ASSESSING THE IMPACTS OF INVASIVE NON-NATIVE AFRICAN SHARPTOOTH CATFISH *CLARIAS GARIEPINUS*

Thesis submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

of

RHODES UNIVERSITY

by

WILBERT TAKAWIRA KADYE

December 2011

"*It is tempting, if the only tool you have is a hammer, to treat everything as if it were a nail*."

Abraham Kaplan's Law of the Instrument

TABLE OF CONTENTS

ACKNOWLEDGMENTS

First and foremost, I would like to express my sincere gratitude to Tony Booth for his relentless effort in supervising this thesis and for providing the necessary assistance in the smooth running of this research. I also want to thank him for introducing me to the art of statistical computing in *R* that became the most convenient data analysis tool.

This study was funded by the National Research Foundation (NRF) of South Africa and Rufford Foundation (UK), and I am thankful for their support. Permission for this study was granted by Eastern Cape's Department of Economic Development and Environmental Affairs, through permit number CRO16/10CR, Rhodes University Ethics committee, through research proposal 2010Q2-1 and Department of Water Affairs, South Africa.

I acknowledge Sven Kaehler (IsoEnvironmental Laboratory, Botany Department, Rhodes University) who assisted with stable isotope analysis. I thank Paul Cowley (SAIAB) who generously provided a VEMCO receiver and hydrophone for the telemetry study. This study benefited immensely from discussions with Andrew Jackson (Trinity College, Dublin) and Aileen Mill (Newcastle University, UK) on stable isotopes, and Carl Schwarz (Simon Fraser University, Canada) on BACI designs. Amber Childs, Rhett Bennett and Carl Huchzermeyer gave insightful advice during planning of the telemetry study.

During field sampling I was assisted by many friends and colleagues who include Albert Chakona, James Merron, Ryan Wasserman, Stanely Tsarwe, Roy Bealey, Warren Bouwer, Thethela Bhokhutlo, Amos Chinomona (Rhodes University), Alison Gocke, Alexander Zuniga and Steven Fauchs (Princeton University). I am grateful for such assistance. Many thanks go to Olaf Weyl (SAIAB) and Nolly Bosman (SANParks) who assisted with many aspects of this study. I also want to thank many farmers and land holders who granted permission to conduct this research.

I acknowledge my wife Rose who assisted with planning of my field trips and for meticulously filing and double-checking my data sets. Without such help, it would have been difficult.

Last but not least, I thank my father and my late mother for all their effort in transforming an enthusiastic footballer. I dedicate this thesis to them.

ABSTRACT

Invasive species are of particular concern as they have the potential to alter community structure and food web relationships within their invaded habitats. African sharptooth catfish *Clarias gariepinus*, a generalist predator, was introduced through an inter-basin water transfer scheme into the Great Fish and Sundays Rivers, Eastern Cape, South Africa, where it threatens the native riverine biota. This thesis assessed its impact from a trophic perspective.

Patterns in catfish distribution and abundance revealed an upstream to downstream gradient that was associated with spatial distribution of most species within the mainstream, and a mainstream to tributary gradient that was associated with the spatial distribution of native minnows. The catfish was predicted to occur widely within the mainstem habitats and to decrease progressively along the mainstrem to tributary gradient with the physico-chemical environment being a good proxy for predicting both its occurrence and abundance. The results suggest the catfish proliferated within mainstem habitats where invasion resistance was possibly reduced due to alteration of flow.

Population dynamics and size structuring of two native cyprinid minnows *Pseudobarbus afer* and *Barbus anoplus*, threatened by catfish, were examined within uninvaded headwater streams in relation to their proximate physical habitats. Their habitats were characterised by seasonal variation in physico-chemical conditions and a spatial variation in substrata compositions. No evidence of differences was found between seasons for density and capture probability for either species. The population size and density for *P. afer* was found to increase with increasing proportion of boulders. In comparison, *B. anoplus* population size and probability of capture increased with increasing proportion of bedrock and bank vegetation, respectively. Size structuring was explained predominantly by seasonality and habitat variables for *P. afer* and *B. anoplus*, respectively.

Stable isotope ratios of carbon and nitrogen were used to compare the spatial variation in both the community-wide and catfish-specific niches and to estimate catfish prey sources from different habitats within the invaded systems. Aquatic community and catfish niches were statistically different among localities, suggesting that each locality had a distinct community-wide trophic structure. Dispersion metrics indicated no evidence of differences in the clustering among individuals, but provided evidence of differences in path trajectories for the comparisons of catfish populations that suggested dietary plasticity within different localities. Dietary studies revealed both ontogenetic shift and omnivory that suggested that catfish may exhibit less pronounced top-down effects within its invaded habitats.

Manipulative experiments were used to test the response of benthic macroinvertebrates within two rivers that were differentially impacted by catfish as a press-type disturbance. Macroinvertebrates were non-responsive to catfish presence within a system where catfish had previously been established. In contrast, excluding catfish in this system indicated a response that suggested the importance of refuge within invaded habitats and the possible recovery pattern of certain macroinvertebrate taxa. By comparison, introduction of catfish within previously uninvaded localities provided evidence of direct catfish impact through elimination of conspicuous taxa.

Acoustic telemetry was used to investigate catfish movement patterns within an invaded lentic habitat and provided evidence that habitat utilisation was non-random. The shallow and structured river mouth habitat, which was most utilised, was probably the most ideal for its breeding and feeding. This inferred potential overlap with native species and suggested the risk of predation and competitive interference. Catfish also exhibited both nocturnal and diurnal activity patterns that were probably related to feeding.

CHAPTER 1

General Introduction

Non-native invasive species in freshwater ecosystems are regarded as major threats to aquatic biodiversity (Courtenay & Stauffer, 1990; Fuller *et al.*, 1999; Canonico *et al.*, 2005) and primary drivers of species loss and biotic homogenisation (Rahel, 2002; Leprieur *et al.*, 2008). Freshwater ecosystems are particularly susceptible to invasions as they are considered to be biogeographic islands that are unsaturated with native species and therefore more likely to favour the establishment of non-natives (Oberdorff *et al.*, 1997; Strayer, 2010). Their connectedness further enhances the potential risk and spread and establishment of invaders, while anthropogenically influenced habitat alteration has been shown to weaken the biotic resistance of native biota (Moyle, 1986; Chapman *et al.*, 1996; Rahel, 2002; Leprieur *et al.*, 2006; Vitule *et al.*, 2009).

Worldwide, many non-native fishes have become established within a wide range of freshwater habitats where they have caused considerable disruptions to native ecosystems at different spatial and temporal scales (Rahel, 2002; Strayer, 2010). Within previously fishless habitats, non-native fishes have established as a new functional group and have had considerable effects on the behaviour, distribution and abundance of the native biota and the broader ecosystem structure (Kiesecker *et al.*, 2001; Simon & Townsend, 2003; Strauss *et al.*, 2006). The most dramatic impacts have been associated with introductions into freshwaters that already contain fish where predation has led to the extirpation of native species (Moyle, 1986; Townsend & Crowl, 1991; Goldschmidt *et al.*, 1993; Lowe-McConnell, 1993). Predation is, however, not the only impact. Non-piscivorous fish have, through their feeding behaviours, caused disturbances through habitat destruction (Miller & Crowl, 2006) and

altered benthic and planktonic food webs (Simon & Townsend, 2003; Baxter *et al*., 2004; Pyke, 2008). Both piscivorous and non-piscivorous non-native fishes have therefore been implicated in indirect impacts such as trophic cascading through eliminating certain taxa and by influencing ecosystem structure and nutrient cycling within their invaded food webs (Flecker & Townsend, 1994; Nyström & McIntosh, 2003; Vander Zanden *et al*., 2003; Meissner & Moutka, 2006; Schmidt *et al*., 2009).

In developing countries, the risk of biological invasions within freshwater ecosystems is high as species introductions continue while their impacts are poorly understood (Vitule *et al*., 2009). In South Africa, at least 41 fish species have been introduced, 16 of these being alien (introduced from outside southern Africa) and 25 translocated (introduced from within southern Africa) (de Moor & Bruton, 1988). The establishment of warm water invasive species such as the North American bass, *Micropterus salmoides*, *M. dolomieu* and *M. punctulatus*, bluegill sunfish, *Lepomis macrochirus*, catfish *Clarias gariepinus* and tilapias, *Tilapia sparrmanii* and *Oreochromis niloticus*, threaten the native species in many rivers and impoundments (de Moor & Bruton, 1988; Skelton, 1990; Cambray, 2003a; 2003b). Cold water trout species, such as *Onchorhynchus mykiss* and *Salmo trutta*, have established in headwater streams (Cambray, 2003b) where they threaten many range-restricted and genetically distinct populations of minnows such as the redfins *Pseudobarbus* spp. and other endemic barbs (Swartz *et al.*, 2004; Tweddle *et al*., 2009). Invasion impact risk is the highest in those rivers with highly endemic yet species-depauperate native ichthyofaunas, such as the Western and Eastern Cape Regions (Tweddle *et al*., 2009). Consequently, in these two regions, at least 24 freshwater fishes, including those that occur within protected national parks (Russell, 2011), are on the IUCN Red List, all being threatened by non-native invasive fishes (Tweddle *et al*., 2009). The endemic cyprinid minnows, especially the redfin minnows

Pseudobarbus spp, most of which are undescribed to species level, dominate the threatened list (Table 1.1).

Table 1.1: Freshwater fish species threatened by non-native invasive fishes within the Cape Floristic Region, South Africa (Tweddle *et al*., 2009).

Family	Species	Threat status
Cyprinidae	*Pseudobarbus afer* (Peters, 1864)	Endangered
	Pseudobarbus sp. "afer Gamtoos"	Endangered
	Pseudobarbus sp. "afer Krom"	Critically endangered
	Pseudobarbus sp. "afer Forest"	Near threatened
	Pseudobarbus tenuis (Barnard, 1938)	Near threatened
	Pseudobarbus sp. "tenuis Keurbooms"	Endangered
	Pseudobarbus asper (Boulenger, 1911)	Endangered
	Pseudobarbus burchelli Smith, 1841	Critically endangered
	Pseudobarbus sp. "burchelli Breede"	Near threatened
	Pseudobarbus sp. "burchelli Heuningnes"	Critically endangered
	Pseudobarbus burgi (Boulenger, 1911)	Endangered
	Pseudobarbus sp. "burgi Verlorenvlei"	Endangered
	Pseudobarbus phlegethon (Barnard, 1938)	Endangered
	Pseudobarbus sp. "phlegethon Doring"	Critically endangered
	Barbus andrewi Barnard, 1937	Endangered
	Barbus serra Peters, 1864	Endangered
	Barbus calidus Barnard, 1938	Vulnerable
	Barbus erubescens Skelton, 1974	Critically endangered
	Labeobarbus capensis (A. Smith, 1841)	Vulnerable
	Labeo seeberi Gilchrist & Thompson, 1911	Endangered
Austroglanididae	*Austroglanis barnardi* (Skelton, 1981)	Endangered
	Austroglanis gilli (Barnard, 1943)	Vulnerable
Galaxiidae	*Galaxias zebratus* Castelnau, 1861	Data deficient
Anabantidae	*Sandelia capensis* (Cuvier, 1831)	Data deficient

The threat to these species not only reflects their high degree of endemicity but their restricted distribution within their natural habitat. Invasion successes and associated impacts within species depauperate systems are usually high due to limited number of native predators and competitors (Simberloff & Von Holle, 1999). Native predators that have co-evolved with these native biota include different species of eels, *Anguilla mossambica*, *A. bicolor* and *A. marmorata* in the Eastern Cape, and eels and large cyprinids such as

Labeobarbus capensis in the Western Cape. These predators feed mostly on macroinvertebrates and small fish (Skelton, 2001). Due to the increasing risk of biotic loss within these regions, the potential impacts associated with establishment of non-native species needs to be investigated.

Figure 1.1: Non-native African sharptooth catfish *Clarias gariepinus* (picture used with permission from SAIAB)

Introduction of African sharptooth catfish

African sharptooth catfish *Clarias gariepinus* (Figure 1.1) is arguably the most latitudinally distributed freshwater fish that naturally occur from the Middle East to the Orange River in South Africa (Daget *et al*., 1984; De Graaf & Janssen, 1996; Skelton, 2001). This species has been widely introduced in many parts of the world through aquaculture and is being cultivated in fish farms throughout Europe, Asia and South America (Lal *et al*., 2003; Vitule *et al*., 2006; Bhakta & Bandyopadhyay, 2007; Radhakrishnan *et al*., 2011). In these regions, it has successfully established within freshwater ecosystems as escapees from aquaculture facilities and through deliberate angler introductions (Cambray, 2005; Vitule *et al*., 2009; Peh, 2010). Although its impact in these regions is unknown, concern has been raised over its

predation and competition impact on local biota (Khan & Panikkar, 2009), and genetic impact through introgression with native catfishes (Lal *et al*., 2003; Senanan *et al*., 2004).

In the Eastern Cape Province, South Africa, catfish was translocated primarily through the Orange/Fish inter-basin water transfer (IBWT) scheme, which was completed in 1976 (Cambray & Jubb, 1977). This species has since spread into several other river and impoundments through secondary IBWT schemes, angler introductions and, to a lesser extent, aquaculture (Cambray, 2003a). Translocated catfish from the Orange River system have established in many Eastern Cape rivers that include the Great Fish, Sundays, Kouga, Swartkops, Keiskamma, Buffalo, Nahoon and Mtata Rivers, and in many reservoirs (de Moor & Bruton, 1988; Cambray, 2003a; Weyl & Booth, 2008; Booth *et al*., 2010). High densities within some reservoirs of the province (Potts *et al.*, 2008) are likely to provide source populations for its continuous spread and establishment.

The sharptooth catfish is fast growing fish that reaches up to 200 mm standard length within a year and attains sexual maturity within two years (Bruton & Allanson, 1980; Skelton, 2001). This species attains total lengths of about 1.4 m and masses exceeding 30 kg, and is generally long lived, surviving for more than 15 years (Bruton, 1976; Weyl & Booth, 2008; Booth *et al*., 2010). Sharptooth catfish is hardy species that tolerates water temperatures from 8 - 35°C, salinity of 0 - 10‰, wide pH ranges (Safriel & Bruton, 1984) and low oxygen concentration partially due to its possession of an arborescent organ (Van de Waal, 1998). This species occurs in a variety of habitats, and thrives in rivers and dams of different sizes and trophic status (Teugels, 1986; Bruton, 1988; Potts *et al.*, 2008). The catfish is generally considered to be a nocturnal benthic feeder that occasionally feed on the surface (Teugels, 1986; Bruton, 1979a) and preys on, among other things, plant matter, plankton, macroinvertebrates, amphibians, reptiles and fish (Groenewald, 1964; Munro, 1967; Bruton, 1979b; Winemiller & Kelso-Winemiller, 1996). Solitary feeding, social hunting and

organised pack-hunting foraging behaviours and feeding migrations have been observed (Bruton, 1979c; Merron, 1993). Ontogenetic diet shifts, with increasing preference to fish in large catfish have been reported (Willoughby & Tweddle, 1978). Munro (1967) also reported that as catfish age, its gill rakers increase in both length and number, thereby increasing its filter feeding efficiency. Large numbers of mature adults migrate upstream, to lakeshores or flooded areas to breed in summer after the rains in both lotic and lentic habitats (Bowmaker, 1973; Bruton, 1979b).

The biological attributes of catfish related to its biology, generalised feeding habits, high mobility and ability to survive in a wide range of habitat raises concern over its impact as an invasive species (de Moor & Bruton, 1988; Cambray, 2003a). The catfish is already linked to local extirpation of endangered Eastern Cape Rocky *Sandelia biansii* in Tyume River and redfin *P. asper* in Gamtoos River (Cambray, 2003a). There is also concern for the conservation of populations of goldie barb *Barbus pallidus* and the endangered Eastern Cape redfin *P. afer* due to its presence, together with largemouth bass *M. salmoides*, in the Sundays River. Potts *et al.* (2008) found that catfish preyed predominantly on fish in some Eastern Cape reservoirs. This species' potential impact on other biota, such as macroinvertebrates, has been noted (de Moor & Bruton, 1988). Its ability to persist in a wide range of habitats and generalised feeding habitats suggests a potential to cause widespread ecosystem impacts. Cambray (2003a) highlighted the need for research and monitoring of the impacts of sharptooth catfish. This species's widespread occurrence within the Great Fish and Sundays Rivers, therefore, provides an opportunity to examine its impact as a non-native invasive species.

Thesis outline

The sharptooth catfish is a generalist predator that, as an invasive species, is likely to maintain high population density because it is not limited by food availability, natural predators or competitors. Due to both its generalised feeding habits and high mobility, it is hypothesised that the catfish will take advantage of locally abundant prey and may prey-switch or even move into other localities once prey becomes less abundant. Little is known about the consequences of such generalised predatory behaviour by catfish within invaded habitats. However, within habitats invaded by generalist predators, prey populations are known to respond in different ways ranging from sub-lethal patterns in behaviour and distribution to more complex patterns that influence trophic interrelationships (Park, 2004; Strayer, 2010; Simberloff, 2011). Therefore, an integrated approach is required in order to assess and predict catfish impact. The primary aim of this thesis was to investigate catfish impact from a trophic perspective within its invaded range, with the focus on quantifying patterns in energy flow and identifying causal patterns, movement and habitat utilisation.

To achieve the above, this thesis is divided into nine chapters. Chapter 1 provides an introduction to the subject and Chapter 2 describes the study area. To identify habitats and species likely threatened by catfish, it is important to assess patterns in its distribution and abundance. In Chapter 3, the distribution and abundance of sharptooth catfish and other fish species within the study systems was assessed. Predictive and correlative methods were used to identify the environmental factors related to catfish distribution, and its association with the ichthyofauna within the invaded systems. Chapter 4 describes the population dynamics of two native cyprinid minnows in relation to their proximate habitat characteristics within uninvaded headwater streams. This chapter describes the use of depletion sampling method to estimate population parameters and their seasonal patterns for the two minnows. These

species are of conservation significance due to the likelihood of catfish invasion into their habitats.

Due to spatial variation in resource availability within the longitudinally series of lotic habitats and between hydrological systems (lotic and lentic) catfish impact is likely to vary in response to these differences. In Chapter 5, dual stable isotope tracers of carbon and nitrogen were used to identify and compare catfish and community niches from different habitats within the invaded systems. This chapter assessed catfish impact from a food web perspective within different spatial localities of the invaded habitats.

Dietary analysis is a direct way of identifying predation impact. In Chapter 6, stomach content analysis and stable isotope tracers were used in complement to investigate the feeding habits and prey sources for catfish. Integrating the two methods provides a robust and synthetic approach of addressing temporal bias in prey availability and digestibility, and increases precision on quantifying and estimating prey sources.

Chapter 7 explored the use of manipulative experiments to identify causal patterns associated with catfish impact. This chapter assessed sharpthooth catfish within a disturbance framework. In this chapter, response patterns in macroinvertebrate communities were tested within rivers that were differentially impacted by catfish.

In Chapter 8, acoustic telemetry was used to identify movement patterns and habitat by catfish within an invaded impoundment. This chapter provides, to my knowledge, the first assessment of its movement patterns and habitat use outside its natural range. Lastly, Chapter 9 integrates the findings of this thesis.

CHAPTER 2

Study Area

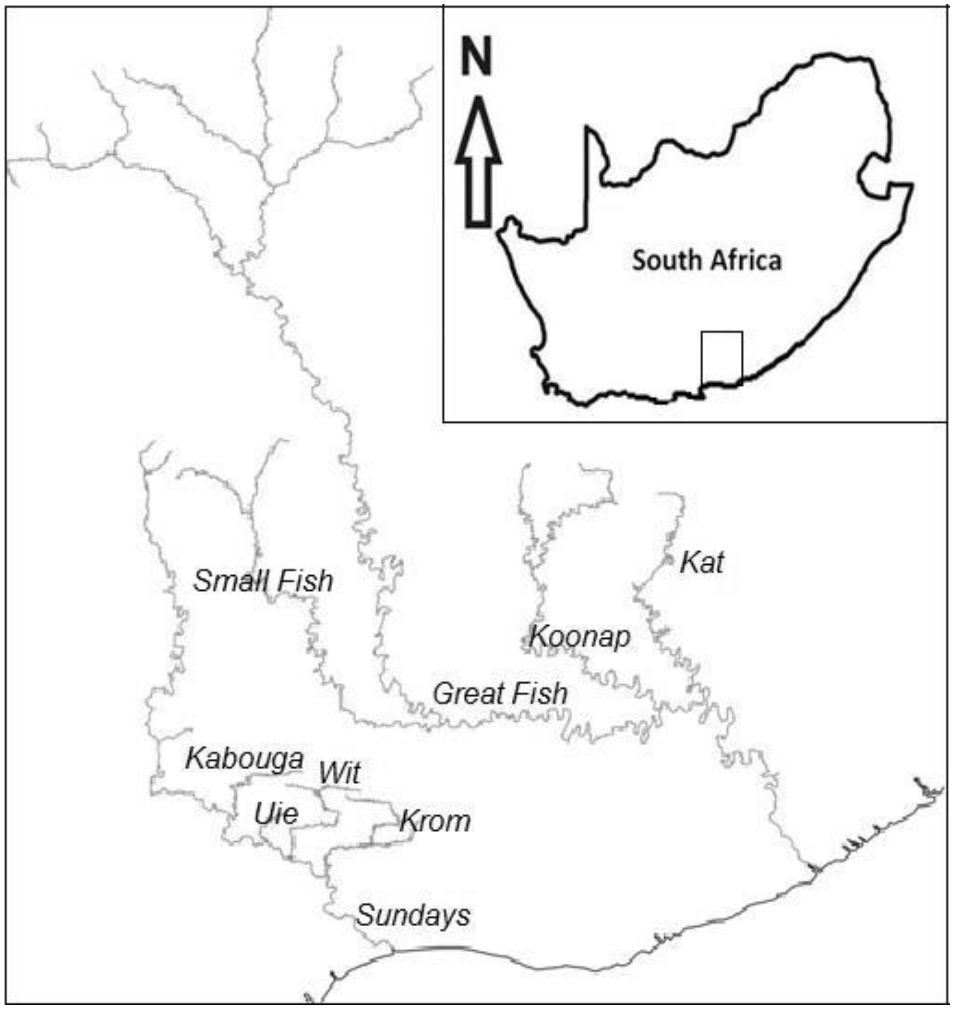

Figure 2.1: Great Fish and Sundays Rivers and their major tributaries within the Eastern Cape, South Africa.

Study systems

The Great Fish and Sundays Rivers are major rivers in the Eastern Cape, South Africa (Figure 2.1). The two rivers originate in the Karoo region of south-central South Africa and flow through the Eastern Cape into the Indian Ocean. The Great Fish River has a longitudinal axis of 650 km and a large catchment of 30 243km^2. The river rises at about 1400 m a.s.l. in

the Tandjiesberg Mountains west of Cradock, and reaches the sea 150 km north of Port Elizabeth (O'Keeffe & de Moor, 1988). Its major tributaries include the Koonap River and the Kat River, that both originate in the Winterberg Mountains, and the Small Fish River. Glen Melville Dam (33°129 S; 26°409 E) is part of the Great Fish River system. The dam, which was constructed in 1992, covers an area of 76 ha and has a maximum depth of 25 m at maximum capacity. The Sundays River has a smaller catchment area of 20 792km^2 and is about 310 km long (Scharler & Baird, 2005). Its main tributaries originate in the Zuurberg Mountains within Addo Elephant National Park (AENP). These include the Kabouga, the Uie and its tributaries, Groot and Klein, the Wit, and the Krom Rivers.

Geology and climate

The geology of both basins comprise of a series of sedimentary strata including sandstones, tillite, marine shales and mudstones (O'Keeffe & de Moor, 1988; Hattingh, 1997). The porosity and permeability of these sedimentary rocks is poor and significant base flow is only found in areas with extensive joints and fractures (O'Keeffe & de Moor, 1988). The underlying geology within the headwaters of Great Fish River, around the Winterberg Mountains, consists of shales and sandstones of the Beaufort series of the Karoo, while the plateau itself comprise of resistant dolerite. Within the headwaters of Sundays River, the Zuurberg Mountains, which are central to the AENP, are part of the Cape Fold Mountains that are predominated by quartzite and sandstone sediments. The greater part of the AENP is dominated by unconsolidated Cretaceous sediments that weather to form deep red to orange-brown fine-grained and relatively fertile soils (Macvicar, 1991).

The climate of the region is warm-temperate. The greater part of the Great Fish catchment is arid, with mean annual rainfall of 350 mm in upper parts and 550 mm in lower parts of the catchment. Summer (October to March) and winter (April to September)

maximum temperatures are 29 and 19°C, respectively (van Zyl, 1994). Frost occurs regularly between April and September, and snow may fall in winter at high altitudes within the Winterberg. Within the Sundays River catchment, mean annual rainfall is relatively low (about 480 mm), falling mostly in late summer (March to May) and late winter (August). Temperatures range from 15 to 45°C in summer and 5 to 18°C in winter, with a mean annual of 18°C (Lombard *et al*., 2001).

Vegetation

Great Fish River valley vegetation comprise of xeric thicket, comprising of leaf-succulent shrubs and evergreen sclerophylls such as *Portulacaria afra*, and *Acacia karoo* thornbush up to 2m high. Prickly pear *Opuntia ficus-indica* has invaded some parts of the valley's vegetation. Vegetation around the major tributaries in the Winterberg is a mosaic of several veld types including thornveld and grassveld that are mixed with dwarf *A. karoo*. A veld type called sourveld, which is dominated by *Thermeda triandra* and other grasses, dominates the plateau slopes (Meadows & Meadows, 1988). Montane forest trees occur in deep soils in valleys and escarpment slopes.

Within the AENP, Sundays River is dominated by xeric thicket. Quartzite and shale fynbos are the dominant vegetation types in the Zuurberg Mountains. Other vegetation types include Karoo-bushveld, mixed-shrub and grassveld (Lombard *et al*., 2001). The lower portion of Sundays River outside AENP is dominated by citrus plantations.

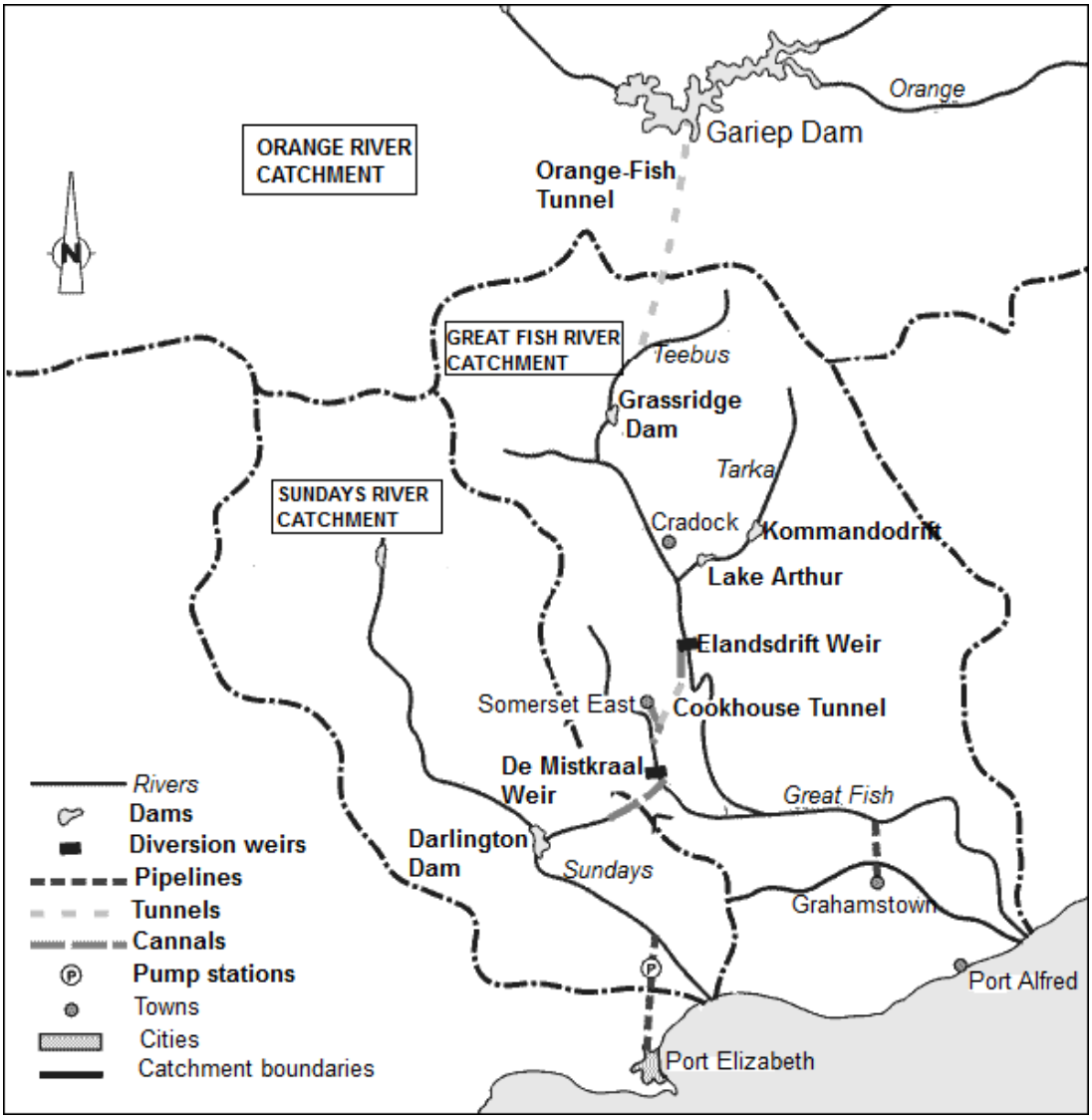

Figure 2.2: Schematic presentation of the Orange River/Great Fish River inter-basin water scheme that transfers Orange River water from the Gariep Dam into both the Great Fish and Sundays Rivers, Eastern Cape, South Africa.

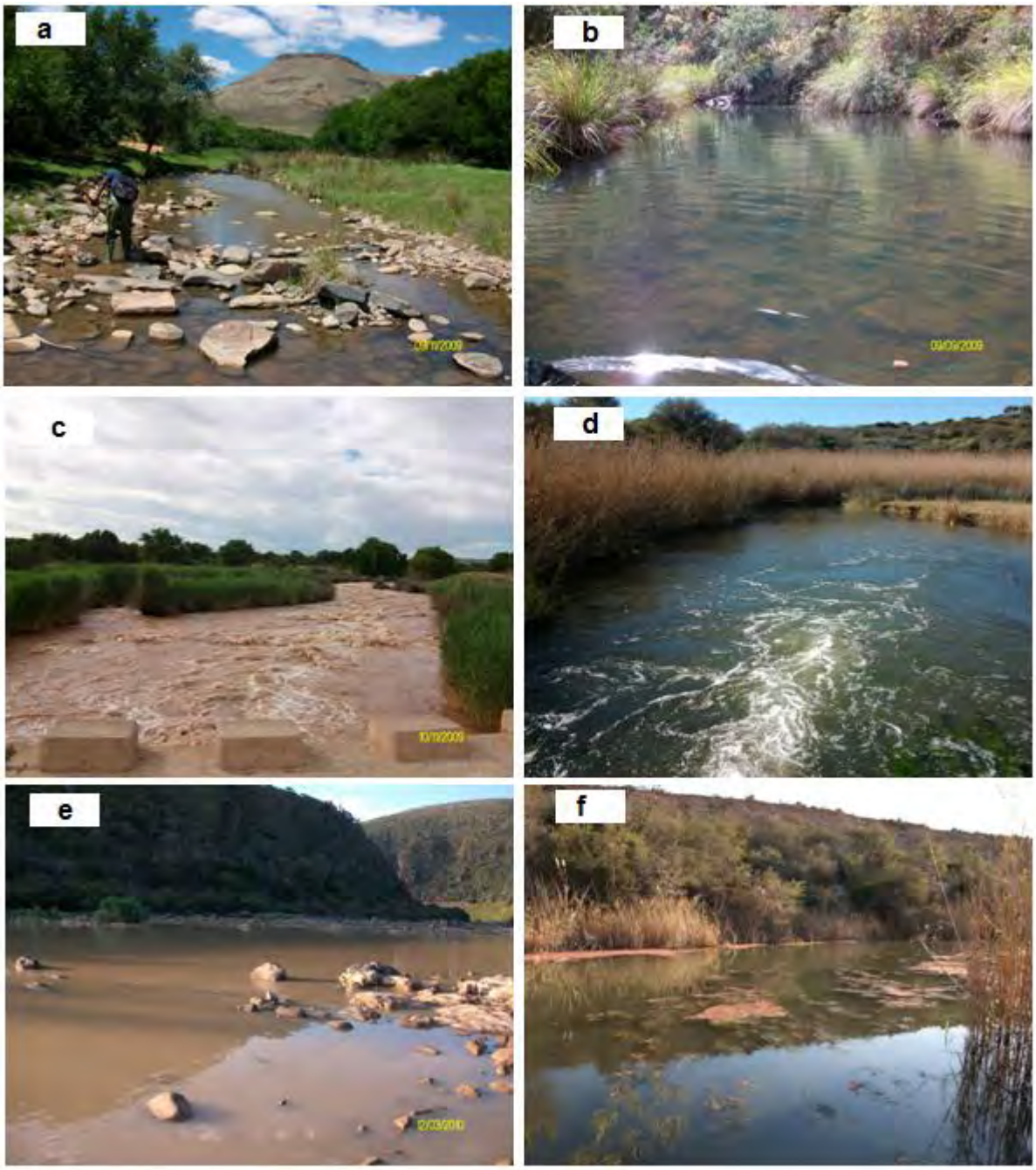

Figure 2.3: The main habitat types showing the headwaters (a) and (b), and turbid (c) and (e) and relatively clear water (d) and (f) mainstreams of the Great Fish (left panel) and the Sundays (right panel) Rivers, respectively, within the Eastern Cape, South Africa.

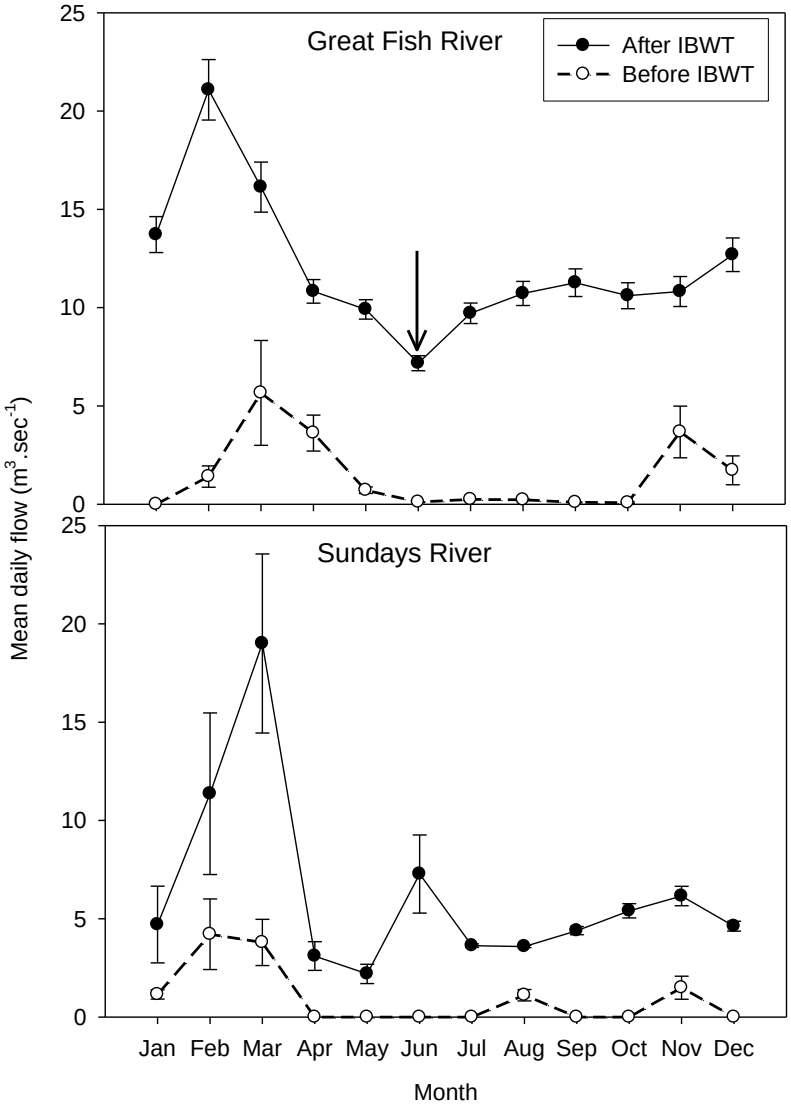

Figure 2.4: Changes in mean (± standard error) flow before the inter-basin water transfer (1973-1974) and during the sampling period (2009-2010) within the Great Fish and Sundays River, Eastern Cape, South Africa. The arrow indicates period of lowest flow within the Great Fish River when the tunnel is closed for maintenance. Data were obtained from the Department of Water Affairs, South Africa.

Inter-basin water transfer schemes

In 1976, the Orange River-Great Fish River inter-basin water transfer (IBWT) scheme was opened (Cambray & Jubb, 1977). It connects the Gariep Dam on Orange River with the headwaters of Great Fish River through an 82 km underground tunnel (Figure 2.2). Water is transferred into a balancing dam, Grassridge Dam, through the Teebus River, from which it is

released into the Great Fish River via the Great Brak River. This IBWT scheme transfers approximately 350×10^6 m^3 water per year (O'Keeffe & de Moor, 1988). Secondary IBWT transfers water from Great Fish River into Sundays River through the Cookhouse tunnel, and into Glen Melville Dam through a tertiary IBWT.

The IBWT scheme has transformed the landscape of the two river systems from seasonal to perennial flow (Figures 2.3 and 2.4). The IBWT tunnel is closed annually in June for maintenance during which flow is lowest but sustained by regulated release from impoundments such as Grassridge Dam. Prior to the IBWT, the natural flow was seasonal and characterised by periodic scouring summer floods with the rivers being reduced to a series of unconnected pools during winter. Presently, only the headwaters still maintain their natural seasonal flow.

CHAPTER 3

An Invader within an Altered Landscape: One Catfish, Two Rivers and an Inter-Basin Water Transfer Scheme

Introduction

Human-mediated introduction of non-native invasive species is a threat to biodiversity (Leprieur *et al*., 2008). Freshwater ecosystems, especially rivers, are vulnerable to the negative impacts of invasive species (Strayer, 2010). Ecological impacts have been shown to range from subtle effects, such as behavioural shifts in native species, to complete alteration of food webs and extirpation of native biota (Cambray, 2003a; 2003b; Simon & Townsend, 2003; Grabowski *et al*., 2005). Several transfer mechanisms, both intentional and accidental, have led to the successful establishment of non-native invasive species outside their natural range. Within the Eastern Cape Province, South Africa, African sharptooth catfish *Clarias gariepinus* was accidentally translocated through the Orange-Fish River inter basin water transfer (IBWT) scheme in 1976 (Cambray & Jubb, 1977). The IBWT scheme, which transfers water from the Orange River into the Great Fish and Sundays Rivers, has not only facilitated the translocation of the Orange River's biota (Laurenson & Hoccutt, 1986), but also transformed the landscape of the two rivers from intermittent to perennial flow with less seasonal variability (O'Keeffe & de Moor, 1988).

The establishment of catfish as an invasive species raises concern as it poses a potential threat because of its generalised feeding habits that could result in ecosystem-wide consequences (Cambray, 2003a; Vitule *et al*., 2006). The catfish has been associated with many negative effects in systems where it is introduced, at both community and ecosystem levels. Such effects can be direct through predation or indirect through influencing trophic and nutrient dynamics within the invaded communities (Khan & Pannikar, 2009; Vitule *et al*.,

2009; Radhakrishan *et al*., 2011). Both direct and indirect ecosystem impacts induced by non-native catfish have the potential to influence the patterns in distribution and abundance of the sympatric native icthyofauna. The catfish is ubiquitous and the alteration of flow regime of the Eastern Cape rivers is likely to favour its spread and establishment in different habitats. Several studies have shown that the alteration of abiotic conditions, such as flow regimes, facilitate the establishment and of spread invasive species and invasion risk (Baltz & Moyle, 1993; Moyle & Light, 1996). Altering a flow regime also reduces biotic resistance to invasion impact as the native biota is typically slow to adjust to changing proximate environmental conditions (Moyle & Light, 1996; Marchetti & Moyle, 2001). In addition to its now widespread occurrence within the Eastern Cape, it also co-occurs with several other non-native species, especially smallmouth yellowfish, *Labeobarbus aeneus*, that has recently become established (Weyl *et al*., 2009a). The interaction between catfish with these other invaders has the potential to exacerbate the negative impacts within its invaded habitats and result in an invasion meltdown through completion or predation, or their interaction.

Assessing the spatial distribution and abundance of non-native species within invaded habitats is necessary to understand the risks associated with invaders such as generalised piscivores. This can be achieved by assessing whether patterns in assemblage composition are independently related to environmental factors (McCullagh & Nelder, 1983; Franklin, 1998, Vayssières *et al*., 2000), or by jointly relating community composition to environmental factors, such as through constrained ordinations (ter Braak & Verdonschot, 1995; Legendre & Legendre, 1998; Peres-Neto *et al*., 2006). Spatial pattern analysis methods usually provide an insight on the distribution patterns, and can identify possible hypotheses relating to the mechanisms of dispersal and the habitats that are more likely to be vulnerable to invasion (Kraft *et al*., 2002). Often, however, non-native species are associated with complex interactions including synergistic effects with their conspecifics. This is more likely

in degraded habitat becomes altered to the extent that they become poor predictors of community composition (Smart *et al*., 2006). An alternative approach to species-habitat relationships is to develop predictive models for the occurrence for the invasive species within altered and degraded habitats (De'ath, 2002).

This study assessed patterns in distribution and abundance of non-native catfish within two rivers that have altered flow regimes, the Great Fish and Sundays Rivers, through a common IBWT scheme. Laurenson & Hocutt (1986) showed that prior to the IBWT scheme, the native ichthyofauna of the Great Fish River was dominated by four anguillid eels, namely *Anguilla bengalensis*, *A. bicolor*, *A. marmorata* and *A. mossambica*, the cyprinids *Barbus anoplus*, *B. pallidus* and *Labeo umbratus*, and an anabantid *Sandelia bainsii*, whereas the lower reaches were dominated by estuarine and marine species of the families Clupeidae, Mugilidae, Sparidae, Monodactylidae and Haemulidae. In addition, other non-native species, such as *Cyprinus carpio*, *Micropterus salmoides*, *Oncorhynchus mykiss*, *Lepomis macrochirus* and *Tilapia sparrmanii* were present due to deliberate angler introduction. Several other species have since been introduced into the system primarily through the IBWT, and have also spread into the Sundays River. These include *L. aeneus*, *L. capensis*, *C. gariepinus* and *Austraglanis sclateri* (Cambray & Jubb, 1977; Laurenson & Hocutt, 1984; 1986). The sharptooth catfish, which is the most widespread, established quickly, and is now the most problematic because it is a generalised piscivore and utilises a wide range of habitats (Cambray, 2003a).

The approach to the current study provides a predictive assessment of the likelihood of catfish occurrence on a broad spatial scale in relation to sympatric fish species and its proximate physico-chemical environment. Since the invasive catfish is widely distributed, with access to all habitat types within the invaded systems, it was hypothesised that (1) the catfish would co-occur with other conspecifics and these associations would indicate

probable impact, and (2) the physico-chemical environment would be a proxy for predicting catfish distribution patterns.

Materials and methods

Data collection

Distribution and abundance data for all fishes were collected from the mainstem and headwater localities of the Great Fish and Sundays Rivers. A total of 11 sites were sampled from the mainstream of each of the two rivers, while 19 and 21 sites were sampled within the headwater streams of the Great Fish and Sundays Rivers, respectively. Mainstem sites were sampled using a variety of gear, which include experimental gill nets, fyke nets and seine nets. Headwater sites were sampled by electric fishing using a 12 V battery-powered Samus 725GN backpack electric fisher. Sampling was conducted monthly from October 2009 to April 2010, and bimonthly from May 2010 to December 2010. A set of six experimental gill nets were used in deep (> 1 m) habitats and six double-ended fyke nets were used in shallow (< 1 m) habitats. Each experimental gill net was 30 m long with three 10 m panels of stretched mesh sizes of 50, 75 and 100 mm. Each fyke net had an 8 m guiding net and a first ring diameter of 55 cm and a 10 mm mesh size. An 8 m seine net with 4 mm mesh size was also used to sample shallow and marginal habitats. Gill nets and fyke nets were set overnight (16h00 - 07h00). Three-pass depletion electric fishing was conducted within the headwater sites. Captured fish were identified to species level and the total number of each species in each gear was recorded. Temperature (°C), pH, total dissolved solids (TDS) and conductivity ($\mu S.cm^{-1}$) were measured with a HANNA HI 98129 Combo meter. Turbidity (NTU) was measured using a HANNA HI 98703 turbidity meter. Daily flow data ($m^3.sec^{-1}$) were obtained for the mainstream habitats from the Department of Water Affairs, South Africa.

Data analysis

Catch per unit effort (CPUE) was expressed as the number of fish collected per gill net or per fyke net, per night. CPUE data from the two sampling gears were treated separately. Data collected using other sampling gear were used to record presence of different species. Two matrices were then constructed - species CPUE by site and species occurrence (presence/absence) by site for each of the rivers. Indirect ordination methods were used to explore distribution patterns using the species occurrence matrix. Detrended correspondence analysis (DCA) was used as a preliminary analysis to assist in the choice of linear versus unimodal multivariate gradient analyses (Jackson & Somers, 1991; Jongman *et al*., 1995). As the range of ordination scores (length of the first gradient) obtained by DCA was more than three standard deviations for both rivers, a unimodal indirect gradient analysis model, correspondence analysis (CA) was used. Logistic regression models were used to predict the occurrence of the catfish and to construct species response curves along the most important ordination axes. The model was of the general form: $\eta(y) = \beta_0 + \sum \beta_j X_j$ where η is the logit-link between the predicted values for the dependent variable y, β the regression coefficients, and X the independent variables.

Classification and regression trees (CART, Breiman *et al*., 1984) were used to predict the distribution and abundance of catfish in each of the rivers. The predictors were physico-chemical variables (temperature, pH, TDS, conductivity and turbidity) and river type (mainstem and tributary). The physico-chemical variables were standardised by z transformation prior to the analyses. CART explains variation of response variables (single or multiple) based on a set of independent explanatory predictors, either numerical or categorical (Breiman *et al*., 1984; Ripley, 1996; De'ath, 2002). The procedure is based on recursive binary partitioning of data into discrete subgroups that are successively homogenous in the values of the response values. Classification trees were constructed using

catfish presence/absence data for each river. Cross-validation (De'ath & Fabricius, 2000) was used to select the best predictive tree with the smallest predicted mean square error. Random forests (RF, Brieman, 2001) were used to improve the accuracy of the predicted tree models. RF is a non-parametric bootstrapping procedure that constructs a set of trees by resampling the data and averaging the predictions from the bootstrap iterations (Brieman, 2001). Within RF trees, heterogeneity between data sets is determined by the Gini index that measures node impurity. A high Gini index indicates an improved ability of the variable to separate groups.

Catfish abundance was also modelled using multivariate regression trees (MRT, De'ath, 2002) based on fyke and gill nets CPUE data, and the most appropriate and parsimonious "pruned" trees were selected using cross-validation. The relationship between abundance and that of the most widespread species was explored using a two-dimensional Kolmogorov-Smirnov test (2DKS, Press *et al*., 1999) based on the bivariate fyke and gill nets CPUE.

Ordination was conducted with *CANOCO v4.5*, whereas classification and the regression trees analyses were conducted in *R* (R Core Development Team, 2011). The following libraries were used for CART analyses - *tree* for classification trees, *randomForest* for random forests, and *mvpart* for multivariate regression trees. CPUE and environmental data were standard normal transformed prior to the *MRT* analysis.

Results

Within the Great Fish River, the main habitat types were distinguished by both their high conductivity and total dissolved solids (TDS) in the mainstem compared to the tributaries (Table 3.1). Conductivity and TDS were highest during the winter period in the mainstem, with mean values of 1512.0±250.8 $\mu S.cm^{-1}$ and 890.0±146.6 ppm, respectively, compared to 508.8±28.6 $\mu S.cm^{-1}$ and 293.7±16.3 ppm in the headwaters of the tributaries. Similarly,

within the Sundays River, conductivity and turbidity were high in the mainstream, with winter averages of 2136.0±843.1 $\mu S.cm^{-1}$ and 1256.0±491.0 ppm, respectively, compared to the tributaries that had winter averages of 413.2±61.1 $\mu S.cm^{-1}$ and 243.8±36.2 ppm. Both rivers were more alkaline in the mainstream compared to the tributaries during each of the sampling periods.

Table 3.1: Physico-chemical variables (mean ± standard deviation) sampled during summer and winter in the Great Fish River and Sundays River, Eastern Cape, South Africa. Dashes (-) indicates that flow data was unavailable in the headwater tributaries.

		Mainstream		Tributary	
		Summer	Winter	Summer	Winter
Great Fish River	Temperature (°C)	25.3±1.7	13.2±1.8	23.1±1.0	10.3±0.6
	pH (range)	8.6 - 8.9	8.7 - 9.2	7.7 - 8.6	8.1 - 8.5
	TDS (ppm)	533.3±261.2	890.0±146.6	135.7±20.0	293.7±16.3
	Conductivity ($\mu S.cm^{-1}$)	887.1±423.2	1512.0±250.8	239.6±36.8	508.8±28.6
	Turbidity (NTU)	242.0±178.9	87.8±21.4	323.0±72.1	13.2±2.3
	Flow ($m^3.sec^{-1}$)	14.4±13.1	9.9±6.7	-	-
Sundays River	Temperature (°C)	23.3±1.1	10.8±0.8	23.6±0.5	14.1±0.3
	pH (range)	7.1 - 8.4	8.5 - 8.6	6.3 - 7.7	6.7 - 9.9
	TDS (ppm)	1141.8±488.3	1256.0±491.0	246.2±29.5	243.8±36.2
	Conductivity ($\mu S.cm^{-1}$)	1886.9±751.5	2136.0±843.1	379.0±30.4	413.2±61.1
	Turbidity (NTU)	26.9±16.2	18.9±12.8	7.2±1.5	2.6±0.5
	Flow ($m^3.sec^{-1}$)	14.0±22.6	3.5±4.5	-	-

Twenty three species belonging to eleven families were sampled (Table 3.2). Seven species, including catfish, were non-native and constituted 30 % of the total richness. The catfish occurred in the mainstream sections and was absent within the headwaters of the tributaries of the two rivers. Within the Great Fish River, most species occurred within the mainstream section, whereas headwater tributaries were occupied by longfin eel *A. mossambica* and chubbyhead barb *B. anoplus*. By comparison, four species, namely *A. mossambica*, *Pseudobarbus afer, B. pallidus* and *Glossogobius callidus*, were sampled within the headwater tributaries of the Sundays River, whereas the majority of the fish species occurred within the mainstem.

Table 3.2: The fish species sampled in the Great Fish River and Sundays River, Eastern Cape, South Africa. Their common names and abbreviations used in the analysis are given. The values indicate the catch per unit effort (CPUE, mean ± standard deviation) from fyke and gill nets (number of fish per net.night^{-1}) in the mainstream and density of minnows (number of fish.m^{-2}) captured by electric fishing (EF) in the tributaries. - indicates that the species was absent and + indicate that the species occurred in the habitat. * indicates non-native in all localities and # indicates non-native only in the Sundays River.

				Great Fish River			Sundays River		
				Mainstream		Tributary	Mainstream		Tributary
Family	Species	Abbreviation	Common name	Fyke	Gill	EF	Fyke	Gill	EF
Anguillidae	*Anguilla mossambica*	Amo	Longfin eel	0.2±0.5	-	-	0.5±0.7	+	+
	Anguilla marmorata	Amar	Mottled eel	0.1±0.3	-	+	+	+	-
Clupeidae	*Gilchristella aestuaria*	Ga	Round-herring	+	+	-	+	+	-
Cyprinidae	*Pseudobarbus afer*	Pa	Eastern Cape redfin	-	-	-	+	+	1.5±1.5
	Barbus anoplus	Ba	Chubbyhead	-	-	0.5±0.6	-	-	-
	Barbus pallidus	Bp	Goldie barb	-	-	-	+	+	+
	*Labeobarbus aeneus**	La	Smallmouth yellowfish	0.8±1.2	1.6±1.6	-	0.4±0.6	0.5±0.7	-
	Labeo umbratus	Lu	Moggel	0.3±0.3	0.9±1.9	-	0.4±0.1	4.0±4.2	-
	*Labeo capensis**	Lc	Mudfish	+	0.1±0.1	-	+	+	-
	*Cyprinus carpio**	Cc	Carp	+	+	-	+	0.1±0.2	-
Clariidae	*Clarias gariepinus**	Cg	Sharptooth catfish	0.5±0.5	0.3±0.4	-	0.2±0.2	0.2±0.3	-
Poeciliidae	*Gambusia affinis**	Ga	Mosquitofish	+	+	-	+	+	-
Cichlidae	*Tilapia sparrmanii**	Ts	Banded tilapia	0.1±0.2	+	-	1.1±1.8	0.3±0.5	-
	Oreochromis mossambicus[#]	Om	Mozambique tilapia	0.1±0.2	+	-	0.5±0.6	1.4±1.9	-
Mugilidae	*Mugil cephalus*	Mce	Flathead mullet	+	+	-	+	+	-
	Myxus capensis	Mca	Freshwater mullet	+	0.1±0.3	-	0.9±3.0	1.3±2.3	-
	Liza macrolepis	Lm	Large-scale mullet	+	+	-	+	+	-
Monodactylidae	*Monodactylus falciformis*	Mf	Cape moony	0.2±0.6	+	-	0.8±1.2	0.2±0.4	-
Gobiidae	*Glossogobius callidus*	Gc	River goby	+	+	-	+	+	+
	Redigobius dewaalii	Rd	Checked goby	+	+	-	-	-	-
	Psammogobius knysnaensis	Pk	Knaysna sandgoby	+	+	-	-	-	-
Haemulidae	*Pomadasys commersonnii*	Pc	Spotted grunter	+	+	-	-	-	-
Sparidae	*Rhabdosargus holubi*	Rh	Cape stimpnose	+	+	-	+	+	-

Correspondence analysis (CA) showed gradients associated with mainstream to tributary patterns on the first ordination axis and upstream to downstream changes on the second ordination axis in both rivers (Figure 3.1). Within the Great Fish River, the upstream to downstream gradient was more pronounced, with the regression indicating a full turnover in the predicted occurrence of most species along the second CA axis. Catfish occurrence was predicted to decrease progressively along both the longitudinal (upstream to downstream) and the mainstem to tributary gradient (Figure 3.1). In contrast to the pattern observed in the Great Fish River, the mainstem to tributary gradient was more pronounced within the Sundays River, with most species exhibiting a full turnover along this gradient. For both rivers, catfish occurrence was predicted to decrease progressively along the mainstem to tributary gradient but showed little variation along the longitudinal axis, indicating that its occurrence within the mainstem section was uniform.

The classification tree for catfish occurrence within the Great Fish River had four branches with splits based on river type (mainstream versus tributary), pH and conductivity (Figure 3.2) and explained 67.8 % of the variation within the data. River type and pH were the dominant predictors for catfish occurrence. Random forests (RF) indicated that river type contributed 12 %, with a Gini index value of 4.7, followed by pH that contributed 5 %, with a Gini index value of 3.9, towards the model predicting the presence of catfish within the Great Fish River (Table 3.3). By comparison, within the Sundays River, classification trees explained 87.3 % of the data variability, and resulted in a three-leaf tree with splits based on river type and conductivity (Figure 3.2). Catfish occurrence was defined by sites with high conductivity. RF showed that conductivity contributed 35 % (Gini index = 7.8 %) followed by river type which contributed 22 % (Gini index = 4.4 %) to predicting catfish occurrence (Table 3.3).

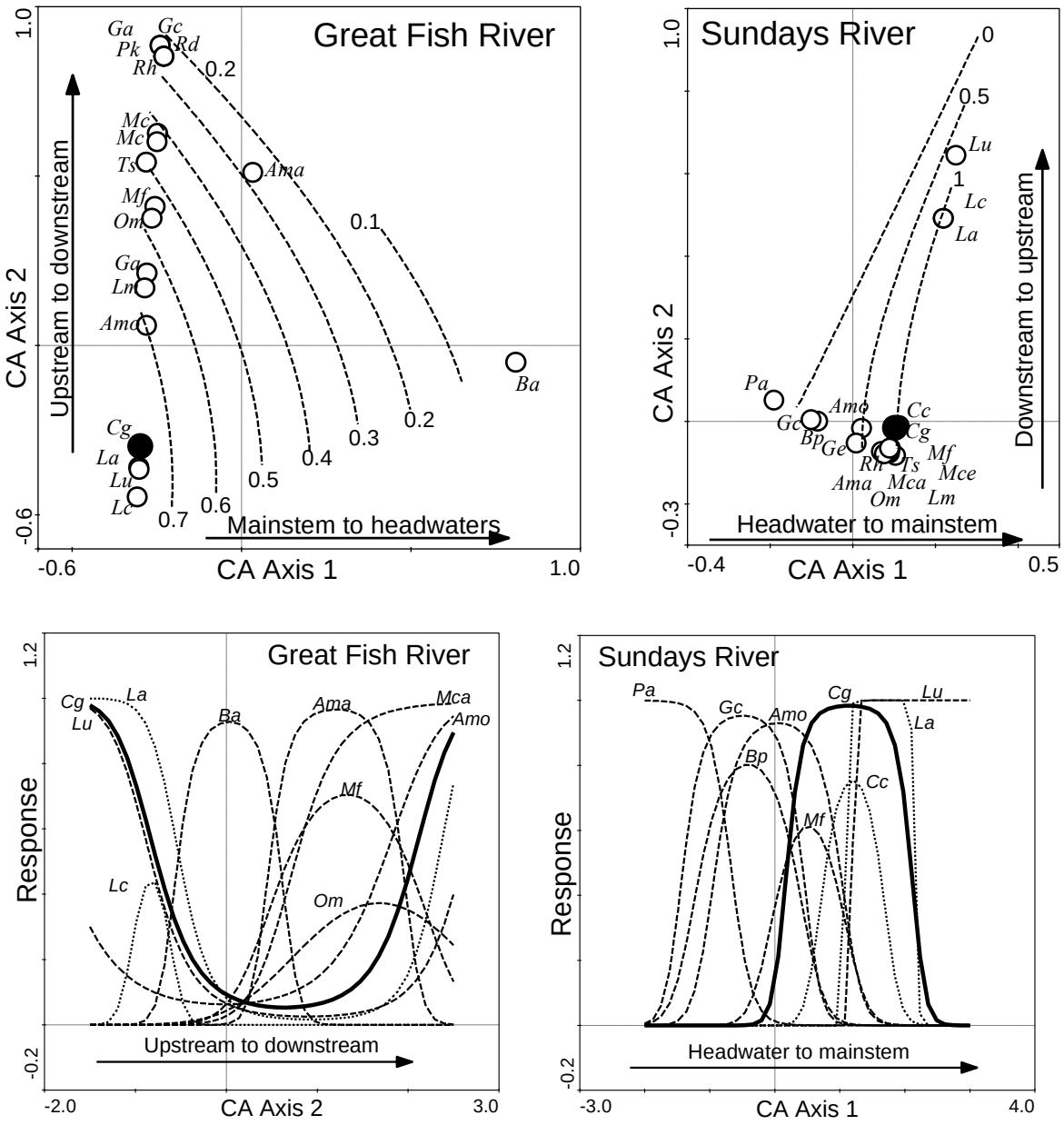

Figure 3.1: Correspondence analysis (CA) ordination plots indicating the patterns in distribution of catfish (closed circles) and other species (open circles) within the Great Fish River (top left) and Sundays River (top right). The dotted contours in the ordination plots indicate the predicted occurrence of catfish based on the resultant logistic regressions from CA. Species response curves based on the logistic regression indicate the predicted occurrence of catfish (bold) and other species (dashed lines) along the ordination gradients within the Great Fish River (bottom left) and Sundays River (bottom right).

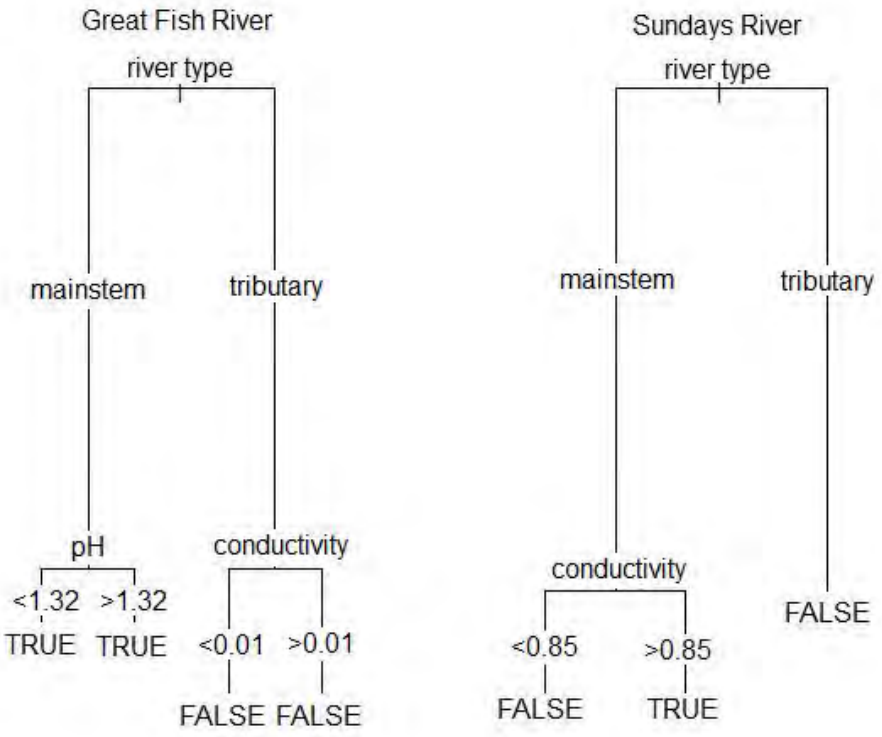

Figure 3.2: Classification trees for catfish presence (TRUE) or absence (FALSE) in relation to physico-chemical variables in the Great Fish and Sundays Rivers. The Great Fish River model has a pseudo-R^2 = 67.8 % and miscalculation error rate (MER) = 8.3 %. The Sundays River model has a pseudo-R^2 = 87.3 % and MER = 3.4 %.

Table 3.3: The relative importance of predictor variables in the ability to predict absence (FALSE), presence (TRUE) or both (MDA (mean decrease accuracy)). MDG (mean decrease Gini) is the Gini index that measures node impurity with high values indicating that a variable is able to group presence or absence into exclusive groups.

	Great Fish River				Sundays River			
	FALSE	TRUE	MDA	MDG	FALSE	TRUE	MDA	MDG
temperature	0.01	0.03	0.01	2.46	0.00	-0.01	0.00	1.37
pH	0.04	0.05	0.04	3.93	0.00	0.04	0.00	1.53
conductivity	0.04	0.04	0.04	3.95	0.07	0.35	0.12	7.84
turbidity	0.00	0.01	0.00	1.27	0.01	0.00	0.00	1.68
river	0.08	0.12	0.09	4.74	0.05	0.22	0.08	4.42

Multivariate regression tree (MRT) analysis, for catfish abundance, within the Great Fish River explained 48.1 % of the standardised fyke and gill net CPUE data (Figure 3.3).

The tree had four branches based on pH, temperature and conductivity. The first split separated five sites with high pH that had a high abundance of catfish in both fyke and gill nets. The second split separated four sites that had both high temperatures (temperature ≥ 1.04) and gill net CPUE. The last split separated tributary sites (n = 34), which had low catfish abundance, from mainstream sites (n = 5) that had high fyke net CPUE. By comparison, within the Sundays River, MRT analysis produced a three-leaf tree based on conductivity and temperature (Figure 3.3). The tree explained 53 % of the variation in standardised catfish CPUE. The first split isolated sites with low conductivity (conductivity < 0.5, n = 40), where catfish abundance was low in both fyke and gill nets, to those with high conductivity (conductivity ≥ 0.5). The second split separated sites with low temperature (n = 7), where catfish CPUE was high in the fyke nets, to sites with high temperature (n = 4) where catfish gill net CPUE was high.

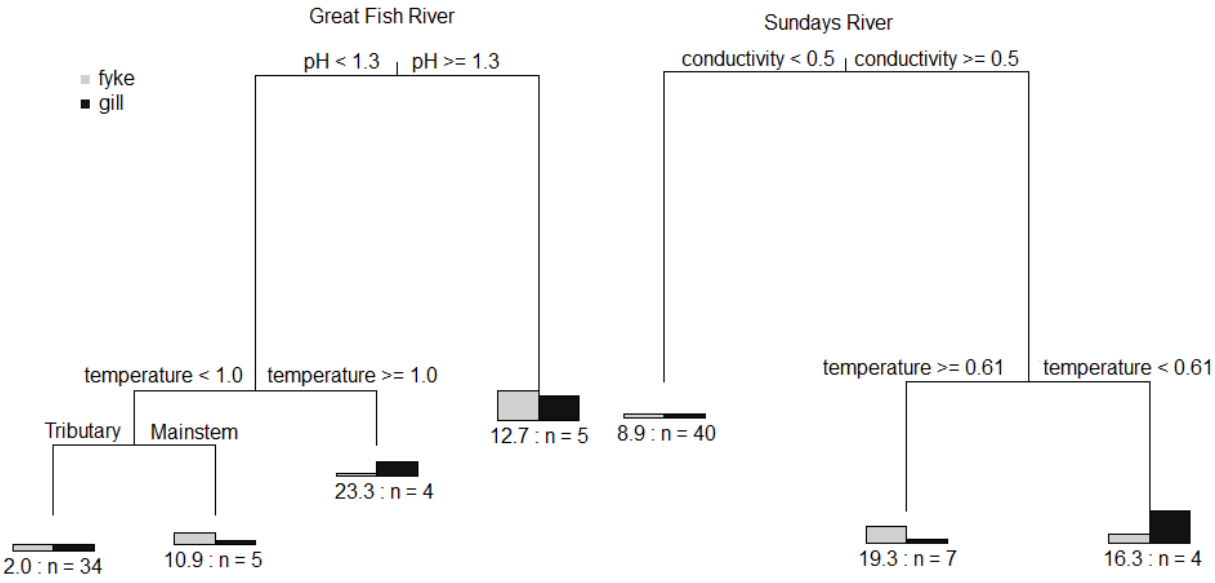

Figure 3.3: Multivariate regression tree for the relationship between CPUE for fyke and gill nets as response variables and physico-chemical variables as predictors for sharptooth catfish *Clarias gariepinus* collected in the Great Fish River and Sundays River, Eastern Cape, South Africa. The pseudo-R^2 for the trees were 48.1 % and 53.0 % for the Great Fish River and

Sundays River, respectively. Under each histogram, *n* is the number of sites in the branch and the value before *n* is the sum of squared errors for the group.

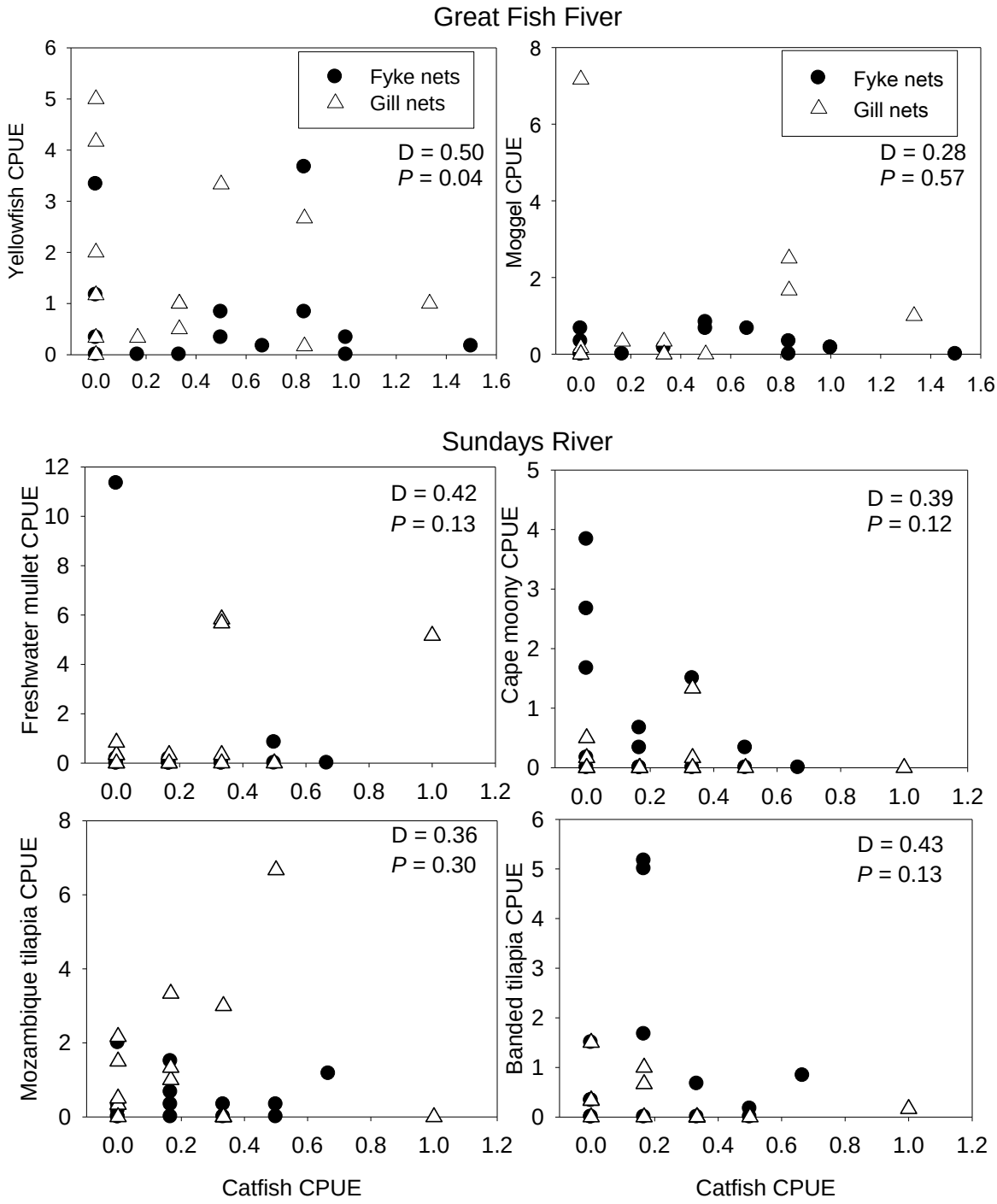

Figure 3.4: Relationships among sharptooth catfish *Clarias gariepinus* catch per unit effort (CPUE) and smallmouth yellowfish *Labeobarbus aeneus* and moggel *Labeo umbratus* CPUE within the Great Fish River, freshwater mullet, *Myxus capensis*, cape moony, *Monodactylus falciformis*, Mozambique tilapia, *Oreochromis mossambicus*, and banded tilapia, *Tilapia sparrmanii*, within the Sundays River collected using gill and fyke nets. Statistical analysis was based on two-dimensional Kolmogorov-Smirnov test.

Within the Great Fish River, two species, non-native smallmouth yellowfish *L. aeneus* and native moggel *L. umbratus*, were widespread and co-occurred with catfish. The CPUE for smallmouth yellowfish was inversely related and significantly different to that of the catfish (2DKS test D = 0.5, $P = 0.04$) (Figure 3.4), indicating that the abundance of the non-native smallmouth yellowfish was low in habitats with high abundance of catfish. In contrast, *moggel* exhibited no discernible pattern in relation to the catfish, with no significant differences in the CPUE for the two species, indicating an overlap in those habitats with both a high moggel and high catfish abundance. By comparison, within the Sundays River, four species were widespread and co-occurred with the catfish. These were freshwater mullet *Myxus capensis*, Cape moony *Monodactylus falciformis*, Mozambique tilapia *Oreochromis mossambicus* and banded tilapia *T. sparrmanii*. Although there was a discernible decrease in the CPUE for *M. capensis*, *M. falciformis* and *T. sparrmanii* with increasing catfish CPUE, there were no statistically significant differences (2DKS test, $P > 0.05$) in the CPUE for pairwise comparisons of these species with catfish (Figure 3.4).

Discussion

Non-native species are considered to be invasive when they cause negative impacts within their invaded range (Simberlorff & Von Holle, 1999). Invasive predators can influence the distribution patterns, population dynamics and community interactions of native species within aquatic ecosystems (Weber & Brown, 2011). Within habitats subjected to anthropogenic modification, invasion resistance is often weakened as native species become exposed to novel conditions to which they are not adapted (Baltz & Moyle, 1993; Byers, 2002; Didham *et al*., 2007). These disturbances can weaken a native species' prior advantage to local environmental conditions accrued over a long evolutionary period (Byers, 2002). This study showed the sharptooth catfish was abundant within mainstem habitats. Within the

Great Fish River, sharptooth catfish was closely associated with smallmouth yellowfish, moggel and mudfish, whereas within the Sundays River, catfish appeared to be associated with a greater number of species, both native and non-native. Mainstem habitats were characterised by altered flow regimes, with most sites having alkaline pH and high conductivity compared to headwater sites of tributaries where native minnows were most abundant. This suggests the proliferation of catfish within flow-altered habitats, supporting the hypothesis of the establishment of invasive non-native species in habitats that are modified (Marchetti & Moyle, 2001; Ricciardi, 2004; Clavero & García-Berthou, 2005; Hermoso *et al*., 2011). Although catfish occurrence was predicted to decrease along the mainstream to tributary gradient, these headwater habitats remain vulnerable to invasion during periods of continuous flow. Within the Sundays River, periodic invasions of the headwater streams by catfish have been reported. Presently, catfish is restricted occurs in lower sections of tributaries within invaded rivers in the Eastern Cape (Ellender *et al*., 2011), as was observed during this study, but periodic incursions into the headwaters have been reported (Traas, 2009).

High catfish abundance was predicted in relation to physico-chemical environmental conditions that were characteristic of the mainstream habitats. Many studies have shown that habitat alteration may drive increase in the abundance and distribution of non-native invaders (Lonsdale, 1999; Lozon & MacIsaac, 1997; Didham *et al*., 2005). Generalist invasive predators, such as catfish, usually reach high densities within modified habitats, and then "spilling-over" into relatively undisturbed habitats of native species (Donovan *et al*., 1997; Rand & Louda, 2006). Often, invasion impact increases in proportion to the abundance of invaders, usually translating into both direct and indirect impacts. Direct impacts are usually related to predation that leads to decrease in prey abundance and, in extreme cases, can result in the extirpation of a prey species (Rahel, 2002; Sax & Gaines, 2008). Within both study

systems, direct impact due to catfish predation is of major concern because the native ichthyofauna is endemic, with some species, especially the native minnows, being endangered. Many of these native minnows are restricted in their distribution in the intermittent headwater tributaries that are often disconnected from the mainstream habitats (Swartz *et al*., 2009; Tweddle *et al*., 2009). The risk of the catfish "spilling-over" into the headwater habitats is most likely when there is continuous flow and flooding. Cambray (2003a) noted that the invasion of sharptooth catfish into the tributaries coincided with a decline and even disappearance of the native minnow *Pseudobarbus asper* in the Gamtoos River and the anabantid *Sandelia bainsii* in the Tyume River. In addition, within the mainstream, many estuarine-dependent species use the freshwater habitats of the lower sections of the study rivers (Wasseman *et al*., 2011), and the catfish is likely to have an impact on their distribution. There is also potential for indirect invasion impacts associated with competition and alteration of food webs and nutrient cycling pathways. Khan & Pannikar (2009) showed that by exerting predation pressure, catfish can alter food webs and ecosystem structure and functioning within invaded habitats through top-down tropic cascade processes.

Fishes that have have similar habitat needs as catfish are likely to be at risk of negative impact. Within the Great Fish River, the native cyprinid, moggel (*L. umbratus*) co-occurred with the catfish at most sites. This indicates that most habitats that were preferred by moggel overlapped with that of catfish. Moggel is benthivorous and known to prefer slow flowing habitats (Skelton, 2001). Predation of moggel by the catfish in these habitats is likely, as has been observed in lentic habitats (Potts *et al*., 2008; Kadye & Booth, 2011). In contrast, non-native smallmouth yellowfish (*L. aeneus*) appeared to be more abundant at low catfish density, which suggests low overlap in habitat use between these two non-native species. Smallmouth yellowfish, which has recently established in the Great Fish River, is

known to prefer fast flowing habitats for feeding and spawning (Weyl *et al*., 2009a), and do poorly in lentic habitats, such as man-made impoundments, in contrast to catfish. The results suggest that smallmouth yellowfish would be abundant in habitats that could potentially act as alternative refugia from the catfish. Within the Sundays River, relatively more species co-existed with catfish. Although there was no evidence of difference in abundance trends, the observed pattern does suggest a general decrease in fish abundance with increasing catfish abundance. This inverse relationships may be indicative of potential exploitation through predation and potential interference through competition when resources become limiting. This suggests a greater community-wide catfish impact on the ichthyofauna of this river.

To conclude, this study indicates the widespread occurrence and abundance of catfish within the mainstream sections of the Great Fish and Sundays Rivers. The potential impact associated with catfish proliferation within the mainstream sections is the risk of predation on native species and the reduced invasion resistance due to alteration of flow regimes. These mainstream habitats would act as sources of invasion into the headwater streams where native species, especially endangered minnows, occur.

CHAPTER 4

Inter-seasonal Persistence and Size-structuring of Two Minnow Species within Headwater Streams in the Eastern Cape, South Africa

Introduction

Conserving stream fishes requires an understanding of factors that influence their population dynamics. In streams, physical habitat is often regarded as the primary factor determining fish population structure (Niaman & Latterell, 2005). The relationship between physical habitat and fish population structure has been demonstrated at various levels, including patterns associated with spatial and temporal changes (Jackson *et al*., 2001; Ostrand & Wilde, 2002), stream size (Zorn *et al*., 2002), microhabitat (Grossman & Ratajczak, 1998), physico-chemical environment (Casatti *et al*., 2006) and spatial autocorrelation (Wilkinson & Edds, 2002). Schlosser (1991) further suggested that, from a landscape ecology perspective, stream physical habitat is a template upon which fish populations are structured. Patterns in fish and habitat relationships are critical in headwater streams that, in addition to being relatively small, are subject to both high temporal and spatial variability.

The population structure of fish inhabiting headwater streams is usually considered to be non-random, and structured as a result of a balance between net energy gain and potential mortality risk (Capone & Kushlan, 1991; Schlosser, 1991). Net energy gain is a trade-off between food availability and swimming costs. Mortality risk is associated with predation and unfavourable conditions especially where streams undergo periodic drying and lose connectivity with adjacent aquatic habitats (Capone & Kushlan, 1991). Predation risk in headwater streams is usually from terrestrial, wading predators, and swimming predators, and can also be size-dependent (Power, 1987; Schlosser, 1988). Stream fish would therefore

select habitats that provide refuge and permit survival from harsh environmental conditions (Koehn *et al*., 1994).

Population stability in headwater streams, based on density and relative abundance may, however, vary in response to severity of environmental conditions in habitats where populations are able to persist in both benign and harsh conditions (Keaton *et al*., 2005). Under extreme environmental fluctuations, temporal variation in physical conditions can be substantial and may be the dominant factor influencing population structure (Ostrand & Wilde, 2002). Survival and maintenance of viable populations under environmental extremes would therefore depend on a species' ability to withstand, as habitat specialists, the environmental fluctuations, or to utilise, as habitat generalists, different habitats or to recolonise when environmental conditions improve (Erős & Grossman, 2005).

The headwater streams of the Great Fish and Sundays Rivers in the Eastern Cape, South Africa provide an excellent opportunity to study fish and their relationship to their proximate habitat. Two native species of cyprinid minnows, Eastern Cape redfin, *Pseudobarbus afer*, which is listed on the IUCN Red List as endangered, and chubbyhead barb, *Barbus anoplus*, are the most widespread in the headwater streams of the Sundays and Great Fish Rivers, respectively. The streams are subject to both spatial and temporal variation in environmental conditions, with most habitats occurring as isolated pools during the dry season. The two minnows are threatened by alien invasive species, especially sharptooth catfish, *Clarias gariepinus*, that has established in the mainstem sections of the two rivers. The piscivorous catfish is potamodramous and migrates upstream when rivers flood. Because of its large body size, the catfish is likely to occupy deeper habitats and persist in the isolated pools that serve as refuge for the minnows during the dry season. This is likely to pose a serious threat to the populations of these native minnows, as observed with the occurrence of other invasive fish in the region (Lowe *et al*., 2008). Previous studies already indicate the

occurrence of catfish in the headwater streams including in habitats with native minnows (Weyl *et al*., 2009b).

The conservation priority criteria proposed by Weyl *et al*. (2009b) indicated that the headwater streams of the Sundays River, in particular, are critical conservation priority areas for the endangered *P. afer*. Continuous monitoring of distribution and density, and an evaluation of the critical habitats for native minnows is therefore essential. A quantitative measure of the fish densities in these headwater streams is crucial for achieving this goal. Depletion sampling provides an opportunity to estimate population parameters, such as absolute densities and probabilities of capture (Peterson *et al*., 2004), and an evaluation of population structure and its environmental correlates. Thus, the objectives of this study were to (1) evaluate the population densities and examine the influence of physical habitats and physico-chemical environment on the two widespread minnows, *P afer* and *B. anoplus*, and (2) examine the size structure of each population in relation to physical habitat and temporal variation.

Materials and methods

Study area and data collection

Pseudobarbus afer was collected in the tributaries of the Sundays River in the Zuurberg Mountains within Addo Elephant National Park (AENP). Three other species, *B. pallidus*, *G. callidus* and *A. mossambica*, co-occurred with *P. afer* in the Zuurberg. *Barbus anoplus* was collected in the headwaters of the Koonap River, a tributary of the Great Fish River, in the Winterberg Mountains. *Anguilla mossambica* was also present in the Winterberg. Three-pass depletion electric fishing was conducted at 21 sites for *P. afer* and 19 sites for *B. anoplus*

(Figure 4.1). Sites were sampled in both summer (February-May 2010) and winter (July-September 2010).

Before sampling, a section of each site was blocked with 4 mm mesh nets that were secured to the streambed. Temperature (°C), pH, total dissolved solids (TDS) (ppm) and conductivity ($\mu S.cm^{-1}$) were measured with a HANNA HI 98129 Combo meter, and turbidity (NTU) was measured using a HANNA HI 98703 turbidity meter. Fish were captured using a Samus 725GN backpack electric fisher powered by a 12V battery. Captured fish from each pass were kept in separate buckets containing stream water. All fish were counted and measured (mm TL [total length] to a maximum of 50 individuals of the total catch per site) and released. To minimise bias, fish were scooped with a hand-held net from the buckets before being measured for length. After fish sampling, ten transects were set perpendicular to the direction of flow at each site, to measure physical habitat variables. The measurements made were depth and substrate types at three points along each transect, width for each transect and total length for the sampled section. Substrate types were categorised following Gorman & Karr (1978) and Schlosser (1982) as silt (< 0.05cm), sand (0.05 - 2cm), gravel (2 - 10cm), pebble (10 - 30cm), boulder (30 - 50cm) and bedrock (> 50cm). The proportion (%) of each category was determined. Bank vegetation was determined from all the points at the end of each transect and expressed as the proportion of points with overhanging or marginal vegetation from the total points. In addition, the total area sampled, average depth and volume were calculated for each site.

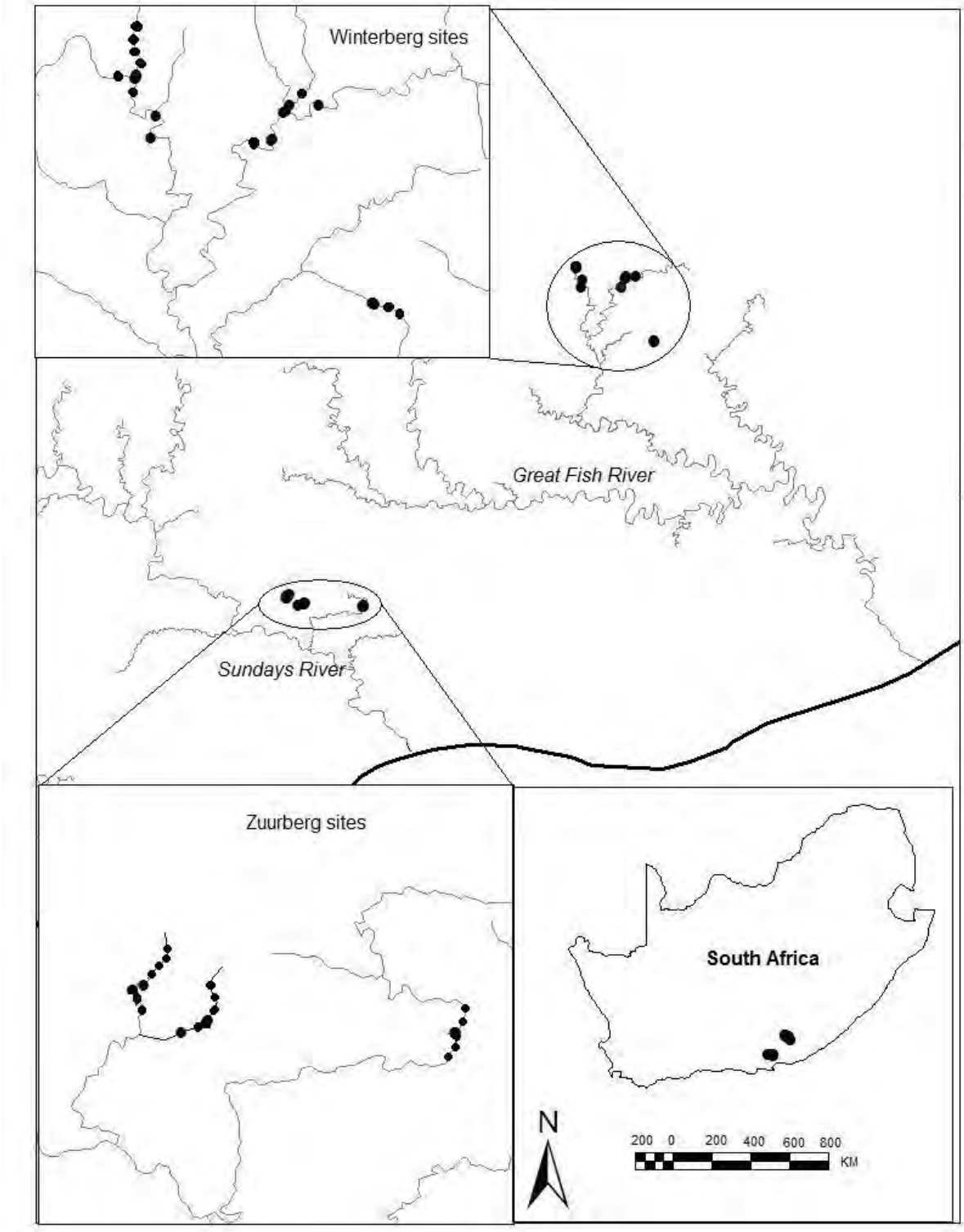

Figure 4.1: Map of the study area showing the sites of fish sampling in the headwater streams in the Zuurberg and Winterberg Mountains, Eastern Cape, South Africa.

Data analysis

Seber's (1982) maximum likelihood approach was used to estimate fish number, $\hat{N}$, and probability of capture, $\hat{p}$. Therefore, given a vector $n=(n_1,n_2,n_3)'$ of three observed removals from a population, the maximum likelihood estimates (MLEs) of $\hat{N}$ and $\hat{p}$ were obtained by maximising a multinomial likelihood function of the form:

$$L(\mathbf{n}|N,k)=\frac{N!}{\left(\prod_{i=1}^{3}n_i!\right)\left(N-\sum_{i=1}^{3}n_i\right)!}p^{n_1}(qp)^{n_2}\left(q^2p\right)^{n_3}\times\left(1-p-qp-q^2p\right)^{N-\sum_{i=1}^{3}n_i},$$

where $q=1-p$. The model assumes that (a) the population being sampled is closed to migration, recruitment and mortality, (b) individuals have the same probability of capture for a unit of effort throughout the experiment and (c) units of effort are independent and additive, and all removals from the population are known (Seber, 1982).

The per capita seasonal change in abundance was calculated as $\Delta N=\ln\left(\hat{N}_{t+1}\right)-\ln\left(\hat{N}_t\right)$ where $\hat{N}_t$ is the estimated abundance in season *t*. Potential density-dependence was examined by the relationship between ΔN and $\ln\hat{N}_t$ (Wootton, 2007).

As the data are dependent of one another, with the same pools sampled in both summer and winter, a linear mixed-effects model assessed the relationships between several environmental factors and population size, density and capture probability. Density, $\hat{D}$, was expressed as number of fish per m^{-2}. Population size, density and capture probability were *z*-transformed prior to the analysis.

Given the i^{th} observation from the p^{th} pool in the s^{th} season, the effect of one or more environmental variables, β_k, the mixed-effect model has the form:

$$y_{iskp}=\mu+\alpha_s+\beta_k+\delta_p+\varepsilon_{iskp}$$

where $\varepsilon_{iskp} \sim N(0,\sigma^2)$ is the residual error, and $\delta_p \sim N(0,\sigma_P^2)$ is the random effect. In the case where β_k is removed from the model, the results are equivalent to a paired *t*-test. The analyses were conducted using package *nlme* in *R* (R Core Development Team, 2011).

To determine size structure, fish length data for each species were categorised into the following arbitrary length classes: L1 (<20 mm TL), L2 (20 – 29 mm TL), L3 (30 – 39 mm TL), L4 (40 – 49 mm TL), L5 (50 – 59 mm TL), L6 (60 – 69 mm TL), L7 (70 – 79 mm TL), L8 (80 – 89 mm TL), L9 (90 – 99 mm TL) and L10 (>100 mm TL). Each length class was expressed as a proportion of all categories. Sexual maturity was 40 mm TL for both species (Cambray & Bruton, 1985; Cambray & Hecht, 1995). Fish were therefore categorised as immature (> 40 mm) or mature (< 40 mm).

A non-linear ordination method, Canonical Correspondence Analysis (CCA), was used on the standardised proportions of length categories and environmental variables to identify fish size and habitat relations. Environmental variables were physical habitat variables (H) and seasonal variable (S). Environmental variables with variance inflation factor (VIF) > 10 indicate multicollinearity with other variables (ter Braak & Smilauer, 1998), and were eliminated from the analysis. A forward selection procedure was then applied to select the best predictor variables. The significant contributions of these variables to the ordination were tested in Monte Carlo simulations at the $P < 0.05$ level. A Monte Carlo permutation test was also used to test the statistical significance of the relationship between species and environmental variables. The total variation in size composition was partitioned using partial CCA as follows: (1) variation accounted for by all environmental variables (using each of the species data and environmental variables (habitat and sampling seasons) $[H \cup S]$), (2) variation accounted for by habitat variables after partialling out the influence of sampling seasons (pure habitat $[H|S]$), (3) variation accounted for by sampling seasons after partialling

out the effect of habitat variables (pure seasonal $[S|H]$), (4) variation jointly explained by both groups $(H \cap S)$and (5) unexplained variation. Each component of the variation was obtained by dividing the canonical eigenvalue of the particular CCA by the total inertia. Ordinations were conducted in *CANOCO v4.5*. All environmental variables were standardised to mean zero and unit variance prior to the analyses.

Results

The maximum estimated population sizes ($\hat{N}$) for *P. afer* were 238 and 284 fish in summer and winter, respectively (Table 4.1), with no significant differences between seasons (Paired-t test, t_{17}= - 0.70, $P > 0.05$). By comparison, maximum estimated population sizes for *B. anoplus* were 128 and 152 fish in summer and winter, respectively, with significant differences between seasons (Paired-t test, t_{14}= 2.64, $P < 0.05$). The number of adults between seasons were not significantly different for *P. afer* but were significantly different (Paired-t test, t_{14} = 2.30, $P < 0.05$) for *B. anoplus*. *Pseudobarbus afer* densities ranged between 0.1 - 5.0 fish per m^{-2} and 0.1 - 5.7 fish per m^{-2} during summer and winter, respectively (Table 4.1), with no significant differences (Paired-t test, t_{17}= 0.11, $P > 0.05$), between the sampling seasons. By comparison, *B. anoplus* densities were lower and ranged between 0.1 - 1.7 fish per m^{-2} during summer and 0.1 - 2.6 fish per m^{-2} during winter (Table 4.1) with no significant differences between seasons. Adult densities were not significantly different between seasons for both species. Capture probability ranged between 0.4 - 0.8 for both *P. afer* and *B. anoplus* (Table 4.1) with no significant differences between seasons. There was no significant relationship between per capita seasonal change in abundance and summer abundance for either species (Figure 4.2).

Table 4.1: Estimated capture parameters, total number of fish captured (N_T), estimated initial population ($\hat{N}$), probability of capture ($\hat{p}$) and density of fish per m^{-2} ($\hat{D}$) and their associated asymptotic standard errors. [a] = water present but no fish sampled within the pools; [b] = pools had dried up, no fish sampled.

		Summer				Winter			
Species	Site	N_T	$\hat{N}$	$\hat{p}$	$\hat{D}$	N_T	$\hat{N}$	$\hat{p}$	$\hat{D}$
Pseudobarbus afer	1	137	147.6±5.6	0.6±0.1	0.8	77	93.2±10.4	0.4±0.1	0.8
	2	7	7.0±0.0	0.8±0.0	0.1	6	6.0±0.0	0.7±0.0	0.1
	3	58	58.0±1.0	0.8±0.1	2.5	26	26.3±1.3	0.7±0.1	0.6
	4	233	238.0±3.0	0.7±0.0	1.8	49	50.6±2.1	0.7±0.1	0.7
	5	57	58.0±1.7	0.7±0.1	1.1	102	104.4±1.3	0.7±0.1	1.8
	6	19	19.0±2.4	0.8±0.3	0.2	59	59.0±1.0	0.8±0.1	0.6
	7	29	29.0±1.2	0.8±0.1	1.5	8	8.0±0.0	0.7±0.0	0.2
	8	40	41.0±1.8	0.7±0.1	0.8	9	9.0±1.6	0.6±0.3	0.1
	9	45	45.1±1.0	0.8±0.1	3.3	29	29.7±1.6	0.7±0.1	0.2
	10	101	102.7±1.9	0.7±0.1	4.9	222	228.6±1.6	0.7±0.0	3.1
	11	111	111.0±0.9	0.8±0.0	3.8	260	262.7±2.2	0.8±0.0	3.5
	12	117	118.9±2.0	0.7±0.0	1.4	267	284.9±6.9	0.6±0.0	5.4
	13	22	22.0±1.3	0.8±0.1	1	49	54.3±4.7	0.5±0.1	1.4
	14	16	16.6±1.8	0.6±0.2	0.3	59	59.0±0.9	0.8±0.1	2.4
	15	81	81.8±1.4	0.8±0.1	1.5	62	62.2±1.1	0.8±0.1	2.2
	16	12	12±1.3	0.7±0.2	0.2	10	10.0±1.3	0.6±0.2	0.3
	17	23	23.0±1.1	0.7±0.1	0.3	22	26.9±6.3	0.4±0.2	0.8
	18	4	4.0±0.0	0.8±0.0	0.1	1	-	-	-
	19	0[a]	-	-	-	19	19.0±1.5	0.8±0.2	4.7
	20	0[a]	-	-	-	24	26.7±3.6	0.5±0.1	1.6
	21	0[a]	-	-	-	13	13.0±1.4	0.7±0.2	0.2
Barbus anoplus	1	36	37.8±2.4	0.6±0.1	0.3	8	8.0±6.3	0.7±1.1	0.1
	2	79	99.6±13.0	0.4±0.1	1.7	28	28.4±1.4	0.7±0.1	0.5
	3	25	25.7±1.7	0.6±0.1	0.5	19	21.8±4.1	0.5±0.2	0.4
	4	56	56.0±0.9	0.8±0.1	0.7	15	15.7±2.0	0.6±0.2	0.2
	5	5	5.0±0.0	0.8±0.0	0.1	2	-	-	0.5
	6	69	71.4±2.5	0.7±0.1	1.1	17	17.0±1.1	0.7±0.1	0.1
	7	126	126.8±1.4	0.8±0.0	1.7	135	152.4±8.6	0.5±0.1	2.6
	8	11	11.0±1.8	0.7±0.3	0.1	1	-	-	0.6
	9	56	60.8±4.1	0.6±0.1	0.7	39	39.8±1.6	0.7±0.1	0.4
	10	74	79.8±4.3	0.6±0.1	0.6	30	32.4±3.1	0.6±0.1	0.3
	11	114	128.1±7.5	0.5±0.1	1.1	22	22.0±1.1	0.7±0.1	0.3
	12	10	10.6±2.1	0.5±0.2	0.4	6	6.0±0.0	0.8±0.0	0.2
	13	16	16.0±1.4	0.7±0.2	0.3	12	12.0±0.0	0.9±0.0	0.3
	14	18	18.0±2.3	0.8±0.3	0.3	16	16.0±1.2	0.7±0.2	0.5
	15	10	10.0±1.7	0.7±0.3	0.3	-[b]	-	-	-
	16	4	4.0±0.0	0.8±0.0	0.1	-[b]	-	-	-
	17	8	8.0±1.5	0.6±0.3	0.1	-[b]	-	-	-
	18	14	14.0±1.2	0.7±0.2	0.1	101	114.5±7.6	0.5±0.1	1.8
	19	5	5.0±0.0	0.7±0.0	0.1	-[b]	-	-	-

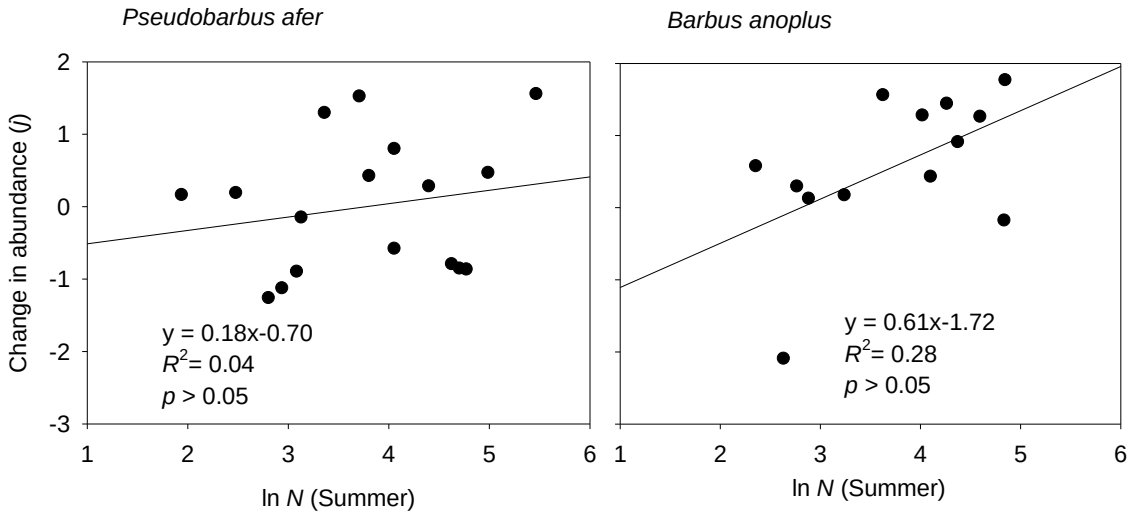

Figure 4.2: The relationship between seasonal per capita rate of change in abundance and summer abundance for *Pseudobarbus afer* and *Barbus anoplus* sampled in the Zuurberg and Winterberg.

Table 4.2: Mean values of physico-chemical and habitat variables (± standard deviation) for *Pseudobarbus afer* and *Barbus anoplus* sampled in the Zuurberg Mountains and Winterberg Mountains during summer and winter.

	Zuurberg		Winterberg	
	Summer	Winter	Summer	Winter
Temperature (°C)	23.6±0.5	14.1±0.3	23.1±1.0	10.3±0.6
pH (range)	8.6 - 8.9	8.7 - 9.2	7.7 - 8.6	8.1 - 8.5
TDS (ppm)	246.2±29.5	243.8±36.2	135.7±20.0	293.7±16.3
Conductivity ($\mu S.cm^{-1}$)	379.0±30.4	413.2±61.1	239.6±36.8	508.8±28.6
Turbidity (NTU)	7.2±1.5	2.6±0.5	323.0±72.1	13.2±2.3
Boulder (%)	12.4±2.9	36.9±4.7	15.6±2.9	15.9±4.4
Gravel (%)	14.6±2.3	2.8±1.3	12.5±3.0	10.9±5.2
Pebble (%)	43.1±4.3	38.3±4.6	31.1±4.7	28.8±5.9
Bedrock (%)	18.3±5.2	16.3±6.0	14.9±5.5	27.9±7.3
Silt (%)	8.1±3.3	5.7±3.8	18.8±7.6	12.6±4.4
Sand (%)	3.3±1.2	0.0±0.0	7.4±1.5	3.9±1.7
Bank vegetation (%)	20.9±3.7	20.6±3.8	13.3±4.2	17.4±4.1
Average depth (cm)	37.6±3.1	34.8±2.8	31.9±2.7	28.8±2.0
Maximum depth (cm)	64.1±5.7	57.5±4.5	54.9±5.6	45.5±2.7
Area (m^2)	58.9±9.5	60.2±7.4	77.8±10.3	71.6±8.5
Volume (m^3)	22.7±4.4	20.2±2.8	26.1±5.7	20.9±2.9

The physico-chemical and habitat variables showed seasonal variation. Water temperatures was higher in summer, with averages of 23.6 and 23.1°C, than winter, with averages of 14.1 and 10.3°C at the Zuurberg and Winterberg sites, respectively (Table 4.2). The pH was neutral in summer (7.1) and alkaline in winter (8.0) in the Zuurberg, and alkaline in both summer and winter (8.1 and 8.4 respectively) in the Winterberg. Total dissolved solids (TDS) and turbidity were all higher in summer than winter in the Zuurberg. By comparison, the Winterberg had higher TDS in winter than summer, while turbidity was higher in summer than winter (Table 4.2). Conductivity was higher during winter than summer in both the Zuurberg and the Winterberg. Except for three pools that had no fish in summer in the Zuurberg, and four pools that were dry in winter in the Winterberg, all pools sampled were persistent in both summer and winter. Substrate type was generally variable among all sites in both the Zuurberg and the Winterberg (Table 4.2). Bank vegetation was present at most sites in both localities.

Pseudobarbus afer's estimated population significantly increased with increasing proportion of boulders and volume (Figure 4.3, Table 4.3). Boulders were also the best predictors for increasing density for *P. afer*. In comparison, *B. anoplus*'s estimated population increased with increasing proportion of bedrock while its density decreased with increasing proportion of silt (Figure 4.3, Table 4.3). Capture probability increased in relation to proportion of bank vegetation and average depth for *B. anoplus* (Figure 4.3, Table 4.3).

Table 4.3: Linear mixed-effects model coefficients for *Pseudobarbus afer* and *Barbus anoplus* relating to estimated number, density and capture probability in relation to several habitat variables.

			Estimate	SE	df	*t*	*p*
Pseudobarbus afer	Number	Boulder	0.63	0.17	16	3.69	0.00
		Volume	0.41	0.17	16	2.42	0.03
		Conductivity	-0.26	0.17	16	-1.52	0.15
	Density	Boulder	0.91	0.25	16	3.63	0.00
Barbus anoplus	Number	Bedrock	0.46	0.16	13	2.83	0.01
	Density	Silt	-0.38	0.17	13	-2.21	0.05
	Capture probability	Vegetation	0.43	0.17	13	2.47	0.03
		Average depth	0.38	0.17	13	2.19	0.05

Length frequencies showed distinct modal peaks for L5 length class for both species in summer, and L3 length class for *B. anoplus* and L5 length class for *P. afer* in winter (Figure 4.4). There was also a higher frequency of the small length class, L2, in summer than winter for both species. Mature *P. afer* were dominant in both summer and winter. By comparison, *B. anoplus* population was dominated by mature fish in summer and immature fish in winter.

The best predictive habitat variables for *P. afer* size structure were pH and temperature, and the seasonal variable, summer, that significantly (Monte Carlo permutations of both first axis and trace, $P < 0.01$) accounted for 13.2% of variation. The explained variation was partitioned as pure habitat $(H|S)$, 2.4%, pure seasonal $(S|H)$, 2.4%, shared/redundant variation $(H \cap S)$, 8.3%, and unexplained, 73.6% (Table 4.4).

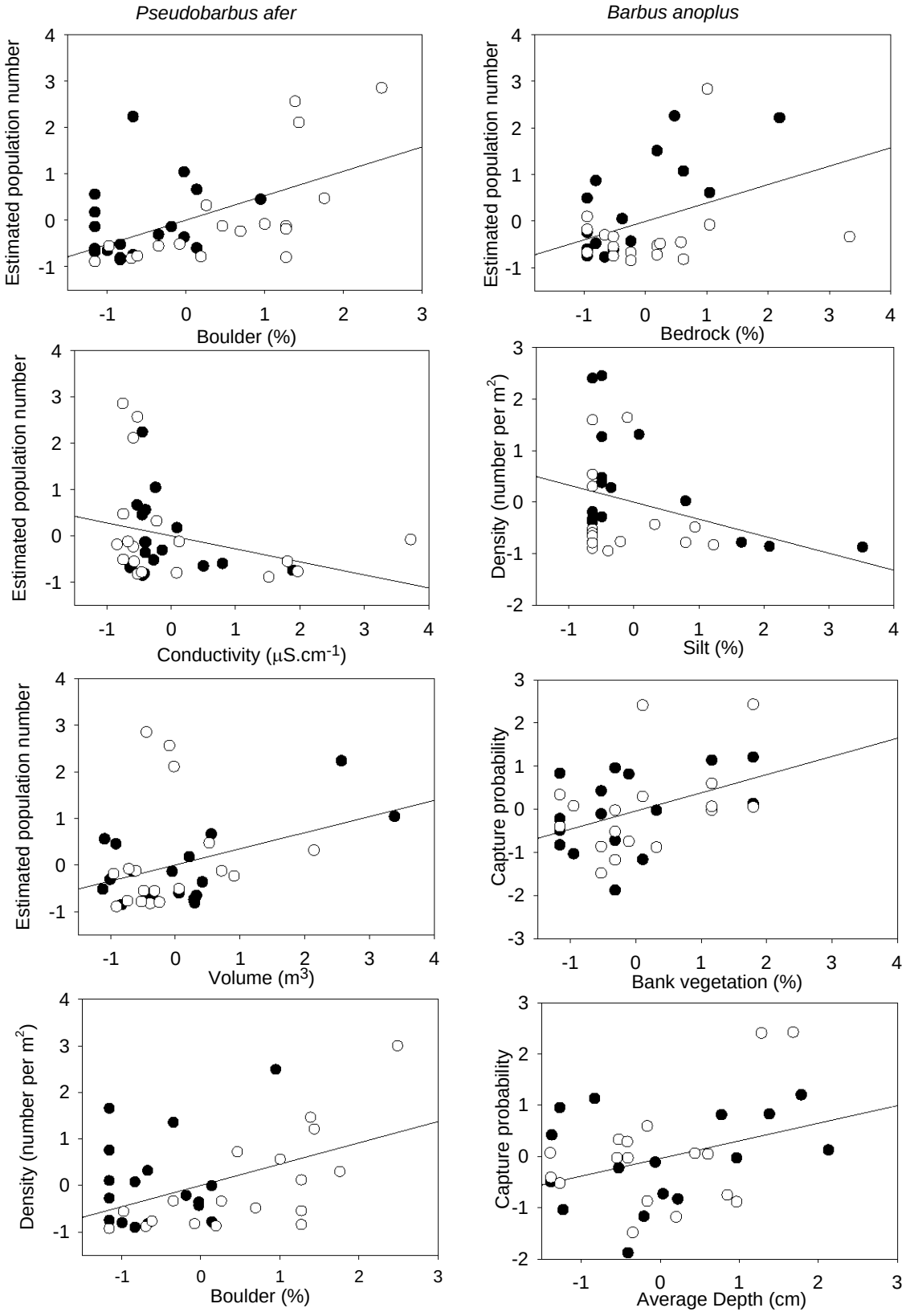

Figure 4.3: *Pseudobarbus afer* and *Barbus anoplus* population number, density and capture probability in relation to important habitat variables in summer (●) and winter (○).

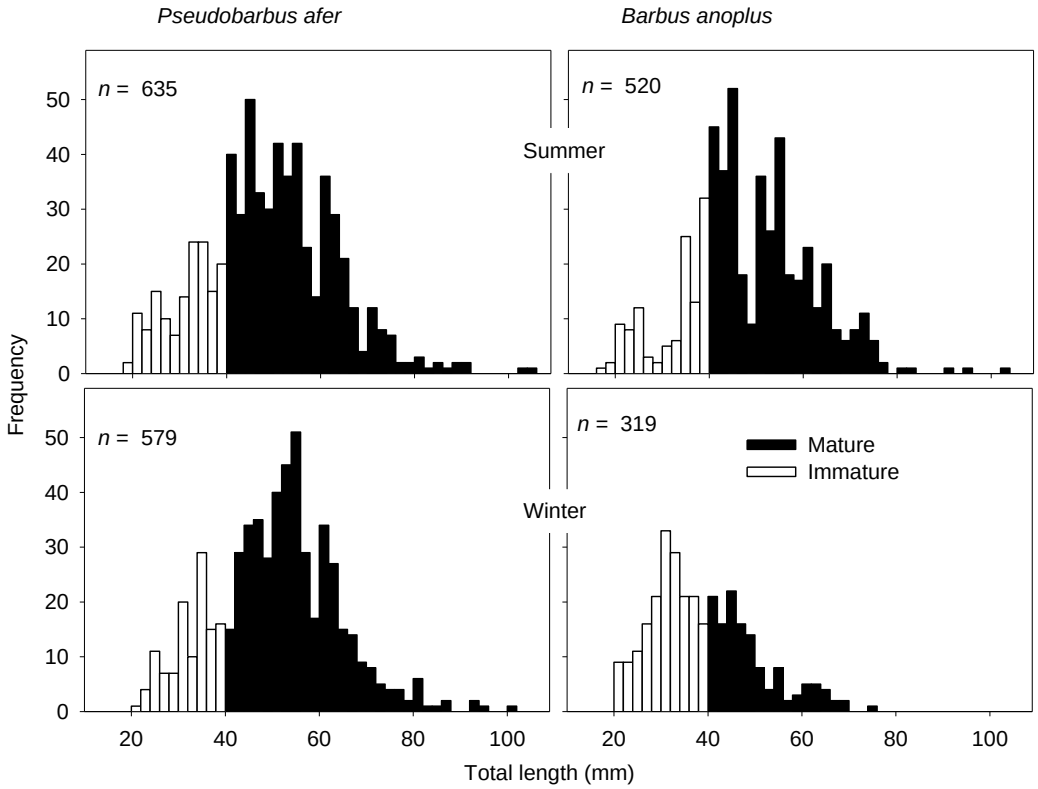

Figure 4.4: Length frequencies of immature and mature *Pseudobarbus afer* and *Barbus anoplus* sampled in the same pools during summer and winter.

Table 4.4: Summary statistics for Canonical Correspondence Analysis for *Pseudobarbus afer* and *Barbus anoplus* ordinations

	Pseudobarbus afer		*Barbus anoplus*	
	Axis 1	Axis 2	Axis 1	Axis 2
Eigenvalues	0.13	0.02	0.14	0.06
Species-environment correlations	0.62	0.28	0.59	0.53
Cumulative percentage variance				
of size class data	10.6	12.0	13.8	19.6
of size class-environment relationship	80.8	91.3	62.5	88.5
Correlations with axes				
pH	0.75	0.62		
Temperature	-0.94	-0.11		
Summer	-0.99	0.10	0.14	-0.37
Bank vegetation			-0.39	0.27
Bedrock			0.45	0.34
Total inertia		1.21		1.01
Sum of all canonical eigenvalues		0.16		0.22
Canonical eigenvalue $H\|S$		0.03		0.18
Canonical eigenvalue $S\|H$		0.03		0.04
Canonical eigenvalue $H \cap S$		0.10		0.00

The first CCA axis suggested an association of smaller length classes (L1, L2 and L3) with seasonal variable summer, and length class L7 with pH, while length classes L4, L5, L6, L8 and L9 appeared to be consistent throughout the sampling seasons (Figure 4.5). Size structure for *B. anoplus* was explained by habitat variables, bedrock and bank vegetation, and the seasonal variables, summer, that significantly (Monte Carlo permutations, $P < 0.05$) accounted for 21.8% of variation (Table 4.4). The explained variation was mostly pure habitat $(H|S)$, which significantly (Monte Carlo permutations, $P < 0.05$) explained 17.8% variation. Pure seasonal variation $(S|H)$ was insignificant (Monte Carlo permutation, $P > 0.05$), explaining only 4.4% of the variation, while no variation was shared between habitat and season $(H \cap S)$. A total of 56.4% of the variation was unexplained (Table 4.4). The first CCA axis showed the association of the length classes L3 and L4 with bank vegetation, and length classes L7 and L8 with bedrock (Figure 4.5). The second CCA axis was a seasonal gradient that indicated an association of the smallest length classes (L1 and L2) and the biggest length classes (L9 and L10) with the seasonal variable, summer (Figure 4.5).

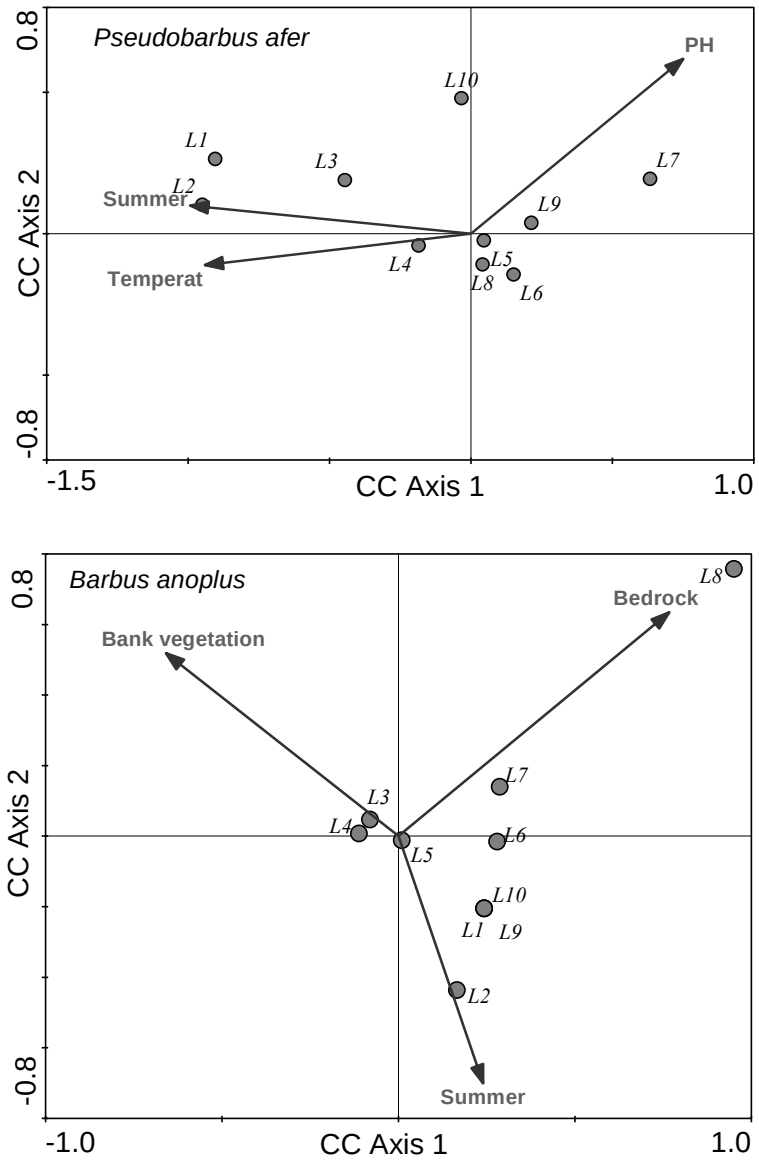

Figure 4.5: Canonical Correspondence Analysis ordination plots for *Pseudobarbus afer* and *Barbus anoplus* size classes. The size classes are L1 (<20mm TL), L2 (20 - 29mm TL), L3 (30 - 39mm TL), L4 (40 - 49mm TL), L5 (50 - 59mm TL), L6 (60 - 69mm TL), L7 (70 - 79mm TL), L8 (80 - 89mm TL), L9 (90 - 99mm TL) and L10 (>100mm TL).

Discussion

The habitats for both *P. afer* and *B. anoplus* were characterised by seasonal variability in all physico-chemical variables, especially temperature, conductivity and turbidity. Spatially, the habitats were heterogeneous with varying proportions of the different substrate types. While the estimated populations were variable between seasons, especially for *B. anoplus*, the

populations of the two species were relatively stable in their habitats with no differences in population parameter estimates for densities and capture probability between seasons. There was no evidence that per capita loss was dependent on summer abundance. This suggests that population dynamics were density-independent and largely driven by extrinsic factors. These factors could be inferred from the substantial seasonal differences in environmental variables, notably substrate in the Zuurberg and physicochemical variables in the Winterberg. During winter, percentage boulder increased in the Zuurberg whereas turbidity decreased substantially as the habitats receded into isolated pool compared to summer when there was high flow. The two minnow species were nonetheless able to persist and maintain stable populations between seasons in the headwater streams where they occur.

Pseudobarbus afer generally occur in relatively stable, clear mountain streams. The genus is, nevertheless, known to persist within unstable and changing environmental conditions, possibly owing to flexible life history patterns, physiological tolerances and both morphological and dietary plasticity (Skelton, 1988; Cambray, 1994). In this study, *P. afer* that were collected in pools that were previously fishless in summer suggested that this species could easily expand its range as habitats become interconnected from upstream founder populations. This opportunistic trait has been observed for many other small cyprinids that occur in headwater streams (Keaton *et al*., 2005). Similarly, *Barbus anoplus* is also known to adapt and persist in unstable environments, such as headwater streams, where it usually occurs as the only fish species (Cambray & Bruton, 1985). Overall, the density patterns documented in this study indicated stable populations of the two species within the headwater streams. This supports observations from other studies indicating that small cyprinids are persistent, and exhibit little seasonal variation in density in headwater streams that are characterised by high spatial and temporal variation in environmental conditions

(Butler & Fairchild, 2005). Such persistence is common in minnows that are habitat generalists (Erős & Grossman, 2005).

Substrate type appeared to be a major determinant for population persistence. The influence of boulders and bedrock on population size and density suggested the importance of large substrate types for the two species. Large substrates are known to provide refuge and result in greater habitat stability, which also produces abundant macroinvertebrates on which fish feed (Guenther & Spacie, 2006; Mueller & Pyron, 2010). It is likely that persistence of *P. afer* during environmental extremes would be mediated by boulder substrate as the most important refugia. By comparison, persistence of *B. anoplus* that was abundant in habitats with bedrock substrate would be mediated by bank vegetation. Bank vegetation would provide a refuge that not only reduces predation risk, especially from terrestrial predators such as birds, but provides food in the form of aquatic invertebrates that are associated with vegetation (Edo & Suzuki, 2003).

Seasonality was the major influence for *P. afer* size structure, shown especially by a high association of smaller length classes with summer. *Pseudobarbus afer* spawn in response to increasing water flow in summer, which is usually between October and November (Cambray, 1994). This suggests that size structuring observed in this study for *P. afer* was mainly recruitment driven. This pattern was reflected as a seasonal gradient of CCA, an observation that was also supported by length frequency analysis where there was a high frequency of small length class, L2, in summer and a gradual shift in length with season. Large length classes, on the other hand, showed high association with pH, which was relatively high in winter suggesting that these were the over-wintering length classes. By comparison, seasonality appeared to have less influence on size structuring for *B. anoplus*. Instead, habitat variables, bedrock and bank vegetation, were the major influence for *B. anoplus* size structure, suggesting the importance of physical habitat and possibly refugia.

Refugia, in the form of bank vegetation, appeared to influence the over-wintering size classes, L3 and L4 for this species. The differences in the importance of seasonality for the two minnow species could be explained by their different spawning strategies. Unlike *P. afer*, *B. anoplus* is known to be iteroparous, with a primary spawning period between November and January and a secondary spawning period between February and March (Cambray & Bruton, 1985). The significance of this strategy, which may have evolved as an adaptation to unstable environments, is to stagger the breeding period to increase net survival probability (Cambray & Bruton, 1985). The first-spawned fish allocate resources towards growth and survival and participate in reproduction in their second summer, whereas the second-spawned fish invest in growth and survival throughout the first spawning season and participate in reproduction in the second spawning season (Cambray & Bruton, 1985). This would suggest that this species was less likely to exhibit seasonal patterns in size structuring as reflected by the occurrence of small length classes throughout the sampling period.

The lack of a relationship between dominant substrates and size structuring was a possible indication of lack of habitat specialisation for the different size classes for both species. This, as hypothesised by Angermeier & Schlosser (1989), may be a consequence of an imbalance between fish and their habitats due to large environmental variation, as they are habitat generalists. Such a strategy would enable species to maintain stable populations as habitat generalists in response to an unstable environment, with refuge availability as the critical factor. Recruitment appeared to be the major driver in size structuring, with the two species, however, having different spawning strategies that are well-studied (e.g. Cambray & Bruton, 1985; Cambray, 1994), and reflected as seasonal gradients in this study. Refugia, especially in the form of boulders, appeared to be important for *P. afer*, while bank vegetation was important for *B. anoplus*'s over-wintering size classes.

To conclude, both *P. afer* and *B. anoplus* are well adapted to the headwater streams that were generally variable in environmental conditions. Additional stressors, especially in the form of alien invasive fishes, may have more confounding effects that would affect the minnows in these habitats. Efforts should be made to protect these minnows especially the endangered *P. afer*, by enforcing the recommendations from previous studies (e.g. Weyl *et al*., 2009b), such as constructing barriers to upstream migration of alien invasive species such as sharptooth catfish *Clarias gariepinus* and largemouth bass *Micropterus salmoides*.

CHAPTER 5

Differential Trophic Niche Responses of an Invasive Non-native Generalist Predator within Invaded Lotic and Lentic Habitats

Introduction

Food webs are a central ecological concept that describes feeding relationships and community trophic structure. Determining food web structure requires a knowledge of the feeding habits and diets of the consumer community. As stable isotopes, especially the isotopic ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$), provide a temporally-integrated measure of a consumer's assimilated diet (Post, 2002; Vizzini *et al*., 2002), they are important in elucidating energy flows and trophic interrelationships (Hobson & Welch, 1992; Vander Zanden *et al*., 1997) because a consumer's stable isotope signatures reflect the isotopic signatures of its prey (DeNiro & Epstein, 1981; Minagawa & Wada, 1984; Hobson, 1993; Cabana & Rasmussen, 1996; Vander Zanden & Rasmussen, 1999; Post, 2002). Stable isotopic ratios can therefore not only determine the energy sources (DeNiro & Epstein, 1978; 1981), but can also estimate trophic positions (Vander Zanden *et al*., 1997; Vander Zanden & Rasmussen, 2001; Anderson & Cabana, 2007) and infer trophic niches (Bearhop *et al*., 2004) of consumers within a food web.

The concept of a trophic niche has gained recent prominence in understanding trophic interrelationships (Layman *et al*., 2007; Newsome *et al*., 2007; Olsson *et al*., 2009). A trophic niche, which is often inferred based on dietary diversity, conceptualises resource use *vis-à-vis* food availability, both on spatial and temporal scale, and any other biotic interactions such as competition and predation (Winemiller *et al*., 2001; Bearhop *et al*., 2004). The trophic niche therefore represents the trophic role of a consumer (Leibold, 1995). Since carbon and nitrogen isotopes vary both spatially and temporally they can be used to assess the trophic

niches of individual populations and communities across different habitats. Several studies have demonstrated the importance of incorporating stable isotope-based trophic niches in ecological studies, which have identified, among other studies, resource partitioning and niche widths of co-occurring species (Flaherty & Ben-David, 2010), niche shifts following invasion (Vander Zanden *et al*., 1999), changes in trophic position following changes in prey availability (Becker & Beissinger, 2006), and both spatial variation in resource use and ontogenetic shift in diets within individual populations (Post, 2003; del Rio *et al*., 2009).

Traditionally, stable isotopes have been used to infer feeding relationships and trophic positions. Most food webs are, however, complex as they include diverse prey sources such as in systems that involve generalist predators (Moreno *et al*., 2010) and in structured habitats where consumers have a wide prey spectrum (McClellan *et al*., 2010). Recent research advocates the use of multi-source isotopic mixing models to estimate and quantify the most probable prey sources for a consumer (Phillips, 2001; Hall-Aspland *et al*., 2005; Inger & Bearhop, 2008; Parnell *et al*., 2010). These mixing models all assume that the consumer's isotopic signature is composed of a weighted mix that reflects the relative contributions of different prey signatures (Phillips, 2001). Consumer isotopic ratios would, therefore, be determined from its prey sources by a set of linear equations that satisfy mass balance if the enrichment rate per trophic level can be determined (Phillips & Gregg, 2001). Mixing models have since evolved from simplified algebraic mass-balance linear equations (Phillips, 2001; Phillips & Gregg, 2001; Phillips & Koch, 2002; Phillips *et al*., 2005) to the use of more complex contemporary Bayesian analyses that can incorporate prior information pertaining to fractionation rates (Jackson *et al*., 2009; Parnell *et al*., 2010), and their associated variability (Moore & Semmens, 2008; Ward *et al*., 2010; Jackson *et al*., 2011). These approaches are critical in providing both qualitative and quantitative information about the relative contributions of different food sources in food webs.

Quantitative knowledge about food web structure has important practical applications in the assessment of the impact of introduced species. Invasion success has been reported to be variable with some invasion events having adverse negative impacts (Vander Zanden *et al*., 2004) whereas other invasive species appear to integrate well into their recipient systems with little or no impact (Tablado *et al*., 2009). In aquatic ecosystems, the major concern is the invasion by non-native fish that have many negative impacts ranging from local extirpation of native species to the alteration of ecosystem processes (Flecker & Townsend, 1994). In particular, invasive non-native fish, especially predators, have been associated with top-down effects and trophic cascading through eliminating of certain prey groups or through competition with sympatric native predators (Nyström & McIntosh, 2003; Phillips *et al*., 2009).

This study focuses on the impact of a non-native sharptooth catfish from a food web perspective. Due to its generalist predatory feeding habits, it poses a potential threat to the depauperate native ichthyofauna. The impact of the catfish may also be related to variation in its food sources. A generalised consumer could have a high probability of invasion success because food availability is unlikely to be limiting. On the other hand, impacts are likely to be influenced by resource availability that would differ among localities within the upstream-downstream stream continuum (Vannote *et al*., 1980), and between different hydrological environments (lotic vs lentic). A common feature for invasive generalist predators appears to be their broad trophic niche with potential to affect a large number of species in the invaded community (Olsson *et al*., 2009). Studies on sharptooth catfish introduced elsewhere indicate that it is an indiscriminant and competitive predator that can alter the food webs of the invaded community (Khan & Pannikar, 2009; Radhakrishan *et al*., 2011).

The primary objectives of this study were to compare the spatial variability in community-wide and catfish-specific trophic niches within different invaded localities, and to

estimate prey sources for the invasive catfish within individual communities. Comparison of isotopic niches was based on a framework of stable isotope niche metrics (Layman *et al*., 2007; Turner *et al*., 2010; Jackson *et al*., 2011), and estimations of catfish prey sources relied on isotope mixing models (Parnell *et al*., 2010). It was hypothesised that the communities would exhibit differences in trophic niche sizes within the longitudinal series of lotic environments (upstream versus downstream) and between hydrological environments (lotic versus lentic). It was hypothesised that due to its generalised feeding, the catfish's trophic niche would expand with a more diverse resource spectrum in downstream reaches.

Materials and methods

Sample collection and preparation

Samples of sharptooth catfish and its potential prey were collected at 11 sites from the Great Fish and Sundays Rivers and three sites from Glen Melville Dam. Sampling for stable isotopes was conducted monthly in summer from October 2009 to April 2010. Fish were captured using gill nets and fyke nets in the Great Fish and Sundays Rivers, and longlines in the Glen Melville Dam, a reservoir connected to the Great Fish River via a secondary IBWT scheme. Use of experimental gill nets and fyke nets was described in Chapter 3. Longlines were constructed of polyethylene rope with 1 m nylon snoods and circle hooks. Hooks were baited with chicken livers. The sampling gear was set overnight (1600h - 0700h). Captured fish were measured to the nearest 1 mm, euthanised by pithing, and dissected. Samples of muscle tissue were collected from fish for stable isotope analysis.

Aquatic macroinvertebrates were sampled using a hand-held scoop net. Benthic macroinvertebrates were sampled by disturbing 1 m^2 of the substratum and collecting the dislodged animals with a hand net. Additional benthic samples were collected by selecting

pebbles and washing them in the hand net. At vegetated localities, macroinvertebrates were sampled by repeatedly sweeping a hand net over the vegetation. Epilithic algae were scrapped from substrates using a scalpel blade and washed. Filamentous algae, macrophytes and other visible organic matter were handpicked. Individual macroinvertebrates were identified to family level and separated in the field. Coarse particulate organic matter (CPOM) was collected by disturbing substratum within an area of 1 m^2 and washing the disloged organic matter into a hand net. All samples were kept in ice in the field.

In the laboratory samples were thawed and dried in an oven to a constant weight at 60°C before being ground into a fine powder. Dry sample material was weighed (1 ± 0.05 mg for animal tissue and 3 ± 0.5 mg for plant tissue) and packed into 8 × 5 mm tin capsules. Samples were analysed using a Europa Scientific INTEGRA isotope ratio mass spectrometer at the IsoEnvironmental Lab, Grahamstown, South Africa. Stable isotope ratios, $\delta^{13}C$ and $\delta^{15}N$, were determined in parts per thousand (‰) relative to Vienna Pee Dee Belemnite and atmospheric nitrogen standards, respectively, according to the formula: $\delta^{13}C$ or $\delta^{15}N = (R_{sample}/R_{standard}-1)\times 1000$, where $R= {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$.

Spatial variability in community and catfish isotopic values

Sampling localities from each river were separated into upper and lower sections based on the dominance of primary freshwater and estuarine fish. Glen Melville Dam, which represented the only lentic environment within the study area, was considered separately. It receives water from the middle section of the Great Fish River. Hence, there were five localities for comparison (Figure 5.1). Within each locality, community data were defined by the $\delta^{13}C$ and $\delta^{15}N$ isotope values of samples, including basal sources of primary production and coarse particulate organic matter (CPOM), diverse macroinvertebrate taxa, and all fish species.

Community structure and niche width

Trophic niche structure were categorised for the five sampled localities at both the community-wide and catfish-specific levels using several metrics proposed by Layman *et al*. (2007) and Jackson *et al*. (2011). The community-wide isotopic niche was based on all potential prey for the catfish that included macroinvertebrates, fish, plants and allochthonous material. Basal source groups of primary production and allochthonous matter were included because recent studies have recognised the importance of these groups in interpreting food web structure (Schmidt *et al*., 2007; Hoeinghaus & Zeug, 2008) and that basal food sources are components of the catfish's diet (Potts *et al*., 2008; Kadye & Booth, 2011). An estimate of each of the metric's variability was obtained by non-parameteric resampling of the observed $\delta^{13}C$ - $\delta^{15}N$ data 250 times with replacement (Efron & Tibshirani, 1993). The metrics used were:

1) $\delta^{15}N$ range (*NR*) - the distance between the most depleted and the most enriched $\delta^{15}N$ values. *NR* provides information about the vertical structure within a food web.
2) $\delta^{13}C$ range (*CR*) - the distance between the most depleted and the most enriched $\delta^{13}C$ values. *CR* provides an estimate of the diversity of basal resources.
3) Small sample size-corrected standard ellipse area (SEA_c) – a robust measure of the the $\delta^{13}C$- $\delta^{15}N$ biplot space that provides a measure of the niche space occupied and food web trophic diversity.
4) Mean of the centroid (*CD*) - the average Euclidean distance of each point to the $\delta^{13}C$-$\delta^{15}N$ centroid, where the centroid in the mean $\delta^{13}C$ and $\delta^{15}N$ for all the points in the food web. *CD* provides additional information about niche width and diversity but also the degree of spacing within the food web.

5) Mean nearest neighbour distance (*MNND*) - the mean of Euclidean distances to each point's nearest neighbour in biplot space and provides a measure of density and clustering within a foodweb.

6) Standard deviation of nearest neighbour (*SDNND*) - that provides a measure of evenness of special density and packing.

Turner *et al*.'s (2010) residual permutation procedure and Hotelling's T^2 test were used to test the null hypotheses that the community-wide or catfish-specific *CD*, *MNND*, distance between two centroid (*D*), path length and path direction ($\theta°$) metrics were equal between sampling localities. For example, it is hypothesised that if the centroids occupied similar positions then their contrasts would not be significantly greater than zero. Path length is defined by cumulative Euclidean distances across centroids whereas path direction is defined by the orientation of scatter, and is based on the first principal component (PC1) of individual samples. Path length is an additional measure of dispersion within individual communities or populations, whereas path direction compares uniformity in the direction of the dispersion between communities and populations (Schmidt *et al*., 2007; Turner *et al*., 2010). Prior to all analyses, outliers were identified using Mahalanobis Distance-based criteria and removed from the data, and Box's M test was used to test the assumption that the variance-covariance matrices were homoscedastic.

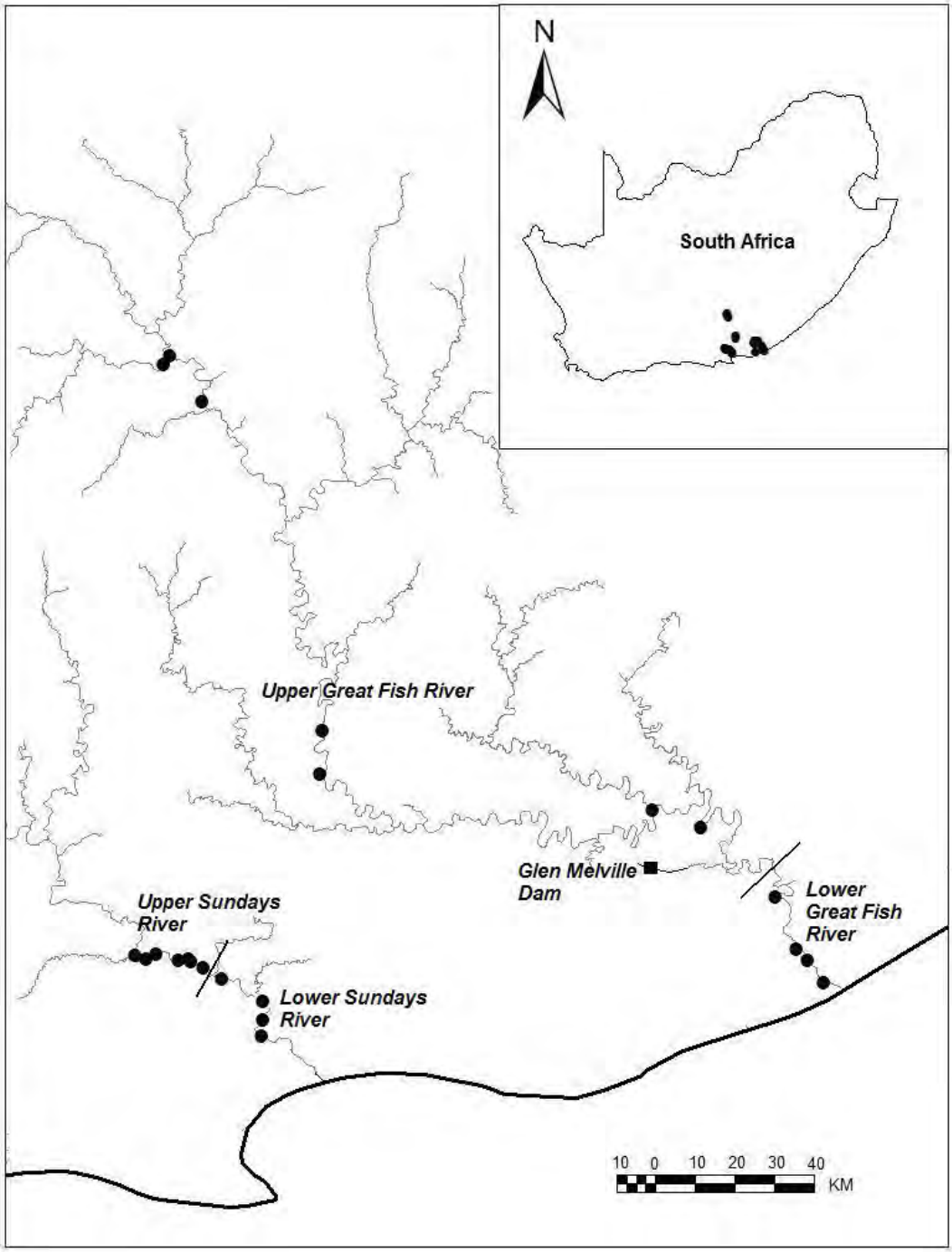

Figure 5.1: Map of the study area of the Eastern Cape, South Africa, showing sites of fish collections and localities that were compared within the Sundays River, Great Fish River and Glen Melville Dam, South Africa.

Isotope mixing model

Stable isotope mixing models require that prey sources are distinct from each other. The k - nearest neighbour distance (KNND) randomisation procedure has been proposed to statistically distinguish different prey sources (Rosing *et al*., 1998; Lubertkin & Simenstad, 2004). Prey sources that fail to meet this criterion should be pooled to reduce trophic redundancy. The KNND statistic was used to discern the different trophic groups within the different localities. Initially, all prey sources were classified into trophic guilds (Tables 5.1 & 5.2) before the KNND randomisation procedure applied to test for statistical differences within and among the trophic guilds in each locality.

A Bayesian isotopic mixing model, SIAR (Stable Isotope Analysis in R, Parnell *et al*., 2010), was used to determine proportional contributions of different prey groups as food sources to the catfish. The SIAR model uses a Markov Chain Monte Carlo simulation using a Dirichlet distribution to produce simulations of plausible values of dietary proportions of sources based on the consumer and potential prey item data. The resulting probability density distributions, with the mean proportion together with the 25, 75 and 95 % credibility intervals (the Bayesian analogue of a confidence interval), were used to compare contributions of different source groups.

All analyses were conducted in *R* (R Core Development Team, 2011). Screening for outliers was done using the library *mvoutlier*. The library *siar v4.1.1* was used for the Layman metrics, ellipses and the isotope mixing model.

Table 5.1: Mean values of carbon and nitrogen stable isotopes (± standard deviation) for fish collected in the Great Fish River, Sunday River and Glen Melville Dam in the Eastern Cape, South Africa. The categories of the trophic guilds were based on Skelton (2001) for freshwater fish, and Whitfield (1998) for estuarine fishes.

	Upper Great Fish River			Lower Great Fish River			Glen Melville Dam			Upper Sundays River			Lower Sundays River			Trophic guild
Species	N	$\delta^{13}C$‰	$\delta^{15}N$‰	N	$\delta^{13}C$‰	$\delta^{15}N$‰	N	$\delta^{13}C$‰	$\delta^{15}N$‰	N	$\delta^{13}C$‰	$\delta^{15}N$‰	N	$\delta^{13}C$‰	$\delta^{15}N$‰	
Anguilla marmorata				5	-23.4±0.3	18.4±0.4							1	-23.3±0.0	18.7±0.0	predator
Anguilla mossambica	3	-25.4±0.9	14.5±0.3	1	-27.5±0.0	20.9±0.0				4	-29.5±1.2	18.8±1.1	1	-27.4±0.0	22.4±0.0	predator
Clarias gariepinus	9	-23.1±0.7	13.7±0.8	20	-26.8±2.0	16.9±0.9	10	-26.4±0.4	18.9±0.5	5	-26.0±2.7	17.7±0.6	19	-27.9±1.3	18.6±2.1	predator
Cyprinus carpio	1	-22.7±0.0	13.8±0.0	3	-24.9±1.4	16.3±1.0	7	-24.6±0.8	16.7±0.5	3	-29.9±1.7	16.6±0.2	2	-30.1±1.5	19.5±0.4	benthivore
Gambusia affinis				5	-23.3±0.2	15.2±1.0										invertivore
Barbus pallidus										7	-28.3±1.3	18.1±1.1	10	-29.3±0.7	18.8±0.8	invertivore
Gilchristella aestuaria				9	-30.0±0.5	18.3±0.4				1	-30.8±0.0	17.0±0.0	1	-29.8±0.0	18.5±0.0	planktivore
Glossogobius callidus				8	-27.7±2.3	15.8±1.8				5	-29.5±0.5	18.0±0.1	7	-28.4±1.5	18.6±0.5	invertivore
Labeobarbus aeneus	36	-25.1±1.2	13.3±0.9	11	-28.0±2.5	14.9±1.0	10	-24.7±0.8	18.2±0.5							benthivore
Labeo umbratus	11	-28.2±1.9	11.6±0.6	15	-29.1±2.0	15.6±1.8	10	-26.8±1.2	15.8±1.1							benthivore
Labeo capensis	1	-26.7±0.0	12.9±0.0	3	-27.0±1.1	13.2±0.4										benthivore
Liza macrolepis				1	-31.6±0.0	17.9±0.0										benthivore
Monodactylus falciformis				4	-26.8±3.4	18.2±1.5							5	-30.6±1.8	19.7±0.9	invertivore
Mugil cephalus				3	-31.7±1.3	16.9±1.4							1	-34.0±0.0	15.6±0.0	benthivore
Myxus capensis				10	-26.2±3.2	16.7±0.6							12	-30.1±2.3	18.0±0.9	benthivore
Pomadasys commersonnii				1	-23.1±0.0	16.7±0.0										invertivore
Psammogobious knaysnaensis				3	-23.4±0.5	16.6±0.2										invertivore
Redigobius dewaali				3	-24.6±0.4	16.7±0.3										invertivore
Rhabdosargus holubi				5	-23.8±2.0	17.2±1.0							1	-28.9±0.0	16.1±0.0	herbivore
Tilapia sparrmanii				6	-27.8±1.3	15.0±0.9				5	-29.8±0.8	15.8±1.1	7	-29.0±1.2	16.6±1.7	herbivore
Oreochromis mossambicus				1	-28.5±0.0	16.8±0.0				6	-29.3±1.7	15.5±1.0	5	-27.2±3.1	16.8±1.1	herbivore

Table 5.2: Mean values of carbon and nitrogen stable isotopes (± standard deviation) for macroinvertebrates, basal sources and other prey collected in the Great Fish River, Sundays River and Glen Melville Dam in the Eastern Cape, South Africa. The categories of the macroinvertebrate trophic guilds was based on Cummins *et al*. (2005).

	Upper Great Fish River		Lower Great Fish River		Glen Melville Dam		Upper Sundays River		Lower Sundays River		Trophic guild
Taxa	$\delta^{13}C‰$	$\delta^{15}N‰$	$\delta^{13}C‰$	$\delta^{15}N‰$	$\delta^{13}C‰$	$\delta^{15}N‰$	$\delta^{13}C‰$	$\delta^{15}N‰$	$\delta^{13}C‰$	$\delta^{15}N‰$	
Baetidae	-28.0±3.4	9.9±0.8	-29.5±1.7	10.4±2.6			-28.1±0.0	14.0±0.0	-30.6±1.0	14.7±0.4	Scraper/collector
Leptophlebiidae	-24.1±0.0	8.6±0.0									Scraper/collector
Belostomatidae	-26.6±0.6	9.4±2.2	-28.1±0.8	13.1±0.1	-28.2±0.0	13.7±0.0					Predator
Coenagrionidae	-28.2±1.8	11.0±0.7	-28.7±2.0	13.6±0.7	-21.4±5.5	13.8±1.1	-28.8±0.4	15.1±1.3	-31.3±0.8	15.8±1.3	Predator
Libellulidae	-27.8±0.0	10.8±0.0	-27.4±0.0	13.2±0.0							Predator
Aeshnidae	-27.6±0.0	10.3±0.0	-28.1±0.0	12.2±0.0			-27.5±0.0	15.3±0.0	-31.0±0.0	15.8±0.0	Predator
Corixidae	-25.4±2.9	9.4±0.5	-24.8±7.2	9.9±1.3	-27.8±0.0	12.6±0.0	-28.7±0.0	13.4±0.0			Herbivore
Planaria									-32.7±0.0	16.3±0.0	Collector
Dytiscidae			-26.3±0.0	9.6±0.0							Predator
Hirudinea			-29.0±0.0	13.9±0.0	-23.3±0.0	18.5±0.0					Predator/collector
Hydropsychidae	-23.9±0.0	10.4±0.0	-27.8±0.0	14.7±0.0					-31.7±0.9	16.7±1.7	Collector/predator
Ancylidae			-14.4±0.0	13.0±0.0							Scraper/grazer
Lymnaeidae			-19.8±0.0	13.0±0.0			-22.1±0.0	13.1±0.0			Scraper/grazer
Notononectidae			-29.3±0.0	12.1±0.0							Predator
Pleidae			-25.8±1.4	10.8±0.0							Predator
Gerridae					-23.5±0.0	14.4±0.0					Predator
Muscidae	-27.0±0.0	11.1±0.0									Collector/filter
Simuliidae	-23.2±0.0	9.0±0.0							-31.4±3.6	12.5±2.8	Collector/filter
Chironomidae					-31.9±0.0	11.8±0.0					Collector
Crab	-24.2±0.0	10.3±0.0	-26.7±0.0	15.5±0.0							Collector/detritivore
Shrimp			-28.2±0.6	17.0±1.5					-24.6±0.4	16.7±1.0	Collector/filter
Zooplankton					-31.2±0.8	13.2±1.1					Planktivore
Tadpole	-26.7±0.0	10.0±0.0			-24.5±0.0	14.3±0.0					Herbivore
Algae	-41.3±0.0	9.2±0.0	-29.5±6.1	11.1±3.3	-33.7±0.1	10.0±0.5	-16.1±0.0	13.4±0.0	-33.3±4.2	12.8±4.2	Primary producer
Macrophytes	-27.5±0.0	10.6±0.0	-18.0±3.6	11.0±3.4			-23.6±0.0	11.3±0.0	-24.1±0.6	14.6±2.5	Primary producer
Periphyton	-23.8±0.2	10.2±1.3	-22.0±0.0	13.9±0.0	-19.1±0.0	8.9±0.0					Primary producer
Allochthnous matter	-26.9±0.0	4.8±0.0	-26.9±0.0	4.8±0.0	-26.1±3.5	10.9±3.4					Secondary producer
Phytoplankton					-25.1±0.3	9.7±1.1					Primary producer

Results

Spatial variation in community and catfish stable isotope data

Comparison of the lotic habitats showed that the lower sections has high species richness, with total fish species counts of 20 and 13, than the upper sections, with total species counts of six and eight, respectively, for the Great Fish and Sundays Rivers. Within the Great Fish River, the $\delta^{13}C$ of the fishes ranged from -28.2‰ to -22.7‰ in the upper section, generally higher than fishes sampled in the lower section that ranged from -31.7‰ to -23.1‰ (Table 5.1). Similarly, within the Sundays River, the fishes sampled in the upper section had higher $\delta^{13}C$ that ranged from -30.8‰ to -26.0‰ compared to the lower section that ranged from -34.0‰ to -27.2‰ for all species except *Anguilla marmorata* that had the highest $\delta^{13}C$ of -23.3‰. This showed that the upper sections of both rivers were more enriched in $\delta^{13}C$ compared to the lower sections. In contrast to $\delta^{13}C$, $\delta^{15}N$ within the Great Fish River was more depleted in the upper section, ranging from 11.6‰ to 14.5‰, compared to the fishes sampled in the lower section that ranged from 13.2‰ to 20.9‰. A similar pattern was observed within the Sundays River where the fishes in the upper section were more depleted in $\delta^{15}N$ isotope values, ranging from 15.5‰ to 18.8‰, compared to the fishes collected in the lower section that ranged from 15.6‰ to 22.4‰. Comparisons of the catfish $\delta^{13}C$ showed enrichment within the upper sections with a maximum of -23.1‰ (Table 5.1). By contrast, catfish $\delta^{15}N$ was enriched within the lower sections with a maximum of 18.6‰. Within all localities, catfish $\delta^{15}N$ values were lower than those of the anguilid eels, *A. marmorata* and *A. mossambica*, which were the native apex predators.

Macroinvertebrate $\delta^{13}C$ was variable within the lower sections where they ranged by 15.1‰ and 8.1‰ in the Great Fish and Sundays Rivers, compared to the upper sections that ranged 5.1‰ and 6.8‰, respectively, (Table 5.2). A similar pattern was observed for $\delta^{15}N$

within the Great Fish and Sundays Rivers that ranged by 7.5‰ and 4.2‰ in the lower sections compared to the upper sections of 2.5‰ and 2.2‰, respectively. Basal food sources comprised of the primary producers algae (filamentous), periphyton (epilithic) and macrophytes, and allochthnous coarse particulate organic matter (CPOM). Among the primary producers, algae was the most depleted in $\delta^{13}C$ within all localities, except the Upper Sundays River, with a minimum of -41.3‰ being observed in the Upper Great Fish River. Periphyton was the most enriched with a maximum of -22.0‰ being observed in the Lower Great Fish River. Periphyton and macrophytes were most enriched in $\delta^{15}N$, with maximum values of 13.9‰ and 14.6‰, within the Great Fish and Sundays Rivers, respectively.

In comparison with the lotic habitats, Glen Melville Dam was characterised by the lowest total fish richness of four species. The isotope values for the fishes had smaller ranges for both $\delta^{13}C$ (-26.8‰ to -24.6‰) and $\delta^{15}N$ (15.8‰ to 18.9‰) compared to the rivers. Macroinvertebrate isotopic range was 10.6‰ and 6.7‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively, and were generally comparable to those within the Great Fish River. Zooplankton constituted an important primary consumer group in addition to the macroinvertebrates. The basal food sources were characterised by presence of phytoplankton in addition to algae, periphyton and CPOM. Macrophytes were absent within the dam. Catfish was the most enriched fish in $\delta^{15}N$ (18.9‰) indicating that it was the apex fish predator.

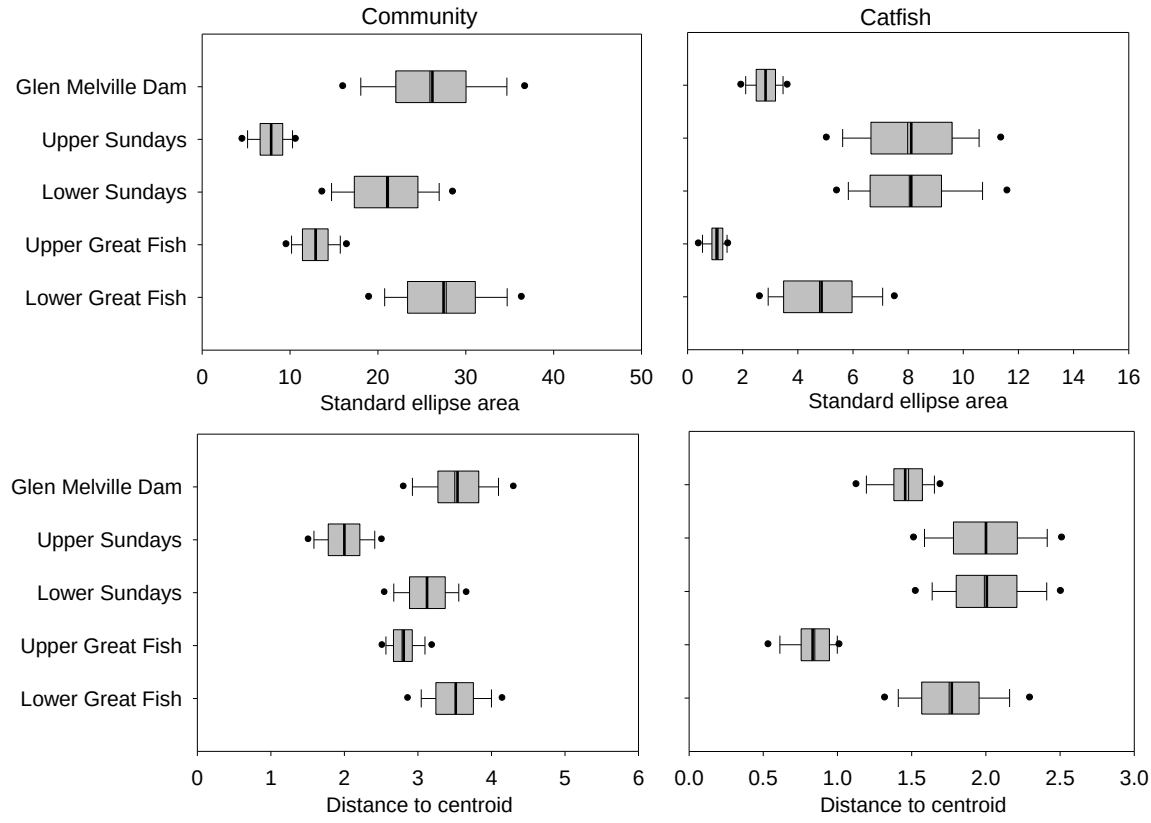

Figure 5.2: Box plots for non-parametric bootstrapped standard ellipse area (SEA_c) and centroid distance (*CD*) for the community and sharptooth catfish, *Clarias gariepinus*, populations collected within different localities of Great Fish River, Sundays River and Glen Melville Dam in the Eastern Cape, South Africa. The vertical solid line within each boxplot indicates the mean.

Spatial variation in isotopic niches

Within the Great Fish River, the community's corrected standard ellipse area (SEA_c), which represented total isotopic niche width, was small in the upper section (12.9‰^{2}) than the lower section (27.5‰^{2}) (Figure 5.2) indicating higher trophic diversity within the lower section compared to the upper section. Trophic diversity corresponded to a wider trophic niche downstream (*NR* = 12.7‰) compared to upstream (*NR* = 8.4‰). Similarly, the *CR* showed a wider breath of carbon sources within the lower section (15.4‰) compared to the upper section (6.0‰). Although the *CD* and *MNND* indicated that, within the upper section, community members were less dispersed and more densely packed than in the lower section

(Figure 5.2 and Figure 5.3), both metrics did not significantly differ (RPP, $P > 0.05$) for contrasts of the communities within the upper and lower sections (Table 4). This showed that the degree of spacing among individuals and clustering within community was similar between the two sections. Further, neither path lengths nor path directions were significantly different for contrasts between upper and lower Great Fish River communities. Euclidean distance between centriods (*D*) however differed significantly from zero for contrasts between communities in the upper and lower section (RPP, $P < 0.01$, Hotelling's T^2, $P < 0.01$) (Table 5.3), indicating that the two communities were ecologically distinct in their overall isotopic composition. These differences were defined by a $\delta^{15}N$ enriched and $\delta^{13}C$ depleted downstream food web compared to that upstream. The patterns in catfish populations mirrored that of the community, with a smaller isotopic niche within the upper section ($SEA_c = 1.3‰^2$) compared to the lower section ($SEA_c = 5.6‰^2$). Likewise, catfish had smaller trophic niche (*NR* = 1.8‰) and carbon breath (*CR* = 1.7‰) in the upper section compared to the lower section that had 2.6‰ and 6.8‰, respectively (Figure 5.3). These data revealed that the catfish population in the lower section had a large trophic range and wider carbon sources compared to the upper section. Individual clustering, based on *CD* showed more spread of the individuals within the lower than the upper section (Figure 5.2), and significantly differed ($P = 0.02$) between the two localities although *MNND* among individuals was not significantly different (RPP, $P > 0.05$) (Table 5.3). The Euclidean distance between centroid locations was significantly different (RPP, $P < 0.01$, Hotelling's T^2, $P < 0.01$) for contrasts of catfish in the upper and lower Great Fish River. Within the Great Fish River, neither the path directions nor path angles for the catfish were significantly greater than zero ($P > 0.05$) for contrasts between the upper and lower sections.

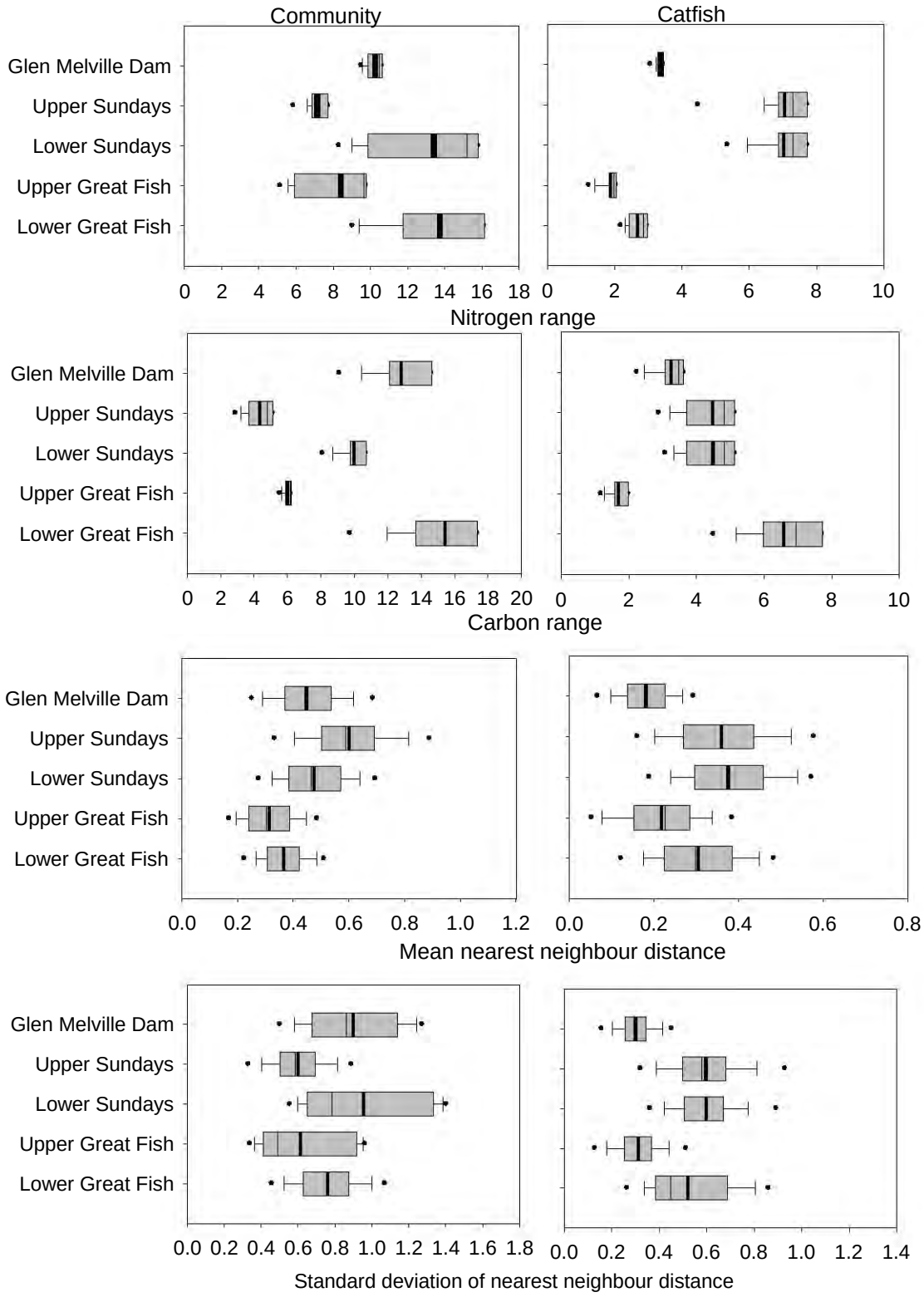

Figure 5.3: Box plots for non-parametric bootstrapped Layman metrics on the community and sharptooth catfish, *Clarias gariepinus*, populations collected within different localities of Great Fish River, Sundays River and Glen Melville Dam in the Eastern Cape, South Africa. The vertical solid line within each boxplot indicates the mean.

Table 5.3: Contrasts for pairwise comparisons of isotopic niche metrics based on the residual permutation procedure (RPP) and multivariate parametric Hotelling's T^2 test for the communities and sharptooth catfish, *Clarias gariepinus*, populations within different localities within the Eastern Cape, South Africa. *CD* = Centroid Distance, *MNND* = Mean Nearest Neighbour Distance, *ECC* = Eccentricity, *D* = Euclidean distance between centroid locations.

	Community					Catfish				
	CD RPP	*MNND* RPP	*ECC* RPP	*D* RPP	Hotelling's T^2	*CD* RPP	*MNND* RPP	*ECC* RPP	*D* RPP	Hotelling's T^2
					Upper Great Fish River vs Lower Great Fish River					
Distance	0.66	0.10	0.44	2.02	39.67	0.93	0.24	0.01	20.32	126.14
P	0.18	0.86	0.02	<0.01	<0.01	0.02	0.45	0.98	<0.01	<0.01
					Upper Great Fish River vs Glen Melville Dam					
Distance	0.70	0.25	0.31	2.52	39.50	0.62	0.08	0.02	15.00	88.25
P	0.17	0.35	0.34	<0.01	<0.01	0.03	0.77	0.86	<0.01	<0.01
					Upper Great Fish River vs upper Sundays River					
Distance	0.74	0.42	0.14	4.33	49.67	1.44	0.70	0.16	35.82	115.12
P	0.17	0.33	0.67	<0.01	<0.01	<0.01	0.17	0.27	<0.01	<0.01
					Lower Great Fish River vs Glen Melville Dam					
Distance	0.04	0.15	0.14	0.08	1.78	0.31	0.32	0.02	1.93	17.76
P	0.95	0.69	0.51	0.43	0.42	0.33	0.09	0.92	<0.01	<0.01
					Lower Great Fish River vs Lower Sundays River					
Distance	0.35	0.22	0.16	1.14	28.04	0.20	0.10	0.19	1.40	13.69
P	0.50	0.36	0.33	<0.01	<0.01	0.63	0.54	0.48	<0.01	<0.01
					Upper Sundays River vs Lower Sundays River					
Distance	0.43	0.10	0.43	0.58	7.56	0.32	0.36	0.35	1.31	5.18
P	0.50	0.86	0.09	0.01	0.03	0.62	0.81	0.45	0.05	0.11
					Upper Sundays River vs Glen Melville Dam					
Distance	0.04	0.17	0.45	0.14	1.66	0.83	0.78	0.14	0.20	0.79
P	0.97	0.72	0.04	0.41	0.45	0.05	0.28	0.37	0.82	0.69
					Lower Sundays River vs Glen Melville Dam					
Distance	0.40	0.07	0.02	1.71	31.76	0.51	0.42	0.21	3.47	31.15
P	0.46	0.82	0.95	<0.01	<0.01	0.18	0.03	0.26	<0.01	<0.01

Trophic diversity within the Sundays River, based on the SEA_c, for the communities was also lower in the upper (7.8‰^{2}) than the lower sections (21.1‰^{2}) (Figure 5.2). *NR* showed that the community spanned 7.1‰ in the upper section compared to 13.4‰ in the lower section. Similarly, *CR* showed a wider breath in carbon sources in the lower section

(9.7‰) than in the upper section (4.3‰) (Figure 5.3). The average distance for community members from their combined centres (*CD*) was high in the upper section where clustering among individuals (*MNND*) was less compared to the lower section. Comparison between upper and lower Sundays River communities for the contrasts for the measures of dispersion (*CD* and *MNND*) showed no significant differences (RPP, $P > 0.05$), but revealed significant differences on the Euclidean distance between centroids (RPP, $P < 0.05$, Hotelling's T^2, $P < 0.05$) (Table 5.3). This showed that, as in the Great Fish River, the communities were ecologically distinct in their isotopic composition. Neither path lengths nor path directions were significantly different (RPP, $P > 0.05$) for the contrasts of upper and lower Sundays River communities. In contrast to the Great Fish River, catfish's SEA_c were similar between upper and lower sections (approximately 8.1‰^{2} in each section). *NR* was wider in the upper compared to lower section, whereas *CR* was comparable between the two sections (Figure 5.3). Comparison of catfish within the upper and lower Sundays Rivers showed that the Euclidean distance between centroids was marginally significant (RPP, $P = 0.05$) whereas the contrasts for the measures of dispersion (*CD* and *MNND*) were not significantly different ($P > 0.05$). Path directions were significantly different (RPP, $P < 0.01$) whereas path lengths were not different ($P = 0.05$) for contrasts between catfish in the upper and lower Sundays River.

In comparison to the lotic habitats, the community within Glen Melville Dam had a relatively large SEA_c (26.2‰^{2}) that was comparable to the lower Great Fish River. The community spanned a wide range of trophic levels (*NR* = 10.2‰) and had a relatively wide range of carbon sources (*CR* = 12.8‰). Community members were on average further away from their combined centres (*CD* = 3.5‰) and the distance among individuals was high (*MNND* =0.45‰) was comparable to the communities in the lower sections of rivers (Figure 5.3). There were no evidence of differences for all pairwise comparisons on measures of

dispersion (*CD* and *MNND*) between the Glen Melville community and all communities from the rivers. By comparison, the community showed significant differences (RPP, $P < 0.01$, Hotelling's T^2, $P < 0.01$) in the Euclidean distance between centriods only for pairwise comparisons with the upper Great Fish River and lower Sundays River communities (Table 5.3). Path lengths for the Glen Melville community was significantly different for contrasts with the upper Great Fish (RPP, $P = 0.03$) and upper Sundays River (RPP, $P = 0.02$), whereas path directions were not different for all pairwise comparisons. Catfish's SEA_c (2.8‰^{2}) within the dam was smaller than river populations except the upper Great Fish River. Catfish, nonetheless, spanned wide trophic levels (*NR* = 3.4‰), being higher than both the Great Fish River populations, and had a relatively wide carbon source base (CR = 3.3‰). The *CD* and *MNND* for the catfish within the dam were comparable to those in the Great Fish River and lower Sundays River. The dam catfish *CD* differed significantly only for contrasts with catfish in the upper Great Fish River (RPP, $P = 0.03$) and upper Sundays River (RPP, $P = 0.05$), whereas the *MNND* only differed from the lower Sundays River (RPP, $P = 0.03$) population (Table 5.3). Path lengths was significantly different (RPP, $P = 0.02$) for contrasts of the Glen Melville Dam and upper Great Fish River catfish populations, whereas the path directions were not (RPP, $P = 0.64$). Conversely, differences in path length was not significantly different (RPP, $P = 0.60$) for catfish within the Glen Melville Dam and upper Sundays River, whereas path directions were marginally different (RPP, $P = 0.05$). The Euclidean distance between centroids differed significantly for catfish within the Glen Melville Dam and the catfish in all river localities (RPP, $P < 0.01$, Hotelling's T^2, $P < 0.01$) except the upper Sundays (Table 5.3).

Isotope mixing model

Five trophic guilds, namely predators, benthivores, invertivores, planktivores and herbivores, were identified among all the fishes. There were no statistically distinguishable intra-guild differences (KNND randomisation test, $P > 0.05$) within different localities except for the fishes within the Glen Melville Dam. Trophic guilds within the dam were therefore considered to be distinct prey categories as they showed significant inter-guild differences ($P < 0.05$). Although macroinvertebrates were initially assigned to several trophic guilds, they exhibited neither intra-guild nor inter-guild statistical differences ($P > 0.05$). Similarly, the basal food sources of primary production (plant matter) did not show either intra-guild or inter-guild statistical differences ($P > 0.05$). Macroinvertebrates and plant matter were, nonetheless, significantly different ($P < 0.05$) from other food sources, and were each treated as distinct trophic groups.

Comparisons of the fish trophic guilds within the rivers showed that eels, which constituted the native predator guild, were the most enriched in $\delta^{15}N$ within all localities and were the apex predators (Figure 5.4). The upper sections were depleted in $\delta^{15}N$ compared to lower section in all food webs. The food chain among the fish trophic guilds within the Great Fish and Sundays Rivers were shorter in the upper sections. Non-native catfish was enriched in $\delta^{13}C$ within the upper sections and depleted within the lower section compared to the eels in both rivers (Figure 5.4). The macroinvertebrates trophic group was depleted in $\delta^{15}N$ when compared to all fish trophic groups indicating that they formed an intermediate trophic group and was considered the primary consumers in all river food webs. Basal food sources comprised of primary producers and coarse particulate organic matter (CPOM). Within the rivers, plant matter spanned a wide range of $\delta^{13}C$ in all localities except the upper Great Fish River. CPOM, which was only present in the Great Fish River, was depleted in $\delta^{15}N$ (4.8‰).

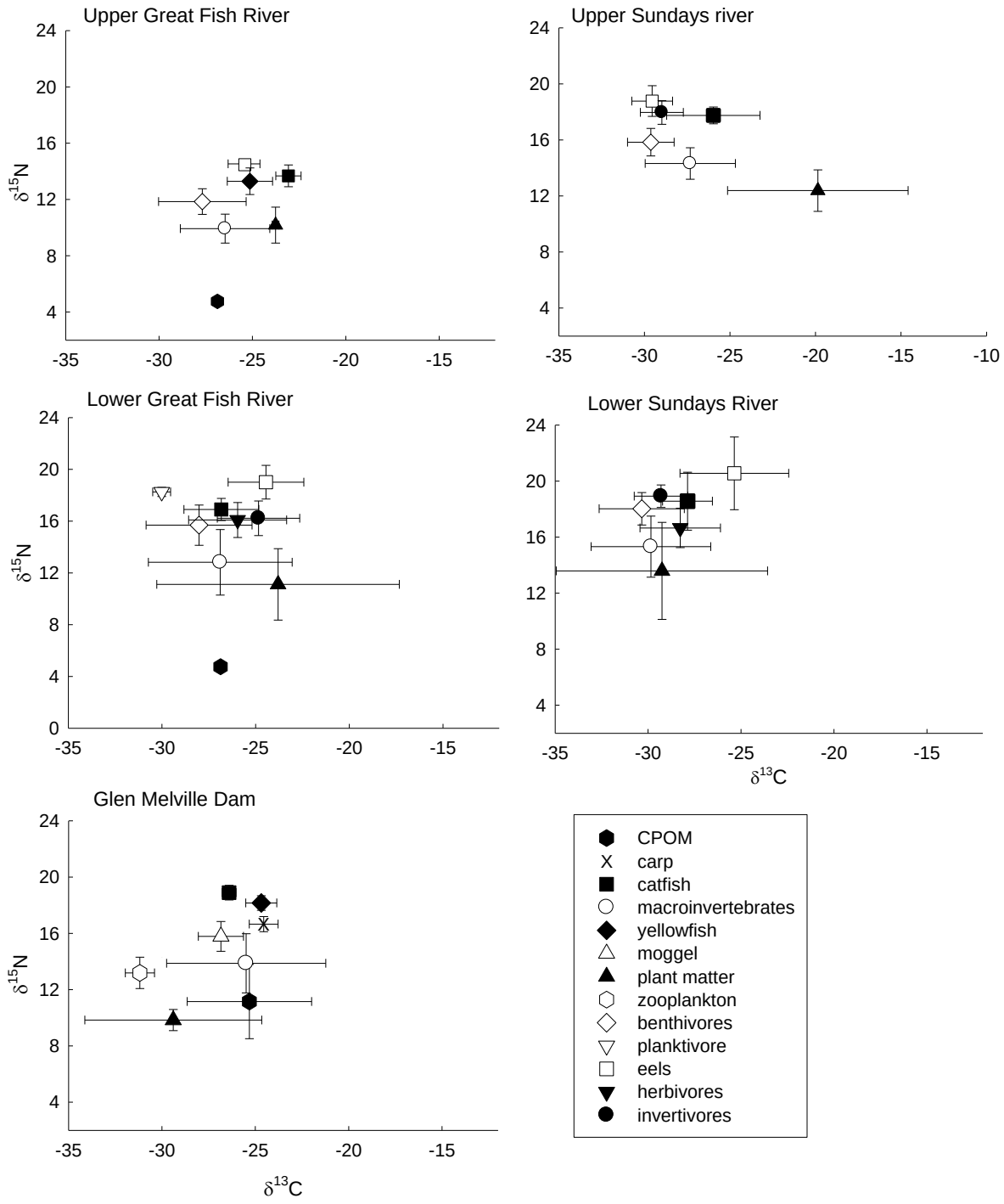

Figure 5.4: Stable isotope biplots of δ13C and δ15N (mean ‰ ± standard deviation) for different trophic guilds of fish, macroinvertebrates, basal food sources and for the sharptooth catfish, *Clarias gariepinus*, sampled in the Great Fish River, Sundays River and Glen Melville Dam in the Eastern Cape, South Africa.

In comparison to the lotic habitats, eels were not sampled within Glen Melville Dam and catfish was considered to be the apex predator ($\delta^{15}N$ = 18.9‰) (Figure 5.4). The catfish within the dam were the most enriched in $\delta^{15}N$ (18.9‰) compared to all river localities. The food chain among the fish trophic guilds, which spanned 3.1‰, was comparable to lower Great Fish River section. Zooplankton was considered the most important pelagic primary consumer within the dam. Of the basal sources sampled, CPOM was enriched in both $\delta^{13}C$ and $\delta^{15}N$ compared to plant matter.

The potential source solutions showed that plant matter made the largest contribution to catfish diets within the upper Great Fish River (mean = 41%, 95% CI = 18 - 70%) and the upper Sundays River (mean = 25%, CI = 8 - 42%) (Figure 5.5). Within the upper Great Fish River, yellowfish were the most important fish prey (mean = 19%, CI = 2 - 40%) followed by eels (mean = 13%, CI = 1 - 30%), whereas within the upper Sundays River, invertivores were the most important fish prey (mean = 19, CI = 0 - 38%) followed by eels (mean = 18%, CI = 2 - 33%) and benthivores (mean = 25%, CI = 0 - 35%). Macroinvertebrates contributed 14% (CI = 0 - 33%) and 20% (CI = 0 - 38%), respectively, to catfish diets in the upper sections of Great Fish and Sundays Rivers. By contrast, macroinvertebrates were the most important prey within the lower sections of the Great Fish and Sundays Rivers, contributing 30% (CI = 16 - 56%) and 24% (CI = 4 - 42%), respectively (Figure 5.5). Benthivores were the most important fish prey to the catfish within the lower sections of both rivers, contributing 18% (CI = 0 - 38%) and 21% (CI = 2 - 39%) in the Great Fish and Sundays Rivers, respectively. In comparison with the lotic habitats, within the Glen Melville Dam, zooplankton was the most important prey for the catfish, and contributed 30% (CI = 26 - 45%) (Figure 5.5). Moggel was the most important fish prey, contributing 20% (CI = 2 - 37%) to catfish diet within the dam.

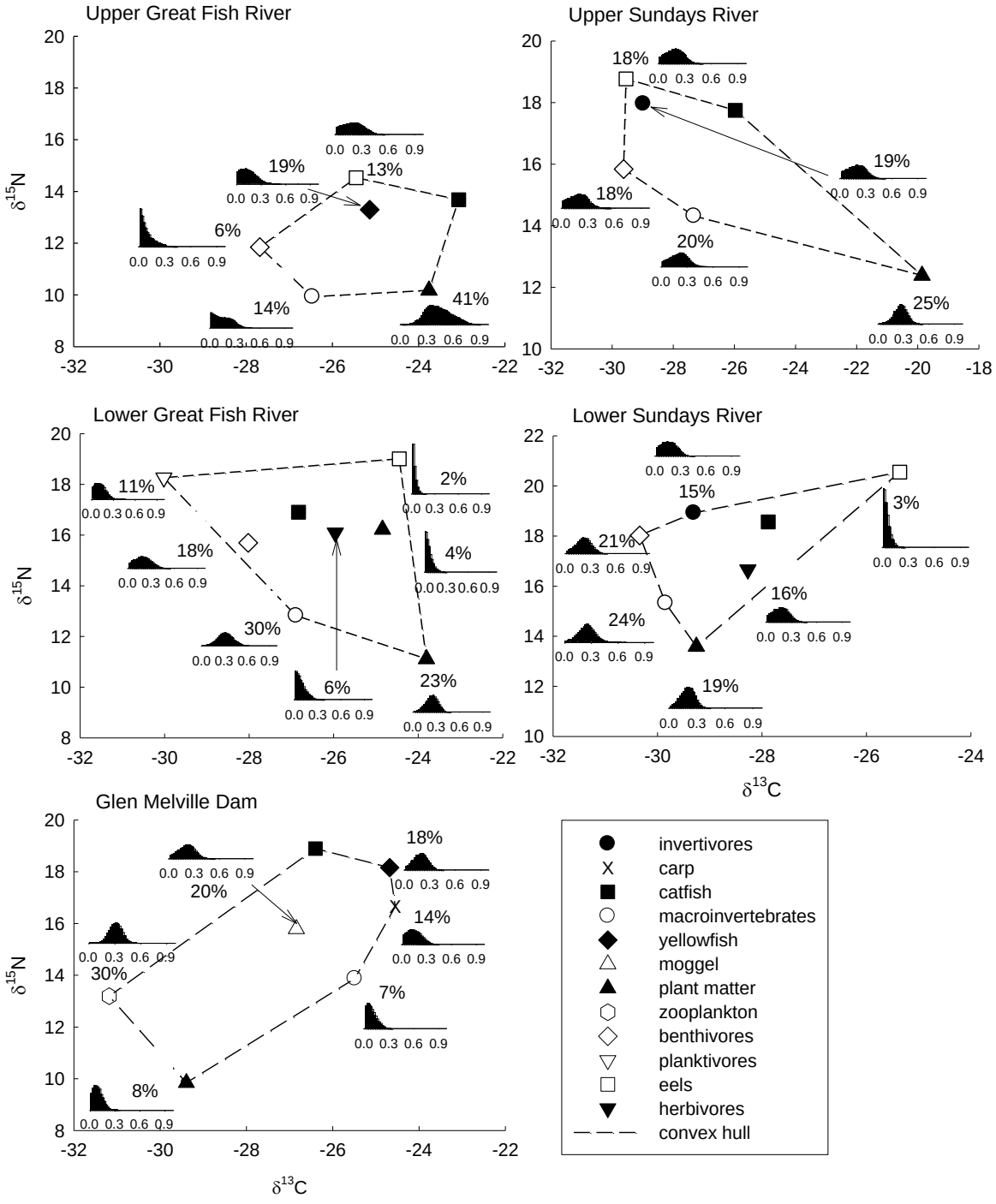

Figure 5.5: Results of SIAR Bayesian mixing model showing the estimates of potential prey for sharptooth catfish, *Clarias gariepinus*, sampled within different localities in the Eastern Cape, South Africa. The values on the histograms indicate the most probable (mean) proportional contribution of each prey item to the catfish diet. The arrows indicate prey groups within the convex hull.

Discussion

Spatial patterns in community and catfish isotope values and niches

Within the rivers, spatial variation in both $\delta^{13}C$ and $\delta^{15}N$ defined the differences in upstream and downstream communities in two ways; $\delta^{13}C$ depletion and $\delta^{15}N$ enrichment downstream compared to upstream localities. $\delta^{13}C$ depletion in downstream consumer groups (both fish and macroinvertebrates) relative to their upstream communities indicates a shift in basal food sources within the rivers. This could be related to a transition from autotroph-driven food webs in the middle and unshaded upstream reaches to heterotroph-driven food webs in the lower reaches (Vannote *et al*., 1980). Consumers in the middle and unshaded upper reaches are driven by primary production, especially epilithic algae. In these habitats, CO_2 is usually limiting due to its increased demand in the autotrophic food webs, and algae becomes enriched in $\delta^{13}C$ due to its decreased discrimination, translating to enriched food webs (Finlay, 2001). By contrast, within the heterotrophic downstream food webs, the primary consumer group is dominated by collectors that rely on fine particulate organic matter (FPOM) and dissolved inorganic carbon (DIC), both often depleted in $\delta^{13}C$ (Finlay, 2001). Consequently, the consumer $\delta^{13}C$ for downstream communities may become depleted, as observed within the rivers in this study. The $\delta^{15}N$ enrichment of downstream communities, as observed in this study, is indicative of a pattern that is characterised longitudinal change within river food webs whereby communities progressively become enriched in $\delta^{15}N$ in downstream reaches (Atkinson *et al*., 2009; Winemiller *et al*., 2011). During this study, differences in the upstream and downstream communities were defined by the contrasts of centriod locations of the dual isotope tracers, suggesting that each locality represented a distinct ecological community for both rivers. Similarly, contrasts of centroid locations for the catfish isotopes suggest diet changes in its prey's isotope values within different localities. The contrasts between centroid locations between the Glen Melville Dam

community and catfish with those in both the rivers showed differences in all but one pairwise comparison, indicating substantial differences between the lotic and lentic habitats.

A comparison of the community structures within the rivers indicated that their downstream communities were characterised by high species richness for both fish and macroinvertebrates. This pattern is characteristic of upstream to downstream gradients within rivers (Payne, 1986). Along such gradients, trophic diversity is expected to increase downstream in relation to increase in community size, energy supply and community stability, often translating to increasing food chain length and wide trophic niches (Pimm, 1982; Schoener,1989; Sabo *et al*., 2010). A similar pattern in niche sizes was observed for the upstream and downstream communities. However, as there was neither evidence of differences in the measures of dispersion (*CD* and *MNND*), nor differences on community trajectories, i.e., path length and angle, suggesting that communities exhibited similar patterns in densities and clustering among individuals regardless of differences in composition, and rather implied that niche expansion was simply by addition of new members that assumed different ecological roles in the community. Comparisons of the community within Glen Melville Dam and those within the rivers also indicated no differences in the measures of dispersion, suggesting similarities in community structuring between lotic and lentic habitats. From a food web perspective, this may suggest that although there were differences in community composition on a spatial scale and between hydrological environments, community structuring was nonetheless similar. Alternatively, this may be indicative of the influence of the non-native invasive predator within the systems. Studies show that in habitats that are strongly influenced by predators, communities show little spatial variation as they become less responsive as they assume new ecological roles (Meissner & Moutka, 2006). Turner *et al*. (2010) noted that a non-native predator can exert top-down pressure on consumer communities to alter their energy sources resulting in the change in consumer

trajectories. Using circular statistics to quantify changes in food web structure, Schmidt *et al*. (2007) demonstrated the shift in native consumer groups from benthic sources to reliance on pelagic food sources following the establishment of invasive freshwater shrimp *Mysis relicta* and lake trout *Salvelinus namaycush* in Lake Tahoe (California-Nevada, USA). A similar niche shift was earlier noted for the consumer community within Lake Tahoe using dual stable isotope plots (Vander Zanden *et al*., 2003). Potential niches shift within catfish invaded communities has also been noted. For instance, Khan & Pannikar (2009) demonstrated, using a mixed trophic impact model, that non-native catfish would impact on almost all fish groups, both native and introduced, potentially changing their feeding patterns and prey preferences, and ultimately modifying the whole invaded community of Keravalapalli Reservoir, India. A probable consequence of invasion pressure on the niche is that individuals of an invaded community, both native and non-native, can either expand their niche to utilise a wide range of available resources, or they shift to utilise a narrow range of resources that are not exploited by their competitors (Olsson *et al*., 2009). Therefore, the influence of non-native invaders is likely to confound natural patterns in community structure on a broad scale. It is possible that, within the catfish invaded systems, such as in this study, that communities in the different localities are subjected to similar invasion pressures due to both predation and access to resources resulting in no detectable differences in community trajectories despite the differences in composition.

Comparison of the SEA_c for catfish showed that it occupied wider trophic niches in the downstream compared to the upstream localities in both rivers. Within the Great Fish River, it exhibited significant differences in spacing of individuals food webs (*CD*), hence niche width, with the downstream population spanning longer trophic levels (*NR*) and showing wide breadth in carbon sources (*CR*) than the upstream population. This suggests that the downstream population was influenced by the availability of resources both along the vertical

structure of the food web and across the spatial range of carbon sources. By contrast, within the Sundays River, the catfish exhibited significant differences in path direction, with the upstream population spanning slightly longer trophic levels but showing similar breadth in carbon sources compared to the downstream population. These differences reveal that within the Sundays River, the upstream catfish population's niche was influenced by the range in trophic diversity, with catfish feeding widely within the food web, whereas the downstream population appeared to be equally influenced by both the range of carbon and nitrogen sources. Spatial variation observed for the niches of the catfish populations was consistent with patterns observed for invasive generalist species that change their niches according to available resources. Olsson *et al*. (2009) observed that non-native crayfish *Pacifastacus leniusculus* adjusted its niche in relation to the biomass of macroinvertebrates, its major prey. Similarly, the predatory invasive sea lamprey *Petromyzon marinus* has been observed to change its niche from feeding on upper trophic level species, such as lake trout, to reliance on lower trophic level species, such as suckers (*Catostomus* spp.) and coregonines (*Coregonus* spp.), within different ecoregions of Lake Superior, North America (Harvey *et al*., 2008). The patterns observed for catfish during this study may be indicative of difference in both resource availability and feeding strategies. For instance, within the Great Fish River, catfish in the lower section appeared to increase its niche by feeding on a wide range of prey sources, as suggested by the wider *NR* and *CR* values compared to those in the upper section. In contrast, within the lower Sundays, catfish niche increased in the downstream without a corresponding increase in either *NR* or *CR*. Comparison of the scatters of the isotope values for the catfish populations within the lower sections of the two rivers showed a wider distribution range in the Sundays River compared to the Great Fish River. A possible explanation for this contrast could be related to diet variability for individuals of the different populations. Bearhop *et al*. (2004) distinguished the feeding patterns of generalist consumers,

and suggested that individuals consuming widely differing proportions of their prey tend to show small variance in their stable isotope ratios than those consuming a constant proportion of each prey type. They further suggested that by feeding randomly, populations with high dietary evenness tend to show high variance in isotope values than populations with low dietary evenness. Prey preference among populations may also vary depending on environmental conditions, such as increasing density that cause increasing competition and result in differential diet specialisation among individuals (Svanbäck & Persson, 2004). The patterns associated with variation in catfish niches may suggest that although the population within the lower Sundays River were variable, prey selection was more consistent, with individuals probably exhibiting some degree of specialisation to certain prey due to a low evenness in diet selection. By comparison, within the lower Great Fish River, the catfish niche suggests a population with individuals feeding on a wide range of prey within the food web.

The Glen Melville Dam catfish had a different niche width (*CD*) compared to those in the upper sections of both rivers, but exhibited no evidence of differences with those in the lower sections. This pattern was similar with that inferred between upstream and downstream populations within the rivers. In particular, the differences in path lengths and path directions, respectively, for comparisons of catfish within the dam and those in the upper sections of both rivers suggests an increase in niche size with increasing habitat size, as was inferred for catfish populations within the rivers. This suggests plasticity in catfish niche in response to availability of prey, which is consistent with its generalised feeding habits (Willoughby & Tweddle, 1978; Kadye & Booth, 2011).

Spatial variation in catfish diets

Catfish diet composition usually reflects the most abundant food items within a given locality (Bruton, 1979). Mixing model results showed spatial differences in the proportions of catfish prey within the different localities. Although it was initially predicted that catfish would exhibit a low degree of prey specialisation, the model indicated dominance of plant matter in the catfish diets within the upstream localities and macroinvertebrates in the downstream in both rivers. While diets of introduced catfish have been reported to be dominated by fish and other animal prey (Vitule *et al*., 2006; Khan & Pannikar, 2009; Radhakrishan *et al*., 2011), this study shows that catfish diets varied in different localities and habitats. Although dominance of plant matter in catfish diets within the upstream sections was surprising, the results may highlight the importance of basal food sources, which are often underestimated. By comparison, the dominance of macroinvertebrates as a dietary item within the lower sections also suggests the importance of this prey group, particularly freshwater shrimps, in both rivers. Differences in catfish diets were also notable in $\delta^{13}C$, with the upstream populations being enriched relative to downstream populations. Comparisons of catfish $\delta^{13}C$ with the native predatory eels showed contrasting pattern in enrichment, with catfish being more enriched in the upstream, which was consistent with dominance of $\delta^{13}C$-enriched plant matter in their diets, whereas eels were enriched in the downstream sections in both rivers, suggesting prey a shift for catfish. Within the Glen Melville Dam, zooplankton dominated the catfish diets, suggesting the importance of the pelagic food web within the lentic habitat in contrast to macroinvertebrates that dominated the primary consumer group within lotic habitats. In addition, the proportional contribution of different fish prey, which ranged between 14 - 20%, suggests a wide feeding niche for the catfish within the dam.

A comparison of the fish prey showed differences in the composition of fish prey in the different localities. In particular, benthivorous fish were the dominant prey group in the

upper Great Fish River, whereas invertivores dominanted in the upper Sundays River. The invertivores comprised of mostly small-bodied fish including goldie barb *Barbus pallidus*. Goldie barb conservation concerns have been raised as it is considered vulnerable in habitats where it co-occurs with catfish (de Moor & Bruton, 1988). Within the Glen Melville Dam, the dominance of benthivorous moggel, *L. umbratus*, in catfish diets may signify the potential impact of catfish on this species. Moggel is known to prefer standing or slow flowing habitats where it feeds on detritus and soft sediments (Skelton, 2001). It is possible that the catfish would exert predation pressure on the moggel population in its preferred habitats, including within the dams and large downstream habitats.

Conclusions

Comparing spatial and temporal changes in food web structure is crucial to elucidating food web linkages within and among habitats. Assessing these differences within a hypothesis-based framework based on dispersion metrics (Layman *et al*., 2007) is important and presents an opportunity to test differences in community or population trajectories (Schmidt *et al*., 2007; Turner *et al*., 2010). The use of isotopic mixing model is complementary because consumer diets can be compared. Using both these approaches, this study highlights the potential impact associated with the non-native invasive catfish within the invaded ecosystems in the Eastern Cape region. In particular, comparisons of community trajectories based on their stable isotope values indicated no evidence of differences among the different localities, which may infer large-scale impacts among habitats that may be ecologically distinct. This observation was complimented by the assessment of catfish diets that showed the dominance of different prey groups in different localities. The risk associated with the spread of catfish is related to its persistence within different habitats. From a food web

perspective, changes in catfish niche and its differential response is reflective of its broad diet that may infer different levels of impacts within different habitats.

CHAPTER 6

Integrating Stomach Contents and Stable Isotope Analyses to Elucidate the Feeding Habits of Non-native Sharptooth Catfish *Clarias gariepinus*

Introduction

Sharptooth catfish, *Clarias gariepinus*, is one of the most widely distributed fish species in Africa and some parts of the Middle East. It occurs naturally from Israel, Syria and southern Turkey in the north and throughout Africa to the Orange River in the south (Daget *et al*., 1984; Skelton, 2001). *Clarias gariepinus* occurs in a variety of habitats that include rivers, swamps, natural lakes and man-made dams (Bruton, 1978; Teugels, 1986; Potts *et al*., 2008). In its natural range, it is an omnivore that feeds on fish, invertebrates, plant material, plankton, reptiles, and amphibians (Munro, 1967; Bruton, 1979a; Merron, 1993; Winemiller & Kelso-Winemiller, 1996; Yalçin *et al*., 2001). The catfish is nonetheless regarded primarily as a piscivore that augments its diet with a wide range of prey items (Groenewald, 1964; Willoughby & Tweddle, 1978; Spataru *et al*., 1987). Its capacity for an amphibious life-style, due to its possession of an arborescent organ, makes it a hardy species that can access, and utilise, a variety of habitats (Cambray, 2003a).

Several introductions of *C. gariepinus* out of its natural range have been reported and it is now established in Brazil, India and China (Cambray, 2005; Vitule *et al*., 2006; Bhakta & Bandyopadhyay, 2007; Khan & Pannikar, 2009; Radhakrishan *et al*., 2011). The impacts associated with the catfish in regions where it is introduced are related to its predatory nature (Vitule *et al*., 2008), its potential to compete with native predators and potentially alter food webs (Khan & Pannikar, 2009), and the risk of introgression with native catfishes (Senanan *et al*., 2004; Peh, 2010). In the Eastern Cape Province, South Africa, *C. gariepinus* was translocated from the Orange River into the Great Fish and Sundays Rivers through the

Orange-Fish inter basin water transfer (IBWT) scheme in 1976 (Cambray & Jubb, 1977; Laurenson & Hocutt, 1986). There are also other populations in many rivers and dams in the Eastern Cape Province through angler introductions. The Eastern Cape region is characterised by a depauperate and mostly endemic ichthyofauna and there is concern over the spread and the potential negative impact of the catfish in the region (de Moor & Bruton, 1988; Potts *et al*., 2008). The endemic minnows of the region such as the endangered Eastern Cape Rocky, *S. biansii*, in Tyume River and the redfin minnow, *P. asper*, in Gamtoos River occur mostly in marginal upstream habitats and rely on main channel pools for refuge in these intermittent rivers. These minnows have however disappeared or reduced in abundance following the spread and establishment of the catfish within the refuge pools (Cambray, 2003a). There is recent concern about potential catfish impacts on populations of the goldie barb, *B. pallidus*, and the endangered Eastern Cape redfin minnow, *P. afer*, due to its presence in the headwaters of the Sundays River where periodic invasions have been reported. The potential role of altered flow regime in exacerbating its spread and associated impact was noted in Chapter 3. Alteration of abiotic conditions, especially flow regimes, has been reported as a factor that contributes to the successful establishment of non-native invasive species while reducing the biotic resistance of native fauna as they adjust to the new conditions (Baltz & Moyle, 1993).

Cambray (2003a) highlighted the need for research and monitoring of catfish impacts in the region. It is therefore critical to understand its feeding habits. Stomach contents analysis is the traditional and most convenient way of addressing this objective, but the method is problematic as it does not adequately consider spatial and temporal variation when drawing dietary inference (Pinnegar & Polunin, 2000), and can underestimate the consumption of highly digestible material (Stapp, 2002). Stable isotope analysis, in contrast, provides a relatively long-term and temporally-integrated measurement of feeding

relationships and diet preferences because it is less influenced by temporal bias (Vizzini *et al*., 2002). By using dual isotope tracers, $\delta^{13}C$ and $\delta^{15}N$, the energy sources and trophic positions, respectively, of organisms can be inferred (DeNiro & Epstein, 1981; Vander Zanden & Rasmussen, 2001; Post, 2002). However, differences in isotopic fractionation between the predator and its prey sources may complicate the interpretation of isotopic values (Gannes *et al*., 1997; Mill *et al*., 2007). In addition, the lack of species-specific fractionation turnover rates often obscures the inferences drawn from stable isotope analysis. Despite the limitations from either method, when combined, stomach contents and stable isotopes provide a robust intergrative approach to analysing the trophic impact of invasive species such as *C. gariepinus*.

This study examined the feeding habits of *C. gariepinus* in the Great Fish and Sundays Rivers. Stomach contents analysis and stable isotope analysis were compared for dietary composition from three localities - the Great Fish River, Sundays River and Glen Melville Dam. Dietary information provides an understanding of the community-wide impact of *C. gariepinus*. As a generalised predator, resource availability is unlikely to be limiting for catfish within invaded habitats. It was predicted that, at population level, catfish feeds from a wide prey range within the food webs of the invaded habitat. It was then hypothesised that, as an invader, catfish would exhibit non-specialised feeding habits and would show no variation in dietary composition among localities and size classes.

Materials and methods

Data collection

Sampling was conducted monthly from October 2009 to April 2010 and bimonthly from May 2010 to December 2010. All samples for stable isotope analysis were collected during the summer period from October 2009 to April 2010 in all localities. Samples of catfish and its

potential prey were collected at 11 sites from each of the two rivers and three sites from Glen Melville Dam (Figure 5.1). The sampling procedure for stable isotopes has been described in Chapter 5. In addition to stable isotope data, catfish stomachs were collected and stored in 10% formalin. In the laboratory, the contents of each stomach were removed and prey were sorted, counted and weighed to the nearest 0.001g. Prey was identified to the lowest taxon possible.

Stomach contents analysis

Cumulative prey curves (Smale, 2005) were constructed to determine whether the number of stomachs sampled was adequate to describe the number of potential prey items in the diet. Dietary composition was assessed using percent by weight and by frequency of occurrence (Hyslop, 1980) defined as:

$\%W_i = \frac{W_i}{\sum_{i=1}^{t} W_i} \times 100$, where $\%W_i$ is the percentage weight of prey *i*, W_i is the total wet weight of prey item *i* from a total of *t* prey items, and

$\%F_i = \frac{F_i}{\sum_{i=1}^{t} F_i} \times 100$, where $\%F_i$ is the percentage frequency of occurrence of prey item *i*,

where $F_i = \frac{n_i}{\sum_{i=1}^{t} n_i}$, where F_i is the frequency of item *i* from the stomachs containing prey item i, n_i, and the total number of stomachs examined.

To facilitate dietary comparison, catfish were categorised into three length classes, which were < 25 cm, 25-50 cm and > 50 cm. Prey items from each fish were assessed by modifying the above equations as:

$\%W_{ij} = \frac{W_{ij}}{\sum_{i=1}^{t} W_{ij}} \times 100$, where $\%W_{ij}$ is the percentage weight of prey *i* in stomach *j*, W_{ij} is the wet weight of prey category *i* in stomach *j*, and

$\%F_{ij} = \frac{F_{ij}}{\sum_{i=1}^{t} F_{ij}} \times 100$, where $\%F_{ij}$ if the percentage frequency of prey *i* in stomach *j*, and F_{ij} is the frequency of prey category *i* in stomach *j*.

Prey items were allocated to diet categories as follows: all fish prey, aquatic invertebrates, terrestrial insects, aquatic macrophytes, filamentous algae and phytoplankton. Prey categories based on $\%W_{ij}$ and $\%F_{ij}$ were analysed using multivariate analysis of variance (MANOVA) to compare localities (L) and size classes nested within localities $(L(S))$. MANOVA was followed by linear discriminant analysis (LDA) using a stepwise procedure to determine the most important prey categories responsible for the differences in catfish diets among the different water bodies. The discrimination power among significant variables was compared using Partial Wilks' λ, with smaller values indicating higher discrimination power. Prey data were arcsine transformed prior to the analyses. Box's M test was used to test the homogeneity of variance-covariance matrices while multivariate normality was tested using a multivariate version of Shapiro-Wilks' test.

Schoener's (1970) index of overlap was used to assess the diet similarity between length classes using wet weight. The index was calculated as: $\alpha_{jk} = 1 - 0.5\sum_{i=1}^{t}\left|p_{ij} - p_{ik}\right|$ where p_{ij} is the proportion of prey item *i* in length class *j*, and p_{ik} is the proportion of prey item *i* in length class *k*. In this index, α ranges from 0 to 1 that corresponds to zero to complete dietary overlap, respectively. Mantel tests (Bahou *et al*., 2007) were conducted to test the

interspecific overlap between length classes based on the proportion of wet weight of prey items.

Stable isotope analysis

Samples preparation and laboratory analysis has been described in Chapter 5. Nested MANOVA models, with explanatory variables similar to those used for prey data were used to compare the differences in catfish isotope values among localities and size classes. The relationship between ontogeny and catfish isotope values was further analysed using linear regressions for both ^{13}C and ^{15}N.

The k - nearest neighbour distance (KNND) randomisation tests (Rosing *et al*., 1998; Lubertkin & Simenstad, 2004) were conducted to evaluate differences in isotopic composition of the different prey categories. Prey categories that were not significantly different were pooled to reduce trophic redundancy. A Bayesian isotopic mixing model, *SIAR* (Stable Isotope Analysis in R, Parnell *et al*., 2010), was then used to determine proportional contributions of different prey groups as catfish food sources. Isotopic fractionation factors were calculated based on Hobson & Welch (1992). The fractionation values for $\delta^{15}N$ and $\delta^{13}C$, respectively, were 3.6 and 0.4 in the Great Fish River, 2.7 and 0.6 in the Sundays River and 3.1 and 0.3 in the Glen Melville Dam. Spearman rank correlations were done to test the relationship between the estimated proportions of fish and aquatic invertebrates prey with those prey weight obtained from stomach contents. Spearman correlations were used instead of Pearson correlations because the data did not fit the assumptions of a parametric correlation analysis.

All analyses were conducted in *R* (R Core Development Team, 2011). The following libraries were used; *ecodist* for Mantel tests, *mvoutlier* for multivariate outliers, *mvnormtest* for multivariate normality and *siar v4.1.1* for the isotope mixing model.

Results

A total of 147 stomachs was analysed from which 67 (46%) were empty. The majority of empty stomachs were from catfish sampled in winter. Of the remaining 80 stomachs, 35, 23 and 22 were from the Great Fish River, Glen Melville Dam and Sundays River, respectively. Cumulative prey curves described a general asymptotic relationship for all locations. Therefore, the stomach contents were considered sufficient to describe both trophic diversity and the main prey items of the catfish. Analysis of the stomach contents revealed total of 32 prey items belonging to five taxonomic groups. These were fish, aquatic invertebrates, terrestrial insects, zooplankton and plant matter (Table 6.1). Among the taxonomic groups, aquatic invertebrates were the most diverse with 20 families belonging to eight orders. These were Ephemeroptera (n = 3), Trichoptera (n = 2), Hemiptera (n = 4), Odonata (n = 2), Diptera (n = 3), Lepidoptera (n = 1), Decapoda (n = 2) and Mollusca (n = 3). Fish were the most abundant diet by weight, contributing 66.2%, 49.2% and 68.7% for Great Fish, Sundays River and Glen Melville Dam catfish, respectively. Fish were also the most frequently consumed prey in these three locations. Three native fish species, goldie barb *B. pallidus*, Mozambique tilapia *O. mossambicus* and moggel *L. umbratus*, and non-native smallmouth yellowfish *L. aeneus*, were among the identified fish prey. Among the aquatic invertebrates, crabs were the most abundant diet by weight and occurrence, contributing 4.9% and 12.8% respectively in the Great Fish River (Table 6.1).

By comparison, hydropsychid caddisflies were the most abundant diet by weight and occurrence, contributing 1.1% and 5.9%, respectively, for catfish in the Sundays River. Aquatic invertebrates were generally less important by weight for catfish diets in Glen Melville Dam. Nevertheless, chironomids and corixids were the most frequently consumed invertebrates in the dam (Table 6.1).

Table 6.1: Dietary composition by percentage weight (% *W*) and percentage frequency of occurrence (% *F*) for sharptooth catfish, *Clarias gariepinus*, sampled in the Great Fish, the Sundays River and Glen Melville Dam, South Africa. Dashes (-) = not sampled.

			Great Fish		Sundays		Glen Melville	
			% *W*	% *F*	% *W*	% *F*	% *W*	% *F*
Fish	Unidentified		53.76	46.15	43.13	23.53	37.49	52.17
	Native	*Barbus pallidus*	-	-	6.05	2.94	-	-
		Oreochromis mossambicus	5.69	2.56	-	-	-	-
		Labeo umbratus	-	-	-	-	26.26	26.09
	Non-native	*Labeobarbus aeneus*	6.78	2.56	-	-	4.99	4.35
Aquatic invertebrates	Ephemeroptera	Baetidae	0.46	10.26	0.34	5.88	-	-
		Leptophlebidae	<0.10	<0.10	-	-	-	-
		Trycorythidae	0.18	2.56	-	-	-	
	Trichoptera	Hydropsychidae	0.31	17.95	1.13	5.88	-	-
		Sericostomatidae	<0.10	<0.10	0.73	2.94	-	-
	Hemiptera	Belostomatidae	<0.10	2.56	0.23	2.94	<0.10	8.70
		Corixidae	<0.10	5.13	-	-	<0.10	13.04
		Naucoridae	<0.10	2.56	-	-	-	-
		Notonectidae	0.26	5.13	-	-	-	-
	Odonata	Libellulidae	0.17	10.26	0.48	5.88	-	-
		Coenagrionidae	<0.10	5.13	0.48	8.82	-	-
	Diptera	Ceratopogonidae	<0.10	2.56	-	-		
		Chironomidae	<0.10	10.26	<0.10	2.94	<0.10	26.09
		Simulidae	<0.10	5.13	-	-	-	-
	Lepidoptera	Pyralidae	0.40	7.69	-	-	-	-
	Decapoda	Crab	4.96	12.82	0.39	2.94	-	-
		Atyidae	-	-	<0.10	2.94	-	-
	Mollusca	Ancylidae	<0.10	2.56	-	-	-	-
		Physidae	<0.10	2.56	-	-	-	-
		Corbiculidae	<0.10	12.82	-	-	-	-
Terestrial insects			5.98	38.46	3.21	11.76	3.74	47.83
Zooplankton			-	-	-	-	<0.10	26.09
Algae			2.59	<0.10	0.70	2.94	4.35	21.74
Phytoplankton			-	-	-	-	9.32	47.83
Macrophytes			14.45	30.76	42.07	20.59	-	-
Detritus			3.43	12.82	1.01	5.88	13.31	43.48
Gravel			0.33	5.13	-	-	0.50	4.35
Total number of stomachs analysed			35		23		22	

Among the plant matter prey, phytoplankton and algae were more abundant in the diets of Glen Melville Dam catfish. Macrophytes were only abundant in the diet of the Sundays River catfish. Terrestrial insects were consumed from all locations (Table 6.1).

Comparisons of catfish diets showed significant differences among localities based on both prey weight (MANOVA $F_{12,132} = 3.72$, $P < 0.01$) and occurrence (MANOVA $F_{12,132} = 5.27$, $P < 0.01$) (Table 6.2). Significant size class differences were also noted based on prey occurrence (MANOVA $F_{30,266} = 3.72$, $P = 0.01$) but not prey weight. The discrimination among localities was highly significant based on both prey weight (Wilks' $\lambda = 0.57$ $F_{12,142} = 3.83$, $P < 0.01$) and prey occurrence (Wilks' $\lambda = 0.57$ $F_{12,142} = 5.12$, $P < 0.01$). The ordination biplots showed the separation along the first LDA of catfish diets from Glen Melville Dam with those from the rivers (Figure 6.2). Based on prey weight, the second LDA axis showed wide variation in the diets of catfish from the Great Fish River compared to those within the Sundays River. Partial Wilks's λ indicates that macrophytes and aquatic invertebrates contributed most to the discrimination among localities (Table 6.3). Macrophytes were absent within the Glen Melville dam and were therefore not recorded in catfish diets from this locality (Figure 6.3). The proportion of aquatic invertebrates in catfish diets were significantly different both among localities based on both prey weight (ANOVA $F_{2,71} = 4.80$, $P = 0.01$) and prey occurrence (ANOVA $F_{2,71} = 3.58$, $P = 0.03$) for the different size classes (nested ANOVA, $P < 0.01$) based on both prey weight and occurrence (Table 6.2). Aquatic invertebrates were the dominant prey group for the smaller size catfish (< 25 cm) especially within the Sundays River (Figure 6.2). In addition, catfish exhibited significant differences in the proportion of phytoplankton among localities based on both weight and occurrence (ANOVA, $P < 0.01$), reflecting the differences between the rivers and the Glen Melville Dam (Table 6.2, Figure 6.3).

Table 6.2: MANOVA and ANOVA results for spatial differences in prey weight and occurrence in sharptooth catfish, *Clarias gariepinus,* diets. Fish were sampled in the Great Fish, the Sundays River and Glen Melville Dam, South Africa. Results are presented by location, L, and Size within Location, $L(S)$.

		Weight				Occurrence			
MANOVA	Variable	df	Wilks' λ	F	P	df	Wilks' λ	F	P
	L	12,132	0.56	3.72	<0.01	12,132	0.46	5.27	<0.01
	$L(S)$	30,266	0.57	1.36	0.11	30,266	0.48	1.81	0.01
ANOVA		df	MS	F	P	df	MS	F	P
Fish	L	2,71	0.46	1.28	0.28	2,71	0.09	0.37	0.69
	$L(S)$	5,71	0.75	2.08	0.08	5,71	0.67	2.79	0.02
Aquatic invertebrates	L	2,71	1.08	4.80	0.01	2,71	0.64	3.58	0.03
	$L(S)$	5,71	0.90	4.03	<0.01	5,71	0.85	4.78	<0.01
Terrestrial insects	L	2,71	0.19	2.38	0.10	2,71	0.10	1.51	0.23
	$L(S)$	5,71	0.05	0.61	0.69	5,71	0.07	1.04	0.40
Macrophytes	L	2,71	0.56	2.79	0.07	2,71	0.56	3.22	0.05
	$L(S)$	5,71	0.19	0.96	0.45	5,71	0.19	1.11	0.36
Phytoplankton	L	2,71	0.25	6.11	<0.01	2,71	0.26	19.54	<0.01
	$L(S)$	5,71	0.01	0.21	0.96	5,71	0.02	1.32	0.26
Algae	L	2,71	0.01	0.10	0.91	2,71	0.01	0.19	0.82
	$L(S)$	5,71	0.02	0.45	0.81	5,71	0.01	0.37	0.87

There was no evidence of differences among the localities in the proportions of fish prey, which was the most abundant dietary item. However, a discernible increase in the proportion of fish prey with size was observed based on prey weight, which indicated an ontogenetic shift. Despite the observed ontogenetic shift, there were no significant differences among all size classes within the different localities (nested ANOVA $P > 0.05$) (Table 6.2).

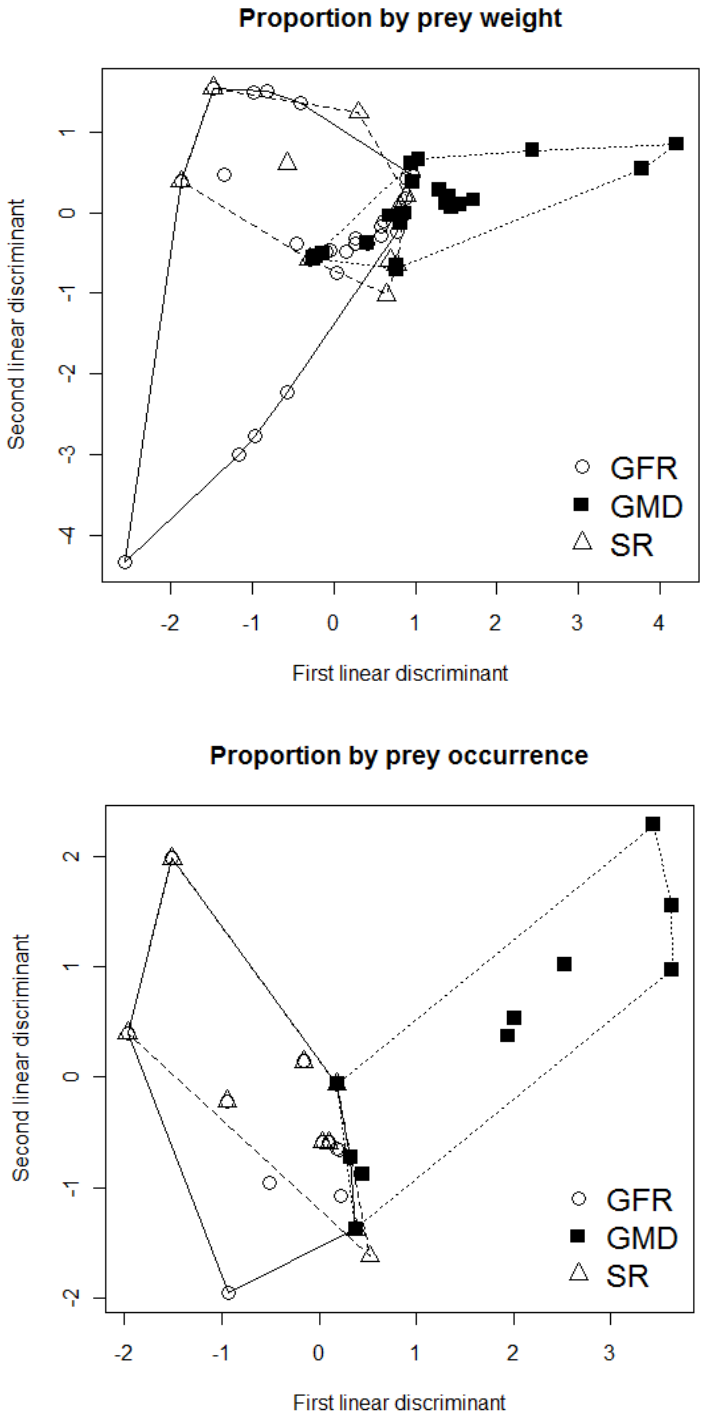

Figure 6.2: Linear discriminant analysis plot of diets of sharptooth catfish, *Clarias gariepinus,* based on prey weight and occurrence. Fish were sampled within the Great Fish River (GFR), Sundays River (SR) and Glen Melville Dam (GMD), South Africa. Convex hulls have been included for each sampling locality.

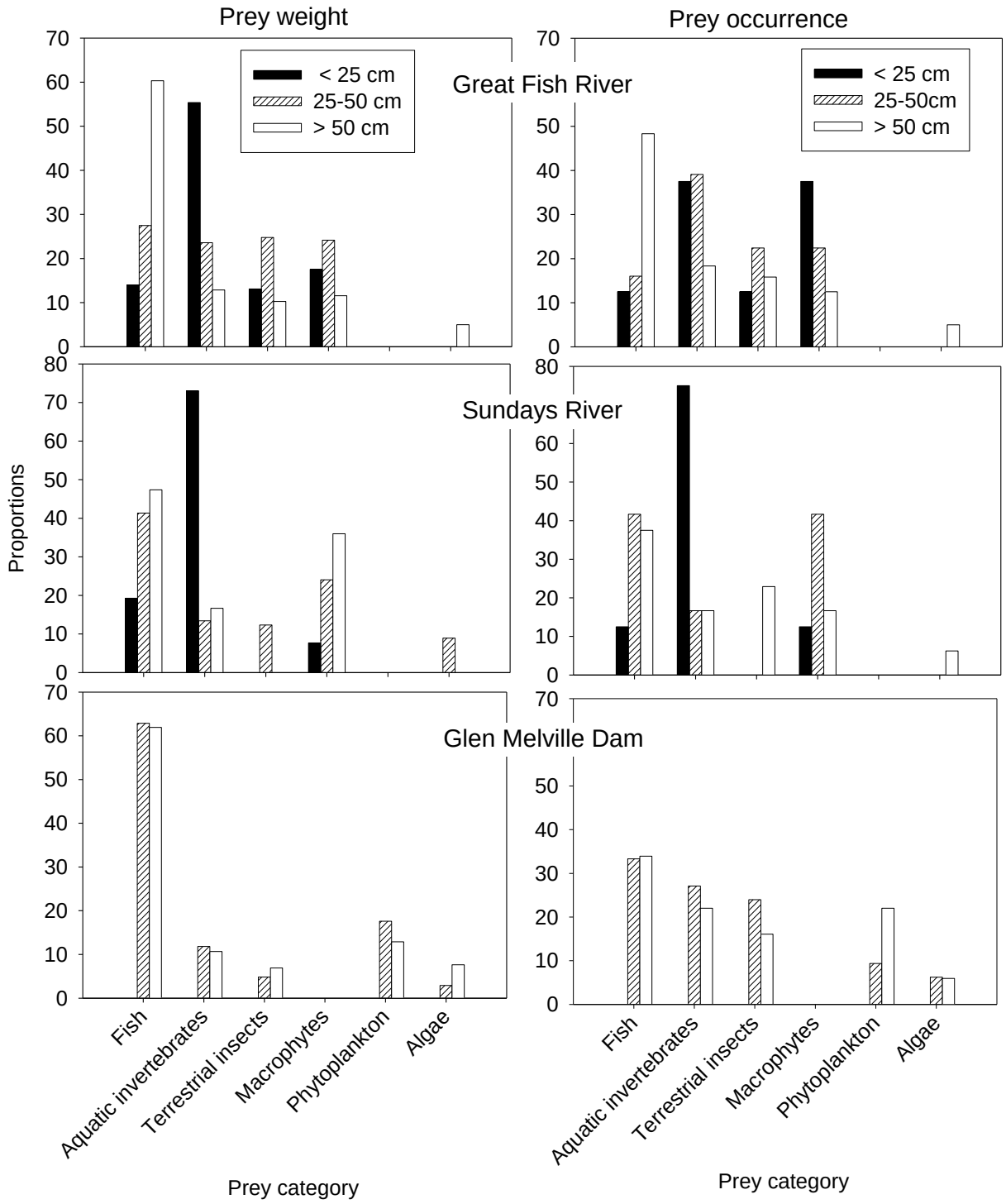

Figure 6.3: Diet comparisons among locations and size classes for sharptooth catfish, *Clarias gariepinus*, sampled at the Great Fish River, Sundays River and Glen Melville Dam, South Africa

Table 6.3: Significance of prey categories in stepwise linear discriminant analysis based on proportion by weight and occurrence sharptooth catfish, *Clarias gariepinus,* diets. Fish were sampled in the Great Fish, the Sundays River and Glen Melville Dam, South Africa.

	Weight				Occurrence			
Prey category	Wilks' λ	Partial Wilks' λ	*F*	*P*	Wilks' λ	Partial Wilks' λ	*F*	*P*
Algae	0.62	0.92	3.21	0.05	0.52	0.95	2.03	0.14
Aquatic invertebrates	0.71	0.80	8.85	<0.01	0.59	0.83	7.46	<0.01
Fish	0.66	0.86	5.65	0.01	0.56	0.86	5.62	0.01
Macrophytes	0.73	0.79	9.62	<0.01	0.60	0.81	8.26	<0.01
Phytoplankton	0.57	1.00	0.01	0.99	0.53	0.92	3.29	0.04
Terrestrial insects	0.69	0.82	7.55	<0.01	0.52	0.94	2.22	0.12

This was supported by the analyses dietary overlap using Schoener's index which showed values > 0.4 with significant overlap (Mantel tests, $P < 0.05$) for all pairwise comparisons in all localities. Significant ontogenetic differences were nonetheless noted for the catfish size classes based on prey occurrence (nested ANOVA $F_{5,72} = 2.79$, $P = 0.02$).

Of the twenty-one fish species collected during this study, seven species were non-native and constituted 33% of the total richness (Table 6.4). Within the Great Fish River and Sundays River, the anguilid eel, *A. marmorata* and the Cape moony *Monodactylus falciformis*, respectively, were the most enriched native fish species in $\delta^{15}N$ with values of 18.4 ‰ ±0.4 and 22.4 ‰ ± 1.8. $\delta^{15}N$ varied widely for native fish species within the Great Fish River (range 13.9 - 18.4 ‰) compared to those within the Sundays River (range 15.6 - 19.7 ‰). Similarly, non-native fish species exhibited a wide range in $\delta^{15}N$ within the Great Fish River where they varied by 2.7 ‰ compared to those within the Sundays River that varied by 2.3 ‰. By contrast, $\delta^{13}C$ for native species varied widely within the Sundays River (range -34.0 to -23.3 ‰) compared to those within the Great Fish River (range -31.7 to -23.1 ‰). The $\delta^{13}C$ for non-native fish nonetheless varied widely within the Great Fish River than the Sunday River. Within the Glen Melville Dam, only one native fish species *L. umbratus* was collected. *Labeo umbratus* was depleted in both $\delta^{15}N$ and $\delta^{13}C$ compared to the non-

native fish species within the dam (Table 6.4). Among the non-fish prey collected within the study localities were the aquatic invertebrates that were more depleted in $\delta^{13}C$ but more enriched in $\delta^{15}N$ in the Sundays River compared to the other locations. Algae were more enriched in $\delta^{13}C$ and more depleted in $\delta^{15}N$ in the Glen Melville Dam compared to other locations (Figure 6.4).

Table 6.4: Carbon and nitrogen stable isotope values for fish collected in the Great Fish River, Sundays River and Glen Melville Dam, South Africa. * indicates non-native fish in all localities and # indicates non-native only within the Sundays River.

	Great Fish River			Sundays River			Glen Melville Dam		
Species	N	$\delta^{13}C$‰	$\delta^{15}N$‰	N	$\delta^{13}C$‰	$\delta^{15}N$‰	N	$\delta^{13}C$‰	$\delta^{15}N$‰
Anguilla marmorata	5	-23.4±0.3	18.4±0.4	1	-23.2±0.0	18.7±0.0	-	-	-
Anguilla mossambica	4	-25.5±1.4	16.1±2.8	5	-29.1±1.3	19.5±1.8	-	-	-
Barbus pallidus	-	-	-	17	-28.8±1.1	18.4±0.9	-	-	-
*Clarias gariepinus**	29	-25.7±2.4	15.9±1.7	24	-27.5±1.8	18.4±1.9	10	-26.4±0.4	18.9±0.5
*Cyprinus carpio**	4	-24.4±1.6	15.7±1.5	5	-30.0±1.4	17.8±1.6	7	-24.6±0.8	16.7±0.5
*Gambusia affinis**	5	-23.3±0.2	15.2±1.0	-	-	-	-	-	-
Gilchristeria aestuaria	9	-30.0±0.5	18.3±0.4	2	-30.3±0.7	17.7±1.1	-	-	-
Glossogobius callidus	8	-27.7±2.3	15.8±1.8	12	-28.8±1.2	18.4±0.5	-	-	-
*Labeo capensis**	4	-26.9±0.9	13.2±0.4	-	-	-	-	-	-
Labeo umbratus	26	-28.7±2.0	13.9±2.4	-	-	-	10	-26.8±1.2	15.8±1.1
*Labeobarbus aeneus**	47	-25.8±2.0	13.7±1.2	-	-	-	10	-24.7±0.8	18.2±0.5
Liza macrolepis	1	-31.6±0.0	17.9±0.0	-	-	-	-	-	-
Monodactylus falciformis	4	-26.8±3.4	18.2±1.5	5	-30.6±1.8	19.7±0.9	-	-	-
Mugil cephalus	3	-31.7±1.3	16.9±1.4	1	-34.0±0.0	15.6±0.0	-	-	-
Myxus capensis	10	-26.2±3.2	16.7±0.6	12	-30.1±2.3	18.0±0.9	-	-	-
Oreochromis mossambicus#	1	-28.5±0.0	16.8±0.0	11	-28.3±2.6	16.1±1.2	-	-	-
Pomadasys commersonnii	1	-23.1±0.0	16.7±0.0	-	-	-	-	-	-
Psammogobious knysnaensis	3	-23.4±0.5	16.6±0.2	-	-	-	-	-	-
Redigobius dewaalii	3	-24.6±0.4	16.7±0.3	-	-	-	-	-	-
Rhabdosargus holubi	5	-23.8±2.0	17.2±1.0	1	-28.9±0.0	16.1±0.0	-	-	-
*Tilapia sparrmanii**	6	-27.8±1.3	15.0±0.9	12	-29.3±1.1	16.3±1.5	-	-	-

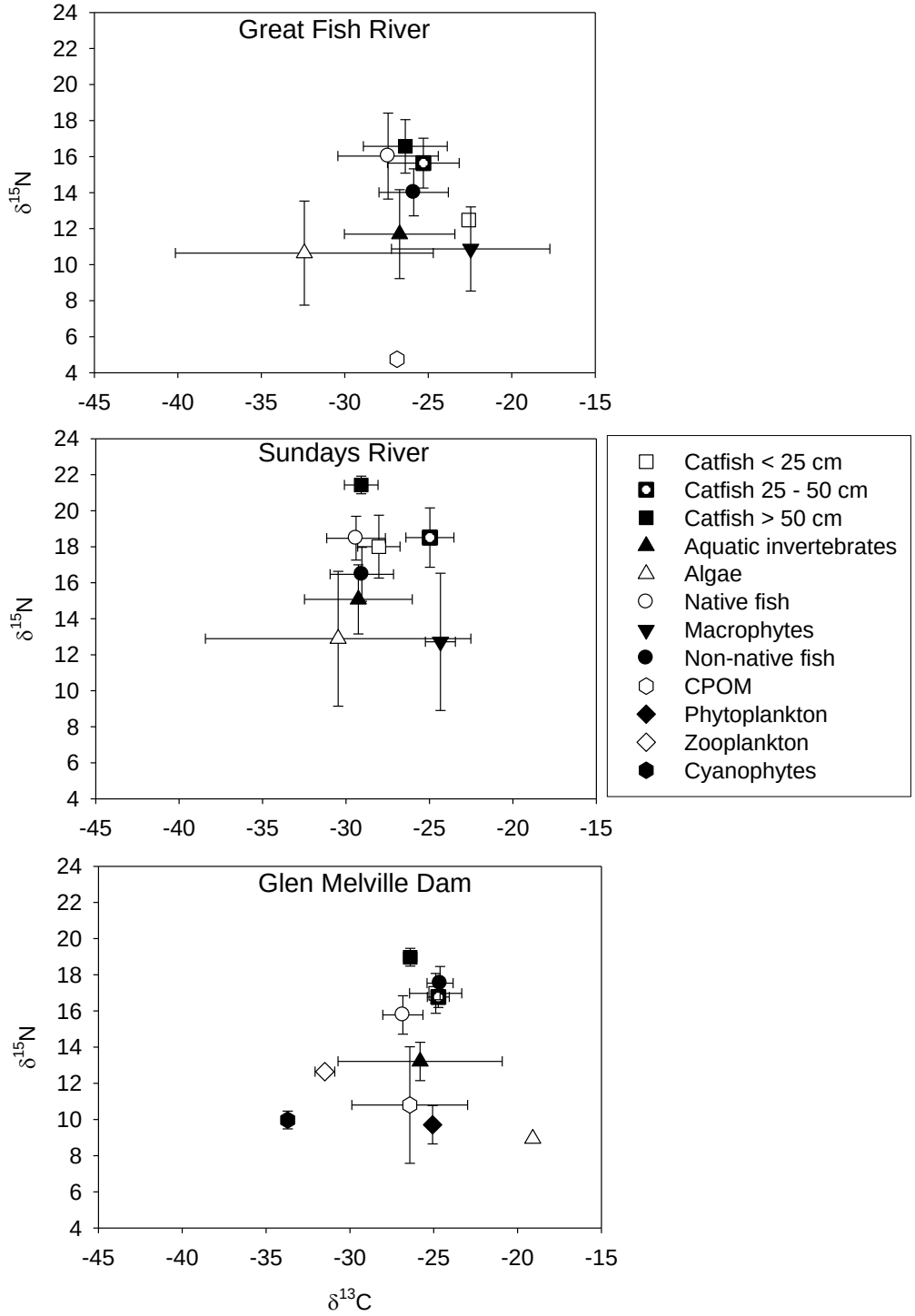

Figure 6.4: Stable isotope biplots of $\delta^{13}C$ and $\delta^{15}N$ values (mean ‰ ± standard deviation) for the different size classes of sharptooth catfish *Clarias gariepinus* and its consumed food items. Samples were collected from the Great Fish River, Sundays River and Glen Melville Dam, South Africa

The stable isotope values of the catfish showed variation among size classes, with the large catfish (> 50 cm) being more enriched in $\delta^{15}N$ in all localities (Figure 6.4). This observation was supported by a significant positive linear relationship for $\delta^{15}N$ and total length in all localities (Figure 6.5). This indicates ontogenetic differences within the food web with large catfish occupying higher trophic levels, whereas the medium and small catfish appeared to feed lower in the food chain. By contrast, catfish exhibited a significant negative relationship between $\delta^{13}C$ and total length indicating that large catfish were depleted in $\delta^{13}C$ compared to small catfish. An exception was for Sundays River catfish that did not exhibit a linear relationship between $\delta^{13}C$ and total length. Catfish, however, exhibited significant differences in the dual stable isotope values (MANOVA $F_{4,120} = 13.7$, $P < 0.001$) among localities. There was also evidence of size class differences in the dual isotope values (nested MANOVA $F_{12,120} = 4.6$, $P < 0.001$) within the different localities.

The KNND randomisation tests showed that the broad prey categories were statistically different. An exception was for the basal food sources of algae, macrophytes, CPOM and phytoplankton, and these sources were pooled as plant matter for analysis in *SIAR*. The *SIAR* mixing models indicates that the catfish diets were diverse among all size classes (Figure 6.6). In contrast to stomach contents analysis, the *SIAR* model showed no clear dominance of particular prey items. However, the *SIAR* estimates showed a discernible increase in native fish prey and decrease in aquatic invertebrates prey with catfish size classes, partially corroborating those observed from stomach contents analysis. In particular, small catfish appeared to have high estimates for aquatic invertebrates, whereas large catfish had high estimates for fish prey in all localities. Spearman's rank correlations, nonetheless, showed no significant relationship ($P > 0.05$) between proportions of fish and aquatic invertebrate prey from the *SIAR* model and those obtained using stomach contents analysis. This indicates that, although the two methods were complimentary, the overall patterns in the

estimates of prey contributions were different. Estimates of basal food sources in the diet appeared to be high for small (< 25 cm) and medium size catfish (25 - 50 cm) compared to large catfish (> 50 cm).

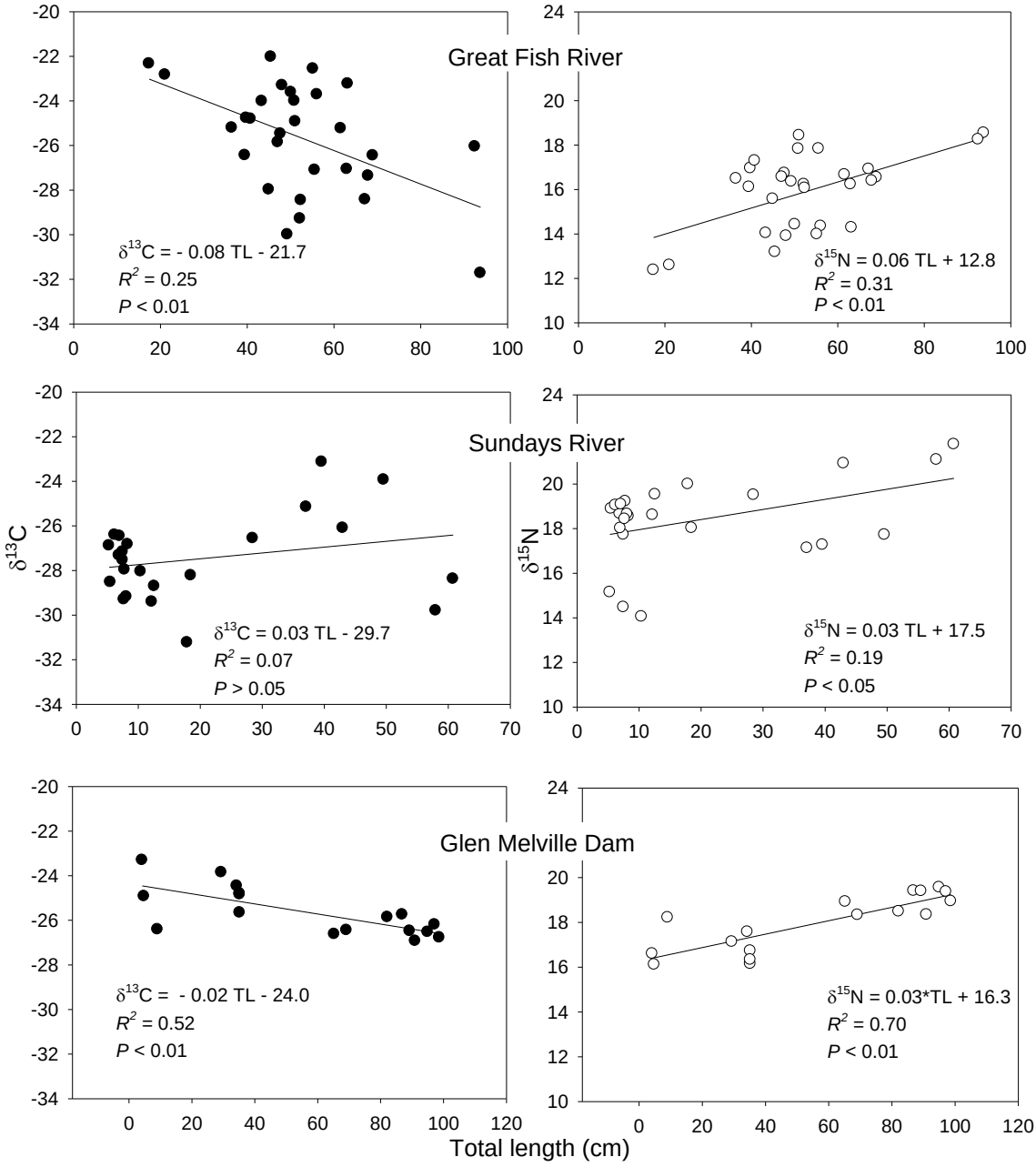

Figure 6.5: The relationship between $\delta^{13}C$ and $\delta^{15}N$ against total length for sharptooth catfish, *Clarias gariepinus*, sampled from the Great Fish River, Sundays River and Glen Melville Dam, South Africa.

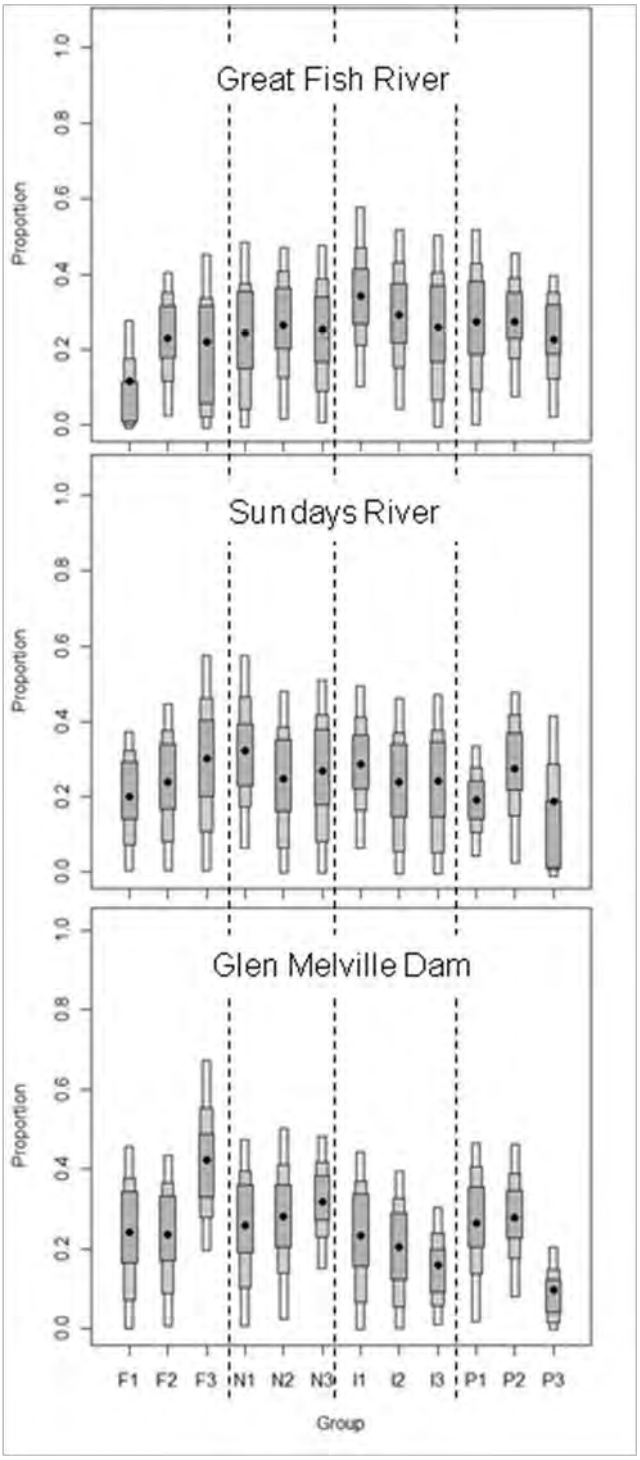

Figure 6.6: Estimated percentage contributions (mean and 95 % credibility intervals) of prey consumed by sharptooth catfish, *Clarias gariepinus,* derived from stable isotopes using *SIAR* from the Great Fish River, Sundays River and Glen Melville Dam, South Africa. Prey groups are abbreviated as F = native fish, N = non native fish, I = aquatic invertebrates and P = plant matter. Catfish size classes are given as 1 = < 25 cm, 2 = 25 - 50 cm and 3 = > 50 cm.

Discussion

Understanding the predation impact associated with generalised predators such as *Clarias gariepinus* is a crucial step towards the assessment of the risks associated with their introductions. Freshwater ecosystems are considered biogeographic islands with specific species pools and limited dispersion from adjacent systems (Oberdorff *et al*., 1997; Leprieur *et al*., 2009). As such, freshwater ecosystems, especially rivers, are considered unsaturated with species and characterised by low interspecific competition, making them more vulnerable to invasions (Leprieur *et al*., 2009). Therefore, the establishment of invasive predators in many freshwater ecosystems has the potential of accelerating the risk of species loss and local extinctions (Chapman *et al*., 1996; Leprieur *et al*., 2006). Invasion risk and species loss is likely to be high in developing and species-rich countries where research is still lagging and impacts often go unnoticed (Vitule *et al*., 2009). This study showed that fish formed the bulk of the catfish diet, which suggests the potential of a predation impact. Concern for impact is great because the study systems are characterised by mostly endemic and species-poor ichthyofaunas. Among the fish prey were three native species, goldie barb in the Sundays River, Mozambique tilapia in the Great Fish River, and moggel from Glen Melville Dam. Goldie barb, which has a restricted distribution in most Eastern Cape rivers, including the Sundays, is reported to be threatened by the presence of catfish (de Moor & Bruton, 1988; Skelton, 2001). While the conservation status of the two other identified prey fish species is considered to be in the category of least concern, these species may be subjected to high predation pressure in less structured habitats where the catfish grow to larger sizes, such as in dams and wider and deeper riverine habitats especially of the Great Fish River. In Glen Melville Dam, Booth *et al*. (2010) reported that large catfish dominate the population and are both highly mobile and aggressive feeders.

Catfish diets broadly revealed differences in habitat types (lotic versus lentic) and dietary diversity (Great Fish River versus Sundays River). These differences were related to the dominance of certain prey, especially macrophytes that were only abundant within the rivers, the aquatic invertebrates, such as crabs that formed the bulk of catfish diets, and other prey including fish that appeared to be more diverse in the Great Fish River compared to other localities. In many rivers of the Eastern Cape, the native crab, *Potamonautes perlatus*, is considered threatened from predation by the catfish (de Moor & Bruton, 1988). In comparison to stomach contents analysis, stable isotope analysis suggests a more generalised feeding pattern, with no clear dominance of particular prey type, neither for particular habitat nor for size class. Stable isotope analysis further revealed that large catfish were top predators in all localities whereas smaller catfish occupied lower trophic levels. This suggests that, by utilising food from different trophic groups, the sharptooth catfish has a complex role as an invasive species in the aquatic food webs. Omnivory was also evident, based on the *SIAR* estimates, with all prey groups being observed in the diets of all size classes. Since stable isotope analysis for muscle tissue represents a temporal integration of carbon and nitrogen assimilated from diet (McCutchan *et al*., 2003; Grey, 2006), this analysis suggests that the catfish feeds on a wide range of prey, including some that may be temporarily unavailable. In contrast, stomach contents analysis provides a "snapshot" of consumed diet depending on the rate of digestion, among other factors (Gearing, 1991). During this study, assessment of seasonal variation in prey consumption based on stomach contents analysis was not feasible because most stomachs were empty in winter. However, the taxonomic resolution of stomach contents analysis provided additional information that was not afforded by stable isotope analysis, such as the consumption of terrestrial insects. The two approaches were therefore complimentary in providing information on catfish feeding.

Observations of catfish feeding habits were consistent with other studies that show diets reflecting the most common prey of suitable size (Groenewald, 1964; Munro, 1967; Bruton, 1979b) and changes in environmental conditions that influence prey availability (Willoughby & Tweddle, 1978; Spataru *et al*., 1987). The catfish's exploitation of a broad prey range in different habitats demonstrates broad niche, which is considered one of the important character for its success to live in a wide range of habitats including those where forage fish are absent (Groenewald, 1964), even in areas where it is introduced (Vitule *et al*., 2009; Radhakrishan *et al*., 2011). In southern Brazil, the catfish has rapidly expanded its range into degraded streams where it is reported to prey on a native amphibian, *Leptodactylus ocellatus*, that is well adapted to habitat disturbance (Vitule *et al*., 2008; 2009). The catfish's ability to feed across all trophic levels may present ecosystem impacts associated with homogenisation of biota. Khan & Pannikar (2009) showed that the non-native catfish exerts a negative predation impact on all fish prey, both native and introduced, resulting in cascading positive impacts on insects and zoobenthos. Such trophic cascading may however be obscured and probably less evident because of the catfish's ability to prey on lower trophic level groups such as insects and zoobenthos. Morphologically, as the catfish grows, both its gape size and number of gill rakers increase, conferring more efficiency to filter feeding (Munro, 1967). Therefore the trophic impacts of invasive catfish may be contrary to those of other piscivores, such as bass and trout species, that cause significant top-down effects within the invaded communities (Flecker & Townsend, 1994; Gratwicke & Marshall, 2001; Cambray, 2003b; Nyström & McIntosh, 2003; Vander Zanden *et al*., 2004; Maezano *et al*., 2005).

Both stomach contents analysis and stable isotope analysis showed an ontogenetic shift towards the preference to fish prey and a general decrease in the dominance of aquatic invertebrates with increasing catfish size. Similar ontogenetic shifts have been observed for

the catfish. For instance, Munro (1967) showed the dominance of aquatic invertebrates, especially chironomid larvae and pupae, in diets of juvenile catfish and the dominance of fish prey in large catfish in Lake McIlwaine, Rhodesia (Zimbabwe). Similarly, Willoughby & Tweddle (1978) found a reduction in the importance of insect larvae in diets of large catfish. Despite the observed ontogenetic shifts in this study, there was evidence of significant dietary overlap among the different size classes. Catfish of nearly all sizes can exploit a wide range of prey. This broad overlap also suggests the risk associated with juvenile catfish, which are more likely to occur in shallow and marginal habitats such the intermittent pools and upstream habitats where native minnows occur, and would exert predation pressure including piscivory. Catfish as small as 16 cm can be piscivorous (Willoughby & Tweddle, 1978). Presently, there are no physical barriers that limit the dispersal of the catfish into headwater streams of the Sundays River where the endangered redfin minnow *P. afer* occurs. Invasion also is possible into the upper Koonap River, a tributary of the Great Fish River, where chubbyhead minnow, *B. anoplus*, is native. The catfish already occurs in the lower sections of the Koonap River. Cambray (2003a) reported that the endangered endemic minnows *P. asper* and *S. bainsii* have disappeared, presumably due to predation, in pools invaded by juvenile catfish in the Gamtoos and Tyume Rivers, respectively.

Moyle & Light (1996) reported that the alteration of hydrological regimes in the highly seasonal Mediterranean streams in California (USA) facilitated the establishment of non-native fish species. Altering the environmental conditions changes biotic interactions among native species, and reduces some populations, ultimately reducing their resistance while increasing the risk of predation and competition by invasive non-native species (Baltz & Moyle, 1993; Moyle & Light, 1996). A similar pattern can be inferred in the Great Fish and Sundays Rivers where IBWT schemes have altered flow regimes. Prior to the opening of the IBWT schemes, the natural flow was seasonal, with the rivers occurring as series of

unconnected pools during the dry season, to which the native fauna was adapted (O'Keeffe & de Moor, 1988). Presently, these rivers are characterised by perennial flow and low seasonality, with the catfish being widely distributed in these systems. In addition, a number of reservoirs within the study systems where the catfish is established, such as the Glen Melville Dam, may act as sources for invasion (e.g. Havel *et al*., 2005).

The IBWT schemes have also facilitated the translocation of other non-native species that were collected during this study. From an ecosystem perspective, the occurrence of other introduced species within the Great Fish and Sundays River has the potential to create "invasion meltdown". Invasion meltdown is a phenomenon in which the joint impact of two or more species would be more severe than that of the single invaders (Simberloff & Von Holle, 1999; Simberloff, 2011). The meltdown could be associated with non-native species, such as common carp, *C. carpio*, smallmouth yellowfish, *L. aeneus* and mudfish, *L. capensis*. Common carp is renowned for altering habitats by uprooting macrophytes and disturbing substrates, thereby directly affecting habitats for other species and influencing nutrient cycling (Miller & Crowl, 2006). Alteration of habitats by common carp may lead to loss of alternative refugia for other species, while resuspension of sediments may increase vulnerability to catfish predation. Smallmouth yellowfish has recently established a breeding population in the Great Fish River (Weyl *et al*., 2009a), whereas mudfish appears to be increasing in abundance. These two species are likely to compete with native moggel *L. umbratus*. The invasive cichlids (*Tilapia sparrmanii* and *O. mossambicus*) are likely to impact the lower reaches of the two rivers where they are abundant.

To conclude, catfish diet reflected spatial differences in prey availability within the study localities. A broad diet and omnivory, which were shown by both stomach contents analysis and stable isotope analysis, may account for the catfish's ability to persist and cause predation impact within the invaded localities. The alteration of flow regimes by the IBWT

schemes probably has influenced invasion success of the catfish, while the occurrence of other non-native invasive species creates the potential for invasion meltdown within the Great Fish and Sundays Rivers. There is also concern over the potential establishment of catfish populations within headwater streams inhabited by endangered endemic minnows.

CHAPTER 7

Detecting Impacts of Invasive Non-native Sharptooth Catfish, *Clarias gariepinus*, Within Invaded and Non-invaded Rivers

Introduction

The response of biological communities to impacts can be categorised as either pulse (short-term and often unpredictable), press (long-term that increase initially before reaching constant levels that are maintained) or ramp (cumulation of impacts over time) disturbances (Underwood, 1991; 1992; Lake, 2000; Parkyn & Collier, 2004; Harper & Peckarsky, 2005). In aquatic ecosystems, disturbances from biological invasions are widely recognised as determinants of community patterns and threats to biodiversity (Johnson *et al*., 2009). Detecting these invasive impacts can unfortunately be confounded by other forms of environmental change that preclude the identification of the invader's role (Underwood, 1994; 1997). In particular, stochastic variability in animal abundance over long temporal scales and their lack of concordance at different localities often result in interactions in trends of abundance over space and time (Grossman *et al*., 1990; Beugly & Pyron, 2010). The underlying principle within an impact study is to be able to distinguish between these natural and stochastic changes from the perceived impact.

Benthic aquatic macroinvertebrates are widely used as indicators in environmental impact studies. Their ubiquity and differential response to different categories of disturbances makes them excellent candidates (Rosenberg & Resh, 1993). In predation impact studies, especially by non-native invasive fish, aquatic macroinvertebrates are highly susceptible and can exhibit responses ranging from simple interactions such as reduction or local extinction of populations, to complex interactions such as trophic cascading (Flecker & Townsend, 1994; Nyström & McIntosh, 2003; Williams & Taylor, 2003). Experimental studies indicate

that when invasive predators become established, they tend to remove the most vulnerable macroinvertebrate taxa, and the resultant community either shows little response to predation-related disturbances (Meissner & Moutka, 2006), or it becomes highly unstable and stochastic (Angeler & Moreno, 2007). Community instabilities in predator-mediated communities are a consequence of a transition to a new alternative stable state during both the disturbance and post-disturbance periods (Suding *et al*., 2004). Such communities are often dominated by macroinvertebrate taxa that can either seek alternative refuge and quickly recolonise from adjacent habitats (Miller & Crowl, 2006), or are not preferred by the predator (Meissner & Moutka, 2006; Effenberger *et al*., 2008). Therefore, the presence of invasive non-native predators can be likened to press disturbances that persistently influence community structure and function. Press disturbances represent environmental perturbations caused by a sustained impact that continuously disrupts community structure and composition (Underwood, 1992; Lake, 2000; Parkyn & Collier, 2004)

Green's (1979) BACI (Before-After Control-Impact) experimental approach has been widely used in impact studies. This experimental design is based on the basic principle of collecting samples Before and After the perceived impact at both Control and Impact locations, hence the name BACI, and was designed to draw inference on ecological impacts based on field experiments. The BACI rubric has been modified at various levels to address concerns related to experimental design, such as pseudo-replication (Hurlbert, 1984) and the confounding effects of spatial and temporal variation (Stewart-Oaten *et al*., 1986; Underwood, 1994; Keough & Mapstone, 1997; Stewart-Oaten & Bence, 2001). Although BACI designs are important in many habitat impact-based studies, they are rarely used in predation impact studies. This study used the BACI design to examine the impact of invasive non-native African sharptooth catfish *Clarias gariepinus* on patterns of benthic macroinvertebrate communities in the Eastern Cape Province, South Africa. This study

examined the impact on benthic macroinvertebrates using short-term enclosure/exclosure experiments within two rivers that were differentially impacted by catfish presence. By comparing community responses in ecosystems with high and low catfish densities, this study provides insights into detection of impact from an invader. By excluding catfish, macroinvertebrates communities should be subjected to minimum predation disturbance and therefore reveal a responses to short-term temporal change to other environmental factors. Within the enclosure/exclosures, the macroinvertebrate communities should reveal more variation when of catfish is present.

Materials and Methods

Sampling localities

The study was conducted in the Koonap River and Brak River, two tributaries of the Great Fish River. The two rivers are in the same ecological region. Tthe experimental localities were within a radius of 20 km, and selected because they were variably impacted by catfish. The Koonap River is larger and contains high ambient densities of the catfish, whereas the smaller Brak River has no catfish. River flow is intermittent and occurs when there are floods, usually in summer. As flow ceases, the aquatic habitats become a series of isolated pools that are usually maintained by base flow. The Koonap River was sampled near its confluence with the Great Fish River. During periods of continuous flow, the catfish migrates into the Koonap River and persists as the dominant predator in the isolated pools, preying on macroinvertebrates and native fish such as anguillid eels and moggel, *L. umbratus*. The Brak River was sampled within its headwaters beyond the migration limits of the catfish from the Glen Melville reservoir. The experimental section was a headwater stretch that periodically receives highly mineralised water from the Great Fish River's tertiary IBWT. Mean water

temperature within the Koonap and Brak Rivers over the study period was 14.5 ± 1.4 °C and 16.5 ± 0.8 °C, respectively. Conductivity was high in the Brak River, with an average of 1290 ± 46 $\mu S.cm^{-1}$ compared to the Koonap River that had an average of 552 ± 30 $\mu S.cm^{-1}$. The pH was less variable and ranged from 7.8 - 8.5 and 8.2 - 8.4 within the Koonap River and Brak River, respectively. Temperature (°C), pH, and conductivity ($\mu S.cm^{-1}$) were measured during each sampling occasion with a HANNA HI 98129 Combo meter. Both rivers had no flow during the study.

Sampling design and data collection

A pilot study was initially conducted over a period of six weeks between July and August 2009 to determine the rate of macroinvertebrate colonisation on different type of artificial substrates. The substrate types used were gravel, large stones, shredded (2 mm) polythene plastic strip, and a mix of stones and polythene plastic strips. Cumulative asymptotes for macroinvertebrate diversity and richness were observed between 3 - 4 weeks. The stone and polythene strips mixed substrate, which provided a better measure of diversity and richness, was then used in this experiment. Each artificial substrate unit consisted of a 4 - mm polythene netting container measuring 10 cm × 10 cm × 3 cm in length, width and height, respectively. The artificial substrates were placed within exclusion cages that each measured 1 m × 1 m × 0.5 m in length, width and height, respectively, and were constructed of 4 - mm diameter polythene netting. Access by larger macroinvertebrates was facilitated by punching 30 additional holes, each measuring between 1 and 2 cm, into each side and bottom of the cage.

In each of the rivers, fish exclosure cages with artificial benthic invertebrate substrates were used to test the treatment effects of the introduction of catfish. The cages, which were

placed in different locations within each of the two rivers, were designed to simulate natural patterns with minimum disturbances for the invertebrates before the impact was assessed. The treatments for the cages were Control (catfish exclusion) and Impact (introduction of the catfish) tested Before and After the impact on multiple locations within each river (MBACI design).

Sampling for the impact experiment was conducted between July and October 2010. A multifactorial experimental design was used in this study. Eight exclusion cages (four were randomly assigned as Controls and four as Impacts) each containing 18 artificial substrates were placed into each of the two rivers for a period of four weeks prior to the start of the experiment to allow for macroinvertebrate colonisation. The cages were placed at a depth between 0.5 - 0.75 m with homogenous pebble and fine silt substrates. Individual cages were placed in different isolated pools within each river. In one large isolated pool within the Koonap River, cages were placed 100 m apart at two sites on either ends of the pool. Treatments (Control and Impact) were randomly assigned to the different sites (cages). After the end of the colonisation period, each river was sampled weekly for three weeks by collecting three artificial substrates from each cage to give a total of 24 samples (8 cages × 3 artificial substrates) during each sampling occasion. This was the Before period. After the third week, two catfish (each measuring between 20 and 40 cm) were introduced into each of the four Impact exclusion cages in each river. Sampling after the introduction of the catfish as an Impact was done weekly for another three weeks and was the After period. For each of the two rivers there were therefore at total of 144 artificial substrates sampled. There was no catfish mortality nor invasion by other fish species into the exclusion cages during the After period.

At the end of each of the six sampling events, the macroinvertebrates on each of the artificial substrates were visually sorted, identified following taxomonic keys of Geber &

Gabriel (2002) to family level under a dissection microscope (magnification 10 ×). The animals were counted and weighed for dry mass (overnight at 60^{o} C) in the laboratory. Each artificial substrate was analysed alive to reduce bias associated with moribund macroinvertebrates and took approximately 30 min to complete. The data from the three artificial substrates samples per treatment cage per sampling period were averaged prior to the analysis.

Statistical analysis

Macroinvertebrate diversity, taxa richness, dry mass and abundance were determined for each treatment sample. Diversity was calculated using Shannon-Wiener's index given as $H' = -\sum_{i=1}^{s} p_i \ln p_i$, where p_i is the proportional abundance of taxon i in the sample and s is the number of taxa. Taxa richness was presented as number of invertebrate taxa per 10 cm^2, while dry mass was expressed as mg per 10 cm^2.

Dry mass and abundance (counts) of individual taxa were ln(x+1) transformed to satisfy the requirements of normally-distributed residuals and homocedasticity. The MBACI analyses contrasted temporal patterns of taxa mean diversity, mean richness, mean ln(dry mass), mean ln(abundance) for both total and selected individual (most abundant) macroinvertebrate taxa at both the Control and Impact sites. The main interaction of interest in an MBACI experimental design for detecting an environmental impact is the interaction between the Control-Impact treatments and Before-After times (Downes *et al*., 2002; Quinn & Keough, 2002). For each river, a linear mixed-effects model estimated the observed in treatment i in period j in Location k and Time l was expressed as:

$$y_{ijkl} = CI \times BA_{ij} + L(CI)_k + T(BA)_l + \varepsilon_{ijkl}$$

where the fixed effects were the Control-Impact treatments (CI - 2 levels) and periods Before-After the introduction of the impact (BA - 2 levels). Random effects were Locations that were nested within CI ($L(CI)$- 8 levels), and Times nested within BA ($T(BA)$ - 6 levels).

The null contrast, for each river, between Control-Impact sites and Before-After times ($CI \times BA$) $H_0 : (\mu_{CA} - \mu_{CB}) - (\mu_{IA} - \mu_{IB}) = 0$, was assumed to be approximately standard-normally (z) distributed such that $z = \frac{(1,-1,-1,1)\boldsymbol{\beta}}{\sqrt{(1,-1,-1,1)\boldsymbol{\Sigma}(1,-1,-1,1)'}}$, where $\boldsymbol{\Sigma}$ is the variance-covariance matrix of the parameter vector $\boldsymbol{\beta} = (\mu_{CA}, \mu_{CB}, \mu_{IA}, \mu_{IB})'$.

The analyses were conducted using the library *lme4* in *R* (R Core Development Team 2011).

Multivariate analysis

The macroinvertebrate counts for each family produced a species assemblage matrix with 23 taxa × 48 averaged samples for each river. Patterns of invertebrate abundance were analysed using redundancy analysis (RDA). RDA is a direct gradient analysis extension of principal components analysis (PCA). Similar to PCA, RDA identifies orthogonal axes that maximally "explain" variation in species composition (Legendre & Legendre, 1998) but unlike PCA the axes are constrained to be linear combinations of explanatory variables. The eigenvalue associated with each axis is a measure of this variation. Thus, RDA can be considered to be a form of multivariate regression (Jongman *et al*., 1995).

As the data were in the form of repeated measurements, a series of ordinations were conducted to test terms analogous to univariate repeated-measures ANOVA. A split-plot design, with a permutation scheme adjusted to suit the repeated-measures design of the data, was used for the analysis. Whole plots were records of each location repeated in time.

Explanatory variables were the same as the fixed- and random-effects in the MBACI analysis. The interaction of treatment (Control and Impact) and time over sampling period were of interest in assessing possible impacts.

To test hypotheses of impact effects, various combinations of variables, covariates and their interactions were used with an appropriate Monte Carlo permutation in the ordination analyses. In the first ordination, variables coding for sampling time and its interaction with treatments were used as explanatory variables, and sampling locations as covariates. This ordination explained variation in macroinvertebrate community attributed to sampling time and treatments occurring through the experiment. In the second ordination, variables coding for interactions between treatments and sampling time were used to explain variation attributed to changes due to the experimental treatment effect. In this analysis, variables coding for locations and time were used as covariates thereby removing the main treatment effects of the experiment. The third ordination was used to explain variation attributed to change in the control treatment after removing the impact treatment effects. The fourth ordination was used to explain variation attributed to change in the impact treatment after removing the control treatment effects. The relationship between individual macroinvertebrate taxa and the treatment effects was further explored using a Generalised Linear Modelling procedure. Ordination analyses were conducted in *CANOCO v4.5*.

Results

Comparison of macroinvertebrate taxa

Twenty-three macroinvertebrate taxa belonging to nine taxonomic groups were sampled during the experiment (Table 7.1). Odonata was the most represented group with six families. Fourteen taxa were collected in the Koonap River that had high ambient densities of catfish

compared to 22 taxa that were collected in the Brak River that had no catfish. Within the Koonap River, leeches (Hirudinea) were the most abundant taxa with a total count of 4205. In comparison, within the Brak River, aquatic oligochaete earthworms were the most abundant with a total count of 2772.

Table 7.1: The total counts combined for all dates and substrates of all macroinvertebrate taxa collected from the Koonap and Brak River, Eastern Cape, South Africa.

Group	Taxa/Family	Koonap River	Brak River
Ephemeroptera	Baetidae	456	12
	Caenidae	0	106
	Leptophlebiidae	0	6
Trichoptera	Hydropsychidae	0	1
Coleoptera	Hydrophilidae	2	4
Hemiptera	Belostomatidae	16	16
	Pleidae	3	9
Odonata	Aeshnidae	2	0
	Gomphidae	0	2
	Libellulidae	0	264
	Chlorocyphidae	0	2
	Coenagrionidae	1	80
	Lestidae	0	2
Diptera	Ceratopogonidae	10	22
	Chironomidae	517	867
	Culicidae	0	11
Turbellaria	Planaria	1	137
Oligochaeta		2951	2772
Hirudinae		4205	72
Mollusca	Ancylidae	0	14
	Lymnaeidae	107	8
	Physidae	1	1
Anthomedusae	Hydridae	45	57

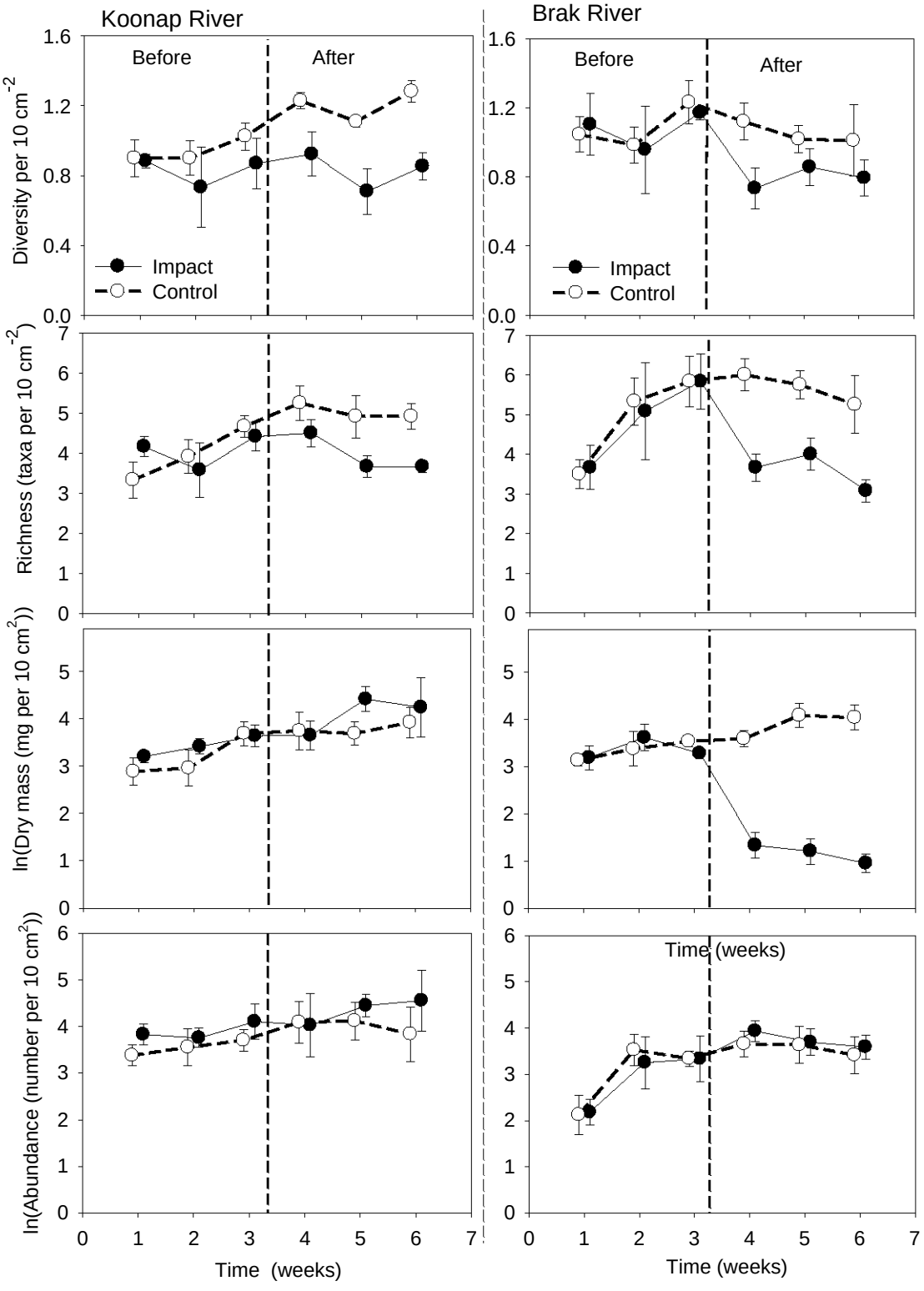

Figure 7.1: Temporal patterns in mean (± standard deviation) Shannon-Wiener diversity, richness and dry mass Before and After the introduction of invasive *Clarias gariepinus* as an Impact within the catfish invaded Koonap River and uninvaded localities in Brak River, Eastern Cape, South Africa.

Temporal patterns in macroinvertebrate mean diversity, mean richness and mean dry mass

A comparison of the temporal patterns indicated that macroinvertebrate mean diversity increased in Control treatment but did not change in the Impact treatment between the sampling periods within the Koonap River (Figure 7.1). This implied that the exclusion of the impact disturbance increased community structure within the localities with high catfish density. A similar pattern was observed for taxa richness that increased in Control treatment relative to the Impact treatment. In contrast, within the Brak River, mean diversity showed no variation in the Control treatment, indicating community stability within localities with minimum catfish disturbance, whereas the Impact treatment exhibited a decline between sampling periods, indicating the direct influence of the predator (Figure 7.1). Taxa richness increased over time but exhibited a more pronounced asymptote in the undisturbed Control treatment within the Brak River. Comparison of mean dry mass for the two rivers indicated similar patterns for the Control treatment where it increased over time. By contrast, the Impact treatment exhibited an increase within the Koonap River, and a dramatic decline within the Brak River following the introduction of the catfish (Figure 7.1). Mean abundance did not vary between the Control and Impact treatments in either river.

MBACI design analysis

The linear mixed-effects model indicated that in both the Koonap and the Brak rivers the treatment and sampling period interaction contrast for mean diversity were significant ($P < 0.05$) (Table 7.2) and implied long-term catfish impacts. For taxon richness, the interaction contrast denoting an impact was significant in both the Koonap River ($P = 0.02$) and the Brak River ($P < 0.01$) suggesting that catfish had a sustained effect on the total number of species over the sampling period (Table 7.2). In the Koonap River, the interaction contrast for mean

dry mass was not statistically significant (P = 0.81) (Table 7.2), indicating failure to detect the impact. By contrast, the interaction was significant in the Brak River (P < 0.01) (Table 7.2), suggesting that dry mass was negatively influenced in localities that were not impacted by catfish.

Among the most abundant macroinvertebrate taxa within the Koonap River, the Baetidae, Belastomatidae and Ceratopogonidae were more abundant in the Control than in the Impact treatments (Figure 7.2). The linear mixed-effects model indicated significant interaction contrasts for Baetidae (P < 0.01), Belastomatidae (P = 0.02) and Ceratopogonidae (P = 0.03) between sampling period and treatments ($CI \times BA$) for these taxa indicating long-term catfish impacts (Table 7.2). By comparison, the Hirudinea, Chironomidae and Oligochaeta were more abundant in the Impact than Control treatments but did not exhibit significant interaction contrasts indicating that these taxa were not negatively influenced by the catfish impact within the Koonap River. Within the Brak River, the odonate taxa Coenagrionidae and Libellulidae, which were more abundant within the Control than the Impact treatments (Figure 7.2), exhibited significant interaction contrasts that inferred long-term catfish impacts (Table 7.2). By comparison, within the Brak River, only the chironomid midges (Chironomidae) were more abundant in the Impact than the Control treatments, with a significant interaction contrast (P < 0.01) indicating a positive catfish impact. While oligochaete earthworms increased in abundance between the sampling periods, they did not exhibit any significant differences (P = 0.87) between treatments.

Table 7.2: Estimates of the Control-Impact and Before-After interaction contrasts from the mixed-linear MBACI model applied to individual taxa and Shannon-Wiener diversity, taxa richness and ln(Dry mass) in both the previously invaded Koonap River and the uninvaded Brak River, Eastern Cape, South Africa. The coefficient of determination (R^2) is provided for each model fit. Dashes denote taxa that were not analysed.

	Koonap River				Brak River			
		Estimate	z	$P(z)$		Estimate	z	$P(z)$
Baetidae	Contrast	8.28	6.87	<0.01	Contrast	-	-	-
	R^2	0.85			R^2	-		
Belostomatidae	Contrast	0.44	2.35	0.02	Contrast	-	-	-
	R^2	0.62			R^2	-		
Caenidae	Contrast	-		-	Contrast	0.44	0.97	0.33
	R^2	-			R^2	0.65		
Ceratopogonidae	Contrast	0.28	2.12	0.03	Contrast	0.22	1.27	0.2
	R^2	0.79			R^2	0.63		
Chironomidae	Contrast	-2.19	-1.55	0.12	Contrast	-14.25	-4.75	<0.01
	R^2	0.94			R^2	0.73		
Coenagrionidae	Contrast	-		-	Contrast	0.89	3	<0.01
	R^2	-			R^2	0.71		
Hirudinea	Contrast	-16.97	-1.49	0.14	Contrast	-	-	-
	R^2	0.69			R^2	-		
Hydridae	Contrast	0.19	0.69	0.49	Contrast	-0.14	-0.46	0.65
	R^2	0.77			R^2	0.73		
Libellulidae	Contrast	-		-	Contrast	3.17	6.66	<0.01
	R^2	-			R^2	0.86		
Oligochaeta	Contrast	-1.69	-0.24	0.81	Contrast	1	0.16	0.87
	R^2	0.78			R^2	0.83		
Planaria	Contrast	-		-	Contrast	0.69	1.99	0.05
	R^2	-			R^2	0.8		
Diversity	Contrast	0.26	2.64	<0.01	Contrast	0.24	2.13	0.03
	R^2	0.83			R^2	0.77		
Richness	Contrast	1.17	3.00	0.02	Contrast	2.06	4.14	<0.01
	R^2	0.88			R^2	0.81		
ln(Dry mass)	Contrast	-0.07	-0.23	0.81	Contrast	2.75	11.95	<0.01
	R^2	0.88			R^2	0.94		
ln(Abundance)	Contrast	-0.01	-0.01	0.99	Contrast	-0.25	-1.22	0.23
	R^2	0.91			R^2	0.90		

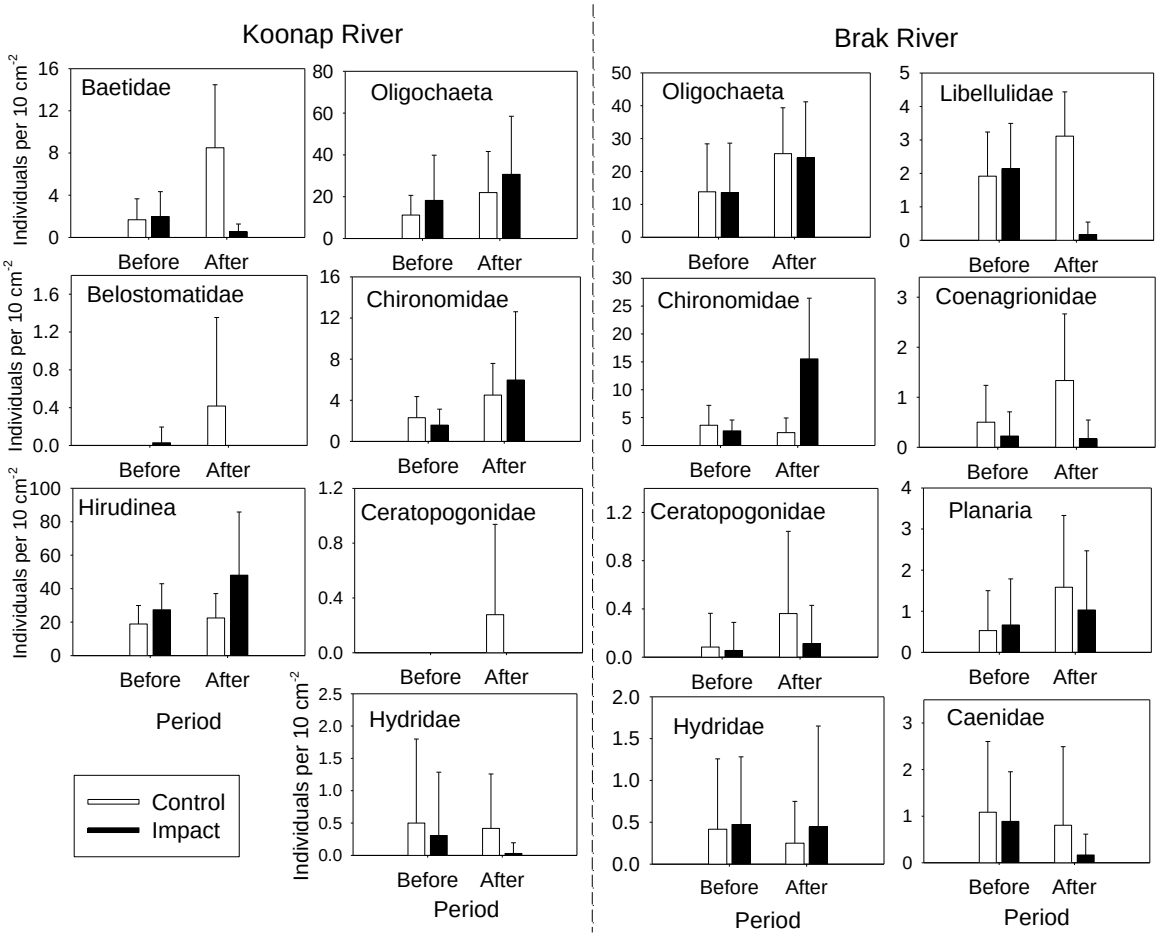

Figure 7.2: Changes in mean (± standard deviation) abundance of aquatic macroinvertebrates in the Control and Impact treatments before and after the introduction of *Clarias gariepinus* in the invaded Koonap River and uninvaded localities in the Brak River, Eastern Cape, South Africa.

Multivariate analysis

From a multivariate perspective, the macroinvertebrate communities underwent significant ($P < 0.01$) directional changes associated with the sampling time and its interaction with treatment effects in the Brak River, whereas non-significant changes were noted in the Koonap River (Table 7.3). These effects explained 21% and 32% of the macroinvertebrate community variation in the Koonap and Brak Rivers, respectively. In the Koonap River, when sampling time was partialled out, the interactive terms of the treatment effects, which

explained the variance due to treatment effects that occurred during the experiment, accounted for 9% of the variation. When considered separately, Control treatment effects explained 12% whereas Impact treatments explained 10%, indicating that there was more variation within the macroinvertebrate composition in the Control than the Impact treatments.

Table 7.3: Partial redundancy analysis examining the patterns of invertebrate communities in the catfish invaded Koonap River and the uninvaded localities in the Brak River, Eastern Cape, South Africa. The F-ratio (*F*) is the ratio statistic test on the trace and its statistical probability (*P*) obtained by a Monte Carlo permutation test with 499 permutations. C = Control, I = Impact (the introduction of *Clarias gariepinus*), T = Time, and × indicates interactions between variables. The corresponding null hypotheses tested were: (1) There are no temporal changes in macroinvertebrate composition, neither common in all treatments, nor specific for particular treatments. (2) Temporal changes in macroinvertebrate composition are independent of treatments. (3) Control (a) or (b) impact treatments have no effect on temporal changes in macroinvertebrate composition.

Hypothesis	Explanatory variables	Covariates	Variation explained (%)	1st axis		All axes	
				F	P(*F*)	*F*	P(*F*)
		Koonap River					
1	T, C×T, I×T	L	21	6.8	0.71	6.6	0.09
2	C×T, I×T	L, T	9			5.9	0.06
3a	C×T	L, I×T	12			7.7	0.05
3b	I×T	L, C×T	10			6.0	0.15
		Brak River					
1	T, C×T, I×T	L	32	14.6	0.00	11.8	<0.001
2	C×T, I×T	L, T	13			9.9	<0.001
3a	C×T	L, I×T	11			8.2	<0.001
3b	I×T	L, C×T	23			16.8	<0.001

The taxa biplot showed the association of most taxa with the Control treatment effects, with the taxa Belastomatidae, Baetidae and Ceratopogonidae being more abundant (Figure 7.3). In contrast, the Impact treatment indicated strong association with the leeches, Hirudinea, whereas the Chironomidae and Oligochaeta increased over time but appeared to be uninfluenced by either treatment. By comparison, within the Brak River, the interaction

terms of the treatment effects were significant ($P < 0.01$) and explained 13% of the variation, indicating a directional change in time and treatment effects for macroinvertebrate composition. When considered separately, both treatment interactions were highly significant ($P < 0.01$), with Control treatment effects explaining 11% while Impact treatments effects explaining 23% (Table 7.3). This indicated that the macroinvertebrate communities were more variable in the Impact than the Control treatments in this river. The biplot indicated that taxa most common within Control treatment plots were odonates (Libellulidae and Coenagrionidae) and Planaria (Figure 7.3). The Impact treatment, by contrast, was strongly associated with Chironomidae, and Oligochaeta appeared uninfluenced by either treatment.

Generalised Linear Modelling illustrated that, within the Koonap River, Baetidae, Belostomatidae, Chironomidae, Hirudinea and Hydridae underwent significant directional changes associated with the treatment effects (Figure 7.4). Baetidae, Belostomatidae and Hydridae increased in abundance in the Control compared to the Impact treatment, whereas Chironomidae and Hirudinae appeared unaffected given that they increased in both treatments. By comparison, within the Brak River, eight taxa (Ancylidae, Belostomatidae, Caenidae, Chironomidae, Coenagrionidae, Libellulidae, Oligochaeta and Planaria) underwent significant ($P < 0.05$) directional change associated with the treatment (Figure 7.4). All taxa, except the Chironomidae and Oligochaeta, increased in abundance within the Control compared to the Impact treatment. The Chironomidae increased in the Impact treatment, while the Oligochaeta were unaffected and increased in both treatments.

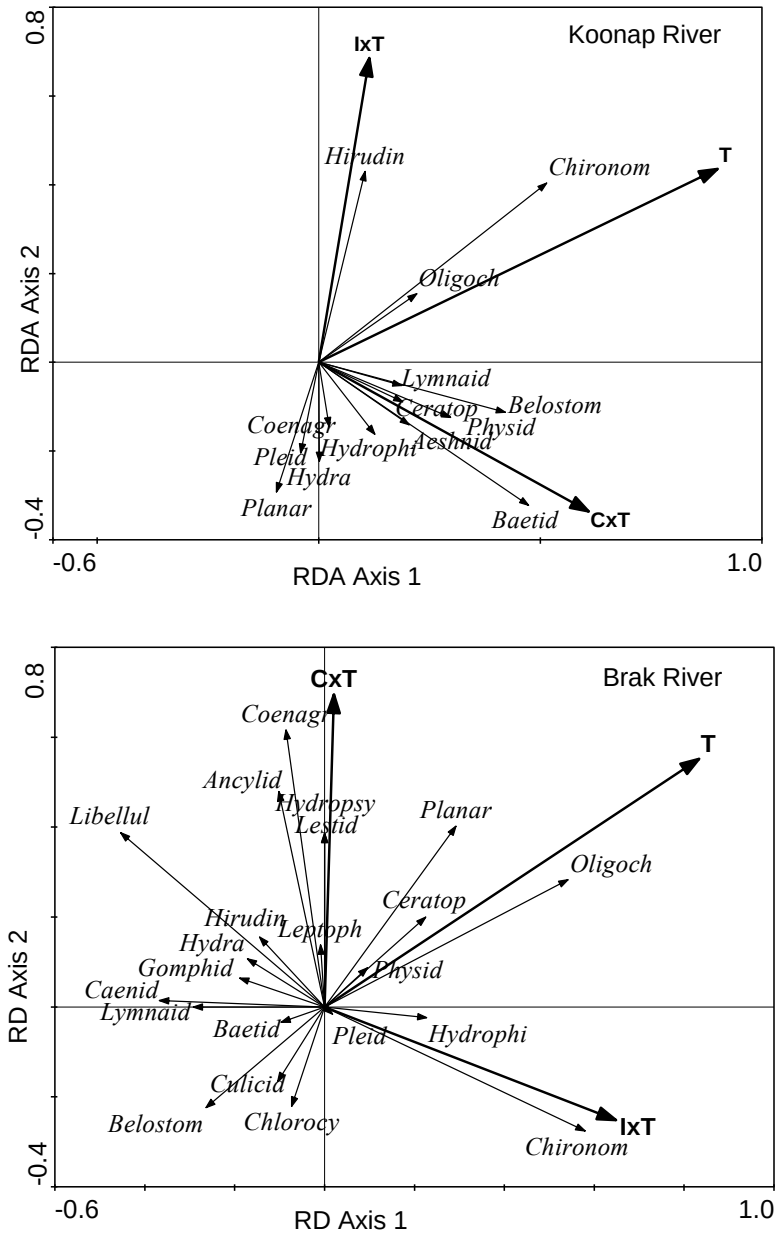

Figure 7.3: Partial RDA ordination plots for total macroinvertebrate counts indicating composition of the invertebrate communities in the catfish invaded Koonap River (top) and uninvaded localities in the Brak Rivers (bottom) in the Eastern Cape, South Africa. C = Control, I = Impact (the introduction of *Clarias gariepinus*), T = Time, and × indicates interactions between variables. The taxa are abbreviated as Aeshnid = Aeshnidae, Ancylid = Ancylidae, Baetid = Baetidae, Belostom = Belostomatidae, Caenid = Caenidae, Ceratop = Ceratopogonidae, Chironom = Chironomidae, Chlorocy = Chlorocyphidae, Coenagr = Coenagrionidae, Culicid = Culicidae, Gomphid = Gomphidae, Hirudin = Hirudinae, Hydroph = Hydrophilidae, Hydropsy = Hydropsychidae, Hydra = Hydridae, Leptoph = Leptophlebiidae, Lestid = Lestidae, Libellul = Libellulidae, Lymnaid = Lymnaeidae, Oligoch = Oligochaeta, Planar = Planaria, Pleid = Pleidae, Physid = Physidae.

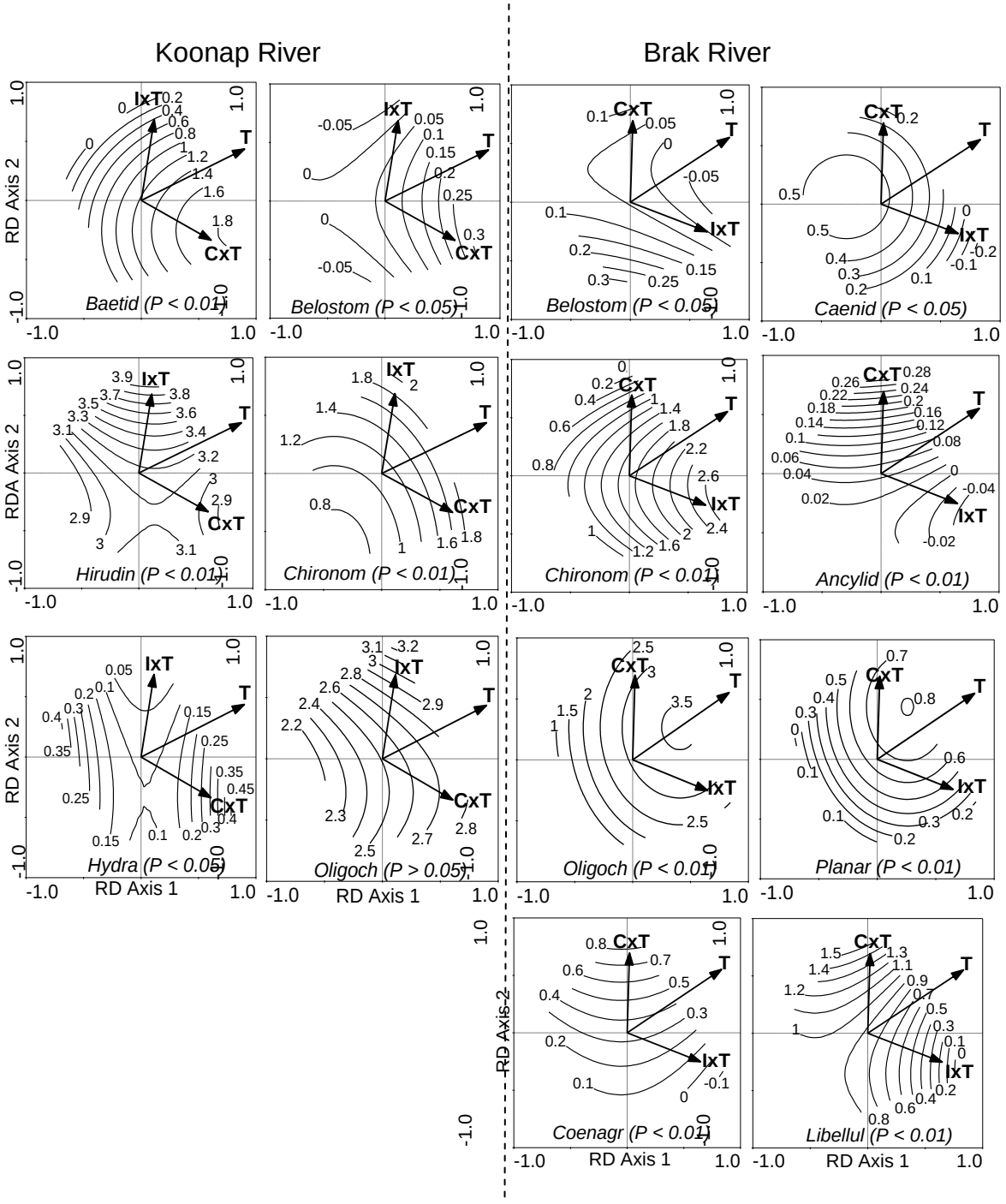

Figure 7.4: Generalised Linear Models indicating the relationship between macroinvertebrate taxa and treatment effects within the Koonap River that had high catfish density, and in the Brak River in localities that had no catfish. Contours on each plot indicate the predicted abundance value for each taxa. C = Control, I = Impact (the introduction of *Clarias gariepinus*), T = Time, and × indicates interactions between variables. Taxa abbreviations are given in Figure 7.3

Discussion

Comparing macroinvertebrate communities between catfish uninvaded and invaded streams revealed contrasting temporal patterns. Within the invaded Koonap River, high taxa diversity and richness were observed in the Control treatment that excluded catfish relative to the Impact treatment with catfish where macroinvertebrates showed less temporal variation. In addition, macroinvertebrate biomass and abundance were uninfluenced by either treatment in the invaded river. This demonstrated that catfish impact was associated with less temporal variation in macroinvertebrate composition, whereas excluding catfish increased community structure within invaded habitats. By comparison, within the univaded Brak River, catfish introduction demonstrated an impact on macroinvertebrate composition, a pattern that was consistent with observations from previous studies on effects of introduced fish on macroinvertebrates in previously uninvaded habitats (Carlisle & Hawkins 1998; Knapp *et al*., 2001; Simon & Townsend, 2003). This study, therefore, revealed different macroinvertebrate responses to the presence of non-native catfish within invaded and uninvaded streams, and suggests that the invader plays a role as a disturbance in influencing macroinvertebrate communities.

Although other studies indicate that macroinvertebrate communities can resist change through rapid colonisation and use of alternative refugia (Collier & Quinn, 2003), this study suggests that adaptive responses to invasive predators may differ in habitats with and without fish. Macroinvertebrates in fish-containing habitats tend to develop anti-predator responses to fish (Schilling *et al*., 2009). In addition, establishment of invasive predators may induce behavioural adaptations to reduce encounters with predators (Simon & Townsed 2003; Abjornsson *et al*., 2004), and morphological adaptations, such as dominance of small-bodied morphs (Meissner & Moutka, 2006; Kitano *et al*., 2008). In contrast, macroinvertebrates within fishless or recently invaded habitats often lack such adaptations and may therefore be

more prone to predation. These contrasts may therefore explain the macroinvertebrate composition and their different responses within the invaded Koonap River and univaded Brak River. In addition to the non-native catfish, the Koonap River had other fish species that include the anguillid eel (*Anguilla mossambica*), moggell (*Labeo umbratus*) and smallmouth yellowfish (*Labeobarbus aeneus*), whereas the Brak River was fishless.

Several studies have shown that introduced fish exert a pronounced effect on macroinvertebrates in recently fishless invaded habitats. This is because of the inability of macroinvertebrate prey to respond to predators that they do not naturally co-exist with (Knapp *et al*., 2001; Stoks *et al*., 2003; Schilling *et al*., 2009), due to a lack of co-evolved adaptive mechanisms (Simon & Townsend, 2003). Within the univaded Brak River, catfish impact was reflected by a decrease in taxa diversity, richness and biomass. Catfish impact was further supported by multivariate analysis that showed high variation in macroinvertebrate composition in Impact treatment plots, which suggests disruption of the community by the invader. Macroinvertebrate abundance was, nonetheless, uninfluenced by catfish presence. Although some studies have demonstrated impacts on macroinvertebrate abundance (Englund & Polhemus, 2001; Maezano *et al*., 2005), others indicate that loss of certain taxa is mediated by an increase in abundance of those taxa that are not negatively affected by the predators, suggesting that impacts are may be related to the response of individual macroinvertebrate taxa (Meissner & Moutka, 2006). Assessment of macroinvertebrate composition within the univaded Brak River reflected the different responses by individual taxa to catfish impact. In particular, the results indicated low abundance of coenagrionid and libellulid odonates in Impact treatment plots, whereas the chironomid midges and oligochaetes appeared uninfluenced by the presence of the predator. The most common response of macroinvertebrates to predation by non-native fish is a decline in the densities of vulnerable taxa (Kadye & Magadza, 2008; Johnson *et al*., 2009; Duxbury

et al., 2010). Large-bodied odonates are usually the most vulnerable group in recently invaded habitats (Englund & Polhemus, 2001; Maezono & Miyashita, 2003; Maezano *et al*., 2005) because invasive predators are opportunistic feeders that tend to target the most conspicuous and accessible prey (Miller & Crowl, 2006; Johnson *et al*., 2009; Weyl *et al*., 2010). Odonates are usually dominant macroinvertebrate predators that play a crucial role as keystone predators, especially in fishless and univaded habitat (Donald & Anderson, 2003). In other studies, the elimination of odonates by invasive predators has been observed to correspond to increase in abundance of their potential prey, which reflects trophic cascades (Maezono & Miyashita, 2003; Phillips *et al*., 2009). Within the Brak River, the high abundance of chironomid midges in Impact treatment relative to the Control treatment may suggest a positive response to low abundance of odonate predators. Other studies have also reported invasion paradoxes whereby the elimination of keystone predators by invasive predators facilitates invasions by other species (Shurin, 2001). Such facilitative invasions can have detrimental effect as the multiple invaders can interact at the expense of the native biota causing invasion "meltdown" (Simberloff & Von Holle, 1999).

In contrast to the univaded stream, within the invaded Koonap River, macroinvertebrates showed little response to catfish impact. Some studies indicate that when non-native predators become established, macroinvertebrates assemblages become less responsive as they become dominated by taxa that are either uninfluenced by the presence of predators (Meissner & Moutka, 2006) or those that show local adaptation to predator presence (Simon & Townsend, 2003; Abjornsson *et al*., 2004). Local adaptations include predator avoidance behaviour, such as altered drift behaviour, use of interstitial spaces for refuge and rapid dispersal by mobile taxa into habitats without predators (Douglas *et al*., 1994; Englund *et al*., 2001; Schilling *et al*., 2009). Within the invaded Koonap River, lack of macroinvertebrate response was particularly reflected by patterns in both abundance and

biomass that did not differ between treatments, and little variation in diversity and richness within Impact treatment, which suggest dominance of resilient taxa. Predator avoidance behaviour may further explain the high taxa diversity and richness observed within Control treatment plots relative to Impact treatment plots in the invaded Koonap River. This avoidance behaviour was also inferred from multivariate analyses that indicated strong association of some taxa, such as Baetidae, Belostomatidae and Hydridae in Control treatment. In particular, the small-bodied baetid mayflies, *Baetis* spp., have been observed to quickly recover under experimentally-induced predation disturbances, and they actively searched for patches that provided more refuge (Meissner & Moutka, 2006). Mayflies are also known to disperse over a large area and to quickly adapt within disturbed environments (Gibbs *et al*., 1998; Knapp *et al*., 2001; Effenberger *et al*., 2008). This observation is consistent with suggestion that disturbance induce instability to macroinvertebrate communities (Muehlbauer *et al*., 2011), which result in assemblages dominated, in addition to the resistant taxa, by those that can either quickly recolonise or find alternative refuge if the disturbance does not impair the habitat (Niemi *et al*., 1990).

Resilient taxa were reflected within Impact treatment for both the invaded and univaded rivers. This was particularly reflected by the abundance of taxa such as leeches (Hirudinea), earthworms (Oligochaeta) and midges (Chironomidae). Assessment of community attributes based on multivariate analysis further indicated the association of the leeches, midges and earthworms with Impact treatment in both rivers. These taxa usually show little negative response to disturbances (Miller & Crowl, 2006), including predation (Knapp *et al*., 2001). Some studies have attributed the resilience of these macroinvertebrate taxa to both their lifestyle because they are less active benthic-dwelling deposit-feeders that are usually not affected by the presence of invasive predators (Carlisle and Hawkins 1998), and indirect positive effects through elimination of their competitors (Knapp *et al*., 2001;

Johnson *et al*., 2009). Maezono *et al.* (2005) also found no evidence of predation pressure on these predation-resilient taxa in experimental treatments with non-native largemouth bass (*Micropterus salmoides*) and bluegill sunfish (*Lepomis macrochirus*) that eliminated large-bodied taxa in Saitama Prefecture, eastern Japan. In the present study, leeches and midges appeared to benefit from predation impact as they were more abundant in the presence of catfish relative to its absence, whereas earthworms appeared to be unaffected group by either treatment. Leeches were unlikely to be negatively affected by the impact because they are generally less mobile and tend to cling firmly to substrates by means of posterior sucker. Some leeches are generally known to feed on a wide range of prey, whereas other leeches are strictly parasitic (Graf *et al.*, 2006; Sket & Trontelj, 2008), although there was no evidence of the latter in this study. Their high abundance during this study suggests that they were probably detritovorous. Similarly oligochaetes and chironomids are detritivores that are known to be abundant within impacted habitats because they tend to burrow into or live within soft substrata, and benefit from both the elimination of their competitors and increased exposure to resources when a detritus-based food web dominates within disturbed environments (Knapp *et al*., 2001; Ruetz *et al*., 2002; Miller & Crowl, 2006; Weyl *et al.*, 2010).

From a disturbance perspective, this study reflected different levels of responses to catfish impact within invaded and univaded streams. The overall impact patterns within the two rivers supports Parkyn and Collier's (2004) observations on press disturbances whose initial impact result in immediate changes in communities, as observed in the Brak River, before they reach a new level comprising of a less responsive community that quickly adapts to the disturbance, and possible maintained following a shift in species composition, as suggested by the patterns observed in the Koonap River. Within the Eastern Cape region, the catfish has established in many rivers and dams, and the main concern is on both its predation

impact and potential to influence trophic interrelationships (Kadye & Booth 2011). Macroinvertebrate recolonisation and within-stream refugia may be compromised as the catfish can utilise a wide range of habitats. Nevertheless, efforts should be made to protect unaffected tributaries and, where possible, eradicate the invasive catfish.

CHAPTER 8

Movement Patterns and Habitat Selection by Non-native African Sharptooth Catfish *Clarias gariepinus* within a Warm-temperate Reservoir

Introduction

Evaluating habitat use by fish is crucial in understanding those factors that influence their distribution and resource use. Habitat selection studies attempt to determine habitat use in relation to its availability (Manly *et al*., 2002; Hirzel *et al*., 2004; Austin, 2007), which in turn, depends upon spatial and temporal resource availability (Mauritzen *et al*., 2003; Mosnier *et al*., 2003; Gillies *et al*., 2006). Habitat use by fish is known to vary with prey abundance (Giannico, 2000), habitat availability (Dauherty & Sutton, 2005), presence of predators and competitors (Brown & Moyle, 1991), and varying environmental conditions. Several studies have also shown non-proportional use of certain habitats in response to disproportionate availability of influential resources (Mysterud & Ims, 1998; Gillies *et al*., 2006; Hansen *et al*., 2009).

Within invaded freshwater habitats, information on fish habitat use is essential because non-native invaders have the potential to influence both resource availability and the distribution of native species (McIntosh *et al*., 1994; Bosch *et al*., 2006; Kadye & Magadza, 2008). By understanding habitat selection and movement of non-native invasive species potential impacts can be determined and management strategies developed in mitigation (Carol *et al*., 2007; Lapointe *et al*., 2010). In the Eastern Cape, South Africa, the African sharptooth catfish *Clarias gariepinus* has established as an invasive species within many rivers and impoundments (de Moor & Bruton, 1988; Laurenson *et al*., 1989; Potts *et al*., 2008). The catfish is a highly mobile and aggressive predator within invaded habitats (Booth

et al., 2010). While the movement patterns of catfish have been studied in both lotic (Willoughby & Tweddle, 1978; Cambray, 1985) and lentic ecosystems (Bruton, 1979a, 1979b; Hocutt, 1989) within its natural range, no information is available for invaded habitats. This catfish's movement patterns and habitat use are primarily driven by foraging needs and reproductive biology (Bowmaker, 1973; Bruton, 1978; 1979c; Merron, 1993). Catfish has been described as an opportunist species that feeds alone or groups (Bruton, 1979a; Merron, 1993). Movements for spawning are often triggered by changes in photoperiod, temperature and water flow, with catfish moving into inundated shallow habitats in lentic habitats and headwaters of lotic habitats to spawn (Bowmaker, 1973; Bruton,1979c; Hocutt, 1989). The catfish exhibits both long and short distance movements, depending on environmental conditions and food availability (Willoughby & Tweddle, 1978; Hocutt, 1989).

This study examined the movement and habitat use of catfish using acoustic telemetry within an invaded impoundment in the Eastern Cape Province, South Africa. Telemetry is a useful tool for determining movement and habitat use, and has been used to study several catfish species within their natural range (Hocutt, 1989; Daugherty & Sutton, 2005; Mitamura *et al*., 2008), and also within habitats where they are non-native (Carol *et al*., 2007). The objectives of this study were to (1) determine the patterns in movement and habitat use and (2) examine any seasonal changes in habitat use or preferences by catfish within the dam. These objectives were tested under the null hypotheses that catfish distribution was random, and was not influenced by seasonal changes, especially in temperature and other physical variables such as depth and habitat complexity.

Materials and methods

Study area

Glen Melville Dam (33°129 S; 26°409 E), constructed in 1992, covers an area of 76 ha and has a maximum depth of 25 m at maximum capacity. The dam is regulated by water transferred from the Great Fish River through an inter-basin water transfer (IBWT) scheme. This regulation involves filling of the dam between February and March and between July and August each year. The substratum is comprised of shale and mud with drowned trees mostly in the former river channel. Water surface temperature ranged from 27°C in February to 14°C in July. The pH was mostly alkaline and ranged between 8 and 9, and turbidity was relatively high, ranging between 129 - 257 NTU during the study period.

Tag implantation and data collection

A pilot experiment was conducted to determine tag loss or expulsion because clariids have been shown to exhibit trans-intestinal expulsion of surgical implants (e.g. Baras & Westerloppe, 1999). Three catfish were surgically equipped with dummy tags that were identical to the acoustic tags (Thelma Biotel, Trondheim, Norway) following methods described by Jespen *et al*. (2002). Fish were anesthetised with clove oil, measured (SL cm) and weighed (g). The fish were 81.9 cm, 65.1 cm and 56.7 cm and weighed 3320 g, 1660 g and 860 g, respectively. The dummy tags were implanted into the peritoneal cavity through a midventral incision that was closed by three separate non-absorbable sutures. Each catfish was maintained in captivity in a separate concrete tank together with two untagged catfish. Catfish were fed daily and monitored weekly for two months from December 2010 to February 2011. The incisions were observed to heal within 7 to 14 days with the sutures

being shed. There was neither evidence of tag expulsion nor signs of deleterious effects of the tags on catfish behaviour or feeding during the experiment.

After the pilot experiment, range tests were conducted to determine the minimum detection distance. This was conducted by detecting three receivers that were randomly placed in different locations within the dam. Ten catfish were captured within the south bay (Figure 8.1) using long lines that were baited with chicken livers. The fish, which were randomly identified as Fish 1 to Fish 10, and measured (84.0, 38.7, 40.6, 58.0, 82.0, 95.8, 100.1, 100.0, 91.8 and 78.0 cm TL) and weighed (3620, 520, 680, 1230, 3380, 5520, 6320, 5960, 5430 and 2960 g), giving tag-to-body mass ratios < 1.08%. Each fish was anesthetised in clove oil and surgically implanted with LP - 13 acoustic tags (Thelma Biotel, Trondheim, Norway) using the dummy tag surgical procedure. After recovery the fish were released at the capture point. The acoustic tags were 13 mm in diameter, weighed 5.6 g (in water), had a pulse interval of 1000 ms and a delay rate of 5 - 15 seconds. Each tag had a guaranteed life span of 4.2 months and an estimated life span of 6.5 months. The tags transmitted at a frequency of 69 kHz. Manual tracking of the tagged fish was conducted in both summer and winter using a VEMCO VR60 receiver with a directional hydrophone (VEMCO, Halifax, Nova Scotia, Canada) approximately every fortnight from February 2011 to June 2011, and once in July 2011. Catfish were tracked from 05h00 to 19h00 and once over a 24 h cycle. During each tracking session, fish positions were sequentially monitored from the dam wall to the river mouth, and the exercise was repeated after every 3 hours. Each tracking period took approximately 2 hours to complete. Each fish's location was recorded using a portable GPS. GPS coordinates outlining the map of the dam and habitats were also taken when the dam was at full capacity, in February 2011. There was little seasonal variation in water level as the dam was refilled to capacity April 2011. Temperature was measured using a HANNA HI 98129 Combo meter during each sampling occasion.

The main habitat types were identified and categorised by depth that was measured from 30 random locations within each habitat. These were river and river mouth (< 3 m depth), upper (3 - 10 m), middle and dam wall (> 15 m), shallow south bay (0 - 8 m), and deep north bay (0 - 15 m) (Figure 8.1). The river mouth was characterised by shallow and inundated habitat with rocky substrate and submerged trees. The upper, south bay and north bay were characterised by submerged trees while the middle and dam wall habitats were deep and generally unstructured.

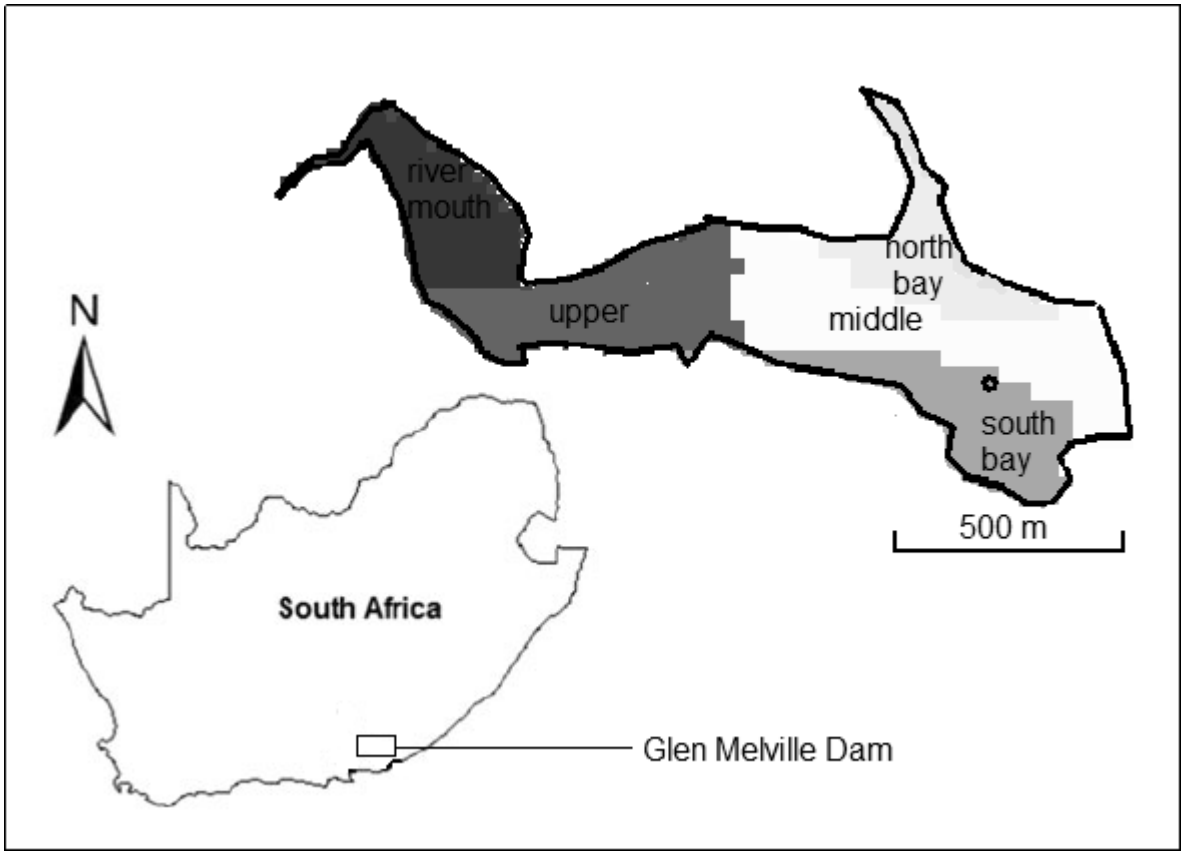

Figure 8.1: Map of the study area illustrating the main habitat types within the Glen Melville Dam, Eastern Cape, South Africa. Increasing intensity in the greyscale inset map indicates decreasing depth. The dot within the south bay represents the joint capture and release point for catfish.

Data analysis

GPS coordinates for catfish relocations were transformed into Universal Transverse Mercator (UTM) units. Home range size for individual catfish was determined using minimum convex polygons (MCP) for 50 to 95 % of the relocations by calculating the smallest convex polygon that encompasses all relocations (Mohr, 1947). Home range sizes were compared for the summer (temperature > 20°C) and winter (temperature < 20°C) periods. Kernel estimation of the utilisation distribution (KUD) (Van Winkle, 1975; Worton, 1989) was used to describe the probability density of the relocations. The smoothing parameter (*h*) for KUD was estimated using least squares cross-validation (LSCV). Ecological-niche factor analysis (ENFA, Hirzel *et al*., 2002) was used to study the patterns in habitat selection by the catfish population. ENFA searches for gradients in ecological space that maximises the differences between the utilised and available habitats or resources (Basille *et al*., 2008). ENFA is based on both marginality and specialisation. Marginality measures the magnitude of deviation between the niche (used space) and available space, and specialisation measures the narrowness of the niche based on the highest variance of the ratio between available and utilised habitat (Calenge, 2006; Basille *et al*., 2008). Compositional analysis was used to test for habitat preference. This was followed by application of Manly *et al*.'s (2002) index, $w_j = \frac{u_j}{a_j}$ to test the selection ratios of the different habitat categories, where u_j is the proportion of use of the habitat category j and a_j is the proportion of availability of this habitat category j. The selection ratios for all habitats were normalised to $B_j = \frac{w_j}{\sum_j w_j}$ (Manly *et al*., 2002). A chi-square test was performed for the Manly's selection ratios to test the null hypothesis of random selection of habitat categories.

Comparison between summer and winter habitat selection was conducted using a Principal Components Analysis-based Outlying Mean Index (OMI, Doledec *et al*., 2000) analysis.

All the analysis were conducted with *R* (R Core Development Team, 2011) using the libraries *adehabitatHR* and *adehabitatHS* (Calenge, 2006).

Results

Movement and habitat selection

All tagged catfish were located 265 times during the sampling period. Most of these locations were further from the common capture and release point near the dam wall. The spatial polygons for catfish relocations indicated both long and short distance movements (Figure 8.2). While the long distance movements were related to those relocations furthest from the capture and release point, the short distance movements showed patterns that appeared to show localised movements within single localities (Fish 2, 4, 5 and 10), widespread movements within single localities (Fish 1, 7, 8 and 9), and localised movements within multiple localities (Fish 3 and 6) (Figure 8.2). A similar pattern was observed for the KUD densities for individual catfish home ranges (Figure 8.3), supporting the observation of spatial relocation polygons. The ENFA biplot, an ordination of habitat utilisation in relation to its availability, showed that distance between the centroid of the available habitat and that of the ecological niche was high, resulting in high marginality (Figure 8.4). This indicates that the optimal space utilised by the catfish population was different from that which was available. By comparison, the specialisation axis showed that the variance of the available habitat was higher that the variance of used habitats. The upper and middle habitat types contributed most

to the marginality whereas the river mouth and south bay habitats contributed to the specialisation.

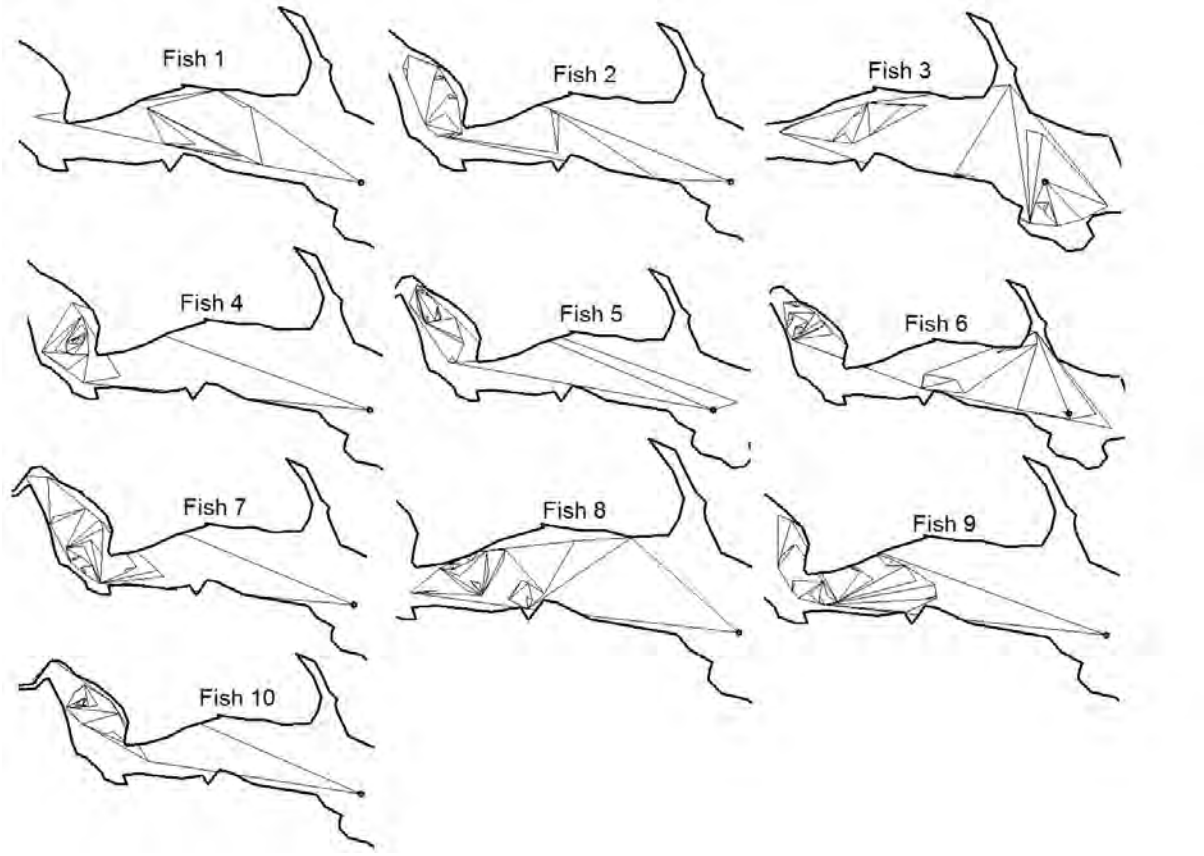

Figure 8.2: Spatial polygons for individual sharptooth catfish *Clarias gariepinus* relocations within Glen Melville Dam, Eastern Cape, South Africa. The dot represents the joint capture and release point for catfish.

Compositional analysis indicated that there was evidence of habitat selection ($\lambda = 0.16$, $P = 0.03$) that was supported by Manly's index of habitat selection ($\chi^2_4 = 211.9$, $P < 0.01$). Manly's index showed significant association (i.e. lack of independence) between catfish and all habitat categories ($P < 0.01$) (Table 8.1). The standardised selection ratios (B_j) indicates that the river mouth and upper sections of the dam were the most preferred habitats (Table 8.1).

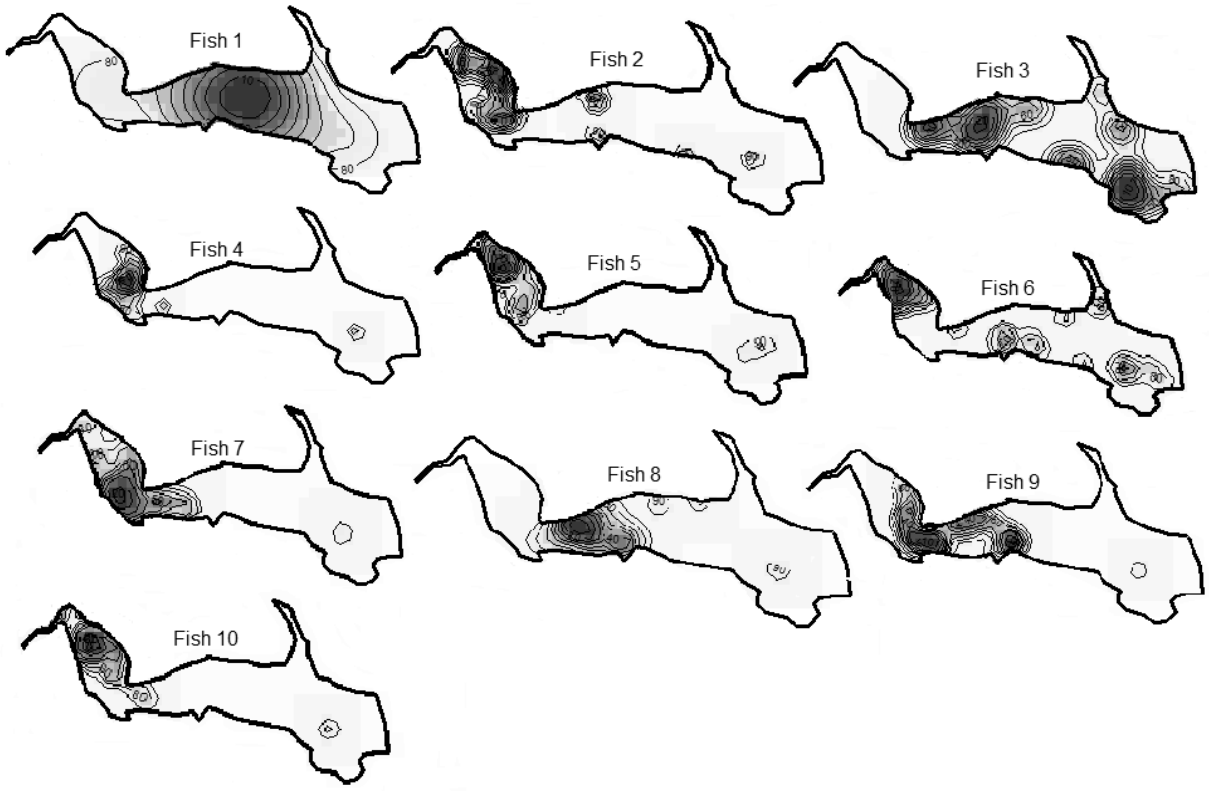

Figure 8.3: Kernel utilisation distribution densities, depicted as raster maps, for individual sharptooth catfish *Clarias gariepinus* within Glen Melville Dam, Eastern Cape, South Africa. Contours illustrate home range size at different probability levels.

Table 8.1: Proportion of used and available habitats and the selection ratios based on Manly's index for tagged sharptooth catfish *Clarias gariepinus* in Glen Melville Dam, Eastern Cape, South Africa. w_j is Manly's selection ratio, $se(w_j)$ is the standard error of the selection ratio, $P(w_j)$ in the statistical probability of the selection ratio and B_j is the standardised selection ratio.

Habitat type	Used	Available	w_j	$se(w_j)$	$P(w_j)$	B_j
River mouth	0.44	0.18	2.52	0.18	0.00	0.50
Upper	0.39	0.24	1.65	0.13	0.00	0.33
South bay	0.10	0.21	0.46	0.09	0.00	0.09
North bay	0.02	0.12	0.19	0.08	0.00	0.04
Middle	0.05	0.26	0.19	0.05	0.00	0.04

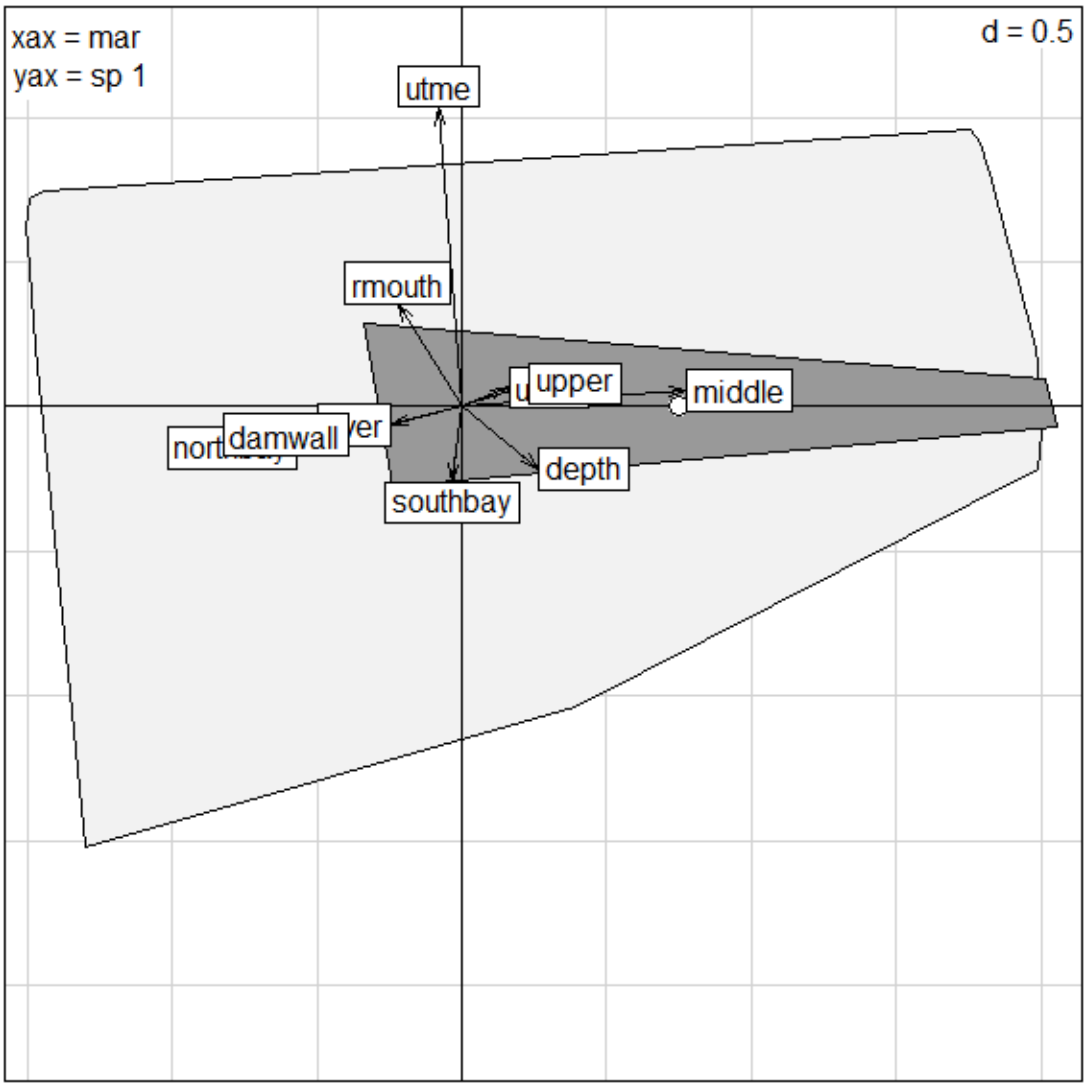

Figure 8.4: Ecological-niche factor analysis (ENFA) for sharptooth catfish *Clarias gariepinus* habitat use within Glen Melville Dam, Eastern Cape, South Africa. The plot indicates factorial maps with the dark grey polygon showing the minimum convex polygon (MCP) of used resource units (RUs) whereas the light grey area depicts the projection MCP of the available sites RUs. The abscissa displays the marginality axis (xax = mar) of ENFA whereas the ordinate displays the first specialisation axis (yax = sp 1) of ENFA. The white dot along the abscissa corresponds to the centroid of the used habitat. The environmental factors include habitat variables and the coordinates of fish locations (utme and utms).

Temporal patterns in habitat selection

Catfish home range sizes for 95% relocations ranged from 2 (Fish 1) to 70 ha (Fish 3) (Figure 8.5). The proportion of home range size for 50 - 95% relocations was large during summer

for most catfish except for three fish (Fish 1, 3 and 6) that had large home ranges during winter (Figure 8.5).

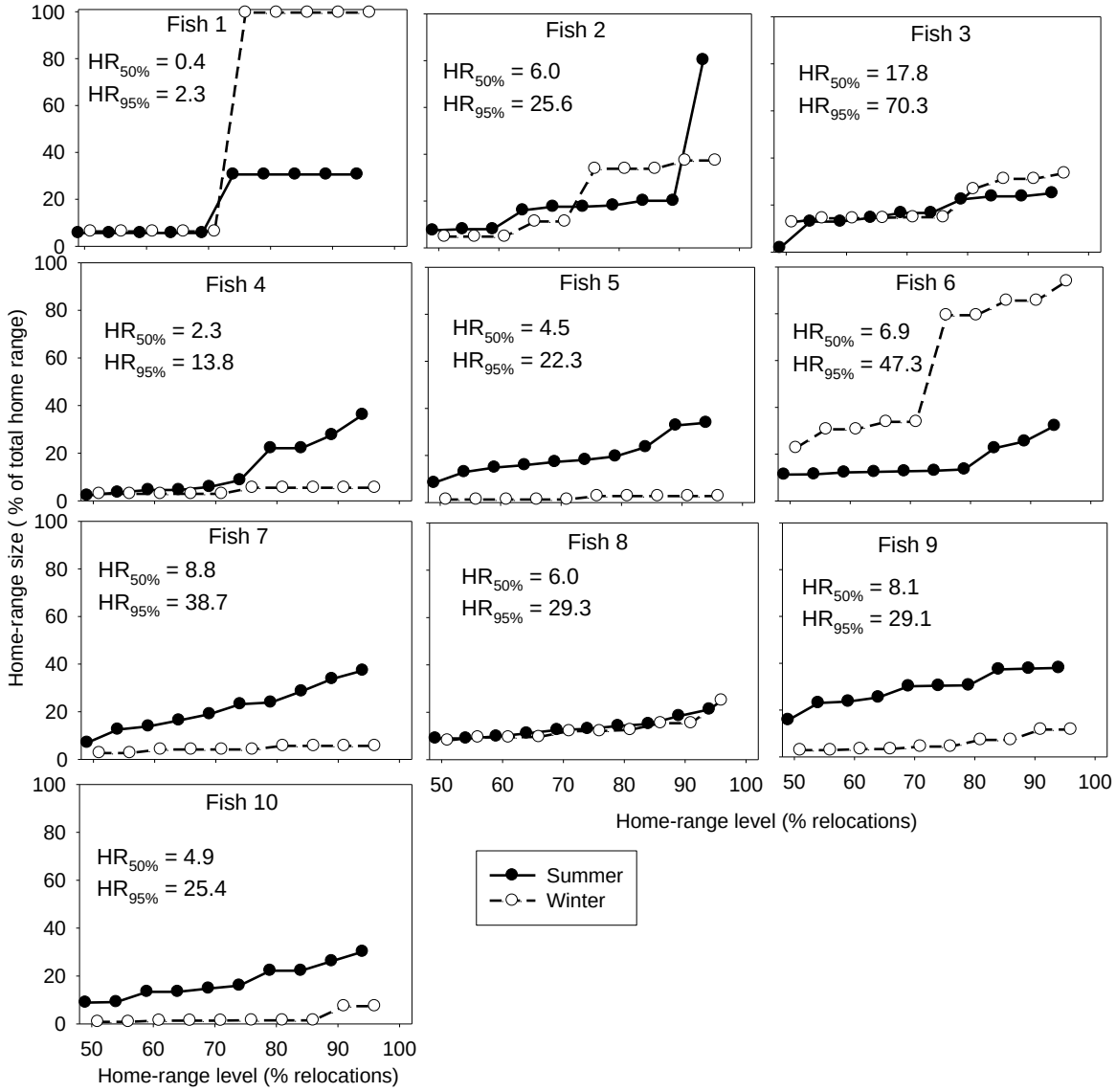

Figure 8.5: Total home range size (hectares) for 50 and 95% relocations of individual sharptooth catfish *Clarias gariepinus* and the proportion of these relocations during both the summer and winter sampling periods within Glen Melville Dam, Eastern Cape, South Africa.

OMI analysis for habitat selection during summer showed that the first and second eigenvalues accounted for 78 and 18 % of the marginality within the data (Table 8.2). The

samples and species plots, depicting the projection of the resource units (samples) and that of the distribution of utilisation weights of individual catfish, showed that six catfish were characterised by a strong selection to the negative values of the first axis (Figure 8.6). The variables plot showed that the negative values corresponded to the river mouth habitat that was strongly correlated to the first axis (Table 8.2). Two catfish (Fish 8 and Fish 9) were strongly associated with the negative values of the second axis on the samples and species plot (Figure 8.6), which corresponded to the upper habitat (Table 8.2). Two other catfish (Fish 1 and Fish 3) were characterised by weak association with either axes. During winter, the first and second axes explained 64 and 29 % of the marginality of the OMI analysis, respectively (Table 8.2). Utilisation weights showed that most catfish were characterised by strong selection to negative values of the first axis where four catfish were associated with river mouth habitat and three others were associated with the upper habitat. A notable shift was observed for Fish 3 and Fish 6 that appeared to utilise the deep and dam wall habitat during the winter compared to summer period.

Table 8.2: Outlying Mean Index eigenvectors and eigenvalues for first and second Principal Components for the habitat variables selected by sharptooth catfish *Clarias gariepinus* within Glen Melville Dam, Eastern Cape, South Africa.

	Summer		Winter		Diel	
	PC1	PC2	PC1	PC2	PC1	PC2
River mouth	-0.61	0.35	-0.36	-0.64	0.59	0.49
Upper	0.02	-0.81	-0.39	0.73	0.07	-0.73
Middle	0.18	0.23	0.22	-0.02	-0.23	0.09
North bay	0.15	0.16	0.18	-0.07	-0.17	0.07
South bay	0.15	0.09	0.26	-0.08	-0.16	-0.01
Dam wall	0.10	0.11	0.13	-0.07	-0.08	0.32
Depth	0.46	0.25	0.48	0.06	-0.48	0.14
Eigenvalue	4.4	1.0	2.4	1.1	4.7	0.2
% variation	77.6	18.2	63.7	28.8	94.3	4.1

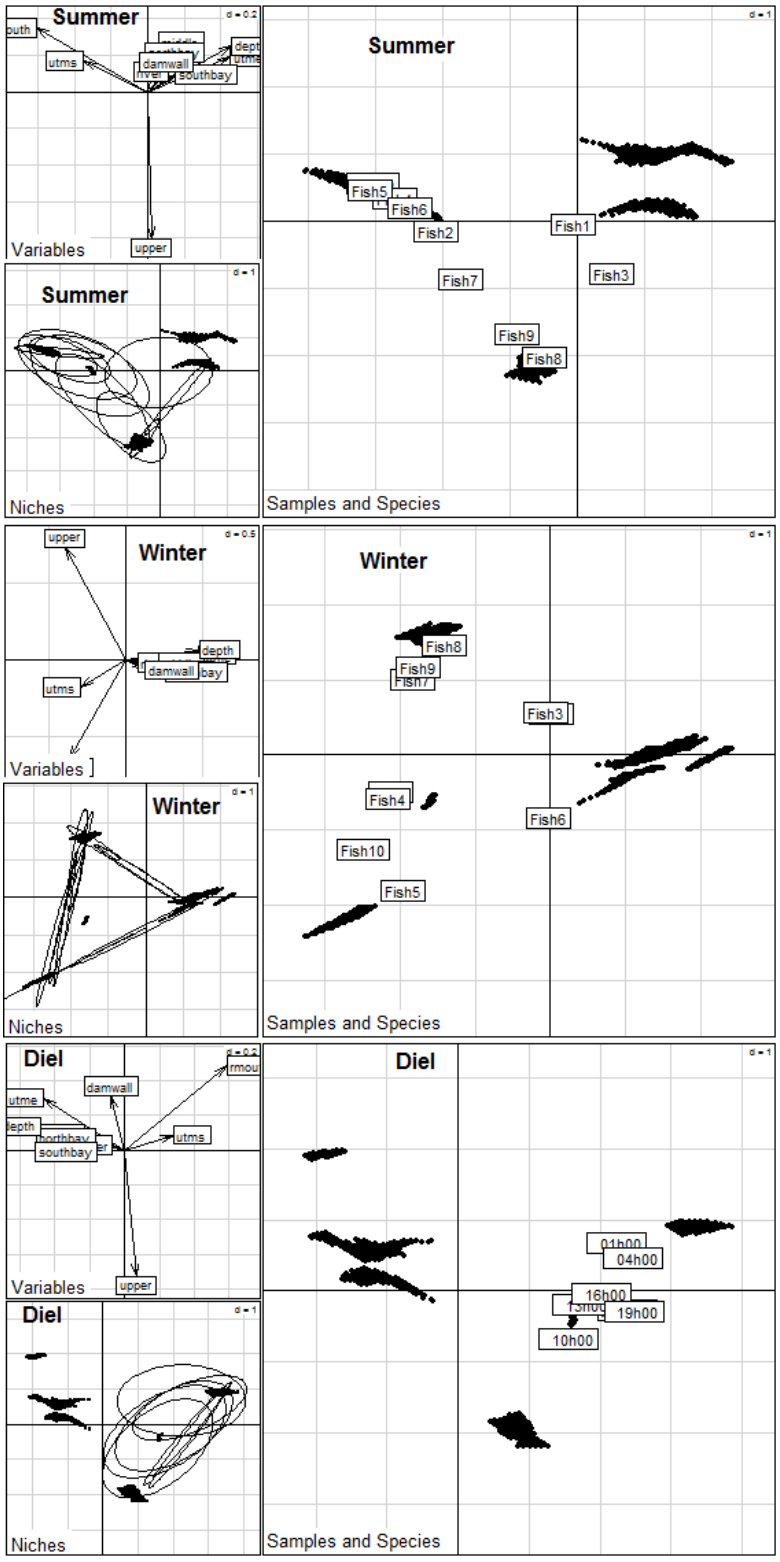

Figure 8.6: Outlying Mean Index (OMI) analysis indicating patterns in sharptooth catfish *Clarias gariepinus* habitat selection during both the summer (temperature > 20°C) and winter (temperature < 20°C) sampling periods and for summer diel movements within Glen Melville Dam, Eastern Cape, South Africa.

Diel patterns in catfish movements showed utilisation of upper habitats during midmorning and the river mouth habitat after midnight (Figure 8.6). These patterns coincided with two peaks in home range size, with wider home ranges being observed during midmorning (10h00) and after midnight (01h00) (Figure 8.7).

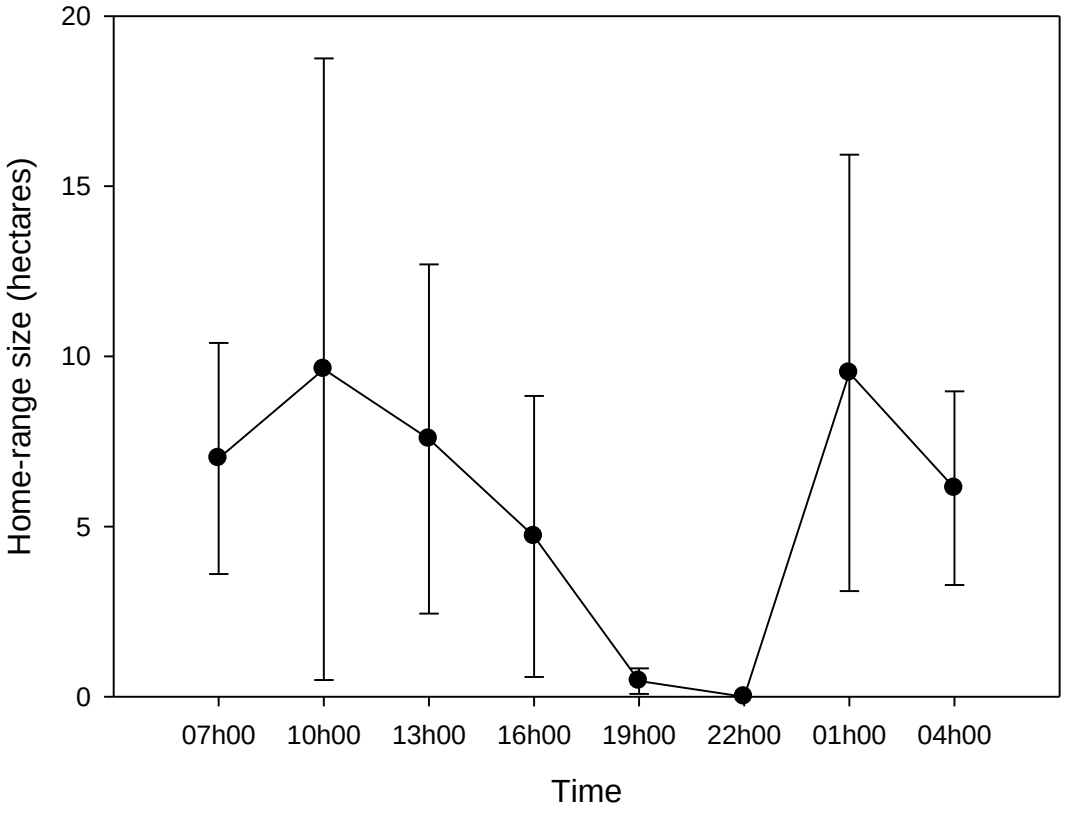

Figure 8.7: Diel changes in home range (mean ± standard deviation) size for all sharptooth catfish *Clarias gariepinus* relocations during summer in Glen Melville Dam, Eastern Cape, South Africa.

Discussion

Catfish exhibited both long and short distance movement patterns within Glen Melville Dam. The short distance patterns suggest that these movements varied from localised relocations on both small and broad spatial scales within single habitats, to localised movements within multiple habitats. Hocutt (1989) described three types of movements in radio-tagged catfish

in Lake Ngezi (Zimbabwe). These were long-distance movements exceeding 200 m, moderate movements within 40 - 200 m and local movements not exceeding 40 m. Long-distance movements in catfish usually coincide with seasonal activity peaks, mostly related to increasing temperature or flood peaks that induce potamodrometic spawning migrations into either headwaters of rivers (Bowmaker, 1973; Cambray, 1985) or lateral movements into shallow and inundated marginal habitats in lakes (Bruton, 1979c; Hocutt, 1989). During this study, catfish were tagged when the dam was at its maximum capacity. Most catfish exhibited long-distance movements immediately after release, and were recorded mostly in the river mouth section and the south bay. These are patterns associated with spawning behaviour. Within the dam, it is likely that the river mouth section would be the most probable spawning habitat for the breeding population of catfish. The sharptooth catfish is, nonetheless, also known to utilise shallow and marginal habitats for feeding (Bruton, 1979a).

In comparison to long-distance movements, short-distance movements strongly defined the home range sizes of catfish during this study. Hocutt (1989) showed that local movements were the dominating mode of behaviour in catfish. Similar patterns have been inferred for the homing behaviour of catfish within lotic habitats within the lower Shire River, Malawi (Willoughby & Tweddle, 1978). Telemetry studies have also shown localised home ranges and territoriality in other catfish species, such as the flathead catfish, *Pylodictis olivaris*, in the St Josephs River, Michigan, USA (Daugherty & Sutton, 2005) and the non-native wels catfish, *Silurus glanis*, in the Flix Reservoir, Ebro River, Spain (Carol *et al*., 2007). Localised movements in catfishes are related to the development of high-use areas of particular habitats where fish would typically exhibit multiple displacements within a small but structured habitat (Daugherty & Sutton, 2005). Catfish home ranges may however change with seasonality, especially due to changes in temperature as fish often utilise deeper habitats during winter (Weller & Winter, 2001). During this study, however, most catfish appeared to

maintain their summer home ranges by exhibiting localised movements. Nonetheless, there were few individuals that showed localised movements in multiple habitats, which suggest a seasonal shift in home ranges.

ENFA is based on the ecological niche concept (Hirzel *et al*., 2002). In ENFA, marginality is used to identify the niche for individuals, populations or species from available resources, whereas specialisation is identified by the narrowness of the niche, depicting the restriction of occurrence to particular environmental variables (Basille *et al*., 2008). During this study ENFA showed both high marginality and specialisation for the tagged catfish population. This was further supported by compositional analysis, which showed strong evidence of habitat selection, and Manly's index of selection ratios, which indicated strong selection for river mouth and upper habitats. The null hypothesis that habitat selected by catfish within the dam was random was rejected providing some evidence of habitat preferences. The upper and river mouth habitats, the most structured habitats, were probably the most suitable for refuge and feeding for both the invasive catfish and native species such as moggel *L. umbratus*. Utilisation of these upstream habitats by catfish suggests a probable impact related to predation and interference competition for feeding and breeding space. Feeding studies indicate that catfish diets are dominated by fish including moggel within the dam (Kadye & Booth, 2011).

Seasonality is an important factor influencing animal distribution. Comparison of habitat utilisation on a temporal scale showed that during summer, most catfish were associated with the river mouth, while few were in the upper habitat. These results corroborated observation on catfish relocations, suggesting that the river mouth was the most important habitat for catfish during summer. Bruton (1979c) observed that during summer, catfish preferred shallow inshore littoral habitats both for breeding and feeding. Within these shallow habitats, individuals would display different foraging strategies such as social

hunting and surface feeding on floating debris, terrestrial insects, plankton and crustaceans, and organised pack hunting for the preferred fish prey (Bruton, 1979c; Merron, 1993). Bruton (1979b) also noted diel incursions into the littoral habitats, and concluded that the sharptooth catfish was an efficient nocturnal feeder, particularly in clear habitats given that it has a poor eyesight. In contrast, Hocutt (1989) reported both diurnal and nocturnal activity in movements, and further suggested that the former was predominant. Although the diel movements were conducted on a small scale during summer in this study, the results indicate two peaks in catfish activity that showed both day and night peaks in movement, corroborating Hocutt's (1989) observations. These observations were partially supported by OMI analysis that showed high marginality with a small but important transition in habitat use that showed diurnal peak in activity within the upper habitat and nocturnal peak within the river mouth. During winter, there was a notable shift in habitat use by some catfish. Three catfish were noted to utilise the middle and deep habitats, while one catfish was observed to have shifted from the river mouth towards the middle of the dam. These observations suggest the influence of seasonality, especially temperature and changing habitat conditions for some individuals. Nonetheless, other catfish appeared to maintain their home ranges, especially in the river mouth and upper habitats.

The results of this study provide an assessment of the probable risk associated with invasive catfish. Invasibility of freshwater ecosystems is usually related to the use of critical habitats that provide refuge and food for native species (Lapointe *et al*., 2010). The potential impact in such habitats would be related to competition for both space and resources between the invaders and native species, and the predation risk for native species associated with predatory invaders. Although the sharptooth catfish occurs in a wide range of habitats within both lotic and lentic ecosystems, its home range behaviour within the invaded impoundment indicates that it was associated with particular habitats. Within Glen Melville Dam, predation

impact by sharptooth catfish could be inferred from the utilisation of specific habitats, especially the river mouth habitat that would probably be a favourable habitat for feeding and spawning for native species such as moggel.

CHAPTER 9

General Discussion

Overview

Catfish introductions are increasing worldwide primarily due to their popularity in aquaculture (De Graaf & Janssen, 1996; Cambray, 2005; Vitule *et al*., 2006; Bhakta & Bandyopadhyay, 2007; Rhadhakrishnan *et al*., 2011). In South Africa, accidental inter-basin water transfer schemes, deliberate angler introductions and aquaculture are the major pathways for its translocation (Cambray & Jubb, 1977; Laurenson & Hocutt, 1984; 1986; Bruton, 1988; de Moor & Bruton, 1988; Skelton, 1990; Cambray, 2003a; Tweddle *et al*., 2009). Since it is a generalised piscivore, the need to understand the impacts associated with sharptooth catfish introductions is imperative. This thesis focused on catfish impacts in the Eastern Cape, South Africa. Previous studies on catfish in the region have focused on its biology (Weyl & Booth, 2008; Wartenberg *et al*., 2011), population dynamics (Booth & Weyl, 2008; Richardson *et al*., 2009; Booth *et al*., 2010) and feeding (Potts *et al*., 2008) within lentic habitats. This study builds on, and extends, this primary research by identifying, and to an extent quantifying, its impact within both invaded lotic and lentic environments.

The primary aim of this thesis was to assess and quantify catfish impacts within invaded freshwater ecosystems from a trophic perspective. This approach was based on the premise that non-native invaders not only create multiple pathways of cause-and-effect but also influence ecosystem processes such as nutrient cycling and energy flow such that they ultimately alter trophic pathways (Vitousek, 1990; Simberlorff, 2011). This thesis integrates several techniques through a collection of complimentary discrete experiments thereby

providing multiple lines of evidence that are a novel approach to invasion biology within freshwater ecosystems. Predictive and correlative studies on its distribution and abundance showed that IBWT schemes potentially facilitated catfish invasions and proliferation (Chapter 3), and suggested a potential risk associated with its invasion into headwater streams where native minnows occur (Chapter 4). From a trophic perspective, stable isotopes and feeding studies showed spatial variation in catfish-specific niches and a wide range in its prey sources in response to resource availability within different food webs (Chapter 5 and 6). Experimental manipulations indicated the role of catfish as a press-type disturbance through the elimination of large-bodies invertebrates together with the proliferation of resilient taxa (Chapter 7). A study on its movement provided evidence of non-random habitat utilisation that suggested interference with native species (Chapter 8). Overall, this thesis provided a more comprehensive understanding on the potential of catfish to eliminate native taxa and potentially homogenise biota within its invaded habitats.

The role of inter-basin transfer scheme in invasion biology

The sharptooth catfish, which was translocated through an inter-basin water transfer scheme that was opened in 1977, established early within the system (Cambray & Jubb, 1977; Laurenson & Hocutt, 1986). From a landscape perspective, it was widespread within mainstem habitats with flow regimes that had been altered from being seasonal to perennial (Chapter 3). As IBWT schemes are increasing both in South Africa and worldwide due to increasing demand for portable water and urbanisation, it is important to note their role in facilitating invasions and to develop, where possible, mitigating measures. The impact of an altered flow regime on the sharptooth catfish's invasion success is twofold. First, the creation of a constant flow regime within both lotic environment and permanent standing water

(lentic) habitats favours the establishment and spread of non-natives (Moyle, 1986; Arthington *et al*., 1990; Moyle & Light, 1996). Most successful invaders are usually adapted to modified flow regimes (Moyle, 1986). Second, native species have evolved life history strategies that include reproduction, growth, spawning and recruitment, which are cued to respond to natural disturbance cycles (Welcomme 1985). For example, Humphries & Lake (2000) proposed that "low flow recruitment" explains the spawning behaviour of fishes within seasonal rivers in southeastern Australia. This reproductive strategy was probably similar to that of the Great Fish and Sundays Rivers prior to the IBWT given that these systems were hydrologically highly variable seasonally. Native species such as moggel that occur in the mainstream are known to migrate after summer rainfall and spawn in shallow flooded areas (Skelton, 2001). Altering these natural flow regimes is likely to weaken the invasion resistance of these native species they may not be able to adapt to new hydrological regimes (Baltz & Moyle, 1993). This, therefore, increases the risk associated with predation, spread and establishment of catfish. While sharptooth catfish is considered problematic, its occurrence together with other translocated non-native species also raises questions on the risk associated with multiple invaders.

Spatial variation in catfish niches and food web sources

Stable isotope analysis has become a useful tool in ecological studies because its use is increasingly becoming cheap, easy and safe compared to other tracers such as radio isotopes (Michener & Lajtha, 2007). Within the context of invasion biology, it has been used to infer dietary composition and trophic positions of invaders (Vander Zanden & Rasmussen, 1999; Britton *et al*., 2010) and to predict the likelihood of their invasion success (Vander Zanden *et al*., 2004). The use of stable isotopes to describe trophic niches has also gained prominence

(Bearhop *et al*., 2004) and has been applied for invasive species (Olsson *et al*., 2009). In this thesis, community-wide and catfish-specific niche changes were assessed within different localities of their invaded habitats (Chapter 4). The results revealed broad diet for the sharptooth catfish. In addition, diet was associated with different habitats. In particular, within the Great Fish River, diet breadth increased within the upstream to downstream continuum. In contrast, within the Sundays River, sharptooth catfish's diet breadth did not change within the upstream to downstream continuum, with metrics on the measures of dispersion suggesting some degree of specialisation in the food web of the lower section of the river. These observations point to catfish's ability as a generalist to easily adjust to different local resource spectrum.

Analysing the diets of non-native species provides direct evidence of predation (Weyl & Lewis, 2006) and provides relevant information on which native species are likely to be consumed (Woodford & Impson, 2004; Kadye & Magadza, 2008; Lowe *et al*., 2008). Catfish diets were compared using both the traditional stomach content analysis approach and a more contemporary stable isotope analysis approach (Chapter 5). The two methods were complimentary in providing information on catfish diets since stomach contents provide a "snapshot" view of ingested diets whereas stable isotope analysis provides a temporally integrated view of ingested prey. Catfish revealed omnivory and ontogenetic diet shifts. Although the sharptooth catfish is considered to be primarily piscivorous, it fed on diverse food resources in these rivers. In particular, and surprisingly, lower trophic groups such as algae, plankton and macroinvertebrates dominated its diet in certain habitats. This evidence suggest that since sharptooth catfish is a relatively non-discriminating consumer, trophic cascades may be less evident within its invaded food webs in contrast to other piscivorous invaders, such as trout and bass that have been shown to cause pronounced top-down effect

within their invaded communities (Flecker & Townsend, 1994; Cambray, 2003b; Nyströ & McIntosh, 2003; Vander Zanden *et al*., 2004; Maezano *et al*., 2005).

The thesis also highlighted that the two dietary analysis methods should be used together to improve on estimation of prey sources as each approach is prone to bias. For instance, while stomach content analysis was important in providing high taxonomic resolution of ingested prey that included terrestrial insects, it could not reliably estimate highly digestible material such as zooplankton that was considered important in the Glen Melville Dam based on stable isotope analysis. Furthermore, a large proportion of catfish stomachs were empty especially in winter, which made it difficult to discern temporal feeding patterns. In contrast, stable isotope analysis was important in integrating the temporal aspect of catfish diets. Nonetheless, this method is likely to be sensitive to differences in species-specific turnover rates, system-specific fractionation rates and different mechanisms in isotopic routing (Gannes *et al*., 1997; Mill *et al*., 2007). Many studies make the generic assumption that systems are in similar states of equilibrium with a constant per-trophic fractionation value of 3.4‰ for $\delta^{15}N$ and ~1‰ for $\delta^{13}C$ (Minagawa & Wada, 1984, Vander Zanden & Rasmussen, 2001; Post, 2002). This assumption has been challenged by others (Pinnegar & Pollunin, 2000; McCutchan *et al*., 2003; Mill *et al*., 2007). It is therefore important to derive system-specific and, where possible, both consumer- and prey-specific fractionation values to improve on the resolution of stable isotope analysis. In this regard, a laboratory experimental approach is required as a complement to field experiments (Gannes *et al*., 1997). This can be achieved by testing assimilation and elimination rates of isotope tracers through diet switch experiments on different body tissuess (Logan *et al*., 2006; MacNeil *et al*., 2006). Precision can also be increased by combining stable isotope analysis with fatty acid trophic tracers. Fatty acids are considered to represent diet composition as most predators are only capable of synthesising certain fatty acids (Williams & Buck, 2010).

Therefore, bias related to source specificity in consumer signatures might be solved through either fatty acid-specific stable isotopes or by using fatty acid-specific tracers (Williams & Buck, 2010; Bec *et al*., 2011).

Identifying causal patterns through experimental manipulation

Experimental exclusion or removal of predators from certain areas and monitoring the response of prey species is one way to test the impact of introduced species. Green (1979) presented the Before-After-Control-Impact (BACI) design to test potential impact and this method has been applied in several studies with various modifications (Stewart-Oaten *et al*., 1986; Underwood, 1994; Keough & Mapstone, 1997; Stewart-Oaten & Bence, 2001; Downes *et al*., 2002). This design is, however, rarely used in predation impact studies. The use of this design in this thesis is, to my knowledge, the first in invasion biology. Its applicability was tested to determine the response of benthic macroinvertebrates to catfish presence as a press-type disturbance in differentially impacted rivers (Chapter 6). The results of this study highlighted that macroinvertebrates were non-responsive to catfish presence within systems where it has previously been established. In contrast, excluding catfish in these systems indicated a response that suggested the importance of refuge within invaded habitats and the possible recovery pattern of certain macroinvertebrate taxa. Therefore, systems can rehabilitate from an invasion perspective. By comparison, introducing catfish within uninvaded localities provided evidence of direct catfish impact through elimination of conspicuous taxa such as odonates.

Use of experimental exclusions in predation studies has been conspicuously absent and therefore should be encouraged. Application of such designs will be important in addressing concerns about sample size, statistical power, scale and timing. If appropriately

designed, these experiments can reveal on the feasibility of eradicating non-native species as part of adaptive management (Park, 2004). Within the context of invasion biology, a BACI design can be used to test hypotheses of planned comparisons or *a priori* predictions on the response of native biota to the presence of both catfish and other invaders in different localities and environments. The invaded localities within the studied systems already represent the impact scenario where predictions about recovery and rehabilitation can be tested in future experiments.

Movement and habitat selection

Telemetry has been used extensively to track wildlife movement and habitat use. For fisheries research surgically implanted transmitters can be used to track fishes by radio, acoustically or by satellite. Radio telemetry relies on radio frequencies while acoustic telemetry relies on sound waves that are transmitted through water to a nearby receiver. Contemporary research, usually on large animals, makes use of transmitters that can communicate with satellites and provide real-time monitoring of fish movement on a broader scale (Eckert, 2002; Hammerschlag *et al*., 2011). In small-scale studies, however, acoustic telemetry is most cost-effective and allows near real-time monitoring as sound travels faster in water that air, and can be applied easily both within freshwater and marine environments. Acoustic telemetry was used to investigate catfish movement patterns within an invaded lentic habitat (Chapter 7). Although the sharptooth catfish is a ubiquitous species in Africa, this study provided evidence that it preferred certain habitats. The shallow river mouth was the most utilised habitat during both summer and winter. This observation was consistent with its observed patterns in habitat use within its natural range (Willoughby & Tweddle, 1978; Bruton, 1979b). The sharptooth catfish also exhibited both nocturnal and diurnal patterns in activity

that were contrary to Bruton's (1979b) observation that it is predominantly nocturnal, but consistent with Hocutt's (1989) observations on tagged catfish that suggested feeding during both day and night. Use of shallow and structured habitats may be associated with predation impact as these habitats may be preferred by native species. Sharptooth catfish also has potential to invade headwater streams where native and endemic cyprinid minnows occur. These minnows include the endangered Eastern Cape redfin, goldie barb, within the Sundays River system, and chubbyhead barb within headwaters of Great Fish River. The population dynamics of these minnows are largely driven by both seasonal variability and environmental fluctuations (Chapter 4). The risk of catfish invasion into their habitats is likely since there are no physical barriers to limit its movements. Periodic incursions have also been reported and these are likely to increase pressure on the stability of minnow populations. Construction of barriers has been recommended to mitigate the movement of catfish into headwater streams (Weyl *et al*., 2009b).

Future perspectives

During this thesis research, the sharptooth catfish was observed not only to be widespread but to co-occur with several non-native species such as smallmouth yellowfish, *Labeobarbus aeneus*, the Orange River mudfish *Labeo capensis,* which were both translocated through the IBWT, and common carp, *Cyprinus carpio*, that was introduced by anglers. Smallmouth yellowfish is a zooplanktivore and invertivore, whereas mudfish and common carp are detritivorous and omnivorous, respectively, with the latter renowned for its ability to transform habitats and influence nutrient cycling (Winker *et al*., 2011). The co-occurrence of catfish and other non-native invaders suggests a risk of more complex impacts such as invasion meltdown. Invasion meltdown is a concept in which the joint impact of two or more

species would be more severe than that of the single invaders (Simberloff & Von Holle, 1999; Simberloff, 2011). A meltdown result from facilitation of one invader population by other invaders or may be a consequence of a mutualistic process among the invaders that results in accrued detrimental effects. Invasion meltdown poses a risk of invasions even by species that would otherwise have more subtle or less obvious impacts as the biotic resistance of the invaded ecosystem becomes weakened. There is therefore a need to understand the role of the multiple invaders within the invaded ecosystem. It will be important to test hypotheses related to feeding, resource partitioning and interaction among, and between, non-native fishes within their invaded habitats. By documenting the potential catfish impact and co-occurrence of other non-native invaders, this study provided baseline data to test this meltdown hypothesis. This can be achieved through stable isotope and gut contents analysis to determine trophic interrelations among the invaders, while further BACI experiments could be extended to assess interaction among different species.

REFERENCES

Abjornsson K., Hansson L. & Bronmark C. (2004) Responses of prey from habitats with different predator regimes: local adaptation and heritability. *Ecology*, **85**, 1859-1866.

Anderson C. & Cabana G. (2009) Estimating the trophic position of aquatic consumers in river food webs using stable nitrogen isotopes. *Journal of the North American Benthological Society*, **26**, 273-285.

Angeler D.G. & Moreno J.M. (2007) Zooplankton community resilience after press-type anthropogenic stress in temporary ponds. *Ecological Applications*, **17**, 1105-1115.

Angermeier P.L. & Schlosser I.J. (1989) Species-area relationships for stream fishes. *Ecology*, **70**, 1450-1462.

Arthington A.H., Hamlet S. & Blühdorn D.R. (1990) The role of habitat disturbance in the establishment of introduced warm-water fishes in Australia. In *Introduced and translocated fishes and their ecological effects* (Pollard D.A. ed.), Bureau of Rural Resources Proceedings No. 8. Australian Government Printing Service, Canberra. 61-66pp.

Atkinson C.L., Golladay S.W., Opsahl S.P. & Covich A.P. (2009) Stream discharge and floodplain connections affect seston quality and stable isotopic signatures in a coastal plain stream. *Journal of the North American Benthological Society*, **28**, 360-370.

Austin M. (2007) Species distribution models and ecological theory: a critical assessment and some possible new approaches. *Ecological Modelling*, **200**, 1-19.

Bahou L., Kone T., N'Douba V., N'Guessan K.J., Kouamelan E.P. & Gouli G.B. (2007) Food composition and feeding habits of little tunny (*Euthynnus alletteratus*) in continental shelf waters of Côte d'Ivoire (West Africa). *International Council for the Exploration of the Sea*, **64**, 1044-1052.

Baltz D.M. & Moyle P.B. (1993) Invasion resistance to introduced species by a native assemblage of California stream fishes. *Ecological Applications*, **3**, 246-255.

Baras E. & Westerloppe L. (1999) Transintestinal expulsion of surgically implanted tags by African catfish *Heterobranchus longifilis* of variable size and age. *Transactions of the American Fisheries Society*, **128**, 737-746.

Basille M., Calenge C., Marboutin E., Andersen R. & Gaillard J.M. (2008) Assessing habitat selection using multivariate statistics: some refinements of the ecological-niche factor analysis. *Ecological Modelling*, **211**, 233-240.

Baxter C.V., Fausch K.D., Murakami M. & Chapman P.L. (2004) Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. *Ecology*, **85**, 2656-2663.

Bearhop S., Adams C.E., Waldron S., Fuller R.A. & Macleod H. (2004) Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology*, **73**, 1007-1012.

Bec A., Perga M, Koussoroplis A., Bardoux G., Desvilettes C., Bourdier G., & Mariotti A. (2011) Assessing the reliability of fatty acid-specific stable isotope analysis for trophic studies. *Methods in Ecology and Evolution*. Early online, DOI 10.1111/j.2041-210X.2011.00111.x

Becker B.H. & Beissinger S.R. (2006) Centennial decline in the trophic level of an endangered seabird after fisheries decline. *Conservation Biology*, **20**, 470-79.

Beugly J. & Pyron M. (2010) Temporal and spatial variation in the long-term functional organization of fish assemblages in a large river. *Hydrobiologia*, **654**,215-226.

Bhakta J.N. & Bandyopadhyay P.K. (2007) Exotic fish biodiversity in Churni River of west Bengal, India. *Electronic Journal of Biology*, **3**, 13-17.

Booth A.J. & Weyl O.L.F. (2008) Retention of T-bar anchor and dart tags by a wild population of African sharptooth catfish, *Clarias gariepinus*. *Fisheries Research*, **92**, 333-339.

Booth A.J., Traas G.R.L. & Weyl O.L.F. (2010) Adult African sharptooth catfish, *Clarias gariepinus*, population dynamics in a small invaded warm-temperate impoundment. *African Zoology*, **45**, 299-308.

Bosch J., Rincon P.A., Boyero L. & Martinez-Salamo I. (2006) Effects of introduced salmonids on a montane population of Iberian frogs. *Conservation Biology*, **20**, 180-189.

Bowmaker A.P. (1973) Potamodromesis in the Mwenda River, Lake Kariba. *Geophysical Monograph*, **17**, 159-164.

Breiman L. (2001) Statistical modelling: the two cultures. *Statistical Science*, **16**, 199-231.

Breiman L., Friedman J.H., Olshen R.A. & Stone, C.G. (1984) *Classification and regression trees*. Wadsworth International Group, Belmont, California, USA.

Britton R.J., Harper D.M., Oyugi D.O. & Grey J. (2010) The introduced *Micropterus salmoides* in an equatorial lake: a paradoxical loser in an invasion meltdown scenario? *Biological Invasions*, **12**, 3439-3448.

Brown L.R. & Moyle P.B. (1991) Changes in habitat and microhabitat partitioning within an assemblage of stream fishes in response to predation by Sacramento squawfish

(*Ptychocheilus grandis*). *Canadian Journal of Fisheries and Aquatic Science*, **48**, 849-856.

Bruton M.N. (1976) On the size reached by *Clarias gariepinus*. *Journal of the Limnological Society of Southern Africa*, **2**, 57-58.

Bruton M.N. (1978) The habitats and habitat preferences of *Clarias gariepinus* (Pisces: Clariidae) in a clear coastal lake (Lake Sibaya, South Africa). *Journal of the Limnological Society of South Africa*, **4**, 81- 88.

Bruton M.N. (1979a) The food and feeding behaviour of *Clarias gariepinus* (Pisces: Clariidae) in Lake Sibaya, South Africa, with emphasis on its role as a predator of cichlids. *Transactions of the Zoological Society of London*, **35**, 47-114.

Bruton M.N. (1979b) The role of diel inshore movements by *Clarias gariepinus* (Pisces, Clariidae) for the capture of fish prey. *Transactions of the Zoological Society of London*, **35**, 115-138.

Bruton M.N. (1979c) The breeding biology and early development of *Clarias gariepinus* (Pisces: Clariidae) in Lake Sibaya, South Africa, with a review of breeding in species of the subgenus *Clarias* (*Clarias*). *Transactions of the Zoological Society of London*, **35**, 1-45.

Bruton M.N. (1988) Systematics and biology of clariid catfish. In *The culture of sharptooth catfish Clarias gariepinus in southern Africa* (Hecht T., Uys W., & Britz P.J. eds.), South African National Scientific Program Report No 153: 1-10 pp.

Bruton M.N. & Allanson B.R. (1980) Growth of *Clarias gariepinus* in Lake Sibaya, South Africa. *South African Journal of Zoology*, **15**, 7-15.

Butler L.H. & Fairchild W.G. (2005) Response of fish assemblages to winter in two adjacent warmwater streams. *American Midland Naturalist*, **154**, 152-165.

Byers J.E. (2002) Impact of non-indigenous species on natives enhanced by anthropogenic alteration of selection regimes. *Oikos*, **97**, 449-458.

Cabana G. & Rasmussen J.B. (1996) Comparing aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Science of the U.S.A.*, **93**, 10844-10847.

Calenge C. (2006) The package "adehabitat" for the R software: a tool for the analysis of space and habitat use by animals. *Ecological Modelling*, **197**, 516-519.

Cambray J.A. (1985) Observations on spawning of *Labeo capensis* and *Clarias gariepinus* in the regulated lower Orange River, South Africa. *South African Journal of Science*, **81**, 18-321.

Cambray J.A. (1994) The comparative reproductive styles of two closely related African minnows (*Pseudobarbus afer* and *P. asper*) inhabiting two different sections of the Gamtoos River system. *Environmental Biology of Fishes*, **41**, 247-268.

Cambray J.A. (2003a) The need for research and monitoring on the impacts of translocated sharptooth catfish, *Clarias gariepinus*, in South Africa. *African Journal of Aquatic Science*, **28**, 191-195.

Cambray J.A. (2003b) Impact on indigenous species biodiversity caused by the globalisation of alien recreational freshwater fisheries. *Hydrobiologia*, **500**, 217-230.

Cambray J.A. (2005) Africa's *Clarias gariepinus* (Teleostei: Clariidae) appears in rivers in Brazil. *African Journal of Aquatic Science*, **30**, 201-202.

Cambray J.A. & Jubb R. (1977) Dispersal of fishes via the Orange-Fish Tunnel, South Africa. *Journal of the Limnological Society of South Africa*, **3**, 33-35.

Cambray J.A. & Bruton M.N. (1985) Age and growth of a colonizing minnow, *Barbus anoplus*, in a man-made lake in South Africa. *Environmental Biology of Fishes*, **12**, 131-141.

Cambray J.A. & Hecht T. (1995) Comparison of the growth of two closely related redfin minnows, *Pseudobarbus afer* (Peters, 1864) and *P. asper* (Boulenger, 1911) (Pisces, Cyprinidae), in the Gamtoos River System, South Africa. *Journal of African Zoology*, **109**, 350-376.

Canonico G.C., Arthington A., McCray J.K. & Thieme, M.L. (2005) The effects of introduced tilapias on native biodiversity. *Aquatic Conservation*: *Marine and Freshwater Ecosystems*, **15**, 463-483.

Capone T.A. & Kushlan J.A. (1991) Fish Community Structure in Dry-Season Stream Pools. *Ecology*, **72**, 983-992.

Carlisle D.M. & Hawkins C.P. (1998) Relationships between invertebrate assemblage structure, 2 trout species, and habitat structure in Utah mountain lakes. *Journal of the North American Benthological Society*, **17**, 286-300.

Carol J., Zamora L. & García-Berthou E. (2007) Preliminary telemetry data on the movement patterns and habitat use of European catfish (*Silurus glanis*) in a reservoir of the River Ebro, Spain. *Ecology of Freshwater Fish*, **16**, 450-456.

Casatti L., Langeani F., Silva A.M. & Castro R.M.C. (2006) Stream fish, water and habitat quality in a pasture dominated basin, southeastern Brazil. *Brazilian Journal of Biology*, **66**, 681-696.

Chapman L.J., Chapman C.A., Ogutu-Ohwayo R., Chandler M., Kaufman L. & Keiter A. (1996) Refugia for endangered fishes from an introduced predator in Lake Nabugabo, Uganda. *Conservation Biology*, **10**, 554-561.

Clavero M. & García-Berthou E. (2005) Invasive species are a leading cause of animal extinction. *Trends in Ecology and Evolution***, 20**,110.

Collier K.J. & Quinn J.M. (2003) Land-use influences macroinvertebrate community response following a pulse disturbance. *Freshwater Biology*, **48**, 1462-1481.

Courtenay W.R. & Stauffer J.R. (1990) The introduced fish problem and the aquarium fish industry. *Journal of World Aquaculture Society*, **21**, 145-159.

Cummins K.W., Merritt R.W. & Andrade P.C.N. (2005) The use of invertebrate functional groups to characterize ecosystem attributes in selected streams and rivers in south Brazil. *Studies on Neotropical Fauna and Environment*, **40**, 69-89.

Daget J., Gosse J.P. & van den Audenaerde T.D. (1984) *Check-list of the Freshwater Fishes of Africa*, Vol. 1. ORSTOM, Paris, 410pp.

Daugherty DJ & Sutton T.M. (2005) Seasonal movement patterns, habitat use, and home range of flathead catfish in the lower St. Joseph River, Michigan. *North American Journal of Fisheries Management*, **25**, 256-269.

De Graaf G. & Janssen H. (1996) *Artificial reproduction and pond rearing of the African catfish* Clarias gariepinus *in Sub-Saharan Africa* - A handbook, vol 362. FAO Fisheries Technical Paper, Rome.

de Moor I. & Bruton M.N. (1988) *Atlas of alien and translocated indigenous aquatic animals in southern Africa.* South African National Scientific Program Report No 144, CSIR, Pretoria, South Africa.

De'ath G. (2002) Multivariate regression trees: a new technique for constrained classification analysis. *Ecology*, **83**,1103-1117.

De'ath G. & Fabricius K.E. (2000) Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology*, **81**, 3178-3192.

del Rio C.M., Sabat P., Anderson-Sprecher R. & Gonzalez S.P. (2009) Dietary and isotopic specialization: the isotopic niche of three *Cinclodes* ovenbirds. *Oecologia*, **161**, 149-159.

DeNiro M. J. & Epstein S. (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, **42**, 495-506.

DeNiro M. J. & Epstein S. (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosochimica Acta*, **45**, 341-351.

Didham R.K., Tylianakis J.M., Hutchinson M.A., Ewers R.M. & Gemmell M.J. (2005) Are invasive species the drivers of ecological change? *Trends in Ecology and Evolution*, **20**, 470-474.

Didham R.K., Tylianakis J.M., Gemmell N.J., Rand T.A. & Ewers R.M. (2007) Interactive effects of habitat modification and species invasion on native species decline. *Trends in Ecology and Evolution*, **22**,489-496.

Doledec S., Chessel D. & Gimaret-Carpentier C. (2000) Niche separation in community analysis: a new method. *Ecology*, **81**, 2914-2927.

Donald D.B. & Anderson S.R. (2003) Resistance of the prey-to-predator ratio to environmental gradients and to biomanipulations. *Ecology*, **84**, 2387-2394.

Donovan T.M., Jones P.W., Annand E.M. & Thompson F.R. (1997) Variation in local-scale edge effects: mechanisms and landscape context. *Ecology*, **78**, 2064-2075.

Douglas P.L., Forrester G.E. & Cooper S.D. (1994) Effects of trout on the diel periodicity of drifting in baetid mayflies. *Oecologia*, **98**, 48-56.

Downes B.J., Barmuta L.A., Fairweather P.G., Faith D.P., Keough M.J., Lake P.S., Mapstone B.D. & Quinn G.P. (2002) *Monitoring ecological impacts: concepts and practice in flowing waters.* Cambridge University Press, Cambridge, UK.

Duxbury C., Holland J. & Pluchino M. (2010) Experimental evaluation of the impacts of the invasive catfish *Hoplosternum littorale* (Hancock, 1828) on aquatic macroinvertebrates. *Aquatic Invasions*, **5**, 97-102.

Eckert S.A., Dolar L.L., Kooyman G.L., Perrin W. & Rahman R.A. (2002) Movements of whale sharks (*Rhincodon typus*) in South-east Asian waters as determined by satellite telemetry. *Journal of Zoology*, **257**, 111-115.

Edo K. & Suzuki K. (2003) Preferable summering habitat of returning adult masu salmon in the natal stream. *Ecological Research*, **18**, 783-791.

Effenberger M., Engel J., Diehl S. & Matthaei C. D. (2008) Disturbance history influences the distribution of stream invertebrates by altering microhabitat parameters: a field experiment. *Freshwater Biology*, **53**, 996-1011.

Efron B. & Tibshirani R. (1993) *An introduction to the bootstrap*. Chapman and Hall, New York.

Ellender B.R., Weyl O.L.F. & Swartz E.R. (2011) Invasion of a headwater stream by non-native fishes in the Swartkops River system, South Africa. *African Zoology*, **46**, 39-46.

Englund R.A. & Polhemus D.A. (2001) Evaluating the effects of introduced rainbow trout (*Oncorhynchus mykiss*) on native stream insects on Kauai Island, Hawaii. *Journal of Insect Conservation*, **5**, 265-281.

Englund G., Cooper S.D. & Sarnelle O. (2001) Application of a model of scale dependence to quantify scale domains in open predation experiments. *Oikos*, **92**, 501-514.

Erős T. & Grossman G.D. (2005) Effects of within-patch habitat structure and variation on fish assemblage characteristics in the Bernecei stream, Hungary. *Ecology of Freshwater Fish*, **14**, 256-266.

Finlay J.C. (2001) Stable-carbon-isotope ratios of river biota: implications for energy flow in lotic food webs. *Ecology*, **82**, 1052-1064.

Flaherty E.A. & Ben-David M. (2010) Overlap and partitioning of the ecological and isotopic niches. *Oikos*, **119**, 1409-1416.

Flecker A.S. & Townsend C.R. (1994) Community-wide consequences of trout introduction in New Zealand streams. *Ecological Applications*, **4**, 798-807.

Franklin J. (1998) Predicting the distribution of shrub species in southern California from climate and terrain-derived variables. *Journal of Vegetation Science*, **9**, 733-748.

Fuller P.L., Nico L.G. & Williams J.D. (1999) *Nonindigenous fishes introduced into inland waters of the United States*. American Fisheries Society Publication 27, Bethesda.

Gannes L.Z., O'Brien D.M. & Del Rio C.M. (1997) Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. *Ecology*, **78**, 1271-1276.

Gearing J.N. (1991) The study of diet and trophic relationships through natural abundance ^{13}C. In *Carbon isotope techniques* (Coleman D.C. & Fry B. eds.), Academic Press, San Diego, 201-218 pp.

Gerber A. & Gabriel M.J.M. (2002) *Aquatic invertebrates of South African rivers: Field guide*. Institute for Water Quality Studies, Pretoria.

Giannico G.R. (2000) Habitat selection by juvenile coho salmon in response to food and woody debris manipulations in suburban and rural stream sections. *Canadian Journal of Fisheries and Aquatic Science*, **57**, 1804-1813.

Gibbs H.L., Gibbs K.E., Siebenmann M. & Collins L. (1998) Genetic differentiation among populations of the rare mayfly *Siphlonisca aerodromia* Needham. *Journal of the North American Benthological Society*, **17**, 464-474.

Gillies C.S., Hebblewhite M., Nielsen S.E., Krawchuk M.A., Aldridge C.L., Frair J.L., Saher D.J., Stevens C.E. & Jerde, C.L. (2006) Application of random effects to the study of resource selection by animals. *Journal of Animal Ecology*, **75**, 887-898.

Gorman O.T. & Karr J.R. (1978) Habitat structure and stream fish communities. *Ecology*, **74**, 1856-1870.

Goudschmidt T., Witte F. & Wanink J. (1993) Cascading effects of the introduced Nile perch on the detritivorous/planktivourous species in the sublittoral ares of Lake Victoria. *Conservation Biology*, **7**, 686-700.

Grabowski J.H., Hughes A.R., Kimbro D.L. & Dolan M.A. (2005) How habitat setting influences restored oyster reef communities. *Ecology*, **86**, 1926-1935.

Graf J., Kikuchi Y. & Rio R.V.M. (2006) Leeches and their microbiota: naturally simple symbiosis models. *Trends in Microbiology*, **14**, 365-371.

Gratwicke B. & Marshall B.E. (2001) The relationship between the exotic predators *Micropterus salmoides* and *Serranochromis robustus* and native stream fishes in Zimbabwe. *Journal of Fish Biology*, **58**, 68-75.

Green R.H. (1979) *Sampling design and statistical methods for environmental biologists*. Wiley Interscience, Chichester, England, UK.

Grey J. (2006) The use of stable isotope analyses in freshwater ecology: current awareness. *Polish Journal of Ecology*, **54**, 563-584.

Groenewald A.A.V.J. (1964) Observations on the food habits of *Clarias gariepinus* Burchell, the South African freshwater barbel (Pisces: Clariidae) in Transvaal. *Hydrobiologia*, **23**, 287-291.

Grossman G.D., Dowd J.F. & Crawford M. (1990) Assemblage stability in stream fishes: a review. *Environmental Management*, **14**, 661-671.

Grossman G.D. & Ratajczak R.E. (1998) Long-term patterns of microhabitat use by fish in a southern Appalachian stream from 1983 to 1992: effects of hydrologic period, season and fish length. *Ecology of Freshwater Fish*, **7**, 108-131.

Guenther C.B. & Spacie A. (2006) Changes in fish assemblage structure upstream of impoundments within the Upper Wabash River basin, Indiana. *Transactions of the American Fisheries Society*, **135**, 570-583.

Hall-Aspland S.A., Hall A. P. & Rogers T. L. (2005) A new approach to the solution of the linear mixing model for a single isotope: application to the case of an opportunistic predator. *Oecologia*, **143**, 143-147.

Hammerschlag N., Gallagher A.J. & Lazarre D.M. (2011) A review of shark satellite tagging studies. *Journal of Experimental Marine Biology and Ecology*, Early online, DOI10.1016/j.jembe.2010.12.012.

Hansen B.B., Herfindal I., Aanes R., Saether B.E. & Henriksen S. (2009) Functional response in habitat selection and the tradeoffs between foraging niche components in a large herbivore. *Oikos*, **118**, 859-872.

Harper M.P. & Peckarsky B. L. (2005) Effects of pulsed and pressed disturbances on the benthic invertebrate community following a coal spill in a small stream in northeastern USA. *Hydrobiologia*, **544**, 241-247.

Harvey C.J., Ebener M.P. & White C. K. (2008) Spatial and ontogenetic variability of sea lamprey diets in Lake Superior. *Journal of Great Lakes Research*, **34**, 434-449.

Hattingh J. (1997) Fluvial response to allocyclic influence during the development of the lower Sundays River, Eastern Cape, South Africa. *Quaternary International*, **33**, 3-10.

Havel J.E., Lee C.E. & Vander Zanden M.J. (2005) Do reservoirs facilitate invasions into landscapes? *BioScience*, **55**, 518-525.

Hermoso V., Clavero M., Blacho-Garrido F. & Prenda J. (2011) Invasive species and habitat degradation in Iberian streams: an analysis of the role in freshwater fish diversity loss. *Ecological Applications*, **21**, 175-188.

Hirzel A.H., Hausser J., Chessel D. & Perrin N. (2002) Ecological-Niche Factor Analysis: how to compute habitat-suitability maps without absence data? *Ecology*, **83**, 2027-2036.

Hirzel A.H., Posse B., Oggier P.A., Crettenand Y., Glenz C. & Arlettaz R. (2004) Ecological requirements of reintroduced species and the implications for release policy: the case of the bearded vulture. *Journal of Applied Ecology*, **41**, 1103-1116.

Hobson K.A. (1993) Trophic relationships among high arctic seabirds: insights from tissue-dependent stable-isotope models. *Marine Ecological Progress Series*, **95**, 7-18.

Hobson K.A. & Welch H.E. (1992) Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $\delta^{15}N$ analysis. *Marine Ecological Progress Series*, **84**, 9-18.

Hocutt C.H. (1989) Seasonal and diel behaviour of radio-tagged *Clarias gariepinus* in Lake Ngezi, Zimbabwe (Pisces: Clariidae). *Journal of Zoology*, **219**, 181-199.

Hoeinghaus D.J., & Zeug S.C. (2008) Can stable isotope ratios provide for community-wide measures of trophic structure? Comment. *Ecology*, **89**, 2353-2357.

Humphries P. & Lake P.S. (2000) Fish larvae and the management of regulated rivers. *Regulated Rivers: Research and Management*, **16**, 421-432.

Hurlbert S.H. (1984) Pseudoreplication and design of ecological field experiments. *Ecological Monographs*, **54**, 187-211.

Hyslop E.J. (1980) Stomach contents analysis-a review of methods and their application. *Journal of Fish Biology*, **17**, 411-430.

Inger R. & Bearhop S. (2008) Applications of stable isotope analyses to avian ecology. *Ibis*, **150**, 447-461.

Jackson A.L., Inger R., Bearhop S. & Parnell A. (2009) Erroneous behaviour of MixSIR, a recently published Bayesian isotope mixing model: a discussion of Moore & Semmens (2008). *Ecology Letters*, **12**, E1-E5.

Jackson A.L., Inger R., Parnell A.C. & Bearhop S. (2011) Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, **80**, 595-602.

Jackson D.A. & Somers K.M. (1991) Putting things in order: the ups and downs of Detrended Correspondence Analysis. *American Naturalist*, **137**, 704-712.

Jackson D.A., Peres-Neto P.R., & Olden J.D. (2001) What controls who is where in freshwater fish communities - the roles of biotic, abiotic, and spatial factors. *Canadian Journal of Fisheries and Aquatic Science*, **58**, 157-170.

Jespen N., Koed A., Thorstad E.B. & Baras E. (2002) Surgical implantation of telemetry transmitters in fish: how much have we learned? *Hydrobiologia*, **483**, 239-248.

Johnson P.T.J., Olden J.D., Solomon C.T. & Vander Zanden M.J. (2009) Interactions among invaders: community and ecosystem effects of multiple invasive species in an experimental aquatic system. *Oecologia*, **159**, 161-170.

Jongman R.H.G., ter Braak C.J.F. & van Tongeren O.F.R. (1995) *Data analysis in community and landscape ecology*. Cambridge University Press, Cambridge, UK.

Kadye W.T. & Magadza C.H.D. (2008) Trout induces a shift from preferred habitat types for indigenous species: the example of the indigenous catfish, *Amphilius uranoscopus* (Pfeffer, 1889), on an African montane plateau. *Hydrobiologia*, **614**,329-337.

Kadye W.T. & Booth A.J. (2011) Integrating stomach content and stable isotope analyses to elucidate the feeding habits of non-native sharptooth catfish *Clarias gariepinus*. *Biological Invasions*: Early online, DOI 10.1007/s10530-011-0116-6.

Keaton M., Haney D. & Andersen B.C. (2005) Impact of drought upon fish assemblage structure in two South Carolina Piedmont streams. *Hydrobiologia*, **545**, 209-223.

Keough M.J. & Mapstone B.D. (1997) Designing environmental monitoring for pulp mills in Australia. *Water Science and Technology*, **35**, 397-404.

Khan M.F. & Panikkar P. (2009) Assessment of impacts of invasive fishes on the food web structure and ecosystem properties of a tropical reservoir in India. *Ecological Modelling*, **220**, 2281-2290.

Kiesecker J.M., Blaustein A.R. & Miller C.L. (2001) Potential mechanisms underlying the displacement of native red-legged frogs by introduced bullfogs. *Ecology*, **82**, 1964-1970.

Kitano J., Bolnick D.I., Beauchamp D.A., Mazur M.M., Mori S., Nakano T. & Peichel C.L. (2009) Reverse evolution of armor plates in the threespine stickleback. *Current Biology*, **18**, 769-774.

Knapp R.A., Matthews K. R. & Sarnelle O. (2001) Resistance and resilience of alpine lake fauna to fish introductions. *Ecological Monographs*, **71**, 401-421.

Koehn J.D., O'Connor N.A. & Jackson P.D. (1994) Seasonal and size-related variation in microhabitat use by a Southern Victorian stream fish assemblage. *Australian Journal of Marine and Freshwater Research*, **45**, 1353-66.

Kraft C.E., Sullivan P.J., Karatayev A.Y., Burlakova L.E., Nekola J.C., Johnson L.E. & Padilla D.K. (2002) Landscape patterns of an aquatic invader: assessing dispersal extent from spatial distributions. *Ecological Applications*, **12**, 749-759.

Lake P.S. (2000) Disturbance, patchiness, and diversity in streams. *Journal of the North American Benthological Society*, **19**, 573-592.

Lal K.K., Singh R.K., Mohindra V., Singh B. & Ponniah A.G. (2003) Genetic makeup of exotic catfish *Clarias gariepinus* in India. *Asian Fisheries Science*, **16**, 229-234.

Lapointe N.W.R., Thorson J.T. & Angermeier P.L. (2010) Seasonal meso- and microhabitat selection by the northern snakehead (*Channa argus*) in the Potomac river system. *Ecology of Freshwater Fish*, **19**, 566-577.

Laurenson L.B. & Hocutt C.H. (1984) Introduction of the rock catfish *Gephyroglanis sclateri* into the Great Fish River via the Orange/Fish Tunnel, South Africa. *The Naturalist*, **28**, 12-15.

Laurenson L.B. & Hoccutt C.H. (1986) Colonisation theory and invasive biota: the Great Fish River, a case history. *Environmental Monitoring and Assessment*, **6**, 71-90.

Laurenson L.B.J., Hocutt C.H. & Hecht T. (1989) An evaluation of the success of invasive fish species of the Great Fish River. *Journal of Applied Ichthyology*, **5**, 28-34.

Layman C.A., Arrington D.A., Montana C.G. & Post D. M. (2007) Can stable isotope ratios provide for community wide measures of trophic structure? *Ecology*, **88**, 42-48.

Legendre P. & Legendre L. (1998) *Numerical ecology* (2nd English edition). Elsevier, Amsterdam, The Netherlands.

Leibold M.A. (1995) The niche concept revisited: mechanistic models and community context. *Ecology*, **76**, 2530-2535.

Leprieur F., Hickey M.A., Arbuckle C.J., Closs G.P., Brosse S. & Townsend C.R. (2006) Hydrological disturbance benefits a native fish at the expense of an exotic fish in a New Zealand River catchment. *Journal of Applied Ecology*, **43**, 930-939.

Leprieur F., Beauchard O., Blanchet S., Oberdorff T. & Brosse S. (2008) Fish invasions in the world's river systems: when natural processes are blurred by human activities. *PLoS Biology*, **6**, e26.

Leprieur F., Brosse S., Garcia-Berthou E., Oberdorff T., Olden J.D. & Townsend C.R. (2009) Scientific uncertainty and the assessment of risks posed by non-native freshwater fishes. *Fish and Fisheries*, **10**, 88-97.

Logan J., Haas H., Deegan L. & Gaines E. (2006) Turnover rates of nitrogen stable isotopes in the salt marsh mummichog, *Fundulus heteroclitus*, following a laboratory diet switch. *Oecologia*, **147**, 315-319.

Lombard A.T., Johnson C.F., Cowling R.M. & Pressey R.L. (2001) Protecting plants from elephants: botanical reserve scenarios within the Addo Elephant National Park, South Africa. *Biological Conservation*, **102**, 191-203.

Lonsdale W.M. (1999) Global patterns of plant invasions and the concept of invasibility. *Ecology*, **80**, 1522-1536.

Lowe S.R., Woodford D.J., Impson D.N. & Day J.A. (2008) The impact of invasive fish and invasive riparian plants on the invertebrate fauna of the Rondegat River, Cape Floristic Region, South Africa. *African Journal of Aquatic Science*, **33**, 51-62.

Lowe-McConnell R.H. (1993) Fish faunas of the African Great Lakes: origins, diversity, and vulnerability. *Conservation Biology*, **7**, 634-643.

Lozon J.D. & MacIsaac H.J. (1997) Biological invasions: are they dependent on disturbance? *Environmental Review*, **5**, 131-144.

Lubetkin S.C. & Simenstad C.A. (2004) Multi-source mixing models to quantify food web sources and pathways. *Journal of Applied Ecology*, **41**, 996-1008.

MacNeil M.A., Drouillard K.G. & Fisk A.T. (2006) Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. *Canadian Journal of Fisheries and Aquatic Science*, **63**, 345-353.

Macvicar C.N. (1991) *Soil classification: A taxonomic system for South Africa*. Memoirs of the agricultural natural resources of South Africa, 15. Department of Agricultural Development, Pretoria, South Africa.

Maezono Y. & Miyashita T. (2003) Community-level impacts induced by introduced largemouth bass and bluegill in farm ponds in Japan. *Biological Conservation*, **109**, 111-121.

Maezono Y., Kobayashi R., Kusahara M. & Miyashita T. (2005) Direct and indirect effects of exotic bass and bluegill on exotic and native organisms in farm ponds. *Ecological Applications*, **15**, 638-650.

Manly B.F.J., McDonald L.L., Thomas D.L., McDonald T.L. & Erickson W.P. (2002) *Resource selection by animals. Statistical design and analysis for field studies* (2nd edition). Kluwer Academic, Dordrecht, The Netherlands.

Marchetti M.P. & Moyle P.B. (2001) Effects of flow regimes on fish assemblages in a regulated Carlifornia stream. *Ecological Applications*, **11**, 530-539.

Mauritzen M., Belikov S.E., Boltunov A.N., Derocher A.E., Hansen E., Ims R.A., Wiig Ø. & Yoccoz N. (2003) Functional responses in polar bear habitat selection. *Oikos*, **100**,112-124.

McClellan C.M., Braun-McNeill J., Avens L., Wallace B.P. & Read A.J. (2010) Stable isotopes confirm a foraging dichotomy in juvenile loggerhead sea turtles. *Journal of Experimental Marine Biology and Ecology*, **387**, 44-51.

McCullagh P. & Nelder J.A. (1983) *Generalized linear models*. Chapman and Hall, London, UK.

McCutchan J.H., Lewis W.M., Kendall C. & Mcgrath C.C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulphur. *Oikos*, **102**, 378-390.

McIntosh A.R., Todd A.C. & Townsend C.R. (1994) Size related impacts of introduced brown trout on the distribution of native common galaxies. *New Zealand Journal of Marine and Freshwater Research*, **28**, 135-144.

Meadows M.E. & Meadows K.F. (1988) Late Quaternary vegetation history of the Winterberg Mountains, Eastern Cape, South Africa. *South Africa Journal of Science*, **84**, 253-259.

Meissner K. & Moutka T. (2006) The role of trout in stream food webs: integrating evidence from field surveys and experiments. *Journal of Animal Ecology*, **75**, 421-433.

Merron G.S. (1993) Pack-hunting in two species of catfish, *Clarias gariepinus* and *C. ngamensis*, in the Okavango Delta, Botswana. *Journal of Fish Biology*, **43**, 575-584.

Michener R. & Lajtha K. (2007) *Stable isotopes in ecology and environment*. Blackwell Publishing, London, UK.

Mill A.C., Pinnegar J.K. & Polunin N.V.C. (2007) Explaining isotope trophic-step fractionation: why herbivorous fish are different. *Functional Ecology*, **21**, 1137-1145.

Miller S.A. & Crowl T.A. (2006) Effects of common carp (*Cyprinus carpio*) on macrophytes and invertebrate communities in a shallow lake. *Freshwater Biology*, **51**, 85-94.

Minagawa M. & Wada E. (1984) Stepwise enrichment of ^{15}N along food-chains - further evidence and the relation between $\delta^{15}N$ and animal age. *Geochimica et Cosmochimica Acta*, **48**, 1135-1140.

Mitamura H., Mitsunaga T., Arai N. & Viputhanumas T. (2008) Movements of immature hatchery-reared Mekong giant catfish *Pangasianodon gigas* released in the Mekong River, measured using acoustic telemetry. *Fisheries Science*, **74**, 1034-1039.

Mohr C. (1947) Table of equivalent populations of North American small mammals. *American Midland Naturalist*, 37, 223-249.

Moore J.W. & Semmens B.X. (2008) Incorporating uncertainty and prior information into stable isotope mixing models. *Ecology Letters*, **11**, 470-480.

Moreno R., Jover L., Munilla I., Velando A. & Sanpera C. (2010) A three-isotope approach to disentangling the diet of a generalist consumer: the yellow-legged gull in northwest Spain. *Marine Biology*, **157**, 545-553.

Mosnier A., Ouellet J-P., Sirois L. & Fournier N. (2003) Habitat selection and home-range dynamics of the Gaspe caribou: a hierarchical analysis. *Canadian Journal of Zoology*, **81**, 1174-1184.

Moyle P.B. (1986) Fish introductions into North America: patterns and ecological impact. In *Ecology of biological invasions of North America and Hawaii* (Mooney H.A. & Drake J.A. eds.), Springer-Verlag, New York, USA. 27-43pp.

Moyle P.B & Light T. (1996). Fish invasions in California: do abiotic factors determine success? *Ecology*, **77**, 1666-1670.

Muehlbauer J.D., Doyle W.M. & Bernhardt E.S. (2011) Macroinvertebrate community response to a dewatering disturbance gradient in a restored stream. *Hydrology and Earth Systems Science*, **15**, 1771-1783.

Mueller R. & Pyron M. (2010) Fish assemblages and substrates in the Middle Wabash River, USA. *Copeia*, **1**, 47-53.

Munro J.L. (1967) The food of a community of East African freshwater fishes. *Journal of Zoology*, **151**, 389-415.

Mysterud A. & Ims R. (1998) Functional responses in habitat use: availability influences relative use in trade-off situations. *Ecology*, **79**, 1435-1441.

Newsome S.D., del Rio C.M., Bearhop S. & Phillips D.L. (2007) A niche for isotopic ecology. *Frontiers in Ecology and the Environment*, **5**, 429-436.

Niaman R.J. & Latterell J.J. (2005) Principles for linking fish habitat to fisheries management and conservation. *Journal of Fish Biology*, **67**, 166-185.

Niemi G.J., DeVore P., Detenbeck N., Taylor D., Lima A., Pastor J., Yount J.D. & Naiman R.J. (1990) Overview of case studies on recovery of aquatic systems to disturbance. *Environmental Management*, **14**, 571-587.

Nyström P. & McIntosh A. R. (2003) Are impacts of an exotic predator on a stream food web influenced by disturbance history? *Oecologia*, **136**, 279-288.

O'Keeffe J.H. & de Moor F.C. (1988) Changes in the physico-chemistry and benthic invertebrates of the Great Fish River, South Africa, following an interbasin transfer of water. *Regulated Rivers: Research and Management*, **2**, 39-55.

Oberdorff T., Hugueny B., Compin A. & Belkessam D. (1997) Non-interactive fish communities in the coastal streams of North-Western France. *Journal of Animal Ecology*, **67**, 472-484.

Olsson K., Stenroth P., Nyström P. & Granéli P. (2009) Invasions and niche width: does niche width of an introduced crayfish differ from a native crayfish? *Freshwater Biology*, **54**, 1731-1740.

Ostrand K.G. & Wilde G.R. (2002) Seasonal and spatial variation in prairie stream-fish assemblage. *Ecology of Freshwater Fish*, **11**, 137-149.

Park K. (2004) Assessment and management of invasive alien predators. *Ecology and Society*, **9**, 12.

Parkyn S.M. & Collier K. J. (2004) Interaction of press and pulse disturbance on crayfish populations: flood impacts in pasture and forest streams. *Hydrobiologia*, **527**, 113-124.

Parnell A.C., Inger R., Bearhop S. & Jackson A.L. (2010) Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE*, **5**, e9672.

Payne A.I. (1986) *The ecology of tropical lakes and rivers*. John Wiley & Son, Chichester.

Peh K.S.H. (2010) Invasive species in south East Asia: the knowledge so far. *Biodiversity and Conservation*, **19**, 1083-1099.

Peres-Neto P.R., Legendre P., Dray S. & Borcard D. (2006) Variation partitioning of species data matrices: estimation and comparison of fraction. *Ecology*, **87**, 2614-2625.

Peterson J.T., Thurow R.F. & Guzevich J.W. (2004) An evaluation of multipass electrofishing for estimating the abundance of stream-dwelling salmonids. *Transactions of the American Fisheries Society*, 133, 462-475.

Phillips D.L. (2001) Mixing models in analyses of diet using multiple stable isotopes: a critique. *Oecologia*, **127**, 166-170.

Phillips D.L. & Gregg J.W. (2003) Source partitioning using stable isotopes: coping with too many sources. *Oecologia*, **136**, 261-269.

Phillips D.L. & Koch P.L. (2002) Incorporating concentration dependence in stable isotope mixing models. *Oecologia*, **130**, 114-125.

Phillips D.L., Newsome S.D. & Gregg J.W. (2005) Combining sources in stable isotope mixing models: alternative methods. *Oecologia*, **144**, 520-527.

Phillips I.D., Vinebrooke R.D. & Turner M.A. (2009) Experimental reintroduction of crayfish species *Orconectes virilis* into formerly acidified Lake 302S (Experimental Lakes Area, Canada). *Canadian Journal of Fisheries and Aquatic Science*, **66**, 1892-1902.

Pimm S.L. (1982) *Food webs*. Chapman and Hall, New York, USA.

Pinnegar J.K. & Polunin N.V.C. (2000) Contributions of stable isotope data to elucidating food webs of Mediterranean rocky littoral fishes. *Oecologia*, **122**, 399-409.

Post D.M. (2002) Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecology*, **83**, 703-718.

Post D.M. (2003) Individual variation in the timing of ontogenetic niche shifts of largemouth bass. *Ecology*, **84**, 1298-1310.

Potts W.M., Hecht T. & Andrew T.G. (2008) Does reservoir trophic status influence the feeding and growth of the sharptooth catfish, *Clarias gariepinus* (Teleostei: Clariidae)? *African Journal of Aquatic Science*, **33**, 149-156.

Power M.E. (1987) Predator avoidance by grazing fishes in temperate and tropical streams: importance of stream depth and prey size. In *Predation: direct and indirect impacts on aquatic communities* (Kerfoot W.C. & Sih A. eds.), University Press of New England, Hanover, NH, pp. 333-353.

Press W.H., Teukolsky S.A., Vetterling W.T. & Flannery B.P. (1999) *Numerical recipes in C: the art of statistical computing*. Cambridge University Press, Cambridge, UK.

Pyke G.H. (2008) Plague minnow or mosquitofish? A review of the biology and impacts of introduced *Gambusia* species. *Annual Review of Ecology, Evolution and Systematics*, **39**, 171-191.

Quinn G. & Keough M. (2002) *Experimental design and data analysis for biologists*. Cambridge University Press, Cambridge, UK.

R Development Core Team (2011) *R*: *A language and environment for statistical computing. Vienna*: R Foundation for Statistical Computing.

Radhakrishnan K.V., Lan Z.J., Zhao J., Qing N. & Huang X.L. (2011) Invasion of the African sharp-tooth catfish *Clarias gariepinus* (Burchell, 1822) in South China. *Biological Invasions*: Early online, DOI 10.1007/s10530-011-0004-0.

Rahel F.J. (2002) Homogenization of freshwater faunas. *Annual Review of Ecology and Systematics*, **33**, 291-315.

Rand T.A. & Louda S.A. (2006) Spillover of agriculturally subsidized predators as a potential threat to native insect herbivores in fragmented landscapes. *Conservation Biology*, **20**, 1720-1729.

Ricciardi A. (2004) Assessing species invasions as a cause of extinction. *Trends in Ecology and Evolution*, **19**, 619.

Richardson T.J., Booth A.J. & Weyl O.L.F. (2009) Rapid biological assessment of the fishery potential of Xonxa Dam, near Queenstown, South Africa. *African Journal of Aquatic Science*, **34**, 87-96.

Ripley B.D. (1996) *Pattern recognition and neural networks*. Cambridge University Press, Cambridge, UK.

Rosenberg D.M. & Resh V.H. (1993) *Freshwater biomonitoring and benthic macroinvertebrates*. Chapman and Hall, New York, USA.

Rosing M.N., Ben-David M. & Barry R.P. (1998) Analysis of stable isotope data: A K Nearest-Neighbors randomization test. *Journal of Wildlife Management*, **62**, 380-388.

Ruetz C.R., Newman R.M. & Vondracek B. (2002) Top-down control in a detritus-based food web: fish, shredders, and leaf breakdown. *Oecologia*, **132**,307-315.

Russell A.I. (2011) Conservation status and distribution of freshwater fishes in South African national parks. *African Zoology*, **46**, 117-132.

Sabo J.L., Finlay J.C., Kennedy T. & Post D.M. (2010) The role of discharge variation in scaling of drainage area and food chain length in rivers. *Science*, **330**, 965-967.

Safriel O. & Bruton M.N. (1984) *Aquaculture in South Africa: a cooperative research programme - a report of the working group for Aquaculture National Programme for Environmental Sciences*. South African National Scientific Programmes Report No. 89.

Sax D.F. & Gaines S.D. (2008) Species invasions and extinction: the future of native biodiversity on islands. *Proceedings of the National Academy of Sciences*, **105**, 11490-11497.

Scharler U.M. & Baird D. (2005) A comparison of selected ecosystem attributes of three South African estuaries with different freshwater inflow regimes, using network analysis. *Journal of Marine Systems*, **56**, 283-308.

Schilling E.G., Loftin C.S. & Huryin A.D. (2009) Effects of introduced fish on macroinvertebrate communities in historically fishless headwater and kettle lakes. *Biological Conservation*, **142**, 3030-3038.

Schlosser I.J. (1982) Fish community structure and function along two habitat gradients in headwater stream. *Ecological Monographs*, **52**, 395-414.

Schlosser I.J. (1988) Predation risk and habitat selection by two size classes of a stream cyprinid: experimental test of a hypothesis. *Oikos*, **52**, 36-40.

Schlosser I.J. (1991) Stream fish ecology: a landscape perspective. *BioScience*, **41**, 704-712.

Schmidt S.N., Olden J.D., Solomon C.T. & Vander Zanden M.J. (2007) Quantitative approaches to the analysis of stable isotope food web data. *Ecology*, **88**, 2793-2802.

Schmidt S.N., Vander Zanden M.J. & Kitchell J.F. (2009) Long-term food web changes in Lake Superior. *Canadian Journal of Fisheries and Aquatic Science*, **66**, 2118-2129.

Schoener T. (1970) Non-synchronous spatial overlap of lizards in patchy habitats. *Ecology*, **51**, 408-418.

Schoener T.W. (1989) Food webs from the small to the large. *Ecology*, **70**, 1559-1598.

Seber G.A.F. (1982) *The estimation of animal abundance* (2nd edition). Charles Griffin & Company, London, UK.

Senanan W., Kapuscinski A.R., Na-Nakorn U. & Miller L. (2004) Genetic impacts of hybrid catfish farming (*Clarias macrocephalus* x *C. gariepinus*) on native catfish populations in central Thailand. *Aquaculture*, **235**, 167-184.

Shurin J.B. (2001) Interactive effects of predation and dispersal on zooplankton communities. *Ecology*, **82**, 3404-3416.

Simberloff D. & Von Holle B. (1999) Positive interaction of nonindigenous species: invasional meltdown. *Biological Invasions*, **1**, 21-32.

Simberloff D. (2011) How common are invasion-induced ecosystem impacts? *Biological Invasions*, **13**, 1255-1268.

Simon K.S. & Townsend C.R. (2003) Impacts of freshwater invaders at different levels of ecological organization, with emphasis on salmonids and ecosystems consequences. *Freshwater Biology*, **48**, 982-994.

Skelton P.H. (1988) A taxonomic revision of the redfin minnows (Pisces, Cyprinidae) from southern Africa. *Annals of Cape Province Museum (Natural History)*, **16**, 201-307.

Skelton P.H. (1990) The conservation and status of threatened fishes in southern Africa. *Journal of Fish Biology*, **37**, 87-95.

Skelton P.H. (2001) A *complete guide to the freshwater fishes of southern Africa* (2nd edition). Struik, Cape Town, South Africa.

Sket B. & Trontelj P. (2008) Global diversity of leeches (Hirudinea) in freshwater. *Hydrobiologia*, **595**, 129-137.

Smale M.J. (2005) The diet of the ragged-tooth shark *Carcharias taurus* Rafinesque 1810 in the Eastern Cape, South Africa. *African Journal of Marine Science*, **27**, 331-335.

Smart S.M., Thompson K., Marrs R.H., Le Duc M.G., Maskell L.C. & Firbank L.G. (2006) Biotic homogenization and changes in species diversity across human-modified ecosystems. *Proceedings of the Royal Society B*, **273**, 2659-2665.

Spataru P., Viveen W.J.A.R. & Gophen M. (1987) Food composition of *Clarias gariepinus* (= *C. lazera*), (Cypriniformes, Clariidae) in Lake Kinneret (Israel). *Hydrobiologia*, **144**, 77-82.

Stapp P. (2002) Stable isotopes reveal evidence of predation by ship rats on seabirds on the Shiant Islands, Scotland. *Journal of Applied Ecology*, **39**, 831-840.

Stewart-Oaten A. & Bence J.R. (2001) Temporal and spatial variation in environmental impact assessment. *Ecological Monographs*, **71**, 305-339.

Stewart-Oaten A., Murdoch W. W. & Parker K. R. (1986) Environmental impact assessment: ‘‘pseudoreplication’’ in time? *Ecology*, **67**, 929-940.

Stoks R., McPeek M.A. & Mitchell J.L. (2003) Evolution of prey behavior in response to changes in predation regime: damselflies in fish and dragonfly lakes. *Evolution*, **57**, 574-585.

Strauss S.Y., Lau J.A. & Carroll S.P. (2006) Evolutionary responses of natives to introduced species: what do introductions tell us about natural communities? *Ecology Letters*, **9**, 357-374.

Strayer D.L. (2010) Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology*, **55**, 152-174.

Suding K.N., Gross K.L. & Houseman G.R. (2004) Alternative states and positive feedbacks in restoration ecology. *Trends in Ecology and Evolution*, **19**, 46-53.

Svanbäck R. & Persson L. (2004) Individual diet specialization, niche width and population dynamics: implication for trophic polymorphisms. *Journal of Animal Ecology*, **73**, 973-982.

Swartz E.R., Flemming A.F. & Mouton P. le F.N. (2004) Contrasting genetic patterns and population histories in three threatened redfin species (Cyprinidae) from the Olifants River System, western South Africa. *Journal of Fish Biology*, **64**, 1153-1167.

Swartz E.R., Skelton P.H. & Bloomer P. (2009) Phylogeny and biogeography of the genus *Pseudobarbus* (Cyprinidae): shedding light on the drainage history of rivers associated with the Cape Floristic Region. *Molecular Phylogenetics and Evolution*, **51**, 75-84.

Tablado Z., Tella J.L., Sánchez-Zapata J.A., Hiraldo F. (2009) The paradox of the long-term positive effects of a North American crayfish on a European community of predators. *Conservation Biology*, **24**, 1230-1238.

ter Braak C.J.F. & Verdonschot P.F.M. (1995) Canonical correspondence analysis and related multivariate methods in aquatic ecology. *Aquatic Sciences*, **55**, 1-35.

ter Braak C.J.F. & Smilauer P. (1998) *CANOCO reference manual and user's guide to CANOCO for Windows: software for canonical community ordination (version 4)*. Microcomputer Power, Ithaca, New York.

Teugels G.G. (1986) *A systematic revision of the African species of the genus Clarias* (Pisces; Clariidae). Annales Musée Royale de l'Afrique Centrale Sciences Zoologiques, 247.

Townsend C. R. & Crowl T.A. (1991) Fragmented population structure in a native New Zealand fish: an effect of introduced brown trout? *Oikos*, **61**, 347–354.

Traas G.R.L. (2009) *The conservation and management of freshwater fishes in the Greater Addo Elephant National Park*. MSc Thesis, Rhodes University.

Turner T.T., Collyer M.L. & Krabbenhoft T.J. (2010) A general hypothesis-testing framework for stable isotope ratios in ecological studies. *Ecology*, **91**, 2227-2233.

Tweddle D., Bills R., Swartz E., Coetzer W., Da Costa L., Engelbrecht J., Cambray J., Marshall B., Impson D., Skelton P.H., Darwall W.R.T. & Smith K.S. (2009) The status and distribution of freshwater fishes. In *The status and distribution of freshwater biodiversity in southern Africa* (Darwall W.R.T., Smith K.G., Tweddle D. & Skelton P.H. eds.), Gland (Switzerland) and Grahamstown (South Africa): IUCN and South African Institute for Aquatic Biodiversity. 21-37pp.

Underwood A.J. (1991) Beyond BACI: experimental designs for detecting human impacts on temporal variations in natural populations. *Australian Journal of Marine and Freshwater Research*, **42**, 569-587.

Underwood A.J. (1992) Beyond BACI: the detection of environmental impacts on populations in the real, but variable, world. *Journal of Experimental Marine Biology and Ecology*, **161**, 145-178.

Underwood A.J. (1994) On Beyond BACI: sampling designs that might reliably detect environmental disturbances. *Ecological Applications*, **4**, 3-15.

Underwood A.J. (1997) *Experiments in ecology: their logical design and interpretation using analysis of variance*. Cambridge University Press, Cambridge, UK.

Van der Waal B.C.W. (1998) Survival strategies of sharptooth catfish *Clarias gariepinus* in desiccating pans in the northern Kruger National Park. *Koedoe*, **41**, 131-138.

van Zyl A.J. (1994) Sex-related local movement in adult rock kestrels in the Eastern Cape Province, South Africa. *The Wilson Bulletin*, **106**, 145-148.

Vander Zanden M.J. & Rasmussen J.B. (1999) Primary consumer $\delta^{15}N$ and $\delta^{13}C$ and the trophic position of aquatic consumers. *Ecology*, **80**, 1395-404.

Vander Zanden M.J. & Rasmussen J.B. (2001) Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic food web studies. *Limnology and Oceanography*, **46**, 2061-2066.

Vander Zanden M.J., Cabana G. & Rasmussen J.B. (1997) Comparing the trophic position of littoral fish estimated using stable nitrogen isotopes ($\delta^{15}N$) and dietary data. *Canadian Journal of Fisheries and Aquatic Science*, **54**, 1142-1158.

Vander Zanden M.J., Shuter B.J., Lester N.P. & Rasmussen J.B. (1999) Patterns of food chain length in lakes: a stable isotope study. *The American Naturalist*, **154**, 406-416.

Vander Zanden M.J., Chandra S., Allen B.C., Reuter J.E. & Goldman C.R. (2003) Historical food web structure and restoration of native aquatic communities in the Lake Tahoe (California-Nevada) Basin. *Ecosystems*, **6**, 274-288.

Vander Zanden M.J., Olden J.D., Thorne J.H. & Mandrak N.E. (2004) Predicting occurrences and impacts of smallmouth bass introductions in north temperate lakes. *Ecological Applications*, **14**, 132-148.

Vannote R.L., Minshall G.W., Cummins K.W., Sedell J.R. & Cushing C.E. (1980) The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences*, **37**, 130-137.

VanWinkle W. (1975) Comparison of several probabilistic home-range models. *Journal of Wildlife Management*, **39**, 118-123.

Vayssiéres M.P., Plant R.E. & Allen-Diaz B.H. (2000) Classification trees: an alternative non-parametric approach for predicting species distributions. *Journal of Vegetation Science*, **11**, 679-694.

Vitousek P.M. (1990) Biological invasions and ecosystem processes: towards an integration of population ecology and ecosystem studies. *Oikos*, **57**, 7-13.

Vitule J.R.S., Umbria S.C. & Aranha J.M.R. (2006) Introduction of the African catfish *Clarias gariepinus* (Burchell, 1822) into Southern Brazil. *Biological Invasions*, **8**, 677-681.

Vitule J.R.S., Umbria S.C. & Aranha J.M.R. (2008) Record of native amphibian predation by the alien African catfish in the Brazilian Atlantic Rain Forest. *Pan-American Journal of Aquatic Science*, **3**, 105-107.

Vitule J.R.S., Freire C.A. & Simberloff D. (2009) Introduction of non-native freshwater fish can certainly be bad. *Fish and Fisheries*, **10**, 98-108.

Vizzini S., Sara G., Michener R.H. & Mazzola A. (2002) The role and contribution of the seagrass *Posidonia oceanica* (L.) Delile organic matter for secondary consumers as revealed by carbon and nitrogen stable isotope analysis. *Acta Oecologia*, **23**, 277-285.

Ward E.J., Semmens B.X. & Schindler D.E. (2010) Including sources uncertainty and prior information in the analysis of stable isotope mixing models. *Environmental Science and Technology*, **44**, 4645-4650.

Wartenberg R., Booth A.J. & Weyl O.L.F. (2011) A comparison of three techniques for fluorochrome marking of juvenile *Clarias gariepinus* otoliths. *African Zoology*, **46**, 72-77.

Wasserman R.J., Weyl O.L.F. & Strydom N.A. (2011) The effects of instream barriers on the distribution of migratory marine-spawned fishes in the lower reaches of the Sundays River, South Africa. *Water SA*, **37**, 495-504.

Weber M.J. & Brown M.L. (2011) Relationships among invasive common carp, native fishes and physico-chemical characteristics in upper Midwest (USA) lakes. *Ecology of Freshwater Fish*, **20**, 270-278.

Welcomme R.L. (1985) *River fisheries*. FAO Fisheries Technical Paper 262. Food and Agriculture Organisation of the United Nations, Rome.

Weller R. R. & Winter J.D. (2001) Seasonal variation in home range size and habitat use of flathead catfish in Buffalo Springs Lake, Texas. *North American Journal of Fisheries Management*, **21**, 792-800.

Weyl O.L.F. & Lewis H. (2006) First record of predation by the alien invasive freshwater fish *Micropterus salmoides* L. (Centrarchidae) on migrating estuarine fishes in South Africa. *African Zoology*, **41**, 294-296.

Weyl O.L.F. & Booth A.J. (2008) Validation of annulus formation in otoliths of a temperate population of adult African sharptooth catfish *Clarias gariepinus* using fluorochrome marking of wild fish. *Journal of Fish Biology*, **73**, 1033-1038.

Weyl O.L.F., Stadtlander T. & Booth A.J. (2009a) Establishment of translocated populations of smallmouth yellowfish, *Labeobarbus aeneus* (Pisces: Cyprinidae), in lentic and lotic habitats in the Great Fish River system, South Africa. *African Zoology*, **44**, 93-105.

Weyl O.L.F., Booth A.J., Traas G.R.L., Swartz E. (2009b) *Determination of critical conservation areas in the Sundays River system for indigenous fish species*. Sundays River Freshwater Fish Research Project ID: PO64438.

Weyl P.S.R., de Moor F.C., Hill M.P. & Weyl O.L.F. (2010) The effect of largemouth bass *Micropterus salmoides* on aquatic macroinvertebrate communities in the Wit River, Eastern Cape, South Africa. *African Journal of Aquatic Science*, **35**, 273-281.

Whitfield A.K. (1998) *Biology and ecology of fishes in southern African estuaries*. J.L.B. Smith Institute of Ichthyology, Grahamstown, South Africa.

Wilkinson C.D. & Edds D.R. (2002) Spatial pattern and environmental correlates of a midwestern stream fish community: including spatial autocorrelation as a factor in community analyses. *The American Midland Naturalist*, **146**, 271-289.

Williams C.T. & Buck C.L. (2010) Using fatty acids as dietary tracers in seabird trophic ecology: theory, application and limitations. *Journal of Ornithology*, **151**, 531-543.

Williams L.R. & Taylor C.M. (2003) Influence of fish predation on assemblage structure of macroinvertebrates in an intermittent stream. *Transactions of the American Fisheries Society*, **132**, 120-130.

Willoughby N.G. & Tweddle D. (1978) The ecology of the catfish *Clarias gariepinus* and *Clarias ngamensis* in the Shire Valley, Malawi. *Journal of Zoology*, **186**, 507-534.

Winemiller K.O. & Kelso-Winemiller L.C. (1996) Comparative ecology of catfishes of the Upper Zambezi River floodplain. *Journal of Fish Biology*, **49**, 1043-1061.

Winemiller K.O., Pianka E.R., Vitt L.J. & Joern A. (2001) Food web laws or niche theory? Six independent empirical tests. *The American Naturalist*, **158**, 193-199.

Winemiller K.O., Hoeinghaus D.J., Pease A.A., Esselman P.C., Honeycutt R.L., Gbanaador D., Carrera E. & Payne J. (2011) Stable isotope analysis reveals food web structure and watershed impacts along fluvial gradient of a Mesoamerican coastal river. *River Research and Applications*, **27**, 791-803.

Winker H., Weyl O.L.F., Booth A.J. & Ellender B.R. (2011) Life history and population dynamics of invasive common carp, *Cyprinus carpio*, in a large turbid African impoundment. *Marine and Freshwater Research*, **62**, 1270-1280.

Woodford D.J. & Impson N.D. (2004) A preliminary assessment of the impact of alien rainbow trout (*Oncorhynchus mykiss*) on indigenous fishes of the upper Berg River, Western Cape Province, South Africa. *African Journal of Aquatic Science*, **29**, 107-111.

Wootton R.J. (2007) Over-wintering growth and losses in a small population of the threespine stickleback, *Gasterosteus aculeatus* (L.), in mid-Wales. *Ecology of Freshwater Fish*, **16**, 476-481.

Worton B. (1989) Kernel methods for estimating the utilization distribution in home-range studies. *Ecology*, **70**, 164-168.

Yalçin S., Akyurt I. & Solak K (2001) Stomach contents of the catfish (*Clarias gariepinus* Burchell, 1822) in the River Asi (Turkey). *Turkish Journal of Zoology*, **25**, 461-468.

Zorn T.G., Seelbach P.W. & Wiley M.J. (2002) Distribution of stream fish and their relationship to stream size and hydrology in Michigan's lower peninsula. *Transactions of American Fisheries Society*, **131**, 70-85.