INVASION STATUS AND IMPACT POTENTIAL OF FLORIDA BASS *MICROPTERUS FLORIDANUS* IN SOUTH AFRICA

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GENERAL ABSTRACT

In South Africa, the introduction of alien fishes was largely driven by the development of recreational fishing opportunities. Government-backed programmes resulted in the introduction of four Black Bass species: *Micropterus salmoides*, *Micropterus dolomieu*, *Micropterus punctulatus* and *Micropterus floridanus*. These species are regarded as the primary threat to native biota and there is an urgent need to assess their spread and impacts. This thesis focuses on: assessing the current distribution of all four Black Basses; using genetic techniques to establish the extent of hybridisation between *M. salmoides* and *M. floridanus,* and using functional responses to assess the impact potential of *M. salmoides* and *M. floridanus* under the context dependence of temperatures and habitat complexity.

Maximum entropy modelling of habitat suitability for Black Bass highlighted that *M. salmoides* had broad climatic suitability across South Africa, while suitability for *M. dolomieu* and *M. punctulatus* was restricted to the Cape Fold Ecoregion. An extensive area-based invasion debt was observed for all *Micropterus* spp. To better understand the dynamics of hybridisation between *M. salmoides* and *M. floridanus*, 38 species-diagnostic single nucleotide polymorphism was used to screen Black Bass populations from the Breede River and Kowie River catchments. *Micropterus salmoides* alleles were more prevalent than *M. floridanus* in both the Breede River catchments (69.1% and 30.9%, respectively) and Kowie River catchments (63.3% and 36.7%, respectively). However, isolated populations found in dams (reservoirs) remained free from hybridisation. To compare resource utilisation (i.e. *per capita* effects) between *M. salmoides* and *M. floridanus*, two experimental chapters were designed. Chapter 4 focuses on how temperature mitigates *per capita* effects between the two Black Basses. There was a significant decrease in *per capita* effects for *M. salmoides* with increasing temperatures, while the converse was true for *M. floridanus*. Chapter 5 gives an account of the influence of habitat complexity on *per capita* effects. The results showed that habitat complexity did mitigate *per capita* effects, specifically on *M. floridanus,* which showed a significant drop in *per capita* effects; however, no significant difference was recorded for *M. salmoides*. In the two experimental chapters, *M. floridanus* exhibited a superior *per capita* effect compared to *M. salmoides*, implying higher predation impact on native biota.

DECLARATION

I, Dumisani Khosa, hereby declare that this thesis submitted in the Department of Ichthyology and Fisheries Science, Rhodes University, is my original work and has not been previously submitted in any form to another university. I have not included ideas, phrases, passages or illustrations from another person's work without acknowledging their authorship.

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PREFACE

This thesis comprises four data chapters which are presented as scientific papers, some of which have been published in peer-reviewed journals, with the remaining papers in press or under review (see below). Included is a general introduction (Chapter 1) and a general synthesis and conclusion (Chapter 6).

- Khosa D, Marr SM, Wasserman RJ, Zengeya TA, Weyl OLF (2019) An evaluation of the current extent and potential spread of Black Bass invasions in South Africa. *Biological Invasions 21*:1721–1736. doi: 10.1007/s10530-019-01930-0.

- Khosa D, Hargrove JS, Peatman E, Weyl OLF (*ready for submission*) The extent of hybridisation between Largemouth Bass and Florida Bass across two river systems in South Africa.

- Khosa D, South J, Cuthbert RN, Wasserman RJ, Weyl OLF (2019) Temperature regime drives differential predatory performance in Largemouth Bass and Florida Bass *Environmental Biology of Fishes 103*: 67–76. doi.org/10.1007/s10641-019-00933-z.

- Khosa D, South J, Wasserman RJ, Weyl OLF (*submitted*) Does habitat structure mitigate predation impact of Black Bass species? (*under review in* ***Environmental Biology of Fishes***).

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Xo hetelela, ndza khensa ka vatswari va mina kuva mi ndzi lehiserile mbilu ka tidyondzo ta mina, ndzi munhu hikwalaho ka n’wina. Ka vamakwerhu va mina, ndzi nkhensa nseketelo wa n’wina na rirhandzu ro pfumala ntangha leri mi ndzi kombaka rona. Yinhla n’wananga, swi tive ku dyonzo i makomba-ndlela, ayi lumi. Rirhandzu ra mina ka n’wina ri pfumala ntangha. Mavona wa khensa a vuyelela.

CHAPTER 1

GENERAL INTRODUCTION

Biological invasion of ecosystems is an important driver of global change (Sala et al. 2000; Leprieur et al. 2008; Gozlan et al. 2010; Ricciardi et al. 2017). Many species have been effectively moved across natural biogeographic barriers and successfully colonised new regions (Dudgeon et al. 2006; Essl et al. 2011; Ricciardi et al. 2017; Meyerson et al. 2019). Anthropogenic activities have been identified as the main drivers of biological invasions (Dudgeon et al. 2006; Leprieur et al. 2008; Courchamp et al. 2017), owing to the rapid expansion of transportation networks for global trade, land transformation, and climate change (Meyer et al. 1999; Seebens et al. 2015, 2017; Early et al. 2016). With the increase in globalisation, there seems to be no decrease in the rate of new biological invasions across different taxa (Seebens et al. 2017), which may result in the increased vulnerability of different ecosystems (Essl et al. 2011; Rouget et al. 2016; Dawson et al. 2017; Meyerson et al. 2019).

Many freshwater fish species have been introduced outside their native ranges (De Moor and Bruton 1988; Welcomme 1988, 1992; Gozlan 2008; Frederico et al. 2019) and have become invasive, threatening native biodiversity, and ultimately reducing the ability of ecosystems to deliver vital ecosystem services (Gozlan et al. 2010; Cucherousset and Olden 2011; van Wilgen and Wilson 2018). Freshwater fishes are the most frequently introduced aquatic animals in the world, with over 624 species introduced worldwide (Gozlan 2008), mainly for aquaculture (51%), ornamental pet trade (21%), sport fishing (12%) and fisheries (7%) (Gozlan 2008, 2017; Gozlan et al. 2010). Non-native fishes are likely to present impacts if they can successfully integrate into the novel ecosystem (*sensu* Blackburn et al. 2011) which may include socio-economic (Pimentel et al. 2004; Pimentel 2007; Arlinghaus and Cooke 2009) and ecological impacts (Cucherousset and Olden 2011; Ellender et al. 2018; van Wilgen and Wilson 2018). Major ecological impacts include high predation pressure and competitive exclusion, hybridisation with congeneric native species, the introduction of parasites and diseases, and alteration of trophic links and behaviour of native biota (Rhymer and Simberloff 1996; Gozlan et al. 2005; Cucherousset and Olden 2011; Jackson et al. 2017; Ellender et al. 2018; Pereira and Vitule 2019).

It is also important to mention that the benefits arising from the introduction of non-native species can be substantial (Schlaepfer et al. 2011; Ellender et al. 2014; Gozlan 2017; Zengeya et al. 2017). Globally, 54% of all the species used in aquaculture and fisheries are native to Asia, thus, the aquaculture and fisheries industry have to continually rely on non-native species to sustain production and generate income (Gozlan 2017). Furthermore, recreational angling species such as salmonids of the genus *Oncorhynchus* and Black Bass species of the genus *Micropterus* are conflict-generating species because of their high negative impacts and benefits in the receiving ecosystems (McDowall 2004; Crawford and Muir 2008; Ellender and Weyl 2014; Ellender et al. 2014). In Chile, for example, introduced Rainbow Trout *Oncorhynchus mykiss* (Walbaum 1792), Brown Trout *Salmo trutta* Linnaeus 1758, and Atlantic Salmon *Salmo salar* Linnaeus 1758 are the focus of a multibillion-dollar fishery that employs about 35 000 people (Gozlan 2017). Similar trends are also evident in South Africa where the Rainbow Trout is worth just over ZAR 63 million annually (van Rensburg et al. 2011). Consequently, managing non-native species, such as salmonids and Black Basses, often results in conflict between the different stakeholders because of polarised perceptions of the impacts and benefits of non-native species (Gozlan et al. 2013; Woodford et al. 2017; Zengeya et al. 2017).

In South Africa, early introductions of non-native fishes were intentional (Coke 1988; De Moor and Bruton 1988; van Rensburg et al. 2011) and were carried out without any consideration of the ecological impact on native biodiversity (Coke 1988; Ellender and Weyl 2014; Marr et al. 2017). South Africa has been identified as one of the fish invasion hotspots (Leprieur et al. 2008) and Ellender and Weyl (2014) reported that 55 fish species (27 non-natives and 28 extralimital) have been introduced into or translocated within the country. Non-native and extralimital species now comprise between 11% and 71%, respectively, of the fish species in 16 major catchments (van Rensburg et al. 2011). The extent of invasion in some areas, such as the Cape Fold Ecoregion (CFE), is severe, with the number of successfully introduced fishes exceeding the original numbers of endemic species (Marr et al. 2009; van der Walt et al. 2016). While most of the introductions appear to have served their purpose, such as creating new recreational angling and aquaculture opportunities (e.g. van Rensburg et al. 2011), the effects on the recipient ecosystems remain poorly understood (Ellender and Weyl 2014). But the available evidence suggests impacts can be detrimental to the recipient ecosystem (Zengeya et al. 2017; Ellender et al. 2018). For example, the non-native salmonid species, Rainbow Trout and Brown Trout, and Black Basses are often introduced into sections of rivers where native fauna is characterised by high levels of endemism and naïveté to predators (Cox and Lima 2006; Woodford et al. 2017), often resulting in the local extirpations of the resident biota (Weyl et al. 2010; Shelton et al. 2017; Van Der Walt et al. 2017; Ellender et al. 2018). Thus, non-native species, such as Black Basses, introduced to create new recreational angling opportunities, create conflicts due to their economic benefits and the potential ecological impacts in the receiving ecosystems (Ellender et al. 2014; Long et al. 2015; Zengeya et al. 2017).

Black Bass fisheries

The genus *Micropterus* is composed of eight freshwater species which are native to North America, east of the Rocky Mountains (Near et al. 2003; Taylor et al. 2019a). Of the eight *Micropterus* species, four have been introduced globally (Largemouth Bass *Micropterus salmoides* (Lacépède 1802), Smallmouth Bass *Micropterus dolomieu* Lacépède 1802, Spotted Bass *Micropterus punctulatus* (Rafinesque 1819), and Florida Bass *Micropterus floridanus* (Lesueur 1822), colonising a broad range of habitats in freshwater ecosystems (Welcomme 1992; Casal 2006; Quinn and Paukert 2009; Long et al. 2015; Shaw 2015). Largemouth Bass, for example, have been introduced into over 72 countries to provide recreational angling opportunities (Welcomme 1988; Casal 2006). In the United States of America (USA), Black Basses are the most targeted fish species (excluding the Great Lakes), attracting a total of 9.6 million of the 29.5 million anglers, and generating a combined fishing effort of up to 117 million days (U.S. Fish and Wildlife Service and U.S. Census Bureau 2016). Furthermore, direct expenditure associated with Black Bass fisheries across the USA (e.g. travel, food, lodging, equipment) was over US$10 billion in the 2015/2016 fishing season (U.S. Fish and Wildlife Service and U.S. Census Bureau 2016). Although angler participation and the economic value of the Black Bass fishery have not been fully quantified globally, anecdotal evidence suggests higher angler participation (McCafferty 2012; Ellender et al. 2014; Hargrove et al. 2015; Long et al. 2015; Taylor et al. 2015) resulting in socio-economic benefits to the local and regional communities (Chen et al. 2003; Cooke and Wilde 2007; Leibold and van Zyl 2008; McCafferty 2012; Ellender et al. 2014; Kinghorn et al. 2014; Weyl and Cowley 2015).

Over the years, recreational angling for Black Bass species has increased substantially (Quinn and Paukert 2009; Hargrove et al. 2015; Long et al. 2015), and so has the movement and stocking across different states (Robbins and MacCrimmon 1974; Jackson 2002; Long et al.

2015). In the USA, stocking Black Bass species outside of their native range has long been a contentious issue owing to the associated genetic implications (Philipp 1991; Maceina and Murphy 1992; Barthel et al. 2010; Hargrove et al. 2019a; Taylor et al. 2019a). This stocking is a major conservation issue for species such as Largemouth Bass and Florida Bass because they readily hybridise when in sympatry, which may lead to the loss of indigenous genetics in their respective native ranges (Barthel et al. 2010; Hargrove et al. 2019a).

Hybridisation between Largemouth Bass and Florida Bass

Largemouth Bass and Florida Bass are the most widespread of the eight Black Bass species because of their recreational and economic importance (Quinn and Paukert 2009; Long et al. 2015). The two Black Basses coexist in introgressed populations throughout the hybrid zone across the south-eastern United States (Figure 1.1; Bailey and Hubbs 1949; Philipp et al. 1983; Barthel et al. 2015; Taylor et al. 2019). Owing to the absence of pre- or postzygotic barriers to isolation, Florida Bass and Largemouth Bass readily hybridise and produce offspring that are morphologically indistinguishable as hybrids, and are only readily identifiable via molecular analysis (Bolnick and Near 2005). As a result, the presence of Florida Bass is hard to detect and they are typically synonymised with Largemouth Bass (Warren 2009). In both their native and introduced ranges, Florida Bass are commonly stocked into existing populations of Largemouth Bass to create trophy fisheries (Barthel et al. 2010; Weyl et al. 2017; Hargrove et al. 2019a), based on the premise that incorporating Florida Bass alleles into the Largemouth Bass populations will enhance the growth rate and so promote trophy fisheries (Myers and Allen 2005; Allen et al. 2008; Myers et al. 2008; Lamothe et al. 2016). Indeed, working in Texas, Myers and Allen (2005) reported a significantly higher prevalence of trophy catches in dams (i.e. reservoirs) stocked with Florida Bass (29%) than in dams containing only Largemouth Bass (4%). Similarly, the increased occurrence of trophy catches after the introduction of Florida Bass has been documented in Arkansas (Lamothe and Johnson 2013), Louisiana (Hughes and Wood 1995), Texas (Forshage and Fries 1995), Oklahoma (Horton and Gilliland 1993) and South Africa (Weyl et al. 2017).

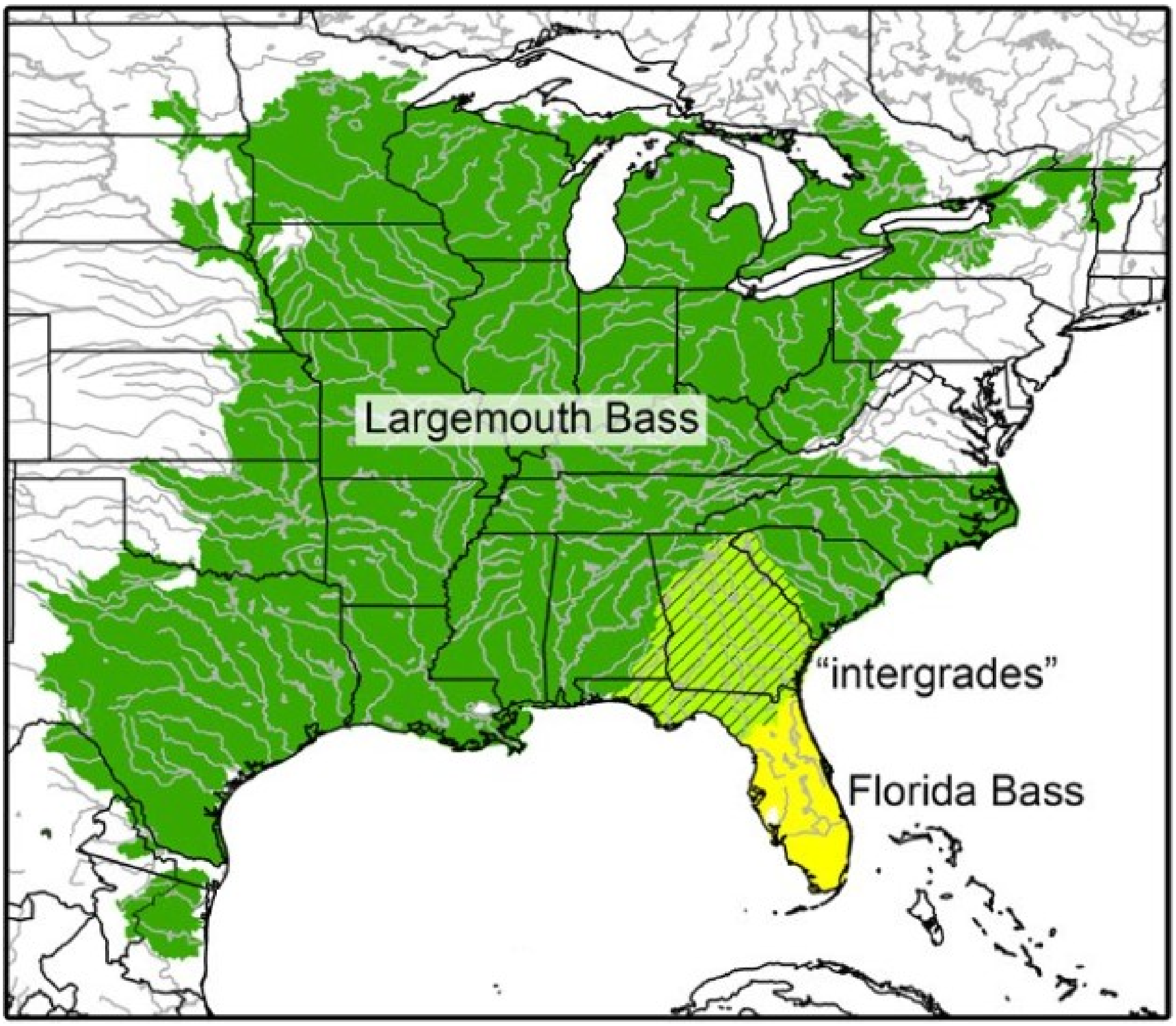

Figure 1.1 Original native distribution of Largemouth Bass and Florida Bass in North America. The intergrade zone between Largemouth Bass and Florida Bass, as described by Bailey and Hubbs (1949). Map from Taylor et al. (2019a)

The benefits derived from recreational angling for Black Bass species often mask the negative ecological impacts associated with their introduction (Philipp 1991; Jackson 2002; Hargrove et al. 2015). The introduction of Florida Bass into Largemouth Bass populations or vice versa often leads to genetic contamination, which may result in the extinction of native genomes by hybridisation and introgression (Philipp et al. 1981, 1983; Rhymer and Simberloff 1996; Barthel et al. 2010; Hargrove et al. 2019a). Several studies have found that the hybrids of both Largemouth Bass and Florida Bass exhibit reduced fitness, while ‘pure’ populations of both species in their respective native ranges exhibit greater growth rate, survival and reproductive success (Philipp and Claussen 1995; Philipp et al. 2002). Further, Cooke et al. (2001), Cooke and Philipp (2005) demonstrated that interstock F_1 hybrids of Largemouth Bass display reduced cardiovascular, swimming, and respiratory performance relative to the pure stock of Largemouth Bass, which was interpreted as a loss of local adaptation. Owing to the detrimental implications of mixing genetic stocks of Largemouth Bass and Florida Bass, some states in the USA have revised their management strategies to prohibit the introduction of non-native Black Bass alleles (Barthel et al. 2010; Hargrove et al. 2019a; Taylor et al. 2019a).

Black Basses in South Africa

Introductions of Black Bass species outside of their native range date back to the late 1800s and were driven by government agencies, anglers and piscatorial societies (De Moor and Bruton 1988; Ellender et al. 2014; Long et al. 2015). Four Black Bass species have been introduced into South Africa to boost recreational angling, namely: Largemouth Bass, Smallmouth Bass, Spotted Bass and Florida Bass (De Moor and Bruton 1988). Largemouth Bass were first introduced into South Africa in 1928 and were imported into the Jonkershoek Hatchery, followed by Smallmouth Bass and Spotted Bass in 1937 and 1939, respectively (Harrison 1936; De Moor and Bruton 1988). Florida Bass were later imported into the Umgeni Hatchery, KwaZulu-Natal, South Africa, in 1980 to enhance the Largemouth Bass fisheries (De Moor and Bruton 1988). Indeed, the introduction of Florida Bass has led to the near doubling of the Largemouth Bass angling record in southern Africa from ca. 4 kg to 8.3 kg (Weyl et al. 2017). The early distribution and spread of all four Black Bass species was facilitated by the government, prior to the cessation of state-enabled active distributions in the early 1990s (McCafferty 2012; Ellender et al. 2014). In South Africa, all four Black Basses are considered conflict species that contribute to the economy of South Africa, but they are also implicated in the freshwater biodiversity crisis and require careful management (Ellender et al. 2014; Woodford et al. 2017; van Wilgen and Wilson 2018).

Largemouth Bass

Largemouth Bass (Figure 1.2) were introduced into South Africa as a game fish for recreational angling in 1928 (De Moor and Bruton 1988). Two shipments of Largemouth Bass fingerlings, totalling 88 individuals, were introduced from Europe into South Africa (Harrison 1936). The first shipment contained 45 juveniles that were placed into the Jonkershoek Hatchery in the Western Cape, and the second shipment comprised 43 fingerlings that were placed into the Pirie Hatchery near King Williams Town in the Eastern Cape (Harrison 1936). Through government-funded stockings and angler-mediated translocations in the early 20th century, Largemouth Bass are now found across all major catchments of South Africa (McCafferty 2012; Ellender et al. 2014). Anecdotal stories suggest anglers went to extraordinary measures to establish local fisheries; De Moor and Bruton (1988) describe a one-armed angler who drove over 250 kilometres from Mbabane in Swaziland to northern Zululand in 1935 to stock Lake Sibaya with 29 individuals of Largemouth Bass. In the Olifants River catchment, in the Western Cape, a single stocking of 30 individuals of Largemouth Bass was sufficient for the successful establishment of breeding populations in the catchment (Harrison 1936).

Figure 1.2 An adult Largemouth Bass from Mankazana Dam, South Africa. Largemouth Bass are morphologically difficult to distinguish from Florida Bass (Photo: Olaf Weyl)

Largemouth Bass are capable invaders with the ability to spread far beyond their initial point of introduction (Harrison 1936; Warren 2009). As a top predator, Largemouth Bass have evolved effective life history strategies characterised by relatively fast growth, delayed maturity, and parental care leading to high-quality offspring (Warren 2009), almost guaranteeing that, where introduced, they establish successfully. Largemouth Bass growth rate and body size at age 1 is highly variable among habitats and across latitudes, ranging from ~33 to 271 mm TL (Clugston 1964; Garvey and Marschall 2003; Garvey et al. 2003). Larger individuals can attain maximum sizes exceeding 600 mm TL (Skelton 2001; Warren 2009). The world angling record for Largemouth Bass was established in Montgomery Lake, Georgia in 1932 with a fish weighing in at 10.1 kg (~787 mm TL) (Warren 2009). It is, however, important to mention that Montgomery Lake is situated at the natural hybrid zone identified by Baily and Hubbs (1949) and Philipp et al. (1983) and, as such, this record catch Largemouth Bass may have been a hybrid of the Largemouth and Florida Bass. Furthermore, the angling record was documented before Kassler et al. (2002) when the Florida Bass was still considered a subspecies of Largemouth Bass. Similarly, the current South African record of Largemouth

Bass was angled in Loskop Dam in 2018, weighing in at 7.2 kg. This record Largemouth Bass is also likely to be a hybrid of the Largemouth Bass and Florida Bass, as it was after the introduction of Florida Bass in 1980 that resulted in the near doubling of the trophy Largemouth Bass from ca. 4 kg to 8.3 kg (Weyl et al. 2017).

Largemouth Bass thrive in a variety of habitats, ranging from dams and slower sections of rivers to coastal lakes and estuaries (Claussen 2015). This species favours clear, standing or slow-flowing waters with submerged and floating vegetation (Skelton 2001; Warren 2009; Claussen 2015). The Largemouth Bass is an opportunistic top carnivore exploiting prey at all levels of the water column (Warren 2009). Fry primarily feed on zooplankton and insect larvae as they grow and their gape increases (Claussen 2015). By the time Largemouth Bass reach 100 mm TL, their diet has transitioned to include fish (Olson et al. 1995; Olson 1996; Brown et al. 2009). Adult Largemouth Bass are primarily piscivorous and consume a variety of prey, including juvenile fishes and minnows (Scott and Crossman 1973; Lasenby and Kerr 2000; Huskey and Turingan 2001; Weyl and Lewis 2006; Taylor et al. 2019c).

Once established, Largemouth Bass have been shown to exert strong predation pressure on native biota (Maezono and Miyashita 2002; Weyl and Lewis 2006; Ellender et al. 2011, 2018; Bezerra et al. 2019; Taylor et al. 2019c), especially in areas in which no predatory fish previously occurred (De Moor and Bruton 1988). In the CFE for example, Gaigher (1973) and van der Walt et al. (2016) showed the complete absence of native species in areas where Largemouth Bass were present, and which survive only above waterfalls that prevent Largemouth Bass from accessing these areas. Similarly, in the Swartkops River system, the endangered Eastern Cape redfin minnow *Pseudobarbus afer* (Peters 1864), has been restricted to a small stretch of the stream above the waterfalls, downstream of which the invasion of

Largemouth Bass has resulted in the extirpation of this endemic native species (Ellender et al. 2011).

Smallmouth Bass

Smallmouth Bass (Figure 1.3) are native to the freshwater systems of central and eastern North America (Carey et al. 2011). Their popularity among anglers has led to the intentional stocking from their native range into over 20 countries, as far afield as Japan and South Africa (De Moor and Bruton 1988; Iguchi et al. 2004). In South Africa, Smallmouth Bass were imported from the Lewistown Hatchery in Maryland, USA, into the Jonkershoek Hatchery in 1937, from where they were propagated and distributed into various waterbodies, specifically into rivers to fill a vacant niche left by Largemouth Bass (De Moor and Bruton 1988). The opening of the Umgeni Hatchery in 1952 led to an increase in the propagation and distribution of Smallmouth Bass fingerlings into various waterbodies in the country (De Moor and Bruton 1988). Several studies have demonstrated introgressive hybridisation between Smallmouth Bass and Largemouth Bass and Spotted Bass when in sympatry (Whitmore and Hellier 1988; Kerr and Grant 1999; Moyle 2002; Fuller 2007; Brewer and Orth 2015; Diedericks et al. 2018). Indeed, in the Olifants River system, Western Cape, South Africa, Diedericks et al. (2018) reported introgressive hybridisation between Largemouth Bass and Smallmouth Bass. Implications of hybridisation on the fitness of the species remain relatively unknown; however, evidence from studies on the impacts of Smallmouth Bass in South Africa suggests a negative association with native fish communities (Woodford et al. 2005; Shelton et al. 2014). Direct predation appears to be the main driver of extirpations of native biota (Woodford et al. 2005), similar to reports in the Canadian Lakes, where predation by Smallmouth Bass was linked to the reduced diversity of small native cyprinids (MacRae and Jackson 2001; Jackson 2002). This predation is of major conservation concern because most of the species that are threatened by Smallmouth

Bass, specifically in the CFE, are listed in the IUCN Red List as vulnerable, endangered or critically endangered (Ellender et al. 2017). Owing to the extent of invasions, it is currently impossible to eradicate Smallmouth Bass in South Africa. Thus, the only practical management strategy is to prevent their further spread into uninvaded catchments by prohibiting stocking (Woodford et al. 2017; van Wilgen and Wilson 2018).

Figure 1.3 An adult Smallmouth Bass (Photo: Leonard Flemming)

Efforts have, however, been made to eradicate Smallmouth Bass in a small stream of South Africa. In the Rondegat River, for example, Weyl et al. (2013) used a piscicide, rotenone, to remove the non-native Smallmouth Bass, on the assumption that removing Smallmouth Bass will increase the amount of habitat available to native fishes, and therefore enhance their chances for recolonisation. Indeed, Smallmouth Bass were successfully removed in the Rondegat River and subsequently, native fishes, including Clanwilliam Redfin *Pseudobarbus calidus* Barnard 1938, Clanwilliam rock catfish *Austroglanis gilli* (Barnard 1943), Clanwilliam yellowfish *Labeobarbus seeberi* (Gilchrist and Thompson 1913), and Fiery redfin minnow

Pseudobarbus phlegethon (Barnard 1938) have returned (Weyl et al. 2013). Prior to the removal of Smallmouth Bass, these fishes were absent from the treatment area, and their presence one year later suggests the presence of Smallmouth Bass was the driver of their absence. To date, this has been the only successful, documented removal of non-native species from a natural ecosystem in South Africa.

Spotted Bass

The Spotted Bass (Figure 1.4) are native to the Mississippi River basin, from southern Ohio and West Virginia, to southwestern Kansas and south to the Gulf of Mexico and Gulf river drainages from Choctawhatchee River, Alabama and Florida, west to the Guadalupe River, Texas (Warren 2009). Spotted Bass are opportunistic carnivores, feeding on diverse prey items found in the water column (Churchill and Bettoli 2015). In South Africa, Spotted Bass were imported from Ohio (USA) by the Cape Piscatorial Society and the Natal Provincial Administration in October 1939 for stocking waters that were too turbid for both Largemouth Bass and Smallmouth Bass (De Moor and Bruton 1988). Of all Black Bass species introduced in South Africa, Spotted Bass appears to be the least successful and has a small distribution range (De Moor and Bruton 1988). Spotted Bass have been recorded as present in sections of the CFE (van der Walt et al. 2016, 2019), Eastern Cape, and KwaZulu-Natal (De Moor and Bruton 1988). However, it is also possible that Spotted Bass catches are misidentified as Largemouth Bass (Churchill and Bettoli 2015), which may result in the underestimation of their distributions. Spotted Bass remains the least-studied species of all Black Basses introduced into South Africa, which makes it difficult to ascertain their impact, but recent evidence suggests that may be similar to those of other introduced Black Bass species (van der Walt et al. 2016, 2019). In the Olifants–Doorn River system, for example, van der Walt et al. (2016) demonstrated the complete absence or low abundance of native fish species in river sections where Spotted Bass were present. Conversely, in the absence of Spotted Bass, native fish species were abundant.

Figure 1.4 An adult Spotted Bass (Photo: Olaf Weyl)

Florida Bass

Pure populations of Florida Bass are native to systems south of the Suwannee River in the Florida peninsula (Philipp et al. 1983) and exist in a natural intergrade zone with Largemouth Bass north of the Suwannee River (Figure 1.1; Bailey and Hubbs 1949; Philipp et al. 1983; Barthel et al. 2010; Claussen 2015). Owing to extensive stocking, Florida Bass and/or hybrids with Largemouth Bass (Figure 1.5) are now found across all continents except Antarctica (Robbins and MacCrimmon 1974; Claussen 2015). In South Africa, Florida Bass was introduced into the Umngeni Hatchery in 1980 to enhance the Largemouth Bass fishery (De Moor and Bruton 1988), and private fish transfers have increased their prevalence across the country (Weyl et al. 2017). From an invasion perspective, the introduction of Florida Bass in South Africa is a cause for concern as the species has been shown to attain the larger maximum size and persist longer than the Largemouth Bass, which may equate to greater biological impacts on native biota (Weyl et al. 2017; Hargrove et al. 2019a).

Figure 1.5 An adult Florida Bass, or a hybrid with Largemouth Bass. South African angling record caught from the Loskop Dam, Mpumalanga (Photo: www.wired2fish.com)

Florida Bass exhibit different life history strategies and preferences from to the Largemouth Bass, despite being closely related (Allen et al. 2002; Crawford et al. 2002; Warren 2009; Barthel et al. 2015; Claussen 2015). Florida Bass's size at age 1 ranges from 116 to 330 mm TL (Allen et al. 2002) which is a relatively faster growth rate than the Largemouth Bass (Warren 2009). The growth rate is highly variable across trophic state and habitat conditions, with faster growth recorded in unvegetated Florida lakes (Cailteux et al. 1996). Female Florida Bass grow faster and live longer than males, with nearly all trophy fishes caught in Florida State being females (Allen et al. 2002; Crawford et al. 2002). The current official Florida State record is 7.85 kg (Warren 2009), with the official world record weighing 10.1 kg, shared between Montgomery Lake (discussed above) and Lake Biwa, Japan (Barthel et al. 2015). Interestingly, the population in Lake Biwa was established with pure Largemouth Bass, however, persistent stocking of Florida Bass resulted in the near-complete replacement of

Largemouth Bass alleles in the Lake (Yokogawa et al. 2005), which is similar to the observation by Hargrove et al. (2019b) in Lake Chicamba, Mozambique.

Florida Bass have also been shown to exhibit higher thermal tolerance than Largemouth Bass (Fields et al. 1987; Beitinger et al. 2000). These differences may have implications for impact potential, with particular relevance for landscapes such as South Africa that are characterised by diverse climate and biogeographic regions. In addition, inherent biological differences between the two Black Bass species may be relevant for future distributions and impacts under climate change. Largemouth Bass and Florida Bass often co-occur and readily hybridise when in sympatry, so understanding individual impacts under different context dependencies is of both ecological and conservation importance (van Wilgen and Wilson 2018). Currently, in South Africa, there is a severe paucity of information on Florida Bass distribution and its impacts. This paucity may have implications for the management of the species (Woodford et al. 2017; van Wilgen and Wilson 2018). As a result, it is important that the distributions and impacts of Florida Bass are established, rather than inferring such from studies based on Largemouth Bass, as is currently the case.

Management of alien invasive species in South Africa

The management of alien invasive species in South Africa is guided by the National Environmental Management: Biodiversity Act (NEM:BA) for alien species (DEA 2014; van Wilgen and Wilson 2018). The NEM:BA legislation lists four different categories (i.e. Category 1a, 1b, 2 or 3) of non-native invasive species and specifies how these species are to be controlled or managed (DEA 2016; Woodford et al. 2017; van Wilgen and Wilson 2018). The listing status of non-native species on the NEM:BA is not only based on scientific evidence, but also focuses on the socio-economic importance of the taxon under consideration

(Woodford et al. 2017; van Wilgen and Wilson 2018). Non-native species placed in Category 1a are identified as species that require compulsory control, and by law, they must be eradicated from the environment (DEA 2016). Currently, none of the introduced non-native fishes are listed in Category 1a in the NEM:BA. The species listed in Category 1b are deemed to have a higher potential of becoming highly invasive if introduced, and their management is context specific; they require compulsory control as part of a non-native invasive species control programme (Table 1.1; Woodford et al. 2017; van Wilgen and Wilson 2018). Species such as the Nile Tilapia *Oreochromis niloticus* (Linnaeus 1758) and Common Carp *Cyprinus carpio* Linnaeus 1758 are listed in Category 1b, exclusively in National Parks, Provincial Reserves, Mountain Catchment Areas and Forestry Reserves. Category 2 non-native species are regulated by area, and they require a permit for import and stocking, breeding and distribution, and buying and selling (van Wilgen and Wilson 2018). Category 3 non-native species are regulated by activity and they may remain in areas where they currently occur. A permit is required for import and stocking, breeding and distribution, and buying and selling (DEA 2016; van Wilgen and Wilson 2018).

With regard to Black Bass species in South Africa, the listing is 'context specific', depending on the area or ecosystem in which they are found (Table 1.1; van Wilgen and Wilson 2018). In protected areas and mountain streams, the species are assigned Category 1b (must be controlled or eradicated, if feasible) while in dams they are listed as Category 2, which means they can be caught, released, and stocked with a permit (Woodford et al. 2017; van Wilgen and Wilson 2018). In wetlands, rivers and estuaries, they are listed as Category 3, implying that the species can remain in these areas; however, stocking and introducing the species is prohibited, otherwise control measures must be implemented (Woodford et al. 2017; van Wilgen and Wilson 2018). The legislation is designed to facilitate socio-economic activities in areas where

Black Bass species currently occur, while prohibiting introduction and further spread into areas where they do not currently occur (Woodford et al. 2017; van Wilgen and Wilson 2018).

To implement the NEM:BA, legislation on non-native Black Bass species requires robust scientific information to guide the decision-making processes (Woodford et al. 2017). Under this current legislation, it is, therefore, important to understand the distribution and the potential spread of all Black Bass species, not only for managing fisheries, but also for understanding the species' invasion success and the possible impacts within South African freshwater ecosystems (Woodford et al. 2017; van Wilgen and Wilson 2018). Furthermore, identifying environmental factors, especially the factors related to anthropogenic perturbations, that promote or mediate their invasion success is imperative in order to develop effective management strategies and to reduce their impacts on freshwater ecosystems (Woodford et al. 2017; Bae et al. 2018).

Table 1.1 The current status of individual non-native Black Bass species and a selected non-native species in the National Environmental Management: Biodiversity Act (NEM:BA) for alien species in South Africa (DEA 2016)

Species	Common name	Category/Area	Scope of exemption and prohibition
Micropterus salmoides	Largemouth Bass	a. Largemouth Bass is listed in Category 2 in National Parks, Provincial Reserves, Mountain Catchment Areas and Forestry Reserves declared in terms of the Protected Areas Act. b. Largemouth Bass is listed in Category 3 in all rivers, wetlands, lakes and estuaries in which it occurs. c. Largemouth Bass is not listed for dams (including for release in dams) within discrete catchment systems in which it occurs.	a. The transfer or release of Largemouth Bass from one discrete catchment system in which it occurs, to another discrete catchment system in which it does not occur; or, from within a part of a discrete catchment system where it does occur to another part where it does not occur as a result of a natural or artificial barrier, is prohibited. b. The release of Largemouth Bass in any rivers, wetlands, lakes and estuaries is prohibited. c. Largemouth Bass listed as Category 2 are exempted for a period of two years from the date upon which this notice takes effect, from requiring a Permit for any restricted activity in terms of the Act or Alien and Invasive Species Regulations, 2014, provided a person is in possession of a valid Provincial Permit issued in terms of Provincial legislation where required for Largemouth Bass. d. Catch and release of Largemouth Bass is exempted in discrete catchment systems in which it occurs.
Micropterus dolomieu *Micropterus punctulatus* *Micropterus floridanus* *Micropterus floridanus* × *Micropterus salmoides*	Smallmouth Bass Spotted Bass Florida Bass Hybrids of Largemouth Bass and Floridanus	a. 1b in National Parks, Provincial Reserves, Mountain Catchment Areas and Forestry Reserves declared in terms of the Protected Areas Act. b. 2 for release into dams within discrete catchment systems in which it occurs. c. 3 in all rivers, wetlands, lakes and estuaries in which it occurs. d. Subject to (b), each listed bass species is not listed for dams within discrete catchment systems in which it (the specific listed bass species) occurs.	a. The transfer or release of a specimen of a listed Black Bass species from one discrete catchment system in which it occurs, to another discrete catchment system in which it does not occur; or, from within a part of a discrete catchment system where it does occur to another part where it does not occur as a result of a natural or artificial barrier, is prohibited. b. The release of the listed bass species in National Parks, Provincial Reserves, Mountain Catchment Areas and Forestry Reserves declared in terms of the Protected Areas Act is prohibited. c. The release of the listed bass species in any rivers, wetlands, lakes and estuaries is prohibited. d. Each bass species listed as Category 2 is exempted for a period of two years from the date upon which this notice takes effect, from requiring a Permit for any restricted activity in terms of the Act or Alien and Invasive Species Regulations, 2014, provided a person is in possession of a valid Provincial Permit issued in terms of Provincial legislation where required for the specific listed bass species. e. Catch and release of the listed Black Bass species is exempted in discrete catchment systems in which they occur.

Oreochromis niloticus	Nile Tilapia	a. Nile Tilapia is listed as a Category 1b in National Parks, Provincial Reserves, Mountain Catchment Areas and Forestry Reserves declared in terms of the Protected Areas Act. b. 2 for aquaculture facilities in the rest of the country. c. 3 in all other discrete catchment system in which it occurs.	a. The transfer or release of a specimen of Nile Tilapia from one discrete catchment system in which it occurs, to a river, wetland, lake or estuary, or a dam that is not an aquaculture facility, in another discrete catchment system in which it does not occur; or, from within a part of a discrete catchment system where it does occur, to a river, wetland, lake or estuary, or a dam that is not an aquaculture facility, in another part where it does not occur as a result of a natural or artificial barrier, is prohibited. b. Release of Nile Tilapia in National Parks, Provincial Reserves, Mountain Catchment Areas and Forestry Reserves declared in terms of the Protected Areas Act is prohibited. c. Catch and release of Nile Tilapia is exempted in discrete catchment systems in which they occur.
Tinca tinca (Linnaeus 1758)	Tench	a. Tench is listed as a Category 1b in National Parks, Provincial Reserves, Mountain Catchment Areas and Forestry Reserves declared in terms of the Protected Areas Act. b. 3 for all other discrete catchment system in which it occurs.	a. The transfer or release of a specimen of Tench from one discrete catchment system in which it occurs, to another discrete catchment system in which it does not occur; or, from within a part of a discrete catchment system where it does occur to another part where it does not occur as a result of a natural or artificial barrier, is prohibited. b. Catch and release of Tench is exempted in discrete catchment systems in which it occurs.

Assessing distribution and impact

Species distribution modelling

Species distribution models (SDM) have become a major tool in biological invasion studies (Hao et al. 2020). Species distribution models attempt to provide detailed predictions of species distributions by relating species presence to the environmental variables associated with site locations (Stockwell and Peterson 2002; Phillips et al. 2004; Elith et al. 2006, 2011). A species will be able to establish itself in a locality if it has a set of appropriate environmental conditions. Broennimann and Guisan (2008), have, however, shown that species can also establish and spread into localities that are climatically distinct from those in their native range. As such, SDM have been used to explore a wide range of questions in order to inform ecological and conservation priorities (Elith et al. 2006; Zengeya et al. 2013). For example, SDM have been used to understand the invasive potential of non-native species (Zengeya et al. 2013; Frederico et al. 2019) and species' past distributions, to inform evolutionary relationships of closely related species (Cicero 2004; Peterson et al. 2004), and predict how climate change will affect future species distributions (Bakkenes et al. 2002; Wang et al. 2017).

There are two main types of SDM, namely correlative models and mechanistic models. In Chapter 2, I use correlative model maximum entropy modelling (MAXENT), which uses species distribution records and environmental variables to predict habitat suitability (Phillips et al. 2006; Elith and Leathwick 2009; van Proosdij et al. 2016). MAXENT has been identified as one of the most frequently utilised presence-only data correlative machine learning tools to model species distributions (Phillips et al. 2006; Elith et al. 2011). For example, Zengeya et al. (2013) used MAXENT to assess the potential invasive range of Nile Tilapia on river systems in southern Africa. They demonstrated that Nile Tilapia has an extensive range in the introduced range which often overlapped with native congenerics, which may have genetic implications for native species such as Mozambique Tilapia *Oreochromis mossambicus* (Peters 1852). Mamun et al. (2018), in the Korean peninsula, also predicted the potential spread and habitat suitability of Largemouth Bass under future climate change scenarios. They demonstrated that Largemouth Bass has the potential to become an invasive species owing to the extensive area that has been identified as potentially suitable.

For species such as Largemouth Bass that readily hybridise with Florida Bass, it is ecologically imperative that future distribution modelling incorporates the genetic status of different populations, as the Largemouth Bass, Florida Bass and their hybrids have been shown to exhibit different tolerances to environmental variables (e.g. temperature) (Fields et al. 1987; Philipp and Whitt 1991; Beitinger et al. 2000). Neglecting the importance of genetic contribution to the distribution of the species is ill-advised and may have implications in predicting future distributions and the invasion debt in the receiving ecosystems.

Molecular tools in invasion biology

Molecular techniques have been used to quantify genetic variation among species (Ellegren and Sheldon 2008; Pauls et al. 2014), to inform their evolutionary history, taxonomic and systematics classification, and identify the sources and routes of biological invasions (Hänfling 2007; Blanchet 2012; Pauls et al. 2014; Chown et al. 2015; Hargrove et al. 2017; Thongda et al. 2019). Recent technical developments in molecular tools, such as next-generation sequencing or gene expression analysis, and the decrease in the cost of more classical methodologies, such as single nucleotide polymorphism analysis (SNP), have largely contributed to the wide adoption and use of molecular tools in invasion biology (DeYoung and Honeycutt 2005; Ellegren and Sheldon 2008; Thongda et al. 2019). For example, Hargrove et al. (2017) used microsatellite markers to trace the invasion history of the Largemouth Bass in South Africa.

In freshwater ecosystems, molecular tools are an alternative (or a complementary approach) to traditional sampling methods, and in many instances, they are used for detecting levels of hybridisation or interbreeding between similar species or subspecies when geographic ranges overlap. Furthermore, molecular tools can be used to estimate the overall spatial distribution of non-native species. As such, the early detection of non-native species is ecologically important to prevent their establishment and potential dispersal (DeYoung and Honeycutt 2005). Indeed, for the Largemouth Bass and Florida Bass, molecular tools play an important role in identifying the two Black Basses and their hybrids (Warren 2009; Kassler et al. 2002). Given the potential for negative impact associated with the introduction of these Black Basses, it is important that their potential distribution and spread is established to inform management and conservation priorities for the native species (Weyl et al. 2017; Hargrove et al. 2019b).

Predicting predation impacts using functional responses

Understanding and predicting the ecological impacts of non-native species is important for risk assessment and management of biodiversity (Dick et al. 2013b, 2017b; Ricciardi et al. 2013). Predictive models are therefore needed to assess the ecological impacts of non-native species in the receiving ecosystem (Ricciardi 2003; Dick et al. 2013a). For high-impacting species, such as Black Basses, understanding their ecological impacts is a conservation and research priority (Jackson 2002; Alexander et al. 2014; Ellender et al. 2018). Largemouth Bass have been a focus for several studies globally, attempting to understand their impacts in the receiving ecosystems (see review by Pereira and Vitule 2019). Most of the impact studies were largely observational fieldwork and theoretical (Pereira and Vitule 2019), with few studies quantifying resource utilisation/predator-prey relationships (functional responses experiments) (Alexander et al. 2014, 2015; Pereira and Vitule 2019). It is important to understand resource utilisation by the Largemouth Bass because, in systems where they occur, localised extirpations and extinctions of native biota have been reported as a result of higher predation pressure (MacRae and Jackson 2001; Weyl et al. 2013, 2014; Ellender et al. 2018; Bezerra et al. 2019). Furthermore, as Florida Bass is often introduced into the Largemouth Bass populations with which they hybridise, and potentially have offspring that may exert higher ecological impact (Weyl et al. 2017), understanding the dynamics that may drive differences/similarities in resource utilisation by the two Black Bass species is ecologically significant.

Functional response (FR) experiments have been used widely as a method of measuring and quantifying impacts of non-native species across a range of trophic and taxonomic groups (Alexander et al. 2014; Sentis and Boukal 2018; Mofu et al. 2019b; South et al. 2019). In the context of predator-prey relationships, FR quantifies the rate of resource utilisation by the predator in relation to resource density (Holling 1959, 1966). Functional responses have been proposed as a useful measure to predict and quantify the *per capita* effects of species (Dick et al. 2013a, 2017a; Alexander et al. 2014, 2015; Cuthbert et al. 2019). The ability to incorporate abiotic dependencies (e.g. temperature and habitat complexity) in the approach makes it a robust and reliable tool to quantify impacts under environmental change (Alexander et al. 2015; Iacarella et al. 2015; Dick et al. 2017a, b; South et al. 2018). Abiotic context dependencies must be incorporated in models of predator-prey dynamics because of their ability to alter interaction strengths (Parker et al. 1999; Iacarella et al. 2015). Non-native species have been shown to exhibit higher *per capita* effects when compared to the native analogous species (Dick et al. 2013a, b; Alexander et al. 2014) and thus resources, such as native prey communities, are vulnerable to potential extirpations or extinctions (MacRae and Jackson 2001; Cucherousset and Olden 2011).

Three categories of FR types have been classified (Figure1.5; Holling 1959). Type I FR describes a constant proportion of the available prey consumed, irrespective of prey density (Figure 1.6), or a linear increase in feeding rate (Figure 1.5) (Holling 1959). Type I FR is predominantly found in filter feeders as they are not limited by handling time (Jeschke et al. 2004). In the Type II FR, predation rate decreases with an increase in prey availability (Figure 1.7) (Holling 1959). The inversely density-dependent Type II FR (Figure 1.6) suggests high resource utilisation at low resource densities and is associated with an inherent risk of resource depletion because rare resources are used at high rates (Alexander et al. 2014; Dick et al. 2017a; South et al. 2017). For the Type III FR, the predation rate decreases at both the low and high prey densities (Figure 1.7), providing a sigmoidal and positively density-dependent relationship (Figure 1.6) (Holling 1959). Type III FRs are associated with learning and switching behaviour by the predator, or caused by other factors such as habitat complexity or temperature change (Holling 1959; Alexander et al. 2013; Iacarella et al. 2015; South and Dick 2017). As such, the FR types exhibited by the consumer are important for understanding the consumer impact and the community dynamics because the response type can contribute to the stability and persistence of the resource (Eggleston et al. 1992; Ward et al. 2008; Dick et al. 2017b).

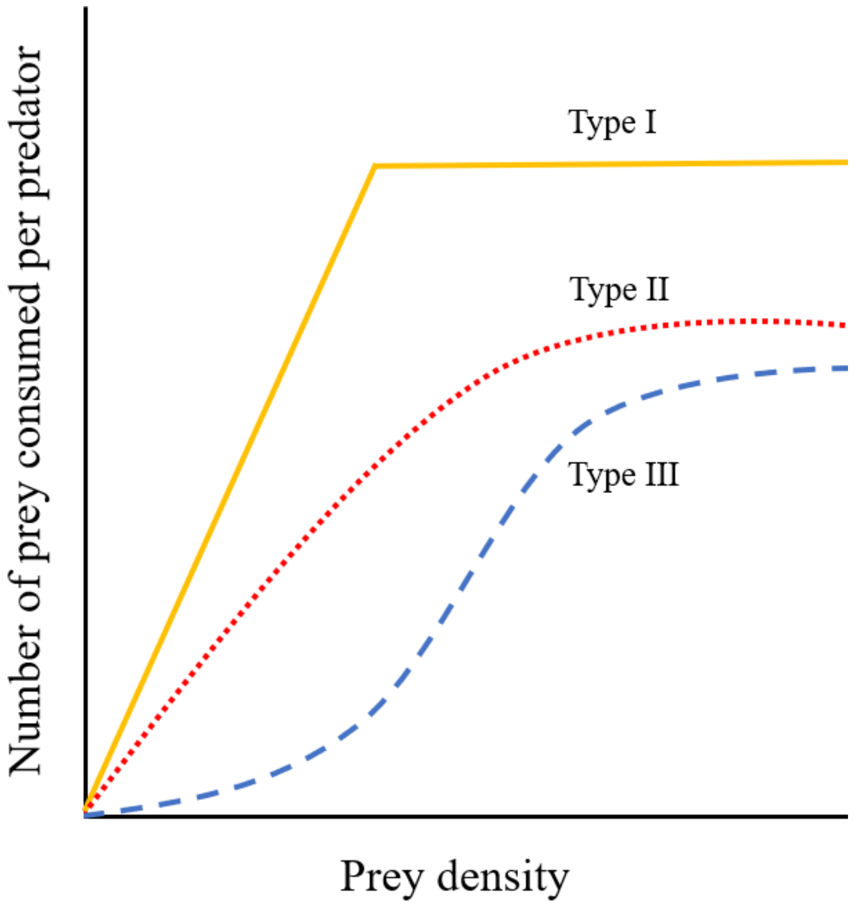

Figure 1.6 Graphs showing categorical functional response types (Type I, Type II and Type III) (Redrawn from Pritchard et al. 2017)

The main consideration of FR analysis is acquiring accurate parameter estimates or the main mechanistic components: the attack rate, handling time and maximum feeding rate (Jeschke et al. 2002; Alexander et al. 2012; Dick et al. 2013b). Owing to the context dependency of FR (Iacarella et al. 2015), the FR magnitudes and types can fluctuate and often switch from Type II to Type III, or vice versa, under different environmental contexts (Dick et al. 2013a; Iacarella et al. 2015). For example, South and Dick (2017) demonstrated the FR of the Dogfish *Scyliorhinus canicula* (Linnaeus 1758) towards amphipod prey *Echinogammarus marinus* (Leach 1815) changed from Type II to Type III with an increase in temperature. This change from population-destabilising Type II to more stabilising Type III could confer protection to prey at low densities, but increase the maximum feeding rate by *S. canicula* in the future.

Furthermore, Hanache et al. (2020) demonstrated that anthropogenic noise can significantly affect the physiological and behavioural patterns of the European minnow *Phoxinus phoxinus* (Linnaeus 1758) towards dipteran larvae (*Chaoborus* sp.). Under two noise conditions, ambient noise and motorboat noise, the European minnow exhibited a Type II FR. They found that boat noise did not affect the handling time, but reduced the attack rate, resulting in a functional response curve of the same height but with a less steep initial slope. In addition, fish exhibited a stress-related response to noise which included increased swimming distance, more social interactions, and altered spatial distribution.

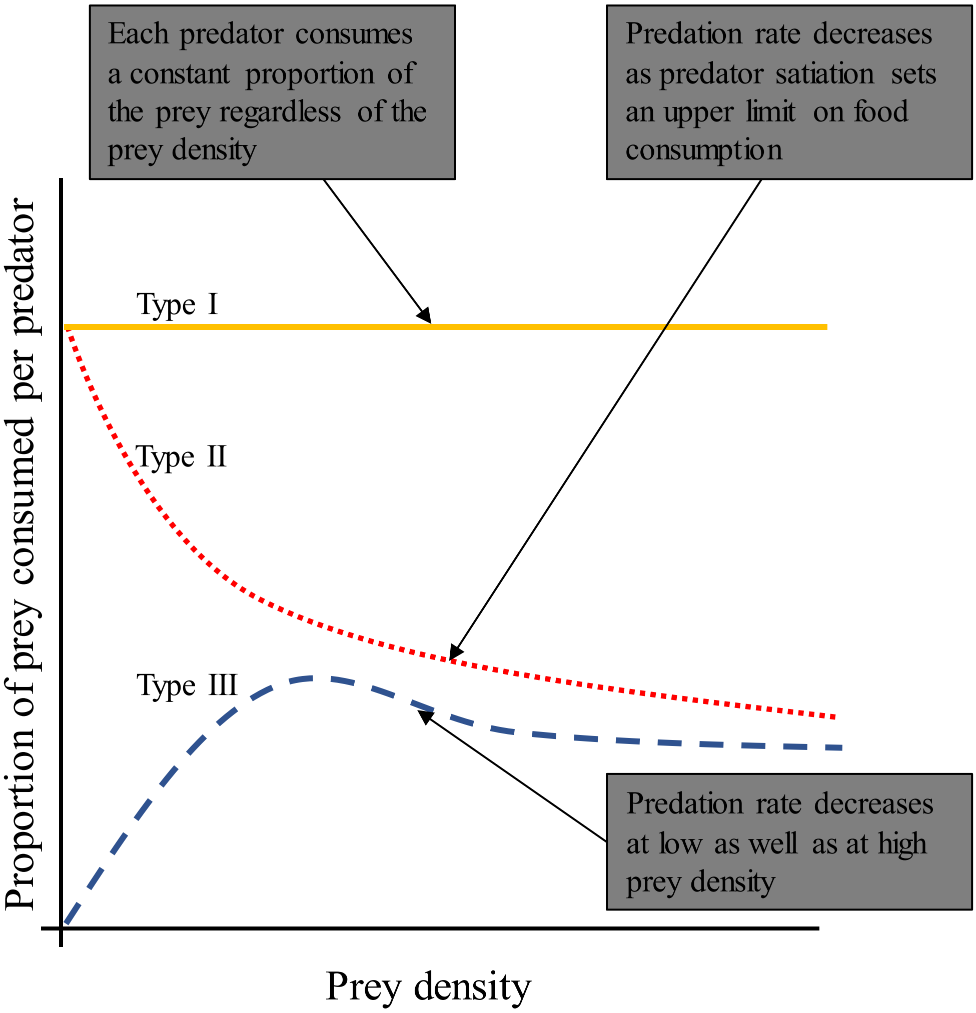

Figure 1.7 Graphs showing a relationship between prey density and proportional consumption across the functional response types (Type I, Type II, Type III) (Redrawn from Pritchard et al. 2017)

Functional response experiments must be optimised to mimic ecologically relevant conditions (Dick et al. 2013a). Doing so involves using a design incorporating replicated feeding trials wherein the total time available is kept constant; the predator is always subject to the same pre- and post-trial conditions; individuals' body sizes must be relatively similar, and there must be several prey densities investigated to allow variability of response to be incorporated into the results (Houck and Strauss 1985; South 2017). After trials, the results can be collected as a binomial distribution of numbers where the number of prey consumed versus the number of prey not consumed can be used to assign weights and then used to estimate maximum likelihood (Houck and Strauss 1985). Various statistical tests can then be used to test for differences between FR curves, including F-test derived from general linear models (GLM), and non-parametric rank sum tests such as the Mann-Whitney Wilcoxon test (Houck and Strauss 1985). The development of the methodology through simplified procedures and the readily available statistical analysis R packages such as the 'frair' has led to an increase in its adoption and use by scientists. For example, Alexander et al. (2014, 2015) used FR experiments to assess the predation impacts of Largemouth Bass, and further show how habitat complexity could mitigate predation efficiency; South et al. (2018) compared the feeding performances of the intertidal shanny *Lipophrys pholis* (Linnaeus 1758) under the predicted future climate scenarios; Guo et al. (2016), Wasserman et al. (2016a), Mofu et al. (2019a) used FR to compare and assess interactions among predators. The predation impact of both Largemouth Bass and Florida Bass can be assessed using this approach (FR experiments), specifically comparing for any differences in resource utilisation between the two Black Bass species.

Thesis outline

The primary aim of this thesis is to deliver insight into the extent of distributions, spread and impact potential of Black Bass species in South Africa, with particular emphasis on addressing the current knowledge gap on Florida Bass. An examination of all Black Bass species with regard to distribution and spread was conducted, while model river systems were used to explore introgression potential between Largemouth Bass and Florida Bass. Similarly, impact potential inferences across environmental context dependencies were explored for the Largemouth Bass and Florida Bass. To this end, this thesis is divided into six chapters, the first being the current introduction, followed by four data chapters (Chapters 2-5) and a final discussion (Chapter 6). The four data chapters explore the following:

- In Chapter 2, I use distribution records collected from both formal and informal information sources to provide new distribution maps of all Black Bass species in South Africa. These distribution records are then used to model the potential spread, habitat suitability, and the potential invasion debt of all Black Bass species in South Africa, using maximum entropy algorithms implemented in MAXENT. This chapter is published as: Khosa D, Marr SM, Wasserman RJ, Zengeya TA, Weyl OLF (2019) An evaluation of the current extent and potential spread of Black Bass invasions in South Africa. *Biological Invasions 21*:1721–1736. doi: 10.1007/s10530-019-01930-0.

- Chapter 3 focuses on specific river systems known to house both Largemouth Bass and Florida Bass with a view to assessing the distribution of both Largemouth Bass, Florida Bass and their hybrids within a catchment setup. This assessment uses Single Nucleotide Polymorphism (SNP) analysis to determine if the introduction of Florida Bass within a catchment area results in hybrids populations.

- In Chapter 4, I use the FR approach to compare resource utilisation (i.e. *per capita* effects) between Largemouth Bass and Florida Bass under the context dependency of temperature. This comparison was carried out to determine temperature effects on predator-prey interaction outcomes. These results are discussed within the context of spatial and temporal temperature heterogeneity. This chapter is published as: Khosa D, South J, Cuthbert RN, Wasserman RJ, Weyl OLF (2019) Temperature regime drives differential predatory performance in Largemouth Bass and Florida Bass. *Environmental Biology of Fishes 103*: 67–76. doi.org/10.1007/s10641-019-00933-z.

- In Chapter 5, I further characterise the FR of both Largemouth Bass and Florida Bass under different habitat complexities to determine if the magnitude of FR of both Black Bass species was influenced by the different levels of habitat complexities. This chapter is under review in the journal *Environmental Biology of Fishes* as: Khosa D, South J, Wasserman RJ, Weyl OLF (*under review*) Does habitat structure mitigate predation impact of Black Bass species?

CHAPTER 2

AN EVALUATION OF THE CURRENT EXTENT AND POTENTIAL SPREAD OF BLACK BASS INVASIONS IN SOUTH AFRICA

Abstract

Black Bass, a collective name for members of the centrarchid genus *Micropterus*, are native to North America, but have been introduced globally to enhance recreational angling. This study assessed the distribution of *Micropterus salmoides*, *M. dolomieu* and *M. punctulatus* in South Africa using both formal (survey-based) and informal (tournament data and social media) information sources. Analysis of the distribution data showed habitat bias between the data sources. Survey data from formal information sources were dominated by locality records in riverine environments while those derived from informal information sources focused more on lacustrine habitats. Presence data were used to develop niche models to identify suitable areas for their establishment. The predicted distribution range of *M. salmoides* revealed a broad suitability over most of South Africa; however, the Cape Fold Ecoregion and all coastal regions were most suitable for the establishment for both *M. dolomieu* and *M. punctulatus*. Flow accumulation and precipitation of coldest quarter were the most important environmental variables associated with the presence of all Black Bass species in South Africa. Anthropogenic disturbance, such as agricultural activities, were associated with the presence of both Smallmouth Bass and Spotted Bass. An extensive area-based invasion debt was observed for all *Micropterus* spp. The potential for further spread of Black Bass in South Africa is of ecological concern because of their impact on native biota.

Introduction

Freshwater ecosystems are threatened by multiple stressors which include habitat destruction and modification, overexploitation, pollution, and the introduction of non-native species (Bellard et al. 2016; Jackson et al. 2016; Venohr et al. 2018). The introduction of non-native fishes is considered one of the least reversible of these stressors (Simberloff 2003; Strayer 2010). Major conservation implications associated with non-native fish introductions are extinctions of native biota related to direct predation and competition, habitat modification, alteration of food webs, and hybridisation with congeneric native species (Eby et al. 2006; Cucherousset and Olden 2011; Jackson et al. 2017).

Most of the introduction and spread of non-native fishes are mediated by human activities (Leprieur et al. 2008; Ellender and Weyl 2014); direct introductions for fisheries enhancement are the most important pathway for new invasions (Gozlan et al. 2010; Ellender and Weyl 2014; Venohr et al. 2018). As a result, considerable effort has gone into documenting non-native fish introductions (e.g. Welcomme 1988; Casal 2006; ISSG 2009; Ellender and Weyl 2014). Despite this, there is still a paucity of information on their subsequent establishment, spread, and abundance which can often be attributed to the substantial cost of large-scale post-introduction assessments (Gillett et al. 2012; Bird et al. 2014; Hargrove et al. 2015).

In South Africa, Black Bass were specifically introduced to develop sport fisheries in areas that were too warm for Rainbow Trout *Oncorhynchus mykiss* (Walbaum 1792*)* and Brown Trout *Salmo trutta* Linnaeus 1758 (Ellender et al. 2014). Largemouth Bass were first imported in 1928 for use mainly in lentic environments, followed by Smallmouth Bass introduced in 1937 for introduction into rivers, and Spotted Bass in 1939 for use in more turbid riverine environments (Ellender et al. 2014). Florida Bass were then introduced in 1980 to enhance

Largemouth Bass fisheries because they attain larger sizes (Weyl et al. 2017). As Florida Bass are morphologically almost identical to, and hybridise with Largemouth Bass, it is currently not possible to distinguish between the two species without genetic verification (Weyl et al. 2017). It was therefore necessary to combine Largemouth Bass and Florida Bass to reduce identification bias, and Largemouth Bass, Florida Bass, and their potential hybrids are hereafter referred to as Largemouth Bass (see Chapter 3).

Following their introduction, Black Bass were subsequently stocked via government-supported stocking programmes (until the early 1990s) and directly by anglers. While these introductions served the purpose of enhancing recreational fisheries, their subsequent invasions have also resulted in negative impacts on native biota (Ellender and Weyl 2014; Ellender et al. 2014) which include the extirpation of native fishes in Black Bass invaded habitats (e.g. Van Der Walt et al. 2016; Ellender et al. 2018). For example, in the CFE, Black Bass species are implicated in the disappearance of several endemic cyprinid species and the anabantid *Sandelia capensis* (Cuvier 1829) (Shelton et al. 2008; Van Der Walt et al. 2016; Ellender et al. 2017). Similar impacts have been reported from Japan (Iguchi et al. 2004; Takamura 2007), the Iberian Peninsula in Europe (Godinho and Ferreira 2000), and other parts of southern Africa (Gratwicke and Marshall 2001).

The development of economically important fisheries around species that impact negatively on native ecosystems often results in conflict between different stakeholders (Ellender et al. 2014; Zengeya et al. 2017) and their management is considered a ‘wicked problem’ (Woodford et al. 2016). South Africa’s legislated management response is to facilitate economic activities in invaded areas while restricting activities such as stock enhancements to prevent further spread (Woodford et al. 2017). As the invasion by non-native fishes is generally irreversible after they have established, and impossible to eradicate without affecting the native biota (Simberloff 2003; Vitule et al. 2009; Cucherousset and Olden 2011), the most practical management strategy is to monitor and document their distribution and promote measures to limit their spread (Iguchi et al. 2004; Zengeya et al. 2013). As a result, understanding the current distribution, and estimating the potential for spread by using correlative methods (e.g. ecological niche modelling) that match occurrence data with environmental variables to identify suitable areas for establishment (Stockwell and Peterson 2002; Phillips and Dudík 2008; Elith et al. 2011) are vital for the effective implementation of the regulations.

In recent years, there has been an increase in the development of algorithms to model ecological niches and species distributions. In this regard, SDM are important as they provide knowledge on the global distributions and evolutionary patterns of biodiversity (Phillips and Dudík 2008). Species distribution models correlate species occurrence to the environmental characteristics (both continuous and categorical) of localities where the species occur (Elith et al. 2006). Of the available algorithms, MAXENT has performed well and is among the most widely used presence-only modelling techniques (Elith et al. 2006, 2011).

For fishes in South Africa, formal sources of information on distributions include occurrence records held by provincial conservation authorities (e.g. CapeNature, Ezemvelo KwaZulu-Natal Wildlife), research institutions (e.g. South African Institute for Aquatic Biodiversity fish collections) and online repositories (e.g. Global Biodiversity Information Facility). A major limitation of such records is that they are often biased towards species and areas of interest to the organisation doing the collecting (e.g. non-native species were, until recently, often not curated in museum collections) and thus do not reflect the full distributional range of many non-native species (Devictor et al. 2010; Tye et al. 2016). As a result, informal data (e.g. blogs, angler databases, and social networks), are increasingly being used to complement formal distribution data (Forrester et al. 2015; Tye et al. 2016). Hargrove et al. (2015) for example, used angling tournament data to assess for the presence, establishment, and stock status of Black Bass in southern African reservoirs, and Gago et al. (2016) used informal online data sources to assess the spatial extent of spread of the European catfish, *Silurus glanis* Linnaeus 1758, in the Iberian Peninsula.

The objectives of the present study were to compile a database of formal and informal distribution records of Black Bass in South Africa to determine the current spatial distribution of different Black Bass species in South Africa; to identify environmental variables that influence their distribution, and to predict areas that are suitable, but from which distribution data are unavailable, to provide the first estimate of potential 'invasion debt' and prioritise future survey efforts. Consequently, I hypothesised that: (1) informal information sources would significantly increase the known extent of occurrence of all Black Bass; (2) all three Black Bass species will have similar distributions across South Africa, and (3) their distributions would be strongly associated with human population density.

Materials and methods

Current distribution

Formal and informal information sources were used to compile a database of occurrence records of all Black Bass species in South Africa (see Supplementary Table I). Formal distribution records housed at the South African Institute for Aquatic Biodiversity (SAIAB, unpublished data), Ezemvelo KwaZulu-Natal Wildlife (EKZN Wildlife, unpublished data), Mpumalanga Tourism and Parks Agency (MTPA, unpublished data) and the CFE (Dallas et al. 2017; CapeNature unpublished data) were used. These were supplemented with data from reviews by De Moor and Bruton (1988), Ellender and Weyl (2014). To incorporate data that may have been omitted by these two reviews, an exhaustive literature search was performed, focusing on recent peer-reviewed publications (2005–2016) dealing with any aspect of the Blackburn et al. (2011) unified framework for biological invasions (transport, introduction, establishment and spread), or documenting any ecological impacts of Black Bass species in South Africa.

The informal information database was compiled from an extensive search for Black Bass records in social network websites (e.g. Facebook), blog sites dedicated specifically to anglers (e.g. www.sabaa.co.za, www.bigbass.co.za), angling magazines (e.g. *The Bass Angler*, *SA BASS*) and from angling tournament records. A Boolean search using AND, OR and NOT as Boolean operators was performed between March and November 2016 using a combination of both common and scientific names for Largemouth Bass, Smallmouth Bass and Spotted Bass. All records were restricted to South Africa. Since informal data are subject to misidentification of species, data were only included in the database if the record included a photograph of the species, and where capture locality could be ascertained (either via available geographic coordinates or by inference from a recognisable geographic feature). Native range distribution records for the three Black Bass species were obtained from the Global Biodiversity Information Facility (http://data.gbif.org). The native ranges of each of the three species were limited to geographic areas as described in Robbins and MacCrimmon (1974), and Near et al. (2003). For each of these taxa, locality records were examined and, where multiple records were available for a single locality, only one was retained for further analysis.

Species distribution modelling

To fit the SDM (Stockwell and Peterson 2002; Phillips et al. 2006), I used distribution data from the native range in North America and complemented these with data from all countries where the three Black Bass species have established (http://data.gbif.org), and from the contemporary South African dataset. To compensate for the lack of accurate and readily available environmental data on water quality variables for the application of niche models in aquatic systems, atmospheric variables are commonly used as proxies in studies on freshwater fishes (see Iguchi et al. 2004; Lübcker et al. 2014; Zengeya et al. 2015; Bae et al. 2018). The environmental variables used for this study were the 19 bioclimatic variables representing annual and seasonal climatic trends (e.g. mean temperature and precipitation) and extreme or limiting environmental variables (e.g. precipitation of the driest and wettest quarter) extracted from the WorldClim version 2.0 at 30 arc-second resolutions (Hijmans et al. 2005). Further, topographic and hydrological data (elevation, slope, topographic index, and flow accumulation) and anthropogenic disturbance data (agricultural land and human population density) were also included to develop the SDM, which was performed using the maximum entropy algorithm that was implemented in MAXENT (version 3.4.0) (Phillips et al. 2006). The predictive ability of ecological niche models is sensitive to the selection of environmental variables utilised to train the models, and various procedures have been suggested to pre-select variables (Peterson and Nakazawa 2008; Merow et al. 2013; Zengeya et al. 2013). This study took advantage of the in-built method of regularisation in MAXENT that deals with the selection of environmental variables (regulating some to zero) which has been shown to perform well, and is thought to out-perform other pre-selection procedures (Elith et al. 2011; Merow et al. 2013).

Models were trained using occurrence records from both native and introduced ranges (Broennimann et al. 2007; Broennimann and Guisan 2008). Since the majority of locality records were from North America, the database was spatially rarefied at 1°, 2°, 3°, 4° and 5° using the SDMtoolbox (Brown 2014) to obtain a better estimate of the species' fundamental niche (Broennimann and Guisan 2008; Zengeya et al. 2013) and improve predictions of the potential future spread of the species, that is, invasion debt (Essl et al. 2011; Rouget et al. 2016). The best performing model at which no spatial auto correlation occurred was at 1°.

As the extent of the spatial background can have a significant effect on the performance of the models (VanDerWal et al. 2009), the background was limited to hydrological basins with known species occurrence points, following the recommendation by Zengeya et al. (2015). For each species, this was achieved by overlaying hydrological basins with occurrence points from both native and introduced ranges using ArcGIS® 10.4 (ArcGIS™; ESRI®, Redlands, CA); a basin formed part of the background if it contained an occurrence point. For each of the three Black Bass species models, the environmental variables were evaluated using correlation analysis to exclude those variables that were highly correlated ($r > 0.8$) (Dormann et al. 2013). For each pair of correlated variables, one variable was retained, based on its biological significance to the species (Supplementary Table II, III and IV; Clugston 1964; Bevelhimer and Breck 2009). The spatial resolution of all environmental variables was 30 arc seconds.

For each species, models were calibrated with 10 000 pseudo-absence points drawn at random from the species' defined background (Phillips et al. 2006; Phillips and Dudík 2008). Occurrence records were randomly partitioned into equal sets (50%) for calibration and validation in MAXENT (Boyce et al. 2002; Phillips et al. 2006). The average performance of the model was obtained by repeating the process for ten iterations for each species. The final distribution map was created as an average of the ten projections. Models were optimised using the following parameters: regularisation multiplier of 1, random test percentage = 50, maximum iterations = 500, convergence threshold = 0.00001; only hinge features were selected and output format was set to logistic. The logistic output indicates the probability of a species presence at a default prevalence of 0.5 (Elith et al. 2011; Merow et al. 2013). Values range from 0, indicating a low probability, to 1, indicating a high probability of a species presence in a given area.

Model evaluation

All model performances were assessed using the area under the receiver operator curve (AUC), which measures the discrimination ability (between presence and background) of the models where values ≤ 0.5 indicate random predictions and values between 0.9–1.0 indicate acceptable predictions (Swets 1988). Although the AUC statistic has been widely used to validate niche models (Phillips et al. 2006; Elith et al. 2011), it is not necessarily an appropriate measure for presence-only model evaluation (Boyce et al. 2002; Lobo et al. 2008). As a result, model performance was further assessed using the Continuous Boyce Index (CBI) (Boyce et al. 2002; Hirzel et al. 2006). The Boyce index evaluates the ability of habitat suitability models to predict species presence in a given area (Boyce et al. 2002). This evaluation is achieved by partitioning the habitat suitability scores from each model output into a number of i classes of equal intervals (Boyce et al. 2002). For each class, the predicted and expected frequencies are calculated. The Predicted Frequency is the number of occurrence points predicted by the model falling into the class i divided by the total number of occurrence points used to build the model. The Expected Frequency is the number of grid cells included in class i, divided by the total number of grid cells in the whole study area. A predicted-to-expected (P/E) ratio is then calculated for each class and a Spearman rank correlation is used to evaluate if the ratio significantly increases as suitability increases (Hirzel et al. 2006). The P/E ratio values may range from -1 to 1, with negative values indicating models that predict worse than random and the positive values indicating models that are consistent with presence distribution in the evaluation dataset (Boyce et al. 2002; Hirzel et al. 2006).

Invasion debt

Invasion debt is broadly defined as the potential increase in the biological invasions that a given region will face over a particular time frame in the absence of any strategic interventions (Rouget et al. 2016). This study examined area-based invasion debt, also known as spread debt, to determine which areas are potentially suitable for invasion by any of the Black Bass species under consideration, but for which no distribution records exist. This area-based invasion debt is determined by (1) the probability that a species will become invasive, (2) the environmental suitability of a region for a species, and (3) the rate of spread (both natural and human-mediated) of that species (Rouget et al. 2016).

The probability that a species will become invasive was assigned using a deductive qualitative threshold based on the current distribution of each of the three species in South Africa. Department of Water Affairs (DWAF) quaternary catchments were used as sampling units because of the coarse scale of the occurrence records. A quaternary catchment constitutes the lowest and most detailed level in a hierarchical system of catchment management in South Africa (Midgeley et al. 1994). There are 1947 quaternary catchments that are further aggregated into 22 Water Management Areas (WMA). A river profile with known occurrence of Black Bass was extracted from each quaternary catchment, and a value of 1 was assigned to river sections with known occurrence, and 0 if the river section did not contain any of the Black Bass species.

Environmental suitability for each of the three Black Bass species was delineated based on the logistic output from the MAXENT models. Areas with a probability above 0.5 were taken as suitable and those below were taken as not suitable. The area (km^2) that was predicted as suitable and occupancy (known occurrence record) was then quantified using ArcGIS® 10.4 (ArcGIS™; ESRI®, Redlands, CA). To provide a first estimate of the rate of spread, the total area of establishment based on occurrence records (excluding stocking data) was regressed against 20-year timeframes as suggested by Rouget et al. (2016). While these data infer spread, they are confounded by a lack of knowledge on sampling effort. As a result, the rate of spread needs to be recognised as a minimum estimate, and invasion debt in the context of this paper is either a true absence or a sampling deficiency. It is included here as it is valuable for directing future survey efforts.

Results

The total database comprised 607 locality records for Black Bass in South Africa (see Supplementary Table I). Of these locality records, 467 (77%) originated from formal information sources and 140 (23%) from informal information sources (Supplementary Table I). The majority (82%) of records from formal information sources were from riverine environments and only 18% originated from lacustrine environments (Table 2.1). By contrast, only five percent of the records from informal information sources originated from riverine environments and 95% of the records were from lacustrine environments (Table 2.1). Largemouth Bass were reported from 379 localities (Figure 2.1), Smallmouth Bass from 146 localities (Figure 2.2) and Spotted Bass from 82 localities (Figure 2.3).

Informal information sources contributed 30% of Largemouth Bass, 15% of Smallmouth Bass and 8% of Spotted Bass records. Largemouth Bass was the most widespread species, recorded in 21 of the 22 WMAs. The exception was the Buffels WMA, an exclusively ephemeral system without any standing water or native fish. Smallmouth Bass and Spotted Bass were less widespread with occurrence records from 17 and 14 WMAs, respectively (Table 2.1). The largest number of locality records were from the Olifants West WMA (102 localities) which is a conservation priority area (Ellender et al. 2017) and has been the focus of directed research on Black Bass impacts (e.g. Woodford et al. 2005; Shelton et al. 2008; Van Der Walt et al. 2016). The smallest number of localities (4) was recorded from the Orange WMA (Supplementary Table I). The spread and occupancy per WMA for all three Black Bass species is presented in Figures 2.1-2.3.

Table 2.1 Summary of records of Black Bass species in different water management areas of South Africa based on the occurrence records obtained from both formal and informal information sources used for this study. See Supplementary Table 1 for informal source details

Water management area	Surface area (km^2)	River length (km)	Formal information sources			Informal information sources		
			Largemouth Bass	Smallmouth Bass	Spotted Bass	Largemouth Bass	Smallmouth Bass	Spotted Bass
A–Limpopo	97 353	12 508	4	1	2	11	0	0
B–Olifants North	65 540	10 376	6	1	0	9	1	0
C–Vaal	179 789	22 885	7	0	0	17	0	0
D–Orange	379 999	41 643	2	0	1	1	0	0
E–Olifants West	46 755	7452	33	44	19	4	1	1
F–Buffels	26 733	3342	0	0	0	0	0	0
G–Berg	2454	3761	10	20	6	40	11	2
H–Breede	15 136	2864	22	16	0	10	5	0
J–Gouritz	43 650	7668	13	1	1	1	1	0
K–Krom	704	1470	7	3	0	4	0	0
L–Gamtoos	33 414	6003	5	7	8	1	1	1
M–Swartkops	256	427	9	1	1	0	0	0
N–Sundays	20 420	3699	5	2	0	0	0	0
P–Bushmans	519	1312	15	0	0	0	0	0
Q–Great Fish	28 930	5746	5	1	2	0	0	0
R – Keiskamma	764	1876	6	1	8	0	1	0
S–Kei	19 550	4670	4	2	0	0	0	0
T–Mzimvubu	44 060	10 452	23	4	7	0	0	0
U–Mkomazi	17 074	4074	50	9	15	6	1	0
V–Tugela	26 770	5380	20	10	5	4	0	0
W–Mfolozi	53 870	10 683	16	0	2	1	0	0
X–Komati	27 960	5585	3	1	1	5	0	0
Total	1 129 246	163 500	265	124	78	114	22	4

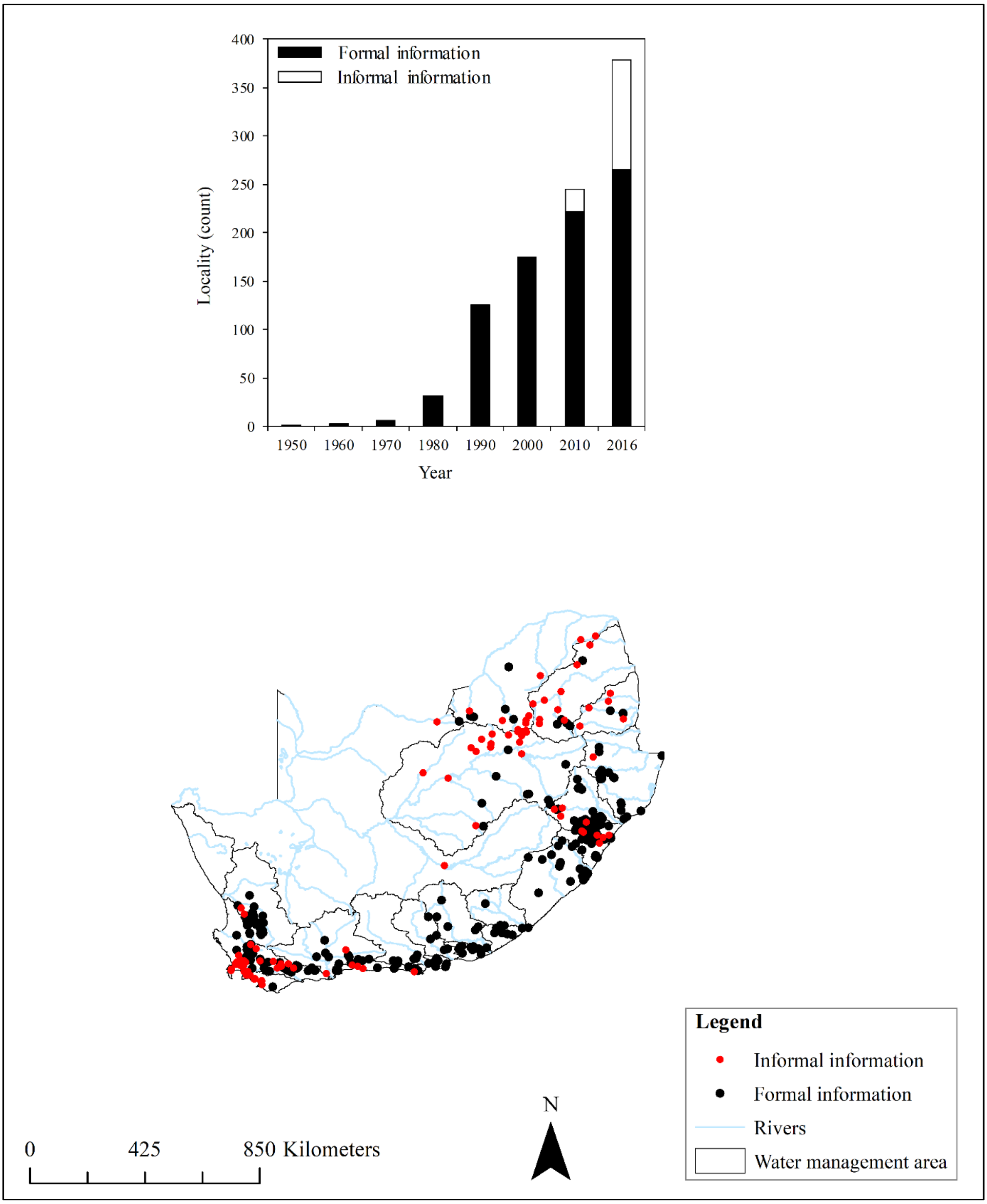

Figure 2.1 The detection rate of the Largemouth Bass in South Africa projected from different information sources

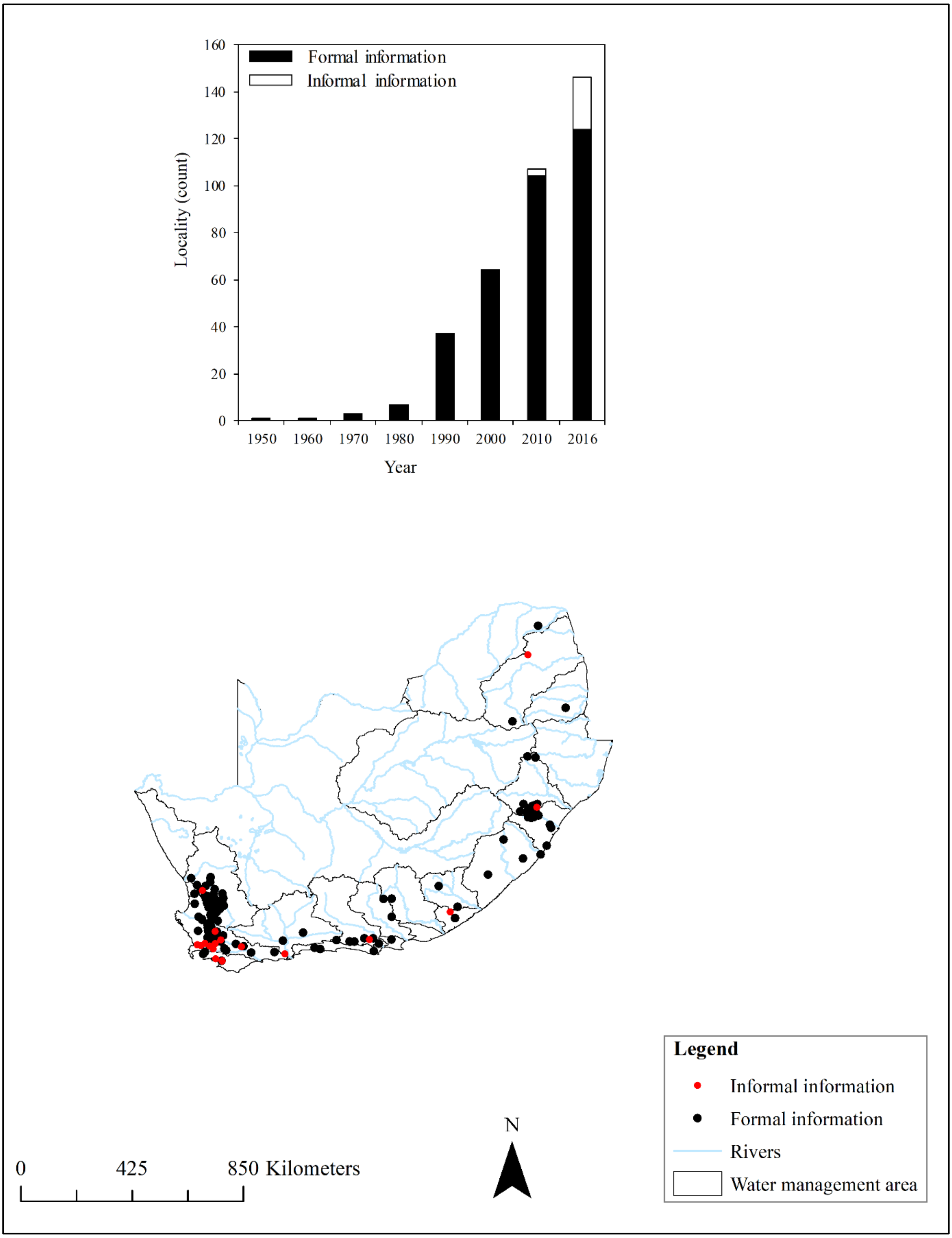

Figure 2.2 The detection rate of the Smallmouth Bass in South Africa projected from different information sources

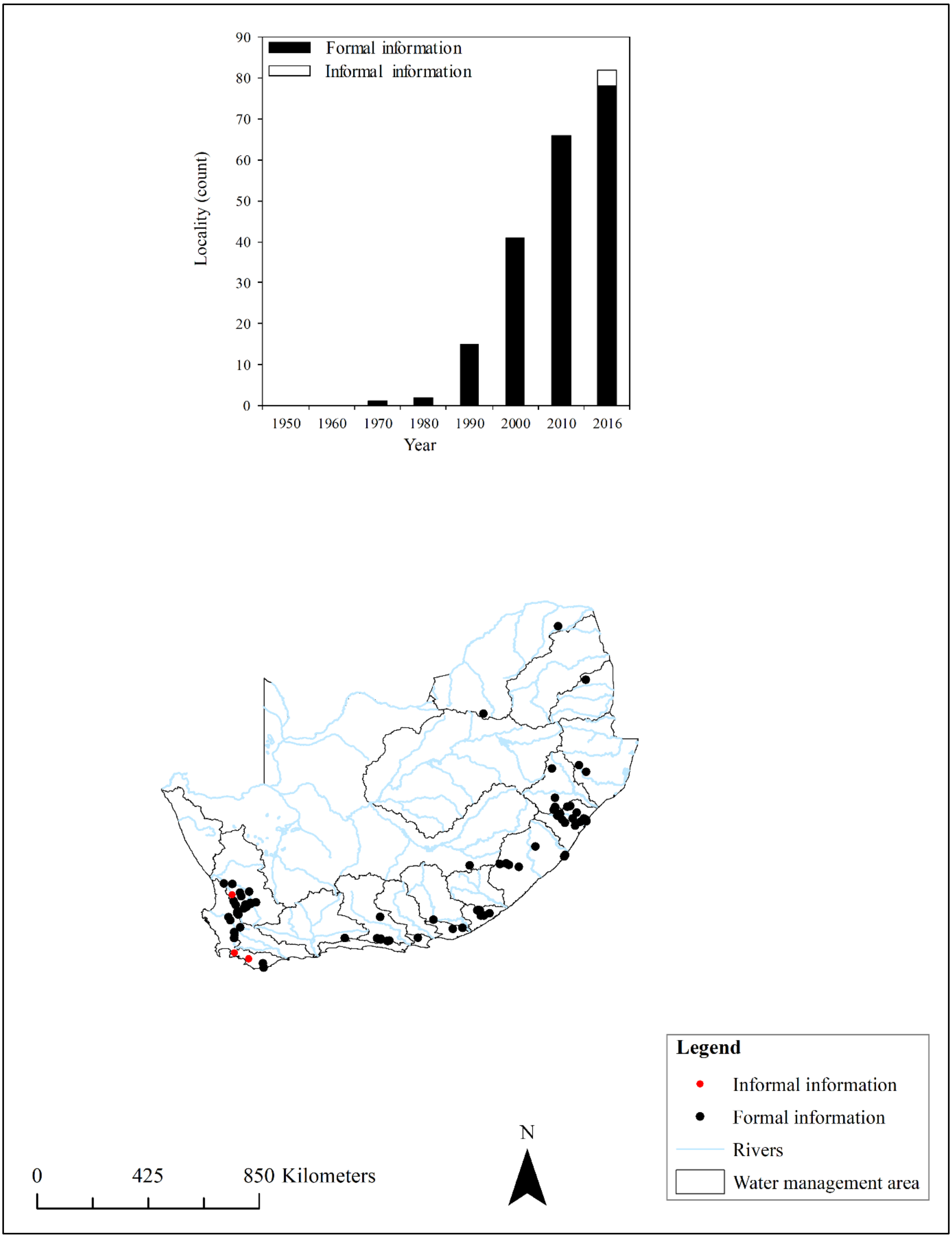

Figure 2.3 The detection rate of the Spotted Bass in South Africa projected from different information sources

Largemouth Bass

For Largemouth Bass, model performance was good (AUC = 0.86; CBI = $p < 0.001$) and the variables contributing most to the model performance were flow accumulation (26.4%), isothermality (height of the day-to-night temperature oscillation relative to the summer-to-winter (annual) oscillations) (25.3%), precipitation of coldest quarter (16.3%), population density (6.4%) and precipitation seasonality (6%) (Table 2.2). Areas that were predicted as highly suitable were mainly associated with maximum temperatures of warmest month between 19 and 30 °C (optimum 26–27.5 °C), high flow accumulation, and population densities (Supplementary Figure 2.2). The jack-knife analysis on training and test gain, and AUC test data showed that the environmental variable with the highest gain, when used in isolation, was flow accumulation, and the environmental variable that decreased the gain the most when it was omitted was flow accumulation. As a result, the areas that were predicted as most suitable (> 0.5) for Largemouth Bass were in coastal areas from the Berg WMA in the CFE and extending up to the Mfolozi WMA in KwaZulu-Natal (Figure 2.4). Inland, sections of the Komati and Olifants North WMA were also predicted as being highly suitable (Figure 2.4).

The total area predicted as suitable for Largemouth Bass was ca. 98 253 km of total river length in an extension of ca. 543 804 km^2 (Table 2.3). Current occupancy (rivers that were predicted as suitable and from which Largemouth Bass records are available) was ca. 27 509 km (28%) (Table 2.3). Subsequent invasion debt was estimated at 72%. The total length of rivers predicted as unsuitable were ca. 75 631 km in an area of ca. 631 463 km^2; and occupancy in this area was only at ca. 3884 km of river length, indicating a 5% modelling error.

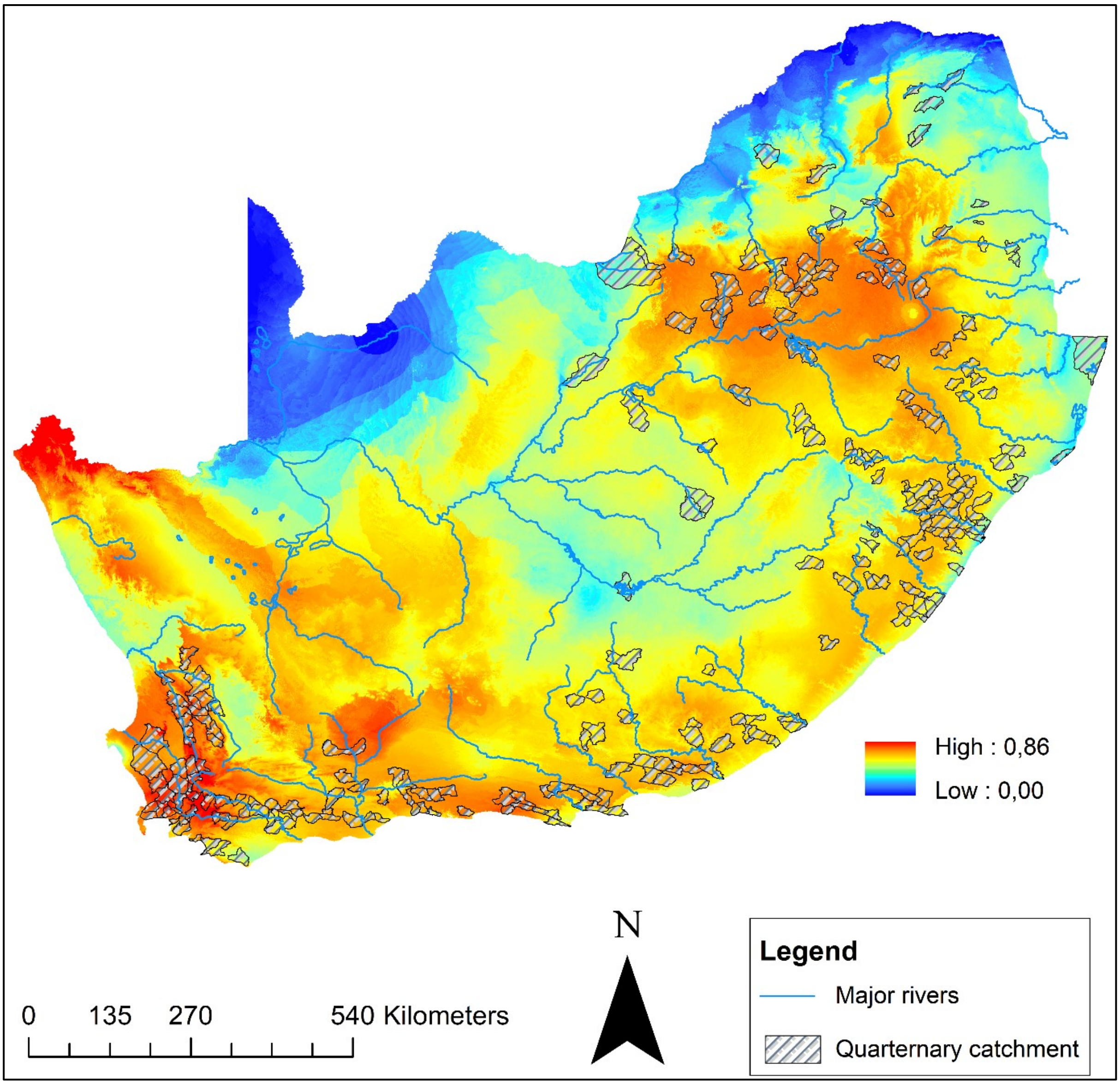

Figure 2.4 The predicted distribution of the Largemouth Bass in South Africa and the quaternary catchments with known Largemouth Bass occurrence

Smallmouth Bass

The Smallmouth Bass model performance was good (AUC = 0.87; CBI = $p < 0.001$); the variables contributing most to the model performance were precipitation of warmest quarter (28.6%), flow accumulation (26%), topographic index (12.9%) and precipitation of coldest quarter (10.3%) (Table 2.2). The response curves show that the areas predicted as highly suitable for Smallmouth Bass were mainly associated with a mean temperature of warmest quarter (range = 19–25 °C, optimum 21–24 °C) and precipitation of the coldest quarter (optimum > 600 mm) (Supplementary Figure 2.3). The jack-knife analysis on training and test gain, and AUC test data showed that the environmental variable with the highest gain when used in isolation was precipitation of warmest quarter (> 100 mm), and the environmental variable that decreased the gain the most when it was omitted was the topographic index. As a result, the areas that were predicted as being suitable (> 0.5) for Smallmouth Bass occurrence were river basins in the CFE (Figure 2.5).

The area predicted as suitable for Smallmouth Bass was estimated at ca. 79976 km of total river length in an extension of ca. 461 163 km^2 (Table 2.3). Currently, occupancy of Smallmouth Bass was only at a total river length of ca. 6356 km (7.9%) and the invasion debt was 92.1%. The area predicted as unsuitable was 93 902 km in an area of ca. 713 637 km^2 with an occupancy of less than 1% of the total area (Table 2.3).

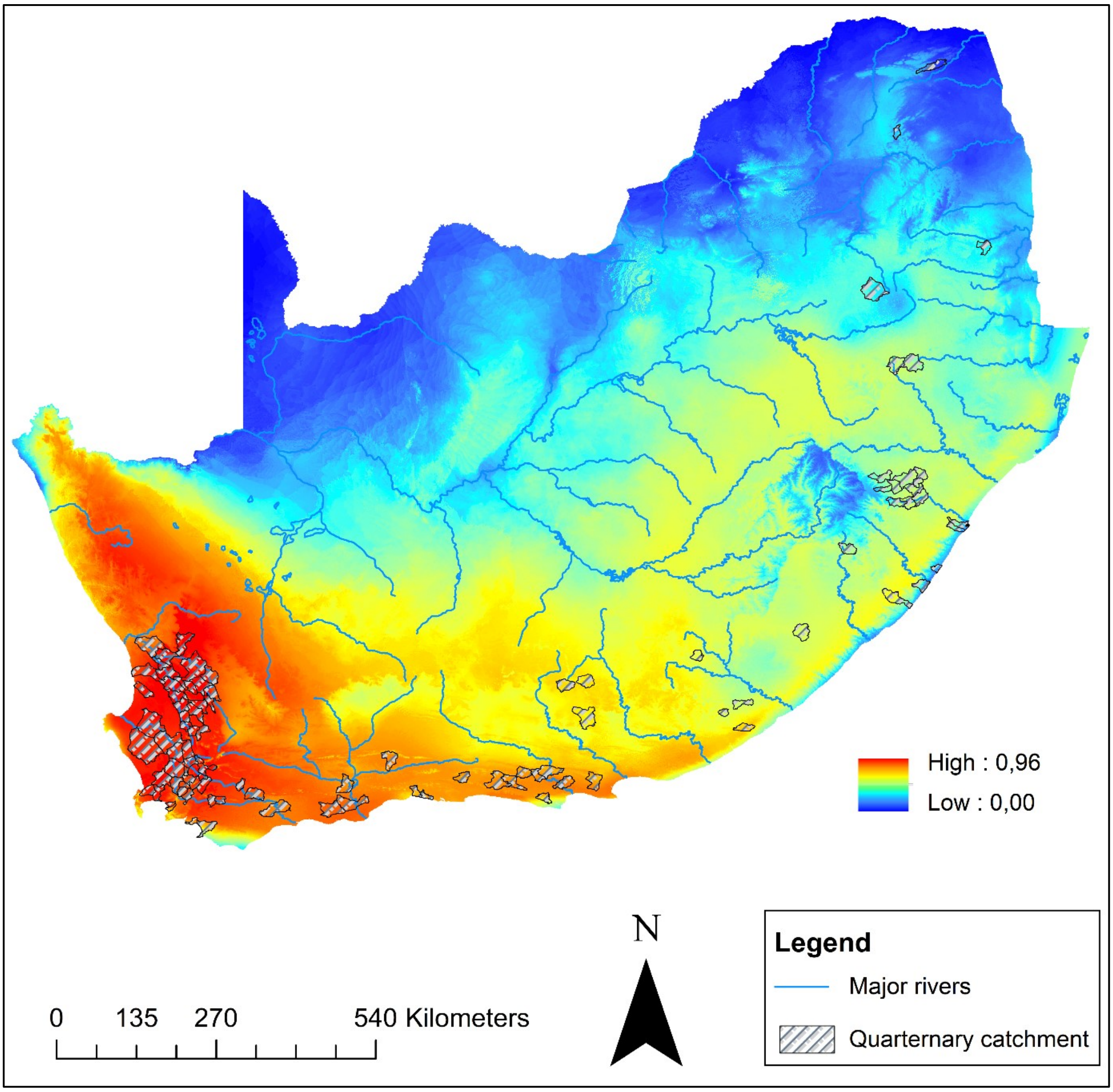

Figure 2.5 The predicted distribution of the Smallmouth Bass in South Africa and the quaternary catchments with known Smallmouth Bass occurrence

Spotted Bass

The model was good (AUC = 0.88; CBI = $p < 0.001$), and the variables that contributed most to model performance were flow accumulation (27.5%), topographic index (18.9%), agricultural land use, annual mean temperature (11.4%) and precipitation of coldest quarter (10.6%) (Table 2.2). The response curves show that the areas that were predicted as highly suitable for the establishment of Spotted Bass were mainly associated with the mean temperature of the driest quarter (range = 20–28 °C, optimum = 23–26 °C) and flow accumulation (Supplementary Figure 2.4). The jack-knife analysis on training and test gain, and AUC test data showed that the environmental variable with the highest gain, when used in isolation (most useful information by itself), was the topographic index, and the environmental variable that decreased the gain the most when it was omitted was the topographic index. The areas that were predicted as suitable (> 0.5) for Spotted Bass were located along the coastal areas of South Africa from the Berg WMA, extending up to the Mfolozi WMA in KwaZulu-Natal (Figure 2.6).

The area predicted as suitable for Spotted Bass was estimated at 18 404 km of total length in river systems in an extension of ca. 85 976 km^2 (Table 2.3). Current occupancy in the suitable area was estimated at 4347 km of the rivers (23.6%) and the invasion debt was 76.4%. Spotted Bass was only recorded in < 1% of the areas that were predicted as unsuitable: 1086 km of the rivers in ca 1 081 612 km^2 of the total area (Table 2.3).

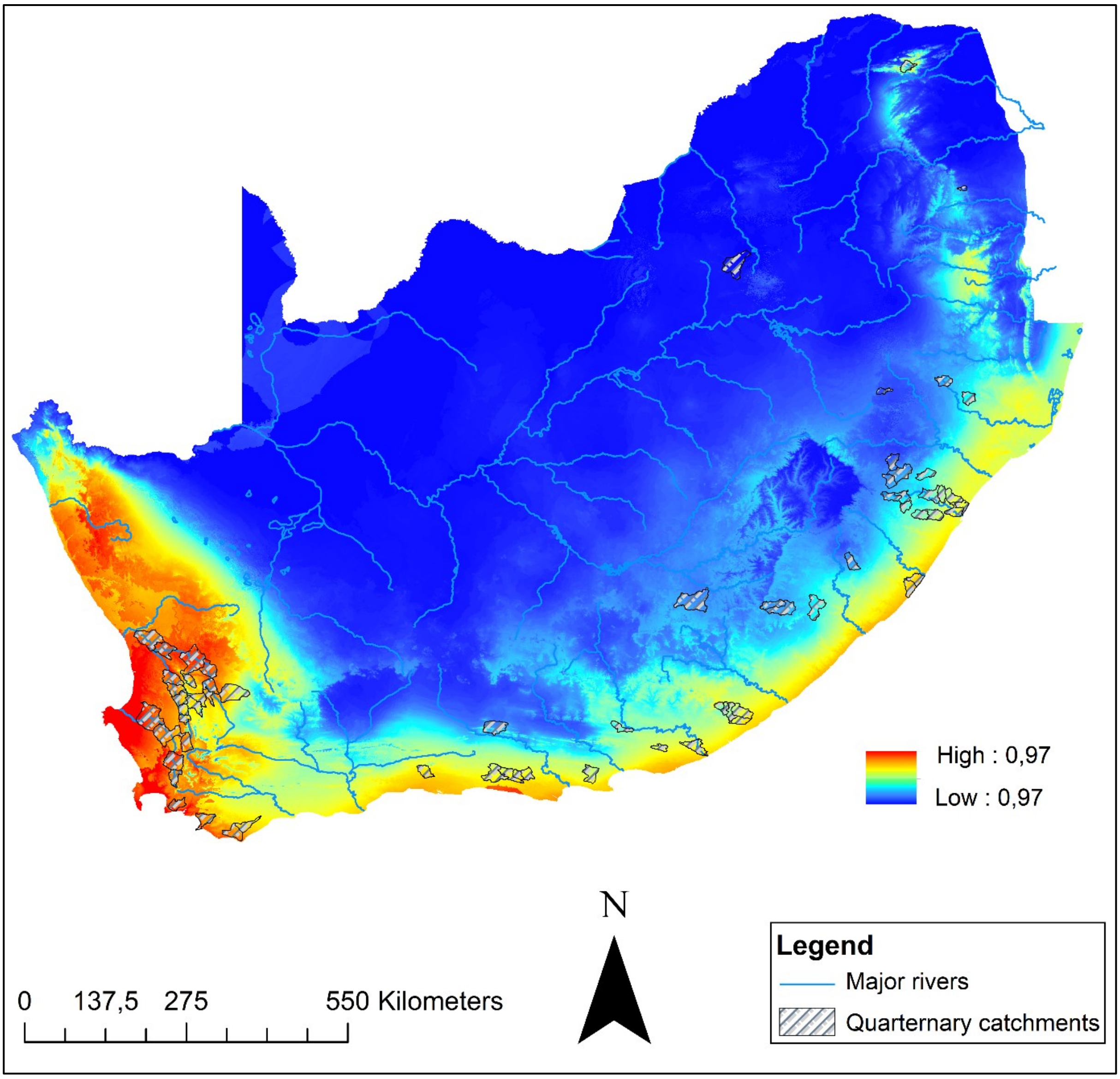

Figure 2.6 The predicted distribution of the Spotted Bass in South Africa and the quaternary catchments with known Spotted Bass occurrence

Table 2.2 Environmental variables and the relative contribution (%) of each variable used to predict the potential spread of Largemouth Bass, Smallmouth Bass and Spotted Bass in South Africa

Environmental variable	Largemouth Bass	Smallmouth Bass	Spotted Bass
Annual mean temperature	-	-	11.4
Isothermality	25.3	2.7	-
Temperature seasonality	-	-	1.6
Temperature annual range	-	-	2.4
Mean temperature of wettest quarter	2.2	1	-
Mean temperature of driest quarter	-	-	1.6
Mean temperature of warmest quarter	-	1.4	-
Annual precipitation	1.5	-	-
Precipitation of driest month	2.9	-	1.7
Precipitation seasonality	6	2.9	-
Precipitation of warmest quarter	4.1	28.6	4
Precipitation of coldest quarter	16.3	10.3	10.6
Flow accumulation	26.4	26	27.5
Population density	6.4	2.1	1
Agricultural land	2.4	8.3	18.2
Topographic index	1.9	12.9	18.9
Slope	1.2	0	0
Elevation	1	0	0

Table 2.3 Summary of the total river length and current occupancy of Black Bass species at different rivers per water management area of South Africa

Water management area	Surface area (km^2)	River length (km)	Suitable river length (km)			Current occupancy (km)		
			Largemouth Bass	Smallmouth Bass	Spotted Bass	Largemouth Bass	Smallmouth Bass	Spotted Bass
A–Limpopo	97 353	12 508	4191	0	0	1945	130	237
B–Olifants North	65 540	10 376	4947	0	0	1641	248	0
C–Vaal	179 789	22 885	13 975	10 009	0	3025	0	0
D–Orange	379 999	41 643	14 198	17 314	392	722	0	237
E–Olifants West	46 755	7452	3385	7452	4684	1584	1756	1561
F–Buffels	26 733	3342	2438	3284	2595	0	0	0
G–Berg	2454	3761	2740	2951	3191	2061	1318	837
H–Breede	15 136	2864	2864	2864	748	1725	890	27
J–Gouritz	43 650	7668	7204	7668	152	1120	162	173
K–Krom	704	1470	1470	1261	1470	450	0	0
L–Gamtoos	33 414	6003	5238	6003	622	864	777	378
M–Swartkops	256	427	427	427	24	316	0	0
N–Sundays	20 420	3699	3395	3699	261	532	261	0
P–Bushmans	519	1312	1312	1312	812	827	0	0
Q–Great Fish	28 930	5746	3852	5746	0	743	129	90
R – Keiskamma	764	1876	1876	1867	923	745	65	146
S–Kei	19 550	4670	1712	2420	147	370	79	0
T–Mzimvubu	44 060	10 452	6901	4591	2077	2307	246	276
U–Mkomazi	17 074	4074	3265	1092	306	2611	153	235
V–Tugela	26 770	5380	5380	16	0	1750	94	38
W–Mfolozi	53 870	10 683	5256	0	0	1555	0	81
X–Komati	27 960	5585	2227	0	0	616	48	31
Total	1 129 246	163 500	98 253	79 976	18 404	27 509	6356	4347

Discussion

Black Bass distributions

This study demonstrated that, with appropriate data quality control measures, informal information sources were a useful tool for extending the knowledge of the occurrence of Black Bass from relatively well-studied riverine environments to poorly studied lacustrine environments. The current dataset used data gathered after 2010, when most records of Black Bass were publicised online, predominantly in anglers' social media and blog platforms. The increase of Black Bass records in informal sources can be attributed mainly to the launch of Facebook in 2004 which facilitated sharing and access to angler catch records (Gago et al. 2016). Incorporating informal information sources into the dataset increased the number of known localities by 30%, thereby supporting the first hypothesis that informal data sources would significantly increase the known extent of occurrence of Black Bass.

As inland fisheries were, until recently, not a priority for South Africa (Weyl et al. 2007), most formal fish surveys in South Africa were conducted with a conservation focus (e.g. Van Der Walt et al. 2016). This disparity is explained by most lentic environments in the country being impoundments that are generally considered of low conservation importance (Beatty et al. 2017), but are used extensively for angling (Hargrove et al. 2015). The current study highlights the value of combining both formal and informal information sources to provide a broader representation of the extent of occurrence of Black Bass in South Africa. This is important because the three species have different habitat preferences which affect the probability of detecting them in lentic and lotic environments. In a survey of 42 tributary streams of the Olifants-Doring River System, Van Der Walt et al. (2016) showed that Smallmouth Bass and Spotted Bass co-occurred in most sections of rivers, inhabiting the fast-flowing riffles and pools, while Largemouth Bass were restricted to slow-moving headwater streams and adjacent ponds. This highlights the importance of including data from both lotic and lentic environments in distributions.

From the analysis of spread/detection rate, it is also evident that detections have increased with increasing survey effort since the 1980s, and the inclusion of informal data (see Figures 2.1–2.3). This increase can be attributed to recent efforts by government and conservation entities to document the spread of non-native species (Ellender and Weyl 2014), and the increased use of online platforms by anglers, both of which facilitate the mining of informal data. The current dataset is the most complete set of distribution records for Black Bass in South Africa and was the basis for the subsequent model of the potential spread of the three Black Bass species in South Africa.

Species distribution modelling

Model outputs closely followed the known distributions of the three species (Figures 2.4–2.6) but only partly supported the second hypothesis that the distribution of all the three Black Bass species was determined by similar environmental factors. While flow accumulation (a variable showing association to mainstream rivers and impoundments) was an equally important variable for all three species, the importance of other environmental variables differed between species. In overcoming the establishment barrier for invasions (Blackburn et al. 2011), climate matching the native is often a significant factor in establishment (Marr et al. 2010). Interspecific differences in the environmental drivers, therefore, need to be viewed in the context of the habitat preferences and requirements of the three species under consideration.

The model outputs for Largemouth Bass indicated that most of the country was environmentally suitable (Figure 2.2). In terms of environment, the response curves show that

Largemouth Bass were likely to occur in warmer areas where maximum temperatures range between 19–30 °C. This was expected as temperature is known to be positively related to growth, spawning, and the survival of eggs and embryos (Clugston 1964; Beamish et al. 2005). Findings from this study are congruent with those of Bae et al. (2018) who found temperature to be the most important predictor of Largemouth Bass distributions. The preference of Largemouth Bass for slow-flowing and lentic environments (Jackson 2002; Claussen 2015) was demonstrated by their high association with flow accumulation. As a result, the presence of more than 3000 impoundments spread across South Africa (Weyl et al. 2007) is likely to facilitate Black Bass invasions, as has been demonstrated for this and other invasive species elsewhere (Johnson et al. 2008; Bae et al. 2018).

For Smallmouth Bass, the SDM showed that the most suitable areas were associated with the Mediterranean climate region in the CFE, with high summer temperatures and high precipitation during the coldest quarter. This fits well within the high maximum temperature tolerance (34.8 °C) and thermal optima for spawning and growth (12.1–21 °C) for this species (Beitinger et al. 2000; Brewer and Orth 2015).

For Spotted Bass, the areas that were predicted as suitable are located between the coast and the escarpment of South Africa, up to mid-KwaZulu-Natal. The most significant climatic variables identified by the models for Spotted Bass were annual mean temperature and precipitation of coldest quarter. Unlike Largemouth Bass and Smallmouth Bass, spawning of Spotted Bass is not stimulated by rising water levels following precipitation (Sammons et al. 1999; Beamish et al. 2005). However, suitable temperatures, between 14–23 °C, are required for successful spawning (Churchill and Bettoli 2015). The significance of temperature was also observed in the response curves where habitat suitable for Spotted Bass was associated with the mean temperature of the driest quarter, with a range of 20–28 °C, which was congruent with the requirements for successful spawning for Spotted Bass (Sammons et al. 1999).

Human activity

The third hypothesis, that because Black Bass are actively introduced to develop opportunities for angling, Black Bass distributions would be strongly associated with human population density, was not supported by the analyses. Population density explained only 6.4% of the distribution of Largemouth Bass, 2% for Smallmouth Bass and 1% for Spotted Bass. This was surprising because Black Bass are popular among anglers, who have been shown to be a major vector for their spread in South Africa (e.g. De Moor 1996; Ellender et al. 2014; Weyl et al. 2017) and elsewhere (Jackson 2002; Long et al. 2015). The lack of direct association with human population density is likely to be a result of the widespread stocking by government agencies and recreational angling societies soon after the introduction of these species (Ellender et al. 2014). Ellender et al. (2014) describe how the hatchery infrastructure, distribution and stocking network developed for trout in the early 1900s was used to introduce Black Bass into parts of almost every major river system in South Africa by 1940. As these government-subsidised stocking events were likely to be informed by the presence of suitable habitat rather than by human population density, the current extent of occurrence of Black Bass populations is only weakly correlated with human population density. The relatively higher association of Smallmouth Bass and Spotted Bass with agricultural land (8-18%), in comparison with Largemouth Bass (1.9%), is likely to be an artefact of their higher occurrence in the CFE, where most low-lying areas are agricultural land.

Invasion debt and survey priorities

Non-native invasions may be characterised by a substantial lag from the time of introduction, subsequent establishment, and spread in the novel environments (Essl et al. 2011; Rouget et al. 2016). These delays are mainly influenced by propagule pressure (Simberloff 2009) and the conditions in the novel environments, that is, competition (De Moor 1996; Jackson et al. 2017) and climate (Jackson and Sax 2009). The results of this study suggest that there is a considerable area-based invasion debt for Largemouth Bass and Smallmouth Bass. For Largemouth Bass, there is an extensive area-based invasion debt across all WMAs. Similarly, besides having lesser areas that are suitable when compared to Largemouth Bass, Smallmouth Bass also have an extensive area that remains unoccupied. I also find overlaps in rivers that are predicted as suitable for both Black Bass species, specifically in the CFE and the Vaal WMA. Although these are based on crude estimates and limited by sampling effort, it is a matter for concern, given the popularity of the species among anglers, so increasing the possibility of their translocation into new areas (Hargrove et al. 2015; Long et al. 2015; Weyl et al. 2015).

Conservation implications

The global spread of Black Bass species has been driven by the desire to create recreational angling opportunities (Jackson 2002; Long et al. 2015), and in some areas, including South Africa, there is still a paucity of information relating to the ongoing introduction and spread of the *Micropterus* species (Ellender et al. 2014; Hargrove et al. 2015, 2017; Weyl et al. 2017). This is a problem because Black Bass exert considerable predation pressure on the biota in invaded environments. In South Africa, this pressure has resulted in the extirpation and fragmentation of native fish communities (Ellender and Weyl 2014; Kimberg et al. 2014; Ellender et al. 2018). Of particular concern is the CFE, which contains 42 native fish taxa, most of which are endemic, and IUCN Red List evaluated as Endangered (Ellender et al. 2017). As all three Black Bass species already occur in the CFE, and environmental conditions in this area are highly suitable for them, action limiting their spread is critical to prevent further impacts on an already imperilled native fish fauna (Ellender et al. 2017).

Limiting the spread and impact of Black Bass in South Africa requires accurate contemporary information on their current distribution. While the current approach of using informal information sources enhanced our understanding of Black Bass distributions, additional survey data will be necessary to implement national legislation attempting to limit the spread of Black Bass into areas that are not already invaded (see Woodford et al. 2017). As this will require considerable resources to implement, I suggest that such surveys initially focus on the 'invasion debt' areas identified in this study. In addition, the need to explore avenues for identification of Florida Bass is critical so that this species can be dealt with discretely from Largemouth Bass. Levels of introgression and hybridisation between the Black Bass species also needs to be explored, given that select species are sympatric in many regions of South Africa.

CHAPTER 3

THE EXTENT OF HYBRIDISATION BETWEEN LARGEMOUTH BASS AND FLORIDA BASS ACROSS TWO RIVER SYSTEMS IN SOUTH AFRICA

Abstract

Native to North America, Largemouth Bass were introduced into South Africa in 1928 for recreational angling purposes. In 1980, Florida Bass were introduced to enhance Largemouth Bass fisheries. Both Largemouth Bass and Florida Bass are listed under the *National Environmental Management: Biodiversity Ac*t (NEM:BA) alien and invasive species lists and regulations. Understanding their spread has implications for the development of management and conservation strategies. To better understand the dynamics of the Florida Bass invasion, fish were sampled from the Breede River catchment (n = 43) and Kowie River catchment (n = 95) and screened using 38 species-diagnostic single nucleotide polymorphism (SNP) to assess the level of genetic admixture. Largemouth Bass alleles were more prevalent than Florida Bass alleles in both the Breede River catchments (69.1% and 30.9%, respectively) and Kowie River catchment (63.3% and 36.7% respectively). The mainstem of both river catchments comprised introgressed populations; in the Breede River, Largemouth Bass alleles and Florida Bass alleles contributed an average of 67.68% and 32.32%, respectively. In the Kowie River, an average of 49.7% and 50.3% of Largemouth Bass alleles and Florida Bass alleles, respectively, were recorded. Pure populations of Largemouth Bass were only found upstream of the Florida Bass invasion. Results of this study indicate that developing independent management strategies for each of the two species may prove difficult; as a result, it is advisable that the two species be managed as one.

Introduction

Biological invasions are a major threat to ecosystems globally (Dawson et al. 2017; Seebens et al. 2017; Blackburn et al. 2019), and freshwater ecosystems and fishes are of particular ecological concern (Ricciardi and Rasmussen 1999; Light and Marchetti 2007). Invasions and the subsequent extinctions have been identified to be more frequent in freshwater ecosystems than in marine and terrestrial ecosystems (Ricciardi and Rasmussen 1999; Ricciardi and Macisaac 2011). The introduction of non-native fishes for recreational angling (Cambray 2003; Cucherousset and Olden 2011) is one of the major drivers of biological invasions (Clarkson et al. 2005; Ellender et al. 2014) and the impacts are a major driver of global biodiversity change (Cambray 2003; Crawford and Muir 2008; Gozlan et al. 2010). Understanding their impacts and managing invasions is therefore a conservation priority in many countries, including Brazil (Vitule et al. 2012; Bezerra et al. 2019), South Africa (Ellender et al. 2014), Europe (Gago et al. 2016; Cucherousset et al. 2018), Asia (Takamura 2007) and North America (Vander Zanden et al. 2004; Long et al. 2015).

Black Basses (genus *Micropterus*) are a group of freshwater fishes native to eastern North America (Warren 2009) and are of substantial economic and ecological significance (Jackson 2002; U.S. Department of the Interior 2011). Owing to their rapid growth and large maximum size, Black Bass have been introduced globally to establish recreational sport fisheries in over 70 countries worldwide (Welcomme 1992; Casal 2006; Ellender et al. 2014; Hargrove et al. 2015). In regions where they have been introduced, Black Bass have formed the basis for economically important recreational fisheries (Taylor et al. 2015). For example, Hargrove et al. (2015) documented 66 reservoirs across southern Africa that hosted tournament angling events for Black Bass. Little information is available on the economic value of Black Bass in the introduced range; however, anglers who specifically target Black Bass use tackle (rods, reels, and lures) and boats that are equivalent to those used in their native range (Taylor et al. 2015; Hargrove et al. 2018). In addition to their economic impacts, Black Bass function as apex predators in many systems into which they are introduced and may exert top-down control in recipient ecosystems (Maezono et al. 2005; Ellender and Weyl 2014; Pereira and Vitule 2019), resulting in the extirpation of small-bodied fishes (e.g. Jackson 2002; Takamura 2007; van der Walt et al. 2016, Ellender et al. 2018). In the Swartkops River, for example, Ellender et al. (2018) demonstrated a negative correlation between the native Eastern Cape redfin *Pseudobarbus afer* (Peters 1864) and the non-native invasive Black Basses, Largemouth Bass and Smallmouth Bass, but which generally co-occurred with the native predators Giant Mottled Eel *Anguilla marmorata* Quoy and Gaimard 1824, and Longfin Eel *A. mossambica* Peters 1852. As a result of the contrast between their economic value and the harm they inflict on some recipient ecosystems, Black Bass management is complicated by the contrasting risk perceptions of different stakeholders (e.g. anglers vs conservationists), and conflicts are common (Ellender et al. 2014; Zengeya et al. 2017).

In South Africa, the main legislative instrument that guides the management of non-native species is the *National Environmental Management: Biodiversity Act* (NEM:BA) (Act 10 of 2004) and the regulations relating to this Act (Republic of South Africa [RSA] 2004). Under the non-native invasive species regulations, Black Basses can be caught, released and stocked with a permit in impoundments where they currently occur; however, where they do not currently occur, their movement and release is prohibited (Woodford et al. 2017). Therefore, knowledge of species distributions is essential if the legislation is to be appropriately applied (Chapter 2). An important question of non-native species management relevance is, “To what extent do Florida Bass spread within a catchment following their initial introduction?” It is well documented that Florida Bass and Largemouth Bass readily hybridise and are commonly co-distributed (e.g. Philipp et al. 1983; Philipp 1991; Barthel et al. 2010; Weyl et al. 2017; Hargrove et al. 2019a, b). Furthermore, efforts to identify the distribution of Largemouth Bass and Florida Bass requires molecular tools which can be costly, time consuming, and require expertise. Thus, it is desirable to know whether sampling a subset of water bodies within a catchment can accurately characterise hybridisation throughout the catchment. In the case of river systems, invasive propagules may enter rivers from a number of potential sources (Woodford et al. 2013). Specifically, flood events may move fish stocked from dams into the riverine environment (Harrison 1936; Hargrove et al. 2019b), or from upstream or downstream stockings in systems lacking barriers to migration (Altermatt 2013; Tonkin et al. 2018a). This is consistent with the asymmetric dispersal, typically observed in dendritic networks, and suggests that dispersal within a catchment scale primarily follows a downstream direction (Vannote et al. 1980; Morrissey and De Kerckhove 2009; Alp et al. 2012; Altermatt 2013).

The current study assessed the distribution of Largemouth Bass, Florida Bass and associated hybrids across multiple river catchments to quantify patterns of hybridisation within and between different water bodies. Specifically, this study sought to address whether the detection of Florida Bass alleles in one portion of a river adequately characterises levels of hybridisation throughout the catchment. It was predicted that non-hybridised Largemouth Bass and Florida Bass would only be detected in disconnected water bodies or isolated river stretches.

Materials and methods

Study area

Kowie River catchment

The Kowie River, a is perennial river with a total length of approximately 70 km (Dalu et al. 2016), is located in the Eastern Cape Province of South Africa and drains a catchment area of approximately 800 km^2 (Heinecken and Grindley 1982). The river originates from the hills of 'Makhanda Heights', at an altitude of ~600 m above sea level, from where it flows in a south-east direction and drains a major part of Bathurst. Its major tributaries are the Bloukrans, Brak and Lushington Rivers, and there are a number of smaller, unnamed streams entering the river along its course (Figure 3.1). Although there are various structures (e.g. weirs) constructed along the mainstem of the Kowie River, these structures do not appear to obstruct the normal flow of the river (Heinecken and Grindley 1982; Whitfield et al. 1994), except for the Kowie weir in the lower reaches of the Kowie River, which may constitute a major barrier for upstream fish migration during low flows (Whitfield et al. 1994; Weyl and Lewis 2006). In a recent survey of the Kowie River catchment, fifteen fish species were identified and documented, including native and non-native, and extralimital species (Table 3.1).

Table 3.1 The list of freshwater fish species collected from both the Kowie River catchment between (2017–2018) and the Breede River catchments in 2016. 0 denotes absence and 1 denotes presence of the species

Species	Common name	Native	Non-native	extralimital	Kowie River catchment	Breede River catchments
Anguilla mossambica	Longfin Eel	X			1	1
Clarias gariepinus	Sharptooth Catfish			X	1	1
Coptodon rendalli	Redbreast Tilapia			X	1	0
Cyprinus carpio	Common Carp		X		1	1
Enteromius anoplus	Chubbyhead Barb	X			1	0
Gilchrestella aestuaria	Estuarine Round Herring	X			1	1
Glossogobius callidus	River Goby	X			1	0
Lepomis macrochirus	Bluegill		X		1	1
Micropterus dolomieu	Smallmouth Bass		X		0	1
Micropterus punctulatus	Spotted Bass		X		1	0
Micropterus salmoides/M. floridanus	Largemouth Bass/Florida Bass		X		1	1
Myxus capensis	Freshwater Mullet	X			1	1
Oreochromis mossambicus	Mozambique Tilapia	X			1	0
Cheilobarbus capensis	Whitefish	X			0	1
Redigobius dewaali	Checkered Goby	X			1	1
Sandelia capensis	Cape Kurper	X			0	1
Tilapia sparrmanii	Banded Tilapia			X	1	1
Tinca tinca	Tench		X		0	1

Sampling sites

Quarry, TCP aquaponics (TCP) and Douglas dams are found at the upper reaches of the Kowie River catchment in the urban settlement of Makhanda. They are connected through a canal system flowing into the Bloukrans River. Frume Dam, which is also located at the upper reaches of the Kowie River catchment, but in an agricultural area on the outskirts of the urban settlement of Makhanda, is also a tributary to the Bloukrans River. The Bloukrans pool, which is located ~5 km upstream of the confluence of the Bloukrans with the Kowie River, was sampled. Terrys and Nature Reserve were the uppermost site in the mainstem of the Kowie River and are ~5 km apart; no barriers for movement were recorded between the two sites. The Kowie Weir and below the weir sites are located at the lower reaches of the Kowie River catchment and are ~12 km upstream of the Kowie Estuary. The Kowie Dam is an off-channel dam that is ~500 m above the Kowie Weir. Leon's Dam impounds an unnamed tributary of the Kowie River on the western side of the Kowie River and it is located 2.5 km before the confluence with the Kowie River. Finally, Delton's Dam is found in the eastern side of the Kowie River impounding an unnamed tributary of the Bloukrans River (Table 3.2).

Black Bass invasion in the Kowie River catchment

The first shipment of Largemouth Bass to Makhanda was received in 1933 from Pirie Hatchery, King Williams Town and consisted of 42 fingerlings (Harrison 1936). Of the 42 fingerlings, nine specimens were placed in the Kowie Weir (Harrison 1936). The second shipment comprised 24 fingerlings from Jonkershoek Hatchery received by the Albany Angling Association in 1934, but it is not clear where the fish were placed after receiving them. In 1992, Florida Bass were obtained from Somerset East, Eastern Cape, and ten individuals were introduced into Frume Dam (C. Frume, personal communication).

Breede River catchment

The Breede River is the largest river in the Western Cape Province, South Africa, and drains a catchment area of approximately 12 600 km^2 (Steynor et al. 2009). The river originates in Skurweberg near Ceres Valley, and flows in a south-easterly direction for approximately 320 km into the Indian Ocean. In the upper reaches, tributaries include the Hex, Wit, Molenaars and Holsloot rivers. The main tributary to the Breede River is the Riviersonderend River which is found in middle reaches. Riviersonderend River originates upstream of Theewaterskloof Dam, in the Hottentots Holland and Franschhoek Mountains, and flows in a southerly direction. The species that are found in the selected sites of the Breede River catchment are listed in Table 3.1. For this study, only the mainstem of the Breede River and two major dams (i.e. Quaggaskloof and Theewaterskloof dams) in the catchment were sampled (Figure 3.1). Sites were therefore partitioned into different reaches based on the distance between the sites. Reach 1 is at the lower reaches of the Breede River, and is ~21 km upstream of the Breede Estuary. Reach 2 is an instream dam in the Breede River and is found near Swellendam, ~11 km downstream of the confluence between the Breede River and Riviersonderend River. Reach 3 is located ~12 km upstream of the confluence between the Breede River and Riviersonderend River. Reach 4 is the uppermost in the mainstem of the Breede River and is ~18 km from Reach 3. Reach 5 is an off-channel dam (Quaggaskloof Dam) used for recreational angling tournaments, and Reach 6 is also a recreational angling tournament venue (Theewaterskloof Dam) impounding Riviersonderend River.

Black Bass invasion in the Breede River catchment

There was no direct introduction of Largemouth Bass into the Breede River mainstem (Harrison 1936). Between 1929 and 1932, a total of 100 Largemouth Bass fingerlings from Jonkershoek Hatchery were introduced in Quaggaskloof Dam, and subsequent flooding events in 1935 led to Largemouth Bass finding their way into the mainstem of the Breede River; by 1938, breeding populations were documented in the river (Harrison 1936). Owing to the lack of formal documentation, it is currently unknown when or where Florida Bass were first introduced into the Breede River catchment as all stocking permit applications list Largemouth Bass as the species being introduced.

Genetic analysis

Fish were collected from the Breede River catchment (n = 43) and Kowie River catchment (n = 95) by angling (hook and line), seine nets, fyke nets and gillnets. Fish were measured for total length (TL) to the nearest mm, and ca. 1 x 1 cm^2 portion of pectoral fin was clipped from each fish captured and preserved in 95% non-denatured ethanol. DNA was extracted from the preserved samples using a 96-well plate extraction protocol (Ivanova et al. 2006). Extracted DNA was quantified using a ND-1000 spectrophotometer (Nanodrop, Wilmington, DE) and diluted to a final concentration of 20 ng/µL. Both positive and negative controls were included in DNA extraction. In total, 38 species-diagnostic SNP markers were used to assess genetic introgression between Largemouth Bass and Florida Bass. The SNP markers used were previously developed by Li et al. (2015) and Zhao et al. (2018) using an RNA-seq based approach in which the transcriptomes of Largemouth Bass, Florida Bass, and their F_1 hybrids were sequenced, and sets of fixed-allelic differences between subspecies were identified. SNP amplification and extension reactions were performed using 10 ng of DNA per sample and utilising the iPLEX Gold Reagent Kit® (Agena Bioscience) according to the manufacturer's protocols. Amplification reactions were performed using the Agena MassARRAY platform (Agena Bioscience, San Diego, CA) and called using the MassARRAY TYPER 4.0 software. Lab work was performed at the Aquatic Genetic and Genomics Laboratory at the School of Fisheries, Aquaculture, and Aquatic Sciences at Auburn University.

The focal research question was to determine if hybridisation occurred at equivalent levels throughout the river landscape, following the introduction of Florida Bass into the catchment. Several approaches were taken to explore the hybridisation dynamics among Florida Bass and Largemouth Bass across sample sites within river catchments. First, genetic composition was summarised in terms of the average percentage of Florida Bass alleles by sample site to ascertain total levels of Florida Bass contributions to the genome. In the Kowie River, potential donor populations (i.e. privately stocked dams in the catchment) were sampled and their profiles were compared to those of sample sites within the river to identify a possible source of Florida Bass alleles.

Patterns of hybridisation within and among populations were visualised using Principal Component Analysis (PCA) as implemented in the *ade4* and *adegenet* packages (Thioulouse et al. 1997; Jombart 2008) in R v 3.5.1 (R Development Core Team 2018). Principal component analysis was performed on a correlation matrix of scaled allele frequencies with genetic variations reduced to two components, allowing individuals to be clustered along axes based on their allele distributions. The graphical output displayed the absolute variance (i.e. eigenvalues) explained by each of the principal components with the X and Y labels representing the percentage of total variance explained by the first and second components, respectively.

The question of whether river systems into which Florida Bass have been introduced could be considered a single panmictic population was evaluated using tests of population structure. Specifically, pairwise estimates of genetic differentiation (F_{ST}) were calculated using GenAlEx v 6.502 (Peakall and Smouse 2012). Additionally, hierarchical population structure was tested using an AMOVA framework in which genetic variance was apportioned among samples, among samples within populations, and among populations (Excoffier et al. 1992). An AMOVA was performed using the R package *poppr* (Kamvar et al. 2014) and statistical significance assessed using the randomisation test (randtest; number of iterations = 10 000) implemented in the *ade*4 package (Dray and Dufour 2007). In the Kowie River, analysis was restricted to river sites and not potential tributary systems.

Lastly, in an effort to understand the hybridisation dynamics occurring within each river catchment, the R package HIest (Fitzpatrick 2012) was used to jointly consider ancestry (*S*, a value that arranges hybrids between two ancestral extremes) and interclass heterozygosity (*H*, a value that distinguishes F_1, F_2, and recombinant inbred lines). While software programmes are available to estimate genetic ancestry (e.g. STRUCTURE; Pritchard et al. 2000, or hybrid class NEWHYBRIDS; Anderson and Thompson 2002), evaluation of these estimates individually may fail to capture important hybridisation patterns present in genotypes. For example, classification of individuals into discrete hybrid classes may be inaccurate in cases where introgression has occurred for many generations (Fitzpatrick 2012) because the correspondence between genotype frequencies and genealogical classes is restricted to the first two generations of admixture (Anderson and Thompson 2002). As a result, joint consideration of ancestry and interclass heterozygosity were used to identify potential differences in hybridisation across populations (Fitzpatrick 2012). Individual estimates of ancestry and heterozygosity were plotted for samples from various locations within the same river catchment to determine if different populations experienced similar levels of Florida Bass genetic introgression over similar numbers of generations.

Results

A total of 138 samples were screened with 38 species-diagnostic SNPs, 95 samples from the Kowie River catchment, and 43 samples from the Breede River catchment (Table 2.2).

Kowie River catchment

Collections in the Kowie River included both sites on the mainstem of the river as well as public and private dams distributed across the catchment (Figure 3.1). In the mainstem of the Kowie River, samples contained an average of 50.3% Florida Bass alleles which varied minimally across sites (S.E. = 2.6; Table 3.2). A slight decrease in Florida Bass influence was observed from the upper reaches of the mainstem (Terrys 56.9% and Bloukrans pool 55.7%) to the lower reaches (below weir 45.3% and Kowie weir 43.8%). The pattern of moderate and consistent levels of hybridisation within the mainstem of the Kowie River was not evident among the dams. Specifically, mean levels of Florida Bass were lower overall (mean = 26.9% Florida Bass alleles) and more variable (S.E. = 9.7; Table 3.2). Levels of Florida Bass alleles were highest at Frume Dam (63.8%) and lowest (< 2%) at Quarry and Douglas dams, all of which are located in the upper reaches of the Kowie River catchment. In the lower reaches, Leon and Kowie dams contained hybrid populations with Florida Bass alleles mean percentage of 39.9% and 42.2%, respectively. Delton Dam was the only site to contain exclusively Largemouth Bass alleles.

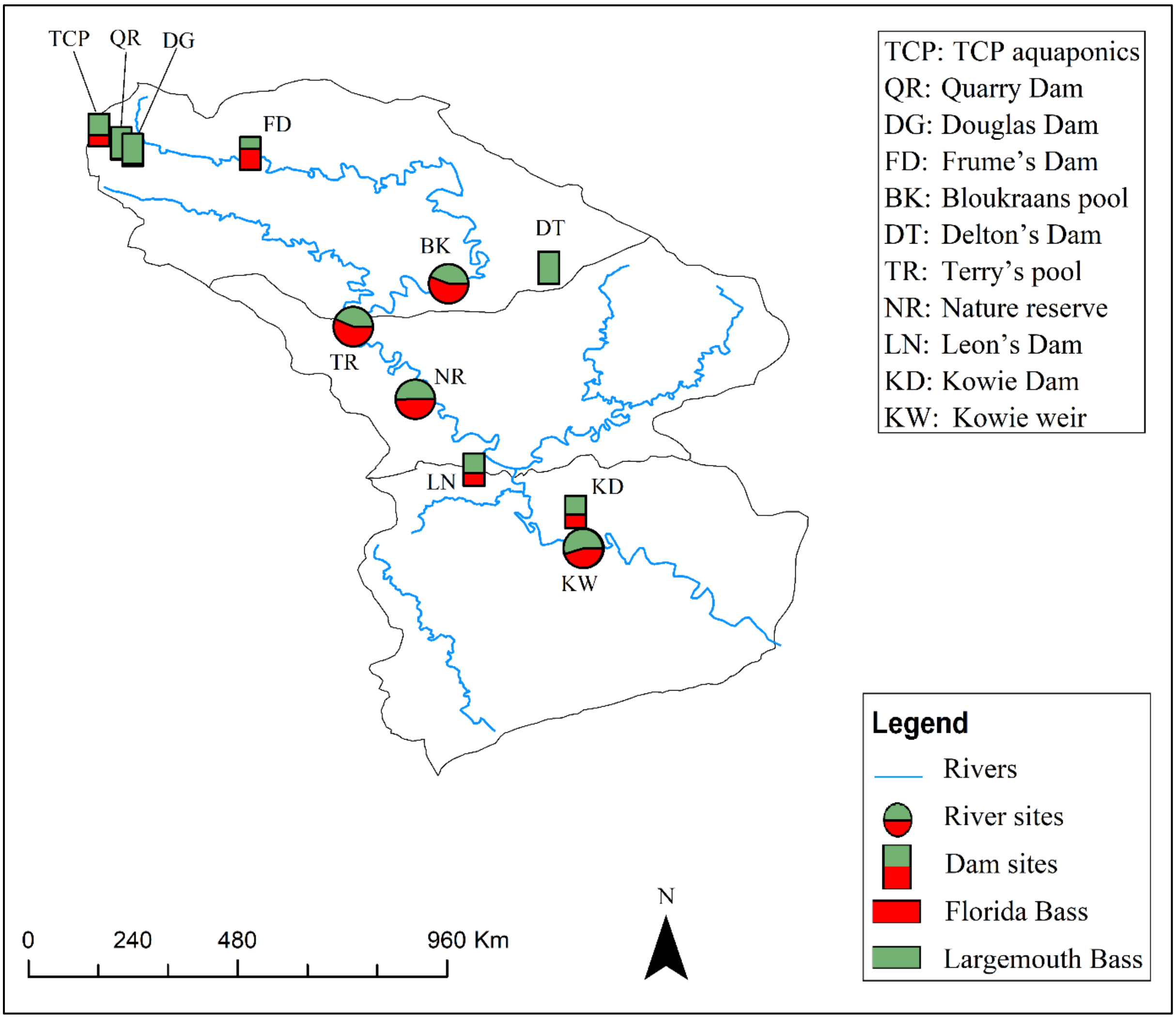

Figure 3.1 The distribution of Largemouth Bass, Florida Bass and their hybrids across the Kowie River catchment in South Africa. Circles represent sites sampled in the mainstem of the river and boxes represent dams. The colou00rs to the box/circle represent the allele percentage contribution by both Largemouth Bass (green) and Florida Bass (red)

A more complex pattern of genetic variation was observed in our PCA for the Kowie River, relative to the Breede River. Whereas sample sites in the Breede River displayed a largely concentric distribution, mainstem sites on the Kowie River were less overlapped (Figure 3.2). The first two components of our PCA of samples from the Kowie River explained 43.5% of the observed genetic variation. Several non-mainstem populations were tightly clustered close to a Largemouth Bass reference sample (e.g. Delton, Douglas, and Quarry dams). In contrast, Frume, Leon, TCP and Kowie dams were widely dispersed, suggesting varying levels of hybridisation.

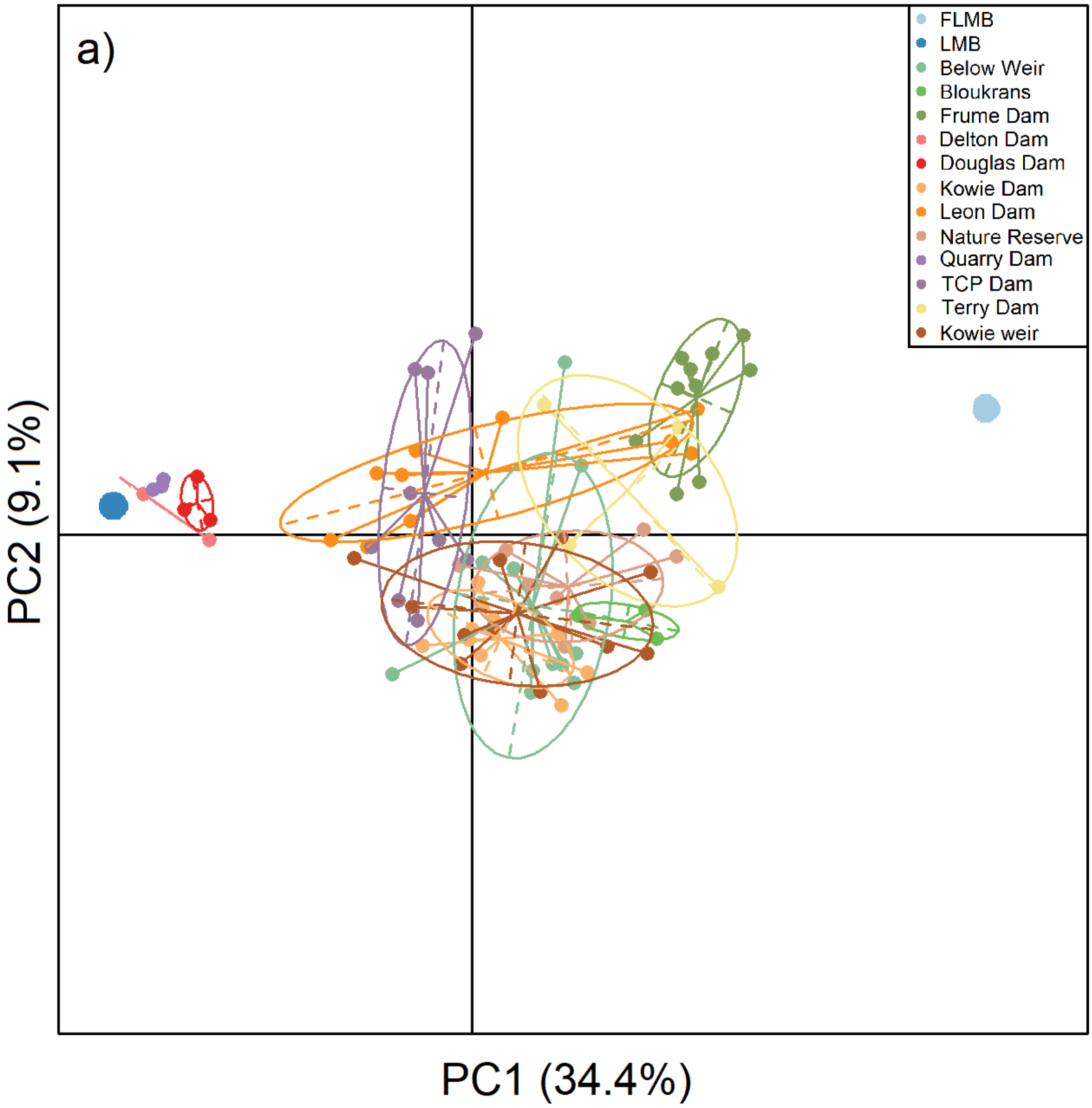

Figure 3.2 Results from the Principal Component Analysis of species-diagnostic SNP markers amplified for Largemouth Bass from the Kowie River catchment to examine patterns of hybridisation with Florida Bass in South Africa

Mean pairwise genetic differentiation (F_{ST}) among all sample sites in the Kowie River (mainstem and tributary sites) was 0.18. Overall levels of genetic differentiation were lower on average and less variable among mainstem sites (F_{ST} = 0.15, S.E. = 0.01) relative to dam populations (F_{ST} = 0.21, S.E. = 0.03; Table 3.2). Hierarchical analysis of population structure performed among river sites revealed the greatest percentage of genetic variation was within individuals (92.4%). Only variance partitions at the population level were statistically significant (Table 3.3).

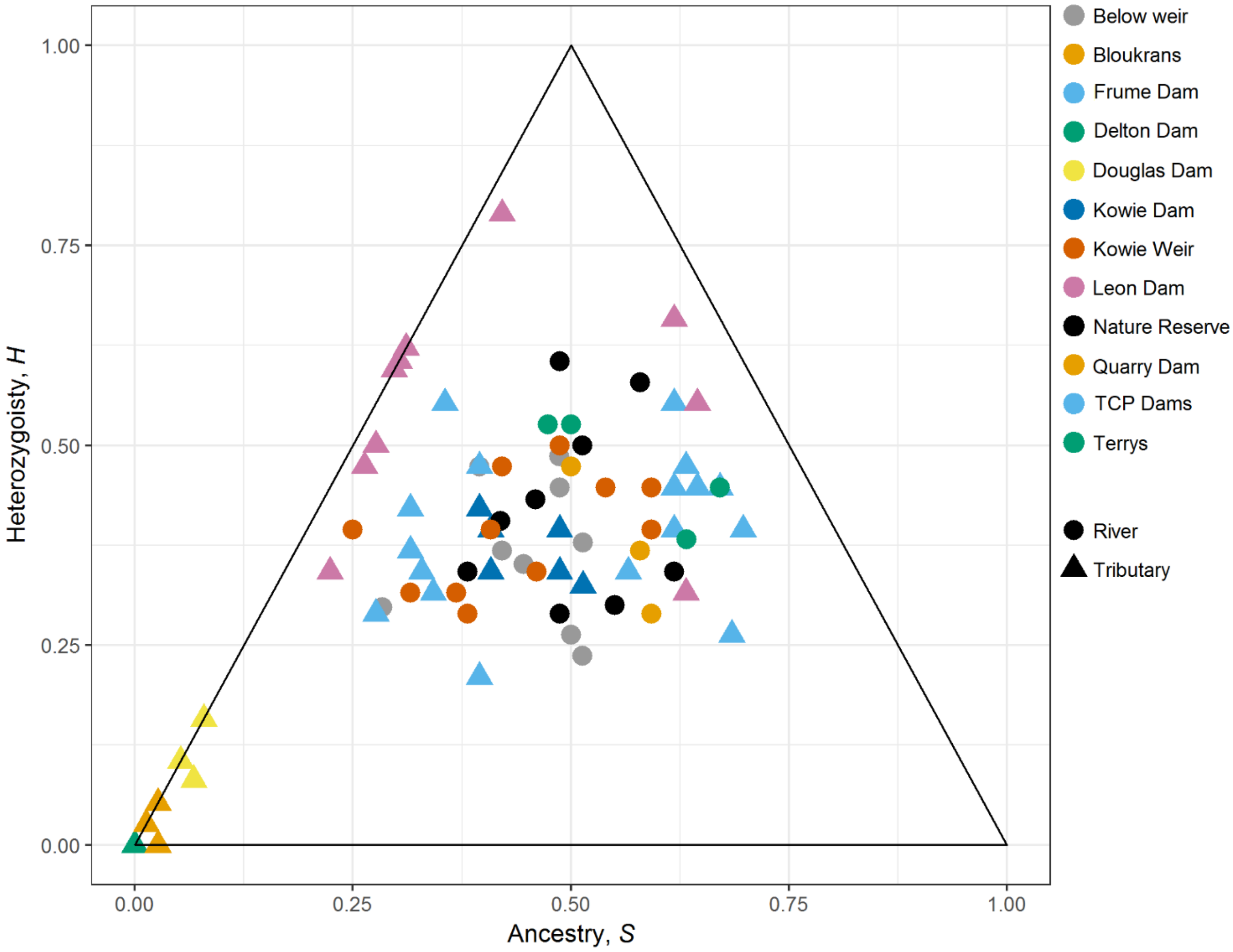

Figure 3.3 Plots of heterozygosity (H) and ancestry (S) of Largemouth Bass sampled from the Kowie River catchment in South Africa. Circles and triangles denote individual fish. Samples with H and S values both equal to 0 correspond to a 'pure' Largemouth Bass; an H value of 0 and S of 1 represents a 'pure' Florida Bass, and an H of 1 and S of 0.5 would be a first generation (F_1) hybrid. Individuals sampled from a tributary system are identified by triangles

Joint evaluation of ancestry and heterozygosity within the Kowie River identified samples from mainstem sample sites as having intermediate values of both Florida Bass ancestry and heterozygosity. The absence of either 'pure' Largemouth Bass or Florida Bass among mainstem sites suggests the river contains a hybrid swarm, in which all individuals are hybrids by varying numbers of generations of backcrossing with parental types and matings among hybrids (Allendorf et al. 2001; Fitzpatrick 2012). Similarly, many dam populations consisted largely of hybrids, with the exception of Delton and Quarry dams which were dominated by Largemouth Bass ancestry and minimal levels of heterozygosity.

Breede River catchment

The mean percentage of Florida Bass alleles measured at the four sample sites lowest in the Breede River catchment was 32.3%, which varied minimally across sites (S.E. = 0.9; Figure 3.4). In contrast, the two upstream reaches contained considerably lower (2.4%; Reach 5) and higher levels (53.6%; Reach 6) of Florida Bass influence (Table 3.2).

The first two principal components in the Breede River catchment explained 39.8% of the observed genetic variance. Samples from reaches 1–4 shared a largely concentric distribution that was intermediate of both reference Largemouth Bass and Florida Bass samples (Figure 3.5). Reach 5 was clustered closely with a reference Largemouth Bass specimen and samples from Reach 6 were more proximate to the Florida Bass reference specimen. The more dispersed nature of samples from Reach 6, relative to other collections, suggested greater variation in hybridisation.

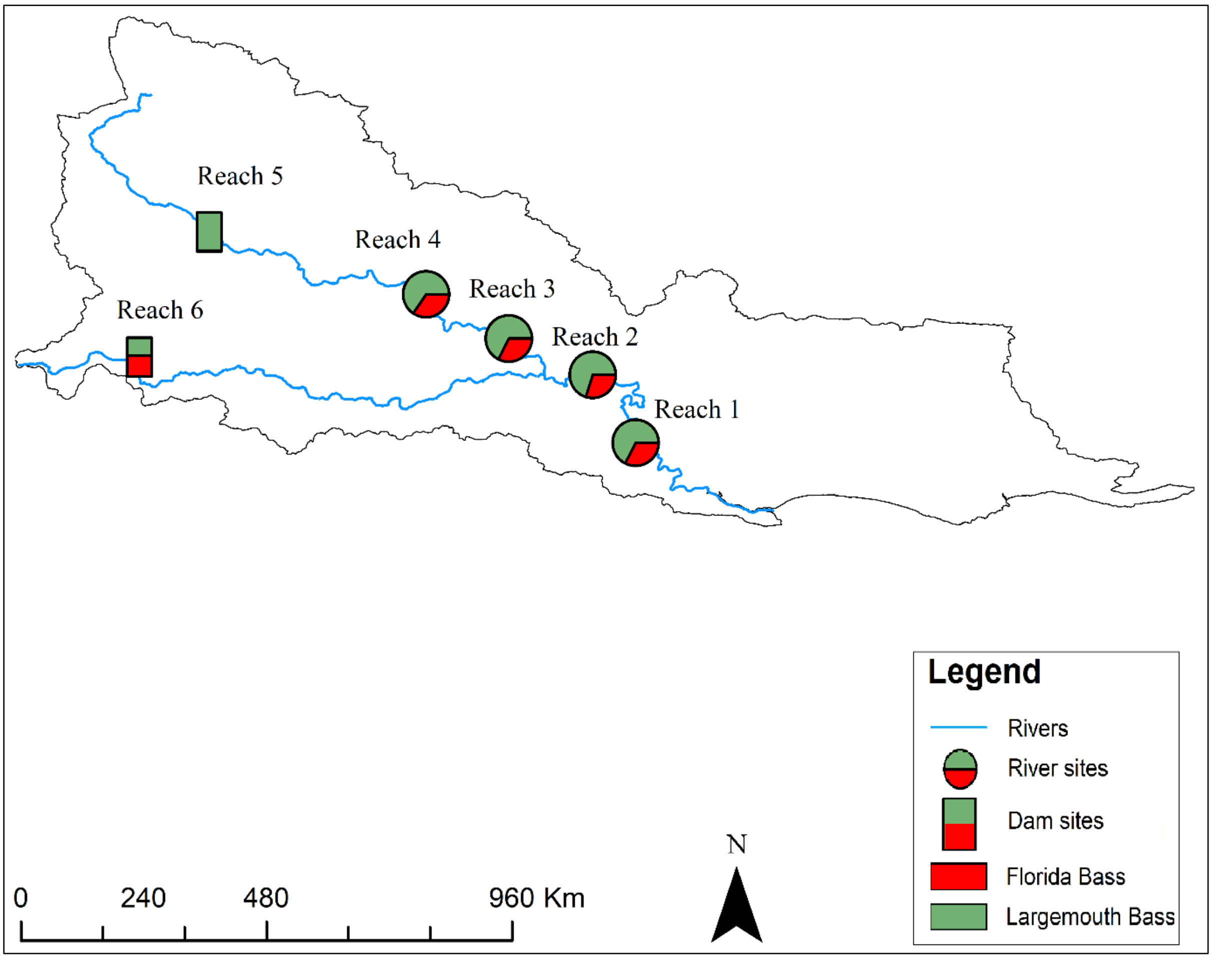

Figure 3.4 The distribution of Largemouth Bass, Florida Bass and their hybrids across the Breede River catchment in South Africa. Circles represent sites sampled in the mainstem of the river, and boxes represent dams. The colours to the box/circle represent the allele percentage contribution by both Largemouth Bass (green) and Florida Bass (red)

Mean pairwise estimates of genetic differentiation across the Breede River catchment were highest for Reach 5 (0.223) followed by Reach 6 (0.158; Table 3.2). In contrast, differentiation was more moderate for Reaches 1–4 (mean = 0.075). Results from hierarchical tests of genetic differentiation identified the largest source of genetic variance was observed within individuals (84.6%). Less variation (11.3%) was explained by organisation at the population level; however, variance partitions at the individual and population level were both statistically significant (Table 3.3).

Joint evaluation of ancestry and observed heterozygosity in the Breede River catchment revealed that, with the exception of samples from Reach 5, all individuals appeared to be Florida Bass × Largemouth Bass hybrids, varying in the number of generations since initial interspecific hybridisation (Figure 3.6). Only samples from Reach 5 exhibited both low levels of heterozygosity and high Largemouth Bass allelic influence.

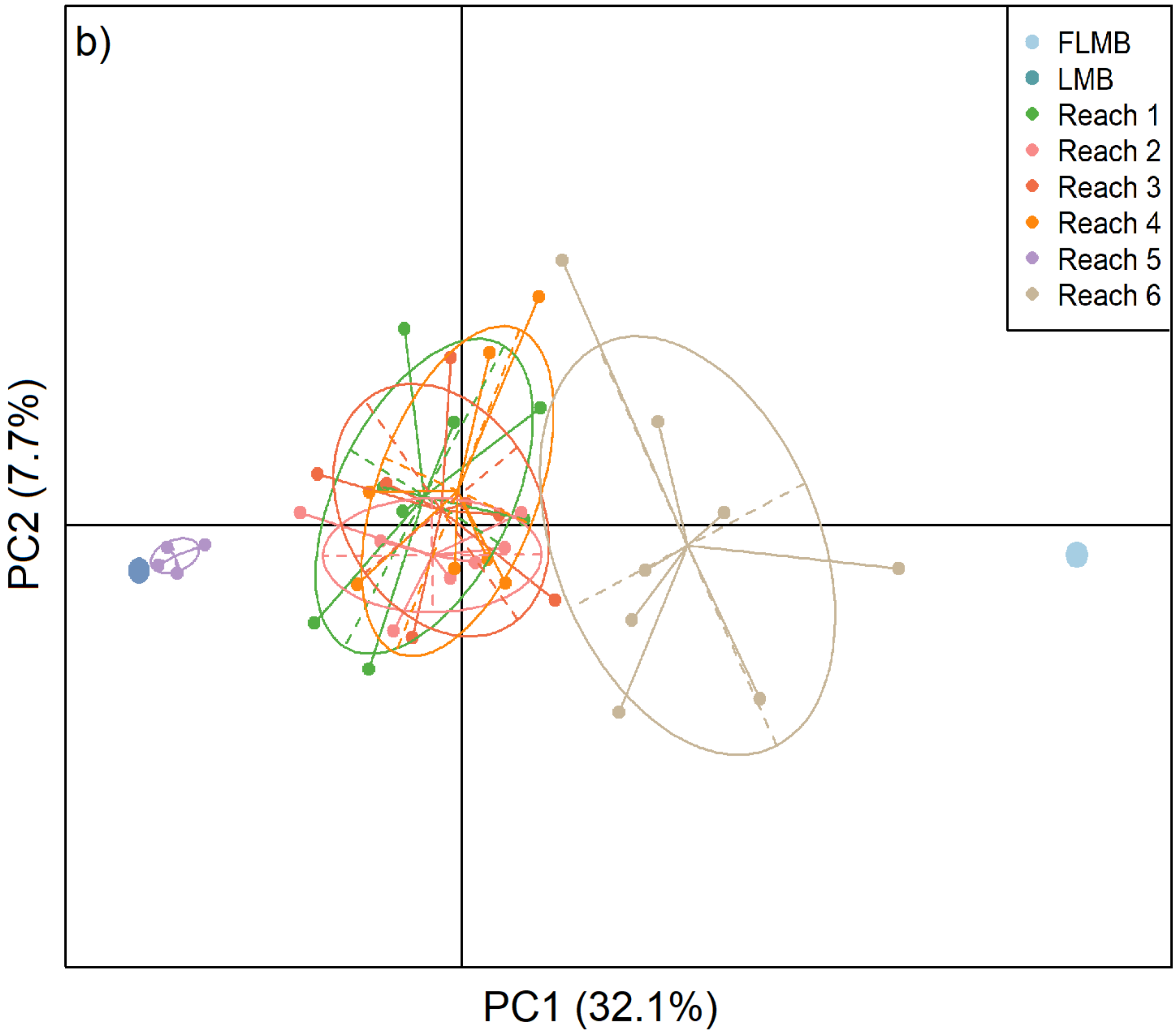

Figure 3.5 Results from the Principal Component Analysis of species-diagnostic SNP markers amplified for Largemouth Bass from the Breede River catchment to examine patterns of hybridisation with Florida Bass in South Africa

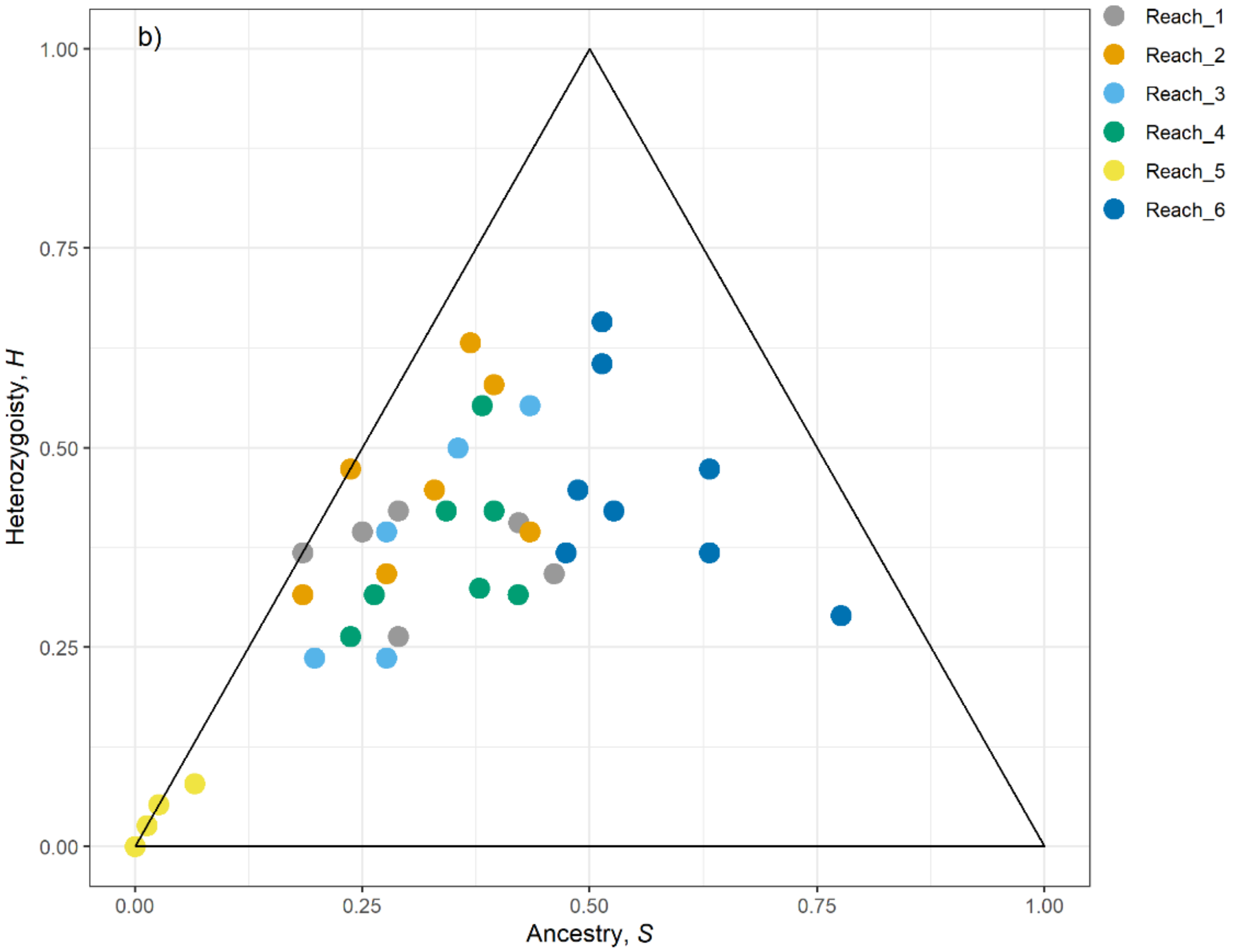

Figure 3.6 Plots of heterozygosity (H) and ancestry (S) of Largemouth Bass sampled from the Breede River catchment in South Africa. Circles denote individual fish. Samples with H and S values both equal to 0 correspond to a ‘pure’ Largemouth Bass; an H value of 0 and S of 1 represents a ‘pure’ Florida Bass, and an H of 1 and S of 0.5 would be a first generation (F_1) hybrid

Table 3.2 The list of catchments, sample sites, and the number of individuals representing either Largemouth Bass (LMB) and Florida Bass (FLB) and the levels of hybridisation across the sampled sites in South Africa. F_{ST} denotes mean value of pairwise fixation index

Catchment	Placement	Population	Latitude (S)	Longitude (E)	N	Mean % LMB	Mean % FLB	% Loci Heterozygous	F_{ST}
Breede River	Mainstem	Reach 1	34°14'13.29"	20°30'39.19"	9	67.5	32.5	37.3	0.070
		Reach 2	34° 4'10.96"	20°24'18.72"	7	70.2	29.8	46.5	0.074
		Reach 3	33°58'49.66"	20°11'56.29"	7	67.5	32.5	42.5	0.077
		Reach 4	33°52'18.42"	19°59'43.36"	7	65.5	34.5	37.3	0.079
		Reach 5	33°45'59.39"	19°27'41.47"	5	97.6	2.4	3.7	0.223
		Reach 6	34° 4'26.65"	19°17'23.86"	8	46.4	53.6	44.7	0.158
Kowie River	Mainstem	Below Weir	33°32'46.15"	26°47'10.83"	11	54.7	45.3	38.7	0.131
		Kowie River	33°32'46.15"	26°47'10.83"	11	56.2	43.8	39.2	0.126
		Nature Reserve	33°27'29.90"	26°41'14.27"	9	50.1	49.9	42.2	0.137
		Terrys	33°25'10.07"	26°39'3.48"	4	43.1	56.9	47.1	0.163
		Bloukrans	33°23'25.32"	26°42'24.19"	3	44.3	55.7	37.7	0.192
	Tributary	Kowie Dam	33°32'8.29"	26°46'50.84"	10	57.8	42.2	38.5	0.139
		Leons	33°30'33.39"	26°43'18.46"	10	60.1	39.9	54.5	0.119
		Deltons	33°23'26.05"	26°45'54.63"	9	100	0.0	0.0	0.329
		Dames Dam	33°19'23.24"	26°35'25.27"	10	36.2	63.8	42.4	0.213
		Douglas	33°19'17.02"	26°31'14.43"	3	93.4	6.6	11.5	0.263
		Quarry	33°19'0.33"	26°30'52.84"	6	98.0	2.0	2.2	0.311
		TCP	33°18'34.17"	26°30'3.11"	9	66.1	33.9	36.8	0.128

Table 3.3 Analysis of molecular variance (AMOVA) performed on Largemouth Bass and Florida Bass samples collected from the Kowie River catchment and Breede River catchment in South Africa. In the Kowie River, comparisons were restricted to samples collected from the mainstem of the river only. SSD = sum of squares, DF = degrees of freedom, italicised *p* values denote significant results controlling for false discovery rate

Catchment	Source of variation	SSD	DF	Estimated Variance	% Variation	*P*-value
Breede River	Among populations	208.83	5	1.89	11.3	*<0.01*
	Among samples within populations	557.12	36	0.69	4.1	0.20
	Within individuals	592.10	42	14.10	84.6	*<0.01*
	Total	1358.05	83	16.7	100	
Kowie River	Among populations	89.67	4	0.52	3.5	*<0.01*
	Among samples within populations	493.26	33	0.62	4.2	0.18
	Within individuals	521.06	38	13.71	92.4	0.09
	Total	1103.99	75	14.85	100	

Discussion

This study represents the first detailed catchment-level evaluation of distributions of Largemouth Bass, Florida Bass and their hybrids in South Africa, using high resolution SNP markers. In both the Breede River catchment and the Kowie River catchment, Largemouth Bass alleles were more prevalent than Florida Bass alleles, and in both catchments, site-specific levels of hybridisation were observed. Specifically, mainstem populations consisted entirely of hybrids and our analysis failed to identify either ‘pure’ Largemouth Bass or ‘pure’ Florida Bass. Further, the extent of hybridisation in both river catchments was influenced by the downstream connectivity between study sites, and individuals that were identified as containing > 97% Largemouth Bass alleles were only sampled from tributaries (dams) that were isolated from hybridised mainstem populations. The data presented here demonstrate that the introduction of Florida Bass in a river catchment containing Largemouth Bass results in hybrid populations, which is largely influenced by connectivity across sites within a catchment, as connectivity facilitates the natural spread of alleles. Further, the presence of hybrid populations in disconnected sites is probably a result of direct stocking (Lamothe et al. 2016; Hargrove et al. 2019a).

Dams have been identified as invasion ‘hubs’ for freshwater invasions to the surrounding interconnected habitats (Johnson et al. 2008), and Black Bass are no exception (Ray et al. 2012; Taylor et al. 2018). Of particular interest in the Breede River catchment was the high prevalence of Florida Bass alleles (53.6%) in Theewaterskloof Dam, while in Quaggaskloof Dam, Florida Bass alleles contributed only 2.4%. Both Theewaterskloof and Quaggaskloof dams are popular Black Bass angling venues (Hargrove et al. 2015). The elevated levels of Florida Bass alleles in Theewaterskloof Dam may be linked to stocking Florida Bass in the dam or into dams in its upstream catchment to enhance the Largemouth Bass fishery (Hargrove et al. 2019b), because the original population was established with Largemouth Bass (Harrison 1936). The dominance by Florida Bass alleles over Largemouth Bass alleles is not unique to Theewaterskloof Dam as Hargrove et al. (2019b) recorded it before in Lake Chicamba in Mozambique. In contrast to the observation in Theewaterskloof Dam, joint evaluation of ancestry and heterozygosity in Quaggaskloof Dam revealed that ‘pure’ Largemouth Bass were present in the dam. For Quaggaskloof Dam, this venue is primarily considered a Smallmouth Bass fishery which may have reduced the motivation for secondary stocking of Florida Bass (M. Bywater, personal communication).

The assessment of genetic composition in the dams across the Kowie River catchment showed significant variation in the prevalence of both Largemouth Bass and Florida Bass alleles. Frume Dam exhibited the highest levels of Florida Bass alleles of any dams sampled in the catchment. This result was expected as Florida Bass were directly introduced into the dam in 1992 from

Somerset East, Eastern Cape (C. Frume, personal communication). The subsequent spread of Florida Bass alleles across the catchment can be linked to the levels of connectivity within the catchment, which may have facilitated their downstream movement (Alp et al. 2012; Tonkin et al. 2018a, b). Of particular interest in the Kowie River catchment is the population in Delton Dam, which contained Largemouth Bass alleles exclusively. The high prevalence of Largemouth Bass alleles in Delton Dam can be associated with its isolation from the mainstem of the Kowie River, or alternatively, may be the lack of secondary introduction into the dam.

Pairwise F_{ST} estimates revealed no significant genetic differences among the populations in the mainstems in either the Breede River catchment or Kowie River catchment. The lack of genetic differences between these populations may indicate a persistent gene flow across the sites in both systems (Allendorf and Lundquist 2003). This assumption is supported by the joint evaluation of ancestry and heterozygosity in the mainstem of both systems which showed that all individuals possessed variable levels of both Largemouth Bass and Florida Bass ancestry, a typical indication of a hybrid swarm (Fitzpatrick 2012). Indeed, stocking permits from CapeNature for the Breede River and the surrounding dams show a continuous stocking of Largemouth Bass into the system without genetic verification, and this may have facilitated the observed genetic composition (M. Jordaan, personal communication).

Conservation implications

Translocation of Black Bass species to enhance freshwater fisheries is a common practice in both native and introduced ranges (Welcomme 1992; Long et al. 2015; Frehse et al. 2019), and often leads to the spread of invasive alleles, resulting in introgressive hybridisation of native genomes (Philipp 1991; Avise et al. 1997; Diedericks et al. 2018; Hargrove et al. 2019a). Introgressive hybridisation is common among Black Bass species. Ray et al. (2012) and

Hargrove et al. (2019a) for example, demonstrated that stocking Florida Bass in central Texas and Tennessee resulted in introgressed populations in both systems. Ultimately, the introduction of invasive alleles in both systems led to a significant shift in the genetic composition of the native Largemouth Bass population. It is postulated that an admixed population may have a significant advantage during the initial stages of colonisation and establishment, facilitated by the emergence of novel genotypes that are absent from the parental population (Rieseberg et al. 1999; Facon et al. 2005, 2006; Sakaguchi et al. 2011; Rius and Darling 2014), and Black Bass are no exception in this regard (Barthel et al. 2010). Recent genetics studies in South Africa have focused on identifying waterbodies that may contain Largemouth Bass, Florida Bass and their hybrids (Hargrove et al. 2017, 2019b; Weyl et al. 2017). From a conservation perspective in South Africa, both Largemouth Bass and Florida Bass are categorised as Category 2 non-native invasive species and are only allowed to be stocked in areas where they already occur (van Wilgen and Wilson 2018).

As Largemouth Bass and Florida Bass are almost indistinguishable morphologically, and readily hybridise when they occur in sympatry (Bailey and Hubbs 1949; Barthel et al. 2010; Weyl et al. 2017), developing independent management strategies for each of the species may prove difficult. As a result, it is advisable that the two species be managed as one in systems where they co-occur in South Africa, while preventing the spread to areas that are not already invaded. Given the current extent of Black Bass distributions and the lack of information regarding Largemouth Bass, Florida Bass, and their hybrids at a national level (Chapter 2), the approach used in this chapter needs to be applied to other river systems to get a better understanding of Black Bass introgression dynamics in South Africa. It is, however, also important that the consequences of the spread of Florida Bass in South Africa are better understood. Elsewhere, Florida Bass introductions have both enhanced and reduced fishery performance because fast growth to larger sizes appears to depend on high temperature climate (see Philipp 1991; Philipp and Whitt 1991; Neal and Noble 2002, 2006; Peterson et al. 2017). As a result, future research into the impact of Florida Bass introductions on fisheries performance in the African context would be interesting. Furthermore, the faster growth, larger size, and longer lifespan of Florida Bass compared to Largemouth Bass infer a potentially higher impact on recipient ecosystems. Research quantifying this impact would be extremely informative for predicting ecosystem responses to the new invader and developing proactive management approaches.

CHAPTER 4

TEMPERATURE REGIME DRIVES DIFFERENTIAL PREDATORY PERFORMANCE IN LARGEMOUTH BASS AND FLORIDA BASS

Abstract

Predation shapes community structures via direct and indirect mechanisms and it is an ecological imperative that predatory impacts are predicted. In this study, comparative functional responses (FRs) were used to compare resource utilisation (i.e. *per capita* effects) between young-of-year Largemouth Bass and Florida Bass across different temperature treatments (18, 24 and 30 °C). *Culex* mosquito larvae were used as prey during the experiments. Across all temperature treatments, Type II FRs were observed, which could cause population-destabilising pressures towards native prey communities due to high predation rates at low prey densities. Attack rates did not differ significantly between the fish species at either temperature. Handling times of Largemouth Bass increased significantly with temperature, while the converse was true for Florida Bass. Largemouth Bass were found to have higher maximum feeding rates at the low temperature (18 °C), driven by significantly shorter handling times compared to Florida Bass. At the intermediate temperature (24 °C), handling times, and thus maximum feeding rates, did not differ significantly between the two species. At high temperature (30 °C), Florida Bass exhibited a significantly higher maximum feeding rate and shorter handling time than Largemouth Bass. These results suggest that temperature drives differential ecological impacts, with Florida Bass exerting higher impact than Largemouth Bass on prey at high temperatures and Largemouth Bass having higher impacts than Florida Bass at lower temperatures.

Introduction

Freshwater ecosystems provide essential services, including the maintenance of ecological structures and provision of ecosystem services (Strayer 2010; Craig et al. 2017). Anthropogenic perturbations such as habitat degradation and species translocation continue to threaten the health and functioning of these ecosystems (Ricciardi et al. 2017; Seebens et al. 2017). One of the major threats to freshwater ecosystems is the introduction of non-native fishes, which contribute to the current global biodiversity crisis (Cucherousset and Olden 2011; Bellard et al. 2016; Venohr et al. 2018). Several studies have documented the negative impacts of non-native fishes on recipient ecosystems (e.g. Takamura 2007; Van Der Walt et al. 2016; Aloo et al. 2017; Ellender et al. 2018). Impacts may range from extirpation of native species (van der Walt et al. 2016; Aloo et al. 2017) to the extinction of congeneric native species through hybridisation (Rhymer and Simberloff 1996). As a result, it is ecologically imperative to predict and quantify the impacts of non-native fishes (Parker et al. 1999), as this will guide management prioritisation and advance the knowledge of the ecological risks of subsequent introductions (Woodford et al. 2017; Zengeya et al. 2017).

Native to North America, centrarchids of the genus *Micropterus* have been introduced globally for the socio-economic benefits derived from sport fishing (Arlinghaus and Cooke 2009; Hargrove et al. 2015; Thompson et al. 2016), but generally without any consideration for the ecological consequences of such actions (Gratwicke and Marshall 2001; Jackson 2002). One such species is the Largemouth Bass, an apex predator capable of altering trophic structures and abundances of aquatic biota (Maezono and Miyashita 2002; Weyl et al. 2010; Bezerra et al. 2019). Largemouth Bass can alter the habitat and foraging behaviour of native species (MacRae and Jackson 2001; Jackson 2002), and in many instances, local extirpation of native species is documented (Gratwicke and Marshall 2001; Jackson 2002; Ellender et al. 2011, 2018). It comes as no surprise that they are now widespread and listed as one of the World's Worst 100 invaders (Lowe et al. 2000; IUCN 2019), with their ecological impacts relatively well documented (e.g. Jackson 2002a; Takamura 2007; Weyl et al. 2010; Bezerra et al. 2019). Hence, predation by Largemouth Bass in systems with high levels of endemicity and imperilment is a cause for concern, because fragmented or localised biota are susceptible to extinction (Fagan et al. 2005; Olden et al. 2006; Ellender et al. 2018).

The Florida Bass, which was previously considered a subspecies of Largemouth Bass (Kassler et al. 2002), has been stocked widely to enhance trophy bass production because of its superior growth performance and larger maximum size relative to Largemouth Bass (Barthel et al. 2010; Weyl et al. 2017). While this species has been introduced widely in regions, including Japan (Takamura 2007) and southern Africa (Weyl et al. 2017; Hargrove et al. 2019b), there is a paucity of studies into their ecological impacts in introduced regions because morphologically, Florida Bass are difficult to distinguish from Largemouth Bass, with which they readily hybridise when they occur in sympatry (Li et al. 2015; Peterson et al. 2017; Hargrove et al. 2019b). This is of concern as the longer lifespan and larger size of Florida Bass (Zolczynski and Davies 1976; Philipp and Whitt 1991; Neal and Noble 2002) may provide a competitive advantage over Largemouth Bass and translate into greater ecological impacts (Maceina et al. 1988; Weyl et al. 2017).

Pure populations of Florida Bass are native to the south of the Suwannee River in the Florida peninsula (Philipp et al. 1983; Barthel et al. 2010), and a natural intergrade zone with Largemouth Bass exists north of the Suwannee River (Bailey and Hubbs 1949; Philipp et al. 1983; Barthel et al. 2010). Conversely, pure populations of Largemouth Bass are primarily restricted to the midwestern and north-eastern United States (Claussen 2015). Florida Bass exhibit superior performance over Largemouth Bass in warmer climates, particularly regarding metabolic and growth rates, while the converse is true for Largemouth Bass in cooler climatic regions (Fields et al. 1987; Philipp and Whitt 1991; Neal and Noble 2002). These discrepancies in performance between the two Black Bass species have been attributed mainly to the thermal environmental history that they encounter naturally within their native ranges (Fields et al. 1987; Neal and Noble 2002).

In fishes, temperature directly influences the distribution (Bae et al. 2018; Khosa et al. 2019), spawning time (Clugston 1964), growth rate (Strawn 1961; Niimi and Beamish 1974), and resource utilisation (Niimi and Beamish 1974; Zweifel et al. 1999). Responses to temperature will also vary across different life history stages (Coutant 1975), and no single temperature can be viewed as optimal; instead, it must be viewed in the context of the life stage or the function that the fish is trying to perform (Kieffer and Cooke 2009). For example, Strawn (1961) showed that feeding rates by Largemouth Bass fry increase substantially at temperatures between 18 °C and 27.5 °C; however, little to no feeding occurred at temperatures below 15 °C. As such, changes in temperature due to climate change may alter these life history strategies which may, in turn, heighten or moderate ecological impacts (Ficke et al. 2007; Pörtner and Knust 2007; Rahel and Olden 2008). One of these impacts is increased predation pressure on native biota as a result of increased metabolic rates at optimum temperatures (Brown et al. 2004). This heightened metabolic demand can exacerbate species *per capita* effects, culminating in the decline of native biota (Legler et al. 2010; Hammock and Johnson 2014; Metcalfe et al. 2016). Consequently, *per capita* effects can be quantified to infer ecological impacts (Parker et al. 1999), and ecological impacts are expected to be higher if the environmental temperatures match species' physiological optima. The converse is also true when the environmental temperatures are above or below optimum (Iacarella et al. 2015).

This study seeks to compare *per capita* effects of young-of-year Largemouth Bass and Florida Bass at different temperatures using comparative FRs. This is important because young-of-year Black Bass prey on plankton and macroinvertebrates before an ontogenic shift to piscivory (Olson 1996; Ludsin and DeVries 1997; García-Berthou 2002; Taylor et al. 2019c), and studies have shown that their predation may have an important top-down effect on lower trophic levels (Maezono and Miyashita 2002; Maezono et al. 2005). These changes can have a cascading effect on trophic interactions through competitive exclusions and alterations to the behaviour of the most susceptible taxa (Maezono and Miyashita 2002; Jackson et al. 2017). It was hypothesised that (1) an increase in temperature will increase the *per capita* effects of both Largemouth Bass and Florida Bass due to enhanced metabolic rates, (2) the warmwater-adapted Florida Bass is expected to exhibit a significantly higher *per capita* effect at the highest temperature treatment, while at lower temperature treatment, Largemouth Bass will have greater feeding rates than Florida Bass.

Materials and methods

Organism collection and maintenance

Young-of-year Largemouth Bass (TL = 35.0 ± 1.0 mm) were collected from an impoundment on the Kariega River system (33°25'11.01"S; 26°29'59.48"E) in November 2017, and Florida Bass (TL = 36.0 ± 2.0 mm) were collected from a small private impoundment on the Kei River (32°39'38.11"S; 27°19'0.12"E) in February 2018, using a seine net (5.0 × 2.0 m long and 1.5 m deep with a 5.0 mm mesh size) (Figure 4.1). It was not possible to collect the desired fish size class (TL = 35.0 mm) of both species at the same time due to differences in spawning seasons (Clugston 1964; Rogers and Allen 2009). Consequently, FR experiments were conducted in November 2017 and February 2018 for Largemouth Bass and Florida Bass, respectively. Single Nucleotide Polymorphism analysis by Hargrove et al. (2019) confirmed population at the impoundment in the Kariega River system to contain > 95% of Largemouth Bass alleles. The population from the Kei River were identified as intergrades containing Florida Bass alleles in excess of > 85% (Khosa unpublished data); for identification purposes, the intergrade populations hereafter will be referred as Florida Bass. After collection, fish were housed in 40 L holding tanks in a temperature-controlled laboratory (25.0 ± 0.2 °C) and under a 14:10 light:dark regime at the SAIAB, Makhanda, South Africa. The holding tanks were fitted with an air supply and filtration system. Prior to the FR experiments, fish were maintained on a diet of larval chironomids supplied by Rainbow Pet Suppliers in Port Elizabeth, South Africa, and were acclimated for seven days in the laboratory. *Culex* mosquito larvae (5.0 ± 1.0 mm) were used as prey for the experiments and were acclimated to the same temperatures as the predators over 24 hours before experimentation.

Figure 4.1 Collecting Florida Bass from a small private impoundment on the Kei River in February 2018 using a seine net

Experimental setup

Experiments were conducted in 20 L cylindrical buckets (width 290 mm × height 400 mm) filled with 6 L of rain water collected from a closed re-circulating system at the Department of Ichthyology and Fisheries Science (DIFS), Rhodes University, Makhanda. Largemouth Bass (*n* = 54) and Florida Bass (*n* = 54) were used for the FR experiments. Individual fish were randomly selected from 24 (i.e. 6 prey densities × 3 replicates + fish-free 6 controls) in the holding tank experimental arenas 24 hours prior to use, and were held without food to allow for standardisation of hunger levels (i.e. 18 fish per temperature treatment). The initial seven days acclimation period to the laboratory conditions was followed by adjusting the temperature to the desired level at a rate of 1.0 °C per day from ambient (25.0 °C), and then held for one day at the required experimental temperature; this was completed simultaneously for both predator and prey species as per South and Dick (2017). Experiments were conducted across three temperature treatments (i.e. 18.0 ± 0.2 °C, 24.0 ± 0.2 °C and 30.0 ± 0.3 °C) which were run independently after fish acclimated to the desired temperature treatment. Larval mosquito prey were supplied at six densities (2, 4, 8, 16, 32 and 64), with three replicates per density across all temperature treatments. Once prey were added, predators were allowed to feed for two hours, and the number of live prey remaining were recorded at the end of this time, following removal of the fishes. Controls were three replicates of each prey density across all temperature treatments in the absence of predators to determine background mortality. No fish was reused in experiments.

Statistical analysis

All statistical analyses were conducted in R v3.5.1 (R Core Team 2018). The R package 'frair' (Pritchard et al. 2017) was used to model the FR type for both Largemouth Bass and Florida Bass using logistic regression that tests for the relationship between the proportion of prey consumed and prey density. Rogers' random predator equation (Rogers 1972) was used to model Type II responses, as prey were not replaced after consumption:

$$N_e = N_0(1 - \exp(a(N_e h - T))) \qquad (1)$$

where N_e is the number of prey eaten, N_0 is the initial prey density, a is the attack rate, h is the handling time and $1/h$ is the maximum feeding estimate. Here, I do not consider the parameters mechanistically, and rather apply them phenomenologically for comparative purposes (Jeschke et al. 2002; Alexander et al. 2012). The data were also non-parametrically bootstrapped (n = 2000) to construct 95% confidence intervals of the fitted FR curves for each treatment. To test between differences in attack and handling time parameters within species across temperature treatments, and between species across temperature treatments, I used the ‘difference method’ outlined in Juliano (2001) via *frair compare*(). Maximum feeding estimates (*1/h*) were calculated from the handling time estimates: if the handling times are significantly different then I can, reciprocally, infer significant difference in maximum feeding estimates.

Results

Prey survival in control treatments was greater than 99% across all temperatures, so I did not adjust feeding rates to account for natural background mortality. Type II FRs were found across all temperature treatments, as indicated by significantly negative first order terms for both predators (Table 4.1; Figure 4.2a-c).

Temperature effects on functional response parameters within species

There was no significant difference in attack rates of Largemouth Bass between 18.0 °C and 24.0 °C ($z = 1.52$, $p = 0.12$; Table 4.1, Figure 4.2), 18.0 °C and 30.0 °C ($z = 1.32$, $p = 0.18$; Table 4.1, Figure 4.2) or 24.0 °C and 30.0 °C ($z = 0.79$, $p = 0.42$; Table 4.1, Figure 4.2). However, handling times were significantly shorter at 18.0 °C compared to 24.0 °C ($z = 3.36$, $p < 0.001$; Table 4.1, Figures 4.2 and 4.3) and 30.0 °C ($z = 3.67$, $p < 0.001$; Table 4.1, Figures 4.2 and 4.3). There was no significant difference in handling time at 24.0 °C compared to 30.0 °C ($z = 0.31$, $p = 0.75$; Table 4.1, Figure 4.2). Maximum feeding rates of Largemouth Bass decreased significantly with increasing temperatures (Table 4.1, Figures 4.2–4.4).

Table 4.1 Logistic regression first order terms to denote functional response Type across all temperature treatments between fish species with associated *p* values. Attack rate *a*, handling time *h*, and maximum feeding rate (1/*h*) estimates generated from Rogers' random predator equation, alongside *p* values.

Species	Temperature (°C)	First order term, *p*	*a* (± SE)	*p*	*h* (± SE)	*p*	1/*h*
Largemouth Bass	18	-0.06, < 0.001	11.24 (± 4.97)	0.024	0.03 (± 0.00)	< 0.001	29.1
Florida Bass	18	-0.05, < 0.001	3.65 (± 0.76)	<0.001	0.05 (± 0.01)	< 0.001	16.9
Largemouth Bass	24	-0.04, < 0.001	3.61 (± 0.65)	<0.001	0.05 (± 0.00)	< 0.001	19.1
Florida Bass	24	-0.03, < 0.001	4.83 (± 1.34)	<0.001	0.04 (± 0.00)	< 0.001	23.3
Largemouth Bass	30	-0.04, < 0.001	4.51 (± 0.92)	<0.001	0.05 (± 0.00)	0.01	18.4
Florida Bass	30	-0.08, < 0.001	13.57 (± 6.08)	0.025	0.02 (± 0.00)	< 0.001	37.8

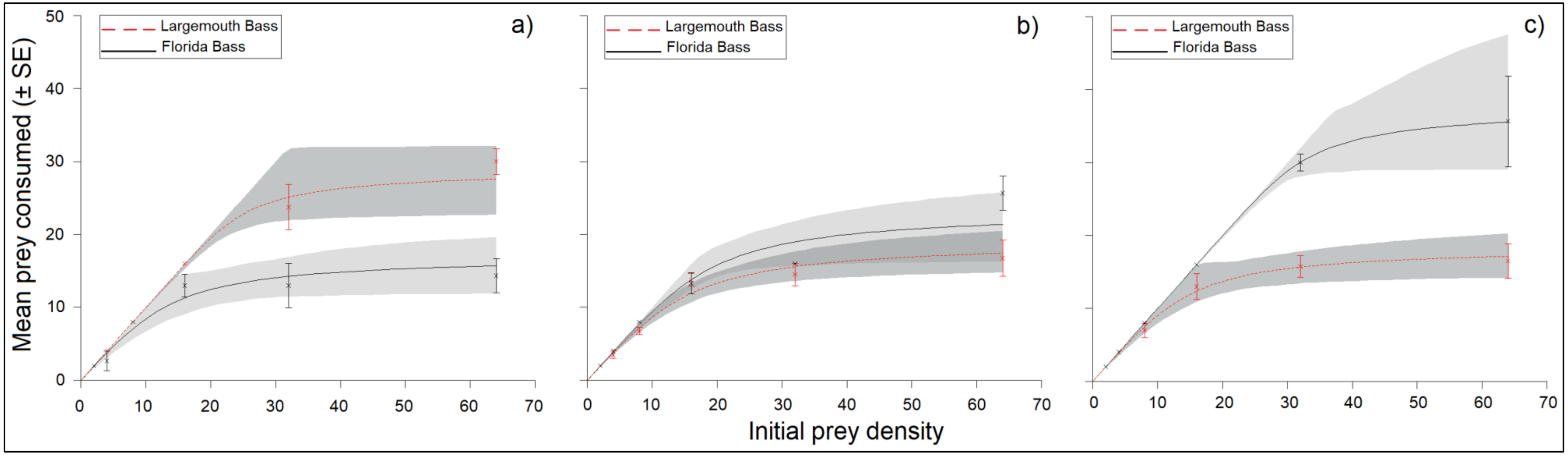

Figure 4.2 Functional responses of Largemouth Bass and Florida Bass across three temperature treatments a) 18 °C, b) 24 °C and c) 30 °C (modelled using the Rogers' random predator equation for a Type II functional response). Points are mean ± SE numbers of prey consumed at each prey density. Shaded areas are bootstrapped 95% confidence intervals

For Florida Bass, attack rates did not differ significantly between 18.0 °C and 24.0 °C ($z = 0.75$, $p = 0.44$; Table 4.1, Figure 4.2), 18.0 °C and 30.0 °C ($z = 1.62$, $p = 0.10$; Table 4.1, Figure 4.2) or 24.0 °C and 30.0 °C ($z = 1.4$, $p = 0.16$; Table 4.1, Figure 4.2). There were significantly shorter handling times at 30.0 °C than at 18.0 °C ($z = 5.11$, $p < 0.001$) and at 24.0 °C ($z = 3.08$, $p = 0.01$; Table 4.1, Figures 4.2 and 4.3) and at 24.0 °C than at 18.0 °C ($z = 2.14$, $p = 0.03$; Table 4.1, Figure 4.2). This shorter handling times resulted in significant increases in maximum feeding rates for Florida Bass at increasing temperatures (Table 4.1, Figure 4.2 and 4.3).

Temperature effects on functional response parameters between species

There were no significant differences in attack rate between Largemouth Bass and Florida Bass at 18.0 °C ($z = 1.50$, $p = 0.13$; Table 4.1, Figure 4.2a), but Largemouth Bass showed a significantly shorter handling time at 18.0 °C than Florida Bass ($z = 3.83$, $p < 0.001$; Table 4.1, Figure 4.2a) resulting in higher maximum feeding rates for Largemouth Bass compared to Florida Bass at 18.0 °C (Figure 4.2a).

At 24.0 °C, Largemouth Bass and Florida Bass FRs were similar with no significant differences with regard to attack rate ($z = 0.81$, $p = 0.41$; Table 4.1, Figure 4.2b) and handling time ($z = 1.43$, $p = 0.15$; Table 4.1, Figure 4.2b); similar maximum feeding rates between the two species were evident (Table 4.1, Figure 4.2b).

At 30.0 °C, Largemouth Bass and Florida Bass had similar attack rates ($z = 1.57$, $p = 0.11$; Table 4.1, Figure 4.2c), but the overall maximum feeding rate of Florida Bass was significantly higher, driven by a significantly shorter handling time compared to Largemouth Bass ($z = 5.41$, $p < 0.001$; Table 4.1, Figure 4.2c).

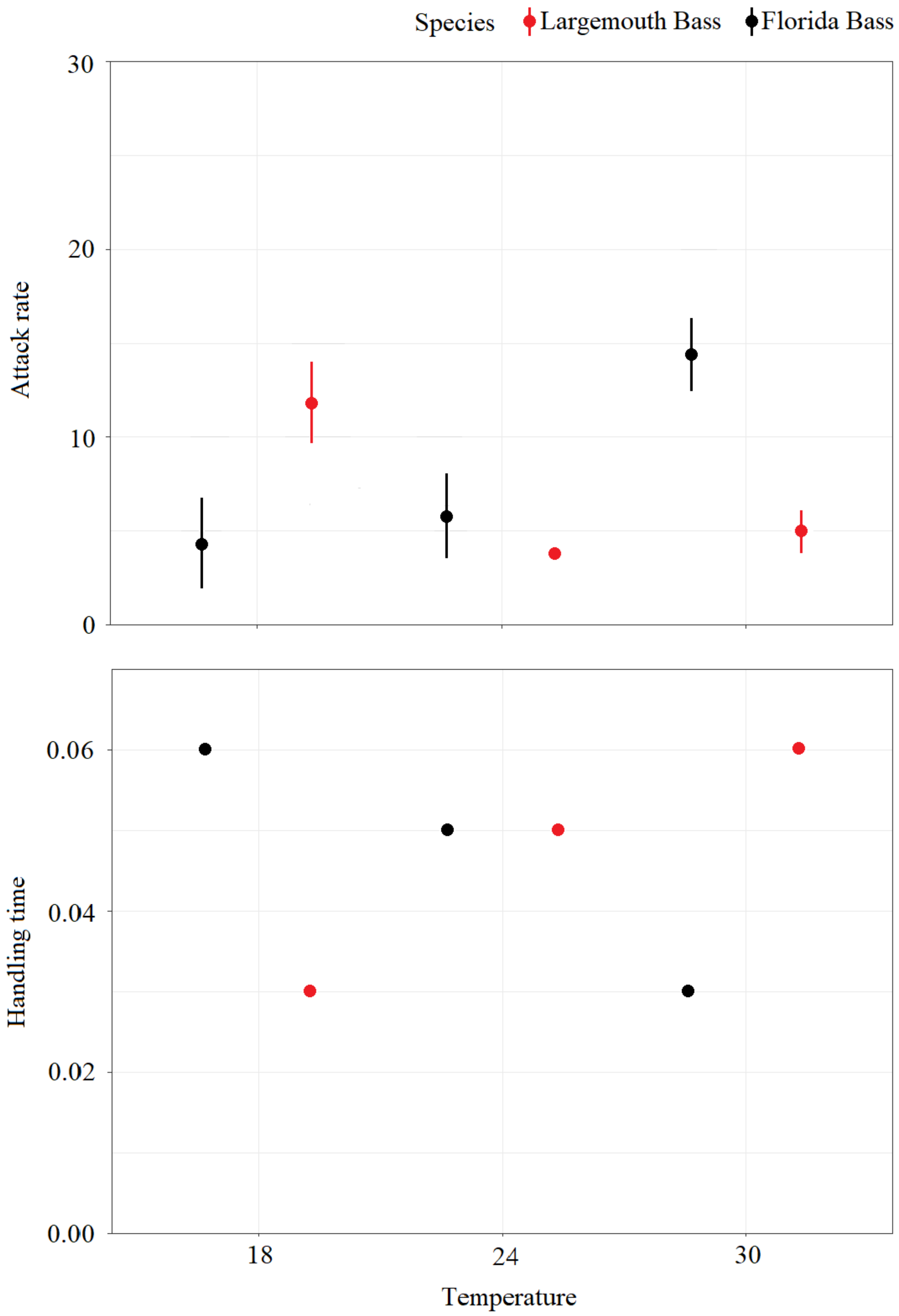

Figure 4.3 Parameter estimates for attack rates and handling times for both Largemouth Bass and Florida Bass on *Culex* mosquito larvae at 18 °C, 24 °C and 30 °C. Points are original MLE values and error bars are bootstrapped 95% confidence intervals

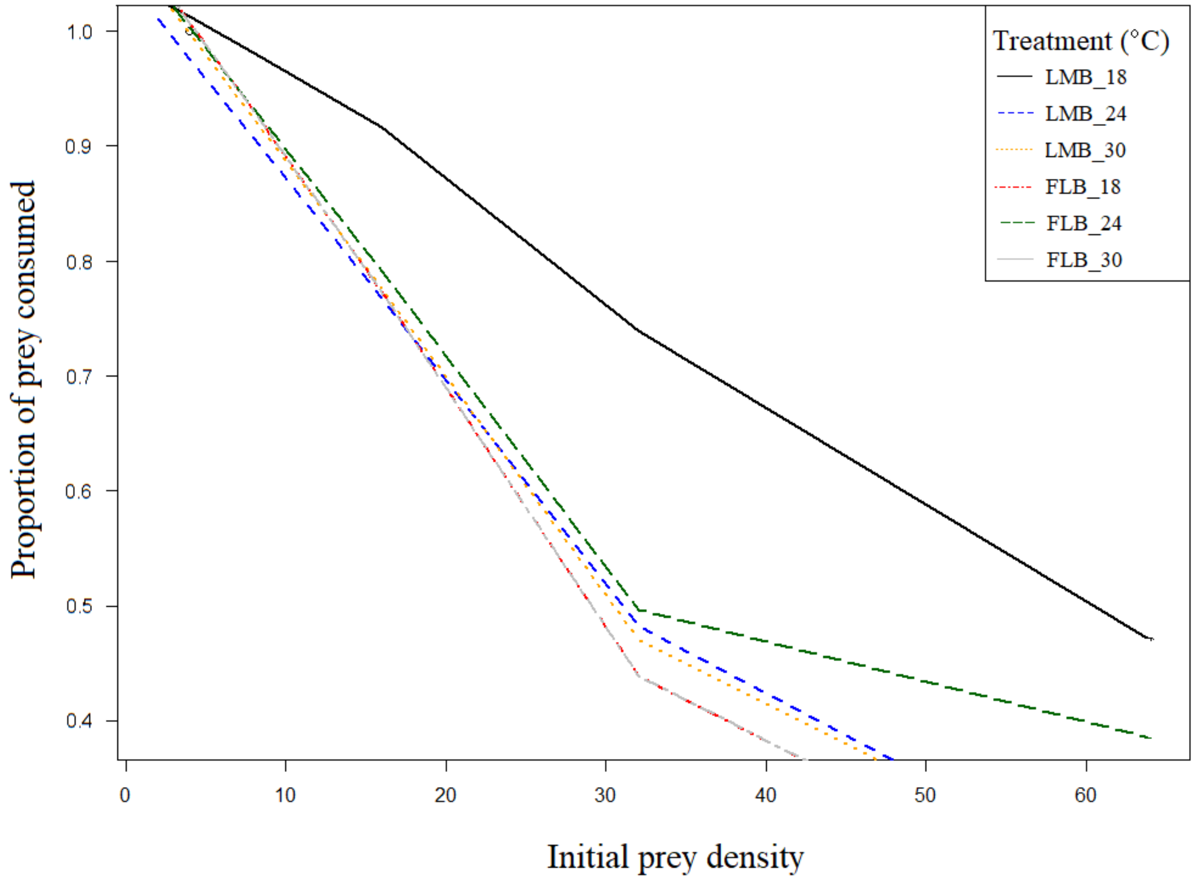

Figure 4.4 LOWESS lines fitted to the proportions of prey consumed by Largemouth Bass (LMB) and Florida Bass (FLB) across the three temperature treatments, 18 °C, 24 °C and 30 °C

Discussion

Predicting ecological impacts of non-native species is context dependent and requires the incorporation of abiotic and biotic factors that alter interaction strength (Parker et al. 1999; Ricciardi et al. 2013). Results from this study demonstrated temperature-dependent differences in the FRs of two morphologically-similar Black Bass species, with *per capita* effects being highest at a lower temperature for Largemouth Bass and highest for Florida Bass at higher temperatures. Further, the abiotic dependency of temperature manifests differently on certain FR parameters (handling time and maximum feeding estimates) of the two Black Bass species. This study thus reflects results outlined in the meta-analysis by Iacarella et al. (2015), who assessed impact within the context of the environmental matching hypothesis. They identify habitat temperature as one of the key drivers of ecological impacts, by showing that performance of non-native species will be optimal if the habitat temperature closely matches their physiological optima.

Many predatory fishes exhibit Type II FRs in the laboratory; however, it is likely that there is both frequency-dependent predation (e.g. prey switching) and habitat-mediated effects *in situ* that allow rare resources to persist by alleviating predation pressure (Murdoch 2014; Cuthbert et al. 2018 a,b). Nevertheless, higher *per capita* effects at low resource densities is common among Black Bass species and represents higher predation pressure on native biota (Alexander et al. 2014). Indeed, such effects have been shown to be independent of differences in habitat structure (Alexander et al. 2015). In this study, both Black Bass species displayed prey population-destabilising Type II FRs across all temperature treatments. The attack rate of predators is a useful measure for comparing predatory efficiency at lower resource densities (Alexander et al. 2014). In this study, the attack rates between Largemouth Bass and Florida Bass were similar across all temperatures, which suggest that differences in *per capita* effects were driven by handling time. The handling time parameter in FRs reflects the time spent pursuing the prey, the efficiency in processing the prey after capture, and the time spent digesting the prey (Jeschke et al. 2002); the lower the handling time, the higher the maximum feeding estimates (i.e. FR asymptote). In the case of Largemouth Bass, the increase in temperature resulted in longer handling time. This was not expected because, in other species, increases in temperature have been reported to enhance the metabolic rate, such that more resources can be assimilated over a given time (Brown et al. 2004; Bevelhimer and Breck 2009). As such, results from this study stand in contrast to other studies that suggest heightened *per capita* effects with increasing temperatures (Brown et al. 2004; Dell et al. 2014), but is similar to Niimi and Beamish (1974) who showed that Largemouth Bass held at 18 °C maintained significantly higher feeding rates relative to the ones held at 25 °C and 30 °C. Further, a bioenergetics model by Zweifel et al. (1999) showed a significant increase in maximum consumption by Largemouth Bass from 18 °C, peaking at 22 °C and then decreasing significantly between 22 °C and 30 °C. These results suggest that, at elevated temperatures, Largemouth Bass individuals might be experiencing thermal stress, hence the decrease in performance (Fields et al. 1987; Philipp and Whitt 1991).

The Florida Bass *per capita* effect was, by contrast, positively correlated with increasing temperatures. In the context of metabolic theory of ecology (Brown et al. 2004), this was expected as increases in temperature enhance the metabolic rate, which may result in increased *per capita* effects (Brown et al. 2004; Bevelhimer and Breck 2009). This finding is further supported by the higher maximum feeding estimates for Florida Bass at higher temperatures than at lower temperatures. Further, these findings corroborate the published literature on thermal growth optima for Florida Bass, which ranges between 24 °C and 30 °C (Strawn 1961; Stuber et al. 1982). These experimental results further support observations that, at lower temperatures, Florida Bass exhibit a slower growth rate and higher over-winter mortalities (Carmichael et al. 1988; Philipp and Whitt 1991). Therefore, these findings are relevant from both a fisheries and conservation perspective. From a fisheries perspective, lower FRs of Florida Bass at lower temperatures infer that cooler climates will incur a larger ecological impact from Largemouth Bass than from Florida Bass, whereas the converse is true at higher temperatures, perhaps leading to increased range expansion and persistence of Florida Bass in systems vulnerable to warming. Although *per capita* predatory effects quantified using comparative FRs can be useful for predicting and comparing ecological impacts under context dependencies (Dick et al. 2014), population-level responses of species to changes in resource availability (i.e. numerical response; Holling 1959) are also critical to consider. Therefore, to decipher whether differential *per capita* effects between Largemouth Bass and Florida Bass persist at the population level, information such as predator abundances may be incorporated in future into the comparative FR approach to enhance predictions of ecological impact (Dick et al. 2017b; Mofu et al. 2019). Further, species *per capita* effects may vary depending on their life stage (i.e. young-of-year, juvenile, and adult stages), as often resource utilisation is proportional to predator size (Jeschke et al. 2002, 2004), and also depends on prey availability and abundance (Olson 1996; Taylor et al. 2019c). While the present study only contrasted small-sized individuals, it effectively highlights differences between the two Black Bass species that may well persist across different size classes.

Florida Bass are introduced by anglers to enhance trophy bass populations (Barthel et al. 2010), a practice that may be counterproductive in regions where temperatures do not exceed 24 °C. However, in the subtropical and warm temperate regions of South Africa (Dallas 2008), indications are that performance and ecological impact will be higher for Florida Bass as the environmental temperature closely matches their native range. In addition, these results suggest that increased mean water temperature under projected climate change scenarios (Schulze 2011) is likely to favour Florida Bass as increased feeding rates are likely to increase growth rates and increase survival (Hunt 1960; Clugston 1964; Stuber et al. 1982). It is therefore likely that the introduction, and subsequent introgression, of Florida Bass into existing Largemouth Bass populations will increase invasion success and impact.

CHAPTER 5

HABITAT STRUCTURE DIFFERENTIALLY MITIGATES PREDATION IMPACT OF JUVENILE LARGEMOUTH BASS AND FLORIDA BASS

Abstract

Habitat complexity plays an important role in structuring species diversity, behaviour and distribution. Further, habitat complexity can mediate the strength of predator-prey interactions through the provision of prey refugia or through enhancing ambush predation. In this study, I use functional responses (FRs) to compare the effect of habitat complexity on resource utilisation between the morphologically Largemouth Bass and Florida Bass predating upon Mozambique Tilapia *Oreochromis mossambicus* fry. Treatments for this study were zero and high habitat complexities, with the latter being artificially constructed using plastic strips attached to a plastic mesh base. Both predator species exhibited a Type II FR in both habitat complexities, with Florida Bass exhibiting significantly higher FR magnitudes compared to Largemouth Bass under both habitat complexity scenarios. Both species experienced dampening of FR magnitudes under high habitat complexity treatments. Florida Bass had persistently lower handling parameters than Largemouth Bass regardless of habitat complexity. While higher habitat complexity may offer prey refugia from Florida Bass, changes in complexity had a negligible effect upon Largemouth Bass predatory efficiency. Measures to enhance natural complexity through system restoration may be a useful conservation measure for endemic fish in rivers invaded by Florida Bass, however, the same mitigation measures may not be as effective for Largemouth Bass.

Introduction

Black Bass (a collective term for members of the genus *Micropterus*) are native to North America and are the focus of a multi-billion dollar sport fishery (Chen et al. 2003; Long et al. 2015). Due to their popularity with recreational anglers they have been introduced to continents outside North America (Pereira and Vitule 2019). Where such introductions have occurred there have been major impacts on native communities due to high predation rates (e.g. Godinho and Ferreira 2000; van der Walt et al. 2016). In the Raia stream in Portugal, for example, Godinho and Ferreira (2000) found that the presence of Largemouth Bass was the best biotic predictor of native species assemblages, with the endemic smaller-sized cyprinids absent in areas where Largemouth Bass were present. Similar results are documented in the Olifants-Doring River system in South Africa where van der Walt et al. (2016) reported the complete absence of native minnows in areas where Black Bass were present.

In South Africa, Largemouth Bass were introduced in 1928 for recreational angling purposes (Ellender and Weyl 2014). In 1980, Florida Bass were then introduced to enhance Largemouth Bass fisheries because they attain larger sizes (Weyl et al. 2017). The two species are difficult to separate morphologically and hybridise when they occur in sympatry (e.g. Warren 2009; Hargrove et al. 2019), as such, they were considered a single species prior to the morphological and genetic reassessments by Kassler et al. (2002) and Near et al. (2003, 2004). Recent genetic research on the spread of Florida Bass in southern Africa suggests that this species is increasing its range and that there is widespread introgression with Largemouth Bass (Weyl et al. 2017; Hargrove et al. 2019). This may have conservation implications because Florida Bass grow faster and live longer than Largemouth Bass (Neal and Noble 2002) and are, therefore, likely to consume more prey than Largemouth Bass (Austin et al. 2012). While this suggests that inferring impacts of Florida Bass from studies on Largemouth Bass may lead to erroneous conclusions, much of the field research documenting predatory impacts of "Largemouth Bass" (see review by Pereira and Vitule 2019) have not distinguished between the two species.

Determining impact is a key component of invasive species assessment and mitigation. A method used extensively to assess relative *per capita* impact is the comparative functional response (FR) approach (Dick et al. 2013), whereupon it is possible to quantify resource consumption (i.e. *per capita* effect) in relation to resource density (Holling 1959). Classically, the FR of a predator can take three forms. The linear Type I FR, in which resource utilisation increases proportional to resource density, is predominantly found in filter feeders as they are not limited by handling time (Jeschke et al. 2004). Type II FRs are inversely density-dependent, characterised by decelerating resource utilisation but with high *per capita* mortality at low resource densities (Alexander et al. 2014; Dunn and Hovel 2020). Type III FRs are sigmoidal in shape, whereby low resource utilisation at low prey densities is observed (Holling 1959; South et al. 2018), thus leading to low prey density refugia with prey population stabilisation implications. The form of FR of a predatory species can, however, be context dependent varying with environmental conditions within a habitat (Iacarella et al. 2015; South et al. 2017). For example, Barrios-O'Neill et al. (2015) demonstrated that an increase in "predator-free" space may have a dampening effect on the FR magnitude of a predator, and oftentimes result in a switch of FR type from a destabilising Type II to a more stabilising Type III FR. The FR can be parsed into three parameters: attack parameter [a], which describes the encounter rate; the handling parameter [h], which is an amalgamation of various processes involving the subduing, processing and digestion of prey (Jeschke et al. 2004; Sentis et al. 2013); and the maximum feeding estimate [$1/h$], which is a function of the handling parameter and the total experimental time (Dick et al. 2017).

Demonstrating the utility of the comparative FR approach, the predatory impacts of Largemouth Bass were tested experimentally by Alexander et al. (2014, 2015). Largemouth Bass had higher *per capita* effect when compared to a functionally analogous native predatory fish (Alexander et al. 2014) and increased habitat complexity mitigated the per capita effects of Largemouth Bass by dampening the maximum feeding estimates via increased handling time (Alexander et al. 2015). In a first attempt to better understand interspecific differences between Largemouth Bass and Florida Bass, in Chapter 4 I used FR to demonstrate that *per capita* effect of young-of-year Largemouth Bass and Florida Bass was temperature dependent and varied in magnitude with species. This further drives the incentive to characterise the other context dependencies of the two Black Bass species in order to infer their ecological impact.

Habitat complexity (i.e. density and diversity of structural elements in a habitat) drives patterns in species diversity, distributions and behaviour (Anderson 1984; Jackson et al. 2001; Shoup and Broderius 2018). In freshwater systems natural habitat structure is provided mainly by woody debris, macrophytes and macroalgae (Everett and Ruiz 2013). Complex habitats can support increased biodiversity in fish and invertebrate communities (Almany 2004b, a; Montoya-Ospina et al. 2020). This is because increasingly complex habitats mediate the strength of predator-prey dynamics (Barrios-O'Neill et al. 2015; Wasserman et al. 2016b; Belgrad and Griffen 2017), through enhancing recruitment and forage capacity (Savino and Stein 1982; Taylor et al. 2019b) and providing refuge for prey communities by reducing encounters rates with predators(Yeager and Hovel 2017; Shoup and Broderius 2018).

Decreased predation risk in more complex habitats is linked to increased refuge availability for prey to evade predators (Alexander et al. 2015; Barrios-O'Neill et al. 2015; Henseler et al. 2020). In small lakes (≤50 ha) in central Ontario, the presence of Smallmouth Bass drives small cyprinids to predominantly utilise complex habitat whereas, in lakes without Smallmouth Bass the cyprinids inhabited a more diverse range of habitats (MacRae and Jackson 2001). Some predators can, however, use complex habitats to their advantage (Savino and Stein 1982, 1989a; Anderson 1984). For Northern Pike *Esox lucius* Linnaeus 1758 for example, increased habitat complexity (vegetation) does not dampen predation efficiency, as they use vegetation to ambush their prey (Savino and Stein 1989b). In such cases, enhancing habitat complexity can also facilitate predation efficiency (Heck and Orth 1980; Anderson 1984; Savino and Stein 1989b). Predators can show plasticity in their hunting behaviour in response to variations in habitat complexity (Annett 1998; Green et al. 2019). Consequently, it is important to understand how different species respond to abiotic changes and determine the mechanisms that drive them, in order to develop measures to predict how these changes may affect predator-prey relationships.

Here we used the comparative FR approach as a hypothesis testing tool (Dick et al. 2013; Alexander et al. 2014) in order to be comparable with other studies, namely Alexander et al. (2015) and Chapter 4. We specifically test juvenile Largemouth Bass and Florida Bass because of their ability to impact on community structure and ecosystem function (Olson et al. 1995; Post et al. 1997, 1998) since at this stage piscine prey is important for their growth and survival (Olson 1996). Furthermore, juvenile fish have been shown to prey upon fish half their size (Olson 1996; Weyl and Lewis 2006) and newly hatched fry (Johnson and Post 1996). We predicted that: (a) increased habitat complexity would mitigate the predation efficiency of both Black Bass species, but (b) Largemouth Bass would have a lower maximum feeding estimates than Florida Bass under both habitat complexities. This is particularly relevant for both species as they occur across a variety of habitat types and both have previously been identified as having interspecific differences in their context dependency (Mesing and Wicker 1986;

Wheeler and Allen 2003). Therefore, abiotic context is likely to be important when assessing localised impact potential.

Materials and methods

Organism collection and maintenance

Experiments were run using juvenile Largemouth Bass and Florida Bass measuring *ca*. 85.0 mm TL. It was not possible to collect the desired fish size class (TL ≈ 85.0 mm) of both species at the same time due to differences in spawning seasons and growth rate (Clugston 1964; Rogers and Allen 2009), hence the differences in fish collections and experimental time. Largemouth Bass (TL ≈ 85.0 ± 0.6 mm) were collected from populations that were tested as being 'pure' Largemouth Bass from a Kariega River impoundment (33°25'11.01"S; 26°29'59.48"E) (Hargrove et al. 2019) in February 2018 using a seine net (5.0 × 2.0 m long and 1.5 m deep with a 5.0 mm mesh size). Florida Bass (TL ≈ 85.0 ± 2.0 mm) were collected from a population that was tested to contain >75% Florida Bass alleles (Khosa unpublished data) from a Kei River impoundment (32°39'38.11"S; 27°19'0.12"E) in November 2017. Fish were housed in 600 L holding tanks in a closed recirculating system at the Department of Ichthyology and Fisheries Science, Rhodes University, Makhanda. Prior to the FR experiments, fish were maintained on a diet of larval chironomids and earthworms supplied by Rainbow pet suppliers in Port Elizabeth, South Africa, and were acclimated to the experimental habitat complexities for seven days. Prey used in the experiment were Mozambique Tilapia (TL ≈ 10.0 ± 2.0 mm) supplied by AquaCulture Innovations, Makhanda. Similarly, prey were also acclimated to the same habitat complexities as predators for seven days.

Experimental setup

In order to be directly comparable, experiments were conducted in the same experimental facility as Alexander et al. (2015). This set up comprised a series of 300 L fibreglass tanks (1000 mm × 1000 mm, depth 370 mm) that were part of the same flow-through system as the holding tanks (water flow 1 L min−1; 24.6 ± 0.2 ºC, mean temperature ± SE) (Figure 5.1). To reduce potential stress on fish during the experiments, 80% shade net cloth was placed on top of the tank during the experiments. To simulate habitat structure through floating vegetation, we used the same habitat structures employed in Alexander et al. (2015) whereupon, plastic strips (400 mm long and 15 mm wide) were attached to a plastic mesh base and weighted to the bottom of the tanks (Figure 5.2). This allowed the artificial vegetation to float upwards and occupy the entire water column in the same way as rooted aquatic plants in freshwater systems (personal observation). Treatments for this study were "zero" and "high" habitat complexities. These habitat complexities were selected because the greatest differences in maximum feeding estimates, i.e. differences in impacts were observed in these treatments (Alexander et al. 2015). High habitat complexity was 2700 blades m^{-2}, while zero habitat complexity was a plastic mesh base without plastic strips (artificial vegetation) (Figure 5.2). Largemouth Bass (n = 56) and Florida Bass (n = 56) were used for the FR experiments.

Before the FR experiments, we conducted pilot feeding trials to quantify how many Mozambique Tilapia were eaten by each Black Bass species at a given time. The feeding trials were partitioned into 1, 2, 3 and 4 hours, from which Largemouth Bass and Florida Bass were allowed to feed on Mozambique Tilapia respectively. The maximum feeding by both Largemouth Bass and Florida Bass plateaued at three hours, and remained constant at four hours, as such, three hours was selected to conducts our FR experiments. Using this time frame also puts a restriction on the total number of live prey used in the experiments and reduces unnecessary mortality. Prey were supplied at seven densities (2, 4, 8, 16, 32, 64, 128), with four replicates per density across the different habitat complexities. Once added, predators were allowed to feed for three hours, and the number of live prey remaining were recorded at the end of this time. Controls were four replicates of each prey density per habitat complexity in the absence of predators to determine background mortality. No predator or prey individuals were reused throughout the experiments.

Figure 5.1 Experimental tanks comprise the closed circulating water where the habitat complexity experiments were conducted

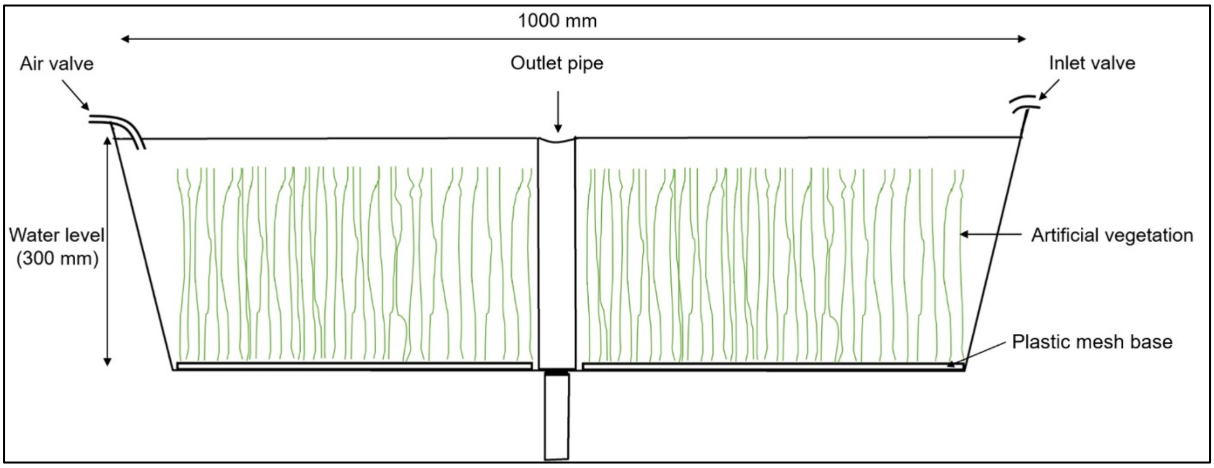

Figure 5.2 Diagram of a cross-section of the experimental arena showing the dimensions and the artificial floating habitat

Statistical analysis

All statistical analyses were conducted in R v3.5.1 (R Development Core Team 2018). Generalised linear models (GLMs) with a quasi-Poisson error distribution were used to analyse whether the raw consumption of prey was affected by fixed factors of “prey density”, “habitat complexity” and “predator species”. We used an Anova and χ^2 to report the effect size of a factor on the dependent variable, i.e. number of prey consumed. If model terms were not significant the model was optimised using a stepwise deletion process to attain the most parsimonious model. Tukey honest significant difference post hoc tests were used for multiple pairwise comparisons using the R package ‘emmeans’ (Lenth et al. 2020).

The R package ‘frair’ (Pritchard et al. 2017) was used to model the FR types of both Largemouth Bass and Florida Bass, using a logistic regression via frair::frair_test to test for the relationship between the proportion of prey consumed and prey density. A Type II FR is characterised by a significantly negative first order term, whilst a Type III FR is characterised by a significantly positive first order term followed by a significantly negative second order term (Solomon 1949; Holling 1959; Juliano 2001). Rogers' random predator equation (Rogers 1972) was used to model Type II responses, as prey were not replaced after consumption:

$$N_e = N_o(1 - \exp\,(a(N_e h - T)) \qquad (1)$$

where N_e is the number of prey eaten, N_0 is the initial prey density, a is the attack rate, h is the handling time and T is the total experiment time (fixed at 1). The data were non-parametrically bootstrapped (n = 2000) to construct 95% confidence intervals of the fitted FR curves for each treatment. To test differences between attack and handling parameters within species across habitat complexities we used the "difference method" outlined in Juliano (2001) via frair::frair_compare. Maximum feeding estimates ($1/h$) were calculated from the handling time estimates, if the handling times are significantly different then we can infer significant difference in maximum feeding estimates.

Results

Prey survival in control treatments was greater than 99%, so experimental deaths were attributed directly to predation. There was a significant interaction effect on the number of prey consumed between "predator species" × "habitat complexity" and between "predator species" × "prey density" (Table 5.1; Figure 5.3). Whereupon Florida Bass consumed significantly more prey than Largemouth Bass at zero habitat complexity (z=2.69, p<0.01; Figure 5.3) but no significant differences in prey consumption ware recorded at high habitat complexity (z=0.21, p=0.83; Figure 5.3). In terms of prey density, Florida Bass consumed significantly more prey than Largemouth Bass at densities 64 (z=6.75, p<0.001; Figure 5.3) and 128 (z=4.54, p<0.001;

Figure 5.3) but at densities below 64 there was no difference in prey consumed (all p>0.05; Figure 5.3).

Table 5.1 Model terms for all factors from a generalised linear model (GLM) with a quasi-Poisson error distribution used to determine differences in prey consumption with regards to factors "predator species", "habitat complexity" and "prey density" using χ^2 to report the effect size of a factor on the dependent variable

Model term	Prey consumed		
	χ^2	df	*p*-value
Species	39.89	1	<0.001
Habitat complexity	23.67	1	<0.001
Density	1306.58	6	<0.001
Species * Habitat complexity	5.07	1	<0.01
Species * Density	27.18	6	<0.001

For both predators, Type II FRs were found across all habitat complexity treatments, indicated by significantly negative first order terms (Table 5.2; Figure 5.4). Largemouth Bass had a significantly higher attack parameter at the zero habitat complexity compared to the high habitat complexity (z=41.41, p<0.001; Table 5.2; Figure 5.4), but there was no difference in the handling parameter between zero and high habitat complexity (z=0.62, p=0.52; Table 5.2; Figure 5.4). Florida Bass, contrastingly, had a marginally significantly lower attack rate at zero habitat complexity compared to high complexity (z=1.92, p=0.05; Table 5.2; Figure 5.4). However, Florida Bass had a significantly longer handling parameter under high habitat complexity compared to zero habitat (z=7.85, p<0.001; Table 5.2; Figure 5.4).

Table 5.2 Logistic regression first order terms to denote functional response type across all habitat types with associated *p* values, the attack rate *a*, handling time *h* and *p* values

Species	Habitat type	First order term, *p*	Attack parameter (*a*), *p*	Handling parameter (*h*), *p*	Max. feeding estimate (1/*h*)
Largemouth Bass	Zero	-0.026, <0.001	32.06, <0.001	0.033, <0.001	30.1
	High	-0.023, <0.001	6.32, <0.001	0.034, <0.001	28.8
Florida Bass	Zero	-0.029, <0.001	4.28, <0.001	0.013, <0.001	75.1
	High	-0.026, <0.001	6.52, <0.001	0.025, <0.001	38.6

In terms of interspecific differences, at zero habitat complexity, Largemouth Bass had a significantly higher attack parameter than Florida Bass (z=90.56, *p*<0.001; Table 5.2; Figure 5.4) but a significantly longer handling parameter (z=14.42, *p*<0.001; Table 5.2; Figure 5.4). At high habitat complexity there were no interspecific differences in the attack parameter (z=0.11, *p*=0.90; Table 5.2; Figure 5.4), however, Florida Bass had a significantly shorter handling time than Largemouth Bass (z=3.51, *p*<0.001; Table 5.2; Figure 5.4).

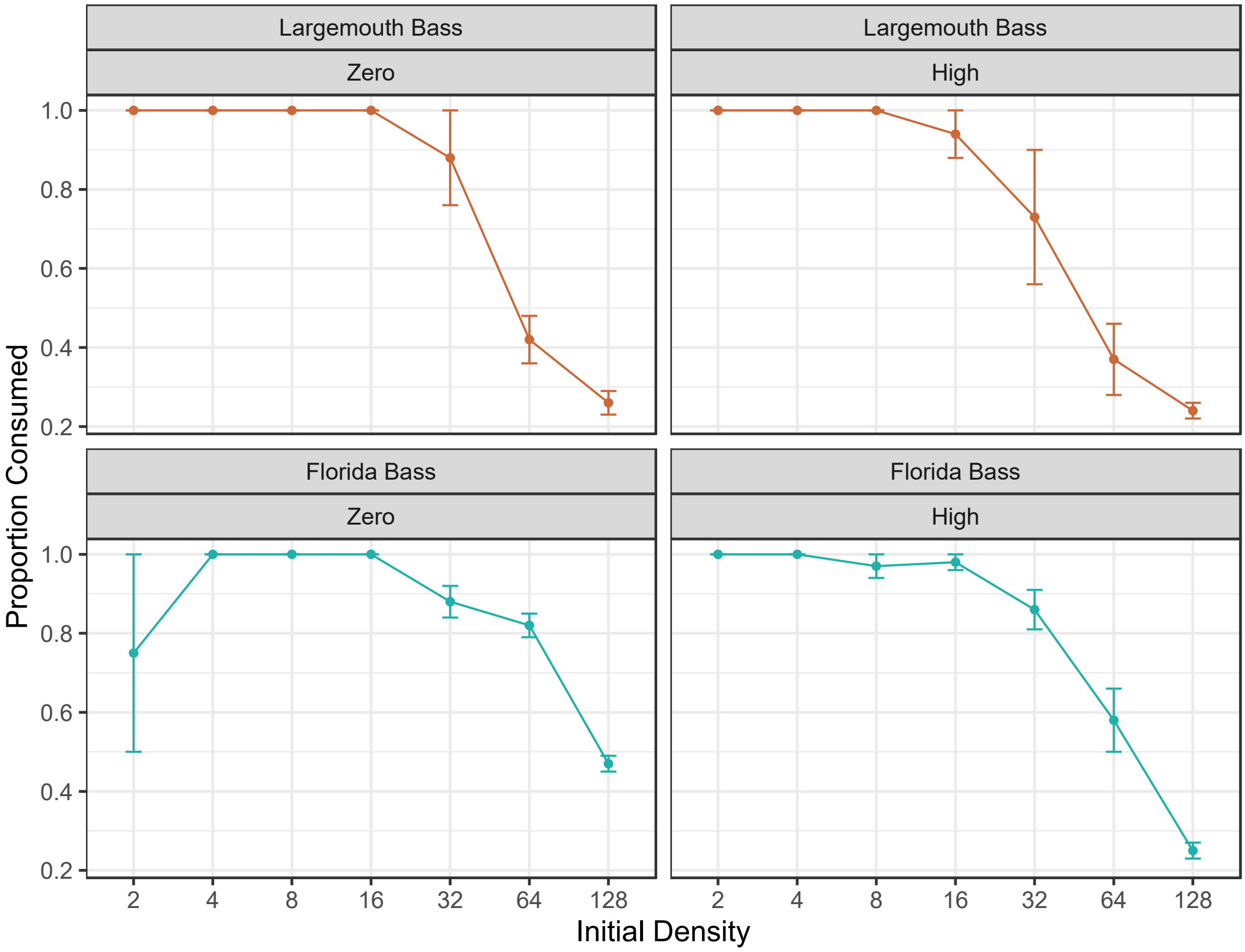

Figure 5.3 Proportion of prey consumed with regards to initial density of prey supplied for Largemouth Bass and Florida Bass at zero and high habitat complexities. Points and error bars indicate mean ± SE proportion consumed

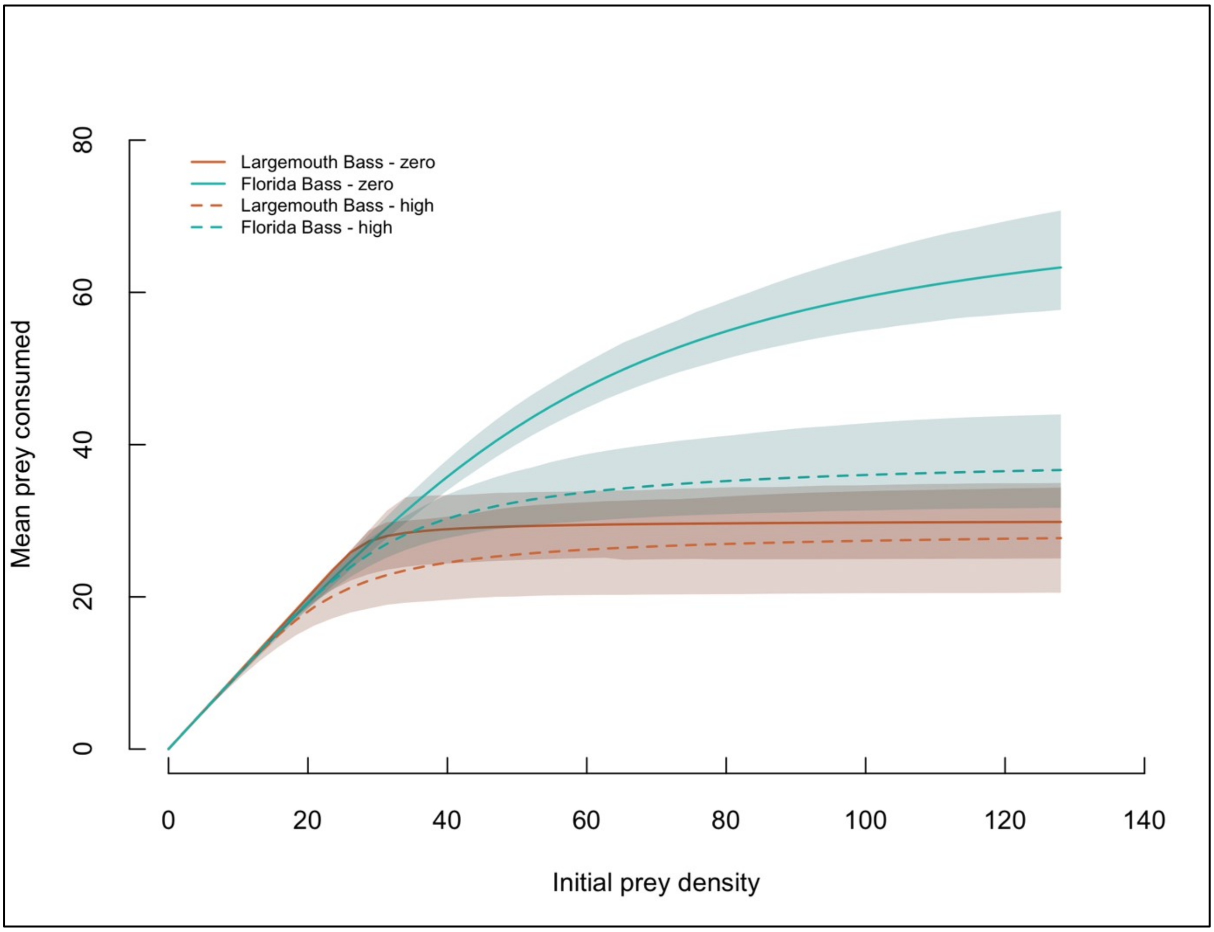

Figure 5.4 Functional response curves of juvenile Largemouth Bass at zero (brown solid line) and high (brown dashed line) habitat complexities and Florida Bass at zero (blue solid line) and zero (blue dashed line) habitat complexities. Curves modelled by the Rogers' random predator equation for a Type II functional response. Shaded areas indicate 95% confidence intervals as calculated by n=2000 non-parametric bootstrapping of the initial fit

Discussion

In this study, we demonstrate that a thorough assessment of interspecific differences in invader predatory performance is essential, especially within the context of invader cryptic diversity. We demonstrate that while habitat complexity does have the capacity to reduce the per capita effect of juvenile Largemouth Bass and Florida Bass, increasing complexity has a disproportionate effect on mitigating the ecological effects of Florida Bass compared to Largemouth Bass. Due to the consistently lower handling time parameter, Florida Bass maintained higher maximum feeding estimates relative to Largemouth Bass across the tested habitat complexities. Unfortunately, due to the nature of laboratory experiments, it is impossible to claim complete realism and it is important to further caveat that these values should be considered as relative differences and specific to juvenile Black Bass under the tested context dependencies. Despite this, we demonstrate that both Largemouth Bass and Florida Bass have a persistent Type II FR towards Mozambique Tilapia fry across the habitat complexities, a classical characteristic of damaging invasive species, due to the high resource utilisation at low resource densities (Dick et al. 2013; Alexander et al. 2015).

Habitat complexity mediates the strength of predator-prey interactions by altering the probability of the predator and prey actually encountering each other (Greene 1986; McCoy and Bell 1991; Barrios-O'Neill et al. 2015). The attack parameter in the FR analysis can be taken as a proxy of encounter rate or search efficiency (Holling 1959; Dick et al. 2017). Black Bass species are active pursuit hunters which means that they are morphologically specialised in searching for their prey items and rely on various cues (Sullivan and Atchison 1978; Luger et al. 2020). The high attack parameter of Largemouth Bass under zero habitat complexity was an unexpected result considering the conclusions drawn from Alexander et al. (2015). However, several studies have demonstrated the Largemouth Bass to be a more aggressive predator relative to Florida Bass (Zolczynski and Davies 1976; Rieger et al. 1978; Kleinsasser et al. 1990). As the two species are morphologically almost identical (Kassler et al. 2002) this suggests that there may be other factors, such as a behavioural difference driving the variation in attack parameter. However, at high habitat complexity, the lack of difference in attack parameter between Largemouth Bass and Florida Bass indicates that there is some disruption in predatory efficiency by way of reducing visual contact with prey items and increasing refugia (Savino and Stein 1982; Warfe and Barmuta 2004; Barrios-O'Neill et al. 2015).

The maximum feeding estimate is determined by the handling parameter of the FR curve. Both species showed an increase in handling parameter values with increasingly complex habitat. Florida Bass, however, exhibited a consistently shorter handling parameter, and consequently higher maximum feeding estimate (per capita effect), across all treatments compared to Largemouth Bass. The foraging efficiency of Florida Bass is congruent with several studies documenting a reduced foraging efficiency of predators due to increasing habitat complexity (e.g Savino and Stein 1982, Warfe and Barmuta 2004, Alexander et al. 2015). This persistence of a higher per capita effect in Florida Bass infers that the ecological effect of this species will be contiguous across a variety of habitats and complexities and suggests a need for a more integrative approach in the management of the two Black Bass species. Predation pressure, especially by invasive species, is considered a major factor threatening South African freshwater biota (Ellender and Weyl 2014; Ellender et al. 2017). Black Bass species are well documented for their negative effects on native and endemic species (Takamura 2007; Kimberg et al. 2014; Ellender et al. 2018). Our data suggest that the spread of Florida Bass may exacerbate the observed predation impacts on native fishes and we recommend further investigation into interspecific differences to better understand how hybridisation between these two Black Bass species may affect ecological impact.

CHAPTER 6

GENERAL DISCUSSION

Overview

South Africa has a long history of fisheries enhancement using non-native fishes, dating back to the 18th century (De Moor and Bruton 1988). In South Africa, recreational angling is primarily based on non-native fishes, including the Black Bass species (van Rensburg et al. 2011; Ellender and Weyl 2014), and these fishes have become a major component of fish assemblages across all major catchments of South Africa (Chapter 2; van Rensburg et al. 2011; Ellender et al. 2014). Government and angler-mediated actions facilitated the rapid spread of Black Bass species before the cessation of stocking in the 1990s (Figure 6.1; McCafferty et al. 2012). The introduction of Black Bass species has had a number of negative ecological impacts on native biota (Ellender et al. 2011, 2018; Shelton et al. 2014; Weyl et al. 2014); however, there is a severe paucity of studies documenting the impacts of these non-native Black Bass species in the receiving freshwater ecosystems of South Africa (Ellender and Weyl 2014; van Wilgen and Wilson 2018). Specifically, in the case of the Florida Bass and their hybrids with Largemouth Bass, their impact remains relatively undocumented because the species are difficult to differentiate from the Largemouth Bass with whom they readily hybridise.

The main purpose of this thesis was to address the severe paucity of studies documenting the impacts of the non-native Florida Bass in the freshwater ecosystems of South Africa (Ellender and Weyl 2014; van Wilgen and Wilson 2018). To do this, I took a multidisciplinary approach by using SDM to establish the current distributions and the invasion debt of all Black Bass species in South Africa (Chapter 2). Previous studies have demonstrated the importance of SDM in guiding priorities for the management of both native and non-native species (Iguchi et al. 2004; Zengeya et al. 2013; Bae et al. 2018; Jarnevich et al. 2018). As such, in Chapter 2, I presented the first analysis of all Black Bass distributions and their invasion debt across all WMA of South Africa. I then surveyed two river catchments (i.e. Breede River catchment and Kowie River catchment; Chapter 3) in order to establish and extend the current understanding of gene flow between the Largemouth Bass and Florida Bass within a river catchment setup. Functional response experiments (FR) were then used to help elucidate the potential impacts by the Largemouth Bass and Florida Bass under the context dependency of temperature and habitat complexity (Chapters 4 and 5).

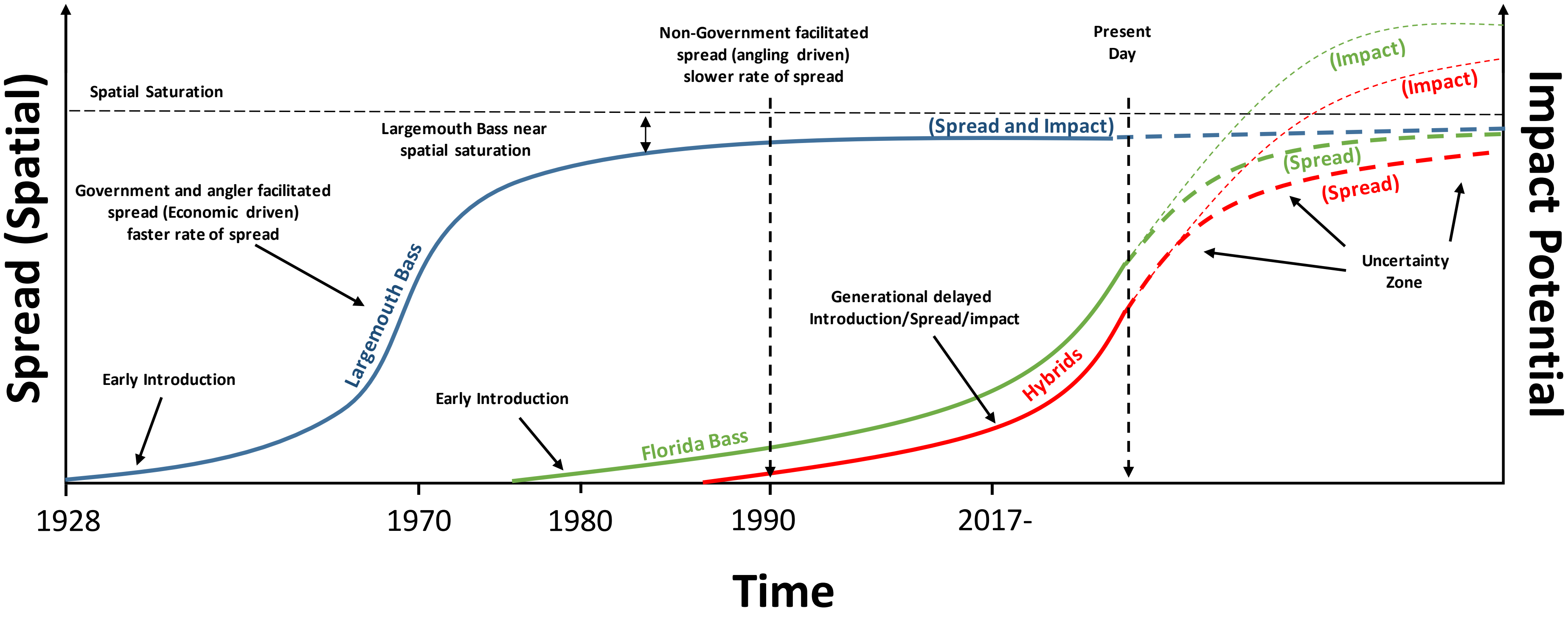

Figure 6.1 Conceptual model outlining invasion dynamics of Largemouth Bass, Florida Bass and their hybrids in South Africa. Largemouth Bass were introduced in 1928 and the early spread and distribution was facilitated by Government and angler-mediated support. As a result, Largemouth Bass are widely distributed and currently are present in all major river catchments across the country. While not certain, the spatial distribution and spread of Florida Bass in South Africa is not yet established; however, recent studies suggest that the species and their hybrids are spreading across all catchments to meet recreational angling purposes (Weyl et al. 2017; Hargrove et al. 2019b). The model systems used show that when occurring in sympatry, the two Black Bass species hybridise, and it is highly likely that, as Florida Bass distribution increases, so will hybridisation levels nationally. Using functional responses experiments, under the context dependency of temperatures and habitat complexity, the pronounced predatory impact for Florida Bass over Largemouth Bass was evident, implying that hybrids may also have elevated impacts under these conditions when compared to pure Largemouth Bass

Main findings

Distributions of Black Bass species in South Africa

Species distribution modelling (SDM) fulfils an important role in the development of conservation and management strategies (Elith et al. 2006; Elith and Leathwick 2009; Zengeya et al. 2013), contributing to the understanding of environmental and anthropogenic drivers that facilitate the distribution and spread of species (Elith et al. 2006; Elith and Leathwick 2009; Hao et al. 2020). However, SDM are susceptible to the quality of environmental and distribution data used to populate them (Elith et al. 2006; Elith and Leathwick 2009; van Proosdij et al. 2016). In Chapter 2, I first demonstrate the locality bias across the different stakeholders (i.e. recreational anglers vs conservation institutions) when collecting Black Bass distribution data. I found that the majority of Black Bass distribution data obtained from recreational anglers (informal) was largely associated with dams, while distribution data from conservation institutions (formal) were largely focused in the riverine environments. The observed locality bias in this study is a clear indication that Black Bass species are of different significance/value for the different stakeholders. Thus, Black Bass species provide recreational opportunities for anglers (Hargrove et al. 2015), hence the high prevalence of distribution data in dams, but are also a nuisance for conservation managers when they invade the rivers because of their ecological impacts on the imperiled native biota that are largely found in the riverine environments and protected areas (Weyl et al. 2014, 2015; Ellender et al. 2018).

From a conservation perspective, understanding the drivers of Black Bass distributions is important (Chapter 2). The response curves derived from SDM show that Black Bass were largely associated with areas of warmer temperatures and higher flow accumulation (dams) (Chapter 2). Similar findings were reported by Bae et al. (2018) who found temperature and the presence of dams to be the most important factor for predicting the distribution of

Largemouth Bass in the Iberian Peninsula. Temperature is a key abiotic factor in habitat suitability for Black Bass, and considering the predicted increases in temperature due to climate change range, expansion for Black Bass species can be expected (Iguchi et al. 2004; Chen et al. 2011; Bae et al. 2015; Mamun et al. 2018; Liu et al. 2019) into the invasion debt areas (Chapter 2). However, such range shifts are a function of intrinsic dispersal capability and landscape connectivity (Chapter 3; Alp et al. 2012; Inoue and Berg 2017; Tonkin et al. 2018a).

In addition to temperature, the large number of dams in South Africa (Weyl et al. 2007) may also serve as invasion hotspots (Johnson et al. 2008; Ricciardi et al. 2013), facilitating the future introduction and spread of Black Bass and other non-native species (Johnson et al. 2008; Ricciardi et al. 2013; Bae et al. 2018). Furthermore, the presence of dams probably modifies the available habitat to the detriment of native species, which are more adapted to the lotic environments (Johnson et al. 2008; van der Walt et al. 2016; Cheng et al. 2018; Ellender et al. 2018; Dudgeon 2019; Reid et al. 2019; Zhang et al. 2020), and provides suitable habitat for the more lentic adapted non-native Black Bass species (Long et al. 2015; Taylor et al. 2019a). Synergistic interaction between dams and climate change may further exacerbate/enhance the impacts of the warm temperature-adapted species (e.g. Florida Bass) (Johnson et al. 2008; Zeng et al. 2017) by constraining the ability of native fishes to make compensatory movements to cooler conditions (Johnson et al. 2008; Kano et al. 2016; Dudgeon 2019), so making them susceptible to predation (see Ellender et al. 2011, 2018; van der Walt et al. 2016), and empirical quantification of resource utilisation by Black Bass suggests higher *per capita* effects (Chapter 4 and 5). As such, improving management efficiency by preventing intentional and unintentional introductions of Black Bass species is imperative for the South African freshwater systems (Woodford et al. 2017; van Wilgen and Wilson 2018).

Genetic distribution of Black Bass species in South Africa

Considering the extent of the current distribution of Largemouth Bass/Florida Bass in South Africa (Chapter 2), the next important step is to provide an understanding of the role that connectivity within a river network plays in facilitating the spread of Largemouth Bass and Florida Bass alleles, and consequently, the levels of hybridisation between the two Black Bass species (Figure 6.3; Chapter 3). In a riverine system, connectivity among populations of species is determined by the dendritic structure of the river network, the spatial position of the population within a network, and the dispersal ability of the species (Fagan 2002; Altermatt 2013; Altermatt and Fronhofer 2018). As such, understanding how dispersal and river network structure interact is important as it enables improved monitoring, management and conservation (Tonkin et al. 2014, 2018a; Altermatt and Fronhofer 2018).

My results in Chapter 3 show that, when Florida Bass is introduced into populations containing Largemouth Bass or vice versa, there is an inherent risk of hybridisation in which individuals would have different proportions of both Largemouth Bass and Florida Bass alleles. Indeed, populations in the mainstems of both the Breede River catchment and Kowie River catchment exhibited different levels of allele contribution from both Largemouth Bass and Florida Bass (Chapter 3). Because Largemouth Bass and Florida Bass evolved in different habitats and under different selective pressures, they are likely to exhibit different fitness levels and their relative fitness will likely differ in different habitats (Philipp et al. 1981; Fields et al. 1987; Philipp and Whitt 1991). Fields et al. (1987) for example, demonstrated that F_1 hybrids of Florida Bass exhibited a significantly higher thermal tolerance than the pure strain of Florida Bass. This high thermal vigour may be advantageous for Florida Bass under the current climate change projections, and may also enhance their predation efficiency, resulting in a higher ecological impact (Chapters 2 and 4).

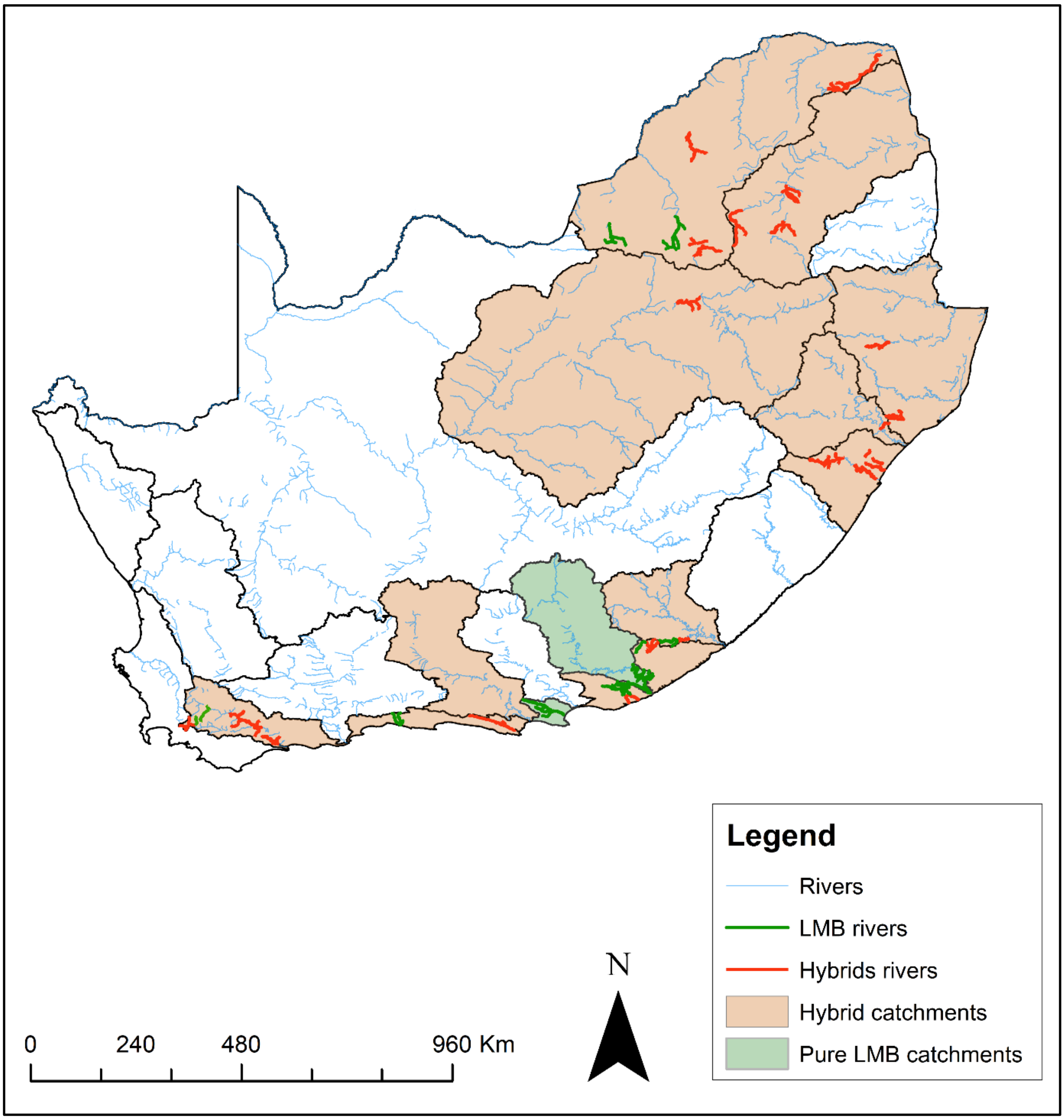

Figure 6.2 The current distribution of Largemouth Bass, Florida Bass and their hybrids across South Africa; genetic status is confirmed in Weyl et al. (2017), Hargrove et al. (2019b). Rivers with pure Largemouth Bass population are identified in green (LMB rivers) and rivers with hybrid populations are represented in red (Hybrid rivers)

Other significant findings from Chapter 3 are that Black Bass populations in isolated upstream dams remain free from hybridisation, probably because there is either no direct propagule pressure from the downstream, or direct introductions of either Largemouth Bass or Florida Bass alleles by people from elsewhere. Intuitively, the two Black Bass species should be managed separately; however, due to the difficulty in distinguishing the two Black Bass species and their hybrids morphologically, the development of separate management strategies remains difficult (Weyl et al. 2017; van Wilgen and Wilson 2018). Considering that Florida Bass alleles have already been documented across the major catchments South Africa (Figure 6.3; Hargrove et al. 2017, 2019b; Weyl et al. 2017), and inferring my findings in Chapter 3, it is likely that the Black Bass populations found across the major river systems of South Africa consist mainly of hybrids of both Largemouth Bass and Florida Bass. As such, I recommend that the two Black Bass species and their hybrids be managed as a single unit.

Inferred impact using functional response experiments

Feeding interactions are fundamental to ecology because survival, growth and reproduction require that all organisms utilise resources of some kind (Dick et al. 2017b; Barrios-O'Neill et al. 2019). These interactions are based on mechanisms that can be experimentally tested in FRs experiments by analysing the rate of resource utilisation as a function of resource availability (Solomon 1949; Holling 1959; Dick et al. 2013a). However, the rate of resource utilisation by a species can be influenced by abiotic contexts, such as temperature and habitat complexity (Englund et al. 2011; Alexander et al. 2015; Iacarella et al. 2015; South et al. 2019), and different species often exhibit different responses to the changing abiotic parameters (Chapters 4 and 5) (Englund et al. 2011). This thesis utilises FR experiments to assess ways in which temperature and habitat complexity can mediate the ecological impacts of Largemouth Bass and Florida Bass.

The use of FRs experiments in this study provided an insight into how Largemouth Bass and Florida Bass are likely to utilise the available resources in the wild. In the two experimental chapters (Chapters 4 and 5), I demonstrate that predation impacts between the Largemouth

Bass and Florida Bass are likely to vary, influenced largely by the abiotic context of temperature and habitat complexity. Iacarella et al. (2015) demonstrated that differences in thermal optima can create competitive imbalances that result in a species being a superior competitor in its optimal habitat conditions but inferior in sub-optimal habitat conditions. Indeed, my results demonstrate that the *per capita* effects of both Largemouth Bass and Florida Bass closely followed their thermal optima and tolerances, with the Florida Bass exhibiting a significantly higher *per capita* effect at a higher temperature (30 °C), while lower temperatures seemed to dampen their *per capita* effects (18 °C); the converse was true for Largemouth Bass (Chapter 4).

The observed differences in *per capita* effects between Largemouth Bass and Florida Bass were driven by the handling time. Florida Bass exhibited significantly lower handling time than Largemouth Bass when the temperature increased (Chapter 4). This finding is in accordance with the expectation of the metabolic theory of ecology (MTE) (Gillooly et al. 2001). The Florida Bass, having evolved in a subtropical climate (Warren 2009), is more adapted to higher temperatures (critical thermal maxima exceeding 41 °C) and more susceptible to low temperatures than the Largemouth Bass (Fields et al. 1987; Philipp and Whitt 1991; Beitinger et al. 2000). Considering that warmer temperatures have been identified as one of the major drivers of Largemouth Bass/Florida Bass distributions in South Africa (Chapter 2), the projected increases in temperature in South Africa due to climate change (Schulze 2011) may incur higher *per capita* effects for Florida Bass than Largemouth Bass as per my results presented in Chapter 4.

Another component of the experimental chapters was to investigate whether habitat complexity can mediate the *per capita* effects of both Largemouth Bass and Florida Bass (Chapter 5).

Across multiple habitat types and complexities, several studies have shown that prey survival is increased significantly with increasing habitat complexity, but often with diminishing returns as habitat complexity continues to increase (Savino and Stein 1982, 1989a; Anderson 1984; Manatunge et al. 2000; Humphries et al. 2011; Alexander et al. 2013, 2015; Toscano and Griffen 2013). Indeed, Savino and Stein (1982, 1989a); Rilov et al. (2007) demonstrated that increasing habitat complexity may facilitate predation efficiency by ambush predators as they can hide from the approaching prey. My results in Chapter 5 describe a variety of responses to habitat complexity by both Largemouth Bass and Florida Bass. In each of the two levels of habitat complexity, both Largemouth Bass and Florida Bass exhibited a Type II FR towards the fish prey. Florida Bass exhibited a significantly lower handling time (i.e. higher maximum feeding estimates) than Largemouth Bass under both zero and high habitat complexity. It is, however, important to point out that the effects of habitat complexity on feeding performances were more pronounced on Florida Bass than Largemouth Bass, as a significant decrease in maximum feeding estimates was only recorded for the Florida Bass (Chapter 5). It is currently difficult to ascertain the drivers that may have led to the observed discrepancies in feeding performance between the Largemouth Bass and Florida Bass, because both species were acclimated to the same habitat complexities for the same amount of time.

Management and conservation recommendations

This study provides a much-needed understanding of Black Bass distributions and identifies the environmental drivers that facilitate for their successful spread. Identifying areas that are suitable for Black Bass invasions and yet to be invaded (invasion debt) (Chapter 2) is important for both conservation and management purposes. Freshwater ecosystems in the Cape Floristic Ecoregion appear to be the most vulnerable to Black Bass invasions. As eradicating Black Bass species in South Africa is not feasible, and is impossible without affecting native species (Weyl et al. 2014), the current NEM:BA legislation is well suited as it advises against the introduction and spread of the non-native Black Bass species into novel environments where they do not currently occur (Woodford et al. 2017; van Wilgen and Wilson 2018). Furthermore, Black Bass species are of socio-economic importance in areas where they currently occur; as such, it is important that regulations designed for their management are carefully implemented (Woodford et al. 2017; Zengeya et al. 2017).

Recommendations for future studies

Climate change is going to have a significant influence on the future distribution of species (Sala et al. 2000; Chen et al. 2011; Shelton et al. 2018). In the CFE for example, Shelton et al. (2018), demonstrated the absence of Rainbow Trout in sections of the rivers where the temperature exceeded 27 °C, but in the cooler regions of the rivers, they were found to be abundant. Future studies should try to establish if the distribution range of all Black Bass species in South Africa is going to expand or shrink under the projected climate change scenarios. This is important because understanding the future distribution range of all Black Bass species will facilitate proactive management strategies, such as identifying the possible introduction pathways within river systems, and will also facilitate the early detection of new invasions, and consequently, their eradication (Chen et al. 2011; Shelton et al. 2018).

One of the major drawbacks of the current study was the use of MAXENT as the only species distribution modelling technique. Recent studies on SDMs suggests that by using multiple modelling techniques (e.g. generalised linear model, generalised additive model, artificial neural networks, random forest and boosted regression trees) can result in better predictions of species distributions (Bae et al. 2018; Ahmad et al. 2019; Hazen et al. 2019; Hao et al. 2020). In addition, by combining the Largemouth Bass and Florida Bass records to model the distributions may have influenced the current predictions of spread and habitat suitability. As such, future SDM studies should separate the two Black Bass to give a clear picture of their distributions.

Results from my genetics assessment of Largemouth Bass and Florida Bass show that the resultant hybrids exhibit variable levels of allele contribution from both Black Bass species (Chapter 3). Fields et al. (1987) have demonstrated higher thermal tolerance on the F_1 generation hybrids of the two Black Bass species; however, this does not persist to the F_2 generation. It will be of ecological importance to predict/assess the predation impacts of the hybrids with different levels of allele contribution. Functional response experiments can be used to assess the impact potential of these hybrids. One of the major drawbacks emanating from this study was the failure to incorporate the numerical response of both Largemouth Bass and Florida Bass (Dick et al. 2017b), and future studies should consider incorporating this factor when conducting the experiments.

The importance of Black Bass species to the inland fisheries of South Africa is undeniable; nonetheless, the status of the fishery remains poorly quantified (McCafferty et al. 2012; Hargrove et al. 2015). A major constraint for assessing the fishery is the lack of reliable fishery-independent data (e.g. scientific surveys) which are often costly and constrained by the lack of personnel and resources (Hargrove et al. 2015), in which case, fisheries assessment must be based on fisheries-dependent data (Maunder and Punt 2004). Fisheries-dependent data are mostly obtained from commercial and recreational fishers, and are usually summarised in the form of CPUE, which is typically used as an index of abundance (Punt et al. 2000; Maunder and Punt 2004; Zhou et al. 2019). As Florida Bass were introduced for the enhancement of the Largemouth Bass fishery and current evidence suggests that Florida Bass are spreading into different catchments (Weyl et al. 2017; Hargrove et al. 2019b), I therefore recommend that future studies should focus on assessing the tournament angling catch data from waterbodies that have been identified to contain either Largemouth Bass, Florida Bass and their hybrids to test if there are any weight differences in Black Bass caught across different dams with known contribution of Florida Bass alleles.

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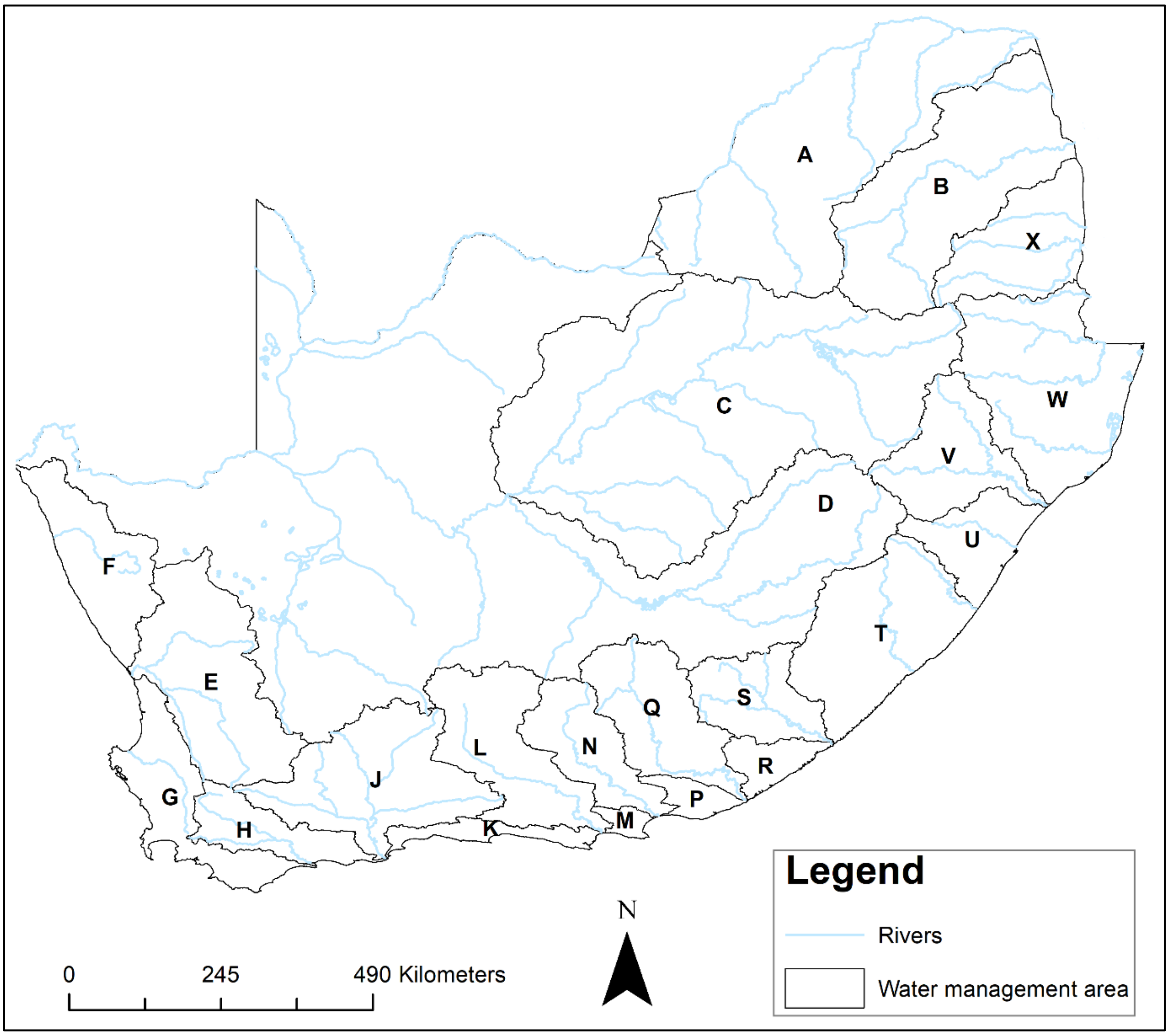
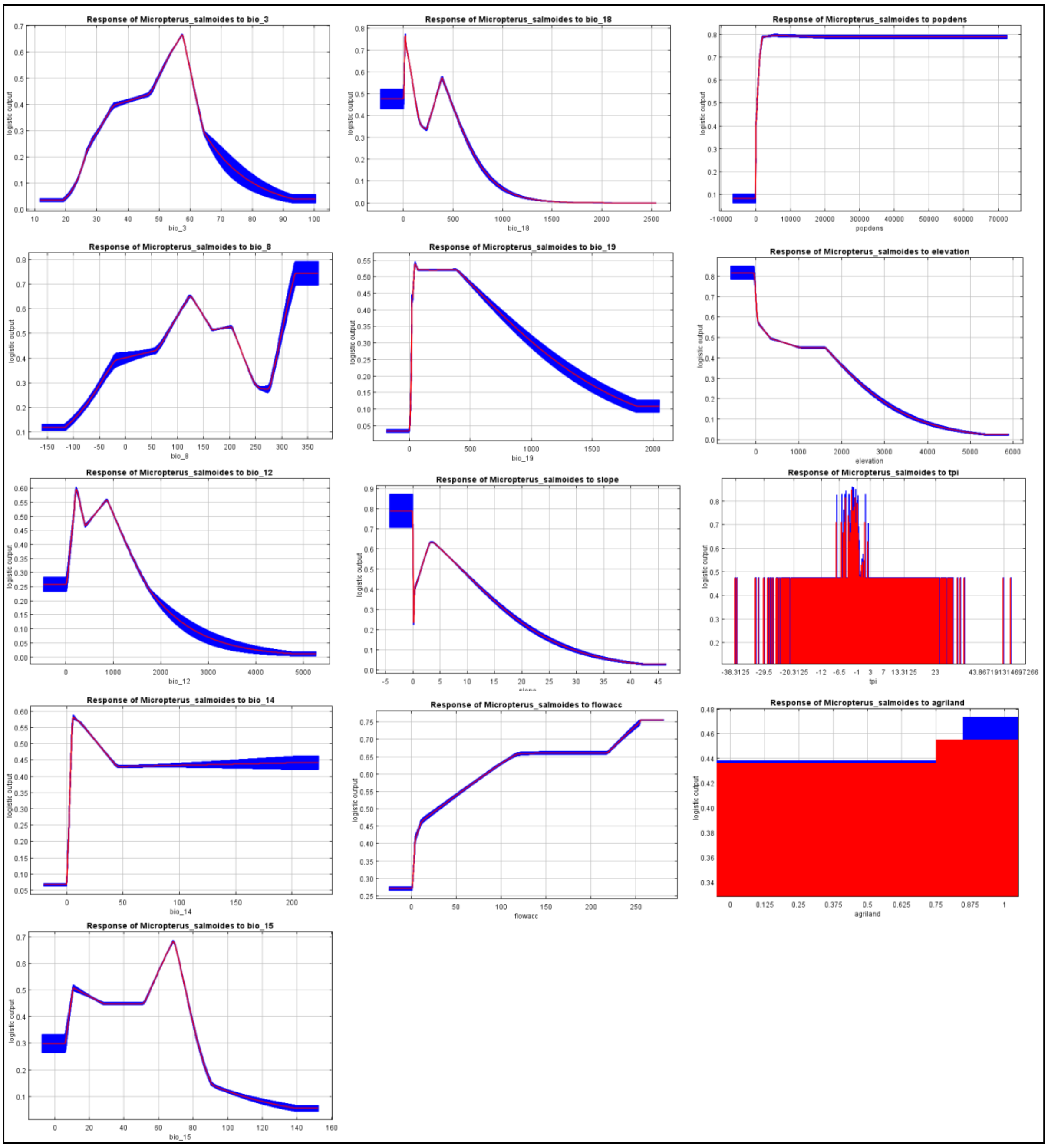
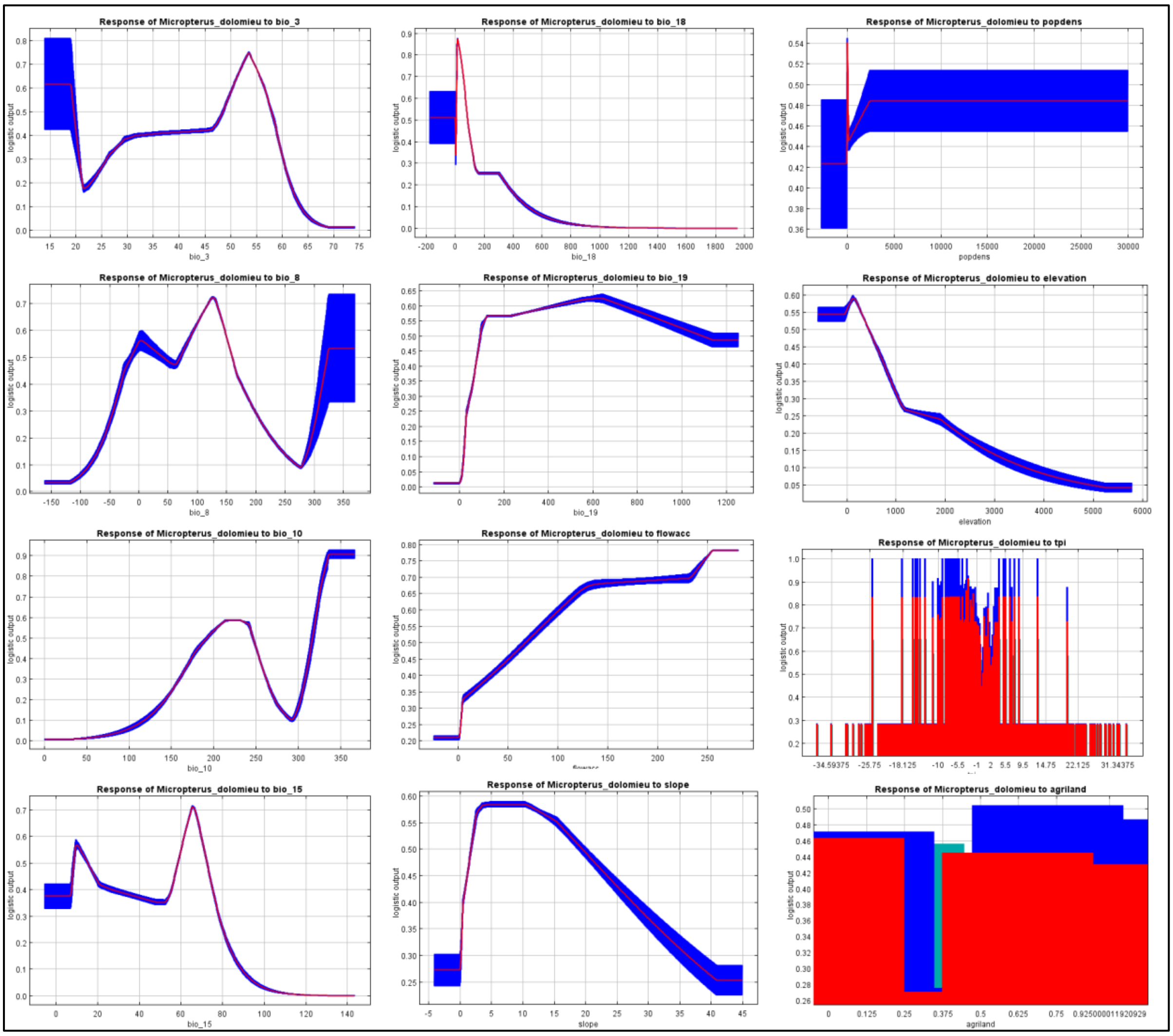
Supplementary materials



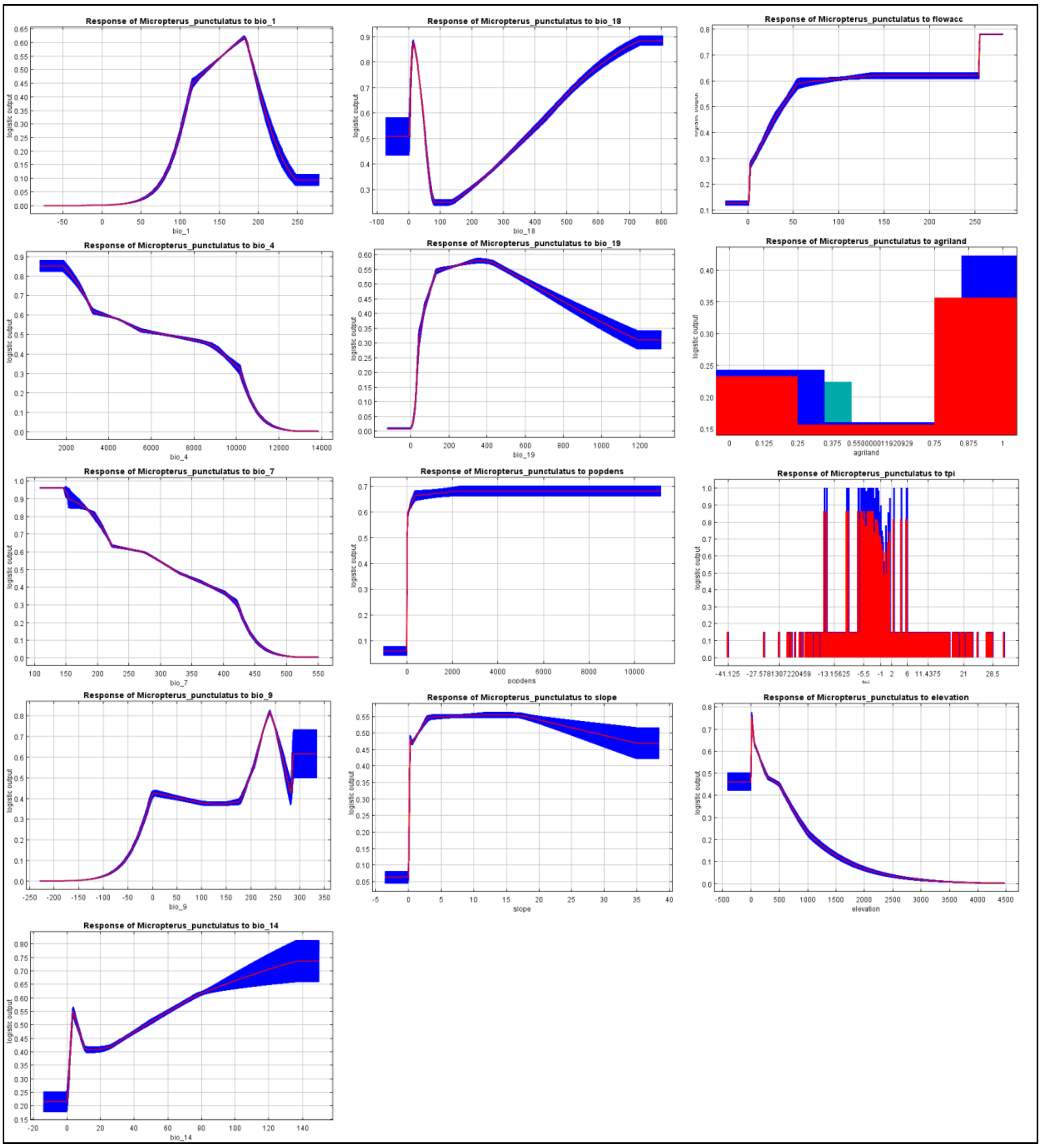
Supplementary Figure 1.I Water management areas of South Africa: A–Limpopo, B–Olifants North, C–Vaal, D–Orange, E–Olifants West, F–Buffels, G–Berg, H–Breede, J–Gouritz, K–Krom, L–Gamtoos, M–Swartkops, N–Sundays, P–Bushmans, Q–Great Fish, R–Keiskamma, S–Kei, T–Mzimvubu, U–Mkomazi, V–Tugela, W–Mfolozi and X–Komati



Supplementary Figure 1.II Species response curves generated by MAXENT for Largemouth Bass *Micropterus salmoides*. Seven Bioclim, topographic and hydrological data (elevation, slope, topographic index and flow accumulation) and anthropogenic disturbance data (agricultural land and human population density) used to project distributions. Bio 3 = Isothermality, Bio 8 = mean temperature of wettest quarter, Bio 12 = annual precipitation, Bio14 = precipitation of driest month, Bio 15 = precipitation seasonality (coefficient of variation), Bio 18 = precipitation of warmest quarter, Bio 19 = precipitation of coldest quarter



Supplementary Figure 1.III Species response curves generated by MAXENT for Smallmouth Bass *Micropterus dolomieu*. Six Bioclim, topographic and hydrological data (elevation, slope, topographic index and flow accumulation) and anthropogenic disturbance data (agricultural land and human population density) used to project distributions. Bio 3 = Isothermality, Bio 8 = mean temperature of wettest quarter, Bio 10 = mean temperature of warmest quarter, Bio 15 = precipitation seasonality, Bio 18 = precipitation of warmest quarter and Bio 19 = precipitation of coldest quarter



Supplementary Figure 1.IV Species response curves generated by MAXENT for Spotted Bass *Micropterus punctulatus*. Seven Bioclim, topographic and hydrological data (elevation, slope, topographic index and flow accumulation) and anthropogenic disturbance data (agricultural land and human population density) used to project distributions. Bio 1 = annual mean temperature, Bio 4 = temperature seasonality, Bio 7 = temperature annual range, Bio 9 = mean temperature of driest quarter, Bio 14 = precipitation of driest month, Bio 18 = precipitation of warmest quarter, Bio 19 = precipitation of coldest quarter

Supplementary Table I Pearson correlation network of the environmental variables used for the development of Largemouth Bass *Micropterus salmoides* MAXENT model. Bio 3 = isothermality, Bio 8 = mean temperature of wettest quarter, Bio 12 = annual precipitation, Bio 14 = precipitation of driest month, Bio 15 = precipitation seasonality, Bio 18 = precipitation of warmest quarter and Bio 19 = precipitation of coldest quarter

Environmental variables	Agri_Land	Bio 12	Bio 14	Bio 15	Bio 18	Bio 19	Bio 3	Bio 8	Elevation	Flow_Acc	Pop_Dens	Slope	Topo_Index
Agri_Land	1.00	0.02	0.12	-0.22	0.02	0.03	-0.34	0.09	-0.35	0.01	0.04	-0.26	0
Bio 12	0.02	1.00	0.49	-0.12	0.76	0.56	0.09	0.13	-0.26	-0.02	0.05	0.21	0.01
Bio 14	0.12	0.49	1.00	-0.78	0.19	0.74	-0.38	-0.26	-0.36	-0.01	0.04	0.04	0.01
Bio 15	-0.22	-0.12	-0.78	1.00	0.14	-0.56	0.57	0.42	0.36	0.01	-0.03	0.04	0
Bio 18	0.02	0.76	0.19	0.14	1.00	0.09	0.04	0.3	-0.07	-0.02	0.05	0.27	0.01
Bio 19	0.03	0.56	0.74	-0.56	0.09	1.00	-0.11	-0.35	-0.27	-0.01	0.03	0.12	0.01
Bio 3	-0.34	0.09	-0.38	0.57	0.04	-0.11	1.00	0.32	0.23	0	-0.05	0.05	0
Bio 8	0.09	0.13	-0.26	0.42	0.3	-0.35	0.32	1.00	-0.35	0.04	0.03	-0.3	-0.04
Elevation	-0.35	-0.26	-0.36	0.36	-0.07	-0.27	0.23	-0.35	1.00	-0.05	-0.05	0.47	0.05
Flow_Acc	0.01	-0.02	-0.01	0.01	-0.02	-0.01	0	0.04	-0.05	1.00	0.01	-0.05	-0.15
Pop_Dens	0.04	0.05	0.04	-0.03	0.05	0.03	-0.05	0.03	-0.05	0.01	1.00	-0.02	0
Slope	-0.26	0.21	0.04	0.04	0.27	0.12	0.05	-0.3	0.47	-0.05	-0.02	1.00	0.05
Topo_Index	0	0.01	0.01	0	0.01	0.01	0	-0.04	0.05	-0.15	0	0.05	1.00

Supplementary Table II Pearson correlation network of the environmental variables used for the development of Smallmouth Bass *Micropterus dolomieu* maxent model. Bio 3 = isothermality, Bio 8 = mean temperature of wettest quarter, Bio 10 = mean temperature of warmest quarter, Bio 15 = precipitation seasonality, Bio 18 = precipitation of warmest quarter and Bio 19 = precipitation of coldest quarter

Environmental variables	Agri_Land	Bio 10	Bio 15	Bio 18	Bio 19	Bio 3	Bio 8	Elevation	Flow_Acc	Pop_Dens	Slope	Topo_Index
Agri_Land	1.00	0.22	-0.09	0.05	-0.04	-0.32	0.21	-0.34	0.01	0.03	-0.30	0.00
Bio 10	0.22	1.00	0.15	0.22	-0.03	0.28	0.71	-0.60	0.04	0.08	-0.33	-0.05
Bio 15	-0.09	0.15	1.00	0.25	-0.57	0.57	0.37	0.33	0.00	0.01	0.11	0.00
Bio 18	0.05	0.22	0.25	1.00	-0.03	0.00	0.39	-0.07	-0.02	0.08	0.25	0.02
Bio 19	-0.04	-0.03	-0.57	-0.03	1.00	-0.22	-0.40	-0.29	-0.01	0.01	0.05	0.01
Bio 3	-0.32	0.28	0.57	0.00	-0.22	1.00	0.20	0.28	0.00	-0.05	0.09	0.00
Bio 8	0.21	0.71	0.37	0.39	-0.40	0.20	1.00	-0.39	0.03	0.06	-0.30	-0.03
Elevation	-0.34	-0.60	0.33	-0.07	-0.29	0.28	-0.39	1.00	-0.05	-0.05	0.53	0.05
Flow_Acc	0.01	0.04	0.00	-0.02	-0.01	0.00	0.03	-0.05	1.00	0.00	-0.05	-0.16
Pop_Dens	0.03	0.08	0.01	0.08	0.01	-0.05	0.06	-0.05	0.00	1.00	-0.02	0.00
Slope	-0.30	-0.33	0.11	0.25	0.05	0.09	-0.30	0.53	-0.05	-0.02	1.00	0.05
Topo_Index	0.00	-0.05	0.00	0.02	0.01	0.00	-0.03	0.05	-0.16	0.00	0.05	1.00

Supplementary Table III Pearson correlation network of the environmental variables used for the development of Spotted Bass *Micropterus punctulatus* maxent model. Bio 1 = annual mean temperature, Bio 4 = temperature seasonality, Bio 7 = temperature annual range, Bio 9 = mean temperature of driest quarter, Bio 14 = precipitation of driest month Bio 18 = precipitation of warmest quarter and Bio 19 = precipitation of coldest quarter

Environmental variable	Agri_Land	Bio 1	Bio 14	Bio 18	Bio 19	Bio 4	Bio 7	Bio 9	Elevation	Flow_Acc	Pop_Dens	Slope	Topo_Index
Agri_Land	1.00	-0.03	0.05	0.26	-0.05	0.36	0.27	-0.25	-0.36	0.01	-0.01	-0.31	0.00
Bio 1	-0.03	1.00	-0.10	0.09	-0.07	-0.69	-0.67	0.68	-0.29	0.03	0.02	-0.30	-0.03
Bio 14	0.05	-0.10	1.00	0.60	0.74	0.21	-0.03	0.05	-0.46	-0.01	0.02	0.01	0.01
Bio 18	0.26	0.09	0.60	1.00	0.30	0.13	-0.09	-0.18	-0.49	-0.02	0.02	-0.19	0.02
Bio 19	-0.05	-0.07	0.74	0.30	1.00	0.01	-0.20	0.29	-0.33	-0.02	0.02	0.20	0.01
Bio 4	0.36	-0.69	0.21	0.13	0.01	1.00	0.93	-0.73	-0.26	0.01	-0.01	-0.17	-0.01
Bio 7	0.27	-0.67	-0.03	-0.09	-0.20	0.93	1.00	-0.70	-0.02	0.02	-0.03	-0.15	-0.02
Bio 9	-0.25	0.68	0.05	-0.18	0.29	-0.73	-0.70	1.00	0.00	0.01	0.01	0.10	-0.01
Elevation	-0.36	-0.29	-0.46	-0.49	-0.33	-0.26	-0.02	0.00	1.00	-0.05	-0.03	0.48	0.05
Flow_Acc	0.01	0.03	-0.01	-0.02	-0.02	0.01	0.02	0.01	-0.05	1.00	0.00	-0.05	-0.15
Pop_Dens	-0.01	0.02	0.02	0.02	0.02	-0.01	-0.03	0.01	-0.03	0.00	1.00	-0.02	0.00
Slope	-0.31	-0.30	0.01	-0.19	0.20	-0.17	-0.15	0.10	0.48	-0.05	-0.02	1.00	0.06
Topo_Index	0.00	-0.03	0.01	0.02	0.01	-0.01	-0.02	-0.01	0.05	-0.15	0.00	0.06	1.00