 metres from site FW2, positioning it furthest downstream in relation to all sites. The channel at this site is wide, with banks being occupied by both shrubs and trees (Figure 2.5).

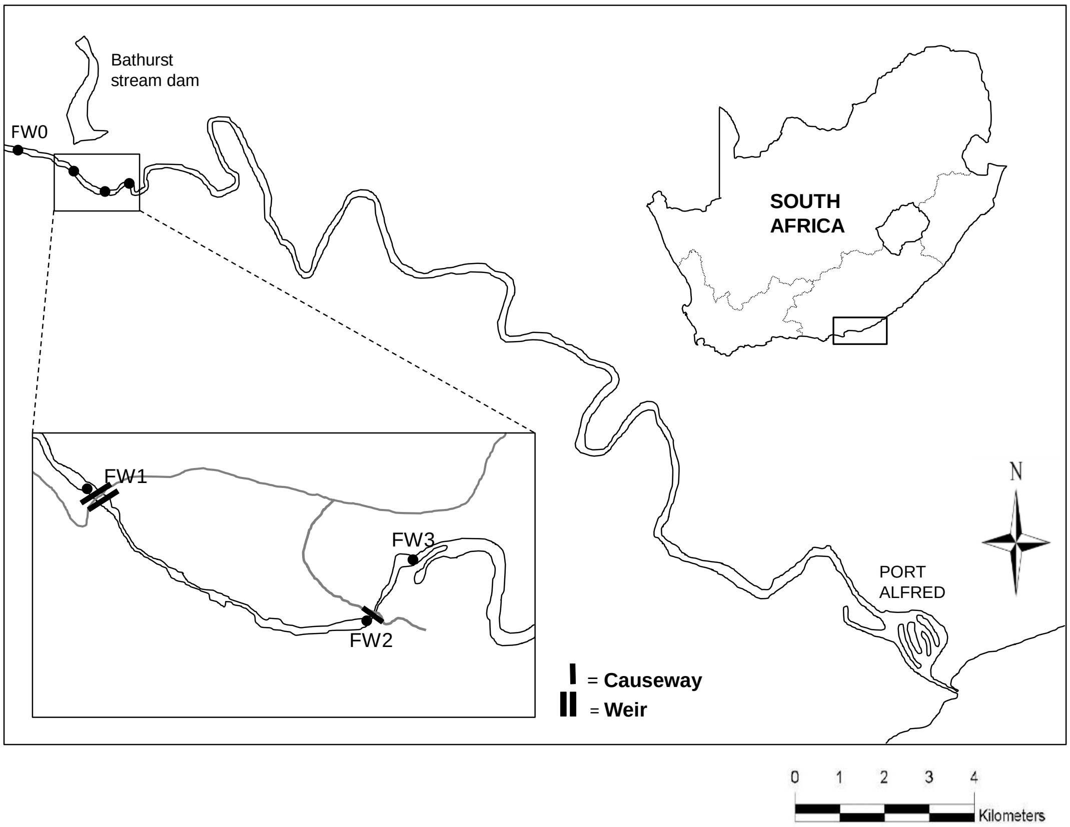

Figure 2.1: Map showing the location of sampling sites FW0 to FW3 in the Kowie River and Estuary, Eastern Cape, South Africa. Also shown are the locations of the weir and the causeway.

Figure 2.2: A view from downstream of site FW0 showing macrophytes and overhanging trees on either side of a deep pool.

Figure 2.3: Side-view of the weir located immediately below site FW1 during high river flow conditions (Picture by AK Whitfield).

Figure 2.4: Lateral view of the lower causeway during low tide in the estuary (to the left of the photograph) with the lower section of site FW2 on the right.

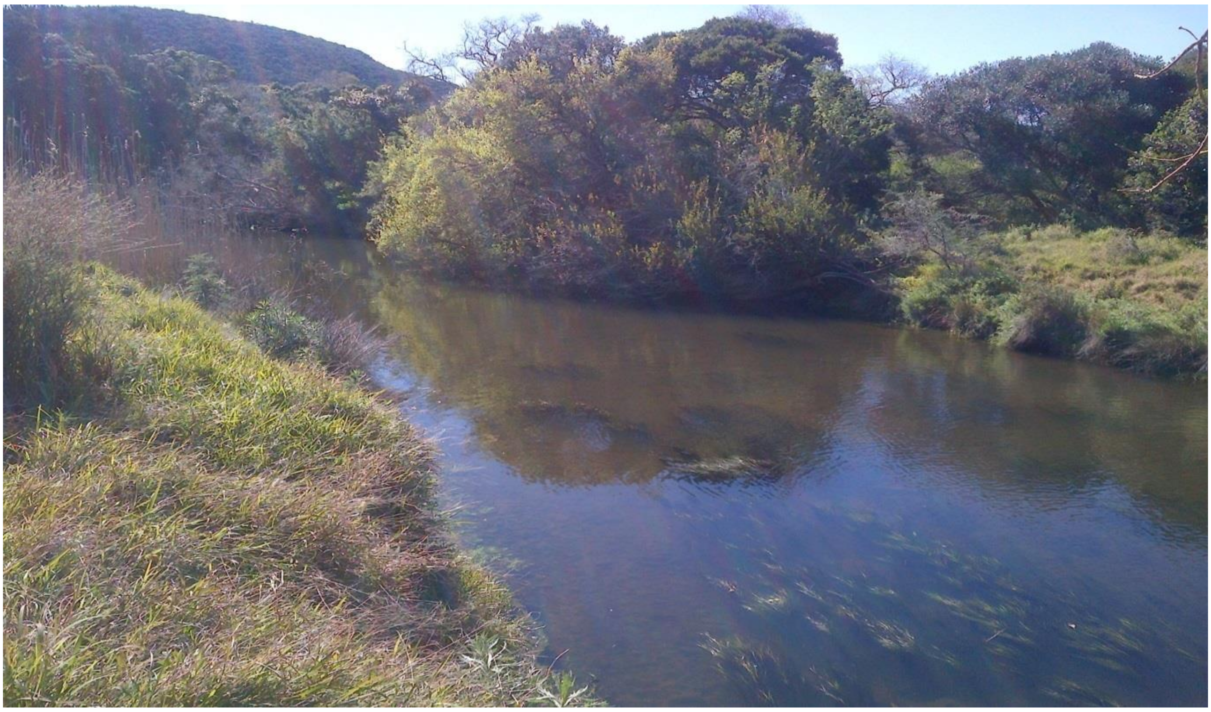

Figure 2.5: A view upstream of site FW3 during a period of relatively high river flow.

2.2. Sample collection

M. salmoides, M. capensis, M. falciformis and *R. holubi* were collected quarterly (once every three months) with a combination of seine nets, gill-nets, dip-nets, cast-nets and conventional rod-fishing. Gobies (*Glossogobius callidus*) were collected were collected by hauling a purse seine net (50 m long x 2 m deep with a 3 cm stretch mesh in the wings and 1 cm stretch mesh in the bag) in a direction perpendicular to the water flow (Leandro Bergamino, unpublished data). Three replicates of macro-invertebrates (*Potamonautes sidneyi*, Aeshnidae, Libellulidae and Coenagronidae) were hand-collected from each site (Sydney Moyo, unpublished data). The time interval between sampling periods was selected so as to allow for the collection of representative samples during all four seasons between April 2012 and March 2013, in order to elucidate seasonal variation in diet. The field sampling, from henceforth occasionally referred to as sampling seasons (C), took place in April 2012 (C1), August 2012 (C2), November 2012 (C3) and February 2013 (C4). Difficulty was experienced in obtaining specimens from all sites, and it was only possible to successfully obtain specimens from all four sites during the fourth sampling season.

Fish were euthanized immediately after capture by immersion in a container filled with ice and then transported back to the Rhodes University laboratory for further analysis. All samples were collected following ethical regulations for collection of animal samples (Rhodes University, Department of Zoology and Entomology Ethics Clearance ZOOL-02-2012, SAIAB Ethics Clearance 2012/04). Due to a failure to obtain sufficient samples with gill nets during the second sampling session (C2, 30 July to 3 August 2013), a fishing competition was organised and fishermen collected the required number of samples using fishing rods. This event took place on 26 August 2012 and involved students from the Ichthyology Department at Rhodes University.

2.3. Laboratory work

2.3.1. Dissections

In the laboratory, fish (*M. salmoides, M. falciformis* and *R. holubi*) were measured (mm) to standard length (SL) and total length (TL), weighed (g) on a Mettler Toledo XP205 electronic scale, and then individually labelled and frozen at -20°C for later processing. All fish dissections occurred within a few weeks of the actual collection date. Stomachs of the predator *M. salmoides* were extracted and then fixed in 10% formalin for a minimum period of seven days before being transferred to 70% ethanol.

For each fish specimen, a total of three pieces of dorsal muscles were removed. The tissue type was selected based on guidelines provided by Budge et al. (2006), as well as for comparisons with SIA studies in the literature, which usually rely on analyses of fish dorsal musculature (Hansson et al., 1997; Pinnegar and Polunin, 1999; Perga and Gerdeaux, 2005; Murdoch et al., 2013). For insects, the whole organism was utilised for stable isotopes analyses, while only muscle tissue extracted from the crabs.

Stable isotope samples were stored in sterilized aluminium foil envelopes, and frozen at -80°C before analysis. The aforementioned envelopes were sterilized by burning in a muffle furnace for 5 hours at 450˚C in order to destroy all traces of organic matter. The samples were then lyophilized with a VirTis BenchTop 2K freeze dryer at -60°C for a minimum of 30 hours. Dried samples were then homogenized by grinding with an ethanol-cleaned mortar and pestle in preparation for stable isotope analyses. Care was also be taken to avoid contamination during all fish dissection process, by using latex gloves and cleaning the dissection board and all utensils used for muscle extraction with 100% ethanol.

2.3.2. Stomach contents

Taking into account the reported seasonal and size-related variations in largemouth bass diet (Clady, 1974; Olson, 1996; Weyl and Lewis, 2006; Weyl and Hecht, 1999; Wasserman et al., 2011), the preserved stomach contents were analysed separately for three size classes of fish for each sampling season: small, medium and large. The fish size classes were determined based on the overall length distribution of fish collected, as well as trends in the dietary composition with fish size observed during the early stages of this study.

Stomach fullness was visually rated as empty, 25%, 50%, 75% or 100% full (Frost and Went, 1940; Wasserman et al., 2011). Stomachs were then cut open and the contents emptied onto a petri dish. The prey items were sorted under a dissecting microscope, identified to the lowest possible taxonomic level, and counted (when appropriate). Unidentified organic matter was not included in calculations of prey relative abundance, as it was not possible to count items individually. Aquatic insects were identified based on Gerber and Gabriel (2002), other invertebrates based on Day (1981), while fish prey were identified using Whitfield (1998).

The volumetric contribution of the different prey categories was then determined for each fish size class and sampling period. A graduated volumetric cylinder was partially filled with water, and the water volume (ml) recorded. Thereafter, the content of the different prey categories were successively added to the cylinder, and their respective volume, as well as the total prey volume, recorded. The volume of each prey category was measured based on their respective displacement volume, by subtracting the initial water volume from the volume obtained after adding each prey category (Pinkas et al., 1971; Hyslop, 1980). Prey items were then filtered onto a fine mesh sieve and transferred back into their respective vials for later gravimetric analysis. For gravimetric analyses, contents of the different prey categories were individually dried in an oven for a minimum period of 12 hours. A drying temperature of 50°C was selected, after considering the possible effect of higher temperatures on carbon content in organic samples (Hyslop, 1980). The dried samples were then weighed using a Mettler Toledo XP205 analytical balance to the nearest milligram.

2.3.3. Preparation of stable isotopes samples

Individual homogenised samples were weighed to approximately 1 mg on a Mettler Toledo XP205 analytical balance and placed in 8×5 mm tin capsules. The capsules were rolled into spheres, placed on a 96 well culture plate and sent to the Rhodes University Department of Botany for mass spectrometry in a Europa Scientific 20-20 IRMS linked to an ANCA SL Prep Unit (analyses performed by Dr Sven Kaehler, IsoEnvironmental cc).

In order to determine if there was any significant effect of lipid presence in isotope samples of *M. salmoides*, lipid extraction was performed on randomly selected samples. This was done by adding a 2:1 chloroform and methanol solution into a test tube containing the isotope sample. The test tubes were capped and placed in a fume cabinet for a minimum of two hours. Thereafter, the 2:1 solution was pipetted out and discarded. The operation was repeated twice to ensure maximum lipid extraction. Samples were then placed uncapped in an oven at 50˚C for a minimum period of 24 hours. The dry samples were removed from the test tubes and re-homogenized with a clean mortar and pestle. A fraction of the sample was then weighed to ±1mg on a Mettler Toledo XP205 analytical balance. These samples were then placed in 8×5mm tin capsules for comparison with *M. salmoides* samples that had not undergone the lipid extraction process.

2.4. Data analysis

2.4.1. Stomach contents data

A length frequency chart was constructed in order to visualise the frequency distribution of the three size classes of fish. For stomach contents analysis, the following equation was utilized to express prey abundance and occurrence:

$$\mathbf{IRI = (\%M + \%V) \times \%\,F}$$

Where:

IRI is the Index of Relative Importance; **%V** is the volume of each food category expressed as a percentage of the total volume of all food items; **%M** is the mass of all individuals in each food category expressed as a percentage of the total mass of all food categories; and **%F** is the number of stomachs containing a certain food item expressed as a percentage of all stomachs in the sample, excluding empty stomachs.

For each prey category, the %IRI was determined as a proportion of the IRI values of all prey categories (i.e. %IRI= (IRI / IRItotal) × 100; Wasserman et al. 2011). For purposes of this particular thesis, data analysis was primarily focused on the %IRI rather than the individual frequency of occurrence, numerical, volumetric and gravimetric values. As previously discussed in the introduction, this compound index, which encompasses all the aforementioned simple indices, is considered to be a more reliable indicator of diet than the individual indices. A size frequency distribution was also constructed for fish collected at each of the four freshwater sites (FW0-FW3) in order to elucidate variation in *M. salmoides* size per site. Samples from site FW3 mainly consisted of small sized fish. Analysis of Variance (ANOVA) ($P = 0.05$) was used to test for significance of variance in stomach fullness between fish collected from the four sites as well as variation between the sampling seasons.

2.4.2. Stable isotopes data

Stable isotope ratios were determined using the following formula:

$$\delta \mathbf{X} = [(\boldsymbol{R}_{\mathbf{sample}}/\boldsymbol{R}_{\mathbf{standard}}) - 1] \times 10^3$$

Where **δX** is $\delta^{13}C$ or $\delta^{15}N$, $\boldsymbol{R}_{\mathbf{sample}}$ is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ and $\boldsymbol{R}_{\mathbf{standard}}$ is the value of the relevant standard (PeeDee limestone for $^{13}C/^{12}C$, and atmospheric air for $^{15}N/^{14}N$). ANOVA was applied to test for variation in isotope signatures of *M. salmoides* between sites, seasons and size classes, after validating conditions of data normality. Linear regression was used to investigate the relationships between $\delta^{13}C$, $\delta^{15}N$ and C:N, according to fish size. The effect of lipid content on isotope signatures was tested using a one-way ANOVA. Two-way ANOVA was employed to test for any synergistic effect of season and site on isotope values. All data was tested for normality and homogeneity of variance prior to analysis. All ANOVA tests were conducted using STATSOFT STATISTICA v10.0 software package. Due to significant variation in the $\delta^{15}N$ and $\delta^{13}C$ values of *M. salmoides*, individual biplots representing food webs were constructed for each site.

The Bayesian based mixing model, Stable Isotope Analysis in R (SIAR) v4.0 package (Parnell et al., 2010) was used to estimate the contributions of each prey item to the diet of *M. salmoides* using $\delta^{13}C$ and $\delta^{15}N$ values. The strength of this particular model, compared to older models such as IsoSource, lies in its integration of uncertainty and variation in parameters such as source isotopic values and trophic enrichment factors (Jones et al., 2010; Parnell et al., 2010). Due to size class, seasonal and spatially related variation in carbon and nitrogen isotope values, the SIAR model was also run using data collected under the aforementioned parameters. The data entered into the model were the $\delta^{15}N$ and $\delta^{13}C$ of consumers, as well as the mean $\delta^{15}N$ and $\delta^{13}C$ values and standard deviations of potential prey. Following suggestions in McCutchan et al. (2003), the Trophic Enrichment factors used were 2.8±0.40‰ for $\delta^{15}N$ and 1.1±0.35‰ for $\delta^{13}C$. Isotope data of individuals belonging to the order Odonata were pooled in order to overcome the reported weaknesses of Bayesian-based mixing models when dealing with a very high number of sources (Fry, 2013).

3. RESULTS

A total of 67 *M. salmoides* were sampled from the four freshwater sites, including 20 small fish (≤150 mm TL; Mean TL=101.5 mm), 18 medium fish (151-300 mm TL; Mean TL= 249.17) and 29 large fish (301-420 mm TL; Mean TL=342.2) (Figure 2.6). Most fish (41%) were collected at site FW1, and most large fish at sites FW1 and FW2 (Table 2.1). A total of 10 fish were collected during April 2012, 19 during August 2012, 5 during November 2012 and 33 during February 2012. The total number of fish collected during the different seasons and at the different sites is shown in table 2.1.

3.1. Stomach contents analysis

3.1.1. Stomach fullness

Small and medium fish had the lowest (14%) and highest (40%) percentage of empty stomachs, respectively. There was no significant variation in mean stomach fullness between the four sampling seasons (ANOVA: F = 0.77579, P > 0.05). Fish collected at site FW0 had the highest stomach fullness when compared to the other sites (ANOVA: F = 3.0862, P < 0.05). Total stomach fullness as well as the variation in stomach fullness between seasons, sites and size classes is shown in figure 2.7.

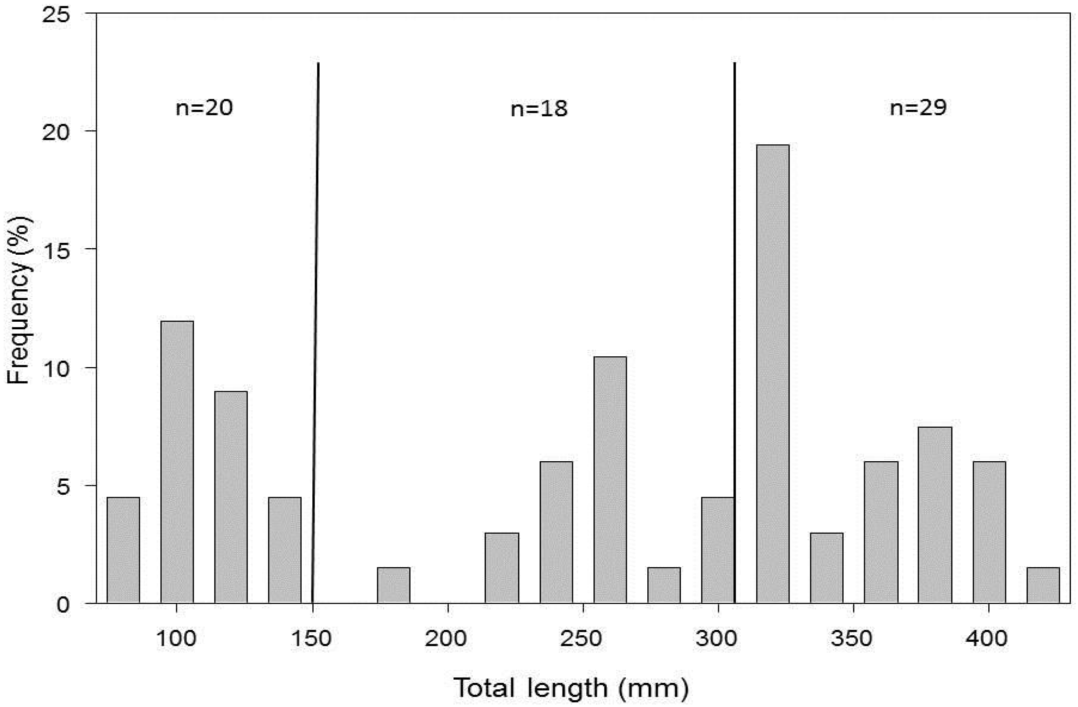

Figure 2.6: Length frequency distribution of largemouth bass, *Micropterus salmoides*, sampled at the lower Kowie River, Eastern Cape, South Africa. Fish were divided into small (≤150 mm TL), medium (151-300 mm TL) and large (301-420 mm TL) size groups.

Table 2.1: Number of fish collected at each site per sampling session showing the minimum and maximum Total Length (mm) as well as the total number of fish.

		FW0	**FW1**	**FW2**	**FW3**
April 2012	Number of fish	0	2	8	0
	Minimum TL	0	135	219	0
	Maximum TL	0	205	337	0
August 2012	Number of fish	0	18	1	0
	Minimum TL	0	253	313	0
	Maximum TL	0	400	313	0
November 2012	Number of fish	0	0	3	2
	Minimum TL	0	0	110	107
	Maximum TL	0	0	175	115
February 2013	Number of fish	8	9	9	7
	Minimum TL	71	228	295	79
	Maximum TL	138	355	402	128
Total fish		8	29	21	9

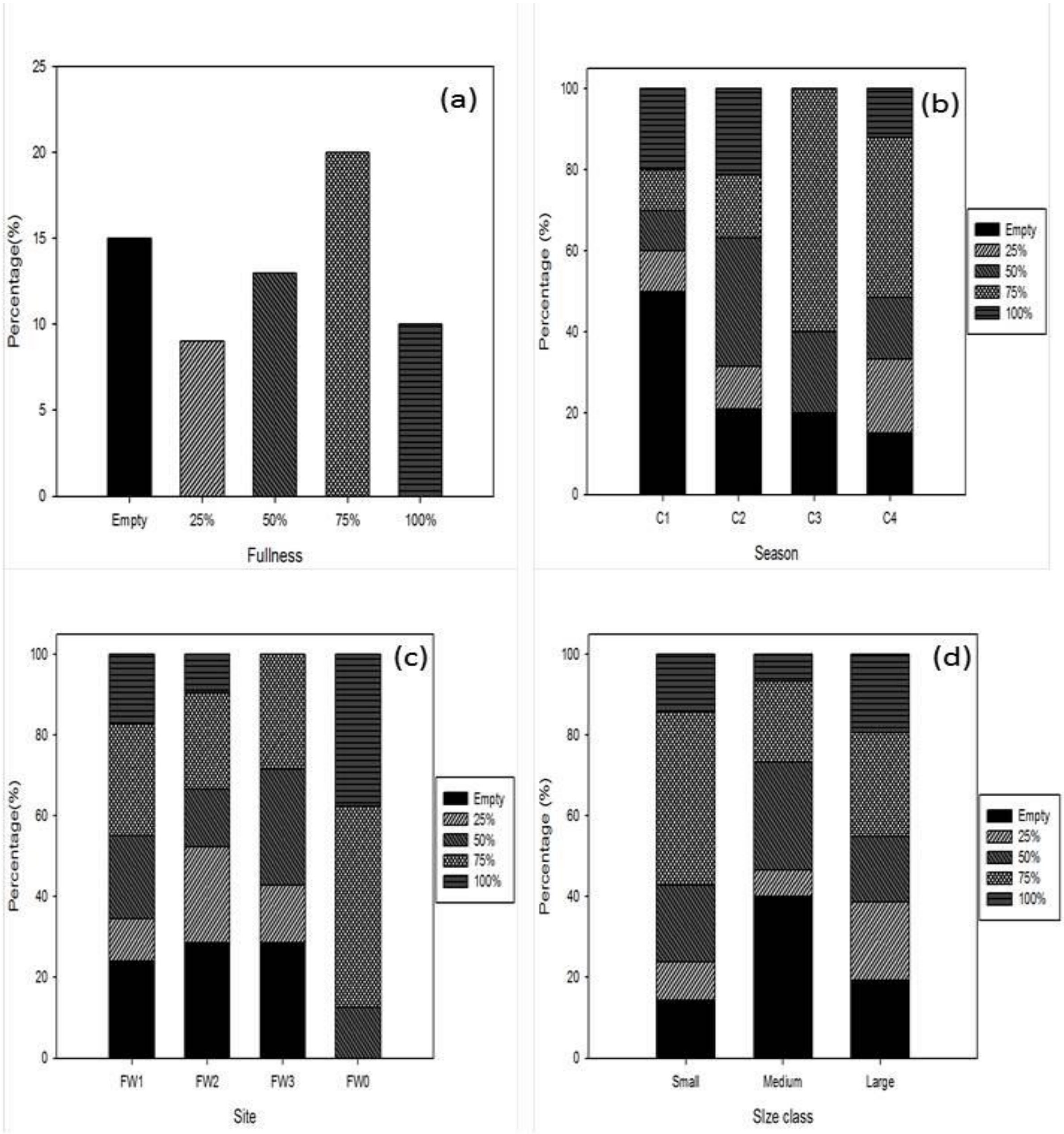

Figure 2.7: Stomach fullness index, of all *Micropterus salmoides* (a), *M. salmoides* collected at each season site (b), each site (c), and per fish size class (d).

3.1.2. General and fish size class related composition of stomach contents

A total of 17 dietary taxa were recorded during the stomach contents analysis. Based on the %IRI, the freshwater crab *Potamonautes sidneyi* was the dominant prey item of *M. salmoides* (Table 2.2), with insects from the family Aeshnidae dominating in terms of both %N and percentage frequency of occurrence. The second most important item in the diet of the largemouth bass was the Cape moony (*Monodactylus falciformis*).

3.1.3. Size and season related composition of stomach contents

A summary of the variation in %IRI between seasons, size classes and sites is presented in table 2.3. Diet composition comparisons between the three size classes of *M. salmoides* indicated that the freshwater mullet dominated the diet of small specimens when compared to the other two size classes. A total of 12 prey taxa were recorded in the stomachs of small sized largemouth bass. Medium sized fish only had five recorded prey taxa and displayed increased cannibalism when compared to the other two groups, with their diets dominated by juvenile *M. salmoides*. Out of the 12 prey taxa detected in the stomach contents of large sized *M. salmoides*, the crab *P. sidneyi* was the most important. Only *M. capensis, M. falciformis* and *P. sidneyi* were common prey in the stomachs of fish belonging to all the size classes.

Data for the seasonal variations in stomach contents is shown in the appendix (Table A1 is for fish collected during April 2012, table A2 for fish collected during August 2012, table A3 for fish collected during November 2012 and table A4 for fish collected during February 2013). Seasonally, *M. faciformis* dominated the diet of fish collected during April 2012, whereas *P. sidneyi* was the most important prey item in August 2012. Freshwater mullet *M. capensis* was dominant during November 2012, while *Caffrogobius* spp. were the most important prey item during February 2013. Crabs were found in 78.9% of fish collected during spring, a period following a severe flood.

3.1.4. Longitudinal variation in composition of stomach contents

Fish dominated the diets of *M. salmoides* collected from site FW2 and site FW3. Insects (Aeshnidae) dominated the diet of fish collected at site FW0. The diet of *M. salmoides* collected at site FW1 was dominated by *P. sidneyi.* The crustacean *P. sidneyi* was not recorded in the stomachs of fish found at the uppermost (FW0) and lowermost (FW3) sites. They were most prevalent in the stomachs of *M. salmoides* collected from site FW1 and only three were found in the stomach of individuals from site FW2. Cannibalism was only observed in *M. salmoides* individuals collected at sites FW1 and FW0. The freshwater mullet *M. capensis* was the only estuary-associated fish species that was recorded in the stomach contents of individuals from all four sampling sites. The detailed data containing all the individual indices employed in the calculation of the %IRI for *M. salmoides* collected from the four individual sites is presented in tables A8 to A11 in the appendix.

Table 2.2: Pooled stomach contents analysis data for 67 *Micropterus salmoides* collected between April 2012 and February 2013 in the lower Kowie River. %N = the number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of stomachs containing a particular food item expressed as a percentage of all stomachs sampled; %M = the mass of each food category expressed as a percentage of the total mass of all food categories; %V = the volume of each food category expressed as a percentage of the total volume of all food items; %IRI = index of relative importance, as a proportion of the total IRI of all prey items.

		Prey category		**Prey abundance, occurrence and importance**					
Class	**Order**	**Family**	**Species**	**N**	**%N**	**%F**	**%M**	**%V**	**%IRI**
Actinopterygii	Mugiliformes	Mugilidae	*Myxus capensis*	8	3.4	11.9	1.5	0.9	0.88
	Perciformes	Centrachidae	*Micropterus salmoides*	6	2.6	6.0	5.9	8.8	2.75
		Gobiidae	*Caffrogobius* sp.	2	0.9	3.0	0.6	2.9	0.33
			Glossogobius callidus	3	1.3	4.5	0.8	4.7	0.76
		Monodactylidae	*Monodactylus falciformis*	10	4.3	13.4	11.9	11.7	9.96
		Sparidae	*Rhabdosargus holubi*	1	0.4	1.5	0.8	4.7	0.26
Amphibia	Anura			1	0.4	1.5	4.5	8.4	0.60
Insecta	Hemiptera	Belostomatidae		1	0.4	1.5	0.0	0.2	0.01
		Naucoridae		3	1.3	3.0	0.1	0.3	0.04
	Odonata	Aeshnidae		54	23.2	10.4	0.6	1.5	0.69
		Coenagrionidae		1	0.4	1.5	0.0	0.0	0.002
		Libellulidae		3	1.3	3.0	0.3	1.1	0.13
Malacostraca	Decaopoda	Potamonautidae	*Potamonautes sidneyi*	32	13.7	26.9	47.7	49.9	82.21
Other			Fish remains	1	0.4	1.5	0.2	0.3	0.02
			Plant leaves	4	1.7	7.5	0.4	0.8	0.29
			Stones	2	0.9	1.5	0.4	0.6	0.05
			Unidentified organic matter	-	-	4.5	0.4	1.3	0.24

Table 2.3: Index of relative importance (%IRI) of prey observed in *M. salmoides* stomachs collected in the lower Kowie River between April 2012 and February 2013. Details for all other indices (%N, %F, %M, %V) are provided in the Appendix (table A1 to table A11).

	Months				**Size classes**			**Sites**			
Prey type	**April**	**August**	**November**	**February**	**Small**	**Medium**	**Large**	**FW0**	**FW1**	**FW3**	**FW4**
Myxus capensis	-	-	82.01	1.37	41.01	1.89	0.09	2.48	0.07	0.81	73.78
Micropterus salmoides	-	-	-	12.11	1.27	33.08	-	4.01	2.94	-	-
Caffrogobius sp.	-	-	-	0.25	-	-	11.35	-	0.23	58.90	-
Glossogobius callidus	-	-	-	53.31	5.63	-	-	17.63	-	-	-
Monodactylus falciformis	68.99	-	-	25.91	6.06	24.66	6.12	-	2.26	23.29	25.25
Rhabdosargus holubi	19.25	-	-	0.03	-	-	0.24	-	0.36	1.37	-
Anura	-	0.91	-	-	-	-	1.01	-	-	-	-
Belostomatidae	1.72	-	-	-	1.14	-	-	-	0.02	-	-
Naucoridae	3.44	-	-	-	1.14	-	0.01	-	0.02	0.08	-
Aeshnidae	6.59	-	-	3.30	29.57	-	0.07	74.57	-	1.43	-
Coenagrionidae	-	-	-	-	0.16	-	-	-	-	-	0.60
Libellulidae	-	0.02	-	0.02	-	9.01	0.02	-	0.02	0.84	-
Potamonautes sidneyi	-	98.54	-	3.59	11.13	31.36	80.64	-	93.57	13.22	-
Fish remains	-	-	17.99	-	2.40	-	-	-	-	-	-
Plant leaves	-	0.22	-	0.10	0.39	-	0.13	1.30	0.21	0.06	-
Stones	-	0.07	-	-	-	-	0.08	-	0.07	-	-
Unidentified organic matter	-	0.23	-	0.01	0.09	-	0.25	-	0.22	-	0.37

3.2. Stable isotope data

3.2.1. Stable isotope values of the consumer and food sources

The comparison between lipid-extracted and non-lipid-extracted isotope samples revealed no significant differences in isotope values (F = 1.80, P = 0.20). Based on this result, only bulk (non-lipid extracted) isotopes samples were analysed further. When all the data was pooled, *M. salmoides* had the most enriched $\delta^{15}N$ value compared to its potential food sources (Mean ± SD; 20.68 ± 1.56‰). The freshwater crab, *P. sidneyi*, exhibited the most depleted $\delta^{13}N$ value (13.2217 ± 1.64‰) compared to all prey samples collected during the course of this study (Figure 2.8). At site FW1, *M. falciformis* had the most enriched $\delta^{15}N$ (21.00 ± 0.47‰), while *M. salmoides* possessed the most enriched $\delta^{15}N$ values at sites FW2 (20.49 ± 1.17‰), FW3 (21.18 ± 0.50‰) and FW0 (23.19 ± 1.06). Libellulidae had the most depleted $\delta^{15}N$ values as sites FW1 (15.74 ± 0.56‰) and FW3 (8.69 ± 0.39‰) while *M. capensis* and *P. sidneyi* had the most depleted $\delta^{15}N$ values at sites FW2 (15.74 ± 3.78‰) and FW0 (15.36 ± 0.08‰), respectively (Figure 2.9).

Overall, *P. sidneyi* exhibited the most enriched $\delta^{13}C$ value (-23.35 ± 0.61‰) of all the prey items, while the Coenagronidae possessed the most depleted value (-27.37 ± 0.99‰). Amongst the sites, the $\delta^{13}C$ value of *M. salmoides* was most enriched at site FW1 (-25.90 ± 1.16), with *P. sidneyi* having the most enriched value at site FW0 (-19.05 ± 0.14‰). The freshwater mullet, *M. capensis,* had the most enriched $\delta^{13}C$ values at sites FW2 (-22.85 ± 1.72‰) and FW3 (-22.24 ± 2.63). The most depleted $\delta^{13}C$ values at sites FW1 (-29.00 ± 0.71), FW2 (-26.49 ± 0.95‰) and FW3 (-28.51 ± 1.40‰) belonged to Coenagronidae, with *M. capensis* having the most depleted $\delta^{13}C$ value amongst the prey organisms sampled at site FW0.

The $\delta^{13}C$ values of *M. salmoides* (Figure 2.10(a)) increased with fish size, while both the $\delta^{15}N$ values and C:N ratio (Figures 2.10(b) and (c)) showed a decreasing trend with increasing fish size. Two-way ANOVA revealed no significant synergistic effect of season and site on isotope values ($F = 0.011$, $P = 0.989$). One-way ANOVA revealed a significant difference in mean δ^{15}N of *M.* salmoides values between seasons ($F = 7.56$, $P < 0.056$), size classes ($F = 15.78$, $P < 0.05$), and sites ($F = 19.54$, $P < 0.05$).

Although significantly different, the mean δ^{15}N values for *M. salmoides* did not exhibit any discernible trend between the seasons. There was also a significant variation in mean δ^{13}C values between season ($F = 8.66$, $P < 0.05$), size class ($F = 14.34$, $P < 0.05$) and sites ($F = 8.91$, $P < 0.05$). The mean δ^{13}C values exhibited a decreasing trend from one sampling season to the next. The C:N ratio did not vary significantly between sites ($F = 1.7$, $P = 0.175901$) or between seasons ($F = 0.7$, $P = 0.54$) but slightly between the three size classes ($F = 6.0$, $P < 0.05$).

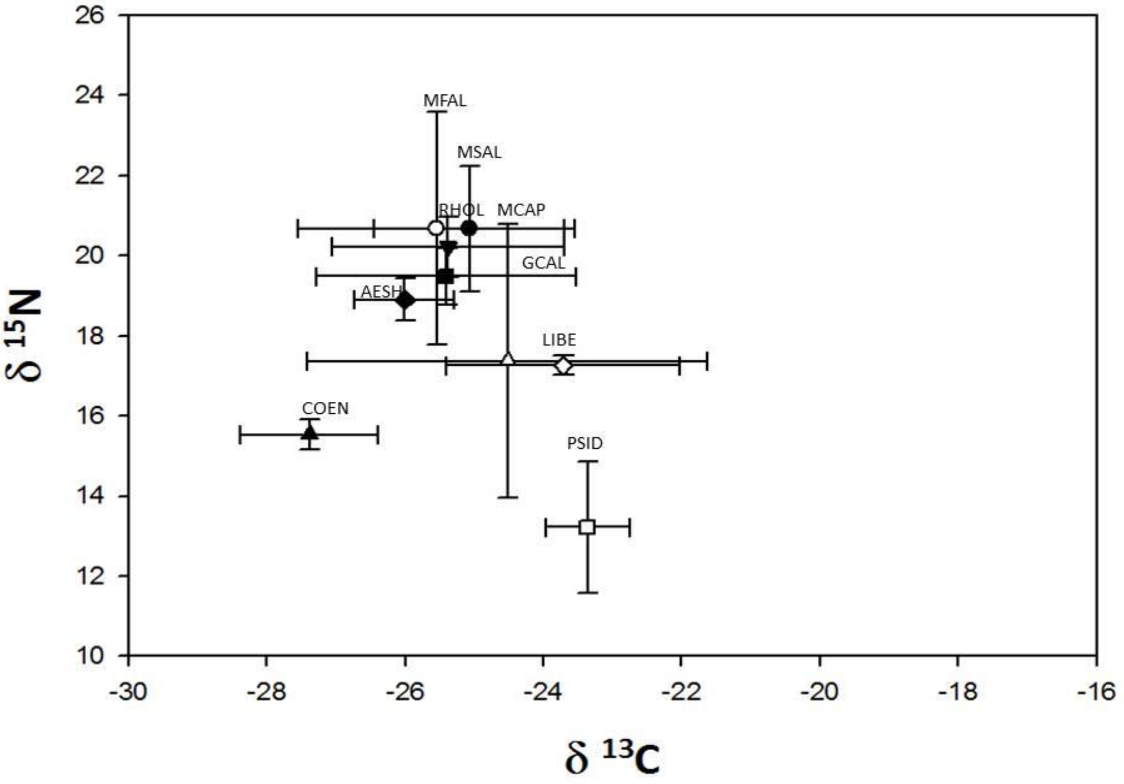

Figure 2.8: Mean (± SD) values of δ^{13}C and δ^{15}N for pooled *M. salmoides* and potential main prey items, collected between April 2012 and February 2013 in the lower reaches of the Kowie River (MSAL: *Micropterus salmoides*; MFAL: *Monodactylus falciformis*; MCAP: *Myxus capensis*; RHOL: *Rhabdosargus holubi*; GCAL: *Glossogobius callidus*; AESH: Aeshnidae; COEN: Coenagrionidae; LIBE: Libellulidae; PSID: *Potamonautes sidneyi*).

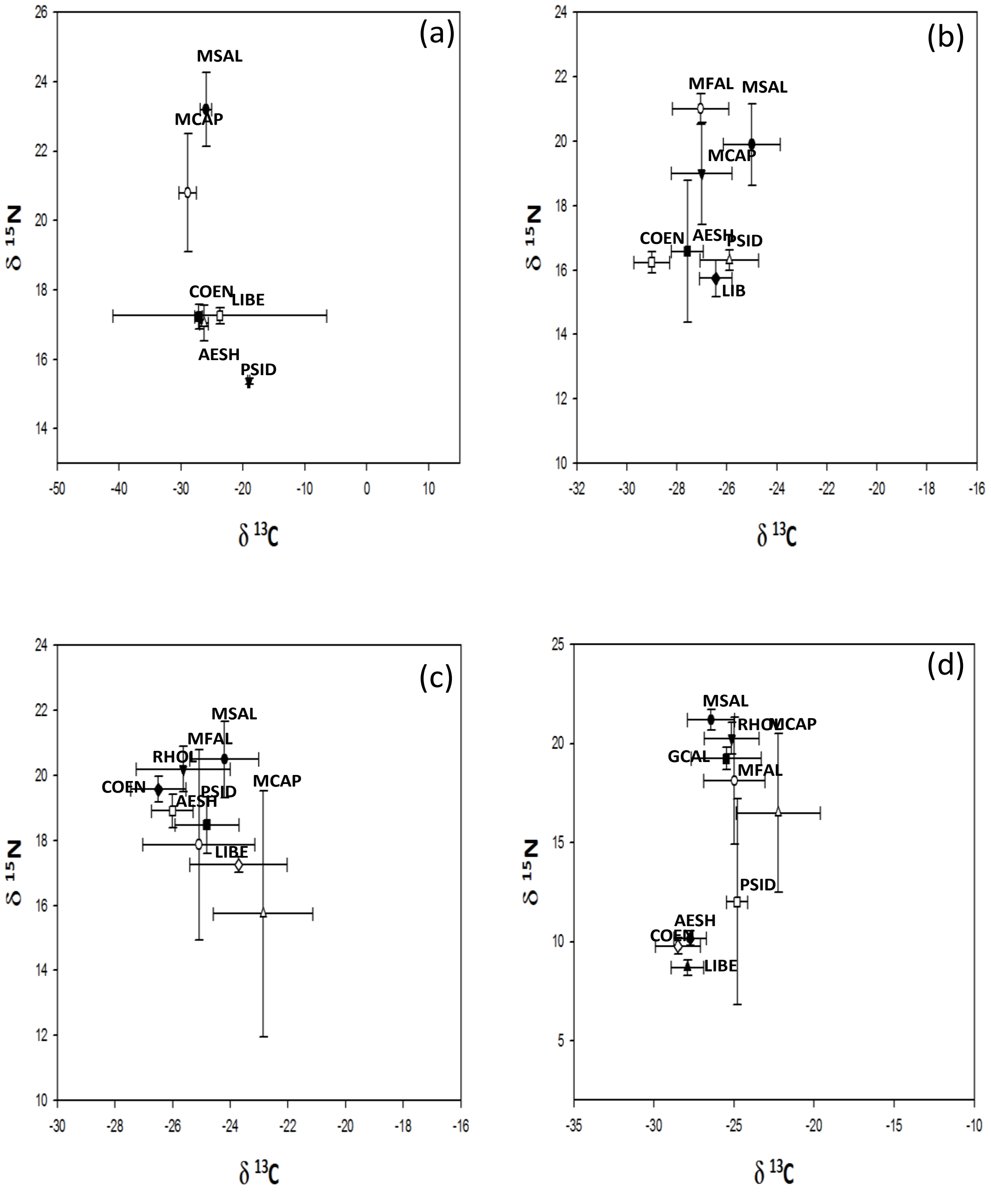

Figure 2.9: Mean (±SD) values of δ^{13}C and δ^{15}N for samples collected at sites FW0 (a), FW1 (b), FW2 (c) and FW3 (d) between April 2012 and February 2013 in the Kowie River (MSAL: *Micropterus salmoides,* MFAL: *Monodactylus falciformis*, MCAP: *Myxus capensis*, RHOL: *Rhabdosargus holubi*, GCAL: *Glossogobius callidus*, PSID: *Potamonautes sidneyi*, AESH: Aeshnidae, COEN: Coenagronidae, LIB: Libellulidae).

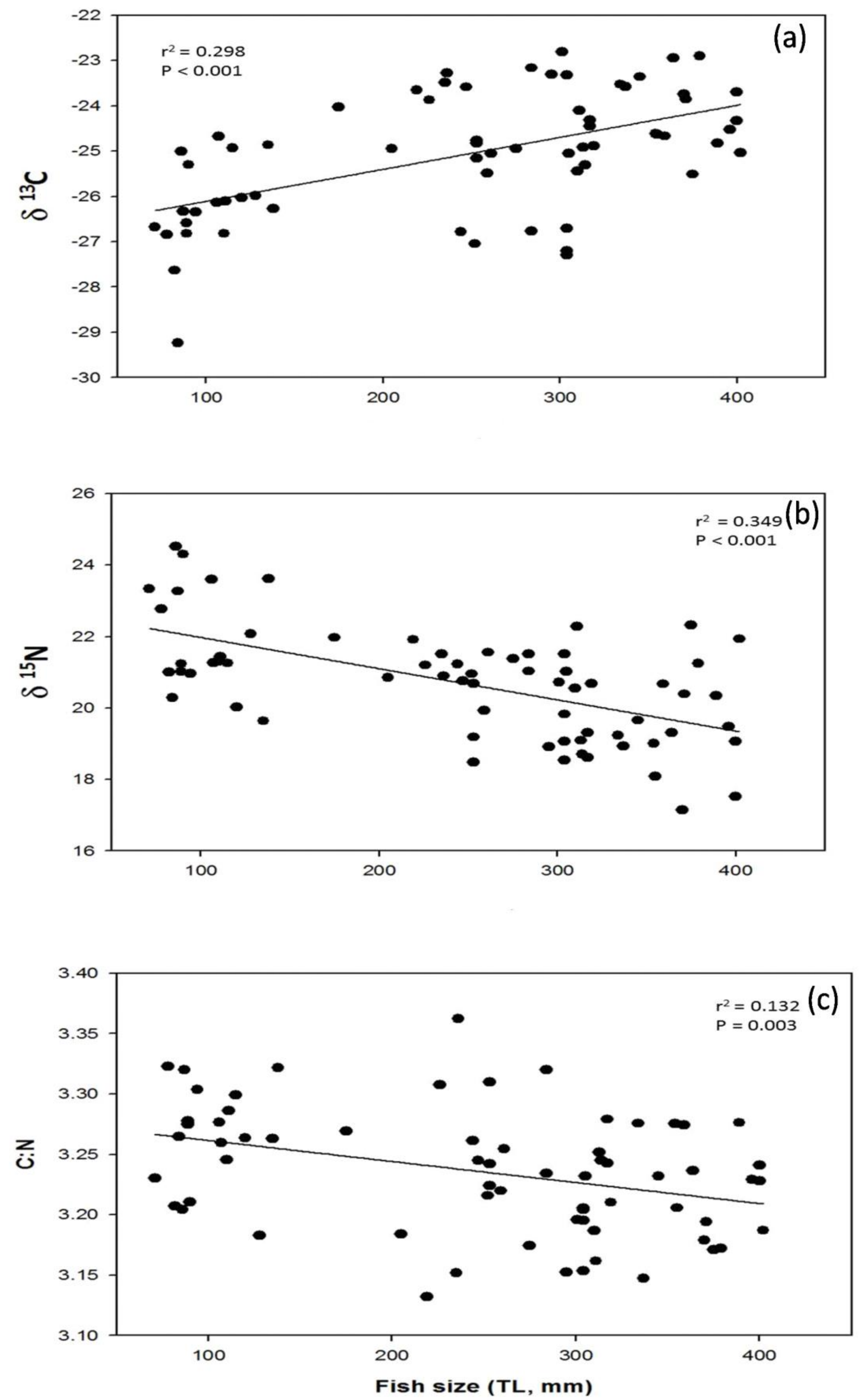

Figure 2.10: Linear regression plots illustrating the relationships of fish size to $\delta^{13}C$ (a), $\delta^{15}N$ (b) and the C:N ratio (c) for *M. salmoides* collected in the lower Kowie River.

3.2.2. Isotopic contributions of potential food sources to largemouth bass diet estimated through a statistical model

Results of the SIAR mixing model using pooled data (figure 2.11) suggest that Odonata form the highest proportion of the diet for *M. salmoides* (52.1%), followed by *M. falciformis* (17.9%) and *R. holubi* (12.4%). Data collected during the first sampling season (April 2012) (Figure 2.12(a)) suggests that *G. callidus* dominated the diet (43.4%), followed by Odonata (27.8%). *M. capensis* contributed 55.3% of the diet during the second sampling season (August 2012) (Figure 2.12(b)), with Odonata and *R. holubi* contributing 32.7% and 30.5% respectively. Gobies *G. callidus* (24.7%), Odonata (19.4%) and *R. holubi* (18.9%) were the three highest contributors to the diet of *M. salmoides* collected during the third season (November 2012) (Figure 2.12(c)). The diet of fish collected during the fourth season (February 2013) (Figure 2.12(d)) was dominated by Odonata (34.7%) and *M. capensis* (31.6%).

The model suggested that *R. holubi* (32.2%), *G. callidus* (24.9%) and *M. falciformis* (21.1%) were the major contributors to the diet of *M. salmoides* collected at site FW0 (Figure 2.13(a)). A fish and a crab species, *M. capensis* and *P. sidneyi,* were the highest contributors (38.9% and 37.4% respectively) to the diet of *M. salmoides* collected from site FW1 (Figure 2.13(b)) while *P. sidneyi* (25.4%), Odonata (23.3%) and *M. falciformis* (18.3%) made the highest contributions in fish collected at site FW2 (Figure 2.13(c)). The diet of fish from site FW3 (Figure 2.13(d)) was dominated by *R. holubi* (29.6%) and *G. callidus* (28.7%).

The diet of small sized *M. salmoides* comprised mainly *M. falciformis* (22.6%), *R. holubi* (22.1%) *G. callidus* (20.8%) and Odonata (18.7%) (Figure 2.14(a)). Odonata contributed the highest proportion (32.9%) to the diet of medium sized *M. salmoides* (figure 2.14(b)), followed by *M. falciformis* (16.7%) and *G. callidus* (15.7%). The model also suggested that 61% of the diet of large sized *M. salmoides* consists of Odonata (Figure 2.14(c)).

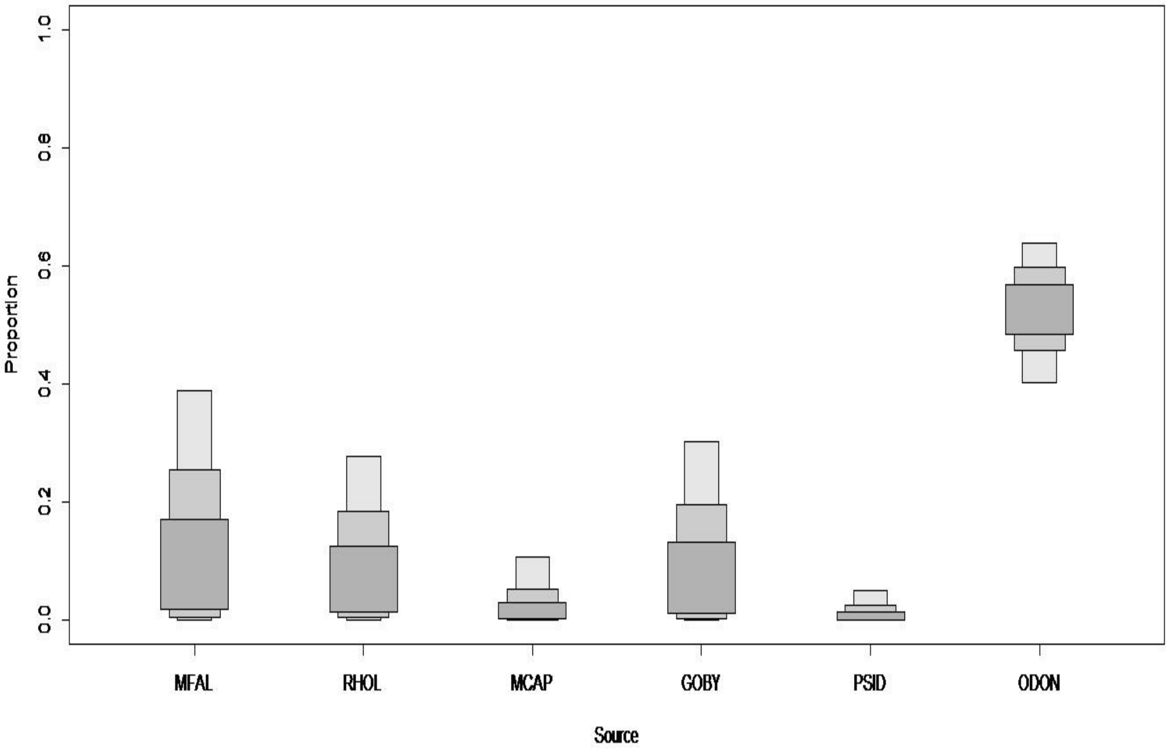

Figure 2.11: SIAR boxplot showing estimated proportions of source contributions to the diet of *M. salmoides* collected from April 2012 to February 2013 in the lower Kowie River. The widths of the bars show the 95, 75 and 50% credibility intervals (MFAL: *Micropterus salmoides*; RHOL: *Rhabdosargus holubi*; MCAP: *Myxus capensis*; GOBY: *Glossogobius callidus*; PSID: *Potamonautes sidneyi*; ODON: Odonata).

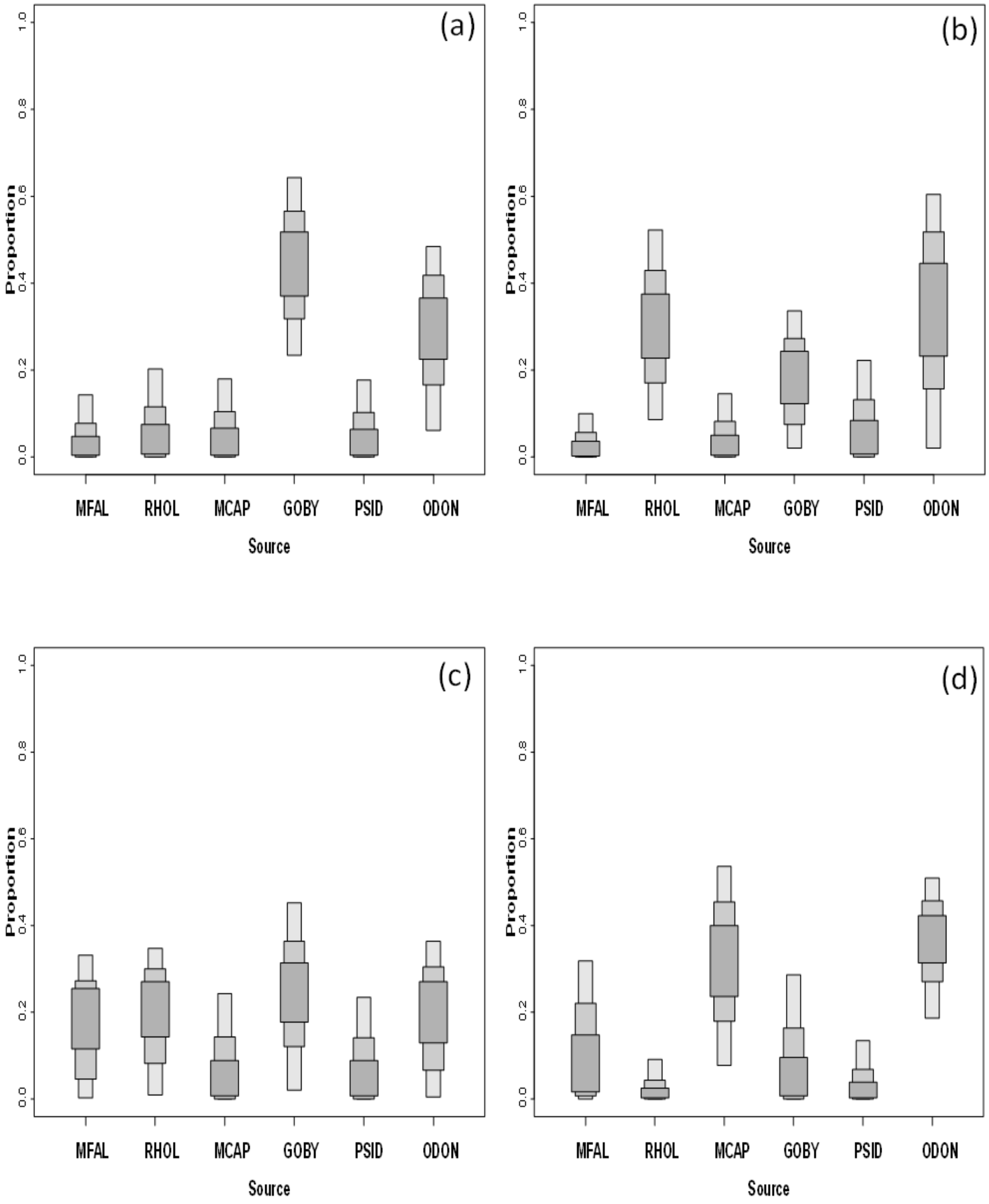

Figure 2.12: SIAR boxplot showing estimated proportions of source contributions to the diet of *M. salmoides* collected during April 2012 (a), August 2012(b), November 2012 (c) and February 2013 (d) in the lower Kowie River, Easter Cape, South Africa. Widths of bars show the 95, 75 and 50% credibility intervals. MFAL: *Micropterus* salmoides; RHOL: *Rhabdosargus holubi*; MCAP: *Myxus capensis*; GOBY: *Glossogobius callidus*; PSID: *Potamonautes sidneyi*; ODON: Odonata.

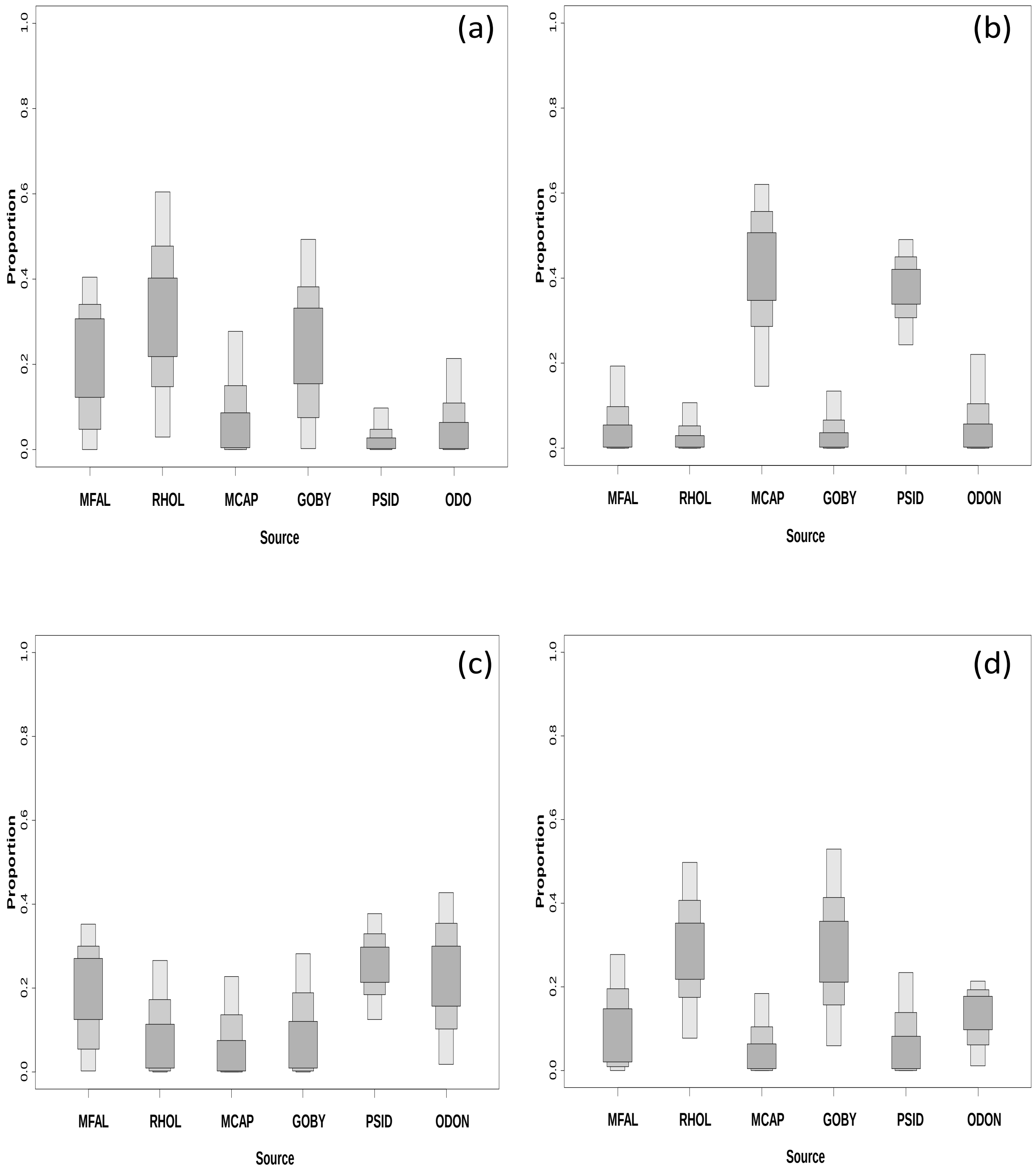

Figure 2.13: SIAR boxplot showing estimated proportions of source contributions to the diet of *M. salmoides* collected at sites FW0 (a), FW1 (b), FW2 (c) and FW3 (d) between April 2012 and February 2013 in the lower Kowie River, Easter Cape, South Africa. Widths of bars show the 95, 75 and 50% credibility intervals. MFAL: *Micropterus* salmoides; RHOL: *Rhabdosargus holubi*; MCAP: *Myxus capensis*; GOBY: *Glossogobius callidus*; PSID: *Potamonautes sidneyi*; ODON: Odonata.

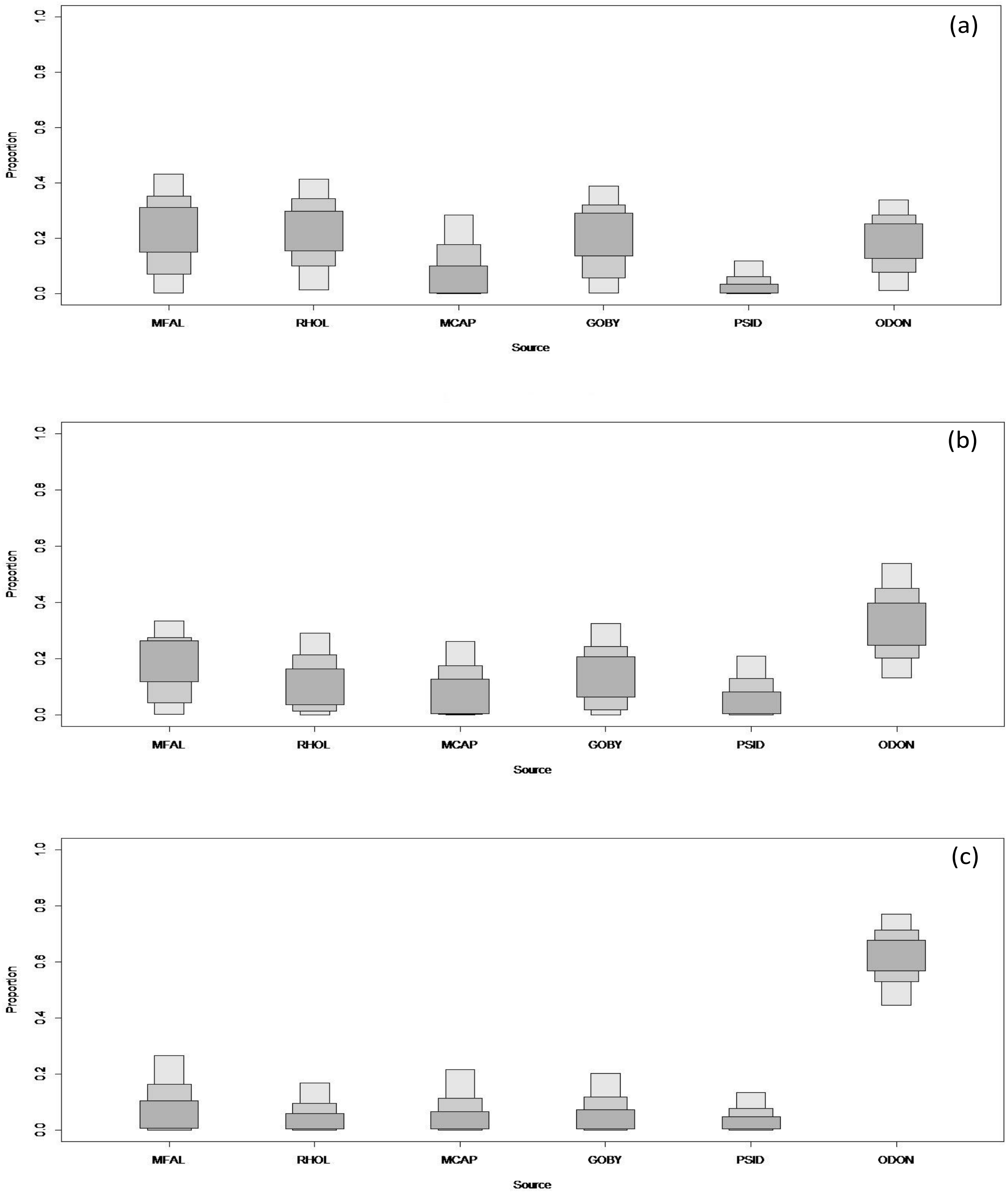

Figure 2.14: SIAR boxplot showing estimated proportions of source contributions to the diet of small (a), medium (b) and large (c) size-class *Micropterus salmoides* collected between April 2012 and February 2013 in the lower Kowie River, Easter Cape, South Africa. Widths of bars show the 95, 75 and 50% credibility intervals. MFAL: *Micropterus* salmoides; RHOL: *Rhabdosargus holubi*; MCAP: *Myxus capensis*; GOBY: *Glossogobius callidus*; PSID: *Potamonautes sidneyi*; ODON: Odonata.

4. DISCUSSION

Stomach content analysis (%IRI) from the pooled *M. salmoides* data suggests that this predatory fish has a particular preference for the freshwater crab, *P. sidneyi*. The dominance of *P. sidneyi* in the diet of largemouth bass is consistent with the results obtained by Wasserman et al. (2011) in the same river system. Based on stomach content analyses, the three estuary-associated fish species, *M. capensis, M. falciformis* and *R. holubi* only contributed 11% to the overall diet of largemouth bass. The mid-water swimming Cape moony was the most important prey fish species, suggesting that the population of *M. falciformis* was more impacted by largemouth bass relative to the other estuary-associated fish. In its native range of North America, fish (especially bluegill *Lepomis macrochirus*) form an integral part of the diet of largemouth bass (Olson et al., 1995; Olson, 1996; Keast, 1985). A study by Clady (1974) in a northern Michigan, USA, lentic system showed that fish form an important portion of large sized *M. salmoides*. Although fish form the dominant portion of its diet, it is not a strict piscivore as insects and aquatic invertebrates are also an integral part of the diet of young *M. salmoides* (García-Berthou, 2002; Wheeler and Allen, 2003).

The gobies *G. callidus* and *Caffrogobius* sp., constituted only 1.1% of largemouth bass diet, which suggests that these benthic cryptic fish species are not targeted as prey items by *M. salmoides*. It is also worth noting that, based on number of individuals, the Aeshnidae were the most abundant prey item of *M. salmoides*. Due to their small size and weight, Aeshnidae %IRI scores were not reflective of such numerical dominance. The biggest portion (98%) of these insects was found in the stomachs of small sized individuals. In Eastern Africa, Hickley et al.(1994) found that insects were an important item in the diet of small *M. salmoides* while similar results have been reported in South-Western Europe (García-Berthou, 2002) and North America (Schramm Jr and Maceina, 1986).

Stomach contents data from the current study clearly indicate that the diet of large sized *M. salmoides* was substantially dominated by *P. sidneyi* with *Caffrogobius* sp. and *M. falciformis* being the most dominant fish species. Both Weyl and Lewis (2006) and Wasserman et al. (2011) also reported the dominance of *P. sidneyi* in the stomachs of large sized *M. salmoides* in the lower Kowie River. The dominance of invertebrates in *M salmoides* diet is not unique to this particular system. Indigenous invertebrates were recorded in the stomachs of *M. salmoides* sampled in the Wit River, Eastern Cape (Weyl et al., 2010). Several authors have also reported the presence of crustaceans such as crayfish in the stomachs of *M. salmoides* (Keast, 1985; Schramm Jr and Maceina, 1986; García-Berthou, 2002; Wheeler and Allen, 2003; Braun and Walser, 2011).

The stomach contents of small *M. salmoides* were dominated by fish, with early juvenile *M. capensis* being the dominant prey item. Juveniles of this species migrate from the estuary to the freshwater areas as part of their life history (Bok, 1979; Beckley, 1984), rendering them vulnerable to predation by largemouth bass. The Aeshnidae and *P. sidneyi* also showed substantial %IRI scores. The dominance of fish in the stomachs of small *M. salmoides* was also reported by Weyl and Lewis (2006). Wheeler and Allen (2003) showed that fish were also the primary prey of small (age-0) largemouth bass in Chipola River, Florida, USA. The Cyprinodontid *Lucania* was an integral component of the diet of small-sized largemouth bass in the lower Santa Fe River (Schramm Jr and Maceina, 1986). In the Mediterranean region, small fish formed an important portion of the food of small and medium sized *M. salmoides* (García-Berthou, 2002).

The most dominant prey items in the stomachs of medium sized largemouth bass were *M. salmoides* and *P. sidneyi*, both of which had nearly identical %IRI values. The Cape moony *M. falciformis* was the third most important prey item, with a %IRI value that was close to both *M. salmoides* and *P. sidneyi*. Throughout the study, cannibalistic feeding on juvenile sized *M. salmoides* was detected in six samples, contributing 2.75% to the overall diet. Cannibalism was only apparent in small and medium sized *M. salmoides*. This is similar to the results obtained by Clady (1974) in two Northern Michigan lakes, who found that juvenile largemouth bass exhibited substantially higher levels of cannibalism than adults. The consumption of young largemouth bass individuals of a bigger size can negatively affect the population through a reduction in recruitment rates (Crass, 1964). It has been suggested that adult male largemouth bass guard nests thus lowering the occurrence of intra-cohort cannibalism while also protecting newly spawned individuals from predation until they reach a 15-30 mm TL (Johnson and Post, 1999; Post, 2003). This nest guarding behaviour can thus also explain the low frequency of cannibalism in large sized *M. salmoides*.

The changes in diet preferences with size (ontogenic niche shift) can also be viewed as a shift in trophic levels, resulting in young bass changing from being potential prey to competing with large sized bass as they mature (Olson, 1996). The species specific timing of a shift from feeding on invertebrates to piscivory can influence the growth rate and alter the degree of fitness of facultative piscivorous fish. An early shift to piscivory is generally deemed a favourable life history characteristic as it gives individuals from a cohort an advantage over their peers through increased growth rates and a decreased probability of being a victim of cannibalism and thus increasing their chances of survival till adulthood (Keast, 1985; Post, 2003). However, if too many individuals from a cohort survive past age-0, this will exert a strain on the population by increasing intraspecific competition and thus stunting growth (Crass, 1964; McDowall, 1968). The stomach contents results from this study seem to suggest that this particular population of largemouth bass undergoes an early switch (<150 mm TL) from an insectivorous to a semi-piscivorous diet, perhaps further highlighting their status as successfully established aliens in the system. Stable isotopes also support this as they suggest that 60% of the diet of small-sized bass consists of fish. It also serves to highlight that individuals from this population begin practicing cannibalism at smaller size class (Post, 2003).

It was also observed during sample collection that small and medium sized largemouth bass were concentrated in the shallow fast flowing habitats while large sized *M. salmoides* were found in deep slow flowing pools. There was also a population of small and medium fish which occupied an area within close proximity to the interface between freshwater section and the upper reaches of the estuary while large *M. salmoides* occupied an adjacent backwater pool. This separation in the sub-populations of the aforementioned size classes may have contributed to the resultant pattern of cannibalism.

Differences in habitat use between different sizes of largemouth bass are not a new phenomenon (Schramm Jr and Maceina, 1986; Olson et al., 1995), although some authors have also reported that all size classes are evenly distributed across all habitats (Wheeler and Allen, 2003). There is need for an assessment of the patterns distribution of the different sizes of largemouth bass as well as the possible driving factors, such as salinity, temperature or habitat preferences and so forth, in this particular system.

The stomach contents also showed variation in feeding preferences between the four sampling sessions. Fish were the most important prey items during April 2012 (*M. falciformis* and *R. holubi*), November 2012 (*M. capensis*) and February 2013 *(M. salmoides, M. falciformis*, *G. callidus* and *Caffrogobius* sp.) while *P. sidneyi* were the most important during August 2012. During the August 2012 sampling session, it was observed that there was a dense prevalence of various species of crabs including *P. sidneyi* along the banks of both the lower river and the upper and middle reaches of the Kowie Estuary. This session was conducted following a severe flood event and thus this might have contributed to the sudden dramatic prevalence of crabs along the banks. There was a trend of an increase in consumption of estuary-associated fish from site FW0 to site FW3. Aeshnidae and *G. callidus* dominated the diet at site FW0, while *M. capensis* and *M. falciformis* were the most important prey at site FW3.

According to the results of the SIAR model, Odonata made the largest contribution to the overall diet of *M. salmoides*, suggesting that largemouth bass have a primarily insectivorous diet in this particular water body. Among the bass fish prey, *M. falciformis, R. holubi* and *G. callidus* made the highest contributions. The high contribution of *R. holubi* to largemouth bass diet is surprising, as it was only detected in one stomach during the current study. However, it is also worth noting that relative to stomach contents, stable isotopes analysis reconstructs diet over a longer time scale (Van der Zanden and Rasmussen, 2002). It can therefore be speculated that largemouth bass consumed significant amounts of *R. holubi* in the months preceding this study and the severe floods that took place since 2012 reduced the abundance of this prey in the freshwater section of the Kowie River.

Estuary-associated fish species made significant contributions to the diet of *M. salmoides* collected from all four sites. The dominance of *M. falciformis* in the diet of largemouth bass collected from site FW2 is also in agreement with the results from the stomach contents analysis. The dominance of fish in the diet of *M. salmoides* at site FW1 is similar to the results of Weyl and Lewis (2006) using only stomach contents analysis. Both the stomach contents and stable isotopes corroborate the claims of the second hypothesis of this thesis, which states that the population of largemouth bass which inhabits a section of the river downstream of the weir (i.e. sites FW2 and FW3) will consume higher quantities of the three aforementioned estuary-associated fish species. Largemouth bass occupying FW3 indeed consumed the highest percentage of the aforementioned fish.

The results of the SIAR mixing model for isotope data on small sized fish were congruent with both the stomach contents results from this study and those reported by Weyl and Lewis (2006), with both showing a preference for feeding on fish (*M. capensis, G. callidus* and *M. falciformis*). Braun and Walser (2011) also reported piscivory in small sized largemouth bass in the lower Boise River, United states. The SIAR results for large sized *M. salmoides* were congruent with those reported by Wasserman et al. (2011), however there was a marked difference with the stomach contents results obtained during this study as these showed that *P. sidneyi* was the most important prey taxon. There also only an agreement between stable isotopes and stomach content analysis in terms of the significant contribution of *M. falciformis* to the diet of medium sized largemouth bass.

Seasonally, Odonata made the most important contribution to the diet of *M. salmoides*. In April 2012, *G. callidus* and Odonata were the most important prey items, while during August 2012 *M. capensis*, Odonata and *R. holubi* were the most important prey items. During November 2012, fish (*G. callidus* and *R. holubi*) were the most important prey, with Odonata also making a significant contribution to the diet. The importance of fish largemouth bass diet is congruent with the results obtained using stomach contents analysis. García-Berthou (2002) also found a variation of diet with season, with fish forming part of largemouth bass diet during the summer season. However, it should be noted that due to the slow turnover rate of stable isotopes in muscle tissue, the model does not necessarily reflect the feeding habits during the season that the sampling was conducted. In February 2013, both *M. capensis* and Odonata were equally important prey items.

The results of this study further demonstrate the generalist feeding behaviour of largemouth bass, highlighting its ability to consume a wide array of food items depending on prey size and availability (Savino and Stein, 1982; Sanderson et al., 2009). For example, the opportunistic feeding on *P. sidneyi* which was visibly prevalent along the river banks after a severe flood during August 2012. The generalist feeding of largemouth bass can also affect, through competition for food sources, indigenous fish species such as *M. falciformis* primarily feeding on invertebrates (Whitfield, 1984; Lorenzoni et al., 2002). Consumption of estuary-associated fish illustrates the connectivity that exists between the marine, estuarine and freshwater ecosystems through exchange of organic matter.

The results also illustrate that fish form an important part of the diet of *M. salmoides* in the lower Kowie River. Due to the implications associated with its carnivorous diet (McDowall, 1968), the stability of this population of largemouth bass partially depends on a continuous supply of fish from the estuary as well as aquatic invertebrates inhabiting the river. Establishment of largemouth bass also depends highly on a stable supply of prey fish, with over exploitation of prey species possibly resulting in the collapse of populations of introduced *M. salmoides* (McDowall, 1968). The prevalence of indigenous fish in the diet is cause for concern as several authors have also illustrated potential threats posed by the presence of *M. salmoides.* Gratwicke and Marshall (2001) attributed altered distribution and density of *Barbus* spp*.* in forty two Zimbabwean streams to alien largemouth bass and *Serranochromis robustus* predation.

Jackson (2002) stated that the presence of largemouth bass in Canadian lakes has a negative effect on the fish community structure by reducing the abundance of small-bodied fish as well as altering the flow of energy between trophic levels. The distribution of small bodied fish also changes in response to the introduction of a new predator, confining these species to a certain portion of the water body and thereby increasing interspecific competition among the native species. Another of his suggestions was that the introduction of predatory fish can result in trophic cascades as reduction of planktivorous fish may also cause a change in the community structures of both zooplankton and phytoplankton. In the western United States, introduced largemouth bass have been shown to reduce the abundance of anadromous coho salmon (*Oncorhynchus kisutch*) through high levels of predation. Largemouth bass were found to be responsible for 98% of the predation on coho salmon relative to nine other introduced fishes and two native species (Bonar et al., 2005).

South Africa's warm climate is favourable for bass as they are not subjected to winter overkill as they would be in their natural habitat. Catch per unit effort data from Wasserman et al. (2011) shows that largemouth bass, smallmouth bass (*Micropterus dolomieu*) and spotted bass (*Micropterus punctulatus*) are the only predatory fish in the lower Kowie River. However, during this study neither *M. dolomieu* nor *M. punctulatus* were detected, which suggests that *M. salmoides* is currently the only predator in the system. Two reasons can be supplied to explain the apparent absence of these species, the first being that *M. dolomieu* and *M. punctulatus* have undergone local extinction within this system, and the second one is that they have shifted their range and only occupy the middle and upper reaches of the Kowie River.

In conclusion this chapter serves to illustrate the importance of using both stomach content and stable isotope analyses in diet reconstruction. Stomach content data also served as an important tool for indicating which potential food sources should be collected for stable isotope analyses. It is therefore recommended that future research on *M salmoides* in the Kowie system should continue to employ a dual approach to dietary assessments of this species.

CHAPTER 3

FATTY ACID ANALYSIS OF LARGEMOUTH BASS AND ITS POTENTIAL PREY IN THE LOWER KOWIE RIVER

1. INTRODUCTION

There are several techniques which are currently used to elucidate the feeding biology of organisms. These include the traditional analysis of stomach contents and contemporary approach using stable isotopes, both of which were employed in Chapter 2. Another modern-day approach applied when reconstructing animal diets involves the use of fatty acids, either through comparison of prey and consumer profiles or using specific fatty acids as molecular biomarkers (Parrish, 2013). Lipids are heterogeneous compounds which are insoluble in polar solvents such as water and methanol but are soluble in nonpolar solvents such as chloroform and hexane (Arts and Wainman, 1999; Voet and Voet, 2004; Budge et al., 2006).

Fatty acids (FA) are a ubiquitous group of lipids that represent the highest proportion of all lipids that are found in the bodies of both plants and animals (Turner and Rooker, 2005). They consist of a straight carbon chain comprising of between 4 and 28 carbon atoms, and zero to six double bonds, with methyl (CH_3) and carboxylic acid (R-COOH) terminals on respective sides (Budge et al., 2006; Budge et al., 2002). Organisms which inhabit aquatic ecosystems possess fatty acids with 14 to 24 carbon atoms (Napolitano, 1999).

There are three major categories of fatty acids, namely: saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. These FA differ in terms of the number of double bonds that exist between their carbon atoms; i.e. saturated fatty acids have no double bonds, monounsaturated fatty acids have one double bond and poly unsaturated fatty acids have two or more double bonds (Parrish, 1999; Budge et al., 2006). There is also another category of fatty acids known as highly unsaturated fatty acids (HUFA). Fatty acids falling under this group are characterised by the possession of more than 20 carbons and more than 3 double bonds (Tocher, 2010). This group includes fatty acids such as eicosapentaenoic acid and docohexaenoic acid.

In nature, fatty acids normally occur esterified to another molecule, with a rare occurrence of free forms known as “free” fatty acids (Parrish, 1999; Voet and Voet, 2004; Budge et al., 2006). The three fatty acid types which are most commonly used in ecological studies are triacylglycerol’s (TAG) (FA esterified to a glycerol back bone), wax esters (WE) (esterified to a fatty alcohol) and phospholipids (PL) (two fatty acids esterified to a glycerol molecule)(Budge et al., 2002). In general, fatty acids serve as a source of energy, while polyunsaturated fatty acids (PUFA) are an integral component of cell membranes in eukaryotes (Watanabe, 1982; De Silva et al., 1998; Metz et al., 2001; Budge et al., 2006; Tocher, 2010; Parrish, 2013).

It is generally accepted that certain fatty acids, such as ω3 and ω6 polyunsaturated fatty acids, are beneficial for human health as their consumption can lower the risk of development of various medical disorders. This group of PUFA is known as essential fatty acids (EFA). Shortage of these fatty acids in the body can result in stunted growth and the development of various diseases, ultimately resulting in the death of the individual (Henderson and Tocher, 1987; Herold and Kinsela, 1986; Rahman et al., 1995; Tocher, 2010). Fish species that are considered to be of commercial importance contain ample proportions of both ω3 and ω6 PUFA and can thus be utilised as a supplement to maintain EFA proportions at an optimal level in the consumer (Herold and Kinsela, 1986; Haliloğlu et al., 2004)

The diets of organisms exert an influence on their fatty acid profiles as certain fatty acids are transferred from the food and are incorporated into the tissue of the consumer. Thus, this can be used to trace the consumer’s primary food sources (Kelly et al., 1958; Stickney and Andrews, 1972; Watanabe, 1982; Turner and Rooker, 2005; Subhadra et al., 2006). The allied relationship between the fatty acid profiles of consumers and their prey allows for qualitative and quantitative descriptions of an animal's diet. The use of fatty acids in studying complex food webs relies on the premise that every species in a particular ecosystem has a unique fatty acid composition. This technique can also serve as a tool for detection of possible seasonal and spatial variability in the diet (Rasoarahona et al., 2005; Budge et al., 2006; Allan et al., 2010).

Recent advances in the field of fatty acid analysis has resulted in the innovative use of certain fatty acids as biomarkers of consumer diets. Essential fatty acids have been used as biomarkers due to their ability to remain relatively unmodified during transfer from prey to predator and throughout the different trophic levels (Napolitano, 1999; Arts et al., 2001; Budge et al., 2006; Allan et al., 2010). In general, PUFA are often used for tracing the diet of fish, due to the fact that these organisms cannot biosynthesize them *de novo*, therefore they are strictly obtained from the diet (Henderson and Tocher, 1987; Iverson et al., 1997; De Silva et al., 1998; Turner and Rooker, 2005). Biomarkers of piscivory include eicosapentaenoic acid (20:5ω3) and docosahexaenoic acid (22:6ω3) (Henderson and Tocher, 1987; Surai et al., 2001). However, it is worth noting that the fatty acids profiles are subject to intraspecific variation based on factors such as locality, individual size and sex (Rahman et al., 1995).

In recent years, FA have been employed in solving questions concerning both the diets of an organism, as well as the transfer of organic matter through food webs (Napolitano, 1999; Iverson et al., 2004; Richoux and Froneman, 2008; Richoux, 2010). The biomarker approach has been successfully used to elucidate the diet, as well as feeding history of fish (Turner and Rooker, 2005; Rooker et al., 2006; Sakdullah and Tsuchiya, 2009; Connan et al., 2010; Elsdon, 2010; van der Bank et al., 2011). Fatty acids have also been used to highlight the connectivity been aquatic and terrestrial ecosystems. For example, Surai et al., (2001) used fatty acids to elucidate the dependence of four bird species (*Morus bassanus, Pelecanus erythrorhynchos, Phalacrocorax auritus and Catharacta skua*) on fish as a food source.

The reliability of this technique is highly reliant on information obtained from other dietary procedures such as stomach contents and stable isotopes analyses (Alfaro et al., 2006). Thus, this study employed all three techniques in an attempt to clearly elucidate the extent of largemouth bass predation of estuary associated fish. Fatty acids samples from the potential prey of *M. salmoides* were collected in the field based on information accumulated during the stomach contents analysis phase of the project.

2. MATERIALS AND METHODS

2.1. Sample collection, preparation and analysis

The procedure for sample collection and muscle tissue preparation has previously been described in chapter 2. The data for *R. holubi, M. capensis* and *M falciformis* was obtained from Dr Laure Carassou (unpublished data). Fatty acid data for gobies (*G. callidus*) was obtained from Leandro Bergamino (unpublished data) while data for *P. sidneyi*, Aeshnidae, Coenagronidae and Libellulidae was obtained from Sydney Moyo (unpublished data). For fish, muscle tissue extracted from the dorsal musculature was utilised in the analysis. The complete insect individual was processed, while only internal muscle tissue of crabs (*P. sidneyi*) was used for fatty acid analysis. For each fish fatty acid sample, 50 to 60 mg dry mass of muscle dorsal tissue was used, depending on the amount of available tissue. Fatty acid analyses followed a protocol developed by Dr N. Richoux (Rhodes University), and adapted from Folch et al. (1957). This protocol differs from the more rapid and commonly used Bligh and Dyer's (1959) and its adaptation from Indarti et al. (2005), in that it involves different solvent ratios for lipid extraction, and an additional column chromatography step in order to isolate neutral lipids in fish muscle tissues.

First, lipids were extracted from the dried and homogenized muscle tissue into 2 ml of chloroform ($CHCl_3$), including 0.01% of butylated hydroxytoluene (BHT), an anti-oxidant allowing for a better preservation of lipids in the solvent. At that stage, 100 µg of internal standard (nonadecanoic acid; 19:0) was added to each sample, to enable quantification of fatty acid methyl esters at a later stage. Samples were then kept at -20°C overnight. Lipids extraction continued with the addition of 1 ml of ice-cold methanol (MeOH), and sonication for 4 minutes, allowing breaking up cell membranes to improve lipids dissolution within the solvents. Samples were then decanted through cotton-wool plugged pipettes to clean up any remaining solids. Successive centrifugations with 0.9% KCl and MeOH allowed separating the organic content of the samples from the aqueous content. A final decantation of the organic phase through pipettes plugged with cotton wool and sodium sulphate (Na_2SO_4) allowed removing any remaining water from the lipid extracts. The organic content was then evaporated to dryness, and preserved at -20°C in 0.5 ml of dried chloroform ($CHCl_3$ with Na_2SO_4).

Following total lipid extractions, neutral lipids were separated from polar lipids using column chromatography (Arts and Wainman, 1999). Phospholipids (or polars) are not readily affected by dietary changes in secondary or higher consumers: they are mostly structural lipids used in cell membranes, whereas neutral lipids are mostly used for storage built up based on an organism’s feeding. Only neutral lipids were therefore used for this particular study (Budge et al., 2006). The separation of neutral lipids was achieved by eluting samples through columns made up with pipettes plugged with glass wool and silica gels, through which different solvents characterized by contrasting polarities were successively decanted. Columns were first cleaned with 6 ml of MeOH and 6 ml of $CHCl_3$. Neutral lipids were then eluted through the columns with 8 ml of 98:1:0.5 $CHCl_3$:MeOH:formic acid, and polar lipids with 6 ml of MeOH. The neutral lipids fractions were then evaporated to dryness, suspended in 1.5 ml of dried methylene chloride (DCM with Na_2SO_4) and stored at -20°C. Polar lipids were archived in MeOH at -20°C for possible future analyses.

The last step for samples being processed for fatty acids analyses involved the production of fatty acid methyl esters (FAME), which can be analysed by gas chromatography (GC). First, 3 ml of Hilditch reagent (a solution with concentrated sulfuric acid and dried MeOH) was added to each sample, and the samples were put in an oven at 100°C for an hour to stimulate the esterification of fatty acids. Then, successive centrifugations with hexane and milliQ water allowed purifying FAMEs, which were evaporated to dryness and suspended in hexane in 2 ml vials stored at -80°C before GC.

GC was performed at the laboratory with an Agilent Technology 7890A GC/Flame Ionisation Detector (FID) and 7000GC/MS (Mass Spectrometer) Triple Quad systems, with helium as the carrier gas. One microliter sample aliquots were injected at 250°C with the oven set at 100°C for the first 3 minutes. The oven temperature was increased to 150°C at a rate of 5°C/min, held for 1 min, and raised to 220°C at 3.5°C/min (the flame ionization detector was set at 260°C).

Peaks were integrated using Agilent ChemStation software and identified using mass spectral (MS) data derived from a subset of samples and by comparing retention times with those of external standards (Supelco 37 Component FAME Mix, PUFA1 Marine Source, PUFA3 Menhaden Oil, Bacterial Acid Methyl Esters Mix). Mass spectral data were processed using Agilent MassHunter WorkStation Qualitative Analysis software and corresponding NIST Mass Spectral Library. Each fatty acid was reported qualitatively as a proportion of total identified fatty acids (%TFA), and quantitatively as $\mu g.mg^{-1}$ of sample dry mass.

2.2. Data analysis

Fatty acids are reported using the short notation A:BωX, where A is the number of carbon atoms in the acyl chain, B is the total number of double bonds and X the position of the first double bond relative to the methyl end of the molecule. The significance of differences in the proportions of SFA, MUFA and PUFA between seasons, sites and size classes for the largemouth bass, and between sites for its prey, was determined using ANOVA.

Multivariate techniques are often employed for analysis of fatty acid data (e.g. Rasoarahona et al., 2005; Allan et al., 2010; Kelly and Scheibling, 2012). For purposes of this thesis, the main focus was on the proportional fatty acid data, with minimal comment on the quantitative data being provided. Accordingly, Principal Components Analysis (PCA) of proportional fatty acid data were used to explore the within-species similarities and variance of *M. salmoides* samples across the three size classes as well as between the four sites. Largemouth bass were separated into three size classes based on the criteria employed in chapter 2. All proportional fatty acid data were arcsine square root transformed in order to minimise the effects of fatty acids which constituted the highest proportions.

The PCA for predator fatty acids was performed separately from the food sources in order to circumvent the effect of different fatty acids profiles on the ordination. A separate PCA was also performed on the proportional fatty acid data of the food sources only. Finally, a third PCA was used to investigate the relationships between the consumer (*M. salmoides*) and the potential food sources taken together. In all cases, only fatty acids which contributed more than 1%TFA were included in the analyses.

3. RESULTS

3.1. Fatty acid profile of consumer

The samples of *M. salmoides* contained a total of 47 fatty acids. These consisted of 17 saturated fatty acids (SFA) (47%), 11 mono unsaturated fatty acids (MUFA) (10%) and 19 polyunsaturated fatty acids (PUFA) (43%). Behenic acid (22:0) was the most abundant SFA while erucic acid (22:1ω9) was the most abundant MUFA. The dominant PUFA was eicosapentaenoic acid (20:5ω3), accounting for 8% of TFA. Essential fatty acids (EFA) constituted 15% of TFA, with linolenic acid (18:3ω3) being the dominant member of the group (6%TFA). However, only 25 fatty acids were detected at proportions >1% TFA in bass. The proportional fatty acid data for *M. salmoides* is presented in table form in the appendix (Table A12).

There were patterns of variation in the relative proportions of SFA, MUFA, PUFA and essential fatty acids (EFA) between seasons (Figure 3.1a), sites (Figure 3.1b), as well as between the three size classes (Figure 3.1c). SFA were most dominant in samples collected during April 2012 (C1) ($F = 93.66$; $P < 0.05$), while MUFA ($F = 13.19$; $P < 0.05$) and PUFA ($F = 85.9347$; $P < 0.05$) were most dominant during August 2012 (C2) and February 2013 (C4) respectively. Samples collected during February 2013 also possessed the highest relative proportion of EFA (F = 11.02, P < 0.05). Samples collected at site FW1 had the highest proportion of SFA (F = 7.62; P < 0.001), while samples collected at site FW3 possessed the highest relative proportion of EFA (F = 2.87, P < 0.05). There were no significant variations in the relative proportions of MUFA and PUFA in samples from all four sites. The fatty acid profile of large-sized *M. salmoides* had the highest proportion of SFA (F = 4.0062; P < 0.05) while there were no significant differences in MUFA and PUFA proportions across all three size classes (Figure 3.1c). Small-sized *M. salmoides* contained the highest relative proportions of EFA (F = 77.14, P < 0.05). The fatty acid profiles of *M. salmoides* for the different seasons, sites and size classes are detailed in the appendix (Tables A13, A14 and A15, respectively). There were also patterns of variation in the concentrations (µg FA mg^{-1} DW) of SFA, MUFA, PUFA and EFA between seasons (Figure 3.2a), sites (Figure 3.2b) and size classes (Figure 3.2c).

Principal component analysis of 25 fatty acids, which individually constituted >1%TFA, from *M. salmoides* produced three distinct groups separated according to sampling season (Figure 3.3). Principal component 1 accounted for 49% of the variability while PC2 accounted for 38%. Bass samples collected in winter (August 2012) were characterized by higher proportions of hexadecanoic (*i*-17:0), behenic (22:0) and cerotic (26:0) acids and lower proportions of montanic (28:0), hexadecanoic (16:2ω4) and linolenic (18:3ω3) acids (variations along PC1, Figure 3.3.). The contrary was true for samples collected in summer (February 2013). Within the summer samples, there were very subtle separations according to sampling site. However, there was no clear separation according to fish size. Samples from other seasons (spring/fall) had intermediate proportions of the aforementioned fatty acids. PC1 was positively loaded with hexadecanoic (*i*-17:0), behenic (22:0) and cerotic (26:0) acids.

The second axis (PC2) was positively loaded with heptadecanoic (17:0), euric (22:1ω9) and eicosatrienoc (20:3ω6) acids. This additionally influenced formation of the group of samples collected during August 2012. PC2 was negatively loaded with methyltetradecanoic (*ai*-15:0), hexadecatrinoic (16:3ω4), eicoapentaenoic (20:5ω3) and docosapentaenoic (22:5ω6) acids. This resulted in the formation of a group consisting of samples collected during April as well as those collected during November 2012. This cluster also exhibited subtle groupings which appear to be influenced by the sampling sites, with samples from site FW0 located closer to samples from site FW1.

3.2. Fatty acid profiles of food sources

The fatty acid profile of *R. holubi* contained a total of 48 fatty acids comprising of sixteen SFA (38%TFA), eleven MUFA (26%TFA) and twenty one PUFA (36%TFA). Only fourteen of these fatty acids were detected at concentrations >1% TFA, with EFA constituting 24%. Palmitic acid (16:0) was the most dominant SFA (21%TFA), oleic acid (18:1ω9) the most dominant MUFA and eicosapentaenoic acid (20:5ω3) the most dominant PUFA (8%TFA) (Table A5). Samples collected from site FW3 had the highest proportion of SFA compared to all the other sites (F = 4.37, P < 0.05), while samples from site FW1 had the highest proportion of PUFA (F =2 879.48, P < 0.05). There were no significant differences in the proportions of MUFA and EFA between sites (P > 0.05 on both cases).

Thirty four fatty acids were detected in *M. capensis*, twenty one of these at >%TFA. The profile consisted of fifteen SFA (59%TFA), five MUFA (8%TFA) and fourteen PUFA (33%) (Table A5). Essential acids constituted 5% of TFA. Methyltetradecenoic acid (i15:0) (13%TFA) was the most dominant SFA, heptadecenoic acid (17:1ω7) the most prominent MUFA (5%TFA) and hexadecanoic acid (16:2ω4) the most dominant PUFA (17%TFA). The most prominent EFA was linolelaidic acid (18:2ω6trans) (2%TFA). Samples collected from site FW1 had significantly higher proportions (F = 60.43, P < 0.05) of SFA compared to samples from the other sites. Samples collected from site FW2 had the highest proportions of both PUFA (F = 24.49, P < 0.05) and EFA (F = 355.32, P < 0.05). The samples from site FW3 had significantly higher proportions of MUFA (F = 268.95, P < 0.05).

Thirty four FA were detected in *M. falciformis*, comprising of fifteen SFA (41%TFA), five MUFA (5%) and fourteen PUFA (54%TFA). The most dominant SFA, MUFA and PUFA were methyltetradecenoic acid (i15:0) (16%TFA), oleic acid (18:1ω9) and linolelaidic acid (18:2ω6 trans) (14%TFA) respectively. There was significant variation in the proportions of SFA between sites ($F = 283.08$, $P < 0.05$), with samples from site FW2 having the highest proportion. There was significant variation in MUFA between sites. Samples collected from site FW1 had the highest proportion of EFA ($F = 102.23$, $P < 0.05$), while samples collected from site FW3 had the highest proportion of PUFA ($F = 802.07$, $P < 0.05$).

Twenty seven fatty acids were detected in samples of *G. callidus*, comprising of ten SFA (41%TFA), five MUFA (23%TFA) and twelve PUFA (35%TFA). Thirteen of these fatty acids were detected at concentrations >1% of the TFA. At 21%, pentadecanoic acid (i16:0) was the most dominant SFA, oleic acid (18:1ω9) the most dominant MUFA (8%TFA), while eicosapentaenoic (20:5ω3) was the most dominant PUFA. In *P. sidneyi*, twenty two fatty acids were detected, with nineteen of these at concentrations >1% TFA. This profile had fourteen SFA (41%TFA), four MUFA (32%TFA) and four PUFA (27%). Palmitic acid (16:0) was the most dominant SFA (22%TFA), palmitoleic acid (16:1ω7) (9%TFA) the most dominant MUFA and eicosapentaenoic acid (20:5ω3) the most dominant PUFA (16%TFA).

Aeshnidae possessed thirty two fatty acids, thirteen of which occurred at >1%TFA. SFA constituted 40% and were dominated by palmitic acid (16:0) (20%TFA), while MUFA contributed 32% with vaccenic acid (18:1ω7) being the most dominant (17%TFA). PUFA constituted 28% and were dominated by arachidonic acid (20:4ω6) (16%), while EFA formed 18%TFA. Libellulidae possessed a total of thirty four fatty acids, thirteen occurring at >1% of TFA. Polyunsaturated fatty acids were marginally prominent (38%) with oleic (18:1ω9) acid being the most dominant in the group. Palmitic acid was the most dominant (16:0) (19%TFA) of the SFA (35%TFA) while eicosapentaenoic acid (20:5ω3) was the most dominant (18%TFA) member of the PUFA group (18%TFA). The EFA constituted 36%TFA.

The Coenagronidae profile consisted of thirty nine fatty acids, twelve of which were detected at >1%TFA. At 38%TFA, PUFA were the most dominant fatty acids, with eicosapentaenoic acid (20:5ω3) being most dominant, constituting 19%TFA. The MUFA formed 29%TFA and EFA 34%TFA. Palmitoleic acid (16:1ω7) was the most dominant MUFA

(10%TFA) while palmitic acid (16:0) was the most dominant (19%TFA) MUFA. The proportional contributions of fatty acids categories for the potential prey are illustrated in Figure 3.4 while the full fatty acids profiles are shown in the appendix (Table A13). The relative proportions of SFA, MUFA, PUFA and EFA for fish are shown in figure 3.4, while the proportions for the invertebrates are shown in figure 3.5.

Principal component analysis of thirty fatty acids occurring at >1TFA in bass prey showed separations according to prey species. PC 1 accounted for 65% of the variability in the data while PC2 accounted for 15% (Figure 3.6). The first axis (PC1) was positively loaded with pentadecanoic (16:0), stearic (18:0), palmitoleic (16:1ω7), oleic (18:1ω9), vaccenic (18:1ω7) and eicosapentaenoic (20:5ω3) acids (Figure 3.6). This axis was negatively loaded with methyltetradecanoic acid (*i*-15:0). The separation between *M. falciformis* from the group containing *R. holubi* and *G. callidus* occurs along this axis. The differentiation between *M. capensis* and the invertebrates (*P. Sidneyi,* Aeshnidae, Libellulidae and Coenagronidae) also occurs on this axis.

The second axis (PC2) was positively loaded with clupanodic (22:5ω3) and docosahexaenoc (22:6ω3) acids and negatively loaded with hexadecanoic (16:2ω4 cis) and linolenic (18:3ω3) acids. The separation of the group containing *R. holubi* and the invertebrates occurs along this axis. This axis also accounted for the separation between the groups for *M. falciformis* and *M. capensis*. The *M. falciformis* were split into two groups, one compact and one consisting of three samples. There was no discernable pattern showing effect of site on sample distribution in all the prey species. Rather, the samples from the different sites appeared to be randomly distributed.

3.3. Relationship between predator and prey fatty acids profiles

Principal components analysis of twenty six fatty acids of *M. salmoides* and its potential prey (*M. falciformis, M. capensis, Holubi, G. callidus, P. sidney*i, Aeshnidae, Coenagronidae and Libellulidae) resulted in two distinct groupings (Figure 3.7). Principal component 1 accounted for 64% of the variability, while principal component 2 accounted for 13%

variability. The first axis (PC1) was positively loaded with palmitic (16:0), stearic (18:0), palmitoleic (16:1ω7), oleic (18:1ω9), vaccenic (18:1ω7) and eicosopentaenoic (20:5ω3) acids, and negatively loaded with methyltetradecanoic (*i*-15:0) and eicosatrienoic (20:3ω3) acids. The second axis (PC2) was positively loaded with eicotetraenoic (20:4ω3), clupanodonic (22:5ω3) and docosahexaenoic (22:6ω3) acids.

The formation of two separate groups took place along the second axis. The first group consisted of samples of *M. salmoides*, *M. falciformis* and *M. capensis*. All samples appeared to be randomly distributed throughout the group. The second group consisted of *R. holubi, G. callidus, P. sidneyi*, Aeshnidae, Libellulidae and Coenagronidae. The influence from the first axis resulted in the apparent partitioning between samples within the second group based on species. The invertebrate prey taxa (*P. sidneyi*, Aeshnidae, Libellulidae and Coenagronidae) were positioned closer to each other, relative to the fish species (*R. holubi and G. callidus*). In summary, the results of the PCA suggest that the fatty acids profile of *M. salmoides* is more similar to those of *M falciformis* and *M. capensis* than the fatty acids profiles of *R. holubi, P. sidneyi,* Aeshnidae, Coenagronidae and Libellulidae.

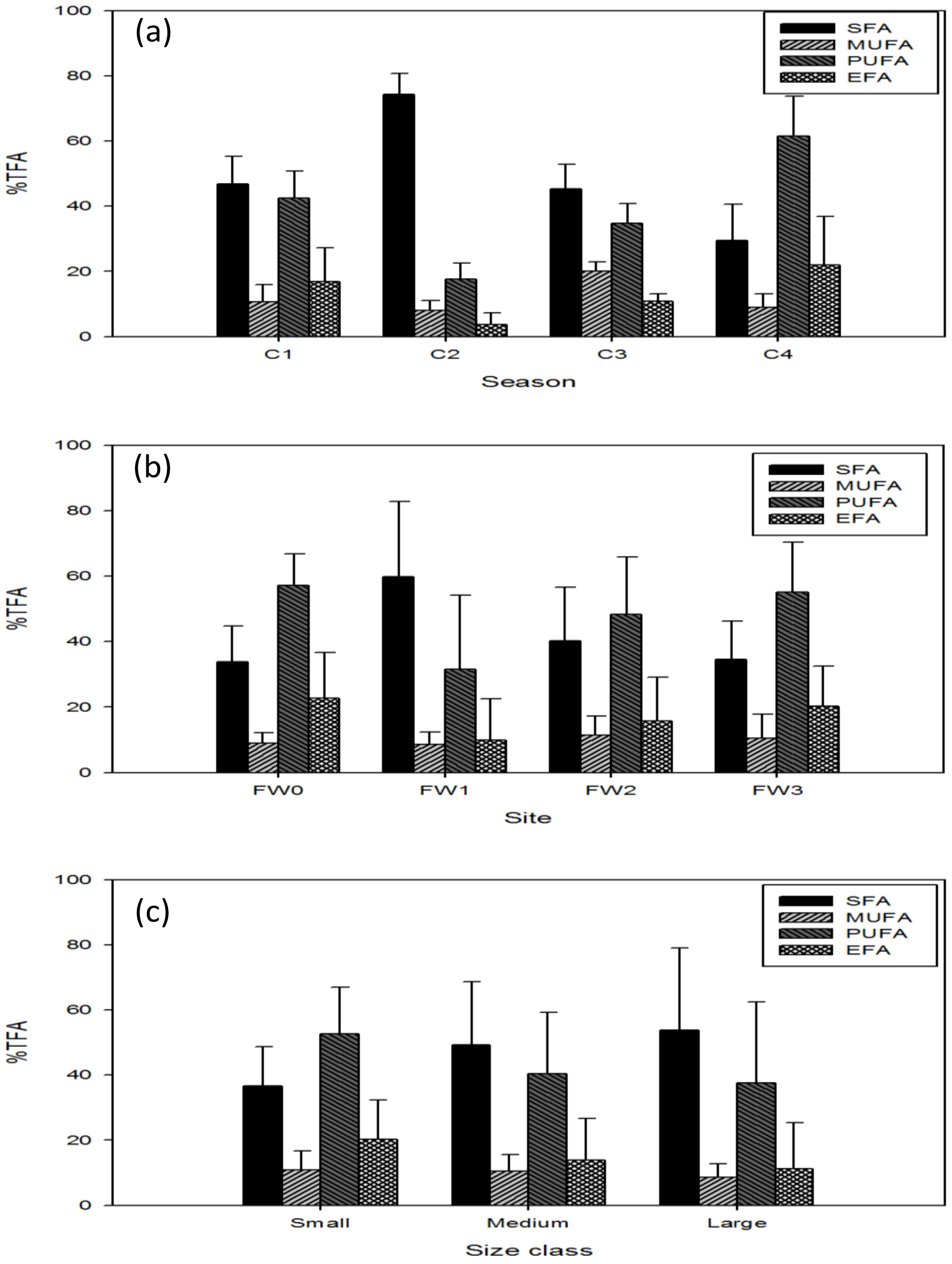

Figure 3.1: Seasonal (a), spatial (b) and size-related (c) changes in the relative proportions of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and essential (EFA) fatty acids in *M. salmoides* collected in the lower Kowie River. Error bars represent standard deviations.

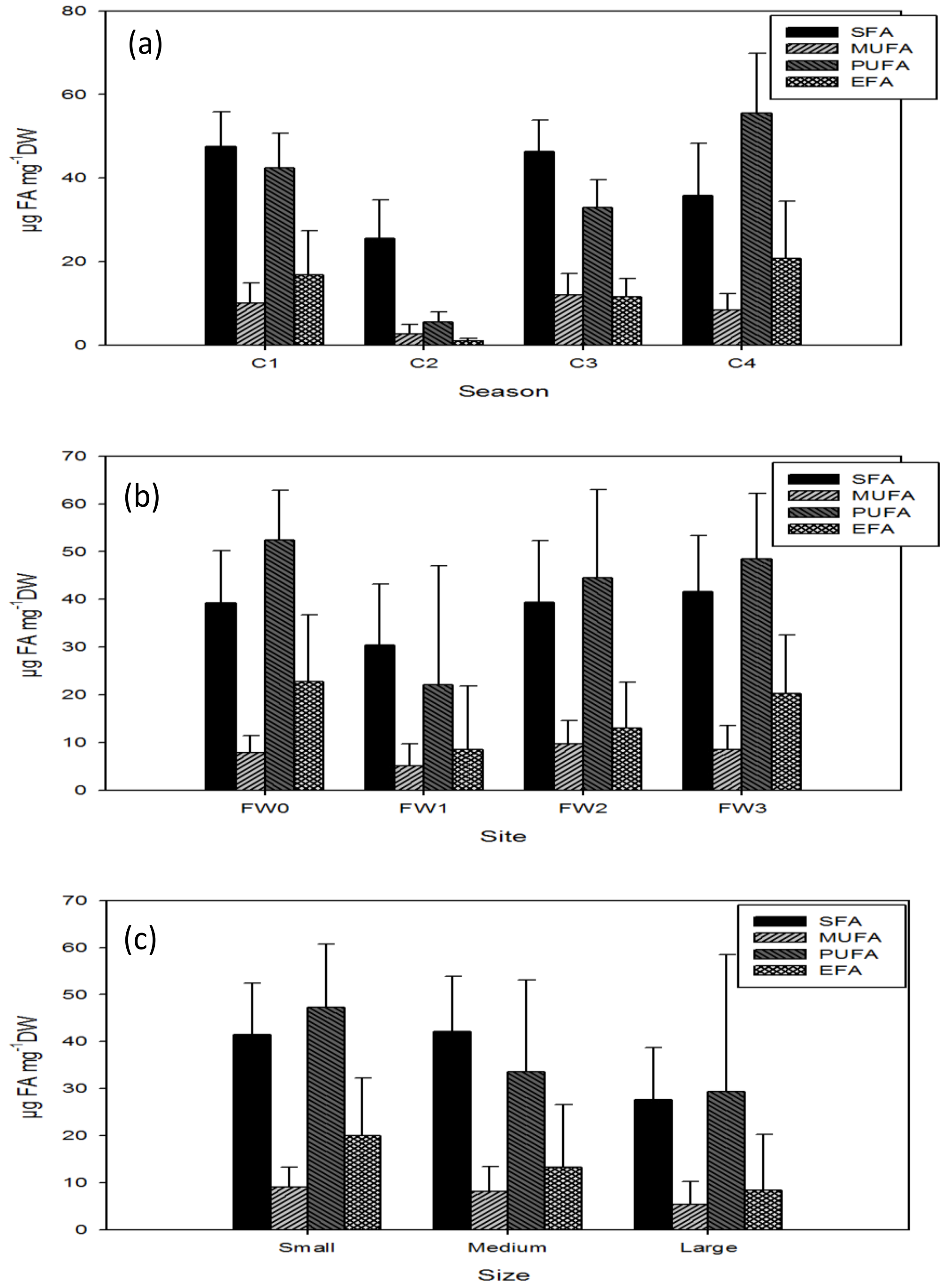

Figure 3.2: Seasonal (a), spatial (b) and size-related (c) changes in the concentrations of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and essential (EFA) fatty acids in *M. salmoides* collected in the lower Kowie River. Error bars represent standard deviations.

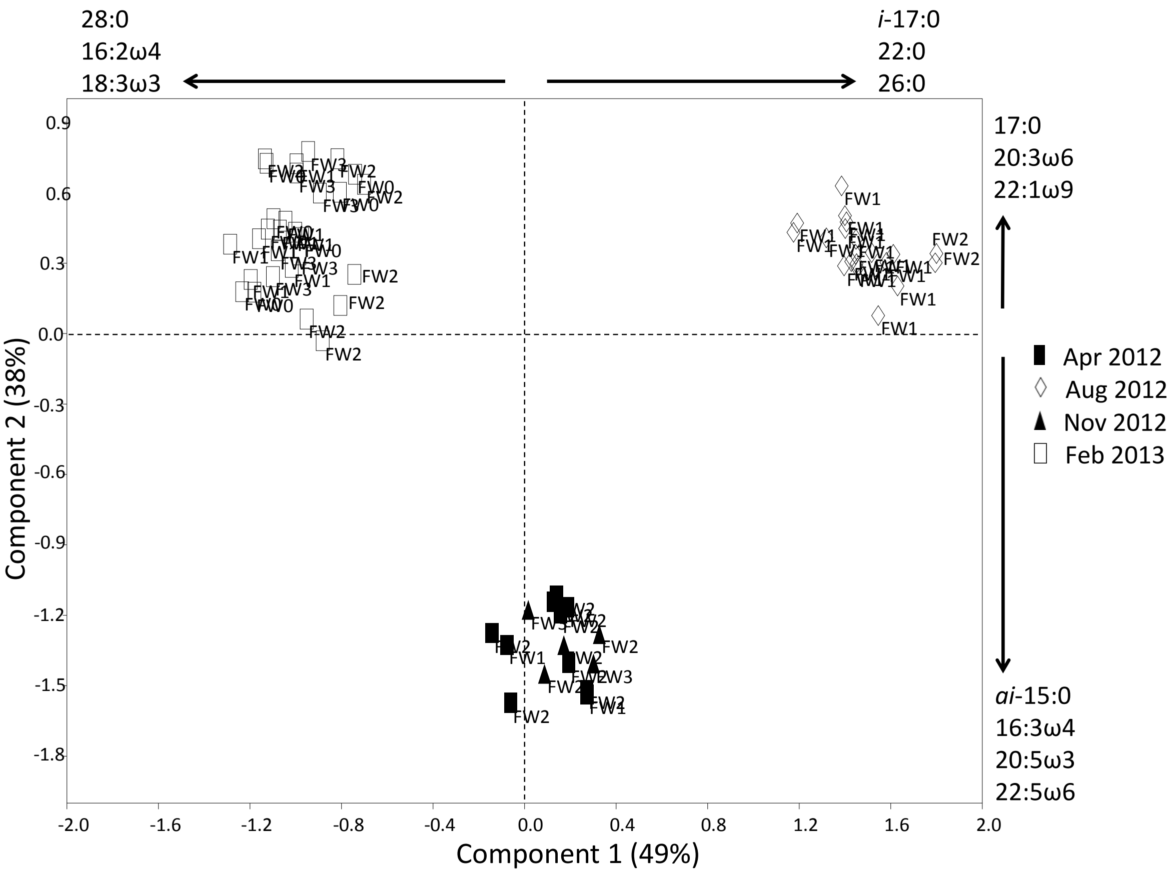

Figure 3.3: Principal component analysis of proportional fatty acid data (%TFA) of *M. salmoides* collected in the lower Kowie River. Only fatty acids which constituted >1%TFA were included in the analysis (25 fatty acids). Numbers adjacent symbols indicate site of collection. Percentages in parentheses represent the variation accounted for by each principal component. Arrows indicate the influence of fatty acids which had loadings >2.0%

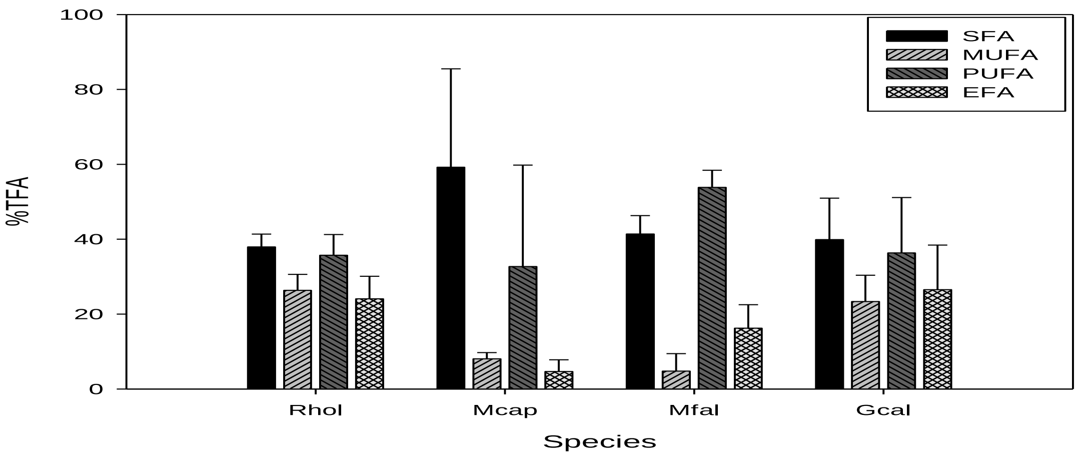

Figure 3.4: Relative proportions of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and essential (EFA) fatty acids in fish prey collected in the lower Kowie River. Error bars represent standard deviations and X-axis key include Rhol = *R. holubi*, Mcap = *M. capensis*, Mfal = *M. falciformis*, Gcal = *G. callidus*.

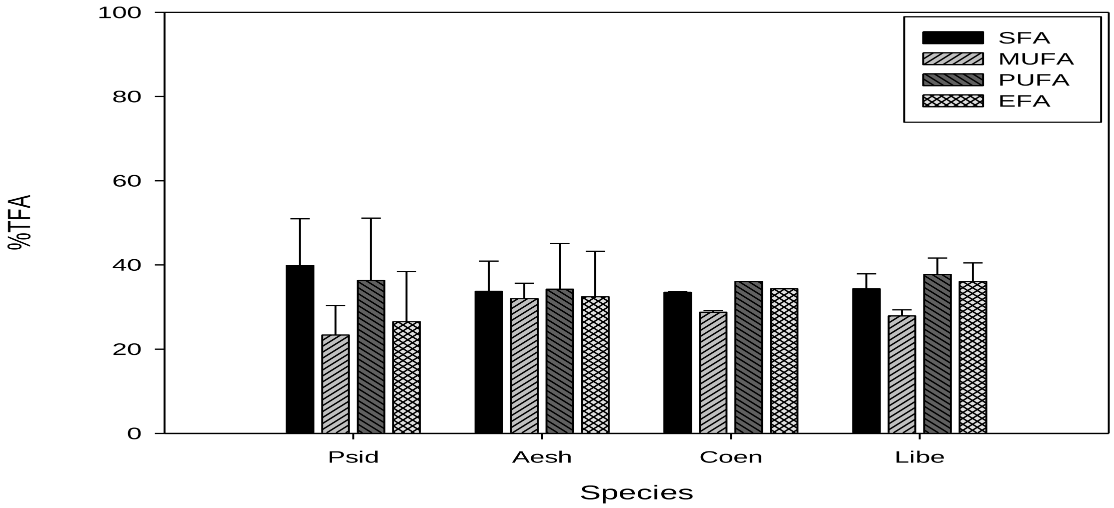

Figure 3.5: Relative proportions of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and essential (EFA) fatty acids in fish prey collected in the lower Kowie River. Error bars represent standard deviations and X-axis key include Psid = *P. sidneyi*, Aesh = Aeshnidae, Coen = Coenagronidae, Libe = Libellulidae.

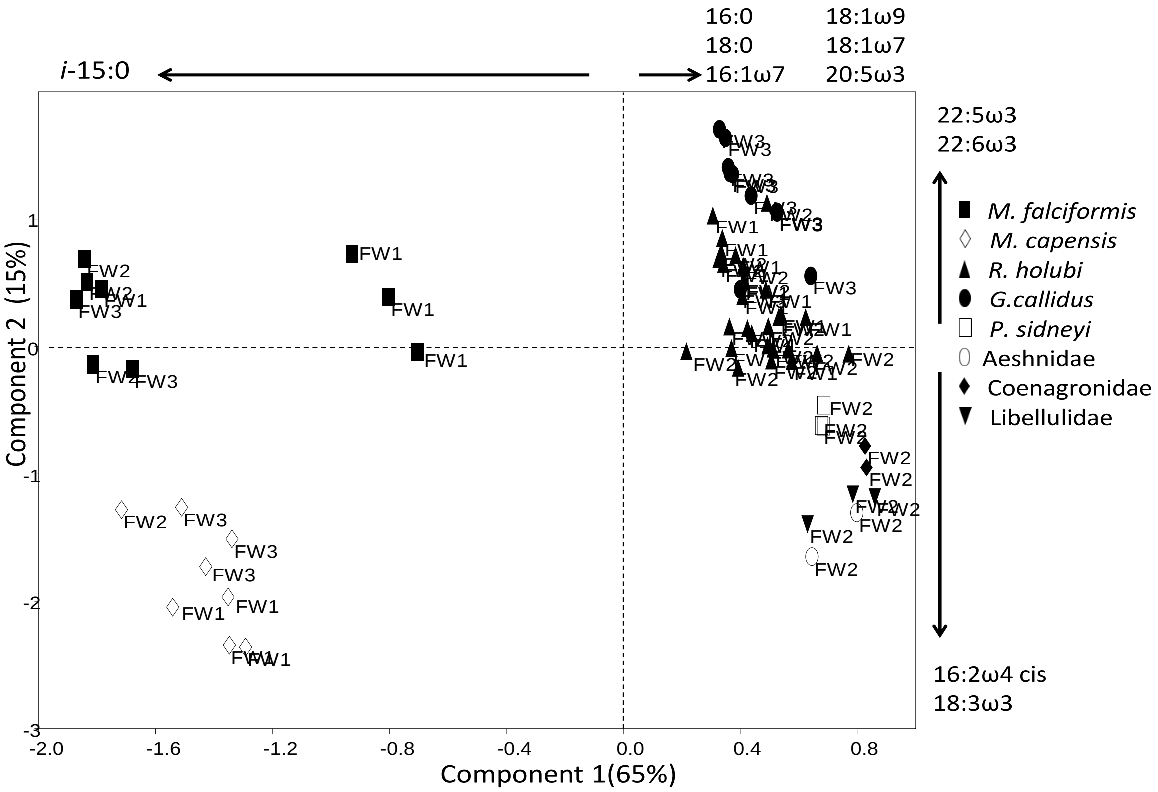

Figure 3.6: Principal component analysis of proportional fatty acid data (%TFA) of prey items collected in the lower Kowie River. Only fatty acids which constituted >1%TFA were included in the analysis (18 fatty acids). Numbers adjacent symbols indicate site of collection. Percentages in parentheses represent the variation accounted for by each principal component. Arrows indicate the influence of fatty acids which had loadings >2.0%.

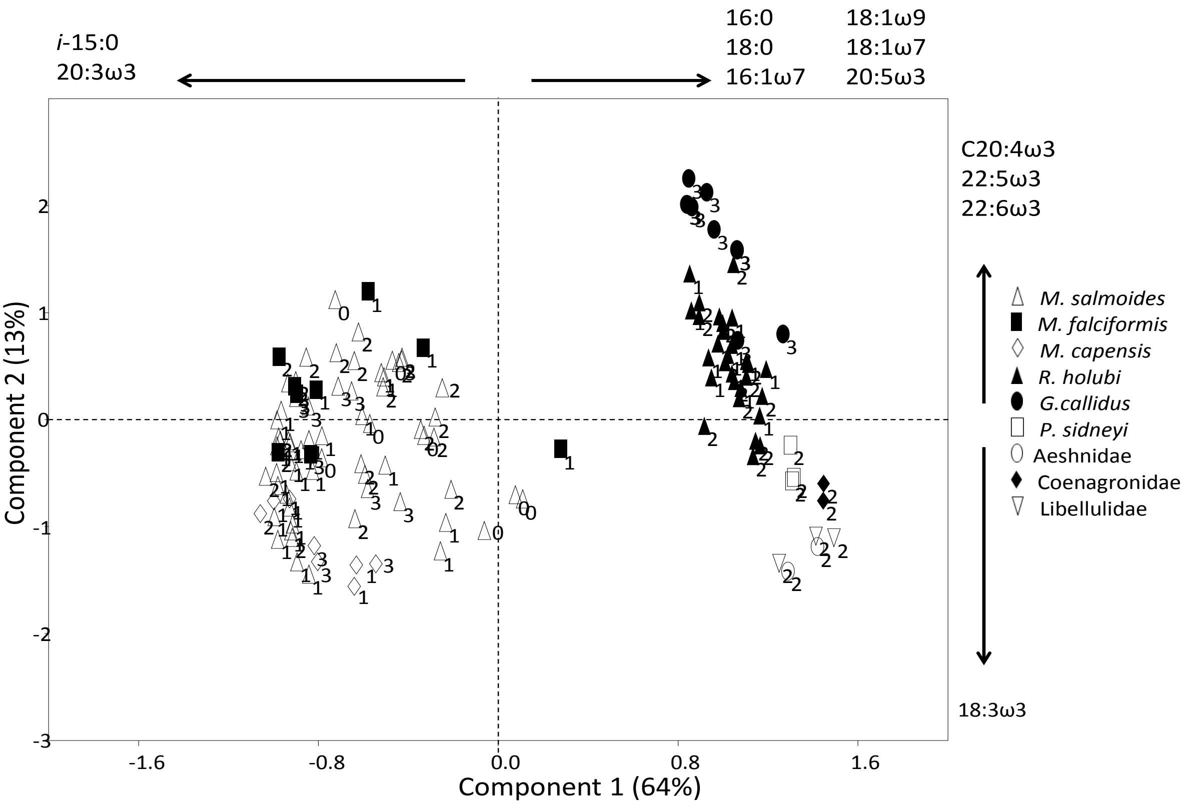

Figure 3.7: Principal component analysis of proportional fatty acid data (%TFA) prey of *M. salmoides* and all potential prey collected in the lower Kowie River. Only fatty acids which constituted >1%TFA were included in the analysis (26 fatty acids). Numbers adjacent symbols indicate site of collection. Percentages in parentheses represent the variation accounted for by each principal component. Arrows indicate the influence of fatty acids which had loadings >2.0%.

4. DISCUSSION

The presence of a high variety of biomarkers in the fatty acids profile of largemouth bass suggests that it currently occupies the position of an upper trophic level predator in this particular system. The biomarkers considered in this study are described by Napolitano (1999) for freshwater inhabiting organisms and Parrish (2013) for marine organisms. There is a discernable pattern of variation in the fatty acids profiles of *M. salmoides* between the sampling seasons, as shown by the principal component analysis. These seasonal variations in fatty acids profiles of *M. salmoides* can either be attributed to the seasonal changes in prey availability or to the effect of water temperature on the rate of synthesis and incorporation of numerous fatty acids, a phenomenon that is associated with poikilothermic organisms such as fish (Henderson and Tocher, 1987; Tocher and Sargent, 1999).

It can thus be hypothesized that when seasonal fluctuations in atmospheric temperatures cause changes in the water temperature this affects the fatty acids profile of *M. salmoides* by altering the rate of *de novo* synthesis and incorporation of SFA, MUFA and PUFA. Rasoarahona et al. (2005) found season related fluctuations in the fatty acids profiles of *Oreochromis niloticus, O. macrochir* and *Tilapia rendalli* sampled in the tropical island of Madagascar. During this current study, it was also found that the ratio of ω3/ω6 PUFA exhibited a trend of decrease from the colder (April and August 2012) to the warmer seasons (November 2012 and February 2013).

It is not clear what fluctuations in this ratio pertain to the current status of physical development of individual fish from this particular largemouth bass population. Subhadra et al. (2006) found that there was no evident effect of the ratio between ω3 and ω6 poly unsaturated fatty acids on the growth rate of largemouth bass based on feeding experiments conducted on samples collected in the United States. Therefore, based on this, it can be advocated that there are currently no definitive implications of the aforementioned ratio in relation to the growth rates of *M. salmoides* individuals inhabiting the lower Kowie River.

However, it is also worth noting that the fatty acid profiles of fish are also subject to fluctuations as a result of changes in the consumer's feeding pattern in relation to water temperature. In essence, the forage behaviour of fish changes between different seasons, and this can also be a contributory factor to changes in the consumer's fatty acid profile (Henderson and Tocher, 1987). The availability and non-availability of prey taxa is another factor that can contribute to changes in the fatty acid content of consumers. For example, starvation can alter the tissue-specific fatty acids profile of fish via the mobilization of fatty acids stored in lipid reserves (Henderson and Tocher, 1987). During this particular study, significant variations in the EFA between seasons in largemouth bass were observed. The relative proportions of EFA showed the second highest proportion during April, subsequently decreasing to the lowest proportion during August. The relative proportions of EFA substantially increased in November 2013 before ultimately reaching their highest value during February 2013.

This can be interpreted as a sign of shifts in the growth rates of individuals from this particular population of largemouth bass. Multivariate analysis using PCA did not demonstrate any discernable separation of *M. salmoides* samples based on size class. Therefore it can be concluded that the rate of growth shifts according to season throughout the population, regardless of any morphometric differences. In essence, ontogeny has no visible influence concerning seasonal fluctuations in EFA proportions, with environmental forces such as water temperature possibly being important.

Taking all issues into considerations, the PCA suggests that the temporal variation in the fatty acids of *M. salmoides* has a stronger effect than spatial variation. It would also appear that the synergism between season and feeding behaviour exerts a stronger influence on the fatty acids profile than the locations of the four sampling sites. The separation of the largemouth bass samples according to site of collection can be explained as having occurred due to either one of two factors; the salinity gradient and/or the differences in prey availability between the four sites. As hypothesized, the availability of estuary-associated fish such as *M. falciformis*, *M, capensis* and *R. holubi* for consumption by largemouth bass will increase with site proximity to the upper reaches of the estuary, thus exerting an influence on feeding patterns and possibly moulding the consumer's fatty acids profile.

The type and concentration/proportions of EFA required by fish are species specific and governed by the environment it inhabits, with warm water dwelling fish generally requiring less EFA compared to warm water inhabitants (Watanabe, 1982). The extent of the effect of salinity on fatty acid composition is also dependent on the type of tissue analysed (Haliloğlu et al., 2004). The fact that small-sized *M. salmoides* had the highest proportion of EFA compared to the other size classes is not surprising as EFA are an important requirement in early developmental stages. The optimal required levels of EFA can be species specific and also vary between the various stages of ontogeny (Tocher, 2010).

Salinity has an appreciable effect on the composition of PUFA, specifically the EFA category. Freshwater fish require types and proportions of EFA which are different from saltwater fish, with the two linolenic acids (18:2ω6 and 18:3ω3) being considered as of particular importance for optimum health (Bell et al., 1986; Bell and Sargent, 2003; Haliloğlu et al., 2004; Tocher, 2010). The general rule is that the proportions of EFA are lower in freshwater fish in comparison to their saltwater counterparts, a direct result of decrease in the proportions of ω3 PUFA in the former habitat (Rahman et al., 1995; Rasoarahona et al., 2005).

Since site FW3 is located closest to the interface between brackish and saltwater, it would be expected that the other three sites would exhibit a lowering of salinity levels as one proceeds upstream, and thus the fatty acids profiles of individuals from these respective sites would follow suit. The results show that samples collected from site FW3 possessed a significantly higher proportion of EFA, with the overall result showing a decreasing trend in EFA proportions between sites FW2 and FW1. Samples from this site also possessed the highest mean proportion of 18:2ω6 and 18:3ω3 (13%) compared to the other three sites. However samples collected at site FW0 exhibited a surprisingly elevated proportion of EFA compared to sites FW2 and FW3. It can therefore be hypothesised that fish occupying site FW0 might be consuming other fish species inhabiting the area.

Freshwater fish do not need to directly acquire C20 and C22 HUFA such as eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids as they can convert the linolenic acids (18:2ω6 and 18:3ω3) into HUFA via desaturation and chain elongation (Tocher, 2010). However, since fish cannot biosynthesise 18:2ω6 and 18:3ω3 *de novo,* due to the lack of Δ12 and Δ15 desaturase enzymes, they can only obtain the two aforementioned EFA from their diet (Henderson and Tocher, 1987).

Taking into account that the population of largemouth bass at sites FW2 and FW3 currently inhabits an area that is subject to fluctuations in salinity as a direct result of the amalgamation between brackish water from the estuary and freshwater from the river, it was expected that their fatty acid profiles would possess a combination of both saltwater and freshwater characteristics. Further, increasing this expectation was the availability of marine and estuarine potential prey including, but not necessarily limited to, the three estuary-associated fish species examined during this particular study. However, the ω3/ω6 ratio of largemouth bass collected from these sites has a freshwater-characterised value (0.86) (calculated based on the suggestions in Napolitano (1999)) and thus does not suggest a direct link to marine food sources. The ratio ω3/ω6 of marine fish has a minimum value of 4.7 and a maximum of 14.4 (Napolitano, 1999)

Principal components analysis of the potential prey suggests that there are close resemblances among the fatty acids profiles of *G. callidus* and *R. holubi*. The most dominant MUFA and PUFA were oleic (18:1ω9) and eicosapentaenoic acids (20:5ω3), respectively, for both these fish species. In the marine environment, oleic acid is regarded as an indicator of carnivory, while eicosapentaenoic acid is considered as an indicator of feeding on diatoms and red macro algae (Parrish, 2013). The fatty acid profiles of these two species also appear to be closely related to those of the invertebrates (*P. sidneyi,* Aeshnidae, Libellulidae and Coenagronidae) as the PCA arranged them within one group. Published work based on stomach contents analyses of *R. holubi* indicate that this species regularly consumes both crustaceans and invertebrates, while *G. callidus* specifically feeds predominantly on invertebrates and chironomid larvae (Whitfield, 1998). Also evident from the PCA ordination is the close relationship between the fatty acids of *M. capensis* and *M. falciformis* relative to the other species. Overall, it appears that there is no discernable pattern regarding the effect of site of collection on the ordination of prey in samples.

Eicosapentaenoic acid (20:5ω3), is regarded as a biomarker of diatom consumption (Parrish, 2013), and was the most dominant PUFA in *M. salmoides*, *R. holubi, G. callidus, P. sidneyi,* Coenagronidae and Libellulidae. The PUFA of *M. falciformis* and Aeshnidae were both dominated by ω6 EFA (18:2ω6 and 20:4ω6, respectively). The results of the PCA conducted using the proportional fatty acid data of *M. salmoides* and all potential prey showed that the fatty acid profile of largemouth bass is closely related to that of *M. falciformis* and *M. capensis*. This clearly suggests that these two estuary-associated fish species form an important component of largemouth diet in the lower Kowie River. However, the PCA does not show a definitive connection between the fatty acid profile of largemouth bass and the primarily herbivorous *R. holubi.*

Also evident from the PCA plot is the lack of a direct similarity between *M. salmoides* fatty acid profile and that of the benthic dwelling *G. callidus*. An interesting observation is the close proximity of *G. callidus* to *R. holubi* in the plot. This relationship is similar to the one elucidated by PCA conducted using the proportional fatty acid data of the potential prey taxa. The weak relationship between the fatty acids profiles of *M. salmoides* and *R. holubi* is congruent with the results obtained from stomach content and stable isotope analyses (Chapter 2). However, fatty acid results partially contradict those obtained in Chapter 2. The PCA does not show a definitive connection between the fatty acid profile of the predatory *M. salmoides* to the fatty acid profiles of the primarily herbivorous *R. holubi,* benthic dweller *G. callidus* or the sampled invertebrates. This highlights the importance using different techniques in assessing the diet of target species. In the current study, stomach content, stable isotope and fatty acid analyses all confirm that largemouth bass consume indigenous estuary-associated fish.

In summary, the results obtained from the fatty acid analysis of *M. salmoides* and its potential food sources suggest that largemouth bass inhabiting the lower Kowie River exhibit an elevated level of piscivory. This statement is based on the similarity of fatty acid profiles of *M. salmoides, M. falciformis* and *M. capensis* as demonstrated by principal component analysis. This finding is corroborated by the fatty acid profile of *M. salmoides* which contained high proportions of the three biomarkers for piscivory, namely 18:2ω6, 18:3ω3 and 20:4ω6 (Napolitano, 1999), and collectively constituted 10% of the total fatty acids in this species.

The most dominant MUFA in largemouth bass was indicative of carnivory, namely oleic acid (18:1ω9) (Parrish, 1999), and further supported this fishes' predatory nature. It also serves to support the hypothesis that largemouth bass prey on indigenous estuary-associated fish. PCA performed using the fatty acid data from all nine taxa did not elucidate a direct connection between largemouth bass and the sampled invertebrates. However, current evidence is insufficient to conclude that invertebrates do not form part of the diet of largemouth bass. As has been elucidated in Chapter 2 through stomach contents and stable isotopes analyses, invertebrates actually form a substantial, if not dominant, portion of largemouth bass diet in this particular system. Therefore, fatty acids cannot form a basis for excluding invertebrates as being part of largemouth bass diet.

In conclusion, the results of the fatty acid analysis support the hypothesis that *M. salmoides* preys on estuary-associated fish in the lower Kowie River. The results clearly demonstrate the connection between largemouth bass and their prey *M. falciformis* and *M. capensis*. However, the results failed to elucidate a direct relationship between largemouth bass and *R. holubi*, thus indicating limited or no predation on this species. Additionally, fatty acid analysis failed to confirm the importance of invertebrates in the diets of largemouth bass, as previously suggested by both stomach contents and stable isotopes analyses. Previous studies in the Kowie River have also confirmed the presence of invertebrates in the diet of M. salmoides (Weyl and Lewis, 2006; Wasserman et al., 2011).

Direct consumption of the two estuary-associated fish species by largemouth bass symbolizes the connectivity that exists between riverine and marine ecosystems, with the estuary serving as an integral corridor, thus facilitating the connection. This connectivity is epitomized by the transfer of organic matter between the ecosystems, with the migration of facultative catadromous fish serving as the "currency" in this instance. The migration of *M. falciformis* from the Indian Ocean into the Kowie Estuary during the post larval phase (Whitfield, 1998) and subsequent upstream migration by juveniles into the freshwater section of the Kowie River serves as a vehicle for the transfer of organic matter. For the downstream scenario, organic matter is transferred from the river to the ocean when *M. falciformis* and *R. holubi* sub adults migrate back to the marine environment.

Largemouth bass have been shown as to pose a threat to the indigenous ichthyofauna when introduced in new aquatic systems (e.g. Gratwicke and Marshall, 2001; Jackson, 2002; Bonar et al., 2005; Ellender et al., 2011). The impacts of bass introductions on native organisms in the United States of America has already been highlighted, e.g. Jackson (2002). This study has concluded that *M. salmoides* in the lower Kowie River serves as a threat to migratory estuary-associated fish which are now forced to adapt to the presence of a new naturalized predator.

CHAPTER 4

MOVEMENTS OF LARGEMOUTH BASS INVESTIGATED USING ACOUSTIC TELEMETRY

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1. INTRODUCTION

Investigation into the area use and movement patterns of fish involves the use of either mark-recapture techniques or attaching electronic transmitter tags, as is the case in studies involving acoustic telemetry (Lucas and Baras, 2000; Simpfendorfer et al., 2010; Campbell et al., 2012). The use of electronic transmitter tags holds several advantages over the more traditional and relatively cheaper mark-recapture approach. For example, electronic tags can also be coupled with sensors which detect changes in the organism's physiology such as heart rate, as well as measuring environmental variables such as temperature, salinity and oxygen levels (Baras and Lagardere, 1995; Lucas and Baras, 2000; Cooke et al., 2002; Cooke et al., 2004).

There are currently two main approaches in the field of acoustic telemetry; one that involves active tracking and another that involves the installation of submerged listening stations in the aquatic system being studied (Cooke et al., 2005; Hanson et al., 2007). The use of fixed stations has advantages over manual or periodic tracking as it encompasses all fine scale events taking place during a full 24-hour cycle throughout the duration of the study and is less labour intensive (Hanson et al., 2007). For example, manual tracking requires the use of a boat while actively tracking fish movements using an ultrasonic receiver.

An important factor when considering the appropriate method for tracking fish is that the method should not cause any permanent physiological impairment to the fish such as altered swimming efficiency or increasing the likelihood of predation (Baras and Lagardere, 1995; Thorstad, 2000; Jepsen et al., 2002). The tracking of fish movements is executed for a plethora of reasons, including elucidating the effect of environmental factors on fish movements (Warden and Lorio, 1975; Childs, Cowley, et al., 2008; Gardner et al., 2013), daily movement patterns (Tulevech and Recksiek, 1994; Cowley et al., 2008), determining the size of a population's home range (Childs, Booth, et al., 2008), as well as investigating the impacts of anthropogenic fish displacement on reproductive success (Cooke et al., 2000). Technological advancements have seen this technique become an important investigative tool, not only in field studies but also in the aquaculture industry (Baras and Lagardere, 1995).

The availability of food has been shown to significantly influence the movement patterns of fish, as fish move greater distances when food availability is at low levels, thus moving outside of their home range (Savitz et al., 1983; Baldwin et al., 2002). The movement patterns of largemouth bass for foraging purposes have been described as alternating in tandem with changes in vegetation density, with foraging success being at its prime during low vegetation density and decreasing with escalating vegetation density (Savino and Stein, 1982). Although there are conflicting reports, largemouth bass have generally been found to exhibit a fidelity to a home range (Lewis and Flickinger, 1967; Ridgway and Shuter, 1996; Sammons et al., 2003). It has previously been reported that largemouth bass also exhibit individual and seasonal variations in movement and home range size and this differs from one population to another (Hanson et al., 2007; Ahrenstorff et al., 2008; Hunter and Maceina, 2008).

Largemouth bass can tolerate salinity levels ranging from 0‰ in rivers and lakes to estuarine waters that can exceed 10‰, however their preferred salinity is generally 3‰ or less (Meador and Kelso, 1989; Lowe et al., 2009; Glover et al., 2012). Largemouth bass are substantially affected by large salinity fluctuations, which has been shown to significantly influence their distribution and movement within a water body (Meador and Kelso, 1989; Glover et al., 2012). High salinity levels have also been shown to hamper the growth rates and reproductive success of *M. salmoides*. Optimum salinity levels of largemouth bass also vary with age and size of an individual (Meador and Kelso, 1989; Glover et al., 2012).

The ability, or lack thereof, of largemouth bass to tolerate a wide salinity range is a key factor in determining food availability, as this will affect their capacity to prey on the juveniles of estuary-associated marine fish species in saline environments. Although generally regarded as a mainly freshwater species, populations of largemouth bass are known to inhabit coastal water bodies such as estuaries and river deltas (Lowe et al., 2009; Glover et al., 2012). Therefore, it was expected that the population assessed during this study would be able to tolerate slight elevations in salinity.

The deleterious impacts of alien largemouth bass predation on indigenous estuary-associated fish species have been highlighted in chapters 2 and 3. The main assumption ascribed in both the aforementioned chapters as well as in previous studies conducted in this system is that largemouth bass remain resident within the freshwater section of the lower Kowie River and only opportunistically consume the juveniles of facultative catadromous fish species when they move above the estuarine headwaters. For this assumption to hold true, the largemouth bass must remain above the ebb and flow region of the estuary. With this in mind, the main aim of this chapter was to investigate (i) whether largemouth bass do move past the ebb and flow region into the Kowie Estuary, (ii) whether they occupy the estuarine portion for any length of time, and (iii) the effect of salinity levels on largemouth bass penetration of the estuary and their ability to therefore prey on a wide variety of estuary-associated marine fish species.

2. Materials and methods

2.1. Study site

The telemetry study was conducted mainly in the lower Kowie River and extended into the estuary (Figure 4.1). The riverine sites were located within the Waters Meeting Nature Reserve and consist of a series of deep wide pools with overhanging trees on steep banks. Photographs of these pools are shown in chapter two (i.e. Figure 2.2 to Figure 2.5). The biological and physical characteristics of the riverine sites have already been discussed in the aforementioned chapter as this is where the specimens for the dietary study were also collected. The majority of the estuarine sites generally encompassed areas dominated by a deeper central channel and shallower littoral zone. The sites located in the upper reaches of the estuary were characterised by overhanging vegetation on either bank while the middle reaches were wider and had mainly emergent aquatic macrophytes along the banks. Sites in the lower reaches of the estuary (22-25) were located adjacent to salt marsh flats, with two sites (26 and 27) situated in the modified mouth of the Kowie Estuary (Kruger and Strydom, 2011; Figure 4.1).

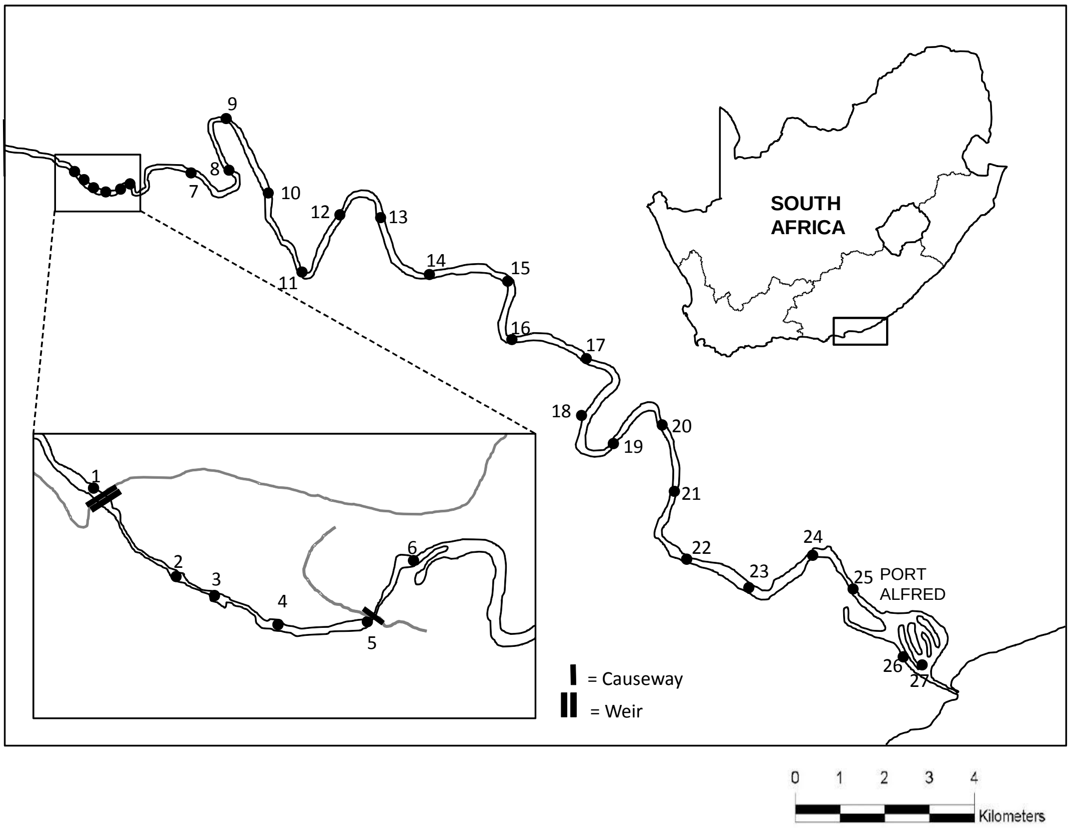

Figure 4.1: Map showing the location of 27 acoustic receivers in the Kowie River and estuary. The enlarged portion shows the area where the 10 largemouth bass used in this study were captured and released.

2.2. Fish collection and transmitter implantation

On 24 January 2013, 10 largemouth bass, ranging in size from 236 to 382 mm standard length, were surgically implanted with acoustic transmitters in order to monitor their movements. All fish were caught with rod and line from the large pools in the lower Kowie River. Surgery was conducted on-site using a floating 'laboratory'. After capture, each fish was placed in a bath containing a solution of river water and 2-phenoxyethanol (approximately 0.7 ml l^{-1}). Fish remained in the bath until they exhibited signs of anaesthesia, as indicated by the loss of equilibrium, usually occurring after an average immersion time of 2 minutes 38 seconds. Each fish was then measured as standard, fork and total length to the nearest millimetre. The fish was placed ventral side up in a wet towel on a specially designed V-shaped high-density foam bed. During surgery, the gills received a continuous supply of oxygenated river water.

Using a surgical blade, a 20 mm incision was made to the ventral part of the abdominal cavity, midway between the anus and pelvic fins. The tags, which were 29 mm long, 9 mm in diameter and weighed approximately 2.9 g, had been sterilised with methylated spirits before being inserted into the peritoneal cavity and positioned above the pelvic girdle. The incision was closed using two independent silk sutures (2/0 Ethicon), followed by the application of an antibiotic powder to the wound. Average surgery duration lasted 2 minutes 55 seconds. The fish was then transferred into a recovery tank filled with fresh oxygenated water from the site before being released back into the river (average recovery time was 2 minutes 47 seconds). All fish were released at their respective sites of capture (i.e. between acoustic receivers 2 and 6 as shown in Figure 4.1). The V9-2L individually coded transmitters (VEMCO Ltd, Halifax, Canada) had a nominal pulse delay of 30 to 90 seconds and an expected battery life of 282 days.

Fish movements were monitored in the Kowie catchment using an array of 27 VEMCO VR2 automated data-logging acoustic receivers, extending from the estuary mouth to 22 km upstream. The location of the individual acoustic receivers is shown in Figure 4.1. A HOBO conductivity/salinity logger was attached to receiver number seven in order to measure fluctuations in salinity at the head of the ebb and flow region. Data on the movement patterns of largemouth bass between 24 January 2013 and 22 July 2013 was downloaded from the acoustic receivers onto a laptop computer and then analysed. The salinity data presented in this chapter was recorded from 24 January 2013 to 3 August 2013.

2.3. Data analysis

All telemetry data was downloaded from each receiver on a monthly basis. The data was arranged according to receiver and fish number and then preliminarily analysed using the VEMCO User Environment (VUE) software. Further analysis was performed in Microsoft Excel. A bubble plot was constructed based on the proportional amount of time each individual fish spent at each receiver. An average proportion of time spent by all fish at each receiver was also calculated in order to elucidate any overall sedentary or active movement patterns.

The salinity data from near bottom water at the head of the estuary (receiver 7) was simultaneously downloaded on the same day as the telemetry data. Since the salinity logger executes measurements on an hourly basis, an average salinity for each day was calculated from the resultant data. Graphical plots were initially constructed using Excel and subsequently refined using SigmaPlot v10.0 (Systat Software, San Jose, California, USA).

3. RESULTS

3.1. Fish movements

The surgery details encompassing the concentration of the anaesthetic, duration of the operation, as well as lengths of the fish tagged and monitored during this study are shown in Table 4.1. The fish were tracked over a period of 192 days with tagged individuals only detected between receivers 2 and 15. No fish were detected at the uppermost receiver (number 1) which was located upstream of a weir containing a fishway. Only fish number eight was detected at receiver 15. In contrast, fish number 10 exhibited a resident lifestyle by remaining in the pool where it was sampled (as detected by receiver 4). Fish number 6 and 9 also exhibited slightly sedentary lifestyles as they spent a substantially higher proportion of time within the vicinity of receivers 2 and 4 respectively. On the last day of the study, these two fish were detected at receivers 10 and 13 respectively. A bubble plot illustrating the proportion of time spent by each fish within the vicinity of each acoustic receiver is presented in Figure 4.2.

Fish number 5 and 8 accomplished the furthest movements in a downstream direction, being detected by receivers 14 and 15 respectively. They were closely followed by fish 4 and 7, reaching areas in the vicinity of receivers 12 and 13 respectively. On the last day of this study, fish number 5 was detected at receiver 12 while fish number 8 was detected in the vicinity of receiver 9. Overall the fish spent the highest proportion of time at acoustic receiver 4 (Figure 4.3) and the smallest proportion of time at acoustic receivers 14 and 15. Fish number 8 was by far the most active individual relative to the other tagged specimens. This is highlighted by fish number 8 having been detected at receivers 2 to 15. All fish that made downstream movements (in the vicinity of receiver 15) only spent a short period of time within this portion of the estuary. All fish that migrated downstream during April returned upstream during the same month and then moved downstream during May. It is noteworthy that all downstream movements into the estuary were of short duration and trips back to the ebb and flow region were made within the same month.

Table 4.1: Surgery details of the 10 largemouth bass *Micropterus salmoides* tagged and monitored in the lower Kowie River on the 24 January.

Fish code	FL (mm)	Anaesthetic concentration (ml/L)	Time in anaesthesition (min:sec)	Surgery Duration (min:sec)	Time to recover (min:sec)
1	236	0.5	02:37	02:54	03:42
2	343	0.5	07:15	03:11	02:42
3	336	0.7	02:38	03:01	02:58
4	289	0.7	02:31	02:40	02:56
5	244	0.7	02:20	03:08	03:08
6	382	0.7	01:34	02:47	02:47
7	320	0.7	02:09	03:05	03:05
8	315	0.7	01:54	02:51	02:51
9	249	0.7	01:15	02:51	02:05
10	322	0.7	02:05	02:42	01:38

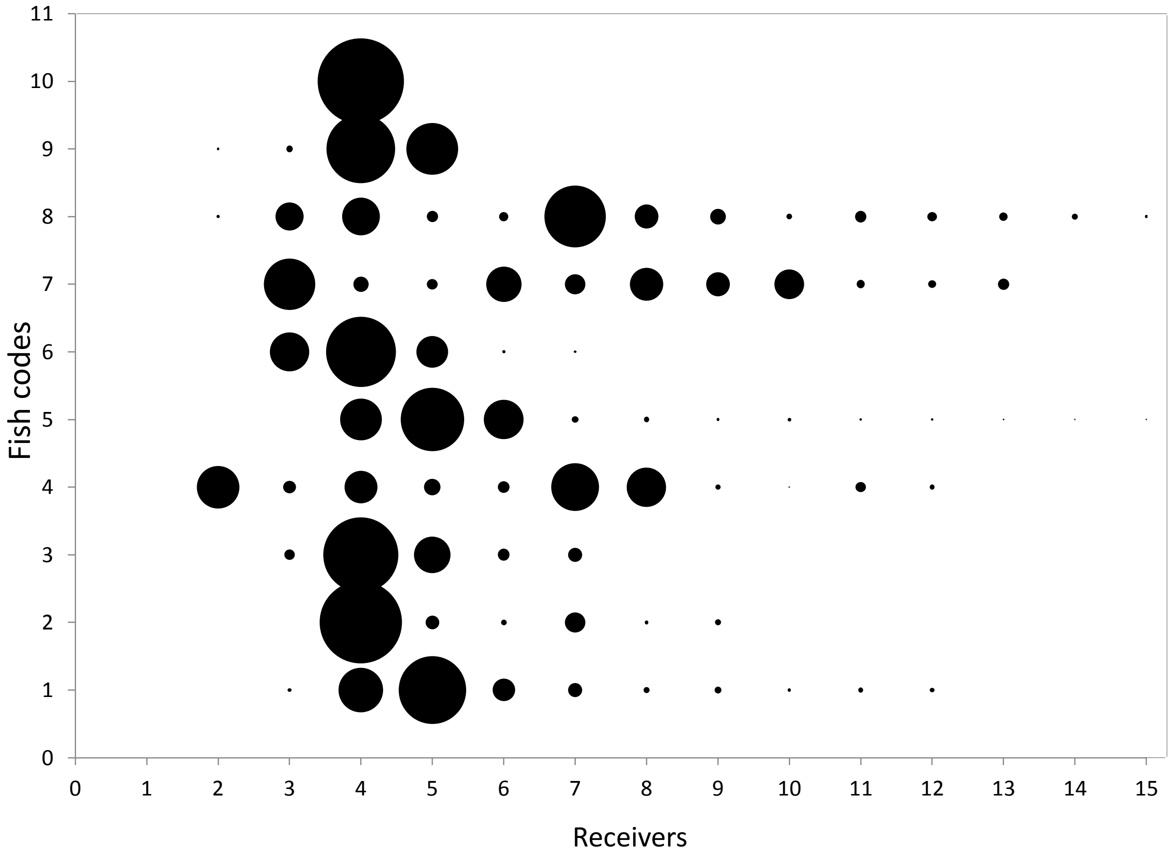

Figure 4.2: Bubble plot showing the total proportion of time each largemouth bass spent at each acoustic receiver in the Kowie River and estuary.

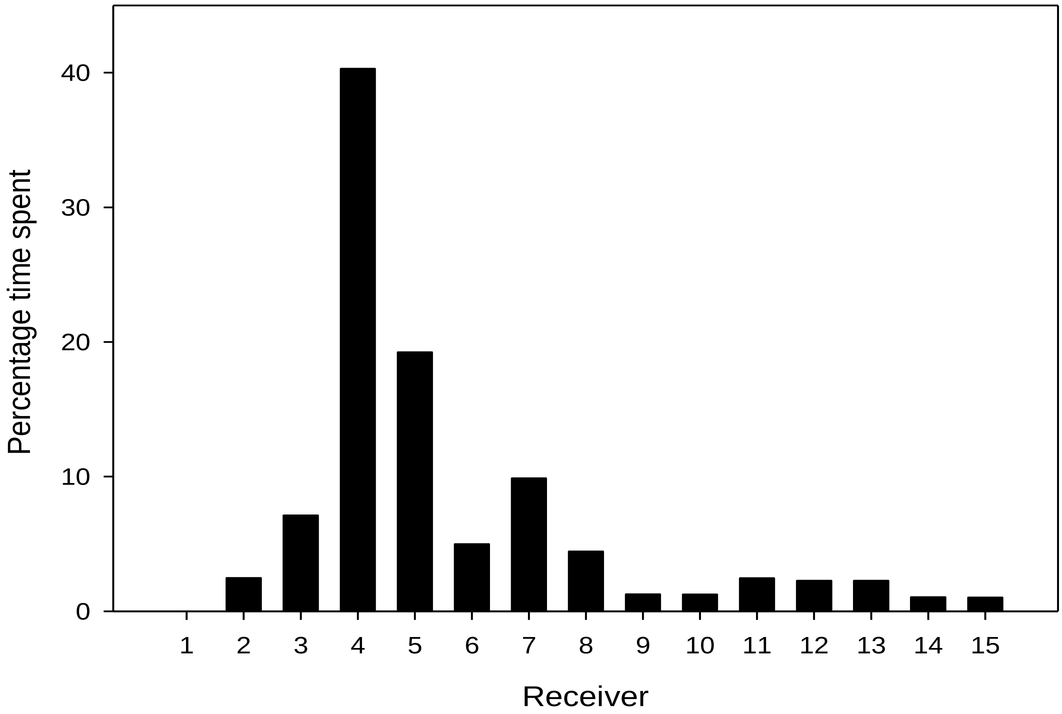

Figure 4.3: Overall average proportional amount of time spent by the largemouth bass at each receiver.

3.2. Salinity

Bottom salinity levels were measured at receiver 7 over a period of 201 days, between the months of January and August 2013. The average salinity measured at this site for the duration of this study was 5.8‰. The highest average monthly salinity was recorded during August (9.8‰) while the lowest occurred during May (2.7‰). The highest mean daily salinity level was recorded on 21 March (14.2‰) while the lowest mean salinity occurred on 11 January (0.01‰). The average daily salinity levels at Station 7 are graphically illustrated in Figure 4.4.

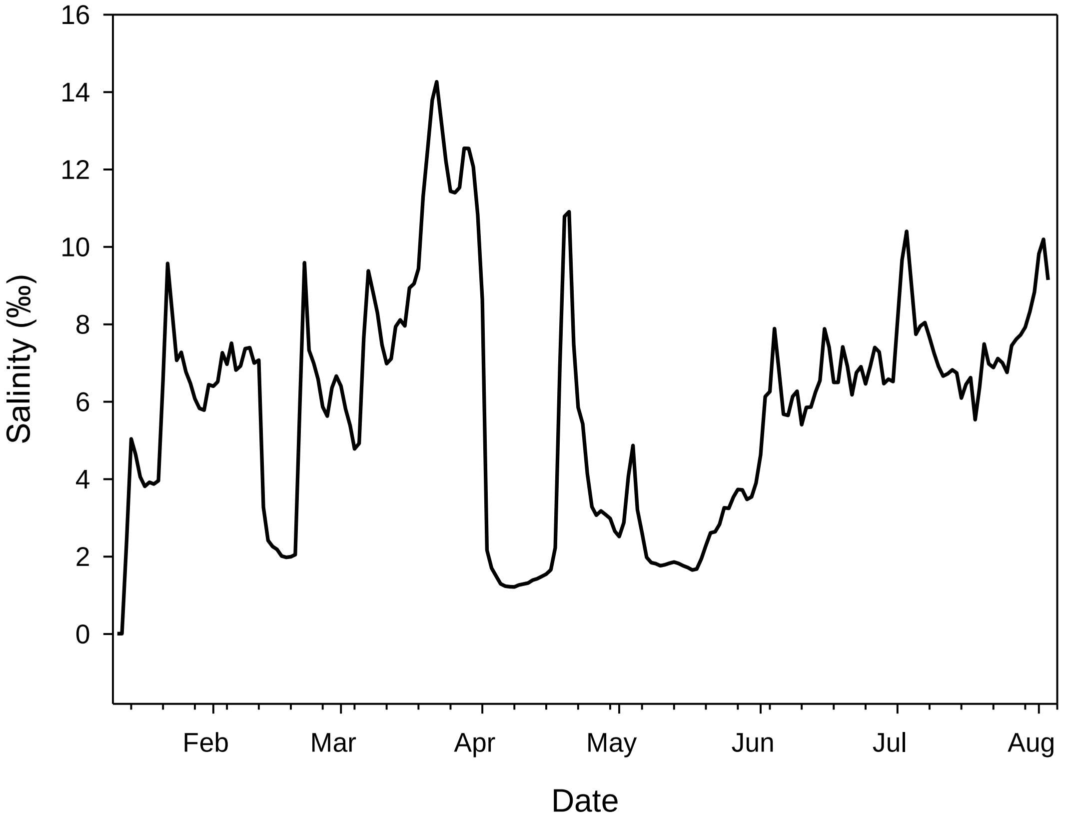

Figure 4.4 Average daily salinity levels during 2013 in the ebb and flow region (receiver 7) of the Kowie Estuary.

4. DISCUSSION

The results obtained from this study seem to suggest that largemouth bass in the Kowie system migrate past the ebb and flow region into the main estuary. The average proportional time spent at each receiver reveal that the fish spent the most time in close proximity to receiver number four. This receiver was situated in a wide pool which is located less than 15 m above a causeway, at the head of the estuary. The depth of the water flowing over the causeway is dependent on both the amount of water coming from the upstream riverine section of the system and the tide level of water from the estuarine section. Under high river flow conditions little or no tidal penetration of estuarine water will occur into the above pool. However, during low river flow conditions, which is the dominant state for this system, saline water from the estuary will enter this pool during the spring tidal cycle.

It was observed that during periods of low tide the water level drops to such an extent that only a thin layer of water flows over the causeway. Therefore, any marine fish movements upstream of the causeway would require a rise in the estuary and/or river water levels such that the causeway is completely inundated. During such conditions, the juvenile estuary-associated marine species that also use the lower riverine area as a nursery can move up into the pools above the causeway. This perhaps renders the pools immediately above the causeway as favourable areas for largemouth bass foraging and may explain their preference for Sites 4 and 5. This is further supported by the dietary results which revealed that *M. salmoides* inhabiting this area had a diet that comprised the highest proportion of estuary-associated fish when compared with *M. salmoides* from sites located further upstream.

During the course of this study, two distinct types of largemouth bass behaviour were observed. The first was a tendency towards a sedentary existence, while the second was a propensity towards a more mobile lifestyle. Largemouth bass were also found to have a combination of sedentary and mobile lifestyles in Mary Lake, north-central Minnesota (Winter, 1977). In addition, Huchzermeyer et al. (2013) found that largemouth bass returned to their original sites after being displaced, thus suggesting that some *M. salmoides* have strong fidelity to specific sites. Hunter and Maceina (2008) also found that *M. salmoides* showed site fidelity in Lake Martin, Alabama. All largemouth bass that migrated into the upper reaches of the Kowie Estuary also made return trips to the more riverine areas above the ebb and flow region. Such events occurred twice during this study period, the first taking place in April and then again in mid-May. This indicates that largemouth bass movement into the estuary is not a chance event.

All fish remained active after returning to the freshwater areas. This in itself suggests that their migration into the estuary did not cause any permanent physiological damage. Lowe et al. (2009) found that age-0 largemouth bass in the Mobile–Tensaw River Delta, Alabama, USA do not migrate into freshwater areas in response to increasing salinity levels. However, Glover et al. (2012) found that elevated salinity levels affected the respiration of largemouth bass as well as resulting in slow growth rates and small body size. As such, it would be expected that the adult individuals from the Kowie's bass population will move upstream towards the freshwater areas when salinity levels rise in the estuarine section during high tide in order to avoid the above mentioned implications that accompany spending lengths of time in saline water. The largemouth bass that moved downstream during April and May also made upstream migrations as they were forced away from areas with elevated salinities.

The fact that no largemouth bass were detected by the receiver located above the weir (receiver 1) suggests that these individuals did not utilise the fishway to migrate upstream of this barrier. However, largemouth bass were also collected from the deep pool located upstream of the weir and fishway during the dietary analysis stage of this study, suggesting that they have the ability to move up and down the fishway. The linear arrangement of the acoustic receivers along the length of the Kowie system during this study renders them incapable of tracking lateral movements of largemouth bass. It has, however, been reported that largemouth bass migrate into shallow waters during the night for foraging purposes (Brown et al., 2009).

The average salinity recorded during the course of this study is well within the tolerance range of largemouth bass (Meador and Kelso, 1989). However, movements into estuarine areas only occurred during April and May, the two months which had the lowest average salinities. As such, Hypothesis C (as listed in Chapter 1) which states that largemouth bass move towards the upper reaches of the Kowie Estuary during periods of high river flow, and is linked to the lowering of salinities as large volumes of freshwater enter the headwaters of the estuary, has been duly supported.

However, this does not serve as a sound basis to conclude largemouth bass cannot survive salinities in excess of 5‰, as they have been reported temporarily inhabiting waters with salinity levels above 20‰ (Brown et al., 2009). Therefore, it can be expected that largemouth bass in the lower Kowie River can swim towards the upper reaches of the estuary even when salinity levels are above the fish's envisaged optimum of 5‰ (Meador and Kelso, 1989; Glover et al., 2012). However, during drought periods when salinities in the upper reaches of the Kowie Estuary approach seawater levels, it is unlikely that this species will enter the estuary.

The movement patterns highlight that the threat of largemouth bass to the juveniles of certain marine fish species is not only confined to the ebb and flow region, but extends well into the estuary during river flooding. The ability of largemouth bass to migrate from the freshwater to the estuarine section has not previously been explored by studies conducted in the Kowie system. This encroachment by largemouth bass into the estuarine waters can be viewed as being synonymous with introducing a new predator directly into the upper reaches of the estuary. Since estuarine-associated fish naturally utilise estuaries as breeding and nursery areas (Whitfield, 1998), expansion of the largemouth bass range can potentially reduce the value of the upper estuary reaches to the aforementioned native fishes (Vermeij, 1996).

Even though largemouth bass in this study were found to move into the estuarine section of the Kowie system, these movements were short-lived and coincided with major freshwater input events. Telemetry results encompassing data collected over a longer time period than was possible during this study are required in order to more fully understand movement patterns by largemouth bass in this system. In particular, their movements during drought years will help identify salinity preferences and tolerances, since polyhaline and euhaline conditions then prevail in the upper reaches of the Kowie Estuary.

The ability of largemouth bass to survive in salinities above 5‰ magnifies their threat to the population integrity of indigenous estuary-associated fish species which use the Kowie system as a nursery area. As described in chapters two and three, largemouth bass in the ebb and flow region prey on indigenous estuary-associated fish entering the lower river sections. If a flood event was to occur, this would increase the influx of freshwater, potentially decreasing salinity levels in the upper reaches of the estuary and thus provide an opportunity for largemouth bass to move into this area. This event would improve the chances of largemouth bass predation on estuary-associated fish.

In summary, the results of this study show that largemouth bass in the lower Kowie River are capable of moving beyond the ebb and flow region as some individuals moved downstream as far as receiver 15. Although three individuals exhibited a resident lifestyle, the other seven individuals appear to have a mobile lifestyle. The individuals that ventured into the brackish water only spent a maximum of three days in the area. This suggests that salinity is a major factor in largemouth bass movement as all the fish made return journeys upstream which coincided with sudden spikes in salinity levels. These individuals appear to move away from the upper reaches of the estuary in response to rising salinity levels. Therefore it can be deduced that largemouth bass in the lower Kowie River are highly affected by changes in salinity and hence only occupy the upper reaches of the estuary during periods of high freshwater influx.

In this chapter, acoustic telemetry was found to be a viable complementary tool for elucidating the extent of the threat posed by largemouth bass towards indigenous estuary-associated marine fish in this system. It has now been documented that largemouth bass are quite capable of occupying the upper reaches of estuaries that are characterised by oligohaline or even polyhaline waters. Besides the various issues associated with acoustic telemetry such as the variable effects of water depth, wind conditions and multiple transmitter interference (Baras and Lagardere, 1995), the results obtained during this study can be used as a reliable tool for understanding the dynamics of largemouth bass movement patterns. Future emphasis needs to be directed towards sampling for largemouth bass in the absence of river flooding and under different upper estuary salinity conditions.

CHAPTER 5

GENERAL DISCUSSION

Alien invasive organisms continue to negatively impact pristine ecosystems around the globe (Vermeij, 1996; Mack et al., 2000; Kennard et al., 2004). Modern day advances in transport have increased the likelihood and frequency of occurrence of human mediated introductions of alien organisms into regions previously out of their reach (Mack et al., 2000; Whinam et al., 2005). Biological invasions not only have deleterious outcomes in terms of the indigenous biota and a reshaping of evolutionary processes in the invaded ecosystem, they can also have detrimental consequences at an economic level (Vermeij, 1996; Kennard et al., 2004). It is therefore of the utmost importance that quick steps are taken to limit and ultimately purge the introductions of invasive organisms from new affected regions. It is also worthwhile to understand the stages involved in the process of introduction and successful integration of alien invasive organisms in order to assist in the development of appropriate eradication programmes (Mack et al., 2000; Van Wilgen et al., 2001).

As was shown in chapters 2 and 3, largemouth bass consume estuary-associated fish as part of their diet. Although estuary-associated fish do not exclusively constitute the diet of largemouth bass, their proportions are of a sufficient magnitude to be a cause for concern. These two chapters also further serve to highlight the importance and relevance of traditional stomach contents analysis as a reference tool for reconstructing diets in tandem with modern techniques such as stable isotope and fatty acid analyses. Stomach content analysis also revealed the existence of cannibalism by largemouth bass in the Kowie system, a trait that has previously been reported in the literature (e.g. Crass, 1964; Clady, 1974; Johnson and Post, 1999). However, since no estimation of the largemouth population in the Kowie system was conducted during this study, the extent of the predation impact by this species on both estuary-associated fish and largemouth bass themselves could not be assessed.

The overall dietary results obtained in this study appear to concur with those obtained by both Weyl and Lewis (2006) and Wasserman et al. (2011). It is also interesting to note that the modern techniques (stable isotopes and fatty acids analyses) are in agreement with traditional stomach contents analysis regarding the consumption of fish by largemouth bass. However, the level of piscivory was found to be lower than that determined by Weyl and Lewis (2006) and Wasserman et al. (2011). Stable isotope analysis using the SIAR package suggested that insects constitute the highest proportion of *M. salmoides* diet.

Also surprising was the indication from SIAR that *R. holubi* constitutes a significantly higher proportion of *M. salmoides* diet than was revealed by stomach content results. Invertebrates constituted a major portion of largemouth bass diet in the Kowie system during the study period. Both stomach content and stable isotope analyses revealed ontogenic differences in dietary preferences of the largemouth bass, with small and medium-sized *M. salmoides* consuming higher proportions of fish than large-sized *M. salmoides.* This is contrary to the normal pattern of piscivory in fishes where juveniles are often more dependent on invertebrates as a prey source and adults feed predominantly on fishes (Hickley et al., 1994; García-Berthou, 2002; Wheeler and Allen, 2003). During sampling, it was observed that juvenile *M. salmoides* occupy a site furthest downstream while adults inhabited deep pools upstream. This may have contributed to the higher consumption of estuary-associated fish by small and medium sized largemouth bass as they had a higher chance of encountering the aforementioned fish compared the large sized *M. salmoides.*

The modern approaches to diet reconstruction such as stable isotope and fatty acid analyses are not without their own inherent shortcomings. These include, but are not limited to, the amount of time involved in the preparation and processing of samples at the laboratory. A considerable amount of effort was also expended on analysing the chromatograms obtained during the fatty acid analysis part of this study. Interpretation of fatty acid data also revealed challenging, as there are few dietary biomarkers that have been described for estuarine and freshwater organisms (Napolitano, 1999) in comparison to their marine counterparts (Budge et al., 2006; Parrish, 2013), thus increasing the level of difficulty when attempting to interpret results based merely on biomarkers.

Principal components analysis of the fatty acid data of largemouth bass and its potential prey suggested a similarity in the profiles of *M. salmoides* with those of *M. falciformis* and *M. capensis*, while failing to find a clear connection with those of *R. holubi, G. callidus, P. sidneyi,* Coenagronidae and Libellulidae, although these prey items were often encountered in bass stomachs, and were identified as a major prey by stable isotopes. One approach that may reveal relationships between the fatty acid profiles of largemouth bass and their potential prey is Quantitative Fatty Acid Signature Analysis (QFASA) as previously described and discussed in detail by Iverson et al. (2004). This statistical model allows for the estimation of proportional contributions of each potential prey item to the overall diet of the consumer. Due to time constraints, and challenges inherent to fatty acids analyses described above, this model could not be used to analyse the data obtained during the present study. Such models shall however be tested in the future.

As for stable isotopes, a major challenge encountered when analysing data collected is that there currently appears to be no universal estimate of trophic enrichment factors (TEF). A case in point is that several authors have suggested varying TEF values for nitrogen isotopes, range between 2 and 4‰ (e.g. DeNiro and Epstein, 1978; Minagawa and Wada, 1986; Peterson and Fry, 1987; Hansson et al., 1997; Vander Zanden et al., 1997). As such, McCutchan et al. (2003) reviewed several TEF values which differ according to the type of stable isotope, as well as the type of consumer and potential prey being analysed. Studies of this kind are very useful tools for researchers seeking to produce results that can be compared with others on an international scale without having to face the challenge of having to develop their own specific TEF estimates. Sadly, due to time constraints, it was not possible to conduct a broad scale review study of TEF estimates within the context of this thesis. Regardless of the aforementioned issue, stable isotopes proved to be a very useful tool in elucidating the proportional contributions of prey items to the diet of largemouth bass.

The results obtained through acoustic telemetry highlight that the threat of largemouth bass is not only confined to the river, but extends well into the mid-section of the estuarine system under high freshwater flow conditions. The ability of largemouth bass to move from the freshwater to the estuarine section has not previously been explored in the Kowie system. This encroachment by *M. salmoides* into the estuarine waters can be viewed as being synonymous with introducing a new predator directly into the upper estuary, an area that usually has a lower abundance of marine piscivorous fishes and higher density of certain estuarine taxa (Whitfield, 1998). Since estuary-associated fish naturally utilise these systems as nursery areas (Whitfield, 1998; Beck et al., 2001; Dolbeth et al., 2008), expansion of largemouth bass ranges into the headwaters and upper reaches could affect the abundance of native estuary-associated fish. In addition, potentially confining the estuary-associated prey fish species to the more saline areas of the estuary could increase the exposure of these taxa to a wider diversity of marine piscivorous species (Vermeij, 1996).

The above scenario serves to support the first hypothesis of this thesis (Hypothesis A), which states that *M. salmoides* acts as a potential barrier to the movement of indigenous estuary-associated fish species into the river, and can also pose as a potential predatory threat to those indigenous fish species occupying the river-estuary interface zone. This apparent propensity of largemouth bass to invade the headwaters of the Kowie Estuary needs to be investigated over a longer timescale than the one used in this study in order to ascertain whether these alien invasive fish repeatedly occupy estuarine areas under lowered salinity conditions (Meador and Kelso, 1989; Lowe et al., 2009). It would appear this population of largemouth bass can survive brackish salinity levels, consistent with previous findings by Brown et al. (2009). It is however worth noting that the migrations into the upper estuarine section of the Kowie Estuary occurred during months when salinity levels dropped to the lowest levels recorded during this study (i.e., April and May 2013), thus supporting Hypothesis C, which stated that largemouth bass move into the upper reaches of the Kowie Estuary during periods of high river flow.

The diet of largemouth bass collected at Site FW3 contained the highest proportion of estuary-associated fish, decreasing in an upstream direction. This supports Hypothesis B, which states that the population of largemouth bass which inhabits a section of the river downstream of the weir will consume higher quantities of the estuary-associated fish species. It can then also be hypothesized that the diet of *M. salmoides* which migrate downstream into the area within the vicinity of acoustic receiver number 15 will consist of an even higher proportion of the aforementioned fish. Therefore, the impact of largemouth bass predation on estuarine fish populations would be more severe the further downstream these predators can migrate.

It is also disconcerting to note that *M. salmoides* seem capable of surviving salinity levels higher than the average 5.8‰ recorded during the course of this study (Meador and Kelso, 1989; Lowe et al., 2009; Glover et al., 2012). This means that largemouth bass have the potential to make downstream movements into the estuary several times a year, especially when high river flow enters the system. It is worth mentioning that largemouth bass have the potential of moving even further down the Kowie Estuary than was recorded in this study, as some coastal populations of *M. salmoides* have been reported surviving in waters with salinities above 20‰ (Brown et al., 2009).

Apart from the negative consequences for the indigenous estuary-associated fish within the estuary itself, a number of these species (e.g. freshwater mullet *M. capensis*) also use the lower river catchment as a nursery area (Whitfield, 1998). The concentration of largemouth bass in the lower reaches of the Kowie River serves as a partial barrier to fish movement upstream of the ebb and flow region. This does not bode well for the connectivity and free movement of indigenous estuary-associated fish between the estuary and river. In the long run, *M. salmoides* might inadvertently play a key role in shaping the evolution of estuary-associated fish in the Kowie as they may have to adapt their lifestyles in order to reduce the impact of this new artificially introduced predator (Vermeij, 1996).

The introduction of non-native fish into new regions can have both positive and negative impacts (Gozlan, 2008). From a commercial standpoint, the introduction of largemouth bass into South African aquatic systems has considerable social and economic benefits linked to recreational angling. These are not limited to social benefits derived by bass anglers but include the financial income derived by land owners who can charge an entrance fee to anglers in order to access fishing areas (Ridgway and Philipp, 2002). Therefore, advocating that the maintenance of the ecological integrity of water bodies is more crucial than the economic benefit that can be derived from the presence of bass in such systems is becoming a highly challenging exercise.

We are living in an era where most people have lost touch with 'nature' and are thus unconcerned about the negative impacts that certain actions have on biodiversity. Therefore, convincing such people about the importance of maintaining natural biodiversity and ecological integrity is no easy task. In addition, the fact that many organisms whose introductions are perceived be detrimental to local biodiversity have actually never been successfully scientifically proven to be a threat, is also used as evidence to support alien introductions (Gozlan, 2008). Therefore, it would appear that most conclusions around the acceptance or rejection of particular alien organisms are based on generalized perceptions that are often not based on research of these particular organisms. As such, scientists need to quote or conduct relevant research when commenting on specific alien introductions and to adopt a balanced approach when assessing the potential impacts (positive and negative) that might result from the introduction of alien organisms. Even though, as a scientist, one might be tempted to dismiss the introduction of any alien organisms into a region, this must not force one to be biased and thus overlook the potential benefits of certain introductions to economic development.

However, the deleterious ecological and environmental implications of introducing alien organisms frequently outweigh any economic benefit derived from such endeavours (Pimentel et al., 2000; Jackson, 2002). This is a highly relevant issue in a country such as South Africa, which has already been invaded by an excessive number of alien organisms to the detriment of its flora and fauna (Van Wilgen et al., 2001). It is of great concern that a variety of alien fish have been detected in aquatic systems which flow through South Africa's national parks (Russell, 2011), thus highlighting the vulnerability and connectivity of aquatic ecosystems. Since the initial introduction of largemouth bass in South African water bodies in 1928, it has continued to spread countrywide as a result of intentional introductions, mainly for sport fishing purposes (De Moor and Bruton, 1988).

Eradication of alien invasive organisms can be a highly costly endeavour, with financial constraints often requiring successful eradication at the very first attempt (Van Wilgen et al., 2001; Cromarty et al., 2002). The eradication of invasive organisms is a far more drastic step than the more subdued actions involved when the control option is chosen. The former action requires that all alien individuals must be removed, or even exterminated from a system, in order for the operation to be considered a success (Cromarty et al., 2002). The use of piscicides, such as rotenone in the Rondegat River in the Western Cape to remove alien largemouth bass, can sometimes yield results which are controversial in the public domain unless all protocol and best practices are observed (Impson et al., 2013). However, the financial costs and moderate success rate of similar operations in other countries, renders this a high risk exercise. Furthermore, some eradication programmes require follow-up programmes in order to confirm their success (Ellender et al., 2011). Thus, there is a strong case to be made for the “prevention is better than cure” approach, but that requires the co-operation of all parties involved and strong enforcement of nature conservation legislation.

Appropriate management practice should involve extensive research being undertaken on the potential positive and negative ecological and economic impacts before any alien fish introductions are implemented (McDowall, 1968; Gozlan, 2008). In the case of the current study area (Waters Meeting Nature Reserve), this would involve assessing the positive benefits of income through gate takings from catch and release bass anglers and the negative effects these invasive alien fish are having on the Kowie aquatic ecosystem. In coming to a management decision, consideration should also be given to the possible long-term impacts of introducing alien organisms to natural systems (Vermeij, 1996), as well as unforeseen consequences that are difficult to measure using current scientific techniques .

In conclusion, invasive largemouth bass introduced into the Kowie River do pose a predation threat to the population integrity and survival of certain indigenous estuary-associated fish species in the headwaters of this system. The bass may also serve as a partial barrier to the upstream migration of juveniles of these estuary-associated fish to riverine nursery areas. At this juncture, the severity of the effect of largemouth bass predation on estuary-associated fish species needs to be quantified through an estimation of the biomass and population size of both the largemouth bass and their prey in this system. In addition, long term acoustic telemetry work is required to determine the magnitude and frequency of *M. salmoides* encroachment into the estuarine section of the Kowie system. This will assist in providing information on whether these migrations are random opportunistic events or a more regular occurrence.

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APPENDIX

Table A1: Stomach contents analysis data for *Micropterus salmoides* (n = 10) collected during April 2012 (session 1) in the lower Kowie system. %N = the number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of stomachs containing a particular food item expressed as a percentage of all stomachs sampled; %M = the mass of each food category expressed as a percentage of the total mass of all food categories; %V = the volume of each food category expressed as a percentage of the total volume of all food items; %IRI = index of relative importance, as a proportion of the total IRI of all prey items.

Prey category				Abundance, occurrence and importance					
Class	**Order**	**Family**	**Species**	**N**	**%N**	**%F**	**%M**	**%V**	**%IRI**
		Monodactylidae	*Monodactylus falciformis*	2	28.6	20.0	70.1	38.0	68.99
		Sparidae	*Rhabdosargus holubi*	1	14.3	10.0	23.2	37.2	19.25
Insecta	Hemiptera	Belostomatidae		1	14.3	10.0	1.3	4.1	1.72
		Naucoridae		2	28.6	20.0	1.3	4.1	3.44
	Odonata	Aeshnidae		1	14.3	10.0	4.1	16.5	6.59

Table A2: Stomach contents analysis data for *Micropterus salmoides* (n = 19) collected during August 2012 in the lower Kowie system. %N = the number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of stomachs containing a particular food item expressed as a percentage of all stomachs sampled; %M = the mass of each food category expressed as a percentage of the total mass of all food categories; %V = the volume of each food category expressed as a percentage of the total volume of all food items; %IRI = index of relative importance, as a proportion of the total IRI of all prey items.

Prey category				**Abundance, occurrence and importance**					
Class	**Order**	**Family**	**Species**	**N**	**%N**	**%F**	**%M**	**%V**	**%IRI**
Amphibia	Anura			1	2.6	5.3	8.9	14.4	0.91
		Libellulidae		2	5.3	5.3	0.3	0.2	0.02
Malacostraca	Decaopoda	Potamonautidae	*Potamonautes sidneyi*	29	76.3	78.9	88.0	80.3	98.54
Other			Stones	2	5.3	5.3	0.9	1.0	0.07
			Plant leaves	3	7.9	15.8	0.7	1.2	0.22
			Unidentified organic matter	-	-	10.5	0.7	2.2	0.23

Table A3: Stomach contents analysis data for *Micropterus salmoides* (n = 5) collected during November 2012 in the lower Kowie system. %N = the number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of stomachs containing a particular food item expressed as a percentage of all stomachs sampled; %M = the mass of each food category expressed as a percentage of the total mass of all food categories; %V = the volume of each food category expressed as a percentage of the total volume of all food items; %IRI = index of relative importance, as a proportion of the total IRI of all prey items.

Prey category				Abundance, occurrence and importance					
Class	**Order**	**Family**	**Species**	**N**	**%N**	**%F**	**%M**	**%V**	**%IRI**
Actinopterygii	Mugiliformes	Mugilidae	*Myxus capensis*	3	75.0	60.0	50.6	70.0	82.01
Other			Fish remains	1	25.0	20.0	49.4	30.0	17.99

Table A4: Stomach contents analysis data for *Micropterus salmoides* (n = 33) collected during February 2013 in the lower Kowie system. %N = the number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of stomachs containing a particular food item expressed as a percentage of all stomachs sampled; %M = the mass of each food category expressed as a percentage of the total mass of all food categories; %V = the volume of each food category expressed as a percentage of the total volume of all food items; %IRI = index of relative importance, as a proportion of the total IRI of all prey items.

Prey category				Abundance, occurrence and importance					
Class	**Order**	**Family**	**Species**	**N**	**%N**	**%F**	**%M**	**%V**	**%IRI**
Actinopterygii	Mugiliformes	Mugilidae	*Myxus capensis*	5	4.8	26.3	0.5	1.9	1.37
	Perciformes	Centrachidae	*Micropterus salmoides*	6	5.8	21.1	2.5	24.6	12.11
		Gobiidae	*Glossogobius callidus*	1	1.0	5.3	0.2	2.0	0.25
			Caffrogobius sp.	5	4.8	21.1	91.3	28.0	53.31
		Monodactylidae	*Monodactylus falciformis*	8	7.7	36.8	4.0	29.1	25.91
Insecta	Hemiptera	Naucoridae		1	1.0	5.3	0.002	0.3	0.03
	Odonata	Aeshnidae		72	69.2	36.8	0.3	4.0	3.30
		Libellulidae		1	1.0	5.3	0.0	0.1	0.02
Malacostraca	Decaopoda	Potamonautidae	*Potamonautes sidneyi*	3	2.9	15.8	1.2	9.6	3.59
Other			Plant leaves	2	1.9	10.5	0.02	0.4	0.10
			Unidentified organic matter	-	-	5.3	0.001	0.1	0.01

Table A5: Stomach contents analysis data for 20 small (≤150 mm TL) *Micropterus salmoides* collected between April 2012 and February 2013 in the lower Kowie system. %N = the number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of stomachs containing a particular food item expressed as a percentage of all stomachs sampled; %M = the mass of each food category expressed as a percentage of the total mass of all food categories; %V = the volume of each food category expressed as a percentage of the total volume of all food items; %IRI = index of relative importance, as a proportion of the total IRI of all prey items.

Prey category				**Abundance, occurrence and importance**					
Class	**Order**	**Family**	**Species**	**N**	**%N**	**%F**	**%M**	**%V**	**%IRI**
Actinopterygii	Mugiliformes	Mugilidae	*Myxus capensis*	6	8.5	28.6	16.1	17.0	41.01
	Perciformes	Centrachidae	*Micropterus salmoides*	1	1.4	4.8	3.0	3.1	1.27
		Gobiidae	*Glossogobius callidus*	3	4.2	4.8	15.7	11.6	5.63
		Monodactylidae	*Monodactylus falciformis*	1	1.4	4.8	3.1	26.3	6.06
Insecta	Hemiptera	Belostomatidae		1	1.4	4.8	1.6	3.9	1.14
		Naucoridae		2	2.8	4.8	1.6	3.9	1.14
	Odonata	Aeshnidae		53	74.6	28.6	12.2	11.6	29.57
		Coenagrionidae		1	1.4	4.8	0.02	0.8	0.16
Malacostraca	Decaopoda	Potamonautidae	*Potamonautes sidneyi*	1	1.4	4.8	38.4	15.4	11.13
Other			Fish remains	1	1.4	4.8	7.0	4.6	2.40
			Plant leaves	1	1.4	4.8	0.34	1.54	0.39
			Unidentified organic matter	-	-	4.8	0.04	0.39	0.09

Table A6: Stomach contents analysis data for 18 medium (151-300 mm TL) *Micropterus salmoides* collected between April 2012 and February 2013 in the lower Kowie system. %N = the number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of stomachs containing a particular food item expressed as a percentage of all stomachs sampled; %M = the mass of each food category expressed as a percentage of the total mass of all food categories; %V = the volume of each food category expressed as a percentage of the total volume of all food items; %IRI = index of relative importance, as a proportion of the total IRI of all prey items.

Prey category				**Prey abundance, occurrence and importance**					
Class	**Order**	**Family**	**Species**	**N**	**%N**	**%F**	**%M**	**%V**	**%IRI**
Actinopterygii	Mugiliformes	Mugilidae	*Myxus capensis*	1	6.7	6.7	0.2	1.3	1.8929
	Perciformes	Centrachidae	*Micropterus salmoides*	5	33.3	20	20.4	57.9	33.077
		Monodactylidae	*Monodactylus falciformis*	2	13.3	13.3	28.7	10.2	24.66
Insecta	Odonata	Libellulidae		1	6.67	6.67	0.53	6.58	9.0117
Malacostraca	Decaopoda	Potamonautidae	*Potamonautes sidneyi*	6	40.0	20.0	50.20	24.01	31.359

Table A7: Stomach contents analysis data for 29 large (301-420 mm TL) *Micropterus salmoides* collected between April 2012 and February 2013 in the lower Kowie system. %N = the number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of stomachs containing a particular food item expressed as a percentage of all stomachs sampled; %M = the mass of each food category expressed as a percentage of the total mass of all food categories; %V = the volume of each food category expressed as a percentage of the total volume of all food items; %IRI = index of relative importance, as a proportion of the total IRI of all prey items.

Prey category				**Abundance, occurrence and importance**					
Class	**Order**	**Family**	**Species**	**N**	**%N**	**%F**	**%M**	**%V**	**%IRI**
Actinopterygii	Mugiliformes	Mugilidae	*Myxus capensis*	1	2.2	3.2	1.4	0.1	0.09
	Perciformes	Gobiidae	*Caffrogobius* sp.	2	4.4	12.9	35.6	12.7	11.35
		Monodactylidae	*Monodactylus falciformis*	7	15.6	19.4	5.4	12.0	6.12
		Sparidae	*Rhabdosargus holubi*	1	2.2	3.2	1.2	2.8	0.24
Amphibia	Anura			1	2.2	3.2	6.6	10.5	1.01
Insecta	Hemiptera	Naucoridae		1	2.2	3.2	0.008	0.1	0.01
	Odonata	Aeshnidae		1	2.2	3.2	0.4	0.9	0.07
		Libellulidae		2	4.44	3.23	0.3	0.12	0.02
Malacostraca	Decaopoda	Potamonautidae	*Potamonautes sidneyi*	25	55.56	45.16	47.1	50.96	80.64
Other			Plant leaves	2	4.44	6.45	0.1	0.93	0.13
			Stones	2	4.44	3.23	0.6	0.74	0.08
			Unidentified organic matter	-	-	6.45	0.5	1.61	0.25

Table A8: Stomach contents analysis data for *Micropterus salmoides* (n = 9) collected at site FW0 between April 2012 and February 2013 in the lower Kowie system. %N = the number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of stomachs containing a particular food item expressed as a percentage of all stomachs sampled; %M = the mass of each food category expressed as a percentage of the total mass of all food categories; %V = the volume of each food category expressed as a percentage of the total volume of all food items; %IRI = index of relative importance, as a proportion of the total IRI of all prey items.

Prey category				**Prey abundance, occurrence and importance**					
Class	**Order**	**Family**	**Species**	**N**	**%N**	**%F**	**%M**	**%V**	**%IRI**
Actinopterygii	Mugiliformes	Mugilidae	*Myxus capensis*	1	1.8	12.5	6.9	5.4	2.48
	Perciformes	Centrachidae	*Micropterus salmoides*	1	1.8	12.5	9.1	10.8	4.01
		Gobiidae	*Glossogobius callidus*	1	1.8	12.5	46.9	40.5	17.63
Insecta	Odonata	Aeshnidae		52	92.9	62.5	36.1	37.8	74.57
Other			Plant leaves	1	1.8	12.5	1.02	5.4	1.30

Table A9: Stomach contents analysis data of *Micropterus salmoides* (n = 29) collected at site FW1 between April 2012 and February 2013 in the lower Kowie system. %N = the number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of stomachs containing a particular food item expressed as a percentage of all stomachs sampled; %M = the mass of each food category expressed as a percentage of the total mass of all food categories; %V = the volume of each food category expressed as a percentage of the total volume of all food items; %IRI = index of relative importance, as a proportion of the total IRI of all prey items.

Prey category				**Prey abundance, occurrence and importance**					
Class	**Order**	**Family**	**Species**	**N**	**%N**	**%F**	**%M**	**%V**	**%IRI**
Actinopterygii	Mugiliformes	Mugilidae	*Myxus capensis*	1	2.0	3.4	1.4	0.1	0.07
	Perciformes	Centrachidae	*Micropterus salmoides*	5	9.8	10.3	8.5	12.0	2.94
		Gobiidae	*Caffrogobius* sp.	1	2.0	3.4	1.4	3.4	0.23
		Monodactylidae	*Monodactylus falciformis*	4	7.8	10.3	12.2	3.5	2.26
Amphibia	Anura			1	2.0	3.4	6.7	0.8	0.36
Insecta	Hemiptera	Belostomatidae		1	2.0	3.4	0.1	0.3	0.02
		Naucoridae		2	3.9	3.4	0.1	0.3	0.02
	Odonata	Libellulidae		2	3.9	3.4	0.3	0.1	0.02
Malacostraca	Decaopoda	Potamonautidae	*Potamonautes sidneyi*	29	56.9	51.7	66.0	64.3	93.57
Other			Plant leaves	3	5.9	10.3	0.5	1.0	0.21
			Stones	2	3.9	3.4	0.6	0.8	0.07
			Unidentified organic matter	-	-	6.9	0.6	1.8	0.22

Table A10: Stomach contents analysis data for *Micropterus salmoides* (n = 20) collected at site FW2 between April 2012 and February 2013 in the lower Kowie system. %N = the number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of stomachs containing a particular food item expressed as a percentage of all stomachs sampled; %M = the mass of each food category expressed as a percentage of the total mass of all food categories; %V = the volume of each food category expressed as a percentage of the total volume of all food items; %IRI = index of relative importance, as a proportion of the total IRI of all prey items.

Prey category				**Prey abundance, occurrence and importance**					
Class	**Order**	**Family**	**Species**	**N**	**%N**	**%F**	**%M**	**%V**	**%IRI**
Actinopterygii	Mugiliformes	Mugilidae	*Myxus capensis*	2	10	9.5	0.6	1.6	0.81
	Perciformes	Gobiidae	*Caffrogobius* sp.	4	20	14.3	75.0	31.3	58.90
		Monodactylidae	*Monodactylus falciformis*	5	25	23.8	11.5	13.7	23.29
		Sparidae	*Rhabdosargus holubi*	1	5	4.8	2.8	4.6	1.37
Insecta	Hemiptera	Naucoridae		1	5	4.8	0.02	0.4	0.08
	Odonata	Aeshnidae		2	10	9.5	0.8	3.0	1.43
		Libellulidae		1	5	4.8	0.50	4.0	0.84
Malacostraca	Decaopoda	Potamonautidae	*Potamonautes sidneyi*	3	15	14.3	8.7	15.2	13.22
Other			Plant leaves	1	5	4.76	0.1	0.2	0.06

Table A11: Stomach contents analysis data for *Micropterus salmoides* (n = 9) collected at site FW3 between April 2012 and February 2013 in the Lower Kowie River. %N = the number of individuals of a particular taxon as a proportion of all prey items recorded; %F = the number of stomachs containing a particular food item expressed as a percentage of all stomachs sampled; %M = the mass of each food category expressed as a percentage of the total mass of all food categories; %V = the volume of each food category expressed as a percentage of the total volume of all food items; %IRI = index of relative importance, as a proportion of the total IRI of all prey items.

Prey category				Prey abundance, occurrence and importance					
Class	**Order**	**Family**	**Species**	**N**	**%N**	**%F**	**%M**	**%V**	**%IRI**
Actinopterygii	Mugiliformes	Mugilidae	*Myxus capensis*	3	60	42.9	74.8	22.0	73.78
		Monodactylidae	*Monodactylus falciformis*	1	20	14.3	24.7	74.7	25.25
Insecta	Odonata	Coenagrionidae		1	20	14.3	0.2	2.2	0.60
Other			Unidentified organic matter	-	-	14	0.4	1.1	0.37

Table A12: Fatty acid composition (mean %TFA ±SD) of *Micropterus salmoides* and potential food sources collected in the lower Kowie River between April 2013 and February 2013.

Fatty acids	***M. salmoides***		***M. falciformis***		***M. capensis***		***R. holubi***		***G. callidus***		**Aeshnidae**		***P. sidneyi***		**Coenagronidae**		**Libellulidae**	
10:0	0.1	±0.3	0		0		0.2	±0.2	0		0		0		0		0	
11:0	0		0.0	±0.1	0.1	±0.1	0		0		0		0		0		0	
12:0	1.0	±1.4	0.2	±0.1	9.4	±18.1	0.3	±0.5	0		0.5	±0.0	0		0.6	±0.1	0.3	±0.1
13:0	0		0		0		0		0		0		0.1	±0.1	0		0	
i14:0	0		0		0		0.1	±0.0	0		0		0		0.1	±0.0	0	
14:0	1.5	±1.8	1.0	±0.7	4.5	±5.8	4.0	±2.3	5.3	±5.5	2.4	±0.1	3.2	±0.2	3.3	±0.2	1.9	±0.1
i15:0	3.1	±6.7	15.2	±7.2	12.9	±22.5	0.2	±0.1	0.6	±0.7	0.2	±0.1	0.5	±0.0	0.2	±0.0	0.1	±0.1
ai15:0	0.7	±3.6	1.8	±1.0	2.0	±2.0	0.1	±0.1	0		0		0.2	±0.0	0.1	±0.0	0.1	±0.0
15:0	1.4	±2.4	3.4	±1.9	6.7	±10.4	0.9	±0.5	1.5	±0.6	0.2	±0.0	0.7	±0.1	0.3	±0.6	0.4	±0.1
i16:0	0		3.1	±4.3	0.4	±0.3	0.2	±0.2	0		0		0.1	±0.2	0.1	±0.0	0	
16:0	0.7	±1.1	0		0		21.8	±3.1	21.2	±3.6	19.9	±7.9	21.9	±1.4	19.3	±1.0	18.6	±3.5
i17:0	5.4	±7.3	0.1	±0.3	11.1	±22.8	0.4	±0.3	0		0.2	±0.3	0.6	±0.0	0		0.3	±0.5
ai17:0	5.7	±9.4	0		0		0.6	±0.6	0		0.3	±0.1	0.9	±0.1	0.1	±0.1	0.4	±0.2
17:0	3.9	±2.8	6.8	±5.7	1.9	±2.5	0.8	±0.5	1.6	±0.1	0.6	±0.2	3.1	±0.1	0.5	±0.1	1.2	±0.2
17:0 cis	0.6	±1.5	3.6	±2.0	4.0	±5.0	0		0		0		0.9	±0.2	0.1	±0.1	0	
18:0	1.4	±2.3	2.3	±2.5	1.5	±1.8	8.4	±2.9	10.4	±2.9	8.7	±0.5	7.1	±0.4	7.1	±0.4	10.1	±1.0
3-OH-14	0		0		0		0		0		0.2	±0.3	0		0		0	
19:0 cis	0		0		0		0		0		5.8	±0.4	0		1.2	±1.8	0	
2-OH-16	0		0		0		0		0		0.5	±0.2	0		0		0	
20:0	3.4	±3.9	0.1	±0.2	3.2	±5.9	0.5	±0.3	0		0.3	±0.1	0.6	±0.1	0.4	±0.1	0.7	±0.1
21:0	0		0		0		0		0		0.1	±0.2	0		0		0	

Table A12 (continued)

Fatty acids	*M. salmoides*		*M. falciformis*		*M. capensis*		*R. holubi*		*G. callidus*		Aeshnidae		*P. sidneyi*		Coenagronidae		Libellulidae	
22:0	8.2	±11.6	0.3	±0.8	0.9	±0.9	0.4	±0.4	0.4	±0.2	0		0.86	±2.8	0.1	±0.4	0.2	±0.1
24:0	3.7	±5.5	0.5	±0.5	0.1	±0.2	0.1	±0.1	0.1	±0.2	0		0		0		0	
26:0	2.1	±4.4	3.1	±1.6	0.4	±0.7	0		0		0		0		0		0	
28:0	4.4	±6.1	0		0		0		0		0		0		0		0	
ΣSFA	47.4	±4.2	41.4	±1.9	59.2	±6.6	39.1	±0.8	41.2	±1.7	40.0	±0.7	40.6	±0.4	33.5	±0.3	34.3	±0.5
14:1ω5	0.2	±0.3	0		0		0.1	±0.1	0		0		0		0.7	±0.2	0.3	±0.1
14:1ω3	0		0		0		0.2	±0.2	0		0		0		0		0	
16:1ω9	0.2	±0.4	0		0		0		0		0.1	±0.2	0		0.2	±0.0	2.6	±3.8
16:1ω7	0.8	±1.3	0.8	±1.1	0.4	±0.3	8.3	±3.7	7.3	±2.3	8.1	±0.9	9.3	±0.6	9.6	±0.9	4.3	±3.6
16:1ω5	0.0	±0.1	0		0		0		0		0.3	±0.1	0		0.4	±0.1	0.4	±0.0
17:1ω7	1.2	±2.7	0.8	±0.5	5.1	±2.7	0.9	±0.6	0.8	±0.9	0		0		0.2	±0.0	0.1	±0.2
18:1ω9	1.8	±1.4	2.3	±5.4	0.3	±0.5	8.4	±3.1	8.0	±3.0	5.8	±0.2	15.5	±0.1	8.0	±0.1	10.0	±0.3
18:1ω7	0.6	±1.4	0.2	±0.7	0.3	±0.2	4.5	±0.9	6.2	±2.4	16.6	±13.5	6.1	±0.4	9.3	±0.5	9.2	±1.6
18:1ω5	0.5	±1.2	0.6	±0.4	1.9	±2.0	0		0		0.4	±0.3	0		0.1	±0.0	0.3	±0.1
20:1ω11	0		0		0		0.6	±0.8	0		0		0		0		0	
20:1ω9	1.4	±1.7	0		0		0.2	±0.3	0.4	±0.4	0.1	±0.1	0.7	±0.2	0.1	±0.0	0.4	±0.1
20:1ω7	0		0		0		0		0		0.1	±0.1	0		0.1	±0.1	0.1	±0.2
20:1ω5	0		0		0		0		0		0		0		0		0.1	±0.2
22:1ω11	0		0		0		0.3	±0.7	0		0		0		0		0	
22:1ω9	2.6	±3.2	0		0		0.1	±0.2	0		0		0		0		0	
24:1ω9	0.4	±1.2	0		0		1.3	±1.9	0		0		0		0		0	
ΣMUFA	9.7	±1.4	4.8	±1.6	8.1	±1.1	24.9	±1.1	22.7	±1.8	31.6	±1.9	31.7	±0.3	28.7	±0.2	27.7	±0.9
16:2ω4	2.6	±5.2	0.1	±0.3	17.2	±23.3	0.6	±0.5	0		1.0	±0.7	0		1.6	±0.2	0.4	±0.4

Table A12 (continued)

Fatty acids	*M. salmoides*		*M. falciformis*		*M. capensis*		*R. holubi*		*G. callidus*		Aeshnidae		*P. sidneyi*		Coenagronidae		Libellulidae	
16:3ω3	0		0		0		0		0		0		0		0.1	±0.1	0	
17:3ω4	0		0		0		0		0		0.3	±0.3	0		0		0.2	±0.1
16:3ω4	1.6	±3.6	3.4	±3.4	1.5	±1.2	0.9	±0.6	0		0.3	±0.1	0		0.3	±0.3	0.2	±0.0
18:2ω9	0.2	±0.4	0		0		0.2	±0.3	0		5.9	±2.9	0		0		0	
18:2ω6cis	3.1	±5.7	4.4	±5.1	1.6	±1.9	4.1	±3.7	1.8	±1.4	0.4	±0.1	6.1	±0.7	5.5	±0.4	5.2	±0.6
18:2ω6trans	0		13.8	±8.7	2.1	±1.9	0		0		0		1.6	±0.4	0.8	±0.0	0.5	±0.1
18:3ω6	0.4	±0.9	0		0		0.6	±0.6	0		0		0		0.4	±0.6	0.7	±0.1
18:3ω3	5.7	±9.4	2.4	±2.5	1.8	±1.9	3.0	±2.7	0.6	±0.8	1.0	±0.3	1.1	±0.3	6.2	±0.5	6.2	±1.8
18:4ω3	0.6	±1.5	0.7	±0.7	0.2	±0.2	1.1	±0.7	0.9	±0.7	0		0		2.1	±0.1	1.1	±0.1
20:2ω9	0		0		0		0.4	±0.4	0		0		0		0		0	
20:2ω6	0.1	±0.9	0		0		0.9	±2.7	0		2.9	±0.2	0		0		0.4	±0.2
20:3ω6	0.9	±2.3	0		0		0.3	±0.3	0		0.1	±0.1	0		0		0	
20:4ω6	1.2	±3.5	4.0	±1.1	0.6	±0.6	3.1	±1.3	3.2	±2.2	16.3	±7.1	2.7	±2.5	1.0	±0.0	4.8	±1.6
20:3ω3	2.3	±2.7	7.4	±6.6	1.4	±1.4	0.1	±0.3	0		0.2	±0.3	0		0		0	
20:4ω3	2.2	±3.1	0.9	±1.5	1.7	±2.4	0.3	±0.4	0		0		0		0.2	±1.2	0	
20:5ω3	7.5	±12.9	3.6	±2.3	0.2	±0.2	8.4	±2.5	12.2	±4.6	0		16.1	±2.3	18.8	±0.1	18.1	±3.9
20:5ω6	1.5	±2.5	0		0		0		0		0		0		0		0	
22:2ω6	0.9	±2.3	0		0		0.1	±0.2	0		0		0		0		0	
22:4ω6	3.1	±4.3	0.7	±0.5	1.3	±1.5	1.0	±0.8	0.3	±0.4	0		0		0		0	
22:5ω6	2.8	±4.8	1.7	±1.0	2.1	±2.6	0.4	±0.4	0.6	±0.5	0		0		0		0	
22:5ω3	3.0	±3.4	9.6	±5.4	0.9	±2.0	4.0	±1.3	3.9	±2.0	0		0		0.6	±0.4	0	
22:6ω3	3.1	±3.2	1.1	±0.6	0.3	±0.7	6.1	±3.9	10.9	±10.7	0		0		0		0	
ΣPUFA	42.8	±3.8	53.8	±2.8	32.7	±3.0	36.1	±1.2	34.5	±2.6	28.4	±1.2	27.7	±1.2	37.7	±0.3	37.9	±0.8
ΣEFA	15.2	±4.3	26.4	±3.1	6.6	±1.2	17.5	±2.5	17.5	±3.2	17.8	±2.5	11.6	±1.0	15.6	±0.2	17.9	±0.8
ΣHUFA	8.8	±7.6	20.6	±5.4	5.5	±1.9	7.1	±3.2	2.4	±1.1	1.5	±0.2	8.9	±0.5	12.5	±0.3	11.9	±0.8

Table A13: Seasonal changes in the fatty acid composition (mean %TFA ±SD) of *Micropterus salmoides* collected in the lower Kowie system. C1 = April 2012; C2 = August 2012; C3 = November 2012; C4 = February 2013.

Fatty acids	C1		C2		C3		C4	
10:0	0.3	±0.4	0		1.0	±0.2	0	
12:0	2.5	±2.7	0.9	±0.4	1.9	±1.2	0.5	±0.5
14:0	3.4	±3.1	0.8	±0.4	3.7	±1.6	0.8	±0.8
i15:0	13.0	±12.6	3.2	±3.9	4.6	±2.6	0	
ai15:0	3.1	±8.9	0.0	±0.1	3.0	±0.4	0	
15:0	1.9	±2.1	0.2	±0.8	1.3	±0.3	2.2	±3.0
16:0	1.8	±1.8	0.1	±0.2	1.4	±1.3	0.6	±0.9
i17:0	1.7	±1.5	14.8	±4.8	6.1	±4.5	0	
ai17:0	0		17.9	±7.6	0		0	
17:0	0.8	±1.1	3.8	±2.1	1.5	±0.2	5.2	±2.9
$17:0^{\Delta}$	1.9	±2.6	0.2	±0.8	3.3	±1.9	0	
18:0	0.6	±0.8	0.0	±0.1	6.8	±0.5	1.6	±2.3
C20:0	2.9	±2.7	4.6	±1.7	0.2	±0.4	3.3	±5.2
C22:0	9.1	±8.0	21.1	±11.2	3.4	±1.0	0	
C24:0	3.9	±3.7	1.1	±2.1	3.1	±0.6	5.8	±7.1
C26:0	0		5.7	±6.1	3.9	±3.4	0	
C28:0	0		0		0		9.6	±5.6
ΣSFA	46.9	±8.5	74.4	±6.3	45.2	±7.6	29.6	±11.1
C14:1ω5	0		0	±0.0	0	±0.0	0.3	±0.3
C16:1ω9	0		0.6	±0.5	0	±0.0	0	
C16:1ω7	0.7	±1.1	0.2	±0.2	4.5	±1.5	0.5	±0.4
C16:1ω5	0		0.1	±0.1	0		0	
C18:1ω9	2.5	±2.2	1.1	±0.5	1.1	±1.3	2.1	±1.4
C18:1ω7	2.1	±1.6	0.0	±0.2	3.8	±1.6	0	
C18:1ω5	2.0	±1.7	0.0	±0.1	2.7	±1.5	0	
C17:1ω7	3.4	±3.3	0.5	±1.7	7.8	±1.1	0	
C20:1ω9	0		2.8	±1.8	0		1.2	±1.3
C22:1ω9	0		1.3	±0.7	0		4.8	±3.5
C24:1ω9	0		1.3	±1.9	0		0	
ΣMUFA	10.7	±5.2	8.0	±3.1	20.0	±2.9	8.9	±4.1
C16:2ω4	0		0		0		5.7	±6.4
C16:3ω4	9.1	±3.8	0.7	±2.9	2.6	±0.6	0	
C18:2ω6	1.7	±2.2	0.4	±0.2	0.2	±0.4	5.9	±7.5
C18:2ω9	0		0.6	±0.4	0		0	
C18:3ω6	0		0		0		0.8	±1.2
C18:3ω3	4.0	±5.5	0.4	±1.0	2.4	±0.5	10.3	±11.8
C18:4ω3	3.1	±2.4	0.0	±0.2	1.0	±0.7	0	
C20:4ω6	2.0	±1.9	0.0	±0.2	0.5	±0.1	1.7	±4.9
C20:2ω6	0		0.4	±1.6	0		0	

Table A13 (Continued)

Fatty acids	**C1**		**C2**		**C3**		**C4**	
C20:3ω6	0		2.3	±0.8	0		0	
C20:3ω3	2.4	±3.5	2.2	±1.5	7.7	±4.1	1.3	±1.5
C20:4ω3	4.2	±2.8	0.1	±0.3	0.8	±0.8	3.3	±3.5
C20:5ω3	4.1	±4.2	0.2	±1.1	3.4	±2.4	14.6	±16.5
C20:5ω6	0		4.8	±1.9	0		0	
C22:2ω6	0		0		0		2.1	±3.0
C22:4ω6	2.1	±2.0	0.8	±0.5	4.1	±0.9	4.8	±5.6
C22:5ω6	6.0	±7.7	0.2	±0.5	4.5	±1.1	3.1	±4.7
C22:5ω3	1.9	±2.9	1.8	±1.2	4.2	±2.2	3.9	±4.4
C22:6ω3	1.9	±2.8	2.6	±1.5	3.5	±0.6	4.0	±4.2
ΣPUFA	42.4	±8.3	17.6	±4.9	34.8	±5.9	61.5	±12.2
ΣEFA	16.9	±10.4	3.7	±3.6	10.9	±2.1	22.0	±14.8
ΣBFA	22.6	±11.7	40.0	±10.1	20.4	±6.2	7.4	±4.4

Table A14: Longitudinal changes in the fatty acid composition (mean %TFA ±SD) of *M. salmoides* collected in the lower Kowie system between April 2012 and February 2013.

Fatty acids	**FW0**		**FW1**		**FW2**		**FW3**	
C10:0	0	±0.0	0.0	±0.3	0.2	±0.4	0.2	±0.4
C12:0	0.5	±0.7	1.1	±1.6	1.2	±1.3	0.9	±1.4
C14:0	0.8	±0.4	0.8	±0.6	2.8	±2.6	1.1	±1.6
iC15:0	0	±0.0	2.5	±5.1	6.4	±9.8	0.6	±1.4
aiC15:0	0	±0.0	0.1	±0.2	2.0	±6.5	0.8	±1.4
C15:0	1.4	±1.9	1.0	±1.9	2.3	±3.3	0.8	±0.5
C16:0	1.3	±1.3	0.2	±0.5	1.3	±1.6	0.2	±0.2
iC17:0	0	±0.0	10.1	±8.2	2.5	±3.8	1.4	±3.4
aiC17:0	0	±0.0	12.3	±10.7	0.7	±3.3	0	±0.0
C17:0	6.3	±3.9	4.0	±2.1	2.1	±2.1	4.9	±3.3
cisC17:0	0	±0.0	0.4	±1.6	0.8	±1.2	1.2	±2.4
C18:0	3.4	±3.2	0.2	±0.4	1.9	±2.6	2.2	±3.0
C20:0	2.2	±2.3	3.4	±2.3	1.8	±2.7	8.8	±7.1
C22:0	0	±0.0	14.1	±12.9	6.2	±10.3	1.0	±1.8
C24:0	4.9	±3.8	2.7	±6.8	4.4	±3.7	4.5	±5.2
C26:0	0	±0.0	4.0	±5.8	0.9	±2.4	0.6	±1.1
C28:0	13.1	±6.0	2.9	±5.6	2.5	±3.9	5.3	±5.2
ΣSFA	33.9	±10.8	59.8	±22.9	40.3	±16.4	34.5	±11.8
C14:1ω5	0.4	±0.5	0.1	±0.2	0.1	±0.3	0.2	±0.2
C16:1ω9	0	±0.0	0.4	±0.5	0.1	±0.3	0	±0.0
C16:1ω7	0.6	±0.2	0.2	±0.3	1.3	±1.6	1.5	±2.2
C16:1ω5	0	±0.0	0.1	±0.1	0.0	±0.0	0	±0.0
C18:1ω9	2.9	±1.8	1.5	±1.3	1.9	±1.5	1.2	±0.9
C18:1ω7	0	±0.0	0.0	±0.2	1.5	±1.7	1.3	±2.4
C18:1ω5	0	±0.0	0.1	±0.6	1.2	±1.5	1.0	±1.9
C17:1ω7	0	±0.0	0.6	±2.0	2.4	±3.3	1.8	±3.3
C20:1ω9	1.0	±0.9	2.2	±1.8	0.6	±1.3	1.1	±1.6
C22:1ω9	4.0	±3.5	2.5	±3.2	2.3	±3.6	2.4	±2.0
C24:1ω9	0	±0.0	0.9	±1.8	0.0	±0.2	0	±0.0
ΣMUFA	8.9	±3.2	8.6	±3.8	11.5	±5.8	10.4	±7.3
C16:2ω4	4.8	±4.8	2.4	±6.5	0.6	±1.4	6.1	±4.2
C16:3ω4	0	±0.0	0.7	±2.6	4.1	±5.0	0.7	±1.4
C18:2ω6	4.1	±4.2	2.0	±3.9	4.4	±8.8	3.1	±2.3
C18:2ω9	0	±0.0	0.4	±0.5	0.0	±0.2	0	±0.0
C18:3ω6	0.7	±1.0	0.3	±0.8	0.0	±0.1	1.1	±1.5
C18:3ω3	9.9	±11.1	4.9	±10.8	3.4	±5.4	9.4	±8.8
C18:4ω3	0	±0.0	0.1	±0.5	1.7	±2.4	0.2	±0.6
C20:4ω6	4.7	±9.1	0.2	±0.3	1.3	±1.6	0.8	±0.6
C20:2ω6	0	±0.0	0.3	±1.3	0	±0.0	0	±0.0
C20:3ω6	0	±0.0	0.3	±1.2	2.2	±3.6	1.0	±2.2
C20:3ω3	1.1	±0.8	1.8	±1.8	3.0	±3.8	3.3	±3.3

Table A14 (Continued)

Fatty acids	**FW0**		**FW1**		**FW2**		**FW3**	
C20:4ω3	1.9	±1.6	1.9	±3.9	2.3	±1.9	3.7	±3.2
C20:5ω3	13.2	±11.5	5.0	±7.2	9.6	±20.2	5.2	±4.5
C20:5ω6	0	±0.0	3.2	±2.7	0.4	±1.6	0	±0.0
C22:2ω6	2.4	±4.2	0.6	±1.4	1.1	±2.6	0.4	±0.3
C22:4ω6	1.6	±1.6	2.1	±2.8	5.2	±6.4	3.3	±1.5
C22:5ω6	5.2	±7.7	1.3	±2.4	4.0	±5.8	3.2	±3.4
C22:5ω3	3.7	±3.8	1.9	±1.2	2.3	±2.2	7.7	±6.2
C22:6ω3	4.0	±5.5	2.4	±1.7	2.8	±2.5	5.8	±4.7
ΣPUFA	57.2	±9.5	31.6	±22.5	48.2	±17.5	55.1	±15.4
ΣEFA	22.7	±14.0	9.8	±12.6	15.7	±13.5	20.3	±12.2

Table A15: Size class related changes in the fatty acid composition (mean %TFA ±SD) of *M. salmoides* collected in the lower Kowie system between April 2012 and February 2013.

Fatty acids	**Small**		**Medium**		**Large**	
C10:0	0.2	±0.5	0.2	±0.4	0.0	±0.1
C12:0	0.7	±1.0	1.6	±2.3	0.9	±0.7
C14:0	1.2	±1.4	2.1	±2.4	1.3	±1.6
iC15:0	2.4	±6.4	6.3	±10.5	1.8	±2.3
aiC15:0	0.7	±1.3	2.1	±7.1	0.0	±0.1
C15:0	1.4	±1.9	1.0	±1.3	1.6	±3.1
C16:0	0.8	±1.1	1.1	±1.6	0.3	±0.7
iC17:0	1.2	±3.1	4.7	±5.9	8.7	±8.5
aiC17:0	0		4.7	±8.8	10.1	±10.8
C17:0	4.9	±3.7	2.9	±2.7	3.7	±2.0
cisC17:0	1.1	±2.4	0.8	±1.3	0.0	±0.2
C18:0	3.1	±3.1	0.8	±1.6	0.5	±1.3
C20:0	4.7	±5.9	2.6	±2.6	3.1	±2.5
C22:0	1.4	±3.1	9.7	±11.0	12.0	±13.7
C24:0	4.4	±4.1	3.0	±2.9	3.6	±7.2
C26:0	0.5	±1.1	2.5	±4.7	3.0	±5.3
C28:0	7.7	±7.1	3.0	±5.7	2.9	±4.8
ΣSFA	36.6	±12.0	49.2	±19.4	53.8	±25.3
C14:1w5	0.3	±0.4	0.1	±0.2	0.1	±0.3
C16:1w9	0		0.2	±0.4	0.3	±0.5
C16:1w7	1.3	1.7	0.8	±1.5	0.4	±0.7
C16:1w5	0		0		0.1	±0.1
C18:1w9	2.0	±1.6	1.9	±1.8	1.5	±1.1
C18:1w7	1.0	±1.9	0.8	±1.1	0.3	±1.2
C18:1w5	0.6	±1.4	1.1	±1.7	0.2	±0.6
C17:1w7	2.2	±3.8	1.6	±2.7	0.3	±0.7
C20:1w9	0.9	±1.2	0.9	±1.3	2.1	±1.9
C22:1w9	2.7	±2.9	2.3	±4.3	2.7	±2.8
C24:1w9	0		0.7	±1.6	0.6	±1.4
ΣMUFA	10.8	±5.9	10.4	±5.2	8.6	±4.1
C16:2w4	4.6	±4.5	4.1	±8.3	0.5	±1.1
C16:3w4	1.2	±2.7	4.5	±5.5	0.4	±1.4
C18:2w6	3.0	±3.3	2.2	±3.9	3.7	±7.7
C18:2w9	0		0.3	±0.5	0.3	±0.4
C18:3w6	0.8	±1.2	0.4	±1.0	0.1	±0.3
C18:3w3	8.8	±9.1	5.2	±9.6	3.8	±9.3
C18:4w3	0.3	±0.6	1.2	±2.0	0.4	±1.5
C20:4w6	2.4	±6.0	0.8	±1.4	0.5	±1.1
C20:2w6	0		0		0.3	±1.3
C20:3w6	1.1	±2.2	1.9	±3.8	0.2	±0.9

Table A15 (Continued)

Fatty acids	**Small**		**Medium**		**Large**	
C20:3w3	2.5	±3.2	2.5	±3.4	2.0	±1.8
C20:4w3	2.5	±2.5	3.6	±4.6	1.3	±2.0
C20:5w3	7.8	±9.2	1.4	±2.7	10.7	±17.1
C20:5w6	0		1.2	±2.1	2.8	±2.9
C22:2w6	1.2	±2.8	0.3	±0.7	1.2	±2.4
C22:4w6	2.6	±1.9	2.4	±2.7	3.9	±5.8
C22:5w6	4.2	±5.5	4.0	±6.5	1.2	±2.0
C22:5w3	5.2	±5.1	2.2	±2.2	1.9	±1.2
C22:6w3	4.6	±4.7	2.5	±2.3	2.6	±1.9
ΣPUFA	52.6	±14.4	40.4	±18.9	37.6	24.9
ΣEFA	20.2	±26.0	13.8	±23.0	11.2	±22.3